Development of Chemical Tools to Probe the Functions of TRIM Proteins

Jessica Katherine Reynolds

University College, University of Oxford

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Prof. Stuart Conway (University of Oxford)

Mr James Dodd (Vertex Pharmaceuticals)

Department of Chemistry

DECLARATION OF AUTHORSHIP

Name: JESSICA REYNOLDS **Candidate number: 200065**

College: UNIVERSITY COLLEGE **Supervisor: PROF. STUART CONWAY**

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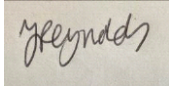
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Abstract

Epigenetic signalling is regulated by 'writer', 'reader', and 'eraser' proteins, which add, identify and remove histone modifications, respectively. Simultaneous recognition of multiple epigenetic marks by proteins containing tandem reader domains increases the complexity of the information transmitted, making these proteins interesting targets for probing epigenetic regulation. Tripartite motif-containing (TRIM) protein 24 and 33 are transcriptional regulators, each containing a C-terminal tandem PHD-bromodomain motif which recognises methylation states and acetylation of modified histone lysine residues. TRIM33 functions within the PARP-dependent DNA damage response pathway, where it is responsible for the timely removal of chromatin-remodelling enzyme ALC1 from sites of DNA damage through its bromodomain. Loss of TRIM33 causes prolonged association of ALC1, and therefore extended chromatin relaxation, which leaves DNA exposed and increases the susceptibility of cells to further DNA damage.

The aim of this study was to develop tool compounds to probe the functions of TRIM33 in epigenetic regulation, and to validate this protein as a therapeutic target in situations where DNA damage is increased. This work describes the two approaches taken; targeting the bromodomain with small molecule ligands, and incorporating these ligands into the design of proteolysis-targeting chimeras (PROTACs) to induce degradation of TRIM33 in cells. Initial work identified **16** as binding to the bromodomains of TRIM33 and TRIM24. Subsequent removal of the sulfonamide moiety and extension of the amine chain in **25** gave selective binding of the TRIM33 bromodomain over the KAc binding pocket of TRIM24. The ionic interaction between E981 and the protonated benzylic amine proved essential for both TRIM33 affinity and selectivity. Dose-response AlphaScreen™, WaterLOGSY NMR experiments, and ITC were used to evaluate ligand binding. Two additional acetyl-lysine mimicking head groups were assessed, but the original benzimidazolone core appeared to produce the optimal orientation of the head group relative to N1039, the residue responsible for recognition of acetylated lysine residues. Probing the hydrophobic region above the KAc binding pocket through lipophilic extension of the head group, sulfonamide projection adjacent to the benzylic amine, and projection of small groups from the

alternative vector, all reduced affinity for TRIM33β. Focusing on the amine chain, aiming to bias the 'pincer' interaction of the two amines around E981, produced several low micromolar ligands, including **114**, with selectivity over TRIM24. These are the first confirmed ligands for the TRIM33 bromodomain.

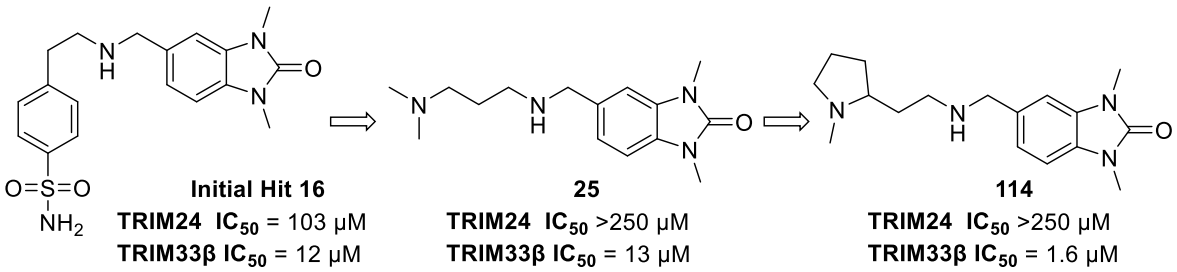


Figure 1. Summary of SAR. IC_{50} values were ascertained using a dose-response AlphaScreen™ with the bromodomain-binding $H3_{1-27}K18Ac$ peptide.

These ligands, and knowledge gained from the SAR studies, were subsequently used in the design of a series of TRIM33-targeting PROTACs. The initial PROTAC series incorporating ligand **25** had low cell permeability, with only three of the 17 PROTACs synthesised appearing in the cell at low concentrations. Consequently, no degradation of TRIM33 was observed in RKO cells. A second series was designed to increase cLogD values by focusing on alkyl linkers and masking hydrogen bond donors. This aimed to increase the concentration of PROTAC within the cell, thus improving the likelihood of ternary complex formation and degradation. Of the six PROTACs synthesised, three were shown to have high cell permeability. Assessment of these three PROTACs in RKO cells revealed that one PROTAC showed time-dependent reduction in TRIM33 levels, with the lowest levels showing around 70% reduction at 16 h after treatment. Therefore, this work describes the discovery of a range of chemical tools that can be used as a basis for further optimisation. This will ultimately enable us to establish the impact of either degrading or inhibiting the bromodomain of TRIM33 in cancer cells, in situations where DNA damage is increased such as radiotherapy.

Additionally, work to determine if TRIM24 can be recruited as an E3 ligase in PROTAC-mediated degradation of BRD4 was also explored, which has the potential to allow context-specific degradation, as TRIM24 is overexpressed in many cancers.

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Abbreviations

Å	Angström
Ac	Acetyl
ÄKTAFPLC	ÄKTA fast protein liquid chromatography
ALC1	Amplified in Liver Cancer 1
AlphaScreen™	Amplified Luminescent Proximity Homogeneous Assay Screen
AMP	Adenosine monophosphate
ATAD2	ATPase family AAA domain-containing protein 2
ATP	Adenosine triphosphate
a.u.	Arbitrary units
BAIB	(Diacetoxyiodo)benzene
BET	Bromodomain and extra terminal domain
BLAST	Basic local alignment search tool
β-ME	β-Mercaptoethanol
Boc	*tert*-Butoxycarbonyl
BPTF	Bromodomain plant homeodomain finger transcription factor
BRD	Bromodomain
BRD4	Bromodomain-containing protein 4
BRPF1	Bromodomain and plant homeodomain finger-containing
BSA	Bovine serum albumin

Bu	Butyl
cal	Calorie
CC	Coiled-coil
CDI	1,1’-Carbonyldiimidazole
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
cIAP1	Cellular Inhibitor of Apoptosis 1
COSY	Correlation spectroscopy
Cp*	Pentamethylcyclopentadiene
CpG	5'–C–phosphate–G–3'
CRABP-I	Cellular retinoic acid-binding protein I
CRABP-II	Cellular retinoic acid-binding protein II
CRBN	Cereblon
CREBBP	cAMP-response element-binding protein binding protein
CV	Column volume
Da	Daltons
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DC_{50}	Half-maximal degradation concentration
DCE	1,2-Dichloroethane
dec.	Decomposition
DIAD	Diisopropyl azodicarboxylate

DIBAL	Diisobutylaluminium hydride
DIPEA	*N*,*N*-Diisopropylethylamine
DMA	Dimethylacetamide
DMAP	*N*,*N*-Dimethyl-4-aminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPA	Diphenylphosphoryl azide
dppf	1,1'-Bis(diphenylphosphino)ferrocene
ε	Molar extinction coefficient
E1	Ubiquitin-activating enzyme
E2	Ubiquitin-conjugating enzyme
E3	Ubiquitin-protein ligase
eq.	Equivalents
ESI	Electrospray ionisation
Et	Ethyl
FAC	Final Assay Concentration
FRET	Förster Resonance Energy Transfer
g	Grams

ΔG	Gibbs free energy changes
GCI	Grand Canonical Integration
GCMC	Grand Canonical Monte Carlo
GROMACS	GROningen MAchine for Chemical Simulations
ΔH	Enthalpy changes
h	Hours
H3	Histone 3
HATs	Histone acetyltransferases
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HDACs	Histone deacetylases
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HIF-1α	Hypoxia-inducible factor 1-alpha
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple bond correlation
HMT	Histone methyltransferase
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
IC_{50}	Half maximal inhibitory concentration

IMAC	Immobilised metal affinity chromatography
IMiDs	Immunomodulatory imide drugs
IPA	Isopropyl alcohol
^{i}Pr	Isopropyl
IPTG	Isopropyl β-D-1-thiogalactopyranoside
ITC	Isothermal Titration Calorimetry
J	Coupling constant
J	Joules
KAc	Acetylated lysine
K_D	Dissociation constant
LE	Ligand efficiency
lit.	Literature value
LOD	Level of detection
LRMS	Low resolution mass spectrometry
M	Molar
m.p.	Melting point
MD	Molecular dynamics
MDM2	Mouse Double Minute 2 homologue
Me	Methyl
mg	Milligrams

MHz	Megahertz
min	Minutes
mL	Millilitres
mol	Moles
MW	Molecular weight
NBS	*N*-Bromosuccinimide
NMP	*N*-Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
Nosyl	Nitrobenzenesulfonyl
OD	Optical density
PAINS	Pan-assay interference compounds
PARP	Poly(ADP-ribose) polymerase
PBS	Phosphate-buffered saline
PDB	Protein Data Bank
PEG	Polyethylene glycol
Ph	Phenyl
PHD	Plant homeodomain
PHF21A	Plant homeodomain finger 21A
PP_i	Pyrophosphate

PPI	Protein-protein interactions
ppm	Parts per million
Pr	Propyl
PROTAC	Proteolysis-targeting chimera
PTMs	Post-translational modifications
RBCC	Collection of Really Interesting New Gene domain, B-Boxes and Coiled-coil region
R_f	Retention factor
RING	Rreally interesting new gene
RKO	Human colon carcinoma cell line
rt	Room temperature
ΔS	Entropy changes
SAR	Structure-activity relationship
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEC	Size-exclusion chromatography
SFI	Solubility forecast index
SGC	Structural Genomics Consortium
shRNA	Short hairpin ribonucleic acid
siRNA	Small interfering ribonucleic acid
SMAD	Mothers Against Decapentaplegic
S_NAr	Nucleophilic aromatic substitution

SPR	Surface plasmon resonance
sRNA	Small ribonucleic acid
*β*TrCP	beta-Transducin repeat containing protein
TBAF	Tetra-n-butylammonium fluoride
t-Bu	*tert*-Butyl
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
Tf	Triflate
TFA	Trifluoroacetic acid
TGF-β	Transforming growth factor-beta
THF	Tetrahydrofuran
TIF1	Transcriptional Intermediary Factor 1
TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
TMS	Trimethylsilyl
ToF	Time of flight
TPSA	Topological polar surface area
TRIM	Tripartite motif-containing
Troc	2,2,2-Trichloroethoxycarbonyl
Ts	Tosyl
Ub	Ubiquitin

UPS	Ubiquitin-proteasome system
UV	Ultraviolet
VHL	Von-Hippel Lindau
VT	Variable temperature
WaterLOGSY	Water-ligand observed *via* gradient spectroscopy
WD	beta-Transducin repeat often terminating in a tryptophan-aspartic acid (W-D) dipeptide
WT	Wild type

Chapter 1 Introduction

1.1 Epigenetics

Epigenetics is the term used to describe heritable changes in phenotype, which are a result of chromosomal modifications, as opposed to alterations in the underlying DNA sequence.[1,2] First described in 1942,[3] the field of epigenetics demonstrates that the information content of the genome extends past the sequence of bases in DNA. This concept helps to explain the diversity of cell type in a human body, where each cell type displays specific characteristics and biological functions, despite possessing the same genetic material.[4] An important class of stable epigenetic modification is covalent DNA methylation which, in mammalian cells, involves methylation of cytosine in CpG dinucleotides at position C5.[5] Around 60–80% of the 29 million CpGs present in the human genome are methylated,[6] and this type of epigenetic mark plays a significant role in development and maintenance of cellular identity, and in disease progression.[7]

Post-translational modifications of histones are another major class of epigenetic modification, which do not directly modify DNA, and help to establish a higher level of genetic diversity.[5] Histones are small, positively charged proteins found in the nuclei of eukaryotic cells. DNA is packaged around histone octamers, which are made up of four histone partners: an H3-H4 tetramer and two H2A-H2B dimers, to form structural units called nucleosomes.[8] These nucleosomes are tightly coiled into a polymer known as chromatin. Chromatin can have two forms: heterochromatin is the densely packed inactive state where transcription is repressed, and euchromatin describes the decondensed form which is transcriptionally active.[9] It is now known that histones are not just involved in the packing of DNA, but also in regulation of gene expression through covalent post-translational modifications of histone tails.[8] There are over 50 types of histone modifications, which include acetylation, methylation, ubiquitination and phosphorylation. It was originally thought that these modifications caused downstream biological effects due to change in charge – for example, acetylation neutralises the positive charge of lysine residues in histone tails, which would reduce the electrostatic interaction with negatively charged

DNA, thus allowing chromatin relaxation and access to DNA for transcription. However, it is now accepted that these modifications also recruit chromatin remodelling enzymes that reposition nucleosomes, and mediate protein-protein interactions.[10] While the N-terminal tails of histones are the main sites for modification, residues found within the globular histone core can also be modified. Disruption of these epigenetic mechanisms can lead to irregular gene expression or silencing, causing major pathologies, including cancer and conditions involving chromosomal instabilities.[11] It is therefore important that these processes are tightly regulated in cells.

Epigenetic signalling is controlled by 'writer', 'reader', and 'eraser' proteins, which add, identify and remove modifications, respectively (**Figure 1.1**). Specific histone marks can act successively or in combination to make up what is known as the 'histone code',[12] which regulates gene expression, leading to diverse and complex downstream effects.[9,13] The recognition of multiple proximal sites of epigenetic marks simultaneously through tandem reader domains in proteins further adds to the complexity of information available without the need for novel reader domains, which is clearly evolutionarily advantageous.

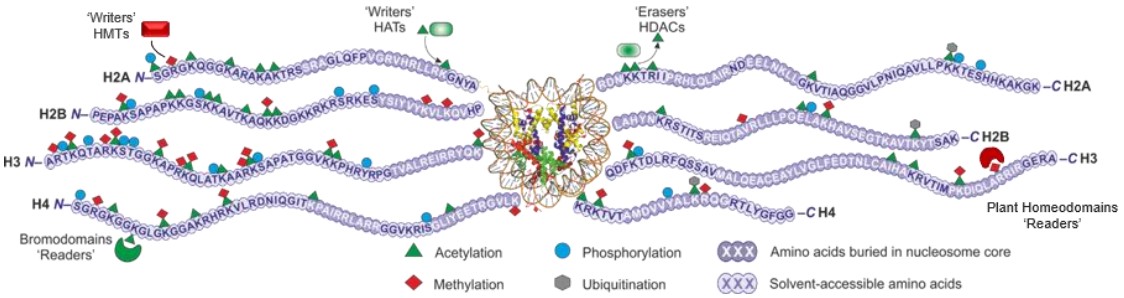


Figure 1.1. A diagram showing a nucleosome which contains DNA wrapped around histone octamers, with the histone tails exposed. Different modifications are shown on the histone tails, with focus on acetyl and methyl groups. Histone acetyltransferases (HATs) and histone methyltransferases (HMT) add, bromodomains (BRDs) and plant homeodomains (PHDs) read and histone deacetylases (HDACs) and histone demethylases remove these marks, respectively. Adapted with permission from Jennings, L. E., Measures, A. R., Wilson, B. G., Conway, S. J., *Future Medicinal Chemistry*, 2014, https://doi.org/10.4155/fmc.13.197. Copyright 2014, Future Science Group.[14]

Addition and removal of acetylation at lysine residues are regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively, whereas bromodomains (BRDs) are responsible for reading this post-translational modification to produce specific biological outcomes.[14] Another ubiquitous epigenetic modification is methylation of histones, the addition of which is catalysed by histone methyltransferases,[15] removed by histone demethylases,[16] and

recognised by plant homeodomain (PHD) fingers.[17] Proteins involved in epigenetic regulation, and therefore gene expression, have wide ranging effects in many diseases such as cancer, inflammation and neuropsychiatric disorders.[4] Exploring the function of these proteins is essential as they are important targets for novel therapeutic studies.[18]

1.2 Epigenetics Readers

1.2.1 Bromodomains (BRDs)

Bromodomains (BRDs) are structurally conserved protein modules, approximately 110 amino acids in size, and are epigenetic readers that bind to acetylated lysine (KAc) residues, commonly on the N-terminal tails of histones.[19] There are 61 known bromodomains in the human proteome, which can be grouped into eight families based on structure alignment (**Figure 1.2**).[20]

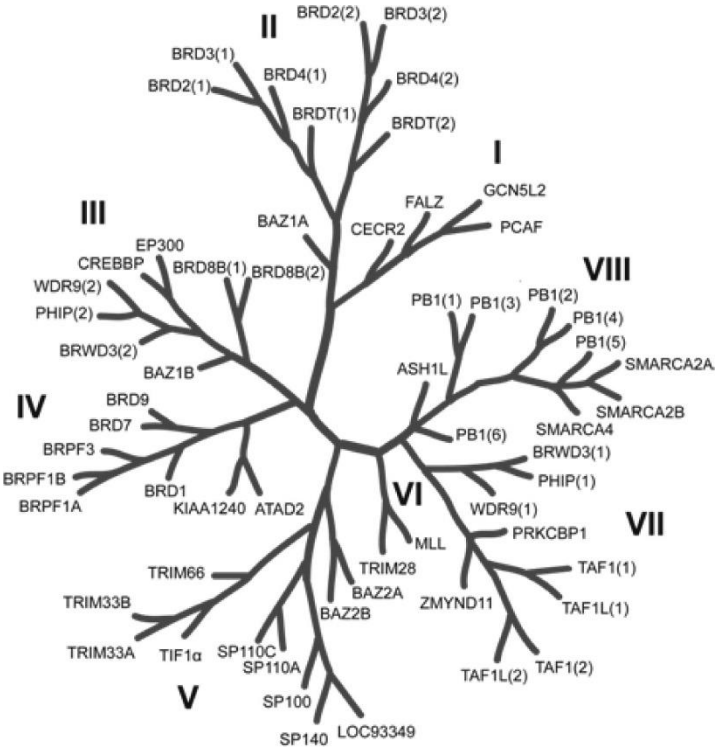


Figure 1.2. Phylogenetic tree showing the eight human bromodomain families, grouped using a structure-based alignment and indicated by the use of Roman numerals. Reprinted with permission from Hewing, D. S., Rooney, T. P. C., Jennings, L. E., Hay, D. A., Schofield, C. J., Brennan, P. E., Knapp, S., Conway, S. J., *J. Med. Chem.* 2012, 55, 22, 9393-9413, https://doi.org/10.1021/jm300915b. Copyright 2012 American Chemical Society.[13]

Bromodomains are characterised by a left-handed bundle of four conserved α-helices, labelled αZ, αA, αB and αC. These are linked by the ZA and BC loops, which have low levels of sequence

homology and account for the different binding partner specificities between bromodomains.[20] Conversely, the characteristic acetylated lysine binding pocket contains highly conserved residues, with most bromodomains containing an asparagine that hydrogen bonds to acetylated lysine residues in modified histones (**Figure 1.3**).[13] Four or five water molecules typically reside in the base of the pocket, despite most binding pockets being hydrophobic in nature.[21]

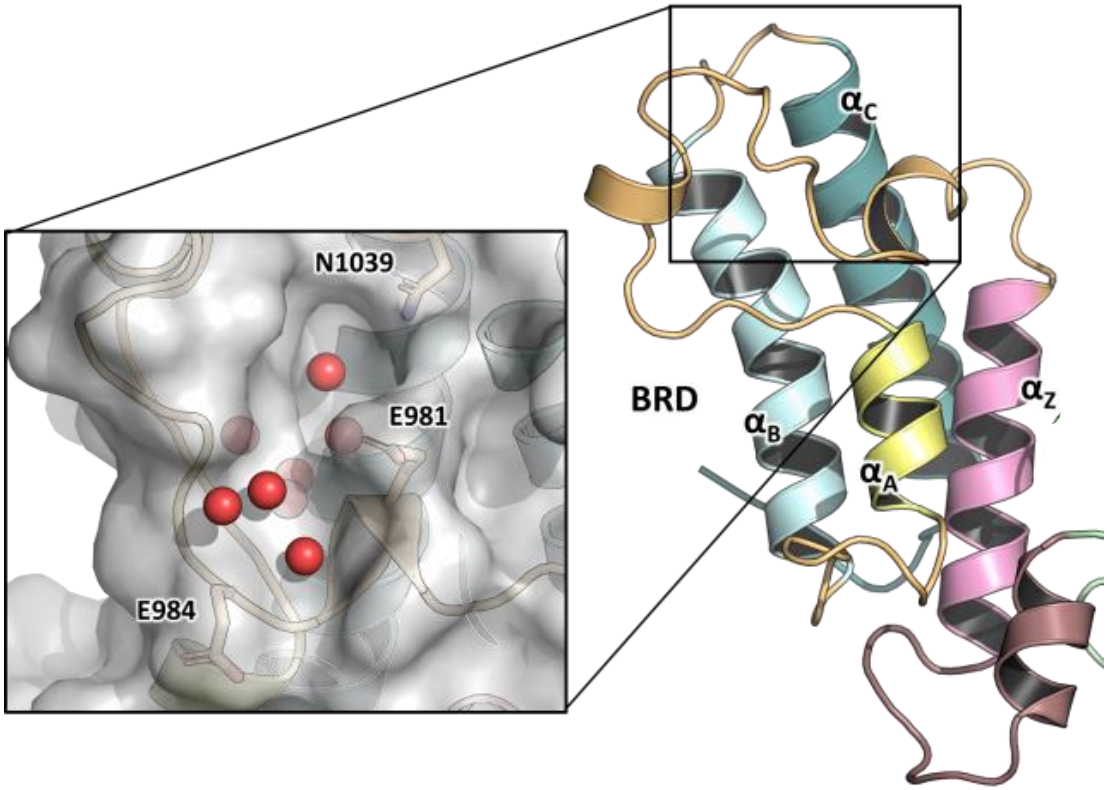


Figure 1.3. Bromodomain structure of TRIM33β (PDB ID: **5MR8**), containing four conserved α-helices, with the acetylated lysine binding pocket highlighted. The binding pocket contains an asparagine residue N1039 and four conserved water molecules.

Bromodomains can act with other protein-interaction domains to ensure targeting of chromatin-modifying enzymes to specific sites.[22] Many bromodomains play significant roles in regulating gene expression, meaning KAc binding pockets have become attractive therapeutic targets.[23]

1.2.2 Plant Homeodomains (PHDs)

PHD fingers are small protein modules, typically containing between 50 and 80 amino acid residues. There is a diverse range of sequences across the PHD finger family, allowing a high level of target specificity.[24] PHD fingers are characterised by a canonical Cys4-His-Cys3 motif which coordinates two zinc ions,[17] and a well-conserved PHD fold, which consists of two

distinctive antiparallel β-sheets (β1 and β2) stabilised by the Zn^{2+} ions and anchored by the Cys4-His-Cys3 motif in a cross-brace topology (**Figure 1.4**).[24]

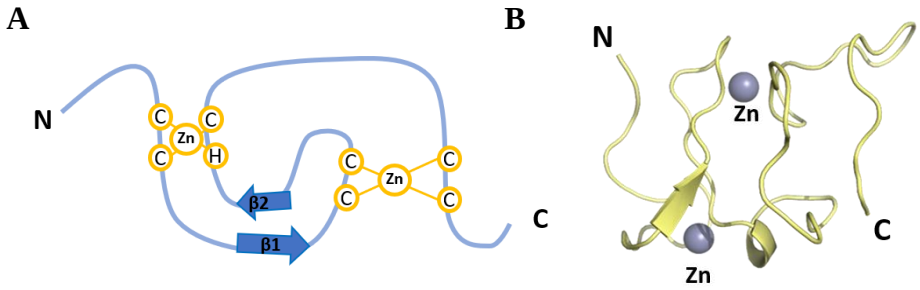


Figure 1.4. A. Schematic of the PHD finger fold, which contains the canonical Cys4-His-Cys3 sequence. This coordinates two zinc ions and forms the two-stranded antiparallel β-sheet structure. **B.** Structure of the PHD finger in TRIM33β (PDB ID: **5MR8**).

Lysine residues have a pK_a value of 10.5, and are therefore protonated at physiological pH. They can be methylated up to three times, so methylation has no effect on the overall residue charge. Nevertheless, methylation increases hydrophobicity and therefore decreases solvation by water molecules.[25] PHD fingers are 'readers' that recognise these different methylation states of lysine residues; trimethylation is frequently recognised through interaction with an aromatic cage structure, as in reader protein BPTF, which is made up of either three or four phenylalanine, tryptophan and tyrosine residues (**Figure 1.5A**).[25] In the same sequence region in group I PHD fingers, unmethylated $H3K4Me_0$ is recognised by interaction with acidic or hydrophobic residues, as in TRIM24 or PHF21A (**Figure 1.5B**).[24,26,27]

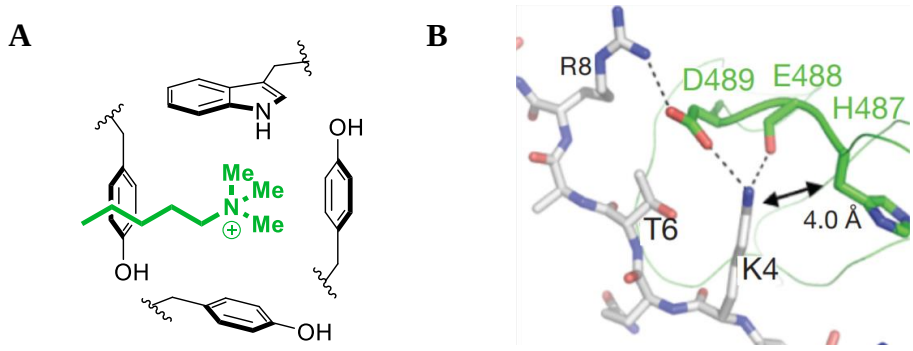


Figure 1.5. A. Representation of the aromatic cage within the PHD finger of BPTF recognising a KMe_3 residue.[28] **B.** PHD finger of PHF21A recognising unmethylated $K4Me_0$ on a H3 peptide through acidic residues D489 and E488. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Recognition of unmethylated histone H3 lysine 4 links BHC80 to LSD1-mediated gene repression, Fei Lan, Robert E. Collins, Rossella De Cegli, Roman Alpatov, John R. Horton, Xiaobing Shi, Or Gozani, Xiaodong Cheng & Yang Shi, 448, 718–722, Copyright Springer Nature, 2007.[27]

PHD fingers are often involved in chromatin remodelling, and work in tandem with other domains to complete functions including mechanisms for inheritance of DNA methylation.[12]

1.3 Small Molecule Ligands Targeting Epigenetic Readers

The 'histone code' hypothesis, introduced above, puts forward the idea that post-translational modifications form a pattern of marks that can be read in succession or simultaneously to produce specific downstream functions. Altering one mark is thought to affect the role of proximal marks.[29] This hypothesis presents opportunities to target 'reader' domains through small molecule inhibition, therefore preventing translation of these modifications into biological outcomes, allowing elucidation of the function of individual protein domains and their place in wider multi-domain processes. Subsequently, this could allow therapeutic intervention in a wide variety of diseases including cancers and inflammation.[30]

1.3.1 Targeting Bromodomains

The first small molecule bromodomain ligand (**1**, **Figure 1.6**) was published in 2007, targeting Human Immunodeficiency Virus 1 (HIV). Transcriptional activation of HIV provirus involves an interaction between the bromodomain of transcriptional co-activator p300/CREB binding protein-associated factor and the acetylated lysine 50 HIV-1 transactivator Tat. Preventing this interaction through bromodomain inhibition reduces Tat-mediated viral transcription.[31,32] Compound **1** does not appear to act as a classic acetyl-lysine mimic, instead gaining affinity from hydrophobic interactions and displacement of water molecules from the binding site.[31] However, it was the publication of the selective and potent inhibitors (+)-JQ1 (**2**)[33] and I-BET762 (**3**)[34] (**Figure 1.6**), and the antiproliferative effects seen in BRD4-dependent cell lines on treatment of (+)-JQ1 (**2**),[33] that sparked significant interest in the field for drug discovery and probe development. These two bromodomain ligands target the bromodomain and extra terminal domain (BET) family of proteins. Subsequently, several groups independently discovered 3,5-dimethylisoxazoles can act as an acetyl-lysine bioisostere.[35,36] Hewings *et al.* published a high affinity BRD4(1) ligand

OXFBD02 (**4**) containing a 3,5-dimethylisoxazole core,[37] which was later optimised to improve affinity and metabolic stability (**OXFBD04** (**5**), **Figure 1.6**).[38]

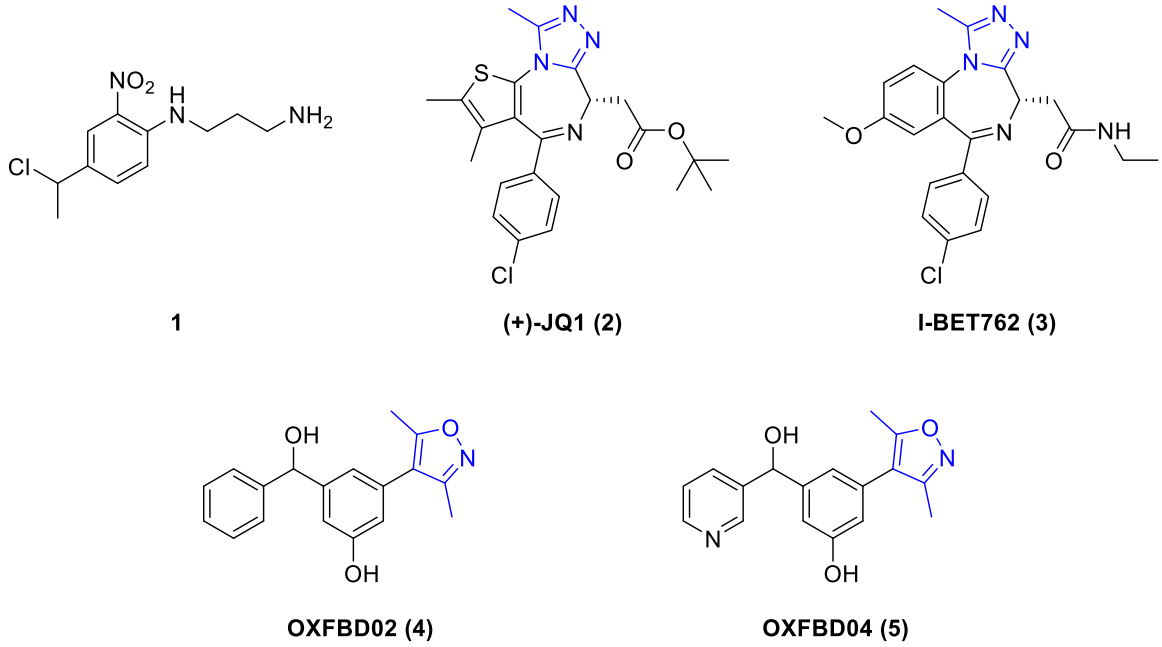


Figure 1.6. Structures of bromodomain ligands,[31,33,34,37,38] with acetyl-lysine mimetics highlighted in blue.

Small molecule inhibitors of non-BET bromodomains have also been reported, including molecules targeting ATPase family AAA domain-containing protein 2 (ATAD2),[39] cAMP-response element-binding protein binding protein (CREBBP),[40] and dual inhibitors of bromodomain and PHD finger containing protein 1 (BRPF1) and tripartite motif-containing protein 24 (TRIM24).[41,42] There are currently many bromodomain-targeting ligands in clinical trials[43,44] for diseases including cancers[45,46] and atherosclerosis.[47] The advances in chemical probes for these domains have helped to validate bromodomains as pharmaceutical targets, and provided a chemical scaffold to further develop drug molecules with good pharmacokinetic properties.[48,49] The ongoing development of ligands for bromodomains is vital for the elucidation of biological mechanisms of action of bromodomain-containing proteins, and remains an important tool in therapeutic target validation.[50,51]

1.4 Tripartite Motif-containing Proteins

Bromodomains and PHDs are found in some of the tripartite motif-containing (TRIM) proteins, which are a large family of more than 80 known proteins.[52] This family is characterised by a

conserved sequence of domains in the N-terminal region, containing one Really Interesting New Gene (RING)-finger domain, one or two zinc-finger domains known as B boxes, and an associated coiled-coil region, collectively known as the RBCC motif (**Figure 1.7**).[53] The majority of the TRIM proteins possess E3 ubiquitin ligase activity through the RING-finger domain, with only eight TRIM proteins not containing this domain.[52] TRIM proteins are further divided into subclasses (C-I to C-XI, and UC) depending on the organisation of their domains.[54] A variety of homo- and heterodimers of the TRIM proteins have been described,[55,56] indicating that a switch in binding partners might contribute to the diverse substrate recognition.[52] The TRIM family play an important part in many cellular processes including intracellular signalling, transcriptional regulation, and roles in diseases including neuropsychiatric disorders such as multiple sclerosis,[57,58] and cancers.[53]



Figure 1.7. Sequence of domains for C-VI subfamily, which comprises TRIM24, TRIM28 and TRIM33.

TRIM24, TRIM28 and TRIM33 belong to the C-VI class of TRIM proteins, containing the characteristic RBCC motif in the N-terminal region and a tandem PHD-bromodomain in the C-terminal region (**Figure 1.7**).[52] TRIM66 also contains the PHD-bromodomain motif, but differs in structure from the other three proteins by the absence of the RING domain.[52] The presence of the adjacent epigenetic reader bromodomain and PHD allows the recognition of multiple histone modifications simultaneously. This combinatorial recognition might allow tighter binding affinities and increased specificity for the target protein.[26] For example, TRIM24 PHD-BRD binds to H3K4Me$_3$K23Ac with a dissociation constant (K_D) of 560 nM, whereas affinity for unmethylated H3K4Me$_0$K23Ac is increased to 96 nM, indicating that unmethylated H3K4 and acetylated H3K23 are read in a combinatorial manner on a single histone peptide by TRIM24.[26] A contrasting biological outcome may also be observed when the pattern of histone marks are different, as binding of these tandem domains to one modification may be dependent on another proximal mark.[59] Sequence alignments of the C-terminal PHD-BRD motif of the C-VI subfamily

indicate TRIM33β contains 68% similarity to TRIM24, 58% similarity to TRIM66, and 25% similarity to TRIM28. The sequence alignments were performed using the Basic Local Alignment Search Tool (BLAST) on https://blast.ncbi.nlm.nih.gov/.[60]

1.4.1 TRIM33

1.4.1.1 Structure

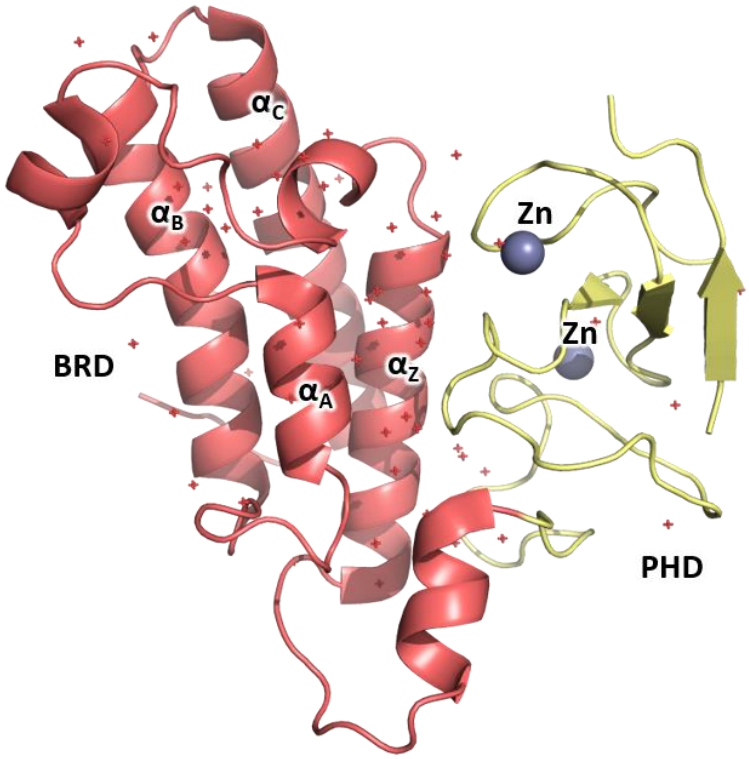


Figure 1.8. Structure of the TRIM33β bromodomain (pink) and PHD finger (yellow), PDB ID: **5MR8**.

TRIM33 (also known as Transcriptional Intermediary Factor TIF1γ) exists as two isoforms, α and β (**Figure 1.8**), with TRIM33α containing an extra 17 amino acids in the BC loop of the bromodomain. Due to this extension, it is hypothesised that TRIM33α has a non-canonical bromodomain, as the conserved asparagine residue known to recognise lysine acetylation is pointing out of the binding pocket (**Figure 1.9**). In contrast, the equivalent asparagine residue in TRIM33β is pointing into the binding pocket, forming a typical bromodomain structure.

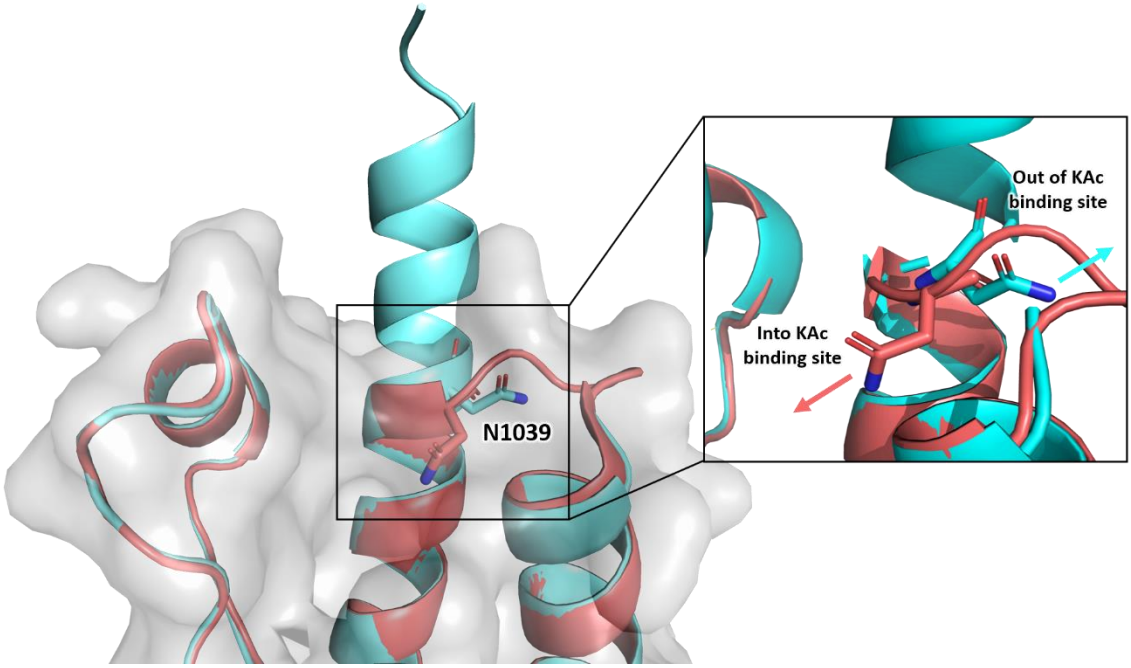


Figure 1.9. Overlay of TRIM33α (light blue, PDB ID: **3U5O**) and TRIM33β (pink, PDB ID: **5MR8**). The conserved asparagine residues responsible for recognising acetylated lysine residues (N1039) in each protein are highlighted. N1039 points out of the binding pocket in TRIM33α, and points into the binding pocket in TRIM33β.

1.4.1.2 Functions

TRIM33 is known to act as a tumour repressor through its role in human chronic myelomonocytic leukemia[61] and liver fibrosis,[62] as well as promoting chromosomal stability after mitosis.[63] TRIM33 also acts as a transforming growth factor beta (TGF-β) superfamily transcriptional repressor,[64] and, through the PHD-BRD motif, can bind to histone 3 (H3) tails when K4 is unmethylated and K18 and K23 are acetylated.[65] Studies indicate that the residence time of activated Mothers Against Decapentaplegic (SMAD) complexes at promoters of TGF-β target genes is controlled by TRIM33 through this chromatin binding event.[65] Binding of the tandem PHD-BRD to modified H3 was also found to activate the E3 ubiquitin ligase activity, where ubiquitination of SMAD4 was observed and not histone ubiquitination.[65] On the other hand, TRIM33 can also function as a tumour promotor by preventing apoptosis in B lymphoblastic leukaemia[66] and pancreatic cancer,[67] demonstrating that TRIM33 has numerous varied biological functions.[68]

Notably, TRIM33 plays a role in the poly(ADP-ribose) polymerase (PARP)-dependent DNA damage response pathway.[69] Activation of PARP1 at sites of DNA breaks allows recruitment of DNA repair proteins, including Amplified in Liver Cancer 1 (ALC1). This chromatin-remodelling enzyme is responsible for the relaxation of chromatin to reveal the damaged DNA to be repaired. Clearly, this process must be tightly regulated as relaxing chromatin for an extended period exposes DNA to further damage. TRIM33 is recruited to DNA damage sites in a PARP1- and ALC1-dependent manner, and interacts with ALC1 after DNA repair to ensure its timely removal.[69] Prolonged retention of ALC1 at DNA damage sites and increased sensitivity to DNA damaging agent bleomycin were observed in TRIM33-deficient cells.[69] On genetic deletion of the TRIM33 bromodomain, localisation of TRIM33 at sites of DNA damage was prevented. This indicates that TRIM33 bromodomain ligands could prevent TRIM33 binding to chromatin and therefore prevent removal of ALC1 from DNA damage sites, leaving the DNA exposed to further impairment by DNA damaging agents. TRIM33 and ALC1 are more highly expressed in a range of cancer cell lines compared to normal cell lines (work carried out in the laboratory of Professor Ester Hammond, unpublished data, **Figure 1.10**). It is hypothesised that targeting TRIM33 should disproportionately affect cancer cells due to ALC1 upregulation. Furthermore, siRNA-mediated knockdown of TRIM33 showed significant DNA damage and reduced colony survival of RKO cell lines in hypoxic conditions (<0.1% O_2). The data discussed indicate that targeting TRIM33 would be a powerful strategy to sensitise therapy-resistant hypoxic tumours to radiotherapy. (Professor Ester Hammond, unpublished data).

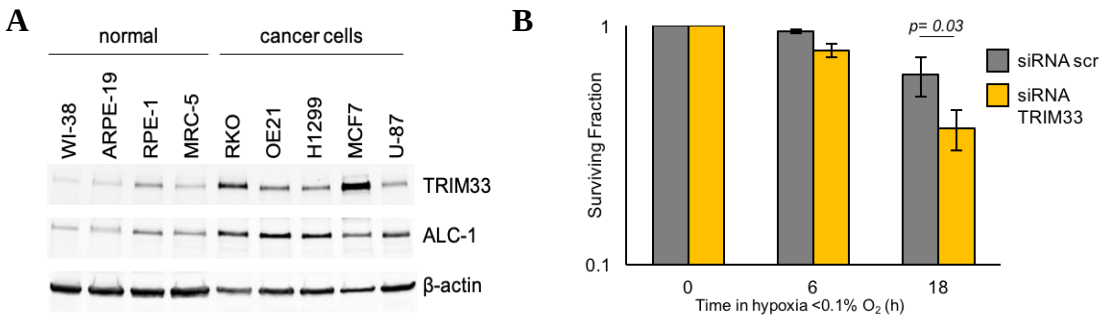


Figure 1.10. Unpublished work from the laboratory of Professor Ester Hammond. **A.** Expression of ALC1 and TRIM33 in normal and cancer cell lines, Western blot shown. **B.** siRNA-mediated knockdown of TRIM33 shows reduced colony survival of RKO cell lines in hypoxic conditions (<0.1% O_2) (scr = scramble).

1.4.1.3 Small Molecule Ligands for the TRIM33 Bromodomain

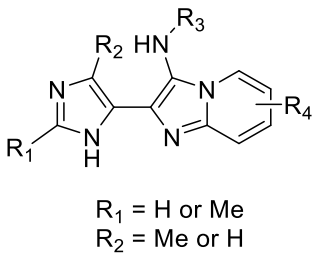


Figure 1.11. General structure of proposed TRIM33 ligands.[70]

In 2018, a patent was published which claimed to have developed high affinity small molecule inhibitors for the TRIM33 bromodomain (**Figure 1.11**).[70] The evidence presented for this claim included an AlphaScreen™ and a crystal structure of the protein with the small molecule bound, although this structure was not enclosed in the patent. Synthesis and biological evaluation of these ligands is discussed in **Chapter 2**. No other ligands have been reported for the TRIM33 bromodomain.

1.4.2 TRIM24

1.4.2.1 Structure

TRIM24 (also known as Transcriptional Intermediary Factor TIF1α) contains the RBCC motif and C-terminal PHD-BRD dual reader domains characteristic of the C-VI TRIM subfamily. The bromodomain has the classical BRD structure of four conserved α-helices connected by ZA and BC loops (**Figure 1.12**).[42] The conserved asparagine residue N980 recognises lysine acetylation K23 on H3 tails. Within the same histone tail, the PHD finger domain recognises unmodified H3K4.[26] This dual binding event increases affinity of TRIM24 for the H3K4Me$_0$K23Ac modified histone, with a K_D value of 96 nM determined by isothermal titration calorimetry (ITC), in contrast with a K_D value of 2.3 μM for unmodified H3K4K23 and a K_D value of 560 nM for H3K4Me$_3$K23Ac.[26] TRIM24 PHD-BRD has also been shown to bind to acetylated H3K9/K14 and H3K9Me$_1$.[26]

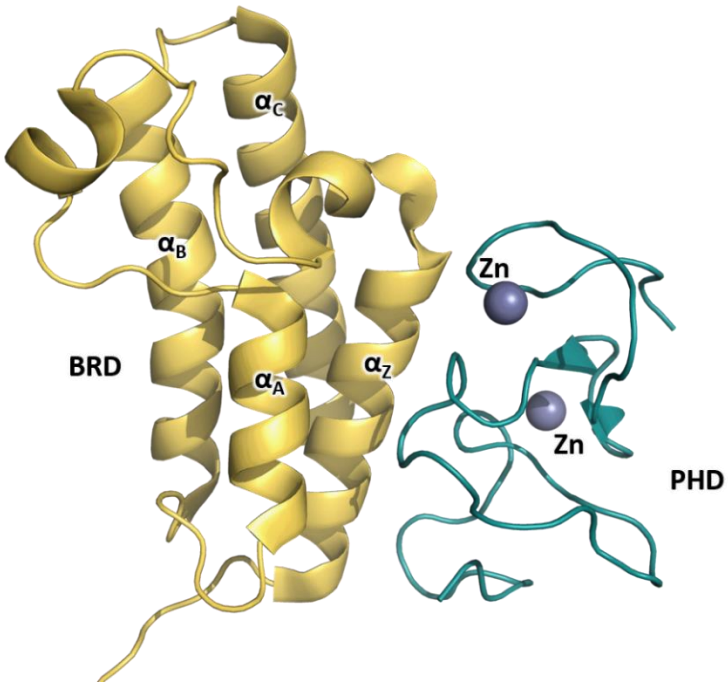


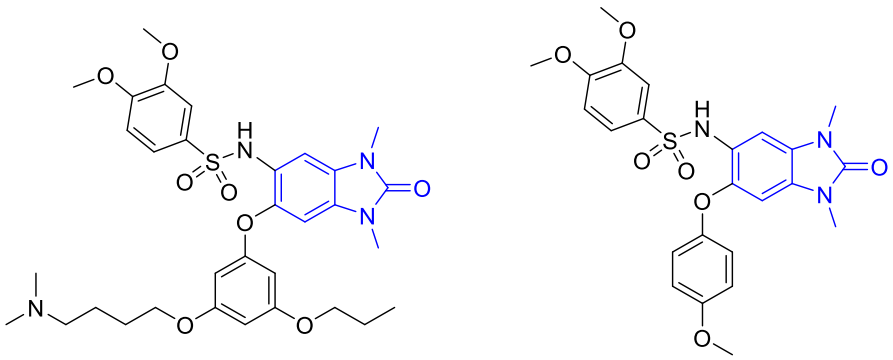
Figure 1.12. Structure of the TRIM24 bromodomain (yellow) and PHD finger (blue), PDB ID: **3O33**.

1.4.2.2 Functions

Through its RING domain, TRIM24 functions as an E3 ubiquitin ligase, targeting p53 for ubiquitination and degradation.[71] The single conserved LxxLL motif is involved with nuclear receptor co-activation or co-repressions in a context-dependent manner.[72] Overexpression of TRIM24 has been connected to tumour progression and poor prognosis in breast cancer,[26] and significant upregulation has been observed in many other cancers including gastric cancer,[73] non-small cell lung cancer,[74] leukaemia,[72] prostate cancer,[75] and hepatocellular carcinoma.[76] Knockdown of TRIM24 in colon cancer cell lines suppressed tumour growth and induced apoptosis.[77] Despite mouse models indicating that TRIM24 can act as a liver-specific tumour suppressor,[78] most studies show that TRIM24 is an oncogene when overexpressed.

1.4.2.3 Small Molecule Ligands for TRIM24

Two small molecule ligands of the TRIM24 bromodomain were published in 2016, both containing a benzimidazolone core as an acetyl-lysine mimic (**Figure 1.13**).[41,42]



IACS9571 (6) **SGC-34 (7)**

Figure 1.13. Structures of the TRIM24 bromodomain ligands. **6** has an IC_{50} value of 8 nM for TRIM24, and **7** has an IC_{50} value of 220 nM. Both structures contain a benzimidazolone core as an acetylated lysine mimic (blue) to interact with the conserved asparagine N980 in the bromodomain binding site.[41,42]

An X-ray crystal structure of ligand **6** (PDB ID: **4YC9**) showed the carbonyl of the head group interacting *via* a hydrogen bond with N980 as predicted, while the *O*-propyl group appears to be projecting into the LAF shelf, acquiring hydrophobic interactions. The terminal tertiary amine is interacting with D926 (**Figure 1.14**).

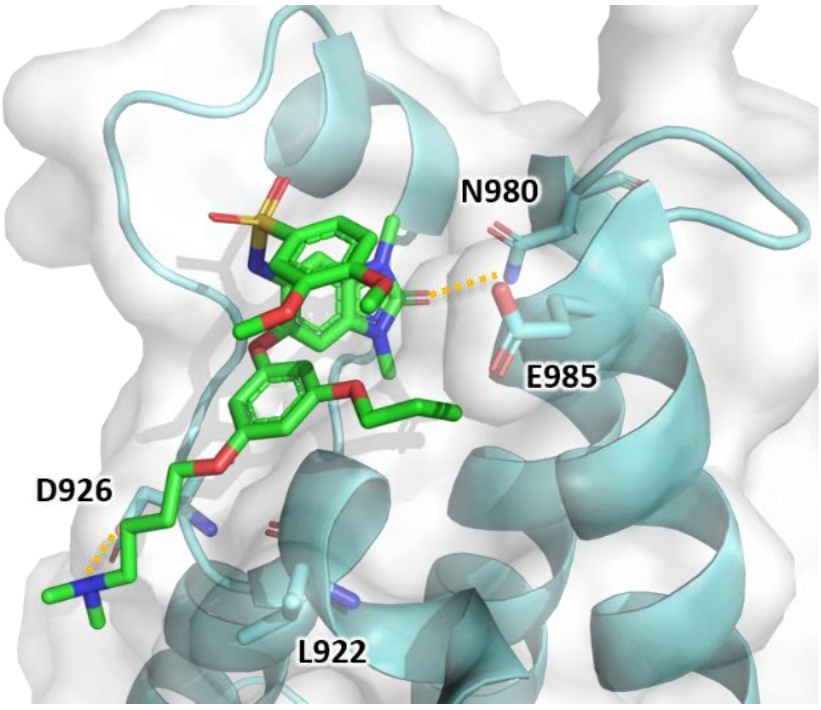


Figure 1.14. Ligand **6** bound to the TRIM24 bromodomain (PDB ID: **4YC9**).

Selectivity of **6** was determined using a BROMOscan® assay, which revealed no binding to the bromodomain of TRIM33.[42] A thermal shift assay indicated that **7** also did not bind to TRIM33.[41]

Despite the selectivity over all other bromodomains, both these ligands are dual inhibitors, binding to BRPF1 as well as TRIM24.[41,42]

1.5 Proteolysis-Targeting Chimeras (PROTACs)

Small molecule ligands are useful tools to probe the role of a certain domain within the context of wider protein function, and further development of these probes has led to clinical trial drug candidates. Conversely, proteolysis-targeting chimera technology is a method for exploring the role of a protein as a whole rather than focusing on a specific domain.

1.5.1 Ubiquitin-Proteasome System (UPS)

Having a designated system to degrade proteins in cells is essential for quality control during protein folding, allows the cell to respond quickly to changing cellular signals, and regulates the pool of accessible amino acids.[79] The majority of proteins are degraded *via* the ubiquitin-proteasome system (UPS). This is a tightly regulated system in which proteins to be degraded are covalently tagged with a small protein known as ubiquitin.[80] This process is carried out by three enzymes, with the first step involving an E1 ubiquitin-activating enzyme consuming ATP to produce the active ubiquitin–adenylate, which covalently attaches to a cysteine residue in the E1 active site (**Figure 1.15**). The subsequent thioester undergoes a transthiolation reaction to an E2 ubiquitin conjugating enzyme, and finally ubiquitin is conjugated to the substrate protein through a bridging E3 ubiquitin ligase, where an amide bond is formed between ubiquitin and lysine residues within the substrate. The cycle continues to allow additional ubiquitin molecules to conjugate to form a polyubiquitin chain. This polyubiquitinated protein is then recognised and degraded into short peptides by the proteasome, a large multi-catalytic protease.[81]

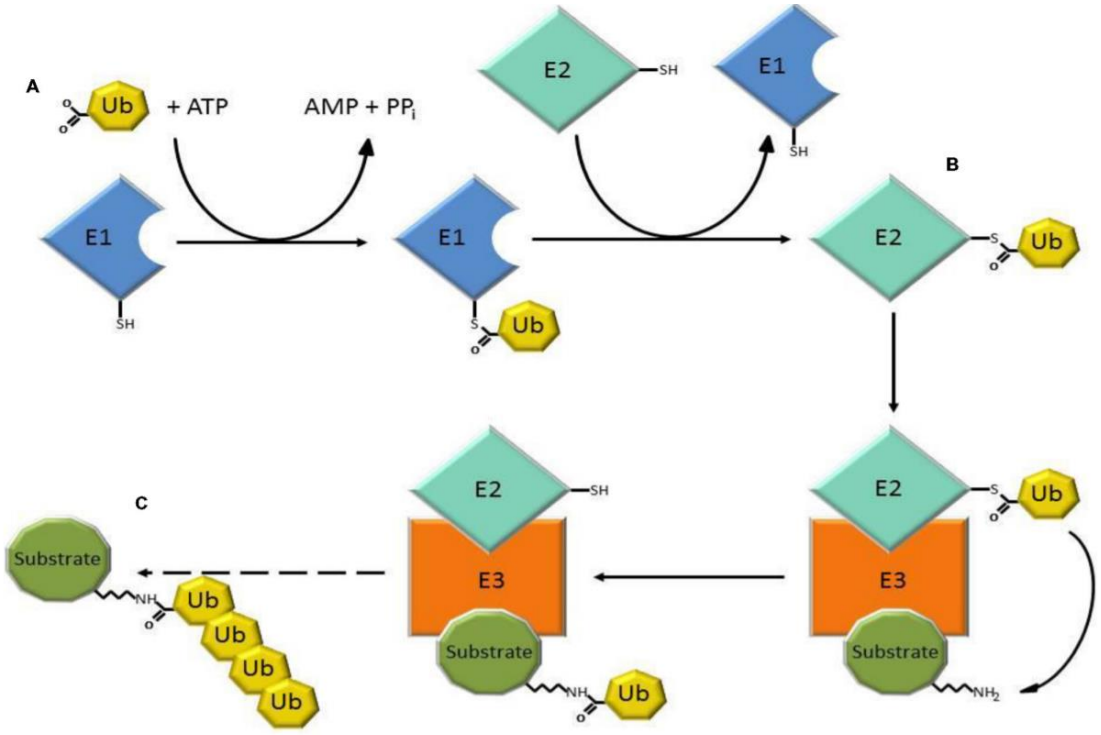


Figure 1.15. The ubiquitin-proteasome system marking a substrate for degradation at the proteasome. An E1 ubiquitin-activating ligase forms a thioester by reacting with ubiquitin. This is shuttled to an E2 ubiquitin conjugating ligase, and finally transfers to the substrate through the E3 ligase, which is responsible for target specificity. Polyubiquitin chains then form and the proteasome degrades the substrate. ATP = adenosine triphosphate, Ub = ubiquitin, AMP = adenosine monophosphate, PP_i = pyrophosphate. Reprinted with permission from Gong, B., Radulovic, M., Figueiredo-Pereira, M. E. & Cardozo, C., *Frontiers in Molecular Neuroscience*, 2016. Copyright 2016 Frontiers in Molecular Neuroscience.[80]

During this process, target specificity is dictated by the E3 ligases, as they recognise specific substrates through protein-protein interactions, and allow close proximity to the ubiquitination machinery.[81] Ubiquitination of proteins does not only induce degradation but, depending on the topology of the polyubiquitin chain, can also regulate many cellular processes, including signal transduction, DNA repair and cell division.[82–84]

1.5.2 PROTAC Mode of Action

Proteolysis-targeting chimeras (PROTACs) are heterobifunctional molecules designed to 'hijack' the ubiquitin-proteasome system.[79] These molecules typically contain a ligand for the protein of interest, which may not be a native substrate of the UPS, linked to a ligand for an E3 ubiquitin ligase.[79,85,86] When in a cellular environment, the PROTAC molecule forms a ternary complex with the protein of interest and the targeted E3 ubiquitin ligase, inducing proximity, which allows

polyubiquitination and subsequent degradation of the target protein at the proteasome (**Figure 1.16**). This process removes the whole protein of interest, allowing study of the entire protein function, as opposed to a single domain, as is the case with small molecule inhibition.[87]

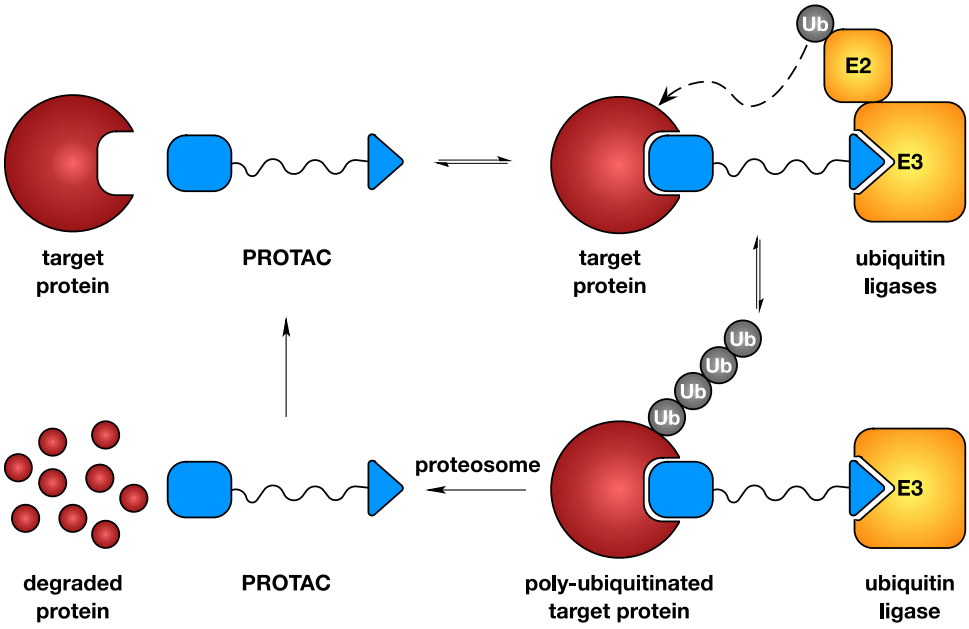


Figure 1.16. The catalytic cycle of a PROTAC molecule degrading a protein of interest. The PROTAC induces proximity between the target protein and an E3 ligase through formation of the ternary complex, which allows polyubiquitination and subsequent degradation at the proteasome. Reprinted with permission from Conway, S. J., *J. Med. Chem*. 2020, 63, 2802−2806. Copyright 2020 American Chemical Society.[88]

1.5.3 E3 Ubiquitin Ligases

Less than 10 of the predicted 600 E3 ligases in the human genome have been validated for PROTAC development.[89,90] The first effective PROTAC employed a phosphopeptide to effectively degrade the F-box and WD domain containing protein βTrCP.[91] The first synthetic heterobifunctional small molecule PROTAC was described in 2008,[92] which showed degradation of androgen receptor using E3 ligase Mouse Double Minute 2 homologue (MDM2). This was closely followed by the discovery of a degrader targeting cellular retinoic acid-binding proteins (CRABP-I and -II) using Cellular Inhibitor of Apoptosis 1 (cIAP1) as the recruited E3 ligase.[93] Subsequently, recruitment of the Von Hippel−Lindau disease tumour suppressor protein (VHL)[94] and Cereblon (CRBN)[95] as E3 ubiquitin ligases have been reported. The most targeted of these E3 ligases are VHL and CRBN due to the discovery of high affinity small molecule ligands for these proteins.[96] The first small molecule VHL ligand was discovered in 2012 (**8**, **Figure 1.17**),[97]

and further development of this compound led to higher affinity ligands **9** and **10**, which are the most commonly used in PROTAC design, with **9** binding to VHL with a K_D value of 0.185 μM.[96,98] Nutlin (**11**) is a *cis*-imidazoline analogue which binds to MDM2 with an IC_{50} of 0.26 μM.[99] Immunomodulatory imide drugs (IMiDs, **Figure 1.17**), including thalidomide (**12**), lenalidomide (**13**), and pomalidomide (**14**), were discovered as CRBN ligands with IC_{50} values of ~30 μM, ~3 μM and ~3 μM respectively, and can be used to recruit CRBN to act as an E3 ligase.[100,101]

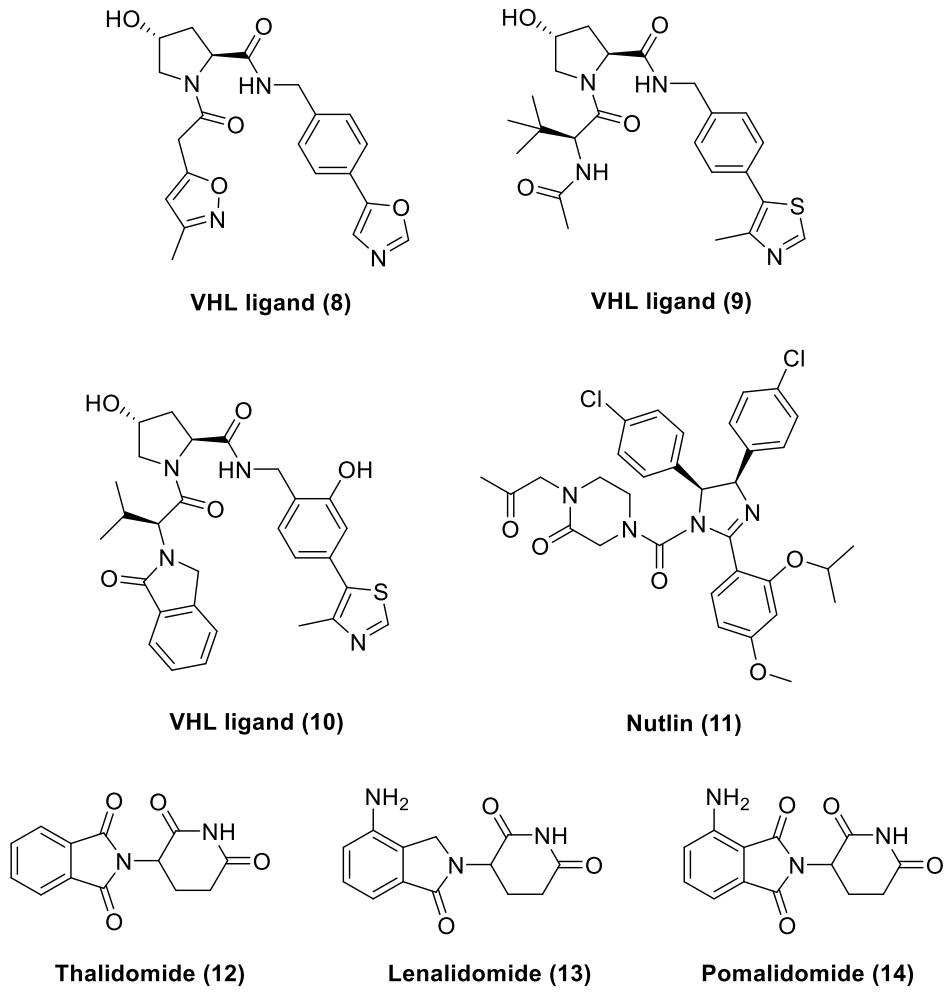


Figure 1.17. Small molecule ligands for E3 ubiquitin ligases used in PROTAC design.[96,98,99,100,101]

Degradation profiles are dependent on the E3 ligase recruited,[102] which is mostly due to the specific nature of the ternary complex, but may also be dependent of the expression patterns of E3 ligases, which varies across cell types. This introduces the possibility of recruiting an E3 ligase that is highly overexpressed in certain contexts to selectively degrade a target protein in that

specific environment. TRIM24, as mentioned previously, is an oncoprotein with E3 ligase activity that is upregulated in a range of cancer cell lines, so recruiting TRIM24 using a PROTAC molecule could allow degradation to occur in a cancer cell-selective manner. Introducing a new E3 ligase for use in PROTAC technology will also offer the opportunity for new, previously 'undruggable' proteins to be targeted selectively.

1.5.4 Importance of the Ternary Complex

The formation and stability of the ternary complex is one of the most important factors determining the success of a PROTAC. Consequently, these heterobifunctional molecules can provide additional selectivity when compared to small molecule ligands. For example, intra-BET selectivity was observed using pan-selective BET targeting ligands.[103] Similarly, Crews *et al.* showed a promiscuous ligand that binds to over 50 kinases can be incorporated into PROTAC molecules that selectively degrade fewer than 15 kinases due to specific stabilising protein-protein interactions.[104] Different degradation profiles were also observed when different E3 ligases were recruited, which has also been demonstrated in other studies.[102] In the same study, Crews *et al.* demonstrated that there is no correlation between affinity of the ligand for the different kinases and the extent of PROTAC-induced degradation, as p38α was efficiently degraded with a half-maximal degradation concentration (DC_{50}) value of 210 nM, which is much lower than the ligand binding affinity (K_D = 11 μM).[104] These data support the hypothesis that the ternary complex stability is more important than small molecule ligand affinity for an effective degrader.

Identity of the ligand chosen to target the protein of interest is also important, which is attributed to the different exit vectors for linker projection changing the orientation of the protein of interest relative to the E3 ligase.[102,105] Changing the orientation of the E3 ligase relative to the target protein to enhance selectivity between protein isoforms has also been explored (**Figure 1.18**). By changing the VHL ligand, and therefore the exit vector for the linker, two PROTACs with the same target protein ligand were found to selectively degrade p38α over p38δ, and vice versa.[96] This is an excellent example of the level of selectivity that can be achieved with PROTAC

technology, and clearly demonstrates the importance of relative orientation of the two proteins in the ternary complex.

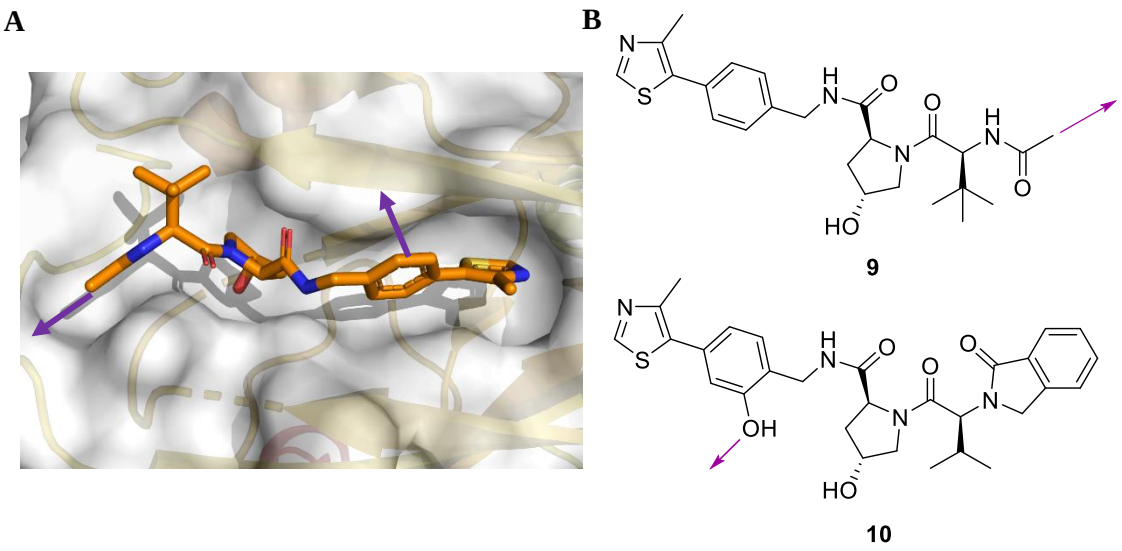


Figure 1.18. A. X-Ray crystal structure (PDB ID: **4W9H**) of VHL-recruiting ligand **9** shown in HIF-1α-binding pocket. Two arrows represent linker attachment points for PROTAC design. **B.** The structures of the two VHL-recruiting ligands, with **9** utilising an amide attachment vector and **10** with a phenyl attachment vector.

The linker length and composition also play a significant role in ternary complex stability. An increase in linker length by a few atoms can impact the selectivity of a PROTAC series.[106] These studies provide evidence that the specific nature of the ternary complex dictates the degradation profile.

1.5.5 Advantages Over Small Molecule Inhibition

Small-molecule drug discovery has traditionally focused on inhibition of proteins through targeting specific accessible active sites. There are many advantages of this approach, including short synthetic routes, easy derivatisation for optimisation and predictable pharmacokinetic properties. However, the method has significant limitations, as the protein of interest must have a 'druggable' site amenable to targeting with a small molecule, and targeting this 'druggable' site specifically – whilst all other domains are functional – must produce the desired therapeutic outcome.[107] There are examples of drug discovery programmes aiming to target a multidomain protein linked to disease phenotypes, only to discover the small molecule binds to an ineffectual domain.[72,108] There is also a need to maintain high systemic exposure to ensure the desired

outcome is achieved, which can increase the potential for off-target effects as high *in vivo* concentrations are frequently needed.[109,110] Furthermore, conformational changes caused by protein mutation can reduce interactions with ligands, introducing a mechanism of resistance.

Using PROTAC molecules to target proteins for degradation has many advantages over traditional small-molecule drug discovery.[111] The entire protein of interest is removed *in vivo*, suggesting the small molecule ligand incorporated into the PROTAC structure is not required to bind to a disease-relevant domain of a protein.[72] This could allow application of ligands that appeared to have no phenotypic effect against disease targets, and allow targeting of previously 'undruggable' proteins, notably proteins that have a disease-relevant domain that is not amenable to small molecule inhibition. Lower doses are often needed, as each PROTAC molecule can be involved in several degradation events and so are catalytic in their mechanism of action. Furthermore, due to the transient and catalytic nature of the interaction between a PROTAC and the target protein, drug resistance resulting from mutations of the target has not been observed.[112] A key step in the progression of the PROTAC field was the announcement of the first PROTAC molecules, ARV-110 and ARV-471, were set to enter Phase I clinical trials for treatment of prostate and breast cancer, respectively.[113,114]

1.5.6 Limitations of PROTAC Technology

With molecular weights typically exceeding 700 Da, PROTAC molecules fall outside of the traditional small molecule drug space.[115] This observation introduces important questions about cell permeability and oral bioavailability if PROTAC molecules are to be brought to market as viable drugs.[116,117] Intravenous administration of potential PROTAC drugs could overcome these issues, although this is one of the least preferable methods of delivery amongst patients.[118]

The hook effect is regularly observed when studying three-component systems such as PROTAC-induced degradation. It is the term used to describe reduced degradation at high PROTAC concentrations.[119] As the concentration of degrader increases to a significantly higher value than the half-maximal degradation concentration (DC_{50}), the binary PROTAC:E3 and

target:PROTAC complexes start to autoinhibit the formation of the ternary target:PROTAC:E3 complexes, so ubiquitination cannot occur and the percentage of degradation of the target protein reduces (**Figure 1.19**).[110] This means dosing of PROTAC molecules may be more challenging when compared to traditional small molecule drugs.[120]

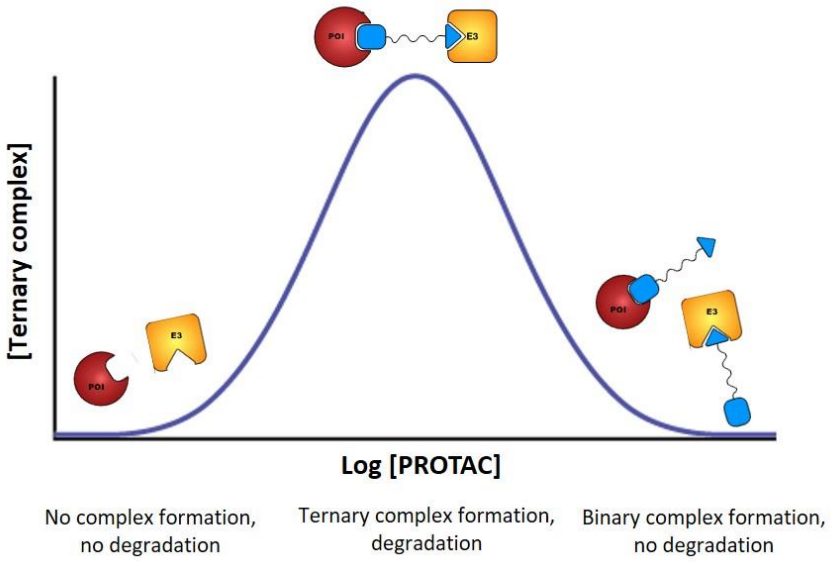


Figure 1.19. A graph indicating concentration of ternary complex as a function of PROTAC concentration. Increasing concentration of PROTAC increases the likelihood of ternary complex formation, until the binary complex begins to outcompete the ternary complex, reducing the degradation seen.

As previously discussed in **Chapter 1.5.4**, the nature of the protein-protein interactions involved in the ternary complex are significant in achieving degradation.[96] Despite this, there are currently very few examples of rational design of linker structure, linker length, and targeted E3 ligase.[121,122] *In silico* modelling still remains a challenge,[123] thus the current method for optimising PROTAC structure involves lengthy and costly synthesis of numerous analogues. Until a reliable and general method for rational design is available, this will remain the biggest challenge for progression of the PROTAC field.

1.5.7 Kinetics

The binding kinetics of ternary complex formation and stability are multifaceted, as three components are required to interact simultaneously. Moreover, the ternary complex stability is not directly proportional to the affinity of each ligand for the target proteins, as there may be

positive or negative cooperative effects of protein-protein interactions between the E3 ligase and the protein of interest.[96,124] In order to quantify the stability of these complexes, Ciulli *et al.* developed a surface plasmon resonance (SPR)-based assay to measure the kinetics of formation and dissociation *in vitro*.[124] Findings showed that positive cooperation for ternary complex formation, and therefore complexes with longer half-lives, correlated with a greater initial rate of intracellular degradation. This method was also used by Crews *et al.* to determine ternary complex affinity and kinetic measurements of an isoform-selective PROTAC, which showed a longer half-life for the p38δ:PROTAC:VHL complex compared to the p38α:PROTAC:VHL complex, and therefore selective degradation of p38δ.[96]

1.5.8 TRIM24 PROTAC

As discussed in **Chapter 1.4.2**, high levels of TRIM24 are associated with disease progression in cancers including breast and prostate cancers,[26,74,75] and genetic knockdown has shown anti-proliferative responses.[77] Despite the discovery of two high affinity TRIM24 bromodomain ligands, these compounds showed no significant anti-proliferative phenotypes,[125] indicating that targeting the bromodomain alone is not an appropriate therapeutic strategy.[41,42] However, when these ineffective TRIM24 bromodomain ligands were incorporated into a PROTAC molecule (**15**, **Figure 1.20**), recruiting VHL as the E3 ubiquitin ligase, effective and selective degradation of TRIM24 was observed.[72] The study also revealed that degradation resulted in increased anti-proliferative response, when compared to bromodomain inhibition. Through this work, TRIM24 was identified as a novel dependency in acute leukaemia.[72] These results indicate that many other selective but ineffective ligands could be incorporated into therapeutic molecules. Interestingly, TRIM24 has E3 ligase activity through the RING domain.[89] However, the study found that TRIM24 degradation was dependent on VHL, and not a consequence of autoubiquitination.[72] TRIM33 also contains a RING domain, and has a high sequence identity compared to TRIM24 (68%, BLAST),[60] thus the study provided evidence that degradation of TRIM33 using this strategy could be viable.

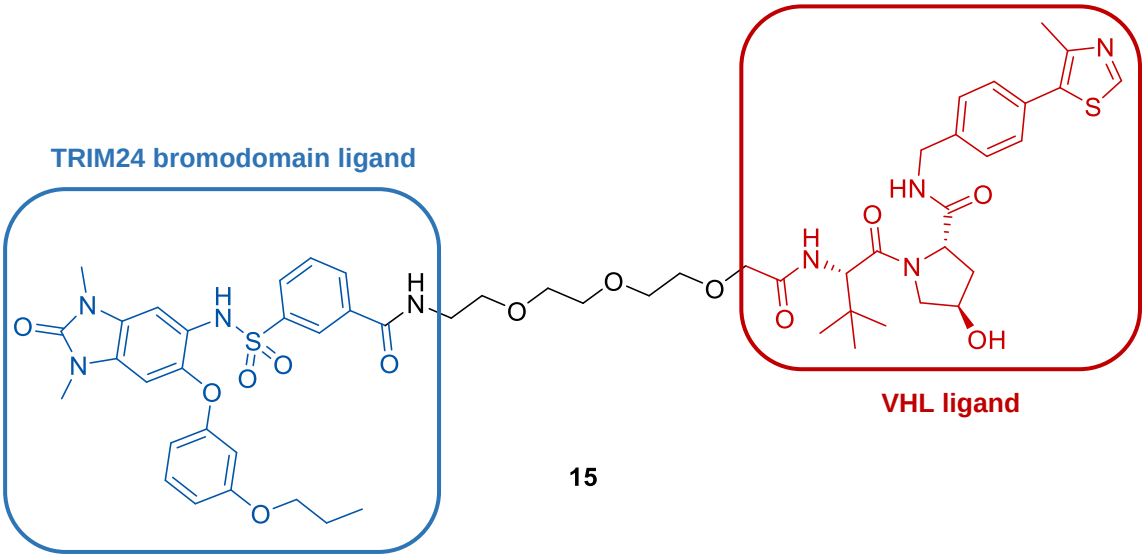


Figure 1.20. Structure of a degrader **15** of TRIM24 using the TRIM24 bromodomain ligand linked *via* a PEG linker to a VHL ligand.[72]

1.6 Assays

When searching for novel ligands for a target protein, assessing candidates using a variety of orthogonal techniques using fundamentally different principles is important to reduce the possibility of receiving a readout that could be misinterpreted. A series of promiscuous compound classes have been identified, known as pan-assay interference compounds (PAINS), which exhibit behaviour that could interfere in assay readouts, for example metal chelation, redox cycling, and protein reactivity.[126] Consequently, compounds that fall into these categories, such as dicyanoalkenes and isothiazolones,[127] should be treated with caution.

1.6.1 AlphaScreen™

1.6.1.1 AlphaScreen™ Principle

AlphaScreen™ (Amplified Luminescent Proximity Homogeneous Assay Screen) is a proximity-based assay, which relies on displacement of a binding partner, giving a fluorescent readout. The donor bead, which contains a phthalocyanine photosensitizer, excites ambient oxygen from a triplet to a singlet state upon irradiation at 680 nm (**Figure 1.21**). This singlet oxygen reacts with thioxene-based groups on the acceptor bead if it is within 200 nm of the donor bead. Dioxetane is formed, which undergoes first-order decay producing a chemiluminescence emission that is

transferred to anthracene. Through Förster Resonance Energy Transfer (FRET), anthracene transfers the signal to rubrene, which produces a fluorescence emission from 520–620 nm.[128,129]

When the target proteins are bromodomains, specific acetylated peptide sequences from histone H3 are used as binding partners, where affinity to the protein is known. The donor beads are streptavidin-coated, which interact with the selected biotinylated peptide. Nickel-chelating acceptor beads interact with the His-tagged protein of interest. Once a ligand is added, displacement of the peptide occurs as the ligand binds to the bromodomain, so the two beads are no longer in close proximity and there will be a reduction in the fluorescent readout. This technique can be used to quantify the affinity of ligands for the protein of interest through dose-response experiments, where the compound concentration is increased, and IC_{50} values can be determined.

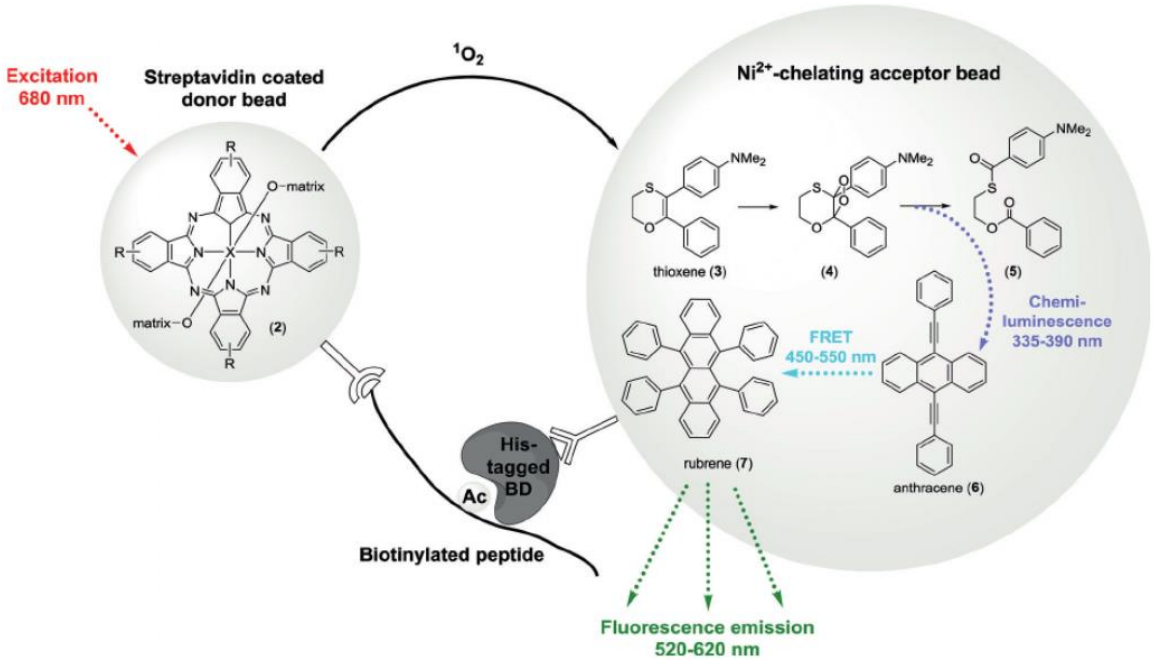


Figure 1.21. Representation of an AlphaScreen™ assay. Upon irradiation, the photosensitiser-containing donor bead produces singlet oxygen which, when in close proximity, interacts with the acceptor bead and produces a chemiluminescent signal which can be measured. The streptavidin-coated donor bead interacts with the biotinylated peptide, and the nickel-chelating acceptor bead interacts with the His-tagged protein. If a ligand is added which displaces the peptide, the donor and acceptor beads are no longer in close proximity meaning the emission is reduced. Reprinted with permission from John Wiley and Sons: Wiley, Angewandte Chemie International Edition, Schiedel, M., Moroglu, M., Ascough, D. M. H., Chamberlain, A. E. R., Kamps, J. J. A. G., Sekirnik, A. R., Conway, S. J., Chemical Epigenetics: The Impact of Chemical and Chemical Biology Techniques on Bromodomain Target Validation, 2019, 58, 50, 17930–17952, https://doi.org/10.1002/anie.201812164, Copyright 2019, John Wiley and Sons.[129]

AlphaScreen™ for use with TRIM24, TRIM33α and TRIM33β was developed and optimised by Dr Angelina Sekirnik[130] and Dr Larissa See[131]. Three histone peptides are biotinylated on the side chain of the C-terminal lysine (K27) and each have different modifications – trimethylated $H3_{1\text{-}27}K9Me_3$ binds to the PHD finger of each TRIM protein, acetylated $H3_{1\text{-}27}K18Ac$ binds to the bromodomain and the trimethylated and acetylated $H3_{1\text{-}27}K9Me_3K18Ac$ binds to both the PHD finger and the bromodomain. Disruption of a specific peptide-protein interaction should therefore indicate location of ligand binding. Compounds are tested against all three proteins with all three peptides, giving a total of nine combinations. However, the focus of this thesis is identifying TRIM33β bromodomain ligands, and therefore discussion will focus on the $H3_{1\text{-}27}K18Ac$ combination with TRIM33β.

Since AlphaScreen™ relies on peptide displacement, the IC_{50} values calculated are relative to the affinity of the peptide for the protein of interest. IC_{50} values of ligands can therefore be compared in the same protein-peptide combination, but not across different combinations.

1.6.1.2 AlphaScreen™ TruHits Assay

AlphaScreen™ is a useful assay as it enables high throughput of compounds. However, assay interference can arise from several compound classes; singlet oxygen quenchers, biotin mimetics, compounds absorbing at the wavelengths mentioned, and insoluble compounds forming aggregates.[128] An assay must therefore be used in tandem with AlphaScreen™ to detect ligands that fall into these classes and produce false readouts. AlphaScreen™ TruHits kit allows identification of different mechanisms of interference through two protocols. The first method involves pre-incubation of streptavidin-coated donor beads and biotinylated acceptor beads, which will produce a strong luminescent signal, followed by addition of compound. Since the assay is performed in the absence of protein and peptide, a reduction in the emission relative to a DMSO control indicates the compound is quenching singlet oxygen. The second method involves pre-incubation of the streptavidin-coated donor beads with the compounds, followed by addition of the biotinylated acceptor beads. Low emission would indicate the compound is a biotin mimetic and is competing for interactions with streptavidin. The interference is quantified by calculating

the emission difference from the DMSO control and normalising against the standard deviation of this control. If the emission is more than two standard deviations greater than the control, it is considered to be interfering with the assay.

1.6.2 NMR WaterLOGSY

WaterLOGSY (Water-Ligand Observed *via* Gradient Spectroscopy) is an 1D NMR-based assay used to identify protein-ligand interactions, relying on intermolecular nuclear Overhauser effect (NOE) and spin diffusion to allow transfer of magnetization.[132] During the experiment, the magnetization of bulk water molecules is excited and transferred *via* NOE signals to the ligand through several different mechanisms.[133] In these mechanisms, the interaction of a bound ligand with water is either through water-ligand-protein or protein-ligand complexes, both of which have a slow tumbling regime and therefore receive negative NOEs (**Figure 1.22**). In the case of a free ligand, the interaction is only through bulk water not binding to the protein, which experiences much faster tumbling and therefore receives positive NOEs. Consequently, non-binding is indicated by negative signals in the WaterLOGSY NMR spectrum of the ligand, while binding is shown by positive signals. Signals in the NMR spectrum are an average of positive and negative NOEs in the free and bound states, respectively, so reduced intensity negative signals relative to the reference spectrum may also indicate weak binding.[134]

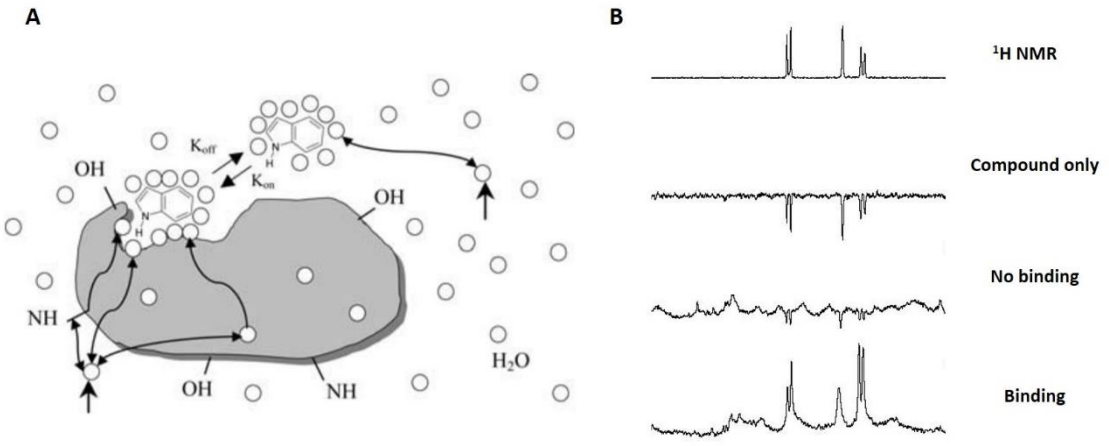


Figure 1.22. WaterLOGSY principle. **A.** A diagram showing the different mechanisms for magnetization transfer to bound and free ligands. Reprinted by permission from Springer Nature: Springer, Journal of Biomolecular NMR, Dalvit, C., Fogliatto, G., Stewart, A., Veronesi, M., Stockman, B., WaterLOGSY as a method for primary NMR screening: Practical aspects and range of applicability, 2001, 21, 349–359, https://doi.org/10.1023/A:1013302231549, Copyright 2001 Springer.[133] **B.** An example of WaterLOGSY NMR spectra. A WaterLOGSY experiment done in the absence of protein shows negative signals. On addition of a protein that the compound does not bind to, negative signals are still observed, but on binding, positive signals are seen.

This method as an orthogonal assay to AlphaScreen™ for the assessment of ligand binding to TRIM24, TRIM33α, and TRIM33β was optimised by Dr Larissa See and Dr Angelina Sekirnik.[130,131] In contrast to AlphaScreen™, WaterLOGSY experiments performed for this work do not give a quantitative readout, only a distinction between binding and non-binding events.

WaterLOGSY is generally used for weak binding ligands with micromolar or millimolar affinity for the protein of interest. Strong binders can appear as false negatives, with slow dissociation rates meaning the negative NOE signal is not transferred to the free population before relaxation. False positive readouts can be observed when compound aggregation occurs, but this is accounted for by running a WaterLOGSY experiment with the compound in the absence of protein. If aggregation is taking place, the NMR peaks will not be inverted, so the sample is diluted until no aggregation is seen.

1.6.3 Isothermal Titration Calorimetry

Isothermal Titration Calorimetry (ITC) is a quantitative technique used to establish thermodynamic parameters of the interaction of small molecules with macromolecules in solution. As the titration is performed at constant pressure and temperature, equilibrium

dissociation constants (K_D), enthalpy changes (ΔH) and binding stoichiometries (*n*) can be measured, which can therefore be used to calculate entropy changes (ΔS) and Gibbs free energy changes (ΔG) of the system.[135]

The experiment set-up consists of a sample cell containing one of the binding partners and a reference cell containing buffer enclosed in an adiabatic jacket (**Figure 1.23**). A constant power is applied to the reference cell, which activates a cell feedback circuit, and temperature differences between the reference cell and the sample cell are monitored. During the course of the experiment, the second binding partner is titrated into the sample cell in known aliquots, causing heat evolution or devolution, depending on the nature of the binding partner interaction. The time-dependent input of power needed to maintain the same temperatures between the sample and reference cells is measured. An exothermic interaction causes a reduction in feedback power to the sample cell in order to maintain equal temperature as the reference cell, and an endothermic interaction causes an increase in feedback power. The power required to maintain the reference and sample cells at the same temperature is plotted as a function of time, with each data point corresponding to one injection.[136]

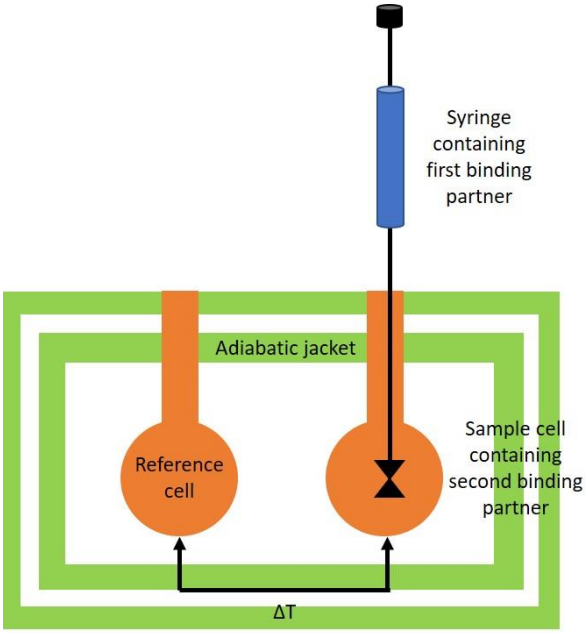


Figure 1.23. Representation of ITC set up, with the reference and sample cells enclosed in an adiabatic jacket. The first binding partner is injected into the sample cell containing the second binding partner, and the power required to maintain the reference and sample cells at the same temperature is measured.

This technique has many advantages, as a complete thermodynamic profile of the protein-ligand interaction is quantified, and there is no requirement for reporter labels or modifications on either protein or ligand. However, the technique is most appropriate for strong binding interactions, so for weaker interactions (for example, in the micromolar region), high concentrations and volumes of protein are necessary.

1.7 Previous Work Towards TRIM33 Bromodomain Ligands

1.7.1 Initial Screen and Optimisation

Initial studies, completed by Dr Angelina Sekirnik[130] and Dr Larissa See,[131] identified benzimidazolone-containing compound **16** from the Protein-Protein Interactions Network[137] (PPI-net) collection as a ligand for the TRIM33 bromodomain using a single-shot AlphaScreen™ (**Figure 1.24**). An orthogonal WaterLOGSY assay using wild-type TRIM33β BRD-PHD and bromodomain mutant TRIM33β-N1039F BRD-PHD validated the ligand as binding in the KAc binding pocket of the TRIM33 bromodomain. IC_{50} values were determined using a dose-response AlphaScreen™, where **16** was found to have an IC_{50} value of 12 μM for TRIM33β, and an IC_{50} value of 103 μM for TRIM24.

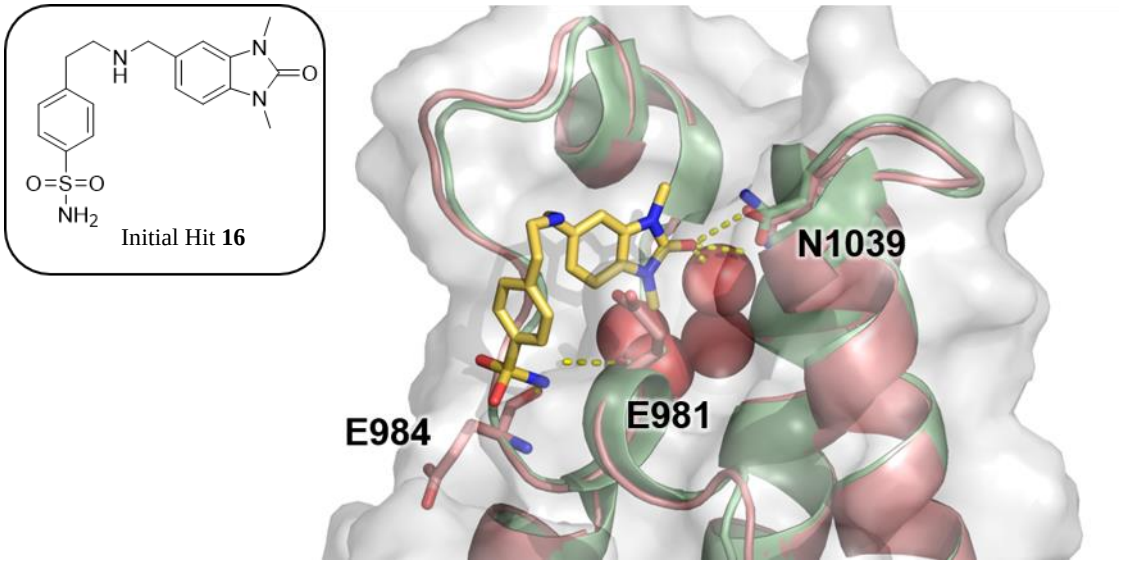


Figure 1.24. Compound **16** docked into TRIM24 (green, PDB ID: **3O33**) and TRIM33β (pink, PDB ID: **5MR8**) using CLC Drug Discovery Workbench 3.0.

From docking (CLC Drug Discovery Workbench 3.0) and molecular dynamic (MD) studies performed by Dr Joe Bluck, compound **16** was hypothesised to bind to both TRIM33β and

TRIM24 as shown in **Figure 1.24**. The carbonyl of the benzimidazolone group is thought to interact with the conserved asparagine residue N1039 in TRIM33β *via* a hydrogen bond, and the benzylic amine is predicted to interact with E981. The terminal sulfonamide appears to be forming a hydrogen bond with E981 or E984. Within the KAc binding pocket of TRIM24, an alanine residue A923 occupies the same space as E981 in TRIM33β, which presents an opportunity to form a hydrogen bond exclusively in TRIM33β, and targeting this residue may be an important gateway to selectivity.

1.7.2 Summary of Initial Findings

Dr Larissa See[131] and Dr Amy Scorah[138] synthesised an array of probe molecules, evaluated using a combination of AlphaScreen™ and WaterLOGSY, to determine key interactions of **16** in the KAc binding pocket (**Figure 1.25**). The probe molecules included an amide analogue **17** of initial hit **16** to determine if the pK_a of the benzylic amine was important – it was hypothesised that the protonation state of the benzylic amine is significant to maintain the interaction with E981, therefore exchanging the amine for an amide significantly reduces the percentage of protonation at this site at physiological pH (pH 7.4). This hypothesis was supported, with **17** showing no binding to TRIM33β using AlphaScreen™ and WaterLOGSY. Amines **18**, **19**, **20**, and **21** were also synthesised to determine if the amine moiety was in the optimal position for gaining an interaction with E981, or if increasing the chain length by one carbon (**18**) may strengthen this interaction. The assay results showed that removal of the sulfonamide moiety to give the small benzylic amine fragment **21** still showed binding to TRIM33β, and now demonstrated no binding to TRIM24. However, the other amine fragments showed either no or very low affinity binding, indicating that the benzylic amine is in the optimal position for interaction with E981. Alcohol **22** was also evaluated to probe whether the nature of the interaction of the benzylic amine with E981 was *via* a hydrogen bond or an ionic interaction. No binding was observed, indicating the pK_a and charge at this position significantly affects the interaction with E981. Alternative groups replacing the benzylic amine (ester **23** and acid **24**) also did not bind to TRIM33β, further demonstrating that a protonated group at this position is important. **25** was also synthesised, as it was

hypothesised that extending the benzylic amine chain with the addition of a tertiary terminal amine could encourage an interaction with E984. **25** showed low micromolar affinity for the TRIM33β bromodomain and no binding to TRIM24.[131,138] This compound appeared to be a good starting point for optimisation and further development to increase affinity for TRIM33β. Removal of the sulfonamide motif that appears in initial hit **16** was important, as this motif appears in the structure of many carbonic anhydrase inhibitors, thus may have led to off-target effects.[139]

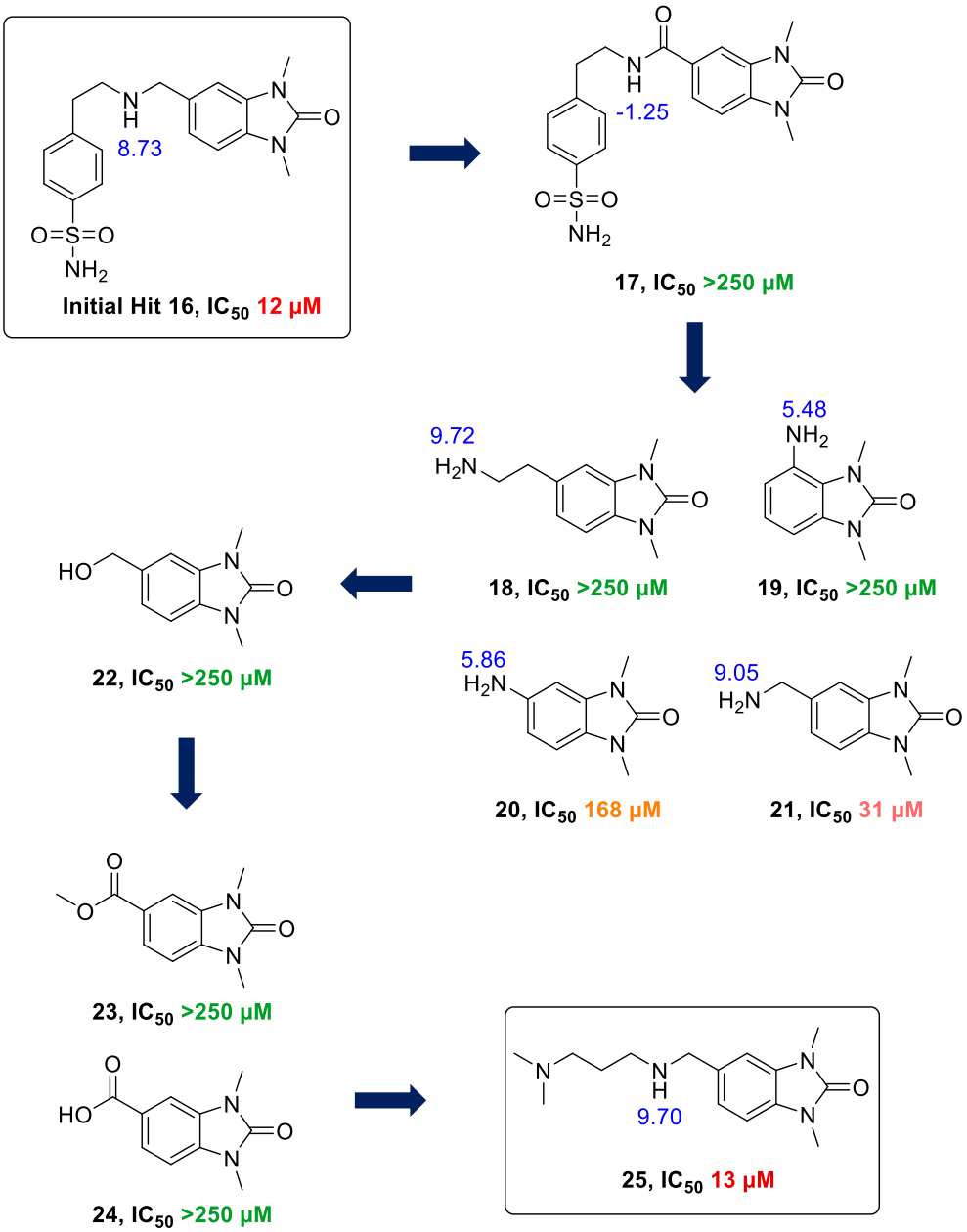


Figure 1.25. Analogues of **16** designed to probe the key interactions with the TRIM33 bromodomain.[131,138] The pK_a of the conjugate acids calculated using ACD I-Lab 2.0 at pH 7.4 are shown in blue. The IC_{50} values determined by a dose-response AlphaScreen™ are shown, with the colour corresponding to the affinity – red indicates higher affinity, and green indicates lower affinity.

1.8 Project Aims

The first aim of this work is to elucidate the biological functions of TRIM33, and therefore validate this protein as a therapeutic target, whilst providing a scaffold probe for further develop into a drug-like compound.

To achieve this, optimisation of the benzimidazolone probe **25** is required to increase affinity for the TRIM33β bromodomain, whilst maintaining selectivity over the similar bromodomain of TRIM24. The crystal structures of TRIM33α (PDB ID: **3U5O**), TRIM33β (PDB ID: **5MR8**), and TRIM24 (PDB ID: **3O33**) are available from the Protein Data Bank, and these will be used in computational methods for the design of compounds. These compounds will then be synthesised, and the affinity for TRIM33α, TRIM33β and TRIM24 will be evaluated using a combination of dose-response AlphaScreen™, WaterLOGSY, and ITC.

On development of a selective TRIM33β ligand, this structure can then be incorporated into PROTAC molecules targeting TRIM33, recruiting VHL and Cereblon as E3 ligases. The structure-activity relationship (SAR) studies performed when developing ligands will be key in designing a PROTAC with an appropriate attachment point for the linker to prevent a decrease in affinity for TRIM33. The differences between degradation of the protein in cells and inhibition of a singular domain can then be assessed. This can be used to reveal the relevance of TRIM33 and its bromodomain in disease, and determine if targeting TRIM33 in tumours in combination with radiotherapy could be an effective therapeutic strategy.

The second aim is to establish TRIM24 as an E3 ubiquitin ligase suitable for use in PROTAC design. These PROTAC molecules have the potential to be selective for cancer cell environments in which TRIM24 is highly overexpressed. For initial studies, bromodomain-containing protein BRD4 has been selected as the target for degradation, as high affinity ligands have been published.[35] This ligand will be linked to a known TRIM24 bromodomain ligand,[42] and several different linker lengths will be trialled to achieve a successful PROTAC capable of degrading BRD4 in a TRIM24-dependent manner. If this aim can be achieved, the results will significantly

progress the PROTAC field, allowing novel protein targets to be studied for medicinal chemistry research.

In summary, the aims for this project are to:

- Develop and optimise small molecule ligands for the TRIM33 bromodomain with selectivity over the similar bromodomain of TRIM24 (Chapter 2).
- Incorporate this TRIM33 bromodomain ligand into the design of a series of PROTAC molecules targeting TRIM33 for degradation and recruiting VHL or Cereblon as an E3 ligases (Chapter 3 and 4).
- Develop a PROTAC capable of degrading BRD4 using TRIM24 as the recruited E3 ligase (Chapter 5).

Chapter 2 Optimisation of TRIM33 Ligands

2.1 Introduction and Aims

Discovery of benzimidazolone-containing compound **16** as the first TRIM33 bromodomain ligand provided a useful starting point for the development and optimisation of high affinity ligands for this domain. It was postulated that the carbonyl of the head group interacts with N1039, as in TRIM24. SAR exploration to determine key interactions between the ligand and acetyl-lysine (KAc)-binding pocket (**Chapter 1.7**) suggested that an essential feature of the ligand is the benzylic amine moiety, which is hypothesised to form an ionic interaction with E981, as the amine is protonated at physiological pH (**Figure 2.1A**, **B**). Furthermore, removal of the sulfonamide chain in fragment **21** led to a loss of affinity for TRIM24. Extension of this benzylic amine to incorporate a terminal tertiary amine (**25**) improved affinity for TRIM33β and maintained selectivity over TRIM24. It was thought that the protonated terminal amine could interact with E984. In TRIM24, A923 occupies the same space as E981 within the protein structure (**Figure 2.1C**), thus the selectivity of fragment **21** and amine **25** for TRIM33 could be accounted for by this additional interaction. Moreover, in TRIM24, there would be an unfavourable interaction of the benzylic amine in **25** with the lipophilic alanine residue, which could further contribute to the observed selectivity.

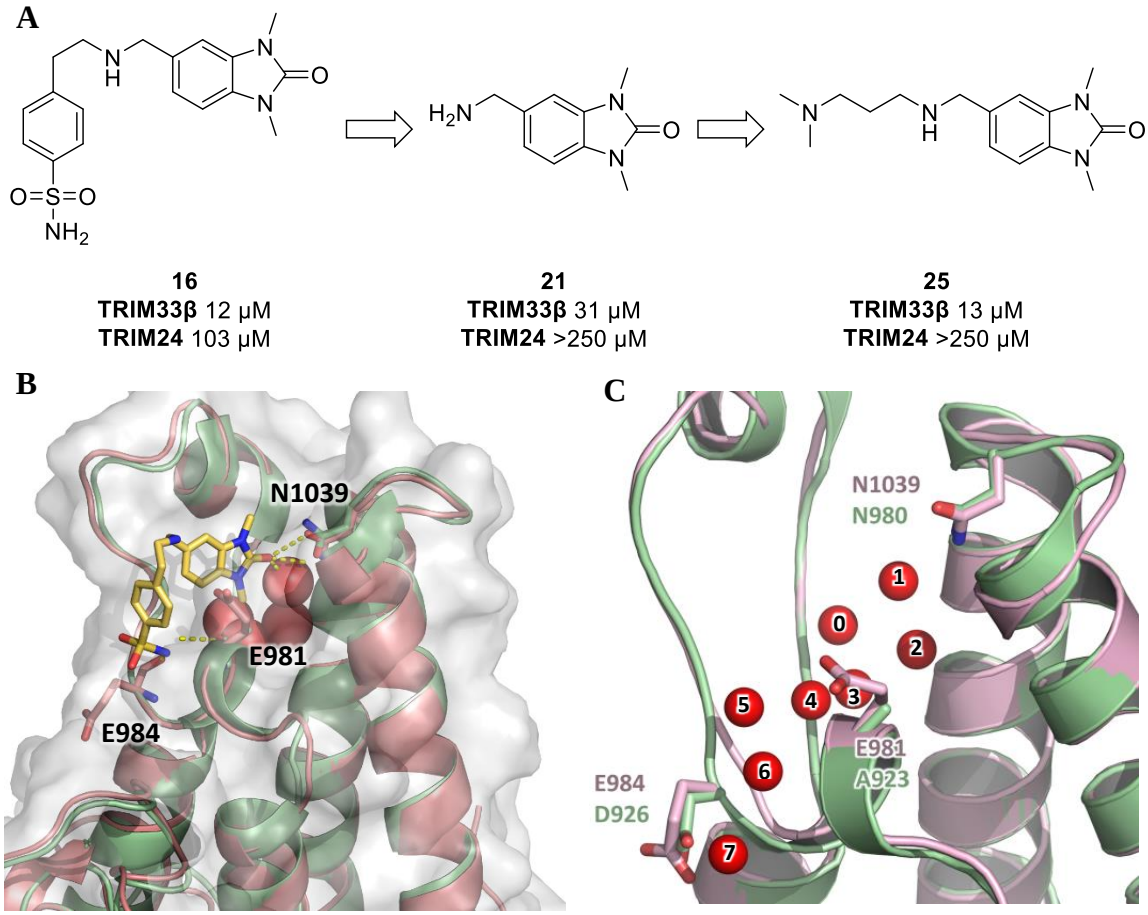


Figure 2.1. A. Initial SAR studies identified key structural features of initial hit **16**, which led to the development of **25**. **B.** Initial hit **16** docked into TRIM24 (green, PDB ID: **3O33**) and TRIM33β (pink, PDB ID: **5MR8**) using CLC Drug Discovery Workbench 3.0, showing key interactions. **C**. TRIM24 (green, PDB ID: **3O33**) and TRIM33β (pink, PDB ID: **5MR8**) with key similar and differing residues highlighted.

The work described in this chapter aimed to optimise TRIM33β ligand **25** to increase affinity for the bromodomain, while maintaining selectivity over the structurally similar TRIM24 bromodomain. A range of acetyl-lysine mimics were investigated in place of the benzimidazolone head group to determine if the interaction with N1039 is optimal, and hydrophobic regions of the KAc binding pocket were explored. Evaluation of the ligands were performed using the orthogonal assays of a dose-response AlphaScreen™, WaterLOGSY, and ITC. Docking and molecular dynamic (MD) studies were also used to direct SAR studies, interpret binding affinities, and calculate binding energies of water molecules residing in the binding site. Finally, different amine chains were explored to probe and maximise the interaction of the ligand with E981. Ligand efficiency (LE), which is a term used to describe the measure of the binding free energy per atom of a ligand and the protein it binds to (**Equation 1**)[140], was also considered in the evaluation of ligands. It is important to account for this metric when exploring small fragments,

as LE provides recognition of beneficial protein-ligand interactions despite the fundamental low affinities of the fragments for their targets. LE typically decreases as the molecular size increases, therefore initial optimisation of compounds should focus on maintaining a high ligand efficiency.

$$\mathrm{LE} = \frac{1.4 \times -log_{10}\mathrm{IC}_{50}}{number\ of\ heavy\ atoms} \quad (1)$$

where heavy atoms refer to all atoms that are not hydrogen.

2.2 Biological Evaluation of Ligands

It is important to use orthogonal assays when assessing affinity of ligands in order to identify false positives or assay interference. IC_{50} values of potential ligands were evaluated using dose-response AlphaScreen[141] and hits were validated using WaterLOGSY NMR experiments. AlphaScreen™ TruHits counter screens were also performed, allowing identification of compounds that interfere with the AlphaScreen™ through various mechanisms.[128]

It is essential to use His-tagged proteins while performing AlphaScreen™, as the protein is required to interact with the nickel-chelating acceptor bead. Protein purification using immobilised metal affinity chromatography (IMAC) also requires an N-terminal His_6 tag. His-tagged TRIM24 protein was expressed and purified by Dr Angelina Sekirnik.[130] TRIM33α and TRIM33β proteins were produced by means of large scale recombinant protein expression in *Escherichia coli* BL21-Gold (DE3). Isopropyl β-D-1-thiogalactopyranoside (IPTG) was used to induce protein expression, and correct folding of the PHD finger was facilitated by the addition of zinc(II) chloride to the growth media. Both proteins were purified *via* IMAC and subsequent size-exclusion chromatography (SEC) using an ÄKTA fast protein liquid chromatography (ÄKTAFPLC) system. Purity was then confirmed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and protein mass spectrometry (**Figure 2.2**).

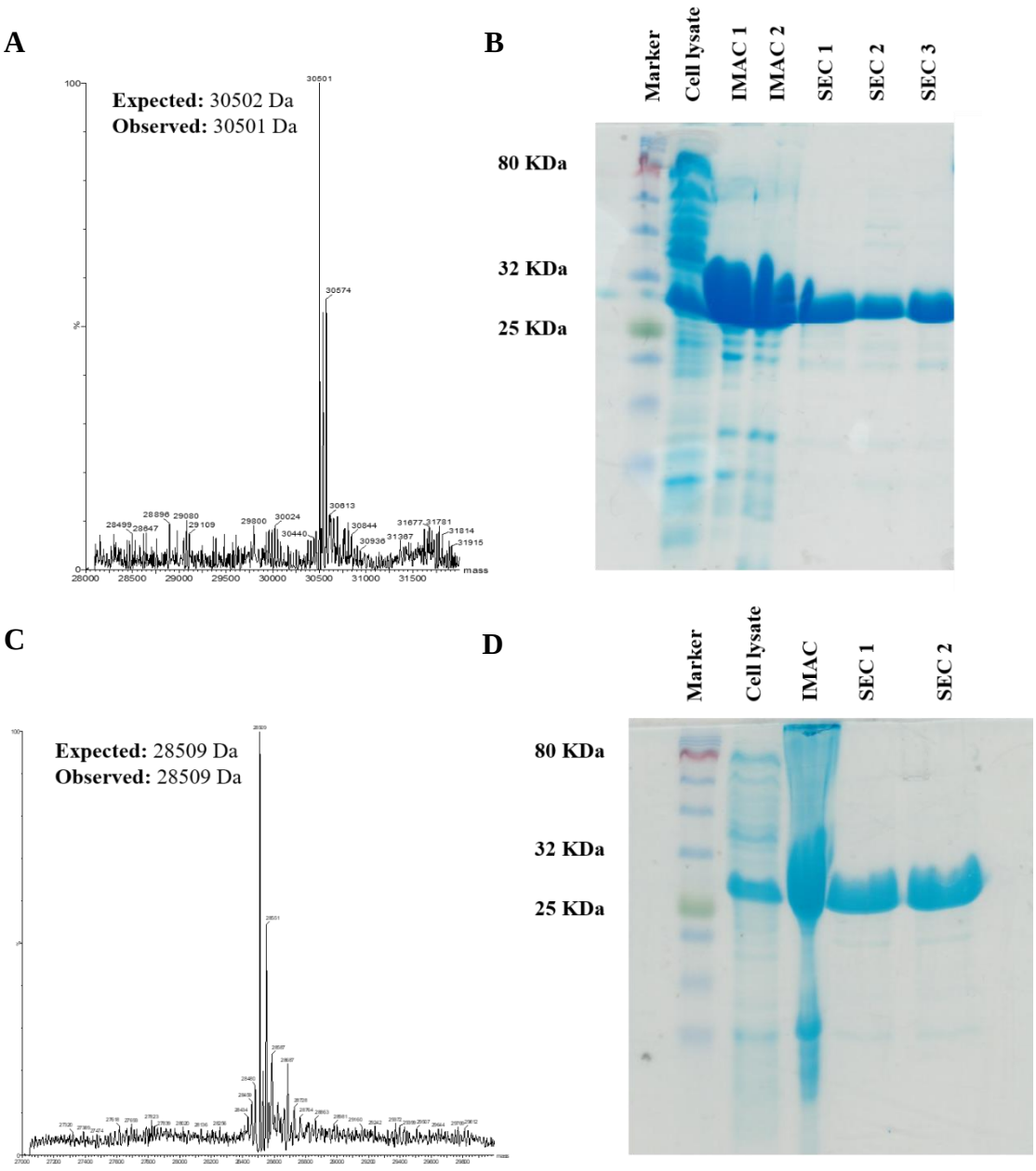


Figure 2.2. Confirmation of purification of TRIM33α and TRIM33β. **A.** Protein mass spectrum of TRIM33α. **B.** SDS-PAGE gel for TRIM33α. **C.** Protein mass spectrum of TRIM33β. **D.** SDS-PAGE gel for TRIM33β.

To optimise AlphaScreen™ conditions for assessment of ligands binding to the respective bromodomains and PHD fingers of TRIM24, TRIM33α and TRIM33β, Dr Angelina Sekirnik and Dr Larissa See screened a variety of biotinylated modified histone H3-mimicking peptides with each protein over a range of concentrations.[130,131] The highest affinity peptides were selected for use in the dose-response AlphaScreen™, which were methylated $H3_{1–27}K9Me_3$, acetylated $H3_{1–27}K18Ac$, and methylated and acetylated $H3_{1–27}K9Me_3K18Ac$. Using peptides with these modifications will allow interpretation of which domain the compound is binding to within the

protein. Each peptide was evaluated in combination with each TRIM24, TRIM33α and TRIM33β, giving a total of nine protein-peptide combinations. Each peptide has a different binding affinity for each protein, and since AlphaScreen™ is a displacement-based assay, the IC_{50} values acquired for ligands binding to proteins are dependent on the affinity of the peptides for the protein. Consequently, IC_{50} values cannot accurately be compared across protein-peptide combinations, only within the same combination. Dr Larissa See[131] performed ITC experiments to determine K_D values for each protein-peptide combination (**Table 2.1**). $H3_{1–27}$K18Ac appeared to have the weakest affinity to TRIM33α (K_D value = 54.7 μM), which indicates that, in general, lower IC_{50} values will be observed in AlphaScreen™ due to the ease of displacement of this peptide.

Table 2.1. K_D values determined by Dr Larissa See using ITC for each protein with each peptide.[131]

	$H3_{1–27}$K9Me$_3$	**$H3_{1–27}$K18Ac**	**$H3_{1–27}$K9Me$_3$K18Ac**
TRIM24	3.90 μM	0.695 μM	0.309 μM
TRIM33α	9.46 μM	54.7 μM	5.58 μM
TRIM33β	9.25 μM	4.92 μM	1.11 μM

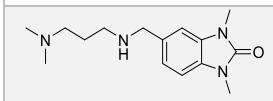
2.3 TRIM33α and TRIM33β

As previously described in **Chapter 1.4.1.1**, TRIM33α contains a 17 amino acid insertion in the BC loop of the bromodomain, causing the conserved asparagine residue N1039 to reside out of the KAc binding pocket. It was therefore hypothesised that TRIM33α has a non-functional bromodomain, as this asparagine residue is responsible for recognition of acetylated lysine residues. However, ligand **25** is still observed to bind to the TRIM33α bromodomain despite loss of the interaction between N1039 and the head group carbonyl in **25**, which could be explained by the 17 amino acid increase in size of binding pocket allowing for additional hydrophobic interactions, and indicating the importance of the benzylic amine interaction with E981.

To confirm the hypothesis that TRIM33α has a non-functional bromodomain, TRIM33α and TRIM33β, each containing a mutation of the conserved N1039 to phenylalanine (N1039F), were expressed by Dr Angelina Sekirnik[130] and Dr Larissa See[131]. Phenylalanine is a bulky amino acid residue with no hydrogen bonding capabilities, thus the residue will occupy a larger area than

N1039 and will not have the ability to hydrogen bond to ligand **25**. Dr Angelina Sekirnik previously showed that this mutation in TRIM33β hindered recognition of histone H3-mimicking peptide $H3_{1–27}$K18Ac, indicating that the binding site is significantly affected by this modification.[130] However, for TRIM33α N1039F, it is hypothesised that the phenylalanine residue should reside out of the binding site, therefore will not affect ligand **25** binding to the KAc site. WaterLOGSY experiments with the mutant proteins confirmed these postulations, with binding of **25** to TRIM33α observed for both the wild type and N1039F mutant, whereas only binding to wild type TRIM33β was seen (**Table 2.2**). The N1039F mutation provides a more useful mutagenesis study than the corresponding N1039A mutation for the TRIM33α and TRIM33β comparison, as the larger size of the group also acts as a blockage in the binding site in addition to removing the hydrogen bonding capabilities.[142]

Table 2.2. Summary of WaterLOGSY results obtained by Dr Angelina Sekirnik and Dr Larissa See for ligand **25** with TRIM33α wild type (WT) and N1039F mutant, and TRIM33β WT and N1039F mutant. Binding is indicated by ✓, and non-binding by ×.

Compound	TRIM33α WT	TRIM33α-N1039F	TRIM33β WT	TRIM33β-N1039F
 **25**	✓	✓	✓	×

The WaterLOGSY data support the hypothesis that in solution, the N1039 residue in TRIM33α is not pointing into the KAc binding pocket and therefore has a non-functional bromodomain, hence work done in this chapter will focus on developing a high affinity ligand for TRIM33β. This hypothesis is also supported by the decreased affinity of peptide $H3_{1–27}$K18Ac for TRIM33α compared to TRIM33β (**Table 2.1**).

2.4 Head Group Optimisation

Initial hit **16** contains a benzimidazolone head group, which acts as an acetyl-lysine mimic by interacting with N1039 through a hydrogen bond with the head group carbonyl. Other acetylated lysine mimetics have been reported in the literature. Therefore, to optimise this interaction with the TRIM33β bromodomain and minimise interaction with TRIM24, different head groups were considered.

2.4.1 Design and Docking Studies

A range of acetyl-lysine mimics that have been shown to bind to bromodomains were identified as potential head groups. Important structural features of initial hit **16** were considered, and it was decided that the aromatic six-membered ring should be maintained, as the rigidity of the head group appears to be essential in directing the acetyl-lysine mimic towards N1039 in the KAc binding pocket (**Figure 2.3**). As shown from initial SAR studies performed by Dr Larissa See[131] and Dr Amy Scorah[138] (**Chapter 1.7**), the relative position of the benzylic amine with respect to the head group is also significant in maintaining the interaction with E981, therefore this was also sustained.

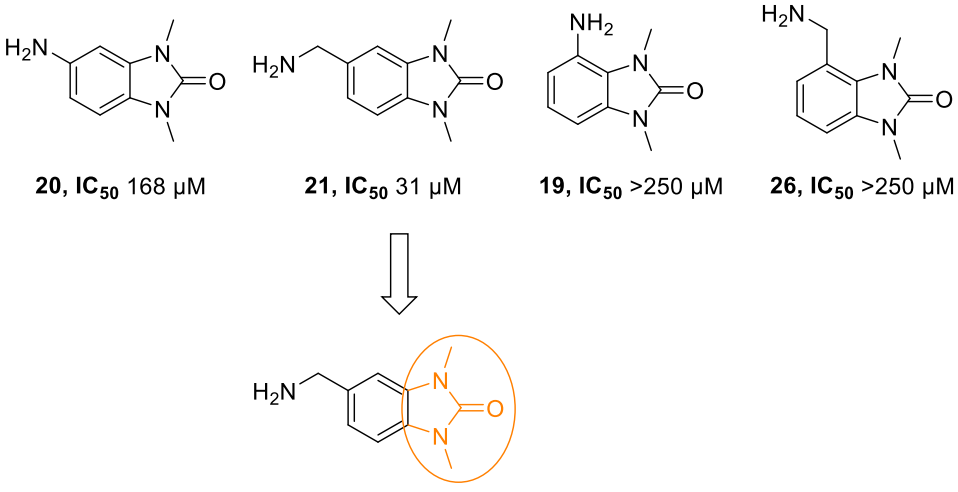


Figure 2.3. SAR studies (**Chapter 1.7**) revealed the importance of the position of the amine moiety, so this was maintained when designing alternative head groups. The highlighted orange head group was the focus of the SAR studies for acetyl-lysine mimic exploration.

With these features in mind, a series of compounds were designed for the *in silico* study (Appendix B). Docking studies were performed using CLC Drug Discovery Workbench 3.0,

using X-ray crystal structures of TRIM24 (PDB ID: **3O33**, resolution 2.0 Å), TRIM33α (PDB ID: **3U5O**, resolution 2.7 Å), and TRIM33β (PDB ID: **5MR8**, resolution 1.7 Å). Amino acid residues in the BC loop are unresolved in the structure of TRIM33α, therefore, due to the importance of this region for the KAc binding pocket, Dr Joe Bluck[143] modelled these residues using MODELLER.[144] Both α and β isoforms of TRIM33 were used for docking. There are five conserved water molecules that reside in the base of the acetyl-lysine binding pocket forming a water network (waters 0–4, **Figure 2.4**), and since a similar network is seen in the crystal structures of TRIM24 bromodomain ligands,[41,42] these water molecules were maintained during docking. There are an additional three water molecules present in the ZA channel (waters 5–7, **Figure 2.4**). During initial docking, binding energies and, consequently, displaceability of these waters were not known, so docking was performed with different combinations of the ZA channel water molecules present in the binding site.

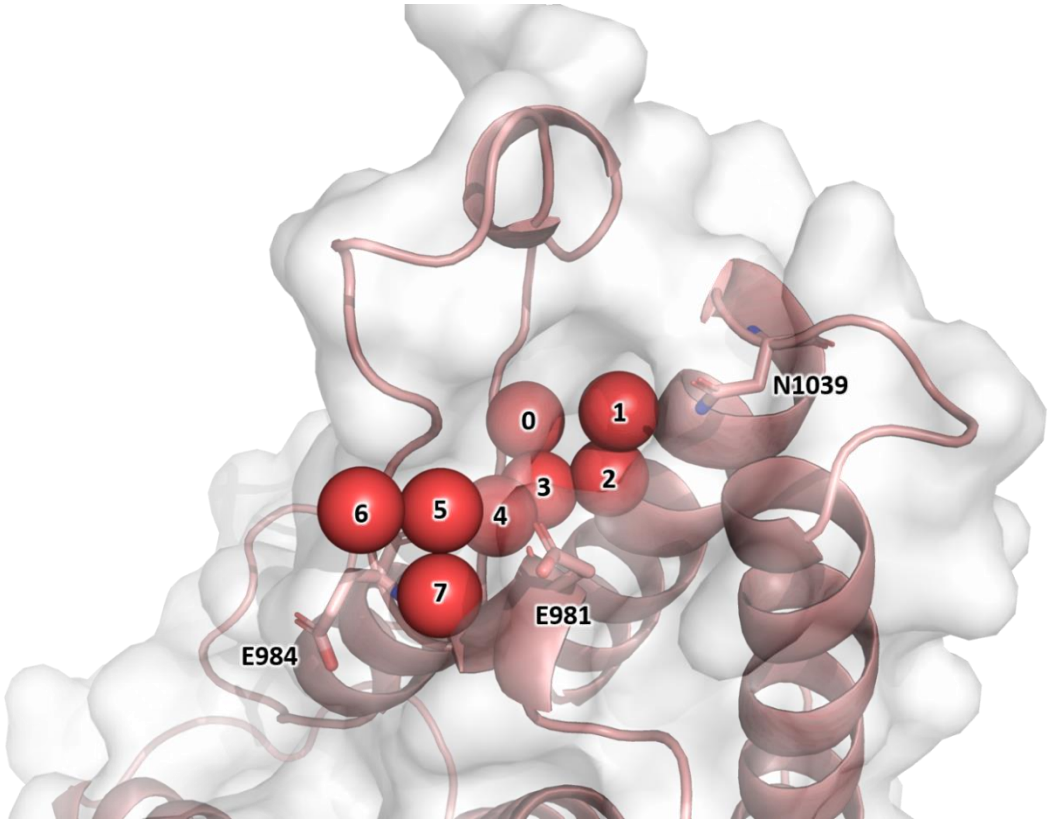


Figure 2.4. TRIM33β acetyl-lysine binding site with key residues highlighted and the water molecules present in the binding site (waters 0–4) and ZA channel (waters 5–7) shown (PDB ID: **5MR8**).

The binding site was set up with a radius of 13 Å centred around E981 to accommodate any ligand interactions with hydrophobic residues surrounding the site. Docking results were visually assessed to select viable ligands to be taken forward for synthesis and biological evaluation.

The compounds selected (Appendix B) were docked using the conditions described above. After visual inspection of binding modes of the most favourable scoring molecules, two head groups **27** and **28** were selected as reasonable substitutions for the benzimidazolone core (**Figure 2.5**). It appeared that the interaction with N1039 was maintained in both cases, and hydrogen bonding to E981 through the benzylic amine was still possible. However, it was decided that these molecules should instead be synthesised with the amine chain present in ligand **25**, as this was the highest affinity ligand with selectivity over TRIM24, aiding detection of binding. Synthetic routes to these two compounds were then designed to synthesise and evaluate these compounds as ligands for TRIM33β.

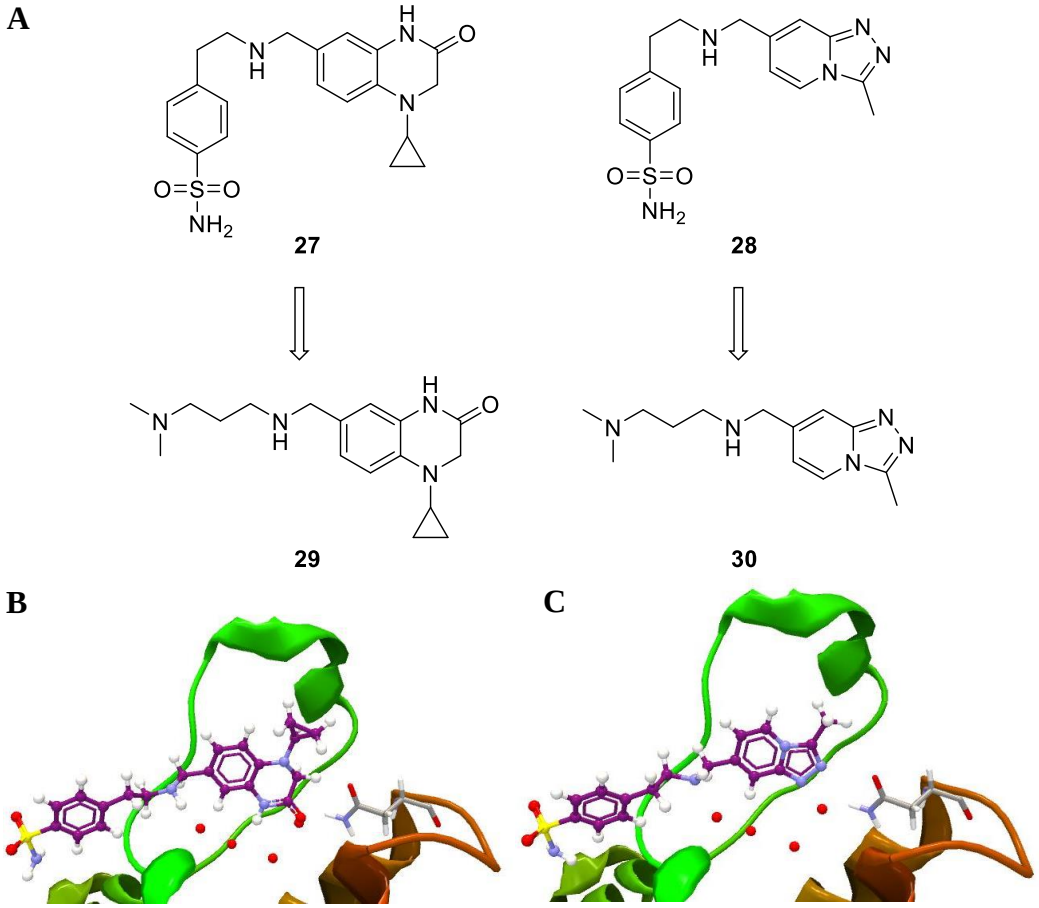
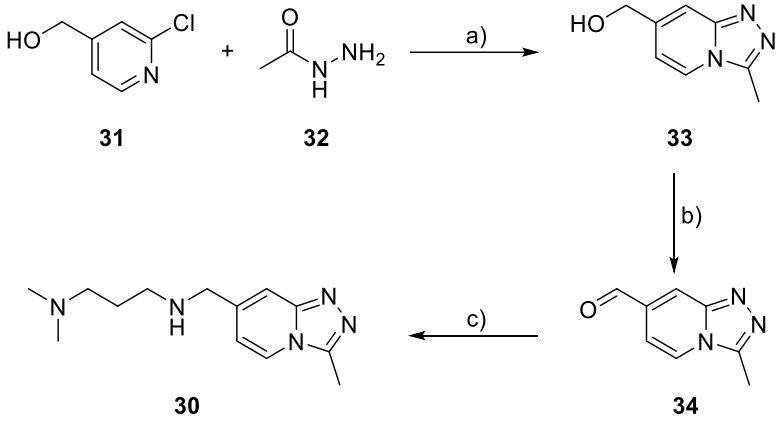


Figure 2.5. A. Structures of two initial hits that appeared to mimic the binding mode of ligand **16**. These were then synthesised with amine chain present in **25** to aid detection of binding. **B.** Ligand **27** docked into TRIM33β (PDB ID: **5MR8**) using CLC Drug Discovery Workbench 3.0. **C.** Ligand **28** docked into TRIM33β.

2.4.2 Synthesis of Triazolopyridine Head Group

Synthesis of the triazolopyridine derivative **30** began with heterocycle formation using (2-chloropyridin-4-yl)methanol **31** and acethydrazide **32** under microwave irradiation (**Scheme 2.1**). Starting material **31** was not completely consumed during the reaction despite increasing reaction time and temperature, which accounted for the moderate yields of desired product **33**. The next step required oxidation of alcohol **33** to aldehyde **34**, which was achieved using manganese(IV) oxide. Subsequently, a reductive amination with 3-(dimethylamino)-1-propylamine yielded final product **30**. The low yield of this reaction was attributed to the difficulty in purification, due to the similarity in R_f of product **30** and the amine starting material. Nevertheless, **30** was produced in a suitable yield for biological evaluation.

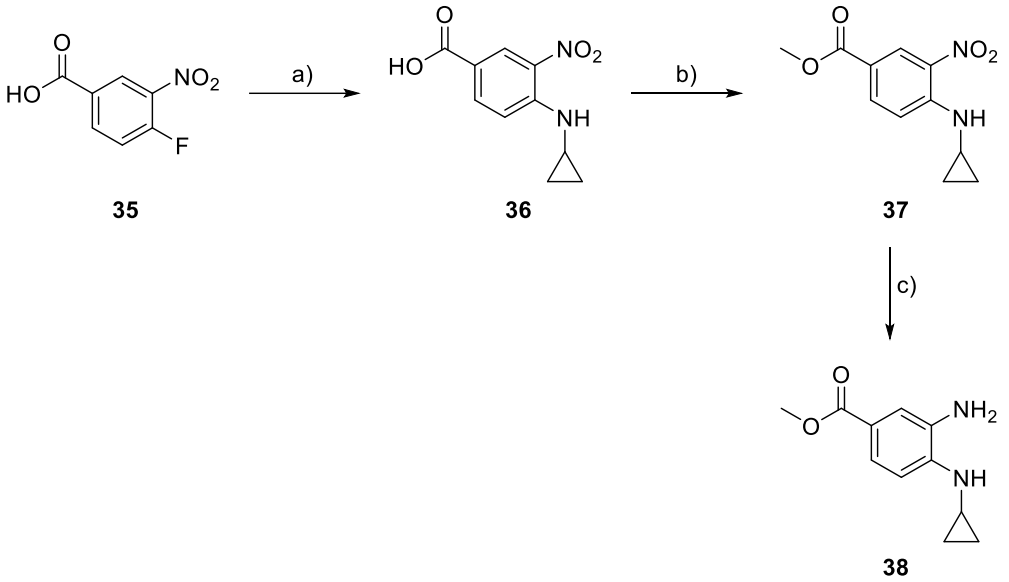


Scheme 2.1. Synthesis of ligand **30**. *Reagents and conditions:* a) *n*-BuOH, 170 ºC, microwave, 5 h, 34–47%, n = 2; b) MnO_2, CH_2Cl_2, rt, 18 h, 44–75%, n = 4; c) i. 3-(Dimethylamino)-1-propylamine, DCE, AcOH, 0 ºC, 4 h, ii. $NaBH_4$, 32%, n = 1.

2.4.3 Synthesis of Dihydroquinoxalinone Head Group

The first step in the synthesis of the dihydroquinoxalinone derivative **29** utilised an S_NAr reaction to install a cyclopropylamine moiety into 4-fluoro-3-nitrobenzoic acid **35** (**Scheme 2.2**). Heating the reaction at 50 ºC for 18 hours only gave 25% yield of desired product **36**. However, on exposure to microwave irradiation at 150 ºC, the reaction went to completion in 20 mins without need for purification. Amine **36** was found to have limited solubility, therefore an esterification was performed to aid solubility as well as protect the carboxylic acid functionality in subsequent steps. This reaction proceeded in consistently high yields of 90–99% to give **37**. The next step

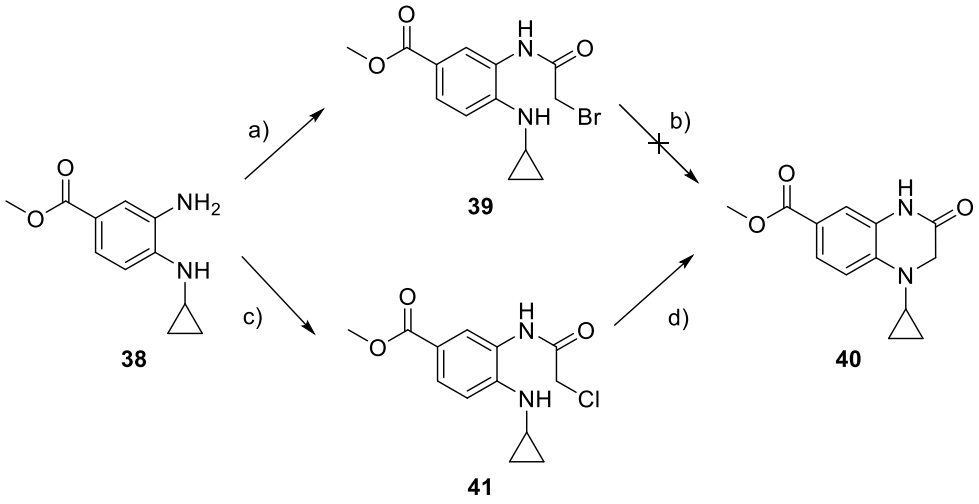
required a reduction of the nitro group, but the resulting amine **38** degraded rapidly at room temperature. This product was therefore used immediately in the following step after filtering through Celite™ without further purification.



35 **36** **37** **38**

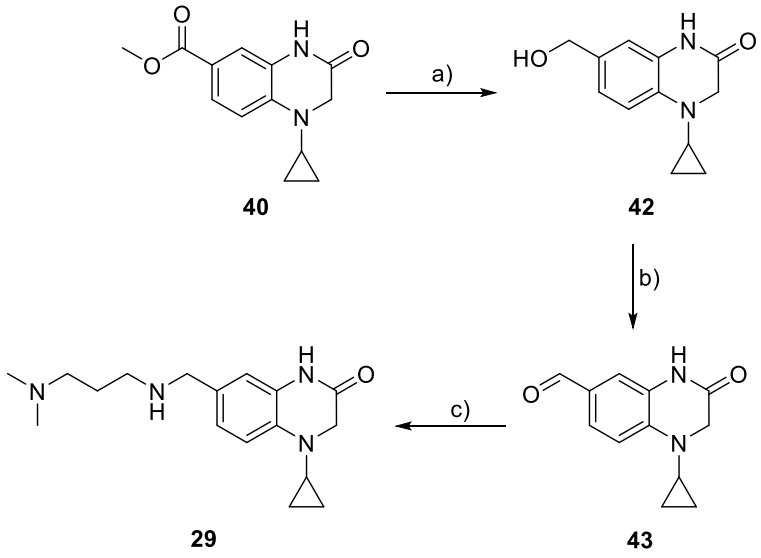
Scheme 2.2. First steps to synthesise intermediate **38**. *Reagents and conditions:* a) Cyclopropylamine, $NaHCO_3$, H_2O, 150 ºC, microwave, 20 min, 88–91%, n = 4; b) $SOCl_2$, MeOH, 50 ºC, 3 h, 90–99%, n = 4; c) $Pd(OH)_2/C$, H_2, EtOAc, rt, 3 h, used directly in next step.

Alkylation of amine **38**, using bromoacetyl bromide, appeared to be successful when monitored by TLC, but the ^{1}H NMR spectrum of the crude product showed very little of desired product **39**, and the characteristic splitting of peaks for bromine isotopes were not seen in LRMS (**Scheme 2.3**). It was thought that decomposition could be occurring due to the reactive nature of the acetyl bromide functionality. The reduction, alkylation and ring closure were attempted successively without isolation or purification steps to prevent decomposition of the two intermediates **38** and **39**, but again this did not yield the desired ring closed product **40**. The reaction was instead repeated with chloroacetyl chloride, which would yield a less reactive intermediate. Compound **41** was produced with good to excellent yields of 50–97%, and was stable to silica gel column chromatography. The ring closure was attempted using DIPEA and sodium iodide in acetonitrile, heating under reflux for two days. This method gave a low yield of 19% as starting material **41** still remained. The problem was solved by subjecting the reaction mixture to microwave irradiation for 5.5 hours at 120 ºC, where higher yields of ring closed product **40** were achieved.



Scheme 2.3. Attempts at ring closure to form **40**. *Reagents and conditions:* a) Bromoacetyl bromide, NEt_3, CH_2Cl_2, 0 ºC, 2 h; b) DIPEA, DMF, reflux, 18 h, no product formed; c) Chloroacetyl chloride, DIPEA, THF, –60 – 20 ºC, 16 h, 50–97%, n = 4; d) Attempt 1: DIPEA, NaI, MeCN, reflux, 22 h, 19%, n = 1, Attempt 2: DIPEA, NaI, MeCN, 120 ºC, microwave, 5.5 h, 51–68%, n = 3.

The next step involved a reduction of ester **40** to alcohol **42**, which was achieved using diisobutylaluminium hydride (DIBAL), followed by an oxidation using Dess–Martin periodinane (DMP) to aldehyde **43**, which proceeded in 55–73% yields (**Scheme 2.4**). The final step required a reductive amination of **43** with 3-(dimethylamino)-1-propylamine. Purification of final compound **29** proved difficult, requiring very polar silica gel column chromatography conditions for elution to occur. Despite this, **29** was synthesised in sufficient yield for biological evaluation.



Scheme 2.4. Final steps to complete synthesis of ligand **29**. *Reagents and conditions:* a) DIBAL, CH_2Cl_2, –78 ºC, 3 h, 80–90%, n = 3; b) DMP, CH_2Cl_2, rt, 3 h, 55–73%, n = 2; c) i. 3-(Dimethylamino)-1-propylamine, DCE, AcOH, 0 ºC, 4 h, ii. $NaBH_4$, 16%, n = 1.

2.4.4 Evaluation of Compounds

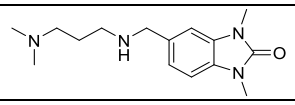
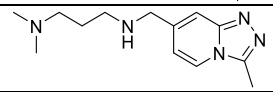
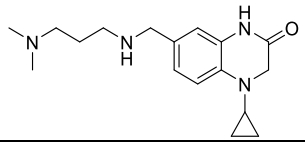
The two compounds **29** and **30** were evaluated using a dose-response AlphaScreen™, starting at a compound concentration of 500 μM and serially diluting 1:2 to give 14 concentrations. Ligand **25** was also included on the assay plate as a positive control. A compound purity of >95% determined using HPLC was required for testing. AlphaScreen™ is a displacement-based assay, where ligand binding is indicated by a reduction in emission, as the biotinylated peptide is displaced from the His-tagged protein. The compounds were tested against TRIM24, TRIM33α and TRIM33β in combination with peptides that bind to the PHD finger ($H3_{1–27}K9Me_3$), bromodomain ($H3_{1–27}K18Ac$) and both domains ($H3_{1–27}K9Me_3K18Ac$) to give a total of nine combinations. The work done in this chapter focused on compounds that bind to the bromodomain, hence data presented and discussed corresponds to results for the $H3_{1–27}K18Ac$ peptide. All data for other protein-peptide combinations can be found in Appendix C. GraphPad Prism 7 software was used to determine IC_{50} values where sigmoidal curves were achieved, by fitting data points to a four-parameter equation (**Equation 2**). In the cases of weak binding where a full IC_{50} curve could not be obtained, binding affinity is shown by >250 μM.

$$Y = Minimum + \frac{Maximum - minimum}{1+10^{(\log IC_{50} - X)(Hill\ slope)}}, where\ X = \log(compound\ concentration) \quad (2)$$

Disappointingly, in the case of **29** and **30**, a full sigmoidal curve was not achieved, suggesting that both compounds **29** and **30** were binding too weakly to the TRIM33β bromodomain to calculate an IC_{50} value (**Table 2.3**). This could be due to a sub-optimal geometry of the acetyl-lysine mimic relative to N1039, and forming this hydrogen bond may pull the benzylic amine away from the optimal position to gain the important interaction with E981. Performing an AlphaScreen™ TruHits experiment showed no assay interference was occurring, confirming that these compounds do not bind to TRIM33β bromodomain. For this reason, WaterLOGSY experiments were not performed on compounds **29** and **30**. On consideration of these data, it was decided that the original benzimidazolone head group supported the correct orientation of carbonyl relative to the benzylic amine in order to maintain a significant interaction with both N1039 and E981, hence this was preserved in subsequent ligand design.

Table 2.3. A table showing IC_{50} values for the compounds **29** and **30** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from a highest compound concentration of 500 μM. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green. Results shown are for each protein with $H3_{1-27}K18Ac$ peptide, with ligand efficiency in brackets. *These IC_{50} values are the mean and standard deviation of multiple repeats (n = 8).

Weaker binding — **Stronger binding**

Compound	Structure	TRIM24 BRD IC_{50} (μM)	TRIM33α BRD IC_{50} (μM)	TRIM33β BRD IC_{50} (μM)
25		>250	3.61* ± 0.352 (0.38)	13.7* ± 1.53 (0.35)
30		>250	>250	>250
29		>250	>250	>250

2.5 Probing Interactions with N1039 and E981

Data collected thus far suggested there were two essential interactions for affinity to the TRIM33β bromodomain; the interaction of benzylic amine with E981, and the hydrogen bond between the benzimidazolone carbonyl and N1039. To confirm these observations, compound **44** was designed, which does not contain a benzimidazolone head group (**Figure 2.6**). Methylating the benzylic amine should probe the importance of the interaction with E981, as hydrogen bonding ability of ligand **45** would be reduced, and pK_a calculations implied this position would only be partially protonated at pH 7.5 (predicted using ACD/I-Lab 2.0). Additionally, **46** was considered to determine if hydrogen bond donor ability would be beneficial in the terminal position. Compounds **44**–**46** were synthesised by Dr Larissa See[131] and Dr Amy Scorah.[138]

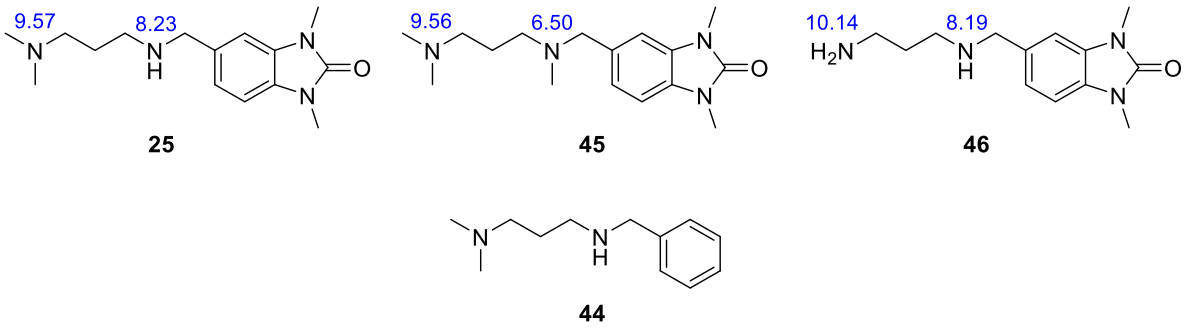
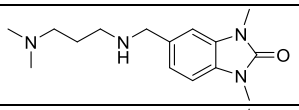
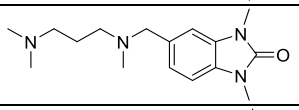
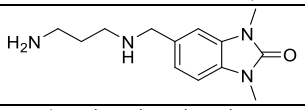
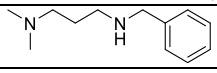


Figure 2.6. Compounds **45** and **46** synthesised to test different methylation states of the amine chain. Compound **44** was synthesised to determine the importance of the N1039 interaction with the head group. Synthesis by Dr Amy Scorah and Dr Larissa See.[131,138] pK_a predictions shown in blue were calculated using ACD/I-Lab 2.0.

Binding was assessed using a dose-response AlphaScreen™ (**Table 2.4**), with the results displayed corresponding to data for the $H3_{1–27}$K18Ac peptide. All data for other protein-peptide combinations can be found in Appendix C.

Table 2.4. A table showing IC_{50} values for the compounds **44–46** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from a highest compound concentration of 500 µM. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green. Results shown are for each protein with $H3_{1-27}$K18Ac peptide, with ligand efficiency in brackets. *These IC_{50} values are the mean and standard deviation of multiple repeats (n = 8).

Weaker binding — **Stronger binding**

Compound	Structure	TRIM24 BRD IC_{50} (µM)	TRIM33α BRD IC_{50} (µM)	TRIM33β BRD IC_{50} (µM)
25		>250	3.61* ± 0.352 (0.38)	13.7* ± 1.53 (0.35)
45		>250	5.69 ± 0.33 (0.35)	23.5 ± 2.80 (0.31)
46		>250	3.73 ± 0.83 (0.42)	9.33 ± 1.50 (0.39)
44		>250	>250	>250

As anticipated, methylated benzylic amine **45** (IC_{50} = 23.5 µM) showed lower affinity for the TRIM33β bromodomain than unmethylated control **25** (IC_{50} = 13.7 µM). The fact that binding was not completely negated indicated that the partially protonated methylated amine **45** was still able to interact with E981 through an ionic interaction. This was consistent with the observation in **Chapter 1.7**, that a benzylic alcohol does not bind to TRIM33β as a hydrogen bond donor alone is not sufficient, and protonation is required. Non-methylated **46** appeared to have a slightly

higher affinity (IC_{50} = 9.33 µM) compared to control **25**. This could be attributed to the increased basicity of the primary amine compared to the tertiary amine, and the increased hydrogen bond ability which has the potential to interact with E984. As expected, **44** did not bind to TRIM33β, signifying the importance of the interaction with N1039 for ligand orientation in the binding pocket. These findings revealed the significance of maintaining the benzimidazolone carbonyl interaction with N1039, and determined that the charge on the benzylic amine is important for ionic interaction with E981. Having a primary amine present in the terminal position of the amine chain appeared to be advantageous, potentially forming an interaction with E984. However, due to the low solubility of **46**, dimethylated **25** was maintained as the control for further development.

2.6 Targeting the Hydrophobic Region

Once the importance of maintaining the key interactions with N1039 and E981 had been established, efforts turned to increasing the affinity of the ligand for the TRIM33β bromodomain. This can potentially be achieved by forming interactions between the ligand and hydrophobic residues in a protein. Computational studies were performed by Dr Joe Bluck[143] to identify target regions for hydrophobic interactions (**Figure 2.7**). Red dots are used to illustrate regions of higher than two times the average possibility of finding an aliphatic carbon in that position. Notably, a large hydrophobic region was recognised, positioned above the acetyl-lysine binding pocket (circled), including residues I990, F1038 and I996. This area revealed an opportunity to increase affinity by extending from the benzimidazolone head group to gain hydrophobic interactions. However, extending from this position removes the line of symmetry in the dimethylated benzimidazolone core, thus the orientation of the ligand in the binding site becomes important.

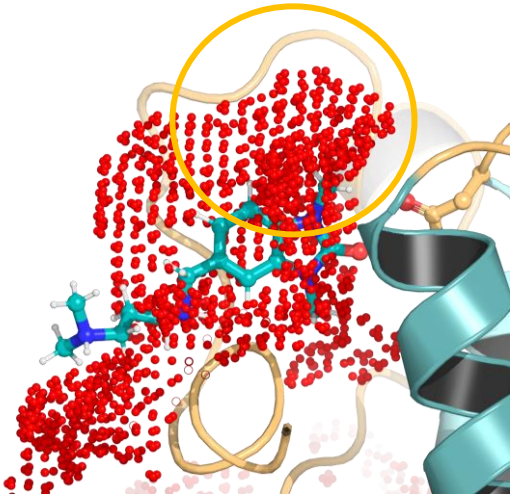


Figure 2.7. An aliphatic carbon propensity map for TRIM33β (PDB ID: **5MR8**) produced by Dr Joe Bluck revealed an opportunity to increase affinity by targeting the region above the binding site (circled).[143] Red dots illustrate regions of higher than 2× average probability of finding an aliphatic carbon at that position.

To determine the preferred orientation of the benzimidazolone-containing compounds in the KAc binding site (**Figure 2.8A**), and therefore decide the most appropriate vector to extend a hydrophobic group from, MD studies were performed and barcode plots were generated by Dr Joe Bluck.[143] Ligand **25** was docked using AutoDock 4, and subsequently simulated using GROMACS 2016.3. The five water molecules residing in the base of the binding pocket were maintained. During the simulations, the terminal amine of **25** was protonated, but the benzylic amine was not, despite pK_a predictions suggesting that both amines would be protonated at pH 7.5. This was due to difficulties observed in getting the ligand to remain in the binding site when both amines were protonated, and this problem could only be solved by singularly protonating the terminal amine. The barcode plots indicate the distance between two defined points during the simulation, which in this instance were between E981 and the benzylic amine (**D1**, **Figure 2.8B**), E981 and the protonated terminal amine (**D2**), and E984 and the protonated terminal amine (**D3**). A stable interaction is characterised by close proximity between the two points over the course of the simulation, shown by a dark purple colour (**Figure 2.8C**).

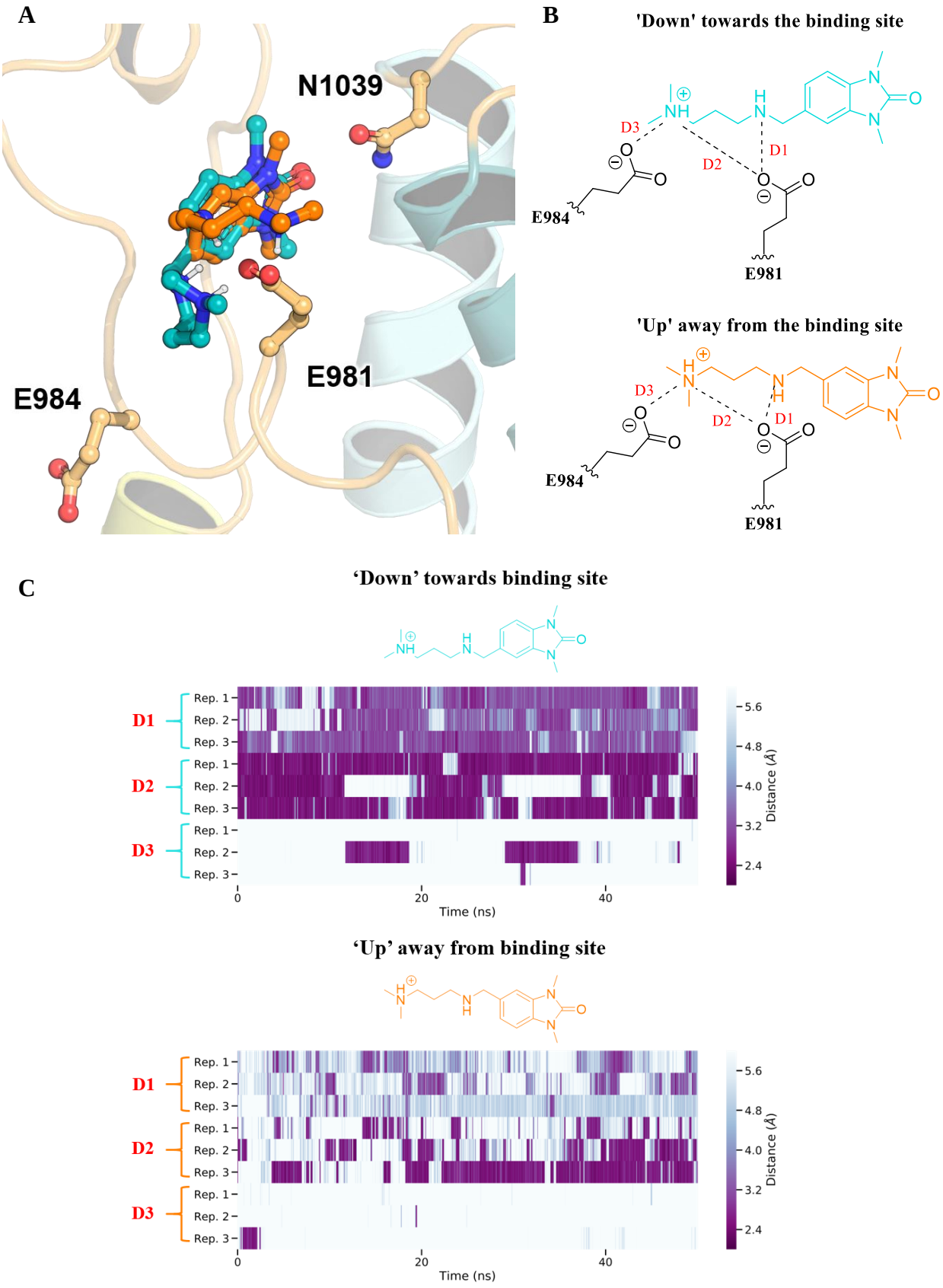


Figure 2.8. A. Ligand **25** docked into TRIM33β bromodomain (PDB ID: **5MR8**) showing the two possible orientations. **B.** Potential poses for ligand **25**, either 'down' towards the binding site (shown in blue) or 'up' away from the binding site (shown in orange), with the interactions labelled D1, D2, and D3. **C.** Barcode plots showing the distance between the two given points labelled D1, D2, and D3. Diagrams by Dr Joe Bluck.[143]

The first barcode plot (**Figure 2.8C**) shows **25** modelled with the amine chain pointing 'down' towards the binding pocket. The interaction between the benzimidazolone carbonyl and N1039 (**D1**) remained constant throughout the simulation, and it appeared that E981 and the benzylic amine (**D2**) also remained in close proximity. The terminal amine is not seen to interact with E984 (**D3**) as hypothesised, but looked to frequently interact with E981 in a pincer-type manner. This could be due to E984 being a largely solvent exposed residue, hence a desolvation penalty would be incurred from any interaction. The second barcode plot shows **25** modelled with the amine chain pointing 'up' away from the binding site, and this appeared to produce weaker interactions with N1039 and E981. Therefore, it appeared that there was a preference for ligand **25** to be 'down' within the binding pocket. This was further confirmed using absolute binding free energy calculations performed by Dr Joe Bluck[143] (**Table 2.5**).

Table 2.5. A table showing the absolute binding free energy calculations performed by Dr Joe Bluck using GROMACS 2016.3. ΔG values represent the mean of three repeats, and the errors reported as 95% confidence intervals. The intervals were calculated by multiplying the standard error by the relevant t-statistic.[143]

Compound	Pose	ΔG_{calc} (kcal/mol)
25	Up, away from binding site	−5.90 ± 3.00
	Down, towards binding site	−8.85 ± 1.23

This result suggested that the best vector to project a hydrophobic group from in order to gain interactions with the region above the KAc binding pocket was as shown in **Figure 2.9**.

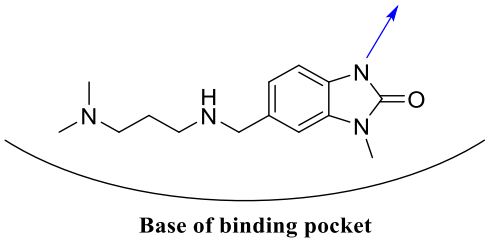


Figure 2.9. Vector chosen to project hydrophobic groups from to gain interactions with region above the KAc binding pocket.

2.6.1 Design of Compounds

After careful consideration, a series of compounds were designed to target the hydrophobic region above the binding pocket (**Figure 2.10**). A range of small saturated groups were proposed, including ethyl (**47**), isopropyl (**48**) and cyclopropyl (**49**) groups, which were extended into larger

cyclopentyl (**50**) and adamantyl (**53**) moieties to probe the size of the hydrophobic region. Hydroxyl-containing **51** was designed to determine if an additional hydrogen bond could be gained with N1039 through a pincer-like interaction. A series of aromatic groups were also envisioned, with ranging flexible and rigid linkers to explore the role of conformation when interacting with the hydrophobic region (**54**–**57**). Sulfonamide **58** and sulfone **60** were considered to investigate rigidity and different conformations. Finally, methylpyrazole-containing **61** was designed to explore the extent of groups that would be tolerated in this region. Compounds labelled with an asterisk were synthesised by Dr Larissa See or Dr Amy Scorah.[131,138]

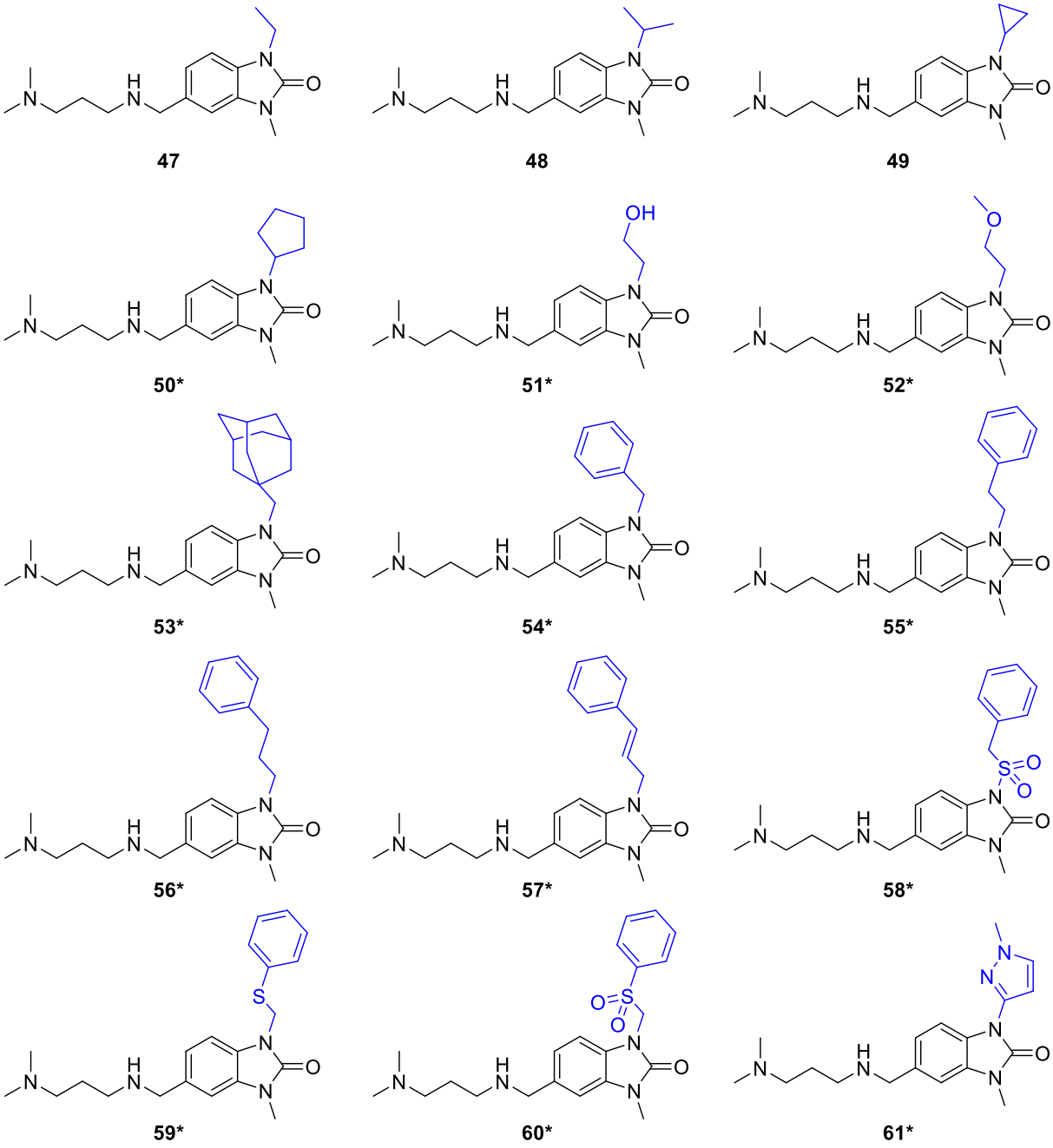


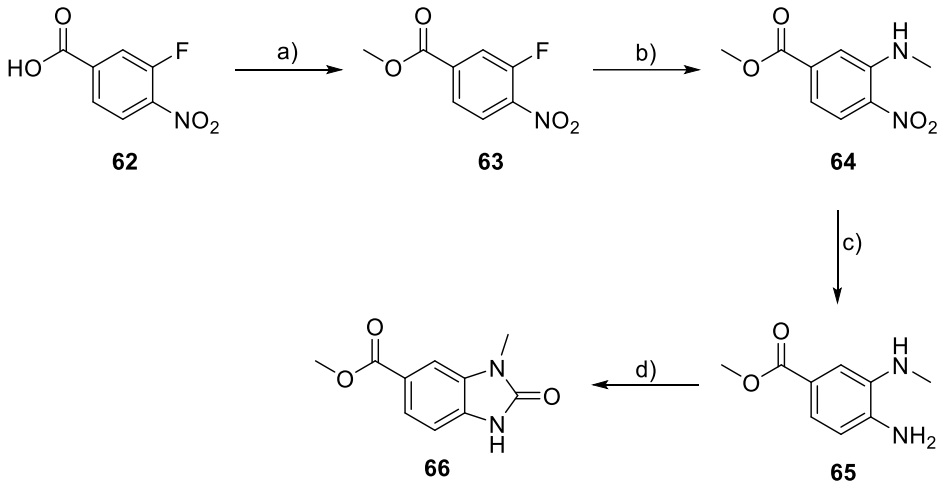
Figure 2.10. Structures of the compound series designed to probe the hydrophobic region above the binding site. *Synthesised by Dr Larissa See or Dr Amy Scorah.[131,138]

2.6.2 Synthesis of Compounds

2.6.2.1 Synthesis of Ethyl and Isopropyl Derivatives

It is important when undertaking SAR studies that the synthetic route chosen permits facile diversification, to allow thorough exploration of the binding pocket. With this in mind, a route was designed to allow late stage modification of the head group. The synthesis began with esterification of acid **62** using iodomethane and potassium carbonate to give methyl ester **63**

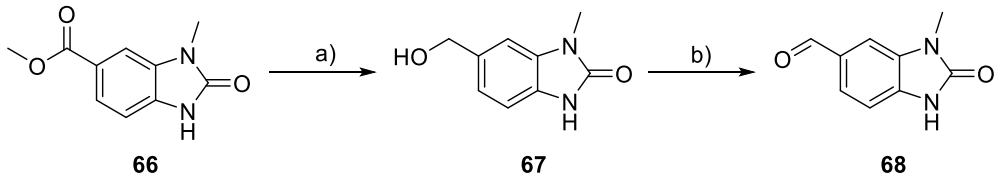
(**Scheme 2.5**). This reaction proceeded with a 91% yield, without need for purification. The next step involved an S_NAr with methylamine hydrochloride, which again gave an excellent yield of amine **64**. Reduction of the nitro group to aniline **65** was achieved using hydrogen and palladium on carbon. However, this product appeared to decompose rapidly at room temperature, so **65** was used immediately in the next step, which involved a ring closing reaction with 1,1'-carbonyldiimidazole (CDI) to form the unsymmetrical head group **66**. This proceeded with good yields, and, due to low solubility, was purified through crystallisation in water and ethanol.



62 **63** **64** **65** **66**

Scheme 2.5. Synthesis of unsymmetrical head group **66**. *Reagents and conditions:* a) MeI, K_2CO_3, DMF, rt, 16 h, 91%, n = 1; b) $MeNH_2$·HCl, DIPEA, DMF, rt, 16 h, 99%, n = 1; c) Pd/C, H_2, EtOAc, EtOH, rt, 16 h; d) CDI, CH_2Cl_2, rt, 5 h, 60% over 2 steps, n = 1.

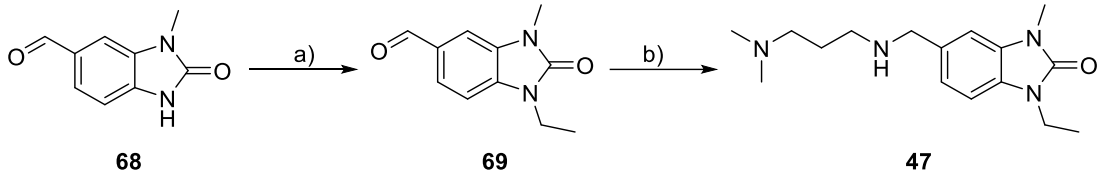
The next step required reduction of ester **66** to alcohol **67** with DIBAL, followed by oxidation using manganese(IV) oxide to give aldehyde **68** in a 71% yield (**Scheme 2.6**). It was decided that diversification should occur at this point in the synthetic route, as the aldehyde moiety was easier to handle and less likely to interfere with the subsequent substitution reaction compared to the amine chain.



66 **67** **68**

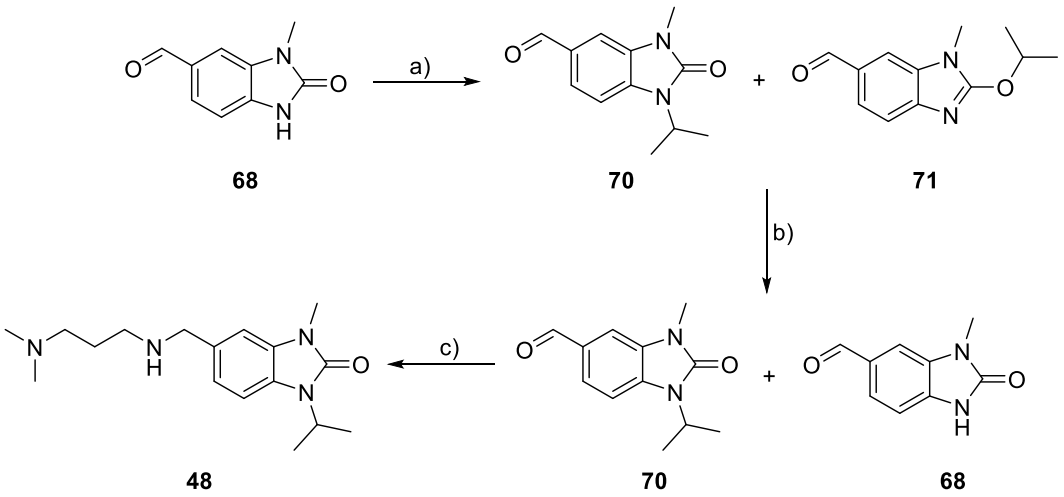
Scheme 2.6. Synthesis of aldehyde **68** ready for diversification. *Reagents and conditions:* a) DIBAL, toluene, rt, 16 h; b) MnO_2, CH_2Cl_2, rt, 16 h, 71% over 2 steps, n = 1.

The substitution reaction began by pre-stirring aldehyde **68** with potassium carbonate, followed by a dropwise addition of bromoethane and subsequent heating to 80 ºC (**Scheme 2.7**). This gave aldehyde **69** in good yield, with no other products isolated. The final step involved a reductive amination with 3-(dimethylamino)-1-propylamine, which, after purification using an Isolute® SCX-2 Amine Catch and Release column, gave the final product **47**.



Scheme 2.7. Synthesis of final product **47**. *Reagents and conditions:* a) EtBr, K_2CO_3, DMF, 80 ºC, 4 h, 71%, n = 1; b) 3-(Dimethylamino)-1-propylamine, AcOH, DCE, $NaBH(OAc)_3$, rt, 16 h, 39%, n = 1.

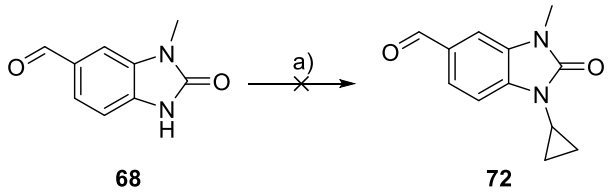
Similarly, substitution using 2-iodopropane was performed by pre-stirring aldehyde **68** with potassium carbonate, followed by addition of the electrophile (**Scheme 2.8**). After complete consumption of starting material and subsequent work up, two aldehydes were observed in the ^{1}H NMR spectrum of the crude material. It is possible that after deprotonation of **68**, both *N*-alkylation and *O*-alkylation occurred, producing two substitution products **70** and **71**. A higher concentration of the desired product **70** was observed relative to *O*-alkylation by-product **71**, which is due to the nitrogen atom being a better nucleophile compared to the oxygen atom. As expected, the R_f values of these two compounds were identical in a range of solvent systems so separation at this point could not be completed. Therefore, the mixture was heated with hydrobromic acid, which caused hydrolysis of the *O*-alkylation product **71** to the thermodynamically more stable starting material **68**, while having no effect on the desired product **70**. The two products **70** and **68** were then easily separable using column chromatography. The final step was a reductive amination with 3-(dimethylamino)-1-propylamine to give product **48**.



Scheme 2.8. Synthesis of final product **48**. *Reagents and conditions:* a) 2-Iodopropane, K_2CO_3, DMF, 80 ºC, 3 h; b) HBr in H_2O, EtOH, 78 ºC, 1 h, 52% over 2 steps, n = 1; c) 3-(Dimethylamino)-1-propylamine, AcOH, DCE, $NaBH(OAc)_3$, rt, 16 h, 53%, n = 1.

2.6.2.2 Synthesis of Cyclopropyl Derivative

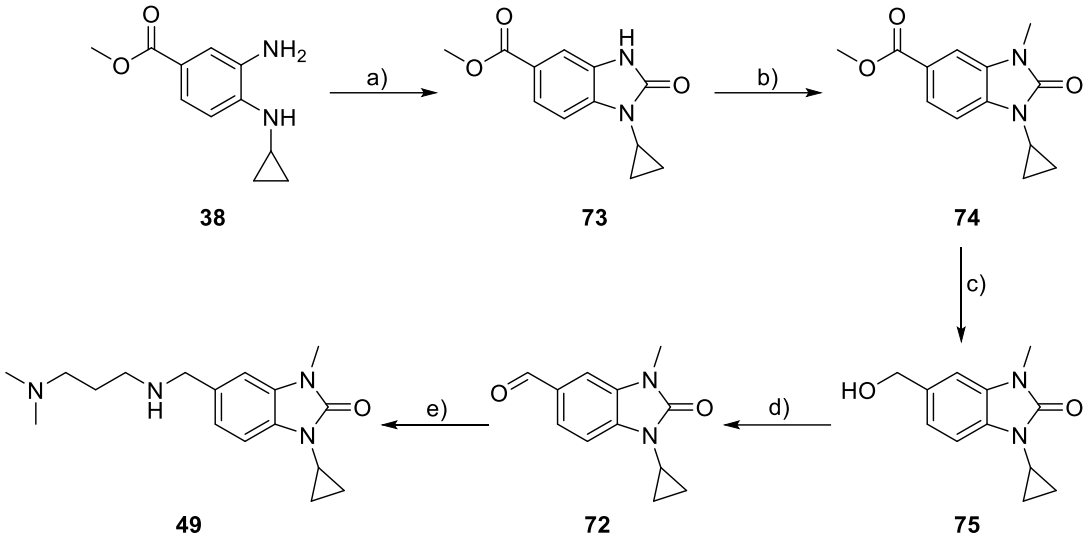
The synthesis of the cyclopropyl derivative was also attempted using a substitution under the same conditions, with bromocyclopropane (**Scheme 2.9**). However, even after an extended reaction time of 16 h at 80 ºC, only starting material remained.



Scheme 2.9. Attempted synthesis of **72** using substitution conditions. *Reagents and conditions:* a) Bromocyclopropane, K_2CO_3, DMF, 80 ºC, 16 h, no reaction.

Due to the structural similarity of previously synthesised six-membered head group **29**, a different synthetic route was proposed to complete the synthesis, which began with common intermediate **38** (**Scheme 2.10**). The ring closure was performed with CDI, and subsequently methylated to yield cyclopropyl-containing head group **74**. The next steps involved reduction of the ester moiety using DIBAL, followed by oxidation using manganese(IV) oxide to give aldehyde **72** in a poor yield. All starting material was consumed during both synthetic steps, thus the poor yield was attributed to insolubility of aldehyde **72**, which could have remained on the Celite® layer used to remove manganese(IV) oxide. Despite this, sufficient product was recovered, and these steps were not further optimised. The final step was a reductive amination with

3-(dimethylamino)-1-propylamine to give final product **49**. This also gave a low yield, which was attributed to difficulty with purification due to the polar nature of the product. However, a sufficient amount of final product **49** was purified for biological evaluation.



Scheme 2.10. Synthesis of final product **49**. *Reagents and conditions:* a) CDI, CH_2Cl_2, rt, 16 h, 29%, n = 1; b) MeI, K_2CO_3, DMF, rt, 36 h, 94%, n = 1; c) DIBAL, CH_2Cl_2, rt, 16 h; d) MnO_2, CH_2Cl_2, rt, 16 h, 26% over 2 steps, n = 1; e) 3-(Dimethylamino)-1-propylamine, AcOH, DCE, $NaBH(OAc)_3$, rt, 16 h, 17%, n = 1.

2.6.3 Evaluation of Compounds

Compounds **50–61** were synthesised by Dr Larissa See[131] and Dr Amy Scorah[138] using the same substitution conditions as detailed for the ethyl and isopropyl derivatives **47** and **48**. The exception was methylpyrazole analogue **61**, which was synthesised using a Chan-Lam coupling.[145]

2.6.3.1 AlphaScreen™ Results

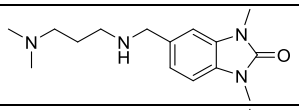
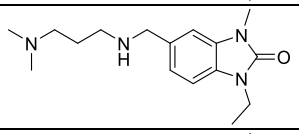
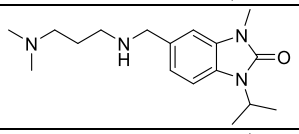
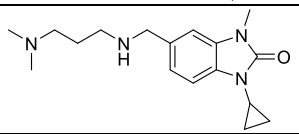
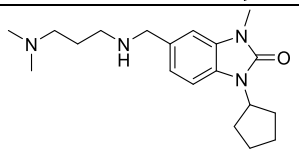
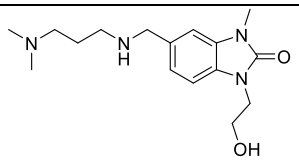
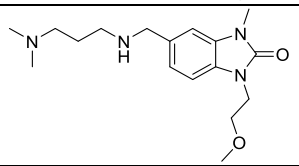
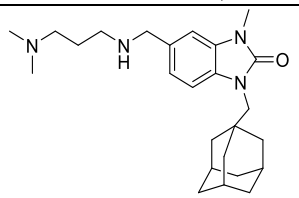
The 15 compounds synthesised were evaluated using a dose-response AlphaScreen™. The compounds were tested against TRIM24, TRIM33α and TRIM33β in combination with peptides that bind to the PHD finger ($H3_{1–27}K9Me_3$), bromodomain ($H3_{1–27}K18Ac$) and both domains ($H3_{1–27}K9Me_3K18Ac$) to give a total of nine combinations. The data presented and discussed corresponds to results of the combination of the bromodomain-binding $H3_{1–27}K18Ac$ peptide with each protein, but all data for other protein-peptide combinations can be found in Appendix C. GraphPad Prism 7 software was used to determine IC_{50} values where sigmoidal curves were achieved, by fitting data points to the four-parameter equation described in **Chapter 2.4.4.1**. The

results for alkyl substituent compounds **47–53** are shown in **Table 2.6**. It appeared that the highest affinity compounds for TRIM33β were those containing smaller alkyl substituents, with ethyl and isopropyl analogues **47** and **48** displaying IC_{50} values of 14.7 μM and 19.6 μM, respectively. However, these were very similar values to the dimethylated control **25** (IC_{50} = 13.7 μM), indicating no advantageous interactions were made through the addition of these small groups. Surprisingly, cyclopropyl analogue **49** appeared to have a low affinity for TRIM24 (IC_{50} = 124 μM), and a much weaker affinity for TRIM33β (IC_{50} = 96.0 μM) compared to the control **25**, despite only containing a small modification. The cyclopentyl analogue **50** appeared to have a higher affinity (IC_{50} = 60.2 μM) than the cyclopropyl analogue **49**, but lower affinity than the ethyl and isopropyl analogues **47** and **48** (IC_{50} = 14.7 μM and 19.6 μM, respectively). During MD studies performed by Dr Joe Bluck, **50** was shown to potentially form an interaction with I990. However, since the IC_{50} value is higher than the control, this possible favourable interaction may be offset by the benzimidazolone head group being pulled away from N1039, which was observed during the MD simulation. This not only reduces the strength of the key carbonyl and N1039 hydrogen bond, but consequently moves the benzylic amine away from E981, which is postulated to be an essential interaction for affinity and selectivity. The hydroxyl containing analogue **51** appeared to have similar affinity (IC_{50} = 20.6 μM) to the isopropyl analogue **48** (IC_{50} = 19.6 μM), and is much higher affinity than the methyl ether **52**, which showed no binding to TRIM33β. This could indicate that the desired additional hydrogen bond to N1039 may be occurring, but affinity is not increased compared to control **25**. Finally, adamantyl analogue **53** showed no binding to any of the three proteins, signifying the hydrophobic region may not be large enough to accommodate this bulky group.

Each compound, excluding the adamantyl analogue **53**, appeared to bind to TRIM33α with low micromolar affinity, comparable with dimethylated control **25**. This could be explained by the additional 17 amino acid residues in the BC loop of TRIM33α compared to TRIM33β resulting in a larger KAc binding site, and therefore the site is more accommodating of additional groups in this region. Furthermore, the weaker affinities of the peptides for TRIM33α suggest they are easier to displace by ligands compared to TRIM33β.

Table 2.6. A table showing IC_{50} values for the compounds **47–53** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from a highest compound concentration of 500 µM. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green. Results shown are for each protein with $H3_{1-27}$K18Ac peptide, with ligand efficiency in brackets. *These IC_{50} values are the mean and standard deviation of multiple repeats (n = 8).

Weaker binding  **Stronger binding**

Compound	Structure	TRIM24 BRD IC_{50} (µM)	TRIM33α BRD IC_{50} (µM)	TRIM33β BRD IC_{50} (µM)
25		>250	3.61* ± 0.352 (0.38)	13.7* ± 1.53 (0.35)
47		>250	2.07 ± 0.200 (0.38)	14.7 ± 1.47 (0.32)
48		>250	3.22 ± 0.246 (0.35)	19.6 ± 1.64 (0.30)
49		124 ± 32.3 (0.25)	9.01 ± 1.66 (0.32)	96.0 ± 24.9 (0.26)
50		>250	4.32 ± 0.351 (0.31)	60.2 ± 7.42 (0.20)
51		>250	8.74 ± 0.819 (0.32)	20.6 ± 1.94 (0.30)
52		>250	16.4 ± 1.08 (0.29)	>250
53		>250	>250	>250

The aromatic analogues were also assessed using dose-response AlphaScreen™ (**Table 2.7**). The benzyl analogue **54** (IC_{50} = 86.0 µM) showed a lower affinity for the TRIM33β bromodomain than the phenethyl analogue **55** (IC_{50} = 59.7 µM), but was still stronger than propyl phenyl analogue **56** (IC_{50} = 100 µM). However, all three of these compounds had considerably weaker

affinities than dimethylated control **25** (IC_{50} = 13.7 µM), signifying that addition of these aromatic groups is not beneficial for increasing affinity to TRIM33β. Rigidifying the linker to form the unsaturated three-carbon phenyl analogue **57** resulted in an increase in affinity for TRIM33β (IC_{50} = 53.5 µM) relative to the flexible propyl phenyl analogue **56** (IC_{50} = 100 µM), which could be due to a smaller entropic loss upon binding. Nevertheless, these compounds were significantly weaker binders than control **25**. For more rigid compounds **58–61**, binding to TRIM33β was abrogated, indicating that these conformations were not suitable for the KAc binding site. On closer observation, MD studies performed by Dr Joe Bluck for methylpyrazole analogue **61** revealed that the group is held in a position just above the KAc binding pocket which appears to disrupt a side-on π-π interaction between phenylalanine F1038 and tyrosine Y993, which would disfavour binding (**Figure 2.11**). This may also be the case for the other rigid aromatic analogues **58–60**.

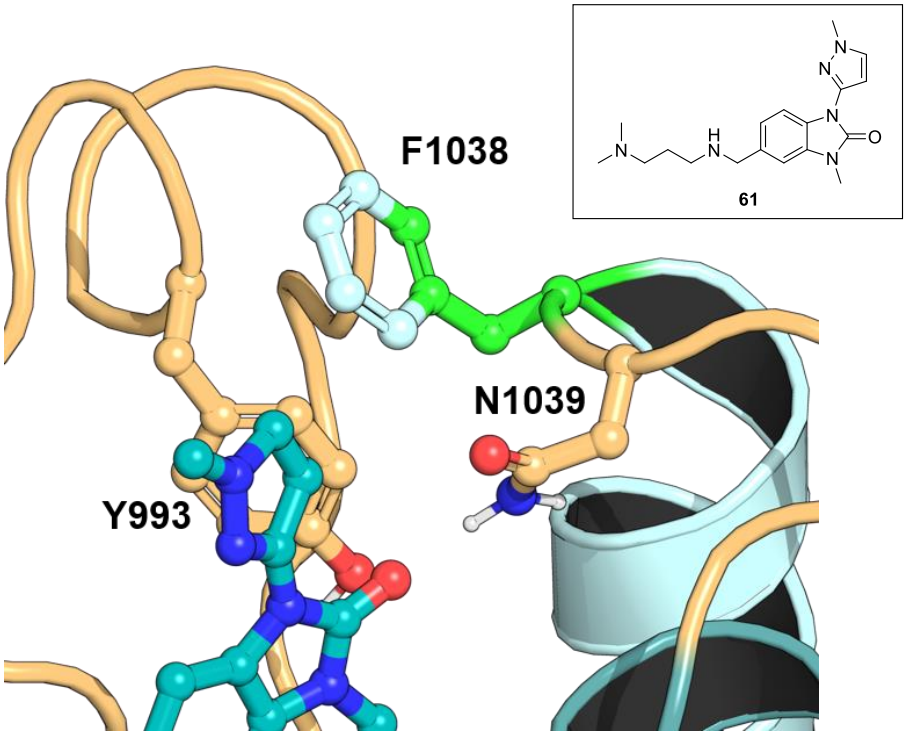
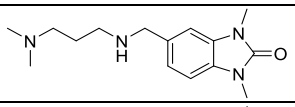
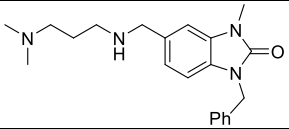
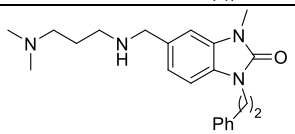
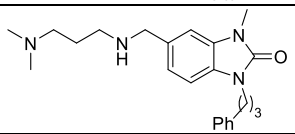
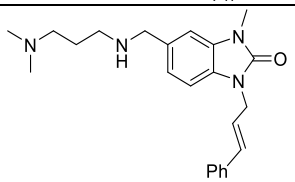
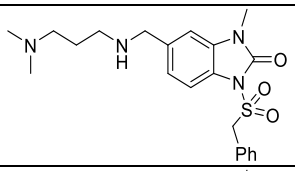
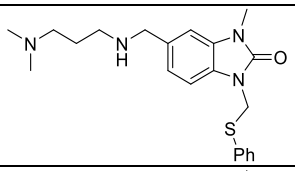
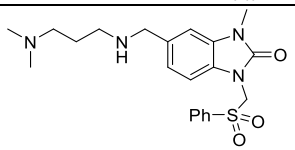
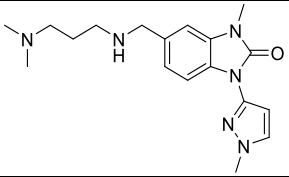


Figure 2.11. Methylpyrazole **61** docked into the TRIM33β bromodomain (PDB ID: **5MR8**). During MD simulations, the interaction between Y993 and F1038 appeared to be disrupted. Diagram by Dr Joe Bluck.

As with alkyl analogues **47–53**, binding to TRIM33α was still observed for compounds **54–57** and **59**, potentially due to the larger binding site and weaker interactions with the peptides to be displaced.

Table 2.7. A table showing IC_{50} values for the compounds **54–61** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from a highest compound concentration of 500 μM. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green. Results shown are for each protein with $H3_{1-27}$K18Ac peptide, with ligand efficiency in brackets. *These IC_{50} values are the mean and standard deviation of multiple repeats.

Weaker binding  **Stronger binding**

Compound	Structure	TRIM24 BRD IC_{50} (μM)	TRIM33α BRD IC_{50} (μM)	TRIM33β BRD IC_{50} (μM)
25		>250	3.61* ± 0.352 (0.38)	13.7* ± 1.53 (0.35)
54		>250	3.64 ± 1.11 (0.29)	86.0 ± 1.15 (0.22)
55		>250	4.34 ± 1.08 (0.28)	59.7 ± 1.12 (0.22)
56		>250	2.47 ± 1.08 (0.28)	100 ± 1.10 (0.20)
57		>250	2.01 ± 0.240 (0.28)	53.3 ± 7.89 (0.21)
58		>250	>250	>250
59		>250	1.42 ± 0.131 (0.30)	>250
60		>250	>250	>250
61		>250	>250	>250

In conclusion, no increases in affinity were observed for the TRIM33β bromodomain compared to the dimethylated control **25**, despite addition of a variety of flexible and rigid hydrophobic

substituents aiming to interact with the region above the KAc binding site. It appeared that projecting a rigid moiety from the head group vector could disrupt the side-on π-π interaction between Y993 and F1038 in the TRIM33β bromodomain. It was hypothesised that the vector of projection for targeting the hydrophobic region may be not be optimal, so different vectors should be considered.

2.6.3.2 WaterLOGSY Results

Despite a reduction in affinity observed for most of the hydrophobic compounds compared to control **25**, the three compounds that showed comparable binding to TRIM33β were validated using an orthogonal WaterLOGSY NMR experiment. As previously discussed, WaterLOGSY is an appropriate method to validate binding of low affinity ligands, typically in the μM to mM region. A ^{1}H NMR experiment is run for each ligand, in which the signals are positive, followed by a compound-only WaterLOGSY experiment, to determine if the compound is forming soluble aggregates. If this is occurring, the signals will remain positive, so the solution must be diluted until no aggregation occurs, indicated by negative peaks in the WaterLOGSY spectrum. On addition of protein, binding is observed if the signals return to positive, and non-binding is revealed by signals remaining negative, therefore still behaving as a small molecule and not as part of a protein-ligand complex. The results from the WaterLOGSY NMR experiments for **47**, **48**, and **51** are shown in **Figure 2.12** (extended spectra shown in Appendix D). In agreement with AlphaScreen™ data, it appeared that all three compounds showed no binding to TRIM24, whereas binding is observed to both TRIM33α and TRIM33β. Performing an orthogonal assay provided confirmation that the binding observed in AlphaScreen™ was not due to interference.

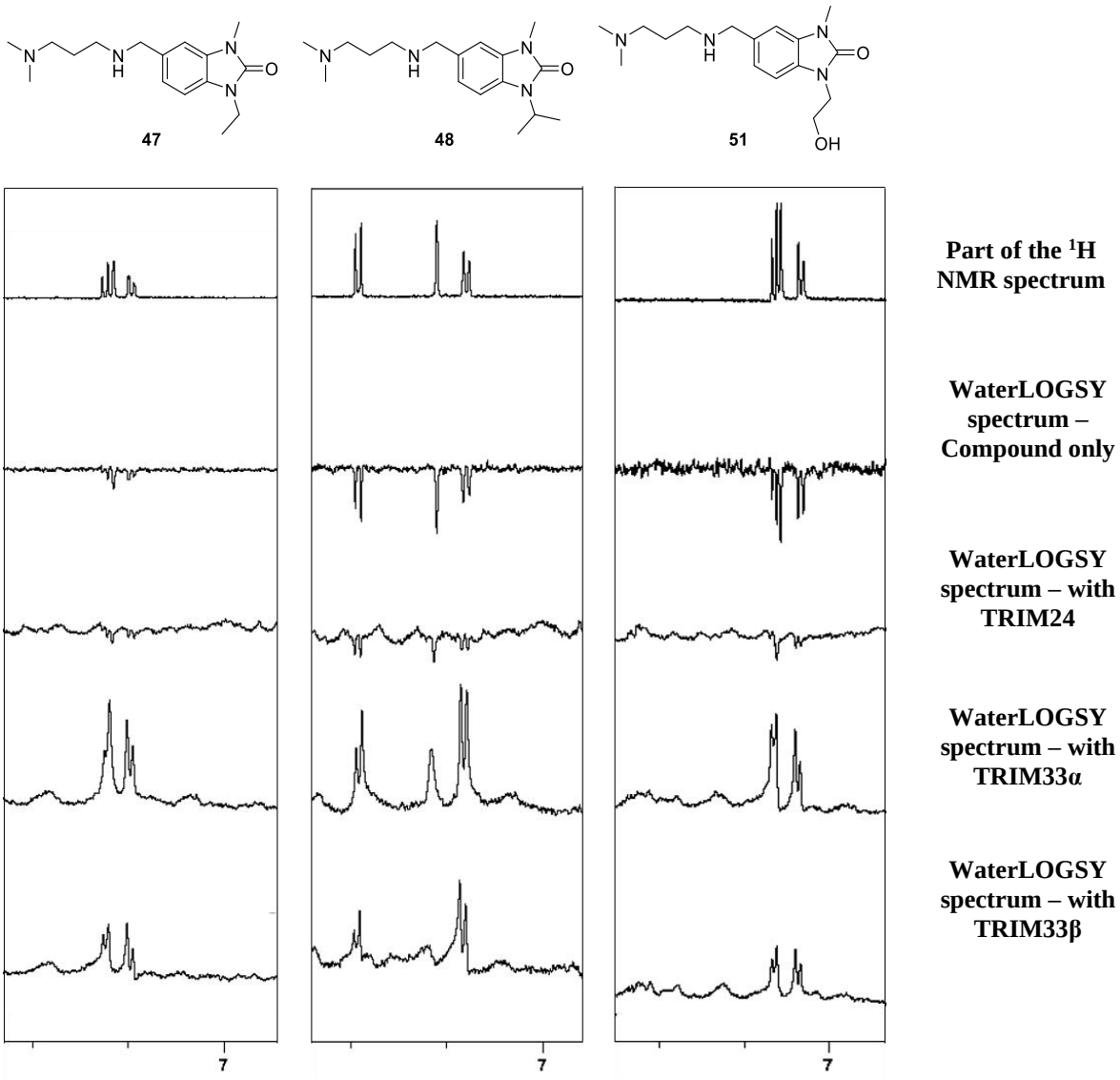
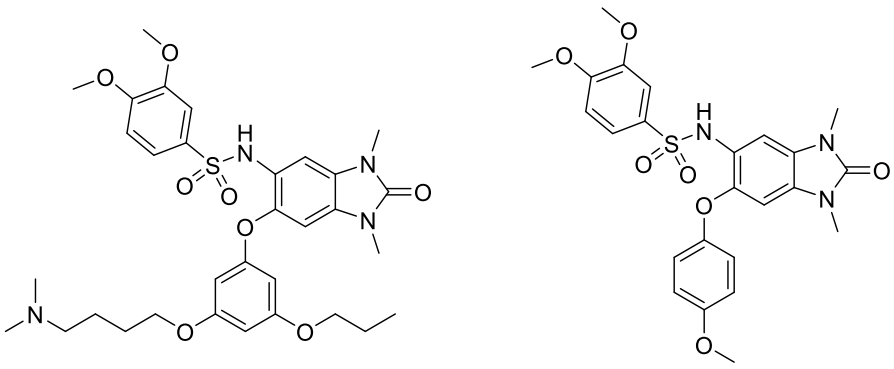


Figure 2.12. Part of the WaterLOGSY spectra of **47**, **48**, and **51**. Spectra stacked, showing ^{1}H NMR spectrum, compound only WaterLOGSY spectrum, and compound in the presence of protein WaterLOGSY spectra. No binding to TRIM24 was observed for all three compounds, whereas binding to both TRIM33α and TRIM33β were seen, indicated by positive signals in the spectra with protein added.

2.7 Targeting the Hydrophobic Region with Sulfonamides

Having established that projection of hydrophobic groups from the benzimidazolone core does not lead to an increase in affinity for the TRIM33β bromodomain, it was hypothesised that a different vector of projection could be explored. Since TRIM33 and TRIM24 have 68% sequence similarity,[60] attention turned to the two published TRIM24 ligands.[41,42] These structures bind to the TRIM24 bromodomain with low nanomolar affinity (**Figure 2.13**). Comparable to the TRIM33 ligands, they also contain the benzimidazolone head group as an acetyl-lysine mimic, and include two groups projected from the aromatic ring: a phenol moiety and a sulfonamide.



6
TRIM24 IC_{50} 8 nM
BRPF IC_{50} 14 nM

7
TRIM24 IC_{50} 220 nM
BRPF IC_{50} 140 nM

Figure 2.13. Structures of the two TRIM24 bromodomain ligands with binding affinities listed below.[41,42]

Palmer *et al*.[42] describe the sulfonamide moiety of **6** making lipophilic contact with V986 to increase affinity for the TRIM24 bromodomain (PDB ID: **4YC9**). TRIM33β has an analogous valine V1045 which occupies the same space in the crystal structure and therefore has the potential to form this interaction (**Figure 2.14A**). Introduction of a sulfonamide moiety into the TRIM33 ligand (**76**) could also position an aromatic ring in the correct binding conformation over the lipophilic region and, depending on the preferred orientation, there is potential to gain an interaction with either valine V1045 or isoleucine I990 (**Figure 2.14B**). With hydrophobic groups projected from this alternative vector, there is a lower chance of disrupting the π-π interaction between Y993 and F1038, which appeared to be a problem when projecting substituents from the head group.

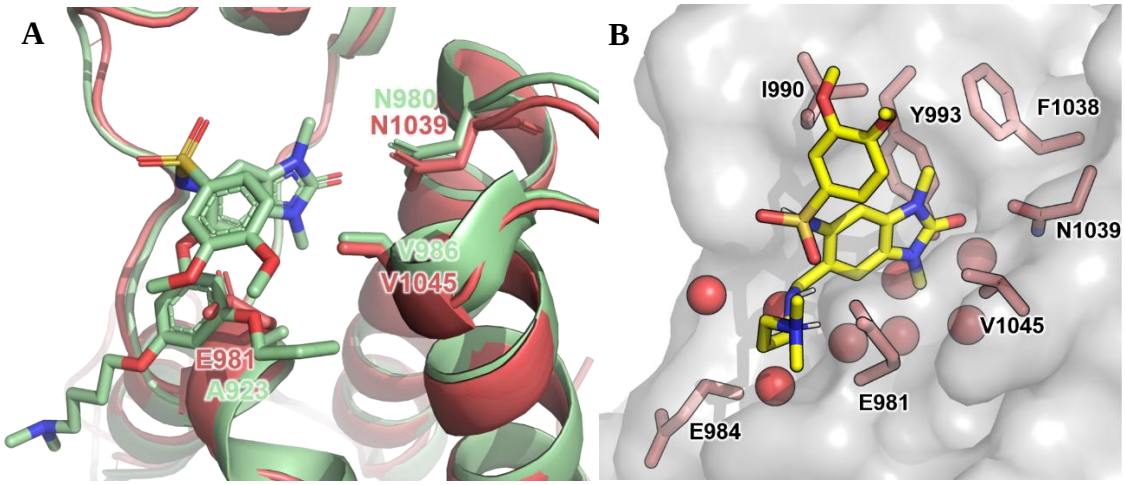


Figure 2.14. A. Crystal structure of TRIM24 containing **6** (PDB ID: **4YC9**) overlaid with the structure of TRIM33β (PDB ID: **5MR8**). **B.** Sulfonamide containing TRIM33 ligand **76**, docked into TRIM33β bromodomain using CLC Drug Discovery Workbench 3.0, with key residues highlighted (Dr Joe Bluck).

2.7.1 Design of Compounds

A series of compounds were designed to incorporate the sulfonamide moiety into the TRIM33 ligand adjacent to the amine chain (**Figure 2.15**). Focus centred on aromatic sulfonamides that appeared to sit flat on top of the hydrophobic region away from Y993 and F1038, potentially forming an interaction with I990 (CLC Drug Discovery Workbench 3.0). Phenyl **77**, methoxyphenyl **78**, dimethoxyphenyl **76**, pyridyl **79** and difluoromethoxyphenyl **80** analogues were all chosen to probe whether the stereoelectronics of the aromatic ring was important. Cyclopropyl **81** was also designed to investigate if a small alkyl group could be beneficial.

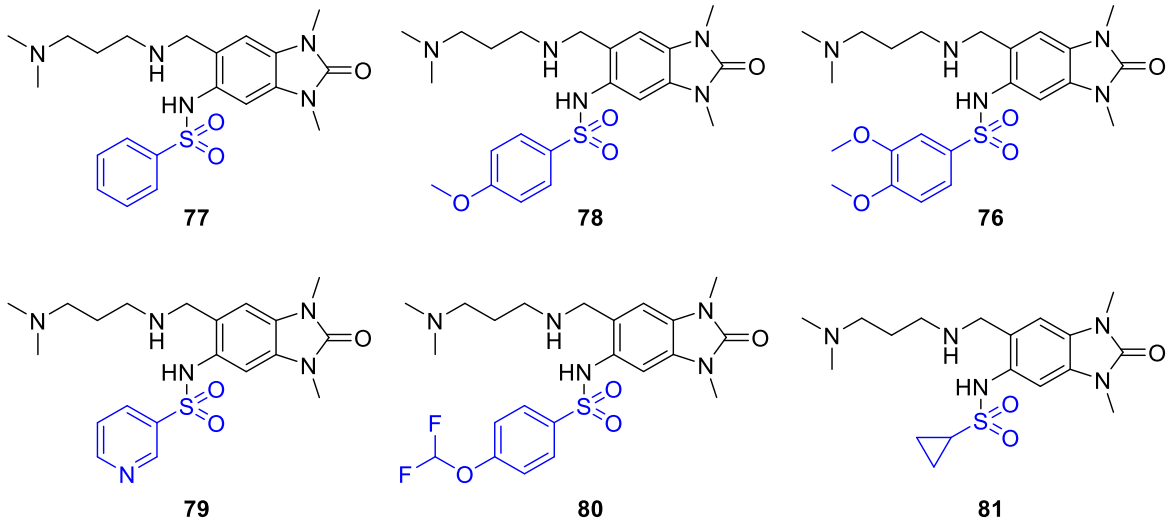
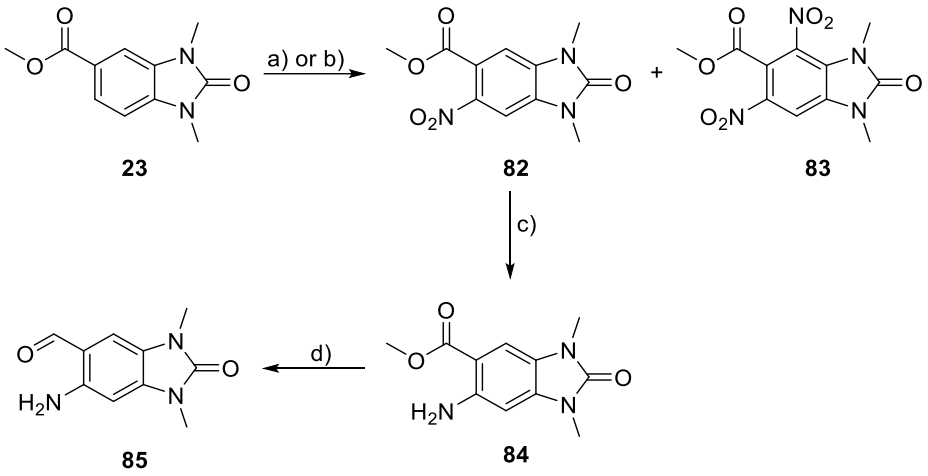


Figure 2.15. Structures **76–81** of the proposed sulfonamide analogues.

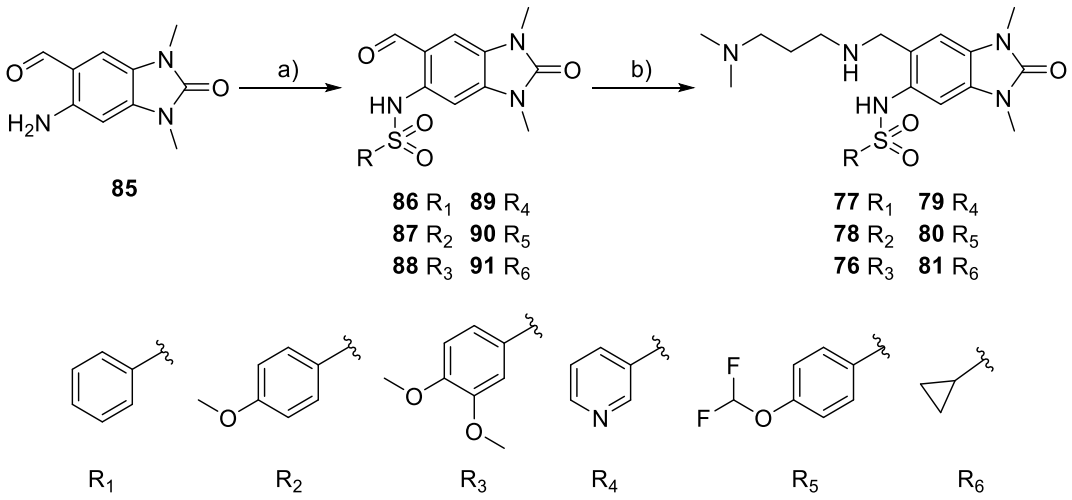
2.7.2 Synthesis of Compounds

A retrosynthetic approach in which a tetrasubstituted benzene could be formed from previously synthesised ester **23** was considered (**Scheme 2.11**). The synthetic route began with a nitration of **23**. This was first attempted using fuming nitric acid at 0 ºC. However, dinitration product **83** was observed, with no desired product **82** detected. Altering the reaction conditions to potassium nitrate in concentrated sulfuric acid yielded the mononitration product **82** in 94–100% yields, with no side products observed. Subsequently, the nitro functionality could be reduced to aniline **84** using hydrogen and palladium on carbon, which proceeded with high yields of 75–93%. The next step involved reduction of ester **84** using DIBAL, followed by oxidation with manganese(IV) oxide, which gave good yields of desired aldehyde **85**.



Scheme 2.11. Synthesis of aldehyde **85**. *Reagents and conditions:* a) HNO_3, 0 ºC, 30 min, no desired product formed, n = 1; b) KNO_3, H_2SO_4, rt, 1 h, 94–100%, n = 4; c) Pd/C, H_2, EtOAc, rt, 4 h, 75–93%, n = 5; d) i. DIBAL, CH_2Cl_2, −78 ºC, 2 h, 55–65%, n = 4, ii. MnO_2, CH_2Cl_2, rt, 16 h, 60–90%, n = 4.

The penultimate step consisted of sulfonamide formation, allowing synthesis of six sulfonamides **86–91** from the corresponding sulfonyl chlorides, which proceeded in a range of yields (**Scheme 2.12**). Finally, a reductive amination of aldehydes **86–91** with 3-(dimethylamino)-1-propylamine installed the amine chain. Initially, sodium acetoxyborohydride was utilised as a reducing agent. However, on observing the ^{1}H NMR spectrum of the crude product after an aqueous work up, it appeared that intermediate imine had been isolated, and the desired product was not seen. Addition of 1.5 equivalent of sodium borohydride also did not effectively reduce the imine. Therefore, the imine was formed, isolated, and subsequently dissolved in methanol and reduced with 5 equivalents of sodium borohydride, which successfully gave the desired products **76–81**. Low yields for some of these reactions could be attributed to difficulty with purification, as these compounds contain several polar functional groups, thus column chromatography required a solvent system of ammonium hydroxide, ethanol and ethyl acetate for elution. Despite this, sufficient quantities of each product were isolated for evaluation by AlphaScreen™.



Scheme 2.12. Final synthetic steps to yield sulfonamide products **76–81**. *Reagents and conditions:* a) R_X-SO_2Cl, pyridine, CH_2Cl_2, rt, 16 h, **86**: 69%, n = 1, **87**: 35%, n = 1, **88**: 54%, n = 1, **89**: 43%, n = 1, **90**: 61%, n = 1, **91**: 25%, n = 1; b) i. 3-(Dimethylamino)-1-propylamine, AcOH, DCE, ii. $NaBH_4$, MeOH, rt, 16 h, **77**: 53%, n = 1, **78**: 37%, n = 1, **76**: 71%, n = 1, **79**: 19%, n = 1, **80**: 43%, n = 1, **81**: 60%, n = 1.

2.7.3 Evaluation of Compounds by AlphaScreen™

Compounds **76–81** were evaluated using a dose-response AlphaScreen™ (**Table 2.8**), using the same assay conditions as previously described. Disappointingly, it appeared the addition of the sulfonamide moiety in five of the six compounds prevented any binding to TRIM33β, and IC_{50} values could not be calculated. Monomethoxy analogue **78** showed weak binding (IC_{50} = 77.7 μM) compared to the control **25** (IC_{50} = 13.7 μM). MD simulations performed by Dr Joe Bluck[143] indicated that the aromatic ring of the sulfonamide moiety made no permanent interactions with any residues in the hydrophobic region, including I990 and V1045, resulting in low binding affinities (**Figure 2.16**). Furthermore, it is predicted (ACD/I-Lab 2.0) that the addition of the sulfonamide reduces the pK_a of the adjacent benzylic amine from 9.70 in the control **25** to 7.80 (for **76**). It was previously established that the ionic interaction between E981 and the benzylic amine was important, so reducing pK_a reduces the percentage of protonation at this site and therefore is likely to reduce binding affinity.

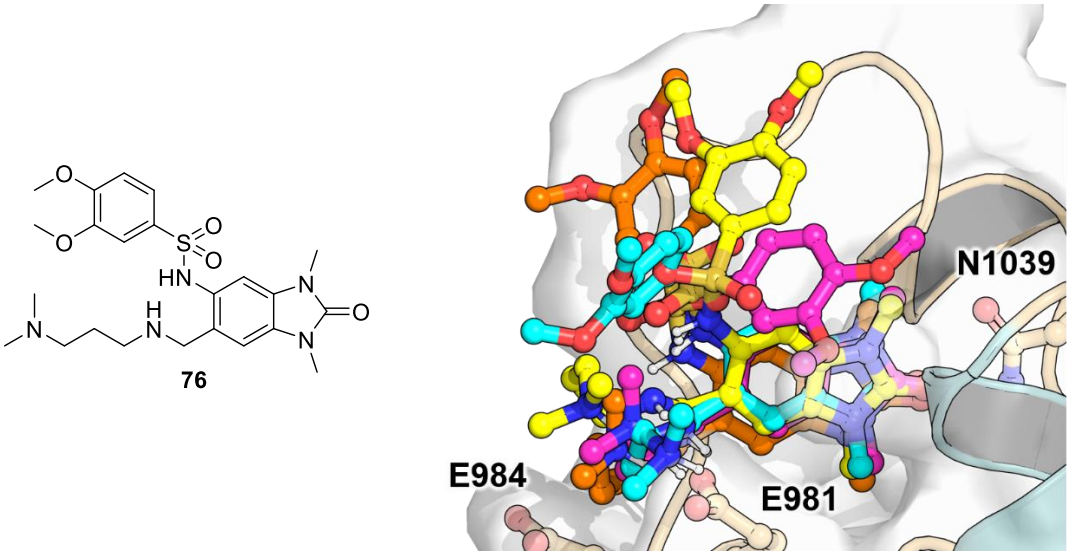
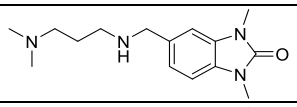
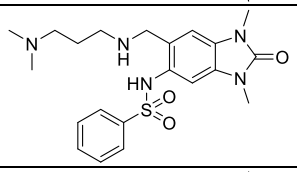
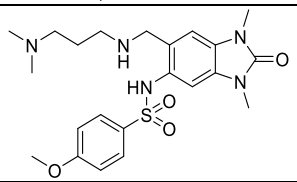
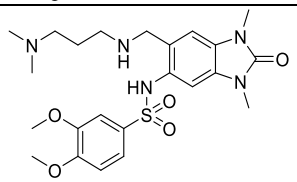
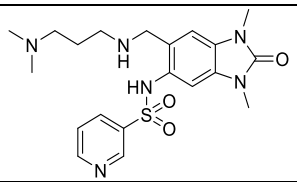
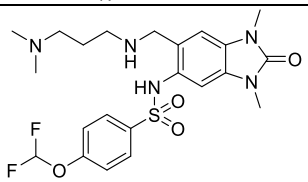
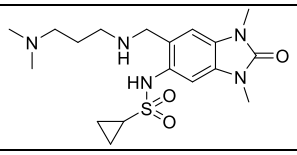


Figure 2.16. Superimposed snapshots of the MD stimulations performed by Dr Joe Bluck indicating that the sulfonamide moiety does not appear to make any permanent interactions. Diagram by Dr Joe Bluck.

Despite this, all six analogues appeared to still bind to TRIM33α with low micromolar affinities. As previously discussed, this could be due to the larger KAc binding site in TRIM33α accommodating the larger ligands, in addition to the weaker peptide-protein interaction allowing easier displacement.

Table 2.8. A table showing IC_{50} values for the compounds **76–81** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from a highest compound concentration of 500 μM. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green. Results shown are for each protein with $H3_{1-27}K18Ac$ peptide, with ligand efficiency in brackets. *These IC_{50} values are the mean and standard deviation of multiple repeats (n = 8).

Weaker binding  **Stronger binding**

Compound	Structure	TRIM24 BRD IC_{50} (μM)	TRIM33α BRD IC_{50} (μM)	TRIM33β BRD IC_{50} (μM)
25		>250	3.61* ± 0.352 (0.38)	13.7* ± 1.53 (0.35)
77		>250	21.5 ± 3.50 (0.22)	>250
78		>250	12.7 ± 1.42 (0.21)	77.7 ± 13.9 (0.18)
76		>250	71.7 ± 7.02 (0.17)	>250
79		>250	11.2 ± 2.10 (0.23)	>250
80		>250	9.52 ± 1.09 (0.21)	>250
81		>250	4.20 ± 0.476 (0.28)	>250

In conclusion, projecting large hydrophobic substituents from the head group or adjacent to the amine chain did not improve affinity, possibly due to the disruption of a key side-on π-π interaction within the protein or the decrease in basicity of the benzylic amine caused by the adjacent sulfonamide. These results also indicated that affinity of TRIM24 ligands **6** and **7** for the bromodomain may be promoted by the π-stacking interaction that can form between the two

adjacent aromatic substituents, which is not the case for the TRIM33 ligands. Hydrophobic interactions are known to play an important role in increasing the affinity of drugs,[146] therefore a different strategy was considered.

2.8 Targeting V986 and I990

On observation of control **25** docked into the TRIM33β crystal structure, it appeared that an alkyl group could be accommodated in an unexplored vector, indicated by the orange arrow (**Figure 2.17**). A small, relatively rigid substituent could be held in the right conformation to interact with I990 and V986, without disrupting residue interactions within the protein.

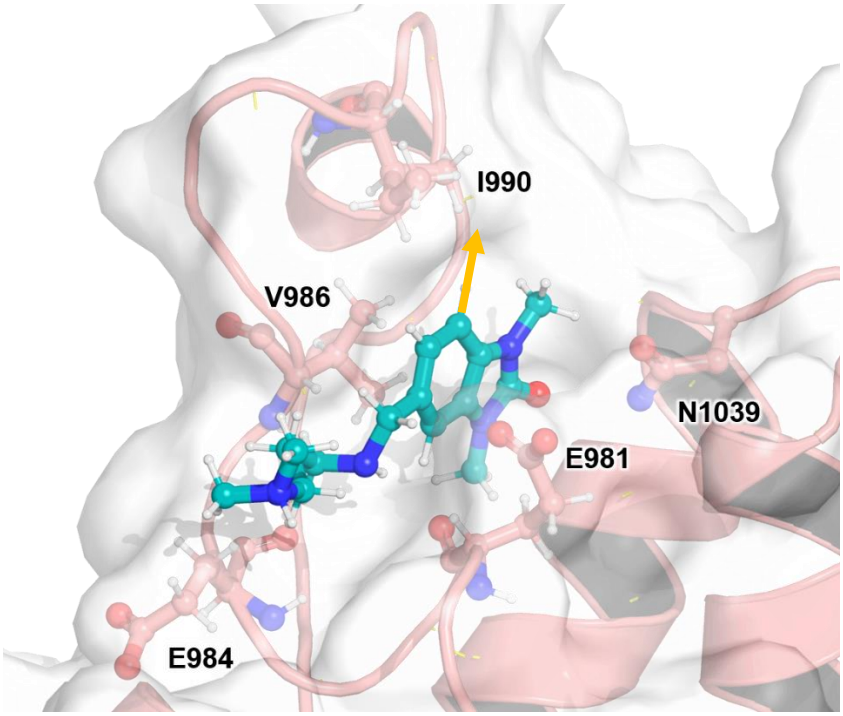
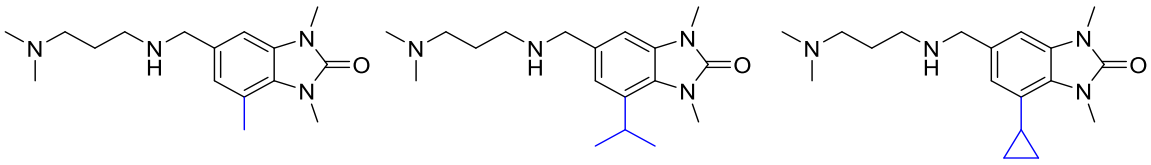


Figure 2.17. Control **25** docked using CLC Drug Discovery Workbench 3.0 into the crystal structure of TRIM33β (PDB ID: **5MR8**). The orange arrow indicates the unexplored vector to project a small alkyl group from to gain interactions with I990 and V986.

2.8.1 Design of Compounds

A small series of ligands were designed to test this hypothesis (**Figure 2.18**). Methyl **92**, isopropyl **93** and cyclopropyl **94** analogues were all considered as appropriate substituents to project from this vector to interact with I990 without disrupting the π-π interaction between Y993 and F1038 that may abrogate binding to TRIM33β.

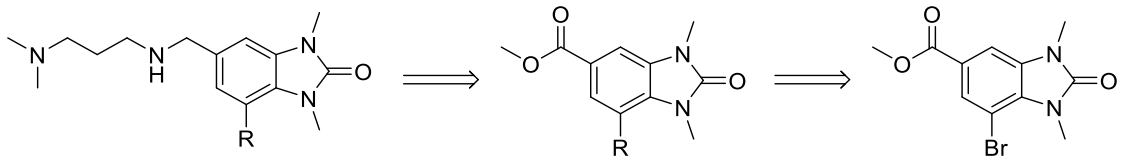


92 **93** **94**

Figure 2.18. Structures of the proposed ligands **92–94**.

2.8.2 Synthesis of Compounds

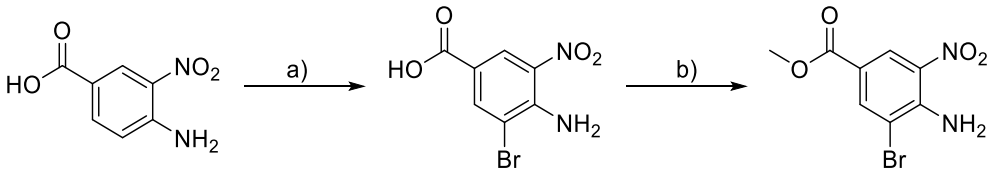
The strategy envisioned for synthesis of compounds **92–94** involved a Suzuki coupling to allow diversification of the small alkyl substituent at a late stage in the synthetic route (**Figure 2.19**).



95 **96** **97**

Figure 2.19. Retrosynthetic analysis of **95** to install small hydrophobic groups, which would proceed through common intermediate **97**.

This approach required synthesis of common intermediate **97**. Synthesis began with a bromination of 4-amino-3-nitrobenzoic acid **98** using *N*-bromosuccinimide following a literature procedure,[147] went to completion by TLC analysis to give product **99** (**Scheme 2.13**). However, the resulting succinimide by-product was difficult to remove, so the reaction was tried using bromine as an alternative brominating agent, again following a literature procedure.[148] This method was successful and gave 98–99% yields of **99**. As **99** was insoluble in a range of solvents, the acid functionality was converted to ester **100** to increase solubility.

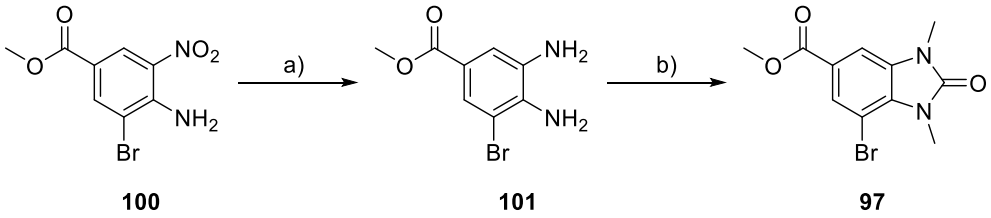


98 **99** **100**

Scheme 2.13. Synthesis of ester **100**. *Reagents and conditions:* a) Br_2, AcOH, rt, 4 h, 98–99%, n = 2; $SOCl_2$, MeOH, 50 ºC, 2 h, 82–84%, n = 2.

The next step required a reduction of the nitro group to an aniline (**Scheme 2.14**). Use of a palladium catalyst to perform this reaction could potentially lead to dehalogenation, thus iron and acetic acid were selected. The reaction appeared to go to completion by TLC analysis, with all

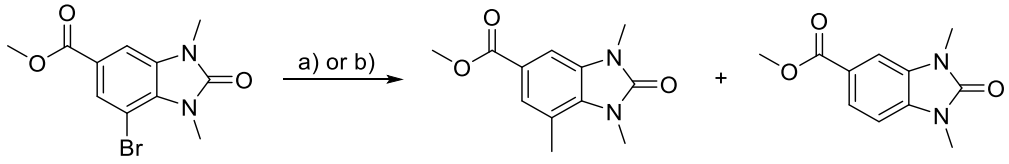
starting material **100** being consumed and a new product spot staining purple when exposed to ninhydrin. However, the ^{1}H NMR spectrum of the crude product showed very weak, broad peaks despite the high concentration of the sample, presumably indicating that the 1,2-diamine product was coordinating iron to form a complex. Therefore, zinc and ammonium chloride were investigated as an alternative, which gave the desired 1,2-diamine **101** in excellent yields. Following this, a CDI ring closure and subsequent methylation gave excellent yields of the benzimidazolone head group **97**.



100 **101** **97**

Scheme 2.14. Final steps to synthesise common intermediate **97**. *Reagents and conditions:* a) Attempt 1: Fe, AcOH, MeOH, rt, 4 h, very broad NMR seen, Attempt 2: Zn, NH_4Cl, MeOH, THF, 40 ºC, 5 h, 94–97%, n = 2.; b) i. CDI, CH_2Cl_2, rt, 1 h, 78–90%, n = 2, ii. MeI, K_2CO_3, DMF, rt, 16 h, 67–95%, n = 2.

With **97** in hand, eight sets of Suzuki coupling conditions were screened, using methylboronic acid or trimethylboroxine as the coupling partner. The desired methylated product **102** was observed in two of the reactions (**Scheme 2.15**), although the dehalogenated product **23** was also seen. The similarity in R_f value of these two products made purification ineffectual by column chromatography. Since the dehalogenated product **23** was produced in all of the reaction conditions screened, a different route was considered.

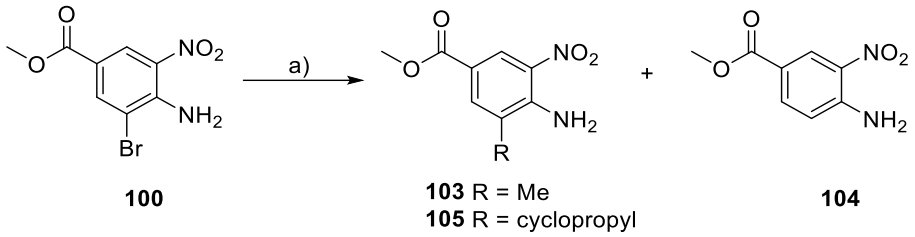


97 **102** **23**

Scheme 2.15. Suzuki reaction producing both desired product **102** and dehalogenated product **23**. *Reagents and conditions:* a) Trimethylboroxine, $Pd(PPh_3)_4$, K_2CO_3, dioxane, 100 ºC, 16 h, ratio 3.2:1 **102**:**23** *via* ^{1}H NMR spectrum of the crude material, b) Methylboronic acid, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, K_2CO_3, dioxane, H_2O, 100 ºC, 2 h, ratio 1:2 **102**:**23** *via* ^{1}H NMR spectrum of the crude material.

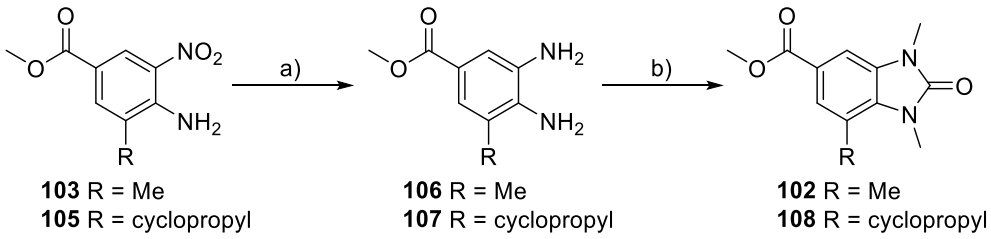
The Suzuki reaction was instead attempted on **100**, in hope that any dehalogenated product would be easier to remove (**Scheme 2.16**). This was found to be the case, with desired product **103** and side product **104** separable by column chromatography. Similarly, the reaction was repeated with

cyclopropylboronic acid, which gave desired product **105**. However, when the same conditions were investigated with isopropylboronic acid, only starting material **100** and dehalogenated product **104** were isolated. Due to time constraints, optimisation for the isopropyl derivative was not explored.



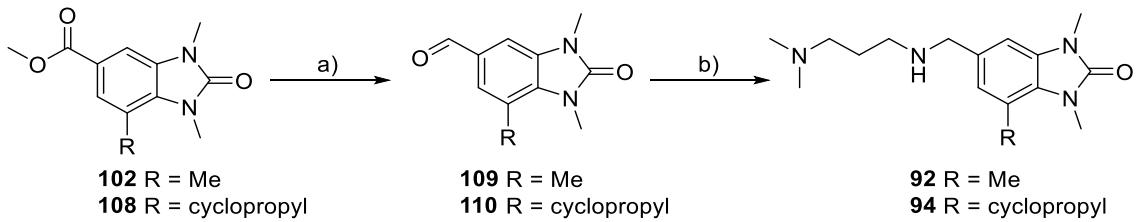
Scheme 2.16. Suzuki reaction producing both desired product and dehalogenated product **104**, which could be separated by column chromatography. *Reagents and conditions:* a) $RB(OH)_2$, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, K_2CO_3, dioxane, H_2O, 80 ºC, 18 h, **103**: 18–20%, n = 2, **105**: 14–20%, n = 2.

Subsequently, reduction of the nitro functionality to the aniline proceeded with excellent yields for both the methyl derivative **106** and the cyclopropyl derivative **107** (**Scheme 2.17**). The next step required a ring closure using CDI followed by a methylation to produce the benzimidazolone head group, which again gave good yields of both **102** and **108**.



Scheme 2.17. Synthesis of benzimidazolone head group. *Reagents and conditions:* a) Zn, NH_4Cl, MeOH, THF, 40 ºC, 2 h, **106**: 96%, n = 1, **107**: 97%, n = 1; b) i. CDI, CH_2Cl_2, rt, 3 h, ii. MeI, K_2CO_3, DMF, rt, 16 h, **102**: 47% over two steps, n = 1, **108**: 39% over two steps, n = 1.

The final steps required reduction and subsequent oxidation of the esters to produce aldehydes **109** and **110**, followed by a reductive amination with 3-(dimethylamino)-1-propylamine which gave the final products **92** and **94** in good yields (**Scheme 2.18**).



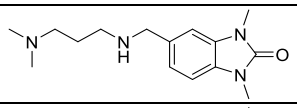
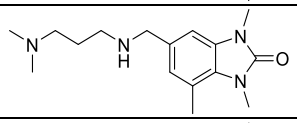
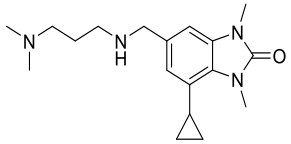
Scheme 2.18. Final steps in the syntheses of desired products **92** and **94**. *Reagents and conditions:* a) i. $LiBH_4$, THF, MeOH, 66 ºC, 4 h, ii. MnO_2, CH_2Cl_2, rt, 16 h, **109**: 87% over two steps, n = 1, **110**: 100% over two steps, n = 1; b) 3-(Dimethylamino)-1-propylamine, AcOH, $NaBH(OAc)_3$, DCE, rt, 16 h, **92**: 54%, n = 1, **94**: 58%, n = 1.

2.8.3 Evaluation of Compounds

A dose-response AlphaScreen™ was used to evaluate compounds **92** and **94** (**Table 2.9**). Unfortunately, the addition of the small hydrophobic groups caused a reduction in affinity for TRIM33β in both **92** (IC_{50} = 29.0 µM) and **94** (IC_{50} = 32.5 µM) compared to the control **25** (IC_{50} = 13.7 µM). This could be due to the angle that the small group is projected, possibly sterically clashing with residues in the binding pocket if the ideal conformation for maximising the interactions with N1039 and E981 is achieved. An AlphaScreen™ TruHits counter screen determined that there was no assay interference occurring. Due to the observed reduction in affinity, further WaterLOGSY NMR experiments were not performed for these compounds.

Table 2.9. A table showing IC_{50} values for the compounds **92** and **94** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from a highest compound concentration of 500 µM. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green. Results shown are for each protein with $H3_{1-27}$K18Ac peptide, with ligand efficiency in brackets. *These IC_{50} values are the mean and standard deviation of multiple repeats (n = 8).

Weaker binding — **Stronger binding**

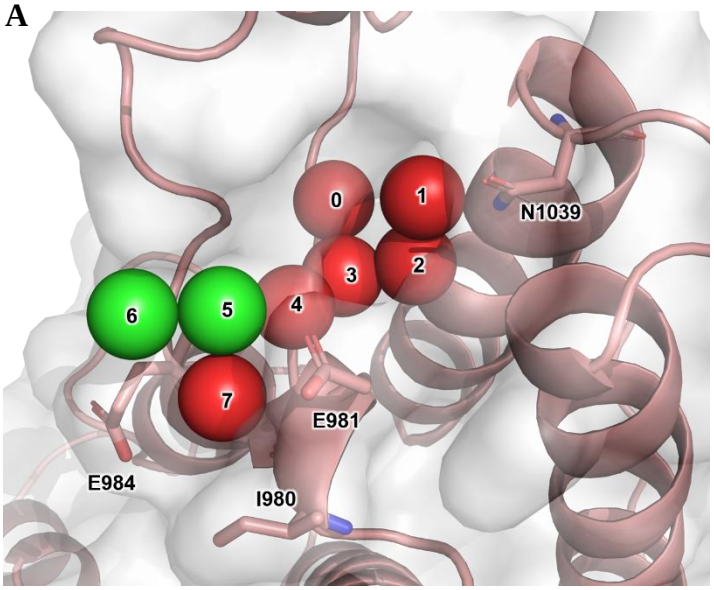
Compound	Structure	TRIM24 BRD IC_{50} (µM)	TRIM33α BRD IC_{50} (µM)	TRIM33β BRD IC_{50} (µM)
25		>250	3.61* ± 0.352 (0.38)	13.7* ± 1.53 (0.35)
92		>250	9.23 ± 1.34 (0.34)	29.0 ± 9.98 (0.30)
94		>250	4.81 ± 0.903 (0.32)	32.5 ± 10.8 (0.27)

2.9 Importance of Waters in ZA Channel

Thus far, modelling and docking studies have been performed with five water molecules present in the binding pocket, and different combinations of the ZA channel waters 5–7 present, as the

binding energies of these molecules were unknown. Water molecules are important when designing ligands for a binding site, particularly in bromodomains,[149,150] as it is essential to determine if displacement is possible, or whether targeting a certain area may cause a reduction in affinity due to the existence of stable water molecules. Consequently, Dr Joe Bluck[143] calculated binding energies of the five water molecules residing in the binding pocket and three located in the ZA channel using Grand Canonical Monte Carlo (GCMC) simulations.[151] The results showed that, as expected, all five water molecules in the base of the binding pocket are stable (waters 0–4, **Figure 2.20**), indicated by the negative binding energies. It was observed that water 7 could have an interaction with the backbones of both I980 and E984, and would not be energetically favourable to displace, with a binding energy of –3.33 kcal mol^{-1}. Significantly, this result is in agreement with the observed outcome of the barcode plots (**Chapter 2.6**), indicating that E984 does not interact with the TRIM33 ligands. Desolvation of E984 and subsequent displacement of water 7 would be necessary for E984 to hydrogen bond to the protonated terminal amine of the amine chain in the ligands, and therefore a large energetic and enthalpic penalty would be associated with this conformation. This explains the 'pincer' interaction of the amine chain around E981 in the TRIM33 ligands observed during the barcode plot simulations.

A



B

Water	Binding energy (kcal mol^{-1})
0	–1.89 ± 0.13
1	–6.35 ± 0.09
2	–2.52 ± 0.14
3	–0.96 ± 0.10
4	–2.03 ± 0.11
5	+1.71 ± 0.09
6	+0.82 ± 0.14
7	–3.33 ± 0.10

Figure 2.20. A. TRIM33β KAc binding site with waters shown (PDB ID: **5MR8**). Red water molecules have stable binding energies, whereas green water molecules have the potential to be displaced. **B.** A table showing the binding energies of each water molecule. A cuboid boundary was set around the ZA channel, in which the GCMC simulation was performed using ProtoMS (version 3.3) that added and removed water molecules over 50M moves.[152] Subsequently, using Grand Canonical Integration (GCI), the energy of moving waters from the ideal gas phase to the regions within the cuboid where crystallographic waters are found could be calculated. This is therefore an estimation the binding energy of the crystallographic waters at the base of the binding pocket and in the ZA channel (Dr Joseph Bluck).[143]

However, waters 5 (+1.71 kcal mol^{-1}) and 6 (+0.82 kcal mol^{-1}) appeared to be less stable, indicated by the positive binding energies. The presence of these water molecules in the ZA channel offers an opportunity for displacement, which could allow a positive entropic contribution to binding. Moreover, an additional hydrogen bond acceptor present in the amine chain could potentially form an interaction with the stable water 7. Careful consideration of the binding energies of water molecules in the vicinity of the binding pocket is crucial to improving ligand affinity.

2.10 Rigidifying the Amine Chain

As it had been established that E984 did not play a role in the binding of the TRIM33 ligands, it was hypothesised that an increase in affinity could be gained by favouring the ‘pincer’ like interaction of the amine chain with E981 through conformational bias of the two amines. Rigidifying the chain may hold the two amines in close proximity to E981, forcing a permanent interaction and therefore increasing the strength of binding.

2.10.1 Design and Biological Evaluation

Several compounds were designed to bias a 'pincer' conformation. Compounds **111–125** were proposed (**Figure 2.21**), as the amine chains are conformationally restricted, which may lock the two amine moieties around E981. Gem-dimethyl **118** was also considered, along with several phenyl-containing amine chains **119–123**, which also have the potential to displace either water 5 or 6 to gain an entropic contribution to binding. The compounds were synthesised by Dr Larissa See[131] and Dr Amy Scorah.[138]

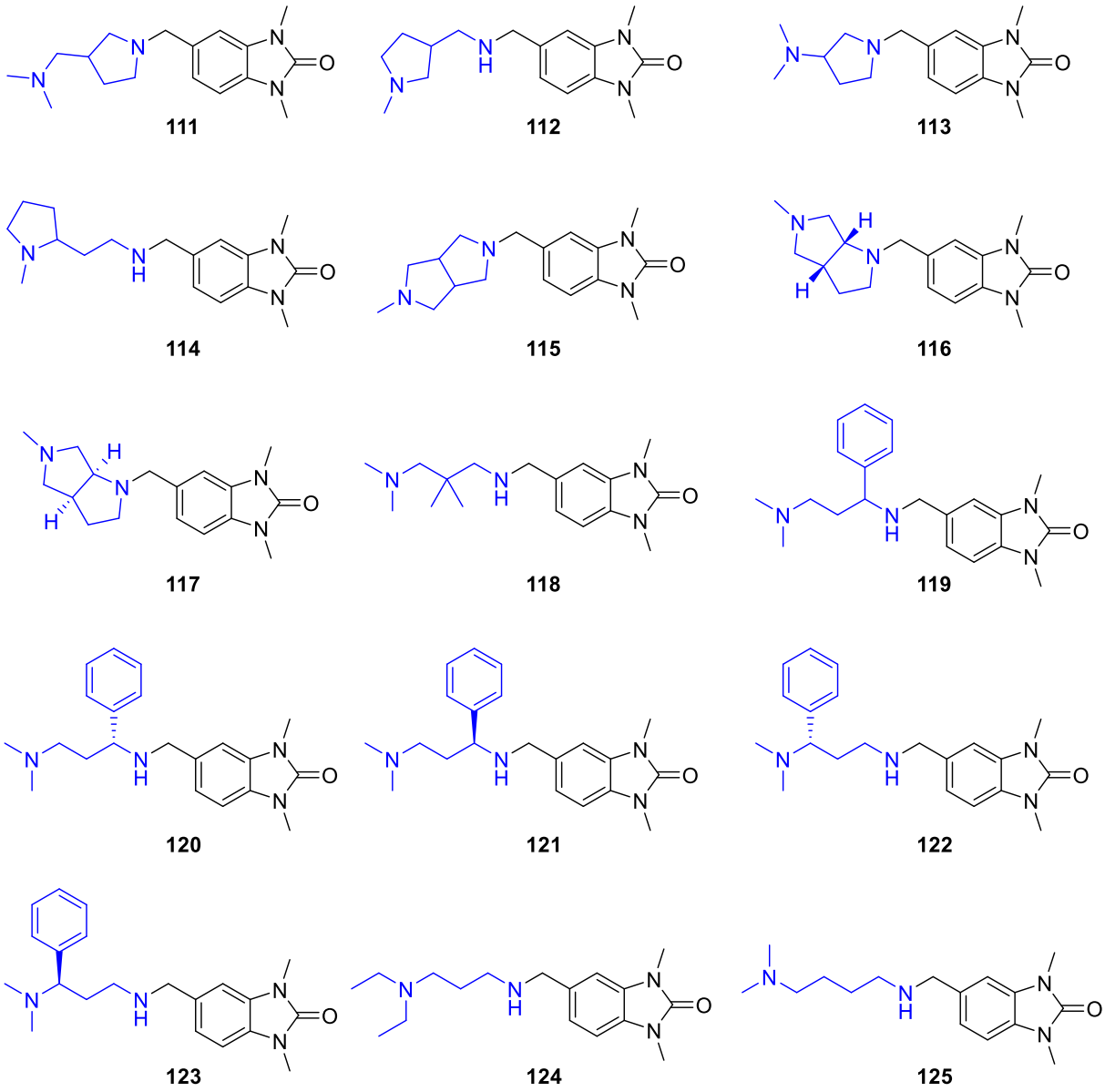


Figure 2.21. Structures of the proposed ligands **111–125** to bias the conformation of the amine chain to favour the 'pincer' interaction around E981, synthesised by Dr Larissa See[131] and Dr Amy Scorah.[138]

The compounds were evaluated using a dose-response AlphaScreen™ (**Table 2.10**). It appeared that five-membered ring containing compounds **113** and **111** had similar binding affinities to control **25**, with IC_{50} values of 12.3 μM and 15.2 μM for TRIM33β, respectively. These results reinforced the previous observation that an ionic interaction must be made between the benzylic amine and E981, which comes from protonation of this amine, thus it appeared both secondary and tertiary amines are tolerated at this position. Compound **114** contained one more carbon in the amine chain and a different pyrrolidine substitution point compared to **112**, and, despite having a similar affinity for TRIM33α, appeared to bind with a higher affinity to TRIM33β (IC_{50} = 1.62 μM, compared to IC_{50} = 8.53 μM for **112**). This is possibly due to an increased propensity for the 'pincer' conformation of **114** around E981. The fused ring system in ligand **115** appeared to reduce affinity for TRIM33β (IC_{50} = 100 μM) compared to control **25**, which may be due to the suboptimal conformation of the two amines. Conversely, fused ring containing **116** and **117**, with different amine orientations with respect to the head group compared to **115**, revealed similar binding to control **25**, with the (*S*)-enantiomer **117** showing slightly higher affinity for TRIM33β (IC_{50} = 9.57 μM) than the (*R*)-enantiomer **116** (IC_{50} = 12.3 μM).

The addition of two methyl groups in the middle of the chain for analogue **18** appeared to reduce affinity for both TRIM33α (IC_{50} = 19.8 μM) and TRIM33β (IC_{50} = 39.2 μM), which may indicate that substituents in this position are not tolerated. On the contrary, addition of a racemic phenyl substituent adjacent to the benzylic amine in analogue **119** showed increased affinities for both TRIM33α (IC_{50} = 1.56 μM) and TRIM33β (IC_{50} = 4.83 μM) compared to control **25**. It was hypothesised that a single enantiomer may have a higher affinity for TRIM33β compared to the racemic analogue **119**, so both (*R*)- and (*S*)-enantiomers, **120** and **121**, were synthesised and evaluated. However, the affinity of these two compounds were very similar for TRIM33β, with the (*S*)-enantiomer **121** having a slightly lower IC_{50} value of 4.52 μM compared to the (*R*)-enantiomer **120** IC_{50} value of 7.75 μM. The increase in affinity of these phenyl-containing compounds compared to control **25** could be a consequence of hydrophobic interactions of the phenyl ring with the protein, or possible displacement of water 6 in the ZA channel. As shown in

Figure 2.22, the phenyl ring can be seen occupying the same space as water 6, and its displacement would cause a favourable increase in entropy.

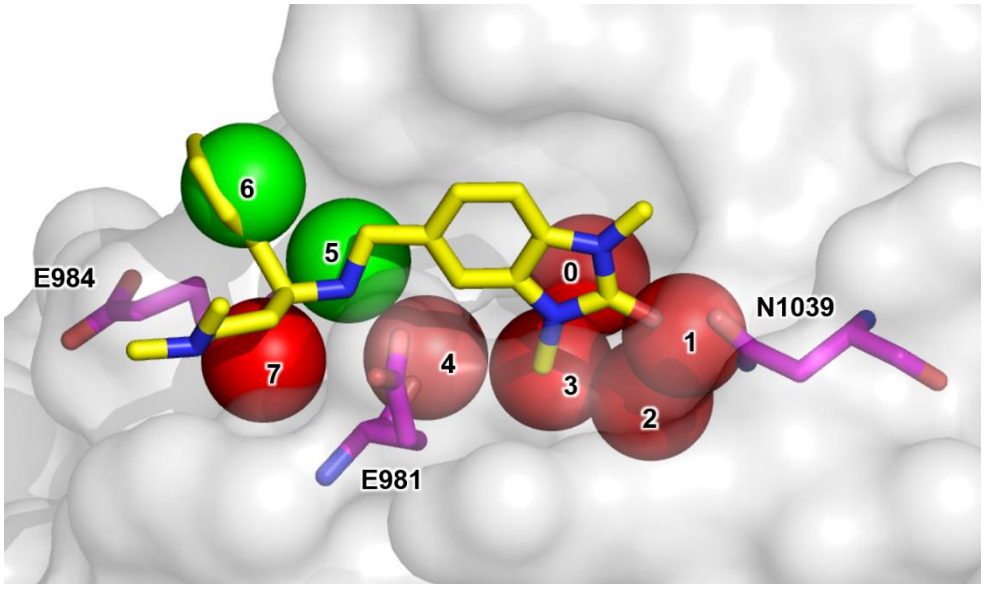


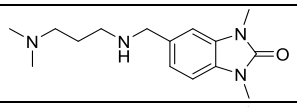
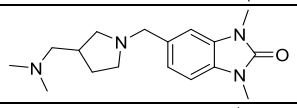
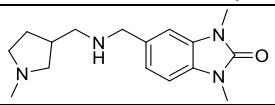
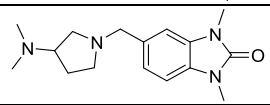
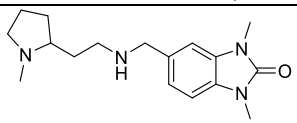
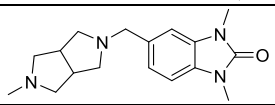
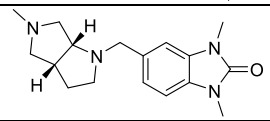
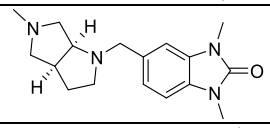
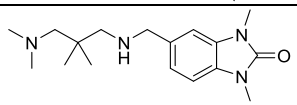
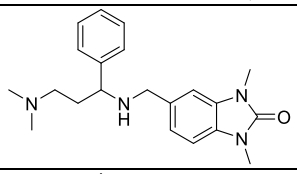
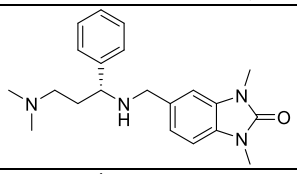
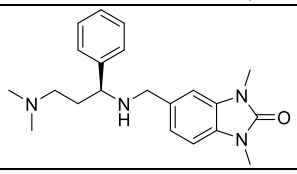
Figure 2.22. (*S*)-Phenyl containing analogue **121** docked using CLC Drug Discovery Workbench 3.0 into TRIM33β (PDB ID: **5MR8**). The phenyl substituent appears to occupy the same space as water 6, which from previous calculations appears to be displaceable. Diagram by Dr Amy Scorah.[138]

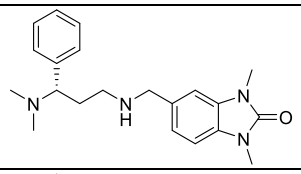
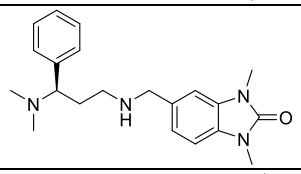
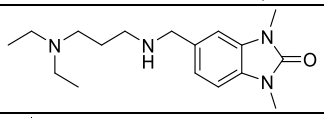
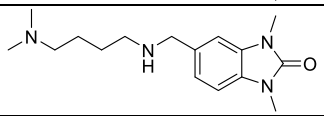
Introducing a phenyl substituent adjacent to the terminal amine was also assessed to determine if substitution could be tolerated at this position. However, both the (*R*)- (**123**) and (*S*)-enantiomers (**122**) appeared to have similar affinity to control **25**, indicating that substitution from this position was not advantageous. This could be attributed to the phenyl group being in closer proximity with water 7, which would be energetically unfavourable to displace.

Extension of the methyl groups on the terminal amine to ethyl substituents in compound **124** led to an increase in affinity (IC_{50} = 2.02 μM) for TRIM33β compared to methylated control **25**, which is presumably due to increased hydrophobic interactions with the protein. Finally, extending the amine chain by one carbon (**125**) also revealed a lower IC_{50} value of 2.73 μM. This could be due to the increased flexibility of the amine chain allowing an ideal 'pincer' conformation around E981.

Table 2.10. A table showing IC_{50} values for the compounds **111–125** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from a highest compound concentration of 500 μM. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green. Results shown are for each protein with $H3_{1-27}$K18Ac peptide, with ligand efficiency in brackets. *These IC_{50} values are the mean and standard deviation of multiple repeats (n = 8).

Weaker binding — **Stronger binding**

Compound	Structure	TRIM24 BRD IC_{50} (μM)	TRIM33α BRD IC_{50} (μM)	TRIM33β BRD IC_{50} (μM)
25		>250	3.61* ± 0.352 (0.38)	13.7* ± 1.53 (0.35)
111		>250	5.81 ± 0.210 (0.33)	15.2 ± 0.787 (0.31)
112		>250	1.60 ± 0.137 (0.39)	8.53 ± 0.558 (0.34)
113		>250	6.61 ± 0.535 (0.35)	12.8 ± 2.09 (0.33)
114		>250	1.52 ± 0.0940 (0.37)	1.62 ± 0.140 (0.37)
115		>250	25.6 ± 6.82 (0.29)	100 ± 20.7 (0.25)
116		>250	3.97 ± 0.858 (0.34)	12.3 ± 3.13 (0.31)
117		>250	5.79 ± 0.841 (0.33)	9.57 ± 1.74 (0.32)
118		>250	19.8 ± 3.56 (0.30)	39.2 ± 8.41 (0.28)
119		>250	1.56 ± 0.100 (0.31)	4.83 ± 0.508 (0.29)
120		>250	2.45 ± 0.241 (0.30)	7.75 ± 0.599 (0.28)
121		>250	1.32 ± 0.0630 (0.32)	4.52 ± 0.596 (0.29)

122		>250	5.94 ± 0.619 (0.28)	9.63 ± 2.00 (0.27)
123		>250	5.56 ± 0.559 (0.28)	12.2 ± 1.55 (0.26)
124		>250	4.74 ± 1.14 (0.34)	2.02 ± 1.06 (0.36)
125		>250	7.96 ± 1.11 (0.34)	2.73 ± 1.14 (0.37)

WaterLOGSY experiments were also performed on compounds **111**, **114**, **119**, **124**, and **125**, which validated binding to both TRIM33α and TRIM33β (spectra shown in Appendix D). In conclusion, the highest affinity compounds for the TRIM33β bromodomain appeared to be five-membered ring containing ligand **114** (IC_{50} = 1.62 μM), (*S*)-phenyl ligand **121** (IC_{50} = 4.52 μM), ethyl ligand **124** (IC_{50} = 2.02 μM) and extended amine chain **125** (IC_{50} = 2.73 μM).

2.11 Isothermal Titration Calorimetry

AlphaScreen™ is a useful assay to determine IC_{50} values of ligands with a relatively high throughput. However, since the assay is based on peptide displacement, the IC_{50} values obtained are relative to the binding affinities of the peptides for the protein. This means that binding affinities cannot be compared across proteins, as each peptide has a different affinity for each protein. Furthermore, this implies, as observed for the ligands with TRIM33α, that low affinity of a peptide for a protein would allow easier displacement and subsequent lower IC_{50} values. In contrast, ITC is a technique that determines thermodynamic parameters of a protein-ligand binding event. The experiment does not require displacement of a binding partner, meaning absolute K_D values can be calculated. It was decided that ITC should be performed on the six highest affinity ligands **25**, **112**, **114**, **121**, **124**, and **125** (**Figure 2.23**), for TRIM33α and TRIM33β from the dose-response AlphaScreen™ to determine K_D values.

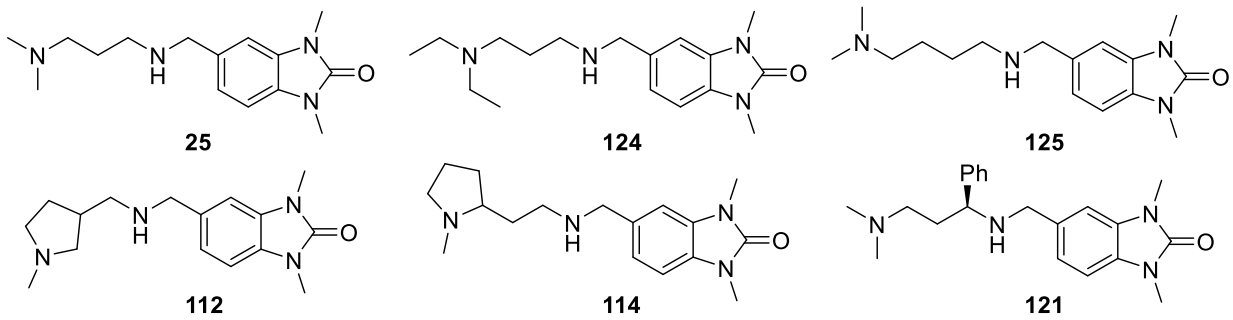


Figure 2.23. Structures of the six highest affinity ligands for TRIM33β to be assessed using ITC.

Both TRIM33α and TRIM33β were dialysed overnight into HEPES buffer (50 mM, 150 mM NaCl) to ensure a buffer match between the protein and ITC buffer. If they are mismatched, this can produce a substantial heat of dilution, which produces unreliable results. The design of a successful ITC experiment requires an estimation of K_D value and binding enthalpy. This is because the unitless *c* value used, which relates to the shape of the sigmoidal binding isotherm, is dependent on K_D value and protein concentration. Aiming for a *c* value between 10 and 500 will allow accurate determination of K_D, producing the best sigmoidal curve. For ligands with binding affinities in the low micromolar range, a higher concentration of protein is required to achieve a *c* value in the desired region. The compounds were first tested at concentrations of 160 μM protein in the syringe and 16 μM compound in the cell. However, sigmoidal curves could not be established as the heat change with each injection was too small, indicating the affinity of these compounds is close to the detection limit by ITC. Increasing protein concentrations to 363 μM for TRIM33α and 368 μM for TRIM33β, and increasing compound concentration to 40 μM, allowed sigmoidal isotherms to be obtained and K_D values to be calculated. ITC isotherms are shown for **25** binding to TRIM33α and TRIM33β in **Figure 2.24**, and all other ITC traces can be found in Appendix E.

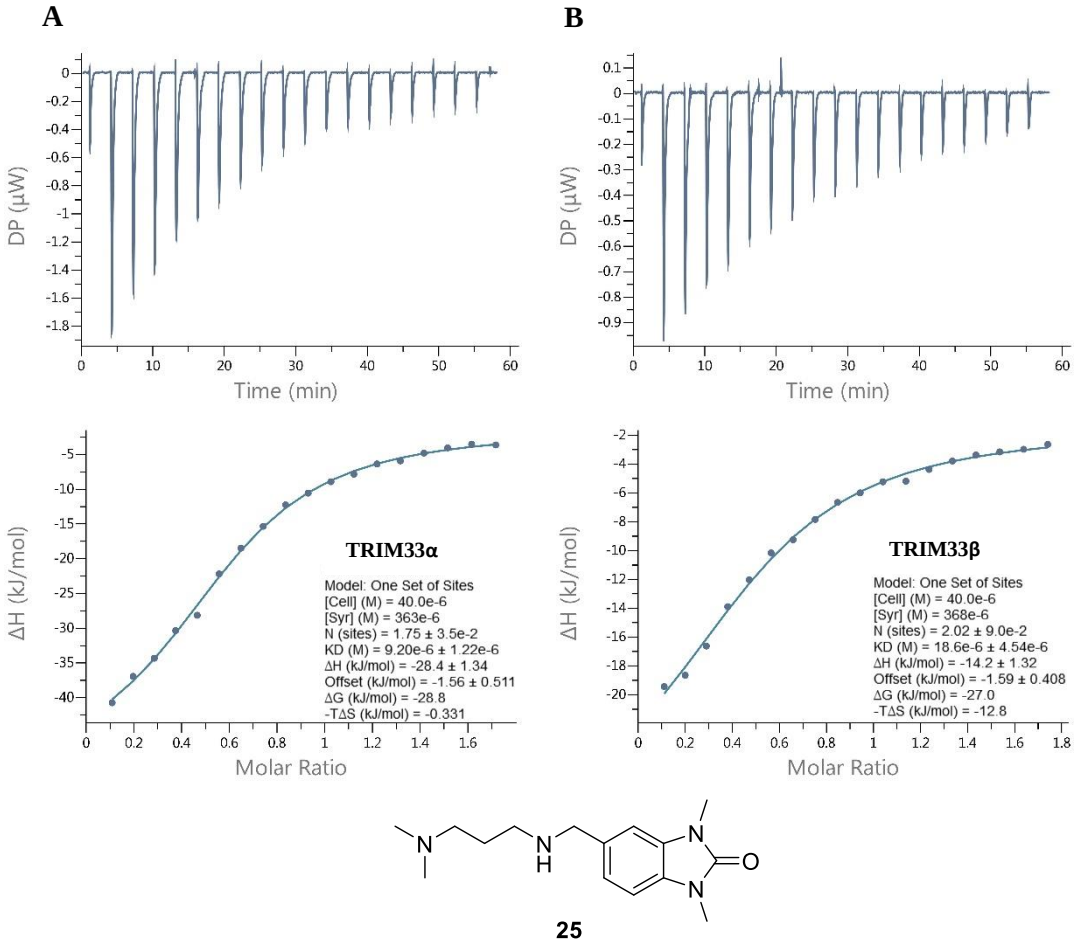
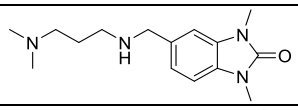
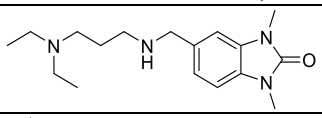
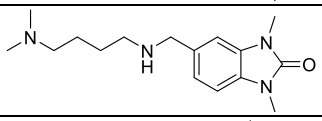
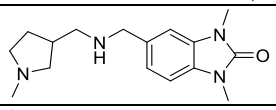
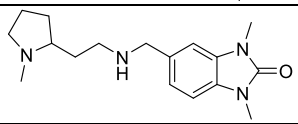
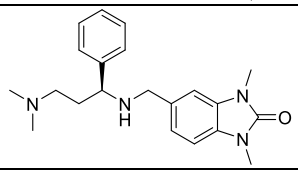


Figure 2.24. ITC isotherms obtained for ligand **25**. **A.** Isotherm showing binding to TRIM33α. **B.** Isotherm showing binding to TRIM33β.

The K_D values obtained by ITC for these six compounds compared with the IC_{50} values obtained by dose-response AlphaScreen™ are summarised in **Table 2.11**. In general, there appears to be reasonable agreement between the K_D and IC_{50} values of each compound, with AlphaScreen™ only slightly overstating the binding affinity in most cases. The ranking order of compounds does change however, as it appears the three highest affinity compounds are phenyl-containing **121** (K_D = 7.33 µM for TRIM33β), and the two five-membered ring containing analogues **112** and **114**, with K_D values of 7.81 and 7.82 µM for TRIM33β, respectively. Interestingly, despite N1039 pointing away from the binding site, all five ligands showed higher affinity for TRIM33α compared to TRIM33β, which could be attributed to the additional 17 amino acid residues in the BC loop creating a larger binding pocket. This could also indicate that the interaction with N1039 is only required for orientation of the ligand and does not contribute to affinity.

Table 2.11. Summary table of the K_D values (μM) obtained using ITC and IC_{50} values (μM) obtained using a dose-response AlphaScreen™ assay (using $H3_{1–27}K18Ac$ to assess bromodomain binding). The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

Weaker binding — **Stronger binding**

Compound	Structure	TRIM33α		TRIM33β	
		K_D (μM)	IC_{50} (μM)	K_D (μM)	IC_{50} (μM)
25		9.20 ± 1.22	3.61 ± 0.352	18.6 ± 4.54	13.7 ± 1.53
124		5.46 ± 0.546	4.74 ± 1.14	11.3 ± 2.55	2.02 ± 1.04
125		9.92 ± 0.819	7.96 ± 1.11	18.3 ± 2.71	2.73 ± 1.14
112		5.26 ± 0.274	1.60 ± 0.140	7.81 ± 1.10	8.53 ± 0.560
114		5.23 ± 0.00530	1.52 ± 0.0940	7.82 ± 3.37	1.62 ± 0.140
121		2.71 ± 0.165	1.32 ± 0.0630	7.33 ± 1.18	4.52 ± 0.596

2.12 Selectivity Over BRD4

Determining selectivity of a chemical probe over similar domains is important, hence why binding to TRIM24 has also been evaluated for each ligand in this chapter. BRD4 is part of the BET family of bromodomain proteins, and a wide range of inhibitors for BRD4 have been published in the literature.[153] Since BRD4 has a strong phenotype,[154] investigating selectivity of a new bromodomain probe over BRD4 is desirable. Ligands **16** and **25** were evaluated using a dose-response AlphaScreen™, using BRD4(1) and $H4_{1–20}(KAc)_4$ as binding partners (**Figure 2.25**), with reported BRD4 ligand **OXBD02** (**4**) used as a positive control.[37] Fortuitously, no binding to BRD4 was observed.

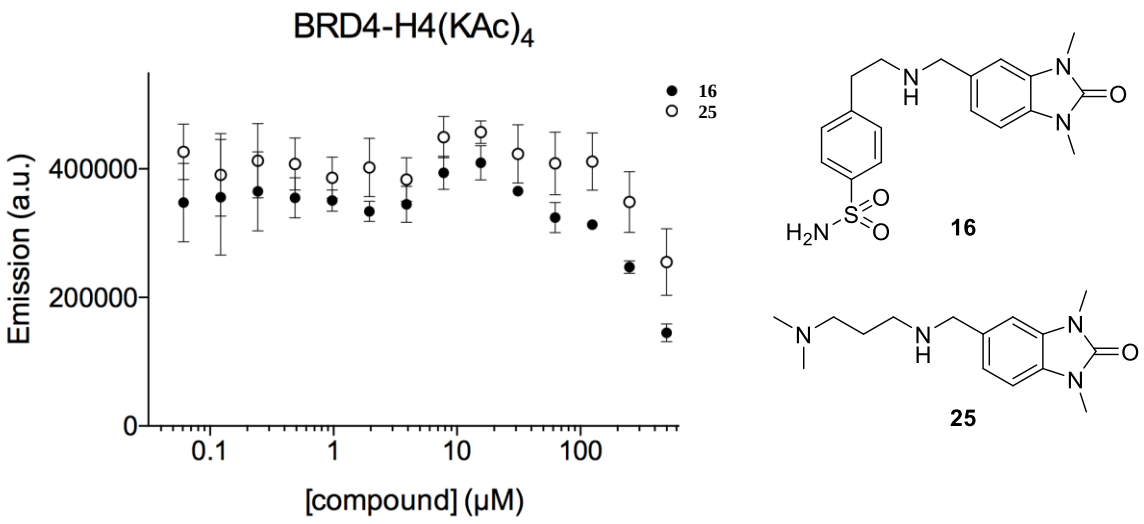


Figure 2.25. Dose-response curves for ligands **16** and **25** evaluated with BRD4 using a dose-response AlphaScreen™. BRD4(1) used with $H4_{1-20}(KAc)_4$ as a binding partner. Compounds were serially diluted 1:2 from a highest compound concentration of 500 μM, and show no binding to BRD4.

2.13 Investigation of Published TRIM33 ligands

2.13.1 Patent Claims

As mentioned in **Chapter 1.4.1.3**, during ligand optimisation for this project, a patent was published describing the discovery of high affinity TRIM33 bromodomain ligands, selective over TRIM24, with the general structure **126** (**Figure 2.26**).[70] The isoform of TRIM33 was not specified. AlphaScreen™ data were given for a selection of compounds, which showed nanomolar affinity for both TRIM33 and TRIM24. Compounds **127** and **129** were said to be selective for TRIM33 over TRIM24, but no affinity data were given. The patent also stated that a crystal structure was resolved to determine binding modes of the compounds, although details of this were not enclosed. To validate these ligands as binding to the TRIM33β bromodomain, five compounds detailed in the patent as high affinity were selected to be synthesised and evaluated (**Figure 2.26**).

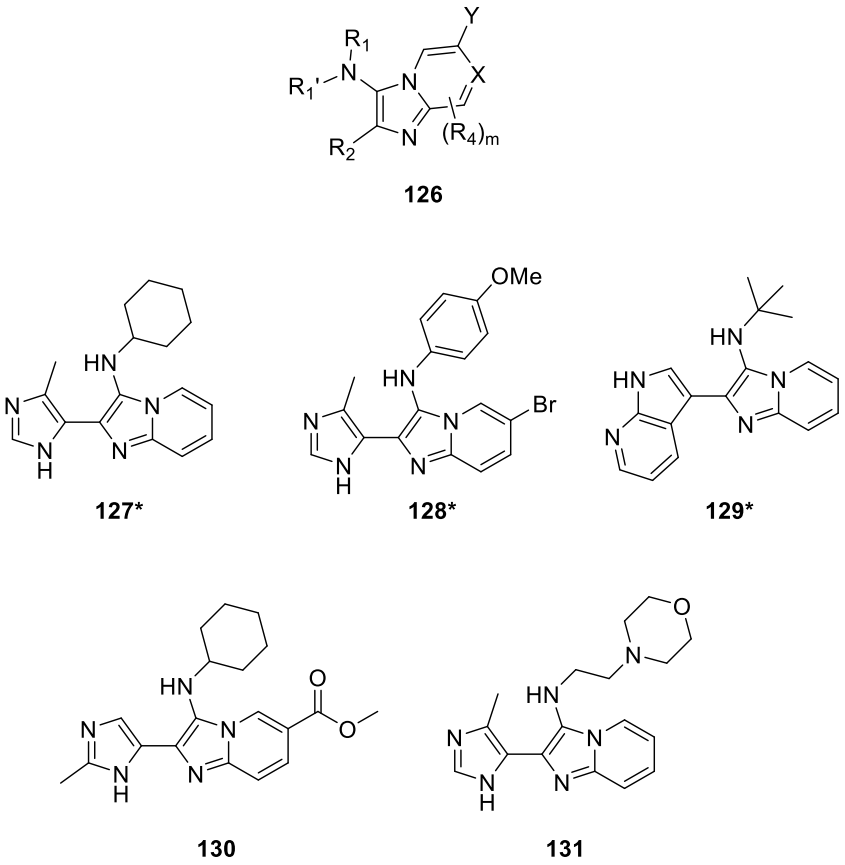
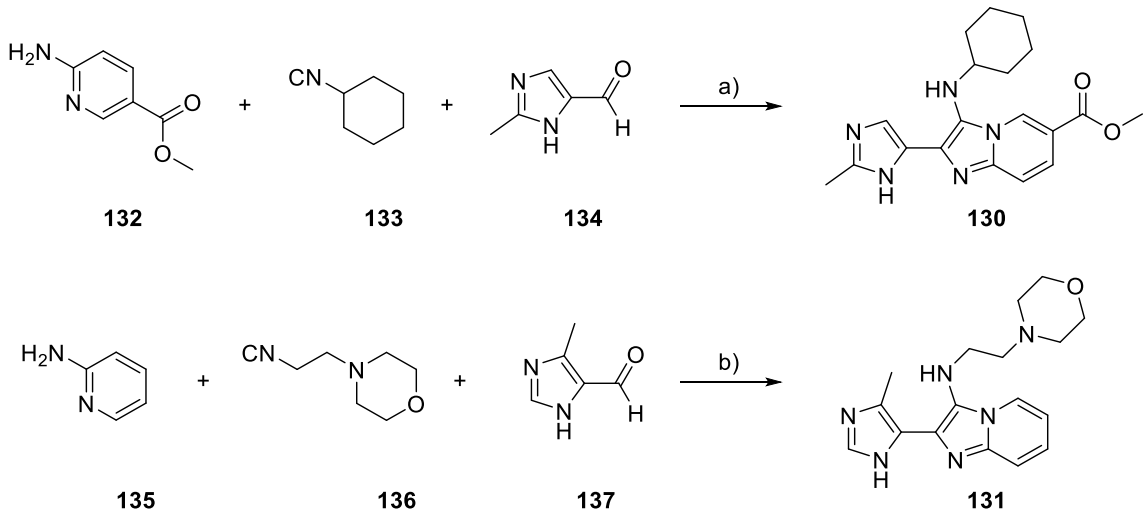


Figure 2.26. General structure **126** of compounds detailed in the patent, where m = 0–2. Structures **127–131** of the compounds selected for synthesis. *Synthesised by Dr Amy Scorah.[70,138]

2.13.2 Synthesis of Compounds

Compounds **130** and **131** described in the patent were synthesised using the Groebke-Blackburn-Bienaymé multicomponent reaction (**Scheme 2.19**).[70] The yields for these reactions were poor, which was attributed to difficulty in purification, and comparable yields were achieved in the patent. Compounds **127–129** were synthesised by Dr Amy Scorah using analogous reaction conditions.



132 **133** **134** **130**

135 **136** **137** **131**

Scheme 2.19. Synthesis of compounds **130** and **131**. *Reagents and conditions:* a) $Sc(OTf)_3$, DMSO, 100 ºC, 16 h, 10%, n = 1; b) $Sc(OTf)_3$, DMSO, 100 ºC, 16 h, 19%, n = 1.

2.13.3 Evaluation of Compounds

2.13.3.1 AlphaScreen™ and TruHits Results

Initially, the five synthesised compounds were evaluated using AlphaScreen™, which was the assay used in the patent. Since it was claimed that compound **127** and **129** were selective against TRIM24, and did not state which isoform of TRIM33 the ligands bound to, **127–131** were tested against all three proteins, with the three different domain-binding peptides to make a total of nine combinations. It appeared that both **127** and **128** bound unselectively to the TRIM24, TRIM33α, and TRIM33β bromodomains with low micromolar IC_{50} values (**Table 2.12**). However, this was also observed for all nine protein-peptide combinations, indicating non-specific binding or assay interference (Appendix F). Compound **129** appeared to have a weak affinity to TRIM24, with no binding observed to either isoform of TRIM33. Binding to TRIM24, TRIM33α and TRIM33β was also observed for compound **130**, with low micromolar IC_{50} value of 7.91 μM for TRIM33β. Finally, **131** appeared to have weak affinity for TRIM33β (IC_{50} = 55.3 μM) but showed no binding to TRIM33α, and higher affinity for TRIM24 (IC_{50} = 18.6 μM). The patent only reported binding data for compounds **128** and **131**, which showed much lower IC_{50} values by almost a factor of 10 compared to the values determined in the AlphaScreen™ performed in this work (**Table 2.13**).

Table 2.12. A table showing IC_{50} values for the patent compounds **127–131** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from a highest compound concentration of 500 μM. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green. Results shown are for each protein with $H3_{1-27}$K18Ac peptide, with ligand efficiency in brackets.

Weaker binding — **Stronger binding**

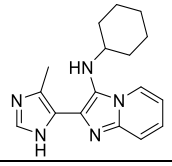
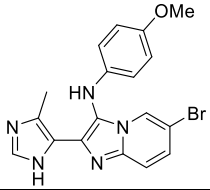
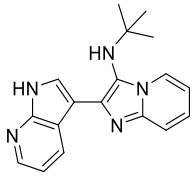
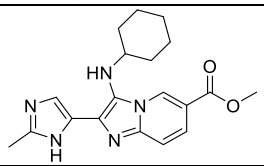
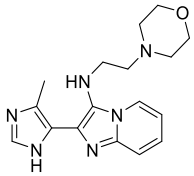
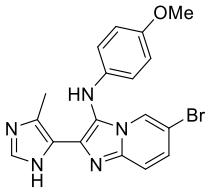
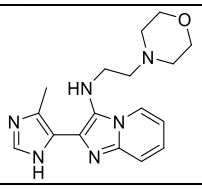
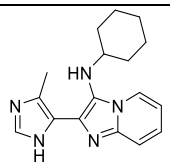
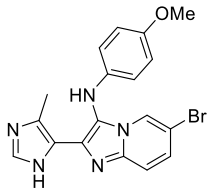
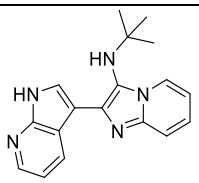
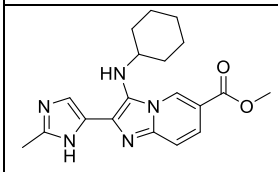
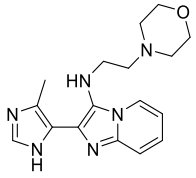
Compound	Structure	TRIM24 BRD IC_{50} (μM)	TRIM33α BRD IC_{50} (μM)	TRIM33β BRD IC_{50} (μM)
127		4.21 ± 0.665 (0.35)	2.74 ± 0.529 (0.35)	3.16 ± 0.545 (0.35)
128		1.76 ± 0.100 (0.32)	1.58 ± 0.165 (0.32)	1.49 ± 0.218 (0.33)
129		43.8 ± 10.9 (0.27)	>250	>250
130		20.0 ± 2.95 (0.25)	16.6 ± 6.10 (0.26)	7.91 ± 1.84 (0.27)
131		18.6 ± 0.727 (0.28)	>250	55.3 ± 4.68 (0.25)

Table 2.13. AlphaScreen™ results reported in the patent.[70] IC_{50} values (μM) shown for TRIM24 and TRIM33 with $H3_{1-20}K9Me_3K18Ac$.

Compound	Structure	TRIM24 IC_{50} (μM)	TRIM33 IC_{50} (μM)
128		0.234	0.136
131		5.484	7.862

As a consequence of **127** and **128** showing no selectivity across all nine protein-peptide combinations, an AlphaScreen™ TruHits experiment was performed. As described in **Chapter 1.6.1.2**, the assay is designed to identify compounds that produce false readouts through various mechanisms. As predicted, the compounds appeared to interfere significantly with the assay relative to the DMSO control, shown by the values highlighted in blue (**Table 2.14**). Compounds **127**, **128**, and **130** appeared to cause the greatest interference, which explained the lowest IC_{50} values seen in AlphaScreen™. Therefore, AlphaScreen™ was clearly not an appropriate assay to assess binding affinities of these compounds, and orthogonal assays must be used.

Table 2.14. AlphaScreen™ TruHits results for patent compounds **127–131**. Five concentrations of the compounds were tested with two incubation formats. Values which are more than two standard deviations greater than the mean DMSO control are highlighted in blue, and indicates assay interference.

Compound	Difference from DMSO average, normalised against standard deviation of DMSO control									
	Pre-incubate donor and acceptor beads, then add compound					Pre-incubate donor beads with compound, then add acceptor beads				
	31.25 µM	62.5 µM	125 µM	250 µM	500 µM	31.25 µM	62.5 µM	125 µM	250 µM	500 µM
 **127**	40.9	42.3	43.0	43.4	43.6	3.84	3.94	4.03	4.04	4.06
 **128**	42.9	43.3	43.6	43.7	43.7	4.00	4.04	4.06	4.06	4.06
 **129**	2.87	8.92	17.8	25.3	32.7	0.792	1.27	1.65	2.28	3.22
 **130**	21.0	19.8	23.8	29.9	35.3	2.09	2.48	3.09	3.55	3.84
 **131**	1.97	2.58	3.96	2.64	4.38	2.07	2.89	3.16	3.00	3.66
Biotin	-	-	-	-	-	3.94	4.00	4.03	4.04	4.06

2.13.3.2 WaterLOGSY

To qualitatively determine whether the compounds were binding to TRIM33 or were in fact false positives, as suggested by the AlphaScreen™ TruHits results, WaterLOGSY NMR experiments were performed on ligands **128** and **131**. These two compounds were chosen for further analysis

as IC_{50} values were reported in the patent for these compounds. **131** clearly displayed no binding, shown by the WaterLOGSY spectrum remaining negative on addition of TRIM33β (**Figure 2.27**).

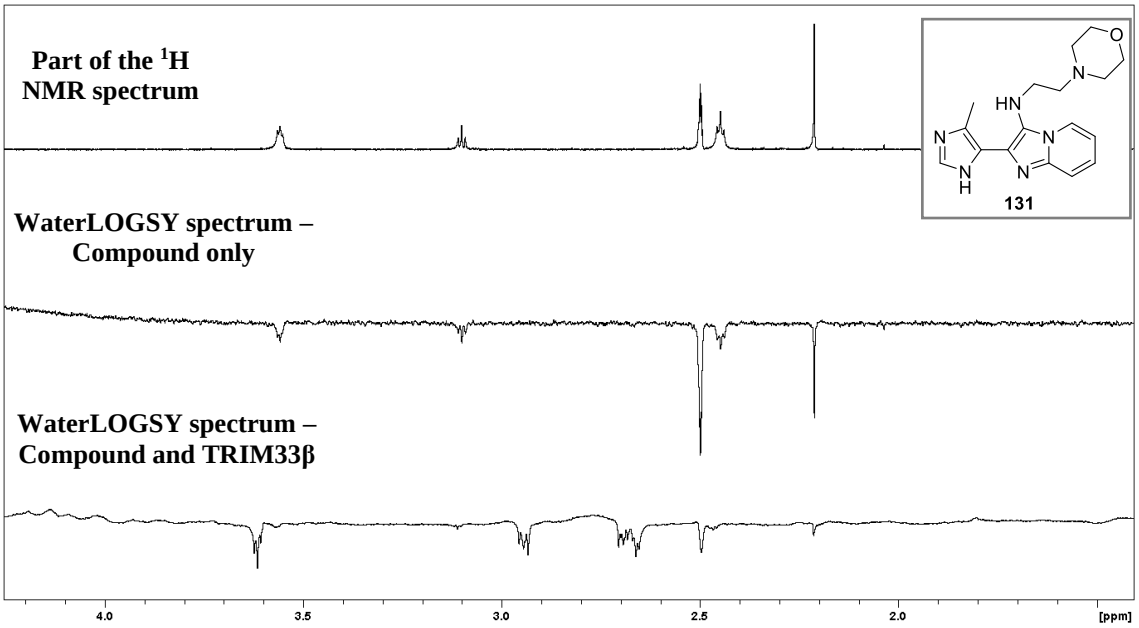


Figure 2.27. Part of the WaterLOGSY spectra for **131**. Spectra stacked, showing ^{1}H NMR spectrum, compound only WaterLOGSY spectrum, and compound and TRIM33β WaterLOGSY spectrum. Absence of binding is indicated by the negative signals in presence of protein.

Compound **128** appeared to form soluble aggregates in the WaterLOGSY NMR spectrum at a concentration of 500 µM in the absence of protein, which was indicated by positive signals (**Figure 2.28**). If the compound was behaving as a small molecule in solution, the signals would have been negative. When the solution was diluted to 250 µM and subsequently 125 µM, aggregation was still observed, so it was concluded that a WaterLOGSY NMR experiment was not an appropriate assay to assess binding of **128**.

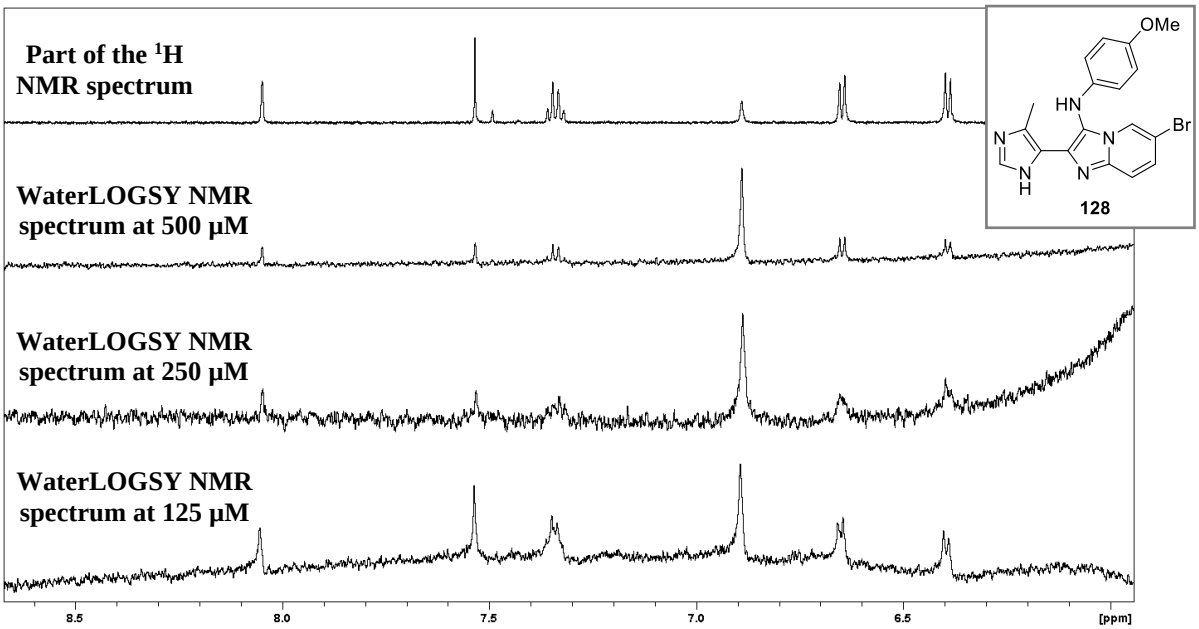


Figure 2.28. Part of the WaterLOGSY spectra for **128**. Spectra stacked, showing ^{1}H NMR spectrum and compound only WaterLOGSY spectra at three concentrations. Positive signals at all three concentrations in absence of protein indicate formation of soluble aggregates.

2.13.3.3 Isothermal Titration Calorimetry

Both WaterLOGSY and AlphaScreen™ TruHits data suggested that these compounds did not bind to TRIM33, and that the initial data shown in the patent was a consequence of assay interference. However, since WaterLOGSY was not a suitable method to assess compound **128**, and because very high affinity ligands can appear as false negatives in a WaterLOGSY experiment, ITC was used to conclusively evaluate binding to TRIM33.

As previously mentioned, design of ITC experiments must be carefully considered. To aid with this design, the unitless *c* value was used, which is dependent on K_D and protein concentration. To ensure the *c* value was between the desired 10 and 500, IC_{50} values quoted in the patent were used as a guide in choosing compound concentration.[70] For both compounds, a titration curve could not be acquired (**Figure 2.29**), whereas a curve was seen for positive control **121**. This indicated that neither **128** nor **131** were binding to TRIM33β, demonstrating that the original affinity data published the patent was a result of assay interference.

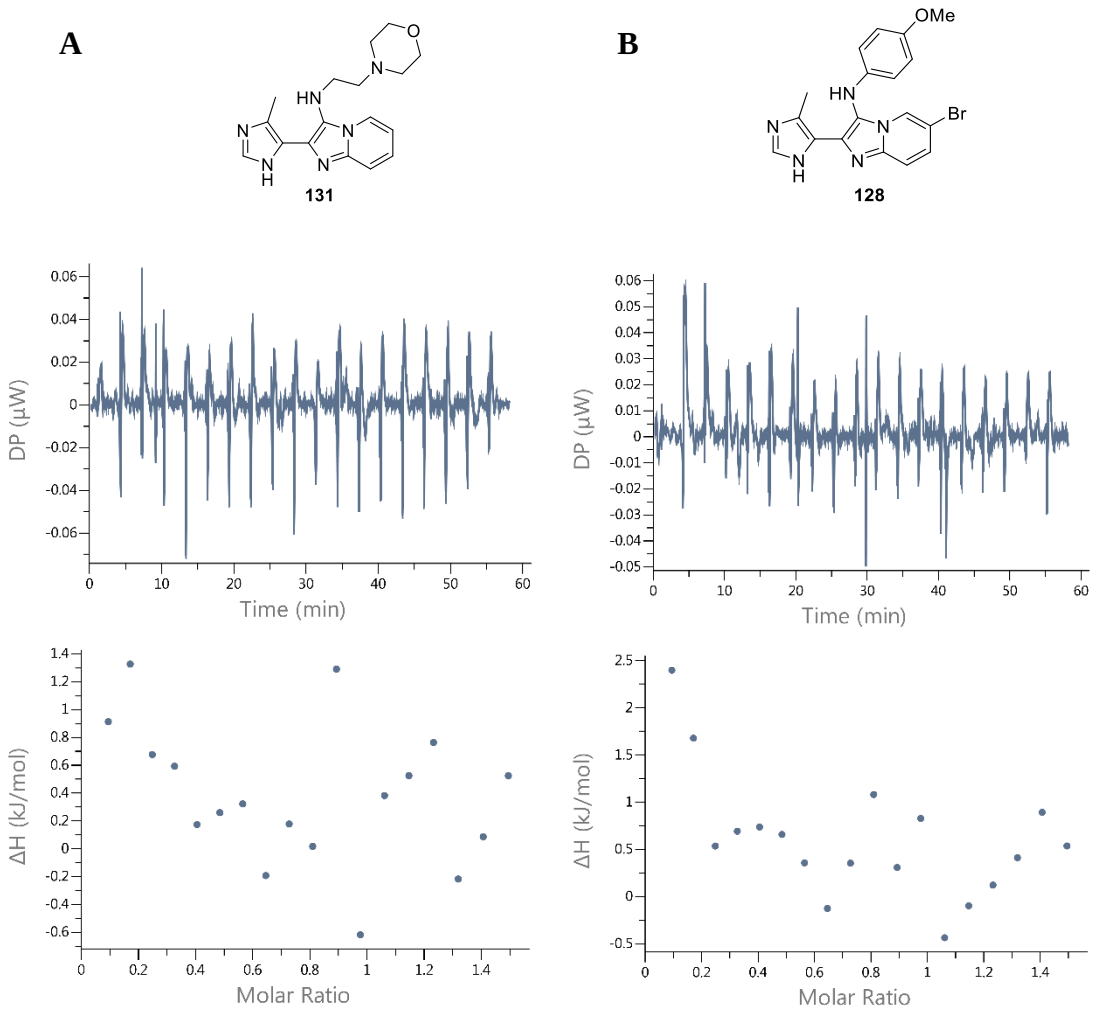


Figure 2.29. ITC traces for **131** and **128** with TRIM33β. **A.** Results for compound **131**, n = 1. **B.** Results for compound **128**, n = 1. The readout shows the power input for each injection, above, and the normalised binding isotherms, below. K_D values could not be determined as no binding is observed.

Contrary to claims made in the patent about discovery of ligands with a high affinity for TRIM33 and selectivity over TRIM24, the data obtained from a variety of orthogonal assays showed that these compounds were not binding to TRIM33. It appeared that assay interference in the initial AlphaScreen™ had shown affinities which could not be reproduced using other methods that do not rely on fluorescence. This work revealed the importance of using orthogonal assays to remove false positive results, as this can lead to time and resources being wasted in development of compounds that are not active.

2.14 Summary and Conclusions

This chapter described the SAR studies performed to probe the acetyl-lysine binding site of the TRIM33β bromodomain and improve the affinity of ligands targeting this site. Focus centred on the β isoform of TRIM33, as previous studies indicated that N1039 – the essential residue for recognising acetylated lysine residues – pointed away from the binding site in TRIM33α, suggesting it is a non-canonical bromodomain. Initial exploration established the importance of the ionic interaction between the benzylic amine of ligand **25** and E981 for affinity to TRIM33β and selectivity over TRIM24. The hydrogen bond between N1039 and the carbonyl of the benzimidazolone head group also appeared to be important, despite ligands still binding to TRIM33α, in which N1039 is pointing out of the binding site. It is hypothesised that this interaction acts as guidance for orientation of the ligand towards E981 rather than contributing to binding affinity. Two alternative acetyl-lysine mimicking head groups were investigated, however, neither showed binding to either isoform of TRIM33, presumably due to the sub-optimal orientation of the head group relative to N1039, which may disrupt the essential benzylic amine interaction with E981.

The hydrophobic region above the acetyl-lysine binding site was explored, in an effort to increase binding affinity. This was first attempted through projection of lipophilic groups from the benzimidazolone core, although in MD simulations performed by Dr Joe Bluck, this appeared to disrupt the side-on π-π interaction between Y993 and F1038 in the TRIM33β bromodomain, and there were subsequently no increases in binding affinity observed for these compounds relative to the dimethylated control. Similarly, addition of a sulfonamide moiety adjacent to the benzylic amine chain did not improve affinity. Finally, placing a small hydrophobic group to target I990 and V986 did not completely negate binding to TRIM33β, but did not appear to be advantageous and a reduction in binding affinity was observed.

Computation exploration of the binding site water network performed by Dr Joe Bluck indicated that six out of the eight water molecules present in and around the binding site are energetically

unfavourable to displace. This supports the hypothesis that E984 does not play a role in interacting with the terminal amine of the amine chain in the ligands, as for this to occur, desolvation and subsequent unfavourable displacement of water 7 would be necessary. Instead, biasing a 'pincer' conformation of the amine chain around E981 was considered, which was probed using a variety of conformationally biased dual amine chains. The highest affinity ligand **114** had an IC_{50} value of 1.62 μM (**Figure 2.30**). Future TRIM33β bromodomain ligand exploration should investigate the binding energies of the ZA channel water molecules in more detail, perhaps determining if water-mediated hydrogen bonds or displacement of water 5 or 6 can improve affinity further.

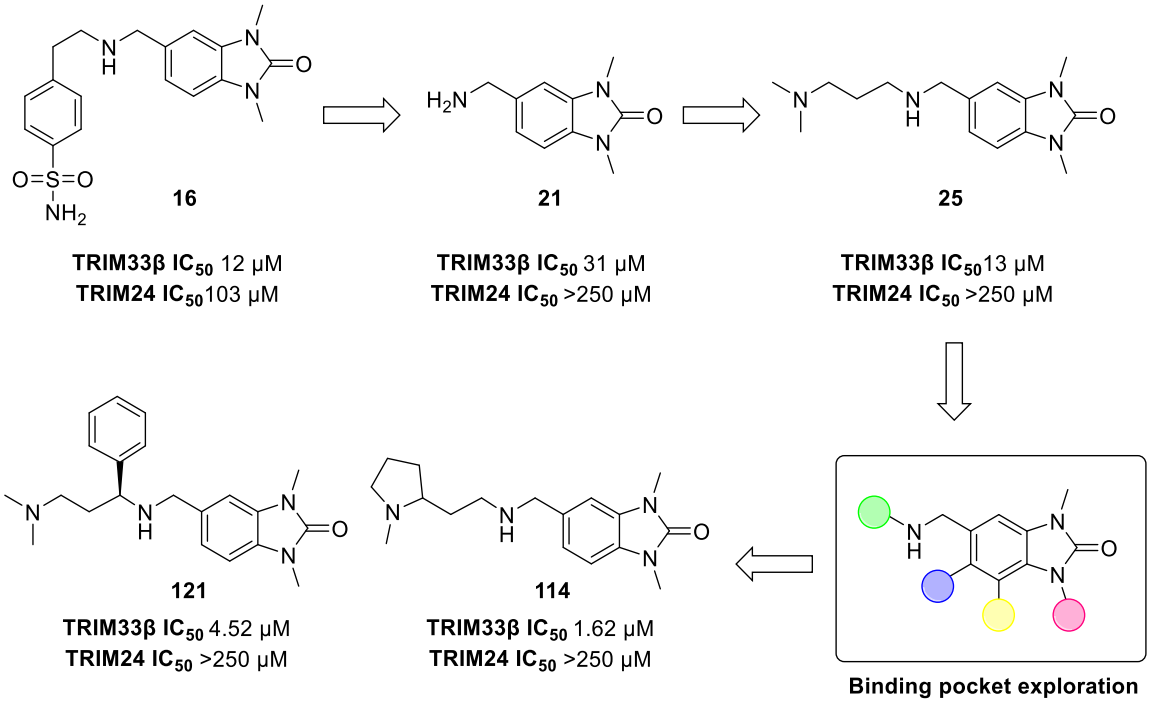


Figure 2.30. Development of initial hit **16**, which gave the highest affinity ligands **114** and **121**.

Isothermal titration calorimetry was performed for the six highest affinity ligands to determine K_D values, which appeared to be in reasonable agreement with the IC_{50} values established using AlphaScreen™. The highest affinity ligand was the phenyl-containing **121**, with an IC_{50} value of 4.52 μM and a K_D value of 7.33 μM.

Although a patent was published describing the discovery of high affinity TRIM33 bromodomain ligands, synthesis and evaluation of these compounds demonstrated that assay interference had led to an unreliable assessment of binding, and orthogonal assays showed the ligands did not bind

to TRIM33. Therefore, this chapter describes the first TRIM33β bromodomain ligands with low micromolar affinity, and selectivity over BRD4 and TRIM24, which provides a strong basis for further ligand development.

Chapter 3 First generation of TRIM33 Proteolysis-Targeting Chimeras (PROTACs)

3.1 Introduction and Aims

A parallel strategy to that described in the previous chapter for probing the biological relevance of TRIM33, is to incorporate the bromodomain ligand into a heterobifunctional PROTAC to degrade TRIM33 in cells. The ligand, containing a benzimidazolone core as an acetyl-lysine mimic, will be linked to a ligand for an E3 ubiquitin ligase. The PROTAC should form a ternary complex with these two proteins, allowing ubiquitination and subsequent degradation of TRIM33. This approach will allow assessment of the differences between whole protein degradation and inhibition of the TRIM33 bromodomain.

Work described in **Chapter 2** provided an increased understanding of the KAc binding pocket of TRIM33β, and the mode of binding of the benzimidazolone ligands. Therefore, the work in this chapter aimed to apply this information to direct the design of PROTAC molecules, as the addition of the linker and E3 ligase ligand must not significantly reduce binding affinity. As introduced in **Chapter 1.5.4**, the nature of the ternary complex is crucial to the degradation of the target protein. A widely applicable computational method to predict the optimal linker composition is yet to be developed, meaning synthesis of a series of PROTACs with different linker lengths and types is required to find a functional PROTAC. Hence, this chapter describes the design, synthesis and evaluation of a series of TRIM33-targeting PROTACs.

3.2 E3 ligase

Two E3 ubiquitin ligases were selected to be recruited by the TRIM33 PROTACs, as each will be held in a different orientation with respect to TRIM33 in the ternary complex.[90] Von-Hippel Lindau (VHL)-recruiting and cereblon-recruiting PROTACs are described in this chapter, with the synthesis of cereblon-targeting PROTACs completed by Dr Amy Scorah.[138]

VHL is a constituent of a protein complex that also contains elongin B, elongin C, and cullin-2, and this complex is responsible for ubiquitination of hypoxia-inducible factors (HIFs).[155,156] Small molecule inhibitors of VHL have been reported (**9** and **10**, **Figure 3.1**),[157,158] with nanomolar binding affinities, and these have been used in a range of PROTAC molecules.[72,96,124] Small molecules that bind to cereblon have also been reported (**12**, **13** and **14**),[100,101] and are commonly used in PROTAC design.[104] Molecules **9**, **12**, and **14** were incorporated into the design for TRIM33-targeting degraders. The usual point of attachment for the linkers are shown in blue, as modification at this point in the compounds does not affect binding to the E3 ligase.

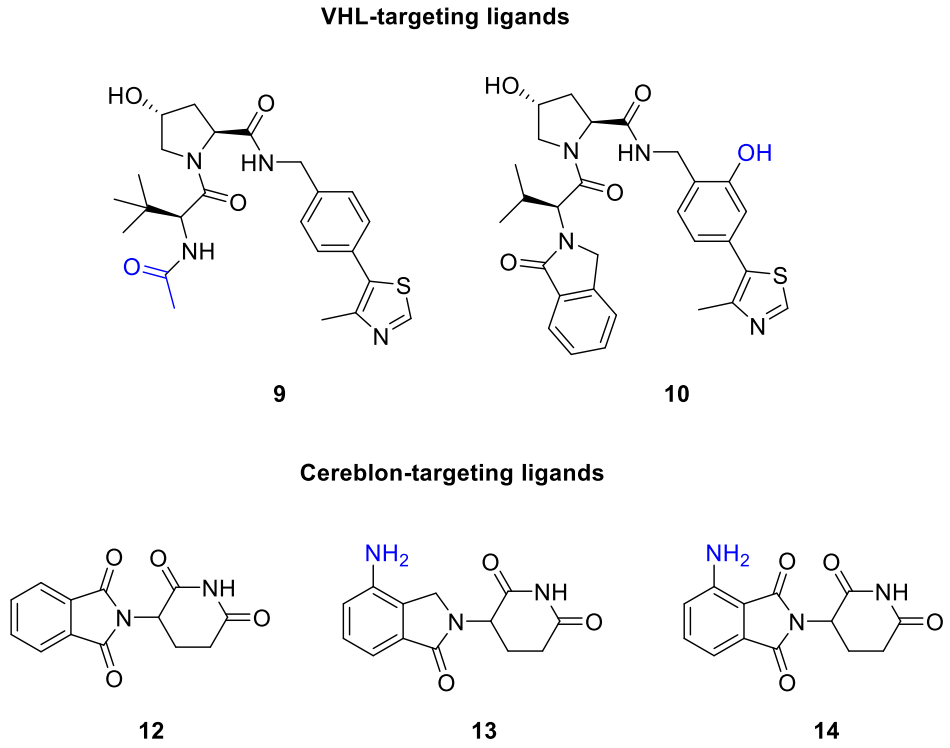


Figure 3.1. Structures of the small molecule inhibitors for VHL (**9** and **10**) and cereblon (**12–14**) which will be used in the design of PROTAC molecules to degrade TRIM33. The point of attachment for the linker is shown in blue, which does not affect binding to the E3 ligase as these regions are largely solvent exposed.[101,158,159]

3.3 Design of VHL-recruiting PROTACs

Ligands for the protein of interest do not need a high affinity to provide degradation when used in a PROTAC molecule.[104] PROTACs function through a catalytic mechanism, where the recruited E3 ligase and target protein only have to transiently be in close proximity to trigger the first transfer of a ubiquitin protein, and subsequent ubiquitination is rapid.[160] Furthermore, on

formation of the ternary complex, stability can be increased by favourable protein-protein interactions which improve the chance of degradation.[96,104] On reviewing the results of the extensive SAR studies performed in **Chapter 2**, and examining the TRIM33 ligand docked into the bromodomain, it appeared the optimum vector for projection of the linker was from the terminal tertiary amine of **25** (**Figure 3.2**), as this region is predicted to project into solvent.

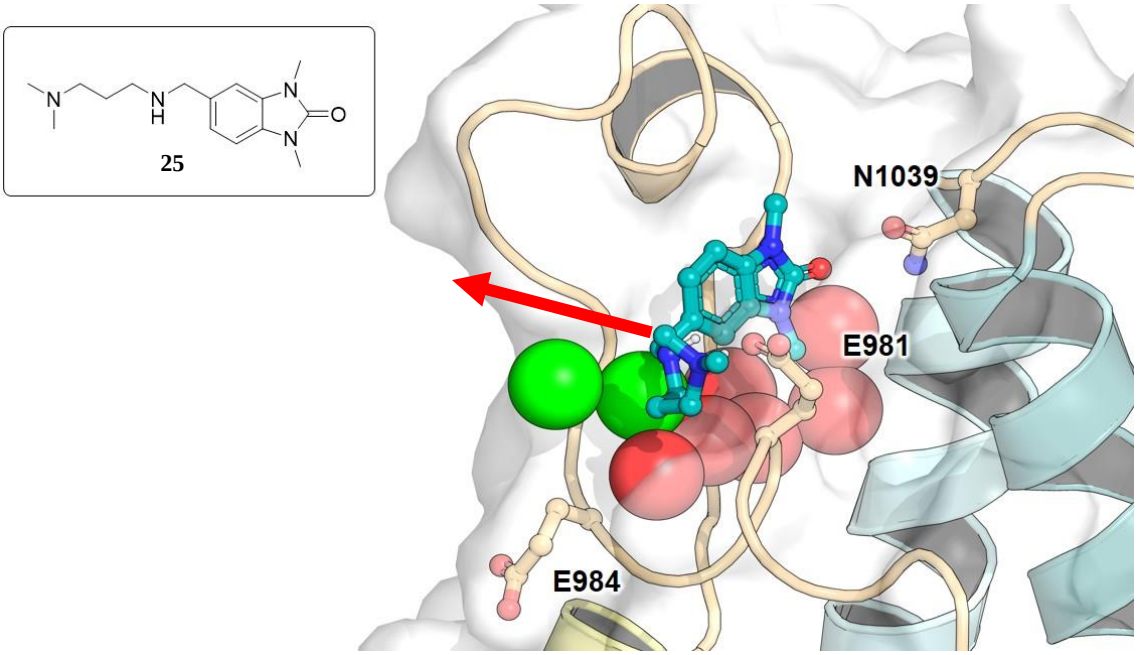
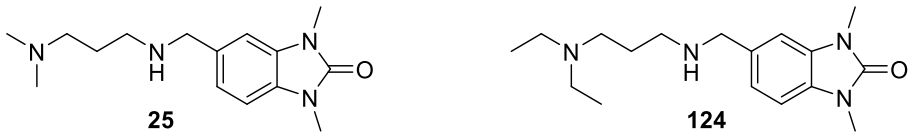


Figure 3.2. Compound **25** docked into the TRIM33β bromodomain (PDB ID: **5MR8**) using CLC Drug Discovery Workbench 3.0. The red arrow shows vector for linker projection to prevent loss in affinity.

During optimisation of the amine chain, it was observed that extending the terminal amine methyl groups to ethyl groups was well tolerated, with compound **124** binding to the TRIM33β bromodomain with a similar affinity to **25** (**Figure 3.3**). This provided evidence that extending from this vector would prevent a significant loss in binding affinity.



TRIM33β IC_{50} = 13.7 μM
TRIM24 IC_{50} = >250 μM

TRIM33β IC_{50} = 2.02 μM
TRIM24 IC_{50} = >250 μM

Figure 3.3. Structures of compounds **25** and **124**. IC_{50} values were determined using a dose-response AlphaScreen™ with $H3_{1–27}$K18Ac peptide, which binds to the TRIM24 and TRIM33 bromodomain.

After studying the literature for VHL-recruiting PROTACs,[72,90,105,161] it appeared that most of the effective molecules incorporated ligand **9** (**Figure 3.1**), with attachment to the linker involving an amide bond in place of the acetyl group. The crystal structure of the ligand bound to the pVHL−ElonginB−ElonginC complex shows that the carbonyl of the acetyl group interacts with a structural water molecule *via* a hydrogen bond, and the methyl of the acetyl group is projected out of the binding site, indicating extending a linker from this position would not affect binding.[158] Therefore, suitable tethering sites for the TRIM33 ligand and the VHL ligand were identified and PROTACs were designed based on this structure (**Figure 3.4**).

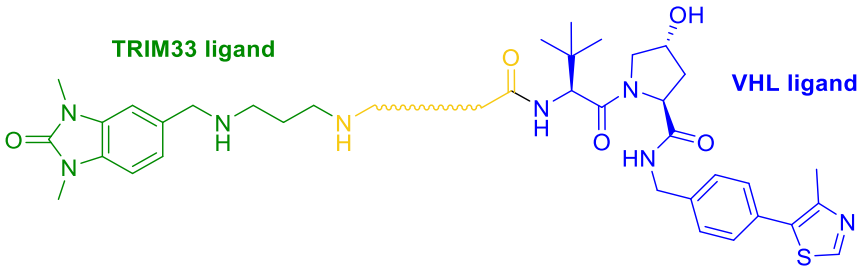


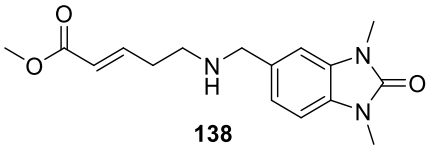
Figure 3.4. Structure of the proposed PROTACs, with the TRIM33 ligand shown in green, the VHL ligand shown in blue, and the linker attachment points shown in yellow.

3.3.1 Linking Strategy

As discussed in **Chapter 1.5.4**, the nature of the ternary complex is one of the most important factors that determines the function of a PROTAC molecule. The specific protein-protein interactions induced on formation of the trimeric species can either be stabilising or destabilising, and for effective ubiquitination, the E3 ligase must have the appropriate orientation relative to the protein of interest. This orientation is controlled by many factors, including linker length and chemical composition, target protein ligand, E3 ligase identity, and points of attachment of linkers. As the TRIM33 bromodomain ligand and E3 ligases were selected, optimisation focused on synthesising of a wide array of analogues varying the linker type and length. The most prevalent linker type used in PROTAC molecules are polyethylene glycol (PEG) chains, to aid solubility. Alkyl linkers contribute to increased cell permeability and triazole-containing linkers bias the conformation of the PROTAC at a specific point in the chain. In this work, PROTACs with a variety of PEG, alkyl and more conformationally biased triazole linkers were designed.

3.3.2 Linker Attachment for TRIM33 Ligand

Two different strategies were chosen for attachment of the linker to the TRIM33 ligand, either through an amide or an amine bond. Compound **138**, synthesised by Dr Larissa See[131] for covalent inhibition of the TRIM33 bromodomain, showed a similar TRIM33β binding affinity to **25**, indicating that a tertiary amine was not required at the terminal position (**Figure 3.5**). Therefore, amide linkage at this position was considered an appropriate strategy.



138

TRIM33β IC_{50} = 12.6 μM

Figure 3.5. Structure of compound **138**. IC_{50} value was determined using a dose-response AlphaScreen™ with $H3_{1–27}$K18Ac peptide. The IC_{50} value indicates that an amide bond could be tolerated in this position.

A series of VHL-recruiting PROTACs were designed with a variety of linkers (**Figure 3.6**). It was hypothesised that the amide-linking series of PROTACs would have a lower binding affinity for the TRIM33β bromodomain compared to the amine-linking series due to the loss of the 'pincer' interaction described in **Chapter 2**, although there may be a slight increase in affinity observed relative to the small molecule ligands due to hydrophobic interactions of the linkers with the surface of the protein.

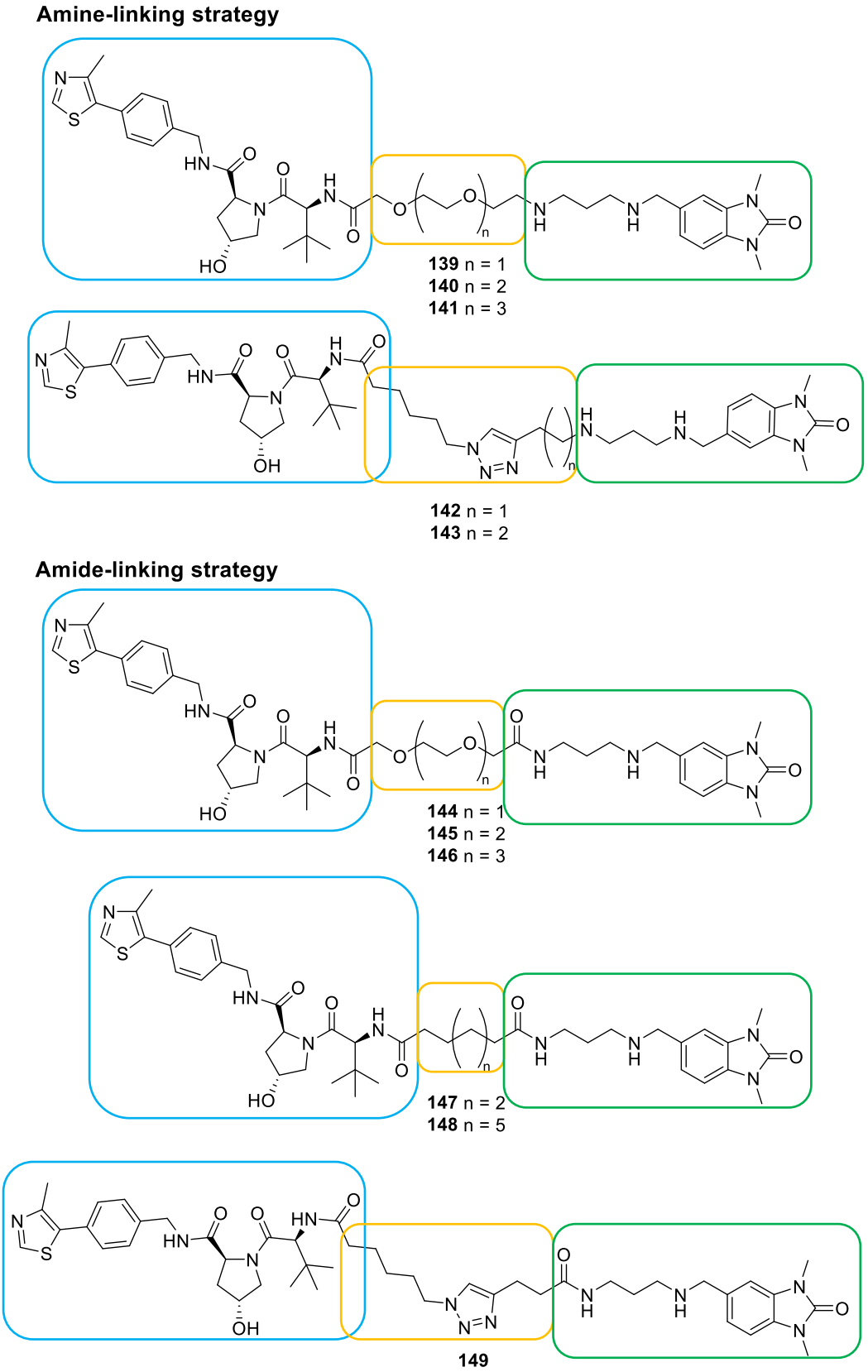


Figure 3.6. Structures of the designed TRIM33-targeting PROTACs. PROTACs **139–143** are linked to the TRIM33 ligand through an amine bond, and **144–149** are linked through an amide bond.

3.3.3 Modelling of Chemical Space

The variety of linkers chosen were designed to result in an array of ternary complex structures, and therefore different orientations of VHL relative to TRIM33, to give the greatest chance of surface lysine ubiquitination. In order to maximise this, it is important to sample a significant amount of conformational space with the PROTAC series. Whilst on placement at Vertex Pharmaceuticals UK (Abingdon), each PROTAC was subjected to computational modelling to determine the lowest energy conformation in an aqueous environment. Conformations were generated with Corina (Molecular Networks GmbH) and minimised in Maestro (Schrödinger, release 2019-2) using MacroModel with the OPLS3e forcefield in implicit water and standard settings (performed by Dr Ronald Knegtel). All compounds were then overlaid on the phenyl and 4-methylthiazole rings of the E3 ligase binding moiety and visualised using PyMOL (**Figure 3.7**).

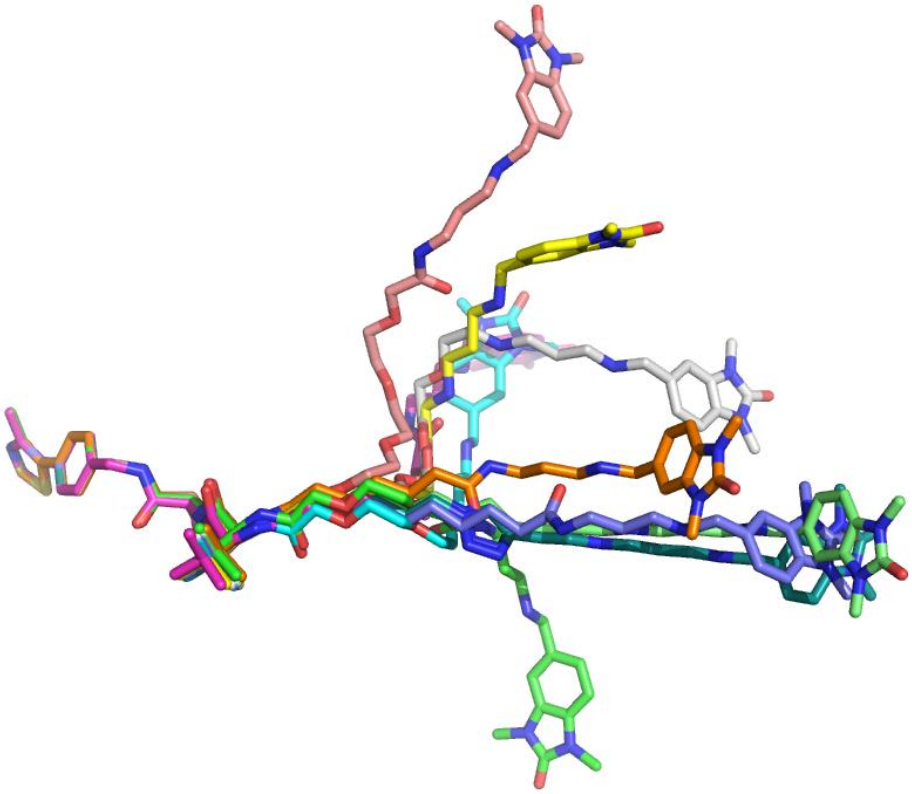


Figure 3.7. PROTAC molecules **139–149** in their lowest energy conformation, all overlaid with respect to the VHL ligand to give an idea of the chemical space being sampled. Diagram produced by Ronald Knegtel.

This modelling indicates that these molecules sample a large area of conformational space. However, due to the large number of low energy conformers that the PROTACs can adopt as a result of the flexible linkers, it is still unclear which conformation would be favoured in the cell,

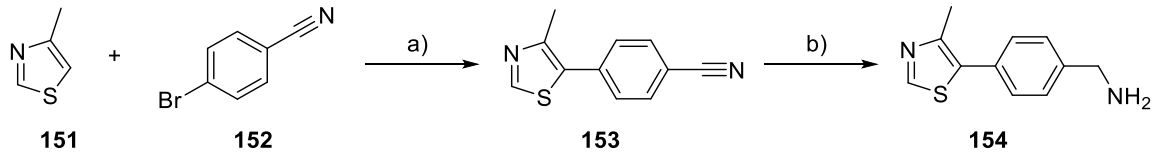
and when bound to the target proteins. Nevertheless, the modelling supported the synthesis of the selected PROTAC molecules **139–149**.

3.4 Synthesis of VHL-Recruiting PROTACs

Since PROTAC molecules are large and typically require a significant number of steps to synthesise, an ideal route would involve syntheses of the TRIM33 and VHL ligands containing functional handles that can be quickly diversified with a range of linkers.

3.4.1 Synthesis of VHL Ligand

VHL ligand **150** was synthesised following the route published by Cuilli and Crews in 2012 (**Scheme 3.1**).[157,158] The first step involved a palladium-catalysed coupling of 4-methylthiazole **151** with 4-bromobenzonitrile **152**, which produced **153** in 83–89% yields. The next step required reduction of the nitrile functional group to primary benzylic amine **154**, which was first attempted with cobalt(II) chloride and sodium borohydride. However, this gave a 37% yield and required multiple purification steps. Switching to lithium aluminium hydride in tetrahydrofuran gave higher yields, with no purification required, thus this method was used going forward.

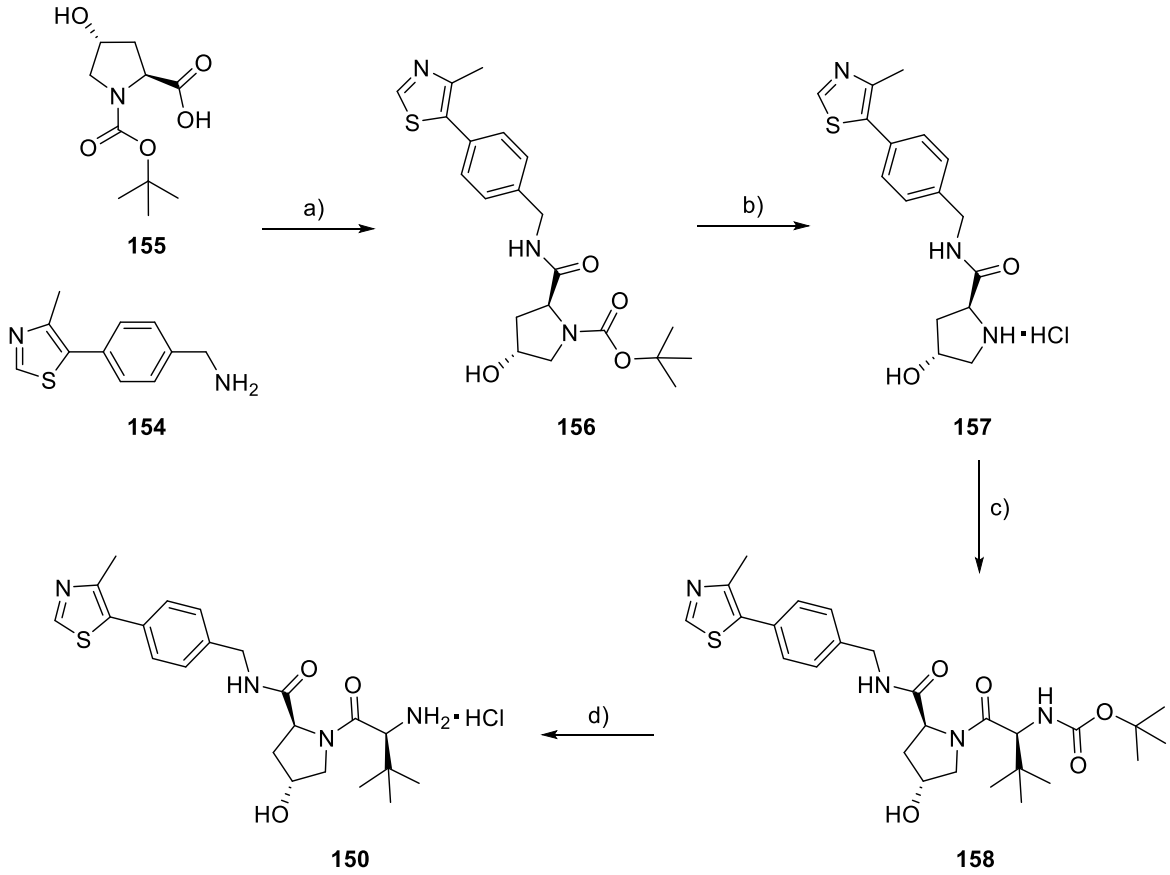


151 **152** **153** **154**

Scheme 3.1. Synthesis of amine **154**. *Reagents and conditions:* a) $Pd(OAc)_2$, KOAc, DMA, 150 ºC, 19 h, 83–89%, n = 3; b) First method: $CoCl_2$, $NaBH_4$, MeOH, 0 ºC, 1.5 h, 37%, n = 1; Second method: $LiAlH_4$, THF, 70 ºC, 5 h, 45–64%, n = 4.

Amine **154** was then coupled with acid **155** to give amide **156**, utilising HATU as a coupling agent (**Scheme 3.2**). Protection of the hydroxyl group was not required, and ester products were not isolated from this reaction. Boc group deprotection of **156** using 4 M hydrochloric acid in dioxane afforded the hydrochloride salt of amine **157**, which was used immediately in the next step without purification. The subsequent amide coupling with HATU and Boc-L-*tert*-leucine installed the final moiety to give the Boc-protected VHL ligand **158** in 53–89% yields. The concluding step was the deprotection of the Boc group with 4 M hydrochloric acid to yield the

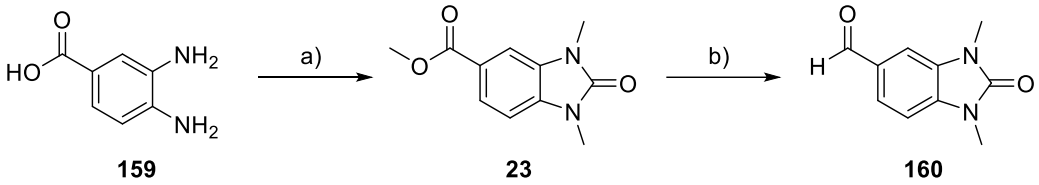
final ligand ready for attachment to the linker. However, the product **150** appeared to degrade at room temperature over time. Therefore, the VHL ligand **150** was stored as Boc-protected **158**, and the deprotection was performed immediately prior to use in the next reaction.



Scheme 3.2. Final steps of the VHL ligand **150** synthesis. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 16 h, 40–80%, n = 4; b) 4 M HCl in dioxane, rt, 1 h, 99–100%, n = 4; c) Boc-L-*tert*-leucine, HATU, DIPEA, DMF, rt, 16 h, 53–89%, n = 3; d) 4 M HCl in dioxane, rt, 1 h, 91–100%, n = 8.

3.4.2 Synthesis of TRIM33 Ligand

Synthesis for the TRIM33 ligand began as in **Chapter 2**, with a CDI ring closure with 3,4-diaminobenzoic acid **159** (**Scheme 3.3**). During this reaction, the carboxylic acid was converted into a methyl ester, as the acid also reacts with CDI and is quenched with methanol. The product was subsequently *N*-methylated with iodomethane to yield the benzimidazolone head group **23**. The ester moiety was reduced with lithium borohydride to yield the benzylic alcohol, which was oxidised to the corresponding aldehyde **160** using manganese(IV) oxide.

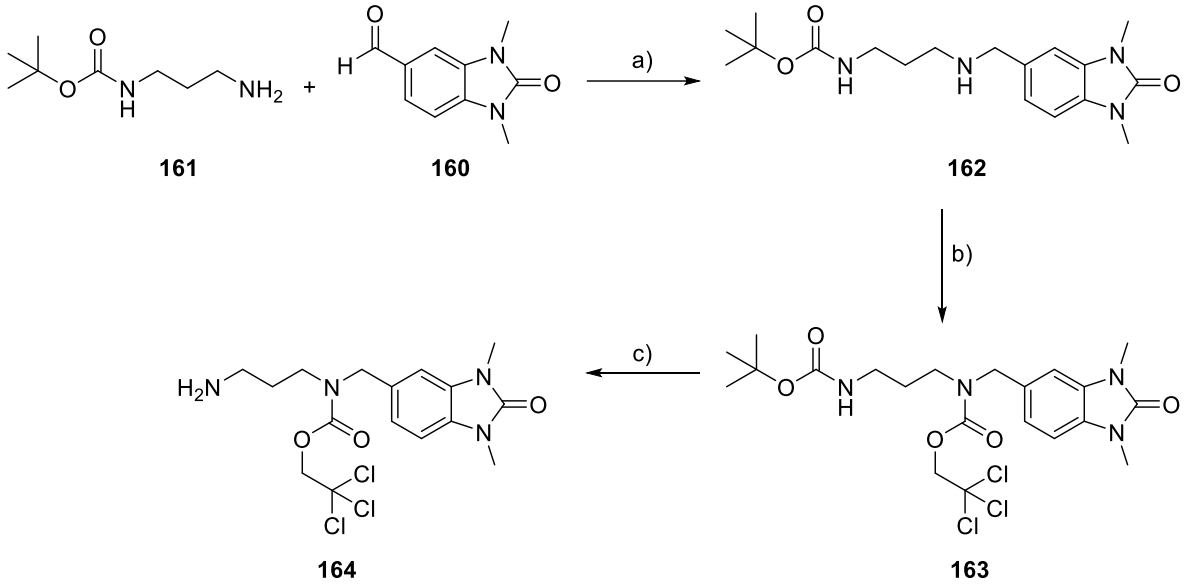


159 **23** **160**

Scheme 3.3. Synthesis of the benzimidazolone core. *Reagents and conditions:* a) i. CDI, CH_2Cl_2, rt, 2 h then MeOH, rt, 1 h, ii. MeI, K_2CO_3, DMF, rt, 16 h, 78–94% over two steps, n = 9; b) i. $LiBH_4$, THF, MeOH, 66 °C, 4 h, ii. MnO_2, CH_2Cl_2, rt, 16 h, 64–87% over two steps, n = 13.

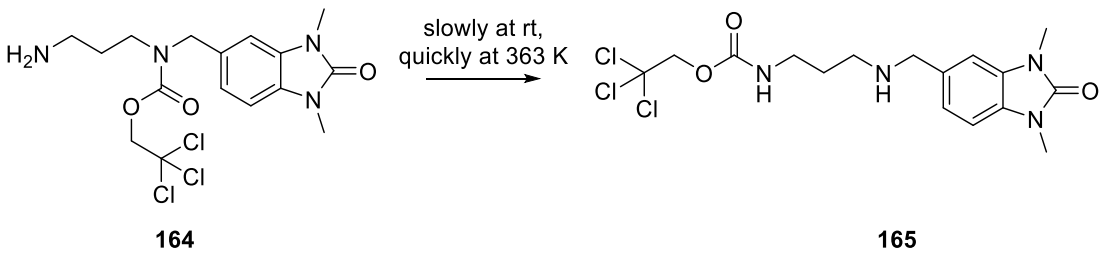
The next step required a reductive amination with Boc-protected amine **161** (**Scheme 3.4**). Aldehyde **160** and amine **161** were pre-stirred to allow imine formation before addition of sodium triacetoxyborohydride, which gave benzylic amine **162** in excellent yields.

At this point, it was important to consider the protecting group strategy. The benzylic amine required a protecting group that is stable to acidic conditions, so that subsequent removal of the Boc group revealed only the terminal primary amine. The chosen group must also be stable to basic conditions, as a later ester hydrolysis step required use of lithium hydroxide. Consequently, a Troc group was selected, and was added to the benzylic amine using 2,2,2-trichloroethoxycarbonyl chloride, giving moderate yields of **163**. Rotamers were observed in the ^{1}H NMR spectrum due to the restricted rotation of the Troc group around the benzylic amine. Variable temperature NMR spectroscopy was performed at 363 K, which resolved the rotamers and showed one set of signals. Before continuing with the synthetic route, it was essential to ensure that the Troc group could be removed, and the conditions would not affect either the TRIM33 ligand or the VHL ligand. On subjecting **163** to zinc(0) and acetic acid, Troc removal was observed. Furthermore, exposing VHL ligand **158** to the same conditions showed no reaction and no change in α_D value, indicating epimerisation of stereocentres was not occurring. Therefore, the route proceeded with a Boc deprotection to reveal the primary amine **164**.



Scheme 3.4. Synthesis of protected TRIM33 ligand **164**. *Reagents and conditions:* a) AcOH, DCE, 3 Å molecular sieves, 0 ºC, 1 h, then $NaBH(OAc)_3$, rt, 16 h, 43–98%, n = 9; b) TrocCl, pyridine, DMAP, CH_2Cl_2, 0 ºC, 2 h, 44–98%, n = 8; c) 4 M HCl in dioxane, rt, 1 h, 57–100%, n = 12.

As with **163**, amine **164** also required variable temperature NMR spectroscopy to resolve the rotamers seen in the ^{1}H and ^{13}C NMR spectra. However, on heating a portion of the sample to 363 K in DMSO-d_6, migration of the Troc group to the terminal amine (**165**) was observed, likely aided by the electrophilic nature of the Troc carbamate and the ability to form a six-membered ring intermediate (**Scheme 3.5**). Reanalysis of the original sample showed that the migration also occurs slowly at room temperature, indicated by two sets of peaks in the ^{1}H NMR spectrum, thus amine **164** was stored as the Boc-protected precursor **163** and prepared immediately prior to the next step.



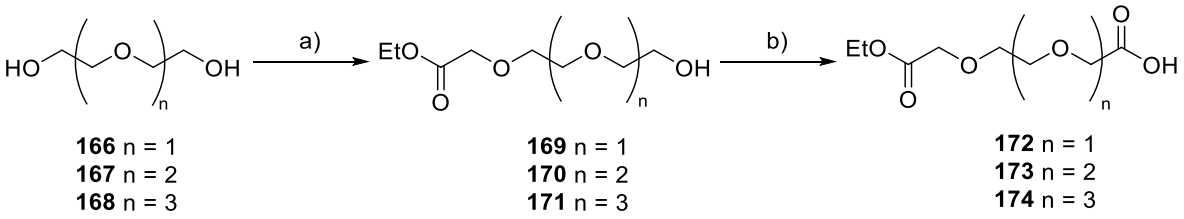
Scheme 3.5. Migration of the Troc protecting group to the terminal amine observed to be occurring quickly at high temperature and slowly at room temperature.

Since performing variable temperature NMR spectroscopy is time consuming and requires a high sample concentration, Dr Amy Scorah[138] explored a route in which no protecting group on the

benzylic amine was used. However, low yields and inseparable mixtures were observed in subsequent steps, thus the Troc protecting strategy was sustained.

3.4.3 Synthesis of Amide-Linked PEG PROTACs

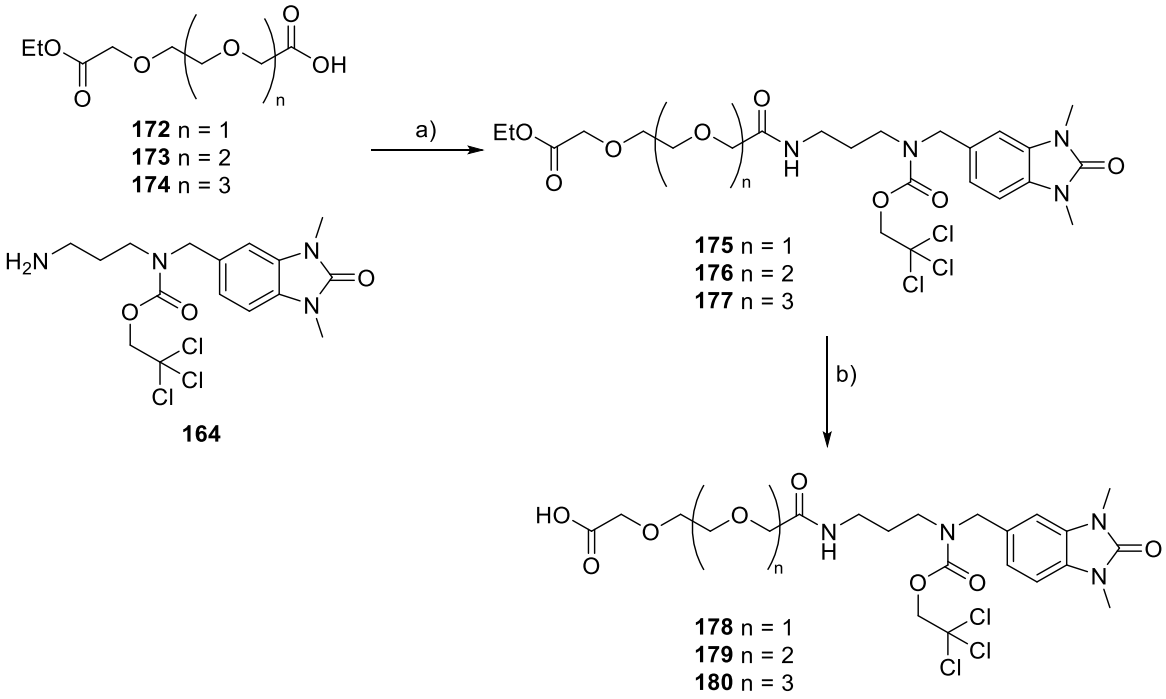
With the VHL ligand and the TRIM33 ligand syntheses completed, focus moved to the linker synthesis. The PEG linkers required two carboxylic acid equivalents to connect to the two amines of the VHL and TRIM33 ligands. Three different linker lengths were envisioned, so three appropriate ethylene glycols **166–168** were selected. Synthesis began with the introduction of an ethyl ester moiety using two equivalents of ethyl diazoacetate and Lewis acid boron trifluoride-diethyl etherate, following a literature procedure (**Scheme 3.7**).[162] This reaction gave monoester products **169–171** in good yields, with a small amount of starting material remaining and the diester also being produced. **169**, **170**, and **171** were oxidised using catalytic TEMPO, with (diacetoxyiodo)benzene (BAIB) as a sacrificial stoichiometric oxidant, to yield carboxylic acids **172**, **173**, and **174**. This reaction completed the linker synthesis, which contained a carboxylic acid and an ester to function as a masked acid equivalent.



Scheme 3.7. Synthesis of PEG linkers for amide linked PROTACs. *Reagents and conditions:* a) Ethyl diazoacetate, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 2 h, **169**: 41–63%, n = 5, **170**: 42–68%, n = 4, **171**: 47–67%, n = 4; b) TEMPO, BAIB, MeCN, H_2O, rt, 30 h, **172**: 53–100%, n = 2, **173**: 24–86%, n = 2, **174**: 64–66%, n = 2.

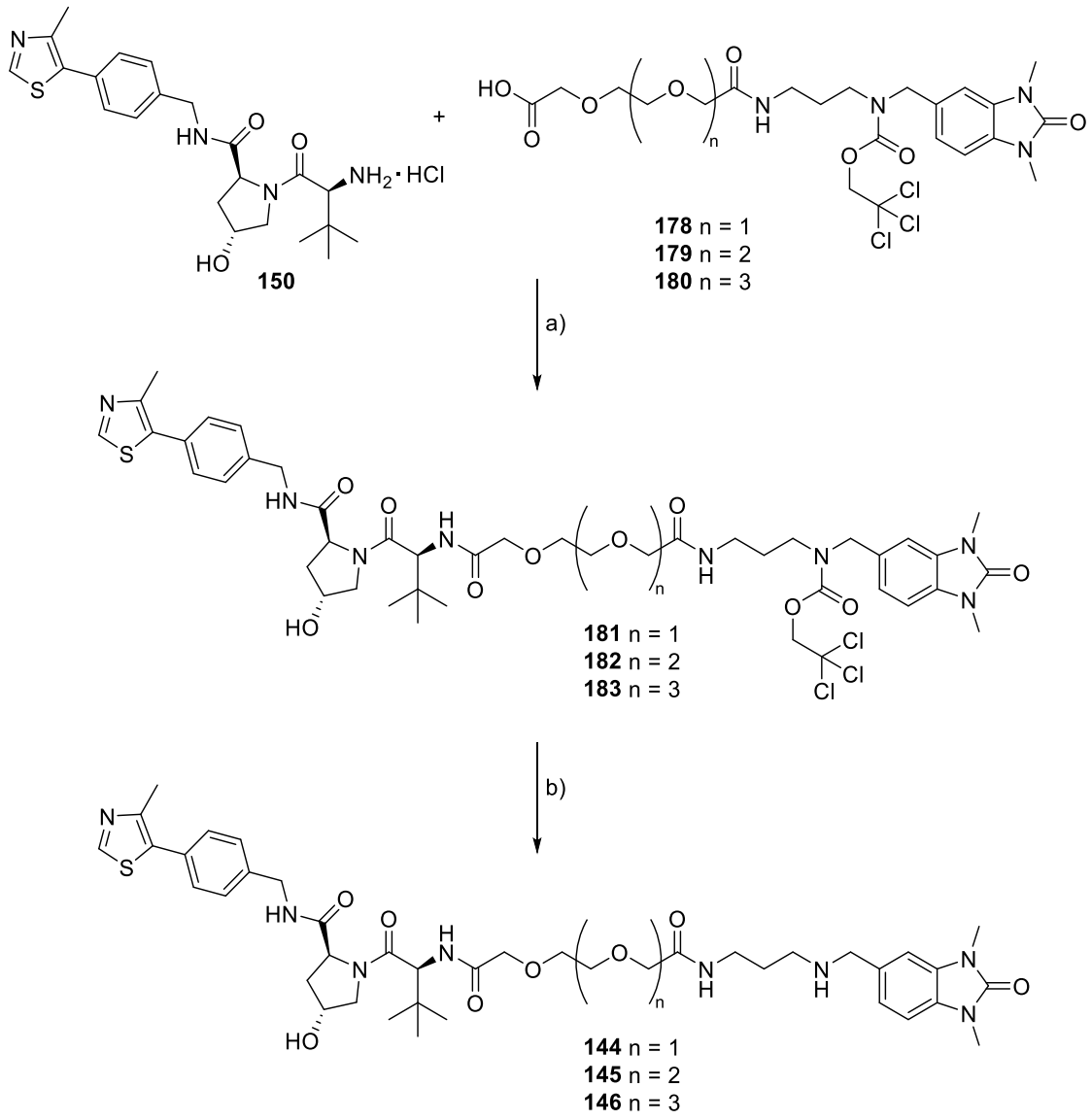
With all three components synthesised, it was decided that the linker would be first coupled to the TRIM33 bromodomain ligand. Amine **164** was prepared just before the amide coupling to the linker acids **172**, **173**, and **174**, which all proceeded with moderate yields of **175–177** (**Scheme 3.8**). The next step involved ester hydrolysis to reveal the carboxylic acid group. Acids **178** and **179** did not require purification, however **180** was purified *via* silica gel column chromatography, which due to the polar nature of the compound may have contributed to the

slight loss in yield. All products **175**–**180** required variable temperature NMR spectroscopy to resolve the rotamers.



Scheme 3.8. Attachment of the PEG linkers to the TRIM33 ligand. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 16 h, **175**: 41–85%, n = 4, **176**: 52–63%, n = 2, **177**: 58–100%, n = 2; b) LiOH, THF, H_2O, MeOH, rt, 1 h, **178**: 83%, n = 1, **179**: 66–86%, n = 3, **180**: 73%, n = 1.

The penultimate step to complete the PROTAC synthesis was an amide coupling with VHL ligand **150**, which was Boc-deprotected to give the amine hydrochloride salt just before use. The couplings proceeded with moderate yields of amides **181**, **182**, and **183** (**Scheme 3.9**). Again, variable temperature NMR was required to observe one set of signals in the ^{1}H and ^{13}C NMR spectra. The final step was deprotection of the Troc group to reveal the benzylic amine. These reactions gave moderate yields, which could be attributed to the reaction not going to completion, with starting material remaining for all three linker lengths. However, sufficient quantities of PROTACs **144–146** were isolated to complete the desired biological assays.

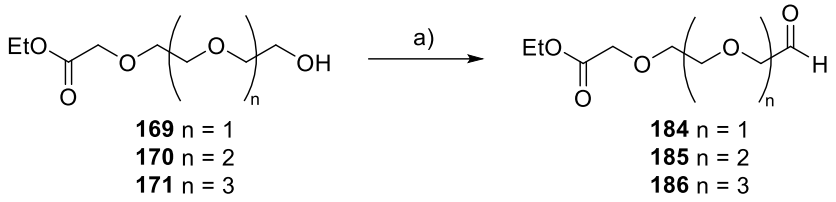


Scheme 3.9. Final steps in the synthesis of the amide-linked PEG PROTACs. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 16 h, **181**: 66%, n = 1, **182**: 49–55%, n = 2, **183**: 32–51%, n = 2; b) Zn, AcOH, THF, rt, 16 h, **144**: 47–64%, n = 2, **145**: 34%, n = 1, **146**: 42%, n = 1.

3.4.4 Synthesis of Amine-Linked PEG PROTACs

3.4.4.1 Reductive Amination Strategy

The first strategy considered for synthesis of amine-linked PEG PROTACs was a reductive amination to attach the PEG linker containing an aldehyde to the TRIM33 ligand terminal amine. Oxidation of the alcohol functional group in the ester PEG linkers **169**, **170**, and **171** gave aldehydes **184**, **185**, and **186**, using Dess Martin Periodinane (DMP) (**Scheme 3.10**). These aldehydes were volatile, but column chromatography could be used for purification as long as care was taken when removing the solvents, and storage in the freezer was necessary.

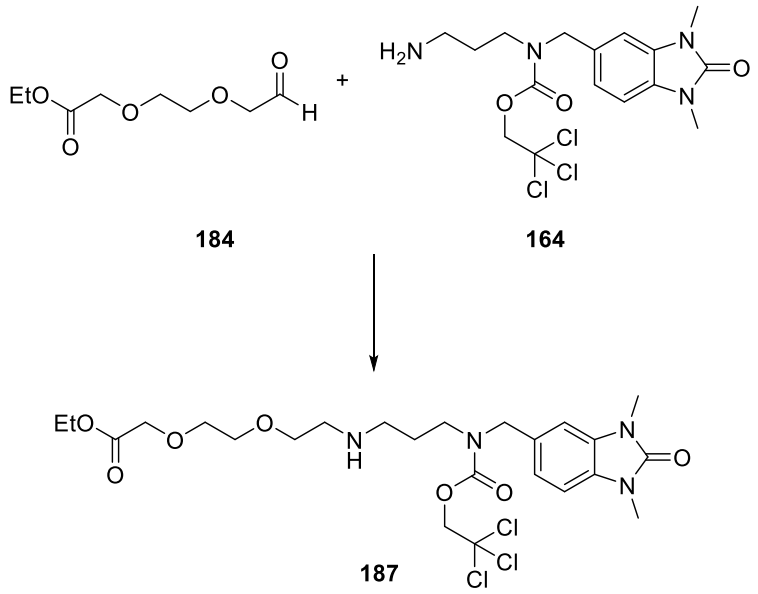


169 n = 1
170 n = 2
171 n = 3

184 n = 1
185 n = 2
186 n = 3

Scheme 3.10. Synthesis of aldehyde PEG linkers **184–186** ready for the reductive amination. *Reagents and conditions:* a) DMP, CH_2Cl_2, rt, 16 h, **184**: 55%, n = 1, **185**: 52%, n = 1, **186**: 42%, n = 1.

It was intended that these aldehydes could be used with TRIM33 ligand amine **164** in a reductive amination to form product **187** (**Scheme 3.11**). The amine of **187** could then be protected, and a subsequent ester hydrolysis would yield an acid ready for amide coupling with VHL ligand **150**.



Scheme 3.11. Proposed reductive amination with aldehyde **184** and amine **164**.

Five sets of conditions were tried, all of which involved pre-stirring aldehyde **184** with amine **164** to promote imine formation, followed by addition of the reducing agent. On the first attempt (**Entry 1**, **Table 3.1**), all aldehyde **184** appeared to have been consumed *via* TLC and ^{1}H NMR after 1 hour, so reducing agent sodium triacetoxyborohydride was added and left to stir for 16 hours. On quenching the reaction, starting material amine **164** was observed by TLC with a new spot. After several attempts of purification using column chromatography, the mixture could not be separated. The second attempt (**Entry 2**, **Table 3.1**) involved reducing the number of equivalents of amine **164** used in an effort to reduce the difficulty of purification. However, this also appeared to give an inseparable mixture with the starting material. The third set of conditions

trialled the reaction under a basic environment using triethylamine (**Entry 3**, **Table 3.1**), which gave rise to the same result. It was observed that the solubility of amine **164** in DCE was low, which could be contributing to the purification issues due to **164** not fully dissolving and therefore not partaking in the reaction. Consequently, the reaction solvent was changed to DMF, which allowed complete dissolution of **164** (**Entry 4**, **Table 3.1**). This reaction gave a product that was separable from the starting material, but did not correspond to the desired product. The ^{1}H NMR spectrum appeared to contain two sets of aromatic peaks, neither of which matched starting material amine **164**, but only one set of the ethyl protons. As this was not the desired reductive amination product, this was not further purified. The final attempt involved using a stronger reducing agent, sodium borohydride, to fully reduce any imine to prevent hydrolysis back to the starting materials (**Entry 5**, **Table 3.1**). However, again an inseparable mixture was obtained. As this method had been unsuccessful, it was decided that a different strategy would be used.

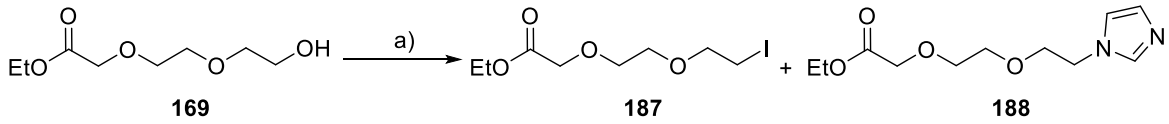
Table 3.1. Conditions used for the proposed reductive amination.

Entry	Solvent	Conditions	Reducing Agent	Observation
1	DCE	AcOH, 0 ºC, 1 eq. **184** and 1.75 eq. **164**	$NaBH(OAc)_3$	Inseparable mixture with starting material.
2	DCE	AcOH, 0 ºC, 1 eq. **184** and 1 eq. **164**	$NaBH(OAc)_3$	Inseparable mixture with starting material.
3	DCE	NEt_3, 0 ºC, 1 eq. **184** and 1 eq. **164**	$NaBH(OAc)_3$	Inseparable mixture with starting material.
4	DMF	NEt_3, 0 ºC, 1 eq. **184** and 1.75 eq. **164**	$NaBH(OAc)_3$	Unknown product made, not desired or double product.
5	DCE	NEt_3, 0 ºC, 1 eq. **184** and 1 eq. **164**	$NaBH_4$	Inseparable mixture with starting material.

3.4.4.2 Substitution Strategy

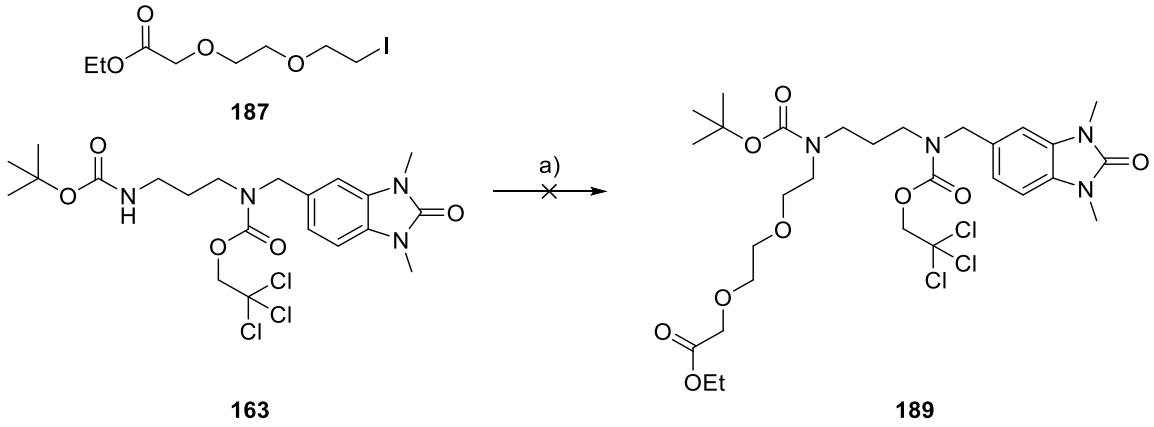
As the Boc-protected TRIM33 ligand **163** had already been synthesised, a substitution using this compound was explored. Literature precedent showed that deprotonation of a Boc-protected amine with sodium hydride followed by addition of an electrophile might produce the desired product **189**.[163,164] This was an attractive route as the product already contains the amine protected,

which reduces a step in the synthesis. The route began with an Appel reaction to convert alcohol **169** into the electrophilic species **187** (**Scheme 3.12**). A small amount of intermediate **188** was also isolated.



Scheme 3.12. Synthesis of an electrophilic partner for the substitution reaction. *Reagents and conditions:* a) PPh_3, imidazole, I_2, toluene, 90 ºC, 2 h, 50% yield of **187**, 13% of **188**, n = 1.

Boc-protected TRIM33 ligand **163** was then pre-stirred with sodium hydride to allow deprotonation (**Scheme 3.13**). The electrophilic species **187** was subsequently added, and after 40 minutes, total consumption of starting material was observed *via* TLC analysis. However, after the work up and purification, 96% of the starting material was recovered, which could indicate that traces of remaining DMF had affected the polarity of the starting material on the TLC. The reaction was repeated, and the mixture was heated to 60 ºC for 16 hours. Again, only starting material was recovered. It was decided that another strategy should be considered.



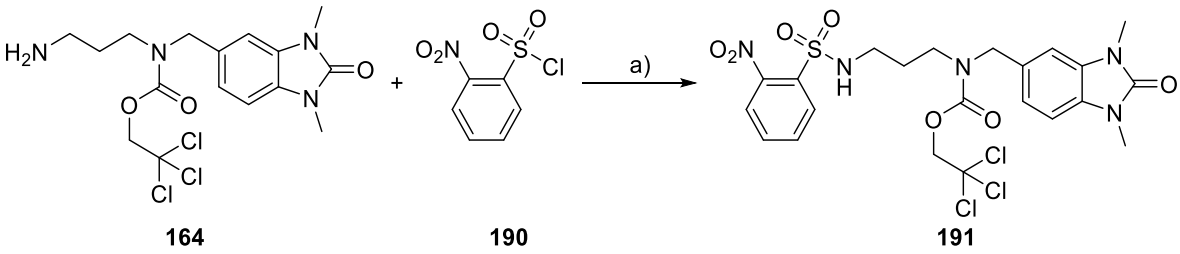
Scheme 3.13. Attempted substitution reaction with protected TRIM33 ligand **163**. *Reagents and conditions:* a) Attempt 1: NaH, DMF, 0 ºC, 2 h, no reaction, Attempt 2: NaH, DMF, 60 ºC, 16 h, no reaction.

3.4.4.3 Mitsunobu Strategy

Despite the substitution strategy failing to yield the desired product, the idea of forming a product with both amines already protected was still desirable. A Mitsunobu reaction was chosen, as this would yield the desired product directly from the alcohol-containing PEG linkers. When considering reaction partners for a Mitsunobu, it is important to note that the pronucleophile

should be acidic ($pK_a < 13$), as the betaine produced from the reaction between triphenylphosphine and azodicarboxylate must remove the proton from the pronucleophile.[165] The Fukuyama amine synthesis provides a method to protect primary amine nucleophiles so that they are appropriate reaction partners in Mitsunobu reactions, using a nitrobenzenesulfonyl (Nosyl) group.[166] This group also prevents multiple alkylations, so once deprotected, the secondary amine can be revealed.

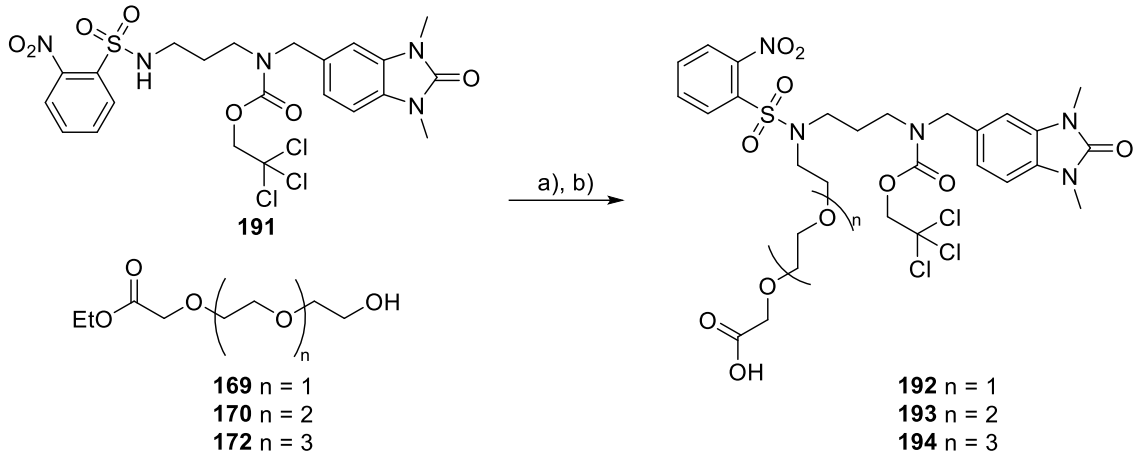
Synthesis began with sulfonamide formation, reacting primary amine **164** with 2-nitrobenzenesulfonyl chloride **190** to yield the Nosyl-protected **191** (**Scheme 3.14**). The predicted pK_a of the sulfonamide proton of **191** is 8.1 (ACD I-Lab 2.0), indicating that this pronucleophile is a suitable candidate for the Mitsunobu reaction.



164 **190** **191**

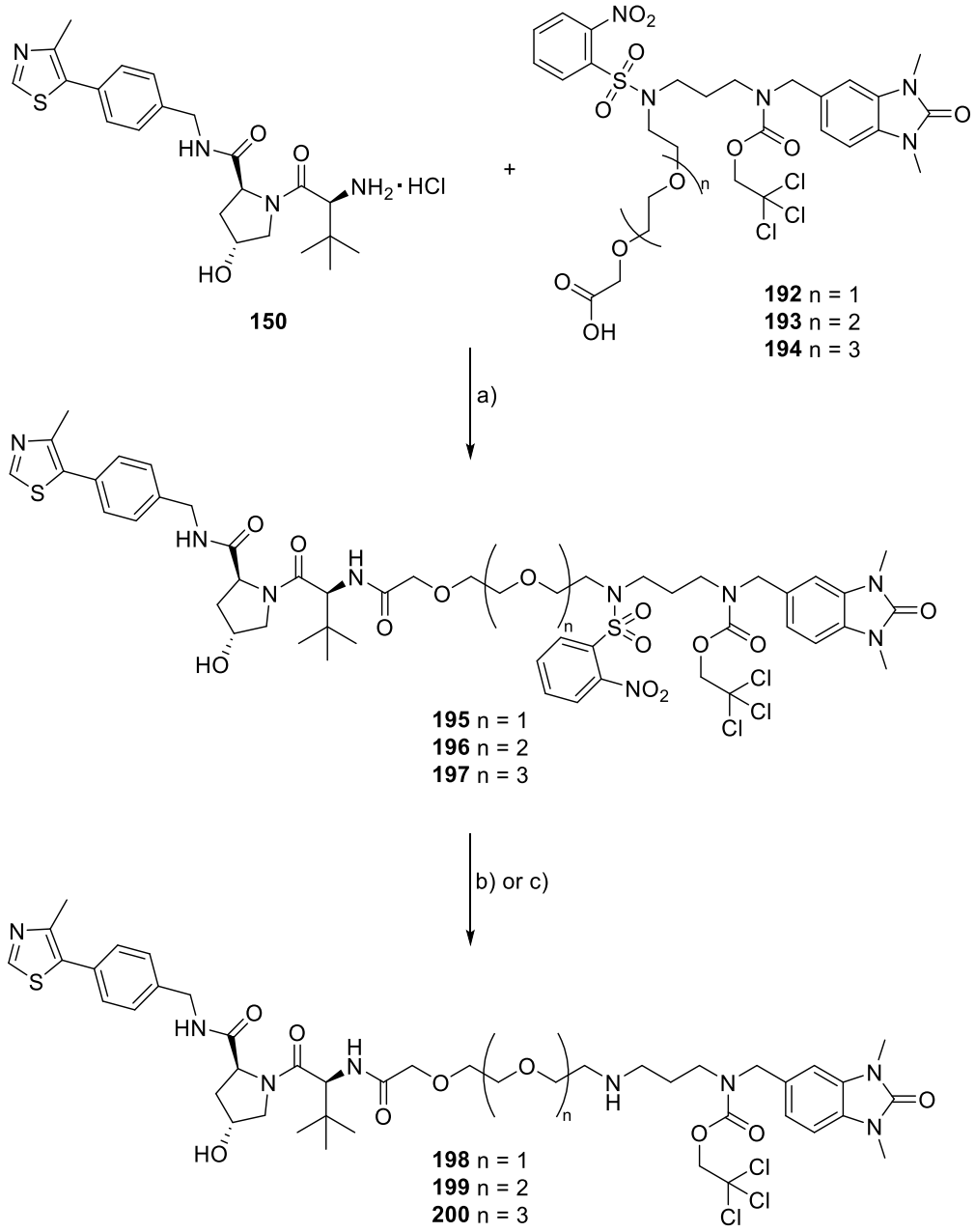
Scheme 3.14. Nosyl protection of the amine in preparation of the Mitsunobu reaction. *Reagents and conditions:* a) NEt_3, CH_2Cl_2, rt, 16 h, 67–100%, n = 6.

The Mitsunobu reaction was performed with alcohol-containing PEG linker **169** (**Scheme 3.15**). Fortunately, the reaction yielded the desired product, although the R_f value of the product was identical to the R_f value of starting material **191** by TLC, making purification difficult. This problem was solved by the addition of 2.5 equivalents each of alcohol **169**, triphenylphosphine, and DIAD, to push the reaction to completion. The reaction was performed for the two longer linker lengths, which yielded the respective desired products. However, the triphenylphosphine oxide by-product was very difficult to remove from these ester products, with crystallisation and several column chromatography attempts proving unsuccessful. Nevertheless, after ester hydrolysis with lithium hydroxide to yield carboxylic acids **192**, **193**, and **194**, the triphenylphosphine oxide was easily removed using column chromatography due to the large polarity change of the product.



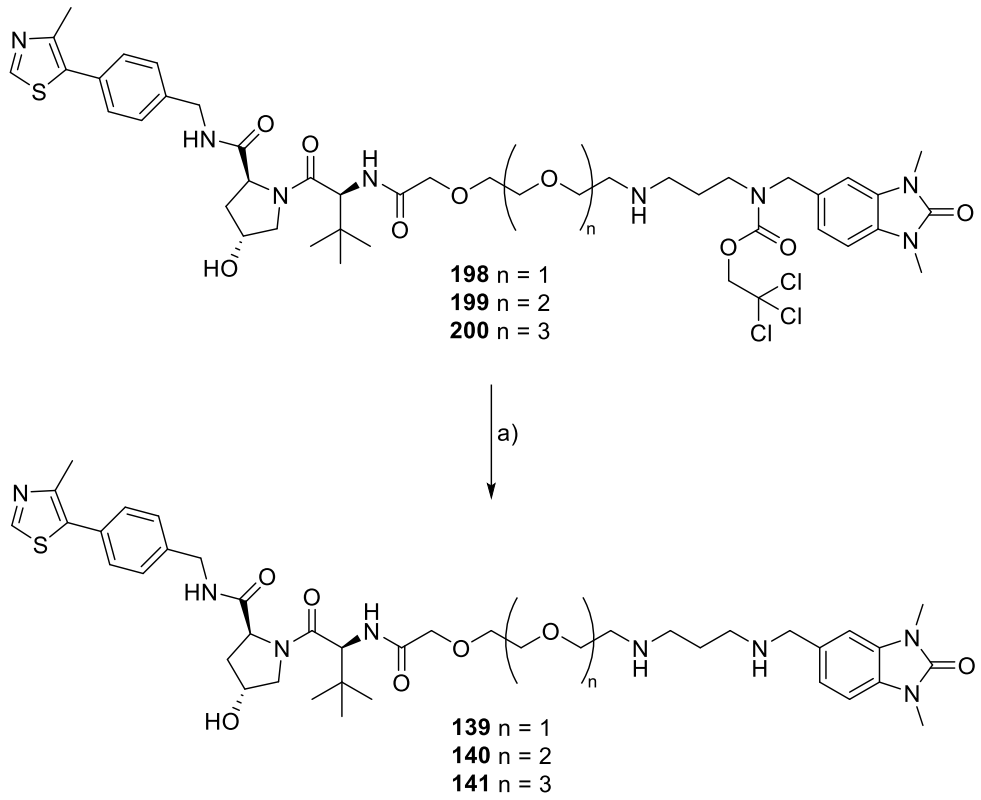
Scheme 3.15. Mitsunobu reaction to attach linkers to TRIM33 ligand **191**. *Reagents and conditions:* a) PPh_3, DIAD, THF, rt, 16 h; b) LiOH, THF, MeOH, H_2O, rt, 1 h, **192**: 50–73% over two steps, n = 3, **193**: 48–49% over two steps, n = 2, **194**: 52% over two steps, n = 1.

It was decided that the Nosyl group would be removed later in the synthesis, so as to keep the amine protected. The next step was the HATU-mediated amide coupling of acids **192**, **193**, and **194** with VHL ligand **150** (**Scheme 3.16**). These reactions proceeded with yields ranging from 20–96%. At this point, the deprotection strategy was considered. The mechanism of deprotection of the Nosyl group is through an S_NAr reaction using a thiol nucleophile, which is facilitated by the nitro group on the aryl ring. If the Troc group was removed first, the reducing conditions required may also reduce the nitro group, meaning the Nosyl group could no longer be removed easily. Thus, it was decided that the Nosyl group should be removed first. Thioglycolic acid was selected as the thiol of choice, as the S_NAr side product could easily be washed out during work up. Compounds **195** and **196** were deprotected under these conditions, with a small amount of starting material remaining. However, the longest linked **197** only returned starting material. This problem was solved by altering the conditions to use thiophenol as the S_NAr nucleophile, which gave the deprotected product **200** in moderate yields. Troc group migration was not observed during variable temperature NMR spectroscopy, presumably because the deprotected amine is now too sterically hindered to form the six-membered intermediate.



Scheme 3.16. Coupling of TRIM33 ligand and VHL ligand with a variety of linker lengths. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 16 h, **195**: 67–96%, n = 3, **196**: 68%, n = 1, **197**: 20–31%, n = 2; b) $HSCH_2CO_2H$, LiOH, DMF, rt, 5 h, **198**: 36–43%, n = 3, **199**: 52%, n = 1; c) PhSH, K_2CO_3, DMF, rt, 2 h, **200**: 87 %, n = 1.

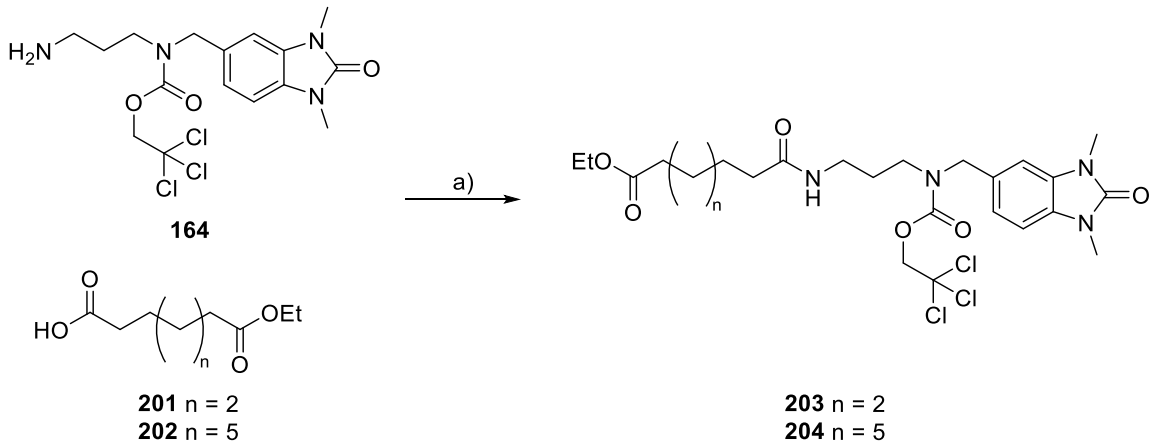
The final step of the synthesis was deprotection of the Troc group using zinc(0) and acetic acid to give the final amine-linked PROTAC molecules **139**, **140**, and **141** (**Scheme 3.17**). None of the three reactions went to completion, however, sufficient quantities of each final PROTAC were isolated for the desired biological assays. Variable temperature NMR spectroscopy was not required on the final products, as the Troc group causing the rotameric centre had been removed.



Scheme 3.17. Final step of the amine linked PROTAC synthesis. *Reagents and conditions:* a) Zn, AcOH, THF, rt, 16 h, **139**: 62%, n = 1, **140**: 54%, n = 1, **141**: 66%, n = 1.

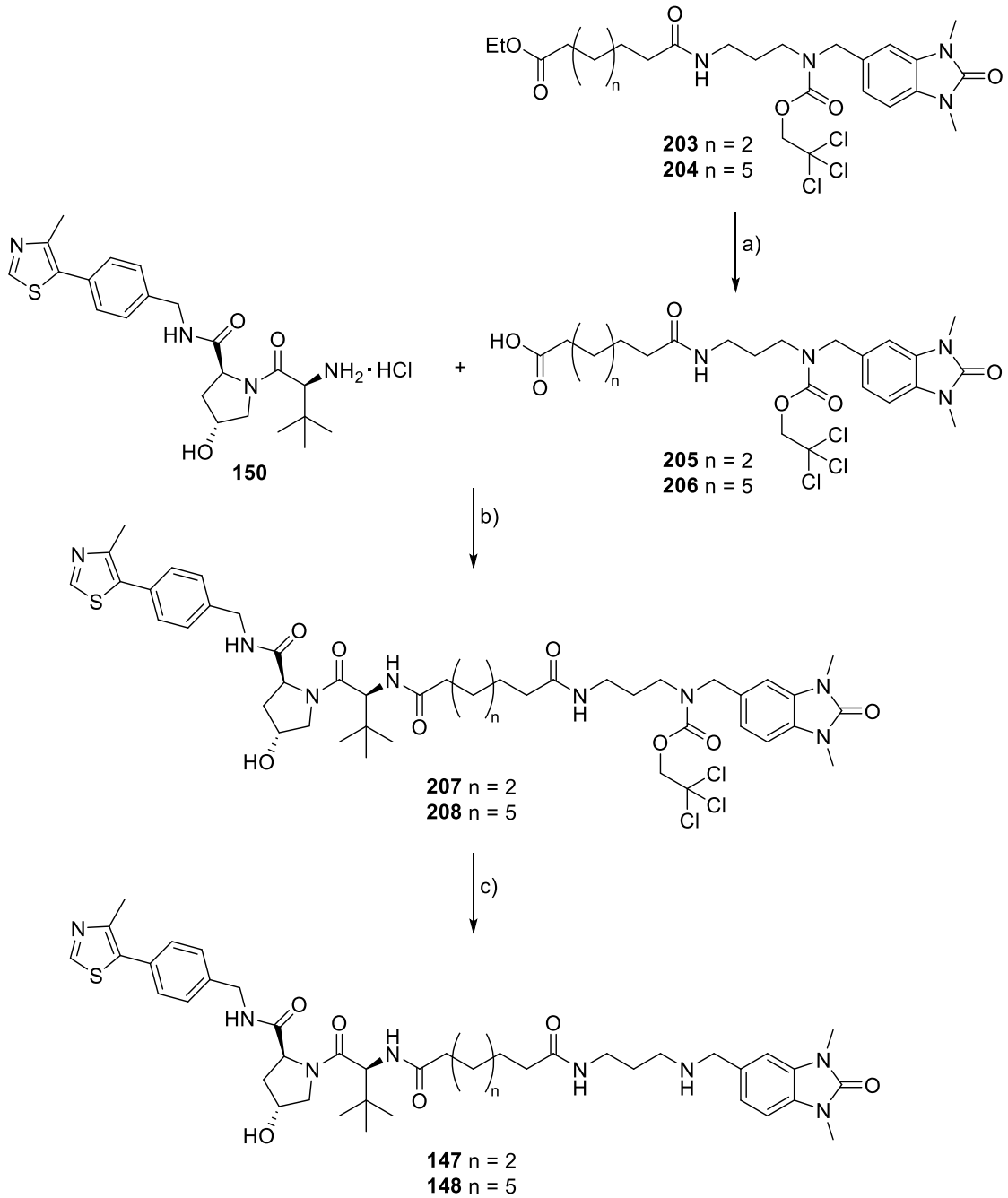
3.4.5 Synthesis of Alkyl PROTACs

The second linking strategy aimed to aid cell permeability by incorporating an alkyl chain. Two alkyl-linked PROTAC molecules were designed, and synthesis began in the same way as for the amide-linked PEG PROTACs, with an amide coupling of acids **201** and **202** with primary amine **164** (**Scheme 3.18**).



Scheme 3.18. Attachment of the alkyl linkers to TRIM33 ligand **164**. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 16 h, **203**: 77%, n = 1, **204**: 80%, n = 1.

Both couplings proceeded with good yields. The next step was to hydrolyse the ethyl esters to reveal the carboxylic acids **205** and **206** (**Scheme 3.19**). Subsequent couplings with VHL ligand **150** gave amides **207** and **208**, and the final Troc deprotection step gave the alkyl linked PROTACs **147** and **148**.



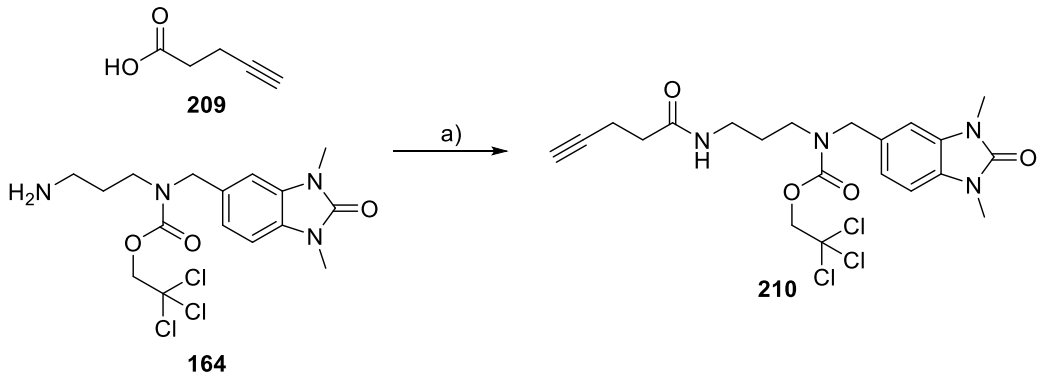
Scheme 3.19. Final steps of alkyl linked PROTAC synthesis. *Reagents and conditions:* a) LiOH, THF, MeOH, H_2O, rt, 1 h, **205**: 80%, n = 1, **206**: 84%, n = 1; b) HATU, DIPEA, DMF, rt, 16 h, **207**: 36%, n = 1, **208**: 32%, n = 1; c) Zn, AcOH, THF, rt, 16 h, **147**: 11%, n = 1, **148**: 47%, n = 1.

3.4.6 Synthesis of Conformationally Biased Triazole PROTACs

The third linkage strategy considered was conformationally biasing the linker, which may alter the orientation of the ternary complex into a favourable conformation to induce degradation. Three triazole linked PROTACs were designed, two that contained amine linkers to the TRIM33 ligand, and one that contained an amide linker.

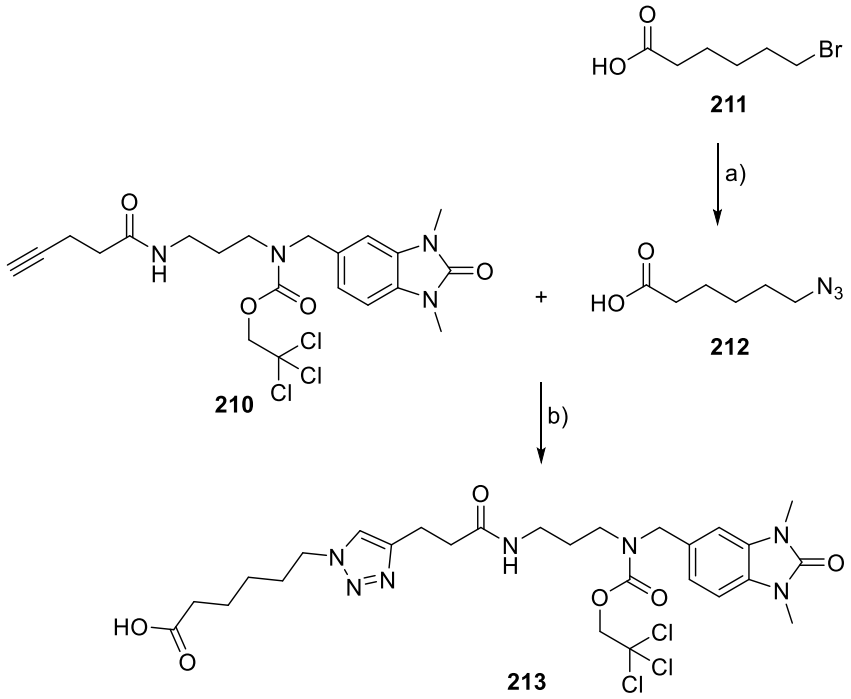
3.4.6.1 Synthesis of Amide-Linked Triazole PROTACs

Synthesis began with an amide coupling of TRIM33 ligand amine **164** and acid **209**, which contains an alkyne functional handle (**Scheme 3.20**). This reaction proceeded with 72–98% yields of desired product **210**.



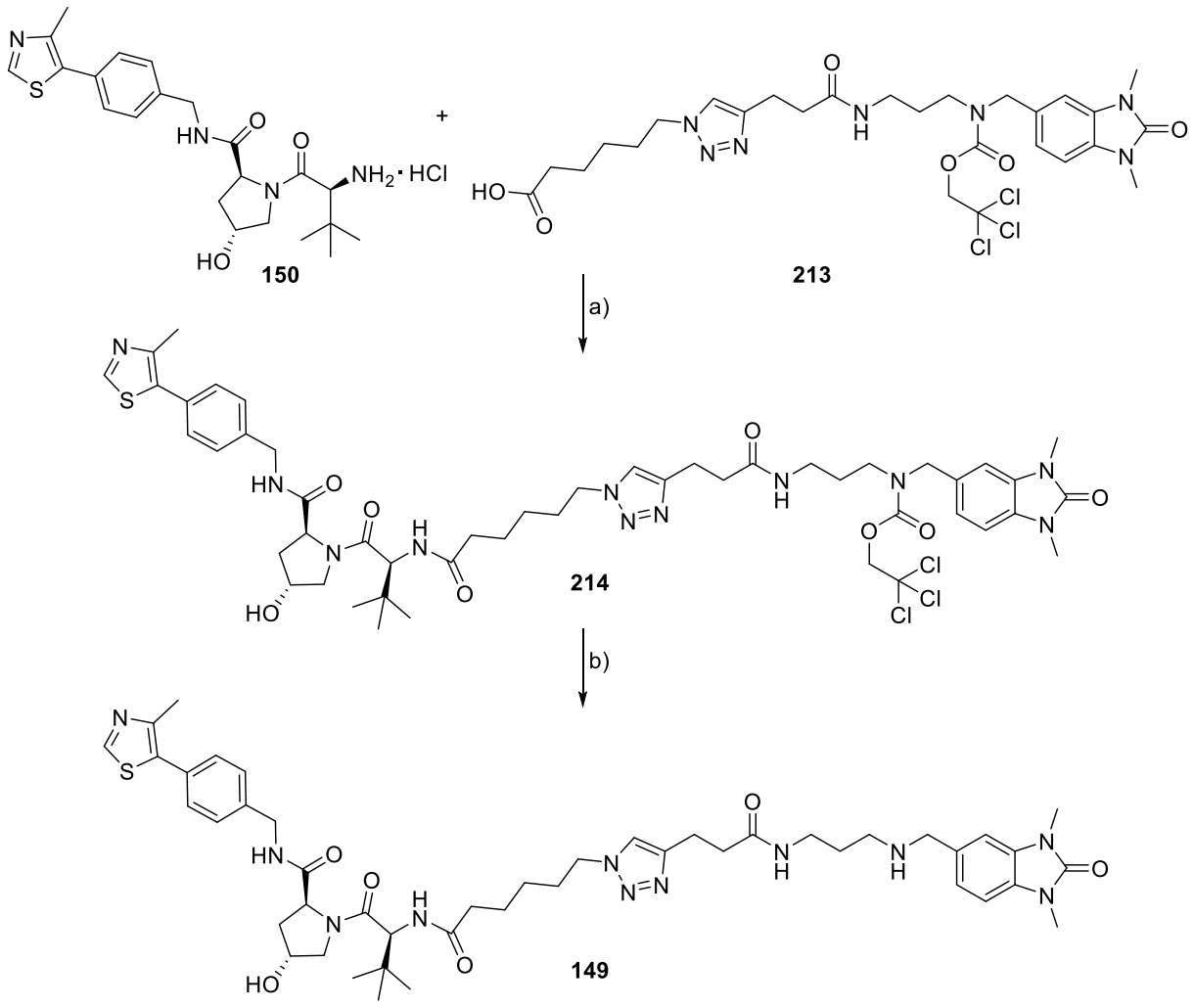
Scheme 3.20. Amide coupling to give amide **210**. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 22 h, 73–98%, n = 2.

The next step required an azide-containing compound to form the triazole with alkyne **210** and complete the linker through a click chemistry reaction. Azide **212** was synthesised with 90–100% yields using an S_N2 reaction of bromide **211** with sodium azide, following a literature procedure (**Scheme 3.21**).[167] **212** was then subjected to the copper-catalysed click reaction conditions with alkyne **210** to give the desired 1,4-triazole **213**. Synthesis of the 1,5-triazole regioisomer was attempted using Cp*RuCl(PPh_3)$_2$ as a catalyst, as this would lock the PROTAC in a different conformation and produce a different ternary complex. However, this reaction only returned starting material after three days, with no new product observed by TLC, hence this idea was not pursued further.



Scheme 3.21. Synthesis of triazole **213**. *Reagents and conditions:* a) NaN_3, DMSO, 80 ºC, 16 h, 90–100%, n = 2; b) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O, ^tBuOH, 50 ºC, 2 h, 59%, n = 1.

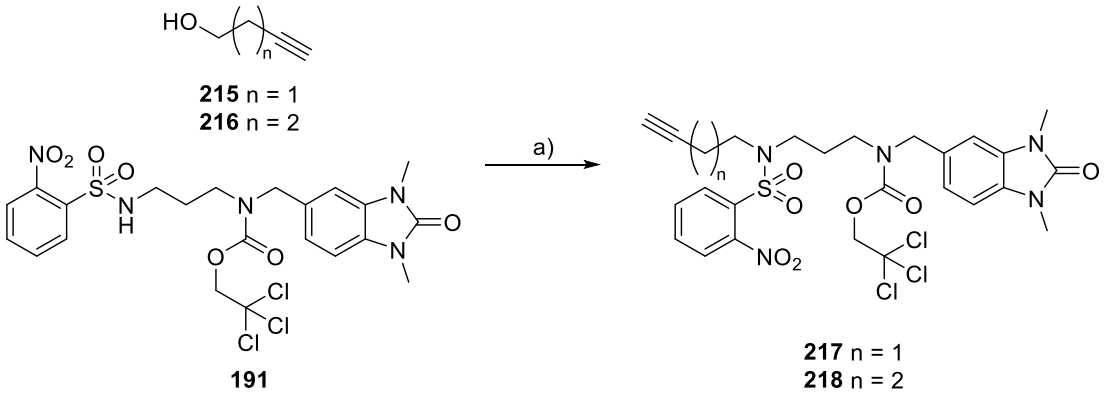
The penultimate step of the synthesis was to couple acid **213** with VHL ligand **150** to give amide **214** (**Scheme 3.22**), which proceeded with low yields of 15–28%, with a large amount of starting material remaining even after 22 hours. The final step involved a Troc deprotection using zinc(0) and acetic acid, which afforded final amide-linked product **149** in moderate yields as some starting material still remained. Neither of these reactions were further optimised, as only a small amount of material was required for the biological assays.



Scheme 3.22. Final steps in the synthesis of PROTAC **149**. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 22 h, 15–28%, n = 2; b) Zn, AcOH, THF, rt, 16 h, 40%, n = 1.

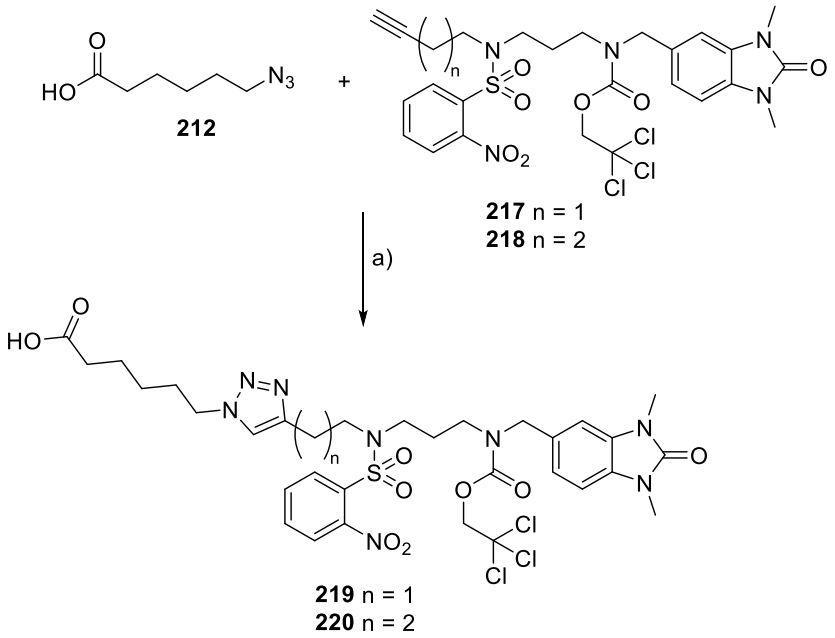
3.4.6.2 Synthesis of Amine-Linked Triazole PROTACs

Synthesis of the amine-linked triazole PROTAC molecules began with the same Fukuyama amine synthesis as the amine-linked PEG PROTACs, with the key step being the Mitsunobu reaction between Nosyl-protected TRIM33 ligand **191** and an alcohol. In this case, the alcohol reacting partners were either but-3-yn-1-ol **215** or pent-4-yn-1-ol **216** (**Scheme 3.23**). These reactions proceeded with moderate yields of 51% and 52% to give compounds **217** and **218**, respectively, which contained the alkyne functional handle.



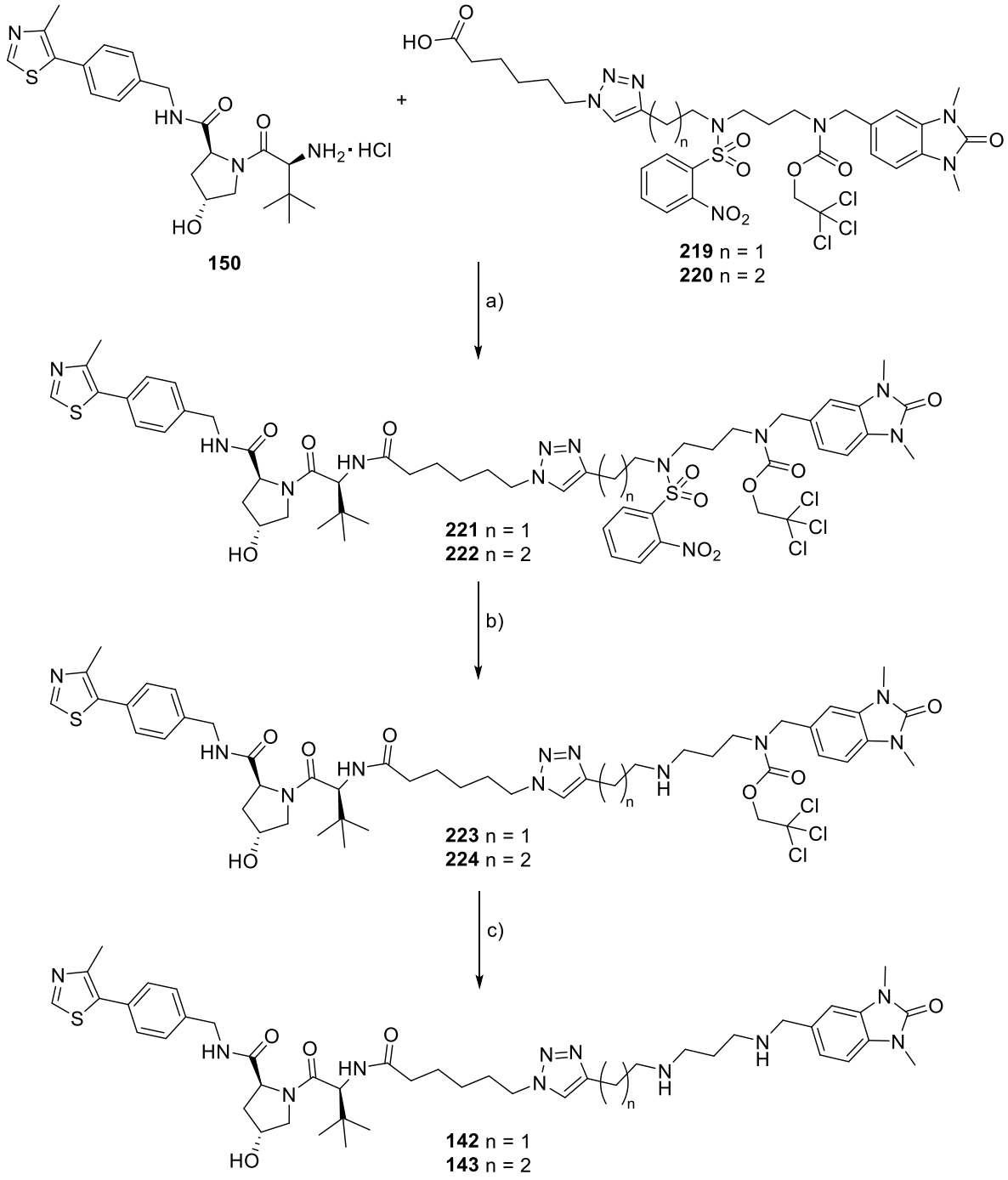
Scheme 3.23. Mitsunobu reaction between Nosyl-protected amine **191** and selected alcohols. *Reagents and conditions:* a) PPh_3, DIAD, THF, rt, 16 h, **217**: 51%, n = 1, **218**: 52%, n = 1.

The next steps were click chemistry reactions between alkynes **217** and **218**, and azide **212**, which afforded desired 1,4-triazoles **219** and **220** in 54% and 58% yields, respectively (**Scheme 3.24**).



Scheme 3.24. Click chemistry reaction between alkynes **217** and **218**, and azide **212**. *Reagents and conditions:* a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O, ^tBuOH, 50 °C, 3 h, **219**: 54% n = 1, **220**: 58%, n = 1.

Subsequently, HATU-mediated coupling between VHL ligand **150** and acids **219** and **220** yielded amides **221** and **222** (**Scheme 3.25**). This was followed by a Nosyl-deprotection step using thiophenol as a nucleophile for the S_NAr reaction. In both cases, all starting material was consumed but only moderate yields of **223** and **224** were seen, which could be attributed to difficult purification. The final step was a Troc group removal using zinc(0) and acetic acid, which gave the final amine-linked triazole PROTAC molecules **142** and **143**.

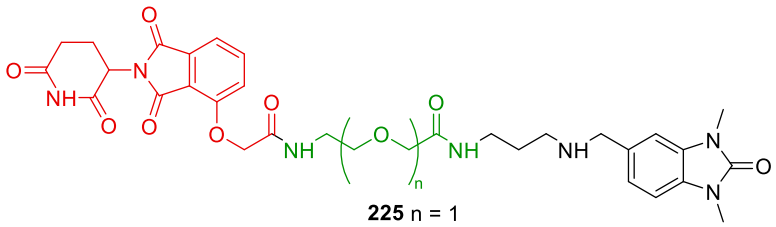


Scheme 3.25. Final steps towards amine-linked triazole PROTACs **142** and **143**. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 22 h, **221**: 75% n = 1, **222**: 49%, n = 1; b) PhSH, K_2CO_3, DMF, rt, 2–5 h, **223**: 56% n = 1, **224**: 54%, n = 1; c) Zn, AcOH, THF, rt, 16 h, **142**: 44% n = 1, **143**: 68%, n = 1.

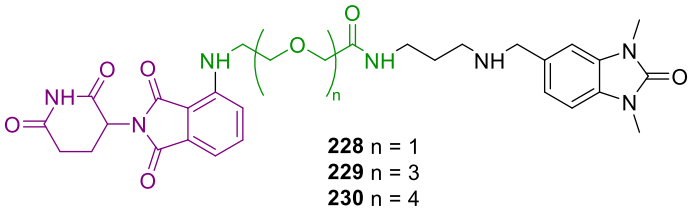
3.5 Cereblon-Recruiting PROTACs

Dr Amy Scorah[138] synthesised six cereblon-recruiting PROTAC molecules, incorporating the same TRIM33 ligand as for the VHL-recruiting PROTACs, with PEG linkers to attach two different cereblon ligands. A summary of all PROTACs synthesised is shown in **Figure 3.8**.

Cereblon-recruiting PROTACs

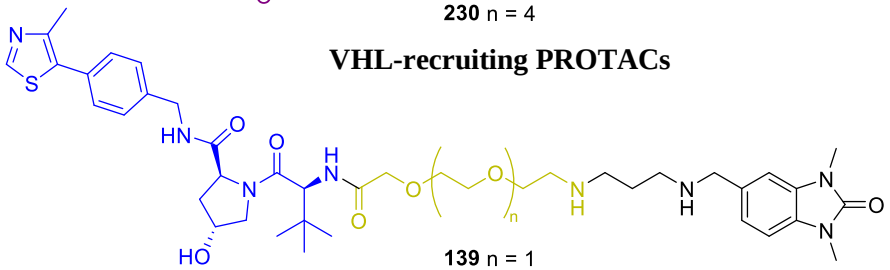


225 n = 1
226 n = 2
227 n = 4

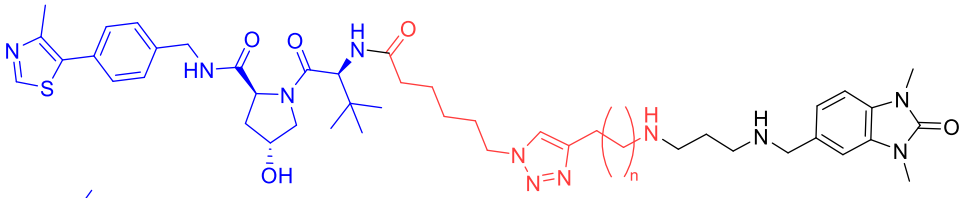


228 n = 1
229 n = 3
230 n = 4

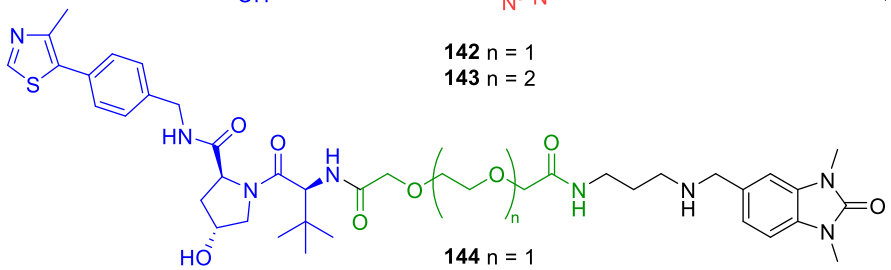
VHL-recruiting PROTACs



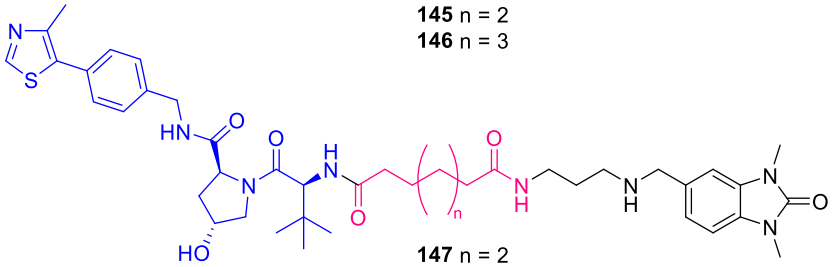
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140 n = 2
141 n = 3



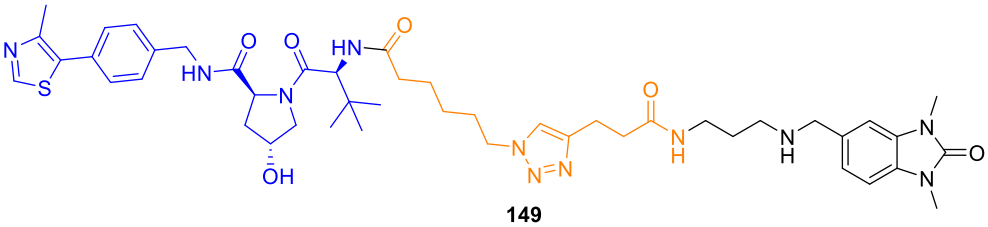
142 n = 1
143 n = 2



144 n = 1
145 n = 2
146 n = 3



147 n = 2
148 n = 5



149

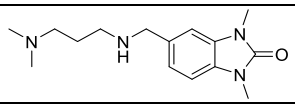
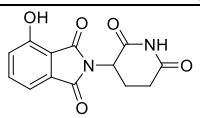
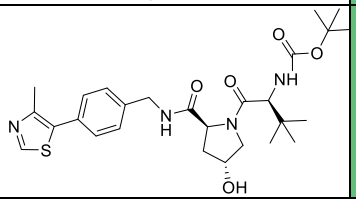
Figure 3.8. Summary of the 17 PROTACs synthesised, including six cereblon-recruiting PROTAC molecules synthesised by Dr Amy Scorah.[138] The varied E3 ligase ligands and linkers are highlighted.

3.6 Evaluation of E3 Ligase Ligands Against TRIM33

Before determining the degradation profiles of the PROTACs synthesised, it was important to first establish that the E3 ubiquitin ligase ligands do not bind to TRIM33, as this could reduce the degradation potential of the PROTAC. Both the VHL and cereblon ligands have potential acetyl-lysine mimicking moieties within their structures, so a dose-response AlphaScreen™ was performed to determine no binding to the TRIM33 bromodomain is observed (**Table 3.2**, all nine protein-peptide combinations are shown in Appendix G). TRIM33 ligand **25** was used as a positive control. The assay showed that neither cereblon ligand **231** nor VHL ligand **158** bind to the TRIM33 bromodomain, indicating that any affinity of the PROTACs for TRIM33 observed will be occurring through the TRIM33 ligand. An assay to determine the affinity of **158** and **231** for proteins VHL and cereblon, respectively, was not performed, as these compounds have been assessed on multiple occasions in the literature.[98,100,101]

Table 3.2. A table showing IC_{50} values for the VHL ligand **158** and cereblon ligand **231** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green. Results shown are protein with $H3_{1-27}K18Ac$ peptide. *These IC_{50} values are the mean and standard deviation of multiple repeats.

Weaker binding — **Stronger binding**

Compound	Structure	TRIM24 BRD IC_{50} (µM)	TRIM33α BRD IC_{50} (µM)	TRIM33β BRD IC_{50} (µM)
25		>250	3.61* ± 0.352	13.7* ± 1.53
231		>250	>250	>250
158		>250	>250	>250

3.7 Evaluation of PROTACs

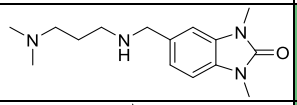
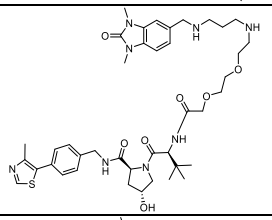
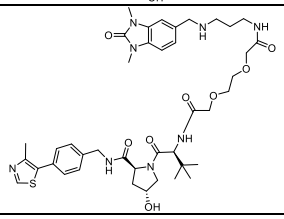
The next important step before assessing degradation of PROTAC molecules was to determine that the PROTAC binds to the target protein, to ensure that the addition of linkers and E3 ligase ligands have not significantly affected affinity.

3.7.1 AlphaScreen™

To assess the affinity of PROTACs **139**–**149** and **225**–**230** for the TRIM33 bromodomain, dose-response AlphaScreen™ assays were performed. This had to be done over several experiments, as only seven compounds including the control can be tested at once. The first AlphaScreen™ indicated VHL-recruiting PROTACs **139** and **144** still had low micromolar affinity for TRIM33 (**Table 3.3**).

Table 3.3. A table showing IC_{50} values for PROTACs **139** and **144** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green. Results shown are protein with $H3_{1-27}$K18Ac peptide. *These IC_{50} values are the mean and standard deviation of multiple repeats.

Weaker binding — **Stronger binding**

Compound	Structure	TRIM24 BRD IC50 (µM)	TRIM33α BRD IC_{50} (µM)	TRIM33β BRD IC_{50} (µM)
25		>250	3.61* ± 0.352	13.7* ± 1.53
139		>250	0.913 ± 0.179	6.15 ± 1.28
144		>250	2.81 ± 0.452	6.26 ± 1.75

However, after performing the AlphaScreen™ on the remaining PROTACs **140–143**, **145–149**, and **225–230**, it appeared there was no binding to TRIM33, including control compound **25**. Several repeats of these experiments displayed the same result. It was considered that the problem

may be the detector, as the emissions observed were much higher than routinely seen, but after using an alternate plate reader, still no binding of the control and PROTACs were observed. A signal-response experiment was performed to determine if the correct concentrations of protein and peptide were being used, but the experiment showed the assay conditions were correct. Unable to find fault with the assay, an AlphaScreen™ TruHits experiment was performed to determine if there was assay interference occurring with the PROTAC molecules. As described in **Chapter 1.6.1.2**, the assay is used to identify compounds that produce false readouts through various mechanisms. The streptavidin coated donor beads and the biotinylated acceptor beads were preincubated, followed by dose-dependent addition of the PROTAC molecules. Unfortunately, interference was detected for all PROTAC compounds compared to the DMSO control (data table shown in Appendix H). This was particularly notable for the VHL-recruiting PROTACs, which indicates that these compounds act as singlet oxygen quenchers or inner filters. However, this does not explain the lack of binding seen in control compound **25**. It was concluded that an alternative method was needed to determine the affinities of these PROTACs for the TRIM33 bromodomain.

3.7.2 Isothermal Titration Calorimetry

An alternative assay to determine affinity of the PROTACs for TRIM33 was ITC. This method offers many advantages over AlphaScreen™, as it measures thermodynamic parameters of the binding event, therefore is not dependent on displacement. Additionally, the assay is not based on fluorescence, so the likelihood of interference by the PROTAC molecules is lower. Since the TRIM33 ligands have low micromolar affinity, high concentrations and volumes of protein are required to accurately determine a K_D value for these compounds. Due to time and protein constraints, only **139**, **148**, and **229** of the 17 PROTAC molecules were evaluated with TRIM33β (**Figure 3.9**). Amine-linked PEG PROTAC **139** displayed a higher affinity than the parent TRIM33 ligand **25**, with a K_D value of 1.76 ± 0.846 μM compared to 18.6 ± 4.54 μM for **25**. This could be attributed to interactions of the linker with the surface of the protein. Amide-linked alkyl PROTAC **148** also showed a higher affinity than control **25**, with a K_D value of 4.19 ± 1.36 μM.

As predicted, the amine-linked PROTAC **139** had a higher affinity for TRIM33β than the amide-linked PROTAC **148**, which was consistent with observations from the SAR studies (**Chapter 2**). The final points on each binding isotherm appear to be anomalous, which could be due to precipitation of the complex as the protein concentration is increased,[168] so these data were omitted from the fitted function.

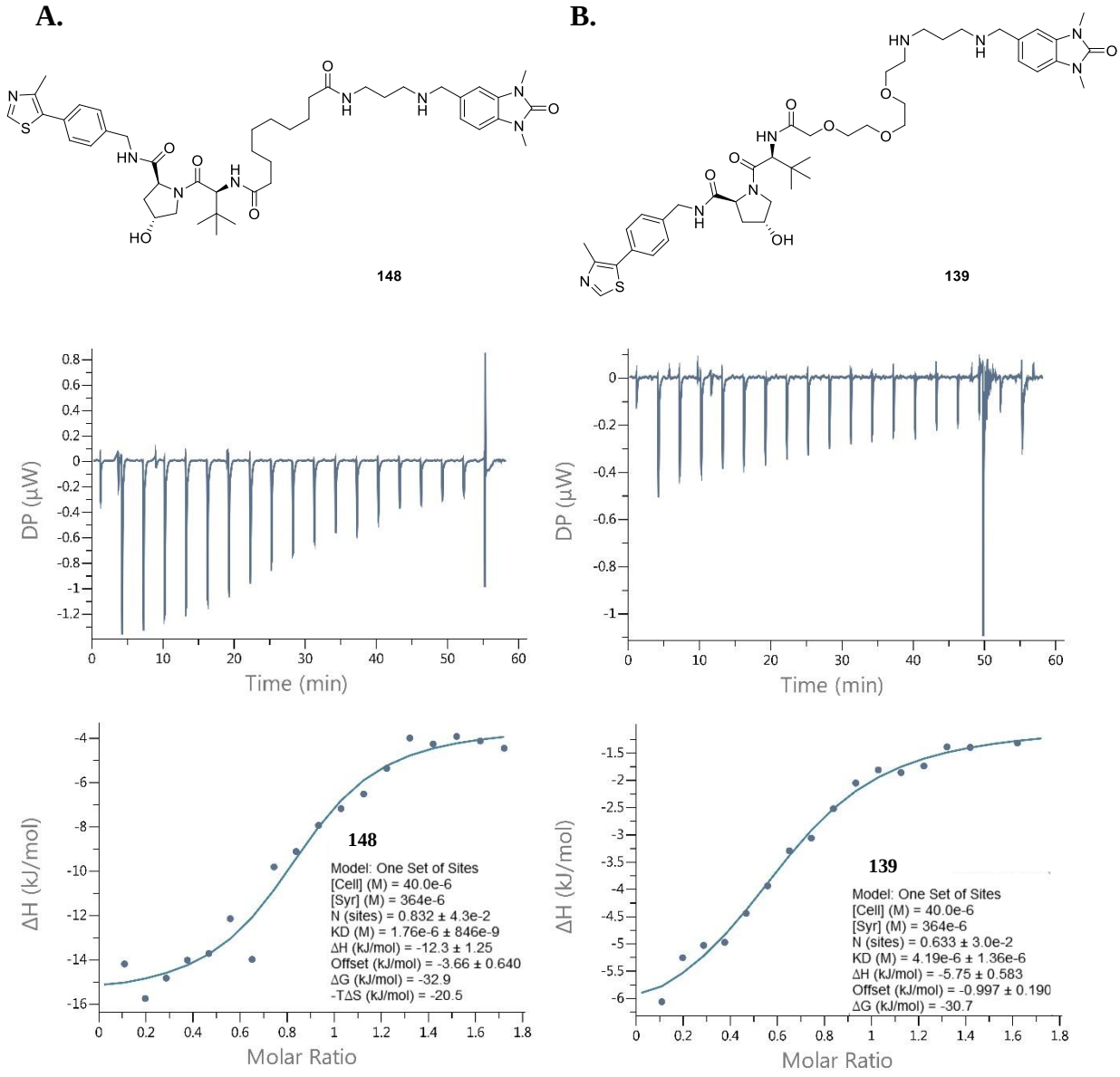


Figure 3.9. A. ITC trace for PROTAC **148**, n = 1. **B.** ITC trace for PROTAC **139**, n = 1. The readout shows the power input for each injection, above, and the normalised binding isotherms, below, including the fitted function which is indicated by the solid line. Anomalous points have been omitted from the fitted function.

ITC was also performed on cereblon-recruiting PROTAC **229**, but a K_D could not accurately be determined (**Figure 3.10**). The graph readout appeared synonymous with one of an insoluble

compound, rather than a compound which does not bind to the protein, as the trace appears to match the initial control run where compound and protein are not present. Again, due to time and protein constraints, this has yet to be repeated with increased DMSO concentration to aid solubility.

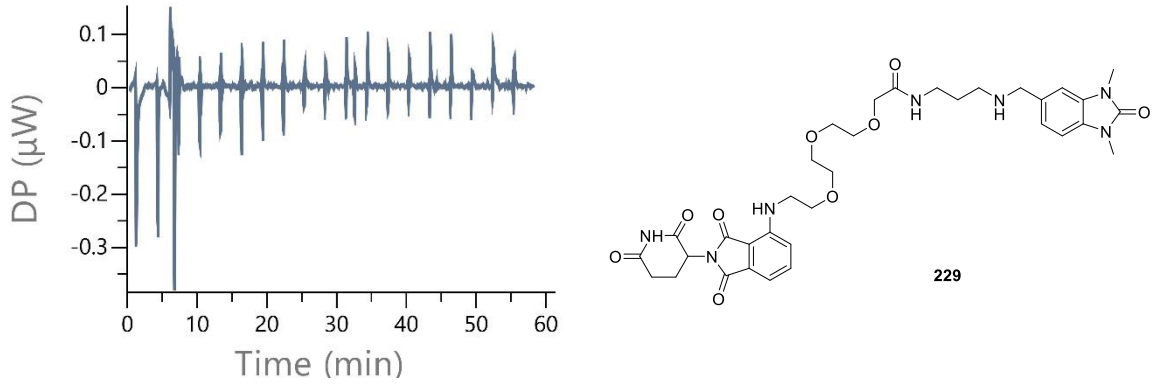


Figure 3.10. ITC trace for cereblon-recruiting PROTAC **229**, n = 1. The readout shows the power input for each injection. A K_D value could not accurately be calculated as there is not a sufficient plateau in the data curve.

Having confirmed that both PEG PROTAC **139** and alkyl PROTAC **148** bind to TRIM33, notably with a higher affinity than the parent TRIM33β ligand, it was decided that the PROTAC molecules should progress to *in vivo* experiments. An assay to determine the affinity of PROTACs **139–149** and **225–230** for VHL and cereblon, respectively, was not performed, as it has been shown in many instances that addition of a linker at the chosen modification points should not significantly reduce affinity.[95,96,104]

3.7.3 Cell Permeability

The next important consideration, before determining the ability of the PROTAC molecules to degrade the target protein, was cell permeability. Understanding uptake of PROTACs in cells is significant for informing the design of future degraders, as it must be ensured that if no degradation is observed, it is a consequence of an insufficient ternary complex and not due to the PROTAC failing to pass through the cell membrane. Experiments to assess cell permeability were performed by Anna Mandel or Dr Hannah Bolland in the group of Professor Ester Hammond. An RKO cell line was treated with each of the 17 PROTACs individually, at a concentration of

10 μM, and incubated for 24 hours. The cells were then lysed, and the lysate was analysed using HPLC to qualitatively determine if the PROTAC was present inside the cell.

Disappointingly, it seemed all 17 PROTAC molecules had very low cell permeability, with only PEG-linked **141**, alkyl-linked **148** and triazole-linked **143** appearing to be present in the cell at low concentrations (shown in orange in **Figure 3.11**, HPLC traces are shown in Appendix I). Permeability can be affected by many factors, including molecular weight, topological polar surface area (TPSA), LogD, intramolecular hydrogen bonding, solubility and compound flexibility. As PROTACs are typically limited to large molecular weights and are relatively flexible by nature, LogD and TPSA values of the PROTAC molecules were considered. In a recent study by Edmondson *et al.*, *in silico* metrics of 38 effective PROTACs recruiting different E3 ligases were collected, which showed an average TPSA of 220 $Å^2$ for cereblon-recruiting PROTACs and 211 $Å^2$ for VHL-recruiting PROTACs,[169] falling outside the classical range for predicted permeability. In contrast, the data showed an average cLogD of 3.2 for cereblon-recruiting PROTACs and 4.2 for VHL-recruiting PROTACs, both of which fall into 'drug-like' space. Experimental LogD determination of **139**, **144**, and **148** at pH 7.4 was performed by Deeyen Karsanji at Vertex Pharmaceuticals UK (Abingdon), and the cLogD and TPSA values of **140–143**, **145–147**, **149**, and **225–230** were predicted using ACD/I-Lab 2.0 (**Figure 3.11**).

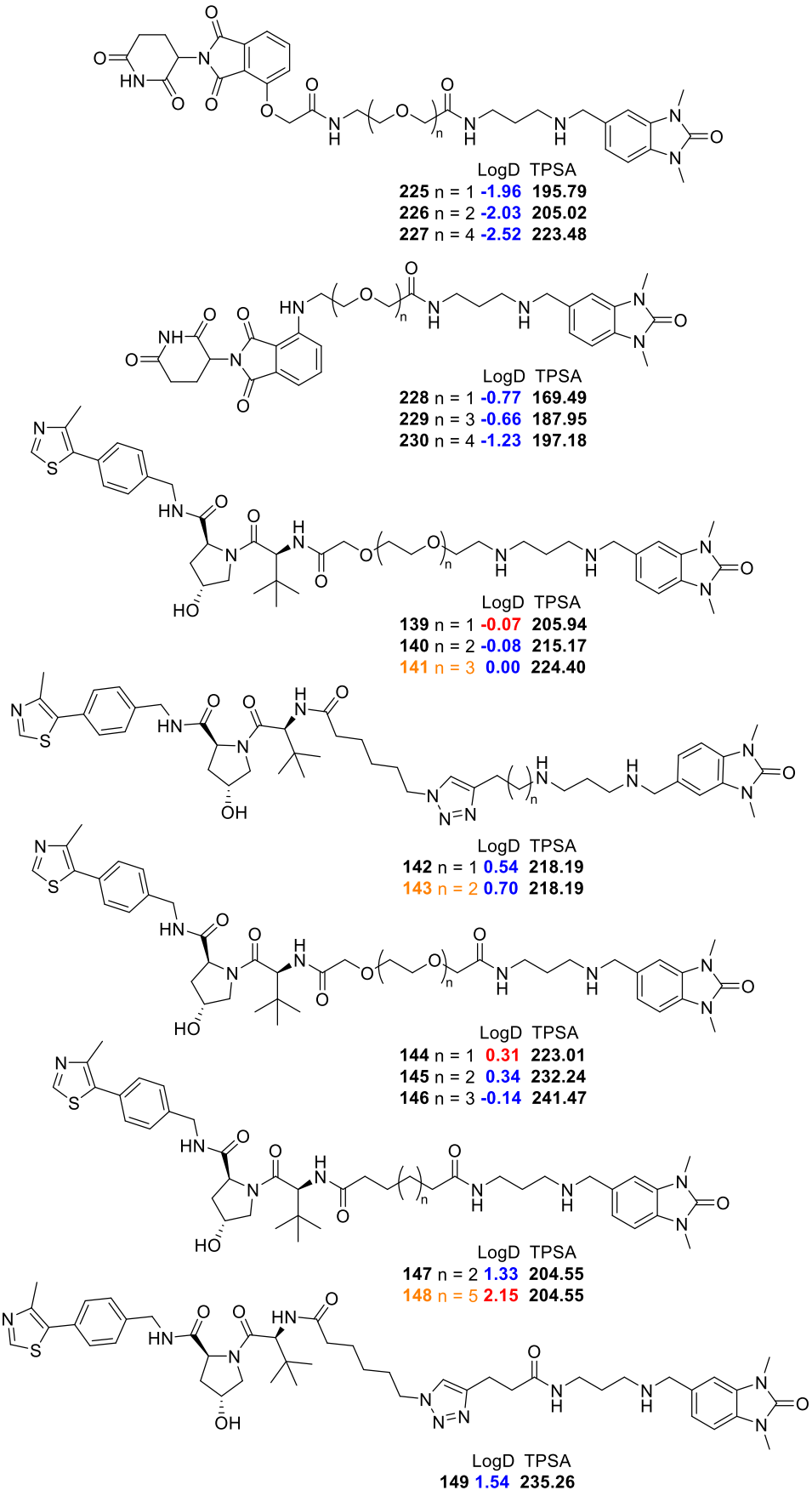


Figure 3.11. Summary of cell permeability results. PROTACs in orange were present at low concentrations in the cell, with all others failing to pass the cell membrane. cLogD values for pH 7.4 in blue and TPSA were predicted using ACD/I-Lab 2.0, LogD values in red were measured experimentally at pH 7.4.

TPSA values for cereblon-recruiting PROTACs **225–230** range from 169–223 Å^2, which, despite being much higher than expected for cell permeable molecules, appears to be below the average 220 Å^2 observed for effective cereblon-recruiting PROTACs. The TPSA range for VHL-recruiting PROTACs **139–149** is higher still, at 204–241 Å^2, presumably due to the increased number of polar atoms within the VHL ligand compared to the cereblon ligands. Again, these values are comparable to the average TPSA of effective VHL-recruiting PROTACs.[169] There does not appear to be a correlation between the TPSA and the membrane permeance of the PROTACs, with **148**, **143**, and **141** having TPSA values of 204, 218, and 224, respectively. TPSA is calculated based on contribution of polar atoms within fragments, therefore does not account for the overall conformation of PROTAC, which may have a lower polar surface area due to the specific 3D structure or internal hydrogen bonding. Since multiple effective PROTACs with an average TPSA values of 211 – 220 Å^2 have been reported,[169] this metric does not seem to be a useful indicator of permeability for PROTAC molecules. This has previously been noted by Gütschow[170] and Crews.[118]

cLogD values for these PROTACs are lower than the typically desired range for cell permeable molecules, and are significantly less than the average cLogD of the reported effective PROTACs,[169] which can be partly attributed to the significant number of polar atoms. Cereblon-recruiting PROTACs **225**–**230** all appeared to have cLogD values below 0, with **227** having a predicted value of -2.52, thus corroborating the observed lack of permeability. The highest value was +2.15 for alkyl-linked **148**, which also appeared to be the most cell permeable from the HPLC lysate data (Appendix I). PEG-linked **141** has a cLogD value of 0.00 but appeared to be present in cells, albeit in low concentrations, whereas compounds such as triazole-linked **149** and alkyl-linked **147** with higher cLogD values were not cell permeable. However, as with TPSA, predicted LogD values do not take into account the conformation of the PROTAC as a whole, and any internal hydrogen bonds that may mask polarity and thus increase permeability. For this reason, caution must be taken not to overinterpret predicted values. Moreover, these observations illustrate that many factors contribute to membrane permeability, not purely TPSA and LogD, but

a general trend of cLogD values can be a useful indicator of the likelihood of permeability, whereas no discernible correlation is observed for TPSA.

3.7.4 Cell Permeability of TRIM33 Ligands

As the majority of the PROTAC series were not membrane permeant, it was important to determine if the parent TRIM33 ligands were cell permeable. An RKO cell line was individually treated with compounds **25** and **121** (**Figure 3.12**), incubated for 24 hours, and subsequently lysed (performed by Anna Mandel in the group of Professor Ester Hammond). The cell lysate was analysed using HPLC to determine if ligands **25** and **121** were present inside the cell (HPLC traces shown in Appendix I). This was observed to be the case, with both **25** and **121** appearing to be in high concentrations. This was an encouraging result, as it indicated that the problem was not the TRIM33 ligand but the high molecular weight and number of hydrogen bond donors and acceptors within the PROTAC. Increasing the lipophilicity and masking non-essential polarity within the PROTAC molecules could therefore aid the cell permeability.

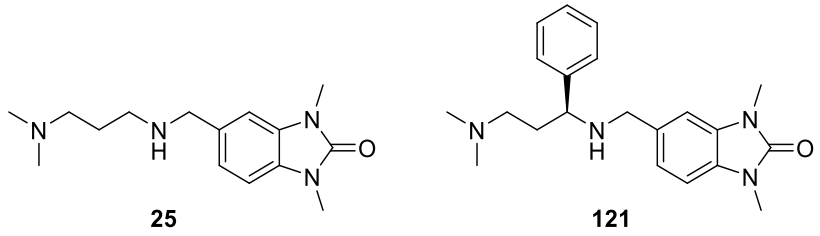


Figure 3.12. Structure of the two TRIM33 ligands **25** and **121** that were confirmed to be cell permeable.

3.7.5 Degradation Assessment by Western Blot

Despite the low permeability observed, it was decided that degradation would still be assessed for PROTACs **141**, **143**, and **148**. RKO cells were treated individually with **141**, **143**, and **148** at 10 μM, and were incubated for 0, 2, 4, 6, 8, 16 or 24 hours, before harvesting at the indicated time points (performed by Dr Hannah Bolland or Anna Mandel in the group of Professor Ester Hammond). Western blots were performed to determine if the PROTACs had an effect on the concentration of TRIM33 in the cell. Incubation at 0 h acted as a control for the normal TRIM33 expression levels in the RKO cell line. β-Actin was used as the loading control to confirm that the samples have been loaded equally into each well. This ensures that any variation in levels of

the protein of interest in different samples is not due to loading variance, but due to expression level differences.[171] For Western blots performed by Dr Hannah Bolland, protein expression levels were quantified using the ImageStudio (LICOR) software, and was normalised relative to the β-actin loading control and expressed as relative fold change over 0 h time points.

On treatment of the cells with PEG-linked PROTAC **141**, a decrease in TRIM33 concentration was observed from 2 to 16 hours relative to t = 0 (**Figure 3.13**). However, there did not seem to be a time-dependence to the reduction, and a fluctuation in TRIM33 concentration was seen after 4 hours of PROTAC exposure. Therefore, from this data, it is difficult to determine if the reduction in concentration of TRIM33 is a consequence of PROTAC-mediated degradation, or if the protein levels are fluctuating in the cell independently of **141**. This experiment was only performed once, thus the assessment should be repeated to establish if PROTAC-dependent degradation is occurring.

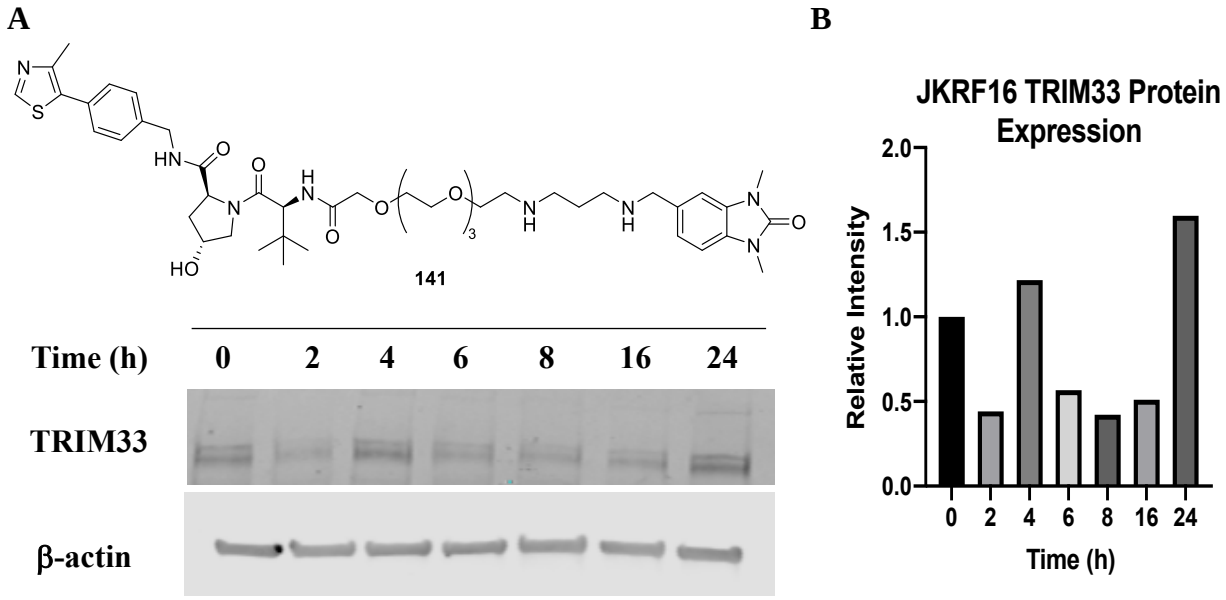
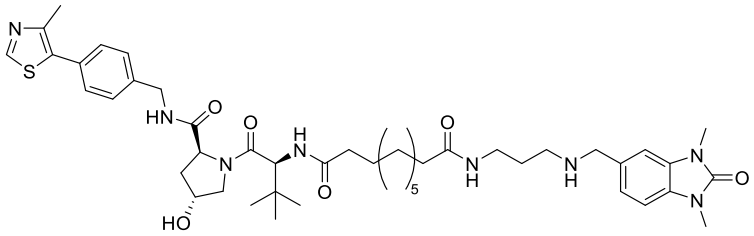


Figure 3.13. A. Time-dependent Western blot showing the concentration of TRIM33 when RKO cells were treated with **141**. Experiment performed by Dr Hannah Bolland, n = 1. **B.** Quantification of degradation relative to t = 0.

Alkyl-linked PROTAC **148** was also assessed. There appeared to be a slight decrease in the concentration of TRIM33 after 4 hours (**Figure 3.14**), although this completely recovered after 8 hours. At 16 and 24 hours, it appeared that the level of TRIM33 was above the normal cellular level observed at t = 0. This could be a consequence of a natural cell cycle or an outside influence increasing the TRIM33 levels independent of **148**. Alternatively, it was hypothesised that the

observed increase could be due to the PROTAC **148** binding to the TRIM33 bromodomain, inhibiting its function and thus causing a feedback mechanism in which cells overexpresses TRIM33, which is a common mechanism for drug resistance.[172] This suggested that some target engagement of the PROTAC with TRIM33 was occurring. Without quantification data, it is difficult to assess the relative levels of degradation, thus the experiment should be repeated to quantify the results. Despite this, the PROTAC does not appear to be significantly degrading TRIM33 in a time-dependent manner.



148

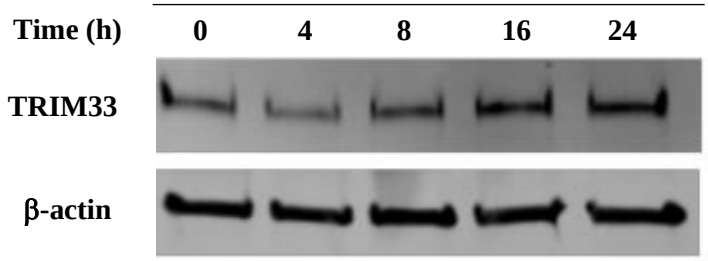
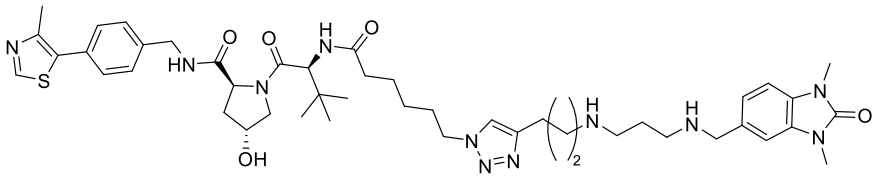


Figure 3.14. Time-dependent Western blot showing the concentration of TRIM33 when RKO cells were treated with **148**. Experiment performed by Anna Mandel, n = 1. No quantification was performed.

Finally, RKO cells were treated with triazole-linked PROTAC **143**. However, an increase in TRIM33 concentration was observed from 4 to 24 hours relative to t = 0 (**Figure 3.15**). Again, this could be a consequence of inhibition of the TRIM33 bromodomain leading to increased expression within the cell. If this is the case, the observed increase suggested that although binary complexes between **143** and TRIM33 are forming, productive ternary complexes with VHL allowing ubiquitination and subsequent degradation are not occurring.



143

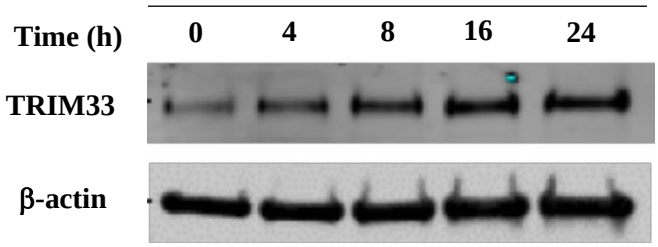


Figure 3.15. Time-dependent Western blot showing the concentration of TRIM33 when RKO cells were treated with **143**. Experiment performed by Anna Mandel, n = 1. No quantification was performed.

The lack of time-dependent degradation observed for **141**, **143** and **148** could be due to the linker length and composition not being optimal for the ternary complexes to produce a suitable orientation between VHL and TRIM33 for effective ubiquitination. Alternatively, there may be insufficient formation of ternary complexes due to very low cellular concentrations of the PROTACs. It was therefore hypothesised that synthesising a series of PROTACs with higher cLogD values may help improve cell permeability, thus increasing the PROTAC cellular concentration, and therefore increasing the likelihood of complete and sustained degradation of TRIM33. These molecules will be designed to mask any non-essential polarity, including the terminal amine of the TRIM33 ligand, and will contain alkyl and rigid linkers to reduce the flexibility of the PROTACs. Unfortunately, molecular weight cannot be reduced, so focus will remain on increasing lipophilicity.

3.8 Summary and Conclusions

In conclusion, 17 potential degraders of TRIM33 were synthesised. To increase the chance of stable ternary complex formation and subsequent degradation, a range of PROTACs were designed, varying linker lengths and compositions, attachment strategies and recruited E3 ligase.

Due to the size and nature of these molecules, syntheses were not trivial, but routes that yielded sufficient quantities of each PROTAC for biological testing were developed. The key step in the

synthesis of the amine-linked PEG PROTACs was a Mitsunobu reaction with a Nosyl-protected amine, which allowed attachment of the linkers to the TRIM33 ligand. Click chemistry was also employed to synthesise three PROTACs sampling conformational space through a triazole moiety. Alkyl-linked and amide-linked PEG PROTACs were synthesised through amide couplings. The protecting group strategy for all of these compounds had to be carefully considered to ensure the PROTACs could be deprotected without affecting other functional groups within the molecule.

A dose-response AlphaScreen™ was performed which confirmed that potential acetyl-lysine mimics VHL ligand **158** and cereblon ligand **231** do not bind to TRIM33. This result established that any affinity for TRIM33 observed from the PROTAC molecules would be a result of the benzimidazolone-containing TRIM33 ligand. Isothermal titration calorimetry was used to determine K_D values, but due to time and protein constraints, only three PROTACs were evaluated. Two VHL-recruiting PROTACs **139** and **148** showed a higher affinity for TRIM33β than the parent ligand, which was thought to be a contribution of the linkers interacting with the surface of the protein. Cereblon-recruiting ligand **229** appeared to be insoluble. Future work should include performing the ITC experiments and repeats on all 17 PROTACs, and repeating the cereblon-recruiting PROTAC **229** experiment at a higher DMSO concentration.

Once binding to TRIM33 was confirmed, cell permeability was investigated by treating an RKO cell line with the PROTACs for 24 hours, using HPLC to establish if the PROTACs had passed through the cell membrane. However, it was found that the series of PROTACs had very low permeability, with only three PROTACs appearing to be in the cell at low concentrations. Many factors affect cell permeability, including molecular weight, flexibility and LogD values. The experimental and calculated LogD values indicated that these molecules were falling outside the range of ideal LogD for cell permeability, and were much lower than the average cLogD values of previous effective PROTAC molecules. However, the TRIM33 parent ligands **25** and **121** were observed to be cell permeable, illustrating that the high molecular weight and addition of multiple

hydrogen bond donors and acceptors within the E3 ligase ligands could be hindering membrane permeability.

Degradation assessment of the moderately cell permeable PROTACs **141**, **143**, and **148** was performed. Of the three PROTACs assessed, only PEG-linked **141** showed a significant reduction in TRIM33 concentration. However, it was not clear whether this was due to PROTAC-mediated degradation as no time-dependence was observed and concentration fluctuations were seen. This result should be repeated to allow significant interpretation. It was hypothesised that TRIM33 was not degraded by these PROTACs either because ubiquitination was not occurring due to non-optimal ternary complex formation, or because of low cellular concentrations of the PROTACs minimising significant ternary complex formation. Therefore, it was decided that a new series of PROTACs should be designed, which would mask all non-essential polarity and hydrogen bond donors, and focus on alkyl and more rigid linkers in order to increase cell permeability to improve the likelihood of complete and sustained degradation.

Chapter 4 Second generation of TRIM33 Proteolysis-Targeting Chimeras (PROTACs)

4.1 Introduction and Aims

Very low cell permeabilities were observed for the 17 PROTACs described in **Chapter 3**. Only three PROTACs **141**, **143**, and **148**, were observed to be in the cell, and at low concentrations. Assessment of the ability of these PROTACs to degrade TRIM33 showed no significant or confirmed degradation up to 24 hours after treatment with each PROTAC. It was hypothesised that this was a consequence of either insufficient ternary complexes or low cellular concentrations of the PROTACs minimising ternary complex formation. Therefore, the work described in this chapter aimed to design and synthesise a second series of TRIM33-targeting PROTAC molecules that have higher cell permeability, to increase the concentration of the PROTACs in cells, and improving the chance of complete and sustained degradation of TRIM33.

4.2 Design of PROTACs

4.2.1 Increasing Cell Permeability

The structure of the original PROTAC series (**Chapter 3**) was considered in the design of the second series of PROTACs (**Figure 4.1**). Alterations could not be made to the VHL or cereblon ligands, as this may affect their affinity for the respective E3 ligases. However, changes to the linker could increase the lipophilicity of the PROTACs, and the extensive SAR studies performed on TRIM33 bromodomain ligand **25** (**Chapter 2**) have proven that alterations can be tolerated that will mask polarity. These studies showed that the benzylic amine was essential for bromodomain binding through the interaction with E981, therefore this group cannot be protected without reducing affinity. However, an adjacent phenyl moiety increases the hydrophobic nature of the ligand and does not affect the interaction with E981, as in **121**. Methylation at the terminal amine of the TRIM33 ligand is well tolerated, so this was also incorporated into the design. Finally, the linkers were considered. Attachment of the linker to the TRIM33 ligand through an amide bond was selected for synthetic ease and to increase rigidity. To improve cell permeability,

alkyl and rigid linkers would be an appropriate choice, to increase lipophilicity and reduce flexibility. However, rigid linkers are difficult to design without computational modelling to determine the most suitable conformation to lock the PROTAC in. Consequently, this chapter will focus on flexible alkyl linkers.

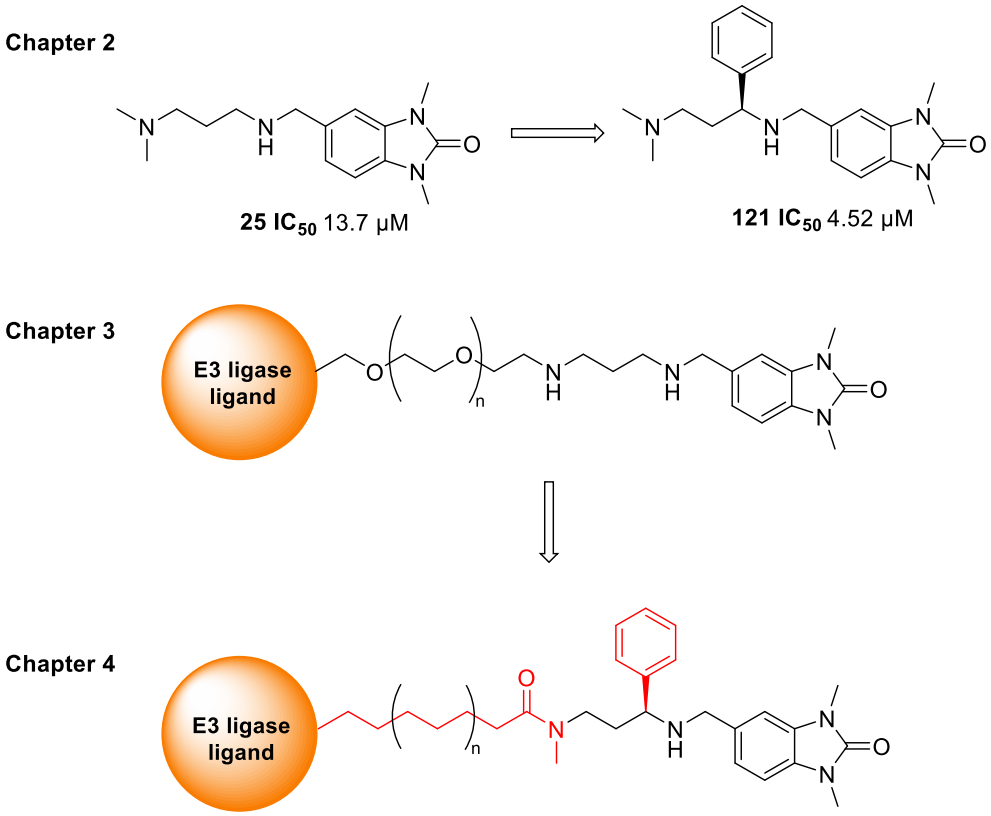


Figure 4.1. Structures of the TRIM33 ligands **25** and **121** (**Chapter 2**), the amine-linked PEG PROTAC molecules (**Chapter 3**) and the structure of the new designed PROTACs (**Chapter 4**). The changes made are highlighted in red.

4.2.2 E3 Ligase Strategy

As with the **Chapter 3** PROTAC design, two E3 ligases, cereblon and VHL, were selected to be recruited by the new PROTAC series. However, previous efforts focused on synthesising PROTAC molecules with a VHL-recruiting ligand that has an amide attachment vector to link to the TRIM33 ligand (Ligand **9**, **Figure 4.2**). It is well documented that the orientation of the recruited E3 ligase is important in determining the degradation profile of PROTACs.[96] Therefore, compounds were designed which also sample a different attachment vector, using a phenyl attachment for linkers (Ligand **10**, **Figure 4.2**) to change the orientation of VHL with respect to

TRIM33 in the ternary complex, offering different surface lysine residues to be ubiquitinated. This could affect the selectivity and viability of the PROTAC series.

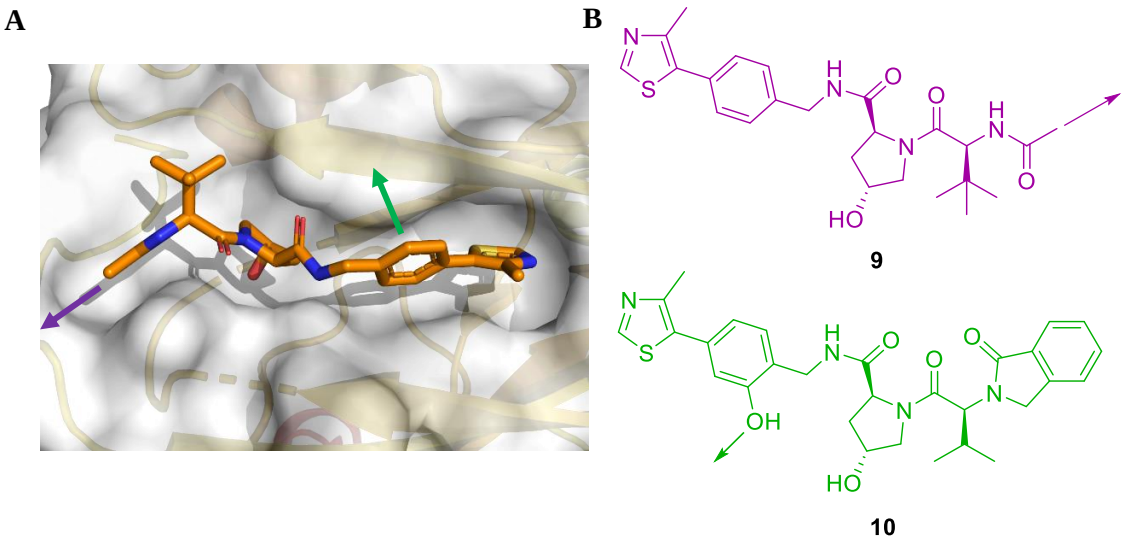


Figure 4.2. A. Crystal structure (PDB ID: **4W9H**) of VHL-recruiting ligand **9** shown in HIF-1α-binding pocket. Two arrows represent linker attachment points for PROTAC design. **B.** The structures of the two VHL-recruiting ligands, with ligand **9** utilising an amide attachment vector (purple) and ligand **10** with a phenyl attachment vector (green).

Therefore, a series of PROTAC molecules were designed which would incorporate all of these considerations (**Figure 4.3**). Despite the stereochemistry of the phenyl group having little effect on affinity, it was decided that the (*S*)-enantiomer should be synthesised to avoid formation of diastereomers once the VHL ligand had been incorporated, as these would be difficult to separate and may cause problems with NMR spectra interpretation. Topological polar surface area (TPSA) and cLogD values at pH 7.4 were predicted for this series using ACD/I-Lab 2.0 (shown in red and blue, respectively, in **Figure 4.3**). As discussed in **Chapter 3**, TPSA did not appear to correlate with the permeability of PROTAC molecules, with many effective PROTACs having TPSAs of greater than 200 Å^2.[169] However, the overall trend showed a reduction in TPSA compared to the previous PROTAC series, largely owing to the lack of polar atoms within the linkers.

The cLogD values for the proposed series are within the typically desired range for cell permeable molecules, with the lowest cLogD at +2.96 for **235**, and are within the scope of the average cLogD values seen in the study of the 38 effective PROTACs.[169] In comparison to the previous series, no

negative cLogD values are observed. However, as these metrics are predicted using computer software, they can only be used as a rough estimate of the permeability properties, and caution must be taken with interpreting specific values.

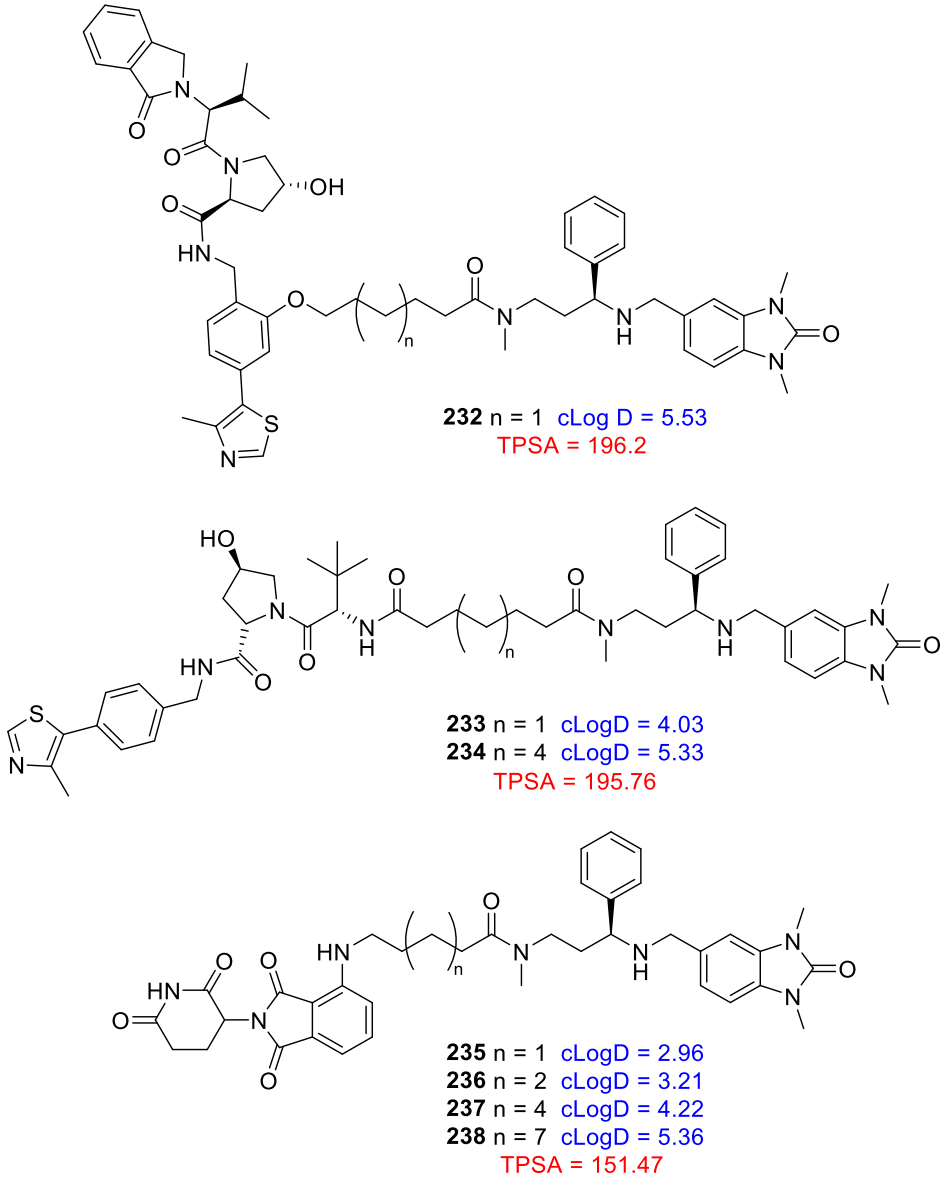
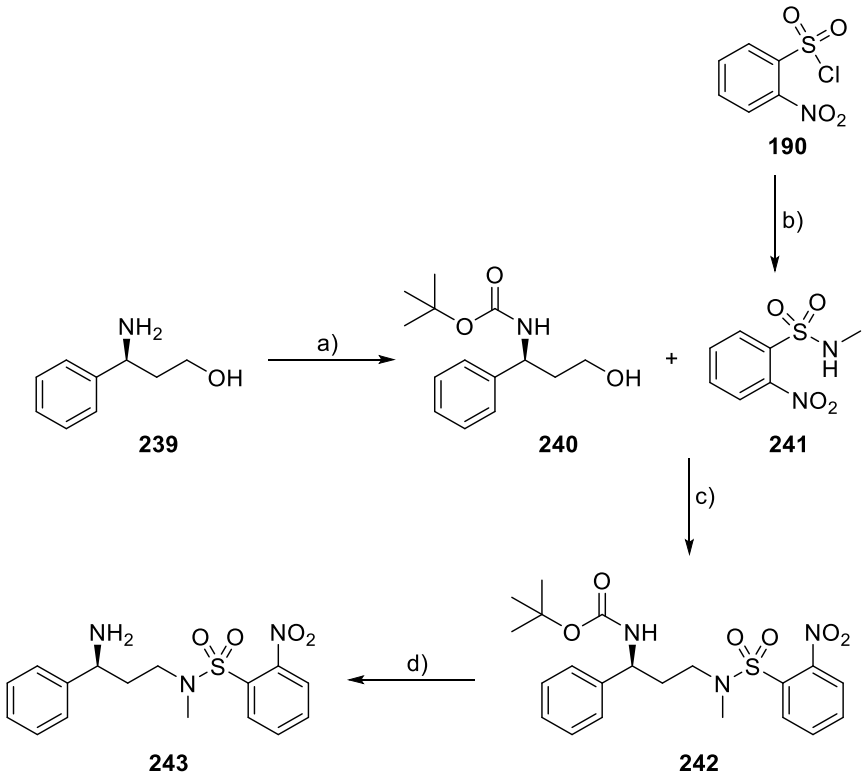


Figure 4.3. Structures of the proposed PROTAC series. Compounds **232–234** are designed to recruit VHL, and **235–238** to recruit cereblon. cLogD values in blue are predicted for pH 7.4 using ACD/I-Lab 2.0, predicted topological polar surface area (TPSA) in Å^2 shown in red for each PROTAC type using ACD/I-Lab 2.0.

4.3 Synthesis of TRIM33 Ligand

Synthesis of the proposed TRIM33 ligand began with a Boc-protection of primary amine (*S*)-3-amino-3-phenylpropan-1-ol **239**, which proceeded in yields of 50–75% (**Scheme 4.1**). The next step required a Mitsunobu coupling partner that could install the Nosyl-protected methyl

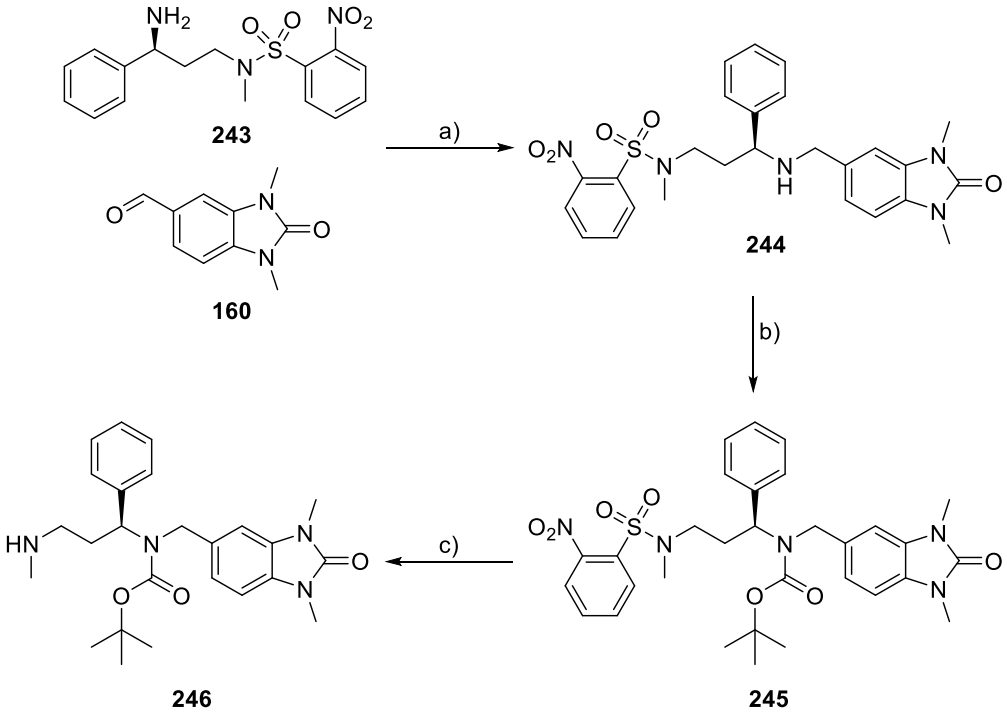
amine moiety. Sulfonamide **241** was synthesised by the reaction of 2-nitrobenzenesulfonyl chloride (**190**) with methylamine, which gave quantitative yields and did not require purification. The Mitsunobu reaction could then be performed, which yielded protected amine chain **242** in yields of 71–82%. **242** was subsequently Boc-deprotected to give the amine **243**.



Scheme 4.1. Synthesis of the amine chain **243**. *Reagents and conditions:* a) Boc_2O, NEt_3, CH_2Cl_2, rt, 1 h, 50–75%, n = 2; b) $MeNH_2$, CH_2Cl_2, rt, 16 h, 100%, n = 2; c) DIAD, PPh_3, THF, rt, 16 h, 71–82%, n = 2; d) 4 M HCl in dioxane, CH_2Cl_2, rt, 1 h, 76–88%, n = 4.

In the next step, a reductive amination was performed. For the first attempt of this reaction, 1 equivalent of aldehyde **160** and 1.5 equivalents of amine **243** were pre-stirred for one hour with acetic acid in DCE to promote imine formation, followed by the addition of sodium triacetoxyborohydride (**Scheme 4.2**). The extra 0.5 equivalents of amine were added to minimise the likelihood of the double reductive amination product forming. Benzylic amine **244** was produced in the reaction, but formed a mixture with starting material amine **243** which could not be separated even after several column chromatography purification steps. The reaction was repeated with 1 equivalent of both aldehyde **160** and amine **243**, but this gave the same inseparable mixture. It was decided that separation would be easier once the benzylic amine was masked using a Boc-protection. The protection was performed, and the product **245** and Boc-protected amine

242 were easily separated using column chromatography. As with the previous PROTAC series, compound **245** required high temperature ^{1}H and ^{13}C NMR spectroscopy to resolve the rotameric doubling of signals. The final step was deprotection of the Nosyl group to reveal the secondary amine, to allow attachment of linkers to the TRIM33 ligand. This deprotection was achieved using thiophenol as a nucleophile for the S_NAr reaction, which gave 74–100% yields of Boc-protected TRIM33 ligand **246**. Boc migration to the terminal amine was not observed while acquiring high temperature NMR spectra as with the previous PROTAC TRIM33 ligand (**Chapter 3**), presumably because the carbamate of the Boc group is more hindered and less electrophilic than the carbamate of a Troc protecting group.



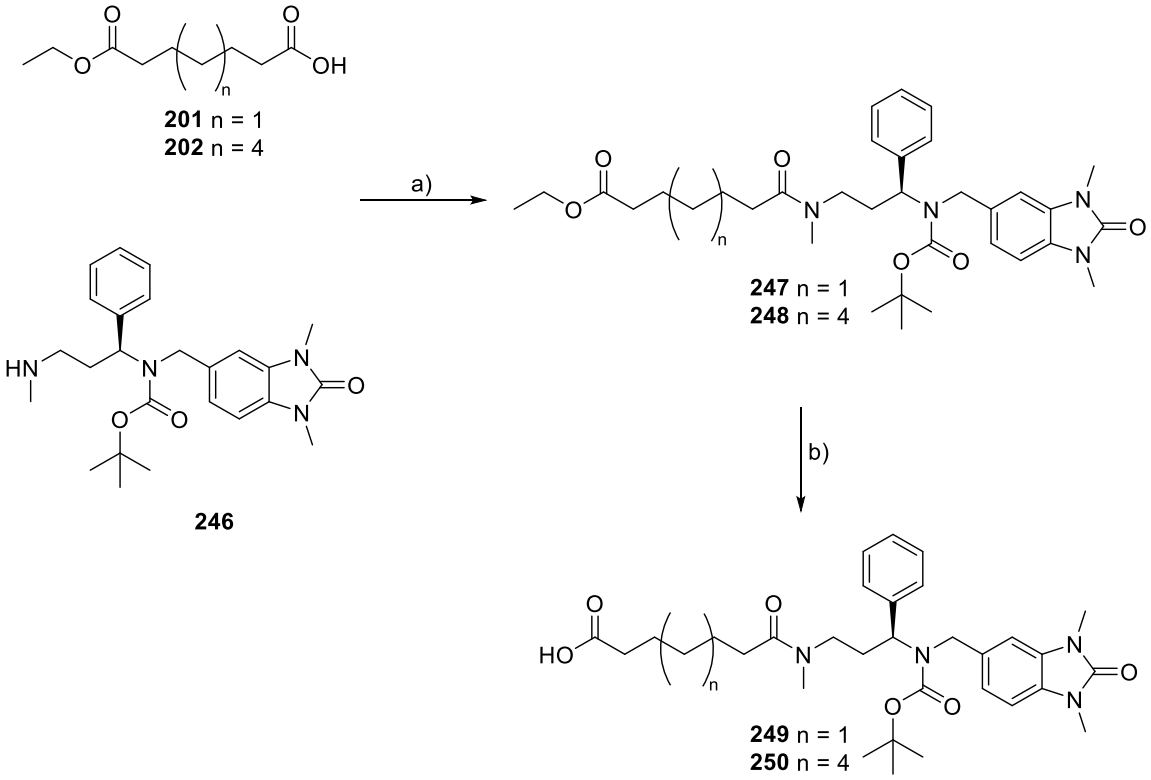
Scheme 4.2. Final steps of the TRIM33 ligand **246** synthesis. *Reagents and conditions:* a) AcOH, DCE, $NaBH(OAc)_3$, rt, 16 h; b) Boc_2O, NEt_3, CH_2Cl_2, rt, 16 h, 27–51% over two steps, n = 2; c) PhSH, K_2CO_3, DMF, rt, 5 h, 74–100%, n = 4.

4.4 Synthesis of VHL-Recruiting PROTACs

4.4.1 Synthesis of PROTAC Molecules Containing VHL Ligand 1

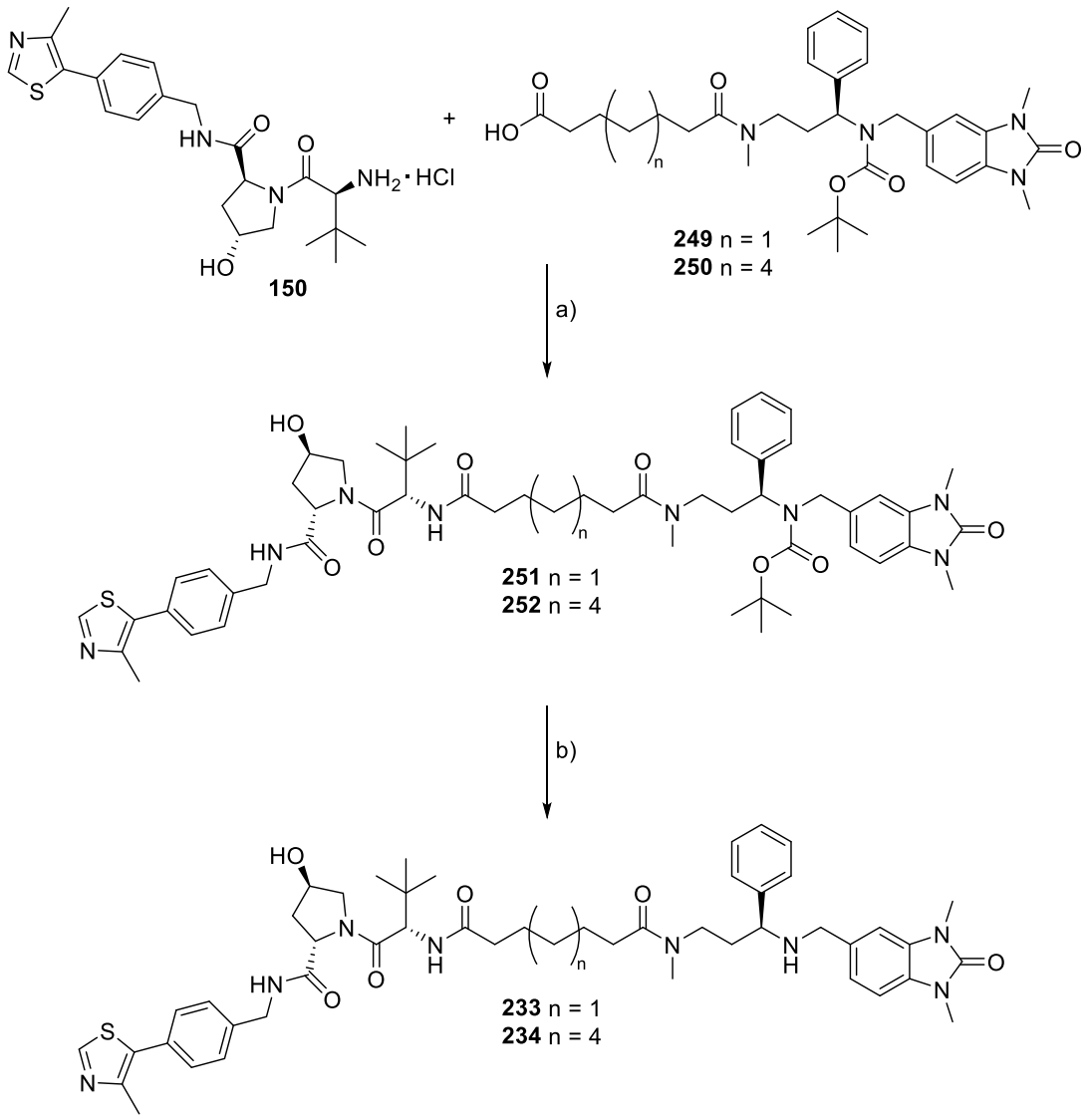
To complete the synthesis of PROTACs containing VHL ligand 1, two alkyl linkers were attached to the TRIM33 ligand **246** through amide couplings with acids **201** and **202**, which proceeded with yields of 50–76% (**Scheme 4.3**). The next step was to hydrolyse the terminal ester to reveal

the acid functional group prior to coupling with VHL ligand **150**. Synthesis of the VHL ligand was performed as in **Chapter 3**.



Scheme 4.3. Attachment of linkers to TRIM33 ligand **246**. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 16 h, **247**: 50–61%, n = 2 **248**: 76% n = 1; b) LiOH, MeOH, THF, H_2O, rt, 1 h, **249**: 97%, n = 1, **250**: 99%, n = 1.

Subsequently, HATU-mediated amide couplings between the VHL ligand and acids **249** and **250** gave protected PROTACs **251** and **252** in yields of 43% and 51%, respectively (**Scheme 4.4**). The final step required a Boc-deprotection, which proceeded to give final PROTACs **233** and **234** in preparation for biological evaluation.

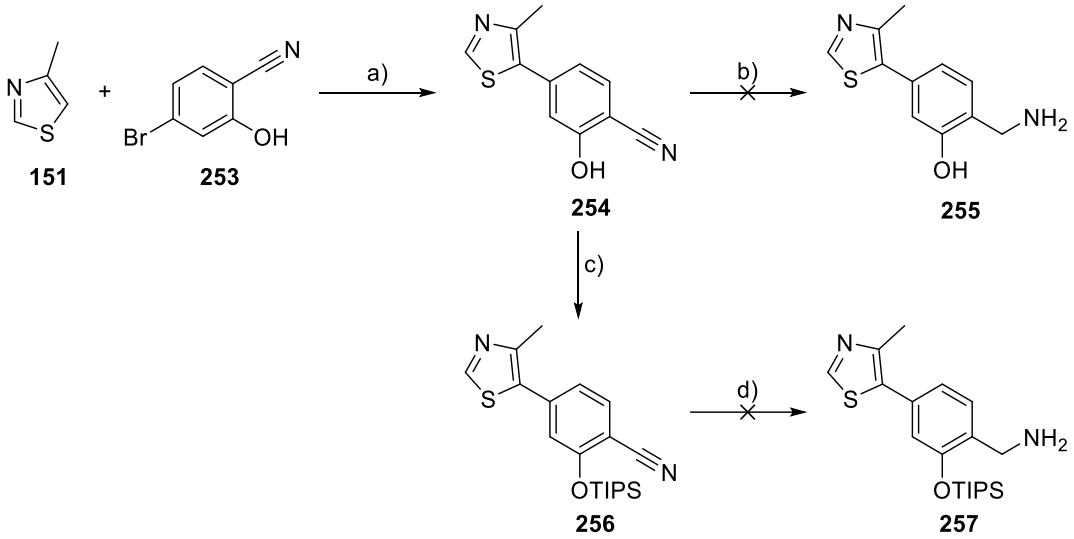


Scheme 4.4. Final steps in the synthesis of VHL-recruiting PROTACs **233** and **234**. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 16 h, **251**: 43%, n = 1, **252**: 51%, n = 1; b) 4 M HCl in dioxane, rt, 30 min, **233**: 54%, n = 1, **234**: 92%, n = 1.

4.4.2 Synthesis of VHL Ligand 2

The alternative VHL ligand **10** contains a different point of attachment for the linker, which alters the orientation of VHL with respect to TRIM33 in the ternary complex. Synthesis of **10** began as in the literature, in a similar way to the original VHL ligand **150**, with a palladium-catalysed coupling of 4-methylthiazole **151** and 4-bromo-2-hydroxybenzonitrile **253**, giving 40–71% yields of **254** (**Scheme 4.5**).[173] The next step required a nitrile reduction to give primary benzylic amine **255**. Literature precedent showed that the reduction could be performed heating under reflux, using 4 equivalents of lithium aluminium hydride in THF for 16 h. However, these conditions

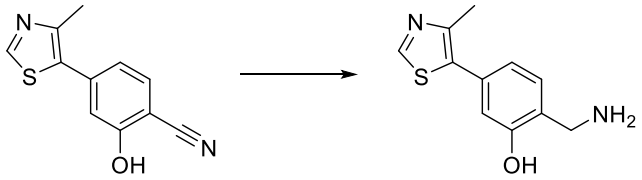
caused decomposition of the starting material and gave a very insoluble mixture of products. The reaction was repeated and quenched after 3 h, however, the same result was achieved. It was thought that the phenolic functional group may need protecting, so TIPS-protected **256** was synthesised, and subjected to the same reduction conditions. Again, a mixture of products was observed, none of which appeared to be the desired primary amine **257**.



Scheme 4.5. First attempts at reduction of nitrile group. *Reagents and conditions:* a) $Pd(OAc)_2$, KOAc, DMA, 150 ºC, 5 h, 40–71%, n = 3; b) Attempt 1: $LiAlH_4$, THF, 66 ºC, 16 h, decomposition, Attempt 2: $LiAlH_4$, THF, 66 ºC, 3 h, decomposition; c) TIPSCl, imidazole, DMF, rt, 16 h, 69–70%, n = 2; d) $LiAlH_4$, THF, 66 ºC, 3 h, decomposition.

Four reducing conditions were therefore screened (**Table 4.1**). Reaction with sodium borohydride using nickel(II) chloride hexahydrate as a Lewis acid only returned starting material. An attempt to produce the hydrochloride salt of amine **255** gave a mixture of products, with no discernible methylene proton peak in the ^{1}H NMR spectrum. Reduction using palladium on carbon and hydrogen was also tried, but after three days, only starting material was recovered. Fortunately, formation of amine **255** was achieved using 1 M lithium aluminium hydride in THF, heated to 50 ºC, instead of reflux, which gave high yields of the desired product **255** without need for purification.

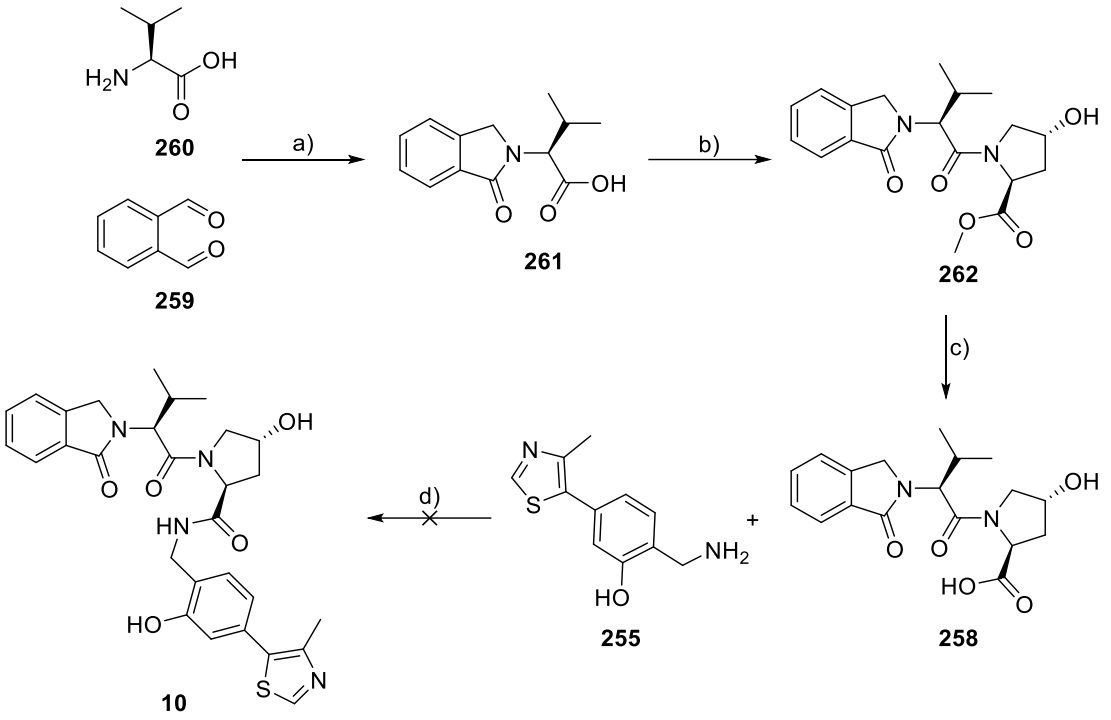
Table 4.1. Conditions trialled for nitrile reduction, with successful conditions yielding primary amine **255**.



254 **255**

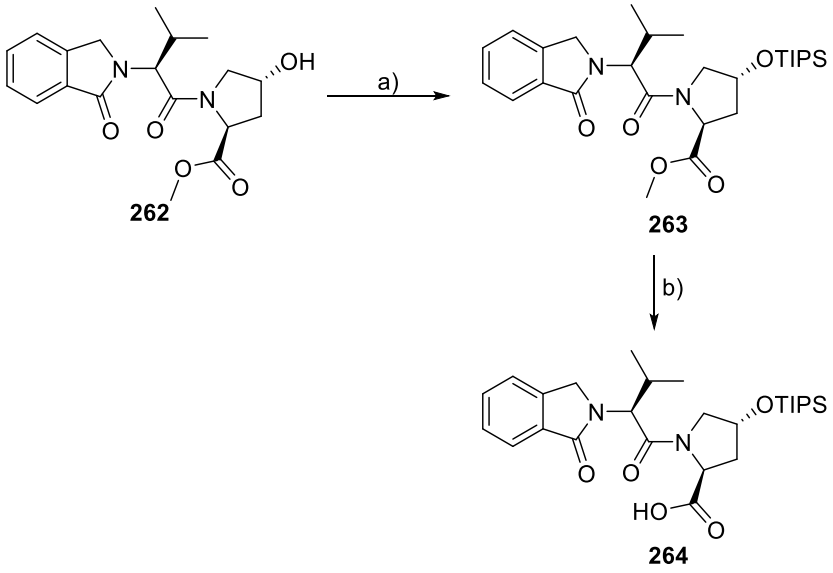
Reagents and Conditions	Result
$NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH, rt, 4 h	Returned starting material
$BH_3 \cdot THF$, THF, 66 ºC, 6 h then 2 M HCl, 66 ºC, 1 h	Mixture of products
Pd/C, H_2, HCl, MeOH, rt, 72 h	Returned starting material
$LiAlH_4$ (1 M in THF), THF, 50 ºC, 6 h	69–96% yield of desired product **255**, n = 4

Once the reduction of **254** had been achieved, attention turned to the synthesis of the amide coupling partner **258**. The first step involved a condensation reaction of phthalaldehyde **259** with L-(+)-valine **260** to produce ring closed **261** (**Scheme 4.6**). This reaction proceeded with an 88% yield and did not require purification. Subsequently, a HATU-mediated amide coupling of acid **261** with L-4-hydroxyproline methyl ester hydrochloride yielded amide **262**, in 63% yield. The next step required an ester hydrolysis to reveal desired carboxylic acid **258**, which proceeded in 82% yield. The amide coupling between acid **258** and amine **255** was then attempted using HATU as a coupling agent. However, a complex mixture of products with very similar R_f values was formed. The product was observed by ^1H NMR but could not be isolated from this mixture.



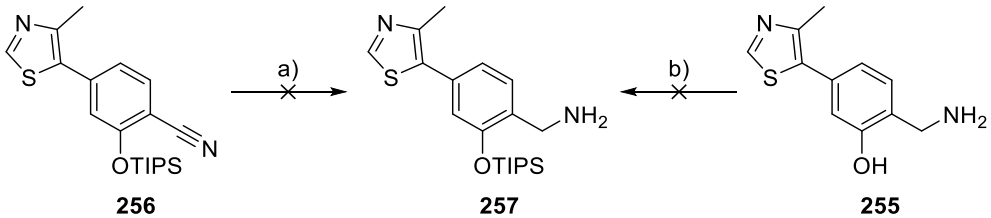
Scheme 4.6. Synthesis of amide coupling partner **258**. *Reagents and conditions:* a) MeCN, 80 ºC, 16 h, 88%, n = 1; b) L-4-hydroxyproline methyl ester hydrochloride, HATU, DIPEA, DMF, rt, 3 h, 63%, n = 1; c) LiOH, THF, H_2O, rt, 1 h, 82%, n = 1; d) HATU, DIPEA, DMF, rt, 1 h, product observed but could not be isolated.

It was hypothesised that the hydroxyl and phenol moieties could be undergoing side reactions. Consequently, a protecting group strategy was considered (**Scheme 4.7**). Ester **262** was protected with a TIPS group using TIPS triflate, which could then be hydrolysed to acid **264**. However, a small set of secondary peaks (5:1) were also observed in the ^{1}H NMR spectrum of acid **264**, indicating formation of a small amount of a diastereomer. This could have occurred through base-mediated epimerisation of either of the two amino acid stereocentres, but is unlikely to be due to epimerisation of the (*R*)-hydroxyl group. Despite this, the synthesis was continued.



Scheme 4.7. Protection strategy for acid **264**. *Reagents and conditions:* a) TIPSOTf, NEt_3, CH_2Cl_2, rt, 6 h, 88%, n = 1; b) LiOH, THF, H_2O, rt, 1 h, 94–99%, n = 2.

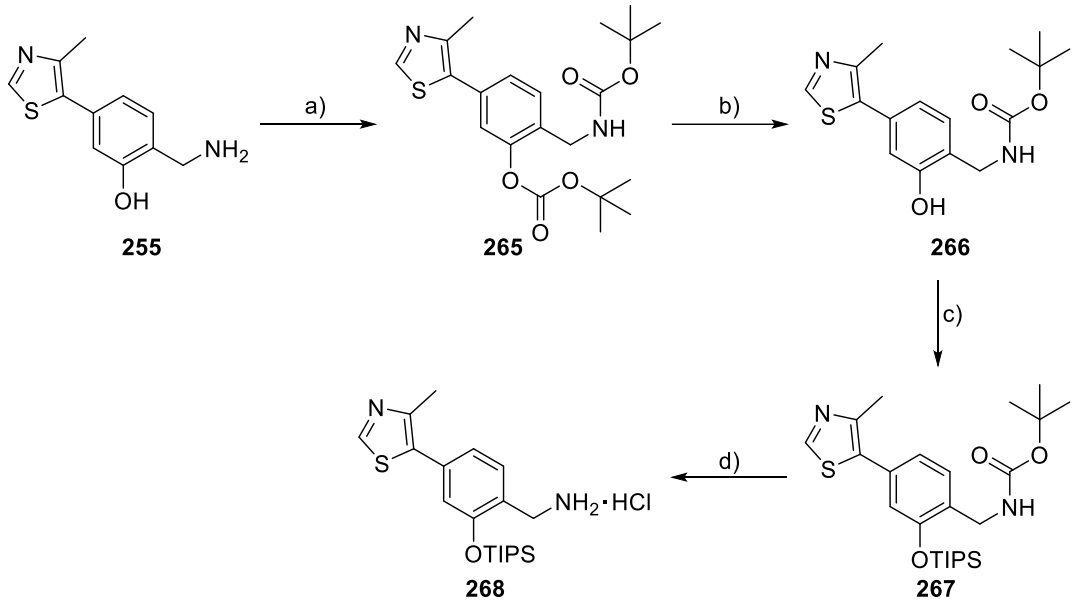
Protection of phenol **254** was also considered. TIPS protected **256**, synthesised in **Scheme 4.5**, was subjected to the reducing conditions used to produce **255** (**Scheme 4.8**). However, during the reaction, the TIPS protecting group was removed, and gave the free phenol-containing reduced amine **255**. Protection of phenol **255** after the reduction was also unsuccessful, as a mixture of products was observed, with only a 2% yield of desired product **257** isolated.



Scheme 4.8. Attempts to protected phenol. *Reagents and conditions:* a) $LiAlH_4$, THF, 50 ºC, 3 h, product **255** isolated; b) TIPSCl, imidazole, DMF, rt, 16 h, 2%, n = 1.

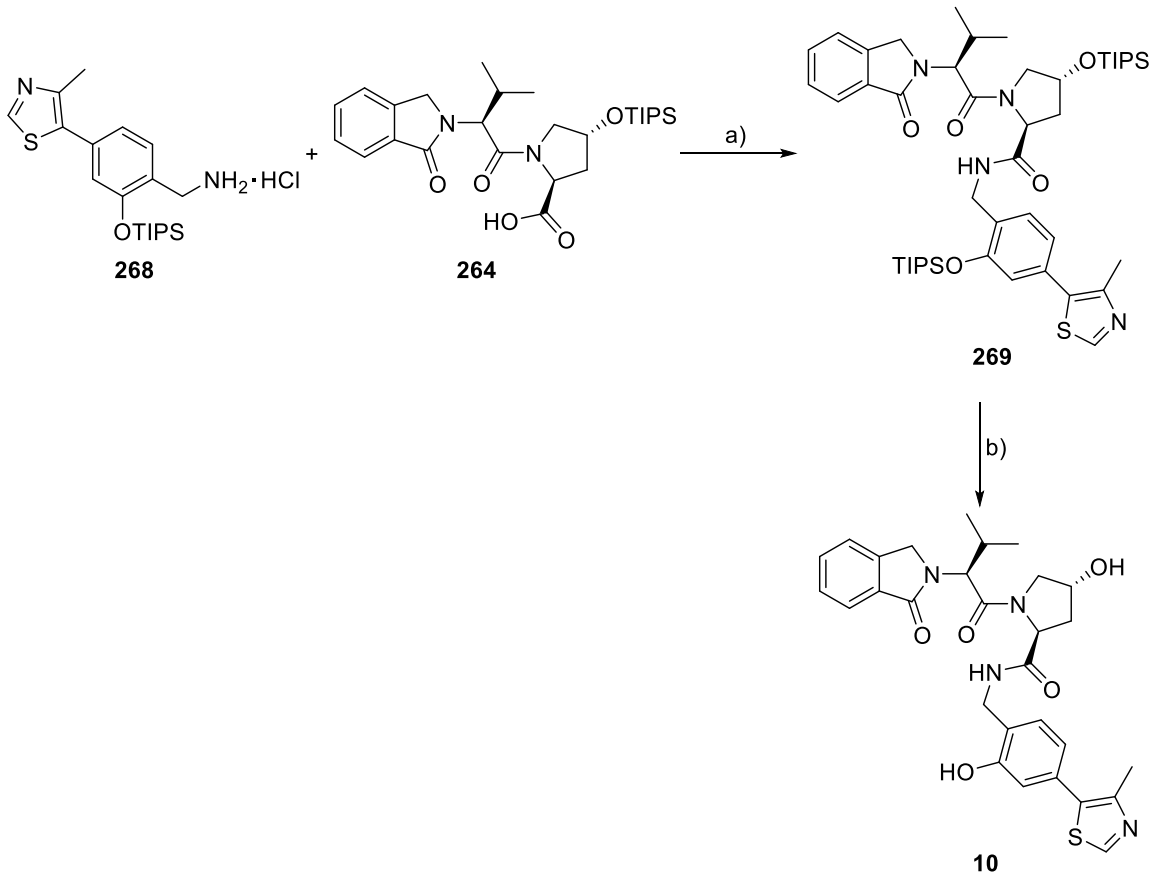
Attempting the amide coupling with protected acid **264** and free phenol-containing amine **255** again led to an inseparable mixture of products, so the protection of **255** was pursued. It was decided that Boc protection of the primary amine was necessary for successful TIPS protection of the phenol moiety, so amine **255** was subjected to Boc anhydride and DIPEA in dichloromethane (**Scheme 4.9**). This produced the double protected product **265**, which could be attributed to the low solubility of amine **255** in dichloromethane, and the increased solubility once Boc protection occurs. It was confirmed that the Boc anhydride had reacted with the phenol, as

an alternative to forming the dicarbamate on the amine, by observation of the ^{1}H NMR spectrum, which showed coupling of the methylene protons to the carbamate proton. The monodeprotection to yield **266** could easily be performed by stirring **265** in piperidine, which removed only the Boc group present on the phenol functional group. Phenol **266** could then be protected with a TIPS group, which produced **267** in 75% yield. However, protected **267** appeared to change colour while dissolved in $CDCl_3$ for assessment by ^{1}H NMR spectroscopy, thus full characterisation was not completed, and **267** was used immediately in the next reaction to prevent decomposition. The final deprotection involved removal of the Boc protecting group to reveal the hydrochloride salt of primary benzylic amine **268**, which proceeded with a quantitative yield.



Scheme 4.9. Synthesis of the TIPS protected free amine **268**. *Reagents and conditions:* a) Boc_2O, DIPEA, CH_2Cl_2, rt, 12 h, 57–77%, n = 2; b) Piperidine, CH_2Cl_2, rt, 2 h, 56%, n = 1; c) TIPSCl, imidazole, DMF, rt, 16 h, 75%, n = 1; d) 4 M HCl in dioxane, rt, 1 h, 100%, n = 1.

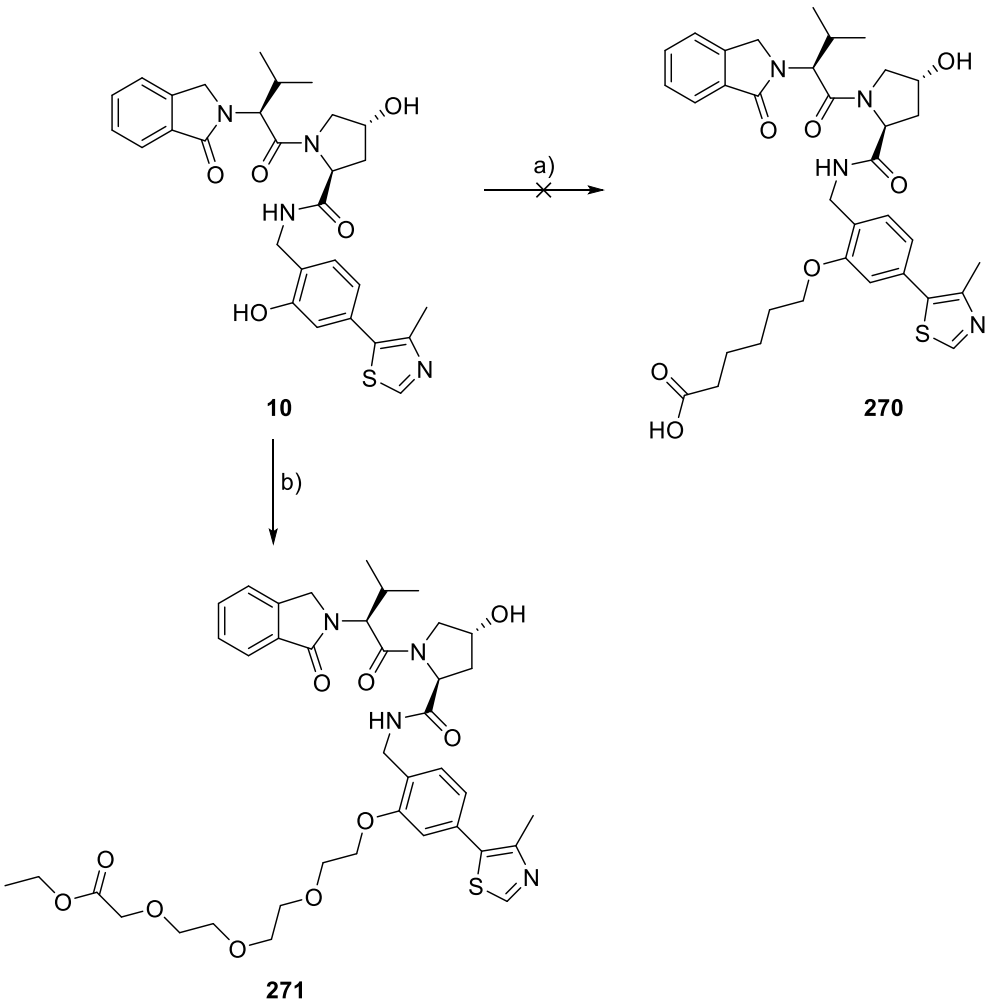
The penultimate step involved an amide coupling between hydrochloride salt **268** and protected acid **264** (**Scheme 4.10**). The reaction proceeded with a 57% yield, producing only one major product which was the desired amide **269**, instead of the mixture of products observed when the amide coupling was performed on the unprotected acid and amine. The final step was the global deprotection of the TIPS groups using TBAF, which gave the VHL ligand **10** in quantitative yields.



Scheme 4.10. Final steps to complete synthesis of VHL ligand **10**. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 3 h, 57%, n = 1; b) TBAF, THF, rt, 40 mins, 100%, n = 1.

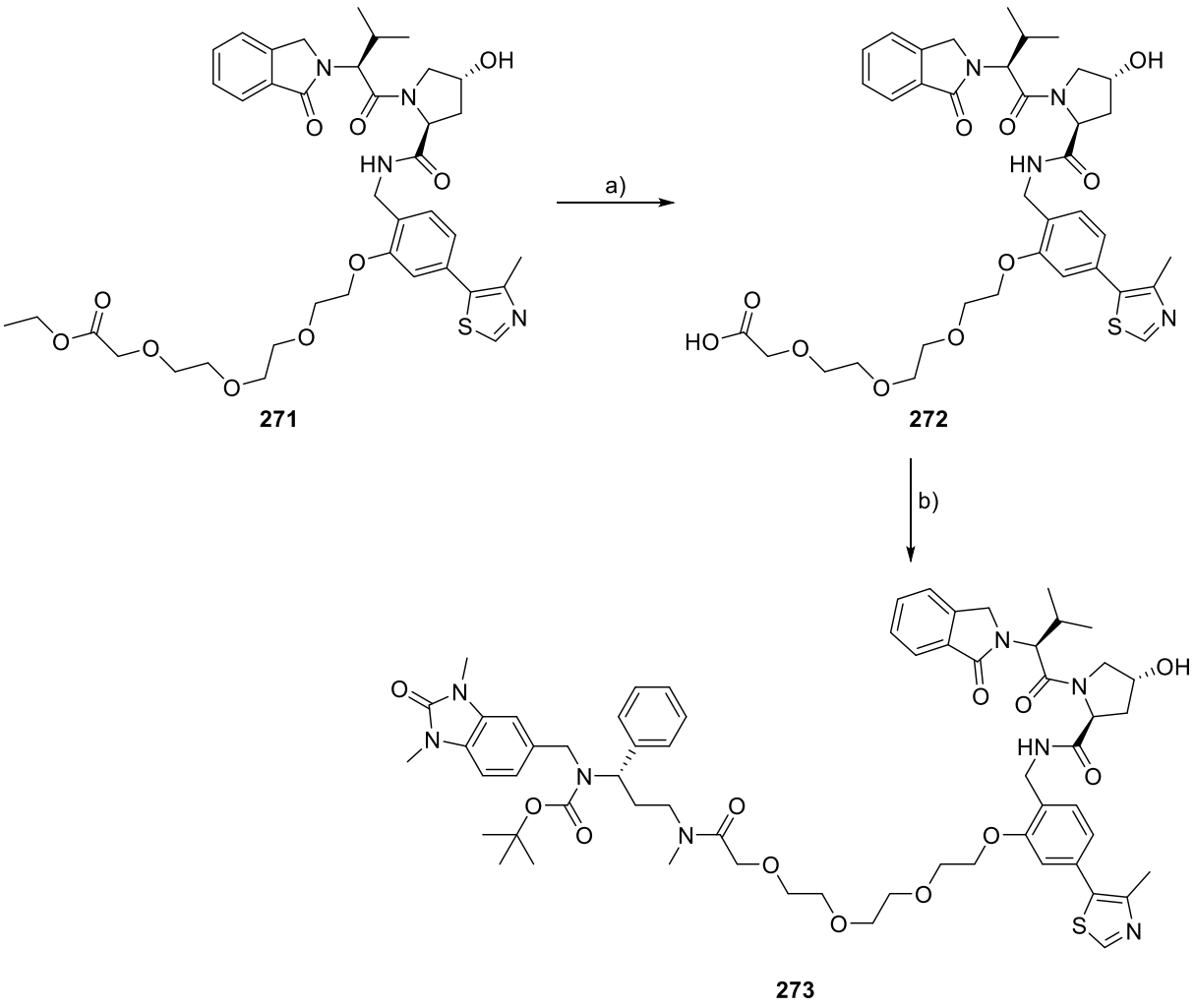
4.4.3 Synthesis of PROTAC Molecules

It was decided that the linker should first be attached to the VHL ligand, followed by attachment to the TRIM33 ligand to reduce the number of compounds that required high temperature NMR experiments, as this is time consuming and requires high sample concentrations. The substitution reaction was first attempted with 6-bromohexanoic acid. However, after 16 hours, only starting material remained and no desired product **270** was isolated (**Scheme 4.11**). Heating the mixture to 60 ºC similarly only returned starting material. Consequently, the reaction was attempted with the previously synthesised tosylated PEG linker, which proceeded with excellent yield to give **271**.



Scheme 4.11. Attempted attachment of linker to VHL ligand. *Reagents and conditions:* a) 6-Bromohexanoic acid, $CsCO_3$, NaI, DMF, rt, 16 h, then 60 ºC, 16 h, starting material returned; b) Ethyl 2-(2-(2-(2-(tosyloxy)ethoxy)ethoxy)ethoxy)acetate, $CsCO_3$, DMF, rt, 16 h, 83%, n = 1.

The next step required hydrolysis of ester **271** using lithium hydroxide to reveal acid **272** in preparation for the amide coupling (**Scheme 4.12**). The reaction was observed to go to completion *via* TLC analysis, but extraction from the aqueous layer during the work up proved difficult, thus only a small amount of material was recovered. Subsequent coupling to TRIM33 ligand **246** appeared to be successful, however, due to the small reaction scale, desired product **273** could not be purified, with only 2 mg of impure product being isolated after column chromatography. As a result of time restrictions, the synthesis was not repeated. Future work should focus on completing the synthesis of this PROTAC for biological evaluation.



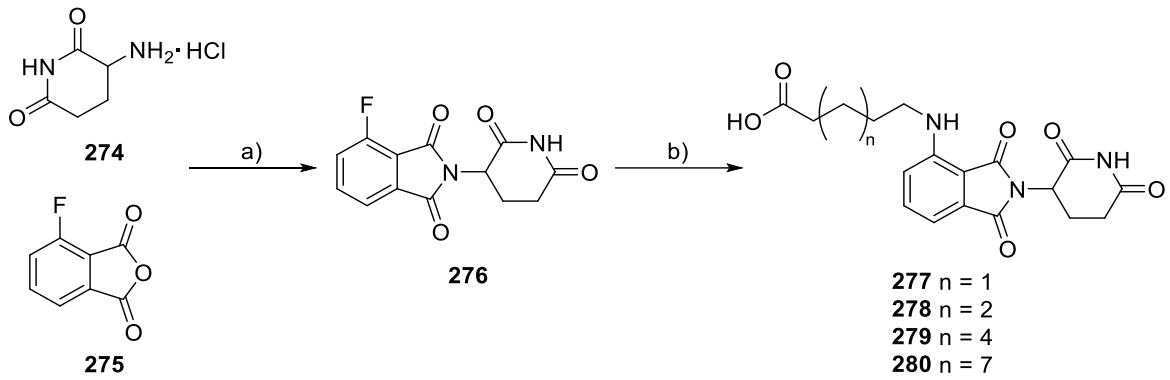
Scheme 4.12. Synthesis of protected PROTAC **273**, which should be the focus for future work. *Reagents and conditions:* a) LiOH, MeOH, THF, H_2O, rt, 30 min, 45%, n = 1; b) TRIM33 ligand **246**, HATU, DIPEA, DMF, rt, 16 h, could not purify.

4.5 Synthesis of Cereblon-Recruiting PROTACs

4.5.1 Synthesis of Pomalidomide Ligand and Linker Attachment

Synthesis of the precursor to the pomalidomide ligand occurred in one step following a literature procedure.[174] This reaction involved the condensation of amine **274** and anhydride **275** to form product **276** in an 89% yield (**Scheme 4.13**). The next step was an S_NAr reaction to allow attachment of the alkyl linkers. This was first attempted with 5-aminovaleric acid and potassium carbonate at 105 ºC in water and DMSO. After 24 hours, some desired product **277** had formed but the majority of the starting material **276** still remained. The reaction was therefore tried with microwave radiation at 110 ºC, using DIPEA as a base. Typically, DMF is used as a solvent for S_NAr reactions. However, *N*-methyl-2-pyrrolidone (NMP) was selected for this reaction as DMF

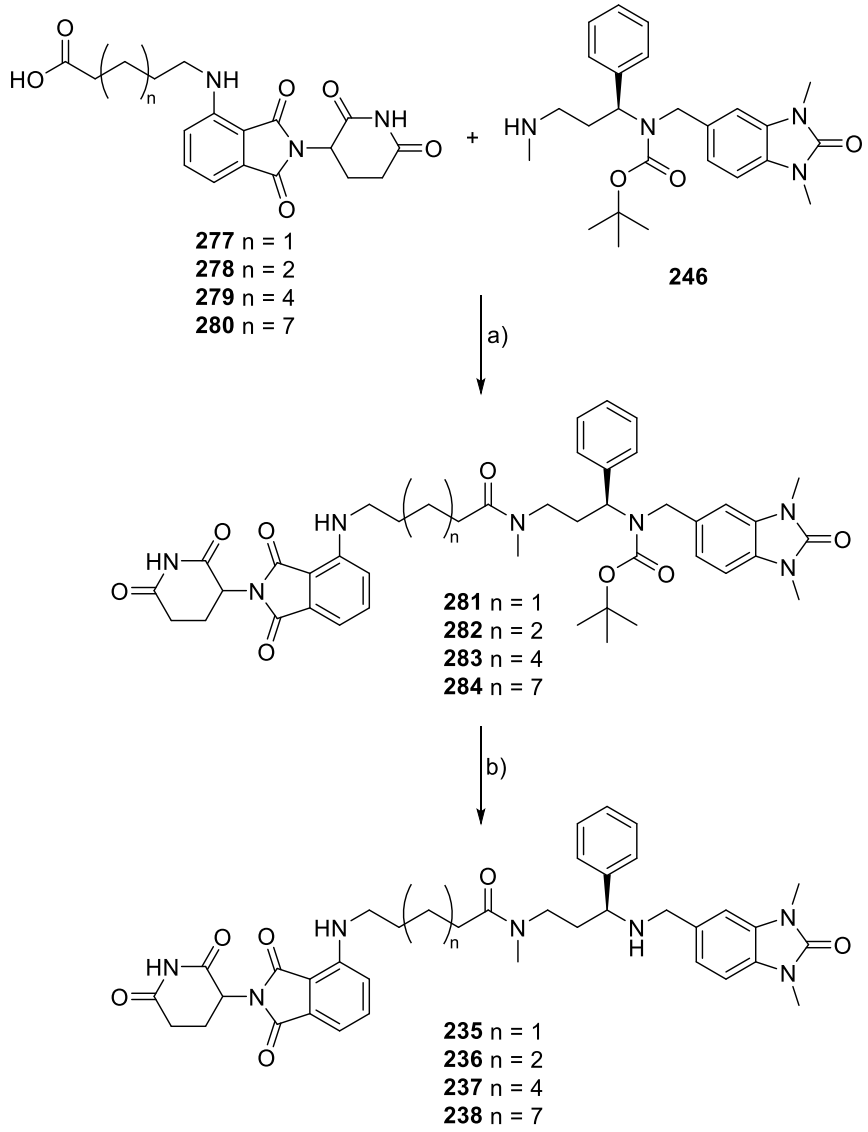
can decompose to produce dimethylamine, which can compete with the amine nucleophile. Desired product **277** was formed in 68% yield, so the reaction was repeated with three more linker lengths to make four acids **277**, **278**, **279**, and **280** ready for coupling to the TRIM33 ligand.



Scheme 4.13. Synthesis of the pomalidomide ligand and linker attachment. *Reagents and conditions:* a) NaOAc, AcOH, 140 ºC, 6 h, 89%, n = 1; b) Amine, DIPEA, DMF, 110 ºC, microwave, 2 h, **277**: 68%, n = 1, **278**: 78%, n = 1, **279**: 93%, n = 1, **280**: 89%, n = 1.

4.5.2 Synthesis of PROTAC Molecules

To complete the syntheses of the cereblon-recruiting PROTAC molecules, an amide coupling of acids **277–280** to TRIM33 ligand **246** was required, which proceeded in 46–74% yields (**Scheme 4.14**). As with the VHL-recruiting PROTACs, the final step was a Boc-deprotection to reveal final PROTACs **235–238**. Two diastereomers were observed in the NMR spectra of the PROTACs, owing to the racemic nature of the cereblon ligand.



Scheme 4.14. Final steps to synthesise PROTACs **235–238**. *Reagents and conditions:* a) HATU, DIPEA, DMF, rt, 16 h, **281**: 64%, n = 1, **282**: 46%, n = 1, **283**: 74%, n = 1, **284**: 51%, n = 1; b) 4 M HCl in dioxane, rt, 1 h, **235**: 40%, n = 1, **236**: 98%, n = 1, **237**: 61%, n = 1, **238**: 72%, n = 1.

4.6 Evaluation of PROTACs

4.6.1 Cell Permeability

A summary of the two VHL-recruiting and four cereblon-recruiting PROTAC molecules synthesised is shown in **Figure 4.4**.

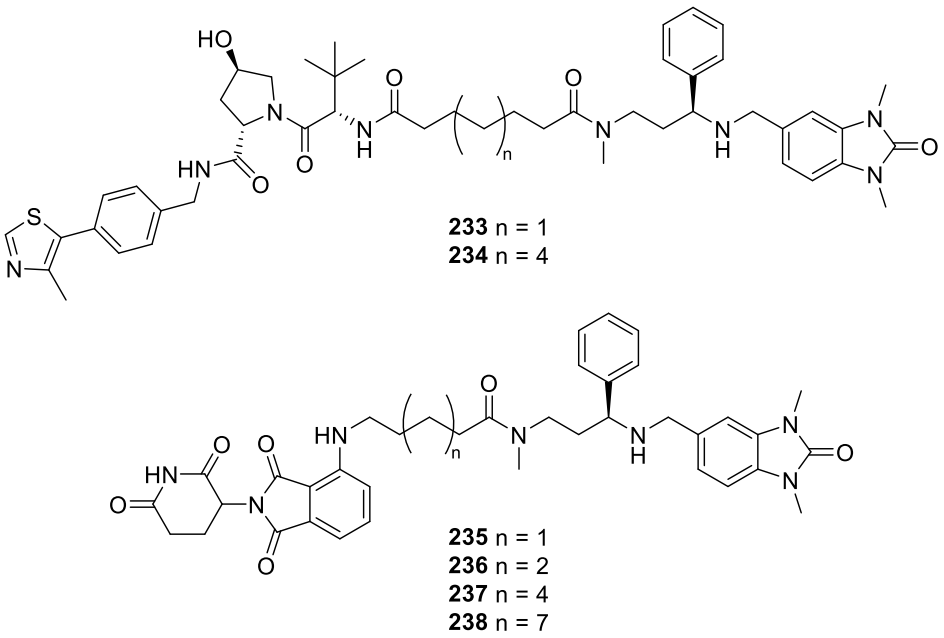
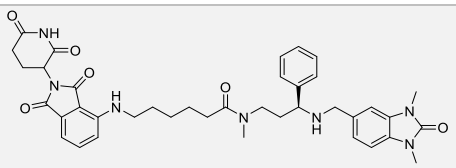
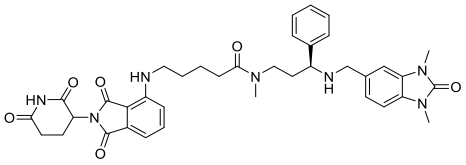
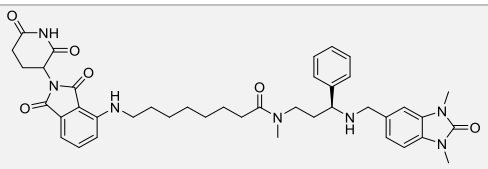
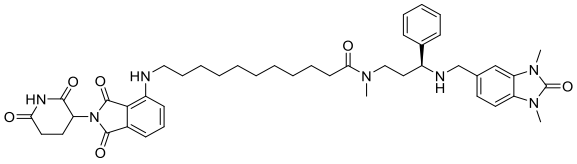
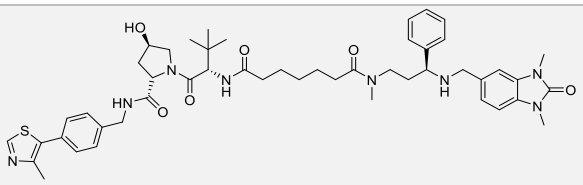
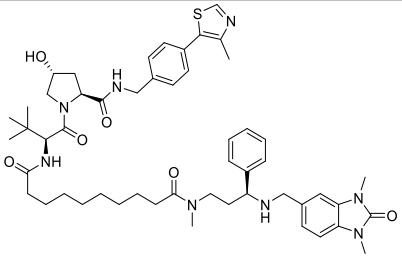


Figure 4.4. A summary of the PROTAC molecules synthesised.

Cell permeability was assessed using the same procedure as in **Chapter 3,** performed by Dr Hannah Bolland in the group of Professor Ester Hammond. An RKO cell line was treated with each of the six PROTACs individually, and incubated for 24 hours. The cells were subsequently lysed, and the lysate was analysed by HPLC to determine if the PROTAC was present inside the cell (**Table 4.2**).

Table 4.2. Cell permeability data for PROTACs **233–238** in an RKO cell line. <LOD = Lower than the level of detection, N/A = experiment not performed due to cell death. cLogD values in blue are predicted for pH 7.4 using ACD/I-Lab 2.0.

	Compound	Moles in 4.5×10^5 cell extract	Moles/cell	Approximate concentration in cell (assuming 1 pL volume)
Cereblon-recruiting	 **236** cLogD = 3.21	<LOD	0	0
	 **235** cLogD = 2.96	0.4 nmoles	0.9 fmoles	0.9 mM
	 **237** cLogD = 4.22	2.8 nmoles	6.2 fmoles	6.2 mM
	 **238** cLogD = 5.36	28 nmoles	62 fmoles	62 mM
VHL-recruiting	 **233** cLogD = 4.03	<LOD	0	0
	 **234** cLogD = 5.33	Cell death	N/A	N/A

Fortunately, three of the four cereblon-recruiting PROTACs **235**, **237**, and **238** appeared in the cell in increasing concentrations of 0.9 mM, 6.2 mM and 62 mM, respectively (**Table 4.2**). Increasing the length of the alkyl chain from seven (**237**) to ten (**238**) carbons gave a tenfold increase in the cellular concentration, indicating that the linker plays a significant role in

PROTAC permeability. **236** was not observed in the HPLC trace of the cell lysate, demonstrating that masking the polarity and increasing lipophilicity of the TRIM33 bromodomain ligand does not appear to make a substantial contribution to permeability relative to linker type and length. A comparison could be made between **237** and cereblon-recruiting PROTAC **229** from the previous PROTAC series, as the linker contained the same number of atoms and the same pomalidomide ligand was employed (**Figure 4.5**). This illustrated that the alterations made to the structure to increase cLogD were beneficial for cell permeability.

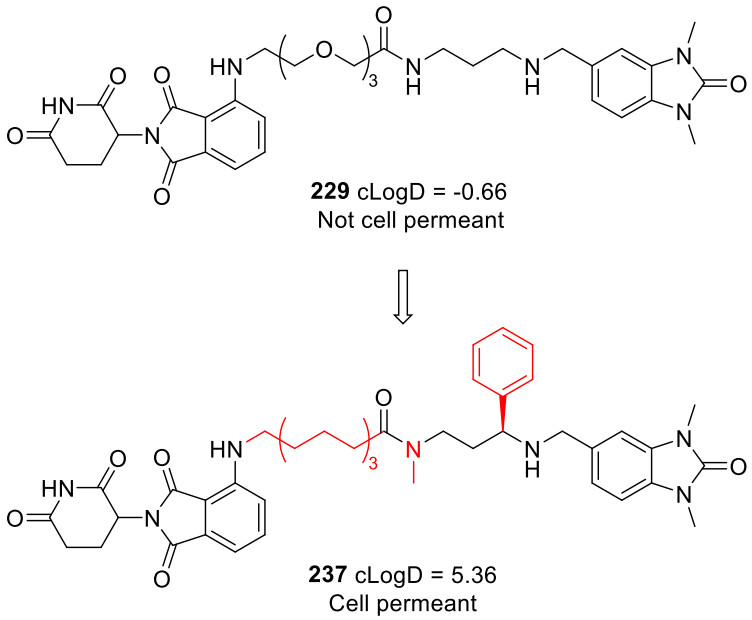


Figure 4.5. Cell permeability of PROTAC **229** improved by the addition of the phenyl moiety adjacent to the benzylic amine, the methyl group on the amide and switching the PEG linker for an alkyl linker. cLogD was determined using ACD/I-Lab 2.0.

The shorter linker VHL-recruiting **233** did not appear to be membrane permeant (**Table 4.2**), as the HPLC trace showed no **233** inside the cell. Of the known E3 ligase ligands used in PROTAC design, VHL-recruiting PROTACs are often reported to show limited cell permeability due to the peptidic nature of the ligand.[169,175] A repeat experiment is required for longer linker **234**, as cell death was observed after the 24 hour incubation period, thus permeability was not assessed.

Despite the lack of membrane permeant VHL-recruiting PROTACs, three cereblon-recruiting PROTACs **235**, **237**, and **238** were deemed permeable and were progressed to degradation assessment.

4.6.2 Degradation Assessment by Western Blot

As with the previous degradation assessment in **Chapter 3**, RKO cells were treated individually with **235**, **237**, and **238** at 10 μM, and were incubated for 0, 2, 4, 6, 8, 16 or 24 hours, before harvesting at the indicated time points (performed by Dr Hannah Bolland in the group of Professor Ester Hammond). Western blots were performed to determine if the PROTACs had an effect on the concentration of TRIM33 in the cell. β-Actin was used as the loading control to confirm that the samples have been loaded equally into each well. Incubation at 0 h acted as a control for the normal TRIM33 expression levels in the RKO cell line. Protein expression levels were quantified using the ImageStudio (LICOR) software, normalised relative to the β-actin loading control and expressed as relative fold change over 0 h time points.

From the Western blot and quantification data, it did not appear that degradation of TRIM33 was occurring after treatment of cells with **235**, as the concentration of TRIM33 remained around the normal cellular levels (**Figure 4.6A, B**). This indicated that there was no formation of a productive ternary complex, which may be a consequence of the short linker preventing close proximity of the target protein and cereblon without steric repulsion. However, after 8, 16, and 24 hours, the level of TRIM33 within the cell appeared to be greater than at t = 0. As discussed in **Chapter 3**, this increase could either be due to a natural cell cycle increasing the TRIM33 levels within the cell, or it could indicate that the PROTAC **235** is binding to the TRIM33 bromodomain, inhibiting its function and therefore causing a feedback mechanism in which cells overexpresses TRIM33.[172]

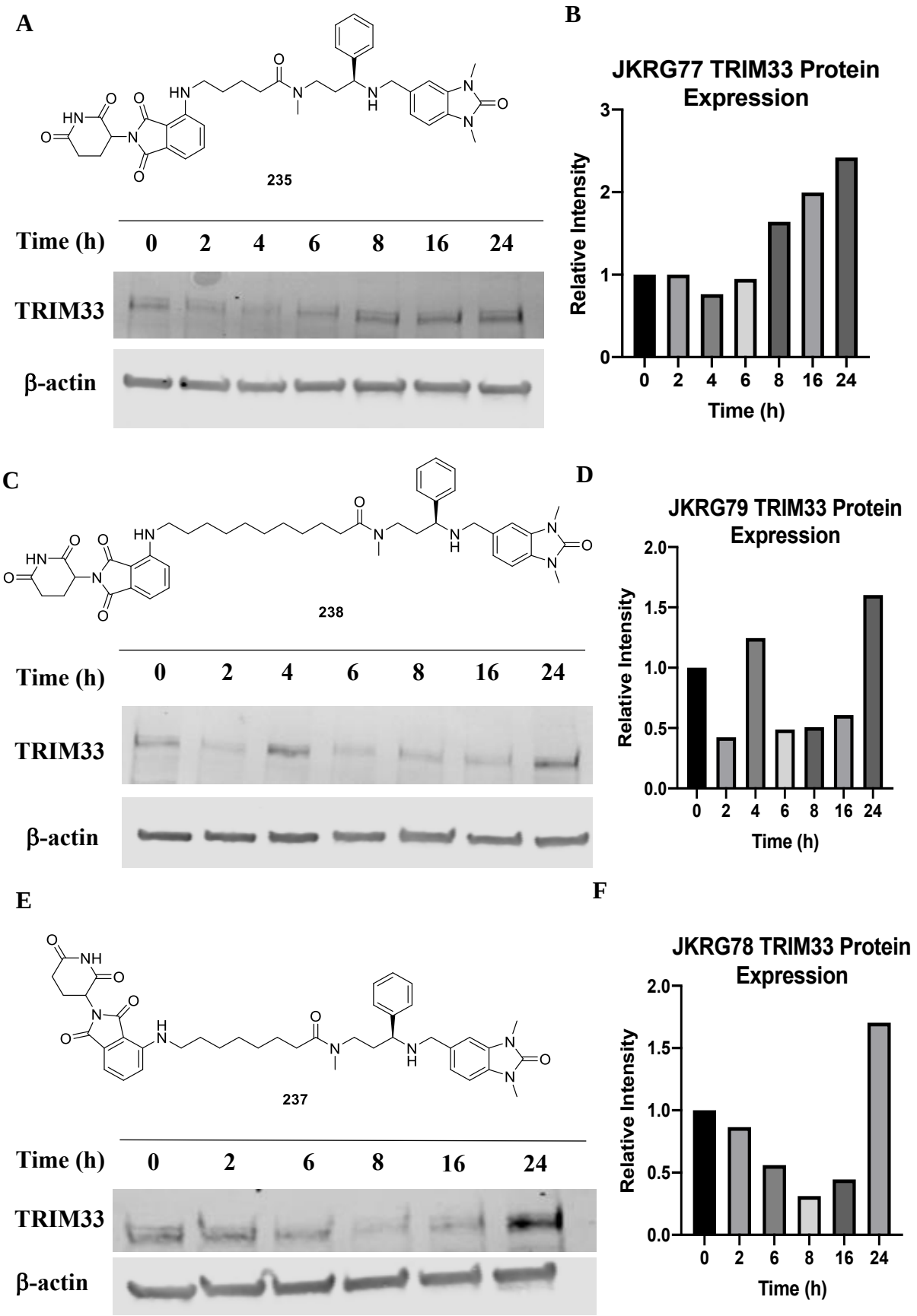


Figure 4.6. A. Time-dependent Western blot showing the concentration of TRIM33 when RKO cells were treated with **235**. Experiment performed by Dr Hannah Bolland, n = 1. **B.** Quantification of degradation relative to t = 0. **C** Time-dependent Western blot on treatment with **238**. Experiment performed by Dr Hannah Bolland, n = 1. **D.** Quantification of degradation relative to t = 0. **E.** Time-dependent Western blot on treatment with **237**. Experiment performed by Dr Hannah Bolland, n = 1. **F.** Quantification of degradation relative to t = 0.

On treatment of the cells with the longest linker PROTAC **238**, a reduction in TRIM33 concentration was observed from 2 to 16 hours (**Figure 4.6C, D**). However, as there is no apparent time-dependence, and there appears to be an anomalous result after 4 hours of PROTAC exposure, it is difficult to significantly interpret this data. Several repeats of the experiment should be performed to determine if the reduction in TRIM33 concentration is a consequence of PROTAC-mediated degradation, or if the protein levels are fluctuating in the cell independently of **238**.

Finally, a distinct time-dependent reduction in TRIM33 levels was observed after treatment with PROTAC **237**, with a steady decrease in protein concentration between 2 and 8 hours, and a maximum reduction of around 70% relative to the 0 h control (**Figure 4.6E, F**). This result suggests a productive ternary complex is forming between TRIM33 and cereblon, leading to degradation. Some recovery of TRIM33 was seen after 16 hours, and an increase in TRIM33 expression above the normal cellular level was detected after 24 hours. Again, this could be a consequence of the natural cell cycle or inhibition leading to overexpression. However, it was hypothesised that since TRIM33 accumulates at sites of DNA damage to allow the timely removal of chromatin-remodelling enzyme ALC1, degrading or inhibiting this protein would lead to prolonged chromatin relaxation. This exposes the DNA to further damage, thus further TRIM33 would be required to accumulate at the site, increasing the concentration. Due to time constraints, the experiment was only performed once, therefore repeats are necessary to confirm the time-dependent concentration reduction observed. Furthermore, due to the catalytic mechanism of action of PROTACs, the increased TRIM33 levels after 24 hours suggests that **237** is being excreted from the cell relatively quickly, thus a time-dependent experiment should be performed over 48 hours, with an additional dose of **237** after 24 hours to determine if the observed protein recovery could be reversed.

Since each PROTAC assessed has a differing linker length, different interactions at the protein−protein interface would be produced, which would influence the stability of the resultant ternary complex. This explains why potential degradation is observed for only one PROTAC,

despite the structural similarities between **235**, **237**, and **238**. These data therefore illustrate the importance of linker length in PROTAC design, as a modification of only a few methylene units can lead to substantial differences in the degradation profiles observed.

Due to time constraints, only one repeat was performed for each experiment, thus further repeats are required to verify the results. Additionally, the degradation was only assessed at one concentration for each PROTAC, so future studies should focus on repeating the experiments varying the concentration to identify the point at which degradation of TRIM33 is maximised, and to recognise where the hook effect is occurring. However, these preliminary data identified PROTAC **237** as a potential degrader of TRIM33, with degradation observed up to 16 hours after administering **237**. TRIM33 is more highly expressed in MCF7 breast cancer cells compared to RKO cells, thus the experiments should also be performed in this cell line to make interpretation of concentration fluctuations more significant.

4.7 Summary and Conclusions

In conclusion, a new series of PROTAC molecules to target TRIM33 was considered, with the aim of increasing cell permeability to increase PROTAC concentration in cells, thus improving the likelihood of total degradation. The PROTACs were designed to mask non-essential polarity through methylation of the terminal amide of the TRIM33 ligand and incorporation of a phenyl group adjacent to the benzylic amine. Alkyl linkers were also utilised to increase lipophilicity. Four cereblon-recruiting and two VHL-recruiting PROTACs were synthesised. In addition to incorporating the VHL ligand used in the previous PROTAC series, PROTACs were designed with a second VHL ligand, which would produce an alternative ternary complex and change the orientation of VHL relative to TRIM33. However, due to time and material constraints, syntheses of these molecules were not completed, and should be prioritised in future work.

Assessing cell permeability showed that increasing the lipophilicity of the new PROTAC series led to improved permeability properties compared to previous PROTACs, with three cereblon-recruiting PROTACs **235**, **237**, and **238** being present in cells in high concentrations. Alkyl linker

length appeared to play a key role, indicated by a tenfold increase in permeability of ten carbon linker PROTAC **238** compared to seven carbon linker PROTAC **237**. The increased cellular concentrations of these PROTACs established that if TRIM33 degradation was not observed, this would not be a consequence of insufficient PROTAC concentration present in the cell.

The degradation profiles of these three PROTACs were subsequently assessed, and **237** appeared to show time-dependent reduction of TRIM33 concentration up to 16 hours after treatment. The lack of time-dependent degradation observed for **238** and **235** demonstrated that, due to the changing linker length, each ternary complex had different protein–protein interactions, thus proving the importance of linker length in PROTAC design. Interestingly, 24 hours after the treatment of PROTACs **238** and **235**, a higher concentration of TRIM33 was observed, possibly due to inhibition leading to increased expression. The same phenomenon was observed for **237**, which could also be due to increased DNA damage leading to accumulation of further TRIM33. This chapter, therefore, describes the discovery of **237**, a potential degrader of TRIM33, which can be used as a basis for further optimisation and ultimately to elucidate the biological roles of TRIM33 in disease.

Future work should focus on repeating the time-dependent degradation experiments to validate these results. Experiments varying the concentrations of the PROTACs should also be performed to identify the concentration at which maximum degradation of TRIM33 is achieved. Furthermore, cell permeability assessment of VHL-recruiting PROTAC **234** should be repeated and degradation of TRIM33 should be evaluated, as this molecule provides a direct comparison with previous series PROTAC **148**. Negative controls should be synthesised to further confirm these observations. Finally, the optimised PROTACs can be used to assess the impact of degrading TRIM33 in situations where DNA damage is increased.

Chapter 5 Investigating TRIM24 as a Novel E3 Ligase for PROTACs

5.1 Introduction and Aims

Since using computational modelling to determine a successful PROTAC is in early stages, development of bifunctional degraders still relies on the synthesis of a library of analogues, varying linker length, type, and recruited E3 ligase. Of the predicted 600 E3 ligases in the human genome,[175] fewer than ten have been successfully utilised in PROTAC technology. Given that the recruited E3 ligase plays an important role in the nature of the ternary complex, and therefore the observed degradation profile, it is imperative that novel E3 ligases are explored. The identification of E3 ligases that are preferentially expressed in a given location or cellular environment could allow the development of PROTACs that degrade the protein of interest selectively in that environment.

Small molecule bromodomain ligands have been developed for TRIM24,[41,42] an oncoprotein that is highly expressed in a range of cancer cell lines. Inhibition of the bromodomain was later shown to be ineffective in a cellular setting.[72] To determine the effect of degrading the whole TRIM24 protein, these ligands were incorporated into a VHL-recruiting TRIM24-degrading PROTAC. This provided a tool for probing the genome-wide consequences of the loss of TRIM24, and identified TRIM24 as a dependency in leukaemia.[72] Interestingly, TRIM24 can also act as an E3 ubiquitin ligase through its RING domain,[71] but its potential to degrade other target proteins in PROTACs has not been explored. Given the observed overexpression of TRIM24 in cancer cell lines,[26,73–76] a TRIM24-recruiting PROTAC could allow selective degradation of a target protein preferentially in specific cancer cell environments (**Figure 5.1**). Clearly, context-dependent degradation could be beneficial to biological studies and therapeutics, so the aim of this work was to design and evaluate a series of PROTAC molecules that recruit TRIM24 as a novel E3 ligase, and determine if degradation occurs only in cancer cells that have high TRIM24 expression levels.

In addition, this work aimed to increase the number of established E3 ligases available for use in PROTAC technology, expanding the toolbox for medicinal chemists.

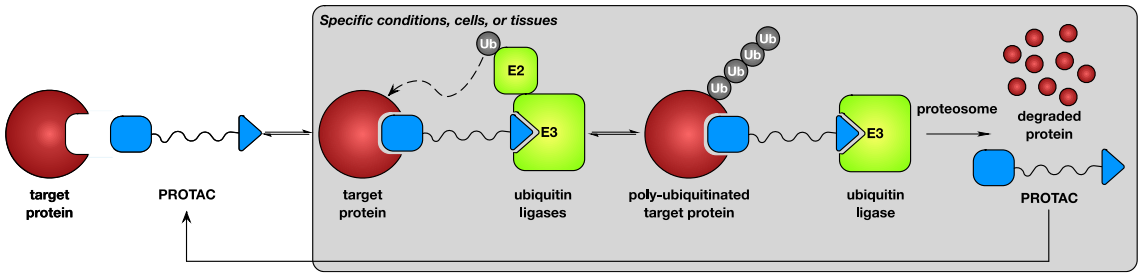
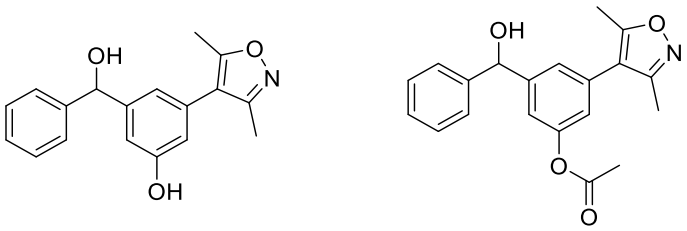


Figure 5.1. Diagram depicting the potential for a PROTAC to recruit an E3 ligase in a specific context, for example if it is overexpressed in cancer cell lines, which would allow selective degradation of the target protein in a particular environment. Diagram by Professor Stuart Conway.

When considering the design of the TRIM24-based PROTAC series, bromodomain-containing protein 4 (BRD4) was selected as the target protein for this proof-of-concept study. BRD4 has relevance to a range of diseases,[176] and its susceptibility to PROTAC-mediated degradation with several other E3 ligases has been demonstrated.[95,177,178] There are many ligands reported that bind to BRD4(1),[179,180] from which 3,5-dimethylisoxazole-containing **OXFBD02 4** (IC_{50} = 382 nM) and **OXFBD03 285** (IC_{50} = 371 nM) were selected for incorporation into the PROTAC series (**Figure 5.2**).[37,38] These two ligands were chosen as they are not synthetically challenging, and the existing co-crystal structure with BRD4 allowed analysis of the most appropriate vector to attach a linker. It had previously been determined that both the (*R*)- and (*S*)-enantiomers of **4** and **285** have similar affinities for BRD4,[37] so a racemic mixture was synthesised.



OXFBD02 4
IC_{50} = 382 nm

OXFBD03 285
IC_{50} = 371 nm

Figure 5.2. Structures of the selected BRD4 ligands to incorporate into the PROTAC design, discovered in the group of Professor Stuart Conway, University of Oxford.[37,38]

On observation of the co-crystal structure (PDB ID: **4J0S**, **Figure 5.3**), the key interactions of the ligand with the protein include the acetyl-lysine mimicking isoxazole forming a hydrogen bond

with asparagine N140, and a conserved water molecule in the binding pocket. The phenol functionality also interacts with a key ZA channel water molecule. Conversely, the secondary alcohol and phenyl group are more solvent exposed, directing out of the binding site (shown by the pink arrow, **Figure 5.3**). Therefore, this was determined to be the most appropriate vector for linker attachment to prevent any loss in binding affinity would be from the racemic alcohol.

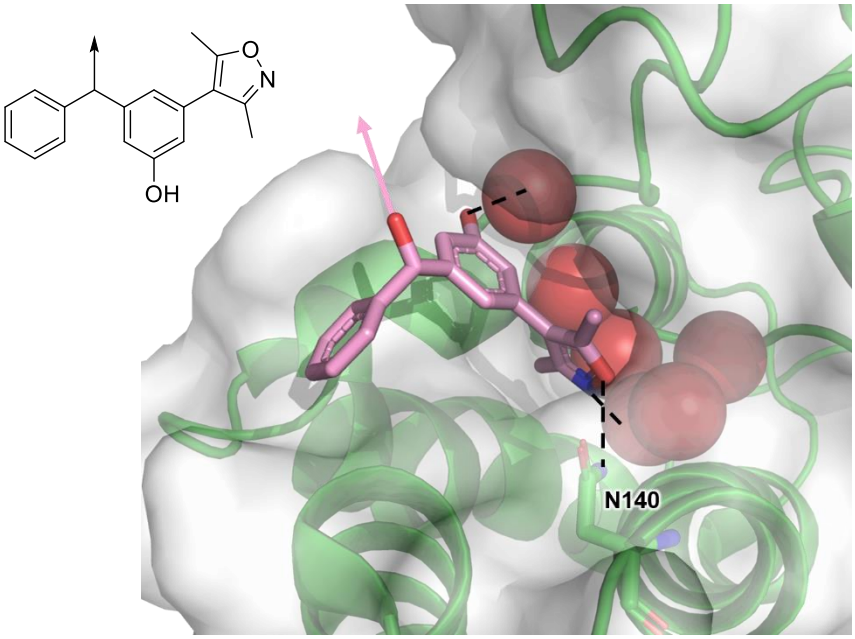
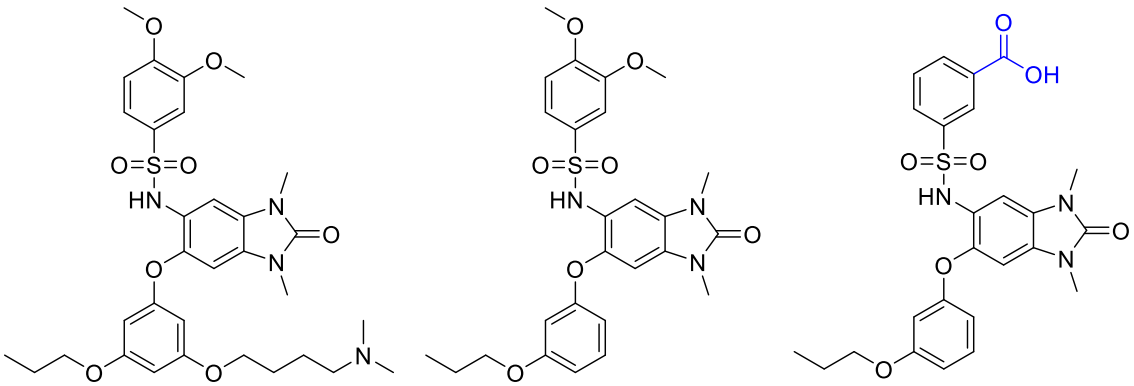


Figure 5.3. X-Ray crystal structure of ligand **4** binding to BRD4(1) (PDB ID: **4J0S**).[37] Dashed lines represent hydrogen bond interactions between the ligand and protein or structural water molecules (red spheres).

Since TRIM24 bromodomain ligand **IACS-9571**[42] **6** has already been incorporated into a PROTAC molecule,[72] this ligand was selected to recruit TRIM24 as modifications to attach a linker clearly did not affect its binding to TRIM24 (**Figure 5.4**). To reduce synthetic complexity, the 4-(dimethylamino)butoxy group was removed from the aryl ether, which only slightly decreases affinity for the TRIM24 bromodomain (**IACS-7e 286**, IC_{50} = 43 nM).[42] As in the TRIM24-VHL PROTAC, the two methoxy groups present on the solvent-exposed benzyl sulfonamide were substituted for a carboxylic acid group to act as a functional handle for attaching the linker through an amide bond.



IACS-9571 6
IC_{50} = 8 nM

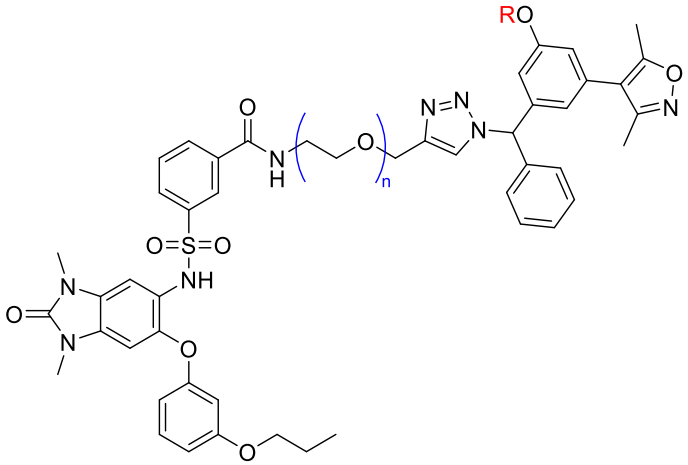
IACS-7e 286
IC_{50} = 43 nM

287

Figure 5.4. Structures and IC_{50} values of TRIM24 bromodomain inhibitors. **IACS-9571 6** has an IC_{50} value of 8 nM, and omitting the 4-(dimethylamino)butoxy group in **IACS-7e 286** does not have a substantial effect on affinity for the bromodomain.[42] Compound **287** will be used in the TRIM24-recruiting PROTAC, with a carboxylic acid group to act as a linker attachment point.

5.2 Previous Work

With attachment points on both the TRIM24 and BRD ligands decided, a series of PROTACs were synthesised by Part II student Oliver Stratton[181] (**Figure 5.5**), utilising a triazole to connect the BRD4 ligand to the linker. Amide attachment of the linker was also attempted, but the corresponding benzylic amine of the BRD4 ligand appeared to degrade quickly. Two PROTACs **288** and **289** were synthesised using **OXFBD02 4** ligand to target BRD4, and one PROTAC **290** was synthesised incorporating **OXFBD03 285** as the BRD4 ligand.



288 n = 2, R = H
289 n = 3, R = H
290 n = 3, R = Ac

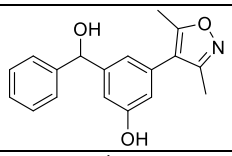
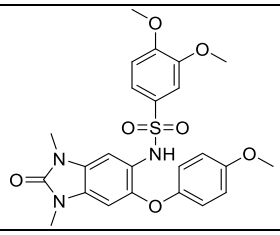
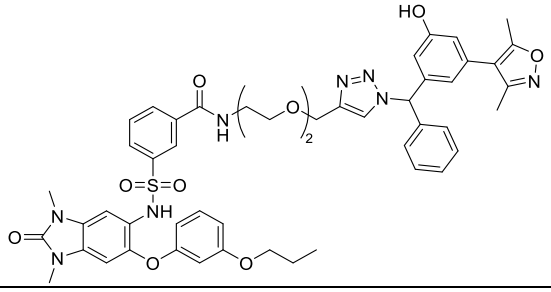
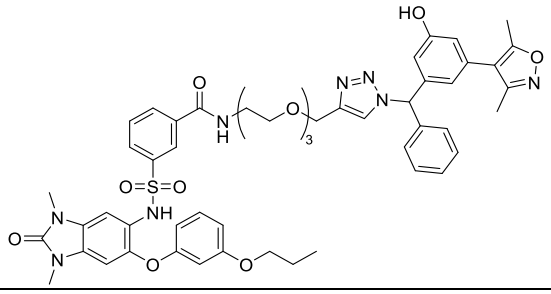
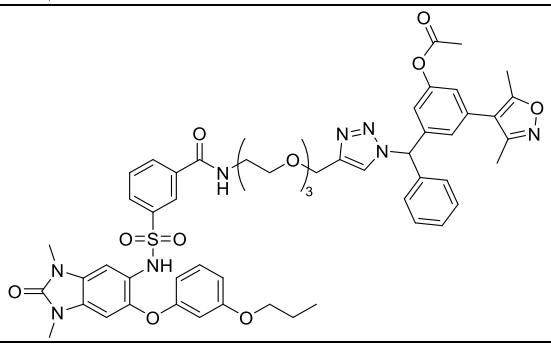
Figure 5.5. Structures of the PROTACs **288–290** synthesised by Part II student Oliver Stratton.[181] A triazole moiety is used to link the BRD4 ligand to the PEG linker.

5.3 AlphaScreen™

The PROTACs were subsequently evaluated using a dose-response AlphaScreen™ to ensure binding to BRD4 and TRIM24 had not been disrupted (performed with Dr Amy Scorah and Oliver Stratton). The results are shown in **Table 5.1**. The $H3_{1–27}K18Ac$ peptide was used as a bromodomain binding partner for TRIM24, and the $H4(KAc)_4$ peptide was used as partner for BRD4. **OXFBD02 4** acted as a positive control for BRD4, and **SGC-34 7** was used as a positive control for TRIM24. The results showed that in general, binding to both BRD4 and TRIM24 were maintained with only a modest loss of affinity. The PROTAC **288** maintained nanomolar affinity for BRD4(1) (IC_{50} = 546 nM) compared to **OXFBD02** control **4** (IC_{50} = 295 nM), while a reduction in affinity was observed for TRIM24 (IC_{50} = 3.66 µM). However, the positive control **7** showed lower affinity for TRIM24 (IC_{50} = 1.98 µM) than the reported literature value (IC_{50} = 222 nM),[41] which may be due to differing assay conditions. The longer PEG linker PROTACs **289** and **290** had lower affinities for BRD4 (IC_{50} = 2.18 and 2.68 µM, respectively) and TRIM24 (IC_{50} = 3.15 and 10.4 µM, respectively) compared to the control compounds. This could be attributed to the decrease in entropy during the binding event not being compensated for by an enthalpic contribution. Curiously, the addition of the acetyl group within the BRD4 ligand in **290** appeared to reduce the affinity for TRIM24 compared to the phenol equivalent **289**. However, this was perhaps due to general AlphaScreen™ experimental error, thus should be repeated. These affinity data provided confirmation that the PROTAC molecules bind to the appropriate targets and were subsequently progressed to cellular experiments.

Table 5.1. A table showing IC_{50} values (μM) for PROTACs **288–290** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. The colour scale is shown below, with low IC_{50} values (stronger binding) shown in red, and high IC_{50} values (weaker binding) shown in green. BRD4 results shown using the $H4(KAc)_4$ peptide as a binding partner, and TRIM24 results shown using the $H3_{1–27}K18Ac$ peptide as a binding partner. Experiment performed with Dr Amy Scorah and Oliver Stratton.

Weaker binding — **Stronger binding**

Compound	Structure	TRIM24 BRD IC_{50} (μM)	BRD4 IC_{50} (μM)
OXFBD02 (4)		N/A	0.295 ± 0.0430
SGC-34 (7)		1.98 ± 0.245	N/A
288		3.66 ± 0.408	0.546 ± 0.196
289		3.15 ± 0.778	2.18 ± 0.289
290		10.4 ± 2.04	2.68 ± 0.219

5.4 Preliminary Cellular Experiments

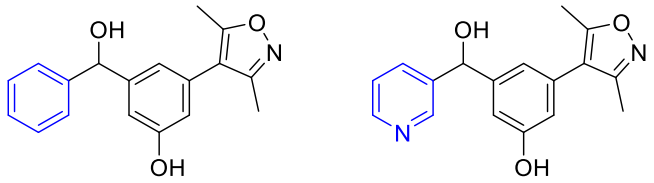
To assess BRD4 degradation, RKO cells were treated individually with PROTACs **288**, **289**, and **290** at 10 μM, and were incubated for 0, 4, 8, 16 or 24 hours, before harvesting at the indicated time points (performed by Anna Mandel in the group of Professor Ester Hammond). Western blots were performed to determine these PROTACs had an effect on the concentration of BRD4 in the cell. The concentration of Myc protein was also assessed, as BRD4 plays a key role in regulation of the expression of this oncogene.[182] It appeared that PROTACs **289** and **290** did not reduce the concentration of BRD4 in cells, which may be due to the incorrect orientation of the ternary complex for effective ubiquitination. However, the concentration of Myc was reduced. This does not indicate that BRD4 has been degraded, but does demonstrates BRD4 target engagement, as inhibition of the BRD4(1) bromodomain through binding to the BRD4 ligand moiety within the PROTACs would reduce Myc concentration.[95] On treatment of the cells with PROTAC **288**, a modest decrease in BRD4 concentration was observed after 4 hours, suggesting the formation of a productive ternary complex and ubiquitination of BRD4 by TRIM24. However, BRD4 levels recovered after 8 hours. Accumulation of BRD4 was observed after 24 hours, which has previously been observed for small molecule BRD4 bromodomain inhibition.[95,183] It was hypothesised that the rapid protein recovery could be due to metabolism of the BRD ligand moiety of the PROTAC. It was postulated that modifying the structure of the BRD4 ligand to reduce susceptibility to metabolism would allow for sustained BRD4 degradation, hence a new TRIM24-recruiting PROTAC was considered.

5.5 Design of PROTACs

Ligand **5** has been reported by Jennings *et al.*[38] to have a higher affinity for BRD4 compared to **OXFBD02 4** (IC_{50} = 166 nM compared to 382 nM for **OXFBD02 4**), and more desirable solubility and metabolic properties.[38] It was hypothesised that a PROTAC incorporating **5** would have improved physicochemical properties, and would have a longer half-life within the cell. Therefore, PROTAC **291** was designed, keeping all other aspects identical to **288** (**Figure 5.6**).

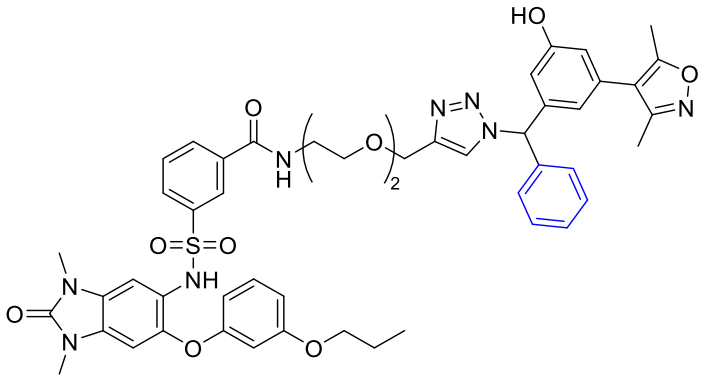
Since Oliver Stratton had only synthesised 1.22 mg of PROTAC **288**,[181] resynthesis was also required in larger quantities to perform experimental repeats.

A

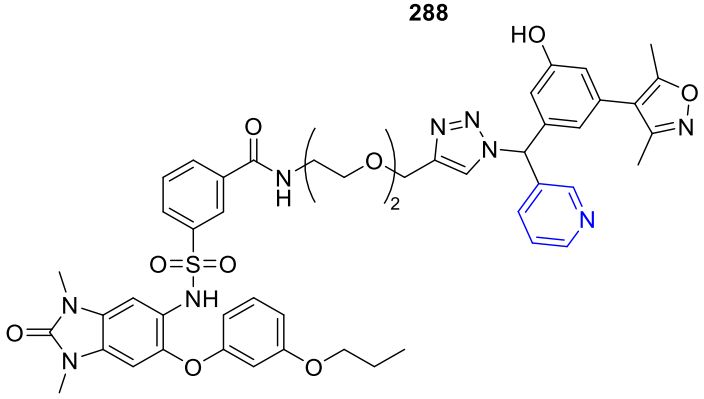


4 **5**

B



288



291

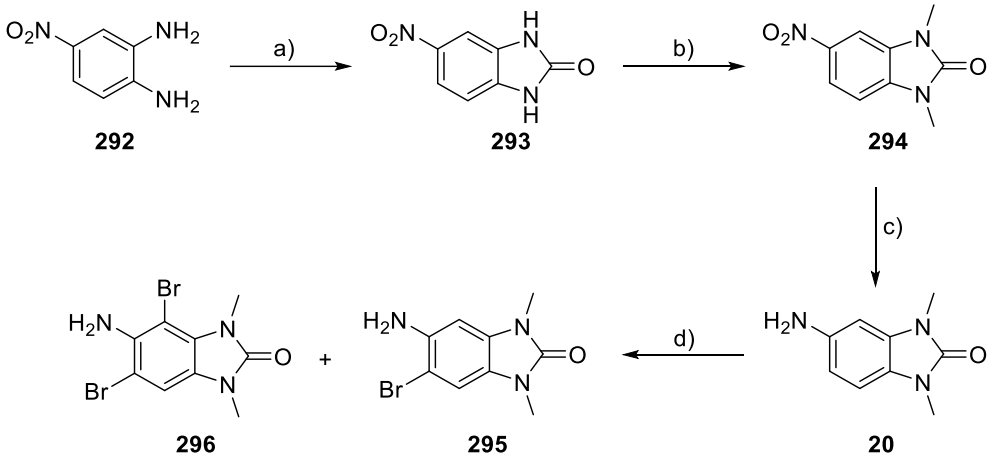
Figure 5.6. A. Structures of two BRD4 ligands, with **5** having more desirable metabolic properties. **B.** Structure of the promising PROTAC molecule **288** to be resynthesised, as well as the proposed PROTAC **291**.

5.6 Synthesis of TRIM24-recruiting PROTACs

5.6.1 Synthesis of TRIM24 Ligand

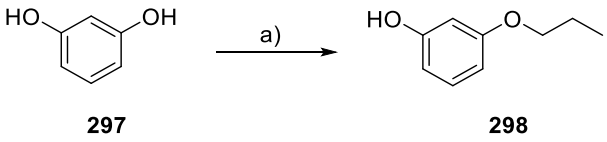
Synthesis of the TRIM24 ligand began as reported in the literature,[42] with a reaction between 4-nitrobenzene-1,2-diamine **292** and CDI to give the ring closed product **293** in 86–100% yields (**Scheme 5.1**). The head group was subsequently methylated using iodomethane to yield the benzimidazolone core **294**. A nitro group reduction to aniline **20** using palladium on carbon with

hydrogen proceeded with 90–100% yields, although the reaction rate was slow, requiring three days for complete conversion. The following step required a bromination *ortho* to the aniline to yield **295**. When the reaction was performed at 0 ºC, a small amount of dibromination product **296** was also observed along with desired product **295**. This side product formation was suppressed by performing the reaction at –20 ºC, giving desired product **295** in high yields.



Scheme 5.1. Synthesis of bromide **295**. *Reagents and conditions:* a) CDI, CH_2Cl_2, rt, 2 h, 86–100%, n = 3; b) MeI, K_2CO_3, DMF, rt, 16 h, 82–90%, n = 3; c) Pd/C, H_2, EtOAc, AcOH, rt, 72 h, 90–100%, n = 4; d) Br_2, AcOH, $CHCl_3$, –20 ºC, 30 min, 69–90%, n = 4.

The route then required the synthesis of phenol **298** to be a partner in the subsequent Ullmann coupling. This was completed by alkylation of 1,3-dihydroxybenzene **297** with 1-bromopropane, which proceeded with 87% yield (**Scheme 5.2**).

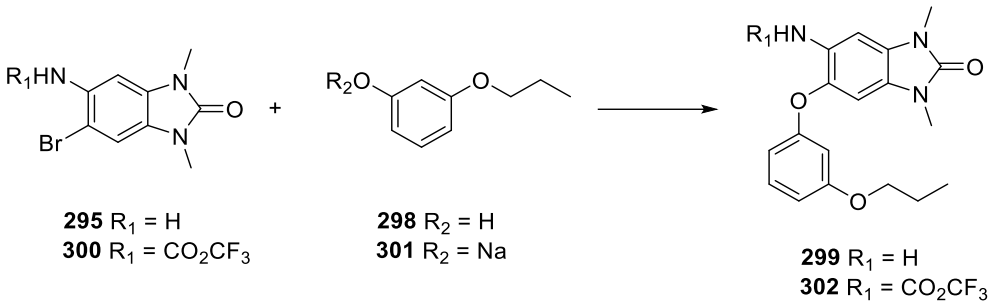


Scheme 5.2. Synthesis of phenol for Ullmann coupling. *Reagents and conditions:* a) 1-Bromopropane, K_2CO_3, DMF, 50 ºC, 18 h, 87%, n = 1.

The next step was a copper-mediated Ullmann coupling to form biaryl ether **299**. Oliver Stratton achieved low yields for this reaction, so to allow viable scale up, optimisation was necessary.[181] Initial conditions involved using copper(I) iodide as a catalyst and 8-hydroxyquinoline as a ligand, with a caesium carbonate base in diethylene glycol dimethyl ether, due to its polar, aprotic nature and high boiling point (**Entry 1**, **Table 5.2**). The copper catalyst and base were dried in an Abderhalden's drying pistol, and the solvent was dried over 3 Å molecular sieves for 48 h prior

to the reaction. However, after 72 h heating at 130 ºC under an argon atmosphere, only a very small amount of product was observed in the ^{1}H NMR spectrum of the crude material, with a large percentage of amine **295** and phenol **298** starting materials remaining. The second attempt involved degassing the diethylene glycol dimethyl ether using several freeze-pump-thaw cycles prior to use. This again led to recovery of starting materials **295** and **298**, and only a minimal amount of product observed (**Entry 2**, **Table 5.2**). Copper(I) chloride and tripotassium phosphate were also investigated (**Entry 3**, **Table 5.2**), but only starting materials were recovered and no product was observed. It was considered that the reaction may be hindered by the aniline moiety in **295**, which may be competing with 8-hydroxyquinoline in the formation of the active catalyst. This group was protected with a trifluoroacetic acid group (**300**), and the coupling was attempted under the original conditions with copper(I) iodide and caesium carbonate (**Entry 4**, **Table 5.2**). Similarly, no product was seen from this reaction. It was decided that pre-deprotonation to form the sodium phenoxide **301** may increase the reaction rate, so phenol **298** was stirred for 1 h with sodium hydroxide in methanol and concentrated. The crude phenoxide **301** was dried under high vacuum for 48 h prior to use. The reaction was then performed with the original conditions and protected amine **300**. Fortunately, a 47% yield of desired product **302** was achieved (**Entry 5**, **Table 5.2**). Due to the increased number of synthetic steps required to protect and subsequently deprotect the amine moiety in **302**, the reaction was tried with free aniline **295**, which proceeded with a higher yield of 81% (**Entry 6**, **Table 5.2**). These were then selected as the conditions of choice for reaction scale up.

Table 5.2. Optimisation of the Ullmann coupling reaction required for synthesis of biaryl ether **299**.



295 R_1 = H
300 R_1 = CO_2CF_3

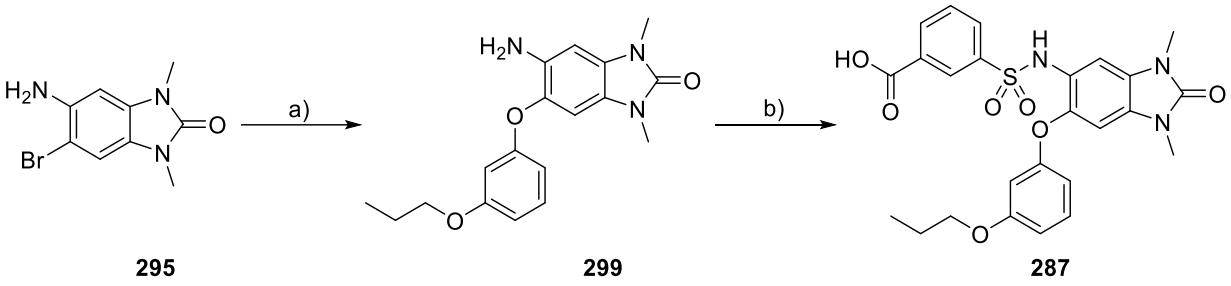
298 R_2 = H
301 R_2 = Na

299 R_1 = H
302 R_1 = CO_2CF_3

Entry	Starting material	Solvent degassed?	Conditions	Result
1	**295** and **298**	No	CuI, Cs_2CO_3, (both dried in an Abderhalden's drying pistol), 8-hydroxyquinoline, diethylene glycol dimethyl ether, (dried over 3 Å molecular sieves), argon atmosphere, 130 ºC, 72 h	Small amount of product observed *via* ^{1}H NMR of the crude material (>4% yield), but could not be recovered
2	**295** and **298**	Yes	CuI, Cs_2CO_3, (both dried in an Abderhalden's drying pistol), 8-hydroxyquinoline, diethylene glycol dimethyl ether, (dried over 3 Å molecular sieves), argon atmosphere, 130 ºC, 72 h	Small amount of product observed *via* ^{1}H NMR spectrum of the crude material (>5% yield), but could not be recovered
3	**295** and **298**	Yes	CuCl, K_3PO_4, (both dried in an Abderhalden's drying pistol), 8-hydroxyquinoline, diethylene glycol dimethyl ether, (dried over 3 Å molecular sieves), argon atmosphere, 130 ºC, 72 h	No product observed, starting material recovered
4	**300** and **298**	Yes	CuI, Cs_2CO_3, (both dried in an Abderhalden's drying pistol), 8-hydroxyquinoline, diethylene glycol dimethyl ether, (dried over 3 Å molecular sieves), argon atmosphere, 130 ºC, 72 h	No product observed, starting material recovered
5	**300** and **301**	Yes	CuI, Cs_2CO_3, (both dried in an Abderhalden's drying pistol), 8-hydroxyquinoline, diethylene glycol dimethyl ether, (dried over 3 Å molecular sieves), argon atmosphere, 130 ºC, 72 h	47% yield of desired product
6	**295** and **301**	Yes	CuI, Cs_2CO_3, (both dried in an Abderhalden's drying pistol), 8-hydroxyquinoline, diethylene glycol dimethyl ether, (dried over 3 Å molecular sieves), argon atmosphere, 130 ºC, 72 h	81% yield of desired product

Once the Ullmann coupling had been optimised, the final step to complete the synthesis of the TRIM24 ligand required sulfonamide formation with 3-(chlorosulfonyl)benzoic acid to yield

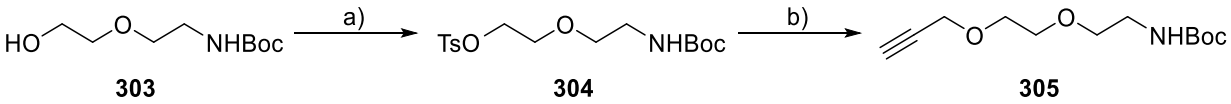
desired product **287** (**Scheme 5.3**). The moderate yields were attributed to difficulty during purification. The carboxylic acid group at the *meta*-position of the sulfonamide could then act as a functional handle ready for linker attachment.



Scheme 5.3. Final steps to synthesise TRIM24 ligand **287**. *Reagents and conditions:* a) Sodium (3-propoxy)phenoxide, CuI, Cs_2CO_3, 8-hydroxyquinoline, diethylene glycol dimethyl ether, 130 ºC, 72 h, 66–81%, n = 2; b) 3-(Chlorosulfonyl)benzoic acid, pyridine, CH_2Cl_2, rt, 16 h, 46%, n = 1.

5.6.2 Synthesis of Linker

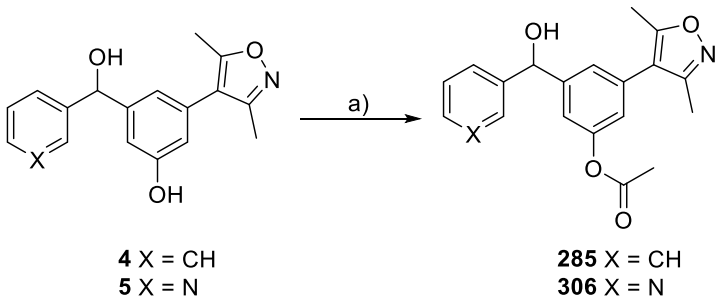
The synthesis of the PEG linkers with the appropriate functional handles proceeded as in the literature.[184] Oliver Stratton had previously attempted the reaction of **303** with propargyl bromide but observed no product formation. Instead, following his successful procedure, Boc-protected **303** was tosylated to convert the alcohol into a good leaving group, giving **304** in a 95% yield (**Scheme 5.4**). This compound **304** was then subjected to a displacement with propargyl alcohol, which proceeded in a 33% yield to give linker **305**, ready for attachment to the two ligands.



Scheme 5.4. Synthesis of PEG linker **305** with appropriate functional handles. *Reagents and conditions:* a) TsCl, NEt_3, CH_2Cl_2, rt, 2 h, 95%, n = 1; b) NaH, propargyl alcohol, THF, 50 ºC, 5 h, 33%, n = 1.

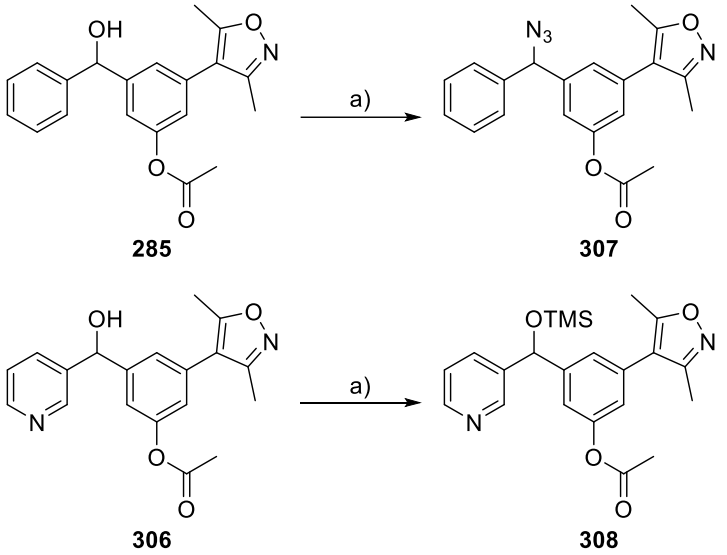
5.6.3 Synthesis of PROTACs

To complete the PROTAC synthesis, BRD4 ligands **4** and **5** were required, which were provided by Michael Platt. From here, the phenolic alcohol was protected with an acetyl group, which proceeded in high yields for both the phenyl **285** and pyridyl **306** compounds (**Scheme 5.5**).



Scheme 5.5. Protection of phenols. *Reagents and conditions:* a) Acetic anhydride, NaOH, IPA, rt, 1 h, **285**: 74%, n =1, **306**: 88%, n = 1.

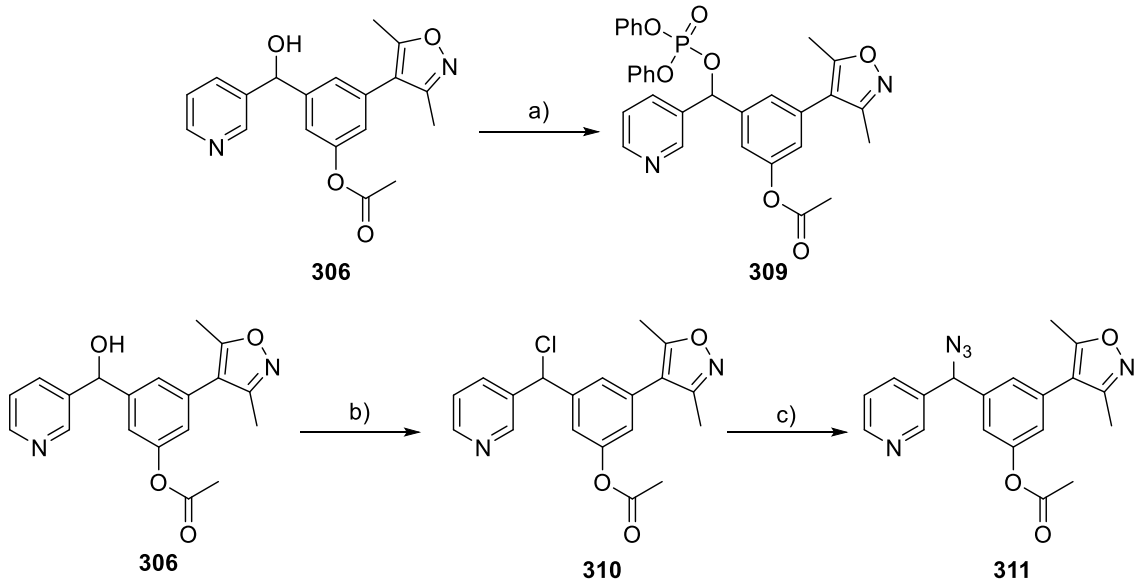
The next step required installation of an azide functionality. The copper-catalysed method allowed direct conversion of the benzylic alcohol to an azide through the formation of the stabilised carbenium ion. The reaction proceeded in high yields of 61–80% to give phenyl-containing **307** (**Scheme 5.6**). However, for pyridyl-containing **306**, the only observed product was the trimethylsilane-protected **308**, even after further addition of copper(II) triflate and azidotrimethylsilane. This result could be due to co-ordination of the pyridyl nitrogen with the copper(II) triflate, disrupting the catalytic cycle.



Scheme 5.6. Formation of azide **307** proceeded with good yields, but under the same reaction conditions, **308** was formed with no desired azide product observed. *Reagents and conditions:* a) $Cu(OTf)_2$, $TMSN_3$, CH_2Cl_2, 40 ºC, 22 h, **307**: 61–80%, n = 2, **308**: 62% of TMS-protected product, n = 1.

An alternative strategy was investigated to form the azide at the benzyl position of pyridyl-containing **306**, using diphenylphosphoryl azide (DPPA) as the azide source and leaving group, and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base. The reaction was stirred for 16 h at room temperature, however, there was no desired product formed. The only isolated compound

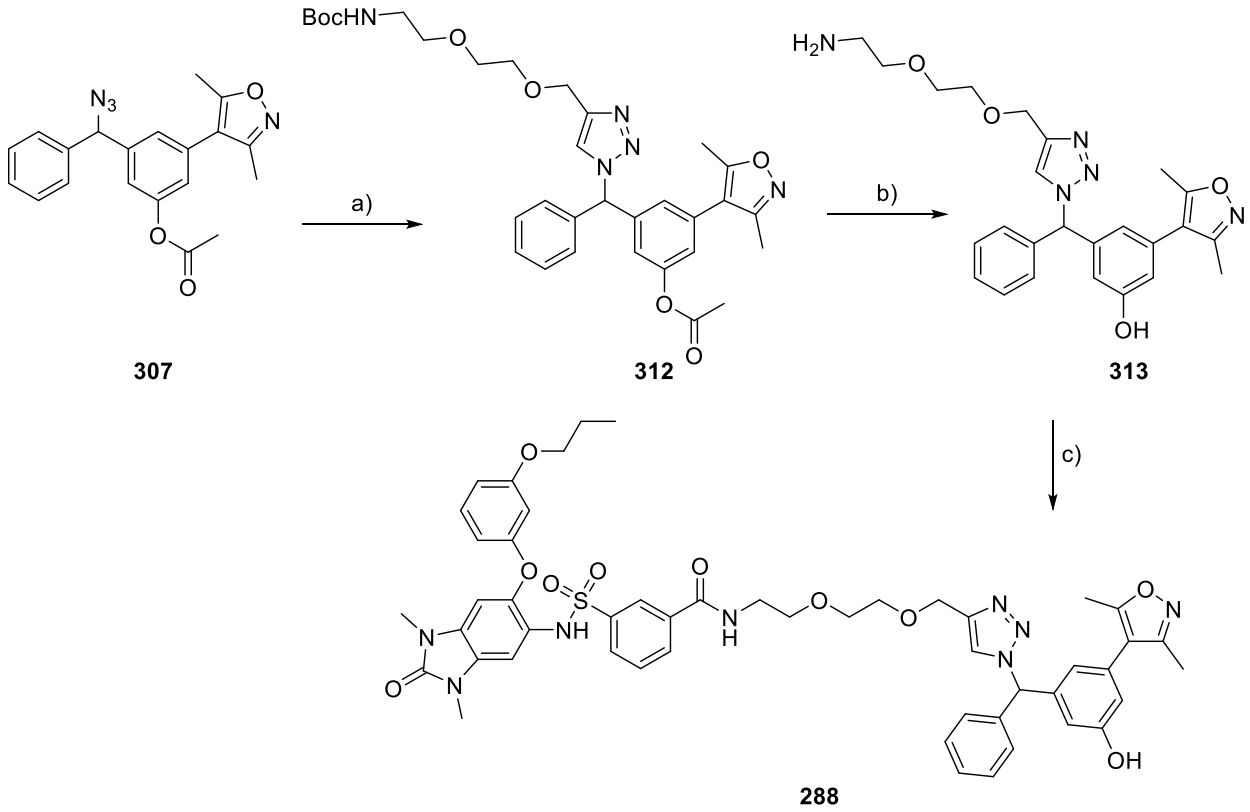
appeared to be the initial product **309** of the substitution between the benzylic alcohol and DPPA (**Scheme 5.7**). The reaction was repeated, instead heating the reaction mixture to 60 ºC for 72 h to facilitate the substitution reaction between **309** and the azide anion. However, again this only gave the initial substitution product **309**. It was decided that an alternate method involving two steps, in which the benzylic alcohol is initially converted into a suitable leaving group, should be used. Thionyl chloride was utilised to produce chloride **310**, which proceeded in 100% yield. This was followed by addition of sodium azide, forming the desired product **311**.



Scheme 5.7. Synthesis of azide **311**. *Reagents and conditions:* a) Attempt 1: DPPA, DBU, toluene, rt, 16 h, Attempt 2: DPPA, DBU, toluene, 60 ºC, 72 h; b) $SOCl_2$, benzene, rt, 1 h, 100%, n = 1; c) NaN_3, DMF, 40 ºC, 4 h, 56%, n = 1.

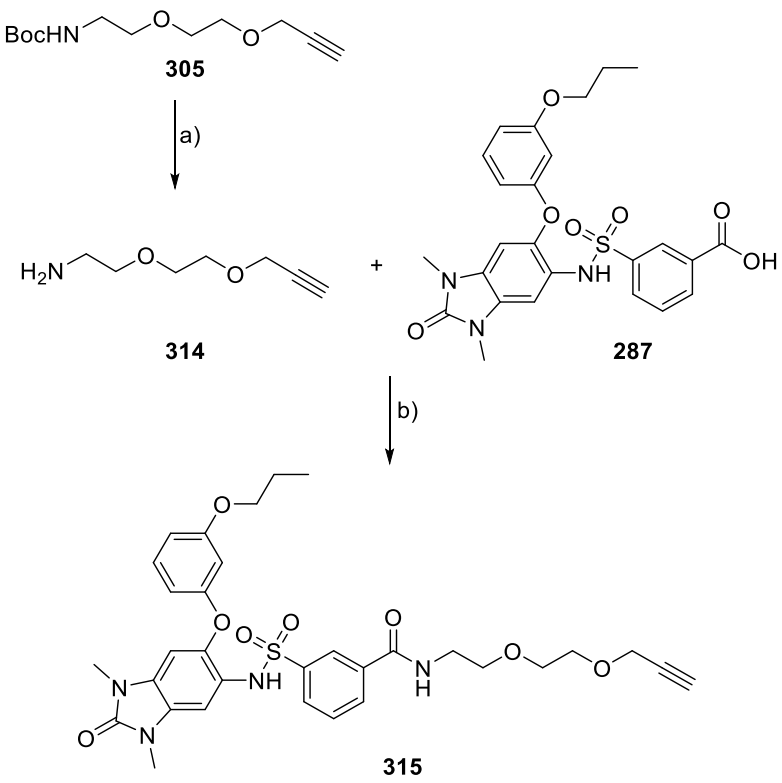
Having synthesised the two desired azides **307** and **311**, the order of attachment of the linker to the BRD4 and TRIM24 ligands was considered. The synthetic route performed by Oliver Stratton first attached the linker to the BRD4 azide through click reaction, which gave 83% yield (**Scheme 5.8**). Subsequent Boc-deprotection of the amine using a methanolic hydrochloric acid solution also allowed methanolysis of the acetyl group, revealing the phenol. However, the following amide coupling to the TRIM24 ligand gave very low yields of 16% with difficult purification, due to the formation of homo-coupled side products, and interference of the free phenol coupling to the acid to form the ester product. It was thought that altering the Boc-

deprotection conditions to prevent acetyl removal and therefore reduce side product formation in the subsequent amide coupling may lead to problems of acetyl migration on to the free amine.



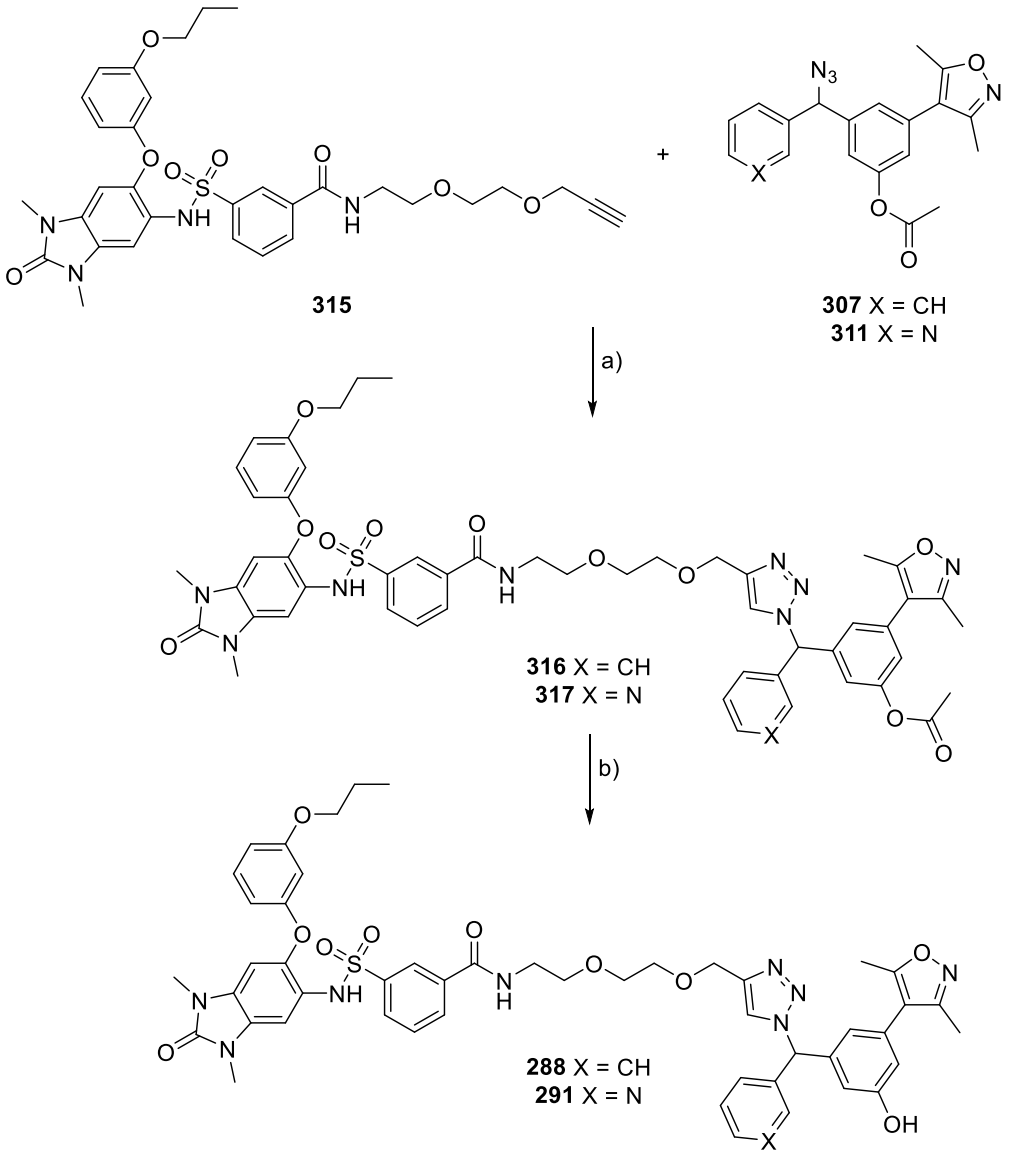
Scheme 5.8. Final synthetic steps to **288** performed by Oliver Stratton, leading to a low final yield and difficult purification. *Reagents and conditions:* a) Linker **305**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF, H_2O, rt, 22 h, 83% n = 1; b) 0.2 M HCl in MeOH, rt, 48 h, 59%, n = 1; c) HATU, DIPEA, DMF, rt, 22 h, 16% n = 1.

For this reason, it was decided that the amide coupling between the linker and the TRIM24 ligand should be performed first, followed by the click reaction and subsequent acetyl deprotection. Boc-deprotection of linker **305** gave 98% yield of desired amine **314** (**Scheme 5.9**). The subsequent amide coupling step with the TRIM24 ligand **287** initially proceeded with a yield of 26% due to the formation of homo-coupled side products, presumably coupling through the sulfonamide nitrogen. It was hypothesised that this occurred during the pre-stirring of HATU and DIPEA with **287** prior to adding amine **314**. The reaction was repeated without the pre-stirring step, adding all the reagents simultaneously. This gave a much higher yield of 75%, with no homo-coupling products observed.



Scheme 5.9. Coupling of the linker **305** to TRIM24 ligand. *Reagents and conditions:* a) 4 M HCl in dioxane, rt, 1 h, 98% n = 1; b) HATU, DIPEA, DMF, rt, 5 h, 26–75%, n = 2.

Subsequently, azides **307** and **311** could be attached to the alkyne functionality in **315** through a copper-mediated click reaction (**Scheme 5.10**). This reaction gave an excellent yield of 93% for phenyl-containing **316**, whereas a moderate yield of 34% was achieved for pyridyl-containing **317**. This lower yield could have been a consequence of stringent washing of the organic components with saturated aqueous EDTA solution to ensure removal of all copper, with the more polar pyridyl **317** being pulled into the aqueous layer. The final step for both PROTACs required removal of the acetyl group, which proceeded with high yields of 74–78%.



Scheme 5.10. Final steps to give PROTACs **288** and **291**. *Reagents and conditions:* a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF, H_2O, rt, 22 h, **316**: 93%, n = 1, **317**: 34%, n = 1; b) LiOH, EtOH, H_2O, rt, 1 h, **288**: 74%, n = 1, **291**: 78%, n = 1.

5.7 Evaluation of PROTACs

5.7.1 AlphaScreen™

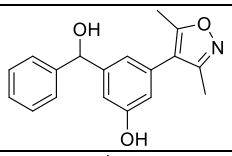
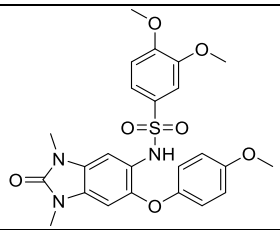
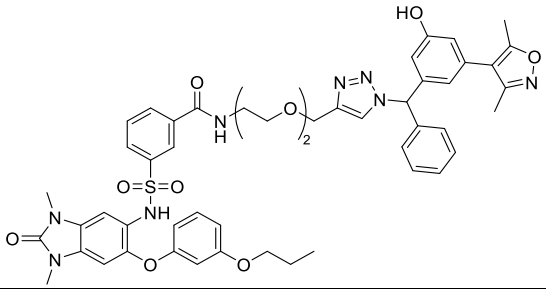
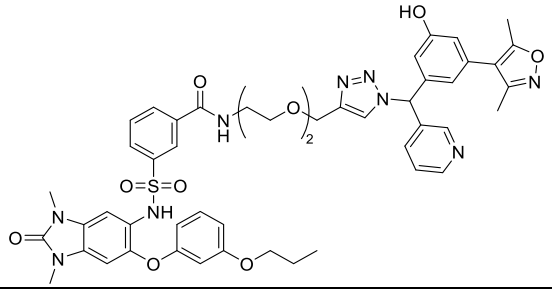
Following the resynthesis of **288** and novel synthesis of **291**, affinity for both BRD4 and TRIM24 were assessed using a dose-response AlphaScreen™. The results are shown in **Table 5.3**. As with the previous AlphaScreen™, the $H3_{1-27}K18Ac$ peptide was used as a bromodomain binding partner for TRIM24, and the $H4(KAc)_4$ peptide was used as partner for BRD4(1). Compounds **OXFBD02 4** and **SGC-34 7** were used as positive controls for BRD4(1) and TRIM24, respectively. As before, the results showed that, in general, binding to both BRD4(1) and TRIM24

was maintained. The **SGC-34 7** control had an affinity for TRIM24 (IC_{50} = 527 nM) closer to the reported literature value (IC_{50} = 222 nM)[41] than was seen in the previous AlphaScreen™. A fresh stock solution of this compound was made just before the assay was performed which could indicate that the previous stock solution was beginning to degrade. The resynthesised phenyl PROTAC **288** had a similar affinity for TRIM24 (IC_{50} = 1.27 µM) to that seen in the previous assay (IC_{50} = 3.66 µM), and the pyridyl PROTAC **291** bound with a higher affinity (IC_{50} = 264 nM) than both **288** and positive control **7**. However, all three IC_{50} curves did not have the ideal plateau at the beginning and end of the dose-response curves, indicating that the values produced will not be 100% accurate and the assay requires some optimisation.

The pyridyl PROTAC **291** had a lower affinity (IC_{50} = 1.75 µM) for BRD4(1) compared to **OXBD02 4** (IC_{50} = 250 nM), but still bound with a reasonable affinity for PROTAC target engagement. The phenyl PROTAC **288** appeared to have a lower affinity for BRD4(1) (IC_{50} = 6.36 µM) compared to the previous AlphaScreen™ (IC_{50} = 546 nM). However, the raw data for BRD4(1) contained several anomalous values which had to be excluded from the final calculations, so this assay should be repeated in the future. Despite this, the data confirmed that both the phenyl **288** and pyridyl **291** PROTACs still bind with reasonable affinity to BRD4(1) and TRIM24, thus assessment of permeability and degradation could be pursued.

Table 5.3. A table showing IC_{50} values (μM) for PROTACs **288** and **291** determined using a dose-response AlphaScreen™. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. The colour scale is shown below, with low IC_{50} values (stronger binding) shown in red, and high IC_{50} values (weaker binding) shown in green. BRD4 results shown using the $H4(KAc)_4$ peptide as a binding partner, and TRIM24 results shown using the $H3_{1–27}K18Ac$ peptide as a binding partner.

Weaker binding — **Stronger binding**

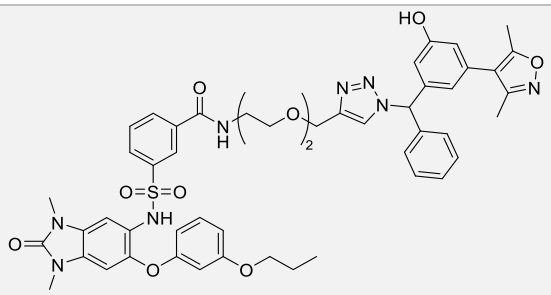
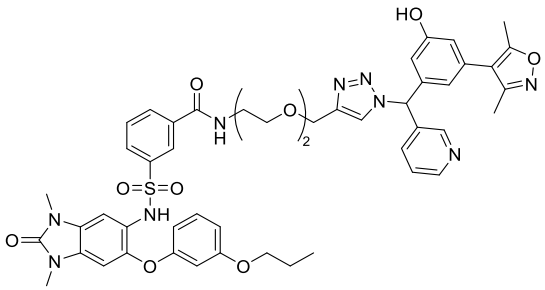
Compound	Structure	TRIM24 BRD IC_{50} (μM)	BRD4 IC_{50} (μM)
OXFBD02 (4)		N/A	0.250 ± 0.0190
SGC-34 (7)		0.527 ± 0.147	N/A
288		1.27 ± 0.374	6.36 ± 1.93
291		0.264 ± 0.0320	1.75 ± 0.198

5.7.2 Cell Permeability

The cell permeability assay was performed by Dr Hannah Bolland in the group of Professor Ester Hammond. An RKO cell line was treated with phenyl and pyridyl PROTACs **288** and **291**, and incubated for 24 hours. The cells were then lysed, and the lysate was analysed using HPLC to determine if the PROTAC was present inside the cell. It was confirmed that both pyridyl **291** and phenyl **288** were present in the cell in concentrations of 33.3 mM and 35.6 mM, respectively (**Table 5.4**). However, this data contradicted the hypothesis that the metabolic instability of

phenyl PROTAC **288** led to the recovery of BRD4 in cells after 8 hours, as **288** appears in a high cellular concentration even after 24 hours. Thus, it was decided that degradation assessment of these compounds was necessary to investigate the rapid BRD4 recovery.

Table 5.4. Cell permeability data for PROTACs **288** and **291** in an RKO cell line. cLogD values in blue are predicted for pH 7.4 using ACD/I-Lab 2.0,

Compounds	Moles in 4.5×10^5 cell extract	Moles/cell	Approximate concentration in cell (assuming 1 pL volume)
 **288** cLogD = 7.41	15 nmoles	33.3 fmoles	33.3 mM
 **291** cLogD = 6.44	16 nmoles	35.6 fmoles	35.6 mM

5.7.3 Degradation Assessment by Western Blot

RKO cells were treated individually with **288** and **291** at 10 µM, and were incubated for 0, 2, 4, 6, 8, 16 or 24 hours, before harvesting at the indicated time points (performed by Dr Hannah Bolland). Western blots were performed to determine if the PROTACs had an effect on the concentration of BRD4 in the cell. β-Actin was used as the loading control to confirm that the samples have been loaded equally into each well. Incubation at 0 h acted as a control for the normal BRD4 expression levels in the RKO cell line. Protein expression levels were quantified using the ImageStudio (LICOR) software, normalised relative to the β-actin loading control and expressed as relative fold change over 0 hour time points.

Degradation assessment of PROTAC **288** was repeated with the resynthesised compound, which showed a decrease in concentration of BRD4 after 2 hours, and recovery after 4 hours (**Figure 5.7**). This was similar to the results seen in the preliminary experiments. However, as the initial concentration of BRD4 in the RKO cells is low, any slight change in protein level may appear significant, and should be interpreted with caution. The experiment should be repeated in a MCF7 breast cancer cell line, in which a high level of BRD4 is expressed, therefore allowing clearer interpretation of observed degradation. Again, 24 hours after treatment of **288**, the concentration of BRD4 increased to a much greater level than was present at t = 0. As previously discussed, the increased concentration of BRD4 after 24 hours is commonly observed on treatment of cells with BRD4 inhibitors.[95,183]

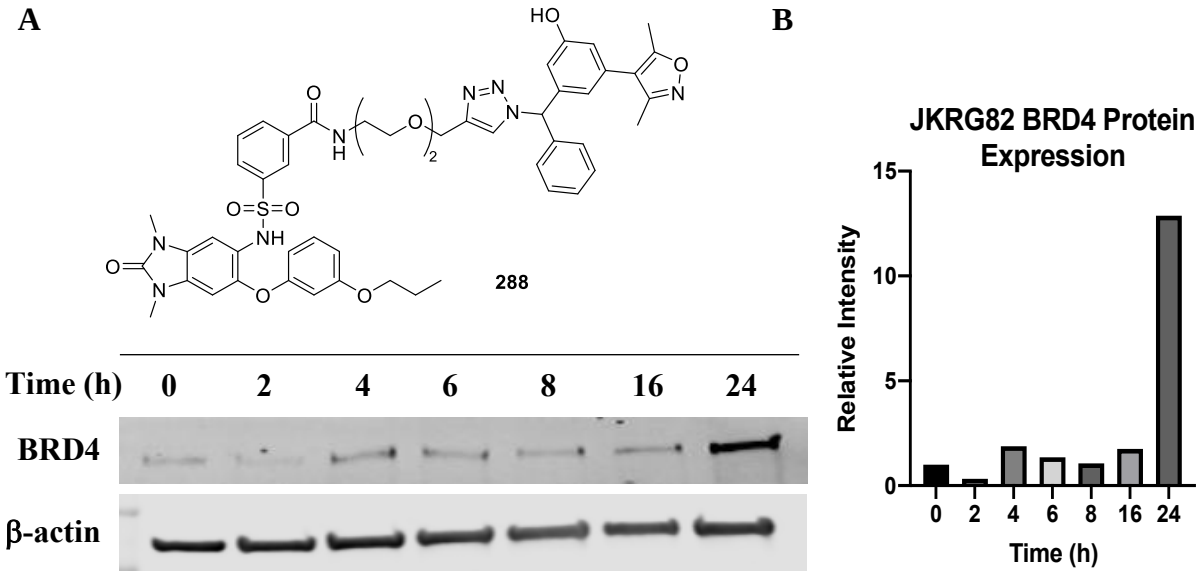


Figure 5.7. A. Time course Western blot of PROTAC **288** degrading BRD4 in RKO cells. Experiment performed by Dr Hannah Bolland, n = 1. **B.** Quantification of degradation relative to t = 0.

Similarly, PROTAC **291** was assessed for its ability to degrade BRD4 (**Figure 5.8**). It appeared that degradation was not observed after 2 hours as with **288**, instead showing varying concentrations of BRD4 until 8 hours, with a small reduction of BRD4 levels relative to t = 0 after 16 hours. Subsequently, a higher concentration of BRD4 was observed after 24 hours. Again, caution should be taken with the interpretation of small changes in protein concentrations, as the initial BRD4 concentration in the RKO cells is low.

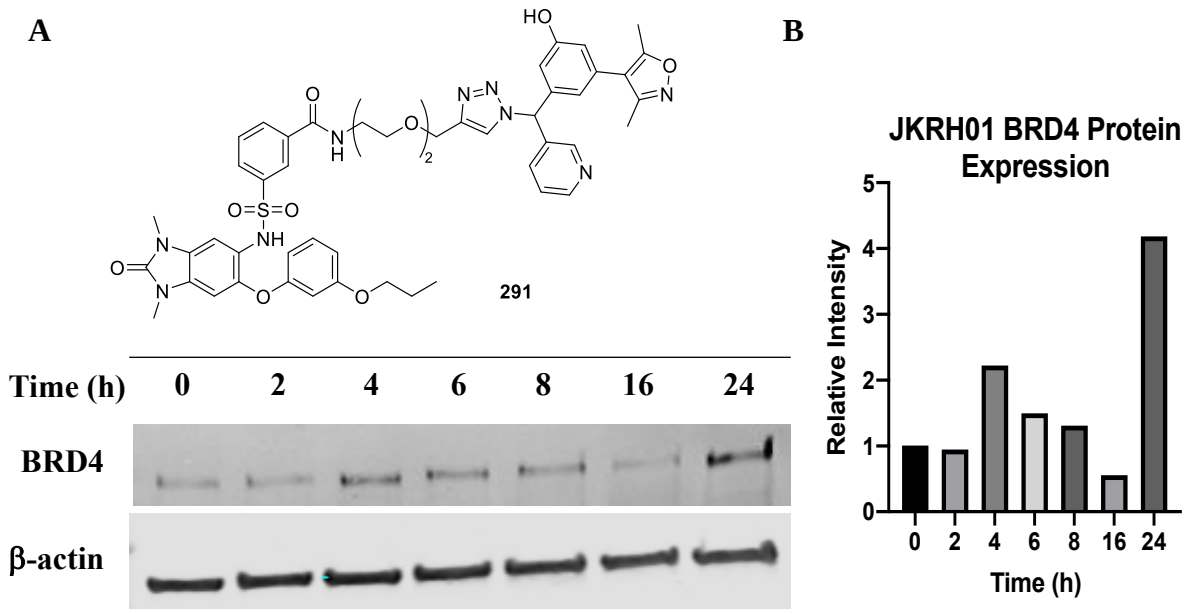


Figure 5.8. A. Time-dependent Western blot of PROTAC **291** degrading BRD4 in RKO cells. Experiment performed by Dr Hannah Bolland, n = 1. **B.** Quantification of degradation relative to t = 0.

The two PROTACs evaluated showed some potential degradation of BRD4, however, further biological testing is required to confirm these results, as varying protein concentrations were observed. As the concentration of both PROTACs remained high in the cells even after 24 hours of incubation, the rapid recovery of BRD4 when treated with **288** may be a consequence of the hook effect, where high PROTAC concentration in the cell leads to the binary complex outcompeting the ternary complex, hence the observed increase in BRD4 levels. Therefore, an assay varying the concentration of the PROTACs should be performed. A negative control would be also essential to determine if there is a natural time-dependent variance of BRD4 levels within the cell unrelated to PROTAC degradation. Due to time constraints, repeats of the time-dependent degradation experiments for **291** were not performed, hence this should be a priority for further studies. Furthermore, conducting these experiments in a cell line expressing high levels of BRD4, for example MCF7, should allow for more meaningful interpretation of changing protein concentration.

5.8 Summary and Conclusions

Context-dependent degradation could be beneficial to biological studies and therapeutics, as a protein recruited for E3 ligase activity that is overexpressed in cancer cell lines has the potential

to selectively degrade in these specific cancer environments. TRIM24 was selected as an E3 ligase in the design of a PROTAC series aiming to degrade BRD4, due to its observed overexpression in many cancers,[26,73–76] and the minimal reported effects of TRIM24 bromodomain inhibition.[72] Preliminary findings suggested that PROTAC **288**, incorporating BRD4 ligand **OXFBD02 4** and TRIM24 ligand **287**, had the potential to partially degrade BRD4 after 4 hours. However, BRD4 production recovered rapidly. It was hypothesised that this recovery was due to metabolic clearance of PROTAC **288**. A PROTAC was designed to incorporate pyridyl-containing BRD4 ligand **5**, which has increased solubility and metabolic stability, to determine if sustained degradation could be achieved. The same linker type and length as PROTAC **288** were included, thus a direct comparison could be made between the two.

The synthetic route to phenyl PROTAC **288** was optimised, as the previous route followed by Oliver Stratton gave very low yields. Slight modifications were made to the route for the synthesis of pyridyl PROTAC **291**, as the copper-mediated conversion of alcohol **306** to the azide **311** did not yield the desired product, thus a two-step approach to this transformation was pursued. Once syntheses of PROTACs **288** and **291** were completed, an AlphaScreen™ was performed, which confirmed that both **288** and **291** bind to TRIM24 and BRD4. Subsequent assessment of the cell permeability determined that both PROTACs were present in the cell after a 24 hour incubation period, although this result opposed the original hypothesis that metabolic instability of phenyl-containing **288** led to rapid BRD4 recovery in cells. Finally, the degradation of BRD4 was evaluated. The results for phenyl PROTAC **288** showed a reduction in BRD4 concentration after 2 hours, which was recovered after 4 hours. BRD4 concentration varied on exposure to pyridyl PROTAC **291**, with a slight decrease relative to t = 0 after 16 h. However, due to low initial BRD4 concentrations in the RKO cell lines, and a lack of experimental repeats, convincing conclusions have not yet been reached.

If PROTAC-mediated degradation of BRD4 by **288** and **291** could be confirmed through further repeats and experiments, TRIM24 could be validated as a novel E3 ligase for use in PROTAC technology, which would expand the toolbox for further PROTAC development. In the future,

the experiments should be repeated in an MCF7 cell line, as high levels of BRD4 are expressed, making interpretation of degradation easier. An assay varying the concentration of the PROTACs should be performed to determine if the hook effect is responsible for the modest BRD4 degradation observed. A focus should also remain on exploring variation of linker length and type to optimise the protein–protein interactions within the ternary complex and maximise BRD4 degradation. A negative control should also be explored, perhaps performing the same experiment in a cell line where TRIM24 has been knocked down to ensure degradation is dependent on TRIM24. Finally, context-dependent degradation should be assessed to determine if selective degradation in cancer cells can be achieved.

Chapter 6 Conclusions and Future Work

6.1 Conclusions

The aim of this study was to develop tool compounds to probe the biological role of TRIM33 in epigenetic regulation, with the intention of validating this protein as a therapeutic target in situations where DNA damage is increased. To achieve this, two approaches were considered; targeting the bromodomain with small molecule ligands, and degrading the whole protein in cells using proteolysis-targeting chimeras. Previous work led to the identification of the benzimidazolone-containing small molecule **16** which was observed to bind to the bromodomain of TRIM33, and TRIM24 (**Figure 6.1**). Synthesis of probe molecules revealed the importance of the ionic interaction between the protonated benzylic amine of the ligand and E981 within the KAc binding pocket of TRIM33. In TRIM24, A923 occupies the same space as E981, which indicates this ionic interaction could be the gateway to selectivity between these similar bromodomains. The hydrogen bond between the acetyl-lysine-mimicking head group of the ligand and N1039 also appeared to be a key interaction for guiding the ligand in the bromodomain.

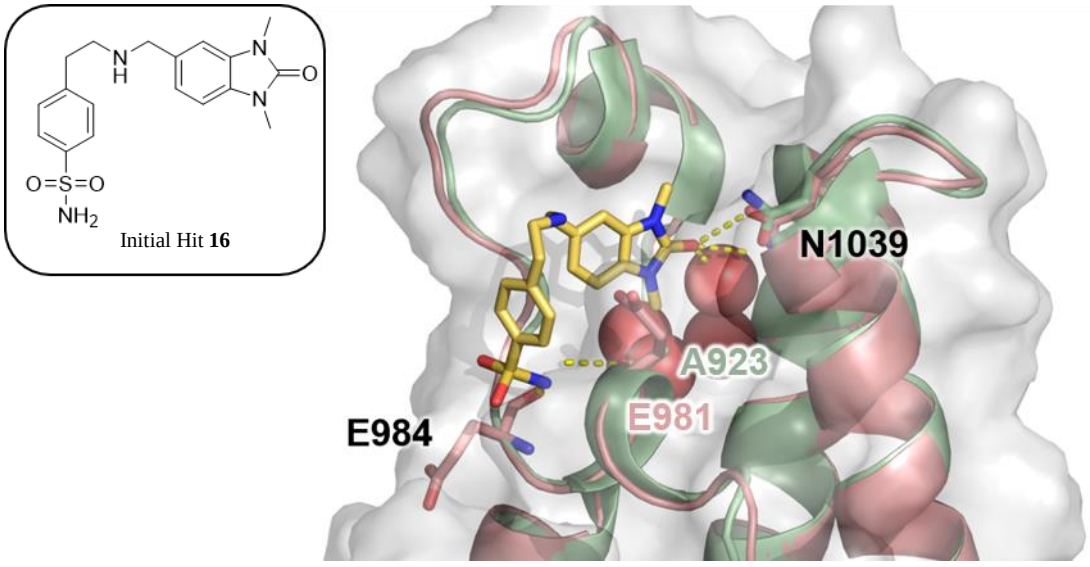


Figure 6.1. Compound **16** docked into TRIM24 (green, PDB ID: **3O33**) and TRIM33β (pink, PDB ID: **5MR8**) using CLC Drug Discovery Workbench 3.0. The key E981 residue in TRIM33β occupies the same space as the A923 residue in TRIM24, presenting an opportunity for selectivity.

The benzylic amine **21** bound to the TRIM33β bromodomain with an IC_{50} value of 31 μM, and no longer showed binding to the TRIM24 bromodomain (**Figure 6.2**). On extension of the amine chain and addition of a terminal dimethylated amine (**25**), an increase in affinity for TRIM33β was observed, and selectivity over TRIM24 was maintained. This ligand served as a useful starting point for SAR studies.

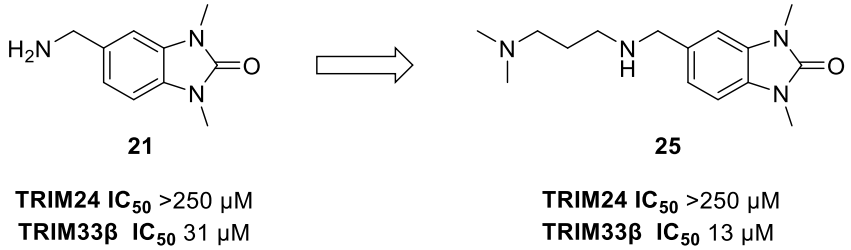


Figure 6.2. Development of benzylic amine fragment **21** to longer chain amine **25**. IC_{50} values were ascertained using a dose-response AlphaScreen™ with the bromodomain-binding $H3_{1–27}$K18Ac peptide.

Chapter 2 explored SAR studies to improve the affinity of the ligand for TRIM33, while maintaining selectivity over the TRIM24 bromodomain. A dose-response AlphaScreen™ was performed to evaluate the activity of the ligands, with WaterLOGSY NMR experiments providing an orthogonal method to confirm binding. There are two isoforms of TRIM33, α and β. It had been previously established that N1039, the residue responsible for recognising acetylated lysine residues, pointed out of the binding site in TRIM33α, indicating a non-functional bromodomain Therefore, these studies focused on the β isoform of TRIM33. Initially, alternative acetyl-lysine-mimicking head groups were investigated. However, both the triazolopyridine and the dihydroquinoxalinone head groups negated binding to both isoforms of TRIM33, and it was hypothesised that these changes had led to a sub-optimal orientation of the head group relative to N1039, disrupting the interaction of the benzylic amine with E981. Exploration of the hydrophobic region above the KAc binding pocket through lipophilic extension of the head group, sulfonamide projection adjacent to the benzylic amine, and projection of small groups from the alternative vector of the core, all reduced affinity for TRIM33β (**Figure 6.3**). It was hypothesised that these groups are either making no permanent interactions with the targeted residues I990 and V986, or disrupting a side-on π-π interaction between Y993 and F1038 in the TRIM33β bromodomain. Computational studies, performed by Dr Joe Bluck, investigating the binding site

water network suggested that a water molecule in the ZA channel was not favourable to displace, which supported the hypothesis that E984 was not involved in binding, and the two amines present in the ligand were forming a 'pincer' interaction around E981. A series of amine chains were therefore designed to bias this 'pincer' interaction, which led to the development of the highest affinity ligands **114** and **121** for TRIM33β, with IC_{50} values of 1.62 µM and 4.52 µM, respectively (**Figure 6.3**). Isothermal titration calorimetry was performed to determine K_D values for the highest affinity ligands, confirming the series as the first low micromolar affinity set of TRIM33 bromodomain ligands. These ligands provide a basis for development of further probe molecules and potential clinical drug candidates.

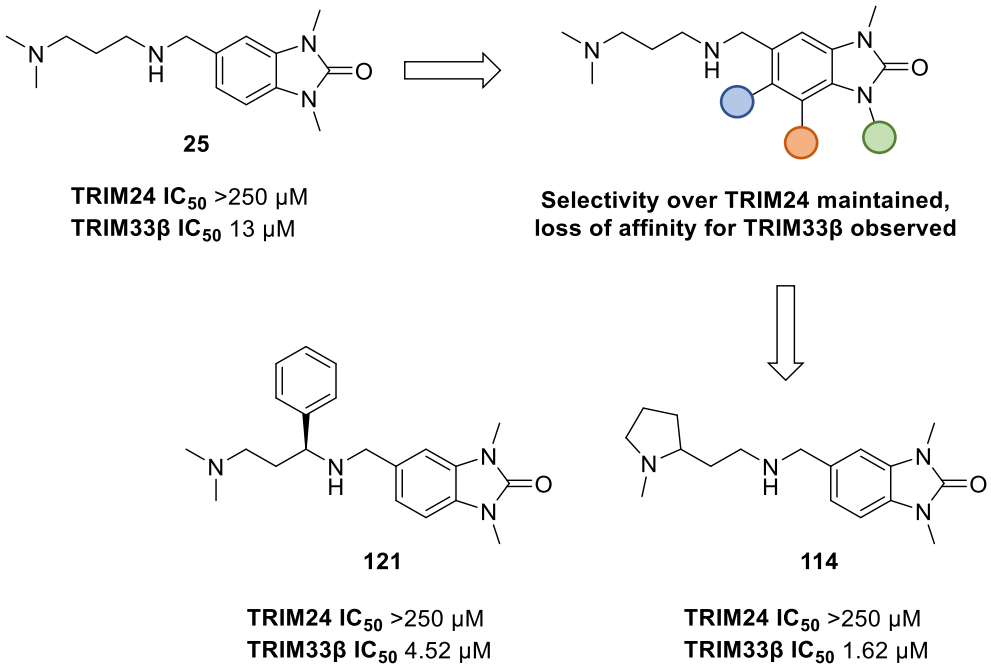


Figure 6.3. Exploration of the hydrophobic region above the KAc binding site led to a reduction in affinity for the TRIM33β bromodomain. The coloured circles indicate the three vectors explored through the projection of lipophilic groups. Subsequent amine chain investigation led to discovery of two low micromolar affinity ligands **114** and **121**. IC_{50} values were ascertained using a dose-response AlphaScreen™ with the bromodomain-binding $H3_{1–27}K18Ac$ peptide.

In 2018, a patent was published describing the discovery of high affinity TRIM33 bromodomain ligands, with IC_{50} values established using AlphaScreen™. Synthesis and evaluation of these ligands using AlphaScreen™ revealed high levels of assay interference, and assessment using ITC and WaterLOGSY as orthogonal assays showed that the compounds did not bind to either isoform of TRIM33.

In Chapter 3, the bromodomain ligand was incorporated into a series of PROTAC molecules, recruiting either VHL or cereblon as the E3 ubiquitin ligase, aiming to degrade the whole TRIM33 protein in cells. Various linker types and lengths were explored, including alkyl, PEG and triazole-containing linkers, to create a variety of potential ternary complexes. Attachment to the TRIM33 bromodomain ligand was through either an amine or amide bond. Synthetic routes to each PROTAC type were developed, and a total of 17 PROTACs were synthesised for biological evaluation. ITC was performed to determine binding to TRIM33 had not been significantly affected. Due to time and protein constraints, only three PROTACs were assessed. It appeared that the affinities of two VHL-recruiting PROTAC molecules **139** and **148** were higher than the parent ligand, with K_D values of 1.76 and 4.19 μM, respectively, which was attributed to hydrophobic interactions of the linker with the surface of the protein. A solubility issue with cereblon-recruiting **229** meant a K_D value could not be determined.

Only three of the 17 PROTACs were membrane permeant, with **141**, **143** and **148** appearing in cells in very low concentrations. Calculated and experimental LogD values for the PROTAC series suggested that these molecules were outside the range of ideal LogD for cell permeability. On treating RKO cells with the parent TRIM33 ligands, membrane permeability was observed, indicating the increased molecular weight and addition of multiple hydrogen bond donors and acceptors within the E3 ligase ligands could be responsible for the lack of permeability of these PROTAC molecules. Despite this, degradation assessment of **141**, **143** and **148** was performed (**Figure 6.4**). However, time-dependent degradation of TRIM33 was not observed – conversely, cells treated with **143** and **148** showed an increase in TRIM33 concentration over time, and treatment with **141** caused apparent fluctuations in protein levels which appeared to have no dependence on the PROTAC, although further repeats of the experiment are necessary to confirm this. It was postulated that these PROTACs do not degrade TRIM33, either because ubiquitination could not occur due to unstable or non-optimal ternary complex formation, or because of low concentrations of the PROTACs within the cell minimising any ternary complex formation.

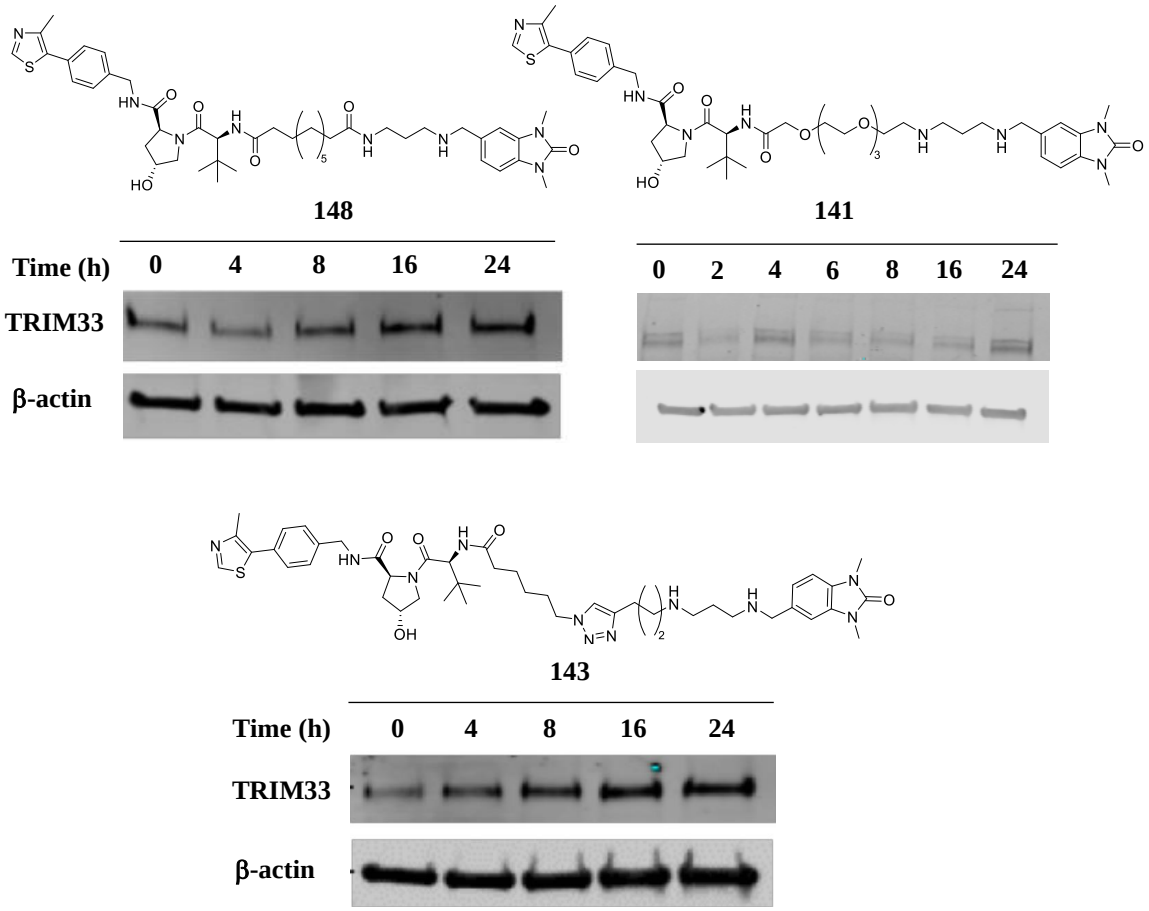


Figure 6.4. Time-dependent Western blots showing the concentration of TRIM33 when RKO cells were treated with **148**, **141**, and **143**. Experiment performed Anna Mandel or Dr Hannah Bolland, n = 1.

Chapter 4 focused on development of membrane permeant PROTAC molecules, aiming to increase the concentration of PROTAC within the cell, thus increasing the likelihood of ternary complex formation and degradation. The series was designed to mask all non-essential polarity and hydrogen bond donors, and focus on flexible alkyl linkers. Ligand **121** was used as the basis for the TRIM33 bromodomain binding motif within the PROTAC series, with the phenyl moiety increasing lipophilicity. Six PROTACs were synthesised, either recruiting VHL or cereblon as E3 ligases, each with a cLogD value of greater than +2.5. On assessment in an RKO cell line, permeability was indeed improved, with three of the four cereblon-recruiting PROTACs appearing in the cell in high concentrations. Alkyl linker length played a key role, illustrated by a tenfold increase in permeability of ten carbon linker PROTAC **238** compared to seven carbon linker PROTAC **237** (structure shown in **Figure 6.5**). The shorter linker cereblon-recruiting **236** was not cell permeable, indicating that the phenyl moiety and methylation of the terminal amide

on the TRIM33 ligand alone were not sufficient to increase membrane permeability. The VHL-recruiting **233** was not observed in the cell, and longer chain **234** requires the experiment to be repeated as cell death was observed after 24 hours, so permeability was not assessed. Assessment of the ability of the three cell permeable PROTACs to degrade TRIM33 in RKO cells revealed one cereblon-recruiting molecule **237** to show time-dependent reduction in TRIM33 levels, with the lowest levels showing around 70% reduction at 16 h after treatment (**Figure 6.5**). Therefore, this work describes the discovery of **237**, a potential degrader of TRIM33, which can be used as a basis for further optimisation and ultimately to establish the impact of degrading TRIM33 in cancer cells, in situations where DNA damage is increased.

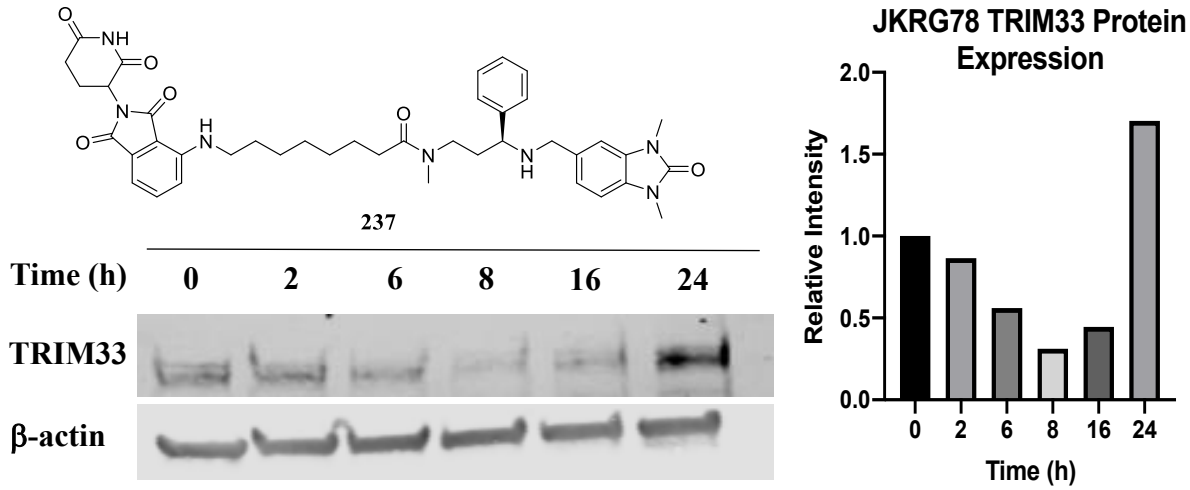


Figure 6.5. Time-dependent Western blot showing the concentration of TRIM33 when RKO cells were treated with **237**, and quantification of degradation relative to t = 0. Experiment performed by Dr Hannah Bolland, n = 1.

Chapter 5 explored 'hijacking' E3 ligase TRIM24 for use in PROTAC-mediated protein degradation. Expanding the toolbox of E3 ligases available for PROTAC design is essential for allowing new proteins of interest to be targeted selectively. Furthermore, as TRIM24 is overexpressed in many cancer cell lines, a TRIM24-recruiting PROTAC could allow selective degradation of a target protein only in specific cancer cell environments. BRD4 was selected as the target protein, due to its relevance in many cancers and its susceptibility to PROTAC-mediated degradation with a number of other E3 ligases. Previous work completed by Part II student Oliver Stratton identified PROTAC **288**, incorporating BRD4 ligand **OXFBD02 4** and TRIM24 ligand **287**, degraded BRD4 after 4 hours, but BRD4 production was quickly recovered. It was postulated

that the PROTAC had high metabolic clearance. Consequently, a PROTAC (**291**) was designed incorporating pyridyl-containing BRD4 ligand **5**, which has a much lower rate of metabolic clearance than the phenyl-containing BRD4 ligand, to determine if sustained degradation could be achieved. The same PEG linker that was incorporated into effective PROTAC **288** was included, thus a direct comparison could be made between the two. Synthetic routes to both PROTACs **288** and **291** were optimised, as previous route used by Oliver Stratton led to a low overall yield. Subsequently, binding to TRIM24 and BRD4 was verified using AlphaScreen™, and permeability of both PROTACs was confirmed in an RKO cell line. Time-dependent degradation assessment of **288** confirmed the results observed with the same molecule synthesised by Oliver Stratton, showing degradation after 2 hours, with quick recovery and subsequent overexpression of BRD4 (**Figure 6.6**). Inhibition of the bromodomain of BRD4(1) also leads to the observed overexpression, indicating BRD4 target engagement. Pyridyl-containing **291** showed fluctuating BRD4 concentrations over the course of 24 hours, with some potential degradation after 16 hours. However, due to the inherent low concentration of BRD4 in RKO cells, the experiments should be repeated in a cell line expressing a higher levels of BRD4, such as MCF7 breast cancer cells, in order to significantly interpret the changes in concentration.

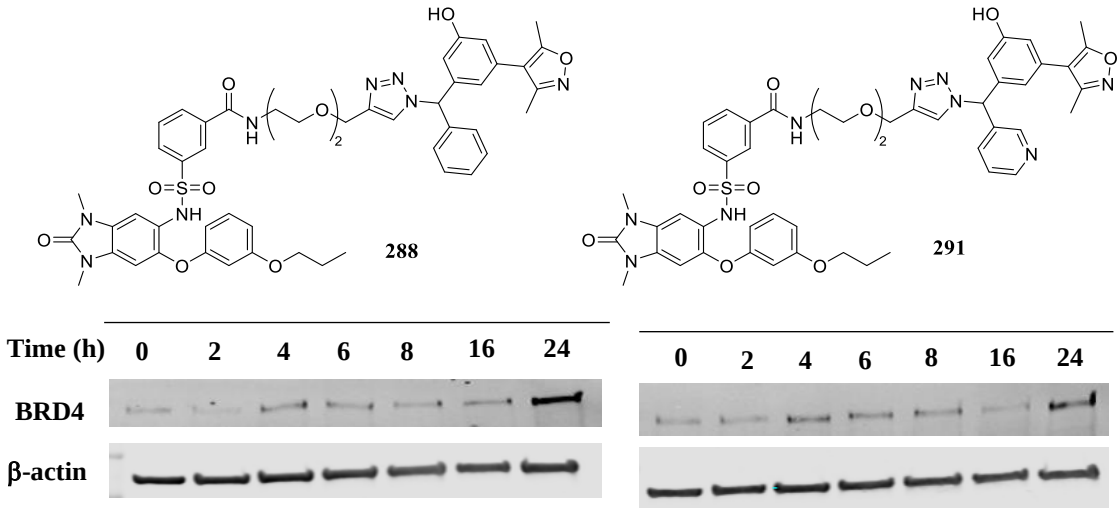


Figure 6.6. Chapter 5 explored the degradation of BRD4 using TRIM24-recruiting PROTACs. The time-dependent Western blots are shown, performed by Dr Hannah Bolland, n = 1.

In summary, this work has allowed the identification of the first series of confirmed TRIM33 bromodomain ligands with low micromolar affinity, and selectivity over the similar bromodomain of TRIM24. These molecules have been incorporated into PROTACs to target TRIM33 for

degradation in cells, with one PROTAC reducing TRIM33 levels in a time-dependent manner. Subsequently, E3 ligase activity of TRIM24 was explored for context-dependent degradation of BRD4.

6.2 Future Work

The work done in Chapter 2 provided a deeper understanding of the TRIM33 bromodomain KAc-binding pocket. Future work should focus on further optimisation of the bromodomain ligands to increase affinity, thus allowing these molecules to be used in mode of action studies. Exploration should focus on the ZA channel water molecules, perhaps determining if water-mediated hydrogen bonds or displacement of water 5 or 6 can improve affinity. As attempted co-crystallisation of the ligands with TRIM33 by Dr Larissa See was not successful, a different method could be explored to rigorously confirm the binding site of these ligands. For example, a photoaffinity label and a functional handle could be added to the ligand to allow photo-crosslinking for a pulldown study.

Having increased the cell permeability of the TRIM33 PROTAC series and identified **237** as a potential degrader of TRIM33, repeats of the time-dependent experiments should be performed to verify these results. Similarly, a time-dependent experiment should be performed over a longer period, perhaps 48 hours, with additional dosing of **237** after 24 hours to determine if sustained degradation could be achieved. Subsequently, different concentrations of **237** should be tested to find the degradation maximum, and to identify at which concentration the hook effect is occurring. Negative controls should be synthesised, which would include the unmethylated head group of the TRIM33 ligand, as this abrogates any binding to the bromodomain. For cereblon-recruiting PROTACs, a negative control requires methylation of the 2,6-piperidinedione to prevent binding. Once an effective degrader has been established, the effect of degrading TRIM33 *in vivo* on DNA damage and cell viability can be evaluated.

The long-term objective of this project is to validate TRIM33 as a therapeutic target, by verifying that inhibition of the bromodomain can lead to increased DNA damage in cancer cells, thus

proving that targeting TRIM33 could be an effective cancer treatment in combination with radiotherapy. Subsequently, PROTAC-mediated degradation of TRIM33 can be performed to assess the differences between bromodomain inhibition and removal of the whole protein. The development of chemical tools for these studies also provides a good starting point for further progression into drug candidates.

Optimisation of the TRIM24-recruiting PROTACs should be the focus of future work, to promote complete and sustained degradation of BRD4. The experiments should also be repeated in MCF7 cells, as high levels of BRD4 are observed in this cell line, making interpretation of the concentration changes more significant. Similarly to TRIM33 PROTAC development, negative controls of the TRIM24-recruiting PROTAC should be synthesised in order to determine degradation of BRD4 is dependent on TRIM24. Alternatively, the experiments could be performed in TRIM24 null cell lines or with TRIM24 knocked down. The next step would be to determine if the PROTAC disproportionately degrades BRD4 in cancer cell lines where TRIM24 is overexpressed, compared to normal cell lines. These experiments will significantly progress the PROTAC field, allowing an additional E3 ligase to be added to the PROTAC toolbox, as well as potentially offering a therapeutic lead in the treatment of BRD4-dependent cancers.

Chapter 7 Experimental

7.1 General Chemistry Experimental

Reagents and solvents used were of commercially available reagent grade quality from Fluorochem and Sigma Aldrich, and were used without further purification, unless stated. All non-aqueous reactions were carried out under a nitrogen or argon atmosphere in a flame-dried flask. Anhydrous solvents were obtained from an MBRAUN Solvent Purification Systems 5 and stored under an argon atmosphere over 3 Å molecular sieves. Concentration *in vacuo* was performed at 40 ˚C for organic solvents using a Buchi™ rotary evaporator. Brine refers to a saturated aqueous solution of sodium chloride. Petroleum ether refers to the fraction of light petroleum ether boiling in the range 40–60 °C. Celite® refers to Celite® 545 filter aid, treated with sodium carbonate, flux-calcined (Sigma Aldrich).

^{1}H NMR spectra were measured on a Bruker AV400 (400 MHz), a Bruker AVII 500 (500 MHz) or a Bruker AV600 (600 MHz) spectrometer in the stated solvents as a reference for the internal deuterium lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ(TMS) = 0.00. The spectra are calibrated using the solvent peak with the data provided by Fulmer *et al.*[185] The multiplicity of each signal is indicated by: s (singlet); bs (broad singlet); d (doublet); t (triplet); q (quartet); p (pentet); sept (sept); m (multiplet) or combinations thereof. The number of protons (n) for a given resonance signal is indicated by nH. Where appropriate, coupling constants (*J*) are quoted in Hz, recorded to the nearest 0.1 Hz, and were determined by analysis using Bruker TopSpin v3.2 software or MestreNova software. Identical coupling constants were averaged. Spectra were assigned using COSY, HSQC and HMBC experiments as necessary. Spectra acquired at high temperature (353 K or 363 K) are indicated.

^{13}C NMR spectra were measured on a Bruker AV400 (101 MHz), a Bruker AVII 500 (126 MHz) or a Bruker AV600 (151 MHz) spectrometer in the stated solvents as a reference for the internal deuterium lock using the standard ^{13}C experiment. The chemical shift data for each signal are

given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ(TMS) = 0.00. The spectra are calibrated using the solvent peak with the data provided by Fulmer *et al.*[185] Signals are quoted to 1 decimal place unless peaks are indistinguishable, in which case 2 decimal places are used. Where appropriate, coupling constants (*J*) are quoted in Hz, recorded to the nearest 0.1 Hz, and were determined by analysis using Bruker TopSpin v3.2 software or MestreNova software. Spectra were assigned using HSQC and HMBC experiments as necessary. Spectra acquired at high temperature (353 K or 363 K) are indicated.

^{19}F NMR spectra were measured on a Bruker AVIII HD 400 (376 MHz) spectrometer in the solvent stated, and are ^{13}C decoupled. Chemical shifts are given as δ in units of parts per million (ppm), to the nearest 0.1 ppm. The multiplicity of each signal is a singlet unless stated otherwise.

Mass spectra were acquired on either an Agilent 6120 (low resolution) or Bruker microToF spectrometer (high resolution) using electrospray ionisation (ESI) from solutions of either methanol or water. *m/z* values are reported in Daltons and are followed by their percentage abundance in parentheses.

Melting points were determined using either a) a Leica Galen III hot stage microscope or b) a Griffin capillary tube melting point apparatus (Registered Design No. 889339) and are uncorrected. The solvent(s) from which the sample was crystallised is given in parentheses. Dec. is used to indicate that the sample decomposed at the stated temperature.

Infrared spectra were obtained from thin films, using a diamond ATR module on a Bruker Tensor 27 spectrometer. Absorption maxima are reported in wavenumbers (cm^{-1}) and reported as s (strong), m (medium), w (weak) or br (broad).

Specific optical rotations were obtained using a Schmidt Haensch Unipol polarimeter, using a sodium lamp at 589 nm and a path length of 1.0 dm at 25 ºC. The concentration (c) is expressed in g/100 mL (equivalent to g/0.1 dm^3). Specific rotations are denoted $[\alpha]_D^T$ and are given in implied units of 10^{-1} deg cm^2 g^{-1} (where superscript T indicates temperature in °C).

Analytical high performance liquid chromatography (HPLC) method 1 was performed on a PerkinElmer Flexar system with a Binary LC Pump and UV/VIS LC Detector set at 254 nm. For determination of purity, a Dionex Acclaim® 120 C18 column [5 μm, 12 Å, 150 mm × 4.6 mm] reverse phase column was used with a constant flow rate of 1.5 mL min^{-1} and gradient method of 10 min from 95:5 H_2O:acetonitrile (0.1% TFA) to 5:95 H_2O:acetonitrile (0.1% TFA) with a 5 min hold. Samples injected were prepared by dissolving in methanol, water or acetonitrile, and filtered. All samples are run with 0.1% TFA added unless indicated.

HPLC (method 2) was performed on a Waters Acquity UPLC-MS with Waters PDA UV detector and Waters QDa mass spectrometer, with detection between 210–400 nm at 45 ºC. A Waters UPLC CSH C18 column [1.7 μm, 2.1 x 50 mm] with Vanguard CSH C18 1.7 μm, 2.1 x 5 mm guard column was used with a constant flow rate of 1.0 mL min^{-1} and a gradient method as follows: 2% acetonitrile, 98% H_2O with 0.1% TFA, 0–1.00 min: 2% acetonitrile to 98% acetonitrile, 1.00–1.35 min: hold at 98% acetonitrile, 1.35–1.40 min: 98% acetonitrile to 2% acetonitrile, 1.40–1.50 min: hold at 2% acetonitrile. Samples injected were prepared by dissolving in methanol, water or acetonitrile, and filtered. All samples are run with 0.1% TFA added unless indicated.

HPLC (method 3) was performed on a Waters Acquity UPLC-MS with Waters PDA UV detector and Waters QDa mass spectrometer, with detection between 210–400 nm at 45 ºC. A Waters UPLC CSH C18 column [1.7 μm, 2.1 x 50 mm] with Vanguard CSH C18 1.7 μm, 2.1 x 5 mm guard column was used with a constant flow rate of 0.6 mL min^{-1} and a gradient method as follows: 2% acetonitrile, 98% H_2O with 0.1% TFA, 0–3.45 min: 2% acetonitrile to 98% acetonitrile gradient, 3.45–3.75 min: hold at 98% acetonitrile, 3.75–3.80 min: 98% acetonitrile to 2% acetonitrile, 3.80–4.00 min: hold at 2% acetonitrile. Samples injected were prepared by dissolving in methanol, water or acetonitrile, and filtered. All samples are run with 0.1% TFA added unless indicated.

HPLC (method 4) was performed on an Atlantis™ C18 reverse phase column [3 μM, 100 Å, 150 mm × 4.6 mm] with detection at 254 nm. This method had a constant flow rate of

1.0 mL min^{-1} and a gradient method of a 2 min hold, followed by 13 min gradient from 100:0 H_2O:acetonitrile (0.1% TFA) to 5:95 H_2O:acetonitrile (0.1% TFA) and a subsequent 5 min hold. Samples injected were prepared by dissolving in methanol or acetonitrile, and filtered.

All biologically assessed compounds had a purity of ≥95%.

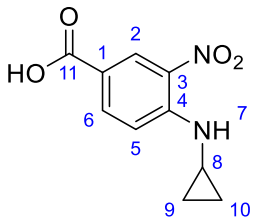
Normal Phase silica gel analytical Thin Layer Chromatography (NP TLC) to monitor reaction progress was carried out on normal phase Merck silica gel 60 F_{254} aluminium-supported thin layer chromatography sheets. Visualisation was carried out by absorption of UV light (λ_{max} = 254 nm and 365 nm) or thermal development after staining in an ethanolic solution of ninhydrin or an aqueous solution of potassium permanganate.

Normal phase silica gel flash column chromatography was carried out manually on Merck Geduran® silica gel 60 (40–63 μm), eluting with solvents as supplied, under a positive pressure of compressed, gaseous nitrogen.

7.2 Procedures and Compound Data

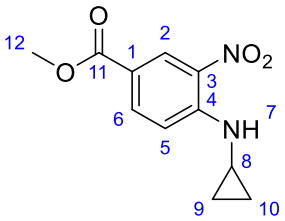
COMPOUNDS FROM CHAPTER 2

4-(Cyclopropylamino)-3-nitrobenzoic acid (36)[186,187]



4-Fluoro-3-nitrobenzoic acid (500 mg, 2.70 mmol, 1.0 eq.) and sodium bicarbonate (363 mg, 5.67 mmol, 2.1 eq.) were dissolved in water (15 mL). Cyclopropyl amine (210 µL, 2.97 mmol, 1.1 eq.) was added and the mixture was heated at 150 ºC for 20 min under microwave irradiation in a sealed microwave tube. The reaction mixture was cooled, poured into 1 M aqueous hydrochloric acid (60 mL) and the resulting precipitate was collected by filtration to yield the title compound **36** as a yellow solid (538 mg, 90%). R_f 0.65 (ethyl acetate); m.p. 238–240 ºC (dec. from EtOAc); ^{1}H NMR (400 MHz, CD_3OD) δ_H 8.78 (1H, d, *J* 2.1, H-2), 8.10 (1H, dd, *J* 9.0, 2.1, H-6), 7.47 (1H, d, *J* 9.0, H-5), 2.71 (1H, tt, *J* 7.0, 3.7, H-8), 1.04 – 0.92 (2H, m, H-9^a and H-10^a), 0.73 – 0.64 (2H, m, H-9^b and H-10^b); *m/z* (ESI$^-$) 221 ([M−H]$^-$, 100%). The spectroscopic data are in good agreement with literature values.[186,187]

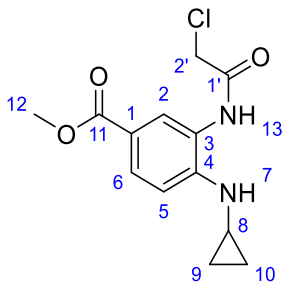
Methyl 4-(cyclopropylamino)-3-nitrobenzoate (37)[188]



36 (900 mg, 4.05 mmol, 1.0 eq.) was dissolved in anhydrous methanol (40.5 mL) and the solution was cooled to 0 ºC. Thionyl chloride (981 µL, 13.4 mmol, 3.3 eq.) was added dropwise and the solution was stirred for 15 min. The reaction mixture was then heated to 50 ºC and thionyl chloride was added in aliquots of 1 mL every 15 min until the starting material was completely consumed

(total of 6.00 mL over 1.5 h). The reaction was quenched with water (40 mL) and extracted with ethyl acetate (3 × 40 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (30–60% dichloromethane in petroleum ether) to yield the title compound **37** as a yellow solid (879 mg, 92%). R_f 0.45 (30% chloroform in petroleum ether); m.p. 80–83 ºC (from CH_2Cl_2) {lit.[188] 80–82 ºC}; $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3386 (s), 1709 (s), 1618 (s), 1567 (m), 1524 (s), 1439 (s), 1424 (m), 1357 (m), 1308 (m), 1284 (s), 1263 (m), 1216 (m), 1189 (s), 1175 (m), 1147 (m), 1122 (m), 1063 (w), 1036 (w), 981 (m), 953 (m), 922 (w), 895 (w), 827 (m), 770 (w), 757 (s), 703 (m), 692 (m), 652 (m), 613 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.87 (1H, d, *J* 2.1, H-2), 8.37 (1H, s, H-7), 8.09 (1H, dd, *J* 9.0, 2.1, H-6), 7.33 (1H, d, *J* 9.0, H-5), 3.91 (3H, s, H-12), 2.68 – 2.60 (1H, m, H-8), 1.02 – 0.93 (2H, m, H-9^a and H-10^a), 0.74 – 0.66 (2H, m, H-9^b and H-10^b); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 165.8 (C-11), 148.8 (C-4), 136.2 (C-6), 131.6 (C-3), 129.3 (C-2), 118.1 (C-1), 115.3 (C-5), 52.3 (C-12), 24.8 (C-8), 8.1 (C-9 and C-10); *m/z* (ESI$^+$) 237 ([M+H]$^+$, 60%), 259 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{11}H_{13}N_2O_4^+$ ([M+H]$^+$) requires 237.08698, found 237.08711; HPLC (method 1) retention time 10.2 min, 100%. The spectroscopic data are in good agreement with literature values.[188]

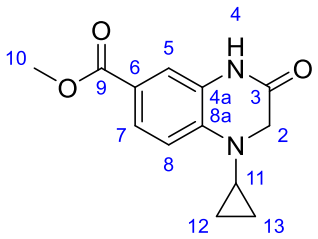
Methyl 3-(2'-chloroacetamido)-4-(cyclopropylamino)benzoate (41)



37 (100 mg, 0.423 mmol, 1.0 eq.) was dissolved in ethyl acetate (2 mL) under a nitrogen atmosphere. Palladium hydroxide on carbon (20% loading, 14.7 mg, 0.0212 mmol, 0.05 eq.) in an ethyl acetate suspension (1 mL) was added to the flask. The reaction vessel was charged with hydrogen and the mixture was stirred at room temperature under a hydrogen atmosphere for 3 h. The reaction mixture was then filtered through Celite® and concentrated *in vacuo*. The crude

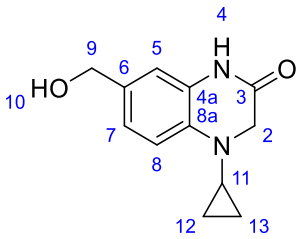
residue was dissolved in anhydrous tetrahydrofuran (3 mL) and *N*,*N*-diisopropylethylamine (80.0 µL, 0.465 mmol, 1.1 eq.) was added. The solution was cooled to −60 ºC and chloroacetyl chloride (36.0 µL, 0.448 mmol, 1.1 eq.) was added dropwise. The solution was stirred at −60 ºC for 3 h and then at room temperature for 16 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with saturated aqueous sodium bicarbonate solution (20 mL), water (20 mL) and brine (20 mL). The organic component was dried over sodium sulfate, and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (10–40% ethyl acetate in petroleum ether) to yield the title compound **41** as a colourless solid (75.0 mg, 62%). R_f 0.87 (1% Triethylamine, 70% ethyl acetate in petroleum ether); m.p. 132–135 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3402 (m), 3253 (m), 2999 (w), 2951 (w), 1688 (m), 1668 (m), 1612 (m), 1577 (w), 1526 (m), 1438 (w), 1423 (w), 1408 (w), 1332 (m), 1304 (s), 1250 (m), 1228 (m), 1198 (m), 1176 (m), 1104 (s), 1128 (s), 1104 (w), 1047 (w), 1028 (w), 988 (w), 974 (w), 934 (w), 899 (w), 847 (w), 821 (m), 796 (w), 764 (s), 709 (w), 681 (w), 647 (w), 611 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.96 – 7.86 (2H, m, H-6 and H-13), 7.82 (1H, d, *J* 2.0, H-2), 7.14 (1H, d, *J* 8.6, H-5), 4.80 (1H, s, H-7), 4.23 (2H, s, H-2'), 3.85 (3H, s, H-12), 2.54 – 2.46 (1H, m, H-8), 0.81 (2H, td, *J* 6.7, 4.8, H-9^a and H-10^a), 0.58 – 0.50 (2H, m, H-9^b and H-10^b); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 166.8 (C-1'), 165.4 (C-11), 147.9 (C-4), 130.5 (C-6), 128.2 (C-2), 120.6 (C-3), 119.3 (C-1), 112.8 (C-5), 51.9 (C-12), 42.9 (C-2'), 25.0 (C-8), 7.7 (C-9 and C-10); *m/z* (ESI$^+$) 283 ([M+H]$^+$, 100%), 305 ([M+Na]$^+$, 90%); HRMS *m/z* (ESI$^+$) $C_{13}H_{16}N_2O_3Cl^+$ ([M+H]$^+$) requires 283.08440, found 283.08446; HPLC (method 1) retention time 8.3 min, 99.3%.

Methyl 1-cyclopropyl-3-oxo-1,2,3,4-tetrahydroquinoxaline-6-carboxylate (40)



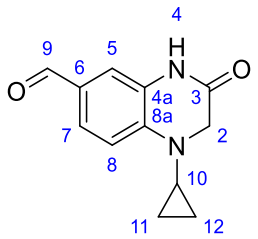
A mixture of **41** (300 mg, 1.1 mmol, 1.0 eq.), sodium iodide (79 mg, 0.53 mmol, 0.5 eq.) and *N*,*N*-diisopropylethylamine (0.24 mL, 1.4 mmol, 1.3 eq.) in acetonitrile (15 mL) was heated to 120 ºC for 5 h under microwave irradiation. The reaction mixture was then cooled to room temperature, concentrated *in vacuo* and diluted with ethyl acetate (200 mL). The mixture was washed with saturated aqueous sodium bicarbonate solution (100 mL) and brine (100 mL), and the organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (20–40% ethyl acetate in petroleum ether) to yield the title compound **40** as a colourless solid (180 mg, 68%). R_f 0.66 (30% ethyl acetate in petroleum ether); m.p. 166–168 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3191 (w), 3100 (w), 2961 (w), 1701 (s), 1673 (s), 1609 (m), 1578 (m), 1530 (w), 1505 (m), 1454 (w), 1438 (m), 1405 (m), 1359 (s), 1301 (s), 1213 (s), 1184 (s), 1130 (m), 1111 (m), 1038 (m), 1016 (w), 990 (w), 958 (w), 936 (w), 900 (m), 881 (m), 839 (m), 819 (m), 798 (m), 774 (m), 763 (s), 722 (m), 676 (m), 630 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.96 (1H, s, H-4), 7.71 (1H, dd, *J* 8.5, 1.9, H-7), 7.48 (1H, d, *J* 1.9, H-5), 7.13 (1H, d, *J* 8.5, H-8), 3.93 (2H, s, H-2), 3.88 (3H, s, H-10), 2.39 (1H, tt, *J* 6.7, 3.7, H-11), 0.97 – 0.87 (2H, m, H-12^a and H-13^a), 0.69 – 0.61 (2H, m, H-12^b and H-13^b); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 167 (C-3), 166.8 (C-9), 140.1 (C-8a), 126.3 (C-7), 126.0 (C-4a), 121.1 (C-6), 116.6 (C-5), 112.6 (C-8), 52.6 (C-2), 52.1 (C-10), 30.7 (C-11), 8.3 (C-12 and C-13); *m/z* (ESI$^+$) 247 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{13}H_{15}N_2O_3^+$ ([M+H]$^+$) requires 247.10772, found 247.10793; HPLC (method 1) retention time 8.0 min, 96.3%.

1-Cyclopropyl-6-(hydroxymethyl)-1,2-dihydroquinoxalin-3(1*H*)-one (42)



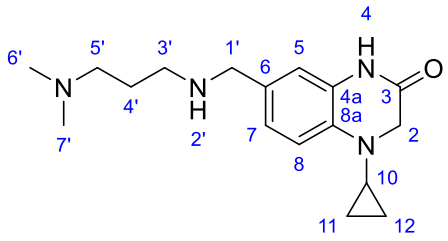
40 (99 mg, 0.40 mmol, 1.0 eq.) was dissolved in anhydrous dichloromethane (4 mL). The solution was cooled to −78 ºC and diisobutylaluminium hydride (1 M in hexanes, 2.8 mL, 2.8 mmol, 7.0 eq.) was added dropwise. The reaction mixture was stirred at −78 ºC for 3 h. After this time, the reaction was quenched with aqueous Rochelle salt solution (20 mL) and stirred at room temperature for 18 h. The aqueous component was extracted with dichloromethane (4 × 20 mL) and the combined organic components were washed with brine (20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (60–90% ethyl acetate in petroleum ether) to yield the title compound **42** as a colourless solid (70 mg, 80%). R_f 0.22 (80% ethyl acetate in petroleum ether); m.p. 126–128 ºC (from EtOAc); $\tilde{\nu}_{max}$ (thin film)/cm^{-1} 3362 (b), 2943 (w), 2783 (w), 1681 (s), 1624 (w), 1594 (w), 1527 (w), 1499 (m), 1455 (m), 1402 (m), 1364 (m), 1318 (m), 1294 (m), 1273 (w), 1219 (w), 1171 (w), 1094 (w), 1022 (m), 999 (w), 946 (w), 934 (w), 876 (m), 815 (m), 802 (m), 784 (w), 741 (w), 679 (m), 632 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.99 (1H, s, H-4), 7.09 (1H, d, *J* 8.2, H-8), 6.98 (1H, dd, *J* 8.2, 1.8, H-7), 6.81 (1H, d, *J* 1.8, H-5), 4.57 (2H, d, *J* 5.4, H-9), 3.76 (2H, s, H-2), 2.34 (1H, t, *J* 5.4, H-10), 2.24 (1H, tt, *J* 6.7, 3.7, H-11), 0.89 – 0.81 (2H, m, H-12^a and H-13^a), 0.61 – 0.52 (2H, m, H-12^b and H-13^b); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 168.1 (C-3), 135.9 (C-8a), 132.7 (C-4a), 126.7 (C-6), 123.1 (C-7), 114.8 (C-5), 113.4 (C-8), 64.9 (C-9), 53.1 (C-2), 30.8 (C-11), 8.1 (C-12 and C-13); *m/z* (ESI$^+$) 219 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{13}H_{15}N_2O_3^+$ ([M+H]$^+$) requires 219.11305, found 219.11280; HPLC (method 1) retention time 6.4 min, 94.8%.

1-Cyclopropyl-3-oxo-1,2,3,4-tetrahydroquinoxaline-6-carbaldehyde (43)



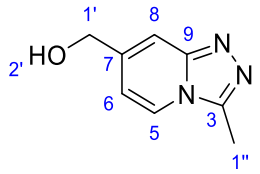
Dess Martin Periodinane (64 mg, 0.15 mmol, 1.1 eq.) was added to a solution of **42** (30 mg, 0.14 mmol, 1.0 eq.) in dichloromethane (2 mL), and the reaction mixture was stirred at room temperature for 4 h. The solution was diluted with dichloromethane (50 mL) and washed with 1:1 10% aqueous sodium thiosulfate: saturated aqueous sodium bicarbonate solution (50 mL), water (30 mL) and brine (50 mL). The organic component was dried over sodium sulfate, and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (10–50% ethyl acetate in petroleum ether) to yield the title compound **43** as a colourless solid (22 mg, 73%). R_f 0.34 (50% ethyl acetate in petroleum ether); m.p. 190–192 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3031 (w), 2850 (w), 2361 (m), 2341 (w), 1684 (s), 1634 (m), 1609 (w), 1536 (w), 1502 (w), 1465 (w), 1441 (w), 1376 (w), 1298 (w), 1170 (w), 1136 (w), 1085 (w), 1039 (w), 947 (w), 905 (w), 832 (w), 806 (w), 779 (w), 721 (m), 670 (w), 650 (w), 609 (w); ^{1}H NMR (500 MHz, acetone-d_6) δ_H 9.78 (1H, s, H-9), 9.59 (1H, s, H-4), 7.53 (1H, dd, *J* 8.3, 1.9, H-7), 7.40 (1H, d, *J* 1.9, H-5), 7.29 (1H, d, *J* 8.3, H-8), 3.91 (2H, s, H-2), 2.53 (1H, tt, *J* 6.7, 3.4, H-10), 1.03 – 0.90 (2H, m, H-11^a and H-12^a), 0.71 – 0.63 (2H, m, H-11^b and H-12^b); ^{13}C NMR (126 MHz, acetone-d_6) δ_C 190.6 (C-9), 166.0 (C-3), 142.4 (C-8a), 129.6 (C-4a), 128.7 (C-6), 128.3 (C-7), 114.6 (C-5), 113.4 (C-8), 52.8 (C-2), 31.4 (C-10), 8.5 (C-11 and C-12); *m/z* (ESI$^+$) 217 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{13}H_{15}N_2O_3^+$ ([M+H]$^+$) requires 217.09715, found 217.09735; HPLC (method 1) retention time 7.2 min, 96.4%.

1-Cyclopropyl-6-({[5'-(dimethylamino)propyl]amino}methyl)-3,4-dihydroquinoxalin-2(1*H*)-one (29)



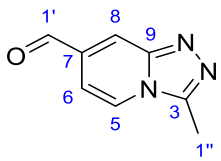
43 (130 mg, 0.601 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (7 mL) with 3 Å molecular sieves. N^1,N^1-Dimethylpropane-1,3-diamine (113 µL, 0.902 mmol, 1.5 eq.) and glacial acetic acid (69.0 µL, 1.20 mmol, 2.0 eq.) were added at 0 ºC and the reaction was warmed to room temperature until imine formation (3 h, monitored by TLC and ^{1}H NMR). Sodium borohydride (34.1 mg, 0.902 mmol, 1.5 eq.) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched with methanol (10 mL), filtered and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (1:2:12 ethyl acetate:ethanol;ammonium hydroxide) to yield the title compound **29** as a colourless oil (64.2 mg, 35%). R_f 0.48 (1:2:12 ethyl acetate:ethanol;ammonium hydroxide); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3488 (w), 2941 (w), 2860 (w), 2820 (w), 2361 (w), 1682 (s), 1624 (w), 1590 (w), 1525 (w), 1493 (w), 1456 (w), 1391 (m), 1364 (m), 1293 (w), 1219 (w), 1169 (w), 1095 (w), 1026 (w), 978 (w), 881 (w), 813 (w), 678 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H δ 7.76 (1H, bs, H-4), 7.09 (1H, d, *J* 8.1, H-8), 6.94 (1H, dd, *J* 8.1, 1.9, H-7), 6.72 (1H, d, *J* 1.9, H-5), 3.81 (2H, s, H-1'), 3.69 (2H, s, H-2), 2.66 (2H, t, *J* 7.1, H-3'), 2.31 (2H, t, *J* 7.1, H-5'), 2.28 – 2.20 (1H, m, H-10), 2.21 (6H, s, H-6' and H-7'), 1.72 – 1.65 (2H, q, *J* 7.1, H-4'), 0.89 – 0.81 (2H, m, H-11^a and H-12^a), 0.63 – 0.57 (2H, m, H-11^b and H-12^b), ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 168.0 (C-3), 135.3 (C-8a) 132.2 (C-6), 126.9 (C-4a), 123.7 (C-7), 115.4 (C-5), 113.3 (C-8), 58.1 (C-5'), 53.5 (C-2), 53.3 (C-1'), 47.9 (C-3'), 45.6 (C-6' and C-7'), 30.8 (C-10), 28.1 (C-4'), 8.0 (C-11 and C-12); *m/z* (ESI$^+$) 303 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{17}H_{27}ON_4^+$ ([M+H]$^+$) requires 303.21794, found 303.21806; HPLC (method 1) retention time 5.3 min, 100%.

(3-Methyl-[1,2,4]triazolo[4,3-a]pyridin-7-yl)methanol (33)



(2-Chloropyridin-4-yl)methanol (150 mg, 1.04 mmol, 1.0 eq.) and acetylhydrazine (116 mg, 1.57 mmol, 1.5 eq.) were dissolved in *n*-butanol (4 mL) and heated to 170 ºC for 4 h under microwave irradiation in a sealed microwave tube. The solvent was removed *in vacuo* and the crude product was purified by silica gel flash column chromatography (10–50% ethanol in ethyl acetate) to yield the title compound **33** as a colourless solid (79.0 mg, 47%). R_f 0.62 (50% ethanol in dichloromethane); m.p. 170–172 ºC (from EtOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3158 (s), 2816 (w), 1647 (m), 1548 (w), 1505 (m), 1447 (m), 1387 (m), 1375 (m), 1336 (m), 1285 (w), 1248 (w), 1145 (m), 1088 (w), 1062 (s), 1039 (m), 1018 (m), 1003 (m), 954 (w), 868 (m), 777 (s), 746 (m), 684 (m), 666 (w), 609 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.25 (1H, dd, *J* 7.2, 1.0, H-5), 7.63 (1H, p, *J* 1.4, H-8), 6.99 (1H, dd, *J* 7.2, 1.5, H-6), 4.69 (2H, d, *J* 1.4, H-1'), 2.75 (3H, s, H-1''); ^{13}C NMR (126 MHz, CD_3OD) δ_c 151.3 (C-9), 145.53 (C-3), 145.49 (C-7), 124.3 (C-5), 114.7 (C-6), 111.0 (C-8), 63.5 (C-1'), 9.7 (C-1''); *m/z* (ESI$^+$) 164 ([M+H]$^+$, 100%), 186 ([M+Na]$^+$, 30%); HRMS *m/z* (ESI$^+$) $C_8H_{10}N_3O^+$ ([M+H]$^+$) requires 164.08184, found 164.08191; HPLC (method 1) retention time 4.1 min, 96.4%.

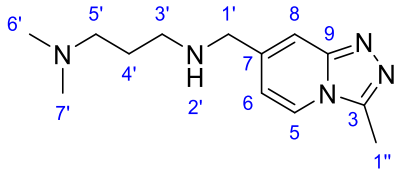
3-Methyl-[1,2,4]triazolo[4,3-a]pyridine-7-carbaldehyde (34)



To a solution of **33** (70 mg, 0.43 mmol, 1.0 eq.) in dichloromethane (5 mL) was added manganese oxide (370 mg, 4.3 mmol, 10.0 eq.) and the reaction was stirred at room temperature for 18 h. The reaction mixture was filtered through Celite® and the filter cake was washed with chloroform (100 mL). The filtrate was concentrated *in vacuo* to yield the title compound **34** as a colourless

solid (52 mg, 75%). R_f 0.73 (50% ethanol in dichloromethane); m.p. 184–186 ºC (dec. from $CHCl_3$); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 1701 (s), 1683 (s), 1633 (w), 1536 (w), 1504 (w), 1465 (w), 1441 (w), 1376 (w), 1337 (w), 1315 (w), 1175 (w), 1167 (w), 1149 (w), 1133 (w), 1084 (m), 1041 (w), 1005 (w), 944 (w), 902 (w), 885 (w), 832 (w), 807 (s), 779 (s), 746 (w), 720 (s), 609 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 10.03 (1H, d, *J* 0.7, H-1'), 8.25 (1H, t, *J* 1.4, H-8), 7.94 (1H, d, *J* 7.2, H-5), 7.36 (1H, dd, *J* 7.2, 1.4, H-6), 2.82 (3H, s, H-1''); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 189.5 (C-1'), 149.5 (C-9), 145.1 (C-3), 134.5 (C-7), 123.8 (C-8), 122.9 (C-5), 109.6 (C-6), 10.5 (C-1''); *m/z* (ESI$^+$) 162 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_8H_8N_3O^+$ ([M+H]$^+$) requires 162.06619, found 162.06628; HPLC (method 1) retention time 4.7 min, 98.8%.

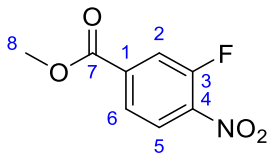
N^1,N^1-Dimethyl-N^3-((3-methyl-[1,2,4]triazolo[4,3-a]pyridin-7-yl)methyl)propane-1,3-diamine (30)



34 (177 mg, 1.11 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (20 mL) with 3 Å molecular sieves. N^1,N^1-Dimethylpropane-1,3-diamine (210 µL, 1.67 mmol, 1.5 eq.) and glacial acetic acid (126 µL, 2.22 mmol, 2.0 eq.) were added at 0 ºC and the reaction was warmed to room temperature until imine formation (3 h, monitored by TLC and ^{1}H NMR). Sodium borohydride (63.4 mg, 1.67 mmol, 2.0 eq.) was added and the reaction mixture was stirred at room temperature for 18 h. The reaction was quenched with methanol (20 mL), filtered, and concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography (1% triethylamine in ethanol) and recrystallised in dichloromethane and hexanes to yield the title compound **30** as a colourless solid (88.3 mg, 32%). R_f 0.01 (1% triethylamine in ethanol); m.p. 76–78 ºC (from EtOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3279 (m), 3063 (w), 2979 (m), 2947 (m), 2858 (m), 2815 (m), 2761 (m), 1650 (s), 1544 (m), 1500 (m), 1443 (m), 1381 (s), 1338 (w), 1296 (w), 1282 (w), 1260 (m), 1229 (w), 1209 (w), 1170 (m), 1116 (m), 1097 (m), 1066 (w), 1040 (m), 1019 (m), 992 (w), 961 (w), 944 (w), 928 (w), 897 (w), 874 (m), 849 (m), 816 (m), 770 (s), 756 (m), 748

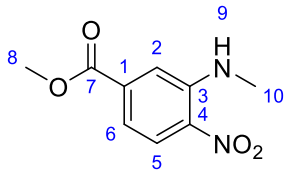
(m), 688 (m), 667 (m), 608 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.25 (1H, dd, *J* 7.1, 1.0, H-5), 7.62 – 7.60 (1H, m, H-8), 7.06 (1H, dd, *J* 7.1, 1.5, H-6), 3.86 (2H, d, *J* 1.2, H-1'), 2.74 (3H, s, H-1''), 2.67 – 2.61 (2H, m, H-3'), 2.43 – 2.35 (2H, m, H-5'), 2.25 (6H, s, H-6' and H-7'), 1.79 – 1.68 (2H, m, H-4'); ^{13}C NMR (126 MHz, CD_3OD) δ_C 149.9 (C-9), 144.1 (C-3), 141.8 (C-7), 122.9 (C-5), 115.0 (C-6), 111.9 (C-8), 57.2 (C-5'), 51.9 (C-1'), 46.8 (C-3'), 44.0 (C-6' and C-7'), 26.8 (C-4'), 8.3 (C-1''); *m/z* (ESI$^+$) 248 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{13}H_{22}N_5^+$ ([M+H]$^+$) requires 248.18697, found 248.18705; HPLC (method 1) retention time 1.6 min, 83.1% (second impurity peak suspected to be the doubly protonated compound, first peak the triply protonated compound).

Methyl 3-fluoro-4-nitrobenzoate (63)[189,190]



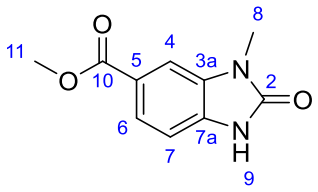
3-Fluoro-4-nitrobenzoic acid (1.50 g, 8.10 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (15 mL). Potassium carbonate (5.60 g, 40.5 mmol, 5.0 eq.) was added followed by dropwise addition of iodomethane (1.01 mL, 16.2 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 16 h, quenched with water (10 mL) and extracted with diethyl ether (4 × 40 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (3 × 40 mL), brine, dried over sodium sulfate, and concentrated *in vacuo* to yield the title compound **63** as a yellow solid (1.47 g, 91%). R_f 0.34 (70% ethyl acetate in petroleum ether); m.p. 46–47 ºC (from EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.14 – 8.08 (1H, m, H-5), 7.99 – 7.93 (2H, m, H-2 and H-6), 3.99 (3H, s, H-8); *m/z* (ESI$^+$) 200 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[189,190]

Methyl 3-(methylamino)-4-nitrobenzoate (64)



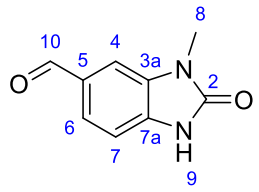
63 (1.30 g, 6.51 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (8 mL). Methylamine hydrochloride (2.64 g, 39.1 mmol, 6.0 eq.) and *N*,*N*-diisopropylethylamine (9.08 mL, 52.1 mmol, 8.0 eq.) were added and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with water (10 mL) and extracted with ethyl acetate (4 × 30 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (3 × 30 mL), dried over sodium sulfate, and concentrated *in vacuo* to yield the title compound **64** as an orange solid (1.35 g, 99%). R_f 0.24 (30% dichloromethane in petroleum ether); m.p. 125–126 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3375 (m), 2935 (w), 1722 (s), 1625 (m), 1575 (s), 1532 (w), 1498 (m), 1449 (m), 1435 (m), 1403 (m), 1347 (m), 1318 (m), 1254 (s), 1222 (s), 1168 (m), 1142 (m), 1108 (m), 1079 (m), 1046 (m), 991 (w), 899 (w), 875 (w), 838 (m), 795 (w), 776 (w), 738 (s), 687 (w), 622 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.21 (1H, d, *J* 8.9, H-5), 8.00 (1H, bs, H-9), 7.55 (1H, d, *J* 1.8, H-2), 7.24 (1H, dd, *J* 8.9, 1.8, H-6), 3.95 (3H, s, H-8), 3.08 (3H, d, *J* 5.1, H-10); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 166.0 (C-7), 145.9 (C-3), 136.7 (C-4), 134.0 (C-1), 127.2 (C-5), 115.5 (C-2), 115.3 (C-6), 52.9 (C-8), 30.0 (C-10); *m/z* (ESI$^+$) 211 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_9H_{11}O_4N_2^+$ ([M+H]$^+$) requires 211.07133, found 211.07151; HPLC (method 1) retention time 9.5 min, 100%.

Methyl 3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (66)



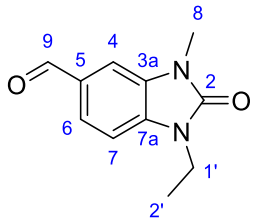
To a solution of **64** (1.43 g, 6.82 mmol, 1.0 eq.) in ethyl acetate (30 mL) and ethanol (15 mL) under an inert nitrogen atmosphere was added 10% palladium on carbon (363 mg, 0.341 mmol, 0.05 eq.). The reaction vessel was charged with hydrogen and the mixture was stirred at room temperature under a hydrogen atmosphere for 16 h. The reaction mixture was filtered through Celite® and concentrated *in vacuo*. The crude residue was dissolved in anhydrous dichloromethane (14 mL) and CDI (1.17 g, 7.21 mmol, 1.3 eq.) was added. The reaction mixture was stirred at room temperature for 5 h and then was cooled to 0 ºC, filtered, and washed with cold diethyl ether. The precipitate was collected and crystallised from water and ethanol to yield the title compound **66** as a colourless solid (850 mg, 60% over two steps). R_f 0.45 (10% methanol in dichloromethane); m.p. 220–221 ºC (from EtOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3066 (w), 1747 (s), 1693 (m), 1624 (m), 1503 (m), 1430 (m), 1391 (m), 1346 (m), 1301 (m), 1253 (s), 1114 (m), 1068 (m), 987 (w), 969 (w), 836 (w), 814 (w), 798 (w), 762 (s), 731 (m), 705 (m), 692 (m); ^{1}H NMR (400 MHz, CD_3OD) δ_H 7.81 (1H, dd, *J* 8.3, 1.6, H-6), 7.73 (1H, d, *J* 1.6, H-4), 7.12 (1H, d, *J* 8.3, H-7), 3.91 (3H, s, H-11), 3.42 (3H, s, H-8); ^{13}C NMR (126 MHz, CD_3OD) δ_C 168.7 (C-10), 157.2 (C-2), 133.8 (C-3a), 132.3 (C-7a), 125.2 (C-6), 124.5 (C-5), 110.0 (C-4), 109.8 (C-7), 52.6 (C-11), 27.1 (C-8); *m/z* (ESI$^+$) 207 ([M+H]$^+$, 100%), 229 ([M+Na]$^+$, 40%); HRMS *m/z* (ESI$^+$) $C_{10}H_{11}O_3N_2^+$ ([M+H]$^+$) requires 207.07642, found 207.07648; HPLC (method 1) retention time 6.5 min, 99.8%.

3-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (68)



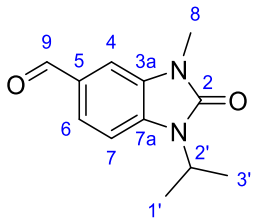
66 (706 mg, 3.43 mmol, 1.0 eq.) was suspended in anhydrous toluene (26 mL) under an argon atmosphere and cooled to 0 ºC. Diisobutylaluminium hydride (1 M in hexanes, 24.0 mL, 23.9 mmol, 7.0 eq.) was added dropwise and the solution was stirred at room temperature for 16 h. The reaction mixture was diluted with diethyl ether (60 mL) at 0 ºC. Water (1.36 mL) was slowly added, followed by 2 M aqueous sodium hydroxide (2.55 mL) and water (2.21 mL), and the mixture was stirred for 15 min. Sodium sulfate was added and stirred for a further 15 min. The mixture was filtered, the salts were stirred in ethanol and filtered again, and the combined organic components were concentrated *in vacuo.* The crude residue (500 mg, 2.82 mmol, 1.0 eq.) was suspended in dichloromethane (10 mL) and manganese(IV) oxide was added (2.45 g, 28.2 mmol, 10.0 eq.). The reaction mixture was stirred at room temperature for 16 h, filtered through Celite®, and concentrated *in vacuo* to yield the title compound **68** as a colourless solid (401 mg, 71% over two steps). R_f 0.19 (5% methanol in dichloromethane); m.p. 211–213 ºC (from CH_2Cl_2); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3167 (w), 1724 (s), 1674 (s), 1619 (s), 1504 (w), 1475 (m), 1441 (m), 1392 (m), 1321 (m), 1295 (m), 1272 (m), 1230 (w), 1170 (m), 1080 (w), 971 (w), 910 (w), 869 (w), 846 (w), 818 (s), 745 (w), 708 (m), 683 (m), 638 (m); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 9.89 (1H, s, H-10), 7.69 – 7.49 (2H, m, H-4 and H-6), 7.15 (1H, d, *J* 7.9, H-7), 3.33 (3H, s, H-8); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 191.8 (C-10), 154.7 (C-2), 134.0 (C-5), 131.5 (C-3a), 129.9 (C-7a), 125.8 (C-6), 108.6 (C-4), 106.9 (C-7), 26.6 (C-8); *m/z* (ESI$^+$) 177 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_9H_9O_2N_2^+$ ([M+H]$^+$) requires 177.06585, found 177.06592; HPLC (method 1) retention time 5.6 min, 99.8%.

1-Ethyl-3-methyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carbaldehyde (69)



68 (100 mg, 0.568 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (2 mL) under an argon atmosphere. Potassium carbonate (236 mg, 1.70 mmol, 3.0 eq.) was added and the mixture was stirred at room temperature for 30 min. Bromoethane (85.0 µL, 1.14 mmol, 2.0 eq.) was added and the mixture was stirred at 80 ºC for 4 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution (10 mL), followed by addition of water (5 mL) and ethyl acetate (10 mL). The mixture was extracted with ethyl acetate (3 × 30 mL) and the combined organic components were washed with 0.5 M aqueous lithium chloride solution (3 × 20 mL), dried over sodium sulfate, and concentrated *in vacuo* to yield the title compound **69** as a colourless solid (82.0 mg, 71%). R_f 0.81 (8% methanol in dichloromethane); m.p. 88–90 ºC (from CH_2Cl_2); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3055 (w), 2977 (w), 1703 (s), 1672 (s), 1618 (m), 1600 (m), 1450 (w), 1405 (w), 1383 (w), 1253 (w), 1182 (m), 1141 (w), 1081 (w), 1032 (w), 915 (w), 889 (w), 803 (s), 748 (w), 723 (w), 666 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.95 (1H, s, H-9), 7.64 (1H, dd, *J* 8.0, 1.5, H-6), 7.54 (1H, d, *J* 1.5, H-4), 7.11 (1H, d, *J* 8.0, H-7), 3.99 (2H, q, *J* 7.2, H-1'), 3.47 (3H, s, H-8), 1.37 (3H, t, *J* 7.2, H-2'); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 191.4 (C-9), 154.5 (C-2), 134.6 (C-7a), 130.9 (C-5), 130.7 (C-3a), 126.9 (C-6), 107.1 (C-7), 106.6 (C-4), 36.5 (C-1'), 27.5 (C-8), 13.8 (C-2'); *m/z* (ESI$^+$) 205 ([M+H]$^+$, 100%); m/z (ESI$^+$) 205 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{11}H_{13}O_2N_2^+$ ([M+H]$^+$) requires 205.09715, found 205.09725; HPLC (method 1) retention time 7.0 min, 100.0%.

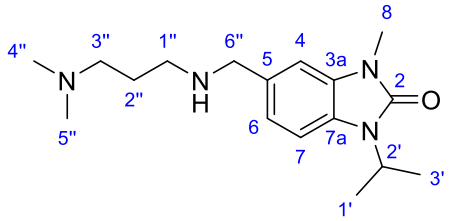
3-Methyl-2-oxo-1-(propan-2-yl)-2,3-dihydro-*1H*-benzimidazole-5-carbaldehyde (70)



68 (50 mg, 0.28 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL) under an argon atmosphere. Potassium carbonate (120 mg, 0.85 mmol, 3.0 eq.) was added and the mixture was stirred at room temperature for 30 min. 2-Iodopropane (57 µL, 0.57 mmol, 2.0 eq.) was added and the mixture was stirred at 80 ºC for 3 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution (5 mL), followed by addition of water (2.5 mL) and ethyl acetate (5 mL). The mixture was extracted with ethyl acetate (3 × 15 mL) and the combined organic components were washed with 0.5 M lithium chloride solution (3 × 10 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was dissolved in ethanol (3 mL) and heated to reflux. Hydrobromic acid (48% in H_2O, 30 µL, 0.28 mmol, 1.0 eq.) was added dropwise and the reaction mixture was stirred at reflux for 1 h. The reaction mixture was then cooled to room temperature and saturated aqueous sodium bicarbonate solution was added (3 mL). The resulting mixture was extracted with ethyl acetate (4 × 10 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude mixture was purified by silica gel flash column chromatography (20–60% ethyl acetate in petroleum ether) to yield the title compound **70** as a colourless solid (33 mg, 52%). R_f 0.81 (8% methanol in dichloromethane); m.p. 120–121 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2979 (w), 2359 (w), 1710 (s), 1686 (s), 1620 (w), 1598 (w), 1503 (w), 1481 (m), 1428 (w), 1389 (w), 1335 (w), 1192 (m), 1143 (w), 1073 (w), 867 (w), 804 (w), 750 (w), 683 (w), 617 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 9.94 (1H, s, H-9), 7.60 (1H, dd, *J* 8.1, 1.5, H-6), 7.52 (1H, d, *J* 1.5, H-4), 7.23 (1H, d, *J* 8.1, H-7), 4.76 (1H, sept, *J* 7.0, H-2'), 3.45 (3H, s, H-8), 1.55 (6H, d, *J* 7.0, H-1' and H-3'); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 191.4 (C-9), 154.2 (C-2), 133.8 (C-7a), 131.0 (C-3a), 130.2 (C-5a), 126.5 (C-6), 108.4 (C-7), 106.6 (C-4), 45.7 (C-2'), 27.4 (C-8), 20.4 (C-1' and C-3'); *m/z* (ESI$^+$) 219 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$)

$C_{12}H_{15}O_2N_2^+$ ([M+H]$^+$) requires 219.11280, found 219.11305; HPLC (method 1) retention time 7.6 min, 97.0%.

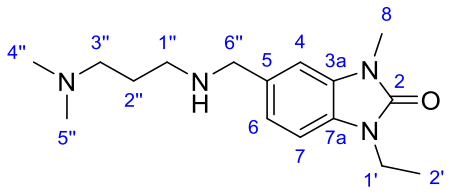
5-({[3''-(Dimethylamino)propyl]amino}methyl)-3-methyl-1-(propan-2'-yl)-1,3-dihydro-*2H*-benzimidazol-2-one (48)



N^1,*N*^{1}-Dimethylpropane-1,3-diamine (69 µL, 0.55 mmol, 2.0 eq.) and acetic acid (31 µL, 0.55 mmol, 2.0 eq.) were added at 0 ºC to a solution of **70** (60 mg, 0.28 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (6 mL) with 3 Å molecular sieves. The reaction mixture was warmed to room temperature for 3 h. Sodium triacetoxyborohydride (290 mg, 1.4 mmol, 5.0 eq.) was added and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (10 mL) and filtered through Celite®. The aqueous components were extracted with dichloromethane (8 × 10 mL), and the organic components were washed with brine (10 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was passed through an Isolute® SCX-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the title compound **48** as a colourless oil (44 mg, 53%). R_f 0.01 (1% triethylamine in ethanol); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3351 (w), 2937 (w), 2814 (w), 1694 (s), 1627 (w), 1604 (w), 1502 (w), 1461 (w), 1429 (w), 1389 (m), 1326 (w), 1257 (w), 1199 (w), 1152 (w), 1132 (w), 1101 (w), 1076 (w), 1041 (w), 1001 (w), 803 (w), 748 (w), 720 (w), 665 (w), 618 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.06 (1H, d, *J* 8.5, H-7), 7.01 – 6.97 (2H, m, H-6 and H-4), 4.72 (1H, sept, *J* 7.0, H-2'), 3.82 (2H, s, H-6''), 3.39 (3H, s, H-8), 2.69 (2H, t, *J* 7.1, H-1''), 2.37 – 2.27 (2H, m, H-3''), 2.21 (6H, s, H-4'' and H-5''), 1.75 – 1.64 (2H, m, H-2''), 1.51 (6H, d, *J* 7.0, H-1' and H-3'); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 154.2 (C-2), 133.4 (C-5), 130.6 (C-3a), 127.3 (C-7a), 120.9 (C-6), 108.7 (C-7), 107.5 (C-4), 58.2 (C-3''), 54.4 (C-6''), 48.1 (C-1''), 45.7 (C-4'' and

C-5’’), 45.1 (C-2’), 28.2 (C-2’’), 27.2 (C-8), 20.5 (C-1’ and C-3’); *m/z* (ESI$^+$) 305 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{17}H_{29}ON_4^+$ ([M+H]$^+$) requires 305.23359, found 305.23319; HPLC retention time 5.2 min, 95.4%.

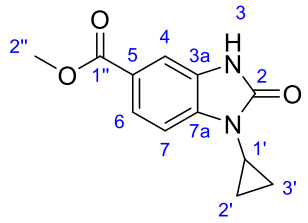
5-({[3’’-(Dimethylamino)propyl]amino}methyl)-1-ethyl-3-methyl-1,3-dihydro-*2H*-benzimidazol-2-one (47)



N^1,*N*^{1}-Dimethylpropane-1,3-diamine (65 µL, 0.52 mmol, 1.5 eq.) and acetic acid (39 µL, 0.69 mmol, 2.0 eq.) were added at 0 ºC to a solution of **69** (70 mg, 0.34 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (7 mL) with 3 Å molecular sieves. The reaction mixture was warmed to room temperature for 3 h. Sodium triacetoxyborohydride (360 mg, 1.7 mmol, 5.0 eq.) was added and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (10 mL) and filtered through Celite®. The aqueous component was extracted with dichloromethane (8 × 10 mL), and the organic components were washed with brine (10 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was passed through an Isolute® SCX-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the title compound **47** as a colourless oil (39 mg, 39%). R_f 0.01 (1% triethylamine in ethanol); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3350 (w), 2937 (w), 2815 (w), 1697 (s), 1628 (w), 1609 (w), 1505 (m), 1460 (m), 1431 (m), 1399 (m), 1354 (w), 1254 (w), 1213 (w), 1142 (w), 1081 (w), 1038 (w), 968 (w), 797 (w), 747 (w), 724 (w), 653 (w), 617 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.02 (1H, dd, *J* 7.9, 1.5, H-6), 6.99 (1H, d, *J* 1.5, H-4), 6.92 (1H, d, *J* 7.9, H-7), 3.92 (2H, q, *J* 7.2, H-1’), 3.82 (2H, s, H-6’’), 3.40 (3H, s, H-8), 2.69 (2H, t, *J* 7.0, H-1’’), 2.36 – 2.28 (2H, m, H-3’’), 2.21 (6H, s, H-4’’ and H-5’’), 1.69 (2H, p, *J* 7.0, H-2’’), 1.31 (3H, t, *J* 7.2, H-2’); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 154.4 (C-2), 133.6 (C-5), 130.5 (C-3a), 128.2 (C-7a), 121.2

(C-6), 107.6 (C-4), 107.2 (C-7), 58.2 (C-3’’), 54.4 (C-6’’), 48.0 (C-1’’), 45.7 (C-4’’ and C-5’’), 36.1 (C-1’), 28.0 (C-2’’), 27.2 (C-8), 13.8 (C-2’); mass not found in *m/z* or HRMS; HPLC (method 1) retention time 4.9 min, 94.9%.

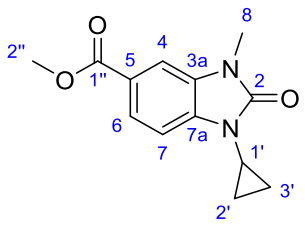
Methyl 1-cyclopropyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carboxylate (73)



To a solution of **37** (1.13 g, 4.79 mmol, 1.0 eq.) in ethyl acetate (50 mL) was added palladium hydroxide on carbon (20% loading, 336 mg, 0.479 mmol, 0.1 eq.) under a nitrogen atmosphere. The reaction vessel was then charged with hydrogen and the reaction mixture was stirred at room temperature under a hydrogen atmosphere for 3 h. The mixture was subsequently filtered through Celite® and concentrated *in vacuo*. The crude residue was dissolved in anhydrous dichloromethane (120 mL) and 1,1'-carbonyldiimidazole (2.41 g, 14.9 mmol, 3.0 eq.) was added. The reaction mixture was stirred at room temperature for 16 h. Water (50 mL) was added and the mixture was extracted with dichloromethane (3 × 100 mL), washed with brine (30 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (20–40% ethyl acetate in dichloromethane) to yield the title compound **73** as a colourless solid (338 mg, 29%). R_f 0.19 (40% ethyl acetate in dichloromethane); m.p. 185–189 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3027 (w), 1690 (s), 1482 (w), 1424 (m), 1401 (m), 1312 (m), 1287 (m), 1229 (s), 1186 (m), 1112 (m), 1085 (w), 1030 (m), 972 (w), 934 (w), 874 (w), 816 (w), 782 (w), 758 (m), 732 (m), 699 (m), 672 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.14 (1H, bs, H-3), 7.87 (1H, dd, *J* 8.3, 1.6, H-6), 7.72 (1H, d, *J* 1.6, H-4), 7.22 (1H, d, *J* 8.3, H-7), 3.92 (3H, s, H-2’’), 2.91 (1H, tt, *J* 7.1, 3.7, H-1’), 1.20 – 1.10 (2H, m, H-2’^{a} and H-3’^{a}), 1.09 – 0.98 (2H, m, H-2’^{b} and H-3’^{b}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 167.2 (C-1’), 156.4 (C-2), 135.2 (C-7a), 127.5 (C-3a), 124.0 (C-6), 123.9 (C-5), 110.9 (C-4), 108.3 (C-7), 52.2 (C-2’’), 22.7 (C-1’), 6.3 (C-2’ and C-3’); *m/z* (ESI$^+$) 233 ([M+H]$^+$, 100%), 255 ([M+Na]$^+$, 80%); HRMS *m/z*

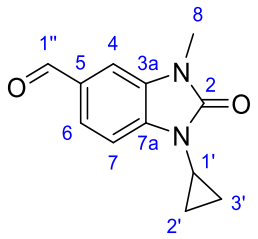
(ESI$^+$) $C_{12}H_{13}O_3N_2^+$ ([M+H]$^+$) requires 233.09207, found 233.09216; HPLC (method 1) retention time 7.2 min, 96.4%.

Methyl 1-cyclopropyl-3-methyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carboxylate (74)



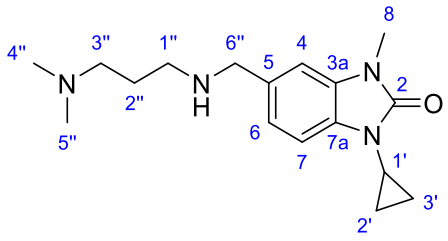
73 (340 mg, 1.38 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (7 mL). Potassium carbonate (954 mg, 6.90 mmol, 5.0 eq.) was added followed by dropwise addition of iodomethane (172 µL, 2.76 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 36 h. The reaction was quenched with water (8 mL) and extracted with diethyl ether (4 × 10 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (3 × 20 mL) and brine (10 mL), dried over sodium sulfate, and concentrated *in vacuo* to yield the title compound **74** as a colourless solid (320 mg, 94%). R_f 0.44 (40% ethyl acetate in dichloromethane); m.p. 142–144 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3070 (w), 2955 (w), 2161 (w), 2020 (w), 1977 (w), 1695 (s), 1622 (w), 1606 (w), 1507 (w), 1474 (m), 1424 (m), 1399 (w), 1350 (w), 1292 (m), 1254 (s), 1218 (w), 1188 (w), 1137 (w), 1118 (w), 1097 (m), 1074 (w), 1052 (w), 1029 (w), 1012 (w), 998 (m), 977 (m), 897 (m), 846 (w), 826 (w), 802 (w), 785 (w), 762 (s), 735 (s), 720 (w), 693 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.86 (1H, dd, *J* 8.2, 1.6, H-6), 7.63 (1H, d, *J* 1.6, H-4), 7.21 (1H, d, *J* 8.2, H-7), 3.93 (3H, s, H-2''), 3.42 (3H, s, H-8), 2.90 (1H, tt, *J* 7.0, 3.7, H-1'), 1.18 – 1.07 (2H, m, H-2'^{a} and H-3'^{a}), 1.06 – 0.96 (2H, m, H-2'^{b} and H-3'^{b}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_c 167.3 (C-1''), 155.0 (C-2), 134.3 (C-5), 129.7 (C-3a), 123.9 (C-6), 123.5 (C-7a), 108.6 (C-4), 107.9 (C-7), 52.3 (C-2''), 27.3 (C-8), 22.9 (C-1'), 6.2 (C-2' and C-3'); *m/z* (ESI$^+$) 247 ([M+H]$^+$, 80%), 269 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{13}H_{15}O_3N_2^+$ ([M+H]$^+$) requires 247.10772, found 247.10775; HPLC (method 1) retention time 8.0 min, 97.3%.

1-Cyclopropyl-3-methyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carbaldehyde (72)



74 (319 mg, 1.30 mmol, 1.0 eq.) was suspended in anhydrous dichloromethane (11 mL) under an argon atmosphere and cooled to 0 ºC. Diisobutylaluminium hydride (1 M in hexanes, 9.07 mL, 9.07 mmol, 7.0 eq.) was added dropwise and the solution was stirred at room temperature for 3 h. Aqueous Rochelle salts solution (30 mL) was added at 0 ºC and the mixture was stirred for 16 h. The aqueous component was extracted with dichloromethane (3 × 40 mL), and the combined organic components were concentrated *in vacuo.* The crude residue (81.9 mg, 0.371 mmol, 1.0 eq.) was suspended in dichloromethane (7 mL) and manganese(IV) oxide (323 mg, 3.71 mmol, 10.0 eq.) was added. The reaction mixture was stirred at room temperature for 16 h. The suspension was filtered through Celite®, washed with dichloromethane, and the filtrate was concentrated *in vacuo* to yield the title compound **72** as an orange solid (71.5 mg, 26% over two steps). R_f 0.52 (80% ethyl acetate in dichloromethane); m.p. 109–111 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2926 (w), 2725 (w), 2360 (w), 1710 (s), 1673 (s), 1618 (m), 1600 (m), 1502 (m), 1478 (m), 1429 (m), 1397 (w), 1373 (w), 1351 (w), 1285 (w), 1259 (m), 1211 (w), 1175 (m), 1127 (w), 1037 (w), 996 (w), 908 (w), 865 (m), 820 (m), 768 (m), 746 (m), 721 (m), 675 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.95 (1H, s, H-1''), 7.63 (1H, dd, *J* 8.0, 1.5, H-6), 7.50 (1H, d, *J* 1.5, H-4), 7.31 (1H, d, *J* 8.0, H-7), 3.43 (3H, s, H-8), 2.92 (1H, tt, *J* 7.1, 3.7, H-1'), 1.22 – 1.12 (2H, m, H-2'^{a} and 3'^{a}), 1.07 – 0.98 (2H, m, H-2'^{b} and H-3'^{b}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 191.5 (C-1''), 155.0 (C-2), 135.9 (C-7a), 130.9 (C-3a), 130.5 (C-5), 126.8 (C-6), 108.3 (C-7), 106.4 (C-4), 27.4 (C-8), 23.0 (C-1'), 6.3 (C-2' and C-3'); *m/z* (ESI$^+$) 217 ([M+H]$^+$, 20%), 455 ([2M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{12}H_{13}O_2N_2^+$ ([M+H]$^+$) requires 217.09715, found 217.09714; HPLC (method 1) retention time 7.0 min, 95.3%.

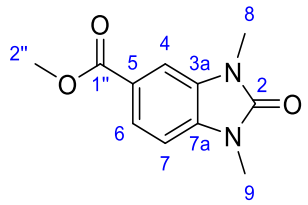
1-Cyclopropyl-5-({[3''-(dimethylamino)propyl]amino}methyl)-3-methyl-1,3-dihydro-*2H*-benzimidazol-2-one (49)



N^1,N^1-Dimethylpropane-1,3-diamine (70 µL, 0.56 mmol, 2.0 eq.) and acetic acid (32 µL, 0.56 mmol, 2.0 eq.) were added to a solution of **72** (60 mg, 0.28 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (6 mL) at 0 ºC. The reaction was warmed to room temperature until imine formation (2 h, followed by TLC and ^{1}H NMR). Sodium triacetoxyborohydride (290 mg, 1.4 mmol, 5.0 eq.) was added at 0 ºC and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (6 mL), filtered through Celite® and the filter cake was washed with water (10 mL) and dichloromethane (15 mL). The organic component was separated, and the aqueous component was extracted with dichloromethane (6 × 10 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution), followed by silica gel column chromatography (6:2:1 ethyl acetate:ethanol:ammonium hydroxide) to yield the title compound **49** as a colourless oil (14 mg, 17%). R_f 0.56 (4:2:1 ethyl acetate:ethanol:ammonium hydroxide); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3498 (w), 2942 (w), 2820 (w), 2360 (w), 1692 (s), 1628 (w), 1504 (m), 1463 (m), 1436 (m), 1401 (m), 1341 (m), 1261 (w), 1217 (w), 1193 (w), 1087 (w), 1031 (w), 1000 (w), 810 (w), 746 (w), 721 (w), 660 (w), 619 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.12 (1H, d, *J* 8.0, H-7), 7.02 (1H, dd, *J* 8.0, 1.6, H-6), 6.96 (1H, d, *J* 1.6, H-4), 3.82 (2H, s, H-6''), 3.37 (3H, s, H-8), 2.86 (1H, tt, *J* 7.0, 3.7, H-1'), 2.68 (2H, t, *J* 7.1, H-3''), 2.36 – 2.27 (2H, m, H-1''), 2.21 (6H, s, H-4'' and H-5''), 1.70 (2H, q, *J* 7.1, H-2''), 1.14 – 1.04 (2H, m, H-2'^{a} and H-3'^{a}), 1.02 – 0.92 (2H, m, H-2'^{b} and H-3'^{b}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 155.1 (C-2), 134.0 (C-5),

130.1 (C-7a), 129.4 (C-3a), 121.1 (C-6), 108.3 (C-7), 107.3 (C-4), 58.2 (C-1’’), 54.4 (C-6’’), 48.1 (C-3’’), 45.7 (C-4’’ and C-5’’), 28.2 (C-2’’), 27.2 (C-8), 22.7 (C-1’), 6.2 (C-2’ and C-3’); *m/z* (ESI$^+$) 303 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{17}H_{27}ON_4^+$ ([M+H]$^+$) requires 303.21794, found 303.21797; HPLC (method 1) retention time 4.9 min, 95.8%.

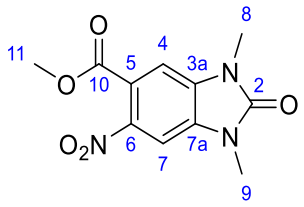
Methyl 1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carboxylate (23)



3,4-Diaminobenzoic acid (5.00 g, 32.9 mmol, 1.0 eq.) was suspended in anhydrous dichloromethane (40 mL) and 1,1'-carbonyldiimidazole (11.7 g, 72.3 mmol, 2.2 eq.) was added. The reaction mixture was stirred at room temperature for 1 h. Methanol (30 mL) was added and the reaction was stirred for 2 h. The solution was cooled to 0 ºC and left for 15 min until a precipitate formed. The precipitate was collected *via* filtration. The filter cake was washed with water (20 mL), and the resulting precipitate was suspended in methanol and concentrated. The crude solid was suspended in anhydrous *N*,*N*-dimethylformamide (20 mL) and potassium carbonate (23.6 g, 170 mmol, 4.0 eq.) was added. The suspension was cooled to 0 ºC and iodomethane (15.9 mL, 256 mmol, 6.0 eq.) was added dropwise. The mixture was warmed to room temperature and stirred for 16 h. After this time, a further addition of iodomethane was necessary (10.6 mL, 170 mmol, 4.0 eq.) and the reaction mixture was stirred at room temperature for a further 16 h. The solution was then diluted with water (80 mL) and extracted with ethyl acetate (4 × 30 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (4 × 20 mL) and brine (60 mL), dried over sodium sulfate, and concentrated *in vacuo* to yield the title compound **23** as a colourless solid (6.07 g, 84%). R_f 0.35 (70% ethyl acetate in petroleum ether); m.p. 146–148 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 1709 (s), 1621 (w), 1605 (w), 1509 (w), 1465 (w), 1445 (m), 1422 (w), 1400 (w), 1352 (w), 1294 (w), 1261 (s), 1233 (m), 1179 (w), 1143 (w), 1103 (m), 1028 (w), 977 (m), 938 (w), 888 (w), 824 (w), 801 (w), 760

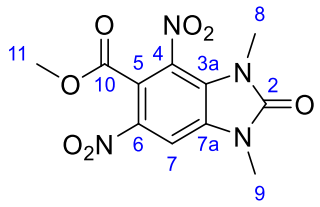
(s), 735 (w), 725 (w), 682 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 7.74 (1H, dd, *J* 8.2, 1.6, H-6), 7.65 (1H, d, *J* 1.6, H-4), 7.23 (1H, d, *J* 8.2, H-7), 3.84 (3H, s, H-2''), 3.37 (3H, s, H-8), 3.35 (3H, s, H-9); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 166.3 (C-1''), 154.0 (C-2), 133.6 (C-3a), 129.5 (C-7a), 123.1 (C-6), 122.1 (C-5), 108.1 (C-4), 107.3 (C-7), 51.9 (C-2''), 27.1, 27.0 (C-8 and C-9); *m/z* (ESI$^+$) 221 ([M+H]$^+$, 100%), 243 ([M+Na]$^+$, 20%); HRMS *m/z* (ESI$^+$) $C_{11}H_{13}O_3N_2^+$ ([M+H]$^+$) requires 221.09207, found 221.09209; HPLC (method 1) retention time 7.2 min, 100%.

Methyl 1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (82)



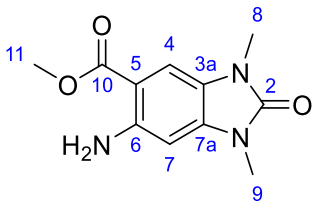
A solution of potassium nitrate (253 mg, 2.50 mmol, 1.1 eq.) in concentrated sulfuric acid (30 mL) was slowly added at room temperature to a solution of **23** (500 mg, 2.27 mmol, 1.0 eq.) in concentrated sulfuric acid (30 mL) and the mixture was stirred for 1 h. The reaction mixture was poured into water (300 mL) and neutralised with ammonium hydroxide solution. The precipitate was filtered, washed with water (100 mL) and dried over sodium sulfate, to yield the title compound **82** as an off-white solid (570 mg, 95%). R_f 0.41 (50% ethyl acetate in dichloromethane); m.p. 130–132 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3073 (w), 1708 (s), 1630 (w), 1608 (w), 1516 (m), 1463 (w), 1441 (w), 1426 (w), 1401 (w), 1346 (m), 1267 (m), 1250 (m), 1188 (w), 1150 (w), 1120 (w), 1047 (w), 989 (w), 958 (m), 890 (w), 836 (w), 809 (w), 779 (w), 760 (w), 744 (w), 728 (w), 697 (w), 654 (w), 636 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.58 (1H, s, H-7), 7.21 (1H, s, H-4), 3.92 (3H, s, H-11), 3.49 (3H, s, H-8), 3.48 (3H, s, H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 166.6 (C-10), 154.8 (C-2), 142.9 (C-6), 133.1 (C-3a), 131.3 (C-7a), 122.7 (C-5), 107.6 (C-4), 103.7 (C-7), 53.5 (C-11), 27.9 (C-8/C-9), 27.8 (C-8/C-9); *m/z* (ESI$^+$) 288 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{11}H_{11}O_5N_3Na^+$ ([M+Na]$^+$) requires 288.05909, found 288.05905; HPLC (method 1) retention time 7.7 min, 98.2%.

Methyl 1,3-dimethyl-4-nitro-6-nitroso-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (83)



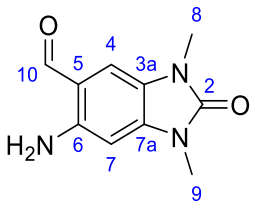
To a flask of fuming nitric acid (0.21 mL) at 0 ºC was slowly added **23** (40 mg, 0.18 mmol, 1.0 eq.) portionwise over 5 min. The solution was stirred at 0 ºC for 30 min and then at room temperature for 1 h. The solution was cooled to 0 ºC and diluted with water (0.20 mL). The mixture was stirred for 30 min and extracted with diethyl ether (3 × 10 mL), washed with brine, dried over sodium sulfate, and concentrated *in vacuo*. The crude mixture was purified by silica gel flash column chromatography (20–60% ethyl acetate in petroleum ether) to yield the title compound **83** as a colourless solid (31 mg, 59%). R_f 0.76 (50% ethyl acetate in petroleum ether); m.p. 185–186 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3086 (w), 2955 (w), 2361 (w), 1736 (s), 1538 (m), 1510 (m), 1467 (m), 1336 (m), 1262 (m), 1112 (m), 1001 (w), 945 (w), 907 (w), 874 (w), 850 (w), 809 (w), 793 (w), 775 (w), 761 (w), 744 (w), 725 (w), 695 (w), 663 (w), 631 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.85 (1H, s, H-7), 3.96 (3H, s, H-11), 3.56 (3H, s, H-9), 3.44 (3H, s, H-8); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 162.9 (C-10), 154.5 (C-2), 140.0 (C-6), 133.0 (C-7a), 131.3 (C-4), 126.5 (C-3a), 118.9 (C-5), 105.3 (C-7), 54.5 (C-11), 29.7 (C-8), 28.4 (C-9); *m/z* (ESI$^+$) 333 ([M+Na]$^+$, 100%), 311 ([M+H]$^+$, 40%); HRMS *m/z* (ESI$^+$) $C_{11}H_{11}O_7N_4^+$ ([M+H]$^+$) requires 311.06223, found 311.06242; HPLC (method 1) retention time 8.8 min, 100%.

Methyl 6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (84)



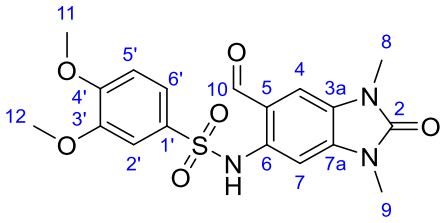
To a solution of **82** (540 mg, 2.04 mmol, 1.0 eq.) in ethyl acetate (15 mL) under an inert nitrogen atmosphere was added 10% palladium on carbon (217 mg, 0.204 mmol, 0.1 eq.). The reaction mixture was charged with hydrogen and stirred at room temperature under a hydrogen atmosphere for 4 h. The reaction mixture was filtered through Celite® and concentrated *in vacuo*. The crude mixture was purified by silica gel flash column chromatography (1% triethylamine, 40–70% ethyl acetate in petroleum ether) to yield the title compound **84** as a colourless solid (446 mg, 93%). R_f 0.30 (80% ethyl acetate in petroleum ether); m.p. 154–155 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3445 (m), 3337 (m), 2945 (w), 1702 (s), 1680 (s), 1504 (m), 1461 (s), 1406 (w), 1266 (m), 1217 (s), 1185 (m), 1095 (m), 1008 (m), 960 (w), 777 (w), 736 (w), 675 (w), 642 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.38 (1H, s, H-4), 6.21 (1H, s, H-7), 5.73 (2H, s, N*H*), 3.87 (3H, s, H-11), 3.35 (3H, s, H-8), 3.32 (3H, s, H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 168.7 (C-10), 155.2 (C-2), 147.9 (C-6), 136.1 (C-7a), 122.1 (C-3a), 108.7 (C-4), 104.2 (C-5), 94.8 (C-7), 51.6 (C-11), 27.3 (C-8 and C-9); *m/z* (ESI$^+$) 258 ([M+Na]$^+$, 100%), 236 ([M+H]$^+$, 40%); HRMS *m/z* (ESI$^+$) $C_{11}H_{14}O_3N_3^+$ ([M+H]$^+$) requires 236.10297, found 236.10289; HPLC (method 1) retention time 5.6 min, 99.0%.

6-Amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (85)



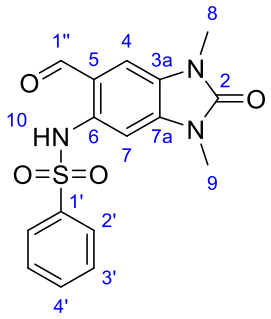
84 (415 mg, 1.76 mmol, 1.0 eq.) was dissolved in anhydrous dichloromethane (15 mL) and cooled to −78 ºC. Diisobutylaluminium hydride (1 M in hexanes, 12.4 mL, 12.4 mmol, 7.0 eq.) was added dropwise to the solution and the reaction mixture was stirred at −78 ºC for 5 h. The reaction was quenched with aqueous Rochelle salt solution (15 mL) and stirred at room temperature for 16 h. The mixture was extracted with dichloromethane (10 × 40 mL), washed with brine (30 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue (210 mg, 1.01 mmol, 1.0 eq.) was dissolved in dichloromethane (12 mL) and manganese(IV) oxide (881 mg, 10.3 mmol, 10.0 eq.) was added. The reaction mixture was stirred at room temperature for 16 h, and was subsequently filtered through Celite® and the filtrate was concentrated *in vacuo*. The crude mixture was purified by silica gel flash column chromatography (70–80% ethyl acetate in petroleum ether) to yield the title compound **85** as a brown solid (136 mg, 42% over two steps). R_f 0.63 (8% methanol in dichloromethane); m.p. 190–192 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3445 (m), 3332 (m), 2981 (w), 2360 (w), 1707 (s), 1648 (s), 1578 (m), 1503 (m), 1395 (w), 1347 (w), 1347 (m), 1282 (w), 1182 (m), 1075 (w), 949 (w), 846 (w), 798 (w), 730 (w), 686 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.78 (1H, s, H-10), 6.92 (1H, s, H-4), 6.20 (2H, bs, N*H*), 6.18 (1H, s, H-7), 3.37 (3H, s, H-8), 3.33 (3H, s, H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 192.0 (C-10), 155.1 (C-2), 148.0 (C-6), 137.0 (C-7a), 122.5 (C-3a), 113.4 (C-5), 112.3 (C-4), 93.8 (C-7), 27.38, 27.35 (C-8 and C-9); *m/z* (ESI$^+$) 206 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{10}H_{12}O_2N_3^+$ ([M+H]$^+$) requires 206.09240, found 206.09236; HPLC (method 1) retention time 5.8 min, 99.7%.

_N_-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-6-yl)-3',4'-dimethoxybenzene-1'-sulfonamide (88)



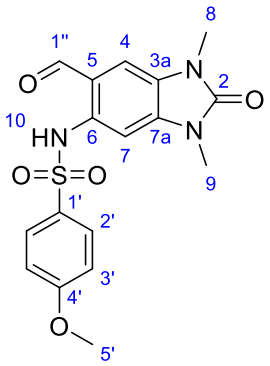
To a solution of **85** (100 mg, 0.49 mmol, 1.0 eq.) in anhydrous dichloromethane (3 mL) were added pyridine (79 µL, 0.97 mmol, 2.0 eq.) and 3,4-dimethoxybenzenesulfonyl chloride (230 mg, 0.97 mmol, 2.0 eq.). The mixture was stirred for 16 h at room temperature and was subsequently quenched with methanol (2 mL), and concentrated *in vacuo*. The crude mixture was purified by silica gel flash column chromatography (60–100% ethyl acetate in petroleum ether) to yield the title compound **88** as a yellow oil (110 mg, 54%). R_f 0.48 (5% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3078 (w), 1715 (s), 1656 (s), 1634 (s), 1600 (m), 1509 (s), 1454 (s), 1394 (w), 1364 (m), 1344 (m), 1264 (s), 1241 (m), 1185 (m), 1152 (w), 1138 (m), 1125 (w), 1093 (w), 1068 (m), 1019 (w), 999 (w), 954 (m), 914 (m), 877 (m), 835 (w), 816 (m), 771 (w), 736 (m), 721 (m), 681 (w), 658 (w), 638 (w), 616 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 10.90 (1H, s, H-10), 9.73 (1H, s, N*H*), 7.43 (1H, dd, *J* 8.5, 2.2, H-6'), 7.40 (1H, s, H-4), 7.27 (1H, d, *J* 2.2, H-2'), 7.04 (1H, s, H-7), 6.80 (1H, d, *J* 8.5, H-5'), 3.87 (3H, s, H-11), 3.85 (3H, s, H-12), 3.44 (3H, s, H-8), 3.41 (3H, s, H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 193.4 (C-10), 154.9 (C-2), 153.0 (C-4'), 149.2 (C-3'), 136.7 (C-7a), 135.9 (C-1'), 130.8 (C-6), 126.3 (C-3a), 121.4 (C-5), 116.8 (C-6'), 113.2 (C-5'), 110.3 (C-2'), 109.5 (C-4), 98.7 (C-7), 56.34, 56.25 (C-11 and C-12), 27.8, 27.5 (C-8 and C-9); *m/z* (ESI$^+$) 428 ([M+Na]$^+$, 100%), 406 ([M+H]$^+$, 20%); HRMS *m/z* (ESI$^+$) $C_{18}H_{20}O_6N_3S^+$ ([M+H]$^+$) requires 406.10673, found 406.10667; HPLC (method 1) retention time 8.1 min, 99.7%.

_N_-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-_1H_-benzimidazol-6-yl)benzenesulfonamide (86)



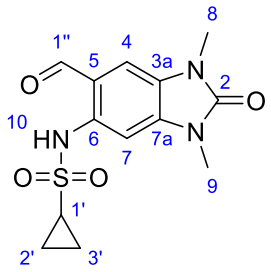
Pyridine (83 µL, 1.0 mmol, 3.0 eq.) and benzenesulfonyl chloride (130 µL, 1.0 mmol, 3.0 eq.) were added to a solution of **85** (70 mg, 0.34 mmol, 1.0 eq.) in anhydrous dichloromethane (3 mL). The reaction mixture was stirred at room temperature for 16 h, after which a further addition of pyridine (55 µL, 0.68 mmol, 2.0 eq.) and benzenesulfonyl chloride (87 µL, 0.68 mmol, 2.0 eq.) were necessary. After a further 16 h, the reaction mixture was quenched with methanol (2 mL), and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (0–1% methanol in dichloromethane) to yield the title compound **86** as an orange solid (81 mg, 69%). R_f 0.63 (8% methanol in dichloromethane); m.p. 164–167 ºC (from CH_2Cl_2); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3065 (w), 2359 (w), 1720 (s), 1656 (s), 1637 (s), 1602 (w), 1514 (m), 1461 (w), 1419 (m), 1396 (w), 1374 (s), 1260 (w), 1184 (m), 1167 (m), 1127 (w), 1091 (w), 1071 (m), 1000 (w), 955 (w), 907 (w), 862 (m), 836 (w), 780 (w), 732 (w), 688 (m), 629 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 10.99 (1H, s, H-10), 9.72 (1H, s, H-1''), 7.86 – 7.80 (2H, m, H-2'), 7.54 – 7.48 (1H, m, H-4'), 7.46 – 7.39 (3H, m, H-7 and H-3'), 7.04 (1H, s, H-4), 3.43 (3H, s, H-9), 3.40 (3H, s, H-8); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 193.3 (C-1''), 154.9 (C-2), 139.3 (C-1'), 136.6 (C-7a), 136.0 (C-6), 133.3 (C-4'), 129.2 (C-3'), 127.2 (C-2'), 126.4 (C-5), 116.7 (C-3a), 113.1 (C-4), 98.5 (C-7), 27.8, 27.5 (C-8 and C-9); *m/z* (ESI$^+$) 368 ([M+Na]$^+$, 100%), 346 ([M+H]$^+$, 10%); HRMS *m/z* (ESI$^+$) $C_{16}H_{15}O_4N_3SNa^+$ ([M+Na]$^+$) requires 368.06755, found 368.06763; HPLC (method 1) retention time 8.3 min, 94.7%.

_N_-(6-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-5-yl)-4'-methoxybenzene-1'-sulfonamide (87)



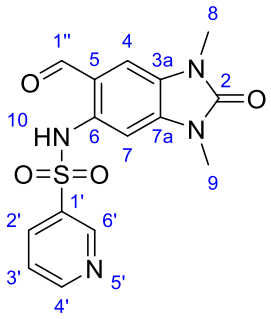
Pyridine (83 µL, 1.0 mmol, 3.0 eq.) and 4-methoxybenzenesulfonyl chloride (210 mg, 1.0 mmol, 3.0 eq.) were added to a solution of **85** (70 mg, 0.34 mmol, 1.0 eq.) in anhydrous dichloromethane (3 mL). The reaction mixture was stirred at room temperature for 16 h, after which a further addition of pyridine (55 µL, 0.68 mmol, 2.0 eq.) and 4-methoxybenzenesulfonyl chloride (140 mg, 0.68 mmol, 2.0 eq.) were necessary. After a further 16 h, the reaction mixture was quenched with methanol (2 mL), and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (0–2% methanol in dichloromethane) to yield the title compound **87** as an orange solid (45 mg, 35%). R_f 0.63 (8% methanol in dichloromethane); m.p. 187–189 ºC (from CH_2Cl_2); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2945 (w), 2845 (w), 2360 (w), 1721 (s), 1656 (s), 1637 (s), 1596 (m), 1514 (m), 1461 (m), 1418 (m), 1396 (w), 1373 (m), 1310 (w), 1262 (m), 1186 (m), 1158 (s), 1127 (w), 1093 (m), 1070 (w), 1023 (w), 1001 (w), 955 (w), 904 (w), 835 (w), 804 (w), 778 (w), 735 (w), 681 (w), 628 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 10.90 (1H, s, H-10), 9.73 (1H, s, H-1''), 7.79 – 7.73 (2H, m, H-2'), 7.39 (1H, s, H-7), 7.04 (1H, s, H-4), 6.91 – 6.83 (2H, m, H-3'), 3.80 (3H, s, H-5'), 3.44 (3H, s, H-8), 3.41 (3H, s, H-9); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 193.3 (C-1''), 163.4 (C-1'), 154.9 (C-2), 136.8 (C-7a), 136.0 (C-1'), 131.0 (C-6), 129.5 (C-2'), 126.3 (C-3a), 116.7 (C-5), 114.4 (C-3'), 113.1 (C-4), 98.5 (C-7), 55.7 (C-5'), 27.8, 27.5 (C-8 and C-9); *m/z* (ESI$^+$) 398 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{17}H_{17}O_5N_3SNa^+$ ([M+Na]$^+$) requires 398.07811, found 398.07817; HPLC (method 1) retention time 8.4 min, 98.7%.

_N_-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-_1H_-benzimidazol-6-yl)cyclopropanesulfonamide (91)



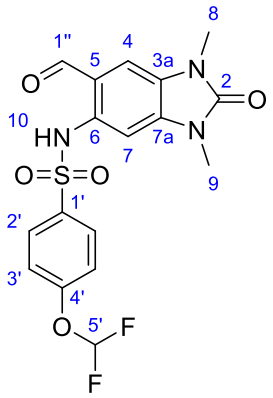
Pyridine (95 µL, 1.2 mmol, 3.0 eq.) and cyclopropanesulfonyl chloride (120 µL, 1.2 mmol, 3.0 eq.) were added to a solution of **85** (80 mg, 0.39 mmol, 1.0 eq.) in anhydrous dichloromethane (4 mL). The reaction mixture was stirred at room temperature for 16 h, after which a further addition of pyridine (30 µL, 0.39 mmol, 1.0 eq.) and cyclopropanesulfonyl chloride (40 µL, 0.39 mmol, 1.0 eq.) were necessary. After a further 3 h, the reaction mixture was quenched with methanol (3 mL), and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (0–0.5% methanol in dichloromethane) to yield the title compound **91** as an orange solid (31 mg, 25%). R_f 0.32 (5% methanol in dichloromethane); m.p. 163–165 ºC (from CH_2Cl_2); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3052 (w), 2558 (w), 2161 (m), 2029 (m), 1976 (m), 1719 (s), 1637 (s), 1601 (w), 1513 (m), 1459 (m), 1417 (m), 1395 (m), 1370 (w), 1346 (w), 1255 (m), 1189 (w), 1151 (m), 1125 (s), 1067 (m), 1040 (w), 1000 (w), 954 (w), 903 (w), 885 (m), 854 (w), 834 (w), 779 (w), 744 (w), 722 (m), 669 (w), 630 (w), 615(w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 10.69 (1H, s, H-10), 9.86 (1H, d, *J* 0.6, H-1''), 7.45 (1H, s, H-4), 7.17 (1H, s, H-7), 3.46 (3H, s, H-8), 3.45 (3H, s, H-9), 2.51 (1H, tt, *J* 8.0, 4.8, H-1'), 1.30 – 1.21 (2H, m, H-2'^{a} and H-3'^{a}), 1.00 – 0.91 (2H, m, H-2'^{b} and H-3'^{b}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 193.4 (C-1''), 154.9 (C-2), 137.6 (C-7a), 136.2 (C-6), 126.3 (C-3a), 116.8 (C-5), 113.2 (C-7), 98.4 (C-4), 30.6 (C-1'), 27.8 (C-8/C-9), 27.6 (C-8/C-9), 6.00 (C-2' and C-3'); *m/z* (ESI$^+$) 332 ([M+Na]$^+$, 100%), 310 ([M+H]$^+$, 20%); HRMS *m/z* (ESI$^+$) $C_{13}H_{15}O_4N_3SNa^+$ ([M+Na]$^+$) requires 332.06755, found 332.06805; HPLC retention time 7.2 min, 98.9%.

_N_-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-_1H_-benzimidazol-6-yl)pyridine-3-sulfonamide (89)



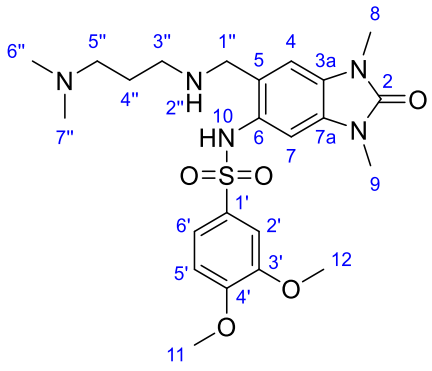
Pyridine (95 µL, 1.2 mmol, 3.0 eq.) and pyridine-3-sulfonyl chloride (140 µL, 1.2 mmol, 3.0 eq.) were added to a solution of **85** (80 mg, 0.39 mmol, 1.0 eq.) in anhydrous dichloromethane (4 mL). The solution was stirred at room temperature for 16 h. The reaction mixture was then quenched with methanol (3 mL), and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (0–2% methanol in dichloromethane) to yield the title compound **89** as a yellow solid (59 mg, 43%). R_f 0.25 (5% methanol in dichloromethane); m.p. 163–165 ºC (from CH_2Cl_2); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3055 (w), 2923 (w), 2852 (w), 2249 (w), 1717 (s), 1656 (s), 1635 (m), 1602 (s), 1573 (w), 1514 (w), 1460 (s), 1418 (s), 1396 (m), 1375 (s), 1328 (w), 1259 (w), 1169 (s), 1124 (w), 1106 (m), 1070 (w), 1021 (w), 1000 (w), 955 (w), 910 (m), 863 (w), 836 (w), 810 (w), 780 (w), 740 (m), 701 (m), 681 (w), 649 (w), 620 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 11.03 (1H, s, H-10), 9.71 (1H, s, H-1''), 8.97 (1H, d, *J* 2.4, H-6'), 8.72 (1H, dd, *J* 4.8, 1.7, H-4'), 8.12 (1H, ddd, *J* 8.2, 2.4, 1.7, H-2'), 7.43 (1H, s, H-7), 7.39 (1H, dd, *J* 8.2, 4.8, H-3'), 7.06 (1H, s, H-4), 3.46 (3H, s, H-8), 3.42 (3H, s, H-9); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 193.5 (C-1''), 154.8 (C-2), 153.9 (C-4'), 148.0 (C-6'), 136.1 (C-1'), 136.0 (C-7a), 135.7 (C-6), 134.9 (C-2'), 126.9 (C-3a), 124.0 (C-3'), 117.0 (C-5), 113.3 (C-4), 99.0 (C-7), 27.9, 27.6 (C-8 and C-9); *m/z* (ESI$^+$) 369 ([M+Na]$^+$, 100%), 347 ([M+H]$^+$, 80%); HRMS *m/z* (ESI$^+$) $C_{15}H_{15}O_4N_4S^+$ ([M+H]$^+$) requires 347.08085, found 347.08097; HPLC (method 1) retention time 7.0 min, 96.0%.

4'-(Difluoromethoxy)-*N*-(5-formyl-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl)benzene-1'-sulfonamide (90)



Pyridine (95 µL, 1.2 mmol, 3.0 eq.) and 4-(difluoromethoxy)benzenesulfonyl chloride (190 µL, 1.2 mmol, 3.0 eq.) were added to a solution of **85** (80 mg, 0.39 mmol, 1.0 eq.) in anhydrous dichloromethane (4 mL). The solution was stirred at room temperature for 16 h. The reaction mixture was then quenched with methanol (3 mL), and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (0–0.5% methanol in dichloromethane) to yield the title compound **90** as an orange solid (99 mg, 61%). R_f 0.44 (5% methanol in dichloromethane); m.p. 145–146 ºC (from CH_2Cl_2); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3105 (w), 2947 (w), 1719 (s), 1657 (m), 1637 (s), 1599 (w), 1514 (m), 1494 (w), 1461 (m), 1418 (w), 1396 (w), 1375 (m), 1301 (w), 1237 (w), 1186 (m), 1163 (s, C-F), 1122 (m), 1092 (m), 1067 (m), 1000 (w), 955 (w), 908 (w), 863 (w), 836 (w), 779 (w), 732 (m), 673 (w), 649 (w), 620 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 10.96 (1H, s, H-1''), 9.72 (1H, s, H-10), 7.86 – 7.79 (2H, m, H-2'), 7.40 (1H, s, H-7), 7.17 – 7.09 (2H, m, H-3'), 7.06 (1H, s, H-4), 6.54 (1H, t, *J* 73.0, H-5'), 3.45 (3H, s, H-8), 3.42 (3H, s, H-9); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 193.4 (C-1''), 154.8 (C-2), 154.5 (t, *J* 2.9, C-4'), 136.3 (C-1'), 136.03 (C-7a), 135.96 (C-6), 129.5 (C-2'), 126.6 (C-3a), 119.5 (C-3'), 116.9 (C-5), 115.2 (t, *J* 262.8, C-5'), 113.2 (C-4), 98.7 (C-7), 27.8, 27.6 (C-8 and C-9); ^{19}F NMR (376 MHz, $CDCl_3$) δ_F −82.27 (2F, d, *J* 73.0, CHF_2); *m/z* (ESI$^+$) 434 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{17}H_{15}O_5N_3F_2SNa^+$ ([M+Na]$^+$) requires 434.05927, found 434.05929; HPLC (method 1) retention time 8.9 min, 97.1%.

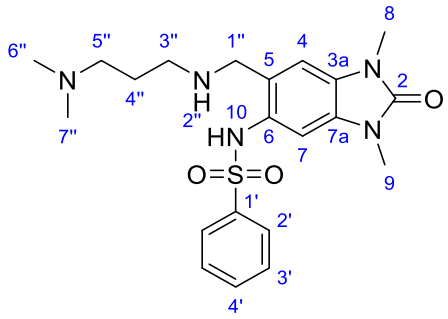
_N_-[6-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl]-3',4'-dimethoxybenzene-1'-sulfonamide (76)



N^1,N^1-Dimethylpropane-1,3-diamine (13 µL, 0.13 mmol, 1.5 eq.) and acetic acid (9.6 µL, 0.17 mmol, 2.0 eq.) were added to a solution of **88** (34 mg, 0.084 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (3 mL) at 0 ºC. The reaction mixture was warmed to room temperature until imine formation was observed (3 h, followed by TLC and ^{1}H NMR). The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (3 mL), filtered through Celite® and the filter cake was washed with water and dichloromethane. The organic component was separated, and the aqueous component was extracted with dichloromethane (6 × 5 mL). The combined organic components were washed with brine (15 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude imine residue was dissolved in anhydrous methanol (3 mL) and sodium borohydride (16 mg, 0.42 mmol, 5.0 eq.) was added at 0 ºC. The reaction mixture was stirred for 1 h, after which the mixture was neutralised to pH 7 with 1 M aqueous hydrochloric acid and concentrated *in vacuo*. The crude residue was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the title compound **76** as a colourless solid (29 mg, 71%). R_f 0.01 (1% triethylamine in ethanol); m.p. 215 ºC (dec. from EtOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3403 (w), 2940 (w), 2360 (w), 1695 (s), 1589 (w), 1507 (m), 1440 (s), 1397 (m), 1327 (m), 1260 (m), 1236 (m), 1153 (m), 1091 (m), 1017 (m), 879 (m), 741 (w), 682 (w), 663 (w), 616 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 7.31 (1H, dd, *J* 8.4, 2.1, H-6'), 7.26 (1H, d, *J* 2.1, H-2'), 6.96 (1H, d, *J* 8.4, H-5'), 6.93 (1H, s, H-4), 6.91 (1H, s, H-7), 3.84 (3H, s, H-12), 3.75 (3H, s, H-11), 3.68 (2H, s, H-1''), 3.35 (3H, s, obscured

by residual methanol peak, H-9), 3.28 (3H, s, H-8), 2.59 (2H, t, *J* 7.3, H-3’’), 2.43 – 2.31 (2H, m, H-5’’), 2.24 (6H, s, H-6’’ and H-7’’), 1.75 – 1.62 (2H, m, H-4’’); ^{13}C NMR (126 MHz, CD_3OD) δ_C 156.4 (C-2), 153.0 (C-4’), 150.2 (C-3’), 139.0 (C-5), 136.9 (C-1’), 130.8 (C-7a), 126.5 (C-6), 126.3 (C-3a), 121.3 (C-6’), 111.9 (C-5’), 111.1 (C-2’), 110.4 (C-4), 105.2 (C-7), 58.6 (C-5’’), 56.5 (C-11 and C-12), 52.3 (C-1’’), 47.7 (C-3’’), 45.4 (C-6’’ and C-7’’), 27.5 (C-4’’), 27.4, 27.3 (C-8 and C-9); *m/z* (ESI$^+$) 492 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{23}H_{34}O_5N_5S^+$ ([M+H]$^+$) requires 492.22752, found 492.22710; HPLC (method 1) retention time 5.7 min, 100%.

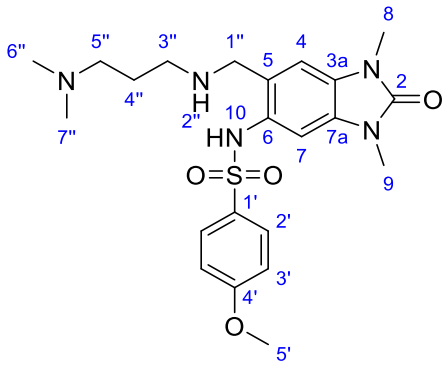
_N_-[5-({[5’’-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]benzenesulfonamide (77)



N^1,N^1-Dimethylpropane-1,3-diamine (33 µL, 0.26 mmol, 1.5 eq.) and acetic acid (20 µL, 0.35 mmol, 2.0 eq.) were added to a solution **86** (60 mg, 0.17 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (6 mL) at 0 ºC. The reaction mixture was warmed to room temperature until imine formation was observed (3 h, followed by TLC and ^{1}H NMR). The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (6 mL), filtered through Celite® and the filter cake was washed with water and dichloromethane. The organic component was separated, and the aqueous component was extracted with dichloromethane (6 × 10 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude imine residue was dissolved in anhydrous methanol (4 mL) and sodium borohydride (33 mg, 0.87 mmol, 5.0 eq.) was added at 0 ºC. The reaction mixture was stirred for 1 h, after which the mixture was neutralised to pH 7 with 1 M aqueous hydrochloric acid and concentrated *in vacuo*. The crude residue was purified using Amine Isolute™ Catch and

Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution), followed by silica gel column chromatography (6:2:1 ethyl acetate:ethanol:ammonium hydroxide) to yield the title compound **77** as a colourless solid (40 mg, 53%). R_f 0.01 (1% triethylamine in ethanol); m.p. 130–133 ºC (from EtOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3268 (w), 2941 (w), 2815 (w), 2766 (w), 2162 (w), 1979 (w), 1693 (s), 1639 (w), 1610 (w), 1509 (m), 1462 (m), 1446 (m), 1400 (m), 1377 (w), 1337 (m), 1257 (m), 1215 (w), 1166, 1121 (s), 1093 (m), 1044 (m), 1013 (m), 999 (w), 978 (w), 960 (w), 942 (w), 869 (m), 845 (m), 780 (m), 744 (s), 732 (s), 681 (s), 627 (m); ^{1}H NMR (500 MHz, CD_3OD) δ_H 7.77 – 7.72 (2H, m, H-2'), 7.57 – 7.51 (1H, m, H-4'), 7.49 – 7.43 (2H, m, H-3'), 6.97 (1H, s, H-4), 6.82 (1H, s, H-7), 3.79 (2H, s, H-1''), 3.34 (3H, s, H-8), 3.25 (3H, s, H-9), 2.78 (2H, t, *J* 7.0, H-3''), 2.52 – 2.44 (2H, m, H-5''), 2.29 (6H, s, H-6''and H-7''), 1.79 (2H, p, *J* 7.1, H-4''); ^{13}C NMR (126 MHz, CD_3OD) δ_C 156.4 (C-2), 143.5 (C-1'), 137.2 (C-5), 133.0 (C-4'), 131.4 (C-7a), 129.9 (C-3'), 128.0 (C-2'), 127.1 (C-6), 123.9 (C-3a), 110.5 (C-4), 105.0 (C-7), 58.4 (C-5''), 51.9 (C-1''), 47.4 (C-3''), 45.3 (C-6'' and C-7''), 27.4, 27.3 (C-8 and C-9), 26.5 (C-4); *m/z* (ESI$^+$) 432 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{21}H_{30}O_3N_5S^+$ ([M+H]$^+$) requires 432.20639, found 432.20616; HPLC (method 1) retention time 5.6 min, 99.4%.

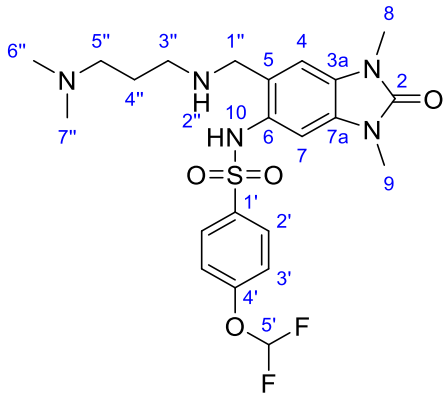
_N_-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]-4'-methoxybenzene-1'-sulfonamide (78)



N^1,N^1-Dimethylpropane-1,3-diamine (20 µL, 0.16 mmol, 2.0 eq.) and acetic acid (9.0 µL, 0.16 mmol, 2.0 eq.) were added to a solution of **87** (30 mg, 0.080 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (3 mL) at 0 ºC. The reaction mixture was warmed to room temperature until imine formation was observed (3 h, followed by TLC and ^{1}H NMR). The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (4 mL), filtered through Celite® and the filter cake was washed with water and dichloromethane. The organic component was separated, and the aqueous component was extracted with dichloromethane (6 × 10 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude imine residue was dissolved in anhydrous methanol (3 mL) and sodium borohydride (15 mg, 0.40 mmol, 5.0 eq.) was added at 0 ºC. The reaction mixture was stirred for 1 h, after which the mixture was neutralised to pH 7 with 1 M aqueous hydrochloric acid and concentrated *in vacuo*. The crude residue was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the title compound **78** as a colourless solid (40 mg, 37%). R_f 0.01 (1% triethylamine in ethanol); m.p. 141–142 ºC (from EtOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3481 (w), 2945 (w), 2825 (w), 1693 (s, C=O), 1638 (w), 1596 (m), 1579 (w), 1499 (m), 1461 (m), 1397 (w), 1332 (w), 1306 (w), 1257 (m), 1157 (s), 1124 (m), 1092 (m), 1020 (w), 935 (w), 836 (w), 804 (w), 741 (w), 665 (w), 629 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.65 – 7.60 (2H, m, H-2'), 7.24 (1H, s, H-4), 6.87 – 6.82 (2H, m, H-3'), 6.55 (1H, s, H-7), 3.81 (3H, s, H-5'), 3.41 (3H, s, H-9), 3.34 (3H, s, H-8), 3.33

(2H, s, H-1'') 2.54 (2H, t, *J* 6.9, H-3''), 2.32 (2H, t, *J* 6.9, H-5''), 2.21 (6H, s, H-6'' and H-7''), 1.66 (2H, p, *J* 6.9, H-4''); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 162.9 (C-4'), 154.9 (C-2), 132.6 (C-1'), 132.2 (C-5), 130.0 (C-7a), 128.9 (C-2'), 127.0 (C-3a), 123.2 (C-6), 114.1 (C-3'), 108.2 (C-7), 104.1 (C-4), 58.1 (C-5''), 55.7 (C-5'), 53.1 (C-1''), 47.6 (C-3''), 45.7 (C-6'' and C-7''), 27.64 (C-4''), 27.57, 27.3 (C-8 and C-9); *m/z* (ESI$^+$) 462 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{22}H_{32}O_4N_5S^+$ ([M+H]$^+$) requires 462.21695, found 462.21694; HPLC (method 1) retention time 5.8 min, 95.1%.

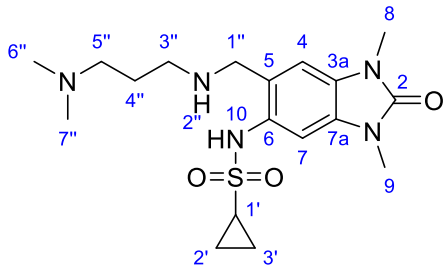
4'-(Difluoromethoxy)-*N*-[5-({[5''-(dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]benzene-1'-sulfonamide (80)



N^1,*N*^{1}-Dimethylpropane-1,3-diamine (43 µL, 0.34 mmol, 2.0 eq.) and acetic acid (19 µL, 0.34 mmol, 2.0 eq.) were added to a solution **90** (70 mg, 0.17 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (7 mL) at 0 ºC. The reaction mixture was warmed to room temperature until imine formation was observed (3 h, followed by TLC and ^{1}H NMR). The reaction was quenched with saturated aqueous sodium bicarbonate solution (8 mL), filtered through Celite® and the filter cake was washed with water and dichloromethane. The organic component was separated, and the aqueous component was extracted with dichloromethane (6 × 10 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude imine residue was dissolved in anhydrous methanol (6 mL) and sodium borohydride (32 mg, 0.85 mmol, 5.0 eq.) was added at 0 ºC. The reaction mixture was stirred for 1 h, after which the mixture was neutralised to pH 7 with 1 M aqueous hydrochloric acid and

concentrated *in vacuo*. The crude residue was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution), followed by silica gel column chromatography (8:2:1 ethyl acetate:ethanol:ammonium hydroxide) to yield the title compound **80** as a colourless solid (37 mg, 43%). R_f 0.01 (1% triethylamine in ethanol); m.p. 110–112 ºC (from EtOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3464 (w), 2949 (w), 2828 (w), 1690 (s), 1637 (w), 1598 (w), 1504 (m), 1462 (m), 1398 (w), 1336 (w), 1241 (m), 1163 (w), 1122 (s, C-F), 1091 (w), 1043 (w), 1022 (w), 1012 (w), 937 (w), 861 (w), 839 (w), 733 (w), 710 (w), 652 (w), 623 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.74 – 7.69 (2H, m, H-2'), 7.22 (1H, s, H-4), 7.13 – 7.08 (2H, m, H-3'), 6.57 (1H, s, H-7), 6.55 (1H, t, *J* 73.0, H-5') 3.41 (3H, s, H-9), 3.35 (2H, s, H-1''), 3.34 (3H, s, H-8), 2.56 (2H, t, *J* 6.8, H-3''), 2.35 (2H, t, *J* 6.8, H-5''), 2.23 (6H, s, H-6'' and H-7''), 1.67 (2H, p, *J* 6.8, H-4''); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 154.9 (C-2), 154.1 (t, *J* 2.6, C-4'), 137.7 (C-1'), 131.9 (C-5), 130.1 (C-7a), 128.9 (C-2'), 127.2 (C-3a), 122.9 (C-6), 119.3 (C-3'), 115.3 (t, *J* 262.4, C-5'), 108.3 (C-7), 103.9 (C-4), 58.0 (C-5''), 53.0 (C-1''), 47.6 (C-3''), 45.6 (C-6'' and C-7''), 27.6, 27.34 (C-8 and C-9), 27.31 (C-4''); ^{19}F NMR (376 MHz, $CDCl_3$) –82.09 (2F, d, *J* 73.0, CHF_2); *m/z* (ESI$^+$) 498 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{22}H_{30}O_4N_5F_2S^+$ ([M+H]$^+$) requires 498.19811, found 498.19786; HPLC (method 1) retention time 6.3 min, 99.8%.

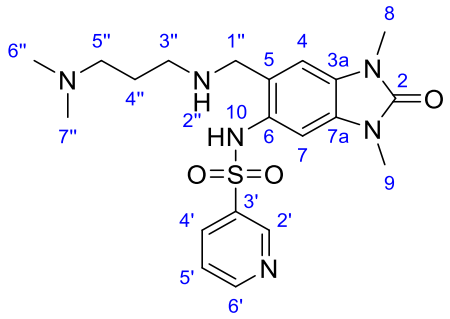
_N_-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]cyclopropanesulfonamide (81)



N^1,N^1-Dimethylpropane-1,3-diamine (20 µL, 0.16 mmol, 2.0 eq.) and acetic acid (9.1 µL, 0.16 mmol, 2.0 eq.) was added to a solution of **91** (25 mg, 0.080 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (3 mL) at 0 ºC. The reaction mixture was warmed to room temperature until

imine formation was observed (3 h, followed by TLC and ^{1}H NMR). The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (5 mL), filtered through Celite® and the filter cake was washed with water and dichloromethane. The organic component was separated, and the aqueous component was extracted with dichloromethane (6 × 8 mL). The combined organic components were washed with brine (20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude imine residue was dissolved in anhydrous methanol (3 mL) and sodium borohydride (12 mg, 0.33 mmol, 5.0 eq.) was added at 0 ºC. The reaction mixture was stirred for 1 h, after which the mixture was neutralised to pH 7 with 1 M aqueous hydrochloric acid and concentrated *in vacuo*. The crude residue was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution), followed by silica gel column chromatography (8:2:1 ethyl acetate:ethanol:ammonium hydroxide) to yield the title compound **81** as a colourless solid (16 mg, 60%). R_f 0.01 (1% triethylamine in ethanol); m.p. 167–168 ºC (from EtOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3285 (w), 3026 (w), 2945 (w), 2813 (w), 2764 (w), 1700 (s), 1638 (w), 1611 (w), 1513 (m), 1451 (m), 1403 (m), 1312 (m), 1254 (w), 1149 (s), 1128 (w), 1039 (w), 1000 (w), 885 (m), 845 (w), 794 (w), 741 (m), 696 (w), 639 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.25 (1H, s, H-4), 6.72 (1H, s, H-7), 3.97 (2H, s, H-1''), 3.40 (3H, s, H-8), 3.38 (3H, s, H-9), 2.67 (2H, t, *J* 6.7, H-3''), 2.44 (1H, tt, *J* 8.0, 4.8, H-4''), 2.32 (2H, t, *J* 6.9, H-5''), 2.20 (6H, s, H-6'' and H-7''), 1.67 (1H, q, *J* 6.8, H-1'), 1.22 – 1.14 (2H, m, H-2'^{a} and H-3'^{a}), 0.97 – 0.86 (2H, m, H-2'^{b} and H-3'^{b}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 154.9 (C-2), 132.7 (C-5), 130.2 (C-7a), 126.9 (C-6), 122.6 (C-3a), 108.4 (C-7), 103.0 (C-4), 58.2 (C-5''), 53.7 (C-1''), 47.7 (C-3''), 45.7 (C-6'' and C-7''), 30.2 (C-4''), 27.7 (C-1'), 27.6, 27.3 (C-8 and C-9), 5.7 (C-2' and C-3'); *m/z* (ESI$^+$) 396 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{18}H_{30}O_3N_5S^+$ ([M+H]$^+$) requires 396.20639, found 396.20684; HPLC (method 1) retention time 4.9 min, 100%.

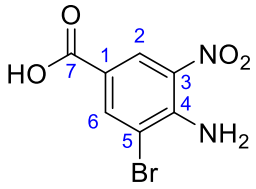
_N_-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]pyridine-3'-sulfonamide (79)



N^1,N^1-Dimethylpropane-1,3-diamine (29 µL, 0.23 mmol, 2.0 eq.) and acetic acid (13 µL, 0.23 mmol, 2.0 eq.) was added to a solution of **89** (40 mg, 0.12 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (4 mL) at 0 ºC. The reaction mixture was warmed to room temperature until imine formation was observed (3 h, followed by TLC and ^{1}H NMR). The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (5 mL), filtered through Celite® and the filter cake was washed with water and dichloromethane. The organic component was separated, and the aqueous component was extracted with dichloromethane (6 × 8 mL). The combined organic components were washed with brine (20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude imine residue was dissolved in anhydrous methanol (5 mL) and sodium borohydride (24 mg, 0.64 mmol, 5.0 eq.) was added at 0 ºC. The reaction mixture was stirred for 1 h, after which the mixture was neutralised to pH 7 with 1 M aqueous hydrochloric acid and concentrated *in vacuo*. The crude residue was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution), followed by silica gel column chromatography (6:2:1 ethyl acetate:ethanol:ammonium hydroxide) to yield the title compound **79** as a yellow oil (11 mg, 19%). R_f 0.01 (1% triethylamine in ethanol); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3439 (m), 2945 (w), 2826 (w), 1687 (s), 1637 (m), 1602 (w), 1579 (w), 1505 (m), 1463 (m), 1414 (w), 1399 (w), 1338 (w), 1246 (m), 1172 (w), 1136 (m), 1103 (m), 1029 (w), 1014 (w), 939 (w), 860 (w), 804 (w), 760 (w), 739 (w), 707 (w), 624 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.94 (1H, d, *J* 2.1, H-2'), 8.74 (1H, dd, *J* 4.9, 1.6, H-4'), 8.05 (1H, dt, *J* 8.0, 2.1, H-6'), 7.38 (1H, ddd, *J* 8.0, 4.9, 0.8, H-5'), 7.15 (1H, s, H-4), 6.63 (1H, s, H-7), 3.47

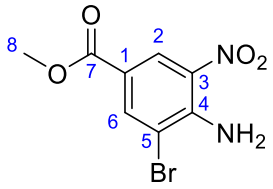
(2H, s, H-1’’), 3.39 (3H, s, H-8), 3.35 (3H, s, H-9), 2.72 – 2.66 (4H, m, H-3’’ and H-5’’), 2.47 (6H, s, H-6’’ and H-7’’), 1.86 (2H, p, *J* 6.6, H-4’’); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 154.8 (C-2), 153.3 (C-4’), 147.8 (C-2’), 137.2 (C-3’), 134.6 (C-6’), 131.3 (C-5), 130.4 (C-7a), 127.5 (C-6), 123.8 (C-5’), 122.2 (C-3a), 108.6 (C-7), 103.7 (C-4), 57.4 (C-5’’), 52.6 (C-1’’), 46.9 (C-3’’), 44.6 (C-6’’ and C-7’’), 27.6, 27.4 (C-8 and C-9), 25.9 (C-4’’); *m/z* (ESI$^+$) 433 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{20}H_{29}O_3N_6S^+$ ([M+H]$^+$) requires 433.20164, found 433.20205; HPLC (method 1) retention time 5.0 min, 100%.

4-Amino-3-nitro-5-bromobenzoic acid (99)[147]



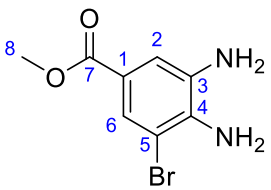
4-Amino-3-nitrobenzoic acid (1.00 g, 5.49 mmol, 1.0 eq.) was dissolved in acetic acid (15 mL). Bromine (309 µL, 6.04 mmol, 1.1 eq.) was added dropwise to the solution at 0 ºC, and the reaction mixture was stirred at room temperature for 4 h. The mixture was poured over ice and the precipitate was collected *via* filtration to yield the title compound **99** as a yellow solid (1.41 g, 98%). R_f 0.52 (10% ethanol in dichloromethane); m.p. >300 ºC (from MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3473 (m), 3353 (m), 3085 (w), 2537 (w), 1687 (m), 1608 (s), 1580 (m), 1549 (m), 1524 (m), 1470 (m), 1425 (m), 1343 (m), 1273 (s), 1158 (m), 1081 (m), 922 (m), 849 (m), 832 (m), 804 (m), 756 (s), 730 (m), 683 (s), 617 (w); ^{1}H NMR (400 MHz, CD_3OD) δ_H 8.75 (1H, d, *J* 2.0, H-2), 8.31 (1H, d, *J* 2.0, H-6), 7.98 (3H, bs, O*H* and N*H*$_2$); *m/z* (ESI$^-$) 259 ([M-H]$^-$, 100%), 261 ([M-H]$^-$, 90%). The spectroscopic data are in good agreement with the literature values.[147,148]

Methyl 4-amino-3-nitro-5-bromobenzoate (100)[191]



99 (1.19 g, 4.55 mmol, 1.0 eq.) was dissolved in anhydrous methanol (52 mL). Thionyl chloride (1.18 mL, 16.2 mmol, 3.0 eq.) was added dropwise at 0 ºC, and the reaction mixture was heated to 50 ºC and stirred for 2 h. After this time, the mixture was cooled to room temperature and quenched with water (50 mL). The resulting solution was extracted with ethyl acetate (3 × 100 mL), and the combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (20–70% dichloromethane in petroleum ether) to yield the title compound **100** as a yellow solid (1.06 g, 84%). R_f 0.40 (50% dichloromethane in petroleum ether); m.p. 94–96 ºC (from CH_2Cl_2) {lit.[191] 106–108 ºC}; $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3472 (w), 3360 (w), 3097 (w), 2954 (w), 2360 (w), 2341 (w), 1717 (s), 1618 (s), 1577 (w), 1556 (w), 1541 (w), 1521 (m), 1458 (w), 1436 (w), 1405 (w), 1363 (w), 1337 (m), 1267 (s), 1194 (w), 1137 (w), 1082 (w), 985 (w), 915 (w), 755 (w), 728 (w), 689 (w), 658 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.84 (1H, d, *J* 2.0, H-2), 8.35 (1H, d, *J* 2.0, H-6), 6.98 (2H, bs, N*H*$_2$), 3.92 (3H, s, H-8); *m/z* (ESI$^+$) 275 ([M+H]$^+$, 100%), 276 ([M+H]$^+$, 90%). The spectroscopic data are in good agreement with the literature values.[191]

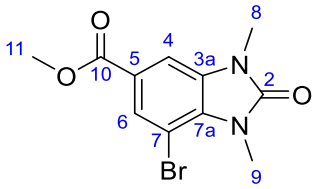
Methyl 3,4-diamino-5-bromobenzoate (101)[192]



100 (100 mg, 0.364 mmol, 1.0 eq.) was dissolved in methanol (2.5 mL) and tetrahydrofuran (0.31 mL), and zinc (238 mg, 3.64 mmol, 10.0 eq.) and ammonium chloride (389 mg, 7.28 mmol, 20.0 eq.) were added. The reaction mixture was heated to 40 ºC and stirred for 4 h. After this time,

zinc (238 mg, 3.64 mmol, 10.0 eq.) and ammonium chloride (389 mg, 7.28 mmol, 20.0 eq.) were added at 40 ºC and the reaction mixture was stirred for a further 1 h. The reaction mixture was then cooled and concentrated *in vacuo*. The residue was dissolved in ethyl acetate (20 mL), saturated aqueous sodium carbonate solution (20 mL) was added and the biphasic mixture was stirred for 10 min. The mixture was filtered, the organic component was washed with water (2 × 20 mL), dried over sodium sulfate, and concentrated *in vacuo* to yield the title compound **101** as an orange oily solid (86.2 mg, 97%). R_f 0.22 (4% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3466 (w), 3425 (w), 3384 (w), 3327 (w), 3196 (w), 2947 (w), 1699 (s), 1624 (s), 1563 (w), 1496 (w), 1434 (s), 1423 (s), 1351 (w), 1319 (s), 1285 (s), 1215 (s), 1098 (m), 1050 (w), 1005 (m), 915 (w), 896 (w), 838 (w), 784 (m), 763 (s), 723 (m), 693 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.73 (1H, d, *J* 1.8, H-6), 7.34 (1H, d, *J* 1.8, H-2), 4.25 (2H, bs, N*H*$_2$), 3.85 (3H, s, H-8), 3.44 (2H, bs, N*H*$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 166.4 (C-7), 138.6 (C-4), 133.7 (C-3), 126.4 (C-6), 121.3 (C-1), 117.3 (C-2), 109.2, (C-5) 52.1 (C-8); *m/z* (ESI$^+$) 245 ([M+H]$^+$, 100%), 247 ([M+H]$^+$, 90%). The spectroscopic data are in good agreement with the literature values.[192]

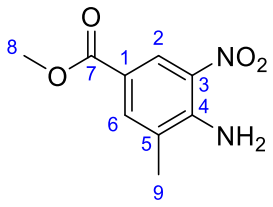
Methyl 7-bromo-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (97)



101 (790 mg, 3.22 mmol, 1.0 eq.) was suspended in anhydrous dichloromethane (26 mL) and 1,1'-carbonyldiimidazole (784 mg, 4.84 mmol, 1.5 eq.) was added. The reaction mixture was stirred at room temperature for 3 h. The mixture was diluted with water (100 mL), and the precipitate was filtered. The filter cake was washed with water (20 mL), and the resulting precipitate was suspended in methanol and concentrated *in vacuo*. The crude solid was suspended in anhydrous *N*,*N*-dimethylformamide (15 mL), and potassium carbonate (1.60 g, 11.6 mmol, 4.0 eq.) was added. The suspension was cooled to 0 ºC and iodomethane (1.08 mL, 17.4 mmol, 6.0 eq.) was added dropwise. The reaction mixture was warmed to room temperature and stirred

for 16 h. After this time, the mixture was diluted with water (40 mL) and extracted with ethyl acetate (4 × 20 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (2 × 20 mL) and brine (60 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–50% ethyl acetate in petroleum ether) to yield the title compound **97** as a colourless solid (641 mg, 67%). R_f 0.47 (60% ethyl acetate in petroleum ether); m.p. 206–208 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2951 (w), 1724 (s), 1701 (s), 1621 (w), 1607 (w), 1582 (w), 1503 (w), 1476 (m), 1442 (m), 1407 (w), 1386 (w), 1327 (w), 1250 (s), 1202 (w), 1144 (w), 1111 (m), 1069 (w), 1026 (w), 991 (m), 946 (w), 875 (w), 820 (w), 785 (w), 763 (m), 744 (m), 695 (w), 637 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.92 (1H, d, *J* 1.5, H-6), 7.54 (1H, d, *J* 1.5, H-4), 3.92 (3H, s, H-11), 3.76 (3H, s, H-9), 3.43 (3H, s, H-8); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 166.0 (C-10), 154.9 (C-2), 131.5 (C-7a), 131.1 (C-3a), 128.4 (C-6), 124.3 (C-1), 107.7 (C-4), 101.0 (C-7), 52.5 (C-11), 30.0 (C-9), 27.7 (C-8); *m/z* (ESI$^+$) 299 ([M+H]$^+$, 100%), 301 ([M+H]$^+$, 90%); HRMS *m/z* (ESI$^+$) $C_{11}H_{12}O_3N_2Br^+$ ([M+H]$^+$) requires 299.0026 and 301.0005, found 299.0027 and 301.0007; HPLC (method 4) retention time 9.33 min, 99.0%.

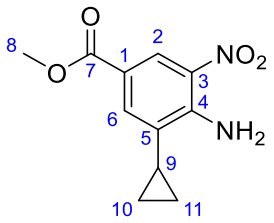
Methyl 4-amino-3-nitro-5-methyl benzoate (103)[193]



100 (400 mg, 1.45 mmol, 1.0 eq.), [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II) (complex with dichloromethane, 119 mg, 0.145 mmol, 0.1 eq.), methylboronic acid (104 mg, 1.75 mmol, 1.2 eq.) and potassium carbonate (601 mg, 4.35 mmol, 3.0 eq.) were added to a microwave vial. The vial was evacuated and filled with nitrogen three times. Degassed dioxane (10 mL) and water (1 mL) were added, and the reaction mixture was heated to 80 ºC for 16 h. After this time, the mixture was cooled to room temperature and partitioned between ethyl acetate (20 mL) and 1 M hydrochloric acid (20 mL). The organic component was dried over sodium sulfate, and

concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% triethylamine in chloroform) to yield the title compound **103** as a yellow solid (56.2 mg, 18%). R_f 0.42 (1% triethylamine, 80% chloroform in petroleum ether); m.p. 130–131 ºC (from $CHCl_3$); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3468 (m), 3356 (m), 2953 (w), 1703 (s), 1624 (s), 1598 (w), 1586 (w), 1565 (m), 1528 (m), 1504 (w), 1475 (w), 1441 (m), 1410 (w), 1387 (w), 1374 (w), 1340 (m), 1266 (s), 1197 (s), 1125 (w), 1111 (w), 1070 (w), 1021 (w), 994 (m), 920 (w), 875 (w), 863 (w), 837 (w), 821 (w), 807 (w), 785 (w), 759 (s), 743 (w), 696 (w), 637 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.73 (1H, d, *J* 2.1, H-2), 7.90 (1H, d, *J* 2.1, H-6), 6.54 (2H, bs, N*H*$_2$), 3.88 (3H, s, H-8), 2.27 (3H, s, H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 165.8 (C-7), 146.4 (C-4), 136.0 (C-2), 131.6 (C-3), 127.2 (C-6), 125.3 (C-5), 117.9 (C-1), 52.2 (C-8), 17.6 (C-9); *m/z* (ESI$^+$) 299 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[193]

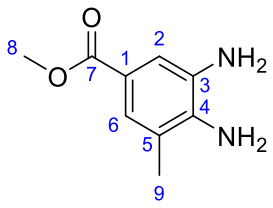
Methyl 4-amino-5-cyclopropyl-3-nitrobenzoate (105)



100 (200 mg, 0.727 mmol, 1.0 eq.), [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II) (complex with dichloromethane, 59.4 mg, 0.0727 mmol, 0.1 eq.), cyclopropylboronic acid (74.9 mg, 0.872 mmol, 1.2 eq.) and potassium carbonate (301 mg, 2.18 mmol, 3.0 eq.) were added to a microwave vial. The vial was evacuated and filled with nitrogen three times. Degassed dioxane (5 mL) and water (0.5 mL) were added, and the reaction mixture was heated to 80 ºC for 16 h. After this time, the mixture was cooled to room temperature and partitioned between ethyl acetate (20 mL) and 1 M hydrochloric acid (20 mL). The organic component was dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% triethylamine in chloroform) to yield the title compound **105** as a yellow solid (33.8 mg, 20%). R_f 0.70 (30% ethyl acetate in petroleum ether); m.p. 150–151 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3496 (m), 3383 (s), 3089 (w), 2950 (w), 2850 (w), 1710 (s), 1631

(m), 1591 (w), 1572 (m), 1525 (m), 1445 (m), 1417 (w), 1360 (w), 1345 (m), 1285 (m), 1244 (m), 1211 (m), 1165 (m), 1110 (w), 1079 (w), 1060 (w), 1040 (w), 998 (w), 935 (w), 913 (w), 898 (w), 847 (w), 825 (w), 806 (w), 758 (m), 733 (w), 697 (w), 623 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.74 (1H, d, *J* 1.9, H-2), 7.91 (1H, dd, *J* 1.9, 1.1, H-6), 6.93 (2H, bs, N*H*$_2$), 3.89 (3H, s, H-8), 1.72 – 1.57 (1H, m, H-9), 1.12 – 0.99 (2H, m, H-10 or H-11), 0.73 – 0.63 (2H, m, H-10 or H-11); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 165.9 (C-7), 147.8 (C-4), 134.9 (C-6), 131.5 (C-3), 130.1 (C-5), 127.4 (C-2), 117.7 (C-1), 52.2 (C-8), 11.3 (C-9), 5.5 (C-10 and C-11); *m/z* (ESI$^+$) 237 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{11}H_{13}O_4N_2^+$ ([M+H]$^+$) requires 237.0870, found 237.0871; HPLC (method 1) retention time 9.89 min, 99.5%.

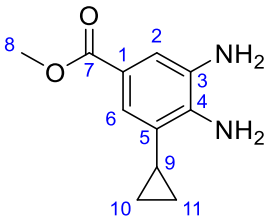
Methyl 3,4-diamino-5-methylbenzoate (106)[193]



103 (50.0 mg, 0.238 mmol, 1.0 eq.) was dissolved in methanol (1.5 mL) and tetrahydrofuran (0.19 mL), and zinc (312 mg, 4.76 mmol, 20.0 eq.) and ammonium chloride (509 mg, 9.52 mmol, 40.0 eq.) were added. The reaction mixture was heated to 40 ºC and stirred for 2 h. After this time, the reaction mixture was cooled and concentrated *in vacuo*. The residue was dissolved in ethyl acetate (20 mL), saturated aqueous sodium carbonate solution (20 mL) was added and the biphasic mixture was stirred for 10 min. The mixture was filtered, the organic component was washed with water (2 × 20 mL), dried over sodium sulfate, and concentrated *in vacuo* to yield the title compound **106** as an orange oil (41.2 mg, 96%). R_f 0.50 (8% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3377 (w), 2949 (w), 2851 (w), 1690 (m), 1620 (m), 1595 (m), 1499 (m), 1433 (s), 1383 (w), 1331 (s), 1219 (s), 1162 (w), 1099 (w), 1021 (m), 992 (w), 959 (w), 888 (w), 769 (s), 723 (w), 698 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.41 – 7.38 (1H, m, H-6), 7.31 (1H, d, *J* 1.9, H-2), 3.83 (3H, s, H-8), 3.63 – 3.22 (4H, bs, 2 × N*H*$_2$), 2.18 (3H, s, H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 167.7 (C-7), 139.2 (C-4), 132.4 (C-3), 124.8 (C-6), 121.8 (C-5), 119.9 (C-1), 116.9

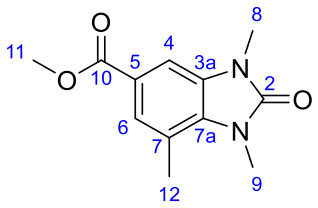
(C-2), 51.7 (C-8), 17.4 (C-9); *m/z* (ESI$^-$) 181 ([M-H]$^-$, 100%). The spectroscopic data are in good agreement with the literature values.[193]

Methyl 3,4-diamino-5-cyclopropylbenzoate (107)



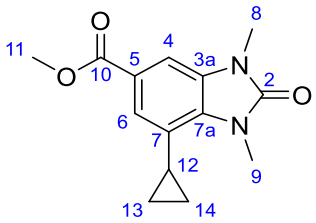
105 (70.0 mg, 0.296 mmol, 1.0 eq.) was dissolved in methanol (2 mL) and tetrahydrofuran (0.3 mL), and zinc (388 mg, 5.93 mmol, 20.0 eq.) and ammonium chloride (633 mg, 11.8 mmol, 40.0 eq.) were added. The reaction mixture was heated to 40 ºC and stirred for 2 h. After this time, the reaction mixture was cooled and concentrated *in vacuo*. The residue was dissolved in ethyl acetate (30 mL), saturated aqueous sodium carbonate solution (30 mL) was added and the biphasic mixture was stirred for 10 min. The mixture was filtered, the organic component was washed with water (2 × 20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–2% methanol in dichloromethane) to yield the title compound **107** as an orange oil (59.2 mg, 97%). R_f 0.29 (4% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3369 (m), 3083 (w), 3003 (w), 2951 (w), 2924 (w), 2849 (w), 1694 (s), 1619 (m), 1597 (m), 1490 (w), 1435 (s), 1380 (w), 1323 (s), 1287 (w), 1225 (s), 1153 (w), 1099 (w), 1072 (w), 1053 (w), 1005 (w), 958 (w), 891 (w), 841 (w), 826 (w), 769 (m), 718 (w), 694 (w), 649 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.38 (1H, dd, *J* 1.9, 0.9, H-6), 7.32 (1H, d, *J* 1.8, H-2), 3.83 (3H, s, H-8), 3.67 (4H, bs, N*H*$_2$ × 2), 1.70 – 1.61 (1H, m, H-9), 0.96 – 0.88 (2H, m, H-10 or H-11), 0.65 – 0.59 (2H, m, H-10 or H-11); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 167.7 (C-7), 140.9 (C-4), 132.3 (C-3), 126.6 (C-5), 123.1 (C-6), 119.6 (C-1), 117.0 (C-2), 51.7 (C-8), 11.4 (C-9), 5.3 (C-10 and C-11); *m/z* (ESI$^+$) 207 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{11}H_{15}O_2N_2^+$ ([M+H]$^+$) requires 207.1128, found 207.1130.

Methyl 1,3,7-trimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (102)



106 (40.0 mg, 0.222 mmol, 1.0 eq.) was suspended in anhydrous dichloromethane (3 mL) and 1,1'-carbonyldiimidazole (54.0 mg, 0.333 mmol, 1.5 eq.) was added. The reaction mixture was stirred at room temperature for 5 h. The mixture was diluted with water (10 mL), extracted with ethyl acetate (3 × 30 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude solid was suspended in anhydrous *N*,*N*-dimethylformamide (2 mL), and potassium carbonate (223 mg, 1.61 mmol, 4.0 eq.) was added. The suspension was cooled to 0 ºC and iodomethane (151 µL, 2.42 mmol, 6.0 eq.) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 16 h. After this time, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (2 × 10 mL) and brine (20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–1% methanol in dichloromethane) to yield the title compound **102** as an orange solid (24.6 mg, 47%). R_f 0.74 (10% methanol in dichloromethane); m.p. 150–152 ºC (from $CHCl_3$); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3523 (w), 3061 (w), 2952 (w), 1698 (s), 1604 (w), 1469 (m), 1436 (w), 1396 (w), 1380 (w), 1343 (w), 1299 (w), 1260 (m), 1225 (m), 1192 (w), 1169 (w), 1117 (w), 1091 (w), 1068 (w), 1012 (w), 991 (w), 976 (w), 904 (w), 877 (w), 792 (w), 770 (w), 745 (w), 700 (w), 658 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.60 – 7.56 (1H, m, H-4), 7.47 (1H, d, *J* 1.6, H-6), 3.90 (3H, s, H-11), 3.67 (3H, s, H-9), 3.41 (3H, s, H-8), 2.63 (3H, s, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 167.2 (C-10), 155.2 (C-2), 131.9 (C-7a), 130.2 (C-3a), 126.7 (C-4), 123.0 (C-5), 119.0 (C-7), 106.9 (C-6), 52.2 (C-11), 30.0 (C-9), 27.5 (C-8), 18.3 (C-12); *m/z* (ESI$^+$) 235 ([M+H]$^+$, 100%), 257 ([M+Na]$^+$, 90%); HRMS *m/z* (ESI$^+$) $C_{12}H_{15}O_3N_2^+$ ([M+H]$^+$) requires 235.10772, found 235.10793; HPLC (method 1) retention time 7.51 min, 93.2%.

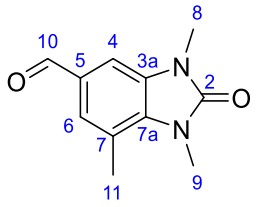
Methyl 7-cyclopropyl-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate (108)



107 (65.0 mg, 0.315 mmol, 1.0 eq.) was suspended in anhydrous dichloromethane (5 mL) and 1,1'-carbonyldiimidazole (76.7 mg, 0.473 mmol, 1.5 eq.) was added. The reaction mixture was stirred at room temperature for 3 h. The mixture was diluted with water (10 mL), extracted with ethyl acetate (3 × 40 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude solid was suspended in anhydrous *N*,*N*-dimethylformamide (2 mL), and potassium carbonate (174 mg, 1.26 mmol, 4.0 eq.) was added. The suspension was cooled to 0 ºC and iodomethane (117 µL, 1.89 mmol, 6.0 eq.) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 16 h. After this time, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (2 × 20 mL) and brine (20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–20% acetone in toluene) to yield the title compound **108** as a colourless solid (13.6 mg, 17%). R_f 0.55 (40% acetone in toluene); m.p. 135–137 ºC (from $CHCl_3$); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3511 (w), 3010 (w), 2953 (w), 1698 (s), 1601 (w), 1474 (m), 1446 (w), 1437 (w), 1396 (w), 1384 (w), 1342 (w), 1286 (w), 1262 (m), 1231 (m), 1192 (w), 1160 (w), 1119 (w), 1081 (w), 1050 (w), 1025 (w), 994 (w), 919 (w), 894 (w), 839 (w), 793 (w), 769 (w), 753 (w), 702 (w), 652 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.58 (1H, dd, *J* 1.6, 0.8, H-4), 7.51 (1H, d, *J* 1.6, H-6), 3.91 (3H, s, H-11), 3.83 (3H, s, H-9), 3.44 (3H, s, H-8), 2.26 (1H, tt, *J* 8.4, 5.3, H-12), 1.05 – 0.98 (2H, m, H-13 or H-14), 0.91 – 0.84 (2H, m, H-13 or H-14); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 167.3 (C-10), 155.3 (C-2), 133.2 (C-7a), 130.0 (C-3a), 124.3 (C-5), 123.8 (C-4), 122.9 (C-7), 107.2 (C-6), 52.2 (C-11), 30.3 (C-9), 27.6 (C-8), 12.2 (C-12), 8.0 (C-13 and C-14); *m/z* (ESI^+) 261 ($[M+H]^+$, 100%); HRMS

m/z (ESI$^+$) $C_{14}H_{17}O_3N_2^+$ ([M+H]$^+$) requires 261.1234, found 261.1235; HPLC (method 1) retention time 8.55 min, 100%.

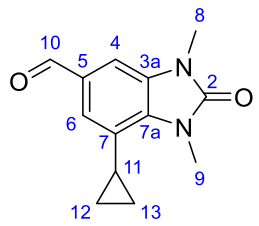
1,3,7-Trimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (109)



102 (20.0 mg, 0.0854 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (2 mL) and the solution was cooled to 0 ºC. Lithium borohydride (2.0 M in tetrahydrofuran, 85.4 µL, 0.171 mmol, 2.0 eq.) was added dropwise, followed by addition of methanol (0.2 mL) dropwise, and the solution was heated to 66 ºC for 4 h. Saturated aqueous sodium bicarbonate solution (10 mL) was added dropwise to the reaction mixture at 0 ºC and the mixture was extracted with dichloromethane (6 × 15 mL). The combined organic components were washed with brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was dissolved in dichloromethane (3 mL), and manganese(IV) oxide (74.2 mg, 0.854 mmol, 10.0 eq.) was added. The reaction mixture was stirred at room temperature for 16 h. The suspension was then filtered through Celite® and the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (50–70% ethyl acetate in petroleum ether) to yield the title compound **109** as a colourless solid (15.1 mg, 87% over two steps). R_f 0.38 (70% ethyl acetate in petroleum ether); m.p. 125–127 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3047 (w), 2927 (w), 2839 (w), 2795 (w), 2716 (w), 1703 (s), 1681 (s), 1612 (w), 1599 (m), 1469 (m), 1413 (w), 1397 (w), 1378 (w), 1339 (w), 1295 (w), 1263 (w), 1244 (w), 1202 (m), 1133 (w), 1094 (m), 1039 (w), 1011 (w), 985 (w), 974 (w), 885 (w), 857 (w), 778 (w), 747 (m), 699 (w), 681 (w), 656 (w), 635 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.89 (1H, s, H-10), 7.40 – 7.33 (2H, m, H-4 and H-6), 3.71 (3H, s, H-9), 3.44 (3H, s, H-8), 2.69 (3H, s, H-11); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 191.4 (C-10), 155.3 (C-2), 133.5 (C-7a), 131.0 (C-3a), 130.5 (C-5), 129.2 (C-4), 119.4 (C-7), 105.2 (C-6), 30.1 (C-9),

27.6 (C-8), 18.3 (C-11); *m/z* (ESI$^+$) 205 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{11}H_{13}O_2N_2^+$ ([M+H]$^+$) requires 205.0972, found 205.0974; HPLC (method 1) retention time 6.65 min, 97.2%.

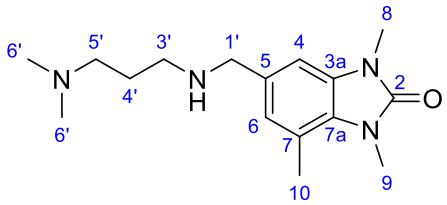
7-Cyclopropyl-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (110)



108 (29.0 mg, 0.111 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (2 mL) and the solution was cooled to 0 ºC. Lithium borohydride (2.0 M in tetrahydrofuran, 167 µL, 0.334 mmol, 2.0 eq.) was added dropwise, followed by addition of methanol (0.2 mL) dropwise, and the solution was heated to 66 ºC for 4 h. Saturated aqueous sodium bicarbonate solution (10 mL) was added dropwise to the reaction mixture at 0 ºC and the mixture was extracted with dichloromethane (6 × 15 mL). The combined organic components were washed with brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was dissolved in dichloromethane (5 mL), and manganese(IV) oxide (97.3 mg, 1.11 mmol, 10.0 eq.) was added. The reaction mixture was stirred at room temperature for 16 h. The suspension was then filtered through Celite® and the filtrate was concentrated *in vacuo* to yield the title compound **110** as a colourless solid (25.5 mg, 100% over two steps). R_f 0.38 (80% ethyl acetate in petroleum ether); m.p. 111–113 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3010 (w), 2951 (w), 1700 (s), 1676 (s), 1625 (w), 1597 (m), 1473 (m), 1449 (m), 1408 (w), 1392 (w), 1373 (w), 1340 (w), 1286 (w), 1264 (w), 1246 (w), 1207 (w), 1192 (w), 1127 (w), 1084 (w), 1051 (w), 1024 (w), 986 (w), 967 (w), 903 (w), 854 (w), 777 (w), 753 (w), 706 (w), 638 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 9.89 (1H, s, H-10), 7.39 – 7.36 (2H, m, H-4 and H-6), 3.86 (3H, s, H-9), 3.46 (3H, s, H-8), 2.30 (1H, tt, *J* 8.4, 5.4, H-11), 1.11 – 1.03 (2H, m, H-12 or H-13), 0.95 – 0.86 (2H, m, H-12 or H-13); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 191.4 (C-10), 155.3 (C-2), 134.8 (C-7a), 130.8 (C-3a), 130.3 (C-5), 126.3 (C-4), 124.8 (C-7), 105.5 (C-6), 30.4 (C-9), 27.6 (C-8), 12.2 (C-11), 8.1 (C-12 and C-13); *m/z*

(ESI$^+$) 231 ([M+H]$^+$, 60%), 253 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{13}H_{15}O_2N_2^+$ ([M+H]$^+$) requires 231.1128, found 231.1130; HPLC (method 1) retention time 7.66 min, 91.6%.

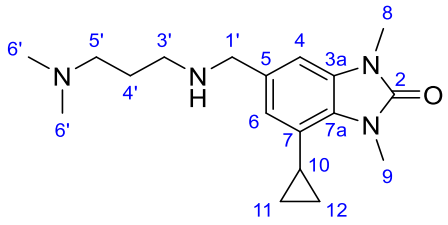
5-({[5'-(Dimethylamino)propyl]amino}methyl)-1,3,7-trimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (92)



N^1,*N*^{1}-Dimethylpropane-1,3-diamine (15 µL, 0.12 mmol, 2.0 eq.) and acetic acid (6.8 µL, 0.12 mmol, 2.0 eq.) were added at 0 ºC to a solution of **109** (12 mg, 0.059 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (1 mL) with 3 Å molecular sieves. The reaction mixture was warmed to room temperature for 2 h. Sodium triacetoxyborohydride (62 mg, 0.29 mmol, 5.0 eq.) was added and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (10 mL) and filtered through Celite®. The aqueous components were extracted with dichloromethane (8 × 10 mL), and the organic components were washed with brine (10 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 25% ethanol in ethyl acetate then 1:2:6 ammonium hydroxide:ethanol:ethyl acetate) to yield the title compound **92** as a colourless oil (9.3 mg, 54%). R_f 0.37 (1:2:6 ammonium hydroxide:ethanol:ethyl acetate); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3434 (w), 2943 (w), 2870 (w), 2827 (w), 2362 (s), 2341 (s), 1690 (s), 1628 (w), 1549 (w), 1468 (m), 1452 (m), 1380 (w), 1344 (w), 1302 (w), 1266 (w), 1213 (w), 1154 (w), 1099 (w), 1069 (w), 1010 (w), 967 (w), 848 (w), 744 (w), 669 (w), 655 (w), 645 (w), 625 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.82 (1H, s, H-6), 6.77 (1H, s, H-4), 3.78 (2H, s, H-1'), 3.64 (3H, s, H-9), 3.38 (3H, s, H-8), 2.70 (2H, t, *J* 7.1, H-3'), 2.59 (3H, s, H-10), 2.33 (2H, t, *J* 7.1, H-5'), 2.22 (6H, s, H-6'), 1.70 (2H, p, *J* 7.1, H-4'); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 155.3 (C-2), 133.2 (C-7a), 130.6 (C-3a), 127.2 (C-5), 124.6 (C-4), 119.3 (C-7), 105.5 (C-6), 58.3 (C-5'), 54.1 (C-1'), 48.1 (C-3'), 45.7 (C-6'), 30.0 (C-9), 27.8 (C-4'), 27.4

(C-8), 18.2 (C-10); *m/z* (ESI$^+$) 313 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{16}H_{27}ON_4^+$ ([M+H]$^+$) requires 291.2179, found 291.2179; HPLC (method 1) retention time 5.12 min, 98.2%.

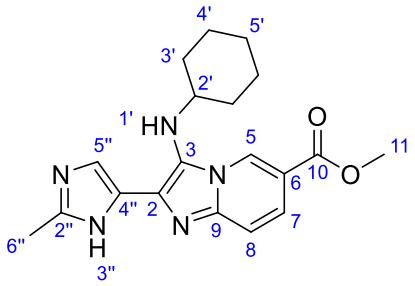
7-Cyclopropyl-5-({[5'-(dimethylamino)propyl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (94)



N^1,*N*^{1}-Dimethylpropane-1,3-diamine (24.0 µL, 0.191 mmol, 2.0 eq.) and acetic acid (10.9 µL, 0.191 mmol, 2.0 eq.) were added at 0 ºC to a solution of **110** (22.0 mg, 0.0955 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (2 mL) with 3 Å molecular sieves. The reaction mixture was warmed to room temperature for 2 h. Sodium triacetoxyborohydride (101 mg, 0.478 mmol, 5.0 eq.) was added and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (20 mL) and filtered through Celite®. The aqueous components were extracted with dichloromethane (8 × 20 mL), and the organic components were washed with brine (20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1:2:12 ammonium hydroxide:ethanol:ethyl acetate) to yield the title compound **94** as a colourless oil (17.4 mg, 58%). R_f 0.59 (1:2:6 ammonium hydroxide:ethanol:ethyl acetate); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3440 (w), 2943 (w), 2858 (w), 2818 (w), 2779 (w), 1694 (s), 1624 (w), 1606 (w), 1469 (m), 1396 (w), 1382 (w), 1338 (w), 1289 (w), 1248 (w), 1192 (w), 1177 (w), 1156 (w), 1099 (w), 1086 (w), 1049 (w), 1026 (w), 980 (w), 895 (w), 841 (w), 749 (w), 671 (w), 652 (w), 616 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.84 (1H, d, *J* 1.6, H-6), 6.75 (1H, dd, *J* 1.6, 0.8, H-4), 3.79 (3H, s, H-9), 3.77 (2H, s, H-1'), 3.39 (3H, s, H-8), 2.69 (2H, t, *J* 7.1, H-3'), 2.33 (2 H, t, *J* 7.1, H-5'), 2.28 – 2.18 (7H, m, H-6' and H-10), 1.70 (2H, p, *J* 7.1, H-4'), 1.00 – 0.94 (2H, m, H-11 or H-12), 0.84 – 0.78 (2H, m, H-11 or H-12); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 155.3 (C-2), 133.1 (C-7a), 130.4 (C-3a), 128.4 (C-5), 124.5 (C-7), 121.3 (C-4), 105.8 (C-6), 58.3 (C-5'), 54.2 (C-1'), 48.1

(C-3'), 45.7 (C-6'), 30.2 (C-9), 27.8 (C-4'), 27.4 (C-8), 12.2 (C-10), 7.9 (C-11 and C-12); *m/z* (ESI$^+$) 317 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{18}H_{29}ON_4^+$ ([M+H]$^+$) requires 317.2336, found 317.2336; HPLC (method 1) retention time 5.13 min, 98.2%.

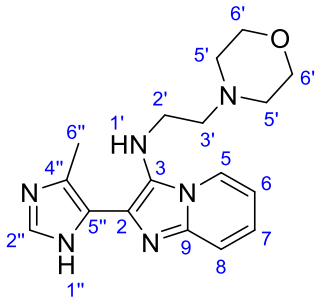
Methyl 3-(cyclohexylamino)-2-(2''-methyl-1*H*-imidazol-4''-yl)imidazo[1,2-a]pyridine-6-carboxylate (130)



2-Methyl-1*H*-imidazole-4-carbaldehyde (109 mg, 0.986 mmol, 1.5 eq.) and cyclohexyl isocyanide (94.7 µL, 0.762 mmol, 1.16 eq.) were added to a solution of methyl 6-aminopyridine-3-carboxylate (100 mg, 0.657 mmol, 1.0 eq.) in anhydrous dimethylsulfoxide (3.0 mL). Scandium(III) triflate (25.9 mg, 0.0526 mmol, 0.08 eq.) was added to the reaction and the mixture was heated to 100 ºC for 16 h. The reaction mixture was then cooled to room temperature and diluted with dichloromethane (20 mL). The solution was washed with water (26 mL), saturated aqueous sodium bicarbonate solution (13 mL) and brine (52 mL). The organic component was dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10% ethanol in ethyl acetate, followed by 1:2:12 ammonium hydroxide:ethanol:ethyl acetate) and further purified by silica gel column chromatography (2–6% methanol in dichloromethane) to yield the title compound **130** as a yellow oil (23.1 mg, 10%). R_f 0.03 (8% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3148 (w), 2928 (m), 2853 (w), 2343 (w), 1724 (m), 1630 (w), 1556 (w), 1437 (w), 1413 (w), 1339 (w), 1290 (s), 1194 (w), 1127 (w), 1062 (w), 1002 (w), 907 (w), 817 (w), 764 (m), 732 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.81 (1H, dd, *J* 1.8, 1.0, H-5), 7.63 (1H, dd, *J* 9.4, 1.8, H-7), 7.40 (1H, d, *J* 9.4, H-8), 7.37 (1H, s, H-5''), 3.96 (3H, s, H-11), 3.07 (1H, tt, *J* 10.4, 3.8, H-2'), 2.41 (3H, s, H-6''), 1.91 – 1.83 (2H, m, H-3'^{a}), 1.78 – 1.68 (2H, m, H-4'^{a}), 1.63 – 1.54 (1H, m, H-5'^{a}), 1.40 – 1.28 (2H, m, H-3'^{b}),

1.28 – 1.15 (3H, m, H-4'^{b} and H-5'^{b}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 165.9 (C-10), 144.8 (C-2''), 142.0 (C-9), 131.0 (C-4'' and C-2), 127.0 (C-5), 126.2 (C-3), 123.3 (C-7), 118.7 (C-5''), 115.9 (C-8), 115.7 (C-6), 56.7 (C-2'), 52.5 (C-11), 34.2 (C-3'), 25.8 (C-5'), 25.0 (C-4'), 14.3 (C-6''); *m/z* (ESI$^+$) 354 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{19}H_{24}O_2N_5^+$ ([M+H]$^+$) requires 354.19245, found 354.19223; HPLC (method 1) retention time 5.9 min, 97.8%.

2-(4''-Methyl-1*H*-imidazol-5''-yl)-*N*-[3'-(morpholin-4'-yl)ethyl]imidazo[1,2-a]pyridin-3-amine (131)

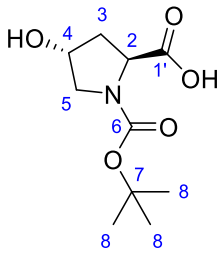


4-Methyl-5-imidazolecarboxaldehyde (263 mg, 2.39 mmol, 1.5 eq.) and 2-morpholinoethyl isocyanide (255 µL, 1.85 mmol, 1.16 eq.) were added to a solution of 2-aminopyridine (150 mg, 1.59 mmol, 1.0 eq.) in anhydrous dimethylsulfoxide (3.0 mL). Scandium(III) triflate (62.7 mg, 0.127 mmol, 0.08 eq.) was added to the reaction and the mixture was heated to 100 ºC for 16 h. The reaction mixture was then cooled to room temperature and diluted with dichloromethane (30 mL). The solution was washed with water (40 mL), saturated aqueous sodium bicarbonate solution (20 mL) and brine (50 mL). The organic component was dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 5–15% ethanol in chloroform) to yield the title compound **131** as a yellow oil (100 mg, 19%). R_f 0.35 (1% ammonium hydroxide, 10% ethanol in chloroform); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3108 (s), 2923 (s), 2857 (s), 2813 (s), 2360 (w), 1650 (m), 1634 (m), 1552 (m), 1500 (m), 1453 (m), 1355 (m), 1298 (m), 1276 (m), 1234 (m), 1203 (m), 1142 (m), 1115 (m), 1034 (w), 917 (m), 857 (m), 755 (m), 733 (m), 636 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.03 (1H, dt, *J* 6.8, 1.3, H-5), 7.60 (1H, s, H-2''), 7.47 (1H, dt, *J* 9.1, 1.2, H-8), 7.09 (1H, ddd, *J* 9.1, 6.7, 1.3, H-7), 6.76 (1H, td, *J* 6.7, 1.1, H-6), 3.68 (4H, t, *J* 4.7, H-6'), 3.14 (2H, t, *J* 6.5, H-2'), 2.57 (3H,

s, H-6''), 2.52 – 2.40 (6H, m, H-5' and H-3'); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 141.5 (C-9), 133.3 (C-6), 129.7 (C-4''), 129.4 (C-5''), 126.1 (C-2), 125.9 (C-3), 123.6 (C-7), 122.4 (C-5), 117.1 (C-8), 111.8 (C-6), 66.8 (C-5'), 58.0 (C-3'), 53.4 (C-6'), 43.3 (C-2'), 12.1 (C-6''); *m/z* (ESI^+) 327 ($[M+H]^+$, 100%); HRMS *m/z* (ESI^+) $C_{17}H_{23}ON_6^+$ ($[M+H]^+$) requires 327.19279, found 327.19264; HPLC (method 1) retention time 1.5 min, 89.0%, retention time 1.9 min, 8.6% (thought to be two protonation states of compound as peaks are broad).

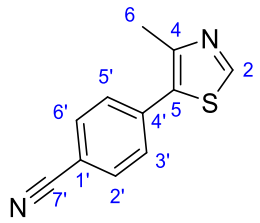
COMPOUNDS FROM CHAPTER 3

(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-hydroxypyrrolidine-2-carboxylic acid (155)[194–196]



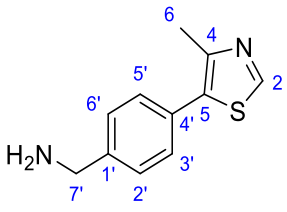
1-*tert*-Butyl 2-methyl (2*S*,4*R*)-4-hydroxypyrrolidine-1,2-dicarboxylate (1.00 g, 4.08 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (25 mL) and methanol (25 mL). A solution of lithium hydroxide (976 mg, 40.8 mmol, 10.0 eq.) in water (25 mL) was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 2 h, and then acidified with 2 M aqueous hydrochloric acid (20 mL). The aqueous component was extracted with ethyl acetate (3 × 50 mL) and the organic components were washed with brine (100 mL), dried over sodium sulfate and concentrated *in vacuo* to yield the title compound **155** as a colourless solid (938 mg, 99%). R_f 0.56 (4:1:1:1 ethyl acetate:methanol:water:acetone); m.p. 120–122 ºC {lit.[194] 126–128 ºC} (from EtOAc); $[\alpha]_D^{25}$= −60.1 (*c* 1.0, MeOH) {lit.[195] −68.1 (*c* 1.3, MeOH)}; ^{1}H NMR (400 MHz, DMSO-d_6, major rotamer assigned) δ_H 12.49 (1H, bs, COO*H*), 5.03 (1H, bs, O*H*), 4.24 (1H, bs, H-2), 4.11 (1H, td, *J* 7.9, 2.4, H-4), 3.42 – 3.34 (1H, m, H-5^a), 3.20 – 3.29 (1H, m, H-5^b), 2.19 – 2.02 (1H, m, H-3^a), 1.95 – 1.81 (1H, m, H-3^b), 1.37 (9H, s, H-8); *m/z* (ESI$^-$) 230 ([M-H]$^-$, 100%). The spectroscopic data are in good agreement with the literature values.[194–196]

4'-(4-Methyl-1,3-thiazol-5-yl)benzonitrile (153)[157,197]



4-Bromobenzonitrile (1.00 g, 5.49 mmol, 1.0 eq.), palladium(II) acetate (49.3 mg, 0.220 mmol, 0.04 eq.) and potassium acetate (1.08 g, 11.0 mmol, 2.0 eq.) were added to a flask, and the flask was purged with nitrogen for 30 min. Degassed dimethylacetamide (5.7 mL) was added, followed by 4-methylthiazole (1.00 mL, 11.0 mmol, 2.0 eq.). The reaction mixture was heated to 150 ºC and stirred for 16 h. After this time, the mixture was cooled to room temperature and quenched with water (60 mL). The aqueous component was extracted with ethyl acetate (4 × 100 mL), and the organic components were washed with brine (70 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (20–40% ethyl acetate in petroleum ether) to yield the title compound **153** as a yellow solid (927 mg, 85%). R_f 0.48 (50% ethyl acetate in petroleum ether); m.p. 69–72 ºC (from EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.76 (1H, s, H-2), 7.76 – 7.70 (2H, m, H-6' and H-2'), 7.60 – 7.54 (2H, m, H-5' and H-3'), 2.57 (3H, s, H-6); *m/z* (ESI^+) 201 ($[M+H]^+$, 100%). The spectroscopic data are in good agreement with the literature values.[157,197]

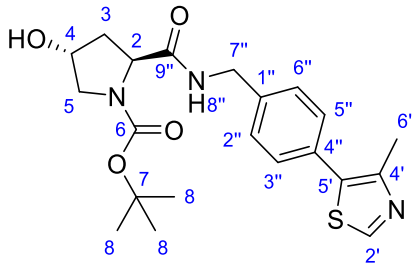
1'-[4'-(4-Methyl-1,3-thiazol-5-yl)phenyl]methanamine (154)[157]



Lithium aluminium hydride (910 mg, 24.0 mmol, 3.0 eq.) was suspended in anhydrous tetrahydrofuran (30 mL) under an argon atmosphere. A solution of **153** (1.60 g, 8.00 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (30 mL) was added dropwise to the suspension at 0 ºC. The reaction mixture was heated to 70 ºC for 5 h. The mixture was then cooled to 0 ºC and diluted

with diethyl ether (50 mL). Water (910 µL) was added, followed by 15% aqueous sodium hydroxide (907 µL) and water (2.73 mL). The reaction mixture was warmed to room temperature and stirred for 15 min. Sodium sulfate was added and the mixture was stirred for a further 15 min. The suspension was then filtered, and the filtrate was concentrated *in vacuo*. The crude residue was dissolved in dichloromethane (20 mL) and stirred with aqueous Rochelle salt solution (30 mL) for 16 h. The aqueous components were extracted with dichloromethane (4 × 70 mL), and the organic components were washed with brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (2–5% methanol in dichloromethane, followed by 1% triethylamine, 5% methanol in dichloromethane) to yield the title compound **154** as a yellow oil (780 mg, 48%). R_f 0.19 (10% [0.5 M ammonia in methanol] in dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.67 (1H, s, H-2), 7.41 (2H, d, *J* 8.3, H-3' and H-5'), 7.37 (2H, d, *J* 8.3, H-2' and H-6'), 3.91 (2H, s, H-7'), 2.53 (3H, s, H-6); *m/z* (ESI$^+$) 205 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[157]

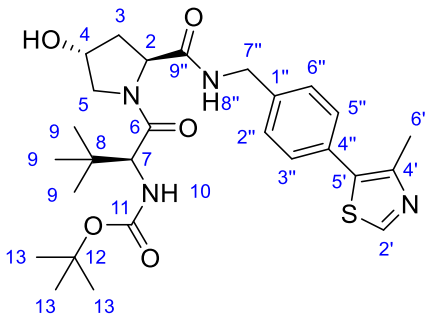
tert-Butyl (2*S*,4*R*)-4-hydroxy-2-({[4''-(4'-methyl-1',3'-thiazol-5'-yl)phenyl]methyl}carbamoyl)pyrrolidine-1-carboxylate (156)[98,157]



HATU (270 mg, 0.710 mmol, 1.1 eq.) was added to a solution of **155** (150 mg, 0.646 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (0.450 mL, 2.58 mmol, 4.0 eq.) in anhydrous *N*,*N*-dimethylformamide (2.5 mL). The solution was stirred for 10 min at room temperature before addition of a solution of **154** (132 mg, 0.646 mmol, 1.0 eq.) in anhydrous *N*,*N*-dimethylformamide (2.5 mL). The reaction mixture was stirred at room temperature for 22 h. The solution was quenched with water (30 mL), and the aqueous component was extracted with ethyl acetate

(3 × 20 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (3 × 20 mL), brine (40 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (0–10% ethanol in dichloromethane) to yield the title compound **156** as a pale yellow oil (120 mg, 45%). R_f 0.44 (10% ethanol in dichloromethane); $[\alpha]_D^{25} = -21.9$ (*c* 1.0, $CHCl_3$); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3290 (w), 2979 (w), 2932 (w), 2546 (w), 2160 (m), 2027 (m), 1977 (m), 1748 (w), 1665 (s), 1541 (m), 1477(w), 1403 (s), 1366 (m), 1255 (w), 1159 (s), 1128 (m), 1087 (w), 1029 (w), 981 (w), 939 (w), 913 (w), 844 (s), 773 (w), 639 (w); ^{1}H NMR (400 MHz, CD_3OD, major rotamer assigned) δ_H 8.87 (1H, s, H-2'), 7.53 – 7.34 (4H, m, H-2'', H-3'', H-5'', and H-6''), 4.58 – 4.26 (4H, m, H-2, H-4, and H-7''), 3.62 – 3.46 (2H, m, H-5), 2.47 (3H, s, H-6'), 2.32 – 2.20 (1H, m, H-3^a), 2.03 (1H, ddd, *J* 13.2, 8.5, 4.6, H-3^b), 1.33 (9H, s, H-8); *m/z* (ESI$^+$) 418 ([M+H]$^+$, 50%). The spectroscopic data are in good agreement with the literature values.[98,157]

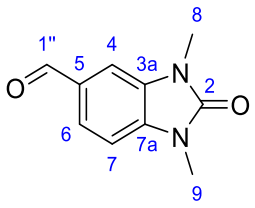
_N_-(*tert*-Butoxycarbonyl)-7-methyl-L-valyl-(4*R*)-4-hydroxy-_N_-{[4''-(4'-methyl-1',3'-thiazol-5'-yl)phenyl]methyl}-L-prolinamide (158)



156 (600 mg, 1.44 mmol, 1.0 eq.) was dissolved in anhydrous dichloromethane (4 mL). 4 M HCl in dioxane (5.40 mL, 21.6 mmol, 15.0 eq.) was added at room temperature and the reaction mixture was stirred for 1 h. The solution was concentrated *in vacuo*. ^{1}H NMR and low resolution mass spectrometry confirmed that the Boc protecting group had been removed (product was the hydrochloride salt of the free amine). The residue (510 mg, 1.44 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (8.5 mL) and *N*,*N*-diisopropylethylamine (1.00 mL, 5.76 mmol, 4.0 eq.) was added. The solution was added to a solution of (S)-*N*-Boc-2-amino-3,3-

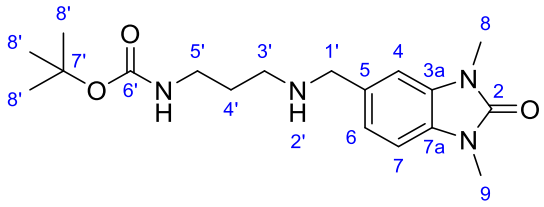
dimethylbutyric acid (366 mg, 1.58 mmol, 1.1 eq.), HATU (602 mg, 1.58 mmol, 1.1 eq.), and *N*,*N*-diisopropylethylamine (1.00 mL, 5.76 mmol, 4.0 eq.) in anhydrous *N*,*N*-dimethylformamide (8.5 mL). The reaction mixture was stirred at room temperature for 22 h. The solution was diluted with ethyl acetate (15 mL) and washed with 0.5 M aqueous lithium chloride solution (3 × 10 mL). The organic component was washed with brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (1% triethylamine, 0–4% methanol in dichloromethane) to yield the title compound **158** as a colourless solid (419 mg, 55%). R_f 0.55 (50% acetone in toluene); $[\alpha]_D^{25}$= −9.0 (*c* 1.0, $CHCl_3$); m.p. 93–95 ºC (from CH_2Cl_2); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3404 (w), 3304 (b), 3075 (w), 2970 (w), 2561 (w), 2185 (m), 2161 (m), 2100 (w), 2026 (m), 1978 (m), 1679 (s), 1628 (s), 1502 (m), 1434 (m), 1392 (m), 1366 (w), 1328 (m), 1276 (w), 1237 (w), 1165 (s), 1086 (w), 1056 (w), 1032 (w), 1011 (w), 967 (w), 938 (w), 920 (w), 852 (w), 802 (w), 730 (w), 694 (w), 646 (w), 618 (w); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.68 (1H, s, H-2'), 7.45 – 7.39 (1H, m, H-8''), 7.38 – 7.32 (4H, m, H-2'', H-3'', H-5'', and H-6''), 5.16 (1H, d, *J* 9.1, H-10), 4.78 (1H, t, *J* 7.8, H-2), 4.58 (1H, dd, *J* 14.9, 6.7, H-7''^{a}), 4.52 (1H, bs, H-4), 4.32 (1H, dd, *J* 14.9, 5.1, H-7''^{b}), 4.15 (1H, d, *J* 9.1, H-7), 4.11 (1H, d, *J* 11.5, H-5^a), 3.56 (1H, dd, *J* 11.5, 3.5, H-5^b), 2.88 (1H, bs, O-H), 2.60 (1H, ddd, *J* 13.5, 7.8, 4.5, H-3^a), 2.51 (3H, s, H-6'), 2.15 – 2.09 (1H, m, H-3^b), 1.40 (9H, s, H-13), 0.91 (9H, s, H-9); ^{13}C NMR (151 MHz, $CDCl_3$) δ_C 173.0 (C-9''), 170.6 (C-6), 156.7 (C-11), 150.4 (C-2'), 148.7 (C-4'), 138.2 (C-1''), 131.7 (C-5'), 131.2 (C-4''), 129.7, 128.3 (C-2'', C-6'', C-3'' and C-5''), 80.7 (C-12), 70.3 (C-4), 59.1 (C-7), 58.3 (C-2), 56.6 (C-5), 43.5 (C-7''), 35.6 (C-3), 34.7 (C-8), 28.5 (C-13), 26.5 (C-9), 16.2 (C-6'); *m/z* (ESI$^+$) 553 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{27}H_{39}O_5N_4S^+$ ([M+H]$^+$) requires 531.26357, found 531.26357; HPLC (method 1) retention time 7.9 min, 97.8%.

1,3-Dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carbaldehyde (160)



23 (1.00 g, 4.54 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (16 mL) and the solution was cooled to 0 ºC. Lithium borohydride (2.0 M in tetrahydrofuran, 4.54 mL, 9.08 mmol, 2.0 eq.) was added dropwise, followed by addition of methanol (0.5 mL) dropwise, and the solution was heated to 66 ºC for 4 h. Saturated aqueous sodium bicarbonate solution (40 mL) was added dropwise to the reaction mixture at 0 ºC and the mixture was extracted with dichloromethane (6 × 20 mL). The combined organic components were washed with brine (50 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude solid was dissolved in dichloromethane (50 mL), and manganese(IV) oxide (3.62 g, 41.6 mmol, 10.0 eq.) was added. The reaction mixture was stirred at room temperature for 16 h. The suspension was then filtered through Celite® and the filtrate was concentrated *in vacuo* to yield the title compound **160** as a colourless solid (778 mg, 95% over two steps). R_f 0.54 (5% methanol in dichloromethane); m.p. 185–189 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2728 (w), 2360 (w), 1705 (s), 1681 (s), 1618 (m), 1605 (m), 1511 (m), 1487 (m), 1463 (m), 1400 (w), 1355 (w), 1271 (w), 1193 (m), 1125 (w), 1081 (w), 1022 (w), 916 (w), 887 (w), 809 (m), 786 (w), 745 (w), 726 (w), 670 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 9.95 (1H, s, H-1''), 7.65 (1H, dd, *J* 8.0, 1.5, H-6), 7.54 (1H, d, *J* 1.5, H-4), 7.08 (1H, d, *J* 8.0, H-7), 3.48 (6H, s, H-8 and H-9); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 191.4 (C-1''), 154.9 (C-2), 135.5 (C-7a), 130.9 (C-5), 130.8 (C-3a), 127.0 (C-6), 107.1 (C-7), 106.5 (C-4), 27.63, 27.55 (C-8 and C-9); *m/z* (ESI$^+$) 191 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{10}H_{11}O_2N_2^+$ ([M+H]$^+$) requires 191.08150, found 191.08168; HPLC (method 1) retention time 6.3 min, 98.4%.

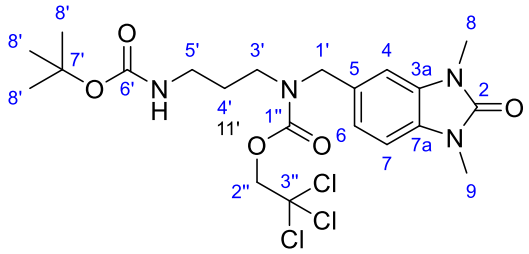
tert-Butyl (3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)carbamate (162)



tert-Butyl (3-aminopropyl)carbamate (1.37 g, 7.89 mmol, 2.0 eq.) and acetic acid (451 µL, 7.89 mmol, 2.0 eq.) were added to a solution of **160** (750 mg, 3.94 mmol, 1.0 eq.) in anhydrous 1,2-dichloroethane (35 mL) at 0 ºC. The reaction mixture was warmed to room temperature until imine formation was observed (2 h, followed by TLC and ^{1}H NMR). Sodium triacetoxyborohydride (4.18 g, 19.7 mmol, 5.0 eq.) was added at 0 ºC and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (40 mL), filtered through Celite® and the filter cake was washed with water (40 mL) and dichloromethane (50 mL). The organic component was separated, and the aqueous component was extracted with dichloromethane (6 × 40 mL). The combined organic components were washed with brine (80 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% triethylamine, 0–5% methanol in dichloromethane) to yield the title compound **162** as a colourless solid (1.14 g, 83%). R_f 0.36 (1% triethylamine, 5% methanol in dichloromethane); m.p. 80–82 ºC (from CH_2Cl_2); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3325 (w), 2935 (w), 2361 (w), 1692 (s), 1509 (w), 1454 (w), 1396 (w), 1365 (w), 1271 (w), 1251 (w), 1171 (w), 807 (w), 745 (w), 728 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.04 (1H, d, *J* 7.9, H-7), 6.99 (1H, s, H-4), 6.89 (1H, d, *J* 7.9, H-6), 5.10 (1H, s, Boc-N*H*), 3.82 (2H, s, H-1'), 3.41 (3H, s, H-8), 3.40 (3H, s, H-9), 3.21 (2H, q, *J* 6.6, H-5'), 2.71 (2H, t, *J* 6.6, H-3'), 2.05 (1H, bs, H-2'), 1.69 (2H, p, *J* 6.6, H-4'), 1.42 (9H, s, H-8'); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 156.3 (C-6'), 154.9 (C-2), 133.3 (C-5), 130.3 (C-7a), 129.3 (C-3a), 121.4 (C-6), 107.5 (C-4), 107.1 (C-7), 79.2 (C-7'), 54.4 (C-1'), 47.2 (C-3'), 39.2 (C-5'), 29.9 (C-4'),

28.6 (C-8'), 27.3 (C-8 and C-9); *m/z* (ESI$^+$) 349 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{18}H_{29}O_3N_4^+$ ([M+H]$^+$) requires 349.22342, found 349.22284; HPLC (method 1) retention time 6.3 min, 95.0%.

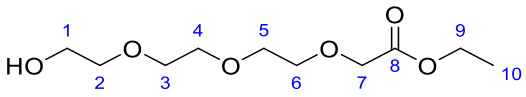
3'',3'',3''-Trichloroethyl {3-[(*tert*-butoxycarbonyl)amino]propyl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (163)



Pyridine (97.2 µL, 1.20 mmol, 1.4 eq.), 4-(dimethylamino)pyridine (20.4 mg, 0.168 mmol, 0.2 eq.) and **162** (300 mg, 0.861 mmol, 1.0 eq.) were dissolved in anhydrous dichloromethane (6 mL). The reaction mixture was cooled to 0 ºC and 2,2,2-trichloroethyl chloroformate (162 µL, 1.20 mmol, 1.4 eq.) was added dropwise. The reaction was stirred at 0 ºC for 2 h. The reaction mixture was then diluted with dichloromethane (10 mL) and washed with water (20 mL), 1 M aqueous hydrochloric acid solution (20 mL), saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL). The organic component was dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–3% methanol in dichloromethane) to yield the title compound **163** as a colourless oil (195 mg, 44%). R_f 0.64 (1% triethylamine, 10% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3344 (w), 2976 (w), 1702 (s), 1510 (w), 1470 (w), 1394 (w), 1365 (w), 1269 (w), 1170 (w), 1140 (w), 811 (w), 718 (w); ^{1}H NMR (400 MHz, DMSO-d_6, major rotamer assigned) δ_H 7.14 – 6.96 (3H, m, H-4, H-6, and H-7), 6.80 – 6.72 (1H, m, N*H*), 4.92 (2H, s, H-2''), 4.53 (2H, s, H-1'), 3.30 (3H, s, H-8, obscured by water peak), 3.29 (3H, s, H-9, obscured by water peak), 3.26 – 3.15 (2H, m, H-3'), 2.96 – 2.81 (2H, m, H-5'), 1.72 – 1.55 (2H, m, H-4'), 1.34 (9H, s, H-8'); ^{1}H NMR (500 MHz, DMSO-d_6, 353 K) δ_H 7.09 – 6.99 (3H, m, H-4, H-6, and H-7), 6.39 (1H, s, N*H*), 4.90 (2H, s, H-2''), 4.55 (2H, s, H-1'), 3.30 (3H, s, H-8, obscured by water peak), 3.29 (3H, s, H-9, obscured by water peak), 3.27 (2H, t, *J* 7.2, H-3'), 2.98 – 2.89 (2H, m, H-5'), 1.68 (2H, p, *J* 7.2,

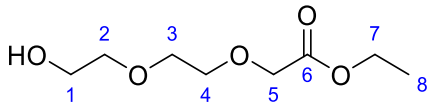
H-4'), 1.36 (9H, s, H-8'); ^{13}C NMR (126 MHz, DMSO-d_6, 353 K) δ_C 155.1 (C-6'), 153.75, 153.74 (C-1'' and C-2), 130.0 (C-7a), 129.7 (C-3a), 129.0 (C-5), 120.1 (C-6), 106.9 (C-7), 106.5 (C-4), 95.8 (C-3''), 77.1 (C-7'), 74.1 (C-2''), 50.0 (C-1'), 44.2 (C-3'), 37.5 (C-5'), 27.86 (C-8'), 27.85 (C-4'), 26.50, 26.46 (C-8 and C-9); *m/z* (ESI$^+$) 545 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{21}H_{29}O_5N_4Cl_3Na^+$ ([M+Na]$^+$) requires 545.10957, found 545.10953; HPLC (method 1) retention time 10.4 min, 96.8%.

Ethyl {2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}acetate (170)[162,198,199]



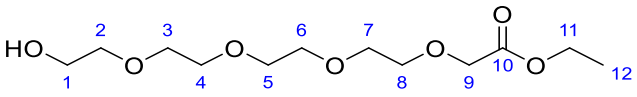
Triethylene glycol (500 µL, 3.74 mmol, 2.0 eq.) and boron trifluoride diethyl etherate (2.00 µL, 0.0162 mmol, 0.01 eq.) were dissolved in anhydrous dichloromethane (7.5 mL). A solution of ethyl diazoacetate (197 µL, 1.87 mmol, 1.0 eq.) in dichloromethane (1.9 mL) was added dropwise at 0 ºC. The reaction mixture was stirred at room temperature for 2 h. The solution was then washed with water (10 mL) and brine (10 mL). The organic component was dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (0–3% methanol in dichloromethane) to yield the title compound **170** as a colourless oil (302 mg, 68%). R_f 0.42 (5% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3427 (m), 2874 (m), 1748 (s), 1453 (w), 1379 (w), 1351 (w), 1283 (w), 1208 (m), 1114 (s), 1069 (m), 1030 (m), 935 (w), 886 (w), 850 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 4.20 (2H, q, *J* 7.1, H-9), 4.13 (2H, s, H-7), 3.75 – 3.63 (10H, m, H-2, H-3, H-4, H-5, and H-6), 3.62 – 3.56 (2H, m, H-1), 2.62 (1H, t, *J* 5.8, O*H*), 1.26 (3H, t, *J* 7.1, H-10); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 170.6 (C-8), 72.7 (C-1), 71.0 (C-2), 70.73 (C-3, C-4, C-5 or C-6), 70.71 (C-3, C-4, C-5 or C-6), 70.5 (C-3, C-4, C-5 or C-6), 68.8 (C-7), 61.9 (C-3, C-4, C-5 or C-6), 61.0 (C-9), 14.3 (C-10); *m/z* (ESI$^+$) 237 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[162,198,199]

Ethyl [2-(2-hydroxyethoxy)ethoxy]acetate (169)[200]



Diethylene glycol (500 µL, 5.27 mmol, 2.0 eq.) and boron trifluoride diethyl etherate (2.00 µL, 0.016 mmol, 0.01 eq.) were dissolved in anhydrous dichloromethane (10.5 mL). A solution of ethyl diazoacetate (277 µL, 2.63 mmol, 1.0 eq.) in dichloromethane (2.7 mL) was added dropwise at 0 ºC. The reaction mixture was stirred at room temperature for 2 h. The solution was then washed with water (10 mL) and brine (10 mL). The organic component was dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (0–2% methanol in dichloromethane) to yield the title compound **169** as a colourless oil (319 mg, 63%). R_f 0.16 (5% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3423 (m), 2876 (w), 1747 (s), 1646 (w), 1449 (w), 1379 (w), 1282 (w), 1208 (m), 1145 (m), 1114 (s), 1068 (m), 1029 (m), 931 (w), 889 (w), 847 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 4.16 (2H, q, *J* 7.1, H-7), 4.08 (2H, s, H-5), 3.72 – 3.62 (6H, m, H-2, H-3, and H-4), 3.59 – 3.53 (2H, m, H-1), 2.82 (1H, t, *J* 5.9, O*H*), 1.23 (3H, t, *J* 7.1, H-8); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.5 (C-6), 72.6 (C-1), 71.0 (C-2), 70.4 (C-3 or C-4), 68.6 (C-5), 61.7 (C-3 or C-4), 60.9 (C-7), 14.2 (C-8); *m/z* (ESI$^+$) 193 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_8H_{17}O_5^+$ ([M+H]$^+$) requires 193.10705, found 193.10704. The spectroscopic data are in good agreement with the literature values.[200]

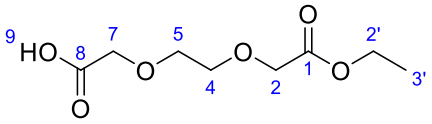
Ethyl 14-hydroxy-3,6,9,12-tetraoxatetradecan-11-oate (171) [201,202]



Tetraethylene glycol (500 µL, 2.90 mmol, 2.0 eq.) and boron trifluoride diethyl etherate (2.00 µL, 0.0162 mmol, 0.01 eq.) were dissolved in anhydrous dichloromethane (5.8 mL). A solution of ethyl diazoacetate (152 µL, 1.45 mmol, 1.0 eq.) in dichloromethane (1.5 mL) was added dropwise at 0 ºC. The reaction mixture was stirred at room temperature for 2 h. The solution was then washed with water (10 mL) and brine (10 mL). The organic component was dried over sodium

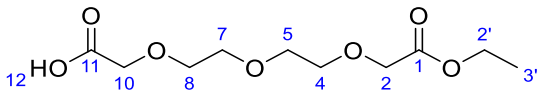
sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel flash column chromatography (0–2% methanol in dichloromethane) to yield the title compound **171** as a colourless oil (269 mg, 67%). R_f 0.24 (5% methanol in dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 4.21 (2H, q, *J* 7.1, H-11), 4.14 (2H, s, H-9), 3.76 – 3.63 (14H, m, H-2, H-3, H-4, H-5, H-6, H-7, and H-8), 3.63 – 3.58 (2H, m, H-1), 1.28 (3H, t, *J* 7.1, H-12); *m/z* (ESI$^+$) 281 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{12}H_{25}O_7^+$ ([M+H]$^+$) requires 281.15948, found 281.15913. The spectroscopic data are in good agreement with the literature values.[201,202]

[2-(2-Ethoxy-2-oxoethoxy)ethoxy]acetic acid (172)



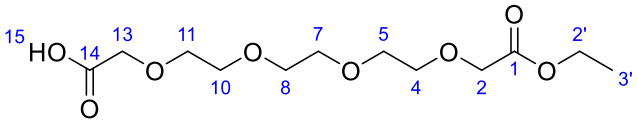
169 (85.1 mg, 0.442 mmol, 1.0 eq.) was dissolved in acetonitrile (2.2 mL) and water (2.2 mL). (Diacetoxyiodo)benzene (313 mg, 0.973 mmol, 2.2 eq.) and TEMPO (15.2 mg, 0.0973 mmol, 0.22 eq.) were added, and the reaction mixture was stirred at room temperature for 30 h. The mixture was then diluted with water (20 mL) and extracted with dichloromethane (3 × 30 mL). The combined organic components were washed with brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% AcOH, 0–8% methanol in dichloromethane) to yield the title compound **172** as a colourless oil (90.9 mg, 99%). R_f 0.23 (1% AcOH, 8% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3523 (w), 2934 (w), 2361 (w), 1744 (s), 1430 (w), 1383 (w), 1211 (s), 1129 (s), 1026 (w), 853 (w), 677 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.64 (1H, bs, H-9), 4.25 – 4.16 (4H, m, H-2’ and H-7), 4.14 (2H, s, H-2), 3.82 – 3.71 (4H, m, H-4 and H-5), 1.27 (3H, t, *J* 7.2, H-3’); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 173.7 (C-8), 170.5 (C-1), 71.2, 70.9 (C-4 and C-5), 68.63 (C-2), 68.60 (C-7), 61.2 (C-2’), 14.3 (C-3’); *m/z* (ESI$^+$) 229 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_8H_{13}O_6^+$ ([M+H]$^+$) requires 205.07176, found 205.07159.

11-Oxo-3,6,9,12-tetraoxatetradecan-1-oic acid (173)



170 (100 mg, 0.423 mmol, 1.0 eq.) was dissolved in acetonitrile (2.2 mL) and water (2.2 mL). (Diacetoxyiodo)benzene (300 mg, 0.910 mmol, 2.2 eq.) and TEMPO (14.5 mg, 0.0931 mmol, 0.22 eq.) were added, and the reaction mixture was stirred at room temperature for 30 h. The mixture was then diluted with water (20 mL) and extracted with dichloromethane (3 × 30 mL). The combined organic components were washed with brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% AcOH, 0–4% methanol in dichloromethane) to yield the title compound **173** as a colourless oil (91.4 mg, 86%). R_f 0.16 (1% AcOH, 4% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3512 (w), 2981 (w), 2909 (w), 1737 (s), 1643 (w), 1432 (w), 1378 (w), 1210 (s), 1144 (s), 1114 (s), 1027 (w), 935 (w), 852 (w), 679 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.34 (1H, bs, H-12), 4.20 (2H, q, *J* 7.1, H-2'), 4.14 (2H, s, H-10), 4.12 (2H, s, H-2), 3.77 – 3.67 (8H, m, H-4, H-5, H-7 and H-8), 1.27 (3H, t, *J* 7.1, H-3'); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 172.9 (C-11), 170.7 (C-1), 71.2, 70.8, 70.6, 70.4 (C-4, C-5, C-7 and C-8), 69.1 (C-2), 68.7 (C-10), 61.2 (C-2'), 14.3 (C-3'); *m/z* (ESI$^+$) 273 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{10}H_{18}O_7Na^+$ ([M+Na]$^+$) requires 273.09447, found 273.09434.

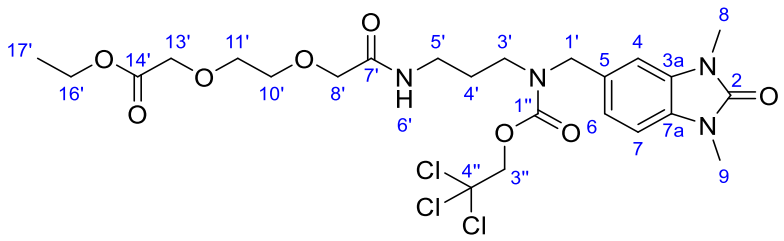
14-Oxo-3,6,9,12,15-pentaoxaheptadecan-1-oic acid (174)



171 (200 mg, 0.713 mmol, 1.0 eq.) was dissolved in acetonitrile (4.4 mL) and water (4.4 mL). (Diacetoxyiodo)benzene (506 mg, 1.57 mmol, 2.2 eq.) and TEMPO (24.5 mg, 0.157 mmol, 0.22 eq.) were added, and the reaction mixture was stirred at room temperature for 30 h. The mixture was then diluted with water (40 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic components were washed with brine (40 mL), dried over sodium sulfate

and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% AcOH, 0–4% methanol in dichloromethane) to yield the title compound **174** as a colourless oil (135 mg, 64%). R_f 0.12 (1% AcOH, 4% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3517 (w), 2877 (w), 1736 (s), 1644 (w), 1449 (w), 1352 (w), 1209 (s), 1099 (s), 1027 (m), 942 (w), 851 (w), 677 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.49 (1H, bs, H-15), 4.20 (2H, q, *J* 7.2, H-2'), 4.13 (2H, s, H-13), 4.12 (2H, s, H-2), 3.76 – 3.61 (12H, m, H-4, H-5, H-7, H-8, H-10 and H-11), 1.26 (3H, t, *J* 7.2, H-3'); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 172.4 (C-14), 170.5 (C-1), 71.1, 70.9, 70.6, 70.5, 70.4, 70.3 (C-4, C-5, C-7, C-8, C-10 and C-11), 69.1 (C-2), 68.7 (C-13), 61.1 (C-2'), 14.3 (C-3'); *m/z* (ESI$^+$) 317 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{12}H_{23}O_8^+$ ([M+H]$^+$) requires 295.13874, found 295.13856.

Ethyl 4'',4'',4''-trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7'-dioxo-2'',9',12'-trioxa-2',6'-diazaheptadecan-14'-oate (175)

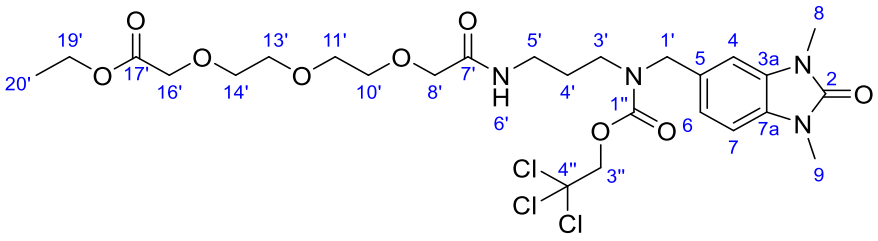


163 (100 mg, 0.191 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (2 mL) and stirred at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo*. The crude residue was passed through an Isolute® SXC-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the deprotected amine product 3'',3'',3''-trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (45.3 mg, 56%). This compound was then used directly in the next step to avoid Troc protecting group migration to the terminal primary amine.

172 (21.9 mg, 0.106 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (73.9 µL, 0.424 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (0.7 mL). HATU (44.3 mg,

0.117 mmol, 1.1 eq.) was added and the solution was stirred at room temperature for 10 min. 3’’,3’’,3’’-Trichloroethyl (3’-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (45.0 mg, 0.106 mmol, 1.0 eq.) in *N*,*N*-dimethylformamide (0.7 mL) was added, and the resulting solution was stirred at room temperature for 22 h. Subsequently, the reaction mixture was quenched with water (5 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (20 mL) and brine (10 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–4% methanol in dichloromethane) to yield the title compound **175** as a yellow oil (42.4 mg, 65%). R_f 0.32 (7% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3365 (w), 2938 (w), 2360 (w), 2342 (w), 1750 (w), 1704 (s), 1539 (w), 1509 (w), 1468 (w), 1444 (w), 1396 (w), 1269 (w), 1212 (m), 1129 (m), 1059 (w), 848 (w), 771 (w), 718 (w), 669 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.41 (1H, bs, H-6’), 7.10 – 7.00 (3H, m, H-4, H-6 and H-7), 4.90 (2H, s, H-3’’), 4.56 (2H, s, H-1’), 4.14 (2H, q, *J* 7.2, H-16’), 4.10 (2H, s, H-13’), 3.86 (2H, s, H-8’), 3.69 – 3.58 (4H, m, H-10’ and H-11’), 3.36 – 3.26 (8H, m, H-8, H-9 and H-3’), 3.14 (2H, q, *J* 6.6, H-5’), 1.74 (2H, p, *J* 7.1, H-4’), 1.21 (3H, t, *J* 7.2, H-17’); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 169.4 (C-14’), 168.6 (C-7’), 153.7 (C-2), 153.6 (C-1’’), 129.9 (C-3a), 129.7 (C-7a), 129.0 (C-5), 120.0 (C-6), 106.9, 106.4 (C-4 and C-7), 95.8 (C-4’’), 74.1 (C-3’’), 69.82, 69.77 (C-10’ and C-11’), 69.6 (C-8’), 67.6 (C-13’), 59.6 (C-16’), 49.9 (C-1’), 44.1 (C-3’), 35.5 (C-5’), 27.4 (C-4’), 26.44, 26.40 (C-8 and C-9), 13.5 (C-17’); *m/z* (ESI$^+$) 632 ([M+Na]$^+$, 70%), 634 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{24}H_{34}O_8N_4Cl_3^+$ ([M+H]$^+$) requires 611.14367 and 613.14072, found 611.14371 and 613.14075; HPLC (method 1) retention time 8.8 min, 99.0%.

Ethyl 4'', 4'', 4''-trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7'-dioxo-2'',9',12',15'-tetraoxa-2',6'-diazaicosan-17'-oate (176)

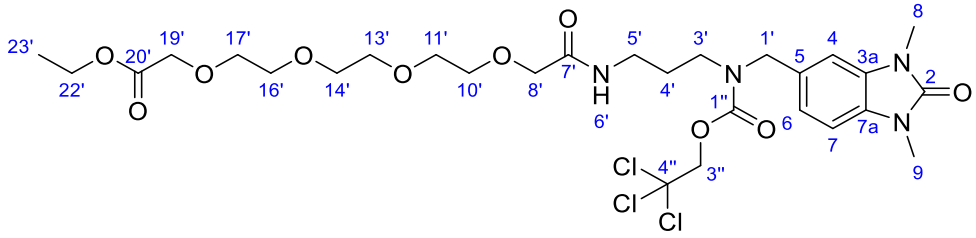


163 (300 mg, 0.570 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (3 mL) and stirred at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo*. The crude residue was passed through an Isolute® SXC-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the deprotected amine product 3'',3'',3''-trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (117 mg, 49%). This compound was then used directly in the next step to avoid Troc protecting group migration to the terminal primary amine.

173 (29.0 mg, 0.116 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (80.8 µL, 0.464 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (0.8 mL). HATU (48.3 mg, 0.127 mmol, 1.1 eq.) was added and the solution was stirred at room temperature for 10 min. 3'',3'',3''-Trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (49.0 mg, 0.116 mmol, 1.0 eq.) in *N*,*N*-dimethylformamide (0.8 mL) was added, and the resulting solution was stirred at room temperature for 22 h. Subsequently, the reaction mixture was quenched with water (7 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (20 mL) and brine (10 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–4% methanol in dichloromethane) to yield the title compound **176** as a yellow oil (47.9 mg, 63%). R_f 0.21 (1% triethylamine, 6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3351 (w), 2940 (w), 2359 (w), 1750 (w), 1705 (s), 1509 (w), 1444 (w), 1396 (w), 1269 (w), 1212 (m), 1127 (m), 847 (w), 718 (w); ^{1}H NMR

(500 MHz, DMSO-d_6, 363 K) δ_H 7.39 (1H, s, H-6'), 7.09 – 7.00 (3H, m, H-4, H-6 and H-7), 4.90 (2H, s, H-3''), 4.56 (2H, s, H-1'), 4.14 (2H, q, *J* 7.1, H-19'), 4.08 (2H, s, H-16'), 3.85 (2H, s, H-8'), 3.66 – 3.52 (8H, m, H-10', H-11', H-13' and H-14'), 3.36 – 3.24 (8H, m, H-8, H-9 and H-3'), 3.14 (2H, q, *J* 6.6, H-5'), 1.74 (2H, p, *J* 7.0, H-4'), 1.21 (3H, t, *J* 7.1, H-20'); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 169.4 (C-17'), 168.6 (C-7'), 153.7 (C-2), 153.6 (C-1''), 129.9 (C-3a), 129.7 (C-7a), 129.0 (C-5), 120.0 (C-6), 106.9 (C-4), 106.4 (C-7), 95.8 (C-4''), 74.1 (C-3''), 70.0 (C-8), 69.80, 69.77, 69.3, 69.2 (C-10', C-11', C-13' and C-14'), 67.6 (C-16'), 59.5 (C-19'), 49.9 (C-1'), 44.1 (C-3'), 35.5 (C-5'), 27.4 (C-4'), 26.44, 26.40 (C-8 and C-9), 13.5 (C-20'); *m/z* (ESI$^+$) 677 ([M+Na]$^+$, 70%), 679 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{26}H_{38}O_9N_4Cl_3^+$ ([M+H]$^+$) requires 655.16989 and 657.16694, found 655.16963 and 657.16652; HPLC (method 1) retention time 8.7 min, 98.0%.

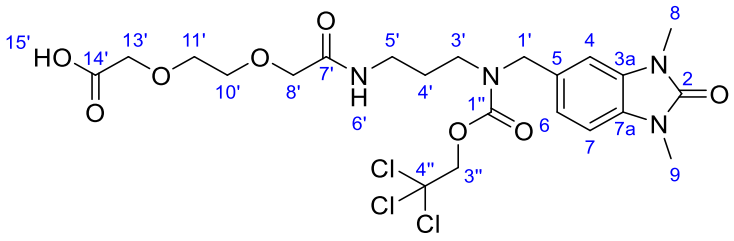
Ethyl 4'',4'',4''-trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7'-dioxo-2'',9',12',15',18'-pentaoxa-2',6'-diazatricosan-20'-oate (177)



163 (300 mg, 0.570 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (3 mL) and stirred at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo*. The crude residue was passed through an Isolute® SXC-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the deprotected amine product 3'',3'',3''-trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (117 mg, 49%). This compound was then used directly in the next step to avoid Troc protecting group migration to the terminal primary amine.

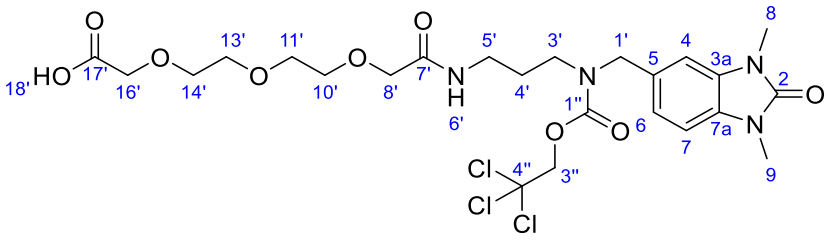
174 (54.8 mg, 0.186 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (130 µL, 0.744 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL). HATU (77.8 mg, 0.205 mmol, 1.1 eq.) was added and the solution was stirred at room temperature for 10 min. 3'',3'',3''-Trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (78.9 mg, 0.186 mmol, 1.0 eq.) in anhydrous *N*,*N*-dimethylformamide (0.5 mL) was added, and the resulting solution was stirred at room temperature for 22 h. Subsequently, the reaction mixture was quenched with water (20 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (40 mL) and brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–4% methanol in dichloromethane) to yield the title compound **177** as a colourless oil (83.1 mg, 64%). R_f 0.21 (1% triethylamine, 6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2937 (w), 1705 (s), 1541(w), 1509 (w), 1470 (w), 1396 (w), 1214 (w), 1127 (m), 843 (s), 718 (w), 637 (w), 622 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.37 (1H, bs, H-6'), 7.10 – 6.99 (3H, m, H-4, H-6 and H-7), 4.90 (2H, s, H-3''), 4.56 (2H, s, H-1'), 4.14 (2H, q, *J* 7.1, H-22'), 4.08 (2H, s, H-19'), 3.85 (2H, s, H-8'), 3.66 – 3.50 (12H, m, H-10', H-11', H-13', H-14', H-16' and H-17'), 3.36 – 3.26 (8H, m, H-8, H-9 and H-3'), 3.14 (2H, q, *J* 6.6, H-5'), 1.74 (2H, p, *J* 7.0, H-4'), 1.22 (3H, t, *J* 7.1, H-23'); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 169.4 (C-20'), 168.6 (C-7'), 153.7 (C-2), 153.6 (C-1''), 129.9 (C-3a), 129.7 (C-7a), 129.0 (C-5), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.8 (C-4''), 74.1 (C-3''), 70.0 (C-8'), 69.79, 69.77, 69.43, 69.40, 69.3, 69.2 (C-10', C-11', C-13', C-14', C-16' and C-17'), 67.6 (C-19'), 59.5 (C-22'), 49.9 (C-1'), 44.1 (C-3'), 35.5 (C-5'), 27.4 (C-4'), 26.42, 26.38 (C-8 and C-9), 13.5 (C-23'); *m/z* (ESI$^+$) 722 ([M+Na]$^+$, 60%), 724 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{28}H_{42}O_{10}N_4Cl_3^+$ ([M+H]$^+$) requires 699.19610 and 701.19315, found 699.19586 and 701.19272; HPLC (method 1) retention time 8.8 min, 99.0%.

4’’,4’’,4’’-Trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1’’,7’-dioxo-2’’,9’,12’-trioxa-2’,6’-diazaheptadecan-14’-oic acid (178)



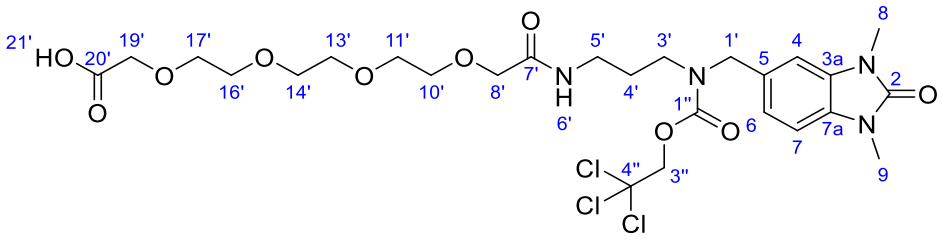
175 (131 mg, 0.214 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (1 mL) and methanol (1 mL). A solution of lithium hydroxide (51.3 mg, 2.14 mmol, 10.0 eq.) in water (1 mL) was added to the solution dropwise at room temperature. The resulting mixture was stirred at room temperature for 1 h. The reaction mixture was then acidified with 1 M aqueous hydrochloric acid (1 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic components were washed with brine (20 mL), dried over sodium sulfate and concentrated *in vacuo* to yield the title compound **178** as a colourless oil (103 mg, 83%). R_f 0.08 (1% AcOH, 4% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3350 (w), 2939 (w), 2362 (w), 1702 (s), 1545 (w), 1509 (m), 1468 (m), 1445 (m), 1398 (w), 1354 (w), 1270 (w), 1220 (m), 1129 (m), 1061 (w), 913 (w), 843 (m), 772 (w), 728 (m), 669 (w), 614 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.41 (1H, s, H-6’), 7.09 – 6.99 (3H, m, H-4, H-6 and H-7), 4.90 (2H, s, H-3’’), 4.56 (2H, s, H-1’), 4.02 (2H, s, H-13’), 3.86 (2H, s, H-8’), 3.68 – 3.58 (4H, m, H-10’ and H-11’), 3.36 – 3.25 (8H, m, H-8, H-9 and H-3’), 3.14 (2H, q, *J* 6.6, H-5’), 1.74 (2H, p, *J* 7.1, H-4’); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 170.7 (C-14’), 168.6 (C-7’), 153.7 (C-2), 153.6 (C-1’’), 129.9 (C-3a), 129.7 (C-7a), 129.0 (C-5), 120.0 (C-6), 106.9 (C-4), 106.4 (C-7), 95.8 (C-4’’), 74.1 (C-3’’), 69.9 (C-8’), 69.8, 69.5 (C-10’ and C-11’), 67.5 (C-13’), 49.9 (C-1’), 44.1 (C-3’), 35.5 (C-5’), 27.4 (C-4’), 26.43, 26.39 (C-8 and C-9); *m/z* (ESI$^+$) 606 ([M+Na]$^+$, 70%), 608 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{22}H_{30}O_8N_4Cl_3^+$ ([M+H]$^+$) requires 583.11237 and 585.10942, found 583.11242 and 585.10938; HPLC (method 1) retention time 6.6 min, 100.0%.

4’’,4’’,4’’-Trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1’’,7’-dioxo-2’’,9’,12’,15’-tetraoxa-2’,6’-diazaicosan-17’-oic acid (179)



176 (40.0 mg, 0.0610 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (0.25 mL) and methanol (0.25 mL). A solution of lithium hydroxide (14.6 mg, 0.610 mmol, 10.0 eq.) in water (0.25 mL) was added to the solution dropwise at room temperature. The resulting mixture was stirred at room temperature for 1 h. The reaction mixture was then acidified with 1 M aqueous hydrochloric acid (0.5 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic components were washed with brine (10 mL), dried over sodium sulfate and concentrated *in vacuo* to yield the title compound **179** as a colourless oil (32.9 mg, 86%). R_f 0.18 (1% AcOH, 6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2938 (w), 2879 (w), 1702 (s), 1547 (w), 1509 (m), 1468 (m), 1446 (m), 1398 (w), 1355 (w), 1270 (w), 1221 (m), 1194 (w), 1138 (m), 1056 (w), 913 (w), 842 (s), 772 (w), 728 (m), 626 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.36 (1H, s, H-6’), 7.09 – 6.97 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3’’), 4.56 (2H, s, H-1’), 4.02 (2H, s, H-16’), 3.85 (2H, s, H-8’), 3.66 – 3.54 (8H, m, H-10’, H-11’, H-13’ and H-14’), 3.36 – 3.25 (8H, m, H-8, H-9 and H-3’), 3.14 (2H, q, *J* 6.6, H-5’), 1.75 (2H, p, *J* 7.1, H-4’); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 170.7 (C-17’), 168.6 (C-7’), 153.7 (C-2), 153.5 (C-1’’), 129.9 (C-3a), 129.7 (C-7a), 128.9 (C-5), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.7 (C-4’’), 74.1 (C-3’’), 69.9 (C-8’), 69.8, 69.6, 69.3, 69.1 (C-10’, C-11’, C-13’ and C-14’), 67.5 (C-16’), 49.9 (C-1’), 44.1 (C-3’), 35.5 (C-5’), 27.4 (C-4’), 26.39, 26.35 (C-8 and C-9); *m/z* (ESI$^+$) 649 ([M+Na]$^+$, 90%), 651 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{24}H_{34}O_9N_4Cl_3^+$ ([M+H]$^+$) requires 627.13859 and 629.13564, found 627.13851 and 629.13539; HPLC (method 1) retention time 5.9 min, 98.8%.

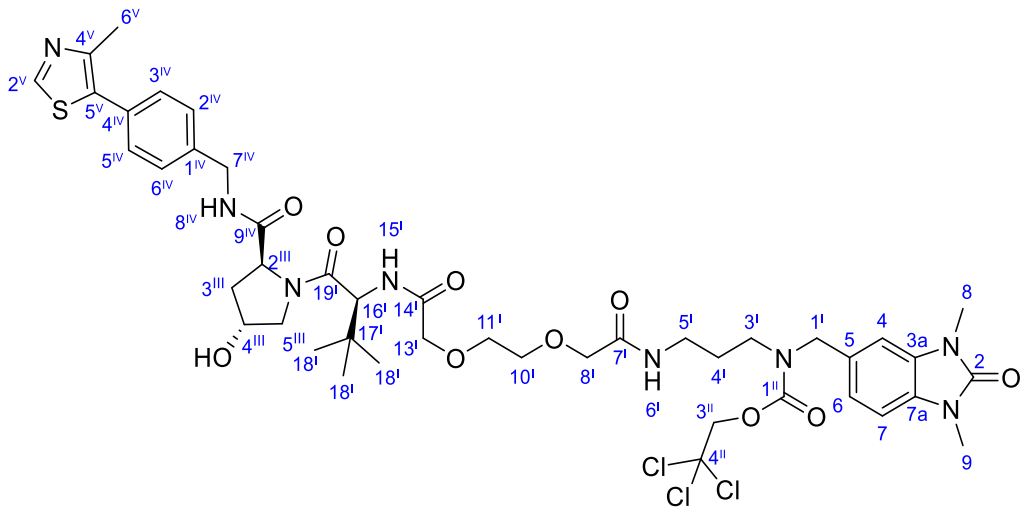
4’’,4’’,4’’-Trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1’’,7’-dioxo-2’’,9’,12’,15’,18’-pentaoxa-2’,6’-diazatricosan-20’-oic acid (180)



177 (39.0 mg, 0.0557 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (0.25 mL) and methanol (0.25 mL). A solution of lithium hydroxide (13.3 mg, 0.557 mmol, 10.0 eq.) in water (0.25 mL) was added to the solution dropwise at room temperature. The resulting mixture was stirred at room temperature for 1 h. The reaction mixture was then acidified with 1 M aqueous hydrochloric acid (0.5 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic components were washed with brine (10 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% AcOH, 2–10% methanol in dichloromethane) to yield the title compound **180** as a colourless oil (27.3 mg, 73%). R_f 0.02 (1% AcOH, 6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3350 (w), 2923 (w), 2367 (w), 1704 (s), 1542 (w), 1509 (w), 1468 (w), 1442 (w), 1396 (w), 1352 (w), 1268 (w), 1218 (w), 1125 (m), 947 (w), 833 (w), 772 (w), 745 (w), 717 (w), 680 (w), 621 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.42 (1H, s, H-6’), 7.11 – 6.99 (3H, m, H-4, H-6 and H-7), 4.90 (2H, s, H-3’’), 4.56 (2H, s, H-1’), 3.96 (2H, s, H-19’), 3.85 (2H, s, H-8’), 3.68 – 3.47 (12H, m, H-10’, H-11’, H-13’, H-14’, H-16’ and H-17’), 3.36 – 3.23 (8H, m, H-8, H-9 and H-3’), 3.13 (2H, partially obscured by residual water peak, q, *J* 6.5, H-5’), 1.79 – 1.68 (2H, m, H-4’); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 170.7 (C-20’), 168.7 (C-7’), 153.8 (C-2), 153.6 (C-1’’), 129.9 (C-3a), 129.7 (C-7a), 129.0 (C-5), 120.0 (C-6), 106.9 (C-4), 106.4 (C-7), 95.8 (C-4’’), 74.1 (C-3’’), 70.0 (C-8’), 69.8, 69.7, 69.44, 69.41, 69.3, 69.1 (C-10’, C-11’, C-13’, C-14’, C-16’ and C-17’), 67.5 (C-19’), 50.8 (C-1’), 44.2 (C-3’), 35.5 (C-5’), 27.4 (C-4’), 26.44, 26.40 (C-8 and C-9); *m/z* (ESI$^+$) 649 ([M+Na]$^+$, 100%), 651 ([M+Na]$^+$, 50%); HRMS *m/z* (ESI$^+$) $C_{24}H_{34}O_9N_4Cl_3^+$

([M+H]$^+$) requires 627.13859 and 629.13564, found 627.13851 and 629.13539; HPLC (method 1) retention time 7.4 min, 99.1%.

3-Methyl-*N*-{4^{II}, 4^{II}, 4^{II} -trichloro-2^{I} -[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-14^{I},7^{I}-dioxo-2^{II},9^{II},12^{II}-trioxa-2^{I},6^{I}-diazaheptadecanan-1^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (181)

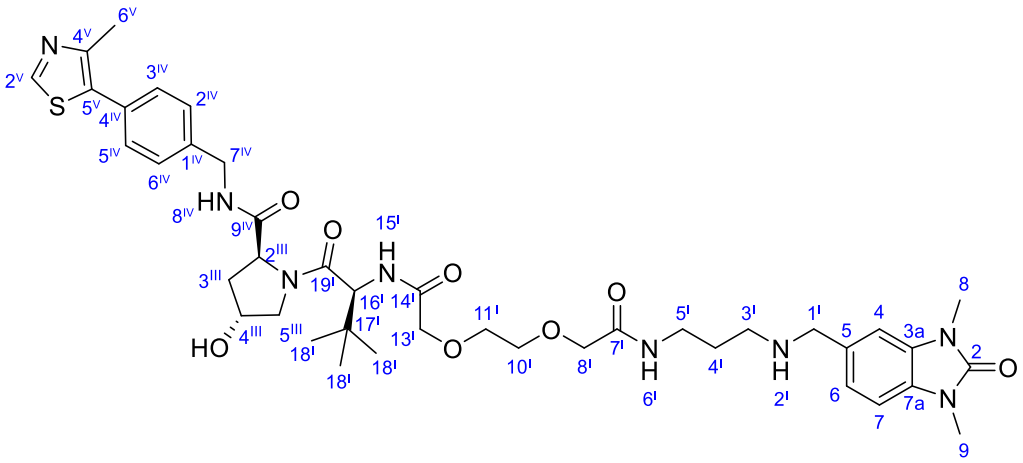


158 (220 mg, 0.415 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (2 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo*. The crude product was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the free amine as a yellow oil (162 mg, 91%). The product was then used immediately in the next step.

178 (62.5 mg, 0.107 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (74.7 µL, 0.428 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL). HATU (44.8 mg, 0.118 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The product from the above Boc deprotection (50.0 mg, 0.107 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with water (5 mL) and extracted with ethyl acetate (4 × 10 mL). The combined organic components were washed with brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude

residue was purified by silica gel column chromatography (0.1% ammonium hydroxide, 2–6% ethanol in chloroform) to yield the title compound **181** as a colourless oil (70.8 mg, 66%). R_f 0.68 (0.1% ammonium hydroxide, 9% ethanol in chloroform); $[\alpha]_D^{25} = -17.8$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3305 (s), 3026 (s), 2925 (s), 2855 (s), 2362 (w), 1685 (s), 1635 (s), 1534 (m), 1510 (m), 1467 (m), 1440 (m), 1274 (m), 1221 (m), 1108 (m), 1029 (m), 852 (w), 718 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.90 (1H, s, H-2^V), 8.17 (1H, t, *J* 5.4, H-8^{IV}), 7.47 – 7.35 (5H, m, H-2^{IV}, H-3^{IV}, H-5^{IV}, H-6^{IV} and H-6^I), 7.28 (1H, d, *J* 9.4, H-15^I), 7.08 – 6.98 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.85 (1H, bs, O*H*), 4.62 – 4.47 (4H, m, H-16^I, H-1^I and H-2^{III}), 4.43 – 4.27 (3H, m, H-7^{IV} and H-4^{III}), 4.04 – 3.93 (2H, m, H-5^{III}), 3.88 (2H, s, H-8^I), 3.76 – 3.58 (6H, m, H-13^I, H-10^I and H-11^I), 3.35 – 3.25 (8H, m, H-8, H-9 and H-3^I), 3.18 – 3.08 (2H, m, H-5^I), 2.45 (3H, s, H-6^V), 2.11 – 1.96 (2H, m, H-3^{III}), 1.74 (2H, p, *J* 7.2, H-4^I), 0.96 (9H, s, H-18^I); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.0 (C-19^I), 169.1 (C-14^I), 168.4 (C-7^I), 168.1 (C-9^{IV}), 153.7 (C-2), 153.6 (C-1^{II}), 150.5 (C-2^V), 147.4 (C-4^V), 138.9 (C-1^{IV}), 130.7 (C-4^{IV}), 129.9 (C-3a), 129.7 (C-7a), 129.5 (C-5^V), 128.9 (C-5), 128.2 (C-5^{IV} and C-3^{IV}), 127.1 (C-6^{IV} and C-2^{IV}), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.8 (C-4^{II}), 74.1 (C-3^{II}), 69.90 (C-8^I), 69.84 (C-10^I), 69.7 (C-13^I), 69.5 (C-5^{III}), 68.4 (C-4^{III}), 58.4 (C-2^{III}), 55.9 (C-11^I), 55.6 (C-16^I), 49.9 (C-1^I), 44.1 (C-3^I), 41.5 (C-7^{IV}), 37.4 (C-3^{III}), 35.5 (C-5^I), 35.0 (C-17^I), 27.3 (C-4^I), 26.42, 26.38, (C-8 and C-9), 25.8 (C-18^I), 15.3 (C-6^V); *m/z* (ESI$^+$) 996 ([M+H]$^+$, 30%), 998 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{44}H_{58}O_{10}N_8Cl_3S^+$ ([M+H]$^+$) requires 995.30567 and 997.30272, found 995.30641 and 997.30377; HPLC (method 1) retention time 6.8 min, 96.9%.

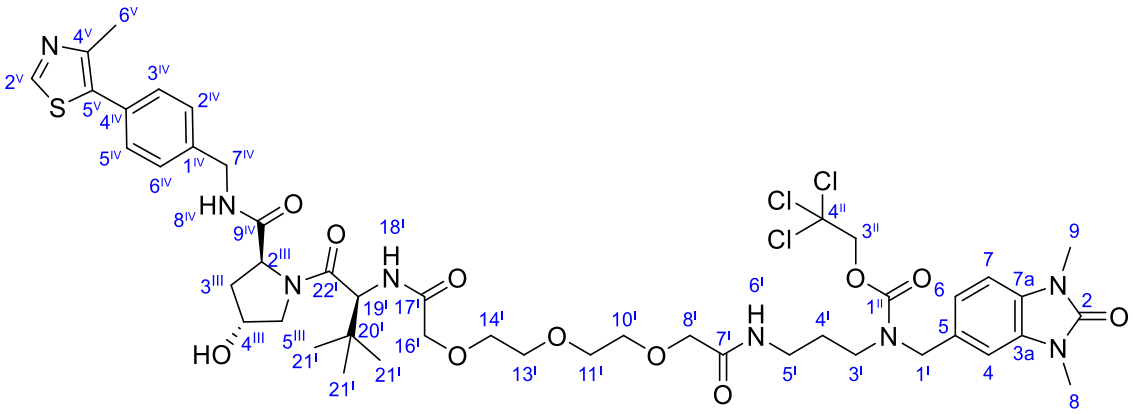
_N_-[2^{I}-(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)-7^{I},14^{I}-dioxo-9^{I},12^{I}-dioxa-2^{I},6^{I}-diazatetradecan-14^{I}-yl]-17^{I}-methyl-L-valyl-(4_R_)-4^{III}-hydroxy-_N_-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (144)



181 (29 mg, 0.029 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.6 mL), and acetic acid (65 µL) and zinc powder (19 mg, 0.29 mmol, 10.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (0.1% ammonium hydroxide, 2–6% ethanol in chloroform) to yield the title compound **144** as a colourless oil (15.3 mg, 64%). R_f 0.50 (1% triethylamine, 8% methanol in dichloromethane); $[\alpha]_D^{25}$= −14.7 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3322 (m), 3057 (w), 2933 (m), 2360 (m), 2341 (w), 1678 (s), 1634 (s), 1537 (m), 1512 (s), 1483 (m), 1445 (s), 1398 (m), 1370 (m), 1340 (m), 1243 (m), 1199 (m), 1114 (w), 1034 (m), 967 (w), 913 (w), 853 (w), 815 (w), 730 (m), 646 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.66 (1H, s, H-2^{V}), 7.76 (1H, t, *J* 6.1, H-8^{IV}), 7.70 (1H, t, *J* 6.3, H-6^{I}), 7.38 – 7.31 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.24 (1H, d, *J* 1.7, H-4), 7.10 (1H, dd, *J* 8.0, 1.7, H-6), 6.89 (1H, d, *J* 8.0, H-7), 4.67 (1H, t, *J* 8.4, H-2^{III}), 4.62 (1H, d, *J* 9.4, H-16^{I}), 4.48 (1H, bs, H-4^{III}), 4.45 – 4.42 (2H, m, H-7^{IV}), 4.09 – 3.83 (7H, m, H-5^{IIIa}, H-1^{I}, H-13^{I} and H-8^{I}), 3.77 – 3.61 (5H, m, H-5^{IIIb}, H-10^{I} and H-11^{I}), 3.39 (8H, m, H-8, H-9 and H-5^{I}), 2.93 – 2.79 (2H, m, H-3^{I}), 2.49 (3H, s, H-6^{V}), 2.32 – 2.17 (2H, m, H-3^{III}), 1.99 (2H, m, H-4^{I}), 0.99 (9H, s, H-18^{I}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.5 (C-19^{I}), 171.3 (C-14^{I}), 170.9 (C-7^{I}), 170.2 (C-9^{IV}), 154.8 (C-2), 150.4 (C-2^{V}), 148.6 (C-4^{V}), 138.5 (C-1^{IV}), 131.7 (C-4^{IV}), 131.0

(C-3a), 130.9 (C-7a), 130.7 (C-5^{V}), 129.5 (C-2^{IV} and C-6^{IV}), 128.2 (C-3^{IV} and C-5^{IV}), 124.1 (C-5), 123.3 (C-6), 109.2 (C-4), 107.4 (C-7), 71.3, 71.2 (C-10^{I} and C-11^{I}), 70.8, 70.4 (C-8^{I} and C-13^{I}), 70.2 (C-4^{III}), 59.4 (C-2^{III}), 57.5 (C-5^{III}), 57.1 (C-16^{I}), 52.2 (C-1^{I}), 44.8 (C-3^{I}), 43.1 (C-7^{IV}), 37.8 (C-3^{III}), 36.3 (C-17^{I}), 36.0 (C-5^{I}), 27.5, 27.4 (C-8 and C-9), 26.8 (C-4^{I}), 26.6 (C-18^{I}), 16.2 (C-6^{V}); *m/z* (ESI^{+}) 843 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{41}H_{57}O_8N_8S^{+}$ ([M+H]$^{+}$) requires 821.40146, found 821.40101; HPLC (method 1) retention time 4.1 min, 99.4%.

20^{I}-Methyl-*N*-{4^{II},4^{II},4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-7^{I},17^{I}-dioxo-2^{II},9^{I},12^{I}, 15^{I}-tetraoxa-2^{I},6^{I}-diazaicosanan-1^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (182)

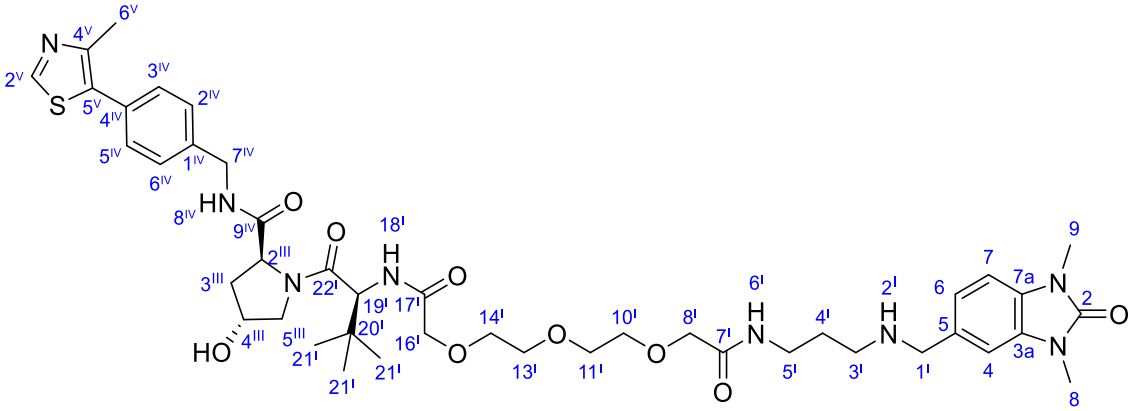


158 (660 mg, 1.24 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (3.11 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo*. The crude product was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the free amine as a yellow oil (491 mg, 92%). The product was then used immediately in the next step.

179 (310 mg, 0.494 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (344 µL, 1.98 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (2 mL). HATU (207 mg, 0.543 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The product from the above Boc deprotection (231 mg, 0.494 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (2 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with water (20 mL) and

extracted with ethyl acetate (4 × 40 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–10% methanol in dichloromethane, then further purified by silica gel column chromatography (1% triethylamine, 40–100% [25% ethanol in ethyl acetate] in heptane) to yield the title compound **182** as a colourless oil (254 mg, 49%). R_f 0.52 (6% methanol in dichloromethane); $[\alpha]_D^{25} = -16.2$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3728 (w), 3626 (w), 3359 (w), 2928 (w), 2858 (w), 2360 (s), 2341 (s), 1710 (s), 1689 (s), 1658 (s), 1510 (m), 1444 (m), 1302 (w), 1177 (w), 1122 (w), 844 (m), 719 (w), 669 (w), 613 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 8.90 (1H, s, H-2^V), 8.17 (1H, t, *J* 6.1, H-8^{IV}), 7.44 – 7.34 (5H, m, H-2^{IV}, H-3^{IV}, H-5^{IV}, H-6^{IV} and H-6^I), 7.24 (1H, d, *J* 9.5, H-18^I), 7.08 – 7.00 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.84 (1H, bs, O*H*), 4.60 – 4.53 (3H, m, H-1^I and H-19^I), 4.51 (1H, t, *J* 7.8, H-2^{III}), 4.42 – 4.35 (2H, m, H-4^{III} and H-7^{IVa}), 4.29 (1H, dd, *J* 15.4, 5.7, H-7^{IVb}), 4.00 – 3.92 (2H, m, H-5^{III}), 3.84 (2H, s, H-8^I), 3.71 (1H, dd, *J* 10.6, 4.5, H-16^{Ia}), 3.66 – 3.57 (9H, m, H-10^I, H-11^I, H-13^I, H-14 and H-16^{Ib}), 3.34 – 3.26 (8H, m, H-8, H-9 and H-3^I), 3.17 – 3.10 (2H, m, H-5^I), 2.45 (3H, s, H-6^V), 2.09 – 1.97 (2H, m, H-3^{III}), 1.74 (2H, p, *J* 7.2, H-4^I), 0.96 (9H, s, H-21^I); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 171.0 (C-22^I), 169.0 (C-17^I), 168.6 (C-7^I), 168.0 (C-9^{IV}), 153.7 (C-2), 153.5 (C-1^{II}), 150.4 (C-2^V), 147.4 (C-4^V), 138.9 (C-1^{IV}), 130.6 (C-4^{IV}), 129.9 (C-3a), 129.7 (C-7a), 129.5 (C-5^V), 128.9 (C-5), 128.2 (C-5^{IV} and C-3^{IV}), 127.2 (C-6^{IV} and C-2^{IV}), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.7 (C-4^{II}), 74.1 (C-3^{II}), 70.1 (C-8^I), 69.9, 69.81, 69.79, 69.5 (C-10^I, C-11^I, C-13^I and C-16^I), 69.2 (C-5^{III}), 68.4 (C-4^{III}), 58.3 (C-2^{III}), 55.8 (C-14^I), 55.5 (C-19^I), 49.9 (C-1^I), 44.1 (C-3^I), 41.5 (C-7^{IV}), 37.3 (C-3^{III}), 35.5 (C-5^I), 35.0 (C-20^I), 27.4 (C-4^I), 26.39, 26.35 (C-8 and C-9), 25.8 (C-21^I), 15.3 (C-6^V); *m/z* (ESI$^+$) 1039 ([M+H]$^+$, 50%), 1041 ([M+H]$^+$, 40%); HRMS *m/z* (ESI$^+$) $C_{46}H_{62}O_{11}N_8Cl_3S^+$ ([M+H]$^+$) requires 1039.33188 and 1041.32893, found 1039.33179 and 1041.32958; HPLC (method 2) retention time 0.80 min, 100%.

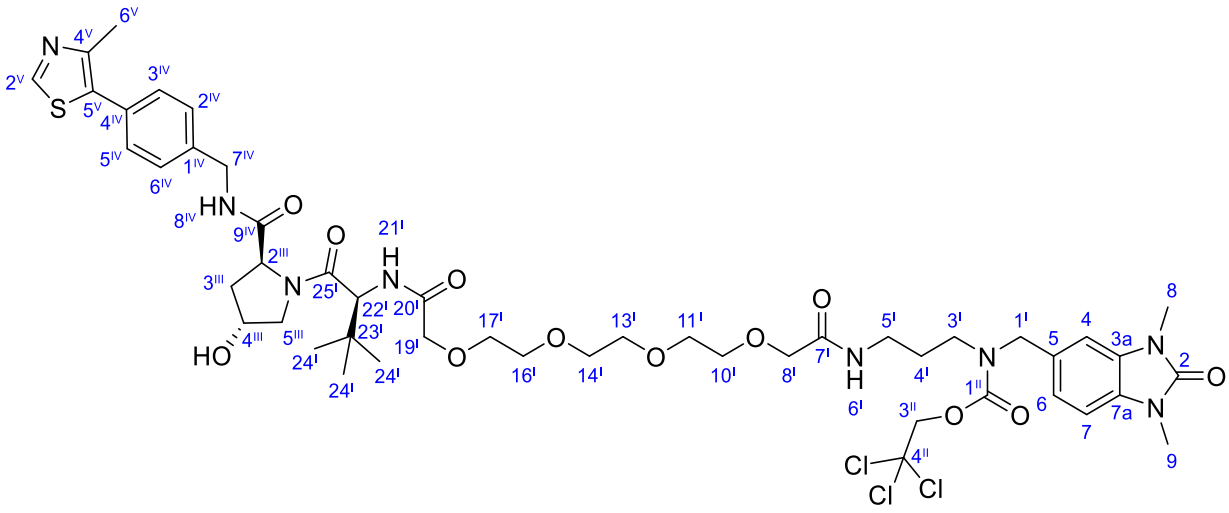
_N_-[2^{I}-(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)-7^{I},17^{I}-dioxo-9^{I},12^{I},15^{I}-trioxa-2^{I},6^{I}-diazaheptadecan-17^{I}-yl]-20^{I}-methyl-L-valyl-(4_R_)-4^{III}-hydroxy-_N_-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (145)



182 (35 mg, 0.034 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.5 mL), and acetic acid (29 µL) and zinc powder (33 mg, 0.50 mmol, 15.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (0.1% ammonium hydroxide, 2–10% methanol in dichloromethane) to yield the title compound **145** as a colourless oil (10 mg, 34%). R_f 0.15 (1% triethylamine, 80% [25% ethanol in ethyl acetate] in heptane); $[\alpha]_D^{25} = -15.7$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3399 (m), 3305 (m), 3076 (w), 2924 (w), 2873 (w), 1668 (s), 1640 (s), 1536 (m), 1509 (m), 1443 (m), 1398 (w), 1370 (w), 1342 (w), 1272 (w), 1242 (w), 1195 (w), 1110 (m), 1031 (w), 967 (w), 938 (w), 853 (w), 811 (w), 744 (w), 728 (w), 660 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.66 (1H, s, H-2^{V}), 7.70 (1H, t, *J* 5.6, H-8^{IV}), 7.42 – 7.31 (5H, m, H-2^{IV}, H-3^{IV}, H-5^{IV}, H-6^{IV} and H-18^{I}), 7.19 (1H, t, *J* 6.4, H-6^{I}), 7.07 – 7.01 (2H, m, H-4 and H-6), 6.89 (1H, d, *J* 8.4, H-7), 4.70 – 4.55 (3H, m, H-7^{IVa}, H-2^{III} and H-19^{I}), 4.44 (1H, bs, H-4^{III}), 4.26 (1H, dd, *J* 15.0, 5.6, H-7^{IVb}), 4.01 (1H, d, *J* 15.7, H-8^{Ia}), 3.92 – 3.84 (3H, m, H-5^{IIIa}, H-8^{Ib} and O*H*), 3.83 – 3.51 (13H, m, H-10^{I}, H-11^{I}, H-13^{I}, H-14^{I}, H-16^{I}, H-5^{IIIb} and H-1^{I}), 3.44 – 3.34 (7H, m, H-8, H-9 and H-5^{Ia}), 3.14 – 3.05 (1H, m, H-5^{Ib}), 2.62 – 2.52 (2H, m, H-3^{I}), 2.51 (3H, s, H-6^{V}), 2.31 (1H, ddd, *J* 13.4, 9.1, 4.3, H-3^{IIIa}), 2.18 – 2.10 (1H, m, H-3^{IIIb}), 1.70 – 1.61 (1H, m, H-4^{Ia}), 1.61 – 1.51 (1H, m, H-4^{Ib}), 0.97 (9H, s,

H-21^{I}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.8 (C-22^{I}), 171.4 (C-17^{I}), 170.4 (C-7^{I}), 169.8 (C-9^{IV}), 155.0 (C-2), 150.3 (C-2^{V}), 148.6 (C-4^{V}), 138.9 (C-1^{IV}), 131.8 (C-4^{IV}), 130.9 (C-3a), 130.5 (C-7a), 129.51 (C-5^{V}), 129.49 (C-2^{IV} and C-6^{IV}), 128.4 (C-3^{IV} and C-5^{IV}), 121.7 (C-6), 107.8 (C-4), 107.2 (C-7), 71.3, 70.9, 70.5, 70.33, 70.30 (C-8^{I}, C-10^{I}, C-11^{I}, C-13^{I}, C-14^{I} and C-16^{I}), 70.2 (C-4^{III}), 59.2 (C-2^{III}), 57.2 (C-5^{III}), 56.7 (C-19^{I}), 53.7 (C-1^{I}), 46.1 (C-3^{I}), 43.3 (C-7^{IV}), 37.4 (C-3^{III}), 37.1 (C-5^{I}), 36.1 (C-20^{I}), 29.3 (C-4^{I}), 27.39, 27.37 (C-8 and C-9), 26.5 (C-21^{I}), 16.2 (C-6^{V}); *m/z* (ESI^{+}) 865 ([M+H]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{43}H_{61}O_9N_8S^{+}$ ([M+H]$^{+}$) requires 865.42767, found 865.42806; HPLC (method 2) retention time 0.69 min, 100%.

23^{I}-Methyl-*N*-{4^{II}, 4^{II}, 4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-7^{I},20^{I}-dioxo-2^{II},9^{I},12^{I}, 15^{I},18^{I}-pentaoxa-2^{I},6^{I}-diazatricosanan-1^{I}-oyl}-L-valyl-(4*R*)-4-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (183)

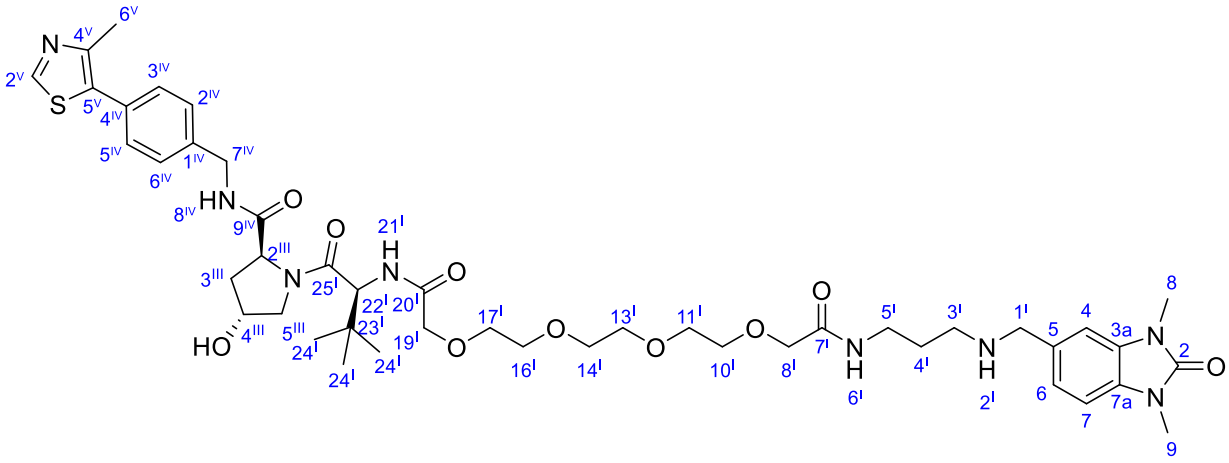


158 (660 mg, 1.24 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (3.11 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo*. The crude product was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the free amine as a yellow oil (491 mg, 92%). The product was then used immediately in the next step.

180 (55 mg, 0.084 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (58 µL, 0.33 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (0.5 mL). HATU (35 mg, 0.092 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The product from the above Boc deprotection (39 mg, 0.084 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (0.5 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with water (10 mL) and extracted with ethyl acetate (4 × 20 mL). The combined organic components were washed with brine (10 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–100% [25% ethanol in ethyl acetate] in heptane) to yield the title compound **183** as a colourless oil (29 mg, 32%). R_f 0.44 (6% methanol in dichloromethane); $[\alpha]_D^{25} = -20.1$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3325 (m), 3120 (w), 3070 (w), 2924 (m), 2342 (w), 1698 (s), 1639 (s), 1531 (m), 1510 (s), 1439 (s), 1397 (m), 1369 (m), 1342 (m), 1301 (m), 1270 (m), 1225 (m), 1193 (m), 1124 (s), 1053 (m), 1027 (s), 1009 (m), 966 (m), 938 (m), 850 (m), 821 (m), 758 (m), 745 (m), 717 (s), 677 (m), 629 (m); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.90 (1H, s, H-2^V), 8.16 (1H, t, *J* 6.1, H-8^{IV}), 7.46 – 7.30 (5H, m, H-2^{IV}, H-3^{IV}, H-5^{IV}, H-6^{IV} and H-6^I), 7.23 (1H, d, *J* 9.4, H-21^I), 7.08 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.83 (1H, s, O*H*), 4.59 – 4.54 (3H, m, H-1^I and H-22^I), 4.52 (1H, t, *J* 7.8, H-2^{III}), 4.43 – 4.35 (2H, m, H-4^{III} and H-7^{IVa}), 4.29 (1H, dd, *J* 15.3, 5.7, H-7^{IVb}), 4.00 – 3.89 (2H, m, H-5^{III}), 3.84 (2H, s, H-8^I), 3.72 (1H, dd, *J* 10.6, 4.5, H-19^{Ia}), 3.66 – 3.52 (13H, m, H-10^I, H-11^I, H-13^I, H-14^I, H-16^I, H-17^I and H-19^{Ib}), 3.34 – 3.26 (8H, m, H-8, H-9 and H-3^I), 3.14 (2H, dt, *J* 6.6, 6.4, H-5^I), 2.45 (3H, s, H-6^V), 2.10 – 1.97 (2H, m, H-3^{III}), 1.74 (2H, p, *J* 7.0, H-4^I), 0.97 (9H, s, H-24^I); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.1 (C-25^I), 169.0 (C-20^I), 168.6 (C-7^I), 168.1 (C-9^{IV}), 153.7 (C-2), 153.5 (C-1^{II}), 150.4 (C-2^V), 147.4 (C-4^V), 138.9 (C-1^{IV}), 130.6 (C-4^{IV}), 129.9 (C-3a), 129.7 (C-7a), 129.5 (C-5^V), 128.9 (C-5), 128.2 (C-5^{IV} and C-3^{IV}), 127.2 (C-6^{IV} and C-2^{IV}), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.7 (C-4^{II}), 74.1 (C-3^{II}), 70.1 (C-8^I), 69.9, 69.8, 69.53, 69.48, 69.42, 69.22 (C-10^I, C-11^I, C-13^I, C-14^I, C-16^I and C-19^I), 69.17 (C-5^{III}), 68.5 (C-4^{III}), 58.3 (C-2^{III}), 55.8 (C-17^I), 55.5 (C-22^I), 49.9 (C-1^I), 44.1 (C-3^I), 41.5 (C-7^{IV}), 37.3 (C-3^{III}), 35.5 (C-5^I), 35.0 (C-23^I), 27.4 (C-4^I), 26.39, 26.35 (C-8 and C-9), 25.8 (C-24^I), 15.2

(C-6^{V}); *m/z* (ESI^{+}) 1083 ([M+H]$^{+}$, 90%), 1085 ([M+H]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{48}H_{66}O_{12}N_8Cl_3S^{+}$ ([M+H]$^{+}$) requires 1083.3581 and 1085.3563, found 1083.3569 and 1085.3547; HPLC (method 2) retention time 0.80 min, 100%.

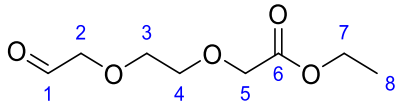
_N_-[2^{I}-(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-7^{I},20^{I}-dioxo-9^{I},12^{I},15^{I},18^{I}-tetraoxa-2^{I},6^{I}-diazaicosan-20^{I}-yl]-23^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (146)



183 (25 mg, 0.023 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.5 mL), and acetic acid (20 µL) and zinc powder (23 mg, 0.35 mmol, 15.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (1% triethylamine, 0–10% methanol in dichloromethane) to yield the title compound **146** as a yellow oil (8.7 mg, 42%). R_f 0.65 (1% triethylamine, 5% methanol in dichloromethane); $[\alpha]_D^{25} = -19.3$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3401 (m), 3069 (w), 2926 (w), 2873 (w), 1663 (s), 1638 (s), 1538 (m), 1512 (m), 1444 (m), 1399 (m), 1370 (w), 1343 (w), 1271 (w), 1244 (w), 1198 (w), 1110 (m), 1031 (w), 967 (w), 940 (w), 853 (w), 808 (w), 745 (w), 728 (w), 656 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.65 (1H, s, H-2^{V}), 7.95 (1H, t, *J* 5.5, H-8^{IV}), 7.63 (1H, t, *J* 5.2, H-6^{I}), 7.39 – 7.31 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.24 (1H, s, H-21^{I}), 7.16 (1H, d, *J* 1.5, H-4), 7.10 (1H, dd, *J* 7.9, 1.5, H-6), 6.89 (1H, d, *J* 7.9, H-7), 4.73 (1H, t, *J* 8.3, H-2^{III}), 4.60 (1H, d, *J* 9.4, H-22^{I}), 4.52 (1H, dd, *J* 15.1, 6.3, H-7^{IVa}), 4.46 (1H, t, *J* 3.9, H-4^{III}), 4.36

(1H, dd, *J* 15.1, 5.4, H-7^{IVb}), 4.06 (1H, d, *J* 15.3, H-8^{Ia}), 4.01 – 3.89 (5H, m, H-1^{Ia}, H-5^{IIIa}, H-19^{I} and H-2^{I}), 3.87 (1H, d, *J* 15.3, H-8^{Ib}), 3.75 – 3.46 (14H, m, H-1^{Ib}, H-5^{IIIb}, H-10^{I}, H-11^{I}, H-13^{I}, H-14^{I}, H-16^{I} and H-17^{I}), 3.43 – 3.30 (7H, m, H-8, H-9 and H-5^{Ia}), 3.29 – 3.17 (1H, m, H-5^{Ib}), 2.74 (2H, t, *J* 6.7, H-3^{I}), 2.49 (3H, s, H-6^{V}), 2.30 (1H, ddd, *J* 13.2, 8.8, 4.3, H-3^{IIIa}), 2.20 (1H, dd, *J* 13.6, 7.8, H-3^{IIIb}), 1.83 (2H, q, *J* 6.8, H-4^{I}), 0.98 (9H, s, H-24^{I}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.6 (C-25^{I}), 171.5 (C-20^{I}), 171.0 (C-7^{I}), 169.7 (C-9^{IV}), 154.9 (C-2), 150.3 (C-2^{V}), 148.6 (C-4^{V}), 138.7 (C-1^{IV}), 131.8 (C-4^{IV}), 130.8 (C-3a), 130.5 (C-7a), 130.1 (C-5^{V}), 129.5 (C-2^{IV} and C-6^{IV}), 128.4 (C-5), 128.2 (C-3^{IV} and C-5^{IV}), 122.6 (C-6), 108.7 (C-4), 107.2 (C-7), 71.0, 70.8, 70.6, 70.5, 70.27, 70.25, 70.2 (C-8^{I}, C-11^{I}, C-13^{I}, C-14^{I}, C-16^{I}, C-17^{I} and C-19^{I}), 70.1 (C-4^{III}), 59.1 (C-2^{III}), 57.4 (C-5^{III}), 56.9 (C-22^{I}), 53.1 (C-1^{I}), 52.7 (C-10^{I}), 45.0 (C-3^{I}), 43.2 (C-7^{IV}), 37.3 (C-3^{III}), 36.5 (C-23^{I}), 36.0 (C-5^{I}), 27.9 (C-4^{I}), 27.43, 27.38 (C-8 and C-9), 26.6 (C-24^{I}), 16.2 (C-6^{V}); *m/z* (ESI$^+$) 910 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{45}H_{65}O_{10}N_8S^+$ ([M+H]$^+$) requires 909.4539, found 909.4518; HPLC (method 3) retention time 1.55 min, 100%.

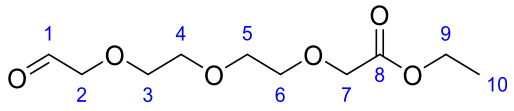
Ethyl [2-(2-oxoethoxy)ethoxy]acetate (184)



Dess Martin Periodinane (130 mg, 0.31 mmol, 1.2 eq.) was added to a solution of **169** (50 mg, 0.26 mmol, 1.0 eq.) in dichloromethane (7.5 mL). The reaction mixture was stirred at room temperature for 16 h. The mixture was then quenched with saturated aqueous sodium bisulfite (3 mL) and saturated aqueous sodium bicarbonate (15 mL) solutions. The organic component was washed with saturated aqueous sodium bicarbonate solution (15 mL), brine (10 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (20–40% acetone in toluene) to yield the title compound **184** as a colourless oil (27 mg, 55%). R_f 0.43 (50% acetone in toluene); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3467 (w), 2912 (w), 1747 (s), 1448 (w), 1381 (w), 1210 (s), 1129 (s), 1028 (m), 852 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.74 (1H, t, *J* 0.9, H-1), 4.22 (2H, q, *J* 7.2, H-7), 4.18 (2H, d, *J* 0.9, H-2), 4.15 (2H, s, H-5), 3.79

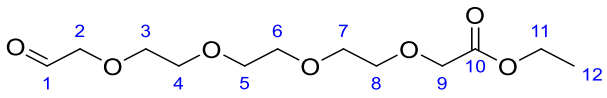
(4H, s, H-3 and H-4), 1.29 (3H, t, *J* 7.2, H-8); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 200.7 (C-1), 170.4 (C-6), 76.9 (C-2), 71.3, 71.1 (C-3 and C-4), 68.8 (C-5), 61.1 (C-7), 14.3 (C-8); *m/z* (ESI$^+$) 213 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_8H_{15}O_5^+$ ([M+H]$^+$) requires 191.09140, found 191.09161.

Ethyl {2-[2-(2-oxoethoxy)ethoxy]ethoxy}acetate (185)



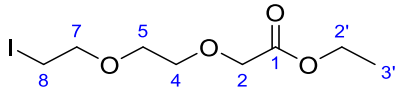
Dess Martin Periodinane (215 mg, 0.508 mmol, 1.2 eq.) was added to a solution of **170** (100 mg, 0.423 mmol, 1.0 eq.) in dichloromethane (14 mL). The reaction mixture was stirred at room temperature for 16 h. The mixture was then quenched with saturated aqueous sodium bisulfite (6 mL) and saturated aqueous sodium bicarbonate (30 mL) solutions. The organic component was washed with saturated aqueous sodium bicarbonate solution (30 mL), brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (25–40% acetone in toluene) to yield the title compound **185** as a colourless oil (51.4 mg, 52%). R_f 0.54 (50% acetone in toluene); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3481 (w), 2912 (w), 1748 (s), 1212 (m), 1117 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.66 (1H, t, *J* 0.8, H-1), 4.14 (2H, q, *J* 7.2, H-9), 4.11 – 4.04 (4H, m, H-2 and H-7), 3.71 – 3.56 (8H, m, H-3, H-4, H-5 and H-6), 1.22 (3H, t, *J* 7.1, H-10); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 201.0 (C-1), 170.5 (C-8), 76.9 (C-2), 71.3, 70.9, 70.8, 70.7 (C-3, C-4, C-5 and C-6), 68.8 (C-7), 60.9 (C-9), 14.3 (C-10); *m/z* (ESI$^+$) 257 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{10}H_{19}O_6^+$ ([M+H]$^+$) requires 235.11761, found 235.11777.

Ethyl 14-oxo-2,4,6,8-tetraoxatetradecan-10-oate (186)



Dess Martin Periodinane (182 mg, 0.428 mmol, 1.2 eq.) was added to a solution of **171** (100 mg, 0.357 mmol, 1.0 eq.) in dichloromethane (12 mL). The reaction mixture was stirred at room temperature for 16 h. The mixture was then quenched with saturated aqueous sodium bisulfite (6 mL) and saturated aqueous sodium bicarbonate (30 mL) solutions. The organic component was washed with saturated aqueous sodium bicarbonate solution (30 mL), brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (20–40% acetone in toluene) to yield the title compound **186** as a colourless oil (41.9 mg, 42%). R_f 0.32 (50% acetone in toluene); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3488 (w), 2875 (w), 1749 (s), 1638 (w), 1457 (w), 1380 (w), 1351 (w), 1288 (w), 1211 (m), 1117 (s), 1032 (m), 944 (w), 852 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.71 (1H, s, H-1), 4.19 (2H, q, *J* 7.1, H-11), 4.15 – 4.10 (4H, m, H-2 and H-9), 3.74 – 3.60 (12H, m, H-3, H-4, H-5, H-6, H-7 and H-8), 1.26 (3H, t, *J* 7.2, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 201.1 (C-1), 170.5 (C-10), 77.0 (C-2), 71.3, 71.0, 70.9, 70.73, 70.72, 70.66 (C-3, C-4, C-5, C-6, C-7 and C-8), 68.8 (C-9), 60.9 (C-11), 14.3 (C-12); *m/z* (ESI$^+$) 301 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{12}H_{23}O_7^+$ ([M+H]$^+$) requires 279.14383, found 279.14397.

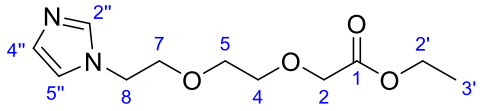
Ethyl [2-(2-iodoethoxy)ethoxy]acetate (187)



Triphenylphosphine (164 mg, 0.624 mmol, 1.2 eq.), imidazole (106 mg, 1.56 mmol, 3.0 eq.), and iodine (172 mg, 0.676 mmol, 1.3 eq.) were added to a solution of **169** (100 mg, 0.520 mmol, 1.0 eq.) in toluene (3 mL). The solution was heated to 90 ºC and stirred for 2 h. The reaction mixture was then cooled to room temperature and concentrated *in vacuo*. The residue was dissolved in dichloromethane (20 mL) and washed with saturated aqueous sodium bicarbonate

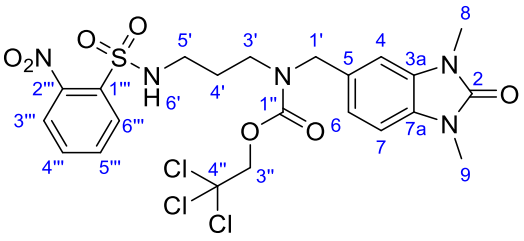
solution (20 mL), water (20 mL) and brine (20 mL). The organic components were dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–3% methanol in dichloromethane, then 20–30% diethyl ether in petroleum ether) to yield the title compound **187** as a colourless oil (79.2 mg, 50%). R_f 0.19 (30% diethyl ether in petroleum ether); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2872 (w), 2360 (w), 1751 (s), 1463 (w), 1376 (w), 1266 (m), 1204 (s), 1145 (s), 1121 (s), 1031 (m), 850 (w), 721 (w), 618 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 4.22 (2H, q, *J* 7.2, H-2'), 4.16 (2H, s, H-2), 3.81 – 3.67 (6H, m, H-7, H-5 and H-4), 3.27 (2H, t, *J* 6.9, H-8), 1.29 (3H, t, *J* 7.2, H-3'); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 170.6 (C-1), 72.1 (C-7), 71.0 (C-5), 70.4 (C-4), 68.9 (C-2), 61.0 (C-2'), 14.4 (C-3'), 2.9 (C-8); *m/z* (ESI$^+$) 325 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_8H_{16}O_4I^+$ ([M+H]$^+$) requires 303.00878, found 303.00876.

Ethyl {2-[2-(1*H*-imidazol-1-yl)ethoxy]ethoxy}acetate (188)



188 was also a product of the above reaction as a colourless oil (29.4 mg, 13%). R_f 0.23 (5% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3587 (w), 3114 (w), 2874 (w), 1747 (s), 1510 (m), 1445 (w), 1379 (w), 1286 (m), 1208 (s), 1146 (s), 1109 (s), 1080 (s), 1029 (m), 916 (m), 824 (m), 747 (m), 667 (m), 626 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.52 (1H, s, H-2''), 7.03 (1H, s, H-5''), 6.98 (1H, s, H-4''), 4.20 (2H, q, *J* 7.1, H-2'), 4.13 – 4.07 (4H, m, H-2 and H-8), 3.74 (2H, t, *J* 5.4, H-7), 3.71 – 3.57 (4H, m, H-4 and H-5), 1.27 (3H, t, *J* 7.1, H-3'); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.4 (C-1), 137.7 (C-2''), 129.4 (C-5''), 119.5 (C-4''), 71.0 (C-7), 70.8 (C-5), 70.7 (C-4), 68.8 (C-2), 61.0 (C-2'), 47.1 (C-8), 14.3 (C-3'); *m/z* (ESI$^+$) 243 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{11}H_{19}O_4N_2^+$ ([M+H]$^+$) requires 243.13503, found 243.13423.

4’’,4’’,4’’-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{2’-[(2’’’-nitrobenzene-1’’’-sulfonyl)amino]propyl}carbamate (191)

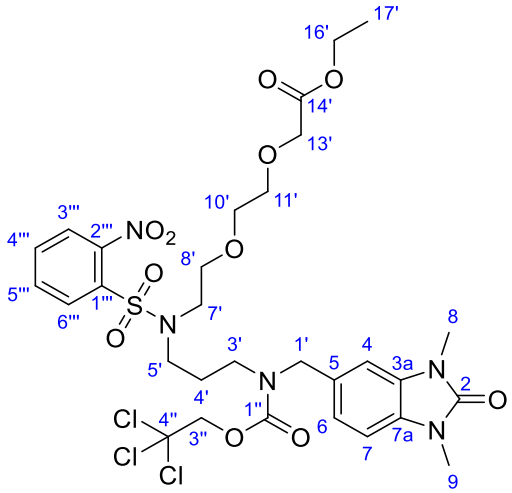


163 (700 mg, 1.34 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (5 mL) and stirred at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo*. The crude residue was passed through an Isolute® SXC-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the deprotected amine product 3’’,3’’,3’’-trichloroethyl (3’-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (547 mg, 97%). This compound was then used directly in the next step to avoid Troc protecting group migration to the terminal primary amine.

3’’,3’’,3’’-Trichloroethyl (3’-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (547 mg, 1.29 mmol, 1.0 eq.) was dissolved in anhydrous dichloromethane (10 mL) and triethylamine (360 µL, 2.58 mmol, 2.0 eq.) was added. A solution of 2-nitrophenylsulfonyl chloride (300 mg, 1.36 mmol, 1.05 eq.) in anhydrous dichloromethane (5 mL) was added dropwise to the solution at 0 ºC. The resulting mixture was stirred at room temperature for 16 h. The solution was then washed with water (40 mL) and the organic component was dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–3% methanol in dichloromethane) to yield the title compound **191** as a yellow solid (592 mg, 75%). R_f 0.44 (6% methanol in dichloromethane); m.p. 65–67 ºC (from MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2845 (w), 2392 (w), 1699 (s), 1541 (m), 1509 (m), 1443 (m), 1342 (m), 1215 (w), 1165 (m), 1126 (m), 729 (m); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 8.02 – 7.93 (1H, m, H-3’’’), 7.91 – 7.86 (1H, m, H-6’’’), 7.86 – 7.79 (2H, m, H-4’’’ and H-5’’’), 7.69 (1H, s, H-6’), 7.09 – 6.97 (3H, m, H-4, H-6 and H-7), 4.87 (2H, s, H-3’’), 4.51

(2H, s, H-1'), 3.35 – 3.26 (8H, m, H-8, H-9 and H-3'), 2.94 (2H, t, *J* 7.1, H-5'), 1.74 (2H, p, *J* 7.1, H-4'); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 153.7 (C-2), 153.5 (C-1''), 147.4 (C-2'''), 133.4 (C-4'''), 132.7 (C-1'''), 131.9 (C-5'''), 129.8 (C-3a), 129.7 (C-7a), 129.1 (C-3'''), 128.9 (C-5), 123.8 (C-6'''), 120.0 (C-6), 106.9, 106.4 (C-4 and C-7), 95.7 (C-4''), 74.1 (C-3''), 50.1 (C-1'), 43.9 (C-3'), 40.2 (C-5'), 27.5 (C-4'), 26.43, 26.38 (C-8 and C-9); *m/z* (ESI$^+$) 632 ([M+Na]$^+$, 100%), 634 ([M+Na]$^+$, 20%); HRMS *m/z* (ESI$^+$) $C_{22}H_{25}O_7N_5Cl_3S^+$ ([M+H]$^+$) requires 608.05348 and 610.05053, found 608.05334 and 610.05023; HPLC (method 1) retention time 9.9 min, 95.1%.

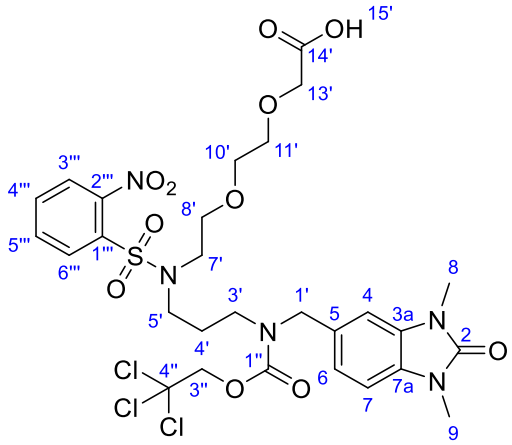
Ethyl 4'',4'',4''-trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6'-(2'''-nitrobenzene-1'''-sulfonyl)-1''-oxo-2'',9',12'-trioxa-2',6'-diazaheptadecan-14'-oate



191 (70.0 mg, 0.115 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (1 mL). The solution was cooled to 0 ºC, and **169** (92.6 mg, 0.287 mmol, 2.5 eq.), triphenylphosphine (75.4 mg, 0.287 mmol, 2.5 eq.) and diisopropyl azodicarboxylate (56.6 µL, 0.287 mmol, 2.5 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The resulting mixture was subsequently taken up in ethyl acetate (20 mL), washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL). The organic component was dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–20% acetone in toluene) and the title compound was isolated as an

inseparable mixture with triphenylphosphine oxide. Triphenylphosphine oxide could be removed after the subsequent ester hydrolysis step *via* silica gel column chromatography. R_f 0.58 (50% acetone in toluene); *m/z* (ESI$^+$) 805 ([M+Na]$^+$, 20%), 807 ([M+Na]$^+$, 50%).

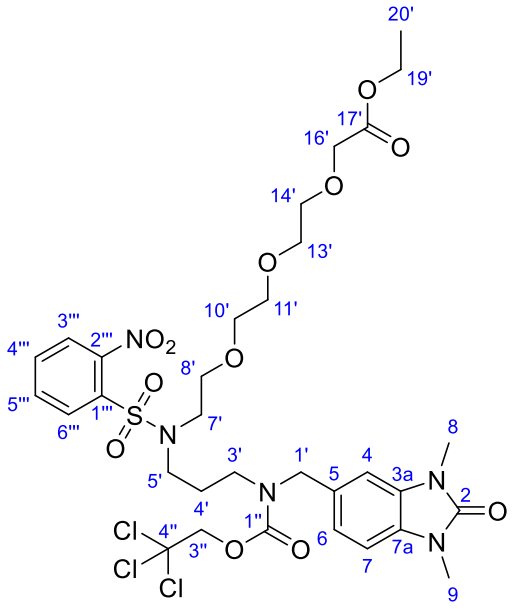
4’’,4’’,4’’-Trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6’-(2’’’-nitrobenzene-1’’’-sulfonyl)-1’’-oxo-2’’,9’,12’-trioxa-2’,6’-diazaheptadecan-14’-oic acid (192)



Ethyl 4’’,4’’,4’’-trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6’-(2’’’-nitrobenzene-1’’’-sulfonyl)-1’’-oxo-2’’,9’,12’-trioxa-2’,6’-diazaheptadecan-14’-oate (1.05 g, 1.34 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (10 mL) and methanol (10 mL). A solution of lithium hydroxide (320 mg, 13.4 mmol, 10.0 eq.) in water (10 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 1 h. The solution was then acidified with 1 M aqueous hydrochloric acid (10 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic components were washed with brine (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% AcOH, 0–3% methanol in dichloromethane) to yield the title compound **192** as a colourless oil (446 mg, 71% over two steps). R_f 0.27 (1% AcOH, 4% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2938 (w), 2359 (w), 2341 (w), 1706 (s), 1543 (m), 1509 (w), 1468 (w), 1440 (w), 1397 (w), 1351 (w), 1302 (w), 1268 (w), 1225 (w), 1160 (m), 1143 (m), 1124 (m), 1060 (w), 1026 (w), 852 (w), 773 (w), 746 (w), 718 (w), 680 (w); ^{1}H NMR (500 MHz,

DMSO-*d*$_6$, 363 K) δ_H 7.96 (1H, d, *J* 7.6, H-3’’’), 7.90 – 7.76 (3H, m, H-4’’’, H-5’’’ and H-6’’’), 7.09 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3’’), 4.53 (2H, s, H-1’), 3.97 (2H, s, H-13’), 3.59 – 3.46 (6H, m, H-8’, H-10’ and H-11’), 3.41 (2H, t, *J* 5.7, H-7’), 3.34 – 3.23 (10H, m, H-8, H-9, H-5’ and H-3’), 1.82 (2H, p, *J* 7.4, H-4’); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 170.7 (C-14’), 153.7 (C-2), 153.5 (C-1’’), 147.4 (C-2’’’), 133.7 (C-5’’’), 131.8 (C-1’’’), 131.7 (C-4’’’), 129.8 (C-3a), 129.7 (C-7a), 129.2 (C-3’’’), 129.0 (C-5), 123.7 (C-6’’’), 120.0 (C-6), 106.9 (C-4), 106.5 (C-7), 95.7 (C-4’’), 74.1 (C-3’’), 69.5, 69.2, 68.1 (C-8’, C-10’ and C-11’), 67.5 (C-13’), 50.1 (C-1’), 46.5 (C-7’), 45.6 (C-5’), 43.9 (C-3’), 26.43, 26.38 (C-8 and C-9), 25.9 (C-4’); *m/z* (ESI$^+$) 776 ([M+Na]$^+$, 80%), 778 ([M+Na]$^+$, 20%); HRMS *m/z* (ESI$^+$) $C_{28}H_{35}O_{11}N_5Cl_3S^+$ ([M+H]$^+$) requires 754.11139 and 756.10844, found 754.11195 and 756.10894; HPLC (method 1) retention time 9.3 min, 100.0%.

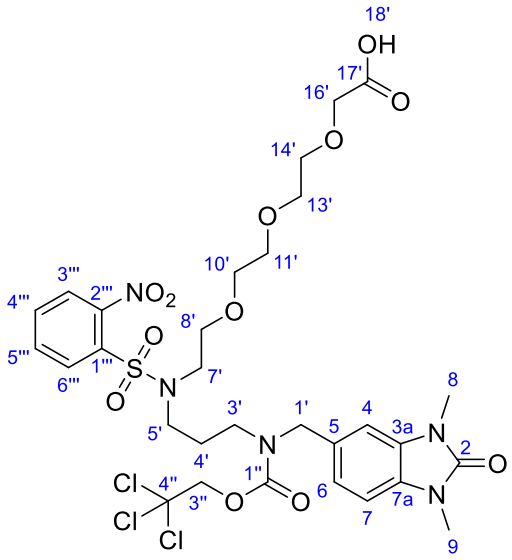
Ethyl 4’’,4’’,4’’-trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6’-(2’’’-nitrobenzene-1’’’-sulfonyl)-1’’-oxo-2’’,9’,12’,15’-tetraoxa-2’,6’-diazaicosan-17’-oate



191 (70.0 mg, 0.115 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (1 mL). The solution was cooled to 0 ºC, and **170** (67.8 mg, 0.287 mmol, 2.5 eq.), triphenylphosphine (75.4 mg, 0.287 mmol, 2.5 eq.) and diisopropyl azodicarboxylate (56.6 µL, 0.287 mmol, 2.5 eq.) were

added. The reaction mixture was stirred at room temperature for 16 h. The resulting mixture was subsequently taken up in ethyl acetate (20 mL), washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL). The organic component was dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–30% acetone in toluene) to yield the title compound as a colourless oil (51.8 mg, 58%). R_f 0.57 (50% acetone in toluene); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2919 (w), 2359 (w), 2341 (w), 1750 (w), 1704 (s), 1544 (m), 1509 (w), 1468 (w), 1441 (w), 1350 (w), 1299 (w), 1267 (w), 1221 (w), 1122 (m), 1060 (w), 1028 (w), 852 (w), 773 (w), 746 (w), 718 (w), 682 (w), 651 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 7.96 (1H, d, *J* 7.7, H-3’’’), 7.90 – 7.76 (3H, m, H-4’’’, H-5’’’ and H-6’’’), 7.10 – 6.97 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3’’), 4.53 (2H, s, H-1’), 4.13 (2H, q, *J* 7.2, H-19’), 4.07 (2H, s, H-16’), 3.64 – 3.37 (12H, m, H-7’, H-8’, H-10’, H-11’, H-13’ and H-14’), 3.36 – 3.22 (10H, m, H-8, H-9, H-3’ and H-5’), 1.82 (2H, p, *J* 7.6, H-4’), 1.21 (3H, t, *J* 7.2, H-20’); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 169.4 (C-17’), 153.7 (C-2), 153.5 (C-1’’), 147.4 (C-2’’’), 133.7 (C-5’’’), 131.8 (C-1’’’), 131.7 (C-4’’’), 129.8 (C-3a), 129.7 (C-7a), 129.2 (C-3’’’), 129.0 (C-5), 123.7 (C-6’’’), 120.1 (C-6), 106.9 (C-4), 106.5 (C-7), 95.7 (C-4’’), 74.1 (C-3’’), 69.8, 69.32, 69.31, 69.2, 68.2 (C-8’, C-10’, C-11’, C-13’ and C-14’), 67.6 (C-16’), 59.5 (C-19’), 50.1 (C-1’), 46.5 (C-7’), 45.6 (C-5’), 43.9 (C-3’), 26.43, 26.37 (C-8 and C-9), 26.1 (C-4’), 13.5 (C-20’); *m/z* (ESI$^+$) 848 ([M+Na]$^+$, 80%), 850 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{32}H_{43}O_{12}N_5Cl_3S^+$ ([M+H]$^+$) requires 826.16890 and 828.16595, found 826.16976 and 828.16706; HPLC (method 1) retention time 10.1 min, 94.8% (impurity is small amount of triphenylphosphine oxide).

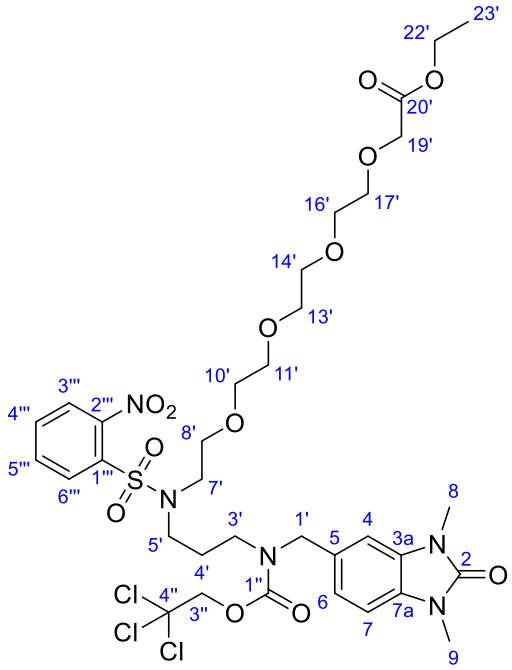
4’’,4’’,4’’-Trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6’-(2’’’-nitrobenzene-1’’’-sulfonyl)-1’’-oxo-2’’,9’,12’,15’-tetraoxa-2’,6’-diazaicosan-17’-oic acid (193)



Ethyl 4’’,4’’,4’’-trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl]-6’-(2’’’-nitrobenzene-1’’’-sulfonyl)-1’’-oxo-2’’,9’,12’,15’-tetraoxa-2’,6’-diazaicosan-17’-oate (50.0 mg, 0.0605 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (0.25 mL) and methanol (0.25 mL). A solution of lithium hydroxide (14.5 mg, 0.605 mmol, 10.0 eq.) in water (0.25 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 1 h. The solution was then acidified with 1 M aqueous hydrochloric acid (1 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic components were washed with brine (10 mL), dried over sodium sulfate and concentrated *in vacuo* to yield the title compound **193** as a colourless oil (41.8 mg, 97%). R_f 0.41 (1% AcOH, 25% ethanol in ethyl acetate); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2942 (w), 2878 (w), 1702 (s), 1543 (m), 1509 (w), 1468 (w), 1441 (w), 1430 (w), 1396 (w), 1350 (w), 1301 (w), 1268 (w), 1223 (m), 1122 (s), 1059 (w), 1025 (w), 998 (w), 959 (w), 852 (w), 836 (w), 817 (w), 748 (s), 717 (m), 681 (w), 666 (w), 652 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.96 (1H, dd, *J* 7.7, 1.5, H-3’’’), 7.90 – 7.76 (3H, m, H-4’’’, H-5’’’ and H-6’’’), 7.09 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3’’), 4.53 (2H, s, H-1’), 3.99 (2H, s, H-16’), 3.64 – 3.37 (12H, m, H-7’, H-8’, H-10’, H-11’, H-13’ and H-14’), 3.36 – 3.22 (10H, m, H-8, H-9, H-3’ and H-5’),

1.82 (2H, p, *J* 7.6, H-4'); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 170.7 (C-17'), 153.7 (C-2), 153.5 (C-1''), 147.4 (C-2'''), 133.7 (C-5'''), 131.8 (C-1'''), 131.7 (C-4'''), 129.8 (C-3a), 129.7 (C-7a), 129.2 (C-3'''), 129.0 (C-5), 123.7 (C-6'''), 120.1 (C-6), 106.9 (C-4), 106.5 (C-7), 95.7 (C-4''), 74.1 (C-3''), 69.6, 69.3, 69.2, 68.1, 67.5 (C-8', C-10', C-11', C-13' and C-14'), 50.1 (C-1'), 46.5 (C-7'), 45.6 (C-5'), 43.9 (C-3'), 26.42, 26.37 (C-8 and C-9), 26.1 (C-4'); *m/z* (ESI$^+$) 820 ([M+Na]$^+$, 80%), 822 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{30}H_{39}O_{12}N_5Cl_3S^+$ ([M+H]$^+$) requires 798.13760 and 800.13465, found 798.13780 and 800.13514; HPLC (method 2) retention time 0.65 min, 100%.

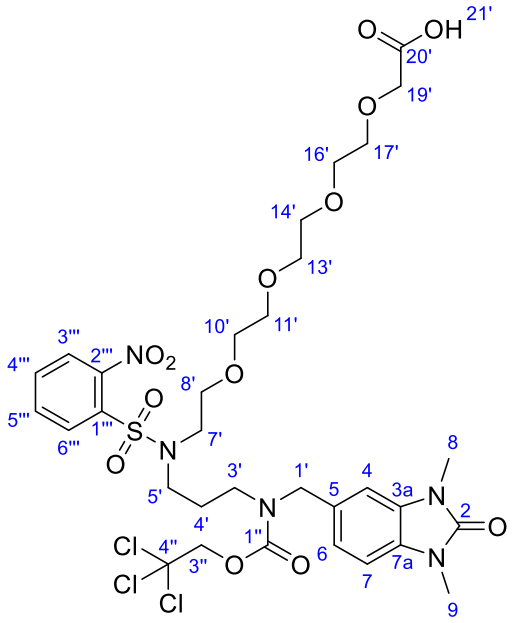
Ethyl 4'',4'',4''-trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6'-(2'''-nitrobenzene-1'''-sulfonyl)-1''-oxo-2'',9',12',15',18'-pentaoxa-2',6'-diazatricosan-20'-oate



191 (304 mg, 0.499 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (5 mL). The solution was cooled to 0 ºC, and **171** (350 mg, 1.25 mmol, 2.5 eq.), triphenylphosphine (328 mg, 1.25 mmol, 2.5 eq.) and diisopropyl azodicarboxylate (246 µL, 1.25 mmol, 2.5 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The resulting mixture was subsequently taken up in ethyl acetate (40 mL), washed with saturated aqueous sodium bicarbonate solution (30 mL)

and brine (30 mL). The organic component was dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–100% [25% ethanol in ethyl acetate] in heptane) to yield the title compound as an inseparable mixture with triphenylphosphine oxide. Triphenylphosphine oxide could be removed after the subsequent ester hydrolysis step *via* silica gel column chromatography. R_f 0.81 (25% ethanol in ethyl acetate); *m/z* (ESI$^+$) 892 ([M+Na]$^+$, 80%), 894 ([M+Na]$^+$, 30%).

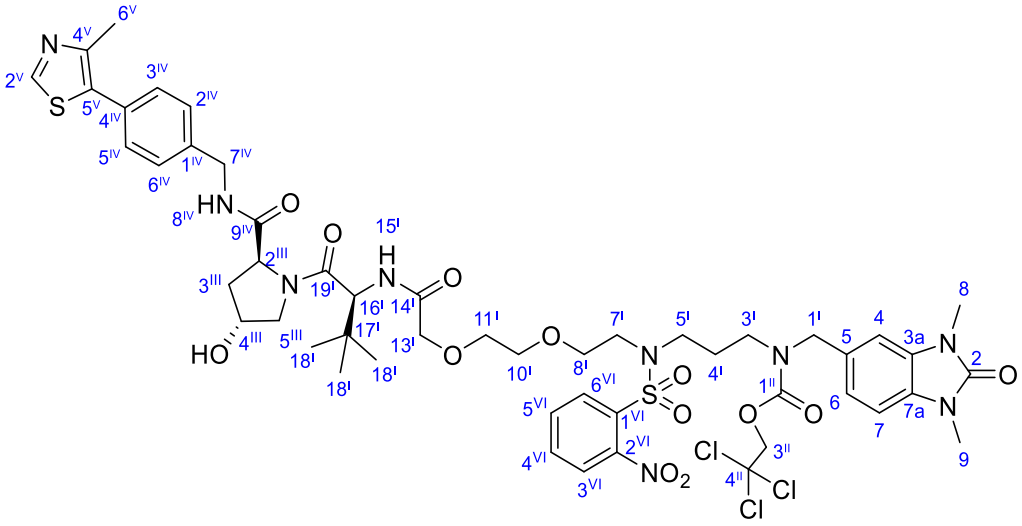
4’’,4’’,4’’-Trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6’-(2’’’-nitrobenzene-1’’’-sulfonyl)-1’’-oxo-2’’,9’,12’,15’,18’-pentaoxa-2’,6’-diazatricosan-20’-oic acid (194)



Ethyl 4’’,4’’,4’’-trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl]-6’-(2’’’-nitrobenzene-1’’’-sulfonyl)-1’’-oxo-2’’,9’,12’,15’,18’-pentaoxa-2’,6’-diazatricosan-20’-oate (370 mg, 0.425 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (2.7 mL) and methanol (2.7 mL). A solution of lithium hydroxide (102 mg, 4.25 mmol, 10.0 eq.) in water (2.7 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 1 h. The solution was then acidified with 1 M aqueous hydrochloric acid (10 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column

chromatography (1% AcOH, 30–100% [25% ethanol in ethyl acetate] in heptane) to yield the title compound **194** as a colourless oil (218 mg, 52% over two steps). R_f 0.09 (1% AcOH, 25% ethanol in ethyl acetate); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3013 (w), 2878 (w), 1702 (s), 1544 (w), 1509 (w), 1468 (w), 1441 (w), 1429 (w), 1397 (w), 1351 (w), 1300 (w), 1268 (w), 1221 (w), 1122 (s), 1060 (w), 1026 (w), 999 (w), 949 (w), 852 (w), 816 (w), 746 (s), 666 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 7.96 (1H, dd, *J* 7.8, 1.4, H-3’’’), 7.88 – 7.76 (3H, m, H-4’’’, H-5’’’ and H-6’’’), 7.07 – 6.99 (3H, m, H-4, H-6 and H-7), 4.88 (2H, s, H-3’’), 4.53 (2H, s, H-1’), 4.00 (2H, s, H-19’), 3.64 – 3.43 (16H, m, H-7’, H-8’, H-10’, H-11’, H-13’, H-14’, H-16’ and H-17’), 3.40 (2H, t, *J* 5.7, H-5’), 3.35 – 3.23 (8H, m, H-8, H-9 and H-3’), 1.82 (2H, p, *J* 7.4, H-4’); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 170.7 (C-20’), 153.7 (C-2), 153.5 (C-1’’), 147.4 (C-2’’’), 133.7 (C-5’’’), 131.8 (C-1’’’), 131.7 (C-4’’’), 129.8 (C-3a), 129.7 (C-7a), 129.2 (C-3’’’), 129.0 (C-5), 123.7 (C-6’’’), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.7 (C-4’’), 74.1 (C-3’’), 69.6, 69.39, 69.37, 69.32, 69.2, 68.1, 67.5 (C-8’, C-10’, C-11’, C-13’, C-14’, C-16’ and C-17’), 50.1 (C-1’), 46.5 (C-7’), 45.6 (C-5’), 43.9 (C-3’), 26.4, 26.3 (C-8 and C-9), 26.1 (C-4’); *m/z* (ESI$^+$) 843 ([M+H]$^+$, 50%), 845 ([M+H]$^+$, 30%), 865 ([M+Na]$^+$, 90%), 867 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{32}H_{43}O_{13}N_5Cl_3S^+$ ([M+H]$^+$) requires 842.16382 and 844.16146, found 842.16368 and 844.16106; HPLC (method 2) retention time 0.66 min, 100%.

17^{I}-Methyl-*N*-{4^{II},4^{II},4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6^{I}-(2^{VI}-nitrobenzene-1^{VI}-sulfonyl)-14^{I}-oxo-2^{II},9^{I},12^{I}-trioxa-2^{I},6^{I}-diazaheptadecanan-14^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (195)

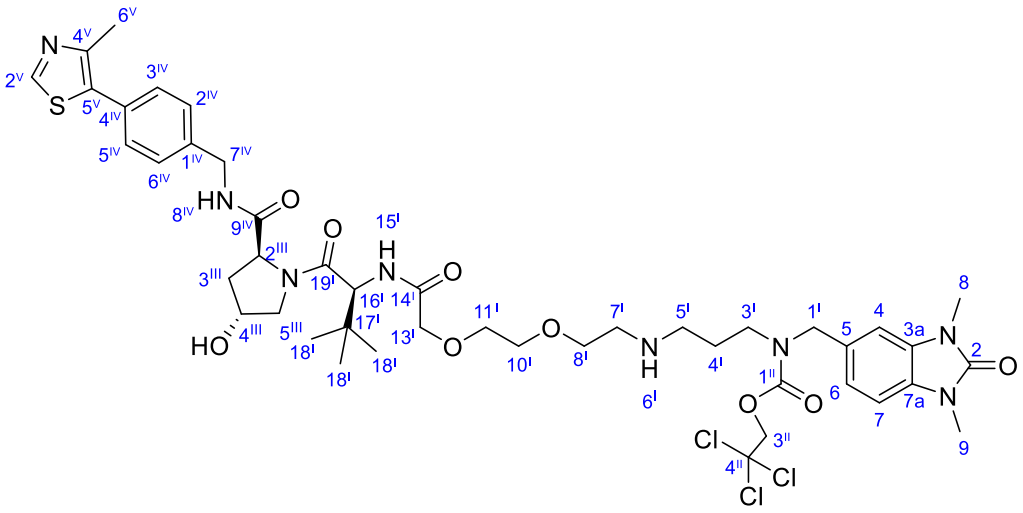


158 (220 mg, 0.415 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (2 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo*. The crude product was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the free amine as a yellow oil (162 mg, 91%). The product was then used immediately in the next step.

192 (109 mg, 0.145 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (101 µL, 0.580 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL). HATU (60.6 mg, 0.160 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The product from the above Boc deprotection (67.8 mg, 0.145 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with water (20 mL) and extracted with ethyl acetate (4 × 40 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 2–4% methanol in

dichloromethane) to yield the title compound **195** as a colourless oil (113 mg, 67%). R_f 0.25 (1% ammonium hydroxide, 6% methanol in dichloromethane); $[\alpha]_D^{25} = -11.7$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3357 (m), 2918 (w), 2616 (w), 2498 (w), 2359 (w), 2342 (w), 2186 (w), 2045 (w), 1703 (s), 1636 (s), 1543 (s), 1509 (m), 1469 (m), 1440 (m), 1397 (w), 1343 (w), 1269 (w), 1225 (w), 1196 (w), 1161 (w), 1123 (m), 1060 (w), 1032 (w), 851 (w), 800 (w), 772 (w), 746 (w), 720 (w), 668 (w), 658 (w), 627 (w), 615 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.90 (1H, s, H-2^V), 8.15 (1H, t, *J* 6.1, H-8^{IV}), 7.95 (1H, dd, *J* 7.7, 1.5, H-6^{VI}), 7.89 – 7.74 (3H, m, H-3^{VI}, H-4^{VI} and H-5^{VI}), 7.44 – 7.35 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.20 (1H, d, *J* 9.5, H-15^I), 7.08 – 6.97 (3H, m, H-4, H-6 and H-7), 4.88 (2H, s, H-3^{II}), 4.84 (1H, s, O*H*), 4.57 (1H, d, *J* 9.5, H-16^I), 4.55 – 4.48 (3H, m, H-1^I and H-2^{III}), 4.42 – 4.27 (3H, m, H-4^{III} and H-7^{IV}), 3.98 – 3.88 (2H, m, H-13^I), 3.72 (1H, dd, *J* 10.7, 4.5, H-5^{IIIa}), 3.63 (1H, d, *J* 10.7, H-5^{IIIb}), 3.59 – 3.46 (6H, m, H-8^I, H-10^I and H-11^I), 3.46 – 3.39 (2H, m, H-7^I), 3.35 – 3.22 (10H, m, H-8, H-9, H-3^I and H-5^I), 2.44 (3H, s, H-6^V), 2.10 – 1.97 (2H, m, H-3^{III}), 1.82 (2H, p, *J* 7.5, H-4^I), 0.95 (9H, s, H-18^I); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.0 (C-9^{IV}), 169.0 (C-19^I), 168.0 (C-14^I), 153.7 (C-1^{II}), 153.5 (C-2), 150.5 (C-2^V), 147.41 (C-2^{VI}), 147.35 (C-4^V), 138.9 (C-1^{IV}), 133.7 (C-5^{VI}), 131.8 (C-5^V), 131.7 (C-4^{VI}), 130.7 (C-3a), 129.8 (C-4^{IV}), 129.7 (C-5), 129.5 (C-7a), 129.2 (C-6^{VI}), 129.0 (C-1^{VI}), 128.2, 127.2 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 123.7 (C-3^{VI}), 120.1 (C-6), 106.9 (C-4), 106.5 (C-7), 95.7 (C-4^{II}), 74.1 (C-3^{II}), 69.9 (C-8^I, C-10^I or C-11^I), 69.5 (C-13^I), 69.1 (C-8^I, C-10^I or C-11^I), 68.5 (C-4^{III}), 68.2 (C-8^I, C-10^I or C-11^I), 58.4 (C-2^{III}), 55.9 (C-5^{III}), 55.5 (C-16^I), 50.1 (C-1^I), 46.4 (C-7^I), 45.6 (C-3^I), 43.9 (C-5^I), 41.5 (C-7^{IV}), 37.3 (C-3^{III}), 35.0 (C-17^I), 26.42, 26.37 (C-8 and C-9), 26.1 (C-4^I), 25.8 (C-18^I), 15.3 (C-6^V); *m/z* (ESI$^+$) 1166 ([M+H]$^+$, 70%), 1168 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{50}H_{63}O_{13}N_9Cl_3S_2^+$ ([M+H]$^+$) requires 1166.30468 and 1168.30386, found 1166.30527 and 1168.30386; HPLC (method 1) retention time 9.2 min, 97.7%.

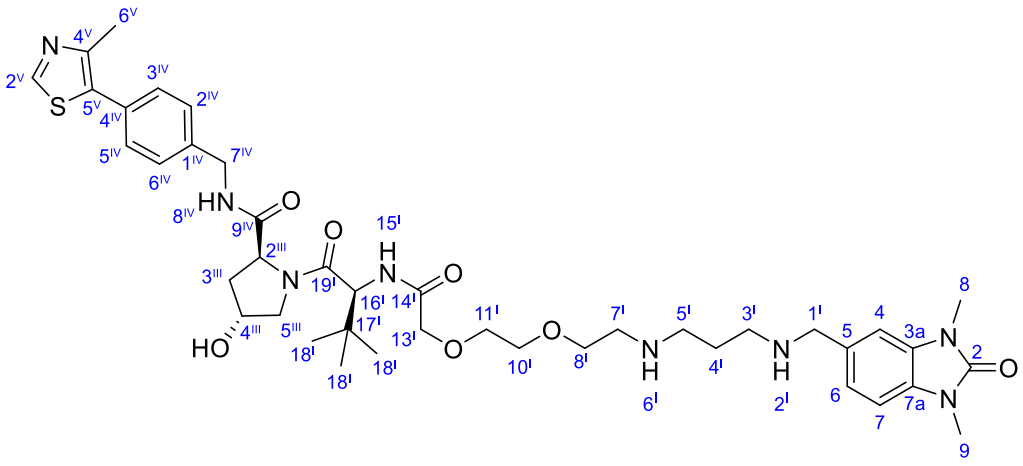
17^{I}-Methyl-*N*-{4^{II},4^{II},4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-14^{I}-oxo-2^{II},9^{I},12^{I}-trioxa-2^{I},6^{I}-diazaheptadecanan-14^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (198)



Thioglycolic acid (12 µL, 0.18 mmol, 2.05 eq.) was added to a solution of **195** (100 mg, 0.086 mmol, 1.0 eq.) and lithium hydroxide (8.2 mg, 0.34 mmol, 4.1 eq.) in degassed anhydrous *N*,*N*-dimethylformamide (2 mL). The resulting mixture was stirred at room temperature for 5 h. Water (10 mL) was then added, and the aqueous solution was extracted with 25% methanol in dichloromethane (3 × 20 mL). The combined organic components were washed with saturated aqueous sodium bicarbonate solution (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 2–6% methanol in dichloromethane) to yield the title compound **198** as a colourless oil (30 mg, 36%). R_f 0.48 (1% triethylamine, 6% methanol in dichloromethane); $[\alpha]_D^{25} = -18.9$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3304 (w), 3067 (w), 2938 (w), 2871 (w), 2361 (w), 1700 (s), 1637 (m), 1509 (m), 1466 (m), 1440 (m), 1398 (w), 1367 (w), 1341 (w), 1303 (w), 1270 (w), 1227 (m), 1117 (m), 1057 (w), 1029 (w), 847 (w), 816 (w), 771 (w), 745 (w), 716 (w), 678 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 8.90 (1H, s, H-2^{V}), 8.18 (1H, t, *J* 6.0, H-8^{IV}), 7.44 – 7.37 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.23 (1H, d, *J* 9.5, H-15^{I}), 7.08 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.61 – 4.46 (4H, m, H-1^{I}, H-16^{I} and H-2^{III}), 4.42 – 4.24 (3H, m, H-4^{III} and H-7^{IV}), 4.00 – 3.89 (2H, m, H-13^{I}), 3.72 (1H, dd, *J* 10.6, 4.5, H-5^{IIIa}), 3.66 – 3.60

(3H, m, H-5^{IIIb} and H-10^{I} or H-11^{I}), 3.59 – 3.54 (2H, m, H-10^{I} or H-11^{I}), 3.48 (2H, td, *J* 5.7, 2.0, H-7^{I}), 3.35 – 3.27 (8H, m, H-8, H-9 and H-3^{I}), 2.66 (2H, t, *J* 5.7, H-8^{I}), 2.54 (2H, t, *J* 6.9, H-5^{I}), 2.45 (3H, s, H-6^{V}), 2.11 – 1.95 (2H, m, H-3^{III}), 1.68 (2H, p, *J* 7.0, H-4^{I}), 0.96 (9H, s, H-18^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.1 (C-9^{IV}), 169.0 (C-19^{I}), 168.1 (C-14^{I}), 153.7 (C-1^{II}), 153.5 (C-2), 150.5 (C-2^{V}), 147.4 (C-4^{V}), 138.9 (C-1^{IV}), 130.7 (C-5^{V}), 130.1 (C-3a), 129.7 (C-4^{IV}), 129.5 (C-5), 128.9 (C-7a), 128.2, 127.2 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.8 (C-4^{II}), 74.1 (C-3^{II}), 70.1 (C-10^{I} or C-11^{I}), 69.7 (C-7^{I}), 69.5 (C-13^{I}), 69.1 (C-10^{I} or C-11^{I}), 68.5 (C-4^{III}), 58.4 (C-2^{III}), 55.9 (C-5^{III}), 55.5 (C-16^{I}), 50.1 (C-1^{I}), 48.1 (C-8^{I}), 46.0 (C-5^{I}), 44.5 (C-3^{I}), 41.5 (C-7^{IV}), 37.3 (C-3^{III}), 35.0 (C-17^{I}), 26.41, 26.38 (C-8 and C-9), 25.9 (C-4^{I}), 25.8 (C-18^{I}), 15.3 (C-6^{V}); *m/z* (ESI$^+$) 981 ([M+H]$^+$, 70%), 983 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{44}H_{60}O_9N_8Cl_3S^+$ ([M+H]$^+$) requires 981.32641 and 983.32345, found 981.32636 and 983.32400; HPLC (method 1) retention time 2.82 min, 100%.

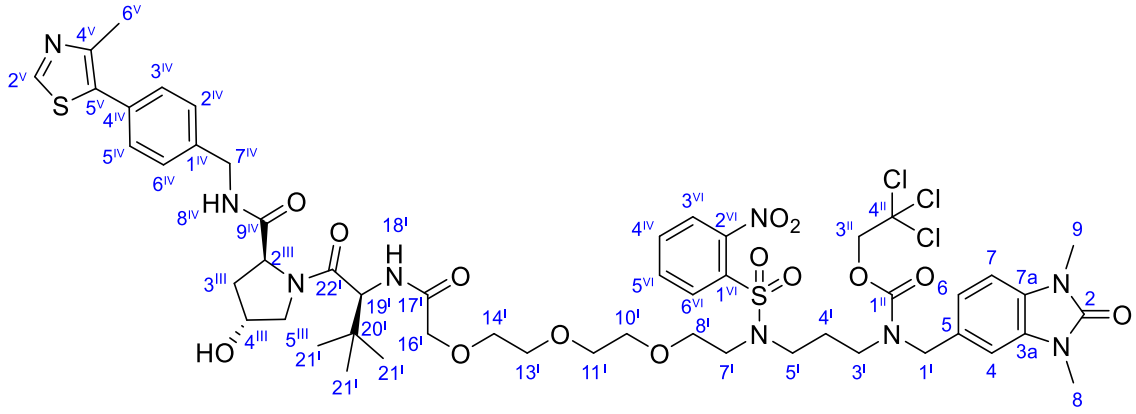
***N*-[2^{I}-(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-14^{I}-oxo-9^{I},12^{I}-dioxa-2^{I},6^{I}-diazatetradecan-14^{I}-yl]-17^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (139)**



198 (27 mg, 0.028 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.9 mL), and acetic acid (65 µL) and zinc powder (18 mg, 0.28 mmol, 10.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column

chromatography (1:2:6 ammonium hydroxide:ethanol:ethyl acetate) to yield the title compound **139** as a colourless oil (14 mg, 62%). R_f 0.17 (1:2:6 ammonium hydroxide:ethanol:ethyl acetate); $[\alpha]_D^{25} = -20.1$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3402 (w), 3292 (w), 3078 (w), 2921 (w), 2874 (w), 1686 (s), 1637 (s), 1509 (m), 1443 (m), 1397 (w), 1371 (w), 1342 (w), 1308 (w), 1268 (w), 1240 (w), 1195 (w), 1108 (m), 1030 (w), 1016 (w), 967 (w), 938 (w), 851 (w), 806 (w), 771 (m), 752 (m), 728 (w), 708 (w), 691 (w), 674 (w), 661 (w), 636 (w), 616 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.67 (1H, s, H-2^V), 7.43 – 7.30 (6H, m, H-2^{IV}, H-3^{IV}, H-5^{IV}, H-6^{IV}, H-8^{IV} and H-15^I), 7.06 – 6.97 (2H, m, H-4 and H-6), 6.89 (1H, d, *J* 7.9, H-7), 4.70 (1H, t, *J* 8.3, H-2^{III}), 4.65 (1H, d, *J* 9.5, H-16^I), 4.53 (1H, dd, *J* 15.0, 6.3, H-7^{IVa}), 4.46 (1H, bs, H-4^{III}), 4.40 (1H, dd, *J* 15.0, 5.5, H-7^{IVb}), 4.11 (1H, d, *J* 15.8, H-13^{Ia}), 4.02 (1H, d, *J* 11.3, H-11^{Ia}), 3.88 (1H, d, *J* 15.8, H-13^{Ib}), 3.82 (2H, s, H-1^I), 3.80 – 3.73 (1H, m, H-10^{Ia}), 3.70 – 3.54 (3H, m, H-5^{III} and H-11^{Ib}), 3.49 – 3.37 (9H, m, H-8, H-9, H-10^{Ib}, H-8^I or H-7^I), 2.76 – 2.62 (6H, m, H-3^I, H-5^I, H-8^I or H-7^I), 2.52 (3H, s, H-6^V), 2.42 (1H, ddd, *J* 13.1, 8.8, 4.1, H-3^{IIIa}), 2.20 – 2.11 (1H, m, H-3^{IIIb}), 1.84 – 1.70 (2H, m, H-4^I), 0.94 (9H, s, H-18^I); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.5 (C-9^{IV}), 171.1 (C-19^I), 170.8 (C-14^I), 155.0 (C-2), 150.4 (C-2^V), 148.7 (C-4^V), 138.3 (C-1^{IV}), 133.3 (C-3a), 131.7 (C-5^V), 131.1 (C-4^{IV}), 130.4 (C-5), 129.7 (C-2^{IV} and C-3^{IV} or C-5^{IV} and C-6^{IV}), 129.4 (C-7a), 128.3 (C-2^{IV} and C-3^{IV} or C-5^{IV} and C-6^{IV}), 121.4 (C-6), 107.6 (C-4), 107.2 (C-7), 71.7 (C-11^I), 71.6 (C-10^I), 71.4 (C-13^I), 70.4 (C-8^I or C-7^I), 69.8 (C-4^{III}), 58.9 (C-2^{III}), 57.2 (C-5^{III}), 56.7 (C-16^I), 54.3 (C-1^I), 49.2 (C-8^I or C-7^I), 48.4 (C-5^I), 47.8 (C-3^I), 43.4 (C-7^{IV}), 36.8 (C-3^{III}), 36.1 (C-17^I), 29.4 (C-4^I), 27.4 (C-8 and C-9), 26.5 (C-18^I), 16.2 (C-6^V); *m/z* (ESI$^+$) 807 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{41}H_{59}O_7N_8S^+$ ([M+H]$^+$) requires 807.42219, found 807.42197; HPLC (method 1) retention time 1.19 min, 100%.

20^{I}-Methyl-*N*-{4^{II},4^{II},4^{II} -trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6^{I}-(2^{VI}-nitrobenzene-1^{VI}-sulfonyl)-17^{I}-oxo-9^{I},12^{I},15^{I},2^{II}-tetraoxa-2^{I},6^{I}-diazaicosanan-17^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (196)

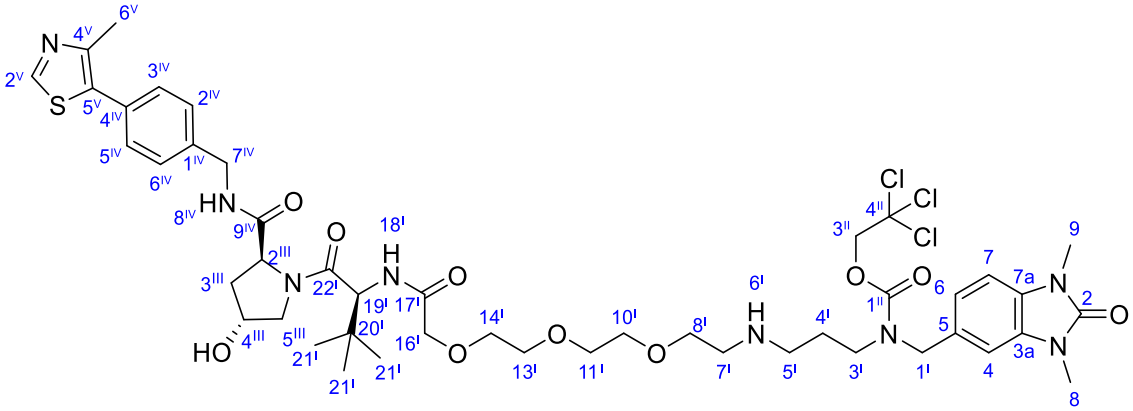


158 (660 mg, 1.24 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (4 mL) and dichloromethane (3 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo* to yield the hydrochloric acid salt of the amine as a yellow solid (579 mg, 94%). The product was then used immediately in the next step.

193 (150 mg, 0.188 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (131 µL, 0.751 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL). HATU (78.5 mg, 0.207 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The product from the above Boc deprotection (87.7 mg, 0.188 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with water (20 mL) and extracted with ethyl acetate (3 × 40 mL). The combined organic components were dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–10% methanol in dichloromethane) to yield the title compound **196** as a colourless oil (93.8 mg, 41%). R_f 0.27 (80% [25% ethanol in ethyl acetate] in heptane); $[\alpha]_D^{25}$= −11.7 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3402 (w), 3065 (w), 2952 (w), 2498 (w), 2363 (w), 2333 (w), 2241 (w), 2210 (w), 2189 (w), 2163 (w), 2042 (w), 1984 (w), 1704 (s), 1638 (m), 1543

(m), 1510 (m), 1439 (m), 1397 (m), 1371 (w), 1346 (w), 1302 (w), 1266 (w), 1225 (w), 1122 (m), 1057 (w), 1027 (w), 852 (w), 800 (w), 747 (w), 717 (w), 681 (w), 631 (w), 617 (w), 608 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.87 (1H, s, H-2^{V}), 8.15 (1H, t, *J* 6.1, H-8^{IV}), 7.94 – 7.90 (1H, m, H-6^{VI}), 7.84 – 7.74 (3H, m, H-3^{VI}, 4^{VI} and H-5^{VI}), 7.42 – 7.34 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.22 (1H, d, *J* 9.4, H-18^{I}), 7.07 – 6.97 (3H, m, H-4, H-6 and H-7), 4.86 (3H, s, H-3^{II} and O*H*), 4.55 (1H, d, *J* 9.5, H-19^{I}), 4.53 – 4.47 (3H, m, H-1^{I} and H-2^{III}), 4.41 – 4.33 (2H, m, H-4^{III} and H-7^{IVa}), 4.28 (1H, dd, *J* 15.4, 5.7, H-7^{IVb}), 3.97 – 3.87 (2H, m, H-16^{I}), 3.70 (1H, dd, *J* 10.6, 4.5, H-5^{IIIa}), 3.65 – 3.57 (3H, m, H-5^{IIIb} and H-14^{I}), 3.57 – 3.52 (2H, m, H-13^{I}), 3.50 – 3.41 (6H, m, H-8^{I}, H-10^{I} and H-11^{I}), 3.37 (2H, t, *J* 5.6, H-7^{I}), 3.32 – 3.20 (10H, m, H-8, H-9, H-3^{I} and H-5^{I}), 2.43 (3H, s, H-6^{V}), 2.09 – 1.96 (2H, m, H-3^{III}), 1.79 (2H, p, *J* 7.4, H-4^{I}), 0.94 (9H, s, H-21^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.0 (C-9^{IV}), 169.0 (C-22^{I}), 168.0 (C-17^{I}), 153.7 (C-1^{II}), 153.4 (C-2), 150.4 (C-2^{V}), 147.39 (C-2^{VI}), 147.35 (C-4^{V}), 138.9 (C-1^{IV}), 133.7 (C-5^{VI}), 131.8 (C-5^{V}), 131.7 (C-4^{VI}), 130.6 (C-3a), 129.8 (C-4^{IV}), 129.7 (C-5), 129.5 (C-7a), 129.1 (C-6^{VI}), 129.0 (C-1^{VI}), 128.2, 127.2 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 123.7 (C-3^{VI}), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.7 (C-4^{II}), 74.1 (C-3^{II}), 70.1 (C-14^{I}), 69.5 (C-16^{I}), 69.31 (C-13^{I}), 69.29, 69.2, 68.4 (C-8^{I}, C-10^{I} and C-11^{I}), 68.1 (C-4^{III}), 58.3 (C-2^{III}), 55.8 (C-5^{III}), 55.5 (C-19^{I}), 50.1 (C-1^{I}), 46.5 (C-7^{I}), 45.6 (C-3^{I}), 43.9 (C-5^{I}), 41.5 (C-7^{IV}), 37.3 (C-3^{III}), 35.0 (C-20^{I}), 26.4, 26.3 (C-8 and C-9), 26.1 (C-4^{I}), 25.8 (C-21^{I}), 15.2 (C-6^{V}); *m/z* (ESI^{+}) 1211 ([M+H]$^{+}$, 80%), 1213 ([M+H]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{52}H_{67}O_{14}N_9Cl_3S_2^{+}$ ([M+H]$^{+}$) requires 1210.33090 and 1212.32930, found 1210.33118 and 1212.32922; HPLC (method 2) retention time 0.91 min, 100%.

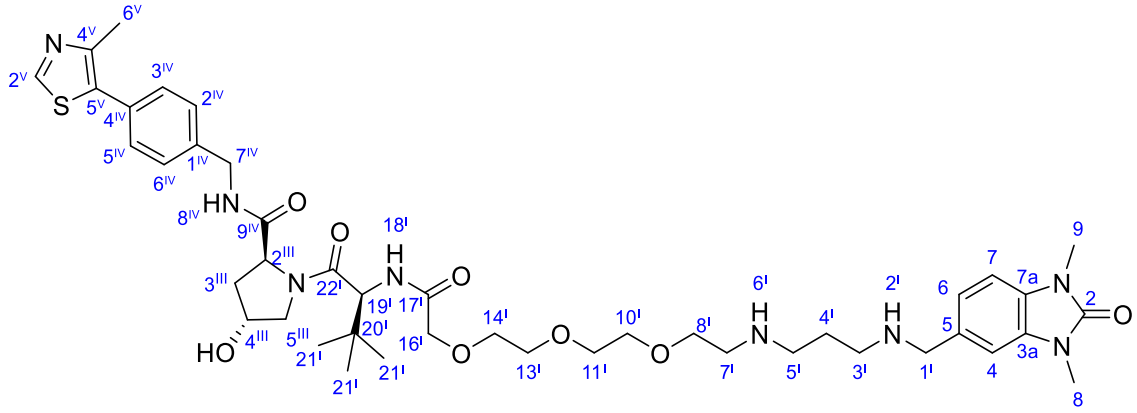
20^{I}-Methyl-*N*-{4^{II},4^{II},4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-17^{I}-oxo-9^{I},12^{I},15^{I},2^{II}-tetraoxa-2^{I},6^{I}-diazaicosanan-17^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (199)



Thioglycolic acid (8.0 µL, 0.12 mmol, 2.0 eq.) and lithium hydroxide (5.5 mg, 0.23 mmol, 4.0 eq.) were stirred for 5 minutes in degassed anhydrous *N*,*N*-dimethylformamide (0.5 mL). This solution was then added to a solution of **196** (70 mg, 0.058 mmol, 1.0 eq.) in degassed anhydrous *N*,*N*-dimethylformamide (2 mL). The resulting mixture was stirred at room temperature for 5 h. Water (10 mL) was then added, and the aqueous solution was extracted with ethyl acetate (3 × 15 mL). The combined organic components were washed with saturated aqueous sodium bicarbonate solution (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% triethylamine, 0–5% methanol in dichloromethane) to yield the title compound **199** as a colourless oil (32 mg, 52%). R_f 0.73 (1% triethylamine, 4% methanol in dichloromethane); $[\alpha]_D^{25}$= −12.7 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3401 (m), 3305 (m), 3072 (m), 2922 (m), 2872 (m), 1695 (s), 1636 (s), 1501 (m), 1442 (m), 1398 (w), 1368 (w), 1350 (w), 1303 (w), 1270 (w), 1229 (w), 1119 (m), 1057 (w), 1029 (w), 966 (w), 937 (w), 848 (w), 816 (w), 804 (w), 771 (w), 758 (w), 745 (w), 717 (w), 677 (w), 638 (w), 624 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 8.90 (1H, s, H-2^{V}), 8.18 (1H, t, *J* 5.4, H-8^{IV}), 7.39 (4H, s, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.23 (1H, d, *J* 9.4, H-18^{I}), 7.08 – 6.98 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.60 – 4.45 (4H, m, H-1^{I}, H-2^{III} and H-19^{I}), 4.44 – 4.34 (2H, m, H-4^{III} and H-7^{IVa}), 4.29 (1H, dd, *J* 15.4, 5.4, H-7^{IVb}), 4.00 – 3.89 (2H, m, H-16^{I}), 3.72

(1H, dd, *J* 10.6, 4.5, H-5^{IIIa}), 3.66 – 3.50 (9H, m, H-5^{IIIb}, H-10^{I}, H-11^{I}, H-13^{I} and H-14^{I}), 3.47 (2H, t, *J* 5.7, H-7^{I}), 3.35 – 3.26 (8H, m, H-8, H-9 and H-3^{I}), 2.69 (2H, t, *J* 5.7, H-8^{I}), 2.59 (2H, t, *J* 6.9, H-5^{I}), 2.45 (3H, s, H-6^{V}), 2.10 – 1.95 (2H, m, H-3^{III}), 1.71 (2H, p, *J* 7.2, H-4^{I}), 0.96 (9H, s, H-21^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.1 (C-9^{IV}), 169.0 (C-22^{I}), 168.1 (C-17^{I}), 153.7 (C-1^{II}), 153.5 (C-2), 150.4 (C-2^{V}), 147.4 (C-4^{V}), 138.9 (C-1^{IV}), 130.6 (C-5^{V}), 130.1 (C-3a), 129.7 (C-4^{IV}), 129.5 (C-5), 128.9 (C-7a), 128.2, 127.2 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.8 (C-4^{II}), 74.1 (C-3^{II}), 70.1 (C-16^{I}), 69.52, 69.49, 69.47, 69.41, 69.2 (C-7^{I}, C-10^{I}, C-11^{I}, C-13^{I} and C-14^{I}), 68.5 (C-4^{III}), 58.6 (C-2^{III}), 55.8 (C-5^{III}), 55.5 (C-19^{I}), 50.1 (C-1^{I}), 48.0 (C-8^{I}), 46.0 (C-5^{I}), 44.5 (C-3^{I}), 41.5 (C-7^{IV}), 37.3 (C-3^{III}), 35.0 (C-20^{I}), 27.5 (C-4^{I}), 26.39, 26.36 (C-8 and C-9), 25.8 (C-21^{I}), 15.2 (C-6^{V}); *m/z* (ESI$^+$) 1025 ([M+H]$^+$, 80%), 1027 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{46}H_{64}O_{10}N_8Cl_3S^+$ ([M+H]$^+$) requires 1025.3526 and 1027.3507, found 1025.3518 and 1027.3495; HPLC (method 2) retention time 0.83 min, 97%.

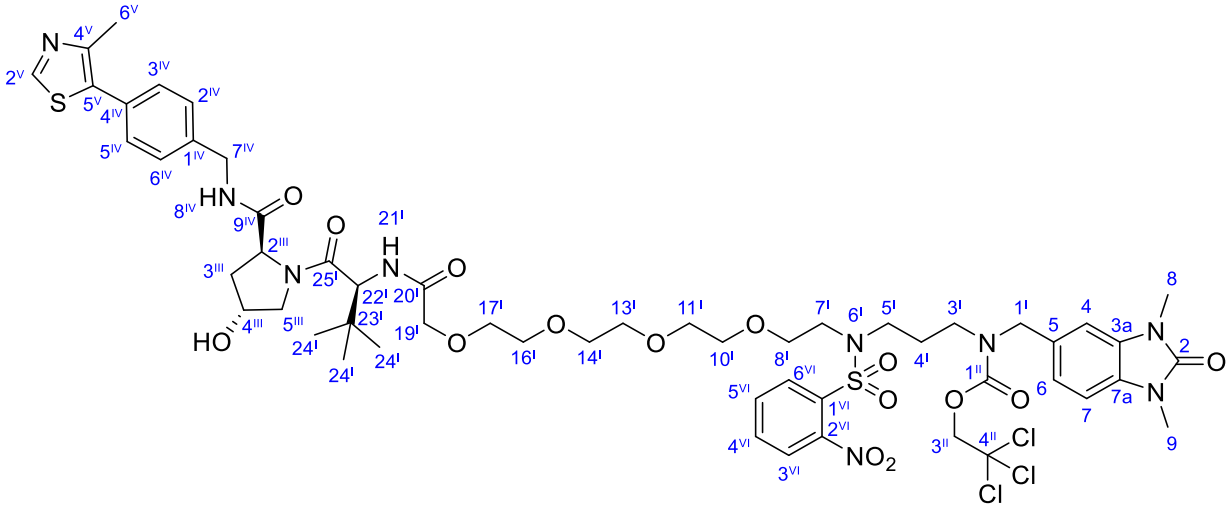
_N_-[2^{I}-(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)-17^{I}-oxo-9^{I},12^{I},15^{I}-trioxa-2^{I},6^{I}-diazaheptadecan-17^{I}-yl]-20^{I}-methyl-L-valyl-(4_R_)-4^{III}-hydroxy-_N_-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (140)



199 (24 mg, 0.023 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.8 mL), and acetic acid (55 µL) and zinc powder (15 mg, 0.23 mmol, 10.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (1:2:12 ammonium hydroxide:ethanol:ethyl acetate) to yield the title compound

140 as a colourless oil (13 mg, 65%). R_f 0.57 (1:2:6 ammonium hydroxide:ethanol:ethyl acetate); $[\alpha]_D^{25} = -13.6$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3404 (m), 3069 (w), 2919 (m), 2871 (w), 2363 (w), 1682 (s), 1634 (s), 1531 (m), 1510 (m), 1446 (m), 1399 (w), 1371 (w), 1345 (w), 1305 (w), 1273 (w), 1243 (w), 1199 (w), 1113 (m), 1031 (w), 967 (w), 938 (w), 852 (w), 813 (w), 745 (w), 728 (w), 660 (w), 632 (w), 623 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.87 (1H, s, H-2^V), 7.48 – 7.37 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.21 – 7.08 (3H, m, H-4, H-6 and H-7), 4.68 (1H, s, H-19^I), 4.58 – 4.47 (3H, m, H-7^{IVa}, H-2^{III} and H-4^{III}), 4.36 (1H, d, *J* 15.4, H-7^{IVb}), 4.08 – 3.97 (2H, m, H-16^I), 3.93 – 3.84 (3H, m, H-1^I and H-5^{IIIa}), 3.80 (1H, dd, *J* 11.0, 3.7, H-5^{IIIb}), 3.72 – 3.55 (12H, m, H-7^I, H-8^I, H-10^I, H-11^I, H-13^I and H-14^I), 3.44 – 3.37 (6H, m, H-8 and H-9), 2.91 – 2.75 (4H, m, H-3^I and H-5^I), 2.46 (3H, s, H-6^V), 2.26 – 2.19 (1H, m, H-3^{IIIa}), 2.08 (1H, ddd, *J* 13.4, 9.5, 4.4, H-3^{IIIb}), 1.78 (2H, p, *J* 6.9, H-4^I), 1.04 (9H, s, H-21^I); ^{13}C NMR (126 MHz, CD_3OD) δ_C 174.4 (C-9^{IV}), 172.0 (C-22^I), 171.7 (C-17^I), 156.4 (C-2), 152.9 (C-2^V), 149.1 (C-4^V), 140.3 (C-1^{IV}), 133.4 (C-5^V), 132.7 (C-3a), 131.5 (C-4^{IV}), 131.4 (C-5), 130.7 (C-7a), 130.4, 129.0 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 123.5 (C-6), 109.5 (C-4), 108.9 (C-7), 72.2, 71.6, 71.4, 71.3 (C-7^I, C-10^I, C-11^I, C-13^I and C-14^I), 71.1 (C-16^I), 71.0 (C-4^{III}), 69.5 (C-7^I, C-10^I, C-11^I, C-13^I or C-14^I), 60.9 (C-2^{III}), 58.24 (C-19^I), 58.16 (C-5^{III}), 54.0 (C-1^I), 48.8 (C-8^I), 48.1 (C-3^I and C-5^I), 43.7 (C-7^{IV}), 39.1 (C-3^{III}), 37.1 (C-20^I), 27.7 (C-4^I), 27.6, 27.5 (C-8 and C-9), 27.0 (C-21^I), 15.8 (C-6^V); *m/z* (ESI$^+$) 851 ([M+H]$^+$, 80%), 873 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{43}H_{63}O_8N_8S^+$ ([M+H]$^+$) requires 851.4484, found 851.4478; HPLC (method 1) retention time 5.07 min, 99.2%.

23^{I}-Methyl-*N*-{4^{II},4^{II},4^{II} -trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6^{I}-(2^{VI}-nitrobenzene-1^{VI}-sulfonyl)-17^{I}-oxo-9^{I},12^{I},15^{I},18^{I},2^{II}-pentaoxa-2^{I},6^{I}-diazatricosanan-20^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (197)

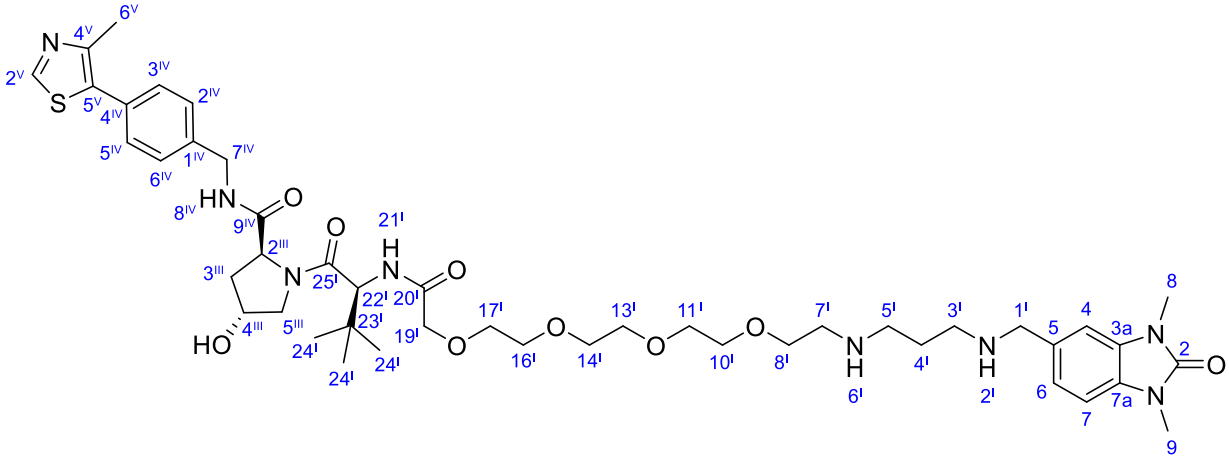


158 (660 mg, 1.24 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (4 mL) and dichloromethane (3 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo* to yield the hydrochloric acid salt of the amine as a yellow solid (579 mg, 94%). The product was then used immediately in the next step.

194 (150 mg, 0.178 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (124 µL, 0.711 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL). HATU (74.4 mg, 0.196 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The product from the above Boc deprotection (83.1 mg, 0.178 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with water (20 mL) and extracted with ethyl acetate (3 × 40 mL). The combined organic components were dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–10% methanol in dichloromethane) to yield the title compound **197** as a colourless oil (74.2 mg, 31%). R_f 0.77 (10% methanol in dichloromethane); $[\alpha]_D^{25}$= –2.2 (*c* 1.0,

$CHCl_3$); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3311 (w), 2950 (w), 2002 (w), 1703 (s), 1637 (m), 1543 (s), 1510 (m), 1440 (m), 1397 (w), 1371 (w), 1349 (w), 1302 (m), 1266 (w), 1225 (w), 1122 (s), 1061 (w), 1030 (w), 965 (w), 852 (w), 802 (w), 754 (s), 717 (w), 666 (w), 610 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.90 (1H, s, H-2^V), 8.17 (1H, t, *J* 5.4, H-8^{IV}), 7.95 (1H, dd, *J* 7.9, 1.4, H-6^{VI}), 7.88 – 7.75 (3H, m, H-3^{VI}, 4^{VI} and H-5^{VI}), 7.45 – 7.35 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.23 (1H, d, *J* 9.3, H-21^I), 7.08 – 6.97 (3H, m, H-4, H-6 and H-7), 4.88 (2H, s, H-3^{II}), 4.84 (1H, s, O*H*), 4.57 (1H, d, *J* 9.4, H-22^I), 4.54 – 4.48 (3H, m, H-1^I and H-2^{III}), 4.43 – 4.34 (2H, m, H-4^{III} and H-7^{IVa}), 4.29 (1H, dd, *J* 15.3, 5.7, H-7^{IVb}), 3.99 – 3.89 (2H, m, H-19^I), 3.72 (1H, dd, *J* 10.6, 4.5, H-5^{IIIa}), 3.65 – 3.42 (15H, m, H-5^{IIIb}, H-8^I, H-10^I, H-11^I, H-13^I, H-14^I, H-16^I and H-17^I), 3.39 (2H, t, *J* 5.7, H-7^I), 3.34 – 3.21 (10H, m, H-8, H-9, H-3^I and H-5^I), 2.45 (3H, s, H-6^V), 2.12 – 1.95 (2H, m, H-3^{III}), 1.81 (2H, p, *J* 7.5, H-4^I), 0.96 (9H, s, H-24^I); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.0 (C-9^{IV}), 169.0 (C-25^I), 168.1 (C-20^I), 153.7 (C-1^{II}), 153.4 (C-2), 150.4 (C-2^V), 147.4 (C-2^{VI}), 147.3 (C-4^V), 138.9 (C-1^{IV}), 133.7 (C-5^{VI}), 131.8 (C-5^V), 131.7 (C-4^{VI}), 130.7 (C-3a), 129.8 (C-4^{IV}), 129.7 (C-5), 129.5 (C-7a), 129.2 (C-6^{VI}), 129.0 (C-1^{VI}), 128.2, 127.1 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 123.7 (C-3^{VI}), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.7 (C-4^{II}), 74.1 (C-3^{II}), 70.1 (C-19^I), 69.52, 69.46, 69.40, 69.3, 69.23, 69.20, 68.4 (C-8^I, C-10^I, C-11^I, C-13^I, C-14^I, C-16^I and C-17^I), 68.1 (C-4^{III}), 58.3 (C-2^{III}), 55.8 (C-5^{III}), 55.5 (C-22^I), 54.1, 50.1 (C-1^I), 46.5 (C-7^I), 45.6 (C-3^I), 43.9 (C-5^I), 41.5 (C-7^{IV}), 37.3 (C-3^{III}), 35.0 (C-23^I), 26.4, 26.3 (C-8 and C-9), 26.1 (C-4^I), 25.8 (C-24^I), 15.2 (C-6^V); *m/z* (ESI$^+$) 1278 ([M+Na]$^+$, 80%), 1280 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{54}H_{71}O_{15}N_9Cl_3S_2^+$ ([M+H]$^+$) requires 1254.35711 and 1256.35561, found 1254.35738 and 1256.35554; HPLC (method 2) retention time 0.91 min, 94%.

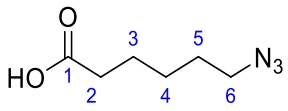
_N_-[2^{I}-(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)-20^{I}-oxo-9^{I},12^{I},15^{I},18^{I}-tetraoxa-2^{I},6^{I}-diazaicosan-20^{I}-yl]-23^{I}-methyl-L-valyl-(4_R_)-4^{III}-hydroxy-_N_-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (141)



Thiophenol (12 µL, 0.12 mmol, 5.0 eq.) and potassium carbonate (9.9 mg, 0.72 mmol, 3.0 eq.) were stirred for 5 minutes in degassed anhydrous *N*,*N*-dimethylformamide (0.3 mL). This solution was then added to a solution of **197** (30 mg, 0.024 mmol, 1.0 eq.) in degassed anhydrous *N*,*N*-dimethylformamide (0.2 mL). The resulting mixture was stirred at room temperature for 2 h, and then concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 4–10% methanol in dichloromethane), to yield the nosyl-deprotected amine **200**. This was used in the next reaction without further purification. **200** (21 mg, 0.020 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.7 mL), and acetic acid (45 µL) and zinc powder (13 mg, 0.20 mmol, 10.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (1:2:6 ammonium hydroxide:ethanol:ethyl acetate) to yield the title compound **141** as a colourless oil (12 mg, 66%). R_f 0.26 (1% triethylamine, 10% methanol in dichloromethane); $[\alpha]_D^{25} = -10.6$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3413 (m), 3307 (m), 3073 (m), 2939 (m), 2908 (m), 2872 (m), 2362 (w), 2343 (w), 1686 (s), 1638 (s), 1541 (m), 1509 (m), 1444 (m), 1397 (w), 1372 (w), 1347 (w), 1304 (w), 1270 (w), 1243 (w), 1193 (w), 1110 (m), 1032 (w), 966 (w), 938 (w), 851 (w), 809 (w), 745 (w), 728 (w), 702 (w), 668 (w), 655 (w); ^{1}H NMR

(500 MHz, CD_3OD) δ_H 8.87 (1H, s, H-2^V), 7.48 – 7.37 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.20 – 7.07 (3H, m, H-4, H-6 and H-7), 4.69 (1H, s, H-22^I), 4.60 – 4.47 (3H, m, H-7^{IVa}, H-2^{III} and H-4^{III}), 4.35 (1H, d, *J* 15.4, H-7^{IVb}), 4.09 – 3.98 (2H, m, H-19^I), 3.89 – 3.83 (3H, m, H-1^I and H-5^{IIIa}), 3.80 (1H, dd, *J* 11.0, 3.7, H-5^{IIIb}), 3.73 – 3.53 (14H, m, H-8^I, H-10^I, H-11^I, H-13^I, H-14^I, H-16^I and H-17^I), 3.44 – 3.38 (6H, m, H-8 and H-9), 2.83 – 2.76 (2H, m, H-3^I), 2.75 – 2.65 (4H, m, H-5^I and H-7^I), 2.47 (3H, s, H-6^V), 2.22 (1H, ddd, *J* 13.3, 7.6, 1.9, H-3^{IIIa}), 2.08 (1H, ddd, *J* 13.3, 9.3, 4.4, H-3^{IIIb}), 1.75 (2H, p, *J* 7.8, H-4^I), 1.04 (9H, s, H-24^I); ^{13}C NMR (126 MHz, CD_3OD) δ_C 174.4 (C-9^{IV}), 172.1 (C-25^I), 171.7 (C-20^I), 156.4 (C-2), 152.9 (C-2^V), 149.1, (C-4^V) 140.3 (C-1^{IV}), 133.9 (C-3a), 133.4 (C-5^V), 131.5 (C-4^{IV}), 131.3 (C-5), 130.5 (C-7a), 130.4, 129.0 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 123.4 (C-6), 109.3 (C-4), 108.8 (C-7), 72.3, 71.60, 71.56, (C-8^I, C-10^I, C-11^I, C-13^I, C-14^I, C-16^I or C-17^I), 71.5 (C-4^{III}), 71.26, 71.25, 71.09 (C-8^I, C-10^I, C-11^I, C-13^I, C-14^I, C-16^I or C-17^I), 71.05 (C-19^I), 70.2 (C-8^I, C-10^I, C-11^I, C-13^I, C-14^I, C-16^I or C-17^I), 60.8 (C-2^{III}), 58.2 (C-22^I), 58.1 (C-5^{III}), 54.4 (C-1^I), 48.8 (C-7^I), 48.6 (C-3^I), 48.0 (C-5^I), 43.7 (C-7^{IV}), 39.0 (C-3^{III}), 37.1 (C-23^I), 29.1 (C-4^I), 27.51, 27.50 (C-8 and C-9), 27.0 (C-24^I), 15.8 (C-6^V); *m/z* (ESI$^+$) 895 ($[M+H]^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{45}H_{67}O_9N_8S^+$ ($[M+H]^+$) requires 895.4746, found 895.4739; HPLC (method 1) retention time 4.92 min, 97.3%.

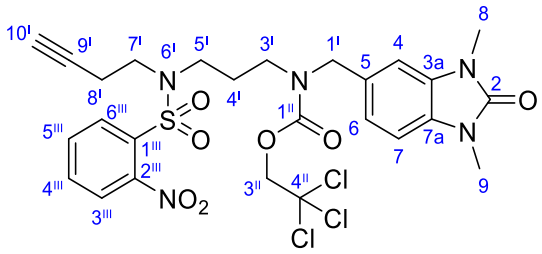
6-Azidohexanoic acid (212)[167]



A solution of 6-bromohexanoic acid (195 mg, 1.00 mmol, 1.0 eq.) in dimethylsulfoxide (1.6 mL) was heated to 40 ºC. Sodium azide (325 mg, 5.00 mmol, 5.0 eq.) was added stepwise. The reaction mixture was then heated to 80 ºC and stirred for 16 h. The temperature was decreased to 40 ºC and concentrated hydrochloric acid (0.43 mL) was added dropwise. The reaction mixture was stirred for a further 7 h, and then allowed to cool to room temperature. Water (3 mL) was added, and the mixture was extracted with diethyl ether (5 × 10 mL). The combined organic components were washed with 10% aqueous sodium hydrogen sulfate (2 × 4 mL), dried over magnesium

sulfate and concentrated under a continuous stream of nitrogen for 1 h to yield the title compound **212** as a colourless oil (141 mg, 90%). R_f 0.46 (1% AcOH, 10% ethanol in chloroform); ^{1}H NMR (400 MHz, CD_3OD) δ_H 3.29 (2H, t, *J* 6.8, H-6), 2.31 (2H, t, *J* 7.3, H-2), 1.69 – 1.56 (4H, m, H-3 and H-5), 1.48 – 1.37 (2H, m, H-4); *m/z* (ESI$^-$) 156 ([M-H]$^-$, 100%). The spectroscopic data are in good agreement with the literature values.[167]

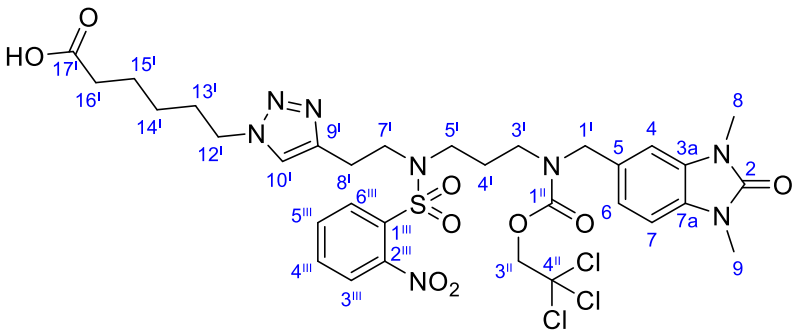
4^{II},4^{II},4^{II}-Trichloroethyl {3-[(but-9^{I}-yn-6^{I}-yl)(2^{III}-nitrobenzene-1^{III}-sulfonyl)amino]propyl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (217)



But-3-yn-1-ol (74.7 µL, 0.985 mmol, 2.0 eq.), triphenylphosphine (259 mg, 0.985 mmol, 2.0 eq.), and diisopropyl azodicarboxylate (191 µL, 0.985 mmol, 2.0 eq.) were added to a solution of **191** (300 mg, 0.493 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (3 mL) at 0 ºC. The reaction mixture was stirred at room temperature for 16 h. After this time, further but-3-yn-1-ol (37.0 µL, 0.493 mmol, 1.0 eq.), triphenylphosphine (129 mg, 0.493 mmol, 1.0 eq.) and diisopropyl azodicarboxylate (95.0 µL, 0.493 mmol, 1.0 eq.) were added, and the reaction mixture was stirred for 2 h. The resulting solution was quenched with water (10 mL), and extracted with ethyl acetate (3 × 20 mL). The organic components were dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–90% ethyl acetate in heptane) and further purified by silica gel column chromatography (0–5% methanol in dichloromethane) to yield the title compound **217** as a yellow oil (165 mg, 51%). R_f 0.60 (80% ethyl acetate in heptane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3300 (w), 2949 (w), 1699 (s), 1630 (w), 1612 (w), 1591 (w), 1543 (m), 1509 (w), 1468 (w), 1441 (w), 1428 (w), 1395 (w), 1372 (w), 1350 (w), 1302 (w), 1268 (w), 1222 (m), 1194 (w), 1162 (m), 1125 (m), 1080 (w), 1058 (w), 1028 (w), 1010 (w),

970 (w), 903 (w), 852 (w), 815 (w), 748 (s), 717 (m), 666 (w), 651 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.96 (1H, dd, *J* 7.6, 1.3, H-3^{III}), 7.89 – 7.77 (3H, m, H-4^{III}, H-5^{III} and H-6^{III}), 7.08 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.54 (2H, s, H-1^{I}), 3.37 (2H, t, *J* 7.3, H-7^{I}), 3.34 – 3.24 (10H, m, H-8, H-9, H-3^{I} and H-5^{I}), 2.63 (1H, t, *J* 2.7, H-10^{I}), 2.37 (2H, td, *J* 7.3, 2.7, H-8^{I}), 1.80 (2H, p, *J* 7.4, H-4^{I}); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 153.7 (C-1^{II}), 153.5 (C-2), 147.3 (C-2^{III}), 133.9 (C-1^{III}), 131.8 (C-5^{III}), 131.5 (C-4^{III}), 129.8 (C-5), 129.7 (C-3a), 129.3 (C-7a), 129.0 (C-6^{III}), 123.8 (C-3^{III}), 120.1 (C-6), 106.8 (C-4), 106.5 (C-7), 95.7 (C-4^{II}), 80.4 (C-9^{I}), 74.1 (C-3^{II}), 71.9 (C-10^{I}), 50.2 (C-1^{I}), 45.8 (C-7^{I}), 45.2 (C-3^{I}), 44.0 (C-5^{I}), 26.39, 26.35 (C-8 and C-9), 26.26 (C-4^{I}), 18.0 (C-8^{I}); *m/z* (ESI^{+}) 660 ([M+H]$^{+}$, 80%), 662 ([M+H]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{26}H_{28}O_7N_5Cl_3S^{+}$ ([M+H]$^{+}$) requires 660.08237 and 662.07964, found 660.08436 and 662.08137; HPLC (method 2) retention time 0.93 min, 97%.

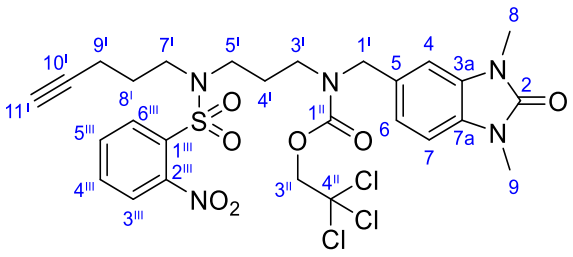
17^{I}-(9^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)(2^{III}-nitrobenzene-1^{III}-sulfonyl)amino]ethyl}-1*H*-1,2,3-triazol-11^{I}-yl)hexanoic acid (219)



A suspension of **217** (50.0 mg, 0.0756 mmol, 1.0 eq.) and **212** (119 mg, 0.756 mmol, 10.0 eq.) in water (1.53 mL) and *tert*-butanol (1.96 mL) was degassed for 5 min under argon. Sodium ascorbate (218 µL of a 35 mM solution in water) and copper(II) sulfate pentahydrate (218 µL of a 12 mM solution in water) were added to the degassed suspension, and the resulting mixture was heated to 50 ºC and stirred for 3 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic components were dried over sodium sulfate and concentrated under a continuous stream of nitrogen for 1 h. The crude residue

was purified by silica gel column chromatography (1% AcOH, 0–6% methanol in dichloromethane) to yield the title compound **219** as a colourless oil (33.7 mg, 54%). R_f 0.38 (7% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2927 (w), 2855 (w), 2359 (w), 2341 (w), 1702 (s), 1544 (m), 1509 (w), 1466 (w), 1440 (w), 1397 (w), 1373 (w), 1347 (w), 1303 (w), 1269 (w), 1224 (m), 1195 (w), 1161 (w), 1141 (w), 1125 (m), 1059 (w), 968 (w), 935 (w), 902 (w), 852 (w), 814 (w), 773 (w), 744 (w), 729 (w), 718 (w), 679 (w), 652 (w), 628 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 7.92 (1H, dd, *J* 7.9, 1.3, H-6^{III}), 7.89 – 7.76 (3H, m, H-3^{III}, H-4^{III} and H-5^{III}), 7.74 (1H, s, H-10^{I}), 7.08 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.54 (2H, s, H-1^{I}), 4.28 (2H, t, *J* 7.1, H-12^{I}), 3.55 – 3.48 (2H, m, H-7^{I}), 3.34 – 3.23 (10H, m, H-8, H-9, H-3^{I} and H-5^{I}), 2.86 – 2.79 (2H, m, H-8^{I}), 2.19 (2H, t, *J* 7.3, H-16^{I}), 1.86 – 1.76 (4H, m, H-13^{I} and H-4^{I}), 1.56 (2H, p, *J* 7.4, H-15^{I}), 1.35 – 1.25 (2H, m, H-14^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 173.5 (C-17^{I}), 153.7 (C-2), 153.4 (C-1^{II}) 147.3 (C-2^{III}), 142.6 (C-9^{I}), 133.9 (C-5^{III}), 131.8 (C-4^{III}), 131.6 (C-1^{III}), 129.8 (C-5), 129.7 (C-3a), 129.2 (C-7a), 129.0 (C-6^{III}), 123.8 (C-3^{III}), 121.6 (C-10^{I}), 120.1 (C-6), 106.9 (C-4), 106.5 (C-7), 95.7 (C-4^{II}), 74.1 (C-3^{II}), 50.2 (C-1^{I}), 48.7 (C-12^{I}), 46.7 (C-7^{I}), 45.1 (C-3^{I}), 44.0 (C-5^{I}), 33.1 (C-16^{I}), 28.8 (C-13^{I}), 26.41, 26.37 (C-8 and C-9), 25.9 (C-4^{I}), 25.1 (C-14^{I}), 24.5 (C-8^{I}), 23.5 (C-15^{I}); *m/z* (ESI$^+$) 818 ([M+H]$^+$, 90%), 820 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{32}H_{40}O_9N_8Cl_3S^+$ ([M+H]$^+$) requires 817.16991 and 819.16747, found 817.16913 and 819.16614; HPLC (method 1) retention time 8.49 min, 100%.

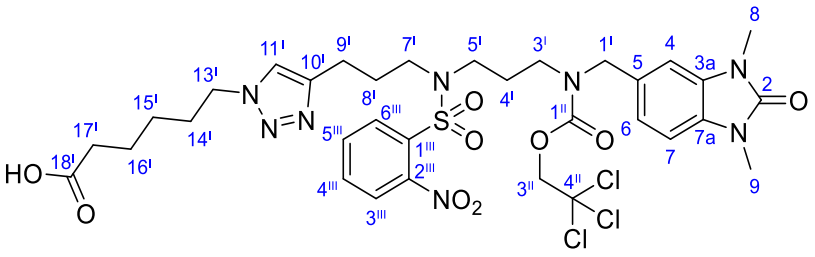
4^{II},4^{II},4^{II}-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{6^{I}-[(2^{III}-nitrobenzene-1^{III}-sulfonyl)(pent-10^{I}-yn-6^{I}-yl)amino]propyl}carbamate (218)



Pent-4-yn-1-ol (30.6 µL, 0.328 mmol, 2.0 eq.), triphenylphosphine (86.1 mg, 0.328 mmol, 2.0 eq.), and diisopropyl azodicarboxylate (63.6 µL, 0.328 mmol, 2.0 eq.) were added to a solution of **191** (100 mg, 0.164 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (1 mL) at 0 ºC. The reaction mixture was stirred at room temperature for 4 h. After this time, further pent-4-yn-1-ol (15.0 µL, 0.164 mmol, 1.0 eq.), triphenylphosphine (43.0 mg, 0.164 mmol, 1.0 eq.) and diisopropyl azodicarboxylate (32.0 µL, 0.164 mmol, 1.0 eq.) were added, and the reaction mixture was stirred for 3 h. The resulting solution was quenched with water (10 mL), and extracted with ethyl acetate (3 × 20 mL). The organic components were dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–100% ethyl acetate in heptane) and further purified by silica gel column chromatography (0–10% acetone in dichloromethane) to yield the title compound **218** as a yellow oil (57.9 mg, 52%). R_f 0.59 (80% ethyl acetate in heptane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3734 (w), 2961 (w), 2930 (w), 2855 (w), 2360 (s), 2342 (s), 1707 (s), 1594(w), 1544 (w), 1508 (w), 1465 (w), 1373 (w), 1273 (w), 1221 (w), 1161 (w), 1124 (w), 1078 (w), 1060 (w), 1019 (w), 956 (w), 913 (w), 852 (w), 816 (w), 772 (w), 746 (w), 718 (w), 668 (w), 656 (m), 637 (w), 625 (w), 608 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.94 (1H, dd, *J* 7.7, 1.4, H-6^{III}), 7.90 – 7.77 (3H, m, H-4^{III}, H-5^{III} and H-3^{III}), 7.09 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.54 (2H, s, H-1^{I}), 3.35 – 3.19 (12H, m, H-8, H-9, H-3^{I}, H-5^{I} and H-7^{I}), 2.57 (1H, t, *J* 2.6, H-11^{I}), 2.11 (2H, td, *J* 7.1, 2.6, H-9^{I}), 1.78 (2H, p, *J* 7.3, H-4^{I}), 1.63 (2H, p, *J* 7.2, H-8^{I}); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 153.7 (C-1^{II}), 153.5 (C-2), 147.3 (C-2^{III}), 133.8 (C-1^{III}), 131.7 (C-5^{III}), 131.5 (C-4^{III}), 129.8 (C-5), 129.7 (C-3a), 129.3

(C-7a), 129.0 (C-6^{III}), 123.8 (C-3^{III}), 120.1 (C-6), 106.8 (C-4), 106.5 (C-7), 95.7 (C-4^{II}), 82.7 (C-10^{I}), 74.1 (C-3^{II}), 70.5 (C-11^{I}), 50.2 (C-1^{I}), 46.2, 45.1, 44.0 (C-3^{I}, C-5^{I} and C-7^{I}), 26.6 (C-8^{I}), 26.39, 26.35 (C-8 and C-9), 26.33 (C-4^{I}), 14.7 (C-9^{I}); *m/z* (ESI$^+$) 674 ([M+H]$^+$, 90%), 696 ([M+Na]$^+$, 100%), 698 ([M+Na]$^+$, 70%); HRMS *m/z* (ESI$^+$) $C_{27}H_{28}O_8N_5Cl_3S^+$ ([M+H]$^+$) requires 674.0997 and 676.0956, found 674.0997 and 676.0970; HPLC (method 2) retention time 0.96 min, 100%.

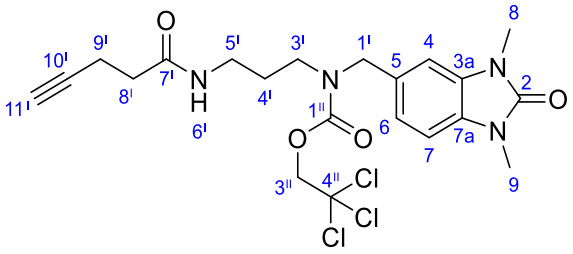
18^{I}-(10^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)(2^{III}-nitrobenzene-1^{III}-sulfonyl)amino]propyl}-1*H*-1,2,3-triazol-12^{I}-yl)hexanoic acid (220)



A suspension of **218** (50.0 mg, 0.0741 mmol, 1.0 eq.) and **212** (116 mg, 0.741 mmol, 10.0 eq.) in water (1.53 mL) and *tert*-butanol (1.96 mL) was degassed for 5 min under argon. Sodium ascorbate (218 µL of a 35 mM solution in water) and copper(II) sulfate pentahydrate (218 µL of a 12 mM solution in water) were added to the degassed suspension, and the resulting mixture was heated to 50 ºC and stirred for 3 h. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic components were dried over sodium sulfate and concentrated under a continuous stream of nitrogen for 1 h. The crude residue was purified by silica gel column chromatography (1% AcOH, 0–5% methanol in dichloromethane) to yield the title compound **220** as a colourless oil (35.8 mg, 58%). R_f 0.35 (7% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3058 (w), 3020 (w), 2940 (w), 2862 (w), 1705 (s), 1544 (m), 1509 (w), 1467 (w), 1441 (w), 1397 (w), 1373 (w), 1346 (w), 1303 (w), 1268 (w), 1224 (w), 1161 (w), 1142 (w), 1125 (w), 1058 (w), 1027 (w), 996 (w), 838 (w), 774 (w), 745 (w), 718 (w), 652 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.91 (1H, dd, *J* 7.8, 1.4, H-6^{III}),

7.88 – 7.75 (3H, m, H-3^{III}, H-4^{III} and H-5^{III}), 7.69 (1H, s, H-11^{I}), 7.08 – 6.99 (3H, m, H-4, H-6 and H-7), 4.88 (2H, s, H-3^{II}), 4.53 (2H, s, H-1^{I}), 4.28 (2H, t, *J* 7.1, H-13^{I}), 3.35 – 3.21 (13H, m, H-8, H-9, H-3^{I}, H-5^{I} and H-7^{I}), 2.59 – 2.53 (2H, m, H-8^{I}), 2.19 (2H, t, *J* 7.3, H-17^{I}), 1.87 – 1.73 (6H, m, H-14^{I}, H-4^{I} and H-9^{I}), 1.56 (2H, tt, *J* 7.4, 7.4, H-16^{I}), 1.35 – 1.26 (2H, m, H-15^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 173.5 (C-18^{I}), 153.7 (C-2), 153.5 (C-1^{II}), 147.3 (C-2^{III}), 145.2 (C-10^{I}), 133.8 (C-5^{III}), 131.7 (C-4^{III}), 131.6 (C-1^{III}), 129.8 (C-5), 129.7 (C-3a), 129.2 (C-7a), 129.0 (C-6^{III}), 123.8 (C-3^{III}), 121.0 (C-11^{I}), 120.1 (C-6), 106.9 (C-4), 106.5 (C-7), 95.7 (C-4^{II}), 74.1 (C-3^{II}), 50.1 (C-1^{I}), 48.7 (C-13^{I}), 46.7 (C-7^{I}), 45.0 (C-3^{I}), 43.9 (C-5^{I}), 33.1 (C-17^{I}), 28.8 (C-14^{I}), 27.2 (C-9^{I}), 26.41, 26.37 (C-8 and C-9), 26.25 (C-4^{I}), 25.1 (C-15^{I}), 23.5 (C-16^{I}), 21.8 (C-8^{I}); *m/z* (ESI$^+$) 832 ([M+H]$^+$, 90%), 834 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{33}H_{42}O_9N_8Cl_3S^+$ ([M+H]$^+$) requires 831.18556 and 833.18315, found 831.18494 and 833.18219; HPLC (method 1) retention time 8.45 min, 100%.

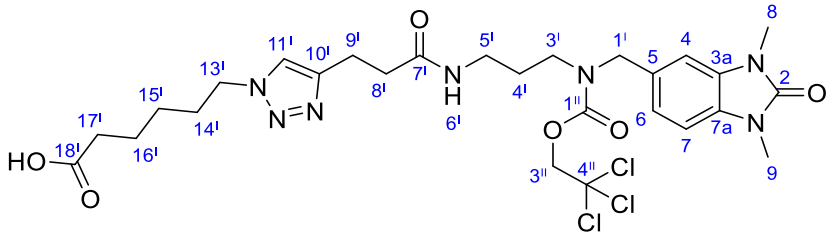
4^{II}, 4^{II}, 4^{II}-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{6^{I}-[(pent-10^{I}-ynoyl)amino]propyl}carbamate (210)



163 (1.00 g, 1.91 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (5 mL) and stirred at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo*. The crude residue was passed through an Isolute® SXC-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the deprotected amine product 3'',3'',3''-trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (877 mg, 100%). This compound was then used directly in the next step to avoid Troc protecting group migration to the terminal primary amine.

Pent-4-ynoic acid (92.6 mg, 0.944 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (658 µL, 3.78 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (2.5 mL). HATU (395 mg, 1.04 mmol, 1.1 eq.) was added and the solution was stirred at room temperature for 10 min. 3'',3'',3''-Trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (395 mg, 0.944 mmol, 1.0 eq.) in anhydrous *N*,*N*-dimethylformamide (2.5 mL) was added, and the resulting solution was stirred at room temperature for 22 h. Subsequently, the reaction mixture was quenched with water (40 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic components were washed with brine (50 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–10% methanol in dichloromethane) to yield the title compound **210** as a colourless oil (346 mg, 73%). R_f 0.33 (6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3305 (w), 3066 (w), 2944 (w), 2886 (w), 1701 (s), 1550 (m), 1509 (w), 1469 (w), 1443 (w), 1428 (w), 1396 (w), 1355 (w), 1300 (w), 1268 (w), 1218 (w), 1193 (w), 1131 (w), 1101 (m), 1060 (w), 961 (w), 842 (w), 813 (w), 772 (w), 743 (w), 717 (w), 679 (w), 635 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.53 – 7.45 (1H, m, H-6^I), 7.09 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.56 (2H, s, H-1^I), 3.37 – 3.25 (8H, m, H-8, H-9 and H-3^I), 3.11 – 3.04 (2H, m, H-5^I), 2.53 – 2.48 (1H, m, partially obscured by DMSO-d_6 peak, H-11^I), 2.36 (2H, td, *J* 6.8, 3.3, H-9^I), 2.29 – 2.22 (2H, m, H-8^I), 1.72 (2H, p, *J* 7.1, H-4^I); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 169.7 (C-7^I), 153.7 (C-2), 153.5 (C-1^{II}), 130.0 (C-5), 129.7 (C-3a), 128.9 (C-7a), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.8 (C-4^{II}), 83.3 (C-10^I), 74.1 (C-3^{II}), 70.1 (C-11^I), 50.1 (C-1^I), 44.3 (C-3^I), 35.9 (C-5^I), 34.0 (C-8^I), 27.4 (C-4^I), 26.37, 26.35 (C-8 and C-9), 13.8 (C-9^I); *m/z* (ESI$^+$) 503 ([M+H]$^+$, 90%), 505 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{21}H_{26}O_4N_4Cl_3^+$ ([M+H]$^+$) requires 503.1014 and 505.0986, found 503.1015 and 505.0985; HPLC (method 2) retention time 0.75 min, 97%.

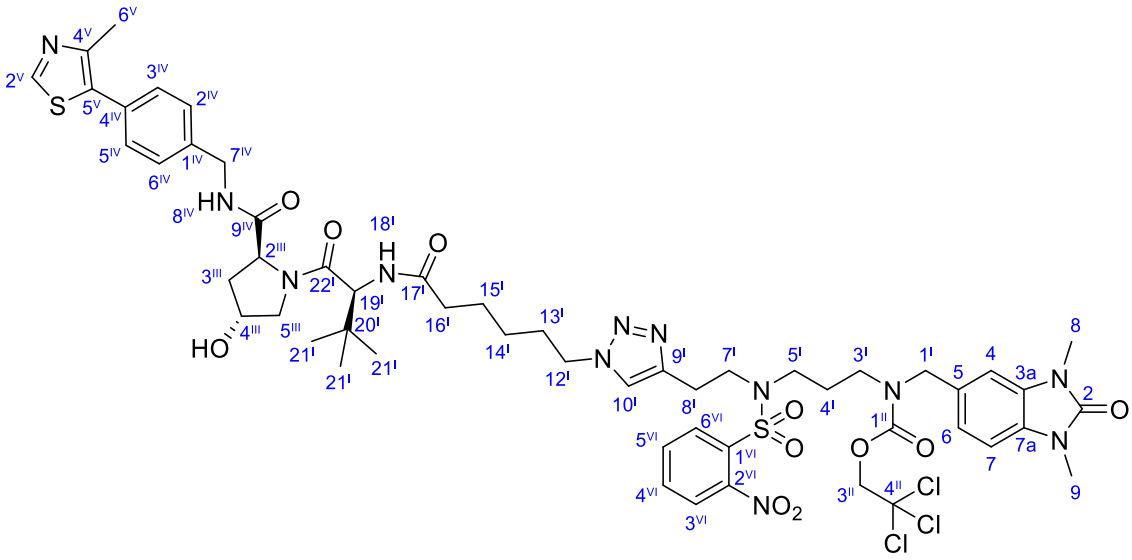
18^{I}-(10^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-7^{I}-oxopropyl}-1*H*-1,2,3-triazol-12^{I}-yl)hexanoic acid (213)



A suspension of **210** (44.9 mg, 0.0891 mmol, 1.0 eq.) and **212** (140 mg, 0.891 mmol, 10.0 eq.) in water (1.83 mL) and *tert*-butanol (2.36 mL) was degassed for 5 min under argon. Sodium ascorbate (262 µL of a 35 mM solution in water) and copper(II) sulfate pentahydrate (262 µL of a 12 mM solution in water) were added to the degassed suspension, and the resulting mixture was heated to 50 ºC and stirred for 2 h, and then 16 h at room temperature. The reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic components were dried over sodium sulfate and concentrated under a continuous stream of nitrogen for 1 h. The crude residue was purified by silica gel column chromatography (1% AcOH, 0–10% ethanol in chloroform) to yield the title compound **213** as a colourless oil (35.0 mg, 59%). R_f 0.14 (6% ethanol in chloroform); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3221 (w), 2874 (m), 2793 (w), 2331 (w), 1705 (s), 1572 (w), 1515 (m), 1487 (m), 1456 (m), 1402 (w), 1367 (w), 1298 (w), 1250 (m), 1142 (m), 1068 (w), 842 (w), 728 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.68 (1H, s, H-11^{I}), 7.53 (1H, t, *J* 4.6, H-6^{I}), 7.09 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.55 (2H, s, H-1^{I}), 4.26 (2H, t, *J* 7.0, H-13^{I}), 3.38 – 3.22 (8H, m, H-8, H-9 and H-3^{I}), 3.11 – 3.02 (2H, m, H-5^{I}), 2.84 (2H, t, *J* 7.6, H-9^{I}), 2.41 (2H, t, *J* 7.6, H-8^{I}), 2.14 (2H, bs, H-17^{I}), 1.80 (2H, p, *J* 7.2, H-14^{I}), 1.70 (2H, p, *J* 7.0, H-4^{I}), 1.59 – 1.48 (2H, d, *J* 9.6, H-16^{I}), 1.30 (2H, tt, *J* 8.0, 7.4, H-15^{I}); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 178.5 (C-18^{I}, observed by HMBC), 170.7 (C-7^{I}), 153.7 (C-1^{II}), 153.6 (C-2), 145.5 (C-10^{I}), 130.0 (C-5), 129.7 (C-3a), 128.9 (C-7a), 121.0 (C-11^{I}), 120.0 (C-6), 106.9 (C-4), 106.4 (C-7), 95.8 (C-4^{II}), 74.1 (C-3^{II}), 50.0 (C-1^{I}), 48.7 (C-13^{I}), 44.3 (C-3^{I}), 35.9 (C-5^{I}), 34.7 (C-8^{I}), 33.9 (C-17^{I}), 28.9 (C-14^{I}), 27.2 (C-4^{I}), 26.43, 26.39 (C-8 and C-9),

25.2 (C-15^{I}), 23.6 (C-16^{I}) 21.0 (C-9^{I}); *m/z* (ESI^{+}) 682 ([M+Na]$^{+}$, 90%), 684 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{25}H_{39}O_6N_7Cl_3^{+}$ ([M+H]$^{+}$) requires 660.18414 and 662.18151, found 660.18478 and 662.18200; HPLC (method 1) retention time 6.45 min, 100%.

_N_-[17^{I}-(9^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)(2^{VI}-nitrobenzene-1^{VI}-sulfonyl)amino]ethyl}-1*H*-1,2,3-triazol-11^{I}-yl)hexanoyl]-20^{I}-methylvalyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (221)

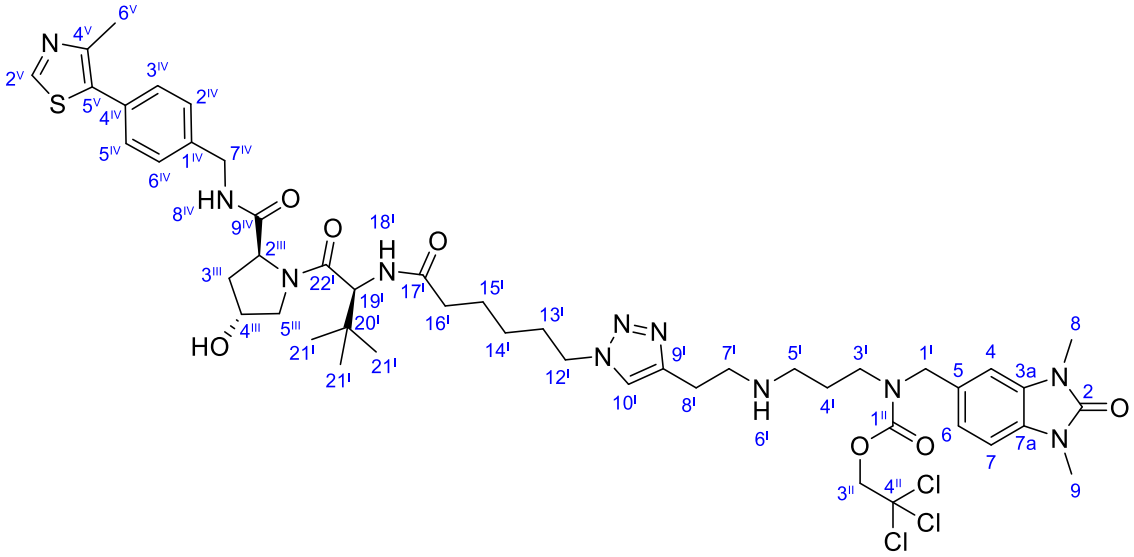


158 (220 mg, 0.415 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (2 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo*. The crude product was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the free amine as a yellow oil (162 mg, 91%). The product was then used immediately in the next step.

219 (32.0 mg, 0.0391 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (27.2 µL, 0.156 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (0.5 mL). HATU (16.3 mg, 0.0430 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The product from the above Boc deprotection (18.3 mg, 0.0391 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (0.5 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with

water (20 mL) and extracted with ethyl acetate (4 × 20 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–6% methanol in dichloromethane) to yield the title compound **221** as a colourless oil (36.0 mg, 75%). R_f 0.25 (6% methanol in dichloromethane); $[\alpha]_D^{25} = -10.2$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3628 (w), 3426 (w), 2949 (w), 2872 (w), 2722 (w), 2363 (w), 2341 (w), 1688 (s), 1631 (s), 1543 (m), 1509 (w), 1465 (w), 1439 (w), 1400 (w), 1373 (w), 1344 (w), 1271 (w), 1226 (w), 1161 (w), 1127 (w), 1061 (w), 1031 (w), 968 (w), 938 (w), 842 (s), 774 (w), 730 (w), 719 (w), 653 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.91 (1H, s, H-2^{V}), 8.14 (1H, s, H-8^{IV}), 7.95 – 7.76 (4H, m, H-3^{VI}, H-4^{VI}, H-5^{VI} and H-6^{VI}), 7.73 (1H, s, H-10^{I}), 7.44 – 7.37 (5H, m, H-2^{IV}, H-3^{IV}, H-5^{IV}, H-6^{IV} and H-18^{I}), 7.08 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.58 – 4.46 (4H, m, H-1^{I}, H-2^{III} and H-19^{I}), 4.45 –4.36 (2H, m, H-4^{III} and H-7^{IVa}), 4.32 – 4.23 (3H, m, H-7^{IVb} and H-12^{I}), 3.76 – 3.59 (2H, m, H-5^{III}), 3.56 – 3.45 (2H, m, H-7^{I}), 3.34 – 3.24 (11H, m, O*H*, H-8, H-9, H-3^{I} and H-5^{I}), 2.88 – 2.76 (2H, m, H-8^{I}), 2.46 (3H, s, H-6^{V}), 2.25 (1H, dt, *J* 14.8, 7.5, H-16^{Ia}), 2.16 (1H, dt, *J* 14.5, 7.3, H-16^{Ib}), 2.09 – 1.95 (2H, m, H-3^{III}), 1.87 – 1.75 (4H, m, H-13^{I} and H-4^{I}), 1.62 – 1.50 (2H, m, H-15^{I}), 1.36 – 1.24 (2H, m, H-14^{I}), 0.96 (9H, s, H-21^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.5 (C-9^{IV}), 171.2 (C-22^{I}), 169.6 (C-17^{I}), 153.7 (C-2), 153.5 (C-1^{II}), 150.5 (C-2^{V}), 147.4 (C-4^{V}), 147.3 (C-2^{VI}), 142.5 (C-9^{I}), 139.0 (C-1^{IV}), 133.9 (C-5^{VI}), 131.8 (C-4^{VI}), 131.6 (C-1^{VI}), 130.7 (C-5^{V}), 129.8 (C-4^{IV}), 129.7 (C-5), 129.2 (C-6^{VI}), 129.03 (C-3a), 129.01 (C-7a), 128.2, 127.2 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 123.8 (C-3^{VI}), 121.5 (C-10^{I}), 120.1 (C-6), 106.9 (C-4), 106.5 (C-7), 95.7 (C-4^{II}), 74.1 (C-3^{II}), 68.5 (C-4^{III}), 58.3 (C-2^{III}), 56.2 (C-19^{I}), 55.7 (C-5^{III}), 50.1 (C-1^{I}), 48.8 (C-12^{I}), 46.7 (C-7^{I}), 45.1 (C-3^{I}), 44.0 (C-5^{I}), 41.6 (C-7^{IV}), 37.3 (C-3^{III}), 34.6 (C-16^{I}), 34.4 (C-20^{I}), 28.8 (C-13^{I}), 26.41, 26.37 (C-8 and C-9), 26.2 (C-4^{I}), 26.0 (C-21^{I}), 25.1 (C-14^{I}), 24.5 (C-8^{I}), 24.2 (C-15^{I}), 15.3 (C-6^{V}); *m/z* (ESI$^+$) 1230 ([M+H]$^+$, 90%), 1232 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{54}H_{68}O_{11}N_{12}Cl_3S_2^+$ ([M+H]$^+$) requires 1229.36320 and 1231.36164, found 1229.36340 and 1231.36145; HPLC (method 1) retention time 7.57 min, 100%.

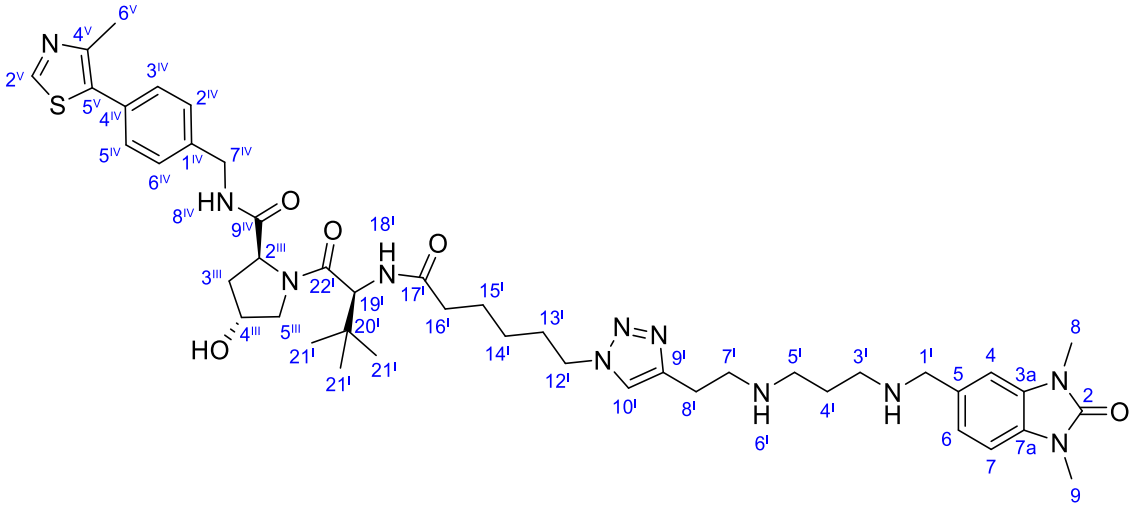
N-[17^{I}-(9^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]ethyl}-1H-1,2,3-triazol-11^{I}-yl)hexanoyl]-20^{I}-methylvalyl-(4R)-4^{III}-hydroxy-N-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (223)



Thiophenol (15 µL, 0.15 mmol, 5.0 eq.) and potassium carbonate (12 mg, 0.088 mmol, 3.0 eq.) were stirred for 5 minutes in degassed anhydrous *N*,*N*-dimethylformamide (0.3 mL). This solution was then added to a solution of **221** (36 mg, 0.029 mmol, 1.0 eq.) in degassed anhydrous *N*,*N*-dimethylformamide (0.2 mL). The resulting mixture was stirred at room temperature for 2 h, and then concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 4–10% methanol in dichloromethane then 1:2:8 ammonium hydroxide:ethanol:ethyl acetate) to yield the title compound **223** as a colourless oil (17 mg, 56%). R_f 0.18 (8% methanol in dichloromethane); $[\alpha]_D^{25} = -11.1$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3630 (w), 3430 (w), 2955 (w), 2868 (w), 2361 (w), 2342 (w), 1686 (s), 1631 (s), 1509 (m), 1465 (m), 1354 (w), 1307 (w), 1272 (w), 1234 (w), 1192 (w), 1138 (w), 1086 (w), 1060 (w), 967 (w), 844 (s), 773 (w), 758 (w), 742 (w), 718 (w), 638 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.87 (1H, s, H-2^{V}), 7.85 (1H, d, *J* 10.6, H-10^{I}), 7.48 – 7.37 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.21 – 7.08 (3H, m, H-4, H-6 and H-7), 4.93 (2H, d, *J* 3.5, H-3^{II}), 4.69 – 4.60 (3H, m, H-1^{I} and H-19^{I}), 4.60 – 4.46 (3H, m, H-2^{III}, H-4^{III} and H-7^{IVa}), 4.42 – 4.32 (3H, m, H-7^{IVb} and H-12^{I}), 3.90

(1H, dt, *J* 11.2, 1.8, H-5^{IIIa}), 3.80 (1H, dd, *J* 11.0, 3.9, H-5^{IIIb}), 3.45 (2H, t, *J* 6.5, H-3^{I}), 3.39 (6H, s, H-8 and H-9), 3.30 – 3.24 (2H, m, H-7^{I}), 3.09 – 2.99 (4H, m, H-5^{I} and H-8^{I}), 2.46 (3H, s, H-6^{V}), 2.34 – 2.18 (3H, m, H-3^{IIIa} and H-16^{I}), 2.09 (1H, ddd, *J* 13.3, 9.1, 4.4, H-3^{IIIb}), 2.00 – 1.85 (4H, m, H-13^{I} and H-4^{I}), 1.63 (2H, p, *J* 7.6, H-15^{I}), 1.39 – 1.25 (2H, m, H-14^{I}), 1.02 (9H, s, H-21^{I}); ^{13}C NMR (126 MHz, CD_3OD) δ_C 175.7 (C-9^{IV}), 174.5 (C-22^{I}), 172.3 (C-17^{I}), 156.6 (C-2), 156.4 (C-1^{II}), 152.8 (C-2^{V}), 149.0 (C-4^{V}), 143.8 (C-9^{I}), 140.3 (C-1^{IV}), 133.4 (C-5^{V}), 132.1 (C-4^{IV}), 131.6 (C-5), 131.5 (C-3a), 130.9 (C-7a), 130.4, 129.0 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 124.2 (C-10^{I}), 122.8 (C-6), 109.0 (C-4), 108.8 (C-7), 97.0 (C-4^{II}), 76.3 (C-3^{II}), 71.1 (C-4^{III}), 60.9 (C-2^{III}), 59.0 (C-19^{I}), 58.0 (C-5^{III}), 51.9 (C-1^{I}), 51.3 (C-12^{I}), 47.9 (C-7^{I}), 46.3 (C-5^{I}), 45.0 (C-3^{I}), 43.7 (C-7^{IV}), 39.0 (C-3^{III}), 36.5 (C-20^{I}), 36.3 (C-16^{I}), 30.9 (C-13^{I}), 27.6, 27.5 (C-8 and C-9), 27.0 (C-21^{I} and C-14^{I}), 26.3 (C-15^{I}), 25.6, (C-4^{I}) 23.2 (C-8^{I}), 15.8 (C-6^{V}); *m/z* (ESI$^+$) 1045 ([M+H]$^+$, 90%), 1047 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{48}H_{65}O_7N_{11}Cl_3S^+$ ([M+H]$^+$) requires 1044.38492 and 1046.38312, found 1044.38475 and 1046.38236; HPLC (method 1) retention time 6.41 min, 95.5%.

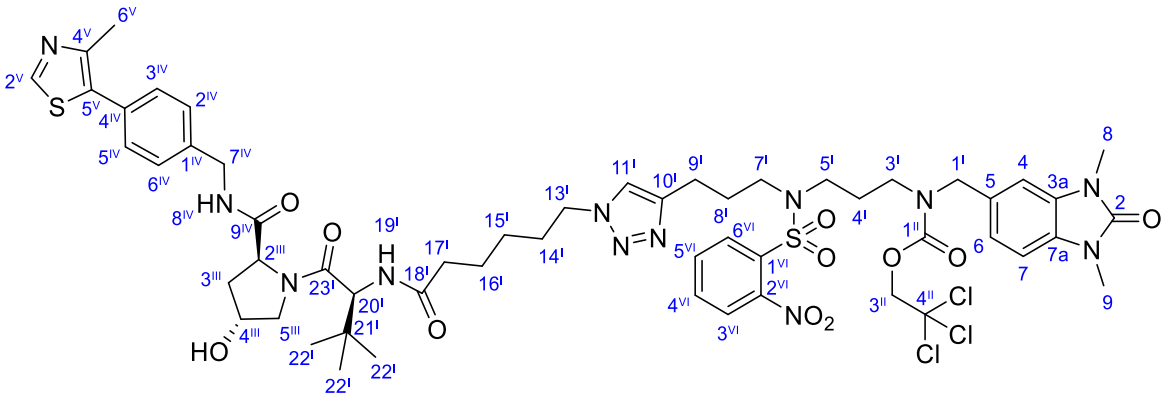
N-[17^{I}-(9^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)amino]ethyl}-1*H*-1,2,3-triazol-11^{I}-yl)hexanoyl]-20^{I}-methylvalyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (142)



223 (16 mg, 0.015 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.7 mL), and acetic acid (35 µL) and zinc powder (10 mg, 0.15 mmol, 10.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 10% methanol in dichloromethane then 1:2:6 ammonium hydroxide:ethanol:ethyl acetate) to yield the title compound **142** as a colourless oil (5.8 mg, 44%). R_f 0.17 (1:2:6 ammonium hydroxide:ethanol:ethyl acetate); $[\alpha]_D^{25}$= −7.3 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3729 (w), 3347 (m), 3088 (m), 2926 (m), 2853 (m), 2361 (s), 2342 (m), 1692 (s), 1633 (s), 1541 (m), 1509 (m), 1446 (m), 1418 (m), 1401 (m), 1370 (w), 1330 (w), 1276 (w), 1225 (w), 1199 (w), 1107 (w), 1087 (w), 1054 (w), 1032 (w), 1016 (w), 920 (w), 898 (w), 849 (w), 807 (w), 746 (w), 728 (w), 706 (w), 689 (w), 669 (w), 654 (w), 632 (w), 619 (w), 610 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.87 (1H, s, H-2^{V}), 7.77 (1H, s, H-10^{I}), 7.48 – 7.39 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.24 – 7.09 (3H, m, H-4, H-6 and H-7), 4.62 (1H, s, H-19^{I}), 4.58 – 4.51 (2H, m, H-2^{III} and H-7^{IVa}), 4.51 – 4.47 (1H, m, H-4^{III}), 4.39 – 4.30 (3H, m, H-7^{IVb} and H-12^{I}), 3.94 – 3.86 (3H, m, H-1^{I} and H-5^{IIIa}), 3.80 (1H, dd, *J* 10.8, 3.7, H-5^{IIIb}),

3.44 – 3.36 (6H, m, H-8 and H-9), 2.95 (2H, t, *J* 6.9, H-7^{I}), 2.89 (2H, t, *J* 6.9, H-8^{I}), 2.84 – 2.73 (4H, m, H-3^{I} and H-5^{I}), 2.47 (3H, s, H-6^{V}), 2.30 – 2.18 (3H, m, H-3^{IIIa} and H-16^{I}), 2.08 (1H, ddd, *J* 13.3, 9.1, 4.4, H-3^{IIIb}), 1.93 – 1.84 (2H, m, H-13^{I}), 1.81 – 1.76 (2H, m, H-4^{I}), 1.67 – 1.57 (2H, m, H-15^{I}), 1.38 – 1.26 (2H, m, H-14^{I}), 1.02 (9H, s, H-21^{I}); ^{13}C NMR (126 MHz, CD_3OD) δ_C 175.6 (C-9^{IV}), 174.5 (C-22^{I}), 172.3 (C-17^{I}), 156.4 (C-2), 152.9 (C-2^{V}), 149.0 (C-4^{V}), 146.4 (C-9^{I}), 140.3 (C-1^{IV}), 133.4 (C-5^{V}), 131.5 (C-4^{IV}), 131.4 (C-5), 130.4 (C-2^{IV} and C-6^{IV} or C-3^{IV} and C-5^{IV}), 129.9 (C-3a), 129.2 (C-7a), 129.0 (C-2^{IV} and C-6^{IV} or C-3^{IV} and C-5^{IV}), 123.7 (C-10^{I}), 123.6 (C-6), 109.5 (C-4), 108.9 (C-7), 71.1 (C-4^{III}), 60.8 (C-2^{III}), 59.0 (C-19^{I}), 58.0 (C-5^{III}), 53.9 (C-1^{I}), 51.1 (C-12^{I}), 49.4 (C-7^{I}), 48.0 (C-3^{I} and C-5^{I}), 43.7 (C-7^{IV}), 39.0 (C-3^{III}), 36.5 (C-16^{I}), 36.2 (C-20^{I}), 30.9 (C-13^{I}), 28.1 (C-4^{I}), 27.5 (C-8 and C-9), 27.0 (C-14^{I} and C-21^{I}), 26.3 (C-15^{I}), 25.8 (C-8^{I}), 15.8 (C-6^{V}); *m/z* (ESI^{+}) 892 ([M+H]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{45}H_{64}O_5N_{11}S^{+}$ ([M+H]$^{+}$) requires 870.48071, found 870.48040; HPLC (method 1) retention time 4.67 min, 95.5%.

_N_-[18^{I}-(10^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)(2^{VI}-nitrobenzene-1^{VI}-sulfonyl)amino]propyl}-1*H*-1,2,3-triazol-12^{I}-yl)hexanoyl]-21^{I}-methylvalyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (222)



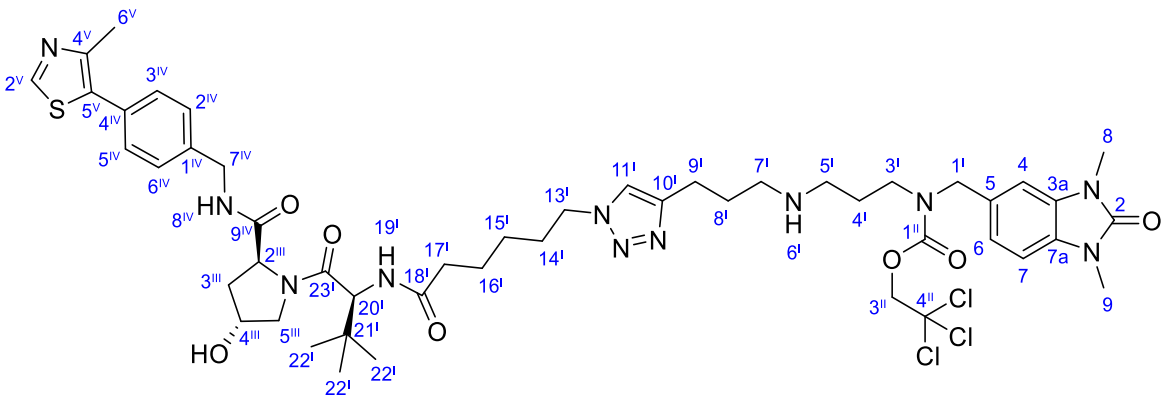
158 (220 mg, 0.415 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (2 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo*. The crude product was purified using Amine Isolute™ Catch and Release SXC-2 column

(10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the free amine as a yellow oil (162 mg, 91%). The product was then used immediately in the next step.

220 (34.0 mg, 0.0409 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (28.5 µL, 0.164 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (0.5 mL). HATU (17.1 mg, 0.0449 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The product from the above Boc deprotection (19.1 mg, 0.0409 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (0.5 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with water (20 mL) and extracted with ethyl acetate (4 × 20 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–8% methanol in dichloromethane) to yield the title compound **222** as a colourless oil (25.0 mg, 49%). R_f 0.21 (6% methanol in dichloromethane); $[\alpha]_D^{25} = -12.3$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3317 (w), 3079 (w), 2936 (w), 2868 (w), 2360 (w), 2342 (w), 1695 (s), 1632 (s), 1543 (s), 1509 (m), 1465 (m), 1439 (m), 1399 (m), 1372 (m), 1347 (m), 1306 (m), 1271 (m), 1225 (m), 1195 (m), 1161 (m), 1142 (m), 1125 (m), 1085 (w), 1059 (w), 1029 (w), 968 (w), 845 (s), 774 (m), 731 (m), 651 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 8.91 (1H, s, H-2^V), 8.13 (1H, t, *J* 5.2, H-8^{IV}), 7.91 (1H, dd, *J* 7.8, 1.3, H-6^{VI}), 7.88 – 7.75 (3H, m, H-3^{VI}, H-4^{VI} and H-5^{VI}), 7.68 (1H, s, H-11^I), 7.45 – 7.34 (5H, m, H-2^{IV}, H-3^{IV}, H-5^{IV}, H-6^{IV} and H-19^I), 7.08 – 6.97 (3H, m, H-4, H-6 and H-7), 4.88 (2H, s, H-3^{II}), 4.57 – 4.46 (4H, m, H-1^I, H-20^I and H-2^{III}), 4.45 – 4.35 (2H, m, H-7^{IVa} and H-4^{III}), 4.31 – 4.22 (3H, m, H-7^{IVb} and H-13^I), 3.73 – 3.61 (2H, m, H-5^{III}), 3.34 – 3.20 (13H, m, O*H*, H-8, H-9, H-3^I, H-5^I and H-7^I), 2.55 (2H, t, *J* 7.5, H-9^I), 2.45 (3H, s, H-6^V), 2.24 (1H, dt, *J* 14.6, 7.5, H-17^{Ia}), 2.15 (1H, dt, *J* 14.6, 7.2, H-17^{Ib}), 2.02 (2H, q, *J* 6.9, 6.3, H-3^{III}), 1.86 – 1.74 (6H, m, H-14^I, H-4^I and H-8^I), 1.55 (2H, hept, *J* 6.5, H-16^I), 1.34 – 1.26 (2H, m, H-15^I), 0.95 (9H, s, H-22^I); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 171.5 (C-9^{IV}), 171.2 (C-23^I), 169.6 (C-18^I), 161.8 (C-1^{II}), 153.7 (C-2), 150.5 (C-2^V), 147.4 (C-4^V), 147.3 (C-2^{VI}), 145.2 (C-10^I), 139.0 (C-1^{IV}), 133.8 (C-5^{VI}), 131.7 (C-4^{VI}), 131.6 (C-1^{VI}), 130.7 (C-5^V), 129.8 (C-5), 129.7 (C-3a), 129.5 (C-7a),

129.2 (C-6^{VI}), 129.0 (C-4^{IV}), 128.2, 127.2 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 123.8 (C-3^{VI}), 121.0 (C-11^{I}), 120.1 (C-6), 106.9 (C-4), 106.5 (C-7), 95.7 (C-4^{II}), 74.1 (C-3^{II}), 68.5 (C-4^{III}), 58.3 (C-2^{III}), 56.2 (C-20^{I}), 55.7 (C-5^{III}), 50.2 (C-1^{I}), 48.7 (C-13^{I}), 46.7 (C-3^{I}), 46.4 (C-7^{I}), 45.0 (C-5^{I}), 41.5 (C-7^{IV}), 37.3 (C-3^{III}), 34.6 (C-17^{I}), 34.4 (C-21^{I}), 28.9 (C-8^{I}), 27.2 (C-14^{I}), 26.43, 26.38 (C-8 and C-9), 26.30 (C-4^{I}), 26.0 (C-22^{I}), 25.2 (C-15^{I}), 24.2 (C-16^{I}), 21.8 (C-9^{I}), 18.4, 15.3 (C-6^{V}); *m/z* (ESI$^+$) 1244 ([M+H]$^+$, 90%), 1246 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{55}H_{70}O_{11}N_{12}Cl_3S_2^+$ ([M+H]$^+$) requires 1243.37885 and 1245.37736, found 1243.37924 and 1245.37725; HPLC (method 1) retention time 7.48 min, 100%.

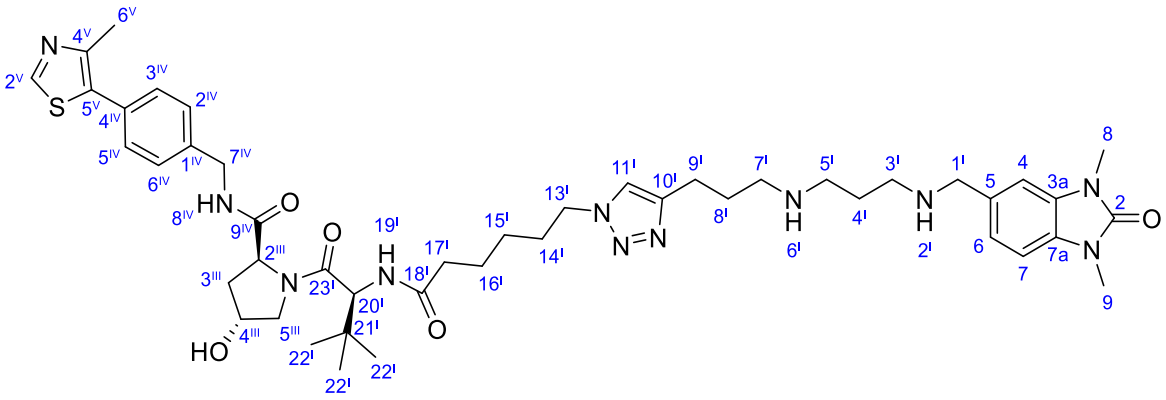
_N_-[18^{I}-(10^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]propyl}-1*H*-1,2,3-triazol-12^{I}-yl)hexanoyl]-21^{I}-methylvalyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (224)



Thiophenol (10 µL, 0.10 mmol, 5.0 eq.) and potassium carbonate (8.3 mg, 0.060 mmol, 3.0 eq.) were stirred for 5 minutes in degassed anhydrous *N*,*N*-dimethylformamide (0.3 mL). This solution was then added to a solution of **222** (25 mg, 0.020 mmol, 1.0 eq.) in degassed anhydrous *N*,*N*-dimethylformamide (0.2 mL). The resulting mixture was stirred at room temperature for 5 h, and then concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 5–10% methanol in dichloromethane then 1:2:4 ammonium hydroxide:ethanol:ethyl acetate) to yield the title compound **224** as a colourless oil (11 mg, 54%). R_f 0.09 (8% methanol in dichloromethane); $[\alpha]_D^{25} = -15.0$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin

film)/cm^{-1} 3422 (w), 2950 (w), 2868 (w), 2434 (w), 2341 (w), 1693 (s), 1632 (s), 1543 (w), 1509 (w), 1445 (m), 1399 (w), 1371 (w), 1354 (w), 1307 (w), 1271 (w), 1230 (w), 1193 (w), 1135 (w), 1087 (w), 1059 (w), 1031 (w), 966 (w), 938 (w), 845 (s), 772 (w), 757 (w), 745 (w), 717 (w), 686 (w), 640 (w), 623 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.87 (1H, s, H-2^{V}), 7.76 (1H, d, *J* 7.2, H-11^{I}), 7.48 – 7.37 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.18 – 7.06 (3H, m, H-4, H-6 and H-7), 4.92 (2H, d, *J* 9.2, H-3^{II}), 4.68 – 4.46 (6H, m, H-1^{I}, H-2^{III}, H-4^{III}, H-20^{I} and H-7^{IVa}), 4.39 – 4.31 (3H, m, H-7^{IVb} and H-13^{I}), 3.89 (1H, dt, *J* 11.2, 1.8, H-5^{IIIa}), 3.80 (1H, dd, *J* 10.9, 3.9, H-5^{IIIb}), 3.45 – 3.33 (8H, m, H-8, H-9 and H-3^{I}), 2.89 – 2.69 (6H, m, H-5^{I}, H-7^{I} and H-9^{I}), 2.47 (3H, s, H-6^{V}), 2.34 – 2.18 (3H, m, H-3^{IIIa} and H-17^{I}), 2.08 (1H, ddd, *J* 13.2, 9.1, 4.4, H-3^{IIIb}), 1.98 – 1.80 (6H, m, H-4^{I}, H-14^{I} and H-8^{I}), 1.64 (2H, p, *J* 7.1, H-16^{I}), 1.38 – 1.27 (2H, m, H-15^{I}), 1.02 (9H, s, H-22^{I}); ^{13}C NMR (126 MHz, CD_3OD) δ_C 175.7 (C-9^{IV}), 174.5 (C-23^{I}), 172.3 (C-18^{I}), 156.38 (C-2), 156.36 (C-1^{II}), 152.8 (C-2^{V}), 149.0 (C-4^{V}), 147.6 (C-10^{I}), 140.3 (C-1^{IV}), 133.4 (C-4^{IV}), 132.2 (C-5^{V}), 131.5 (C-5), 131.0 (C-3a), 130.9 (C-5a), 130.3, 129.0 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 123.4 (C-11^{I}), 122.7 (C-6), 109.0 (C-4), 108.7 (C-7), 97.1 (C-4^{II}), 76.3 (C-3^{II}), 71.1 (C-4^{III}), 60.8 (C-2^{III}), 59.0 (C-20^{I}), 58.0 (C-5^{III}), 51.7 (C-1^{I}), 51.2 (C-13^{I}), 47.3 (C-5^{I}), 46.7 (C-7^{I}), 45.5 (C-3^{I}), 43.7 (C-7^{IV}), 39.0 (C-3^{III}), 36.5 (C-17^{I}), 36.3 (C-21^{I}), 31.0 (C-8^{I}), 28.4 (C-4^{I}), 28.0 (C-14^{I}), 27.6, 27.5 (C-8 and C-9), 27.0 (C-22^{I}), 26.6 (C-15^{I}), 26.3 (C-16^{I}), 23.5 (C-9^{I}), 15.8 (C-6^{V}); *m/z* (ESI^{+}) 1059 ([M+H]$^{+}$, 90%), 1061 ([M+H]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{55}H_{70}O_{11}N_{12}Cl_3S_2^{+}$ ([M+H]$^{+}$) requires 1058.40057 and 1060.39883, found 1058.40015 and 1060.39783; HPLC (method 1) retention time 6.34 min, 93.9%.

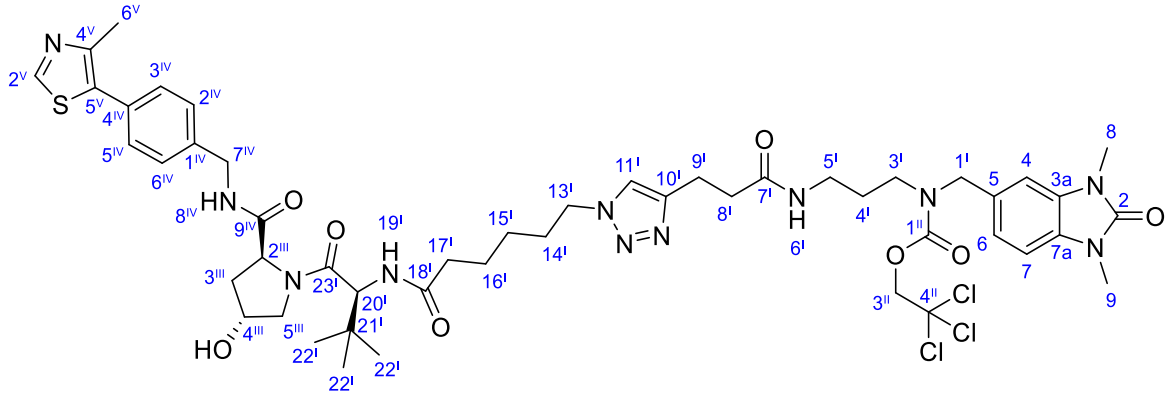
N-[18^{I}-(10^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl]amino}propyl)amino]propyl}-1H-1,2,3-triazol-12^{I}-yl)hexanoyl]-21^{I}-methylvalyl-(4R)-4^{III}-hydroxy-N-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (143)



224 (10 mg, 0.0095 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.5 mL), and acetic acid (22 µL) and zinc powder (6.2 mg, 0.095 mmol, 10.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 10% methanol in dichloromethane then 1:2:6 ammonium hydroxide:ethanol:ethyl acetate) to yield the title compound **143** as a colourless oil (5.7 mg, 68%). R_f 0.17 (1:2:6 ammonium hydroxide:ethanol:ethyl acetate); $[\alpha]_D^{25} = -10.4$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3301 (m), 3079 (w), 2930 (m), 2867 (m), 2361 (s), 2341 (s), 1690 (s), 1634 (s), 1539 (m), 1508 (m), 1446 (m), 1398 (w), 1374 (w), 1340 (w), 1319 (w), 1272 (w), 1241 (w), 1196 (w), 1133 (w), 1111 (w), 1086 (w), 1059 (w), 1036 (w), 1028 (w), 967 (w), 940 (w), 850 (w), 813 (w), 745 (w), 728 (w), 669 (w), 657 (w), 636 (w), 612 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.87 (1H, s, H-2^{V}), 7.72 (1H, s, H-11^{I}), 7.49 – 7.37 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.24 – 7.06 (3H, m, H-4, H-6 and H-7), 4.62 (1H, s, H-20^{I}), 4.60 – 4.47 (3H, m, H-2^{III}, H-4^{III} and H-7^{IVa}), 4.39 – 4.30 (3H, m, H-7^{IVb} and H-13^{I}), 3.92 – 3.86 (3H, m, H-1^{I} and H-5^{IIIa}), 3.80 (1H, dd, *J* 10.9, 3.9, H-5^{IIIb}), 3.40 (6H, s, H-8 and H-9), 2.88 – 2.73 (6H, m, H-3^{I}, H-5^{I} and H-7^{I}), 2.68 (2H, t, *J* 7.5, H-9^{I}), 2.47 (3H, s, H-6^{V}), 2.31 – 2.17 (3H, m, H-3^{IIIa} and H-17^{I}), 2.08

(1H, ddd, *J* 13.2, 9.1, 4.5, H-3^{IIIb}), 1.95 – 1.84 (4H, m, H-14^{I} and H-8^{I}), 1.83 – 1.76 (2H, m, H-4^{I}), 1.64 (2H, p, *J* 7.3, H-16^{I}), 1.36 – 1.27 (2H, m, H-15^{I}), 1.02 (9H, s, H-22^{I}); ^{13}C NMR (126 MHz, CD_3OD) δ_C 175.7 (C-9^{IV}), 174.5 (C-23^{I}), 172.3 (C-18^{I}), 156.4 (C-2), 152.9 (C-2^{V}), 149.0 (C-4^{V}), 147.9 (C-10^{I}), 140.3 (C-1^{IV}), 133.4 (C-4^{IV}), 131.5 (C-5^{V}), 131.4 (C-5), 130.4 (C-2^{IV} and C-6^{IV} or C-3^{IV} and C-5^{IV}), 129.9 (C-3a), 129.2 (C-7a), 129.0 (C-2^{IV} and C-6^{IV} or C-3^{IV} and C-5^{IV}), 123.5 (C-11^{I}), 123.2 (C-6), 109.4 (C-4), 108.9 (C-7), 71.1 (C-4^{III}), 60.8 (C-2^{III}), 59.0 (C-20^{I}), 58.0 (C-5^{III}), 54.1 (C-1^{I}), 51.1 (C-13^{I}), 49.0 (C-3^{I}), 48.9 (C-5^{I}), 48.0 (C-7^{I}), 43.7 (C-7^{IV}), 39.0 (C-3^{III}), 36.5 (C-17^{I}), 36.3 (C-21^{I}), 31.0 (C-8^{I}), 29.0 (C-14^{I}), 27.6 (C-4^{I}), 27.5 (C-8 and C-9), 27.0 (C-22^{I} and C-15^{I}), 26.3 (C-16^{I}), 23.7 (C-9^{I}), 15.8 (C-6^{V}); *m/z* (ESI$^+$) 884 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{46}H_{66}O_5N_{11}S^+$ ([M+H]$^+$) requires 884.49636, found 884.49585; HPLC (method 1) retention time 4.65 min, 91.2%.

_N_-[18^{I}-(10^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-7^{I}-oxopropyl}-1*H*-1,2,3-triazol-12^{I}-yl)hexanoyl]-21^{I}-methylvalyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (214)

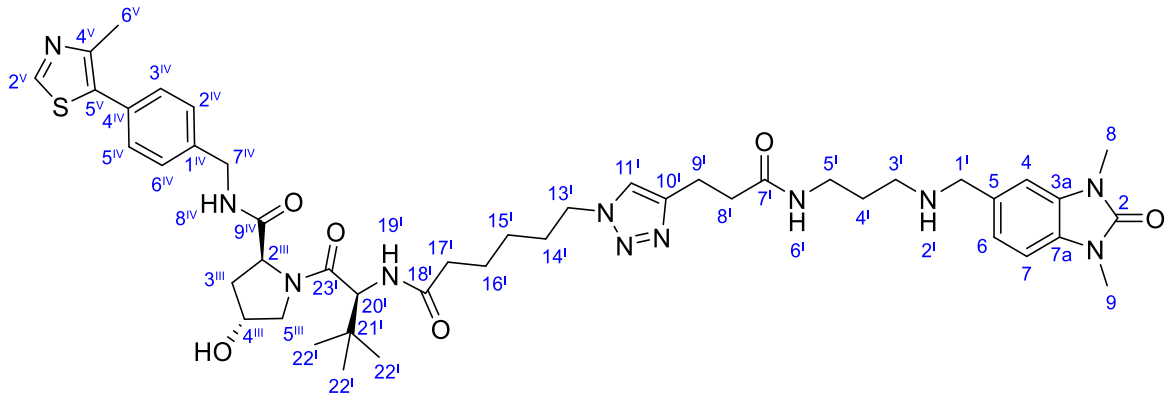


158 (220 mg, 0.415 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (2 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo*. The crude product was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the free amine as a yellow oil (162 mg, 91%). The product was then used immediately in the next step.

213 (45.0 mg, 0.0681 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (47.0 µL, 0.272 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL). HATU (28.5 mg, 0.0749 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The product from the above Boc deprotection (31.8 mg, 0.0681 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with water (20 mL) and extracted with ethyl acetate (4 × 20 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–10% methanol in dichloromethane) and further purified by silica gel column chromatography (5–10% methanol in dichloromethane) to yield the title compound **214** as a colourless oil (11.2 mg, 15%). R_f 0.04 (1:2:12 ammonium hydroxide:ethanol:ethyl acetate); $[\alpha]_D^{25} = -13.4$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3419 (w), 3073 (w), 2939 (w), 2867 (w), 2360 (w), 2342 (w), 2259 (w), 1693 (s), 1638 (s), 1537 (m), 1510 (m), 1466. (m), 1439 (m), 1399 (w), 1370 (w), 1336 (w), 1306 (w), 1271 (w), 1220 (w), 1194 (w), 1136 (w), 1087 (w), 1052 (w), 1026 (s), 1007 (s), 843 (s), 763 (w), 744 (w), 717 (w), 624 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.91 (1H, s, H-2^V), 8.16 (1H, t, *J* 5.2, H-8^{IV}), 7.67 (1H, s, H-11^I), 7.52 (1H, bs, H-19^I), 7.45 – 7.35 (5H, m, H-2^{IV}, H-3^{IV}, H-5^{IV}, H-6^{IV} and H-6^I), 7.09 – 6.98 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.83 (1H, s, O*H*), 4.59 – 4.45 (4H, m, H-1^I, H-2^{III} and H-20^I), 4.44 – 4.35 (2H, m, H-4^{III} and H-7^{IVa}), 4.33 – 4.19 (3H, m, H-7^{IVb} and H-13^I), 3.74 – 3.61 (2H, m, H-5^{III}), 3.36 – 3.23 (8H, m, H-8, H-9 and H-3^I), 3.10 – 3.02 (2H, m, partially obscured by residual water peak, H-5^I), 2.83 (2H, t, *J* 7.7, H-9^I), 2.45 (3H, s, H-6^V), 2.41 (2H, t, *J* 7.7, H-8^I), 2.27 – 2.19 (1H, m, H-17^{Ia}), 2.15 (1H, dt, *J* 14.4, 7.3, H-17^{Ib}), 2.07 – 1.96 (2H, m, H-3^{III}), 1.80 (2H, p, *J* 7.3, H-14^I), 1.74 – 1.62 (2H, m, H-4^I), 1.60 – 1.48 (2H, m, H-16^I), 1.33 – 1.20 (2H, m, H-15^I), 0.95 (9H, s, H-22^I); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.5 (C-9^{IV}), 171.3 (C-23^I), 170.7 (C-7^I), 169.6 (C-18^I), 153.8 (C-2), 153.5 (C-1^{II}), 150.6 (C-2^V), 147.4 (C-4^V), 145.6 (C-10^I), 139.0 (C-1^{IV}), 130.7 (C-4^{IV}), 130.0 (C-5^V), 129.7 (C-5), 129.5 (C-3a), 129.0 (C-7a), 128.3, 127.2 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 121.0 (C-11^I), 120.1 (C-6), 106.9 (C-4), 106.5 (C-7), 95.8 (C-4^{II}), 74.1 (C-3^{II}), 68.5 (C-4^{III}), 58.4 (C-2^{III}), 56.2 (C-20^I), 55.7 (C-5^{III}), 50.0

(C-1^{I}), 48.7 (C-13^{I}), 44.3 (C-3^{I}), 41.5 (C-7^{IV}), 37.4 (C-3^{III}), 35.9 (C-5^{I}), 34.7 (C-8^{I}), 34.6 (C-21^{I}), 34.5 (C-17^{I}), 28.9 (C-14^{I}), 27.4 (C-4^{I}), 26.5, 26.4 (C-8 and C-9), 26.0 (C-22^{I}), 25.2 (C-15^{I}), 24.3 (C-16^{I}), 21.0 (C-9^{I}), 15.4 (C-6^{V}); *m/z* (ESI^{+}) 1073 ([M+H]$^{+}$, 40%), 1075 ([M+H]$^{+}$, 50%), 1095 ([M+Na]$^{+}$, 90%, 1097 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{49}H_{64}O_8N_{11}Cl_3S^{+}$ ([M+H]$^{+}$) requires 1072.38093 and 1074.37917, found 1072.38004 and 1074.37774; HPLC (method 1) retention time 6.98 min, 93.9%.

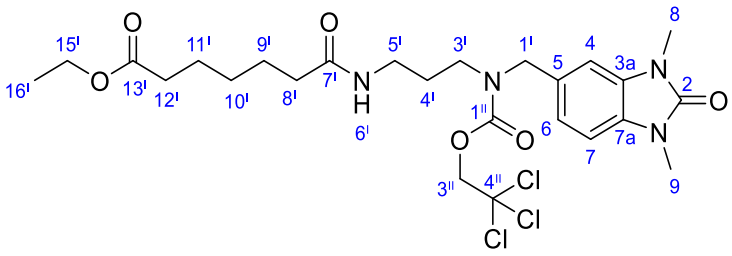
_N_-[18^{I}-(10^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)amino]-7^{I}-oxopropyl}-1*H*-1,2,3-triazol-12^{I}-yl)hexanoyl]-21^{I}-methylvalyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (149)



214 (14 mg, 0.019 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.7 mL), and acetic acid (43 µL) and zinc powder (12 mg, 0.186 mmol, 10.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 3–10% methanol in dichloromethane then 1:2:6 ammonium hydroxide:ethanol:ethyl acetate) to yield the title compound **149** as a colourless oil (6.6 mg, 40%). R_f 0.37 (1% ammonium hydroxide, 10% methanol in dichloromethane); $[\alpha]_D^{25}$= −26.0 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3391 (w), 3296 (w), 3077 (w), 2937 (w), 2868 (w), 2431 (w), 1632 (s), 1547 (m), 1509 (m), 1446 (m), 1398 (m), 1372. (m), 1343 (w), 1271 (w), 1239 (w), 1195 (w), 1139 (w), 1086 (w), 1058 (w), 1030 (w), 967 (w), 939 (w), 852 (w), 808 (w),

744 (w), 728 (w), 700 (w), 657 (w), 618 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.87 (1H, s, H-2^{V}), 7.69 (1H, s, H-11^{I}), 7.48 – 7.37 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.18 – 7.06 (3H, m, H-4, H-6 and H-7), 4.62 (1H, s, H-20^{I}), 4.59 – 4.47 (3H, m, H-2^{III}, H-4^{III} and H-7^{IVa}), 4.38 – 4.30 (3H, m, H-7^{IVb} and H-13^{I}), 3.90 (1H, dt, *J* 11.3, 1.8, H-5^{IIIa}), 3.82 – 3.76 (3H, m, H-5^{IIIb} and H-1^{I}), 3.42 (3H, s, H-8 or H-9), 3.39 (3H, s, H-8 or H-9), 3.21 (2H, t, *J* 6.7, H-5^{I}), 2.92 (2H, t, *J* 7.5, H-9^{I}), 2.58 (2H, t, *J* 7.2, H-3^{I}), 2.50 (2H, t, *J* 7.5, H-8^{I}), 2.46 (3H, s, H-6^{V}), 2.32 – 2.17 (3H, m, H-3^{IIIa} and H-17^{I}), 2.08 (1H, ddd, *J* 13.3, 9.1, 4.5, H-3^{IIIb}), 1.91 – 1.82 (2H, m, H-14^{I}), 1.73 – 1.57 (4H, m, H-16^{I} and H-4^{I}), 1.34 – 1.24 (2H, m, H-15^{I}), 1.02 (9H, s, H-22^{I}); ^{13}C NMR (126 MHz, CD_3OD) δ_C 175.6 (C-9^{IV}), 174.7 (C-23^{I}), 174.5 (C-7^{I}), 172.3 (C-18^{I}), 156.4 (C-2), 152.8 (C-2^{V}), 149.0 (C-4^{V}), 147.6 (C-10^{I}), 140.3 (C-1^{IV}), 133.9 (C-4^{IV}), 133.4 (C-5^{V}), 131.5 (C-5), 131.3 (C-3a), 130.5 (C-7a), 130.4, 129.0 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 123.4 (C-6 and C-11^{I}), 109.3 (C-4), 108.8 (C-7), 71.1 (C-4^{III}), 60.8 (C-2^{III}), 59.0 (C-20^{I}), 58.0 (C-5^{III}), 54.5 (C-1^{I}), 51.1 (C-13^{I}), 46.8 (C-3^{I}), 43.7 (C-7^{IV}), 39.0 (C-3^{III}), 38.0 (C-5^{I}), 36.5 (C-8^{I}), 36.4 (C-17^{I}), 36.2 (C-21^{I}), 30.9 (C-14^{I}), 30.0 (C-4^{I}), 27.50, 27.49 (C-8 and C-9), 27.03 (C-22^{I}), 27.00 (C-15^{I}), 26.2 (C-16^{I}), 22.6 (C-9^{I}), 15.8 (C-6^{V}); *m/z* (ESI$^+$) 922 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{46}H_{64}O_6N_{11}S^+$ ([M+H]$^+$) requires 898.47563, found 898.47528; HPLC (method 1) retention time 5.14 min, 100%.

Ethyl 7^{I}-[(2^{I}-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-13^{I}-oxoheptanoate (203)

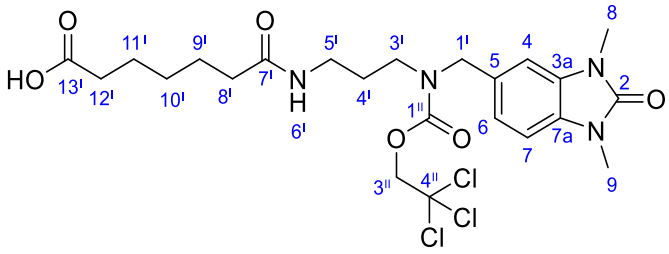


163 (1.00 g, 1.91 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (5 mL) and stirred at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo*. The crude residue was passed through an Isolute® SXC-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the deprotected amine product 3'',3'',3''-trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (877 mg, 100%). This compound was then used directly in the next step to avoid Troc protecting group migration to the terminal primary amine.

7-Ethoxy-7-oxo-heptanoic acid (191 mg, 1.02 mmol, 1.1 eq.) and *N*,*N*-diisopropylethylamine (525 µL, 4.06 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (5 mL). HATU (424 mg, 1.12 mmol, 1.2 eq.) was added and the solution was stirred at room temperature for 10 min. 3'',3'',3''-Trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (400 mg, 0.944 mmol, 1.0 eq.) in *N*,*N*-dimethylformamide (5 mL) was added, and the resulting solution was stirred at room temperature for 22 h. Subsequently, the reaction mixture was quenched with water (40 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (50 mL) and brine (50 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (60–100% ethyl acetate in heptane) to yield the title compound **203** as a colourless oil (429 mg, 77%). R_f 0.35 (6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3305 (w), 2941 (w), 2867 (w), 1706 (s), 1651 (m), 1542 (w), 1510 (m), 1467 (m), 1444 (m), 1396 (w), 1353 (w), 1300 (w), 1268 (w), 1216 (m), 1192 (w),

1134 (m), 1062 (w), 1029 (w), 844 (s), 771 (w), 757 (w), 745 (w), 718 (w), 681 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 7.35 (1H, s, H-6^{I}), 7.08 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.56 (2H, s, H-1^{I}), 4.07 (2H, q, *J* 7.2, H-15^{I}), 3.36 – 3.24 (8H, m, H-8, H-9 and H-3^{I}), 3.06 (2H, q, *J* 6.5, H-5^{I}), 2.24 (2H, t, *J* 7.4, H-12^{I}), 2.04 (2H, t, *J* 7.4, H-8^{I}), 1.71 (2H, p, *J* 7.1, H-4^{I}), 1.57 – 1.45 (4H, m, H-10^{I} and H-11^{I}), 1.35 – 1.22 (2H, m, H-9^{I}), 1.19 (3H, t, *J* 7.2, H-16^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 172.1 (C-7^{I}), 171.4 (C-13^{I}), 153.7 (C-2), 153.5 (C-1^{II}), 129.9 (C-5), 129.7 (C-3a), 128.9 (C-7a), 119.9 (C-6), 106.8 (C-4), 106.3 (C-7), 95.8 (C-4^{II}), 74.1 (C-3^{II}), 58.9 (C-15^{I}), 50.0 (C-1^{I}), 44.3 (C-3^{I}), 35.8 (C-5^{I}), 34.9 (C-8^{I}), 33.1 (C-12^{I}), 27.7 (C-9^{I}), 27.5 (C-4^{I}), 26.38, 26.35 (C-8 and C-9), 24.3 (C-11^{I}), 23.7 (C-10^{I}), 13.5 (C-16^{I}); *m/z* (ESI^{+}) 593 ([M+H]$^{+}$, 90%), 595 ([M+H]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{25}H_{36}O_6N_4Cl_3^{+}$ ([M+H]$^{+}$) requires 593.1695 and 595.1668, found 593.1696 and 595.1667; HPLC (method 2) retention time 0.84 min, 100%.

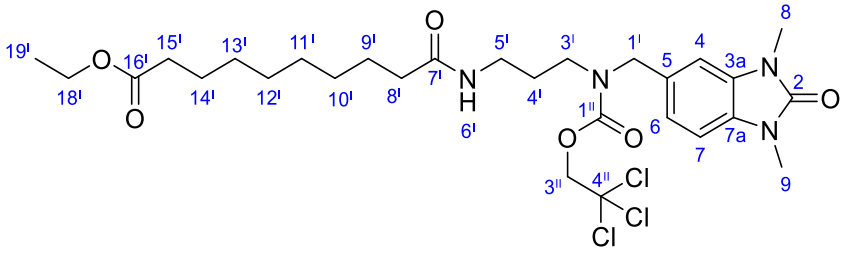
7^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II}, 4^{II}, 4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-13^{I}-oxoheptanoic acid (205)



203 (387 mg, 0.651 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (3 mL) and methanol (3 mL). A solution of lithium hydroxide (156 mg, 6.51 mmol, 10.0 eq.) in water (3 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 1 h. The solution was then acidified with 1 M aqueous hydrochloric acid (10 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% AcOH, 0–5% methanol in dichloromethane) to yield the title compound **205** as a colourless oil (296 mg, 80%). R_f 0.74 (1% AcOH, 6% methanol in dichloromethane); $\bar{\nu}_{max}$

(thin film)/cm^{-1} 3315 (w), 2943 (w), 2868 (w), 2360 (w), 2341 (w), 1704 (s), 1558 (w), 1509 (w), 1467 (w), 1397 (w), 1355 (w), 1299 (w), 1269 (w), 1218 (w), 1193 (w), 1137 (w), 1060 (w), 958 (w), 913 (w), 839 (w), 771 (w), 757 (w), 744 (w), 717 (w), 669 (w), 646 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 11.51 (1H, s, COO*H*), 7.37 (1H, s, H-6^{I}), 7.09 – 6.98 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.55 (2H, s, H-1^{I}), 3.35 – 3.25 (8H, m, H-8, H-9 and H-3^{I}), 3.06 (2H, q, *J* 6.6, H-5^{I}), 2.18 (2H, t, *J* 7.4, H-12^{I}), 2.04 (2H, t, *J* 7.4, H-8^{I}), 1.70 (2H, p, *J* 7.3, H-4^{I}), 1.56 – 1.45 (4H, m, H-10^{I} and H-11^{I}), 1.34 – 1.24 (2H, m, H-9^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 173.5 (C-13^{I}), 171.5 (C-7^{I}), 153.7 (C-2), 153.5 (C-1^{II}), 129.9 (C-5), 129.7 (C-3a), 128.9 (C-7a), 119.9 (C-6), 106.8 (C-4), 106.4 (C-7), 95.8 (C-4^{II}), 74.1 (C-3^{II}), 50.0 (C-1^{I}), 44.3 (C-3^{I}), 35.8 (C-5^{I}), 34.9 (C-8^{I}), 33.2 (C-12^{I}), 27.8 (C-9^{I}), 27.5 (C-4^{I}), 26.41, 26.37 (C-8 and C-9), 24.4 (C-11^{I}), 23.8 (C-10^{I}); *m/z* (ESI^{+}) 587 ([M+Na]$^{+}$, 90%), 589 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{23}H_{32}O_6N_4Cl_3^{+}$ ([M+H]$^{+}$) requires 565.1382 and 567.1354, found 565.1380 and 567.1351; HPLC (method 2) retention time 0.53 min, 100%.

Ethyl 7^{I}-[(2^{I}-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II}, 4^{II}, 4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-16^{I}-oxodecanoate (204)



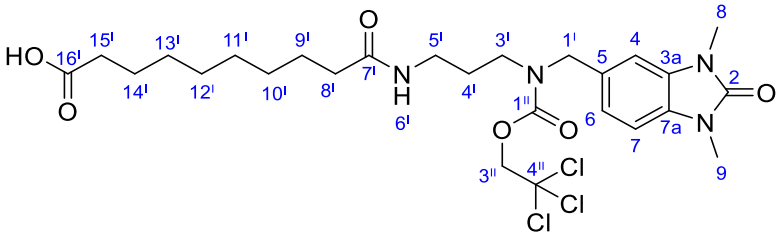
163 (1.00 g, 1.91 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (5 mL) and stirred at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo*. The crude residue was passed through an Isolute® SXC-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the deprotected amine product 3'',3'',3''-trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (877 mg, 100%). This compound was

then used directly in the next step to avoid Troc protecting group migration to the terminal primary amine.

10-Ethoxy-10-oxo-decanoic acid (141 mg, 0.614 mmol, 1.1 eq.) and *N*,*N*-diisopropylethylamine (398 µL, 2.29 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (4 mL). HATU (257 mg, 0.675 mmol, 1.2 eq.) was added and the solution was stirred at room temperature for 10 min. 3'',3'',3''-Trichloroethyl (3'-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (263 mg, 0.571 mmol, 1.0 eq.) in *N*,*N*-dimethylformamide (4 mL) was added, and the resulting solution was stirred at room temperature for 22 h. Subsequently, the reaction mixture was quenched with water (40 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic components were washed with 0.5 M aqueous lithium chloride solution (50 mL) and brine (50 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–5% methanol in dichloromethane) to yield the title compound **204** as a colourless oil (297 mg, 80%). R_f 0.60 (5% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3408 (w), 3300 (w),2931 (w), 2857 (w), 2255 (w), 2128 (w), 1702 (s), 1656 (w), 1546 (w), 1509 (w), 1467 (w), 1444 (w), 1428 (w), 1396 (w), 1353 (w), 1300 (w), 1267 (w), 1216 (w), 1191 (w), 1131 (w), 1051 (s), 1025 (s), 1005 (s), 846 (w), 824 (m), 761 (m), 716 (m), 625 (m); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.34 (1H, s, H-6^I), 7.08 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.55 (2H, s, H-1^I), 4.07 (2H, q, *J* 7.0, H-18^I), 3.37 – 3.24 (8H, m, H-8, H-9 and H-3^I), 3.06 (2H, q, *J* 6.3, H-5^I), 2.25 (2H, t, *J* 7.3, H-15^I), 2.03 (2H, t, *J* 7.4, H-8^I), 1.71 (2H, p, *J* 7.2, H-4^I), 1.58 – 1.44 (4H, m, H-9^I and H-14^I), 1.32 – 1.22 (8H, m, H-10^I, H-11^I, H-12^I and H-13^I), 1.19 (3H, t, *J* 7.1, H-19^I); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 172.3 (C-7^I), 171.5 (C-16^I), 153.7 (C-2), 153.5 (C-1^{II}), 129.9 (C-5), 129.7 (C-3a), 128.9 (C-7a), 119.9 (C-6), 106.8 (C-4), 106.4 (C-7), 95.7 (C-4^{II}), 74.1 (C-3^{II}), 58.9 (C-18^I), 50.0 (C-1^I), 44.3 (C-3^I), 35.8 (C-5^I), 35.1 (C-8^I), 33.2 (C-15^I), 28.1, 28.0, 27.89, 27.85 (C-10^I, C-11^I, C-12^I and C-13^I), 27.5 (C-4^I), 26.4, 26.3 (C-8 and C-9), 24.6 (C-9^I), 23.9 (C-14^I), 13.5 (C-19^I); *m/z* (ESI$^+$) 657 ([M+Na]$^+$, 90%), 659 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$)

$C_{28}H_{41}O_6N_4Cl_3^+$ ([M+H]$^+$) requires 635.21404 and 637.21140, found 635.21607 and 637.21318; HPLC (method 2) retention time 0.94 min, 98%.

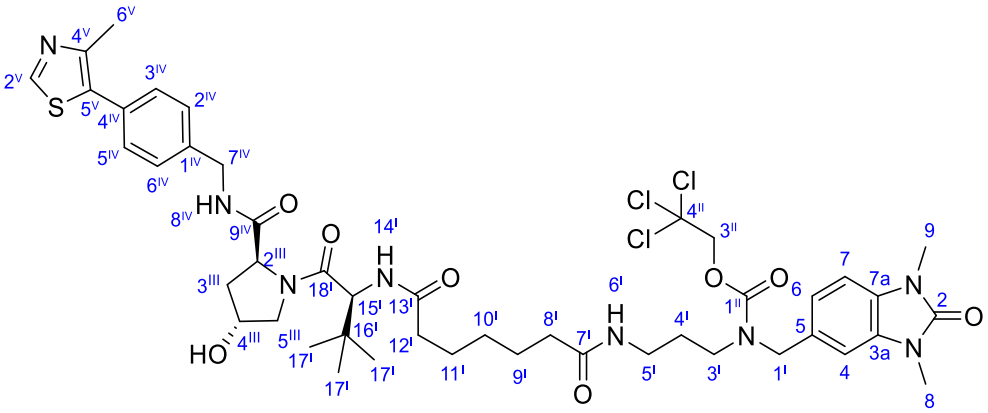
7^{I}-[(2^{I}-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II}, 4^{II}, 4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-16^{I}-oxodecanoic acid (206)



204 (260 mg, 0.409 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (2 mL) and methanol (2 mL). A solution of lithium hydroxide (97.9 mg, 4.09 mmol, 10.0 eq.) in water (2 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 1 h. The solution was then acidified with 1 M aqueous hydrochloric acid (7 mL) and extracted with ethyl acetate (3 × 40 mL). The combined organic components were washed with brine (20 mL), dried over sodium sulfate and concentrated *in vacuo.* The crude residue was purified by silica gel column chromatography (1% AcOH, 0–10% methanol in dichloromethane) to yield the title compound **206** as a colourless oil (215 mg, 84%). R_f 0.71 (1% AcOH, 6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3328 (w), 3159 (w), 2926 (w), 2850 (w), 2160 (w), 2029 (w), 1730 (s), 1705 (s), 1683 (s), 1635 (s), 1537 (m), 1509 (m), 1466 (m), 1446 (m), 1428 (m), 1398 (m), 1381 (w), 1352 (w), 1305 (w), 1272 (m), 1225 (s), 1189 (m), 1170 (m), 1134 (s), 1121 (m), 1088 (w), 1054 (w), 1040 (w), 1028 (w), 1010 (w), 967 (w), 930 (w), 868 (w), 856 (w), 831 (s), 817 (m), 776 (w), 757 (w), 740 (w), 730 (w), 713 (s), 683 (w), 659 (w), 634 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 11.49 (1H, s, COO*H*), 7.34 (1H, s, H-6^{I}), 7.10 – 6.97 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.55 (2H, s, H-1^{I}), 3.36 – 3.25 (8H, m, H-8, H-9 and H-3^{I}), 3.06 (2H, q, *J* 6.5, H-5^{I}), 2.18 (2H, t, *J* 7.3, H-15^{I}), 2.03 (2H, t, *J* 7.4, H-8^{I}), 1.71 (2H, p, *J* 7.0, H-4^{I}), 1.56 – 1.44 (4H, m, H-9^{I} and H-14^{I}), 1.33 – 1.20 (8H, m, H-10^{I}, H-11^{I}, H-12^{I} and H-13^{I}); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ_C 173.5 (C-16^{I}), 171.6 (C-7^{I}), 153.7 (C-2), 153.5 (C-1^{II}), 129.9 (C-5), 129.7 (C-3a), 128.9 (C-7a), 119.9 (C-6), 106.8 (C-4), 106.4 (C-7), 95.8 (C-4^{II}), 74.1 (C-3^{II}), 50.0 (C-1^{I}), 44.3 (C-3^{I}),

35.8 (C-5^{I}), 35.1 (C-8^{I}), 33.3 (C-15^{I}), 28.1, 28.02, 28.00, 27.98 (C-10^{I}, C-11^{I}, C-12^{I} and C-13^{I}), 27.5 (C-4^{I}), 26.39, 26.35 (C-8 and C-9), 24.7 (C-9^{I}), 24.0 (C-14^{I}); *m/z* (ESI^{+}) 629 ([M+Na]$^{+}$, 90%), 631 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{26}H_{37}O_6N_4Cl_3^{+}$ ([M+H]$^{+}$) requires 607.18274 and 609.18005, found 607.18505 and 609.18220; HPLC (method 2) retention time 0.57 min, 97%.

_N_-{7^{I}-[(2^{I}-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-13^{I}-oxoheptanoyl}-16^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (207)

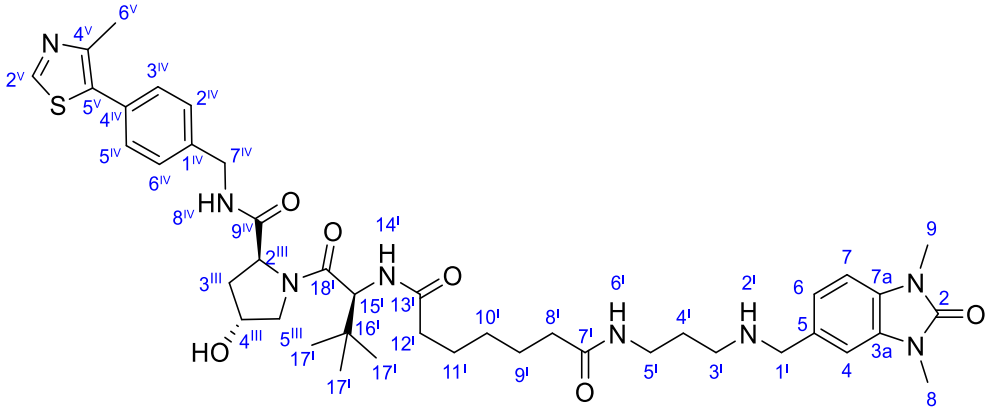


158 (220 mg, 0.415 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (2 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo*. The crude product was purified using Amine Isolute™ Catch and Release SXC-2 column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the free amine as a yellow oil (162 mg, 91%). The product was then used immediately in the next step.

205 (220 mg, 0.389 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (271 µL, 1.56 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (2 mL). HATU (163 mg, 0.428 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The product from the above Boc deprotection (182 mg, 0.389 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (2 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with water (40 mL) and extracted with ethyl acetate (4 × 40 mL). The combined organic components were washed with

brine (30 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–10% methanol in dichloromethane) to yield the title compound **207** as a colourless oil (141 mg, 36%). R_f 0.60 (5% methanol in dichloromethane); $[\alpha]_D^{25} = -9.0$ (*c* 1.0, $CHCl_3$); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3311 (m), 3072 (w), 2957 (m), 1628 (s), 1537 (m), 1480 (m), 1437 (m), 1419 (m), 1372 (w), 1328 (w), 1276 (w), 1236 (w), 1198 (w), 1123 (w), 1088 (w), 1061 (w), 1033 (w), 967 (w), 938 (w), 852 (w), 802 (w), 754 (m), 717 (w), 664 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.90 (1H, s, H-2^V), 8.11 (1H, s, H-8^{IV}), 7.43 – 7.29 (6H, m, H-2^{IV}, H-3^{IV}, H-5^{IV}, H-6^{IV}, H-14^I and H-6^I), 7.07 – 6.99 (3H, m, H-4, H-6 and H-7), 4.89 (2H, s, H-3^{II}), 4.78 (1H, s, O*H*), 4.58 – 4.45 (4H, m, H-1^I, H-2^{III} and H-15^I), 4.44 – 4.35 (2H, m, H-4^{III} and H-7^{IVa}), 4.28 (1H, dd, *J* 15.4, 5.7, H-7^{IVb}), 3.74 – 3.62 (2H, m, H-5^{III}), 3.34 – 3.24 (8H, m, H-8, H-9 and H-3^I), 3.05 (2H, q, *J* 6.5, H-5^I), 2.45 (3H, s, H-6^V), 2.23 (1H, ddd, *J* 14.8, 7.4, 7.4, H-3^{IIIa}), 2.13 (1H, ddd, *J* 14.8, 7.4, 7.4, H-3^{IIIb}), 2.06 – 1.96 (4H, m, H-8^I and H-12^I), 1.70 (2H, p, *J* 7.0, H-4^I), 1.56 – 1.43 (4H, m, H-10^I and H-11^I), 1.27 (2H, p, *J* 7.6, H-9^I), 0.95 (9H, s, H-17^I); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.5 (C-9^{IV}), 171.18 (C-18^I), 171.16 (C-7^I), 169.6 (C-13^I), 153.7 (C-2), 153.5 (C-1^{II}), 150.4 (C-2^V), 147.4 (C-4^V), 138.9 (C-1^{IV}), 130.7 (C-4^{IV}), 129.9 (C-5^V), 129.7 (C-5), 129.5 (C-3a), 128.9 (C-7a), 128.2, 127.1 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 120.0 (C-6), 106.8 (C-4), 106.4 (C-7), 95.8 (C-4^{II}), 74.1 (C-3^{II}), 68.5 (C-4^{III}), 58.3 (C-2^{III}), 56.2 (C-15^I), 55.6 (C-5^{III}), 50.0 (C-1^I), 44.3 (C-3^I), 41.51 (C-7^{IV}), 41.46 (C-16^I), 37.3 (C-8^I), 35.8 (C-5^I), 35.0 (C-12^I), 34.6 (C-3^{III}), 27.9 (C-9^I), 27.4 (C-4^I), 26.39, 26.35 (C-8 and C-9), 26.0 (C-17^I), 24.6, 24.5 (C-10^I and C-11^I), 15.2 (C-6^V); *m/z* (ESI$^+$) 978 ([M+H]$^+$, 90%), 980 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{45}H_{60}O_8N_8Cl_3S^+$ ([M+H]$^+$) requires 977.33149 and 979.32956, found 977.33118 and 979.32904; HPLC (method 2) retention time 0.80 min, 96%.

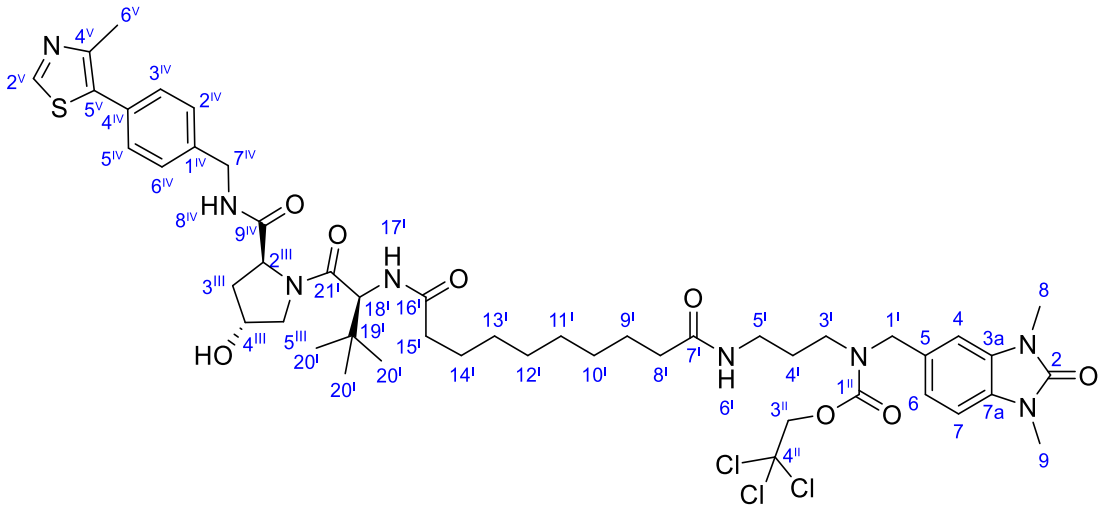
_N_-{7^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)amino]-13^{I}-oxoheptanoyl}-16^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-_N_-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (147)



207 (110 mg, 0.112 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (1 mL), and acetic acid (95.8 µL) and zinc powder (110 mg, 1.69 mmol, 15.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 4–10% methanol in dichloromethane) to yield the title compound **147** as a colourless oil (10.2 mg, 11%). R_f 0.27 (1% ammonium hydroxide, 10% methanol in dichloromethane); $[\alpha]_D^{25} = -13.9$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3408 (m), 3297 (m), 3073 (w), 2934 (m), 2868 (w), 2361 (m), 2342 (m), 1689 (s), 1634 (s), 1541 (m), 1509 (m), 1447 (m), 1399 (w), 1371 (w), 1340 (w), 1316 (w), 1271 (w), 1241 (w), 1195 (w), 1141 (w), 1086 (w), 1063 (w), 1030 (w), 967 (w), 939 (w), 853 (w), 810 (w), 745 (w), 728 (w), 669 (w), 655 (w), 624 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.67 (1H, s, H-2^{V}), 7.41 – 7.30 (5H, m, H-2^{IV}, H-3^{IV}, H-5^{IV}, H-6^{IV} and H-8^{IV}), 7.07 – 6.99 (2H, m, H-4 and H-6), 6.91 (1H, d, *J* 7.9, H-7), 6.78 (1H, t, *J* 5.3, H-6^{I}), 6.25 (1H, d, *J* 8.8, H-14^{I}), 4.70 (1H, t, *J* 8.0, H-2^{III}), 4.59 – 4.45 (3H, m, H-4^{III}, H-15^{I} and H-7^{IVa}), 4.34 (1H, dd, *J* 15.0, 5.3, H-7^{IVb}), 3.99 (1H, d, *J* 11.3, H-5^{IIIa}), 3.85 (2H, s, H-1^{I}), 3.58 (1H, dd, *J* 11.3, 3.6, H-5^{IIIb}), 3.42 – 3.37 (6H, m, H-8 and H-9), 3.35 – 3.24 (2H, m, H-5^{I}), 2.76 (2H, t, *J* 6.5, H-3^{I}), 2.52 – 2.42 (4H, m, H-6^{V} and H-3^{IIIa}), 2.22 – 2.03 (5H, m, H-3^{IIIb}, H-8^{I} and H-12^{I}), 1.73 (2H, p, *J* 6.5, H-4^{I}), 1.62 – 1.45 (4H, m, H-10^{I} and H-11^{I}), 1.31 – 1.18 (2H, m, H-9^{I}),

0.92 (9H, s, H-17^{I}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 173.6 (C-9^{IV}), 173.5 (C-18^{I}), 172.0 (C-7^{I}), 171.0 (C-13^{I}), 155.0 (C-2), 150.4 (C-2^{V}), 148.7 (C-4^{V}), 138.3 (C-1^{IV}), 131.7 (C-4^{IV}), 131.1 (C-5^{V}), 130.5 (C-5), 130.2 (C-3a), 129.70 (C-7a), 129.65, 128.3 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 121.8 (C-6), 107.9 (C-4), 107.3 (C-7), 70.1 (C-4^{III}), 58.8 (C-2^{III}), 57.6 (C-15^{I}), 57.1 (C-5^{III}), 54.0 (C-1^{I}), 47.2 (C-3^{I}), 43.4 (C-7^{IV}), 38.3 (C-5^{I}), 36.4 (C-8^{I}), 36.3 (C-3^{III}), 36.0 (C-12^{I}), 35.4 (C-16^{I}), 28.6 (C-4^{I}), 28.5 (C-9^{I}), 27.41, 27.40 (C-8 and C-9), 26.6 (C-17^{I}), 25.1, 24.9 (C-10^{I} and C-11^{I}), 16.2 (C-6^{V}); *m/z* (ESI^{+}) 803 ([M+H]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{42}H_{59}O_6N_8S^{+}$ ([M+H]$^{+}$) requires 803.4273, found 803.4261; HPLC (method 3) retention time 1.52 min, 95%.

_N_-{7^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-16^{I}-oxodecanoyl}-19^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (208)



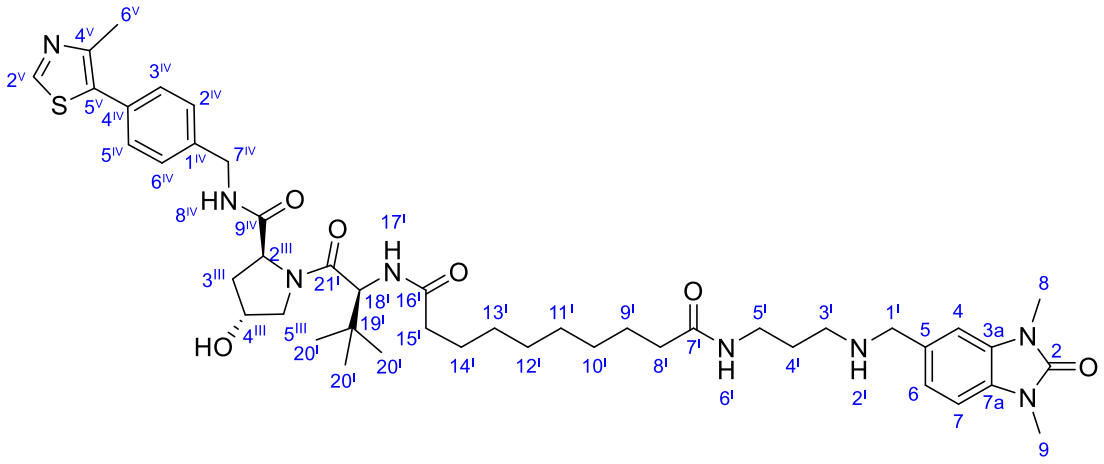
158 (670 mg, 1.26 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (3.16 mL), and the reaction mixture was stirred at room temperature for 1 h. The solution was concentrated *in vacuo* to yield the hydrochloric acid salt of the amine as a yellow solid (589 mg, 100%). The product was then used immediately in the next step.

206 (170 mg, 0.280 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (195 µL, 1.12 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (2 mL). HATU (117 mg, 0.308 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. The

product from the above Boc deprotection (131 mg, 0.280 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (2 mL) was added to the solution and the reaction mixture was stirred at room temperature for 22 h. After this time, the mixture was quenched with water (40 mL) and extracted with ethyl acetate (4 × 50 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–10% methanol in dichloromethane) to yield the title compound **208** as a colourless oil (92.0 mg, 32%). R_f 0.64 (8% methanol in dichloromethane); $[\alpha]_D^{25} = -7.0$ (*c* 1.0, $CHCl_3$); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3309 (m), 3071 (w), 2932 (m), 2857 (w), 1698 (s), 1636 (s), 1538 (m), 1509 (m), 1466 (m), 1439 (m), 1397 (w), 1371 (w), 1302 (w), 1270 (w), 1220 (w), 1135 (w), 1088 (w), 1061 (w), 1030 (w), 966 (w), 938 (w), 851 (w), 813 (w), 754 (m), 718 (w), 665 (w), 625 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.97 (1H, s, H-2^V), 8.54 (1H, t, *J* 6.1, H-8^{IV}), 7.80 (1H, d, *J* 9.3, H-17^I), 7.70 (1H, t, *J* 5.7, H-6^I), 7.44 – 7.35 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.11 – 6.96 (3H, m, H-4, H-6 and H-7), 5.10 (1H, d, *J* 3.6, O*H*), 4.91 (2H, d, *J* 9.3, H-3^{II}), 4.57 – 4.48 (3H, m, H-1^I and H-18^I), 4.46 – 4.39 (2H, m, H-2^{III} and H-7^{IVa}), 4.35 (1H, bs, H-4^{III}), 4.22 (1H, dd, *J* 15.9, 5.5, H-7^{IVb}), 3.70 – 3.60 (2H, m, H-5^{III}), 3.33 – 3.26 (6H, m, partially obscured by residual water peak, H-8 and H-9), 3.20 (2H, q, *J* 7.9, H-3^I), 3.01 (2H, d, *J* 6.4, H-5^I), 2.44 (3H, s, H-6^V), 2.28 – 2.20 (1H, m, H-15^{Ia}), 2.13 – 1.95 (4H, m, H-3^{IIIa}, H-15^{Ib} and H-8^I), 1.90 (1H, ddd, *J* 12.9, 8.5, 4.7, H-3^{IIIb}), 1.72 – 1.56 (2H, m, H-4^I), 1.53 – 1.36 (4H, m, H-9^I and H-14^I), 1.29 – 1.12 (8H, m, H-10^I, H-11^I, H-12^I and H-13^I), 0.93 (9H, s, H-20^I); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 175.0 (C-9^{IV}), 171.7 (C-21^I), 171.6 (C-7^I), 169.6 (C-16^I), 153.7 (C-2), 153.5 (C-1^{II}), 150.5 (C-2^V), 147.4 (C-4^V), 138.9 (C-1^{IV}), 130.7 (C-4^{IV}), 130.0 (C-5^V), 129.7 (C-5), 129.5 (C-3a), 129.0 (C-7a), 128.2, 127.2 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 120.0 (C-6), 106.9 (C-4), 106.4 (C-7), 95.8 (C-4^{II}), 74.2 (C-3^{II}), 68.5 (C-4^{III}), 58.3 (C-2^{III}), 56.2 (C-18^I), 55.6 (C-5^{III}), 50.0 (C-1^I), 44.3 (C-3^I), 41.5 (C-7^{IV}), 37.3 (C-8^I), 35.8 (C-5^I), 35.1 (C-15^I), 34.8 (C-19^I), 34.6 (C-3^{III}), 28.18, 28.16, 28.14, 28.11 (C-10^I, C-11^I, C-12^I and C-13^I), 27.4 (C-4^I), 26.43, 26.39 (C-8 and C-9), 26.0 (C-20^I), 24.9, 24.7 (C-9^I and C-14^I), 15.3 (C-6^V); *m/z* (ESI$^+$) 1020 ([M+H]$^+$, 90%), 1022 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{48}H_{66}O_8N_8Cl_3S^+$ ([M+H]$^+$)

requires 1019.37844 and 1021.37662, found 1019.37854 and 1021.37610; HPLC (method 2) retention time 0.86 min, 100%.

_N_-{7^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)amino]-16^{I}-oxodecanoyl}-19^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide (148)

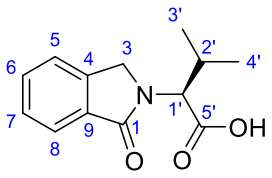


208 (60.0 mg, 0.0588 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.55 mL), and acetic acid (50.1 µL) and zinc powder (57.7 mg, 0.882 mmol, 15.0 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then filtered through Celite® and the filtrate was concentrated. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 4–10% methanol in dichloromethane) to yield the title compound **148** as a colourless oil (23.4 mg, 47%). R_f 0.27 (1% ammonium hydroxide, 10% methanol in dichloromethane); $[\alpha]_D^{25} = -16.3$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3294 (m), 3068 (w), 2928 (m), 2856 (w), 1694 (s), 1632 (s), 1543 (m), 1512 (m), 1481 (m), 1445 (m), 1418 (m), 1399 (m), 1338 (m), 1271 (w), 1242 (w), 1199 (w), 1141 (w), 1087 (w), 1029 (w), 1017 (w), 967 (w), 938 (w), 920 (w), 853 (w), 814 (w), 789 (w), 745 (w), 728 (w), 654 (w), 620 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.87 (1H, s, H-2^{V}), 7.49 – 7.38 (4H, m, H-2^{IV}, H-3^{IV}, H-5^{IV} and H-6^{IV}), 7.29 (1H, d, *J* 1.6, H-4), 7.26 (1H, dd, *J* 8.0, 1.6, H-6), 7.18 (1H, d, *J* 8.0, H-7), 4.63 (1H, s, H-18^{I}), 4.60 – 4.47 (3H, m, H-2^{III}, H-4^{III} and H-7^{IVa}), 4.36 (1H, d, *J* 15.5, H-7^{IVb}), 4.19 (2H, s, H-1^{I}), 3.90 (1H, d, *J* 11.0, H-5^{IIIa}), 3.80 (1H, dd, *J* 11.0, 3.9, H-5^{IIIb}), 3.44 (3H, s, H-8 or H-9), 3.42 (3H, s,

H-8 or H-9), 3.27 (2H, t, *J* 6.5, H-5^{I}), 2.98 (2H, t, *J* 7.2, H-3^{I}), 2.47 (3H, s, H-6^{V}), 2.34 – 2.14 (5H, m, H-3^{IIIa}, H-8^{I} and H-15^{I}), 2.09 (1H, ddd, *J* 13.2, 9.0, 4.5, H-3^{IIIb}), 1.88 (2H, p, *J* 6.8, H-4^{I}), 1.65 – 1.50 (4H, m, H-9^{I} and H-14^{I}), 1.36 – 1.23 (8H, m, H-10^{I}, H-11^{I}, H-12^{I} and H-13^{I}), 1.03 (9H, s, H-20^{I}); ^{13}C NMR (126 MHz, CD_3OD) δ_C 177.3 (C-9^{IV}), 176.0 (C-21^{I}), 174.4 (C-7^{I}), 172.3 (C-16^{I}), 156.4 (C-2), 152.8 (C-2^{V}), 149.0 (C-4^{V}), 140.3 (C-1^{IV}), 133.4 (C-4^{IV}), 132.0 (C-5^{V}), 131.6 (C-5), 131.5 (C-3a), 130.3, 129.0 (C-2^{IV}, C-3^{IV}, C-5^{IV} and C-6^{IV}), 126.6 (C-7a), 124.6 (C-6), 110.4 (C-4), 109.2 (C-7), 71.1 (C-4^{III}), 60.8 (C-2^{III}), 59.0 (C-18^{I}), 58.0 (C-5^{III}), 52.8 (C-1^{I}), 45.7 (C-3^{I}), 43.7 (C-7^{IV}), 38.9 (C-3^{III}), 36.92 (C-5^{I}), 36.91 (C-19^{I}), 36.7 (C-8^{I}), 36.6 (C-15^{I}), 30.25, 30.24, 30.22, 30.19 (C-10^{I}, C-11^{I}, C-12^{I} and C-13^{I}), 27.9 (C-4^{I}), 27.6 (C-8 and C-9), 27.04 (C-20^{I}), 26.95, 26.90 (C-9^{I} and C-14^{I}), 15.8 (C-6^{V}); *m/z* (ESI^{+}) 845 ([M+H]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{45}H_{65}O_6N_8S^{+}$ ([M+H]$^{+}$) requires 845.4742, found 845.4751; HPLC (method 3) retention time 1.76 min, 100%.

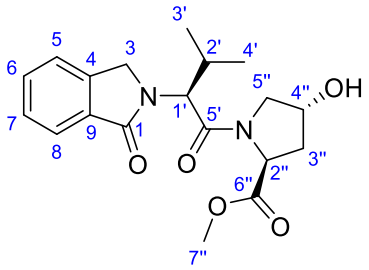
COMPOUNDS FROM CHAPTER 4

(2*S*)-3-Methyl-2-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoic acid (261)[203]



(2*S*)-2-Amino-3-methyl-butanoic acid (2.62 g, 22.4 mmol, 1.0 eq.) was added to a solution of phthalaldehyde (3.00 g, 22.4 mmol, 1.0 eq.) in acetonitrile (62 mL). The resulting mixture was heated to reflux for 16 h. The reaction mixture was then cooled to room temperature and filtered to yield the title compound **261** as a yellow solid (4.57 g, 88%). R_f 0.75 (1% AcOH, 8% methanol in dichloromethane); m.p. 159–161 ºC (from MeCN) {lit.[203] 163–165 ºC}; $[\alpha]_D^{25}$= −45.3 (*c* 1.0, MeOH) {lit.[203] –41.8 (c 1.0, MeOH)} ; $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2963 (w), 2875 (w), 2566 (w), 2161 (w), 1978 (w), 1729 (s), 1634 (s), 1592 (m), 1476 (m), 1456 (m), 1424 (w), 1390 (w), 1368 (w), 1349 (w), 1326 (w), 1311 (w), 1259 (w), 1234 (w), 1203 (s), 1178 (s), 1114 (m), 1023 (w), 1005 (w), 970 (w), 858 (m), 774 (m), 754 (s), 735 (s), 691 (s), 633 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 12.94 (1H, s, COO*H*), 7.71 (1H, dt, *J* 7.6, 0.7, H-5), 7.65 – 7.60 (2H, m, H-7 and H-8), 7.53 – 7.47 (1H, m, H-6), 4.63 (1H, d, *J* 17.6, H-3^a), 4.55 – 4.49 (2H, m, H-3^b and H-1'), 2.29 (1H, dsept, *J* 9.6, 6.7, H-2'), 1.02 (3H, d, *J* 6.6, H-3' or H-4'), 0.85 (3H, d, *J* 6.7, H-3' or H-4'); *m/z* (ESI$^+$) 234 ([M+H]$^+$, 100%), 256 ([M+Na]$^+$, 20%). The spectroscopic data are in good agreement with the literature values.[203]

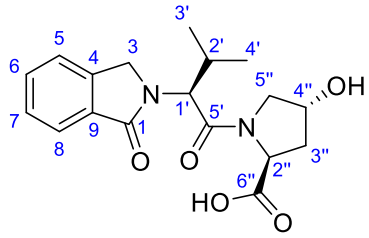
Methyl (4*R*)-4''-hydroxy-1''-[(2*S*)-2''-methyl-2-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoyl]-L-prolinate (262)[204]



HATU (3.91 g, 10.3 mmol, 1.2 eq.) was added at 0 ºC to a solution of **261** (2.00 g, 8.57 mmol, 1.0 eq.), *N*,*N*-diisopropylethylamine (3.58 mL, 20.6 mmol, 2.4 eq.) and L-4-hydroxyproline methyl ester hydrochloride (1.87 g, 10.3 mmol, 1.2 eq.) in anhydrous *N*,*N*-dimethylformamide (12 mL).The resulting mixture was stirred at room temperature for 3 h. The reaction mixture was then partitioned between ethyl acetate (50 mL) and water (50 mL). The organic component was separated, and the aqueous component was extracted with ethyl acetate (3 × 30 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–4% methanol in dichloromethane) and further purified by silica gel column chromatography (50–100% ethyl acetate in petroleum ether) to yield the title compound **262** as a colourless solid (1.94 g, 63%). R_f 0.48 (6% methanol in dichloromethane); m.p. 122–125 ºC (from EtOAc); $[\alpha]_D^{25}$= −130.9 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3481 (w), 2968 (w), 2927 (w), 2571 (w), 2549 (w), 2182 (m), 2161 (m), 2023 (m), 1993 (m), 1977 (m), 1738 (m), 1672 (s), 1640 (s), 1528 (m), 1471 (m), 1441 (m), 1411 (m), 1391 (m), 1326 (m), 1303 (m), 1276 (m), 1262 (m), 1249 (m), 1197 (m), 1169 (s), 1137 (m), 1107 (m), 1089 (m), 1060 (m), 1029 (m), 1010 (m), 944 (m), 924 (m), 883 (m), 872 (m), 856 (m), 833 (m), 796 (m), 782 (m), 732 (s), 681 (m), 661 (m), 625 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.69 (1H, d, *J* 7.5, H-8), 7.41 (1H, ddd, *J* 7.4, 7.4, 1.2, H-6), 7.35 (1H, ddd, *J* 7.4, 7.4, 1.1, H-7), 7.26 – 7.23 (1H, m, H-5), 5.71 (1H, bs, O*H*), 4.77 (1H, d, *J* 11.0, H-1'), 4.68 (1H, d, *J* 18.0, H-3^a), 4.61 (1H, dd, *J* 9.6, 7.6, H-2''), 4.54 (1H, t, *J* 4.0, H-4''), 4.40 – 4.28 (2H, m, H-3^b and H-5''^{a}), 3.83 (1H, dd, *J* 11.2, 3.5, H-5''^{b}), 3.75 (3H, s, H-7''), 2.47 – 2.36

(1H, m, H-2'), 2.37 – 2.28 (1H, m, H-3''[a]), 1.98 (1H, ddd, *J* 13.5, 9.6, 4.3, H-3''[b]), 1.09 (3H, d, *J* 6.6, H-3' or H-4'), 0.83 (3H, d, *J* 6.6, H-3' or H-4'); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 172.7 (C-5'), 169.8 (C-6''), 169.2 (C-1), 142.2 (C-4), 131.9 (C-9), 131.1 (C-6), 128.0 (C-7), 123.9 (C-8), 122.8 (C-5), 70.2 (C-4''), 58.7 (C-1'), 58.1 (C-2''), 56.3 (C-5''), 52.3 (C-7''), 47.7 (C-3), 37.7 (C-3''), 29.2 (C-2'), 19.0, 18.9 (C-3' and C-4'); *m/z* (ESI$^+$) 383 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[204]

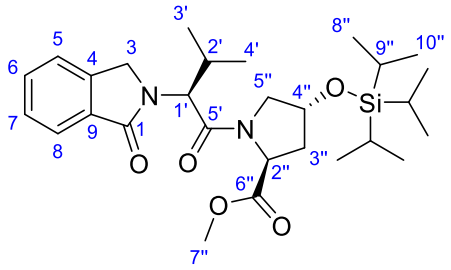
(4*R*)-4''-Hydroxy-1''-[(2*S*)-2''-methyl-2-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoyl]-L-proline (258)[204]



A solution of lithium hydroxide (116 mg, 2.77 mmol, 2.0 eq.) in water (5 mL) was added at 0 ºC to a solution of **262** (500 mg, 1.39 mmol, 1.0 eq.) in tetrahydrofuran (10 mL). The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was then acidified to pH 1 with 1 M aqueous hydrochloric acid (6 mL), and extracted with ethyl acetate (3 × 40 mL). The organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% AcOH, 4–10% methanol in dichloromethane) to yield the title compound **258** as a colourless solid (394 mg, 82%). R_f 0.23 (1% AcOH, 8% methanol in dichloromethane); m.p. 130–132 ºC (from MeOH); $[\alpha]_D^{25} = -145.3$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3415 (w), 2962 (w), 2935 (w), 2874 (w), 2522 (w), 2161 (w), 2027 (w), 1978 (w), 1731 (m), 1623 (s), 1470 (m), 1446 (m), 1408 (m), 1323 (w), 1306 (w), 1263 (m), 1214 (m), 1188 (m), 1106 (w), 1083 (m), 1030 (w), 985 (w), 911 (w), 880 (w), 849 (m), 797 (w), 774 (w), 732 (s), 681 (w), 654 (w), 619 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.73 (1H, dd, *J* 7.6, 1.1, H-8), 7.49 (1H, dd, *J* 7.5, 1.2, H-6), 7.42 – 7.34 (2H, m, H-5 and H-7), 4.83 (1H, d, *J* 11.1, H-1'), 4.73 (1H, d, *J* 17.9, H-3[a]), 4.61 (1H, t, *J* 8.4, H-2''), 4.54 (1H, bs, H-4''), 4.44 – 4.33 (2H, m, H-3[b] and H-5''[a]), 3.81 (1H, dd, *J* 11.4, 3.6, H-3''[b]), 2.50 – 2.36 (1H, m, H-2'),

2.37 – 2.26 (1H, m, H-3’’[a]), 2.16 (1H, ddd, *J* 13.4, 9.0, 4.4, H-3’’[b]), 1.05 (3H, d, *J* 6.6, H-3’ or H-4’), 0.86 (3H, d, *J* 6.6, H-3’ or H-4’); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 174.2 (C-6’’), 170.5 (C-5’), 169.9 (C-1), 142.3 (C-4), 132.1 (C-6), 131.3 (C-9), 128.1 (C-7), 123.9 (C-8), 123.1 (C-5), 69.9 (C-4’’), 58.7 (C-1’), 58.5 (C-2’’), 56.4 (C-5’’), 47.7 (C-3), 37.1 (C-3’’), 29.0 (C-2’), 19.1, 19.0 (C-3’ and C-4’); *m/z* (ESI$^+$) 369 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[204]

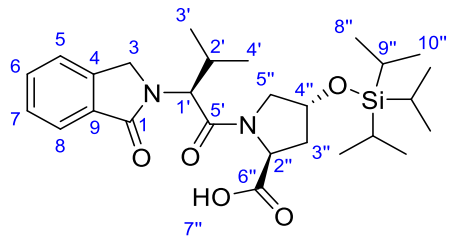
Methyl (4*R*)-1’’-[(2*S*)-2’-methyl-2-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoyl]-4’’-{[tri(propan-9’’-yl)silyl]oxy}-L-prolinate (263)



Triethylamine (203 µL, 0.666 mmol, 1.2 eq.) and triisopropylsilyl trifluoromethanesulfonate (164 µL, 0.610 mmol, 1.1 eq.) were added to a solution of **262** (200 mg, 0.555 mmol, 1.0 eq.) in anhydrous dichloromethane (3 mL) and the resulting mixture was stirred at room temperature. After 2 h, further triisopropylsilyl trifluoromethanesulfonate (100 µL, 0.372 mmol, 0.6 eq.) was added and the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was then concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (30–50% ethyl acetate in petroleum ether) to yield the title compound **263** as a colourless oil (235 mg, 88%). R_f 0.68 (80% ethyl acetate in petroleum ether); $[\alpha]_D^{25}$= −115.3 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3023 (m), 2943 (m), 2866 (w), 2361 (w), 2342 (w), 1750 (m), 1687 (s), 1649 (s), 1469 (m), 1436 (m), 1402 (m), 1356 (w), 1321 (w), 1302 (w), 1259 (w), 1198 (m), 1174 (m), 1099 (m), 1070 (w), 1018 (m), 922 (w), 882 (m), 820 (w), 793 (w), 736 (m), 682 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.80 (1H, d, *J* 7.5, H-8), 7.51 (1H, dd, *J* 7.4, 1.2, H-6), 7.45 – 7.37 (2H, m, H-5 and H-7), 4.83 (1H, d, *J* 11.0, H-1’), 4.59 – 4.48 (3H, m, H-3[a], H-2’’ and H-4’’), 4.36 (1H, d, *J* 17.6, H-3[b]), 4.01 (1H, dt, *J* 10.8, 1.9, H-5’’[a]), 3.83 (1H, dd, *J* 10.8, 3.7, H-5’’[b]), 3.76 (3H, s,

H-7''), 2.51 – 2.40 (1H, m, H-2'), 2.24 (1H, ddd, *J* 12.6, 7.5, 2.2, H-3''[a]), 1.98 (1H, ddd, *J* 12.6, 9.3, 4.0, H-3''[b]), 1.09 – 0.97 (6H, m, H-9' and H-3' or H-4'), 0.95 – 0.77 (21H, m, H-8', H-10' and H-3' or H-4'); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 172.7 (C-5'), 169.3 (C-6''), 168.6 (C-1), 142.1 (C-4), 132.1 (C-9), 131.6 (C-6), 127.9 (C-7), 124.0 (C-8), 123.0 (C-5), 71.2 (C-4''), 58.4 (C-1'), 58.1 (C-2''), 56.1 (C-5''), 52.4 (C-7''), 47.1 (C-3), 38.8 (C-3''), 28.8 (C-2'), 19.3, 18.6 (C-3' and C-4'), 17.9, 17.8 (C-8' and C-9'), 12.0 (C-9'); *m/z* (ESI$^+$) 539 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{28}H_{45}O_5N_2Si^+$ ([M+H]$^+$) requires 517.30530, found 517.30526; HPLC (method 1) retention time 13.2 min, 99.7%.

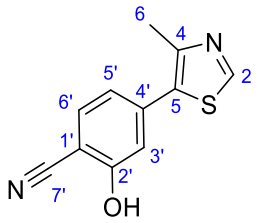
(4*R*)-1-[(2*S*)-2'-Methyl-2-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoyl]-4''-{[tri(propan-9''-yl)silyl]oxy}-L-proline (264)



Lithium hydroxide (8.6 mg, 0.34 mmol, 2.0 eq.) dissolved in water (1 mL) was added at 0 ºC to **263** (88 mg, 0.17 mmol, 1.0 eq.) dissolved in tetrahydrofuran (2 mL). The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was then acidified to pH 1 using 1 M aqueous hydrochloric acid (1 mL), and extracted with ethyl acetate (3 × 10 mL). The organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% acetic acid, 0–3% methanol in dichloromethane) to yield the title compound **264** as a colourless foam (81 mg, 94%). R_f 0.15 (1% acetic acid, 4% methanol in dichloromethane); $[\alpha]_D^{25}$= −115.3 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2960 (m), 2943 (m), 2866 (m), 1741 (m), 1686 (s), 1645 (s), 1469 (m), 1451 (m), 1407 (m), 1356 (w), 1323 (w), 1304 (w), 1255 (w), 1213 (w), 1189 (m), 1164 (m), 1099 (m), 1070 (w), 1034 (w), 1015 (w), 996 (w), 921 (w), 882 (m), 851 (w), 818 (w), 796 (w), 784 (w), 734 (s), 682 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.82 (1H, d, *J* 7.6, H-8), 7.56 – 7.50 (1H, m, H-6), 7.48 – 7.40 (2H, m, H-5 and H-7), 4.85 (1H, d, *J* 10.9, H-1'), 4.64 – 4.49 (3H, m, H-2'', H-4'' and H-3[a]), 4.39

(1H, d, *J* 17.6, H-3[b]), 4.05 (1H, d, *J* 10.9, H-5''[a]), 3.79 (1H, dd, *J* 10.9, 3.9, H-5''[b]), 2.54 – 2.42 (1H, m, H-2'), 2.30 – 2.18 (2H, m, H-3''), 1.06 – 1.00 (6H, m, H-9' and H-3' or H-4'), 0.92 – 0.81 (21H, m, H-8'', H-10'' and H-3' or H-4'); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 174.5 (C-6''), 170.7 (C-5'), 168.7 (C-1), 142.0 (C-4), 131.9 (C-9), 131.7 (C-6), 128.0 (C-7), 124.1 (C-8), 123.0 (C-5), 70.8 (C-4''), 58.6 (C-1'), 58.4 (C-2''), 56.4 (C-5''), 47.2 (C-3), 38.1 (C-3''), 28.9 (C-2'), 19.3, 18.6 (C-3' and C-4'), 17.88, 17.85 (C-8'' and C-10''), 12.0 (C-9'); *m/z* (ESI$^+$) 503 ([M+H]$^+$, 20%), 525 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{27}H_{43}O_5N_2Si^+$ ([M+H]$^+$) requires 503.29358, found 503.29324; HPLC (method 1) retention time 11.7 min, 98.0%.

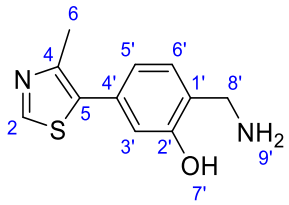
2'-Hydroxy-4'-(4-methyl-1,3-thiazol-5-yl)benzonitrile (254)[205]



2-Hydroxy-4-bromobenzonitrile (3.00 g, 15.2 mmol, 1.0 eq.), palladium(II) acetate (102 mg, 0.454 mmol, 0.03 eq.) and potassium acetate (2.97 g, 30.3 mmol, 2.0 eq.) were added to a flask and the flask was purged with nitrogen for 30 min. Degassed anhydrous *N*,*N*-dimethylacetamide (70 mL) was added, followed by 4-methylthiazole (3.00 g, 30.3 mmol, 2.0 eq.). The reaction mixture was heated to 150 ºC for 3 h. The reaction was then cooled to room temperature and quenched with water (120 mL). The aqueous component was extracted with ethyl acetate (4 × 120 mL), and the combined organic components were washed with water (100 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–70% ethyl acetate in heptane) to yield the title compound **254** as a yellow solid (2.51 g, 77%). R_f 0.52 (50% ethyl acetate in heptane); m.p. 150–152 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3351 (m), 3084 (w), 2488 (w), 2361 (w), 2224 (s), 1606 (s), 1578 (m), 1423 (s), 1303 (m), 1271 (w), 1240 (m), 1119 (w), 1054 (w), 1033 (w), 948 (w), 869 (m), 815 (m), 738 (w), 666 (w), 631 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.95 (1H, s, H-2), 7.64 – 7.57 (1H, m, H-5'), 7.09 – 7.04 (2H, m, H-3' and H-6'), 2.53 (3H, s, H-6); ^{13}C NMR

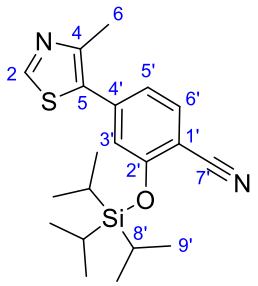
(126 MHz, CD_3OD) δ_C 161.8 (C-2'), 154.0 (C-2), 150.5 (C-4), 139.3 (C-4'), 134.9 (C-5'), 132.0 (C-5), 121.6 (C-6'), 117.4 (C-7'), 117.3 (C-3'), 100.4 (C-1'), 16.2 (C-6); *m/z* (ESI^+) 217 ($[M+H]^+$, 100%). The spectroscopic data are in good agreement with the literature values.[205]

2-(Aminomethyl)-5-(4-methyl-1,3-thiazol-5-yl)phenol (255)[204]



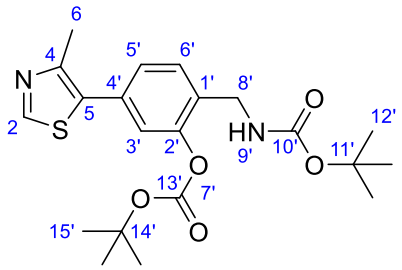
A solution of lithium aluminium hydride (132 mg, 3.47 mmol, 3.0 eq.) in anhydrous tetrahydrofuran (3.48 mL) was added dropwise at 0 ºC to a stirred solution of **254** (250 mg, 1.16 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (8.52 mL). The reaction mixture was heated to 50 ºC for 5 h. After this time, the reaction mixture was cooled to 0 ºC. Water (132 µL) was added, followed by 15% aqueous sodium hydroxide (132 µL), and then water (396 µL), and the suspension was stirred for 15 min at room temperature. Sodium sulfate was added, the suspension was stirred for a further 15 min, filtered, and concentrated *in vacuo* to yield the title compound **255** as a yellow oil (208 mg, 82%). R_f 0.69 (6:2:1 ethyl acetate:ethanol:ammonium hydroxide); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3378 (s), 2362 (w), 1640 (s), 1571 (s), 1500 (m), 1411 (m), 1340 (s), 1232 (w), 1103 (m), 946 (w), 869 (w), 826 (w), 667 (w); ^1H NMR (500 MHz, CD_3OD) δ_H 8.75 (1H, s, H-2), 7.07 (1H, d, *J* 7.6, H-6'), 6.75 (1H, d, *J* 1.9, H-3'), 6.50 (1H, dd, *J* 7.6, 1.9, H-5'), 3.76 (2H, s, H-8'), 2.48 (3H, s, H-6); *m/z* (ESI^+) 204 ($[M-NH_2]^+$, 100%). The spectroscopic data are in good agreement with the literature values.[204]

4'-(4-Methyl-1,3-thiazol-5-yl)-2'-{[tri(propan-8'-yl)silyl]oxy}benzonitrile (256)



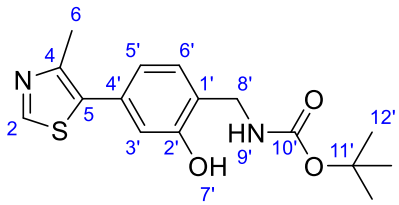
Triisopropylsilyl chloride (257 µL, 1.20 mmol, 1.3 eq.) was added to a solution of **254** (200 mg, 0.925 mmol, 1.0 eq.) and imidazole (195 mg, 2.87 mmol, 3.1 eq.) in anhydrous *N*,*N*-dimethylformamide (3 mL) at room temperature. The reaction mixture was stirred at room temperature for 3 h. TLC analysis indicated starting material remained, so a further 100 µL of triisopropylsilyl chloride was added and the reaction mixture was stirred at room temperature for 1 h. The mixture was diluted with ethyl acetate (20 mL) and washed with water (20 mL) and saturated aqueous sodium bicarbonate solution (30 mL). The organic component was dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–20% ethyl acetate in petroleum ether) to yield the title compound **256** as a colourless solid (238 mg, 69%). R_f 0.73 (40% ethyl acetate in petroleum ether); m.p. 60–61 ºC (from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3063 (w), 2945 (m), 2867 (m), 2224 (m), 2161 (w), 2027 (w), 1975 (w), 1599 (m), 1554 (m), 1531 (w), 1486 (m), 1464 (m), 1419 (w), 1302 (s), 1274 (s), 1248 (s), 1205 (s), 1190 (w), 1124 (w), 1064 (w), 1027 (w), 993 (w), 943 (w), 916 (s), 880 (s), 843 (s), 820 (s), 783 (s), 702 (s), 683 (s), 666 (s), 632 (m), 606 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.73 (1H, s, H-2), 7.56 (1H, d, *J* 8.0, H-6'), 7.07 (1H, dd, *J* 8.0, 1.6, H-5'), 7.00 (1H, d, *J* 1.6, H-3'), 2.55 (3H, s, H-6), 1.36 (3H, hept, *J* 7.3, H-8'), 1.16 (18H, d, *J* 7.3, H-9'); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 158.6 (C-2'), 151.5 (C-2), 149.9 (C-4), 138.2 (C-4'), 134.0 (C-6'), 130.5 (C-5), 122.0 (C-5'), 119.8 (C-3'), 116.8 (C-7'), 104.3 (C-1'), 18.0 (C-9'), 16.6 (C-6), 13.0 (C-8'); *m/z* (ESI$^+$) 373 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{20}H_{29}ON_2SSi^+$ ([M+H]$^+$) requires 373.17644, found 373.17638; HPLC (method 1) retention time 14.41 min, 100%.

1'-{[(*tert*-Butoxycarbonyl)amino]methyl}-4'-(4-methyl-1,3-thiazol-5-yl)-2'-phenyl *tert*-butyl carbonate (265)



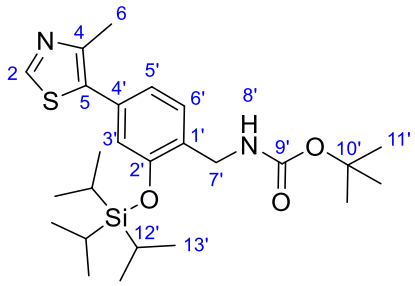
255 (100 mg, 0.454 mmol, 1.0 eq.) was dissolved in anhydrous dichloromethane (2 mL). *N*,*N*-Diisopropylethylamine (158 µL, 0.908 mmol, 2.0 eq.) followed by di-*tert*-butyl decarbonate (99.1 mg, 0.454 mmol, 1.0 eq.) were added and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then diluted with ethyl acetate (30 mL) and washed with 0.1 M aqueous hydrochloric acid (10 mL) and saturated aqueous sodium bicarbonate solution (20 mL). The organic component was dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (30% ethyl acetate in petroleum ether) to yield the title compound **265** as a colourless oil (109 mg, 57% and 16% recovered starting material). R_f 0.68 (80% ethyl acetate in petroleum ether); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3358 (w), 2978 (w), 2932 (w), 2361 (w), 1757 (m), 1711 (m), 1620 (w), 1503 (w), 1455 (w), 1416 (w), 1393 (w), 1368 (m), 1271 (m), 1249 (s), 1145 (s), 1120 (s), 1049 (w), 937 (w), 904 (w), 852 (w), 781 (w), 732 (w), 647 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.68 (1H, s, H-2), 7.45 (1H, d, *J* 8.0, H-6'), 7.30 (1H, dd, *J* 8.0, 1.8, H-5'), 7.22 (1H, d, *J* 1.8, H-3'), 4.93 (1H, bs, H-9'), 4.31 (2H, d, *J* 5.8, H-8'), 2.53 (3H, s, H-6), 1.56 (9H, s, H-15'), 1.45 (9H, s, H-12'); *m/z* (ESI$^+$) 421 ([M+H]$^+$, 90%), 321 ([M-Boc+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{21}H_{29}O_5N_2S^+$ ([M+H]$^+$) requires 421.17917, found 421.17873.

tert-Butyl {[2'-hydroxy-4'-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}carbamate (266)



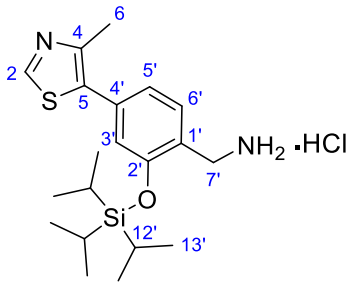
265 (108 mg, 0.257 mmol, 1.0 eq.) was dissolved in piperidine (3.81 mL, 38.5 mmol, 150.0 eq.) and dichloromethane (3.91 mL), and the reaction mixture was stirred at room temperature for 2 h. After this time, the reaction was added to ethyl acetate (30 mL), and the organic component was washed with 4% aqueous potassium bisulfate solution (50 mL), water (20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–50% ethyl acetate in petroleum ether) to yield the title compound **266** as a colourless oil (45.8 mg, 56%). R_f 0.44 (40% ethyl acetate in petroleum ether); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3306 (w), 3080 (w), 2978 (w), 1678 (m), 1615 (w), 1584 (w), 1514 (m), 1421 (m), 1393 (w), 1366 (m), 1287 (m), 1250 (m), 1164 (s), 1124 (w), 1046 (w), 1029 (w), 946 (w), 930 (w), 914 (w), 883 (w), 862 (w), 821 (w), 791 (w), 732 (m), 664 (w), 649 (w), 626 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.66 (1H, s, H-2), 7.11 (1H, d, *J* 7.8, H-6'), 7.03 (1H, d, *J* 1.8, H-3'), 6.89 (1H, dd, *J* 7.8, 1.8, H-5'), 5.38 (1H, t, *J* 6.7, H-9'), 4.26 (2H, d, *J* 6.7, H-8'), 2.53 (3H, s, H-6), 1.45 (9H, s, H-12'); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 158.6 (C-10'), 156.1 (C-2'), 150.5 (C-2), 148.6 (C-4), 133.5 (C-5), 131.8 (C-4'), 131.0 (C-6'), 124.9 (C-1'), 120.8 (C-5'), 118.5 (C-3'), 81.5 (C-11'), 41.1 (C-8'), 28.5 (C-12'), 16.3 (C-6); *m/z* (ESI$^+$) 321 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{16}H_{21}O_3N_2S^+$ ([M+H]$^+$) requires 321.12674, found 321.12674; HPLC (method 1) retention time 6.86 min, 100%.

tert-Butyl {[4'-(4-methyl-1,3-thiazol-5-yl)-2'-{[tri(propan-2-yl)silyl]oxy}phenyl]methyl}carbamate (267)



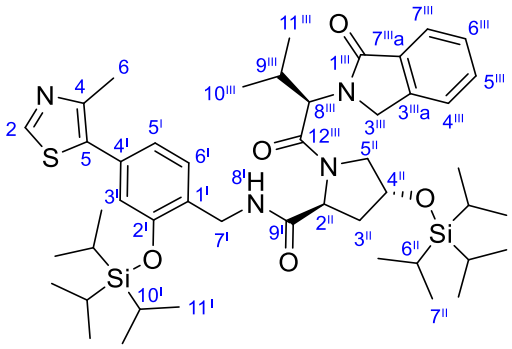
Triisopropylsilyl chloride (39 µL, 0.18 mmol, 1.3 eq.) was added to a solution of **266** (45 mg, 0.14 mmol, 1.0 eq.) and imidazole (30 mg, 0.44 mmol, 3.1 eq.) in anhydrous *N*,*N*-dimethylformamide (3 mL) at room temperature. The reaction mixture was stirred at room temperature for 3 h. TLC analysis indicated starting material remained. Further triisopropylsilyl chloride (50 µL, 0.23 mmol, 1.7 eq.) was added and the reaction mixture was stirred at room temperature for 16 h. The mixture was diluted with ethyl acetate (20 mL) and washed with water (20 mL) and saturated aqueous sodium bicarbonate solution (20 mL). The organic component was dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (5–15% ethyl acetate in petroleum ether) to yield the title compound **267** as a colourless oil (50 mg, 75%). The oil rapidly changed from colourless to brown after dissolving in deuterated chloroform for NMR analysis, so the compound was prepared immediately before use. R_f 0.57 (30% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.70 (1H, s, H-2), 7.30 (1H, d, *J* 7.8, H-6'), 6.99 (1H, dd, *J* 7.8, 1.7, H-5'), 6.88 (1H, d, *J* 1.7, H-3'), 4.91 (1H, bs, H-8'), 4.35 (2H, d, *J* 5.9, H-7'), 2.54 (3H, s, H-6), 1.46 (9H, s, H-11'), 1.32 (3H, hept, *J* 7.5, H-12'), 1.13 (18H, d, *J* 7.5, H-13'); *m/z* (ESI$^+$) 477 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{25}H_{41}O_3N_2SSi^+$ ([M+H]$^+$) requires 477.26017, found 477.26044.

1'-[4'-(4-Methyl-1,3-thiazol-5-yl)-2'-{[tri(propan-2-yl)silyl]oxy}phenyl]methanamine hydrochloride (268)



267 (49 mg, 0.10 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (1 mL) and stirred at room temperature for 1 h. The reaction mixture was concentrated *in vacuo* to yield the Boc deprotected **268** as a brown oil, which was then used directly in the next step without further purification. R_f 0.91 (6:2:1 ethyl acetate:ethanol:ammonium hydroxide); ^{1}H NMR (400 MHz, CD_3OD) δ_H 9.68 (1H, s, H-2), 7.58 (1H, d, *J* 7.9, H-3'), 7.27 (1H, dd, *J* 7.9, 1.7, H-5'), 7.09 (1H, d, *J* 1.7, H-6'), 4.25 (2H, s, H-7'), 2.59 (3H, s, H-6), 1.49 – 1.36 (3H, m, H-12'), 1.18 (18H, d, *J* 7.5, H-13'); *m/z* (ESI$^+$) 377 ([M+H]$^+$, 30%).

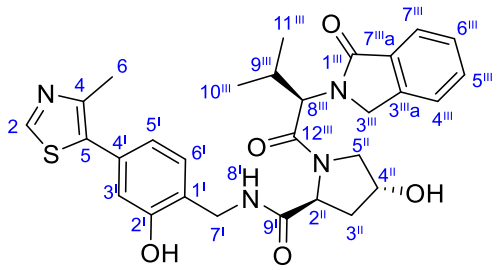
(4*R*)-1-[(2*R*)-9^{III}-Methyl-8^{III}-(1^{III}-oxo-1^{III},3^{III}-dihydro-2*H*-isoindol-2^{III}-yl)butanoyl]-*N*-{[4^{I}-(4-methyl-1,3-thiazol-5-yl)-2^{I}-{[tri(propan-2-yl)silyl]oxy}phenyl]methyl}-4^{II}-{[tri(propan-2-yl)silyl]oxy}-L-prolinamide (269)



264 (63.3 mg, 0.126 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (87.8 µL, 0.504 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (0.5 mL). HATU (52.6 mg, 0.138 mmol, 1.1 eq.) was added slowly, and the solution was stirred at room temperature for 10 min. **268** (52.0 mg, 0.126 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide

(0.5 mL) was added to the solution and the reaction mixture was stirred at room temperature for 3 h. After this time, the mixture was quenched with water (20 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic components were washed with brine (30 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–2% methanol in dichloromethane) and further purified by silica gel column chromatography (10–35% ethyl acetate in petroleum ether) to yield the title compound **269** as a colourless oil (61.5 mg, 57%). R_f 0.48 (40% ethyl acetate in petroleum ether); $[\alpha]_D^{25}$= −80.6 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3325 (w), 3062 (w), 2944 (s), 2892 (m), 2867 (s), 1688 (s), 1647 (s), 1540 (w), 1499 (w), 1468 (m), 1414 (m), 1359 (w), 1322 (w), 1285 (w), 1263 (w), 1229 (w), 1180 (w), 1125 (w), 1101 (w), 1069 (w), 1032 (w), 1014 (w), 920 (m), 883 (m), 809 (w), 735 (m), 683 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.67 (1H, s, H-2), 7.81 (1H, dt, *J* 7.5, 1.2, H-7^{III}), 7.51 (1H, ddd, *J* 7.5, 7.5, 1.2, H-5^{III}), 7.46 – 7.37 (2H, m, H-4^{III} and H-6^{III}), 7.31 (1H, d, *J* 7.8, H-6^{I}), 7.06 (1H, t, *J* 5.9, H-8^{I}), 6.97 (1H, dd, *J* 7.8, 1.7, H-5^{I}), 6.88 (1H, d, *J* 1.7, H-3^{I}), 4.82 (1H, d, *J* 10.9, H-8^{III}), 4.64 – 4.43 (5H, m, H-4^{II}, H-2^{II}, H-3^{IIIa} and H-7^{I}), 4.36 (1H, d, *J* 17.6, H-3^{IIIb}), 3.95 (1H, dd, *J* 10.8, 1.5, H-5^{IIa}), 3.76 (1H, dd, *J* 10.8, 4.4, H-5^{IIb}), 2.52 (3H, s, H-6), 2.48 – 2.36 (2H, m, H-9^{III} and H-3^{IIa}), 2.02 (1H, dddd, *J* 13.0, 7.9, 4.0, 1.4, H-3^{IIb}), 1.33 (3H, hept, *J* 7.6, H-10^{I}), 1.13 (18H, d, *J* 7.6, H-11^{I}), 0.93 – 0.79 (27H, m, H-10^{III}, H-6^{II} and H-7^{II}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.8 (C-12^{III}), 170.4 (C-9^{I}), 168.5 (C-1^{III}), 154.0 (C-2^{I}), 150.3 (C-2), 148.3 (C-3^{III}a), 142.1 (C-4), 132.1 (C-5), 131.97 (C-4^{I}), 131.95 (C-7^{III}a), 131.6 (C-5^{III}), 129.6 (C-6^{I}), 128.2 (C-1^{I}), 128.0 (C-6^{III}), 124.0 (C-7^{III}), 122.9 (C-4^{III}), 122.1 (C-5^{I}), 118.8 (C-3^{I}), 71.0 (C-4^{II}), 59.0 (C-2^{II}), 58.7 (C-8^{III}), 56.1 (C-5^{II}), 47.2 (C-3^{III}), 39.0 (C-7^{I}), 37.5 (C-3^{II}), 29.1 (C-9^{III}), 19.3, 18.6 (C-10^{III} and C-11^{III}), 18.19, 17.90, (C-7^{II} and C-11^{I}), 16.2 (C-6), 13.2 (C-10^{I}), 12.1 (C-6^{II}); *m/z* (ESI$^+$) 862 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{47}H_{73}O_5N_4SSi_2^+$ ([M+H]$^+$) requires 861.48347, found 861.48254.

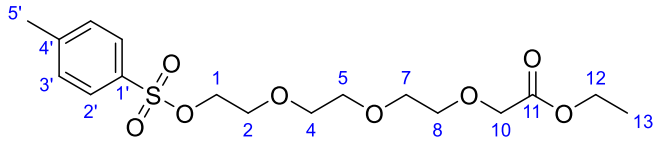
(4*R*)-4^{II}-Hydroxy-*N*-{[2^{I}-hydroxy-4^{I}-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}-1^{I}-[(2*R*)-9^{III}-methyl-8^{III}-(1^{III}-oxo-1^{III},3^{III}-dihydro-2*H*-isoindol-2^{III}-yl)butanoyl]-L-prolinamide (10)



Tetrabutylammonium fluoride (1 M in tetrahydrofuran, 139 µL, 0.139 mmol, 4.0 eq.) was added to a solution of **269** (30.0 mg, 0.0348 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (1.5 mL). The reaction mixture was stirred at room temperature for 40 min. The solvent was removed *in vacuo*, and the resulting residue was dissolved in ethyl acetate (20 mL). The organic component was washed with water (20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–6% methanol in dichloromethane) to yield the title compound **10** as a colourless solid (20.2 mg, 100%). R_f 0.27 (8% methanol in dichloromethane); m.p. 125–126 ºC (from MeOH); $[\alpha]_D^{25}$= −101.2 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3296 (w), 3081 (w), 2961 (w), 2931 (w), 2874 (w), 2360 (w), 2342 (w), 1638 (s), 1559 (w), 1470 (m), 1445 (m), 1409 (m), 1353 (w), 1324 (w), 1304 (w), 1286 (w), 1225 (w), 1200 (w), 1163 (w), 1122 (w), 1087 (w), 1025 (w), 979 (w), 945 (w), 881 (w), 797 (w), 736 (m), 684 (w), 665 (w), 624 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.32 (1H, s, O*H*), 8.70 (1H, s, H-2), 8.08 (1H, t, *J* 6.4, H-8^{I}), 7.73 (1H, dd, *J* 7.6, 1.2, H-7^{III}), 7.48 (1H, ddd, *J* 7.5, 7.5, 1.1, H-5^{III}), 7.42 – 7.31 (2H, m, H-4^{III} and H-6^{III}), 7.12 (1H, d, *J* 7.8, H-6^{I}), 7.00 (1H, d, *J* 1.8, H-3^{I}), 6.87 (1H, dd, *J* 7.8, 1.8, H-5^{I}), 4.75 – 4.58 (3H, m, H-2^{II}, H-8^{III} and H-3^{IIIa}), 4.46 (1H, bs, H-4^{II}), 4.42 – 4.26 (4H, m, H-7^{I}, H-3^{IIIb} and H-5^{IIa}), 3.63 (1H, dd, *J* 11.4, 3.6, H-5^{IIb}), 2.52 (3H, s, H-6), 2.43 – 2.28 (2H, m, H-9^{III} and H-3^{IIa}), 1.94 (1H, ddd, *J* 13.6, 8.0, 1.9, H-3^{IIb}), 0.86 (6H, d, *J* 6.4, H-10^{III} and H-11^{III}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 173.1 (C-12^{III}), 170.9 (C-9^{I}), 169.7 (C-1^{III}), 156.1 (C-2^{I}), 150.6 (C-2), 148.8 (C-3^{III}a) 142.1 (C-4), 133.5 (C-5), 132.0 (C-5^{III}), 131.5 (C-4^{I}), 131.3 (C-6^{I}), 128.22 (C-7^{III}a), 128.20 (C-6^{III}), 124.1 (C-1^{I}), 123.9 (C-7^{III}), 123.0 (C-4^{III}), 120.9 (C-5^{I}), 118.6 (C-3^{I}),

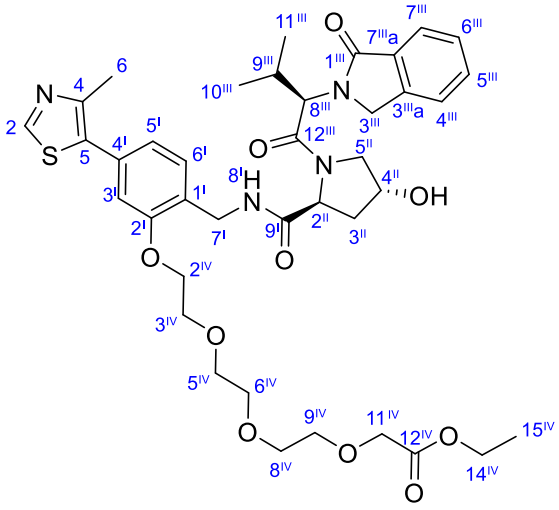
69.9 (C-4^{II}), 58.8 (C-2^{II}), 58.2 (C-8^{III}), 56.2 (C-5^{II}), 47.6 (C-3^{III}), 40.3 (C-7^{I}), 35.9 (C-3^{II}), 29.0 (C-9^{III}), 19.2, 18.9 (C-10^{III} and C-11^{III}), 16.2 (C-6); *m/z* (ESI$^+$) 549 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{29}H_{33}O_5N_4S^+$ ([M+H]$^+$) requires 549.21662, found 549.21643; HPLC (method 1) retention time 5.48 min, 98.2%.

Ethyl [7-(4-{1-[(4'-methylbenzene-1'-sulfonyl)oxy]ethoxy}ethoxy)ethoxy]acetate



A solution of 4-toluenesulfonyl chloride (194 mg, 1.02 mmol, 1.2 eq.) in tetrahydrofuran (3.2 mL) was added dropwise to a solution of triethylamine (739 µL, 2.42 mmol, 2.9 eq.) and **170** (200 mg, 0.847 mmol, 1.0 eq.) in tetrahydrofuran (3.2 mL). The reaction mixture was stirred at room temperature for 3 h. After this time, further 4-toluenesulfonyl chloride (150 mg, 0.789 mmol, 0.93 eq.) was added, and the reaction mixture was stirred at room temperature for 16 h. Dichloromethane (30 mL) was added, and the organic component was washed with 1 M aqueous hydrochloric acid (3 × 20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (20–50% dichloromethane in petroleum ether, then 5% methanol in dichloromethane) to yield the title compound as a colourless oil (297 mg, 90%). R_f 0.18 (6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2874 (w), 2362 (w), 1750 (m), 1598 (w), 1451 (w), 1354 (m), 1291 (w), 1207 (w), 1189 (m), 1175 (s), 1097 (s), 1018 (m), 917 (s), 816 (m), 775 (m), 731 (w), 706 (w), 690 (w), 663 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.83 – 7.77 (2H, m, H-3'), 7.38 – 7.31 (2H, m, H-2'), 4.26 – 4.14 (4H, m, H-12 and H-8), 4.13 (2H, s, H-10), 3.75 – 3.63 (6H, m, H-1, H-2 and H-7), 3.59 (4H, s, H-4 and H-5), 2.45 (3H, s, H-5'), 1.28 (3H, t, *J* 7.1, H-13); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 170.4 (C-11), 144.8 (C-4'), 133.2 (C-1'), 129.9 (C-2'), 128.0 (C-3'), 70.9, 70.8 (C-4 and C-5), 70.7, 70.6 (C-1 and C-7), 69.3 (C-8), 68.7 (C-10 and C-2), 60.8 (C-12), 21.6 (C-5'), 14.2 (C-13); *m/z* (ESI$^+$) 413 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{17}H_{27}O_8S^+$ ([M+H]$^+$) requires 391.14211, found 391.14203.

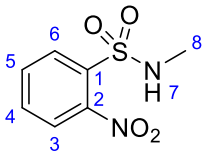
Ethyl {8^{IV}-[5^{IV}-(2^{IV}-{1^{I}-[({(4*R*)-4^{II}-hydroxy-1^{II}-[(2*R*)-9^{III}-methyl-8^{III}-(1^{III}-oxo-1^{III},3^{III}-dihydro-2*H*-isoindol-2^{III}-yl)butanoyl]-L-prolyl}amino)methyl]-4^{I}-(4-methyl-1,3-thiazol-5-yl)phenoxy}ethoxy)ethoxy]ethoxy}acetate (271)



Caesium carbonate (13 mg, 0.040 mmol, 2.0 eq.) was added to a solution of ethyl [7-(4-{1-[(4'-methylbenzene-1'-sulfonyl)oxy]ethoxy}ethoxy)ethoxy]acetate (9.8 mg, 0.025 mmol, 1.25 eq.) and **10** (11 mg, 0.020 mmol, 1.0 eq.) in anhydrous *N*,*N*-dimethylformamide (1 mL). The reaction mixture was stirred at room temperature for 16 h. After this time, the reaction was diluted with ethyl acetate (20 mL) and washed with water (2 × 20 mL). The organic component was dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (3–10% ethanol in ethyl acetate) to yield the title compound **271** as a colourless oil (13 mg, 83%). R_f 0.18 (6% ethanol in ethyl acetate); $[\alpha]_D^{25}$ = −41.4 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3316 (w), 3067 (w), 2927 (m), 2872 (w), 1750 (m), 1732 (m), 1672 (s), 1644 (s), 1572 (w), 1541 (w), 1501 (w), 1470 (m), 1447 (m), 1409 (s), 1376 (w), 1357 (w), 1324 (w), 1302 (w), 1282 (w), 1265 (w), 1211 (s), 1178 (w), 1125 (s), 1057 (w), 1029 (w), 982 (w), 940 (w), 855 (w), 798 (w), 737 (m), 684 (w), 663 (w), 620 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.67 (1H, s, H-2), 7.83 – 7.74 (1H, m, H-4^{III}), 7.53 (1H, td, *J* 7.5, 1.2, H-5^{III}), 7.47 – 7.40 (2H, m, H-7^{III} and H-6^{III}), 7.36 (1H, t, *J* 6.1, H-8^{I}), 7.32 (1H, d, *J* 7.7, H-6^{I}), 6.98 (1H, dd, *J* 7.7, 1.6, H-5^{I}), 6.90 (1H, d, *J* 1.6, H-3^{I}), 4.81 – 4.71 (2H, m, H-3^{IIIa} and H-8^{III}), 4.61 – 4.47 (3H, m, H-7^{Ia}, H-4^{II} and H-2^{II}), 4.46 – 4.35 (3H, m, H-7^{Ib}, H-3^{IIIb} and H-5^{IIa}), 4.26 – 4.12 (4H, m, H-14^{IV} and H-2^{IV}), 4.06 (2H, s,

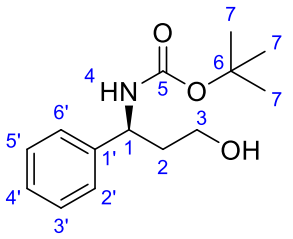
H-11^{IV}), 3.99 – 3.85 (2H, m, H-3^{IV}), 3.79 – 3.57 (9H, m, H-5^{IIb}, H-5^{IV}, H-6^{IV}, H-8^{IV} and H-9^{IV}), 2.53 (3H, s, H-6), 2.45 – 2.30 (2H, m, H-3^{IIa} and H-9^{III}), 2.10 (1H, ddt, *J* 13.2, 7.8, 1.8, H-3^{IIb}), 1.25 (3H, t, *J* 7.1, H-15^{IV}), 0.96 (3H, d, *J* 6.5, H-10^{III} or H-11^{III}), 0.88 (3H, d, *J* 6.6, H-10^{III} or H-11^{III}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 170.8 (C-12^{III}), 170.6 (C-12^{IV}), 170.2 (C-9^{I}), 169.5 (C-1^{III}), 157.0 (C-2^{I}), 150.4 (C-2), 148.7 (C-4), 142.3 (C-3^{IIIa}), 132.4 (C-1^{I} and C-5), 131.9 (C-5^{III}), 129.9 (C-6^{I}), 128.1 (C-6^{III} and C-7^{IIIa}), 127.1 (C-4^{I}), 123.9 (C-4^{III}), 123.0 (C-7^{III}), 122.2 (C-5^{I}), 112.9 (C-3^{I}), 71.0, 70.9, 70.7, 70.6 (C-5^{IV}, C-6^{IV}, C-8^{IV} and C-9^{IV}), 70.2 (C-4^{II}), 69.8 (C-3^{IV}), 68.7 (C-11^{IV}), 68.1 (C-2^{IV}), 61.0 (C-14^{IV}), 58.8 (C-2^{II}), 58.7 (C-8^{III}), 56.2 (C-5^{II}), 47.6 (C-3^{III}), 39.4 (C-7^{I}), 36.6 (C-3^{II}), 29.0 (C-9^{III}), 19.2 (C-10^{III} and C-11^{III}), 16.3 (C-6), 14.3 (C-15^{IV}); *m/z* (ESI$^+$) 789 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{39}H_{51}O_{10}N_4S^+$ ([M+H]$^+$) requires 767.3320, found 767.3316; HPLC (method 1) retention time 7.63 min, 94.8%.

_N_-Methyl-2-nitrobenzene-1-sulfonamide (241)[206,207]



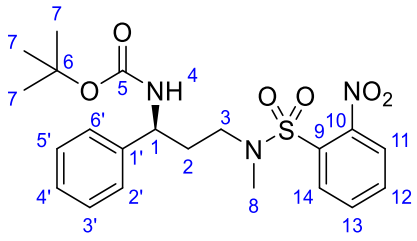
Methylamine (33% in ethanol, 2.80 mL, 22.6 mmol, 2.5 eq.) was slowly added to 2-nitrobenzenesulfonyl chloride (2.00 g, 9.02 mmol, 1.0 eq.) dissolved in dichloromethane (28 mL). The reaction mixture was stirred at room temperature for 30 min. After this time, the organic component was washed with 1 M hydrochloric acid (30 mL), followed by saturated aqueous sodium carbonate solution (30 mL), dried over sodium sulfate, and concentrated *in vacuo* to yield the title compound **241** as a pale yellow solid (2.00 g, 100%). R_f 0.52 (60% ethyl acetate in petroleum ether); m.p. 97–97 ºC (from EtOAc) {lit.[206] 106 ºC}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.17 – 8.11 (1H, m, H-3), 7.90 – 7.84 (1H, m, H-6), 7.79 – 7.72 (2H, m, H-4 and H-5), 5.22 (1H, bs, H-7), 2.80 (3H, d, *J* 5.3, H-8); *m/z* (ESI$^-$) 215 ([M-H]$^-$, 100%). The spectroscopic data are in good agreement with the literature values.[206,207]

tert-Butyl [(1*S*)-3-hydroxy-1-phenylpropyl]carbamate (240)[208–210]



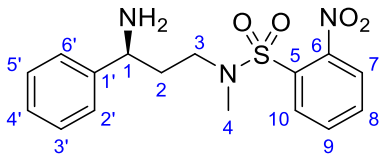
(S)-3-Amino-3-phenylpropan-1-ol (750 mg, 4.96 mmol, 1.0 eq.) was dissolved in anhydrous dichloromethane (15 mL). Triethylamine (812 µL, 5.95 mmol, 1.2 eq.) and di-*tert*-butyl dicarbonate (1.52 g, 6.94 mmol, 1.4 eq.) were added at 0 ºC, and the resulting mixture was stirred at room temperature for 1 h. After this time, the reaction mixture was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (20–50% ethyl acetate in petroleum ether) to yield the title compound **240** as a colourless oil (940 mg, 75%). R_f 0.46 (50% ethyl acetate in petroleum ether); $[\alpha]_D^{25}$= −55.4 (*c* 1.0, MeOH) {lit.[208] –59.8 (*c* 1.0, MeOH)}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.40 – 7.26 (5H, m, H-2', H-3', H-4', H-5' and H-6'), 5.01 – 4.85 (1H, m, H-1), 3.71 (2H, dd, *J* 7.7, 3.7, H-3), 2.14 – 2.00 (1H, m, H-2^a), 1.93 – 1.78 (1H, m, H-2^b), 1.44 (9H, s, H-7); *m/z* (ESI$^+$) 274 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[208–210]

tert-Butyl {(1*S*)-3-[methyl(10-nitrobenzene-9-sulfonyl)amino]-1-phenylpropyl}carbamate (242)



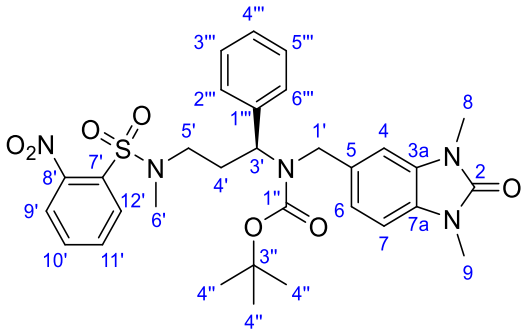
240 (761 mg, 3.03 mmol, 1.0 eq.) and **241** (982 mg, 4.54 mmol, 1.5 eq.) were dissolved in anhydrous tetrahydrofuran (15 mL). The solution was cooled to 0 ºC, and triphenylphosphine (1.19 g, 4.54 mmol, 1.5 eq.) and diisopropyl azodicarboxylate (894 µL, 4.54 mmol, 1.5 eq.) were added. The reaction mixture was stirred at room temperature for 16 h. After this time, ethyl acetate (30 mL) was added, and the organic component was washed with saturated aqueous sodium bicarbonate solution (40 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (50–70% diethyl ether in petroleum ether) to yield the title compound **242** as a colourless oil (1.12 g, 82%). R_f 0.20 (50% diethyl ether in petroleum ether); $[\alpha]_D^{25} = -19.4$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3395 (w), 2978 (w), 2932 (w), 2362 (w), 2340 (w), 1699 (s), 1590 (w), 1544 (s), 1515 (w), 1455 (w), 1440 (w), 1367 (s), 1351 (s), 1282 (w), 1248 (w), 1165 (s), 1125 (w), 1058 (w), 1027 (w), 975 (w), 946 (w), 872 (w), 852 (w), 761 (m), 702 (m), 651 (w), 617 (w); ^{1}H NMR (400 MHz, CD_3OD) δ_H 7.96 – 7.90 (1H, m, H-11), 7.82 – 7.71 (3H, m, H-12, H-13 and H-14), 7.34 – 7.26 (4H, m, H-2', H-3', H-5' and H-6'), 7.26 – 7.19 (1H, m, H-4'), 4.59 (1H, t, *J* 7.5, H-1), 3.27 (2H, t, *J* 7.5, H-3), 2.90 (3H, s, H-8), 1.98 (2H, q, *J* 7.5, H-2), 1.41 (9H, s, H-7); ^{13}C NMR (101 MHz, CD_3OD) δ_C 157.8 (C-5), 149.8 (C-10), 144.4 (C-1'), 135.2 (C-13), 132.9 (C-12), 132.6 (C-9), 131.6 (C-11), 129.5 (C-3' and C-5'), 128.2 (C-4'), 127.3 (C-2' and C-6'), 125.4 (C-14), 80.2 (C-6), 53.7 (C-1), 48.8 (C-3), 36.2 (C-2), 35.4 (C-8), 28.8 (C-7); *m/z* (ESI$^+$) 472 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{21}H_{27}O_6N_3SNa^+$ ([M+Na]$^+$) requires 472.15128, found 472.15115; HPLC (method 4) retention time 11.17 min, 95.0%.

_N_-[(3_S_)-1-Amino-1-phenylpropyl]-_N_-methyl-6-nitrobenzene-5-sulfonamide (243)



242 (850 mg, 1.89 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (15 mL) and stirred at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo*. The crude residue was passed through an Isolute® SXC-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the title compound **243** as a colourless oil (501 mg, 76%). R_f 0.76 (6:2:1 ethyl acetate:ethanol:ammonium hydroxide); $[\alpha]_D^{25}$= −16.8 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3089 (w), 3028 (w), 2931 (w), 2884 (w), 2361 (w), 2342 (w), 1650 (w), 1589 (w), 1542 (s), 1493 (w), 1455 (w), 1439 (w), 1372 (m), 1344 (m), 1297 (w), 1265 (w), 1204 (w), 1161 (m), 1125 (w), 1059 (w), 1029 (w), 1001 (w), 965 (w), 852 (w), 754 (m), 702 (m), 651 (w), 617 (w); ^{1}H NMR (400 MHz, CD_3OD) δ_H 7.92 – 7.88 (1H, m, H-7), 7.81 – 7.72 (3H, m, H-8, H-9 and H-10), 7.36 – 7.31 (4H, m, H-2', H-3', H-5' and H-6'), 7.29 – 7.22 (1H, m, H-4'), 3.90 (1H, t, *J* 7.2, H-1), 3.22 (2H, td, *J* 7.2, 5.5, H-3), 2.88 (3H, s, H-4), 1.97 (2H, q, *J* 7.2, H-2); ^{13}C NMR (101 MHz, CD_3OD) δ_C 149.8 (C-6), 145.9 (C-1'), 135.3 (C-9), 133.0 (C-8), 132.6 (C-5), 131.5 (C-7), 129.7 (C-3' and C-5'), 128.4 (C-4'), 127.6 (C-2' and C-6'), 125.4 (C-10), 54.4 (C-1), 48.7 (C-3), 37.8 (C-2), 35.0 (C-4); *m/z* (ESI$^+$) 372 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{16}H_{20}O_4N_3S^+$ ([M+H]$^+$) requires 350.11690, found 350.11688; HPLC (method 4) retention time 7.50 min, 98.6%.

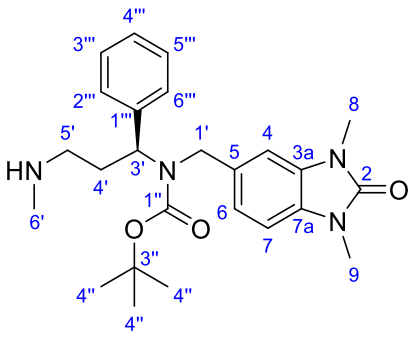
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{(1*S*)-5'-[methyl(8'-nitrobenzene-7'-sulfonyl)amino]-3'-phenylpropyl}carbamate (245)



243 (622 mg, 1.78 mmol, 1.3 eq.) was dissolved in 1,2-dichloroethane (14 mL). **160** (257 mg, 1.35 mmol, 1.0 eq.) and acetic acid (116 µL, 2.02 mmol, 1.5 eq.) were added at 0 ºC, and the reaction mixture was stirred at room temperature for 1 h. After this time, sodium triacetoxyborohydride (910 mg, 4.30 mmol, 3.2 eq.) was added, and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (20 mL). The organic component was separated, and the aqueous component was extracted with dichloromethane (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% triethylamine, 1–2% methanol in dichloromethane) followed by further purification by silica gel column chromatography (40–80% [3:1 ethyl acetate:ethanol] in petroleum ether) to yield the desired reductive amination product as an inseparable mixture with starting material **243**. The mixture dissolved in anhydrous dichloromethane (15 mL), and triethylamine was added (281 µL, 2.06 mmol, 1.2 eq.). The solution was cooled to 0 ºC and di-*tert*-butyl dicarbonate (750 mg, 3.44 mmol, 2.0 eq.) was added. The reaction mixture was stirred at room temperature for 16 h. After this time, the reaction mixture was diluted with dichloromethane (30 mL) and washed with water (30 mL). The organic component was dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–20% acetone in toluene) to yield the title compound **245** as a colourless foam (545 mg, 65% over two steps). R_f 0.53 (40% acetone in

toluene); $[\alpha]_D^{25}$= −27.7 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3437 (w), 2973 (w), 2936 (w), 2787 (w), 2436 (w), 1686 (s), 1633 (w), 1612 (w), 1509 (m), 1454 (m), 1397 (m), 1365 (w), 1333 (w), 1306 (w), 1270 (w), 1248 (w), 1190 (w), 1161 (m), 1110 (w), 1083 (w), 1021 (w), 961 (w), 922 (w), 866 (w), 811 (w), 767 (w), 744 (w), 729 (w), 703 (w), 653 (w), 623 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 7.91 – 7.74 (4H, m, H-9', H-10', H-11' and H-12'), 7.38 – 7.29 (4H, m, H-2''', H-3''', H-5''' and H-6'''), 7.28 – 7.23 (1H, m, H-4'''), 6.93 (1H, d, *J* 7.8, H-7), 6.86 (1H, d, *J* 7.8, H-6), 6.71 (1H, s, H-4), 5.11 (1H, t, *J* 7.9, H-3'), 4.37 (1H, d, *J* 15.5, H-1'^{a}), 4.26 (1H, d, *J* 15.5, H-1'^{b}), 3.28 (3H, s, H-8 or H-9), 3.21 (3H, s, H-8 or H-9), 3.16 – 3.02 (2H, m, H-5'), 2.75 (3H, s, H-6'), 2.34 – 2.15 (2H, m, H-4'), 1.40 (9H, s, H-4''); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 154.8 (C-1''), 153.7 (C-2), 147.5 (C-8'), 139.8 (C-1'''), 133.8 (C-11'), 131.8 (C-7'), 131.6 (C-10'), 130.4 (C-5), 129.24 (C-7a), 129.22 (C-9'), 128.3 (C-7a), 127.8 (C-3''' and C-5'''), 127.1 (C-2''' and C-6'''), 126.8 (C-4'''), 123.8 (C-12'), 119.9 (C-6), 106.4 (C-4), 106.2 (C-7), 79.1 (C-3''), 56.7 (C-3'), 47.6 (C-1'), 47.2 (C-5'), 33.9 (C-6'), 29.5 (C-4'), 27.6 (C-4''), 26.4, 26.2 (C-8 and C-9); *m/z* (ESI$^+$) 624 ([M+H]$^+$, 60%), 646 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{31}H_{38}O_7N_5S^+$ ([M+H]$^+$) requires 624.24865, found 624.24829; HPLC (method 4) retention time 8.11 min, 98.5%.

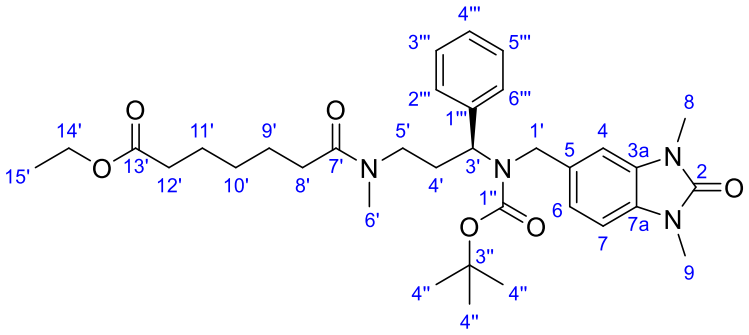
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(1*S*)-3-(methylamino)-1-phenylpropyl]carbamate (246)



Thiophenol (144 µL, 1.40 mmol, 5.0 eq.) and potassium carbonate (116 mg, 0.842 mmol, 3.0 eq.) were added to a solution of **245** (175 mg, 0.281 mmol, 1.0 eq.) in anhydrous *N*,*N*-dimethylformamide (5 mL). The reaction mixture was stirred at room temperature for 5 h.

After this time, the solvent was removed *in vacuo*. The resulting residue was dissolved in dichloromethane (30 mL), filtered and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% ammonium hydroxide, 5–15% methanol in dichloromethane) to yield the title compound **246** as a colourless oil (91.1 mg, 74%). R_f 0.27 (1% ammonium hydroxide, 8% methanol in dichloromethane); $[\alpha]_D^{25} = -48.9$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3437 (w), 2973 (w), 2936 (w), 2787 (w), 2436 (w), 1686 (s), 1633 (w), 1612 (w), 1509 (m), 1454 (m), 1397 (m), 1365 (m), 1333 (w), 1306 (w), 1270 (w), 1248 (m), 1190 (w), 1161 (m), 1110 (w), 1083 (w), 1020 (w), 961 (w), 922 (w), 866 (w), 811 (w), 767 (w), 744 (w), 729 (w), 704 (w), 653 (w), 623 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 7.40 – 7.28 (4H, m, H-2’’’, H-3’’’, H-5’’’ and H-6’’’), 7.27 – 7.21 (1H, m, H-4’’’), 6.95 (1H, d, *J* 8.0, H-7), 6.86 (1H, d, *J* 8.0, H-6), 6.68 (1H, s, H-4), 5.19 (1H, t, *J* 7.6, H-3’), 4.37 (1H, d, *J* 15.6, H-1’^{a}), 4.26 (1H, d, *J* 15.6, H-1’^{b}), 3.29 (3H, s, H-8 or H-9), 3.22 (3H, s, H-8 or H-9), 2.49 – 2.40 (2H, m, H-5’), 2.24 (3H, s, H-6’), 2.16 – 2.02 (2H, m, H-4’), 1.39 (9H, s, H-4’’); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 155.0 (C-1’’), 153.7 (C-2), 140.6 (C-1’’’), 132.1 (C-5), 129.2 (C-3a), 128.2 (C-7a), 127.7 (C-3’’’ and C-5’’’), 127.3 (C-2’’’ and C-6’’’), 126.7 (C-4’’’), 119.8 (C-6), 106.3 (C-4), 106.1 (C-7), 78.8 (C-3’’), 56.9 (C-3’), 48.1 (C-5’), 47.4 (C-1’), 35.1 (C-6’), 30.7 (C-4’), 27.7 (C-4’’), 26.4, 26.2 (C-8 and C-9); *m/z* (ESI$^+$) 439 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{25}H_{35}O_3N_4^+$ ([M+H]$^+$) requires 439.2704, found 439.2700; HPLC (method 4) retention time 8.24 min, 93.7%.

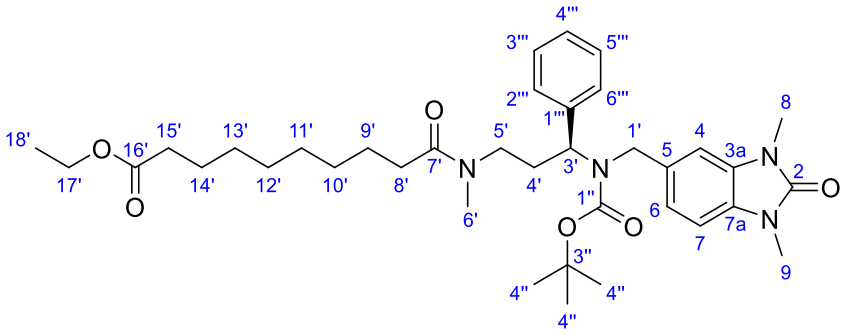
Ethyl 13'-{[(3*S*)-2'-{(*tert*-butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3'-phenylpropyl](methyl)amino}-7'-oxoheptanoate (247)



Ethyl hydrogen pimelate (12.9 mg, 0.0684 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (47.7 µL, 0.274 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (2 mL). HATU (28.6 mg, 0.0752 mmol, 1.1 eq.) was added and the solution was stirred at room temperature for 10 min. **246** (30.0 mg, 0.0684 mmol, 1.0 eq.) was added, and the reaction mixture was stirred at room temperature for 16 h. After this time, the solution was diluted with water (10 mL) and ethyl acetate (10 mL), and the aqueous component was extracted with ethyl acetate (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (50–100% ethyl acetate in petroleum ether) to yield the title compound **247** as a colourless oil (20.8 mg, 50%). R_f 0.14 (80% ethyl acetate in petroleum ether); $[\alpha]_D^{25}$= −40.1 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2929 (w), 2857 (w), 1687 (s), 1636 (m), 1509 (m), 1454 (m), 1400 (m), 1366 (m), 1329 (m), 1303 (w), 1267 (w), 1249 (m), 1162 (m), 1142 (m), 1082 (w), 1030 (w), 960 (w), 874 (w), 844 (m), 803 (w), 766 (w), 744 (w), 729.11 (w), 703 (w), 670 (w), 661 (w), 648 (w), 629 (w), 609 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 7.40 – 7.29 (4H, m, H-2''', H-3''', H-5''' and H-6'''), 7.28 – 7.23 (1H, m, H-4'''), 6.96 (1H, d, *J* 8.0, H-7), 6.88 (1H, d, *J* 8.0, H-6), 6.71 (1H, s, H-4), 5.09 (1H, t, *J* 7.6, H-3'), 4.39 (1H, d, *J* 15.6, H-1'^{a}), 4.28 (1H, d, *J* 15.6, H-1'^{b}), 4.07 (2H, q, *J* 7.1, H-15'), 3.29 (3H, s, H-8), 3.23 (3H, s, H-9), 3.21 – 3.11 (2H, m, H-5'), 2.86 – 2.64 (3H, bs, H-6'), 2.29 – 1.99 (6H, m, H-4', H-8' and H-12'), 1.58 – 1.41 (4H, m, H-9' and H-11'), 1.40 (9H, s, H-4''), 1.33 – 1.23 (2H, m, H-10'), 1.19 (3H, t, *J* 7.1, H-16'); ^{13}C NMR (126 MHz, DMSO-d_6,

363 K) δ_C 172.3 (C-13'), 171.3 (C-7'), 155.0 (C-1''), 153.8 (C-2), 140.1 (C-1'''), 132.1 (C-5), 129.3 (C-3a), 128.3 (C-7a), 127.8, 127.3 (C-2''', C-3''', C-5''' and C-6'''), 126.9 (C-4'''), 119.9 (C-6), 106.4 (C-7), 106.2 (C-4), 79.0 (C-3''), 59.1 (C-15'), 57.0 (C-3'), 47.5 (C-1'), 44.9 (C-5'), 34.5 (C-6'), 33.2 (C-12'), 31.8 (C-4'), 29.1 (C-8'), 27.8 (C-10'), 27.7 (C-4''), 26.4 (C-8), 26.3 (C-9), 23.9 (C-9' and C-11'), 13.6 (C-16'); *m/z* (ESI$^+$) 631 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{34}H_{49}O_6N_4^+$ ([M+H]$^+$) requires 609.3647, found 609.3645; HPLC (method 1) retention time 10.9 min, 100%.

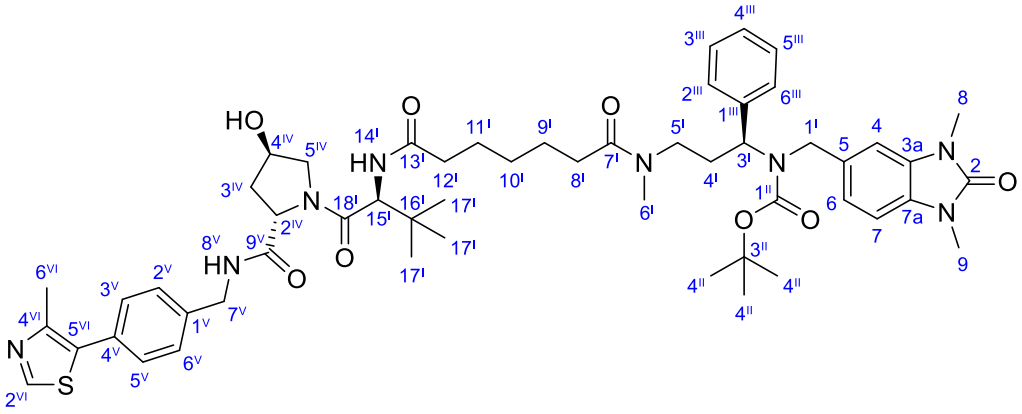
Ethyl 16'-{[(3*S*)-2'-{(*tert*-butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3'-phenylpropyl](methyl)amino}-7'-oxodecanoate (248)



Ethyl hydrogen sebacate (21.0 mg, 0.0912 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (63.5 µL, 0.365 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (2 mL). HATU (38.1 mg, 0.100 mmol, 1.1 eq.) was added and the solution was stirred at room temperature for 10 min. **246** (40.0 mg, 0.0912 mmol, 1.0 eq.) was added, and the reaction mixture was stirred at room temperature for 16 h. After this time, the solution was diluted with water (10 mL) and ethyl acetate (10 mL), and the aqueous component was extracted with ethyl acetate (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (50–80% ethyl acetate in petroleum ether) to yield the title compound **248** as a colourless oil (45.0 mg, 76%). R_f 0.31 (80% ethyl acetate in petroleum ether); $[\alpha]_D^{25}$ = −41.5 (*c* 1.0, $CHCl_3$); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3515 (w), 2931 (m), 2856 (w), 2362 (w), 2224 (w), 1996 (w), 1711 (s), 1688 (s), 1645 (m), 1509 (m), 1454 (m), 1397 (m), 1366 (m), 1328 (m), 1303 (m), 1248 (m), 1163 (m), 1031 (w), 960 (w), 874 (w),

805 (w), 763 (w), 745 (w), 728 (w), 703 (w), 623 (w); ^{1}H NMR (500 MHz, toluene-d_8, 363 K) δ_H 7.34 (2H, s, H-2''' and H-6'''), 7.18 – 7.02 (3H, m, partially obscured by toluene-d_8 peak, H-3''', H-4''' and H-5'''), 6.81 (1H, s, H-6), 6.56 (1H, s, H-4), 6.43 (1H, s, H-7), 5.29 (1H, s, H-3'), 4.40 (1H, d, *J* 15.5, H-1'^{a}), 4.27 (1H, d, *J* 15.8, H-1'^{b}), 4.04 – 3.92 (2H, m, H-17'), 3.46 – 3.12 (2H, m, H-5'), 3.03 (3H, s, H-8), 2.94 (3H, s, H-9), 2.53 (3H, s, H-6'), 2.27 – 2.07 (4H, m, partially obscured by toluene-d_8 peak, H-4' and H-8'), 1.99 (2H, s, H-15'), 1.69 – 1.50 (4H, m, H-9' and H-14'), 1.44 (9H, s, H-4''), 1.31 – 1.17 (8H, m, H-10', H-11', H-12' and H-13'), 1.08 – 1.00 (3H, m, H-18'); ^{13}C NMR (126 MHz, toluene-d_8, 363 K) δ_C 172.8 (C-16'), 172.0 (C-7'), 156.5 (C-1''), 154.9 (C-2), 141.5 (C-1'''), 133.3 (C-3a), 131.1 (C-7a), 130.0 (C-5), 129.3 (C-2''' and C-6'''), 128.6 (C-3''' and C-5'''), 128.4 (C-4'''), 120.9 (C-6), 107.2 (C-4), 106.5 (C-7), 80.1 (C-3''), 59.9 (C-17'), 58.5 (C-3'), 49.0 (C-1'), 47.0 (C-5'), 34.63, 34.62 (C-8' and C-15'), 33.5 (C-6'), 30.7 (C-4'), 30.0, 29.8, 29.7, 28.7 (C-10', C-11', C-12' and C-13'), 26.8, 26.7 (C-8 and C-9), 25.6 (C-9'), 25.5 (C-14'), 14.4 (C-18'); *m/z* (ESI$^+$) 673 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{37}H_{55}O_6N_4^+$ ([M+H]$^+$) requires 651.4116, found 651.4114; HPLC (method 4) retention time 12.5 min, 98.6%.

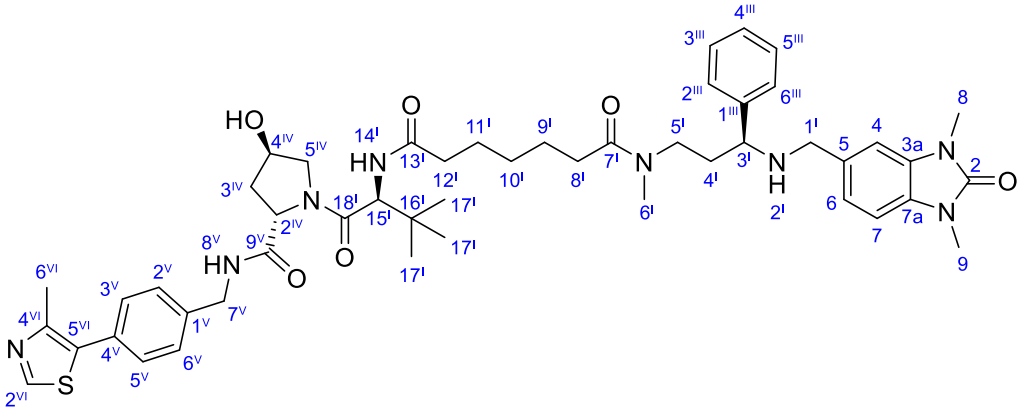
N-(14^{I}-{[(3*S*)-2^{I}-{(*tert*-Butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl](methyl)amino}-7^{I}-oxoheptanoyl)-16^{I}-methyl-L-valyl-(4*R*)-4^{IV}-hydroxy-N-{[4^{V}-(4^{VI}-methyl-1^{VI},3^{VI}-thiazol-5^{VI}-yl)phenyl]methyl}-L-prolinamide (251)



A solution of lithium hydroxide (14.8 mg, 0.616 mmol, 5.0 eq.) in water (2 mL) was added to a solution of **247** (75.0 mg, 0.123 mmol, 1.0 eq.) in methanol (2 mL) and tetrahydrofuran (2 mL). The reaction mixture was stirred at room temperature for 1 h. The solution was then acidified to pH 1 using 1 M aqueous hydrochloric acid, and extracted with ethyl acetate (3 × 30 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue (69.0 mg, 0.119 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (1.5 mL), and HATU (49.7 mg, 0.131 mmol, 1.1 eq.) and *N*,*N*-diisopropylethylamine (82.9 µL, 0.476 mmol, 4.0 eq.) were added. The solution was stirred at room temperature for 10 min. After this time, VHL amine (55.6 mg, 0.119 mmol, 1.0 eq.) dissolved in *N*,*N*-dimethylformamide (1.5 mL) was added and the reaction mixture was stirred at room temperature for 16 h. The solution was diluted with water (20 mL) and ethyl acetate (20 mL), and the aqueous component was extracted with ethyl acetate (3 × 30 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–20% ethanol in ethyl acetate) to yield the title compound **251** as a colourless oil (51.1 mg, 43%). R_f 0.14 (10% ethanol in ethyl acetate); $[\alpha]_D^{25} = -41.7$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3433 (m), 3413 (m), 3298 (m), 3073 (m), 2943

(m), 2362 (w), 2337 (w), 1685 (s), 1629 (s), 1536 (m), 1509 (m), 1451 (m), 1402 (m), 1369 (w), 1330 (w), 1243 (w), 1161 (m), 1084 (w), 1031 (w), 967 (w), 852 (w), 804 (w), 764 (w), 745 (w), 702 (w), 649 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.90 (1H, s, H-2^{VI}), 8.21 – 8.10 (1H, m, H-8^{V}), 7.47 – 7.21 (10H, m, H-2^{III}, H-3^{III}, H-4^{III}, H-5^{III}, H-6^{III}, H-2^{V}, H-3^{V}, H-5^{V}, H-6^{V} and H-14^{I}), 6.95 (1H, d, *J* 8.0, H-7), 6.87 (1H, d, *J* 8.0, H-6), 6.71 (1H, s, H-4), 5.09 (1H, t, *J* 7.5, H-3^{I}), 4.81 (1H, s, O*H*), 4.60 – 4.46 (2H, m, H-2^{IV} and H-15^{I}), 4.46 – 4.34 (3H, m, H-4^{IV}, H-7Va and H-1^{Ia}), 4.33 – 4.22 (2H, m, H-7^{Vb} and H-1^{Ib}), 3.76 – 3.61 (2H, m, H-5^{IV}), 3.32 – 3.10 (8H, m, H-8, H-9 and H-5^{I}), 2.76 (3H, s, H-6^{I}), 2.45 (3H, s, H-6^{VI}), 2.29 – 1.94 (8H, m, H-4^{I}, H-3^{IV}, H-8^{I} and H-12^{I}), 1.57 – 1.34 (13H, m, H-4^{II}, H-9^{I} and H-11^{I}), 1.30 – 1.16 (2H, m, H-10^{I}), 0.96 (9H, s, H-17^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.61 (C-9^{V}), 171.58 (C-7^{I}), 171.3 (C-18^{I}), 169.6 (C-13^{I}), 154.9 (C-2), 153.7 (C-1^{II}), 150.5 (C-2^{VI}), 147.4 (C-4^{VI}), 140.0 (C-1^{III}), 139.0 (C-1^{V}), 132.0 (C-5^{VI}), 130.7 (C-7a), 129.5 (C-4^{V}), 129.2 (C-3a), 128.3 (C-5), 128.2 (C-2^{V} and C-6^{V} or C-3^{V} and C-5^{V}), 127.8 (C-2^{III} and C-6^{III} or C-3^{III} and C-5^{III}), 127.3 (C-2^{V} and C-6^{V} or C-3^{V} and C-5^{V}), 127.2 (C-2^{III} and C-6^{III} or C-3^{III} and C-5^{III}), 126.8 (C-4^{III}), 119.8 (C-6), 106.4 (C-4), 106.2 (C-7), 79.0 (C-3^{II}), 68.5 (C-4^{IV}), 58.3 (C-2^{IV}), 57.0 (C-3^{I}), 56.2 (C-15^{I}), 55.7 (C-5^{IV}), 47.5 (C-1^{I}), 45.0 (C-5^{I}), 41.5 (C-7^{V}), 39.6 (C-16^{I}), 37.3 (C-8^{I} and C-12^{I}), 34.6 (C-3^{IV} and C-6^{I}), 31.9 (C-4^{I}), 28.0 (C-10^{I}), 27.7 (C-4^{II}), 26.4, 26.2 (C-8 and C-9), 26.0 (C-17^{I}), 24.7, 24.0 (C-9^{I} and C-11^{I}), 15.3 (C-6^{VI}); *m/z* (ESI^{+}) 994 ([M+H]$^{+}$, 90%), 1016 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{54}H_{73}O_8N_8S^{+}$ ([M+H]$^{+}$) requires 993.5267, found 993.5256; HPLC (method 1) retention time 8.88 min, 91.0%.

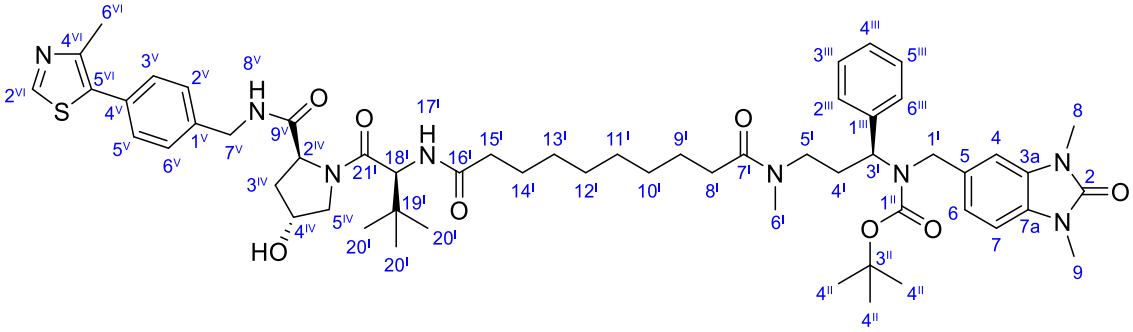
N-(14^{I}-{[(3*S*)-1^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl](methyl)amino}-7^{I}-oxoheptanoyl)-16^{I}-methyl-L-valyl-(4*R*)-4^{IV}-hydroxy-*N*-{[4^{V}-(4^{VI}-methyl-1^{VI},3^{VI}-thiazol-5^{VI}-yl)phenyl]methyl}-L-prolinamide (233)



251 (40 mg, 0.040 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (1 mL), and the reaction mixture was stirred at room temperature for 1 h. After this time, the mixture was neutralised to pH 7 with 2 M aqueous sodium hydroxide solution. The solution was extracted with dichloromethane (3 × 20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (5–10% [1:2 ammonium hydroxide:ethanol] in ethyl actate) to yield the title compound **233** as a colourless oil (9.3 mg, 54%). R_f 0.27 (10% [1:2 ammonium hydroxide:ethanol] in ethyl actate); $[\alpha]_D^{25}$= −36.2 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3393 (w), 3063 (w), 3029 (w), 2938 (w), 2870 (w), 2465 (w), 2419 (w), 1688 (s), 1626 (s), 1541 (w), 1507 (w), 1449 (m), 1419 (m), 1399 (w), 1371 (w), 1343 (w), 1308 (w), 1270 (w), 1240 (w), 1191 (w), 1138 (w), 1120 (w), 1085 (w), 1029 (w), 967 (w), 938 (w), 851 (w), 807 (w), 768 (w), 745 (w), 727 (w), 704 (w), 617 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.86 (1H, s, H-2^{VI}), 7.48 – 7.23 (9H, m, H-2^{III}, H-3^{III}, H-4^{III}, H-5^{III}, H-6^{III}, H-2^{V}, H-3^{V}, H-5^{V} and H-6^{V}), 7.08 – 6.96 (3H, m, H-4, H-6 and H-7), 4.63 (1H, s, H-15^{I}), 4.60 – 4.46 (3H, m, H-4^{IV}, H-3^{I} and H-7Va), 4.35 (1H, d, *J* 15.5, H-7^{Vb}), 3.90 (1H, d, *J* 11.1, H-5^{IVa}), 3.79 (1H, dd, *J* 11.1, 3.9, H-5^{IVb}), 3.68 – 3.48 (3H, m, H-2^{IV} and H-1^{I}), 3.42 – 3.35 (6H, m, H-8 and H-9), 3.30 – 3.21 (1H, m, H-5^{Ia}), 3.12 (1H, ddd, *J* 14.9, 10.3, 5.1, H-5^{Ib}), 2.90 (2H, s, H-6^{I}), 2.81 (1H, s, H-6^{I}), 2.46 (3H, s, H-6^{VI}), 2.33 – 2.17 (4H, m, H-4^{Ia}, H-3^{IVa} and H-8^{I}), 2.16 – 2.01 (3H, m, H-4^{Ib}, H-3^{IVb} and

H-12^{Ia}), 1.98 – 1.88 (1H, m, H-12^{Ib}), 1.67 – 1.14 (6H, m, H-9^{I}, H-10^{I} and H-11^{I}), 1.03 (9H, s, H-17^{I}); ^{13}C NMR (126 MHz, CD_3OD) δ_C 175.9 (C-9^{V}), 175.5 (C-7^{I}), 174.5 (C-18^{I}), 172.3 (C-13^{I}), 156.4 (C-2), 152.8 (C-2^{VI}), 149.0 (C-4^{VI}), 144.0 (C-1^{III}), 140.3 (C-1^{V}), 134.9 (C-7a), 133.4 (C-5^{VI}), 131.5 (C-3a), 131.2 (C-5), 130.4 (C-2^{III} and C-6^{III} or C-3^{III} and C-5^{III}), 129.9 (C-2^{V} and C-6^{V} or C-3^{V} and C-5^{V}), 129.7 (C-2^{V} and C-6^{V} or C-3^{V} and C-5^{V}), 129.0 (C-2^{III} and C-6^{III} or C-3^{III} and C-5^{III}), 128.7 (C-4^{III}), 128.5 (C-4^{V}), 123.3 (C-6), 109.4 (C-4), 108.6 (C-7), 71.1 (C-4^{IV}), 61.1 (C-2^{IV}), 60.8 (C-3^{I}), 59.0 (C-15^{I}), 58.0 (C-5^{IV}), 52.2 (C-1^{I}), 46.1 (C-5^{I}), 43.7 (C-7^{V}), 38.9 (C-12^{I}), 36.9 (C-16^{I}), 36.6 (C-8^{I}), 35.8 (C-4^{I}), 34.1 (C-6^{I}), 33.7 (C-3^{IV}), 33.5, 29.9 (C-10^{I}), 27.5 (C-17^{I}), 27.1, 27.0 (C-8 and C-9), 26.7, 25.9 (C-9^{I} and C-11^{I}), 15.8 (C-6^{VI}); *m/z* (ESI$^+$) 894 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{49}H_{65}O_6N_8S^+$ ([M+H]$^+$) requires 893.4742, found 893.4733; HPLC (method 1) retention time 6.76 min, 100%.

_N_-(17^{I}-{[(3*S*)-2^{I}-{(*tert*-Butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl](methyl)amino}-7^{I}-oxodecanoyl)-19^{I}-methyl-L-valyl-(4*R*)-4^{IV}-hydroxy-*N*-{[4^{V}-(4^{VI}-methyl-1^{VI},3^{VI}-thiazol-5^{VI}-yl)phenyl]methyl}-L-prolinamide (252)

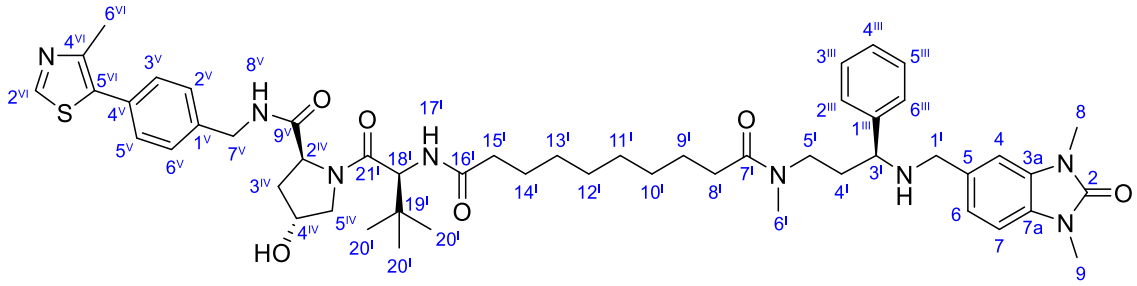


A solution of lithium hydroxide (6.99 mg, 0.292 mmol, 5.0 eq.) in water (0.25 mL) was added to a solution of **248** (38.0 mg, 0.0584 mmol, 1.0 eq.) in methanol (0.25 mL) and tetrahydrofuran (0.25 mL). The reaction mixture was stirred at room temperature for 1 h. The solution was then acidified to pH 1 using 1 M aqueous hydrochloric acid, and extracted with ethyl acetate (3 × 10 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue (30.0 mg, 0.0482 mmol, 1.0 eq.) was dissolved in

anhydrous *N*,*N*-dimethylformamide (1 mL), and HATU (20.1 mg, 0.0530 mmol, 1.1 eq.) and *N*,*N*-diisopropylethylamine (33.6 µL, 0.193 mmol, 4.0 eq.) were added. The solution was stirred at room temperature for 10 min. After this time, VHL amine (24.3 mg, 0.0482 mmol, 1.0 eq.) dissolved in *N*,*N*-dimethylformamide (1 mL) was added and the reaction mixture was stirred at room temperature for 16 h. The solution was diluted with water (20 mL) and ethyl acetate (20 mL), and the aqueous component was extracted with ethyl acetate (3 × 30 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (30–60% acetone in toluene) and further purified by silica gel column chromatography (2–20% ethanol in ethyl acetate) to yield the title compound **252** as a colourless oil (25.5 mg, 51%). R_f 0.36 (50% acetone in toluene); $[\alpha]_D^{25} = -40.8$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3398 (w), 2928 (w), 2856 (w), 1684 (s), 1629 (s), 1540 (w), 1508 (w), 1451 (m), 1401 (w), 1366 (w), 1330 (w), 1273 (w), 1244 (w), 1161 (w), 1085 (w), 1029 (w), 853 (w), 767 (w), 745 (w), 702 (w), 622 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 8.91 (1H, s, H-2^{VI}), 8.16 (1H, s, H-8^{V}), 7.46 – 7.20 (10H, m, H-17^{I}, H-2^{III}, H-3^{III}, H-4^{III}, H-5^{III}, H-6^{III}, H-2^{V}, H-3^{V}, H-5^{V} and H-6^{V}), 6.95 (1H, d, *J* 8.0, H-7), 6.87 (1H, d, *J* 8.0, H-6), 6.71 (1H, s, H-4), 5.09 (1H, t, *J* 7.6, H-3^{I}), 4.81 (1H, s, O*H*), 4.59 – 4.34 (5H, m, H-18^{I}, H-4^{IV}, H-2^{IV}, H-7Va and H-1^{Ia}), 4.32 – 4.22 (2H, m, H-7^{Vb} and H-1^{Ib}), 3.75 – 3.60 (2H, m, H-5^{IV}), 3.33 – 3.10 (8H, m, H-8, H-9 and H-5^{I}), 2.76 (3H, s, H-6^{I}), 2.45 (3H, s, H-6^{VI}), 2.29 – 1.91 (8H, m, H-4^{I}, H-3^{IV}, H-8^{I} and H-15^{I}), 1.58 – 1.34 (13H, m, H-4^{II}, H-9^{I} and H-14^{I}), 1.32 – 1.13 (8H, m, H-10^{I}, H-11^{I}, H-12^{I} and H-13^{I}), 0.95 (9H, s, H-20^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.6 (C-9^{V}), 171.3 (C-7^{I}), 171.2 (C-21^{I}), 169.6 (C-16^{I}), 154.9 (C-1^{II}), 153.7 (C-2), 150.5 (C-2^{VI}), 147.4 (C-4^{VI}), 140.0 (C-1^{III}), 139.0 (C-1^{V}), 132.0 (C-7a), 130.7 (C-5^{VI}), 129.5 (C-4^{V}), 129.2 (C-3a), 128.3 (C-5), 128.2 (C-2^{V} and C-6^{V} or C-3^{V} and C-5^{V}), 127.8 (C-2^{III} and C-6^{III} or C-3^{III} and C-5^{III}), 127.3 (C-2^{V} and C-6^{V} or C-3^{V} and C-5^{V}), 127.2 (C-2^{III} and C-6^{III} or C-3^{III} and C-5^{III}), 126.8 (C-4^{III}), 119.8 (C-6), 106.4 (C-7), 106.2 (C-4), 79.0 (C-3^{II}), 68.5 (C-4^{IV}), 58.3 (C-2^{IV}), 57.0 (C-3^{I}), 56.2 (C-18^{I}), 55.6 (C-5^{IV}), 47.5 (C-1^{I}), 45.0 (C-5^{I}), 41.5 (C-7^{V}), 39.5 (obscured by DMSO-*d*$_6$ peak, C-6^{I}), 37.3 (C-3^{IV}), 34.7 (C-19^{I}), 34.6 (C-8^{I} and C-15^{I}), 31.9 (C-4^{I}), 28.24, 28.16, 28.14 (C-10^{I}, C-11^{I}, C-12^{I} and C-13^{I}), 27.7 (C-4^{II}), 26.4, 26.2 (C-8 and C-9), 26.0 (C-20^{I}), 24.9, 24.2 (C-9^{I} and C-14^{I}), 15.3

(C-6^{VI}); *m/z* (ESI$^+$) 1035 ([M+H]$^+$, 90%), 1057 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{57}H_{79}O_8N_8S^+$ ([M+H]$^+$) requires 1035.5736, found 1035.5726; HPLC (method 1) retention time 9.63 min, 94.7%.

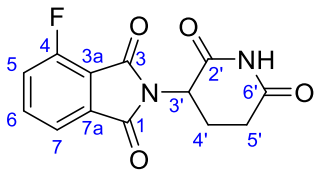
_N_-(10-{[(3*S*)-3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3-phenylpropyl](methyl)amino}-10-oxodecanoyl)-3-methyl-L-valyl-(4*R*)-4-hydroxy-*N*-{[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}-L-prolinamide (234)



252 (20 mg, 0.019 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (1 mL), and the reaction mixture was stirred at room temperature for 1 h. After this time, the mixture was neutralised to pH 7 with 2 M aqueous sodium hydroxide solution. The solution was extracted with dichloromethane (3 × 20 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (3–10% [1:2 ammonium hydroxide:ethanol] in ethyl actate) to yield the title compound **234** as a colourless oil (17 mg, 92%). R_f 0.14 (10% {1:2 ammonium hydroxide:ethanol} in ethyl actate); $[\alpha]_D^{25}$= −29.1 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3387 (w), 3061 (w), 2930 (w), 2856 (w), 2406 (w), 2365 (w), 2342 (w), 1694 (m), 1629 (s), 1507 (w), 1452 (m), 1419 (m), 1371 (w), 1343 (w), 1309 (w), 1270 (w), 1240 (w), 1190 (w), 1138 (w), 1087 (w), 1029 (w), 966 (w), 938 (w), 851 (w), 808 (w), 769 (w), 745 (w), 727 (w), 704 (w), 659 (w), 640 (w), 614 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.86 (1H, s, H-2^{VI}), 7.49 – 7.26 (9H, m, H-2^{III}, H-3^{III}, H-4^{III}, H-5^{III}, H-6^{III}, H-2^V, H-3^V, H-5^V and H-6^V), 7.10 – 6.98 (3H, m, H-4, H-6 and H-7), 4.64 (1H, d, *J* 2.9, H-18^I), 4.60 – 4.51 (2H, m, H-7Va and H-3^I), 4.51 – 4.47 (1H, m, H-4^{IV}), 4.35 (1H, dd, *J* 15.6, 2.5, H-7^{Vb}), 3.90 (1H, d, *J* 11.0, H-5^{IVa}), 3.80 (1H, dd, *J* 11.0, 3.9, H-5^{IVb}), 3.69 – 3.52 (3H, m, H-2^{IV} and H-1^I), 3.45 – 3.34 (6H, m, H-8 and H-9), 3.29 – 3.21 (1H, m, H-5^{Ia}), 3.19 – 3.10 (1H, m, H-5^{Ib}), 2.91 (2H, s, H-6^I), 2.81 (1H, s, H-6^I),

2.46 (3H, s, H-6^{VI}), 2.35 – 2.17 (4H, m, H-3^{IVa}, H-4^{Ia} and H-15^{I}), 2.17 – 1.85 (4H, m, H-3^{IVb}, H-4^{Ib} and H-8^{I}), 1.65 – 1.49 (3H, m, H-14^{I} and H-9^{Ia}), 1.47 – 1.10 (9H, m, H-9^{Ib}, H-10^{I}, H-11^{I}, H-12^{I} and H-13^{I}), 1.03 (9H, s, H-20^{I}); ^{13}C NMR (126 MHz, CD_3OD) δ_C 176.0 (C-9^{V}), 175.5 (C-7^{I}), 174.5 (C-21^{I}), 172.3 (C-16^{I}), 156.4 (C-2), 152.8 (C-2^{VI}), 149.0 (C-4^{VI}), 143.7 (C-1^{III}), 140.3 (C-1^{V}), 134.8 (C-7a), 133.4 (C-5^{VI}), 131.5 (C-3a), 131.2 (C-5), 130.4 (C-2^{V} and C-6^{V} or C-3^{V} and C-5^{V}), 129.9 (C-4^{IV}), 129.8 (C-2^{III} and C-6^{III} or C-3^{III} and C-5^{III}), 129.0 (C-2^{V} and C-6^{V} or C-3^{V} and C-5^{V}), 128.8 (C-4^{III}), 128.7 (C-2^{III} and C-6^{III} or C-3^{III} and C-5^{III}), 123.4 (C-6), 109.4 (C-4), 108.7 (C-7), 71.1 (C-4^{IV}), 61.1 (C-2^{IV}), 60.8 (C-3^{I}), 58.9 (C-18^{I}), 58.0 (C-5^{IV}), 52.2 (C-1^{I}), 46.0 (C-5^{I}), 43.7 (C-7^{V}), 38.9 (C-15^{I}), 36.8 (C-19^{I}), 36.63 (C-4^{I}), 36.58 (C-8^{I}), 35.9 (C-6^{I}), 34.3 (C-3^{IV}), 30.4, 30.31, 30.26, 30.24 (C-10^{I}, C-11^{I}, C-12^{I} and C-13^{I}), 27.5 (C-20^{I}), 27.04, 26.99 (C-8 and C-9), 26.6 (C-9^{I}), 26.3 (C-14^{I}), 15.8 (C-6^{VI}); *m/z* (ESI$^+$) 935 ([M+H]$^+$, 100%), 957 ([M+Na]$^+$, 60%); HRMS *m/z* (ESI$^+$) $C_{52}H_{71}O_6N_8S^+$ ([M+H]$^+$) requires 935.5212, found 935.5198; HPLC (method 1) retention time 7.38 min, 100%.

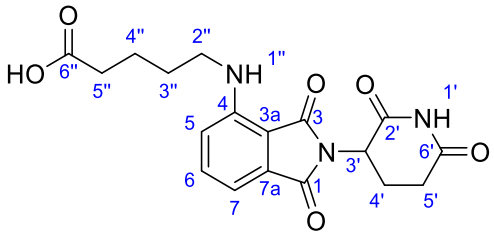
2-(2',6'-Dioxopiperidin-3'-yl)-4-fluoro-1*H*-isoindole-1,3(2*H*)-dione (276)[211,212]



3-Fluorophthalic anhydride (2.00 g, 12.0 mmol, 1.0 eq.) was dissolved in glacial acetic acid (36 mL). 3-Aminopiperidine-2,6-dione (1.98 g, 12.0 mmol, 1.0 eq.) and sodium acetate (2.36 g, 28.8 mmol, 2.4 eq.) were added to the solution, and the reaction mixture was stirred at 140 ºC for 6 h. The reaction mixture was then cooled to room temperature and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (5–10% methanol in dichloromethane) to yield the title compound **276** as a colourless solid (2.93 g, 89%). R_f 0.38 (10% methanol in dichloromethane); m.p. 235–237 ºC (from MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3281 (w), 2162 (w), 1780 (w), 1701 (s), 1611 (w), 1479 (w), 1443 (m), 1385 (s), 1351 (m), 1328 (m), 1303 (w), 1257 (s), 1200 (s), 1116 (m), 1019 (w), 992 (w), 950 (w), 825 (w), 773 (w), 743 (s), 668 (w), 637 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.14 (1H, s, N*H*), 7.95 (1H, ddd,

J 8.4, 7.3, 4.6, H-6), 7.79 (1H, d, *J* 7.3, H-7), 7.74 (1H, ddd, *J* 9.2, 8.4, 0.8, H-5), 5.16 (1H, dd, *J* 12.8, 5.4, H-3'), 2.89 (1H, ddd, *J* 17.0, 13.9, 5.5, H-5'^{a}), 2.65 – 2.44 (2H, m, H-5'^{b} and H-4'^{a}), 2.11 – 2.01 (1H, m, H-4'^{b}); ^{19}F NMR (377 MHz, $CDCl_3$) δ_F –114.69 (dd, *J* 9.5, 4.6); *m/z* (ESI$^+$) 299 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[211,212]

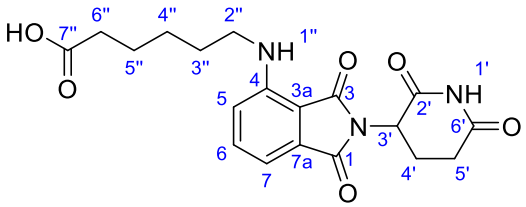
6''-{[2-(2',6'-Dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}pentanoic acid (277)[213]



276 (300 mg, 1.09 mmol, 1.0 eq.), 5-aminovaleric acid (152 mg, 1.30 mmol, 1.2 eq.) and *N*,*N*-diisopropylethylamine (570 µL, 3.27 mmol, 3.0 eq.) were dissolved in *N*-methyl-2-pyrrolidone (7.5 mL) and the mixture was heated at 110 ºC for 2 h under microwave irradiation in a sealed microwave tube. The reaction mixture was then cooled and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–10% methanol in dichloromethane, followed by 1% acetic acid, 10% methanol in dichloromethane) to yield the title compound **277** as a yellow oil (276 mg, 68%). R_f 0.07 (3% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2956 (w), 2927 (w), 1696 (s), 1625 (w), 1509 (w), 1409 (w), 1360 (w), 1326 (w), 1303 (w), 1261 (w), 1199 (w), 1112 (w), 1024 (w), 989 (w), 914 (w), 880 (w), 815 (w), 747 (w), 667 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.79 (1H, bs, H-1'), 7.48 (1H, dd, *J* 8.5, 7.1, H-6), 7.08 (1H, d, *J* 7.1, H-5), 6.87 (1H, d, *J* 8.5, H-7), 6.26 (1H, t, *J* 5.5, H-1''), 4.99 – 4.85 (1H, m, H-3'), 3.34 – 3.23 (2H, m, H-2''), 2.96 – 2.66 (3H, m, H-4'^{a} and H-5'), 2.41 (2H, t, *J* 6.7, H-5''), 2.17 – 2.01 (1H, m, H-4'^{b}), 1.81 – 1.66 (4H, m, H-3'' and H-4''); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 178.4 (C-6''), 171.8 (C-2'), 169.6 (C-6'), 168.9 (C-3), 167.8 (C-1), 147.0 (C-4), 136.3 (C-6), 132.6 (C-7a), 116.7 (C-7), 111.7 (C-5), 110.2 (C-3a), 49.0 (C-3'), 42.4 (C-2''), 33.6 (C-5''), 31.5

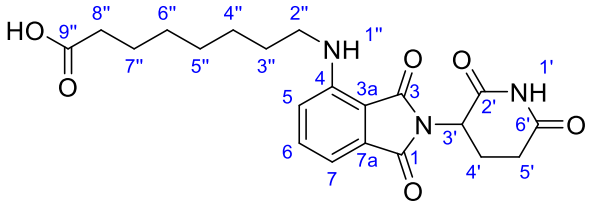
(C-5’), 28.7 (C-3’’), 22.9 (C-4’), 22.1 (C-4’’); *m/z* (ESI$^-$) 372 ([M-H]$^-$, 100%). The spectroscopic data are in good agreement with the literature values.[213]

7’’-{[2-(2’,6’-Dioxopiperidin-3’-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}hexanoic acid (278)[213]



276 (300 mg, 1.09 mmol, 1.0 eq.), 6-aminohexanoic acid (171 mg, 1.30 mmol, 1.2 eq.) and *N*,*N*-diisopropylethylamine (570 µL, 3.27 mmol, 3.0 eq.) were dissolved in *N*-methyl-2-pyrrolidone (7.5 mL) and the mixture was heated at 110 ºC for 2 h under microwave irradiation in a sealed microwave tube. The reaction mixture was then cooled and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (2–4% methanol in dichloromethane) to yield the title compound **278** as a yellow oil (328 mg, 78%). R_f 0.14 (5% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3393 (w), 3237 (w), 3100 (w), 2936 (w), 2863 (w), 1694 (s), 1624 (w), 1510 (w), 1471 (w), 1409 (w), 1360 (w), 1324 (w), 1260 (w), 1197 (w), 1180 (w), 1112 (w), 1037 (w), 1021 (w), 989 (w), 912 (w), 814 (w), 746 (w), 680 (w), 648 (w), 609 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.69 (1H, s, H-1’), 7.47 (1H, dd, *J* 8.5, 7.1, H-6), 7.07 (1H, d, *J* 7.1, H-5), 6.86 (1H, d, *J* 8.5, H-7), 6.23 (1H, t, *J* 5.7, H-1’’), 4.98 – 4.84 (1H, m, H-3’), 3.26 (2H, q, *J* 6.4, H-2’’), 2.93 – 2.66 (3H, m, H-4’^{a} and H-5’), 2.34 (2H, t, *J* 7.4, H-6’’), 2.20 – 2.03 (1H, m, H-4’^{b}), 1.73 – 1.61 (4H, m, H-3’’ and H-5’’), 1.51 – 1.40 (2H, m, H-4’’); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 178.4 (C-7’’), 171.6 (C-2’), 169.6 (C-6’), 168.9 (C-3), 167.8 (C-1), 147.1 (C-4), 136.3 (C-6), 132.6 (C-7a), 116.8 (C-7), 111.6 (C-5), 110.0 (C-3a), 49.0 (C-3’), 42.6 (C-2’’), 34.3 (C-6’’), 31.5 (C-5’), 29.0 (C-3’’), 26.5 (C-4’’), 24.7 (C-5’’), 22.9 (C-4’); *m/z* (ESI$^+$) 410 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[213]

8''-{[2-(2',6'-Dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}octanoic acid (279)[213]

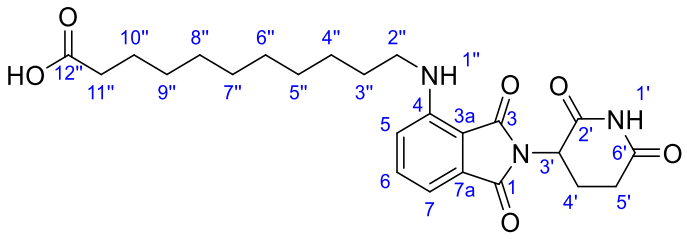


8-[(*tert*-Butoxycarbonyl)amino]octanoic acid (300 mg, 1.16 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (4 mL) and stirred at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo* to yield the Boc-deprotected product 8-aminooctanoic acid hydrochloride (213 mg, 94%), which was used immediately in the next step.

276 (300 mg, 1.09 mmol, 1.0 eq.), 8-aminooctanoic acid hydrochloride (213 mg, 1.09 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (759 µL, 4.36 mmol, 4.0 eq.) were dissolved in *N*-methyl-2-pyrrolidone (7.5 mL) and the mixture was heated at 110 ºC for 2 h under microwave irradiation in a sealed microwave tube. The reaction mixture was then cooled and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–3% methanol in dichloromethane) to yield the title compound **279** as a yellow oil (421 mg, 93%). R_f 0.16 (6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3394 (w), 3195 (w), 3093 (w), 2929 (w), 2858 (w), 2547 (w), 2160 (w), 2018 (w), 1976 (w), 1694 (s), 1648 (m), 1629 (m), 1509 (w), 1475 (w), 1464 (w), 1407 (m), 1362 (s), 1323 (m), 1307 (m), 1259 (m), 1199 (s), 1172 (s), 1114 (m), 1038 (w), 1022 (w), 994 (w), 967 (w), 920 (w), 875 (w), 856 (w), 813 (w), 740 (s), 708 (w), 679 (w), 665 (w), 606 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.18 (1H, s, H-1'), 7.42 (1H, dd, *J* 8.6, 7.1, H-6), 7.00 (1H, d, *J* 7.1, H-5), 6.82 (1H, d, *J* 8.6, H-7), 6.18 (1H, t, *J* 5.6, H-1''), 4.92 – 4.83 (1H, m, H-3'), 3.19 (2H, q, *J* 6.9, H-2''), 2.86 – 2.62 (3H, m, H-4'^{a} and H-5'), 2.27 (2H, t, *J* 7.4, H-8''), 2.11 – 2.02 (1H, m, H-4'^{b}), 1.65 – 1.50 (4H, m, H-3'' and H-7''), 1.43 – 1.20 (6H, m, H-4'', H-5'' and H-6''); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 177.7 (C-9''), 172.0 (C-2'), 169.5 (C-6'), 169.0 (C-3), 167.7 (C-1), 146.9 (C-4), 136.1 (C-6), 132.5 (C-7a), 116.6 (C-7), 111.2 (C-5), 109.8 (C-3a), 48.8 (C-3'), 42.6 (C-2''), 34.0 (C-8''), 31.4 (C-5'), 29.1 (C-3'' or C-7''), 29.6, 28.9, 26.7 (C-4'',

C-5'' and C-6''), 24.6 (C-3'' or C-7''), 22.7 (C-4'); *m/z* (ESI$^+$) 438 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[213]

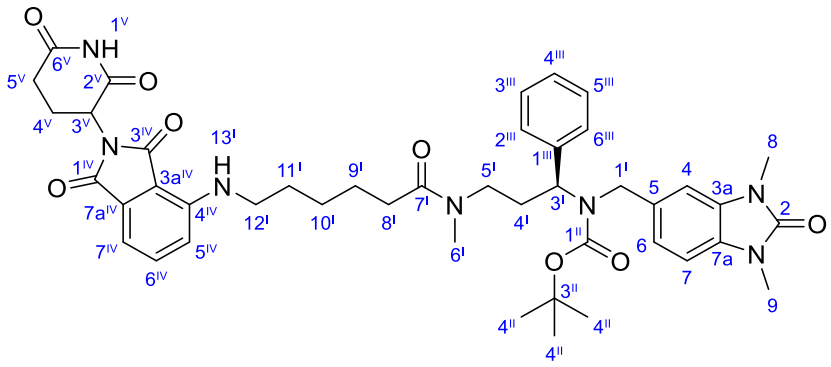
11''-{[2-(2',6'-Dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}undecanoic acid (280)[213]



276 (300 mg, 1.09 mmol, 1.0 eq.), 11-aminoundecanoic acid (262 mg, 1.30 mmol, 1.2 eq.) and *N*,*N*-diisopropylethylamine (759 µL, 4.36 mmol, 4.0 eq.) were dissolved in *N*-methyl-2-pyrrolidone (7.5 mL) and the mixture was heated at 110 ºC for 2 h under microwave irradiation in a sealed microwave tube. The reaction mixture was then cooled and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (3% methanol in dichloromethane) to yield the title compound **280** as a yellow oil (444 mg, 89%). R_f 0.40 (5% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2924 (m), 2854 (w), 2368 (w), 2333 (w), 1756 (w), 1698 (s), 1625 (w), 1603 (w), 1509 (w), 1483 (w), 1466 (w), 1409 (w), 1360 (m), 1325 (w), 1303 (w), 1261 (w), 1198 (w), 1177 (w), 1114 (w), 1053 (w), 1035 (w), 1023 (w), 990 (w), 880 (w), 815 (w), 799 (w), 747 (w), 722 (m), 686 (w), 669 (w), 657 (w), 621 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.23 (1H, s, H-1'), 7.42 (1H, dd, *J* 8.6, 7.1, H-6), 7.00 (1H, d, *J* 7.1, H-5), 6.82 (1H, d, *J* 8.6, H-7), 6.19 (1H, t, *J* 5.5, H-1''), 4.93 – 4.84 (1H, m, H-3'), 3.24 – 3.13 (2H, m, H-2''), 2.84 – 2.66 (3H, m, H-4'^{a} and H-5'), 2.26 (2H, t, *J* 7.5, H-11'), 2.12 – 2.02 (1H, m, H-4'^{b}), 1.65 – 1.50 (4H, m, H-3'' and H-10''), 1.39 – 1.14 (12H, m, H-4'', H-5'', H-6'', H-7'', H-8'' and H-9''); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 178.1 (C-12''), 172.0 (C-2'), 169.5 (C-6'), 169.1 (C-3), 167.7 (C-1), 146.9 (C-4), 136.0 (C-6), 132.4 (C-7a), 116.6 (C-7), 111.2 (C-5), 109.7 (C-3a), 48.8 (C-3'), 42.6 (C-2''), 34.0 (C-11''), 31.3 (C-5'), 29.34 (C-3'' or C-10''), 29.23, 29.19, 29.15, 29.13, 29.0, 26.8 (C-4'', C-5'', C-6'', C-7'', C-8'' and C-9''), 24.7 (C-3'' or C-10''), 22.7 (C-4');

m/z (ESI$^+$) 480 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[213]

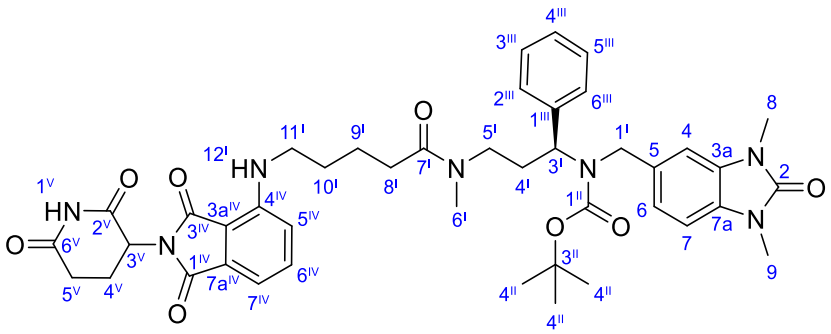
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{(1*S*)-3^{I}-[(13^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}hexanoyl)(methyl)amino]-3^{I}-phenylpropyl}carbamate (282)



278 (39.7 mg, 0.103 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (71.8 µL, 0.412 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (1 mL). HATU (43.1 mg, 0.113 mmol, 1.1 eq.) was added and the solution was stirred at room temperature for 10 min. **246** (45.0 mg, 0.103 mmol, 1.0 eq.) was added, and the reaction mixture was stirred at room temperature for 16 h. After this time, the solution was diluted with water (10 mL) and ethyl acetate (10 mL), and the aqueous component was extracted with ethyl acetate (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–5% methanol in dichloromethane) and further purified by silica gel column chromatography (30–50% acetone in toluene) to yield the title compound **282** as a yellow oil (38.1 mg, 46%). R_f 0.20 (50% acetone in toluene); $[\alpha]_D^{25}$= −38.7 (*c* 1.0, $CHCl_3$); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3393 (w), 2933 (w), 2856 (w), 2360 (w), 1756 (w), 1697 (s), 1625 (w), 1509 (w), 1454 (w), 1407 (m), 1362 (m), 1326 (w), 1303 (w), 1262 (w), 1196 (w), 1162 (w), 1122 (w), 1083 (w), 1054 (w), 1027 (w), 875 (w), 815 (w), 748 (m), 704 (w), 665 (w), 611 (w); ^{1}H NMR (500 MHz, toluene-*d*$_8$, 363 K) δ_H 8.82 (1H, s, H-1^{V}), 7.48 – 7.29 (2H, m, H-3^{III} and H-5^{III}), 7.13 (2H, t, *J* 7.4, H-2^{III} and H-6^{III}), 7.12 – 7.03 (2H, m, partially obscured by toluene-*d*$_8$ peak, H-4^{III} and H-6^{IV}), 6.94 (1H, d, *J* 7.1, H-5^{IV}), 6.83 (1H, d, *J* 7.9, H-6), 6.61 (1H,

s, H-4), 6.55 (1H, d, *J* 8.4, H-7^{IV}), 6.48 (1H, d, *J* 7.9, H-7), 6.24 (1H, t, *J* 6.0, H-13^{I}), 5.30 (1H, s, H-3^{I}), 4.71 (1H, dd, *J* 12.6, 5.4, H-3^{V}), 4.43 (1H, d, *J* 15.5, H-1^{Ia}), 4.28 (1H, d, *J* 15.5, H-1^{Ib}), 3.24 (2H, bs, H-5^{I}), 3.08 (3H, s, H-8 or H-9), 2.99 (3H, s, H-8 or H-9), 2.93 (2H, q, *J* 6.0, H-12^{I}), 2.74 – 2.43 (4H, m, H-4^{Va} and H-6^{I}), 2.30 (1H, dt, *J* 17.6, 3.8, H-5^{Va}), 2.26 – 2.15 (2H, m, H-4^{I}), 2.09 – 1.92 (3H, m, H-5^{Vb} and H-8^{I}), 1.60 (2H, p, *J* 7.6, H-9^{I}), 1.55 – 1.23 (14H, m, H-4^{Vb}, H-4^{II}, H-11^{I} and H-10^{I}); ^{13}C NMR (126 MHz, toluene-d_8, 363 K) δ_C 172.0 (C-7^{I}), 170.9 (C-2^{V}), 170.4 (C-6^{V}), 168.8 (C-1^{IV}), 168.0 (C-3^{IV}), 156.7 (C-1^{II}), 155.1 (C-2), 147.8 (C-4^{IV}), 141.6 (C-1^{III}), 136.2 (C-6^{IV}), 133.8 (C-7^{IVa}), 131.3 (C-7a), 130.2 (C-3a), 129.5 (C-5), 129.0 (C-2^{III} and C-6^{III}), 128.8 (C-3^{III} and C-5^{III}), 128.5 (C-4^{III}), 121.1 (C-6), 116.8 (C-7^{IV}), 111.6 (C-3^{IVa}), 111.5 (C-5^{IV}), 107.4 (C-4), 106.8 (C-7), 80.3 (C-3^{II}), 58.6 (C-3^{I}), 50.0 (C-3^{V}), 49.2 (C-1^{I}), 47.2 (C-5^{I}), 43.1 (C-12^{I}), 35.3 (C-6^{I}), 33.4 (C-8^{I}), 31.8 (C-5^{V}), 30.9 (C-4^{I}), 29.8 (C-10^{I} or C-11^{I}), 28.9 (C-4^{II}), 27.4 (C-10^{I} or C-11^{I}), 27.1, 27.0 (C-8 and C-9), 25.3 (C-9^{I}), 23.3 (C-4^{V}); *m/z* (ESI$^+$) 830 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{44}H_{54}O_8N_7^+$ ([M+H]$^+$) requires 808.4028, found 808.4023; HPLC (method 4) retention time 11.0 min, 96.8%.

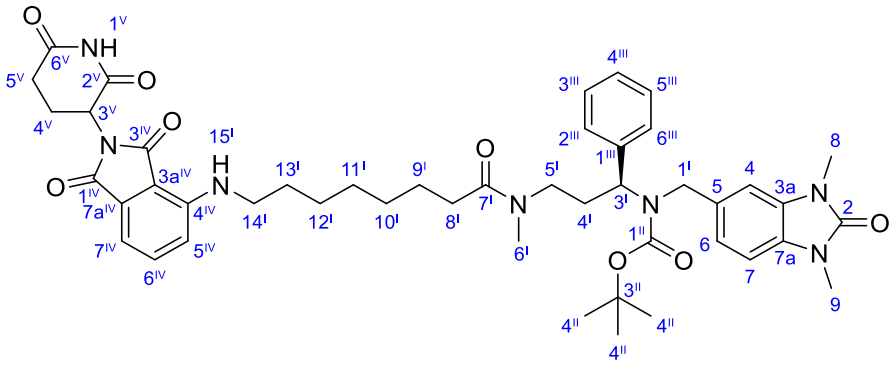
***tert*-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{(1*S*)-3^{I}-[(12^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}pentanoyl)(methyl)amino]-3^{I}-phenylpropyl}carbamate (281)**



277 (25.5 mg, 0.0684 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (47.7 µL, 0.274 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (2 mL). HATU (28.6 mg, 0.0752 mmol, 1.1 eq.) was added and the solution was stirred at room temperature for 10 min. **246** (30.0 mg, 0.0684 mmol, 1.0 eq.) was added, and the reaction mixture was stirred at room

temperature for 16 h. After this time, the solution was diluted with water (10 mL) and ethyl acetate (10 mL), and the aqueous component was extracted with ethyl acetate (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–4% methanol in dichloromethane) to yield the title compound **281** as a yellow oil (50.3 mg, 93%). R_f 0.15 (5% methanol in dichloromethane); $[\alpha]_D^{25}$= −19.1 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3393 (w), 2933 (w), 2856 (w), 2360 (w), 1756 (w), 1697 (s), 1625 (m), 1509 (w), 1454 (w), 1407 (m), 1362 (m), 1326 (w), 1303 (w), 1262 (w), 1196 (w), 1162 (m), 1122 (w), 1083 (w), 1054 (w), 1027 (w), 875 (w), 815 (w), 748 (m), 704 (w), 665 (w), 611 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 10.76 (1H, s, H-1^V), 7.56 (1H, dd, *J* 8.6, 7.0, H-6^{IV}), 7.41 – 7.29 (4H, m, H-2^{III}, H-3^{III}, H-5^{III} and H-6^{III}), 7.29 – 7.21 (1H, m, H-4^{III}), 7.06 (1H, d, *J* 8.6, H-7^{IV}), 7.01 (1H, d, *J* 7.0, H-5^{IV}), 6.95 (1H, d, *J* 8.1, H-7), 6.87 (1H, d, *J* 8.1, H-6), 6.70 (1H, s, H-4), 6.40 (1H, t, *J* 5.0, H-12^I), 5.09 (1H, t, *J* 7.5, H-3^I), 5.00 (1H, dd, *J* 12.5, 5.5, H-3^V), 4.38 (1H, d, *J* 15.6, H-1^{Ia}), 4.27 (1H, d, *J* 15.6, H-1^{Ib}), 3.36 – 3.12 (10H, m, H-8, H-9, H-5^I and H-11^I), 2.92 – 2.67 (4H, m, H-6^I and H-8^{Ia}), 2.66 – 2.53 (2H, m, H-4Va and H-8^{Ib}), 2.33 – 2.02 (5H, m, H-4^I, H-4^{Vb} and H-5^V), 1.57 (4H, bs, H-9^I and H-10^I), 1.39 (9H, s, H-4^{II}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.9 (C-2^V), 171.1 (C-7^I), 169.2 (C-6^V), 168.6 (C-1^{IV}), 166.8 (C-3^{IV}), 154.9 (C-1^{II}), 153.7 (C-2), 146.3 (C-4^{IV}), 140.0 (C-1^{III}), 135.7 (C-6^{IV}), 132.0 (C-7a), 131.9 (C-7a^{IV}), 129.2 (C-3a), 128.3 (C-5), 127.8, 127.3 (C-2^{III}, C-3^{III}, C-5^{III} and C-6^{III}), 126.8 (C-4^{III}), 119.8 (C-6), 116.7 (C-7^{IV}), 110.0 (C-5^{IV}), 109.1 (C-3a^{IV}), 106.4 (C-7), 106.2 (C-4), 79.0 (C-3^{II}), 57.0 (C-3^I), 48.4 (C-3^V), 47.5 (C-1^I), 44.9 (C-5^I), 41.5 (C-11^I), 39.3 (obscured by DMSO-*d*$_6$ peak, C-6^I), 31.9 (C-4^I), 30.6 (C-8^I), 29.2 (C-5^V), 28.1 (C-10^I), 27.7 (C-4^{II}), 26.4, 26.3 (C-8 and C-9), 21.8 (C-4^V), 21.6 (C-9^I); *m/z* (ESI$^+$) 816 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{43}H_{52}O_8N_7^+$ ([M+H]$^+$) requires 794.3872, found 794.3866; HPLC (method 1) retention time 10.17 min, 100%.

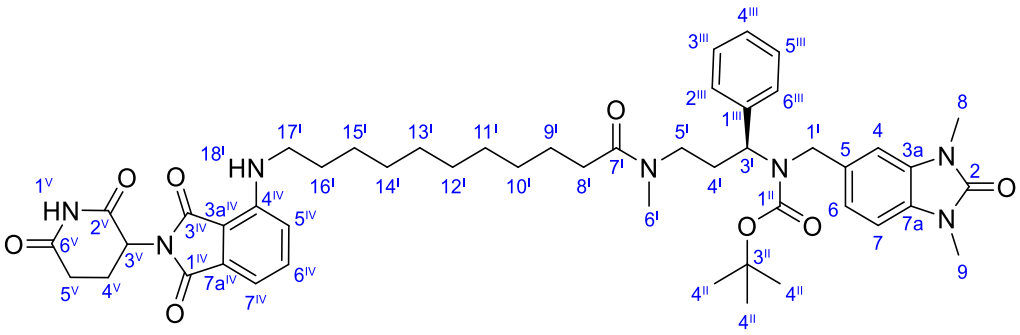
***tert*-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{(1*S*)-3^I-[(15^I-{[2^{IV}-(2^V,6^V-dioxopiperidin-3^V-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}octanoyl)(methyl)amino]-3^I-phenylpropyl}carbamate (283)**



279 (66.5 mg, 0.160 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (111 µL, 0.640 mmol, 4.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (4 mL). HATU (66.9 mg, 0.176 mmol, 1.1 eq.) was added and the solution was stirred at room temperature for 10 min. **246** (70.0 mg, 0.160 mmol, 1.0 eq.) was added, and the reaction mixture was stirred at room temperature for 16 h. After this time, the solution was diluted with water (20 mL) and ethyl acetate (20 mL), and the aqueous component was extracted with ethyl acetate (3 × 30 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–4% methanol in dichloromethane) and further purified by silica gel column chromatography (30–50% acetone in toluene) to yield the title compound **283** as a yellow oil (98.9 mg, 74%). R_f 0.21 (6% methanol in dichloromethane); $[\alpha]_D^{25} = -10.4$ (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3639 (w), 3400 (w), 2934 (w), 2859 (w), 1755 (w), 1698 (s), 1626 (w), 1509 (w), 1455 (w), 1408 (w), 1364 (w), 1329 (w), 1305 (w), 1264 (w), 1199 (w), 1162 (w), 1122 (w), 1053 (w), 1028 (w), 1009 (w), 843 (s), 766 (w), 748 (w), 704 (w), 631 (w), 620 (w), 609 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ_H 10.75 (1H, s, H-1^V), 7.57 (1H, dd, *J* 8.6, 7.1, H-6^{IV}), 7.40 – 7.28 (4H, m, H-2^{III}, H-3^{III}, H-5^{III} and H-6^{III}), 7.28 – 7.21 (1H, m, H-4^{III}), 7.06 (1H, d, *J* 8.6, H-7^{IV}), 7.01 (1H, d, *J* 7.1, H-5^{IV}), 6.95 (1H, d, *J* 8.0, H-7), 6.87 (1H, d, *J* 8.0, H-6), 6.71 (1H, s, H-4), 6.39 (1H, t, *J* 5.9, H-15^I), 5.09 (1H, t, *J* 7.6, H-3^I), 5.04 – 4.97 (1H, m, H-3^V), 4.38 (1H, d, *J* 15.6, H-1^{Ia}), 4.27 (1H, d, *J* 15.6, H-1^{Ib}), 3.34 – 3.11 (10H, m, H-8, H-9, H-5^I and H-14^I), 2.92 – 2.82 (1H, m, H-5Va), 2.76 (3H, s, H-6^I), 2.66 – 2.52 (2H, m, H-4Va and H-5^{Vb}), 2.29 – 1.96 (5H, m, H-4^I, H-4^{Vb} and H-8^I), 1.60 (2H, p, *J* 7.1, H-13^I), 1.52 – 1.13 (17H, m, H-4^{II}, H-9^I, H-10^I, H-11^I and H-12^I); ^{13}C NMR (126 MHz,

DMSO-d_6, 363 K) δ_C 171.9 (C-2^{V}), 171.3 (C-7^{I}), 169.2 (C-6^{V}), 168.6 (C-1^{IV}), 166.8 (C-3^{IV}), 154.9 (C-1^{II}), 153.7 (C-2), 146.3 (C-4^{IV}), 140.1 (C-1^{III}), 135.7 (C-6^{IV}), 132.0 (C-7a), 131.9 (C-$7a^{IV}$), 129.3 (C-3a), 128.4 (C-5), 127.8, 127.3 (C-2^{III}, C-3^{III}, C-5^{III} and C-6^{III}), 126.8 (C-4^{III}), 119.9 (C-6), 116.7 (C-7^{IV}), 110.0 (C-5^{IV}), 109.1 (C-$3a^{IV}$), 106.4 (C-7), 106.2 (C-4), 79.0 (C-3^{II}), 57.0 (C-3^{I}), 48.4 (C-3^{V}), 47.5 (C-1^{I}), 45.0 (C-5^{I}), 41.7 (C-14^{I}), 39.3 (obscured by DMSO-d_6 peak, C-6^{I}), 31.9 (C-4^{I}), 30.6 (C-5^{V}), 30.0 (C-8^{I}), 28.4 (C-13^{I}), 28.2, 28.1 (C-9^{I} and C-12^{I}), 27.7 (C-4^{II}), 26.4, 26.3 (C-8 and C-9), 25.8, 24.2 (C-10^{I} and C-11^{I}), 21.8 (C-4^{V}); *m/z* (ESI$^+$) 858 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{46}H_{58}O_8N_7^+$ ([M+H]$^+$) requires 836.4341, found 836.4331; HPLC (method 1) retention time 11.16 min, 99.2%.

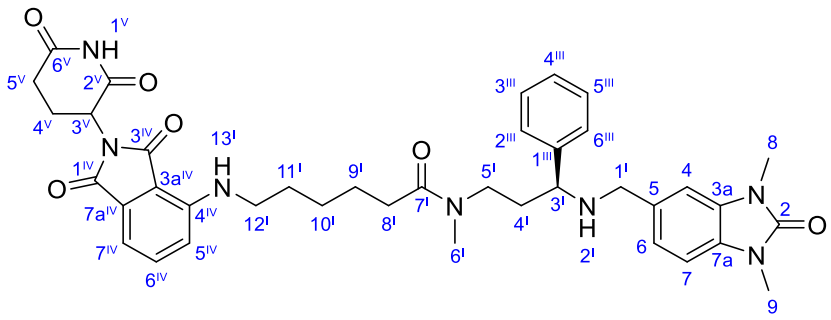
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{(1*S*)-3^{I}-[(18^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}undecanoyl)(methyl)amino]-3^{I}-phenylpropyl}carbamate (284)



280 (73.2 mg, 0.160 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (55.7 µL, 0.320 mmol, 2.0 eq.) were dissolved in anhydrous *N*,*N*-dimethylformamide (4 mL). HATU (66.9 mg, 0.176 mmol, 1.1 eq.) was added and the solution was stirred at room temperature for 10 min. **246** (70.0 mg, 0.160 mmol, 1.0 eq.) was added, and the reaction mixture was stirred at room temperature for 16 h. After this time, the solution was diluted with water (20 mL) and ethyl acetate (20 mL), and the aqueous component was extracted with ethyl acetate (3 × 30 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (20–35% acetone in toluene) to yield the title compound **284** as a yellow oil (71.2 mg, 51%). R_f 0.55 (40% acetone in toluene); $[\alpha]_D^{25} = -15.5$ (*c*

1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3402 (w), 2929 (w), 2854 (w), 1757 (w), 1697 (s), 1625 (m), 1509 (w), 1455 (w), 1407 (w), 1362 (w), 1328 (w), 1304 (w), 1262 (w), 1197 (w), 1164 (w), 1115 (w), 1053 (w), 1028 (w), 1010 (w), 843 (s), 765 (w), 747 (w), 704 (w), 649 (w), 609 (w); ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 363 K) δ_H 10.71 (1H, s, H-1^{V}), 7.56 (1H, dd, *J* 8.6, 7.1, H-6^{IV}), 7.38 – 7.29 (4H, m, H-2^{III}, H-3^{III}, H-5^{III} and H-6^{III}), 7.27 – 7.22 (1H, m, H-4^{III}), 7.06 (1H, d, *J* 8.6, H-7^{IV}), 7.01 (1H, d, *J* 7.1, H-5^{IV}), 6.94 (1H, d, *J* 8.0, H-7), 6.88 (1H, d, *J* 8.0, H-6), 6.71 (1H, s, H-4), 6.37 (1H, t, *J* 5.9, H-18^{I}), 5.09 (1H, t, *J* 7.6, H-3^{I}), 5.00 (1H, dd, *J* 12.5, 5.5, H-3^{V}), 4.39 (1H, d, *J* 15.6, H-1^{Ia}), 4.28 (1H, d, *J* 15.6, H-1^{Ib}), 3.36 – 3.12 (10H, m, H-8, H-9, H-5^{I} and H-17^{I}), 2.91 – 2.82 (1H, m, H-5Va), 2.77 (3H, s, H-6^{I}), 2.67 – 2.52 (2H, m, H-4Va and H-5^{Vb}), 2.28 – 1.98 (5H, m, H-4^{I}, H-4^{Vb} and H-8^{I}), 1.62 (2H, p, *J* 7.1, H-16^{I}), 1.51 – 1.13 (23H, m, H-4^{II}, H-9^{I}, H-10^{I}, H-11^{I}, H-12^{I}, H-13^{I}, H-14^{I} and H-15^{I}); ^{13}C NMR (126 MHz, DMSO-*d*$_6$, 363 K) δ_C 171.8 (C-2^{V}), 171.3 (C-7^{I}), 169.1 (C-6^{V}), 168.6 (C-1^{IV}), 166.8 (C-3^{IV}), 154.9 (C-1^{II}), 153.7 (C-2), 146.3 (C-4^{IV}), 140.1 (C-1^{III}), 135.6 (C-6^{IV}), 132.0 (C-7a), 131.9 (C-7a^{IV}), 129.3 (C-3a), 128.3 (C-5), 127.7, 127.2 (C-2^{III}, C-3^{III}, C-5^{III} and C-6^{III}), 126.8 (C-4^{III}), 119.8 (C-6), 116.7 (C-7^{IV}), 109.9 (C-5^{IV}), 109.1 (C-3a^{IV}), 106.3 (C-7), 106.2 (C-4), 79.0 (C-3^{II}), 57.0 (C-3^{I}), 48.4 (C-3^{V}), 48.1 (C-5^{I}), 47.5 (C-1^{I}), 41.7 (C-17^{I}), 39.3 (obscured by DMSO-*d*$_6$ peak, C-6^{I}), 31.9 (C-4^{I}), 30.6 (C-5^{V}), 29.6 (C-8^{I}), 28.33 (C-16^{I}), 28.27, 28.22, 28.1 (C-9^{I}, C-10^{I}, C-11^{I}, C-14^{I} and C-15^{I}), 27.6 (C-4^{II}), 26.3, 26.2 (C-8 and C-9), 25.8, 24.2 (C-12^{I} and C-13^{I}), 21.8 (C-4^{V}); *m/z* (ESI^{+}) 900 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{49}H_{64}O_8N_7^{+}$ ([M+H]$^{+}$) requires 878.4811, found 878.4806; HPLC (method 1) retention time 12.39 min, 100%.

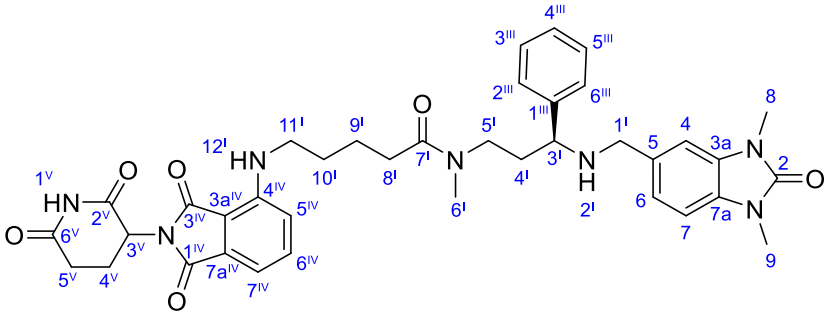
_N_-[(3_S_)-3^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-4^{IV}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1_H_-isoindol-4^{IV}-yl]amino}-_N_-methylhexanamide (236)



282 (36.0 mg, 0.0446 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (0.5 mL) and dichloromethane (0.5 mL), and was stirred at room temperature for 2 h. The reaction mixture was then neutralised with 2 M aqueous sodium hydroxide solution and extracted with dichloromethane (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% triethylamine, 1–3% ethanol in dichloromethane) to yield the title compound **236** as a yellow oil (31.0 mg, 98%). R_f 0.53 (1% triethylamine, 8% methanol in dichloromethane); $[\alpha]_D^{25}$= −15.0 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2936 (w), 2857 (w), 2359 (w), 2338 (w), 1755 (w), 1696 (s), 1624 (m), 1496 (w), 1463 (w), 1452 (w), 1407 (w), 1355 (w), 1324 (w), 1264 (w), 1191 (w), 1150 (w), 1114 (w), 1086 (w), 1043 (w), 1027 (w), 955 (w), 885 (w), 861 (w), 814 (w), 768 (w), 747 (w), 727 (w), 704 (w), 681 (w), 637 (w), 625 (w), 606 (w); ^{1}H NMR (500 MHz, CD_3OD, two diastereomers observed, second diastereomer indicated by asterisk) δ_H 7.55 – 7.45 (1H, m, H-6^{IV}), 7.40 – 7.32 (4H, m, H-2^{III}, H-3^{III}, H-5^{III} and H-6^{III}), 7.30 – 7.26 (1H, m, H-4^{III}), 7.06 – 6.94 (5H, m, H-4, H-6, H-7, H-5^{IV} and H-7^{IV}), 5.06 – 4.96 (1H, m, H-3^{V}), 3.67 – 3.41 (4H, m, H-3^{I}, H-1^{I} and H-5^{Ia}), 3.38 (3H, s, H-8), 3.37 (3H, s, H-9), 3.31 (1H, obscured by CD_3OD peak, H-12^{Ia}), 3.28 – 3.21 (2H, m, H-12^{Ib} and H-5^{Ib}), 2.92 (1.5H, d, *J* 2.4, H-6^{I} diastereomer 1), 2.87 – 2.76 (2.5H, m, H-5Va and H-6^{I} diastereomer 2), 2.75 – 2.57 (2H, m, H-4Va and H-5^{Vb}), 2.35 – 2.28 (2H, m, H-8^{I}), 2.16 – 2.00 (3H, m, H-4^{Vb} and H-4^{I}), 1.72 – 1.41 (5H, m, H-9^{I}, H-11^{I} and H-10^{Ia}), 1.35 – 1.25 (1H, m, H-10^{Ib}); ^{13}C NMR (126 MHz, CD_3OD) δ_C 175.4, 175.3* (C-7^{I}), 174.6 (C-1^{IV}), 171.57,

171.55* (C-3^{IV}), 170.82, 170.75* (C-2^{V}), 169.28, 169.26* (C-6^{V}), 156.4 (C-2), 148.22, 148.20* (C-4^{IV}), 144.0, 143.8* (C-1^{III}), 137.24, 137.18* (C-6^{IV}), 134.9, 134.7* (C-5), 133.91, 133.88* (C-3a), 131.2, 131.1* (C-7a), 130.21, 130.20* (C-7a^{IV}), 129.9, 129.7* (C-2^{III} and C-6^{III}), 128.8, 128.7* (C-3^{III} and C-5^{III}), 128.5 (C-4^{III}), 123.3, 123.1* (C-6), 117.92, 117.89* (C-7^{IV}), 111.7, 111.6* (C-5^{IV}), 111.2, 111.0* (C-4), 109.4, 109.1* (C-3a^{IV}), 108.64, 108.61* (C-7), 61.2, 60.9* (C-3^{I}), 52.43, 52.37* (C-1^{I}), 50.2, 50.1* (C-3^{V}), 45.92, 45.87* (C-5^{I}), 43.3, 43.2* (C-12^{I}), 35.78, 35.76* (C-6^{I}), 34.1, 34.0* (C-8^{I}), 33.62, 33.58* (C-4^{I}), 32.20, 32.17* (C-5^{V}), 30.1 29.9* (C-11^{I}), 27.78, 27.74* (C-10^{I}), 27.69, 27.64, 27.60*, 27.5* (C-8 and C-9), 26.2, 25.9* (C-9^{I}), 23.8, 23.7* (C-4^{V}); *m/z* (ESI^{+}) 708 ([M+H]$^{+}$, 40%), 730 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{39}H_{46}O_6N_7^{+}$ ([M+H]$^{+}$) requires 708.3504, found 708.3496; HPLC (method 1) retention time 7.61 min, 100%.

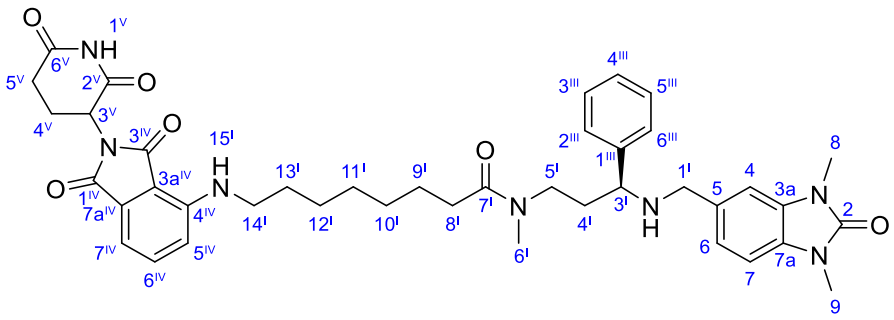
_N_-[(3*S*)-3^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-4^{IV}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}-*N*-methylpentanamide (235)



281 (28.0 mg, 0.0353 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (0.5 mL) and dichloromethane (0.5 mL), and was stirred at room temperature for 2 h. The reaction mixture was then neutralised with 2 M aqueous sodium hydroxide solution and extracted with dichloromethane (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1% triethylamine, 1–2% methanol in dichloromethane) to yield the title compound **235** as a yellow oil (9.90 mg, 40%). R_f 0.27 (1% triethylamine, 7% methanol in dichloromethane); $[\alpha]_D^{25}$= −1.4 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3428 (w), 2933 (w), 2852 (w), 1755 (w), 1696 (s), 1624

(m), 1509 (w), 1496 (w), 1458 (w), 1409 (w), 1359 (w), 1324 (w), 1264 (w), 1198 (w), 1188 (w), 1149 (w), 1115 (w), 1087 (w), 1052 (w), 1040 (w), 1025 (w), 989 (w), 927 (w), 875 (w), 815 (w), 769 (w), 747 (w), 729 (w), 705 (w), 681 (w), 657 (w), 622 (w), 607 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 7.55 – 7.31 (6H, m, H-6^{IV}, H-2^{III}, H-3^{III}, H-4^{III}, H-5^{III} and H-6^{III}), 7.14 (1H, s, H-4), 7.11 – 6.96 (4H, m, H-5^{IV}, H-7^{IV}, H-6 and H-7), 5.08 – 4.94 (1H, m, H-3^{V}), 3.98 (1H, t, *J* 6.2, H-3^{I}), 3.88 – 3.80 (1H, m, H-1^{Ia}), 3.80 – 3.68 (1H, m, H-1^{Ib}), 3.68 – 3.56 (1H, m, H-5^{Ia}), 3.42 – 3.33 (7H, m, H-8, H-9 and H-11^{Ia}), 3.26 – 3.15 (2H, m, H-5^{Ib} and H-11^{Ib}), 2.98 (2H, s, H-6^{I} diastereomer 1), 2.86 – 2.58 (4H, m, H-4Va, H-5^{V} and H-6^{I} diastereomer 2), 2.47 – 2.40 (1H, m, H-8^{Ia}), 2.21 – 2.14 (2H, m, H-4^{Ia} and H-8^{Ib}), 2.14 – 2.07 (1H, m, H-4^{Ib}), 2.07 – 1.98 (1H, m, H-4^{Vb}), 1.80 – 1.65 (2H, m, H-9^{I}), 1.40 – 1.23 (2H, m, H-10^{I}); ^{13}C NMR (126 MHz, CD_3OD, two diastereomers observed, second diastereomer indicated by asterisk) δ_C 175.0 (C-7^{I}), 174.7, 174.6* (C-1^{IV}), 171.62, 171.55* (C-3^{IV}), 170.8 (C-2^{V}), 169.3, 169.2* (C-6^{V}), 156.4 (C-2), 148.2, 148.1* (C-4^{IV}), 137.3 (C-6^{IV}), 137.2 (C-1^{III}), 133.92, 133.89* (C-7a^{IV}), 131.4 (C-5), 131.3 (C-3a), 130.4 (C-2^{III} and C-6^{III} or C-3^{III} and C-5^{III}), 130.2 (C-7a), 129.2 (C-2^{III} and C-6^{III} or C-3^{III} and C-5^{III}), 128.9 (C-4^{III}), 124.2, 123.5* (C-6), 118.0 (C-7^{IV}), 111.8 (C-5^{IV}), 111.05, 111.04* (C-3a^{IV}), 110.1, 109.5* (C-4), 109.0, 108.8* (C-7), 61.2, 61.1* (C-3^{I}), 51.9, 51.5* (C-1^{I}), 50.20, 50.16* (C-3^{V}), 45.7 (C-5^{I}), 43.1, 43.0* (C-11^{I}), 36.0, 35.7* (C-6^{I}), 33.9, 33.7* (C-8^{I}), 33.5, 33.4* (C-4^{I}), 32.21, 32.17* (C-5^{V}), 29.88, 29.86* (C-10^{I}), 27.56, 27.54, 27.50*, 27.49* (C-8 and C-9), 23.79, 23.75* (C-4^{V}), 23.40, 23.39* (C-9^{I}); *m/z* (ESI^{+}) 694 ([M+H]$^{+}$, 100%), 716 ([M+Na]$^{+}$, 40%); HRMS *m/z* (ESI^{+}) $C_{38}H_{44}O_6N_7^{+}$ ([M+H]$^{+}$) requires 694.3348, found 694.3339; HPLC (method 1) retention time 7.29 min, 100%.

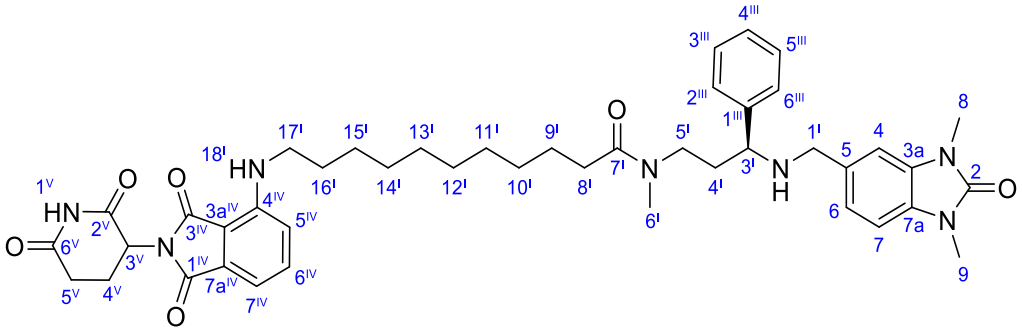
_N_-[(3_S_)-1^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-7^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1_H_-isoindol-4^{IV}-yl]amino}-_N_-methyloctanamide (237)



283 (40.0 mg, 0.0478 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (1 mL), and was stirred at room temperature for 2 h. The reaction mixture was then neutralised with 2 M aqueous sodium hydroxide solution and extracted with dichloromethane (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (2–4% [1:2 ammonium hydroxide:ethanol] in ethyl acetate) to yield the title compound **237** as a yellow oil (21.4 mg, 61%). R_f 0.42 (10% [1:2 ammonium hydroxide:ethanol] in ethyl acetate); $[\alpha]_D^{25}$= −15.9 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2930 (w), 2855 (w), 1756 (w), 1694 (s), 1623 (m), 1495 (w), 1453 (w), 1407 (w), 1356 (w), 1323 (w), 1264 (w), 1189 (w), 1148 (w), 1114 (w), 1086 (w), 1041 (w), 1025 (w), 984 (w), 952 (w), 909 (w), 873 (w), 857 (w), 814 (w), 768 (w), 746 (w), 728 (w), 704 (w), 680 (w), 651 (w), 606 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 7.55 – 7.46 (1H, m, H-6^{IV}), 7.41 – 7.32 (4H, m, H-2^{III}, H-3^{III}, H-5^{III} and H-6^{III}), 7.33 – 7.25 (1H, m, H-4^{III}), 7.06 – 6.95 (5H, m, H-4, H-6, H-7, H-7^{IV} and H-5^{IV}), 5.08 – 4.95 (1H, m, H-3^{V}), 3.67 – 3.47 (3H, m, H-3^{I} and H-1^{I}), 3.46 – 3.36 (7H, m, H-8, H-9 and H-14^{Ia}), 3.30 – 3.10 (3H, m, H-5^{I} and H-14^{Ib}), 2.91 (1.5H, d, *J* 1.9, H-6^{I} diastereomer 1), 2.88 – 2.76 (2.5H, m, H-6^{I} diastereomer 2 and H-5Va), 2.76 – 2.60 (2H, m, H-4Va and H-5^{Vb}), 2.28 (1H, td, *J* 7.5, 3.4, H-8^{Ia}), 2.18 – 1.86 (4H, m, H-4^{Vb}, H-4^{I} and H-8^{Ib}), 1.70 – 1.50 (3H, m, H-9^{Ia} and H-13^{I}), 1.48 – 1.12 (7H, m, H-9^{Ib}, H-10^{I}, H-11^{I} and H-12^{I}); ^{13}C NMR (126 MHz, CD_3OD, two diastereomers observed, second diastereomer indicated by asterisk) δ_C 175.7, 175.5* (C-7^{I}), 174.64, 174.62* (C-1^{IV}), 171.59, 171.55* (C-3^{IV}), 170.82,

170.76* (C-2^{V}), 169.29, 169.27* (C-6^{V}), 156.4 (C-2), 148.25, 148.20* (C-4^{IV}), 143.83, 143.79* (C-1^{III}), 137.22, 137.14***** (C-6^{IV}), 134.44, 134.39* (C-5), 133.9, 133.8* (C-7a^{IV}), 131.2, 131.1* (C-3a), 130.23, 130.21* (C-7a), 129.9, 129.7*, 128.8, 128.7* (C-2^{III}, C-3^{III}, C-5^{III} and C-6^{III}), 128.6 (C-4^{III}), 123.4, 123.2* (C-6), 117.95, 117.88* (C-7^{IV}), 111.71, 111.61* (C-5^{IV}), 111.0, 110.9* (C-3a^{IV}), 109.4, 109.1* (C-4), 108.64, 108.61* (C-7), 61.1, 61.0* (C-3^{I}), 52.3, 52.2* (C-1^{I}), 50.18, 50.15* (C-3^{V}), 45.91, 45.88* (C-14^{I}), 43.34, 43.32* (C-5^{I}), 36.9 (C-4^{I}), 35.81, 35.74* (C-6^{I}), 34.18, 34.16* (C-8^{I}), 32.21, 32.18* (C-5^{V}), 30.31, 30.29, 30.24, 30.19*, 30.16*, 30.0* (C-9^{I}, C-10^{I} and C-11^{I}), 27.83, 27.78* (C-12^{I}), 27.5 (C-8 and C-9), 26.4, 26.1* (C-13^{I}), 23.8, 23.7* (C-4^{V}); *m/z* (ESI^{+}) 758 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{41}H_{50}O_6N_7^{+}$ ([M+H]$^{+}$) requires 736.3817, found 736.3812; HPLC (method 1) retention time 8.40 min, 96.8%.

_N_-[(3_S_)-1^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-7^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1_H_-isoindol-4^{IV}-yl]amino}-_N_-methylundecanamide (238)

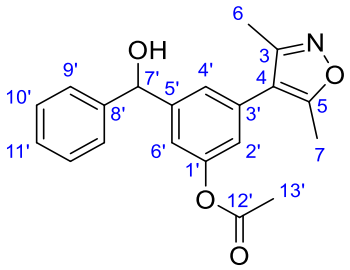


284 (30.0 mg, 0.0339 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (1 mL), and was stirred at room temperature for 2 h. The reaction mixture was then neutralised with 2 M aqueous sodium hydroxide solution and extracted with dichloromethane (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (2–4% [1:2 ammonium hydroxide:ethanol] in ethyl acetate) to yield the title compound **238** as a yellow oil (19.0 mg, 72%). R_f 0.49 (10% [1:2 ammonium hydroxide:ethanol] in ethyl acetate); $[\alpha]_D^{25}$= −13.4 (*c* 1.0, MeOH); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2926 (w), 2853 (w), 2359 (w), 1755 (w), 1698 (s), 1626 (m), 1558

(w), 1508 (w), 1480 (w), 1465 (w), 1407 (w), 1359 (w), 1324 (w), 1301 (w), 1262 (w), 1198 (w), 1179 (w), 1111 (w), 1091 (w), 1030 (w), 857 (w), 802 (w), 771 (w), 746 (w), 731 (w), 704 (w), 682 (w), 645 (w), 629 (w), 614 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 7.55 – 7.49 (1H, m, H-6^{IV}), 7.41 – 7.33 (4H, m, H-2^{III}, H-3^{III}, H-5^{III} and H-6^{III}), 7.32 – 7.26 (1H, m, H-4^{III}), 7.09 – 6.97 (5H, m, H-4, H-6, H-7, H-5^{IV} and H-7^{IV}), 5.03 (1H, ddd, *J* 12.9, 5.6, 2.9, H-3^{V}), 3.68 – 3.50 (3H, m, H-3^{I} and H-1^{I}), 3.48 – 3.36 (7H, m, H-8, H-9 and H-17^{Ia}), 3.30 – 3.12 (3H, m, H-17^{Ib} and H-5^{I}), 2.91 (2H, s, H-6^{I} diastereomer 1), 2.88 – 2.79 (2H, m, H-6^{I} diastereomer 2 and H-5Va), 2.76 – 2.65 (2H, m, H-4Va and H-5^{Vb}), 2.27 (1H, t, *J* 7.5, H-8^{Ia}), 2.17 – 1.87 (4H, m, H-4^{Vb}, H-4^{I} and H-8^{Ib}), 1.64 (2H, dp, *J* 14.4, 7.1, H-16^{I}), 1.57 – 1.49 (1H, m, H-9^{Ia}), 1.47 – 1.10 (13H, m, H-9^{Ib}, H-10^{I}, H-11^{I}, H-12^{I}, H-13^{I}, H-14^{I} and H-15^{I}); ^{13}C NMR (126 MHz, CD_3OD) δ_C 175.8, 175.6* (C-7^{I}), 174.64, 174.62* (C-1^{IV}), 171.6 (C-3^{IV}), 170.81, 170.80* (C-2^{V}), 169.3 (C-6^{V}), 156.4 (C-2), 148.29, 148.28* (C-4^{IV}), 143.79, 143.76* (C-1^{III}), 137.21, 137.19* (C-6^{IV}), 134.8 (C-5), 133.9 (C-7a^{IV}), 131.21, 131.19* (C-3a), 130.3, 130.2* (C-7a), 129.9, 129.7*, 128.8, 128.7* (C-2^{III}, C-3^{III}, C-5^{III} and C-6^{III}), 128.6 (C-4^{III}), 123.4, 123.2* (C-6), 118.0, 117.9* (C-7^{IV}), 111.69, 111.68* (C-5^{IV}), 110.96, 110.95* (C-3a^{IV}), 109.4, 109.1* (C-4), 108.7, 108.6* (C-7), 61.1, 61.0* (C-3^{I}), 52.3, 52.2* (C-1^{I}), 50.2 (C-3^{V}), 46.0 (C-17^{I}), 43.4 (C-5^{I}), 36.9 (C-4^{I}), 35.8, 35.7* (C-6^{I}), 33.8, 33.6 (C-8^{I}), 32.2 (C-5^{V}), 30.51, 30.48, 30.45, 30.41, 30.39, 30.35, 30.33*, 30.28*, 30.26*, 30.23*, 27.91*, 27.89* (C-11^{I}, C-12^{I}, C-13^{I}, C-14^{I}, C-15^{I} and C-16^{I}), 27.5 (C-8 and C-9), 26.6, 26.3 (C-9^{I} and C-10^{I}), 23.8 (C-4^{V}); *m/z* (ESI^{+}) 778 ([M+H]$^{+}$, 60%), 800 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{44}H_{56}O_6N_7^{+}$ ([M+H]$^{+}$) requires 778.4287, found 778.4278; HPLC (method 1) retention time 9.56 min, 100%.

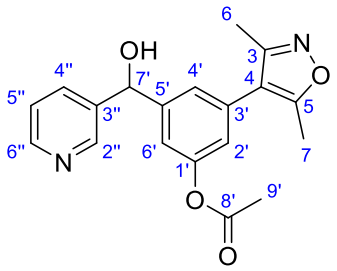
COMPOUNDS FROM CHAPTER 5

3'-(3,5-Dimethyl-1,2-oxazol-4-yl)-5'-[hydroxy(phenyl)methyl]phenyl acetate (285)[37]



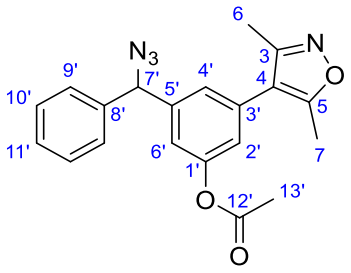
Sodium hydroxide (1 M in water, 2.54 mL, 2.54 mmol, 3.0 eq.) and acetic anhydride (240 µL, 2.54 mmol, 3.0 eq.) were added to a solution of 3-(3,5-dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(phenyl)methyl]phenol (250 mg, 0.846 mmol, 1.0 eq.) in isopropyl alcohol (15 mL), and the reaction mixture was stirred at room temperature for 1 h. After this time, the reaction was neutralised with the addition of 1 M aqueous hydrochloric acid and extracted with diethyl ether (3 × 30 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (30–50% ethyl acetate in petroleum ether) to yield the title compound **285** as a colourless oil (210 mg, 74%). R_f 0.76 (70% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.41 – 7.33 (4H, m, H-9' and H-10'), 7.33 – 7.28 (1H, m, H-11'), 7.16 – 7.13 (1H, m, H-2'), 7.11 (1H, dt, *J* 2.3, 1.1, H-4'), 6.90 (1H, t, *J* 1.9, H-6'), 5.87 (1H, d, *J* 2.6, H-7'), 2.37 (3H, s, H-7), 2.30 (3H, s, H-13'), 2.23 (3H, s, H-6); *m/z* (ESI$^+$) 338 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[37]

3’-(3,5-Dimethyl-1,2-oxazol-4-yl)-5’-[hydroxy(pyridin-3’’-yl)methyl]phenyl acetate (306)



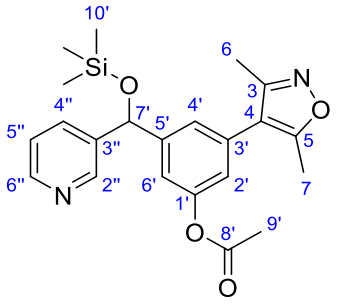
Sodium hydroxide (1 M in water, 2.02 mL, 2.02 mmol, 3.0 eq.) and acetic anhydride (191 µL, 2.02 mmol, 3.0 eq.) were added to a solution of 3’-(3,5-dimethyl-1,2-oxazol-4-yl)-5’-[hydroxy(pyridin-3’’-yl)methyl]phenol (200 mg, 0.675 mmol, 1.0 eq.) in isopropyl alcohol (15 mL), and the reaction mixture was stirred at room temperature for 1 h. After this time, the reaction was neutralised with the addition of 1 M aqueous hydrochloric acid and extracted with diethyl ether (3 × 30 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (2–4% methanol in dichloromethane) to yield the title compound **306** as a colourless oil (201 mg, 88%). R_f 0.25 (6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3244 (w), 2931 (w), 2850 (w), 2365 (w), 2342 (w), 1766 (m), 1633 (w), 1591 (w), 1473 (w), 1418 (m), 1369 (w), 1311 (w), 1253 (w), 1205 (s), 1138 (w), 1085 (w), 1043 (w), 1018 (m), 967 (w), 900 (w), 871 (w), 841 (w), 806 (w), 763 (w), 723 (w), 650 (w), 635 (w), 621 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.48 (1H, d, *J* 2.2, H-2’’), 8.37 (1H, dd, *J* 4.9, 1.7, H-6’’), 7.69 (1H, dt, *J* 7.9, 2.0, H-4’’), 7.24 (1H, ddd, *J* 7.9, 4.8, 0.9, H-5’’), 7.13 – 7.06 (2H, m, H-4’ and H-6’), 6.88 (1H, t, *J* 1.9, H-2’), 5.85 (1H, s, H-7’), 2.32 (3H, s, H-7), 2.27 (3H, s, H-9’), 2.17 (3H, s, H-6); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.4 (C-8’), 165.7 (C-5), 158.4 (C-3), 151.2 (C-1’), 148.6 (C-6’’), 148.0 (C-2’’), 145.8 (C-5’), 139.6 (C-3’’), 134.7 (C-4’’), 132.1 (C-3’), 124.5 (C-4’), 123.8 (C-5’’), 121.5 (C-2’), 118.9 (C-6’), 115.8 (C-4), 73.1 (C-7’), 21.2 (C-9’), 11.7 (C-7), 10.8 (C-6); *m/z* (ESI$^+$) 339 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{19}H_{19}O_4N_2^+$ ([M+H]$^+$) requires 339.1339, found 339.1338; HPLC (method 1) retention time 6.01 min, 99.2%.

5'-[Azido(phenyl)methyl]-3'-(3,5-dimethyl-1,2-oxazol-4-yl)phenyl acetate (307)



Azidotrimethylsilane (118 µL, 0.889 mmol, 3.0 eq.) and copper(II) triflate (10.7 mg, 0.0296 mmol, 0.1 eq.) were added at 0 ºC to a solution of **285** (100 mg, 0.296 mmol, 1.0 eq.) in anhydrous dichloromethane (2 mL). The reaction mixture was warmed to room temperature and stirred for 2 h. The mixture was then heated to 40 ºC for 22 h. After this time, additional azidotrimethylsilane (39.3 µL, 0.296 mmol, 1.0 eq.) was added and the mixture was stirred for a further 2 h. The mixture was then quenched with saturated aqueous EDTA solution (3 mL), diluted with water (20 mL), and extracted with dichloromethane (3 × 30 mL). The combined organic components were dried over sodium sulfate, and concentrated under a continuous stream of nitrogen gas. The crude residue was purified by silica gel column chromatography (10–20% ethyl acetate in petroleum ether) to yield the title compound **307** as a colourless oil (85.1 mg, 80%). R_f 0.67 (30% ethyl acetate in petroleum ether); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3065 (w), 3031 (w), 2929 (w), 2100 (s), 1767 (s), 1633 (w), 1590 (m), 1495 (w), 1469 (m), 1453 (m), 1415 (m), 1368 (m), 1316 (w), 1252 (w), 1202 (s), 1141 (w), 1078 (w), 1041 (m), 1018 (w), 1001 (w), 985 (w), 913 (w), 892 (w), 873 (w), 840 (w), 764 (w), 706 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 – 7.29 (5H, m, H-9', H-10' and H-11'), 7.07 (1H, ddd, *J* 2.3, 1.6, 0.7, H-6'), 7.04 (1H, td, *J* 1.6, 0.7, H-4'), 6.96 (1H, t, *J* 1.9, H-2'), 5.74 (1H, s, H-7'), 2.38 (3H, s, H-7), 2.30 (3H, s, H-13'), 2.24 (3H, s, H-6); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.2 (C-12'), 165.8 (C-5), 158.5 (C-3), 151.2 (C-1'), 142.1 (C-3'), 138.9 (C-8'), 132.3 (C-5'), 129.1 (C-10'), 128.7 (C-11'), 127.7 (C-9'), 125.2 (C-4'), 121.8 (C-2'), 119.7 (C-6'), 115.7 (C-4), 68.0 (C-7'), 21.3 (C-13'), 11.7 (C-6), 10.9 (C-7); *m/z* (ESI$^+$) 363 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{20}H_{19}O_3N_4^+$ ([M+H]$^+$) requires 363.1452, found 363.1452; HPLC (method 1) retention time 11.36 min, 98.3%.

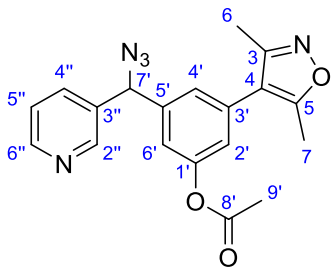
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-{(pyridin-3-yl)[(trimethylsilyl)oxy]methyl}phenyl acetate (308)



Azidotrimethylsilane (212 µL, 1.60 mmol, 3.0 eq.) and copper(II) triflate (19.2 mg, 0.0532 mmol, 0.1 eq.) were added at 0 ºC to a solution of **306** (180 mg, 0.532 mmol, 1.0 eq.) in anhydrous dichloromethane (4 mL). The reaction mixture was warmed to room temperature and stirred for 2 h. The mixture was then heated to 40 ºC for 22 h. After this time, the mixture was quenched with saturated aqueous EDTA solution (6 mL), diluted with water (30 mL), and extracted with dichloromethane (3 × 50 mL). The combined organic components were dried over sodium sulfate, and concentrated under a continuous stream of nitrogen gas. The crude residue was purified by silica gel column chromatography (70% ethyl acetate in petroleum ether) to yield the title compound **308** as a colourless oil (135 mg, 62%). R_f 0.40 (5% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3202 (w), 2929 (w), 2855 (w), 1766 (m), 1743 (w), 1665 (w), 1633 (w), 1610 (w), 1591 (w), 1472 (w), 1417 (m), 1369 (w), 1312 (w), 1253 (w), 1204 (s), 1138 (w), 1086 (w), 1043 (w), 1017 (m), 969 (w), 883 (m), 843 (m), 804 (w), 763 (w), 750 (w), 722 (m), 650 (w), 620 (w), 607 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.60 (1H, s, H-2’’), 8.49 (1H, d, *J* 3.8, H-6’’), 7.67 – 7.61 (1H, m, H-4’’), 7.29 – 7.20 (1H, m, H-5’’), 7.08 (1H, td, *J* 1.6, 0.7, H-4’), 7.04 (1H, ddd, *J* 2.2, 1.6, 0.7, H-6’), 6.88 (1H, dd, *J* 2.2, 1.6, H-2’), 5.79 (1H, s, H-7’), 2.35 (3H, s, H-7), 2.28 (3H, s, H-9’), 2.22 (3H, s, H-6), 0.09 (9H, s, H-10’); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 169.2 (C-8’), 165.6 (C-5), 158.5 (C-3), 151.1 (C-1’), 149.0 (C-6’’), 148.2 (C-2’’), 146.3 (C-5’), 139.7 (C-3’’), 134.2 (C-4’’), 131.9 (C-3’), 124.2 (C-4’), 123.6 (C-5’’), 121.3 (C-2’), 118.7 (C-6’), 115.9 (C-4), 73.9 (C-7’), 21.2 (C-9’), 11.7 (C-7), 10.9 (C-6), 0.1 (C-10’); *m/z* (ESI$^+$) 411 ([M+H]$^+$, 100%);

HRMS *m/z* (ESI$^+$) $C_{22}H_{27}O_4N_2Si^+$ ([M+H]$^+$) requires 411.1735, found 411.1732; HPLC (method 1) retention time 6.00 min, 86.7%.

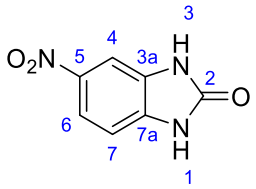
5'-[Azido(pyridin-3''-yl)methyl]-3'-(3,5-dimethyl-1,2-oxazol-4-yl)phenyl acetate (311)



Thionyl chloride (6.47 µL, 0.0887 mmol, 2.0 eq.) was added dropwise at 0 ºC to a solution of **306** (15.0 mg, 0.0443 mmol, 1.0 eq.) in anhydrous benzene (0.5 mL). The reaction mixture was warmed to room temperature and stirred for 1 h. The solvent was removed *in vacuo*, and the residue was dissolved in dichloromethane (20 mL). The organic component was washed with saturated aqueous sodium bicarbonate solution (20 mL) and water (20 mL), dried over sodium sulfate and concentrated *in vacuo*. The crude residue was dissolved in anhydrous *N*,*N*-dimethylformamide (0.5 mL), and sodium azide (5.76 mg, 0.0887 mmol, 2.0 eq.) was added. The reaction mixture was heated to 40 ºC for 4 h. After this time, the mixture was cooled and diluted with water (20 mL). The aqueous component was extracted with diethyl ether (3 × 20 mL), and the combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (30–60% ethyl acetate in petroleum ether) to yield the title compound **311** as a colourless oil (9.00 mg, 56% over two steps). R_f 0.51 (60% ethyl acetate in petroleum ether); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 2927 (w), 2101 (s), 1767 (s), 1633 (w), 1610 (w), 1591 (w), 1476 (w), 1417 (m), 1368 (w), 1317 (w), 1253 (m), 1202 (s), 1142 (w), 1081 (w), 1042 (w), 1019 (w), 1001 (w), 985 (w), 915 (w), 892 (w), 874 (w), 842 (w), 804 (w), 764 (w), 720 (m), 616 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.67 – 8.55 (2H, m, H-2'' and H-6''), 7.65 (1H, dt, *J* 8.0, 1.8, H-4''), 7.34 (1H, dd, *J* 8.0, 4.8, H-5''), 7.07 – 7.02 (2H, m, H-4' and H-6'), 6.99 (1H, t, *J* 1.8, H-2'), 5.78 (1H, s, H-7'), 2.39 (3H, s, H-7), 2.31 (3H, s, H-9'), 2.25 (3H, s, H-6); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 169.2 (C-8'), 165.9 (C-5), 158.4 (C-3), 151.4

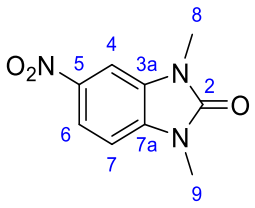
(C-1'), 150.0 (C-6''), 148.9 (C-2''), 141.0 (C-5'), 134.99 (C-3''), 134.98 (C-4''), 132.8 (C-3'), 125.1 (C-4'), 124.0 (C-5''), 122.4 (C-2'), 119.7 (C-6'), 115.6 (C-4), 65.7 (C-7'), 21.3 (C-9'), 11.8 (C-7), 10.9 (C-6); *m/z* (ESI$^+$) 336 ([M-N_2+H]$^+$, 100%), 364 ([M+H]$^+$, 20%); HRMS *m/z* (ESI$^+$) $C_{19}H_{18}O_3N_5^+$ ([M+H]$^+$) requires 364.1404, found 364.1405; HPLC (method 1) retention time 7.03 min, 93.1%.

5-Nitro-1,3-dihydro-2*H*-benzimidazol-2-one (293)[214–216]



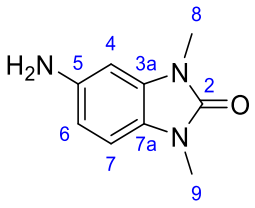
4-Nitro-*o*-phenyldiamine (1.00 g, 6.53 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (10 mL) and 1,1'-carbonyldiimidazole (2.12 g, 13.1 mmol, 2.0 eq.) was added at room temperature. The reaction mixture was stirred for 16 h and was then diluted with water (200 mL). The precipitate was collected and washed with water (3 × 50 mL) to yield the title compound **293** as a yellow solid (1.06 g, 91%). R_f 0.57 (1% triethylamine, 10% methanol in dichloromethane); m.p. 306 ºC (dec. from CH_2Cl_2) {lit.[214] 308–309 °C}; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.40 (1H, bs, H-1 or H-3), 11.18 (1H, bs, H-1 or H-3), 7.94 (1H, dd, *J* 8.7, 2.3, H-6), 7.71 (1H, d, *J* 2.3, H-4), 7.10 (1H, d, *J* 8.7, H-7); *m/z* (ESI$^+$) 180 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[214–216]

1,3-Dimethyl-5-nitro-1,3-dihydro-2*H*-benzimidazol-2-one (294)[217,218]



293 (1.00 g, 5.92 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (10 mL), and potassium carbonate (3.27 g, 35.5 mmol, 4.0 eq.) and iodomethane (1.47 mL, 35.5 mmol, 4.0 eq.) were added at 0 ºC. The reaction mixture was warmed to room temperature and was stirred for 16 h. The reaction mixture was then diluted with water (200 mL). The precipitate was collected and washed with water (3 × 50 mL) to yield the title compound **294** as a yellow solid (1.05 g, 90%). R_f 0.83 (1% triethylamine, 5% methanol in dichloromethane); m.p. 203–204 ºC (from CH_2Cl_2) {lit.[217] 202–204 ºC, lit.[218] 220 ºC}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.12 (1H, dd, *J* 8.7, 2.2, H-6), 7.89 (1H, d, *J* 2.2, H-4), 7.02 (1H, d, *J* 8.7, H-7), 3.50 (3H, s, H-8), 3.49 (3H, s, H-9); *m/z* (ESI$^+$) 208 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[217,218]

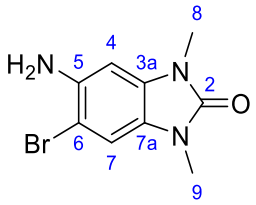
5-Amino-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (20)[218]



To a solution of **294** (1.04 g, 5.00 mmol, 1.0 eq.) in ethyl acetate (14 mL) under an inert nitrogen atmosphere was added 10% palladium on carbon (534 mg, 0.500 mmol, 0.1 eq.). The reaction mixture was charged with hydrogen gas and stirred at room temperature under a hydrogen atmosphere for 16 h. The reaction mixture was filtered through Celite® and concentrated *in vacuo* to yield the title compound **20** as a yellow solid (818 mg, 92%). R_f 0.11 (5% methanol in dichloromethane); m.p. 149–151 ºC (from EtOAc) {lit.[218] 160 ºC}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.75 (1H, d, *J* 8.2, H-7), 6.45 (1H, dd, *J* 8.2, 2.2, H-6), 6.37 (1H, d, *J* 2.2, H-4), 3.58 (2H, s,

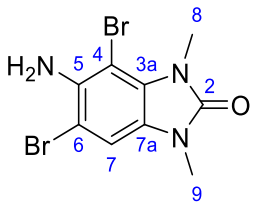
NH_2), 3.36 (3H, s, H-8), 3.35 (3H, s, H-9); *m/z* (ESI^+) 178 ($[M+H]^+$, 100%). The spectroscopic data are in good agreement with literature values.[218]

5-Amino-6-bromo-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (295)[219]



A solution of **20** (800 mg, 4.51 mmol, 1.0 eq.) in chloroform (5.5 mL) and acetic acid (5.5 mL) was cooled to –20 ºC, and bromine (0.230 mL, 4.51 mmol, 1.0 eq.) was added dropwise. The reaction mixture was stirred at room temperature for 30 min and the solution was concentrated. The crude product was purified by silica gel flash column chromatography (20–50% ethyl acetate in dichloromethane) to yield the title compound **295** as a brown solid (900 mg, 90%). R_f 0.32 (8% methanol in dichloromethane); m.p. 169–170 ºC (dec. from EtOAc) {lit.[219] 209 ºC}; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.01 (1H, s, H-7), 6.44 (1H, s, H-4), 3.95 (2H, s, NH_2), 3.34 (3H, s, H-8), 3.33 (3H, s, H-9); *m/z* (ESI^+) 256, 258 ($[M+H]^+$, 100%). The spectroscopic data are in good agreement with literature values.[42,219]

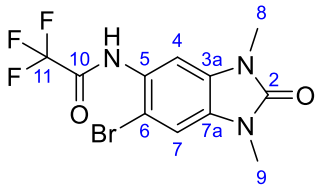
5-Amino-4,6-dibromo-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (296)



5-Amino-4,6-dibromo-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**296**) was also formed in the above reaction when done at 0 ºC (103 mg, 5%). R_f 0.54 (8% methanol in dichloromethane); m.p. 165–166 ºC (dec. from EtOAc); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3413 (m), 3302 (m), 2942 (w), 1712 (s), 1601 (m), 1490 (m), 1452 (m), 1389 (m), 1323 (w), 1275 (w), 1239 (m), 1160 (w), 1087 (m), 983 (m), 943 (w), 855 (w), 829 (m), 797 (w), 773 (w), 740 (m), 720 (w), 684 (w), 643 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.98 (1H, s, H-7), 4.38 (2H, s, N*H*$_2$), 3.71 (3H, s, H-8), 3.33 (3H, s,

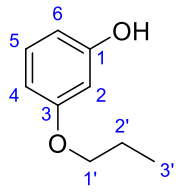
H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 154.7 (C-2), 137.1 (C-5), 128.2 (C-3a), 124.2 (C-7a), 110.5 (C-7), 100.9 (C-6), 90.7 (C-4), 30.0 (C-8), 27.4 (C-9); *m/z* (ESI$^+$) 336, 338 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_9H_{10}ON_3Br_2^+$ ([M+H]$^+$) requires 335.91647, found 335.91649; HPLC retention time 8.3 min, 95.7%.

_N_-(6-Bromo-1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)-11,11,11-trifluoroacetamide (300)[42]



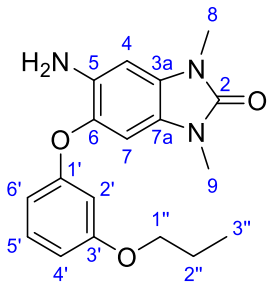
To a solution of **295** (1.60 g, 6.24 mmol, 1.0 eq.) in anhydrous dichloromethane (45 mL) was added trifluoroacetic anhydride (955 µL, 6.87 mmol, 1.1 eq.) and triethylamine (1.74 mL, 12.5 mmol, 2.0 eq.) at 0 ºC. The reaction mixture was warmed to room temperature and stirred for 2 h. After this time, water (70 mL) and dichloromethane (50 mL) were added, and the organic component was separated. The aqueous component was extracted with dichloromethane (3 × 50 mL), and the combined organic components were dried over sodium sulfate, and concentrated *in vacuo* to yield the title compound **300** as an orange solid (2.17 g, 99%). R_f 0.42 (6% methanol in dichloromethane); m.p. 198–200 ºC (from MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.43 (1H, bs, N*H*), 8.00 (1H, s, H-4), 7.18 (1H, s, H-7), 3.43 (3H, s, H-8 or H-9), 3.41 (3H, s, H-8 or H-9); ^{19}F NMR (377 MHz, $CDCl_3$) δ_F –75.8 (d, *J* 1.5); *m/z* (ESI$^+$) 352 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{11}H_{10}O_2N_3BrF_3^+$ ([M+H]$^+$) requires 351.9903 and 353.9883, found 351.9904 and 353.9884. The spectroscopic data are in good agreement with the literature values.[42]

3-Propoxyphenol (298)[220]



To a solution of resorcinol (5.50 g, 50.0 mmol, 3.0 eq.) in anhydrous *N*,*N*-dimethylformamide (15 mL) was added potassium carbonate (6.91 g, 50.0 mmol, 3.0 eq.) and 1-bromopropane (1.50 mL, 16.7 mmol, 1.0 eq.) at room temperature. The reaction mixture was heated to 50 ºC and stirred for 16 h. The reaction mixture was then quenched with slow addition of 1 M aqueous hydrochloric acid (75 mL), and extracted with ethyl acetate (3 × 50 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–20% ethyl acetate in petroleum ether) to yield the title compound **298** as a yellow oil (2.21 g, 87%). R_f 0.52 (30% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.15 – 7.08 (1H, m, H-5), 6.49 (1H, ddd, *J* 8.3, 2.2, 0.9, H-4), 6.42 – 6.38 (2H, m, H-2 and H-6), 4.69 (1H, s, O*H*), 3.89 (2H, t, *J* 6.6, H-1'), 1.79 (2H, dt, *J* 13.9, 7.4, H-2'), 1.03 (3H, t, *J* 7.4, H-3'); *m/z* (ESI$^+$) could not be detected; HRMS *m/z* (ESI$^+$) $C_9H_{13}O_2^+$ ([M+H]$^+$) requires 153.0910, found 153.0909. The spectroscopic data are in good agreement with the literature values.[220]

5-Amino-1,3-dimethyl-6-(3'-propoxyphenoxy)-1,3-dihydro-2*H*-benzimidazol-2-one (299)[42]

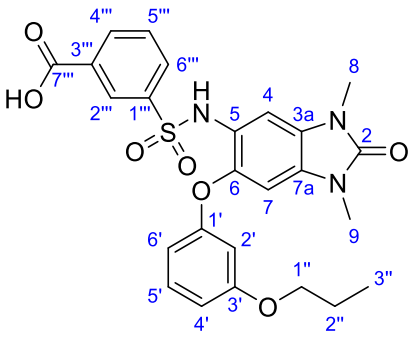


Sodium hydroxide (78.8 mg, 1.97 mmol, 1.0 eq.) was added to a solution of 3-propoxyphenol **298** (300 mg, 1.97 mmol, 1.0 eq.) in methanol (2 mL). The reaction mixture was stirred at room temperature for 1 h. The mixture was the concentrated *in vacuo*, and the resulting solid was dissolved in toluene and concentrated *in vacuo* to yield sodium 3-propoxyphenolate as a colourless solid (341 mg, 100%). This product was used directly in the next step.

Copper(I) iodide and caesium carbonate were dried using an Abderhalden's drying pistol at 160 ºC under high vacuum for 48 h prior to the reaction. All other reagents were dried at room temperature for 48 h under high vacuum. **295** (100 mg, 0.390 mmol, 1.0 eq.), sodium 3-propoxyphenolate (102 mg, 0.586 mmol, 1.5 eq.), copper(I) iodide (7.43 mg, 0.0390 mmol, 0.1 eq.), caesium carbonate (381 mg, 1.17 mmol, 3.0 eq.) and quinolin-8-ol (11.3 mg, 0.0780 mmol, 0.2 eq.) were added to a flame dried microwave vial, which was subsequently sealed and the atmosphere was purged with nitrogen through three successive evacuate/fill cycles. Anhydrous diethylene glycol dimethyl ether (5 mL) was degassed through three successive freeze-pump-thaw cycles under argon before addition to the mixture. The reaction vessel was covered in foil. The reaction was then heated to 130 ºC for 72 h. After this time, the reaction mixture was cooled, filtered through a silica plug, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–2% methanol in dichloromethane) to yield the title compound **299** as a brown solid (108 mg, 81%). R_f 0.13 (2% methanol in dichloromethane); m.p. 137–139 ºC (from CH_2Cl_2) {lit.[181] 144–147 ºC}; $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3437 (w), 3344 (w), 3216 (w), 2964 (w), 2936 (w), 2878 (w), 1688 (s), 1645 (m), 1603 (m), 1506 (s), 1489 (m), 1466 (s), 1394 (m), 1326 (w), 1260 (m), 1183 (m), 1136 (s), 1079 (w), 1049 (w),

1026 (m), 1006 (w), 953 (w), 910 (w), 846 (w), 764 (w), 733 (m), 686 (w), 637 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.17 (1H, t, *J* 8.1, H-5'), 6.63 (1H, s, H-7), 6.58 (1H, d, *J* 8.1, H-6'), 6.54 – 6.40 (3H, m, H-4, H-2' and H-4'), 3.87 (2H, t, *J* 6.5, H-1''), 3.36 (3H, s, H-8), 3.30 (3H, s, H-9), 1.78 (2H, p, *J* 7.0, H-2''), 1.01 (3H, t, *J* 7.3, H-3''); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 160.7 (C-3'), 159.6 (C-1'), 155.0 (C-2), 137.5 (C-6), 134.1 (C-5), 130.2 (C-5'), 127.6 (C-7a), 122.9 (C-3a), 108.4, 108.3 (C-4' and C-6'), 103.1 (C-2'), 101.8 (C-7), 96.7 (C-4), 69.7 (C-1''), 27.34, 27.27 (C-8 and C-9), 22.6 (C-2''), 10.6 (C-3''); *m/z* (ESI$^+$) 328 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[42]

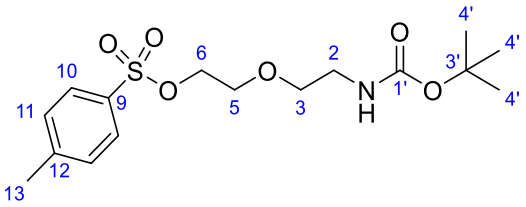
3'''-{[1,3-Dimethyl-2-oxo-6-(3'-propoxyphenoxy)-2,3-dihydro-1*H*-benzimidazol-5-yl]sulfamoyl}benzoic acid (287)[221]



3-(Chlorosulfonyl)benzoic acid (97.2 mg, 0.440 mmol, 1.2 eq.) and pyridine (59.3 µL, 0.734 mmol, 2.0 eq.) were added to a solution of **299** (120 mg, 0.367 mmol, 1.0 eq.) in anhydrous dichloromethane (3 mL) at room temperature. The reaction mixture was stirred at room temperature for 16 h. The mixture was then quenched with 1 M aqueous hydrochloric acid (4 mL) and extracted with 25% methanol in dichloromethane (3 × 30 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (0–10% methanol in dichloromethane) to yield the title compound **287** as a yellow solid (86.5 mg, 46%). R_f 0.14 (6% methanol in dichloromethane); m.p. 116–118 ºC (from CH_2Cl_2) {lit.[181] 111–115 ºC}; $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3075 (w), 2936 (w), 1692 (s), 1602 (m), 1541 (s), 1507 (m), 1489 (m), 1465 (w), 1397 (w), 1344 (w), 1318 (w), 1259 (m), 1230 (w), 1168 (s), 1131 (s), 1079 (w), 1048 (w), 1025 (w), 1005 (w), 959 (w), 902 (w), 841

(w), 834 (w), 755 (w), 735 (m), 700 (w), 681 (w), 662 (w), 653 (w), 613 (w); ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.26 (1H, t, *J* 1.8, H-2’’’), 8.05 (1H, dt, *J* 7.7, 1.4, H-4’’’), 7.80 (1H, dt, *J* 7.9, 1.3, H-6’’’), 7.41 – 7.33 (2H, m, H-4 and H-5’’’), 7.00 (1H, t, *J* 8.2, H-5’), 6.57 (1H, s, H-7), 6.51 (1H, dd, *J* 8.2, 2.4, H-4’), 6.09 (1H, dd, *J* 8.2, 2.4, H-6’), 5.90 (1H, t, *J* 2.4, H-2’), 3.78 (2H, t, *J* 7.0, H-1’’), 3.43 (3H, s, H-8), 3.23 (3H, s, H-9), 1.75 (2H, h, *J* 7.0, H-2’’), 1.02 (3H, t, *J* 7.0, H-3’’); ^{13}C NMR (126 MHz, CD_3OD) δ_C 168.0 (C-7’’’), 161.7 (C-3’), 159.6 (C-1’), 156.5 (C-2), 146.5 (C-6), 141.9 (C-1’’’), 134.6 (C-4’’’), 133.0 (C-3’’’), 132.2 (C-6’’’), 131.0 (C-5’), 130.1 (C-5’’’), 129.9 (C-3a), 129.4 (C-2’’’), 127.3 (C-7a), 123.1 (C-5), 110.5 (C-6’), 110.1 (C-4’), 108.3 (C-4), 105.0 (C-2’), 100.9 (C-7), 70.5 (C-1’’), 27.6, 27.5 (C-8 and C-9), 23.6 (C-2’’), 10.8 (C-3’’); *m/z* (ESI$^+$) 512 ([M+H]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{25}H_{26}O_7N_3S^+$ ([M+H]$^+$) requires 512.1486, found 512.1486. The spectroscopic data are in good agreement with the literature values.[221]

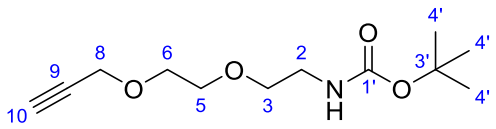
7-{5-[(*tert*-Butoxycarbonyl)amino]ethoxy}ethyl 12-methylbenzene-9-sulfonate (304)[222]



tert-Butyl [2-(2-hydroxyethoxy)ethyl]carbamate (500 mg, 2.44 mmol, 1.0 eq.) and triethylamine (998 µL, 7.32 mmol, 3.0 eq.) were dissolved in anhydrous dichloromethane (2.5 mL). 4-Methylbenzene-1-sulfonyl chloride (929 mg, 4.87 mmol, 2.0 eq.) was added at 0 ºC, and the resulting mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was then concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (1–2% methanol in dichloromethane) to yield the title compound **304** as a yellow oil (833 mg, 95%). R_f 0.62 (5% methanol in dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.83 – 7.78 (2H, m, H-10), 7.38 – 7.33 (2H, m, H-11), 4.80 (1H, bs, N*H*), 4.19 – 4.13 (2H, m, H-6), 3.65 – 3.60 (2H, m, H-5), 3.45 (2H, t, *J* 5.3, H-3), 3.24 (2H, q, *J* 5.4, H-2), 2.45 (3H, s, H-13),

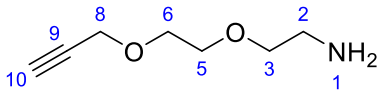
1.45 (9H, s, H-4'); *m/z* (ESI$^+$) 360 ([M+H]$^+$, 70%), 382 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[222]

***tert*-Butyl (2-{2-[(prop-2-yn-1-yl)oxy]ethoxy}ethyl)carbamate (305)**[184]



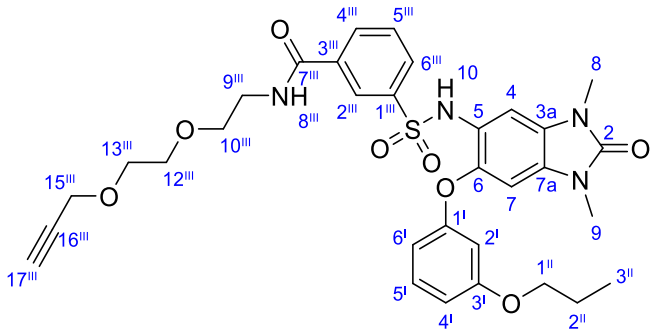
Sodium hydride (60% in mineral dispersion, 178 mg, 4.45 mmol, 2.0 eq.) was added slowly to a solution of propargyl alcohol (259 µL, 4.45 mmol, 2.0 eq.) in anhydrous tetrahydrofuran (20 mL) at 0 ºC and stirred for 30 min. **304** (800 mg, 2.23 mmol, 1.0 eq.) was subsequently added, and the reaction mixture was stirred at 50 ºC for 5 h. After this time, the mixture was cooled to room temperature and quenched with isopropyl alcohol (5 mL). 1 M aqueous hydrochloric acid (10 mL) was added, and the aqueous component was extracted with ethyl acetate (3 × 30 mL). The combined organic components were washed with water (40 mL), dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (10–25% ethyl acetate in petroleum ether) to yield the title compound **305** as a colourless oil (179 mg, 33%). R_f 0.46 (6% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3352 (w), 3295 (w), 3254 (w), 2976 (w), 2931 (w), 2870 (w), 2363 (w), 2115 (w), 1700 (s), 1511 (m), 1456 (w), 1392 (w), 1366 (m), 1272 (m), 1249 (m), 1169 (s), 1131 (s), 1095 (s), 1035 (w), 1004 (w), 968 (w), 920 (w), 864 (w), 842 (w), 781 (w), 758 (w), 665 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.00 (1H, s, N*H*), 4.14 (2H, d, *J* 2.4, H-8), 3.66 – 3.54 (4H, m, H-5 and H-6), 3.48 (2H, t, *J* 5.2, H-3), 3.25 (2H, q, *J* 5.3, H-2), 2.41 (1H, t, *J* 2.4, H-10), 1.38 (9H, s, H-4'); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 156.0 (C-1'), 79.6 (C-3'), 79.1 (C-9), 74.7 (C-10), 70.3 (C-3), 70.1, 69.0 (C-5 and C-6), 58.4 (C-8), 40.4 (C-2), 28.4 (C-4'); *m/z* (ESI$^+$) 266 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[184]

2-{2-[(Prop-2-yn-1-yl)oxy]ethoxy}ethan-1-amine (314)[223]



305 (140 mg, 0.575 mmol, 1.0 eq.) was dissolved in a 4 M HCl in dioxane solution (2 mL) and stirred at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo*. The crude residue was passed through an Isolute® SXC-2 Catch and Release for Scavenging Basic Compounds column (10 mL methanol followed by 10 mL 2 M ammonia in methanol solution) to yield the title compound **314** as a colourless oil (80.3 mg, 98%). R_f 0.11 (6% methanol in dichloromethane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 4.22 (2H, d, *J* 2.4, H-8), 3.74 – 3.62 (4H, m, H-5 and H-6), 3.57 – 3.51 (2H, t, *J* 5.2, H-3), 2.91 (2H, t, *J* 5.2, H-2), 2.44 (1H, t, *J* 2.4, H-10); *m/z* (ESI$^+$) 144 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with the literature values.[223]

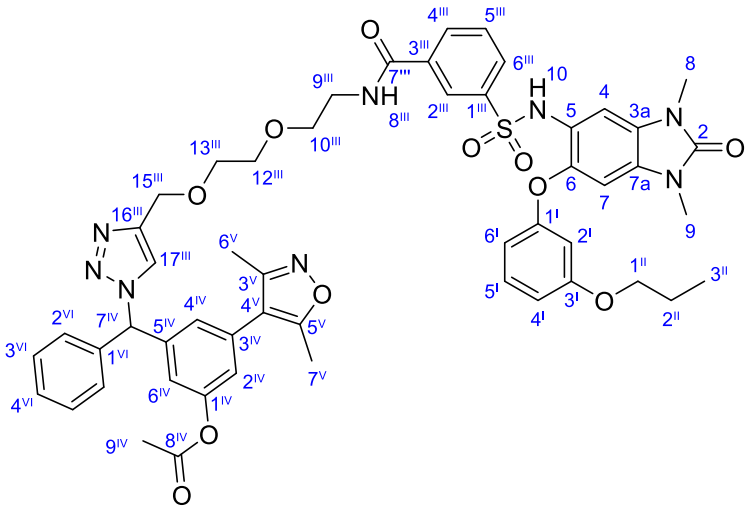
3^{III}-{[1,3-Dimethyl-2-oxo-6-(3^{I}-propoxyphenoxy)-2,3-dihydro-1*H*-benzimidazol-5-yl]sulfamoyl}-*N*-(2-{2-[(prop-2-yn-1-yl)oxy]ethoxy}ethyl)benzamide (315)



HATU (81.6 mg, 0.215 mmol, 1.1 eq.), *N*,*N*-diisopropylethylamine (67.9 µL, 0.390 mmol, 2.0 eq.), and **314** (36.4 mg, 0.254 mmol, 1.3 eq.) were added to a solution of **287** (120 mg, 0.195 mmol, 1.0 eq.) in anhydrous *N*,*N*-dimethylformamide (4 mL). The reaction mixture was stirred at room temperature for 5 h. After this time, the reaction mixture was diluted with water (20 mL) and ethyl acetate (20 mL), and the aqueous component was extracted with ethyl acetate (3 × 30 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (30%

acetone in toluene) to yield the title compound **315** as an orange oil (93.4 mg, 75%). R_f 0.34 (8% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3274 (w), 3072 (w), 2936 (w), 2876 (w), 1694 (s), 1659 (m), 1604 (m), 1538 (m), 1508 (s), 1489 (m), 1462 (m), 1417 (m), 1397 (m), 1344 (m), 1319 (m), 1260 (m), 1230 (m), 1174 (m), 1147 (s), 1132 (s), 1097 (m), 1047 (w), 1028 (w), 1005 (m), 959 (w), 913 (w), 854 (w), 837 (w), 739 (m), 685 (m), 661 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.09 (1H, t, *J* 1.6, H-2^{III}), 7.96 (1H, dt, *J* 8.0, 1.6, H-4^{III}), 7.72 (1H, dt, *J* 8.0, 1.6, H-6^{III}), 7.39 (1H, t, *J* 8.0, H-5^{III}), 7.33 (1H, s, H-4), 7.05 (1H, t, *J* 8.1, H-5^{I}), 6.94 (1H, s, H-10), 6.67 (1H, t, *J* 5.1, H-8^{III}), 6.56 (1H, dd, *J* 8.3, 2.4, H-6^{I}), 6.40 (1H, s, H-7), 6.09 – 6.02 (2H, m, H-2^{I} and H-4^{I}), 4.19 (2H, d, *J* 2.4, H-15^{III}), 3.82 (2H, t, *J* 7.0, H-1^{II}), 3.71 – 3.58 (8H, m, H-9^{III}, H-10^{III}, H-12^{III} and H-13^{III}), 3.44 (3H, s, H-8), 3.25 (3H, s, H-9), 2.42 (1H, t, *J* 2.4, H-17^{III}), 1.77 (2H, h, *J* 7.0, H-2^{II}), 1.02 (3H, t, *J* 7.0, H-3^{II}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 165.5 (C-7^{III}), 160.6 (C-3^{I}), 158.0 (C-1^{I}), 154.9 (C-2), 143.0 (C-6), 139.4 (C-1^{III}), 135.6 (C-3^{III}), 132.1 (C-4^{III}), 130.5 (C-5^{I}), 129.9 (C-6^{III}), 129.3 (C-5^{III}), 128.6 (C-3a), 126.8 (C-7a), 125.6 (C-2^{III}), 121.6 (C-5), 109.4 (C-6^{I}), 109.2 (C-4^{I}), 104.4 (C-4), 104.0 (C-2^{I}), 99.6 (C-7), 79.6 (C-16^{III}), 75.0 (C-17^{III}), 70.1 (C-10^{III}, C-12^{III} or C-13^{III}), 69.8 (C-1^{II}), 69.6 (C-10^{III}, C-12^{III} or C-13^{III}), 69.1 (C-10^{III}, C-12^{III} or C-13^{III}), 58.5 (C-15^{III}), 40.0 (C-9^{III}), 27.6 (C-8), 27.5 (C-9), 22.6 (C-2^{II}), 10.7 (C-3^{II}); *m/z* (ESI$^+$) 659 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{32}H_{37}O_8N_4S^+$ ([M+H]$^+$) requires 637.2327, found 637.2324; HPLC (method 1) retention time 9.42 min, 98.4%.

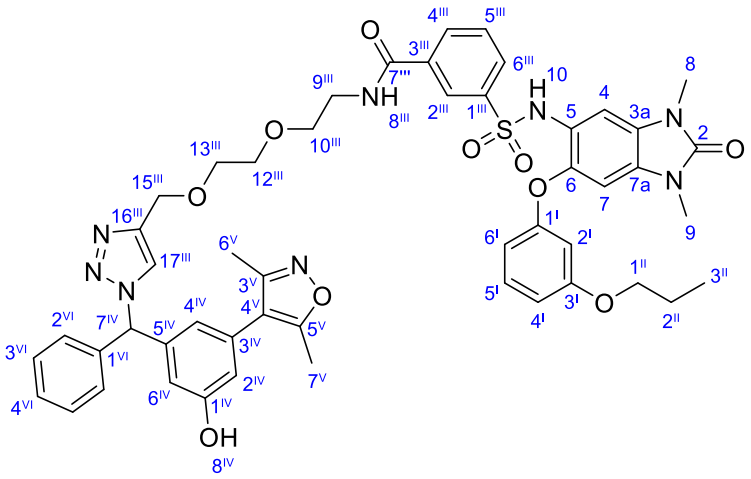
3^{IV}-(3^{V},5^{V}-Dimethyl-1^{V},2^{V}-oxazol-4^{V}-yl)-5^{IV}-{[4-({2-[2-(3-{[1,3-dimethyl-2-oxo-6-(3^{I}-propoxyphenoxy)-2,3-dihydro-1*H*-benzimidazol-5-yl]sulfamoyl}benzamido)ethoxy]ethoxy}methyl)-1*H*-1,2,3-triazol-1-yl](phenyl)methyl}phenyl acetate (316)



307 (18.8 mg, 0.0518 mmol, 1.1 eq.) was added to a solution of **315** (30.0 mg, 0.0471 mmol, 1.0 eq.) in tetrahydrofuran (0.5 mL), followed by addition of a solution of copper(II) sulfate pentahydrate (1.20 mg, 0.00471 mmol, 0.1 eq.) and sodium ascorbate (0.933 mg, 0.00471 mmol, 0.1 eq.) in water (0.5 mL). The reaction mixture was stirred at room temperature for 22 h. Saturated aqueous EDTA solution (10 mL) was added, followed by water (10 mL). The aqueous component was extracted with dichloromethane (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (60–100% ethyl acetate in petroleum ether, followed by 100% ethanol) to yield the title compound **316** as a colourless oil (43.7 mg, 93%). R_f 0.38 (40% acetone in toluene); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3070 (w), 2963 (w), 2936 (w), 2875 (w), 2243 (w), 1768 (w), 1699 (s), 1658 (m), 1604 (m), 1592 (m), 1538 (w), 1508 (s), 1489 (w), 1455 (s), 1416 (w), 1397 (w), 1369 (w), 1343 (w), 1317 (m), 1258 (m), 1203 (s), 1180 (s), 1147 (s), 1132 (s), 1085 (w), 1047 (w), 1018 (w), 1006 (w), 959 (w), 912 (m), 876 (w), 852 (w), 836 (w), 733 (s), 702 (w), 686 (w), 662 (w), 648 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.20 (1H, t, *J* 1.7, H-2^{III}), 8.00 (1H, dt, *J* 7.9, 1.7, H-4^{III}), 7.67 (1H, dt, *J* 7.9, 1.7, H-6^{III}), 7.50 (1H, s, H-17^{III}), 7.47 – 7.32 (5H, m, H-5^{III},

H-3^{VI}, H-4^{VI} and H-10), 7.30 (1H, s, H-4), 7.22 – 7.16 (2H, m, H-2^{VI}), 7.14 – 7.10 (2H, m, H-8^{III} and H-7^{IV}), 7.03 – 6.97 (2H, m, H-5^{I} and H-2^{IV}), 6.83 (1H, d, *J* 1.9, H-6^{IV}), 6.78 (1H, d, *J* 1.9, H-4^{IV}), 6.53 (1H, dd, *J* 8.3, 1.9, H-6^{I}), 6.36 (1H, s, H-7), 6.03 – 5.98 (2H, m, H-2^{I} and H-4^{I}), 4.69 (2H, s, H-15^{III}), 3.79 (2H, t, *J* 7.0, H-1^{II}), 3.70 – 3.51 (8H, m, H-9^{III}, H-10^{III}, H-12^{III} and H-13^{III}), 3.42 (3H, s, H-8), 3.23 (3H, s, H-9), 2.32 (3H, s, H-7^{V}), 2.27 (3H, s, H-9^{IV}), 2.17 (3H, s, H-6^{V}), 1.75 (2H, h, *J* 7.0, H-2^{II}), 1.01 (3H, t, *J* 7.0, H-3^{II}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 169.2 (C-8^{IV}), 165.9 (C-5^{V}), 165.5 (C-7^{III}), 160.5 (C-3^{I}), 158.4 (C-3^{V}), 158.1 (C-1^{I}), 154.9 (C-2), 151.3 (C-1^{IV}), 144.8 (C-16^{III}), 143.2 (C-6), 140.5 (C-1^{VI}), 139.4 (C-1^{III}), 137.2 (C-5^{IV}), 135.5 (C-3^{III}), 132.5 (C-3^{IV}), 132.4 (C-4^{III}), 130.3 (C-5^{I}), 129.9 (C-6^{III}), 129.3 (C-3^{VI}), 129.2 (C-4^{VI}), 129.1 (C-5^{III}), 128.51 (C-7a), 128.45 (C-2^{VI}), 126.6 (C-3a), 125.8 (C-4^{IV}), 125.5 (C-2^{III}), 123.2 (C-17^{III}), 122.3 (C-2^{IV}), 121.7 (C-5), 120.3 (C-6^{IV}), 115.4 (C-4^{V}), 109.3 (C-6^{I}), 109.2 (C-4^{I}), 104.5 (C-4), 104.1 (C-2^{I}), 99.4 (C-7), 70.6, 69.8, 69.70 (C-10^{III}, C-12^{III} and C-13^{III}), 69.66 (C-1^{II}), 67.7 (C-7^{IV}), 64.4 (C-15^{III}), 40.1 (C-9^{III}), 27.6 (C-8), 27.4 (C-9), 22.6 (C-2^{II}), 21.3 (C-9^{IV}), 11.8 (C-7^{V}), 10.9 (C-6^{V}), 10.7 (C-3^{II}); *m/z* (ESI$^+$) 999 ([M+H]$^+$, 60%), 1021 ([M+Na]$^+$, 100%); HRMS *m/z* (ESI$^+$) $C_{52}H_{55}O_{11}N_8S^+$ ([M+H]$^+$) requires 999.3706, found 999.3696; HPLC (method 1) retention time 10.52 min, 95.5%.

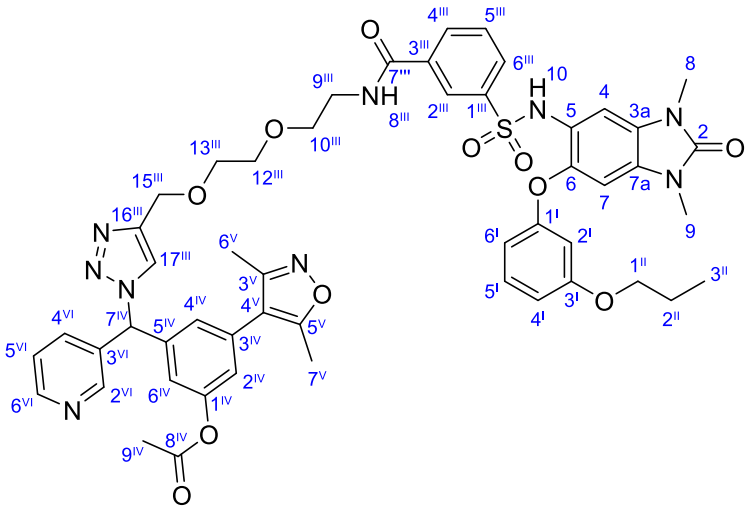
N-(10^{III}-{13^{III}-[(5^{IV}-{[3^{IV}-(3^{V},5^{V}-Dimethyl-1^{V},2^{V}-oxazol-4^{V}-yl)-1^{IV}-hydroxyphenyl](phenyl)methyl}-1*H*-1,2,3-triazol-4-yl)methoxy]ethoxy}ethyl)-1^{III}-{[1,3-dimethyl-2-oxo-6-(3^{I}-propoxyphenoxy)-2,3-dihydro-1*H*-benzimidazol-5-yl]sulfamoyl}benzamide (288)



A solution of lithium hydroxide (4.79 mg, 0.200 mmol, 5.0 eq.) in water (1 mL) was added to a solution of **316** (40.0 mg, 0.0400 mmol, 1.0 eq.) in ethanol (1 mL), and the reaction mixture was stirred at room temperature for 1 h. After this time, the reaction mixture was neutralised to pH 7 with 1 M aqueous hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (2–5% ethanol in ethyl acetate) to yield the title compound **288** as a colourless oil (28.3 mg, 74%). R_f 0.41 (7% methanol in dichloromethane); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3153 (w), 2966 (w), 2932 (w), 2877 (w0, 2361 (w), 2341 (w), 1693 (s), 1654 (m), 1641 (m), 1600 (s), 1541 (w), 1508 (s), 1490 (m), 1456 (s), 1421 (w), 1397 (w), 1323 (m), 1259 (m), 1230 (w), 1174 (m), 1148 (s), 1131 (s), 1085 (w), 1049 (w), 1030 (w), 1005 (w), 959 (w), 911 (w), 859 (w), 837 (w), 816 (w), 762 (w), 733 (s), 711 (w), 701 (w), 685 (w), 661 (w), 648 (w), 607 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.12 (1H, t, *J* 1.8, H-2^{III}), 8.02 – 7.96 (2H, m, H-4^{III} and H-8^{IV}), 7.72 (1H, dt, *J* 7.8, 1.4, H-6^{III}), 7.43 (1H, s, H-10), 7.41 (1H, s, H-17^{III}), 7.38 (1H, t, *J* 7.8, H-5^{III}), 7.35 – 7.30 (3H, m, H-3^{VI} and H-4^{VI}), 7.30 (1H, s, H-4), 7.17 – 7.09 (3H, m, H-8^{III} and H-2^{VI}), 7.06 – 7.00 (1H, m, H-5^{I}), 6.96 (1H, s, H-7^{IV}), 6.75 (1H, dd, *J* 2.2, 1.6, H-2^{IV}),

6.55 (1H, ddd, *J* 8.3, 2.3, 1.0, H-6^{I}), 6.45 (1H, d, *J* 1.6, H-4^{IV}), 6.42 (1H, t, *J* 2.2, H-6^{IV}), 6.40 (1H, s, H-7), 6.07 – 6.03 (2H, m, H-2^{I} and H-4^{I}), 4.69 (1H, d, *J* 12.3, H-15^{IIIa}), 4.63 (1 H, d, *J* 12.3, H-15^{IIIb}), 3.80 (2H, t, *J* 7.0, H-1^{II}), 3.74 – 3.55 (6H, m, H-10^{III}, H-12^{III} and H-13^{III}), 3.56 – 3.49 (2H, m, H-9^{III}), 3.40 (3H, s, H-8), 3.23 (3H, s, H-9), 2.30 (3H, s, H-7^{V}), 2.15 (3H, s, H-6^{V}), 1.77 (2H, h, *J* 7.0, H-2^{II}), 1.01 (3H, t, *J* 7.0, H-3^{II}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 166.0 (C-7^{III}), 165.6 (C-5^{V}), 160.6 (C-3^{I}), 158.6 (C-3^{V}), 158.0 (C-1^{I}), 157.8 (C-1^{IV}), 155.0 (C-2), 144.6 (C-16^{III}), 143.7 (C-6), 140.1 (C-1^{VI}), 139.5 (C-1^{III}), 137.6 (C-5^{IV}), 135.2 (C-3^{III}), 132.6 (C-3^{IV}), 132.5 (C-4^{III}), 130.4 (C-5^{I}), 130.1 (C-6^{III}), 129.4 (C-5^{III}), 129.1 (C-3^{VI}), 129.0 (C-4^{VI}), 128.6 (C-7a), 128.3 (C-2^{VI}), 126.5 (C-3a), 125.4 (C-2^{III}), 123.1 (C-17^{III}), 121.5 (C-5), 120.6 (C-4^{IV}), 116.6 (C-2^{IV}), 116.1 (C-4^{V}), 113.7 (C-6^{IV}), 109.5 (C-4^{I}), 109.4 (C-6^{I}), 105.1 (C-4), 104.3 (C-2^{I}), 99.5 (C-7), 70.4, 70.0, 69.8 (C-10^{III}, C-12^{III} and C-13^{III}), 69.7 (C-1^{II}), 67.9 (C-7^{IV}), 64.5 (C-15^{III}), 40.3 (C-9^{III}), 27.7 (C-8), 27.5 (C-9), 22.6 (C-2^{II}), 11.8 (C-7^{V}), 11.0 (C-6^{V}), 10.7 (C-3^{II}); *m/z* (ESI^{+}) 957 ([M+H]$^{+}$, 50%), 979 ([M+Na]$^{+}$, 100%); HRMS *m/z* (ESI^{+}) $C_{50}H_{53}O_{10}N_8S^{+}$ ([M+H]$^{+}$) requires 957.3600, found 957.3593; HPLC (method 1) retention time 9.96 min, 100%.

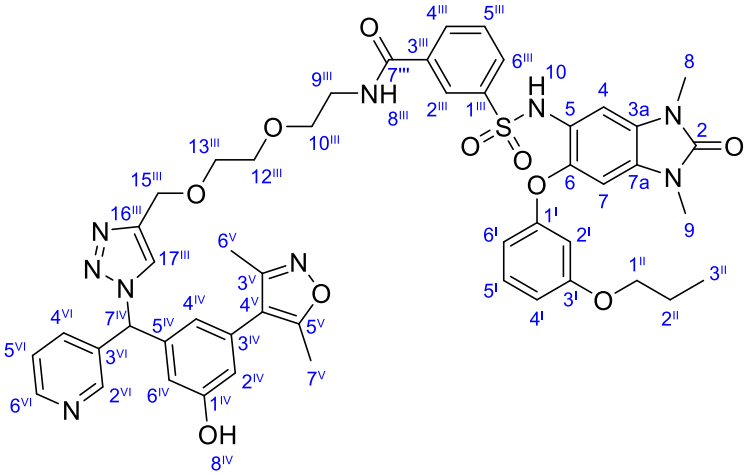
3^{IV}-(3^{V},5^{V}-Dimethyl-1^{V},2^{V}-oxazol-4^{V}-yl)-5^{IV}-{[4-({2-[2-(3^{III}-{[1,3-dimethyl-2-oxo-6-(3^{I}-propoxyphenoxy)-2,3-dihydro-1*H*-benzimidazol-5-yl]sulfamoyl}benzamido)ethoxy]ethoxy}methyl)-1*H*-1,2,3-triazol-1-yl](pyridin-3^{IV}-yl)methyl}phenyl acetate (317)



311 (10.0 mg, 0.0275 mmol, 1.1 eq.) was added to a solution of **315** (15.9 mg, 0.0250 mmol, 1.0 eq.) in tetrahydrofuran (0.5 mL), followed by addition of a solution of copper(II) sulfate pentahydrate (0.624 mg, 0.00250 mmol, 0.1 eq.) and sodium ascorbate (0.495 mg, 0.00250 mmol, 0.1 eq.) in water (0.5 mL). The reaction mixture was stirred at room temperature for 22 h. Saturated aqueous EDTA solution (10 mL) was added, followed by water (10 mL). The aqueous component was extracted with dichloromethane (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (20–60% acetone in toluene) to yield the title compound **317** as a colourless oil (8.40 mg, 34%). R_f 0.18 (40% acetone in toluene); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3068 (w), 2960 (w), 2927 (w), 2875 (w), 2357 (w), 2246 (w), 1768 (w), 1698 (s), 1655 (m), 1604 (m), 1592 (m), 1541 (w), 1508 (m), 1489 (m), 1458 (m), 1418 (m), 1397 (m), 1369 (w), 1342 (w), 1317 (m), 1258 (m), 1202 (s), 1177 (m), 1147 (s), 1132 (s), 1085 (m), 1047 (w), 1024 (w), 1006 (w), 959 (w), 911 (m), 878 (w), 854 (w), 838 (w), 730 (s), 685 (w), 661 (w), 647 (w), 607 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.60 (1H, d, *J* 4.8, H-2^{VI}), 8.51 (1H, d, *J* 2.4, H-6^{VI}), 8.17 (1H, t, *J* 1.6, H-2^{III}), 8.01 (1H, dt, *J* 7.9, 1.6, H-4^{III}), 7.66 (1H, dt, *J* 7.9, 1.6, H-6^{III}), 7.62 (1H, s, H-17^{III}),

7.57 (1H, dt, *J* 8.0, 2.0, H-5^{VI}), 7.51 (1H, s, H-10), 7.39 – 7.31 (2H, m, H-4^{VI} and H-5^{III}), 7.29 (1H, s, H-4), 7.17 – 7.11 (2H, m, H-7^{IV} and H-8^{III}), 7.04 – 6.98 (2H, m, H-5^{I} and H-2^{IV}), 6.89 (1H, t, *J* 2.0, H-6^{IV}), 6.86 (1H, t, *J* 1.6, H-4^{IV}), 6.55 – 6.51 (1H, m, H-6^{I}), 6.37 (1H, s, H-7), 6.04 – 5.99 (2H, m, H-2^{I} and H-4^{I}), 4.70 (2H, s, H-15^{III}), 3.80 (2H, t, *J* 7.0, H-1^{II}), 3.70 – 3.53 (8H, m, H-9^{III}, H-10^{III}, H-12^{III} and H-13^{III}), 3.43 (3H, s, H-8), 3.24 (3H, s, H-9), 2.34 (3H, s, H-7^{V}), 2.28 (3H, s, H-9^{IV}), 2.18 (3H, s, H-6^{V}), 1.76 (2H, h, *J* 7.0, H-2^{II}), 1.01 (3H, t, *J* 7.0, H-3^{II}); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 169.2 (C-8^{IV}), 166.0 (C-5^{V}), 165.5 (C-7^{III}), 160.5 (C-3^{I}), 158.3 (C-3^{V}), 158.1 (C-1^{I}), 154.9 (C-2), 151.5 (C-1^{IV}), 150.4 (C-2^{VI}), 149.6 (C-6^{VI}), 145.3 (C-16^{III}), 143.3 (C-6), 139.41 (C-1^{III}), 139.35 (C-3^{VI}), 136.1 (C-5^{VI}), 135.6 (C-3^{III}), 133.4 (C-5^{IV}), 133.0 (C-3^{IV}), 132.5 (C-4^{III}), 130.4 (C-5^{I}), 129.9 (C-6^{III}), 129.2 (C-5^{III}), 128.6 (C-7a), 126.7 (C-3a), 125.8 (C-4^{IV}), 125.4 (C-2^{III}), 124.0 (C-4^{VI}), 123.2 (C-17^{III}), 122.8 (C-2^{IV}), 121.6 (C-5), 120.4 (C-6^{IV}), 115.3 (C-4^{V}), 109.3 (C-4^{I}), 109.2 (C-6^{I}), 104.6 (C-4), 104.2 (C-2^{I}), 99.5 (C-7), 70.6, 69.9, 69.8 (C-10^{III}, C-12^{III} and C-13^{III}), 69.7 (C-1^{II}), 65.6 (C-7^{IV}), 64.4 (C-15^{III}), 40.1 (C-9^{III}), 27.6 (C-8), 27.5 (C-9), 22.7 (C-2^{II}), 21.3 (C-9^{IV}), 11.8 (C-7^{V}), 11.0 (C-6^{V}), 10.7 (C-3^{II}); *m/z* (ESI$^+$) 1000 ([M+H]$^+$, 100%), 1022 ([M+Na]$^+$, 90%); HRMS *m/z* (ESI$^+$) $C_{51}H_{54}O_{11}N_9S^+$ ([M+H]$^+$) requires 1000.3658, found 1000.3651; HPLC (method 1) retention time 8.53 min, 97.7%.

N-(10^{III}-{13^{III}-[(7^{IV}-{[3^{IV}-(3^{V},5^{V}-dimethyl-1^{V},2^{V}-oxazol-4^{V}-yl)-1^{IV}-hydroxyphenyl](pyridin-3^{VI}-yl)methyl}-1*H*-1,2,3-triazol-4-yl)methoxy]ethoxy}ethyl)-1^{III}-{[1,3-dimethyl-2-oxo-6-(3^{I}-propoxyphenoxy)-2,3-dihydro-1*H*-benzimidazol-5-yl]sulfamoyl}benzamide (291)



A solution of lithium hydroxide (0.84 mg, 0.035 mmol, 5.0 eq.) in water (0.5 mL) was added to a solution of **317** (7.0 mg, 0.0067 mmol, 1.0 eq.) in ethanol (0.5 mL), and the reaction mixture was stirred at room temperature for 1.5 h. After this time, the reaction mixture was neutralised to pH 7 with 1 M hydrochloric acid and extracted with ethyl acetate (3 × 20 mL). The combined organic components were dried over sodium sulfate, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (60–70% acetone in toluene) to yield the title compound **291** as a colourless oil (5.0 mg, 78%). R_f 0.19 (60% acetone in toluene); $\bar{\nu}_{max}$ (thin film)/cm^{-1} 3148 (w), 2961 (w), 2929 (w), 2874 (w), 2367 (w), 2358 (w), 2338 (w), 2248 (w), 1693 (s), 1655 (w), 1640 (w), 1598 (m), 1542 (w), 1508 (m), 1489 (w), 1460 (m), 1422 (w), 1397 (w), 1324 (m), 1259 (m), 1230 (w), 1173 (m), 1147 (s), 1130 (s), 1084 (m), 1048 (w), 1028 (w), 1004 (w), 958 (w), 909 (m), 858 (w), 842 (w), 728 (s), 684 (m), 667 (w), 646 (w), 630 (w), 608 (w); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.55 (1H, s, H-2^{VI}), 8.42 (1H, s, H-6^{VI}), 8.20 (1H, s, H-10), 8.10 (1H, t, *J* 1.8, H-2^{III}), 7.99 (1H, dt, *J* 7.8, 1.4, H-4^{III}), 7.72 (1H, dt, *J* 8.0, 1.4, H-6^{III}), 7.55 – 7.43 (3H, m, H-5^{VI}, H-17^{III} and H-8^{IV}), 7.39 (1H, t, *J* 7.8, H-5^{III}), 7.31 – 7.27 (2H, m, H-4 and H-4^{VI}), 7.11 (1H, t, *J* 5.4, H-8^{III}), 7.04 (1H, t, *J* 8.2, H-5^{I}), 6.98 (1H, s, H-7^{IV}), 6.77 (1H, t, *J* 1.6, H-2^{IV}), 6.55 (1H, ddd, *J* 8.2, 2.3, 0.9, H-6^{I}), 6.52 – 6.46 (2H, m, H-4^{IV} and H-6^{IV}), 6.40 (1H, s, H-7),

6.08 – 6.00 (2H, m, H-2^{I} and H-4^{I}), 4.69 (1H, d, *J* 12.3, H-15^{IIIa}), 4.65 (1H, d, *J* 12.3, H-15^{IIIb}), 3.80 (2H, t, *J* 6.6, H-1^{II}), 3.74 – 3.57 (6H, m, H-10^{III}, H-12^{III} and H-13^{III}), 3.56 – 3.49 (2H, m, H-9^{III}), 3.42 (3H, s, H-8), 3.24 (3H, s, H-9), 2.32 (3H, s, H-7^{V}), 2.18 (3H, s, H-6^{V}), 1.81 – 1.71 (2H, m, H-2^{II}), 1.01 (3H, t, *J* 7.4, H-3^{II}); ^{13}C NMR (151 MHz, $CDCl_3$) δ_C 166.0 (C-7^{III}), 165.6 (C-5^{V}), 160.6 (C-3^{I}), 158.5 (C-3^{V}), 158.1 (C-1^{I}), 158.0 (C-1^{IV}), 155.0 (C-2), 150.1 (C-2^{VI}), 149.3 (C-6^{VI}), 145.2 (C-16^{III}), 143.7 (C-6), 139.5 (C-1^{III}), 139.0 (C-3^{VI}), 136.0 (C-5^{VI}), 135.3 (C-3^{III}), 133.8 (C-5^{IV}), 133.1 (C-3^{IV}), 132.5 (C-4^{III}), 130.5 (C-5^{I}), 130.1 (C-6^{III}), 129.5 (C-5^{III}), 128.7 (C-7a), 126.6 (C-3a), 125.4 (C-2^{III}), 123.9 (C-4^{VI}), 123.1 (C-17^{III}), 121.5 (C-5), 120.4 (C-4^{IV}), 117.2 (C-2^{IV}), 115.9 (C-4^{V}), 113.8 (C-6^{IV}), 109.5 (C-4^{I}), 109.4 (C-6^{I}), 105.1 (C-4), 104.4 (C-2^{I}), 99.5 (C-7), 70.4, 70.0, 69.8 (C-10^{III}, C-12^{III} and C-13^{III}), 69.7 (C-1^{II}), 65.9 (C-7^{IV}), 64.5 (C-15^{III}), 40.3 (C-9^{III}), 27.7 (C-8), 27.5 (C-9), 22.7 (C-2^{II}), 11.8 (C-7^{V}), 11.0 (C-6^{V}), 10.7 (C-3^{II}); *m/z* (ESI^+) 958 ($[M+H]^+$, 100%), 980 ($[M+Na]^+$, 80%); HRMS *m/z* (ESI^+) $C_{49}H_{52}O_{10}N_9S^+$ ($[M+H]^+$) requires 958.3552, found 958.3547; HPLC (method 1) retention time 8.31 min, 96.9%.

7.3 General Biology Methods

7.3.1 pH Measurements

The pH of buffers was monitored using an Oakton PH 550 Benchtop pH Meter Kit. Calibration was performed using standard solutions provided by Oakton at pH 4.0 (water, potassium acid phthalate, cetylpyridinium chloride and methyl red solution), pH 7.0 (water, potassium dihydrogen phosphate, cetylpyridinium chloride, sodium hydrogen phosphate, methyl orange solution) and pH 10.0 (water, sodium carbonate, sodium bicarbonate, thymol blue solution). Electrodes were stored at pH 4.0 in a phthalate buffer solution.

7.3.2 Concentration of Protein Measurement

Concentration of protein samples were determined using the Beer-Lambert law, using the extinction coefficients as follows: TRIM24, $\varepsilon = 25160\ M^{-1}cm^{-1}$; TRIM33α, $\varepsilon = 26650\ M^{-1}cm^{-1}$; TRIM33β, $\varepsilon = 25160\ M^{-1}cm^{-1}$). Absorbance was measured using Thermo Scientific Nanodrop Lite at 280 nm. Molecular weights and molar extinction coefficients of the proteins were calculated using ProtParam on the ExPASy Bioinformatics Resource Portal.

7.3.3 Dose Response AlphaScreen™ Experimental

The peptides (**Table 7.1**) were supplied at >95% purity by either Cambridge Biochemicals or GenScript and were stored as either 1 mM or 100 μM stocks in Milli-Q water at –80 °C.

Table 7.1. Amino acid sequences of peptides used in AlphaScreen™.

Peptide	Amino Acid Sequence
H3K9Me$_3$ (H3PHD)	H_2N-ARTKQTARK(Me3)STGGKAPRKQLATKAARKY-CO_2H
H3K18Ac (H3BRD)	H_2N-ARTKQTARKSTGGKAPRK(Ac)QLATKAARKY-CO_2H
H3K9Me$_3$K18Ac (H3DUAL)	H_2N-ARTKQTARK(Me3)STGGKAPRK(Ac)QLATKAARKY-CO_2H
H4KAc$_4$	H_2N-YSGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RHRK-$CONH_2$

The assay buffer (**Table 7.2**, pH 7.6) was filtered through a 0.22 μm filter before use. The buffer is stored at 4 ºC as a 5× solution without bovine serum albumin (BSA), which was added on the day of use. For the incubation steps, the plates were sealed, shaken for 10 seconds at 600 rpm on a plate oscillator, and stored at room temperature in darkness. The compounds, proteins, and

peptides were dispensed into a ProxiPlate-384 Plus (Perkin Elmer) using a Thermo-Fisher electronic multi-channel pipette. Plates were read using a Synergy™ 2 MultiMode Microplate Reader using the inbuilt AlphaScreen™ 384 ProxiPlate function: excitation 680 nm, 0.18 sec; emission 570 nm, 0.37 sec. Ni^{2+} chelate acceptor and streptavidin donor beads were prepared as a mixture in a 1:300 dilution (0.007 mg/mL final assay concentration).

Table 7.2. Composition of assay buffer used in AlphaScreen™ experiments, adjusted to pH 7.6 using an aqueous NaOH solution.

	Composition	**Mass (mg) for 50 mL**
HEPES	25 mM	297.9
NaCl	100 mM	292.2
CHAPS	0.05% (w/v)	25
BSA	0.1% (w/v)	50

50 mM DMSO stock solutions of the compounds were serially diluted, and dispensed into wells (5 μL). The protein-peptide mixes (7 μL) for each combination were prepared following the specified final assay concentration (**Table 7.3**), and dispensed into wells. Assay beads (8 μL) were added to attain a final assay volume of 20 μL. Dose response curves were obtained for each compound in triplicate, with serial 1:2 dilutions. Data was processed by fitting a four-parameter equation to calculate IC_{50} values using Prism software.

Table 7.3. Final assay concentrations of each peptide-protein combination. $H3K9Me_3$ is a methylated peptide which binds to the PHD of the given protein, H3K18Ac is an acetylated peptide which binds to the BRD, and $H3K9Me_3K18Ac$ is a dual acetylated and methylated peptide that binds to both the BRD and the PHD.

Protein	**Peptide**	**Protein FAC (nM)**	**Peptide FAC (nM)**
TRIM24	$H3K9Me_3$ (H3PHD)	100	4
	H3K18Ac (H3BRD)	25	8
	$H3K9Me_3K18Ac$ (H3DUAL)	25	4
TRIM33α	$H3K9Me_3$ (H3PHD)	500	15
	H3K18Ac (H3BRD)	500	15
	$H3K9Me_3K18Ac$ (H3DUAL)	250	8
TRIM33β	$H3K9Me_3$ (H3PHD)	500	8
	H3K18Ac (H3BRD)	125	15
	$H3K9Me_3K18Ac$ (H3DUAL)	125	8

7.3.4 AlphaScreen™ TruHits Experimental

50 mM DMSO stock solutions of the compounds were serially diluted 1:2 from 500 μM and dispensed into wells (5 μL). For the first method, 15 μL of the provided assay beads were preincubated for 30 min, added to the plate using a Thermo-Fisher electronic multi-channel pipette, and subsequently incubated for 10 min. For the second method, the compounds were incubated with 7.5 μL of streptavidin-coated donor beads for 30 min. 7.5 μL of biotinylated acceptor beads were added, and the plate incubated for 30 min. The plate was read using a SynergyTM 2 MultiMode Microplate Reader, and processed by calculating the percentage decrease in emission intensity with respect to the average DMSO control.

7.3.5 NMR WaterLOGSY Experimental

Proteins were buffer exchanged from HEPES storage buffer into 10 mM Na/K phosphate buffer (0.9% NaCl, pH 7.6) using GE Healthcare PD SpinTrap G-25 columns. Protein concentration was measured using Thermo Scientific Nanodrop Lite (TRIM24, ε = 25160 M^{-1} cm^{-1}; TRIM33α, ε = 26650 M^{-1} cm^{-1}; TRIM33β, ε = 25160 M^{-1} cm^{-1}). For each compound, a water-suppressed ^{1}H NMR spectrum was recorded as a reference, followed by a WaterLOGSY spectrum of the compound in the absence of protein, and then another WaterLOGSY spectrum in the presence of the specified protein. Samples were prepared to a total volume of 160 μL in Invitrogen 10 mM Na/K phosphate buffer (0.9% NaCl pH 7.6), with 500 μM compound from 50 mM DMSO-d_6 stock solutions, 10% D_2O for signal locking, and 20 μM protein for samples with protein. If the compounds are seen to form soluble aggregates in the initial ^{1}H NMR spectrum, the compound and protein concentration were decreased. After loading into 3 mm Hilgenberg NMR tubes for Bruker Match™ system, samples were briefly centrifuged in a manual benchtop rotor. Spectra were recorded on a Bruker AVIII 700 MHz spectrometer with inverse TCI cryoprobe using a standard WaterLOGSY pulse sequence.

7.3.6 Isothermal Titration Calorimetry (ITC)

Experiments were performed using a MicroCal PEAQ-ITC Automated (Malvern) and analysed using the MicroCal PEAQ-ITC Analysis software (Malvern 1.1.0.1262) using the single binding site model. The buffer was made up as in **Table 7.4**, and was subsequently filtered through a 0.2 μM filter paper (Sartorius UK) before use. Proteins and peptides were dialysed to provide a buffer match at 4 °C overnight using a Slide-A-Lyzer™ Mini Dialysis Device (2K MWCO, Thermo Fisher Scientific) into the ITC HEPES buffer, with 0.2% DMSO. The proteins were then centrifuged for 3 min at 3000 rpm to remove any aggregates. Concentrations were determined by measuring the absorbance at 280 nM using a Thermo Scientific Nanodrop Lite. 50 mM DMSO stock solution of the required compounds were diluted to the appropriate concentration using the dialysis buffer. The cell temperature was maintained at 25 ºC, and was stirred at 750 rpm, with the reference power set to 5 μcals^{-1}. After an initial 60 s delay, 20 × 2 μL injections (first injection 0.4 μL) were completed with pauses of 150 s. Heats of dilutions were measured under the same conditions and subtracted during analysis. The compound solutions were in the calorimetric cell (250 μL, 40 μM, 0.2% DMSO), and were titrated with the protein solutions in the syringe (60 μL, 363 μM).

Table 7.4. Composition of ITC buffer, adjusted to pH 7.6.

	Composition	**Mass (g) for 1 L**
HEPES	50 mM	11.9
NaCl	150 mM	8.77

7.3.7 Protein Production

Plasmids

Dr Angelina Sekirnik provided the pNIC28-Bsa4 TRIM24, pNIC28-Bsa4 TRIM33α and pNIC28-Bsa4 TRIM33β plasmid constructs used for protein production (**Figure 7.1**).

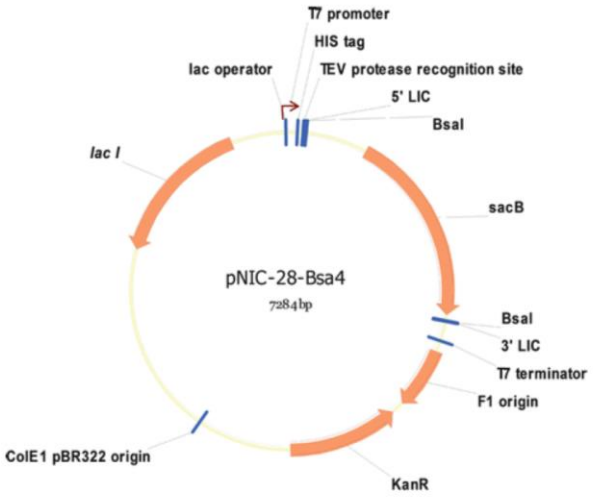


Figure 7.1. Plasmid map.

Bacterial transformation

Escherichia Coli BL21 (DE3) competent cells were thawed on ice, followed by the addition of plasmid DNA for the specific protein. This was flicked 10 times to allow mixing and then was incubated on ice for 20 minutes. The cells were then placed in 42 °C water bath for 30 seconds then returned to ice for 5 minutes. 500 µL SOC outgrowth media was added and the cells were incubated at 37 °C for 1 hour. The bacteria (10 µL and 40 µL) were then streaked on solid 2× TY medium (**Table 7.5**) with 50 µgmL^{-1} kanamycin under aseptic conditions using sterile equipment. The plate cultures were inverted to prevent condensation reaching the agar and grown overnight at 37 °C, and subsequently stored at 4 °C.

Table 7.5. Composition of 2× TY media.

Media	Reagent	Amount required (g)
2× TY (1 L)	Tryptone	16.0
	Yeast extract	10.0
	NaCl	5.0
	Agar bacteriological (Oxoid Ltd)	15.0

Protein expression

All media were autoclaved before use using an *Thermo Live Sciences* MAT90 LEI autoclave at 121 °C. Small growths for starter cultures (100 mL of 2× TY media containing 50 µgmL^{-1} kanamycin in 500 mL flask), were inoculated with one colony from an agar plate and incubated

at 37 °C for 16 h. Large-scale recombinant protein expression was performed using 2000 mL PYREX® narrow-mouth baffled Tunair™ flasks containing 1000 mL of 2× TY medium containing 50 μgmL^{-1} kanamycin. Flasks were inoculated with 1% by volume (10 mL) of the bacterial starter culture and grown at 37 °C (180 r.p.m.) until they reached an OD_{600} of 1.0. Optical density readings were taken in 1.0 mL cuvettes with 2× dilution in 2× TY against a reference sample of the 2× TY growth media. The absorbance was measured using a Novaspec® II spectrophotometer at 600 nm. After reaching the desired optical density, cultures were equilibrated to 16 °C and induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) (0.1 mM) and $ZnCl_2$ (0.2 mM), before incubation for 16 h (180 r.p.m.). 21 hours after the IPTG induction, cultures were centrifuged at 11325 ×g, 4 °C for 5 minutes using an Avanti™ J-25 centrifuge with a Beckman F10-6 × 500y rotor, and the resulting bacterial pellet was weighed and frozen in a resealable plastic bag at –80 °C.

7.3.8 Protein Purification

Table 7.6. Composition of IMAC and SEC buffers.

Reagents	**IMAC buffers (pH 7.6)**			**SEC buffer (pH 7.6)**
	Binding	**Elution**	**Strip**	**Gel filtration**
HEPES	50 mM	50 mM	50 mM	50 mM
NaCl	500 mM	500 mM	500 mM	500 mM
Glycerol	10% *v/v*	10% *v/v*	–	–
Imidazole	–	500 mM	–	–
EDTA	–	–	50 mM	–

All buffers (**Table 7.6**) were stored at 4 °C and filtered under vacuum through a 0.2 μm filter paper. Cell pellets were resuspended in Extraction Buffer (1 Sigma*FAST*™ protease inhibitor cocktail tablet EDTA free (Sigma Aldrich) dissolved in 100 mL Binding Buffer), at a ratio of 100 mL per 20 g cell mass. 10 μL Benzonase® nuclease (Novagen®) per 7 mL was added, and the cells were sonicated (50% amplitude), thirty cycles of 5 second bursts interrupted by 5 second breaks (Fisherbrand™ Q500 Sonicator). Polyethyleneimine (Sigma Aldrich) (0.15% *v/v*) was added from a 5% (*v/v*) stock and the mixture was incubated on ice for 15 minutes to precipitate

DNA. The lysate was centrifuged (25000 ×g, 4 ºC for 15 minutes), the supernatant was collected and subsequently filtered through a 0.45 μm syringe filter. The filtrate was diluted with Binding Buffer to 100 mL, and then 10 mM β-mercaptoethanol (β-ME) (Bio-Rad Laboratories) and 20 mM imidazole were added.

The protein was purified by Immobilised Metal Affinity Chromatography (IMAC) followed by size-exclusion chromatography (SEC) operated through a computerised ÄKTA fast protein liquid chromatography (ÄKTAFPLC) system (GE Healthcare), all performed in a room set at 4 ºC. IMAC was performed with a HisTrap™ HP 5 mL column (GE Healthcare). The column was rinsed with 50 mL (10 column volumes) Milli-Q water, followed by 10 mL (2 column volumes) of 0.1 M $NiSO_4$. The column was then equilibrated with 50 mL (10 column volumes) of Binding Buffer (containing 10 mM β-ME) before loading the cell lysate at 1 mL/min. The column was then washed with a gradient of 50 mL (10 column volumes) 9% Elution Buffer in Binding Buffer (45 mM imidazole) and 50 mL (10 column volumes) 12% Elution Buffer in Binding Buffer (60 mM imidazole), then 20 mL (4 column volumes) 60% Elution Buffer in Binding Buffer (300 mM imidazole) followed by 10 mL (2 column volumes) 100% Elution Buffer (500 mM imidazole). The protein was collected in 2 mL fractions. 50 mL (10 column volumes) Milli-Q water was used to wash the column, and then 50 mL (10 column volumes) of Strip Buffer was used to strip the bound Ni^{2+} ions off the affinity column. The fractions containing the protein were collected.

Further purification was completed using Size Exclusion Chromatography (SEC). The protein fractions from the IMAC purification were concentrated using Corning® Spin-X® UF Centrifugal Concentrator, 10k MWCO in a Beckman Coulter Allegra X-12R benchtop centrifuge at 3270 ×g, 4 °C until it reached 6 mL. 2 mL of the concentrated protein was loaded onto a gel filtration column XK 16/70 (GE Healthcare) packed with 120 mL Superdex 75 resin (GE Healthcare) (previously rinsed with 150 mL degassed Milli-Q water and equilibrated with 150 mL degassed gel filtration buffer). Protein elution occurred with 150 mL degassed gel filtration buffer with a flow rate of 1 mL/min and collected in 5 mL fractions. Fractions containing pure protein

(determined from the SEC trace) were spin concentrated using Corning® Spin-X® UF Centrifugal Concentrator, 10k MWCO in a Beckman Coulter Allegra X-12R benchtop centrifuge at 3270 ×g, 4 °C then aliquoted, frozen and stored at –80 °C. Protein mass spectrometry was carried out on a Waters LCT Premier mass spectrometer diluted to 0.02 mg/mL and 0.1 mg/mL with methanol or gel filtration buffer.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

Table 7.7. Gel compositions (volumes shown calculated for 2 gels).

Reagents	Resolving Gel (16%)	Stacking Gel (4%)
Milli-Q water	2.1 mL	2.9 mL
1.5 M Tris-HCl, pH 8.8	2.5 mL	–
0.5 M Tris-HCl, pH 6.8	–	1.3 mL
30% Acrylamide/bis	5.3 mL	0.7 mL
10% Sodium dodecyl sulfate (SDS)	100 µL	50 µL
TEMED	8 µL	5 µL
10% APS	100 µL	50 µL

Protein purity was determined using SDS-PAGE 16% (**Table 7.7**) with Coomassie Blue staining. Gels were prepared using 70 mm × 100 mm glass plates, with 0.75 mm spacers. TEMED and 10% ammonium persulfate (APS) were added just before casting gels. The resolving gel also contained a layer of methanol before casting. The methanol was removed after polymerization of the gel and the boundary was rinsed with Milli-Q water before casting the stacking gel. For protein denaturation, samples were prepared by mixing with Laemmli sample loading buffer (4×, with addition of 10% β-ME), and incubating at 100 °C for 10 min. Gels were loaded with colour protein standard, broad range (NEB) molecular weight ladder then run on a mini-PROTEAN Tetra Electrophoresis System (Bio-Rad) at a constant potential of 100 V. Following electrophoresis, gels were stained for 15 minutes with InstantBlue™ Coomasie® stain (Expedeon) and rinsed with Milli-Q water then destained multiple times with destaining solution (40% methanol and 10% acetic acid in Milli-Q water).

7.3.9 LogD Determination Protocol at pH 7.40

Sodium phosphate buffer (10 mM, pH 7.40) was made up by adding 3.56 g $HNa_2O_4P.2H_2O$, followed by 17.54 g NaCl to 2 L Milli-Q water, and the pH was adjusted to 7.40 ± 0.02 using dilute NaOH or dilute HCl. This buffer was stored at 4 °C and kept for no longer than one month after the day of preparation. Octanol (250 mL) and sodium phosphate buffer (250 mL) were added to a 1 L Duran bottle and left for at least 12 h to allow phase separation.

10 mM test compounds in DMSO were added to a 384 well plate with a positive control with a known LogD value. The plates were centrifuged at 1000 rpm for 2 mins prior to placing on the deck of a Hamilton STAR robot. LCMS optimisation runs are performed to ensure compounds can be recognised. 750 µL buffer was added to a new assay plate, followed by 750 µL octanol. Test compounds were then spiked in by adding 1.5 µL of 10 mM DMSO stocks from the 384 well compound plates. Once complete, the plates were sealed using an Aglient Velocity II Thermal Microplate Sealer (seal time 1.5 seconds, temperature 180 °C), and attached to a Stuart rotator driver, set to rotate at 18–20 rpm for 20–24 hours at room temperature. The plates are then centrifuged for 10 min (4000 rpm, 20°C). 250 µL octanol from assay plates was transferred to fresh Corning 300 µL separation plates. The remaining octanol (top layer) was removed to expose buffer. 250 µL buffer was transferred to fresh Corning 300 µL separation plates. Compounds were combined in groups of four (that have very different masses), resulting in a 20.5 fold dilution, in a 2 mL NUNC plate. Initial compound dilution was further diluted 100 fold *via* 2× dilutions in firstly a 2 mL NUNC plate, then a 1 mL Waters analysis plate. These plates contained both replicates of Octanol and Buffer samples at both dilutions. LCMS was then used to determine concentration of compound in each layer.

Table 7.8. Concentrations and dilutions for the assay.

Reagent	Test compound
Stock Concentration	10 mM in DMSO
Assay Concentration	10 µM (1.5µL of 10 mM + 750µL of Octanol + 750µL of Aqueous buffer)
Initial dilution concentration (results in cassetting 4x compounds) **(A)**	40 µL of assay sample [Octanol OR Aqueous] + 120 µL assay samples + 660 µL of 1:1:1 H_2O:MeCN:MeOH 20.5× fold dilution of original assay samples
Intermediate dilution **(B)**	100 µL of Initial dilution (A) + 900 µL of 1:1:1 H_2O:MeCN:MeOH 205× fold dilution of original assay samples
Final dilution **(C)**	50 µL of Intermediate dilution (B) + 450 µL of 1:1:1 H_2O:MeCN:MeOH 2050× fold dilution of original assay samples

For following calculation is used for calculating LogD:

$$LogD = LOG10\frac{[Peak\ area\ in\ Buffer\ sample]}{[Peak\ area\ in\ Octanol\ sample]}$$

7.3.10 Cell Permeability Assay

These experiments were performed by Dr Hannah Bolland or Anna Mandel in the group of Professor Ester Hammond. RKO cells were seeded in a 6 cm dish. The cells were treated with 10 µM PROTAC overnight. The media was then removed, and X3 rinsed in ice cold PBS. The cells were scraped in 1 mL PBS and spun down for 5 mins (2000 rpm, 4°C). The supernatant was removed and the pellet was resuspended in 100 µL MeCN. This was then spun 13,000 xg, (15 min, 4 °C), and the supernatant was analysed by HPLC.

HPLC (Waters 2695 system) system used was comprised an RPB column (5 μm, 100 mm × 3.2 mm, 35 °C). Separation was achieved at a flow rate of 0.5 mL/min with a gradient of 5–95% acetonitrile in 0.1% aqueous trifluoroacetic acid over 12 minutes, returning to starting conditions over 0.1 minute. Detection used a photodiode array spectrophotometer (Waters 2996). Injections of 10 μL were made. Data was expressed as moles in cell extract, moles in a single cell and concentration in a single cell (assuming a volume of 1 pL).

7.3.11 Degradation Assessment

RKO cells were obtained from ATCC and cultured in RPMI supplemented with 10% FBS. Cells were seeded onto 6 well plates and treated with 10 μM of each PROTAC for 0, 2, 4, 6, 16, or 24 hours and harvested at the indicated time points in UTB lysis buffer. Samples were then sonicated, and protein quantified using the Nanodrop One system. 50 μg of protein was loaded for immunoblotting and samples were separated *via* electrophoresis on a 4–20% gradient gel. Proteins were transferred onto a nitrocellulose membrane using the BioRad Turbo Transfer System (BioRad). Membranes were blocked for 1 hour using Intercept (TBS) Blocking Buffer (LI-COR) and incubated with primary antibody overnight at 4 °C. Primary antibodies and the following dilutions were used in the study were TRIM33, 1:500 (A301-060A, Bethyl Laboratories), BRD4, 1:500, (Bethyl Laboratories, A301-985A100) and β-actin (Santa Cruz, sc69879). Secondary antibodies used were IR Dye 800CW goat anti-rabbit IgG (926-32211; LI-COR, Lincoln, Nebraska, USA), IR Dye 680RD goat anti-mouse IgG (926-68070; LI-COR, Lincoln, Nebraska, USA). Western blots were visualised using an Odyssey infrared imaging system (LI-COR, software version 3.0). Protein expression was quantified using the ImageStudio (LICOR) software, protein expression was normalised to the β-actin loading control and expressed as relative fold change over 0 hour time points. These experiments were performed by Dr Hannah Bolland in the group of Professor Ester Hammond.

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Appendices

Appendix A: Protein Characterisation

Table A.1. TRIM33α characterisation.

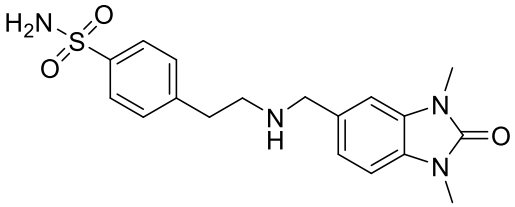
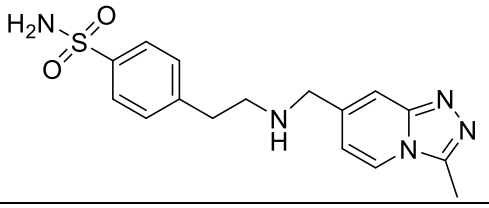
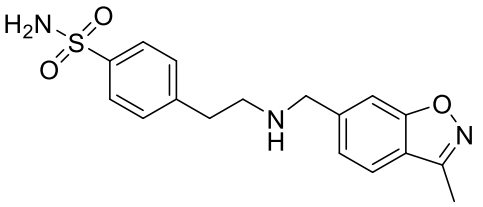
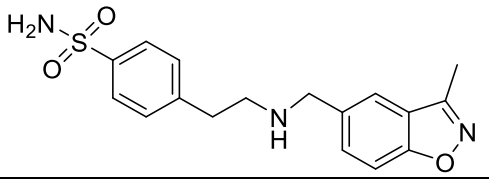
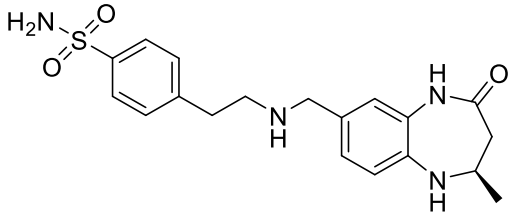
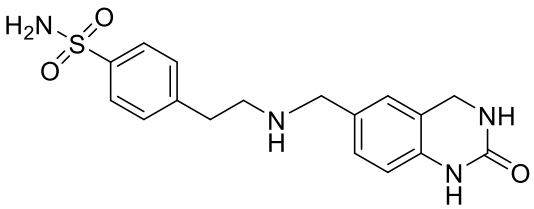
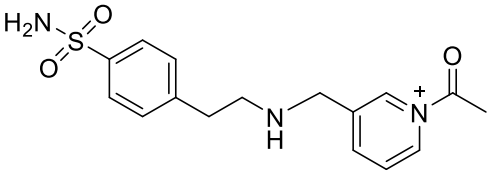
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Plasmid vector	pNIC28-Bsa4 (Kanamycin)
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Amino Acid sequence	MHHHHHHSSGVDLGTENLYFQSMDWCAVCQNGGDLLCCEKC PKVFHLTCHVPTLLSFPSGDWICTFCRDIGKPEVEYDCDNLQHS KKGKTAQGLSPVDQRKCERLLLYLYCHELSIEFQEPVPASIPNY YKIIKKPMDLSTVKKKLQKKHSQHYQIPDDFVADVRLIFKNCE RFNEMMKVVQVYADTQEINLKADSEVAQAGKAVALYFEDKL TEIYSDRTFAPLPEFEQEEDDGEVTEDSDEDFIQPRRKRLKSDER PVHIK
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ε (M^{-1} cm^{-1})	26650

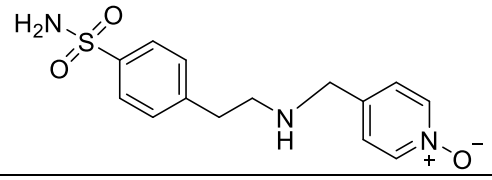
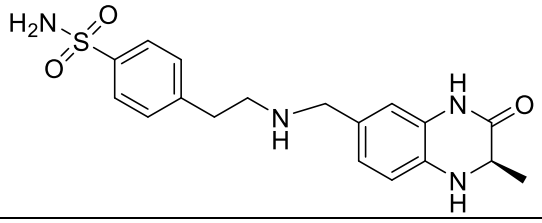
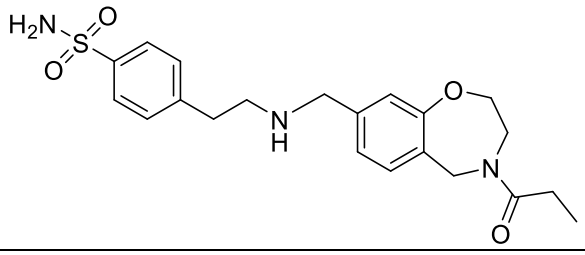
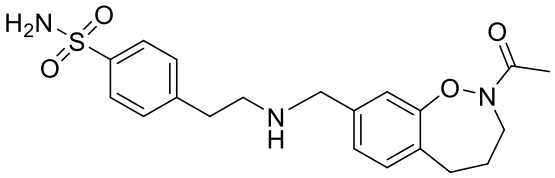
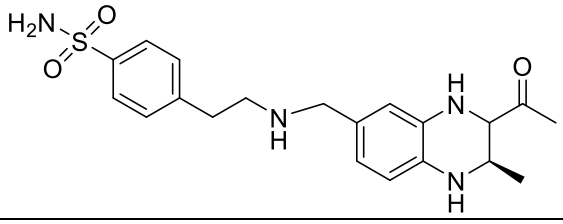
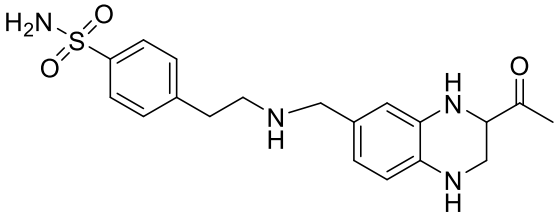
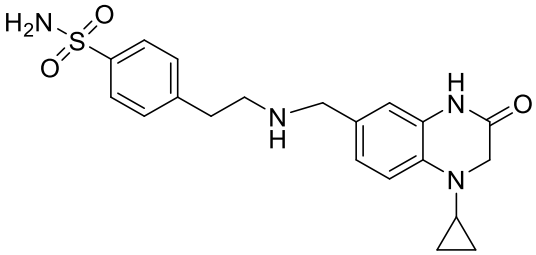
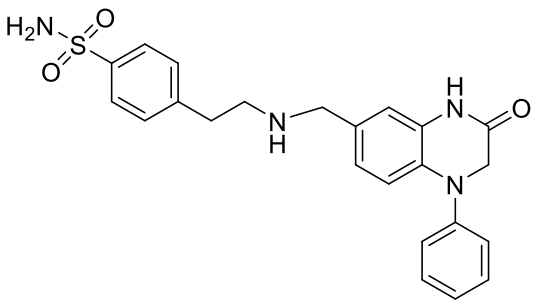
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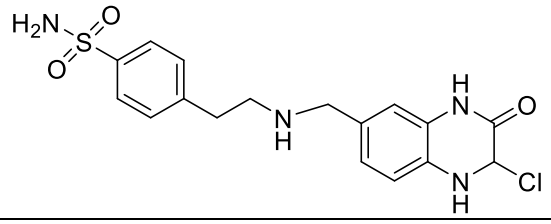
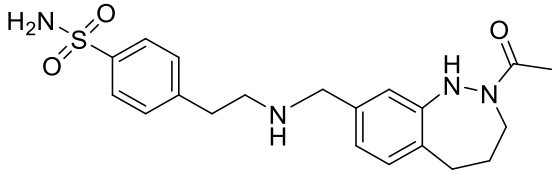
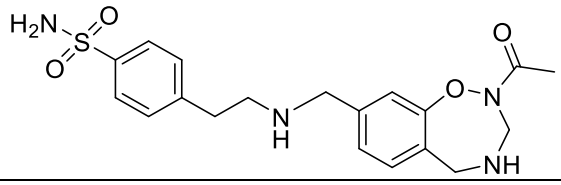
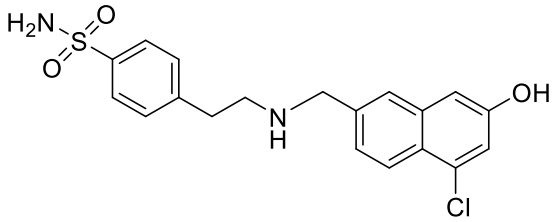
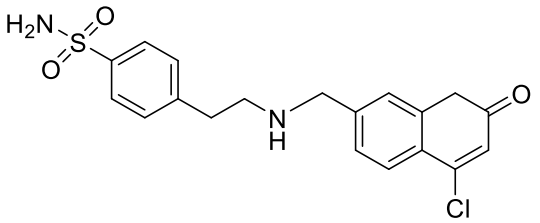
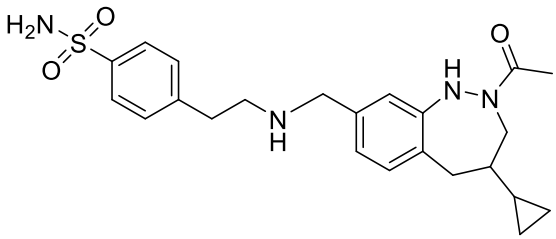
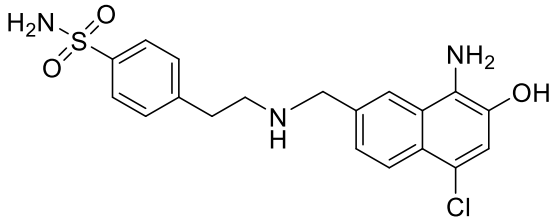
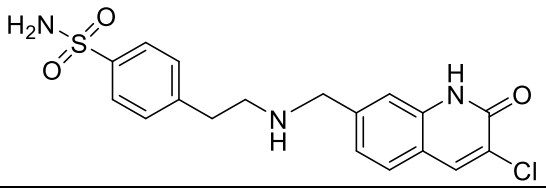
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ε (M^{-1} cm^{-1})	25160

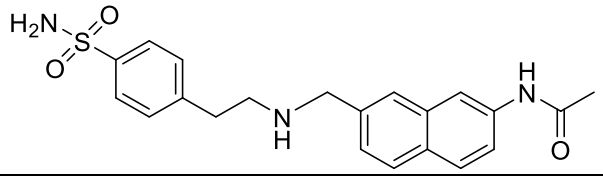
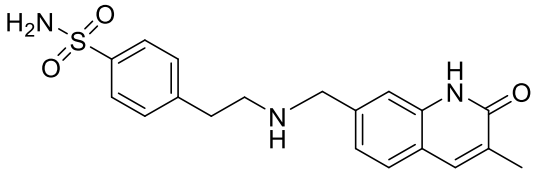
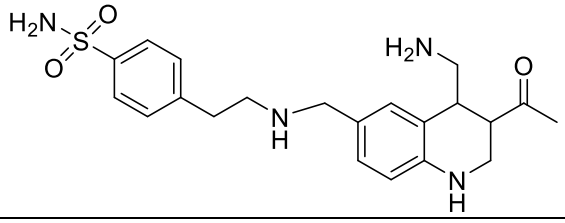
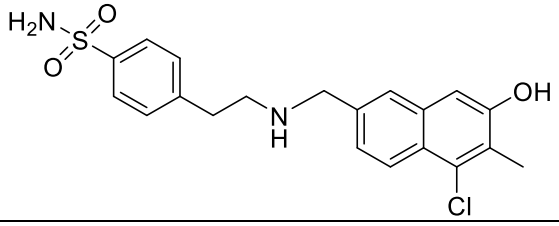
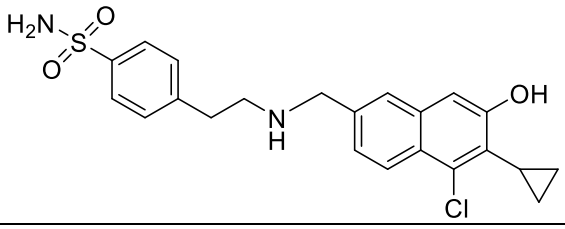
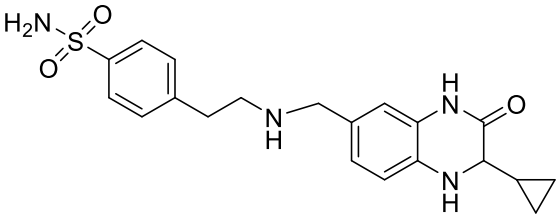
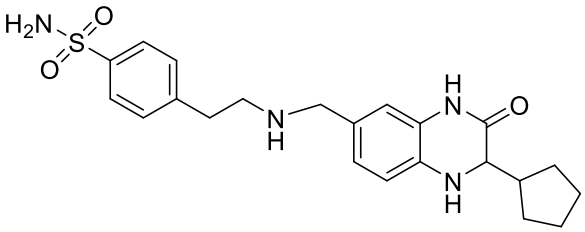
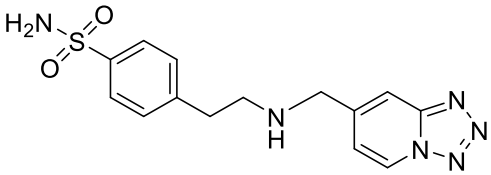
Appendix B: Docking Data (Chapter 2)

Table B.1. Structures of potential KAc mimicking head groups, docked using CLC Drug Discovery Workbench. LogD at pH 7.4, number of aromatic rings, solubility forecast index (SFI) and docking scores were generated using CLC Drug Discovery Workbench Version 3.0, (https://www.qiagenbioinformatics.com/), using the X-ray crystal structure of TRIM33β (PDB ID: **5MR8**). Those highlighted in red were selected for synthesis based on docking score and visual analysis of binding mode.

Number	Compound	LogD (7.4)	Aromatic rings	SFI	Docking Score
1		−0.28	2	1.72	−65.73
2		−1.17	2	0.83	−54.00
3		−0.24	2	1.76	−49.68
4		−0.26	2	1.74	−60.41
5		−0.53	2	1.47	−54.22
6		−0.82	2	1.18	−52.02
7		−4.2	2	-2.2	−54.40

8		−1.73	2	0.27	−53.06
9		−0.61	2	1.39	−55.56
10		−0.09	2	1.91	−50.38
11		−0.16	2	1.84	No binding
12		−0.07	2	1.93	−54.64
13		−0.72	2	1.28	−55.14
14		−1.14	2	0.86	−66.98
15		−0.07	2	1.93	−62.81

16		1.07	2	3.07	−53.94
17		0.96	2	2.96	−54.08
18		−0.06	2	1.94	No binding
19		−0.96	2	1.04	−63.29
20		1.54	3	4.54	−60.05
21		0.85	2	2.85	−58.04
22		0.58	2	2.58	−66.46
23		0.58	3	3.58	−56.11

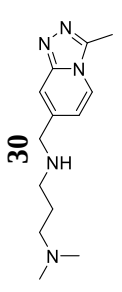
24		0.57	2	2.57	−58.95
25		0.29	3	3.29	−55.06
26		0.29	2	2.29	−55.49
27		−3.31	2	1.31	−56.25
28		1.95	3	4.95	−61.77
29		2.21	3	5.21	−58.36
30		−0.18	2	1.82	−54.51
31		0.71	2	2.71	−69.03

Appendix C: AlphaScreen™ Data (Chapter 2)

Table C.1. Dose-response AlphaScreen™ IC_{50} values of compounds **29** and **30** for all nine protein-peptide combinations, corresponding to **Table 2.3**. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. PHD refers to peptide H3K9Me$_3$, BRD refers to peptide H3K18Ac, and DUAL refers to peptide H3K9Me$_3$K18Ac. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

Weaker binding — **Stronger binding**

AlphaScreen™ IC_{50} (μM)			Compound 30	Compound 29
TRIM24	PHD		>250	>250
	BRD		>250	>250
	DUAL		>250	>250
TRIM33α	PHD		>250	>250
	BRD		>250	>250
	DUAL		>250	>250
TRIM33β	PHD		>250	>250
	BRD		>250	>250
	DUAL		>250	>250



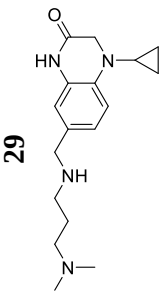


Table C.2. Dose-response AlphaScreen™ IC_{50} values of compounds **44–46** for all nine protein-peptide combinations, corresponding to **Table 2.4**. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. PHD refers to peptide H3K9Me_3, BRD refers to peptide H3K18Ac, and DUAL refers to peptide H3K9Me_3K18Ac. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

Weaker binding — **Stronger binding**

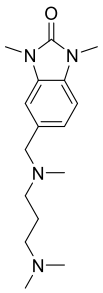
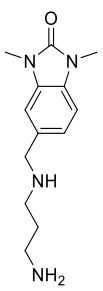
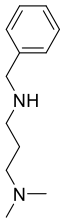
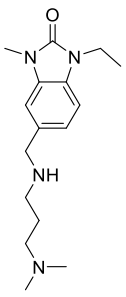
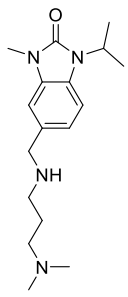
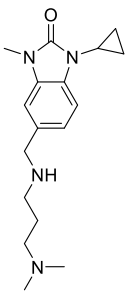
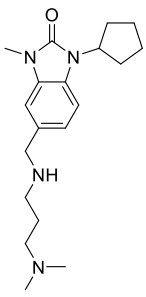
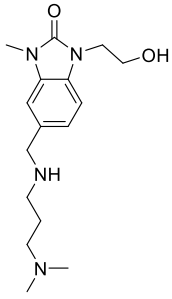
Compound	AlphaScreen™ IC_{50} (μM)									
	TRIM24			TRIM33α			TRIM33β			
	PHD	BRD	DUAL	PHD	BRD	DUAL	PHD	BRD	DUAL	
45 	>250	>250	>250	9.42 ± 1.14	5.69 ± 0.33	12.8 ± 1.32	15.5 ± 1.79	23.5 ± 2.80	>250	
46 	>250	>250	>250	>250	3.73 ± 0.38	>250	7.71 ± 0.79	13.8 ± 1.00	37.5 ± 8.01	
44 	>250	>250	>250	>250	>250	>250	>250	>250	>250	

Table C.3. Dose-response AlphaScreen™ IC_{50} values of compounds **47–53** for all nine protein-peptide combinations, corresponding to **Table 2.6**. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. PHD refers to peptide H3K9Me$_3$, BRD refers to peptide H3K18Ac, and DUAL refers to peptide H3K9Me$_3$K18Ac. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

Weaker binding — **Stronger binding**

Compound	AlphaScreen™ IC_{50} (μM)									
	TRIM24			TRIM33α			TRIM33β		
	PHD	BRD	DUAL	PHD	BRD	DUAL	PHD	BRD	DUAL
47 	>250	>250	>250	>250	2.07 ± 0.20	5.99 ± 0.41	10.5 ± 1.31	14.7 ± 1.47	>250
48 	>250	>250	>250	>250	3.22 ± 0.25	7.47 ± 0.59	15.6 ± 2.71	19.6 ± 1.64	>250
49 	>250	123 ± 32.2	155 ± 36.7	9.51 ± 1.04	9.01 ± 1.66	22.1 ± 3.59	81.3 ± 16.6	96.0 ± 24.9	>250
50 	>250	>250	>250	7.83 ± 2.16	4.32 ± 0.35	15.6 ± 1.93	58.9 ± 20.3	60.2 ± 7.42	>250
51 	>250	>250	>250	>250	8.74 ± 0.82	29.6 ± 3.22	14.4 ± 2.93	20.6 ± 1.94	>250

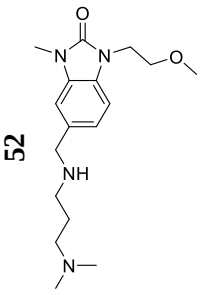
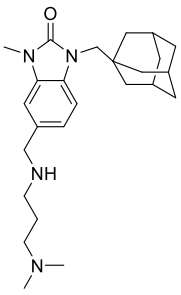
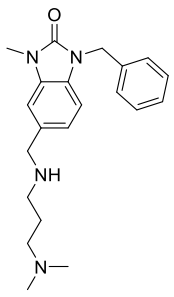
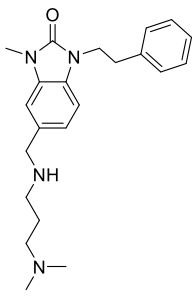
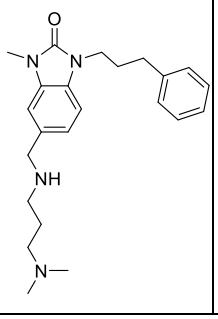
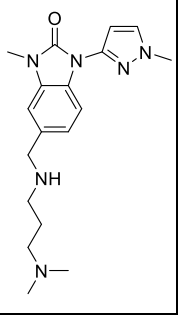
52 	>250	>250	>250	>250	16.4 ± 1.08	28.0 ± 8.35	>250	>250
53 	>250	>250	>250	>250	250	23.8 ± 4.58	>250	>250

Table C.4. Dose-response AlphaScreen™ IC_{50} values of compounds **54–61** for all nine protein-peptide combinations, corresponding to **Table 2.7**. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. PHD refers to peptide H3K9Me$_3$, BRD refers to peptide H3K18Ac, and DUAL refers to peptide H3K9Me$_3$K18Ac. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

Weaker binding — **Stronger binding**

Compound	AlphaScreen™ IC_{50} (μM)									
	TRIM24			TRIM33α			TRIM33β			
	PHD	BRD	DUAL	PHD	BRD	DUAL	PHD	BRD	DUAL	
54 	>250	>250	>250	2.39 ± 1.20	3.64 ± 1.11	5.77 ± 1.10	35.7 ± 1.12	86.0 ± 1.15	>250	
55 	>250	>250	>250	2.81 ± 1.19	4.34 ± 1.08	6.07 ± 1.10	19.2 ± 1.30	59.7 ± 1.12	150 ± 1.26	
56 	>250	>250	>250	0.710 ± 2.56	2.47 ± 1.08	3.89 ± 1.05	27.8 ± 1.14	100 ± 1.10	>250	
61 	>250	>250	>250	>250	>250	>250	>250	>250	>250	

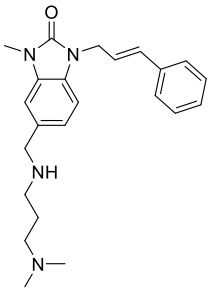
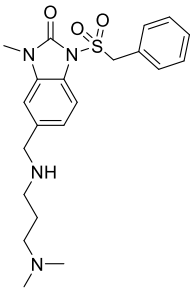
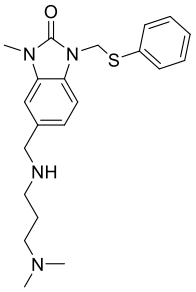
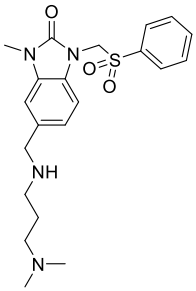
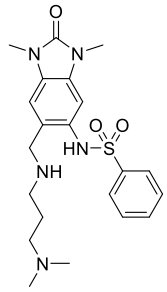
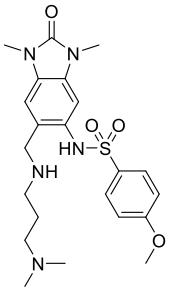
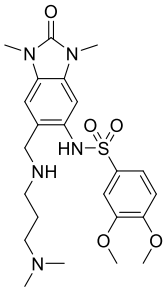
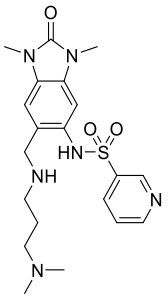
57 	>250	>250	2.01 ± 0.24	5.94 ± 0.61	>250	53.3 ± 7.89	>250	>250
58 	>250	>250	>250	>250	>250	>250	>250	>250
59 	>250	>250	>250	1.42 ± 0.13	3.62 ± 0.24	>250	>250	>250
60 	>250	>250	>250	>250	>250	>250	>250	>250

Table C.5. Dose-response AlphaScreen™ IC_{50} values of compounds **76–81** for all nine protein-peptide combinations, corresponding to **Table 2.8**. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. PHD refers to peptide H3K9Me$_3$, BRD refers to peptide H3K18Ac, and DUAL refers to peptide H3K9Me$_3$K18Ac. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

Weaker binding — **Stronger binding**

Compound	AlphaScreen™ IC_{50} (μM)									
	TRIM24			TRIM33α			TRIM33β			
	PHD	BRD	DUAL	PHD	BRD	DUAL	PHD	BRD	DUAL	
77 	>250	>250	>250	>250	21.5 ± 3.50	56.3 ± 10.7	>250	>250	>250	
78 	>250	>250	>250	>250	12.7 ± 1.42	42.5 ± 6.56	>250	77.7 ± 13.9	>250	
76 	>250	>250	>250	>250	71.7 ± 7.02	150	>250	>250	>250	
79 	>250	>250	>250	>250	11.2 ± 2.10	63.1 ± 21.7	>250	>250	>250	

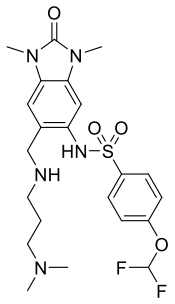
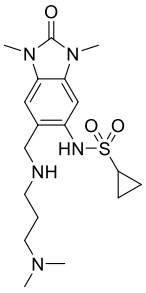
80		>250	>250	>250	>250	9.52 ± 1.09	26.3 ± 3.42	>250	>250	>250
81		>250	>250	>250	>250	4.20 ± 0.48	16.8 ± 4.08	>250	>250	>250

Table C.6. Dose-response AlphaScreen™ IC_{50} values of compounds **92** and **94** for all nine protein-peptide combinations, corresponding to **Table 2.9**. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. PHD refers to peptide H3K9Me$_3$, BRD refers to peptide H3K18Ac, and DUAL refers to peptide H3K9Me$_3$K18Ac. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

Weaker binding — **Stronger binding**

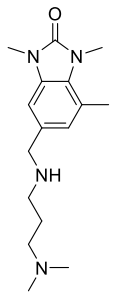
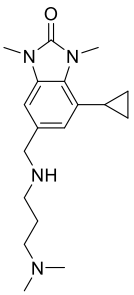
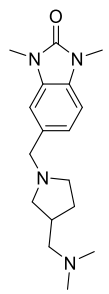
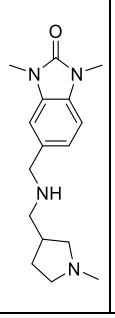
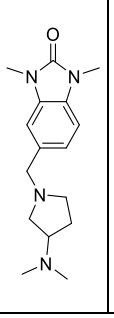
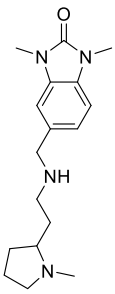
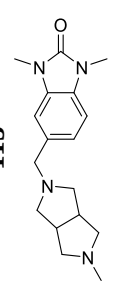
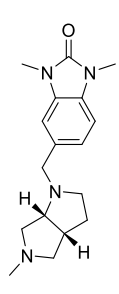
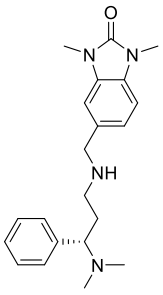
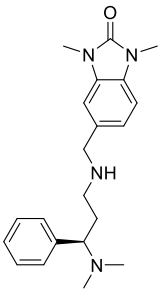
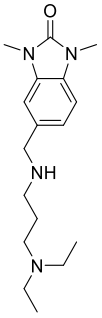
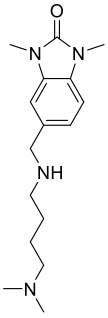
Compound	AlphaScreen™ IC_{50} (μM)									
	TRIM24			TRIM33α			TRIM33β			
	PHD	BRD	DUAL	PHD	BRD	DUAL	PHD	BRD	DUAL
92 	>250	>250	>250	>250	9.25 ± 1.34	8.03 ± 1.58	>250	29.0 ± 9.98	38.8 ± 7.76
94 	>250	>250	>250	>250	4.81 ± 0.90	7.19 ± 1.64	>250	32.5 ± 10.8	23.6 ± 6.37

Table C.7. Dose-response AlphaScreen™ IC_{50} values of compounds **111–125** for all nine protein-peptide combinations, corresponding to **Table 2.10**. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. PHD refers to peptide $H3K9Me_3$, BRD refers to peptide H3K18Ac, and DUAL refers to peptide $H3K9Me_3K18Ac$. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

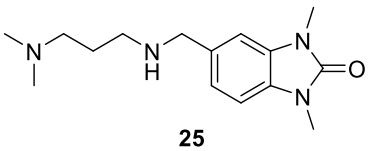
Weaker binding — **Stronger binding**

Compound	AlphaScreen™ IC_{50} (μM)									
	TRIM24			TRIM33α			TRIM33β			
	PHD	BRD	DUAL	PHD	BRD	DUAL	PHD	BRD	DUAL
111 	>250	>250	>250	11.9 ± 1.71	5.81 ± 0.21	11.6 ± 0.97	18.3 ± 4.38	15.2 ± 0.79	72.8 ± 30.1
112 	>250	>250	>250	2.84 ± 0.47	1.60 ± 0.14	4.58 ± 0.23	7.50 ± 0.54	8.53 ± 0.56	29.7 ± 3.37
113 	>250	>250	>250	>250	6.61 ± 0.54	15.2 ± 3.61	12.6 ± 1.04	12.8 ± 2.09	38.5 ± 17.3
114 	>250	>250	>250	4.97 ± 0.74	1.52 ± 0.10	2.29 ± 0.26	2.83 ± 0.20	1.62 ± 0.24	7.50 ± 0.54
115 	>250	>250	>250	43.0 ± 7.00	25.6 ± 6.82	70.3 ± 12.2	>250	100 ± 20.7	>250
116 	45.7 ± 5.04	44.3 ± 3.43	55.6 ± 7.54	4.51 ± 0.62	3.97 ± 0.86	16.2 ± 1.41	13.8 ± 3.74	12.3 ± 3.13	>250

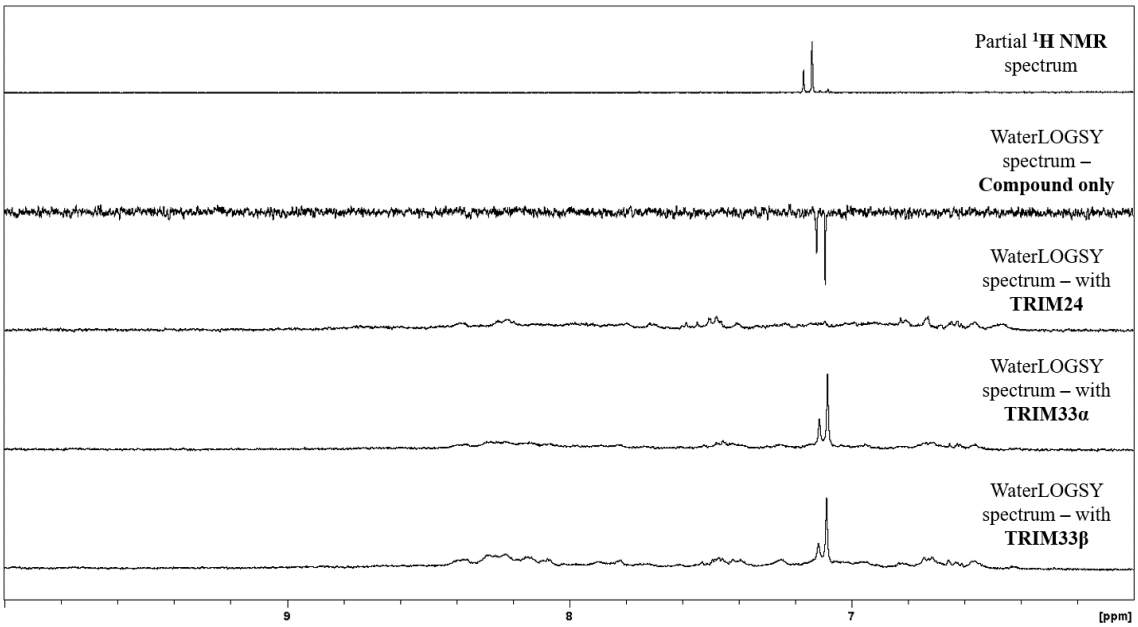
117	>250	>250	>250	7.91 ± 0.81	5.79 ± 0.84	10.3 ± 2.21	12.8 ± 3.04	9.57 ± 1.74	>250
118	>250	>250	>250	>250	19.8 ± 3.56	43.3 ± 11.7	51.6 ± 5.24	39.2 ± 8.41	>250
119	>250	>250	>250	6.56 ± 1.63	1.56 ± 0.10	3.61 ± 0.29	5.36 ± 0.50	4.83 ± 0.51	12.1 ± 1.39
120	>250	>250	>250	4.44 ± 0.65	2.45 ± 0.24	5.89 ± 0.65	9.21 ± 2.60	7.75 ± 0.60	>250
121	>250	>250	>250	1.97 ± 0.10	1.33 ± 0.06	2.70 ± 0.24	4.32 ± 1.25	4.52 ± 0.60	29.0 ± 2.69

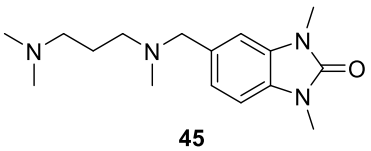
122 	>250	>250	>250	7.13 ± 0.68	4.31 ± 0.24	12.3 ± 1.85	21.6 ± 6.45	16.9 ± 2.25	>250
123 	>250	>250	>250	>250	5.56 ± 0.56	>250	15.8 ± 1.46	12.2 ± 1.55	105 ± 25.8
124 	>250	>250	>250	2.38 ± 1.12	4.74 ± 1.14	1.65 ± 1.08	1.58 ± 1.28	2.02 ±1.06	6.24 ± 1.04
125 	>250	>250	>250	4.21 ± 1.11	7.96 ± 1.11	2.39 ± 1.12	2.67 ± 1.22	2.73 ± 1.14	3.86 ± 1.07

Appendix D: WaterLOGSY Data (Chapter 2)

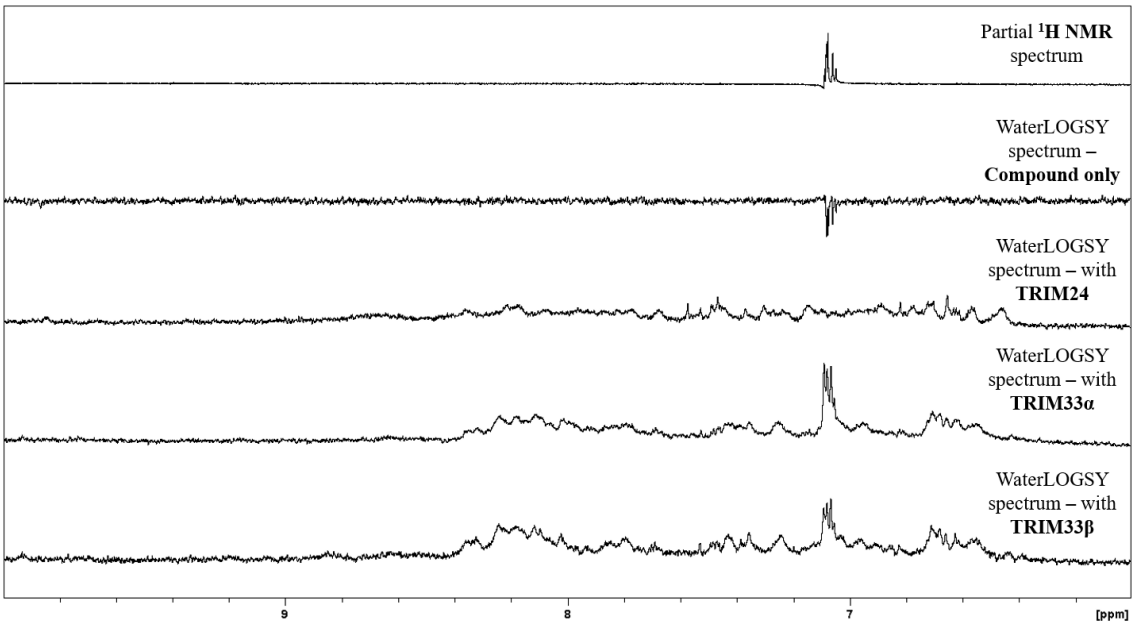


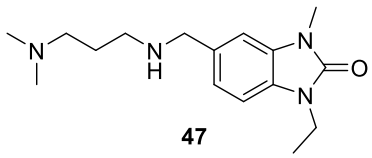
25


Partial ¹H NMR spectrum
WaterLOGSY spectrum – Compound only
WaterLOGSY spectrum – with TRIM24
WaterLOGSY spectrum – with TRIM33α
WaterLOGSY spectrum – with TRIM33β
9
8
7
[ppm]

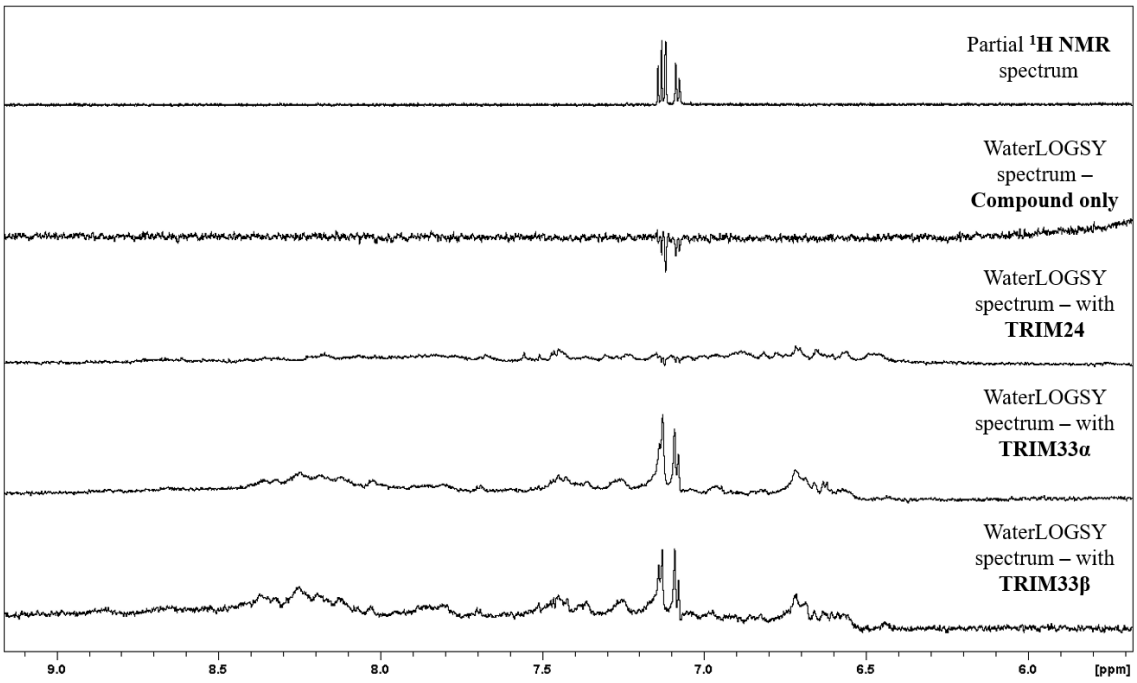


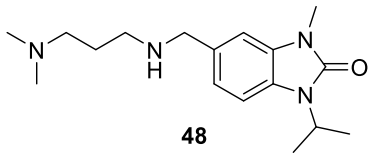
45


Partial ¹H NMR spectrum
WaterLOGSY spectrum – Compound only
WaterLOGSY spectrum – with TRIM24
WaterLOGSY spectrum – with TRIM33α
WaterLOGSY spectrum – with TRIM33β
9
8
7
[ppm]

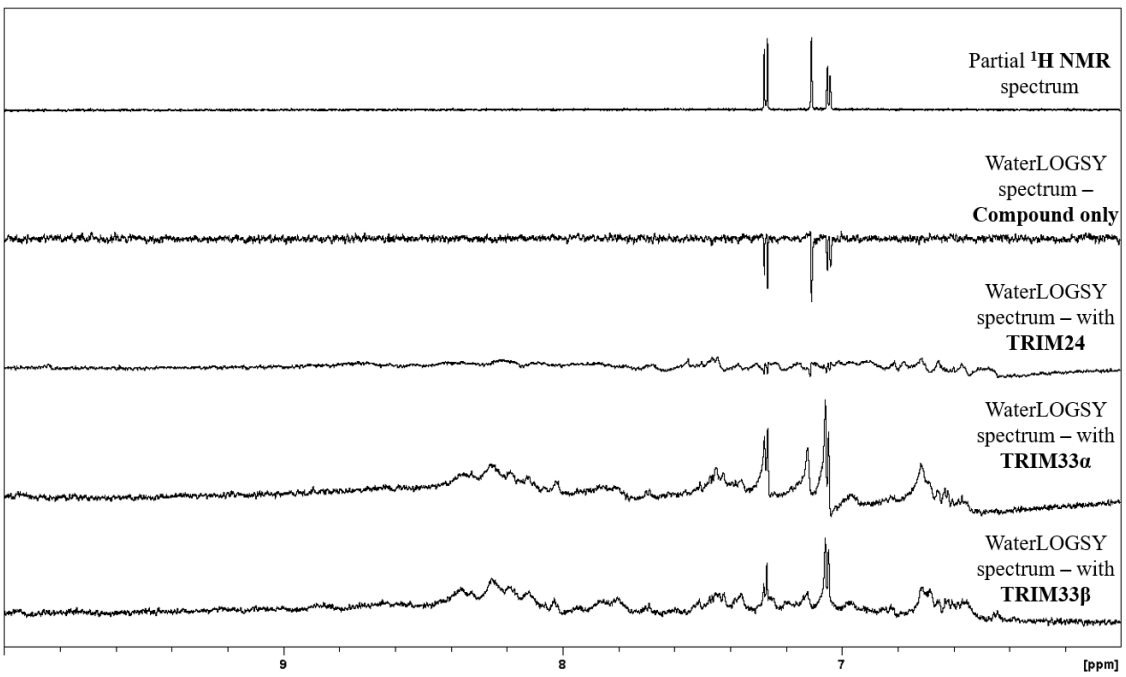


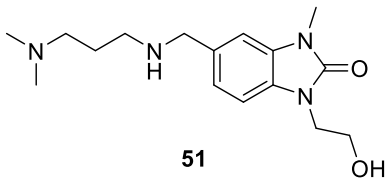
47


Partial ¹H NMR spectrum
WaterLOGSY spectrum – Compound only
WaterLOGSY spectrum – with TRIM24
WaterLOGSY spectrum – with TRIM33α
WaterLOGSY spectrum – with TRIM33β
9.0
8.5
8.0
7.5
7.0
6.5
6.0
[ppm]

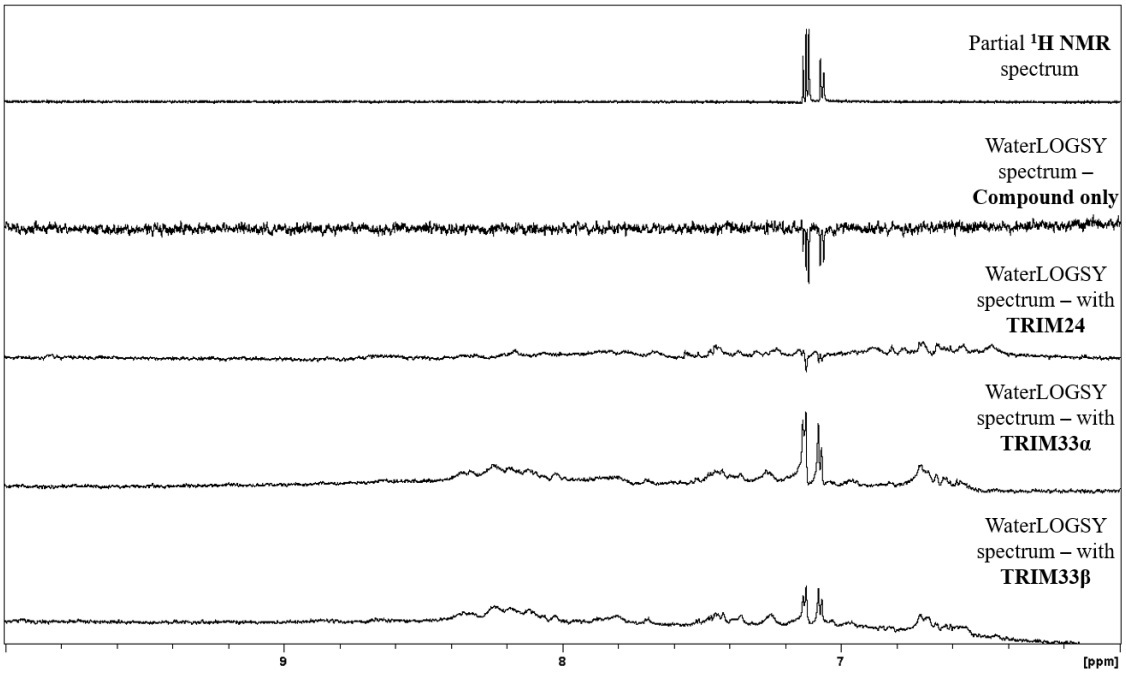


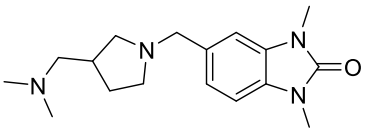
48


Partial ¹H NMR spectrum
WaterLOGSY spectrum – Compound only
WaterLOGSY spectrum – with TRIM24
WaterLOGSY spectrum – with TRIM33α
WaterLOGSY spectrum – with TRIM33β
9
8
7
[ppm]

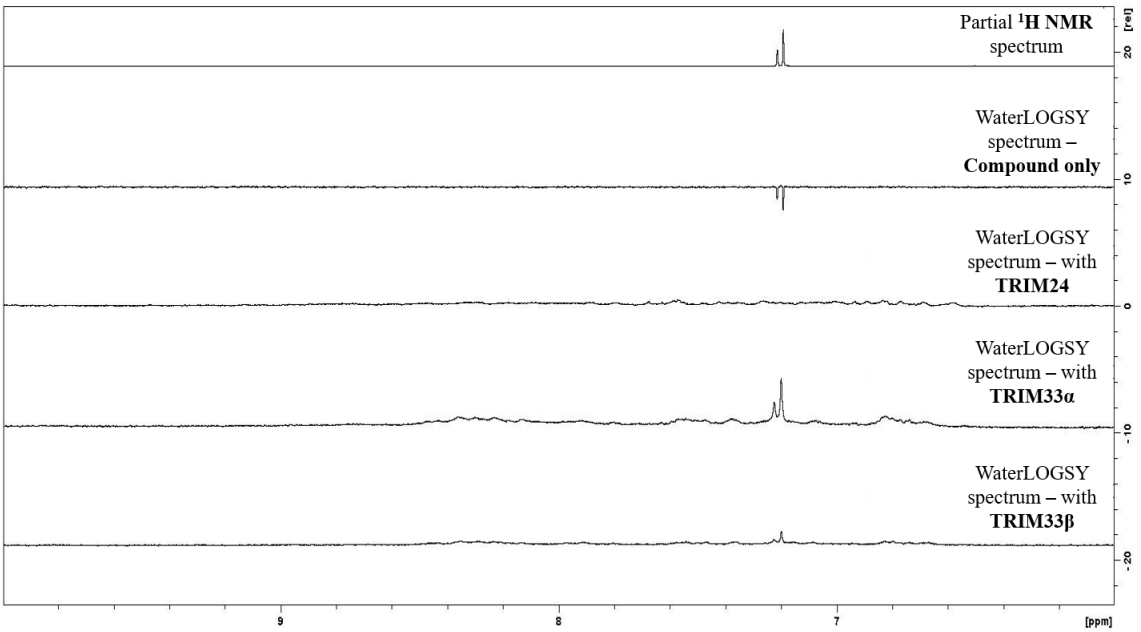


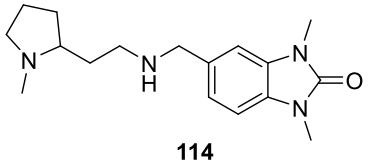
51


Partial ¹H NMR spectrum
WaterLOGSY spectrum – Compound only
WaterLOGSY spectrum – with TRIM24
WaterLOGSY spectrum – with TRIM33α
WaterLOGSY spectrum – with TRIM33β
9
8
7
[ppm]

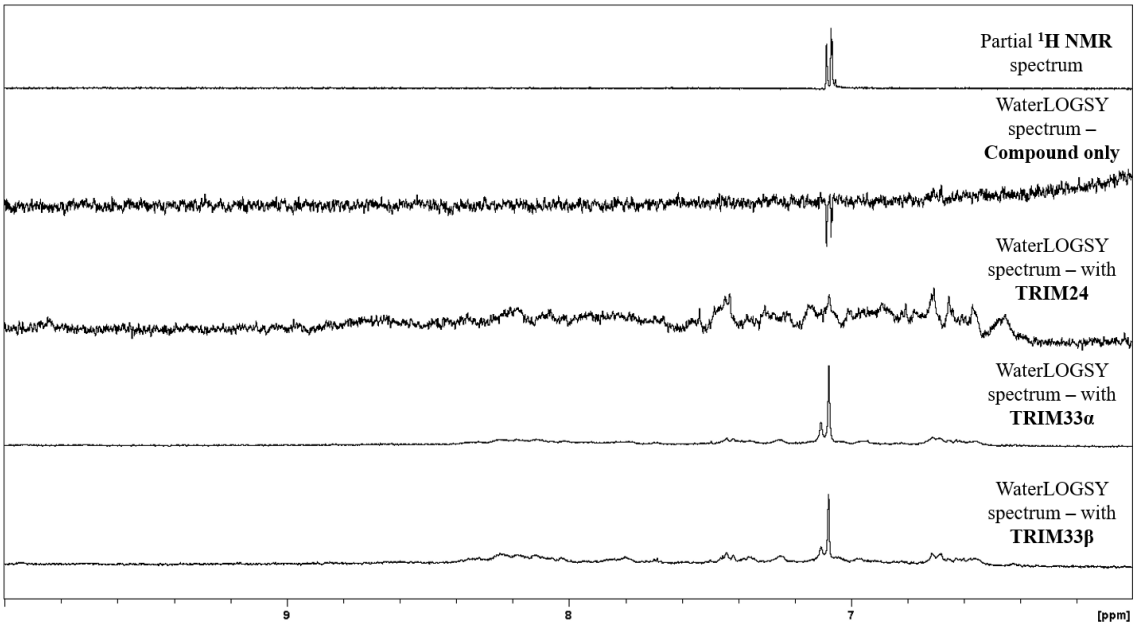


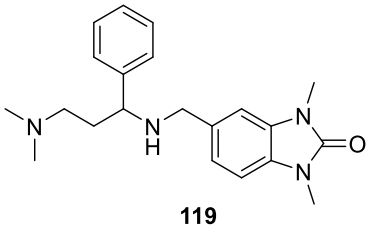
111


Partial ¹H NMR spectrum
WaterLOGSY spectrum – Compound only
WaterLOGSY spectrum – with TRIM24
WaterLOGSY spectrum – with TRIM33α
WaterLOGSY spectrum – with TRIM33β
[rel]
20
10
0
-10
-20
9
8
7
[ppm]

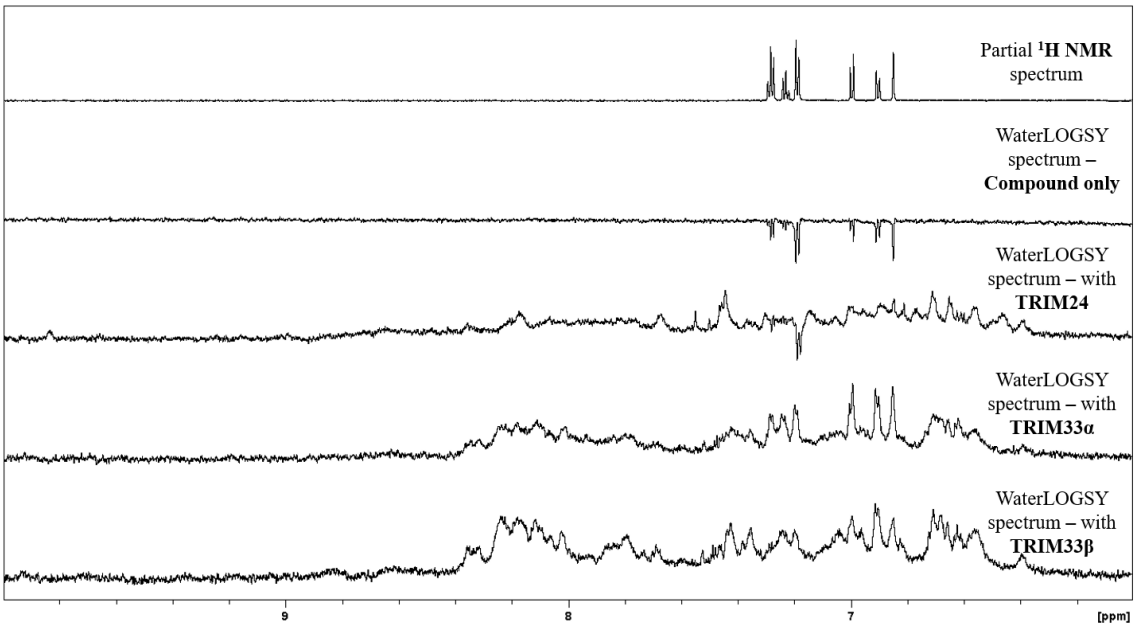


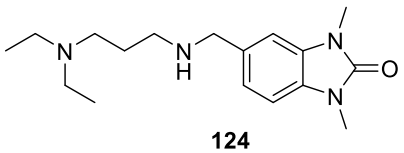
114


Partial ¹H NMR spectrum
WaterLOGSY spectrum – Compound only
WaterLOGSY spectrum – with TRIM24
WaterLOGSY spectrum – with TRIM33α
WaterLOGSY spectrum – with TRIM33β
9
8
7
[ppm]

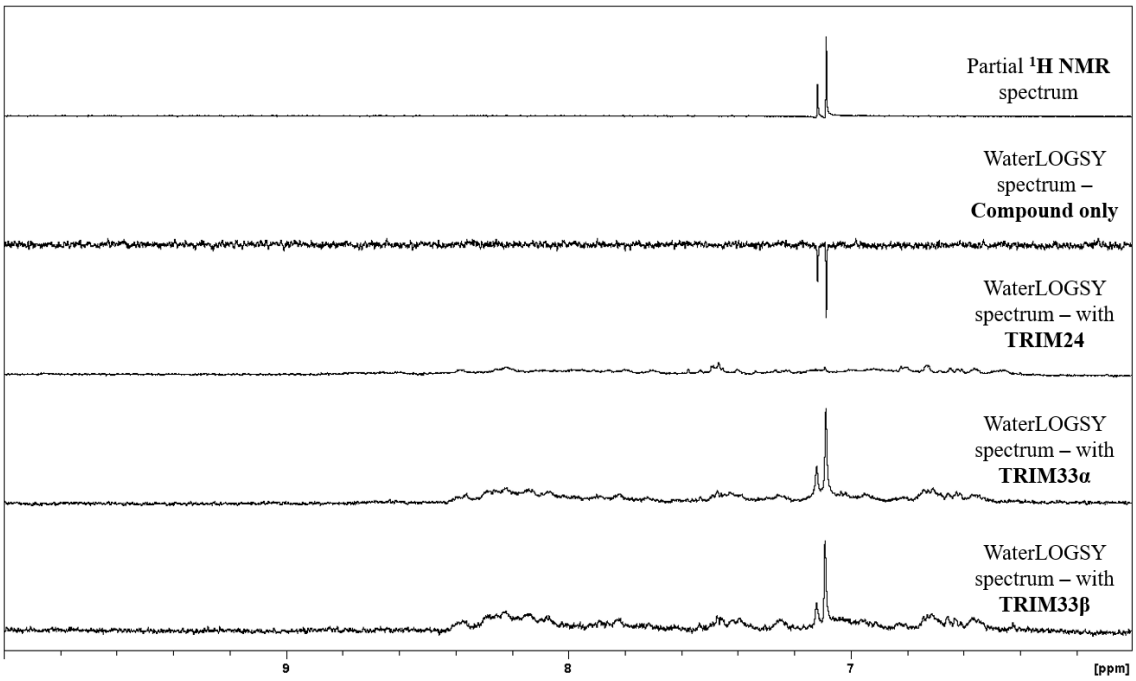


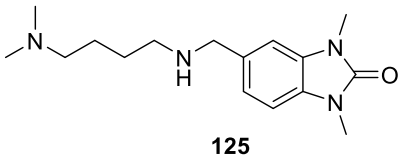
119


Partial ¹H NMR spectrum
WaterLOGSY spectrum – Compound only
WaterLOGSY spectrum – with TRIM24
WaterLOGSY spectrum – with TRIM33α
WaterLOGSY spectrum – with TRIM33β
9
8
7
[ppm]

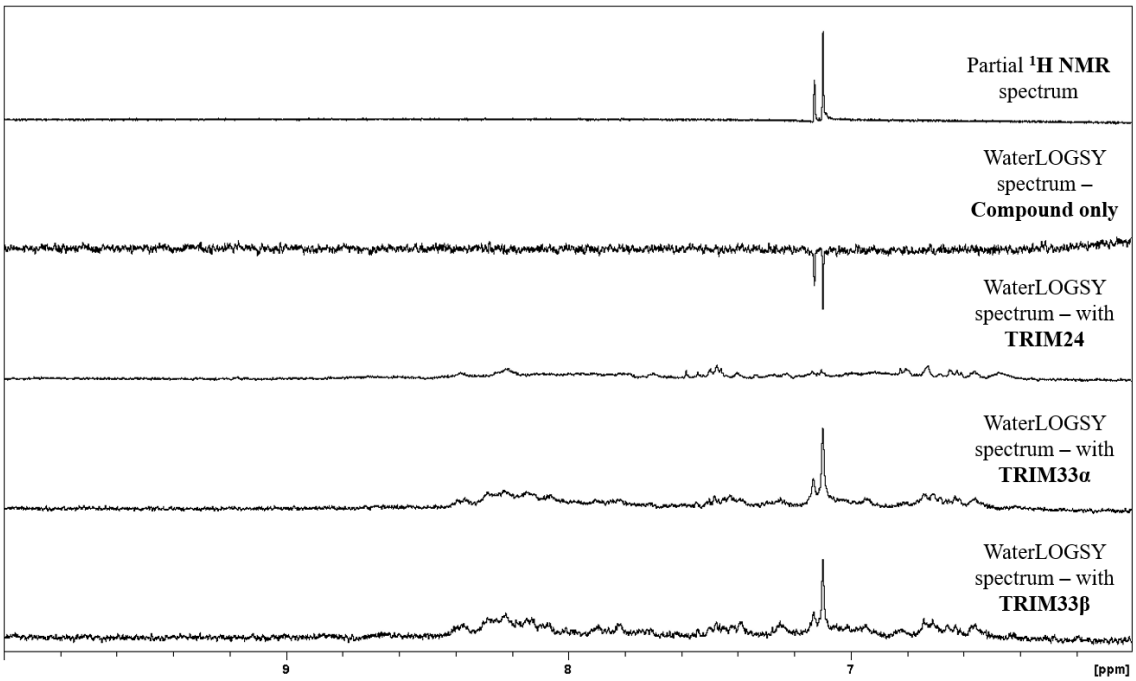


124

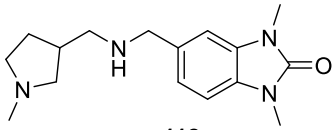

Partial ¹H NMR spectrum
WaterLOGSY spectrum – Compound only
WaterLOGSY spectrum – with TRIM24
WaterLOGSY spectrum – with TRIM33α
WaterLOGSY spectrum – with TRIM33β
9
8
7
[ppm]



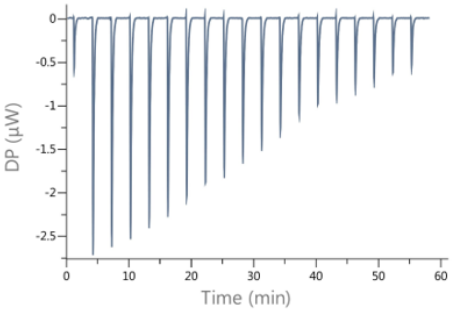
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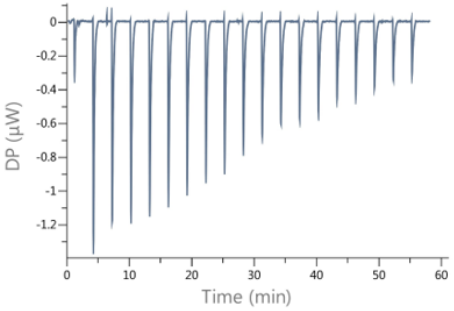

Partial ¹H NMR spectrum
WaterLOGSY spectrum – Compound only
WaterLOGSY spectrum – with TRIM24
WaterLOGSY spectrum – with TRIM33α
WaterLOGSY spectrum – with TRIM33β
9
8
7
[ppm]

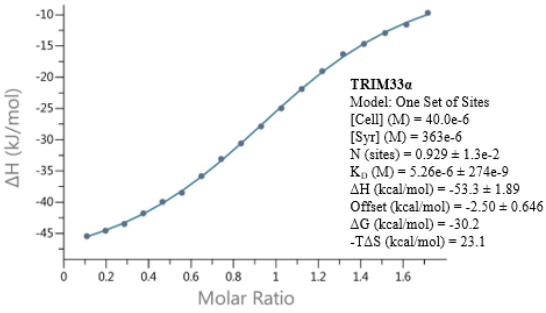
Appendix E: Isothermal Titration Calorimetry Data (Chapter 2)

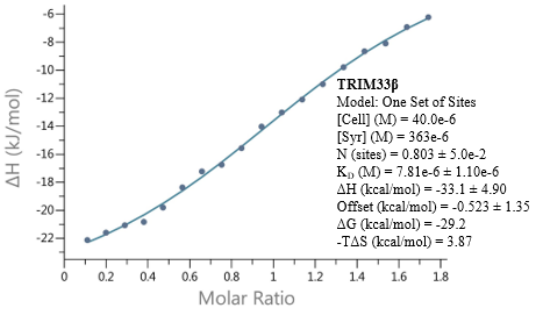


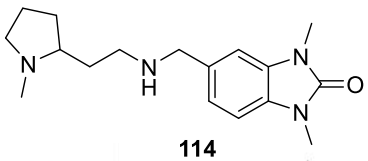
112


DP (µW)
Time (min)

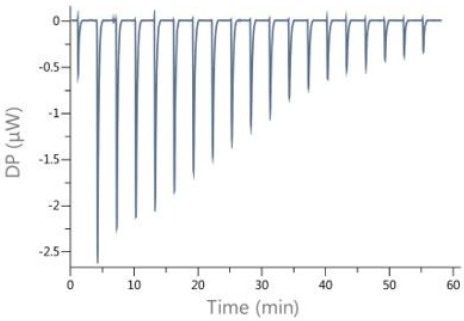

DP (µW)
Time (min)

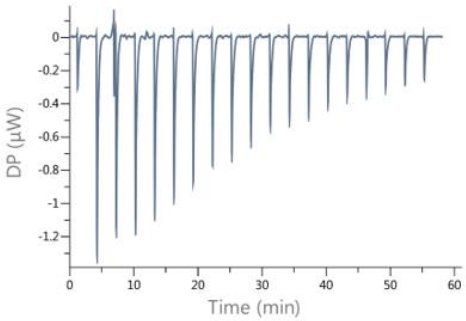

ΔH (kJ/mol)
Molar Ratio
TRIM33α
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 0.929 ± 1.3e-2
K_D (M) = 5.26e-6 ± 274e-9
ΔH (kcal/mol) = -53.3 ± 1.89
Offset (kcal/mol) = -2.50 ± 0.646
ΔG (kcal/mol) = -30.2
-TΔS (kcal/mol) = 23.1

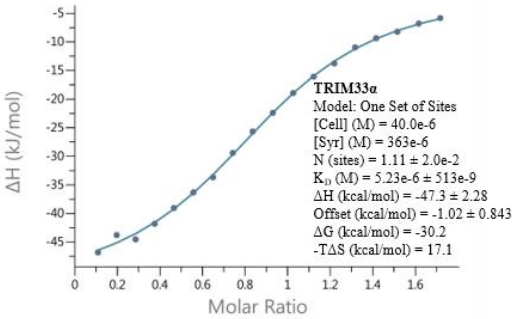

ΔH (kJ/mol)
Molar Ratio
TRIM33β
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 0.803 ± 5.0e-2
K_D (M) = 7.81e-6 ± 1.10e-6
ΔH (kcal/mol) = -33.1 ± 4.90
Offset (kcal/mol) = -0.523 ± 1.35
ΔG (kcal/mol) = -29.2
-TΔS (kcal/mol) = 3.87

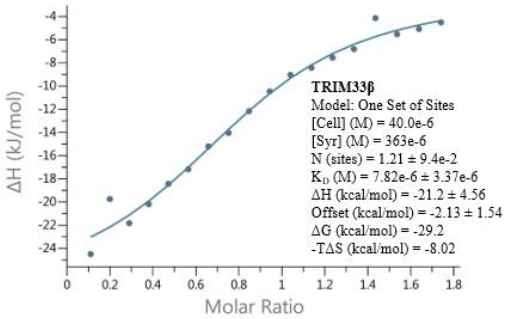


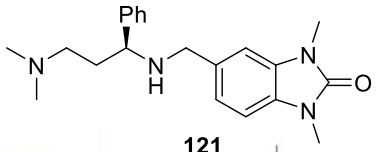
114


DP (µW)
Time (min)

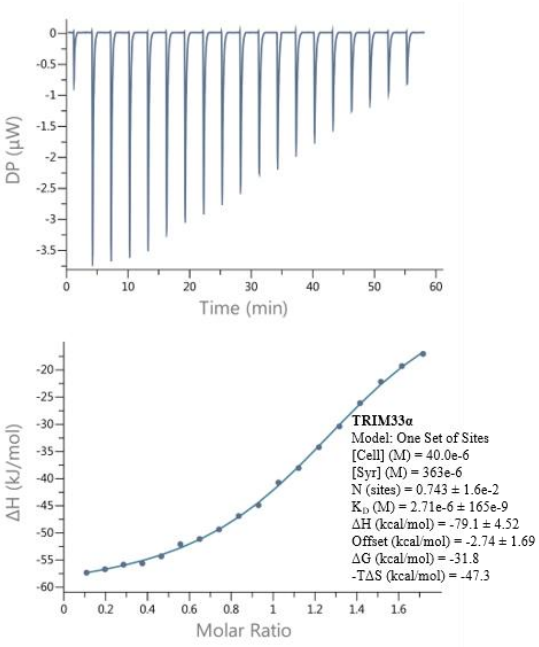

DP (µW)
Time (min)

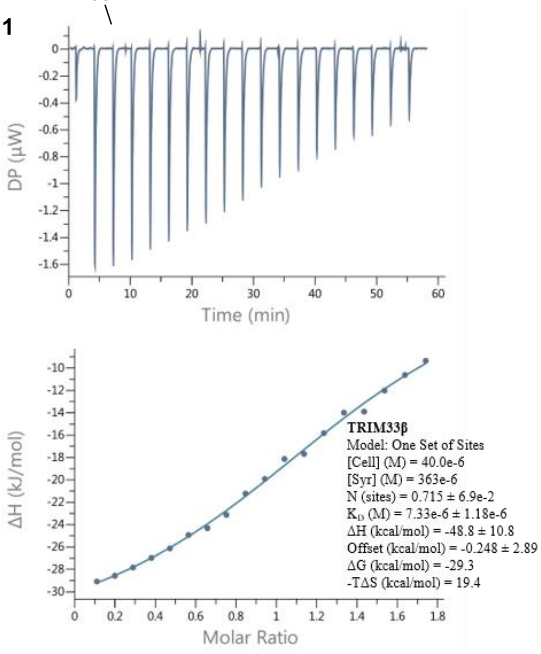

ΔH (kJ/mol)
Molar Ratio
TRIM33α
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.11 ± 2.0e-2
K_D (M) = 5.23e-6 ± 513e-9
ΔH (kcal/mol) = -47.3 ± 2.28
Offset (kcal/mol) = -1.02 ± 0.843
ΔG (kcal/mol) = -30.2
-TΔS (kcal/mol) = 17.1

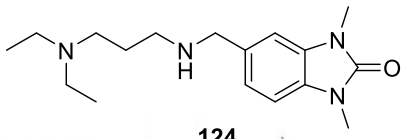

ΔH (kJ/mol)
Molar Ratio
TRIM33β
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.21 ± 9.4e-2
K_D (M) = 7.82e-6 ± 3.37e-6
ΔH (kcal/mol) = -21.2 ± 4.56
Offset (kcal/mol) = -2.13 ± 1.54
ΔG (kcal/mol) = -29.2
-TΔS (kcal/mol) = -8.02



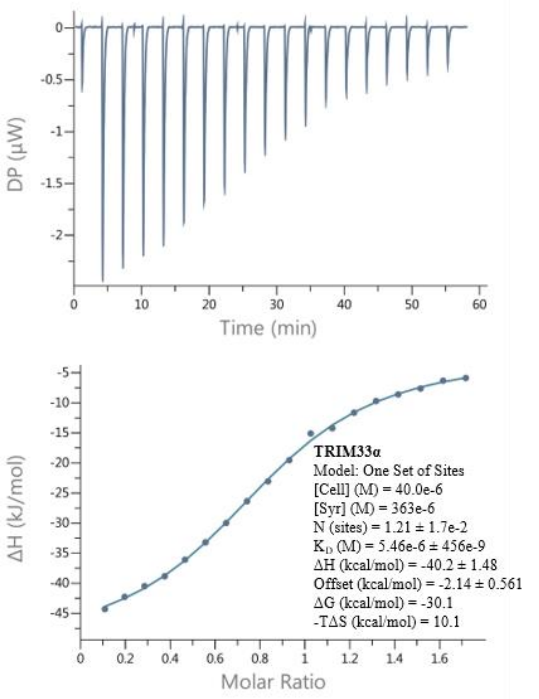
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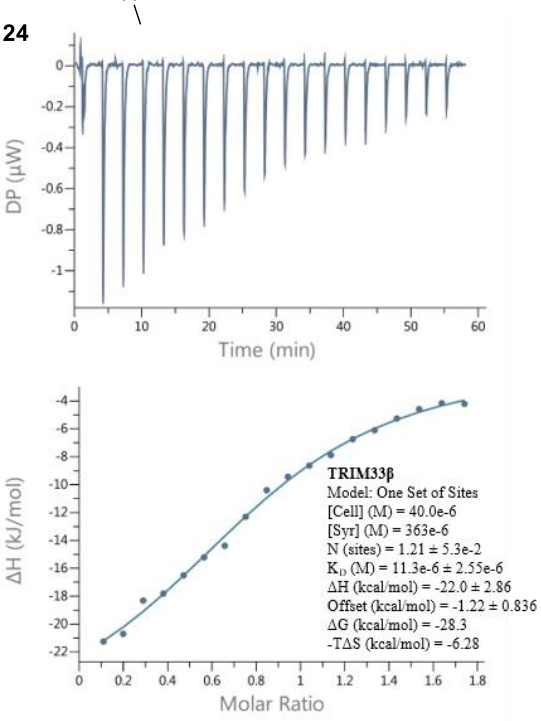

DP (µW)
Time (min)
ΔH (kJ/mol)
Molar Ratio
TRIM33α
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 0.743 ± 1.6e-2
K_D (M) = 2.71e-6 ± 165e-9
ΔH (kcal/mol) = -79.1 ± 4.52
Offset (kcal/mol) = -2.74 ± 1.69
ΔG (kcal/mol) = -31.8
-TΔS (kcal/mol) = -47.3

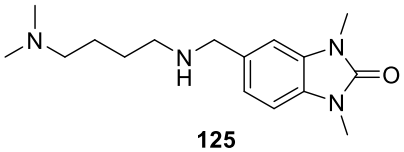

DP (µW)
Time (min)
ΔH (kJ/mol)
Molar Ratio
TRIM33β
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 0.715 ± 6.9e-2
K_D (M) = 7.33e-6 ± 1.18e-6
ΔH (kcal/mol) = -48.8 ± 10.8
Offset (kcal/mol) = -0.248 ± 2.89
ΔG (kcal/mol) = -29.3
-TΔS (kcal/mol) = 19.4



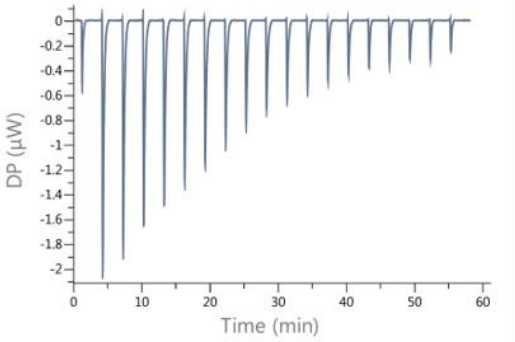
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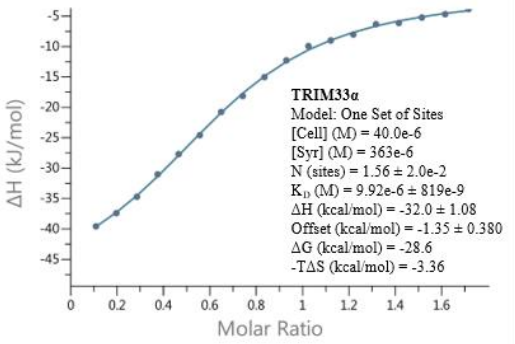

DP (µW)
Time (min)
ΔH (kJ/mol)
Molar Ratio
TRIM33α
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.21 ± 1.7e-2
K_D (M) = 5.46e-6 ± 456e-9
ΔH (kcal/mol) = -40.2 ± 1.48
Offset (kcal/mol) = -2.14 ± 0.561
ΔG (kcal/mol) = -30.1
-TΔS (kcal/mol) = 10.1

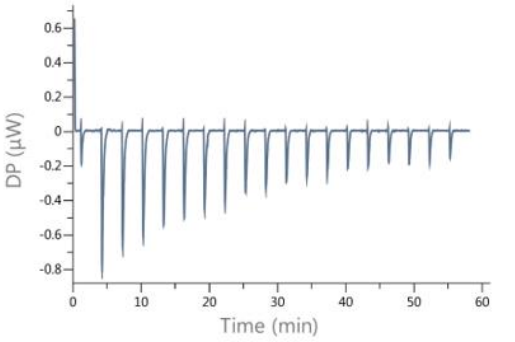

DP (µW)
Time (min)
ΔH (kJ/mol)
Molar Ratio
TRIM33β
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.21 ± 5.3e-2
K_D (M) = 11.3e-6 ± 2.55e-6
ΔH (kcal/mol) = -22.0 ± 2.86
Offset (kcal/mol) = -1.22 ± 0.836
ΔG (kcal/mol) = -28.3
-TΔS (kcal/mol) = -6.28

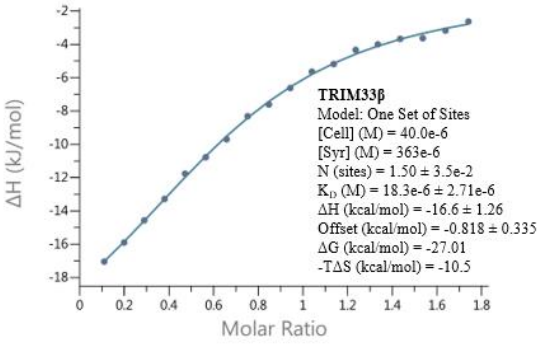


125


DP (µW)
Time (min)


ΔH (kJ/mol)
Molar Ratio
TRIM33α
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.56 ± 2.0e-2
K_D (M) = 9.92e-6 ± 819e-9
ΔH (kcal/mol) = -32.0 ± 1.08
Offset (kcal/mol) = -1.35 ± 0.380
ΔG (kcal/mol) = -28.6
-TΔS (kcal/mol) = -3.36

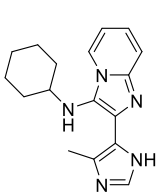
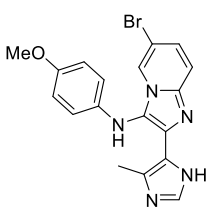
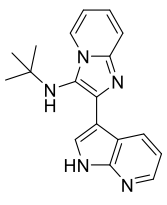
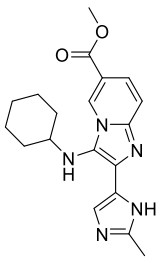

DP (µW)
Time (min)

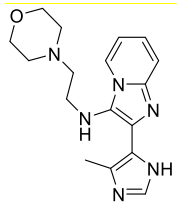

ΔH (kJ/mol)
Molar Ratio
TRIM33β
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.50 ± 3.5e-2
K_D (M) = 18.3e-6 ± 2.71e-6
ΔH (kcal/mol) = -16.6 ± 1.26
Offset (kcal/mol) = -0.818 ± 0.335
ΔG (kcal/mol) = -27.01
-TΔS (kcal/mol) = -10.5

Appendix F: Patent Compounds AlphaScreen™ Data (Chapter 2)

Table F.1. Dose-response AlphaScreen™ IC_{50} values of compounds **127–131** for all nine protein-peptide combinations, corresponding to **Table 2.12**. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. PHD refers to peptide H3K9Me_3, BRD refers to peptide H3K18Ac, and DUAL refers to peptide H3K9Me_3K18Ac. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

Weaker binding — **Stronger binding**

Compound	AlphaScreen™ IC_{50} (μM)									
	TRIM24			TRIM33α			TRIM33β			
	PHD	BRD	DUAL	PHD	BRD	DUAL	PHD	BRD	DUAL
127 	3.57 ± 0.52	4.21 ± 0.67	3.33 ± 0.23	2.71 ± 0.17	2.74 ± 0.53	1.68 ± 0.41	3.37 ± 0.62	3.16 ± 0.55	3.11 ± 0.34
128 	1.86 ± 0.18	1.76 ± 0.10	1.46 ± 0.08	1.89 ± 0.12	1.58 ± 0.17	1.15 ± 0.12	1.40 ± 0.19	1.49 ± 0.22	1.82 ± 0.21
129 	105 ± 26.7	43.8 ± 10.9	58.0 ± 20.3	>250	>250	>250	>250	>250	>250
130 	17.1 ± 6.15	20.0 ± 2.95	11.1 ± 1.81	>250	16.6 ± 6.10	75.1 ± 20.5	6.70 ± 1.79	7.91 ± 1.84	8.86 ± 2.41

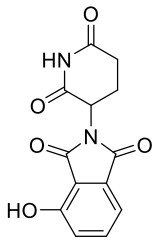
131 	52.0 ± 5.56	18.6 ± 0.73	23.1 ± 0.74	>250	>250	>250	>250	55.3 ± 4.68	58.7 ± 6.00

Appendix G: PROTACs AlphaScreen™ Data (Chapter 3)

Table G.1. Dose-response AlphaScreen™ IC_{50} values of compounds **231** and **158** for all nine protein-peptide combinations, corresponding to **Table 3.2**. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. PHD refers to peptide H3K9Me_3, BRD refers to peptide H3K18Ac, and DUAL refers to peptide H3K9Me_3K18Ac. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

Weaker binding — **Stronger binding**

AlphaScreen™ IC_{50} (μM)			Compound	
			231	**158**
TRIM24	**PHD**		>250	>250
	BRD		>250	>250
	DUAL		>250	>250
TRIM33α	**PHD**		>250	>250
	BRD		>250	>250
	DUAL		>250	>250
TRIM33β	**PHD**		>250	>250
	BRD		>250	>250
	DUAL		>250	>250



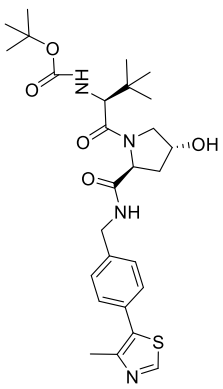
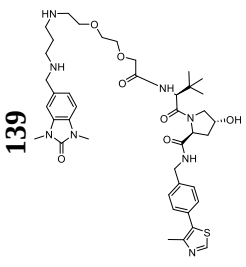
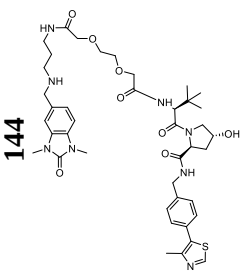


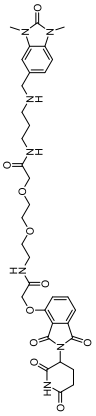
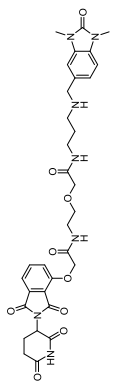
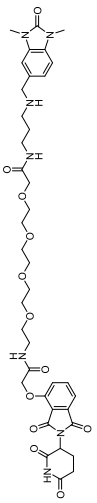
Table G.2. Dose-response AlphaScreen™ IC_{50} values of compounds **139** and **144** for all nine protein-peptide combinations, corresponding to **Table 3.3**. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. PHD refers to peptide H3K9Me_3, BRD refers to peptide H3K18Ac, and DUAL refers to peptide H3K9Me_3K18Ac. The colour scale is shown below, with low IC_{50} values shown in red, and high IC_{50} values shown in green.

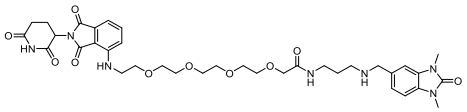
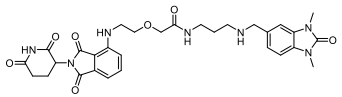
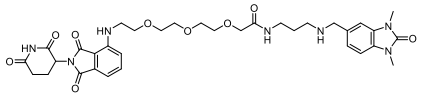
Weaker binding — **Stronger binding**

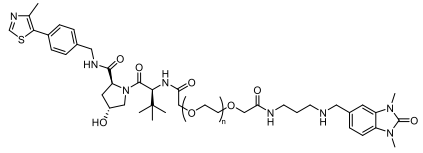
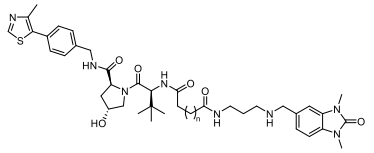
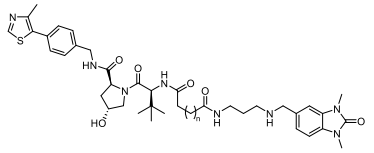
Compound	AlphaScreen™ IC_{50} (μM)									
	TRIM24			TRIM33α			TRIM33β			
	PHD	BRD	DUAL	PHD	BRD	DUAL	PHD	BRD	DUAL
139 	>250	>250	>250	>250	0.913 ± 0.18	3.58 ± 0.57	15.5 ± 4.64	6.15 ± 1.28	>250
144 	>250	>250	>250	>250	2.81 ± 0.45	12.3 ± 3.00	>250	6.26 ± 1.75	>250

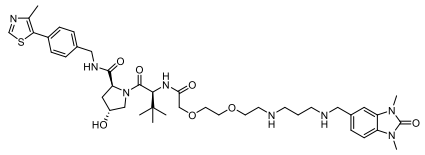
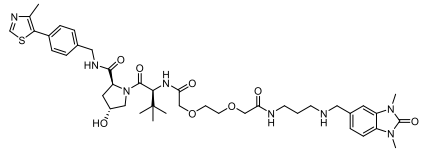
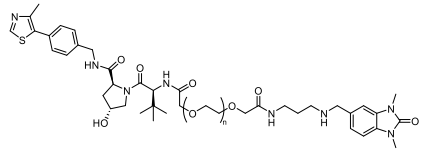
Appendix H: AlphaScreen™ TruHits Data (Chapter 3)

Table H.1: AlphaScreen™ TruHits results for PROTAC molecules **139**, **144–148** and **225–230**. Five concentrations of the compounds were tested with two incubation formats. Values which are more than two standard deviations greater than the mean DMSO control are highlighted in blue, and indicates interference.

Compound	Difference from DMSO average, normalised against standard deviation of DMSO control										
	Pre-incubate beads					Pre-incubate Donor beads with compound					
	31.25 µM	62.5 µM	125 µM	250 µM	500 µM	31.25 µM	62.5 µM	125 µM	250 µM	500 µM	
 **226**	1.62	0.920	0.0850	1.12	3.82	1.31	1.77	1.35	0.619	0.141	
 **225**	2.09	0.942	0.0610	1.14	4.58	0.665	1.50	0.786	0.165	0.076	
 **227**	1.44	0.100	0.0420	1.60	5.93	0.814	1.29	0.710	0.242	0.781	

Compound	Difference from DMSO average, normalised against standard deviation of DMSO control									
	Pre-incubate beads					Pre-incubate Donor beads with compound				
	31.25 µM	**62.5 µM**	**125 µM**	**250 µM**	**500 µM**	**31.25 µM**	**62.5 µM**	**125 µM**	**250 µM**	**500 µM**
 **230**	3.55	3.78	3.25	3.64	7.37	0.0520	0.576	0.0610	0.622	0.681
 **228**	0.967	0.789	1.51	1.36	2.00	0.863	0.846	0.426	1.06	1.41
 **229**	0.828	1.04	1.78	1.74	1.22	0.690	0.432	0.124	0.421	0.501

Compound	Difference from DMSO average, normalised against standard deviation of DMSO control									
	Pre-incubate beads					Pre-incubate Donor beads with compound				
	31.25 µM	62.5 µM	125 µM	250 µM	500 µM	31.25 µM	62.5 µM	125 µM	250 µM	500 µM
 n = 3 **146**	0.435	1.02	1.60	2.67	7.34	0.655	0.438	0.461	0.539	1.11
 n = 7 **148**	0.0798	0.566	1.51	2.70	7.21	0.469	0.254	0.190	0.620	1.10
 n = 4 **147**	0.531	1.07	2.68	4.95	10.6	0.0490	0.0210	0.123	0.601	1.25

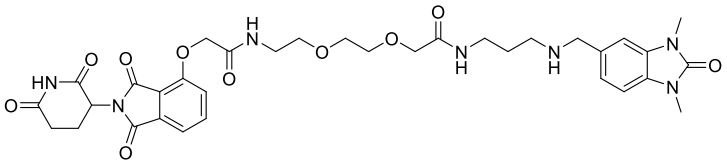
Compound	Difference from DMSO average, normalised against standard deviation of DMSO control									
	Pre-incubate beads					Pre-incubate Donor beads with compound				
	31.25 µM	62.5 µM	125 µM	250 µM	500 µM	31.25 µM	62.5 µM	125 µM	250 µM	500 µM
 **139**	3.22	4.09	5.45	7.30	10.8	1.58	1.62	1.07	1.73	1.59
 **144**	0.649	1.11	1.87	3.10	6.34	1.14	1.04	0.708	1.08	1.04
 **n = 2** **145**	3.16	4.30	5.88	10.0	15.8	0.775	0.931	0.728	1.22	1.61
Biotin	-	-	-	-	-	1.44	2.72	3.62	3.99	4.08

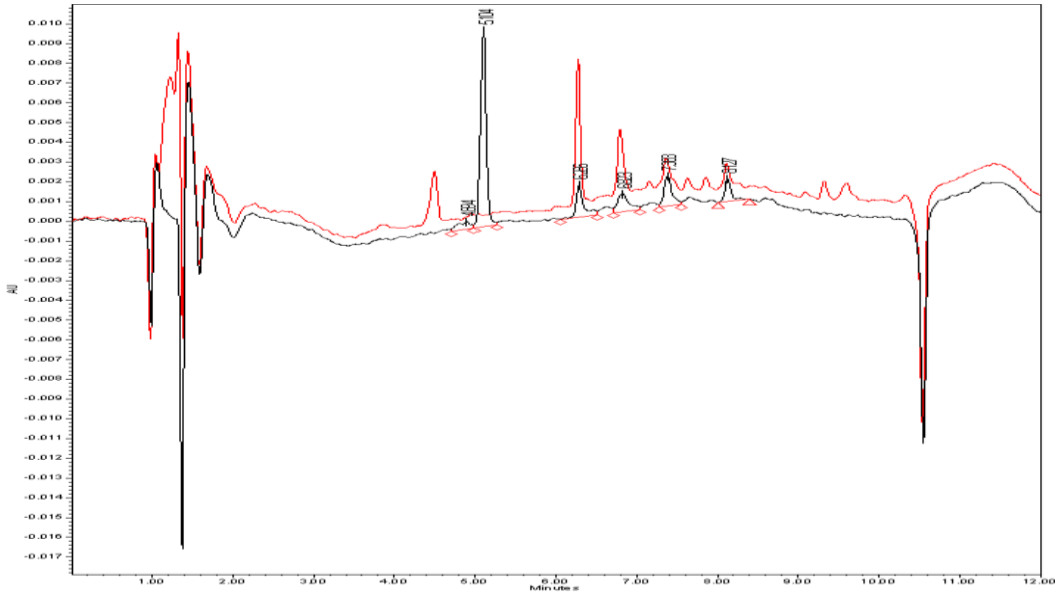
Appendix I: PROTAC Cell Permeability Data (Chapter 3)

Table I.1. Summary of whether the PROTAC molecules were observed to enter cells (using HPLC).

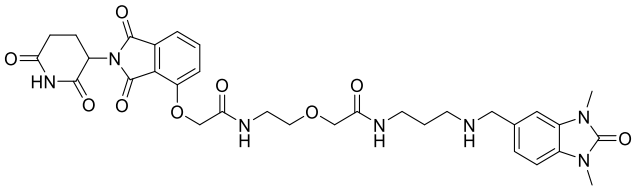
Name	E3 ligase	Target protein	MW	Enters cells (HPLC)
226	CRBN	TRIM33	821.76	No
225	CRBN	TRIM33	777.69	No
227	CRBN	TRIM33	909.85	No
230	CRBN	TRIM33	851.81	No
228	CRBN	TRIM33	719.65	No
229	CRBN	TRIM33	807.76	No
144	VHL	TRIM33	821.01	No
139	VHL	TRIM33	807.02	No
140	VHL	TRIM33	851.08	No
146	VHL	TRIM33	909.11	No
145	VHL	TRIM33	865.06	No
147	VHL	TRIM33	803.04	No
149	VHL	TRIM33	898.14	No
142	VHL	TRIM33	870.13	No
148	VHL	TRIM33	845.12	To some extent
143	VHL	TRIM33	884.15	To some extent
141	VHL	TRIM33	895.13	To some extent

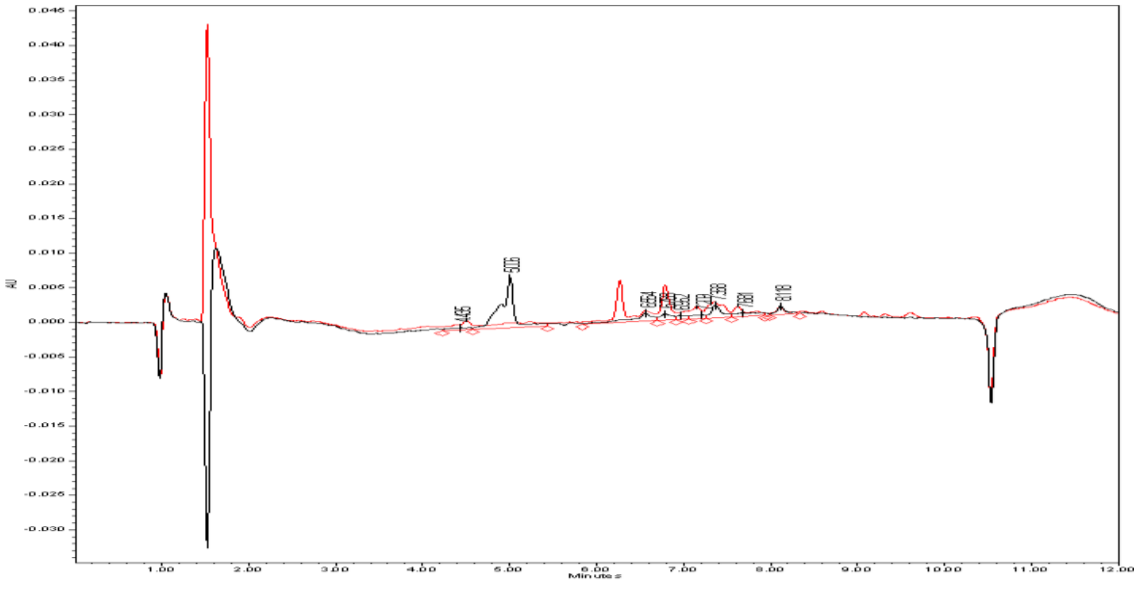
RKO cells were treated with either 10 μM of the PROTAC or control dimethyl sulfoxide (DMSO), incubated for 24 hours, and lysed in acetonitrile. The lysed samples were then prepared for HPLC. Reverse-phased HPLC was carried out using 0.1% TFA as mobile-phase additive.



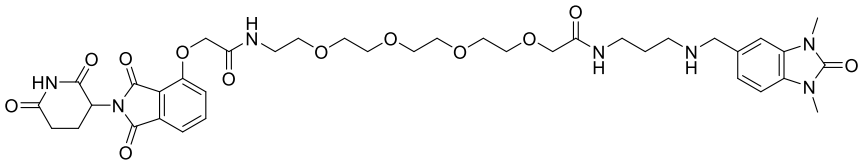


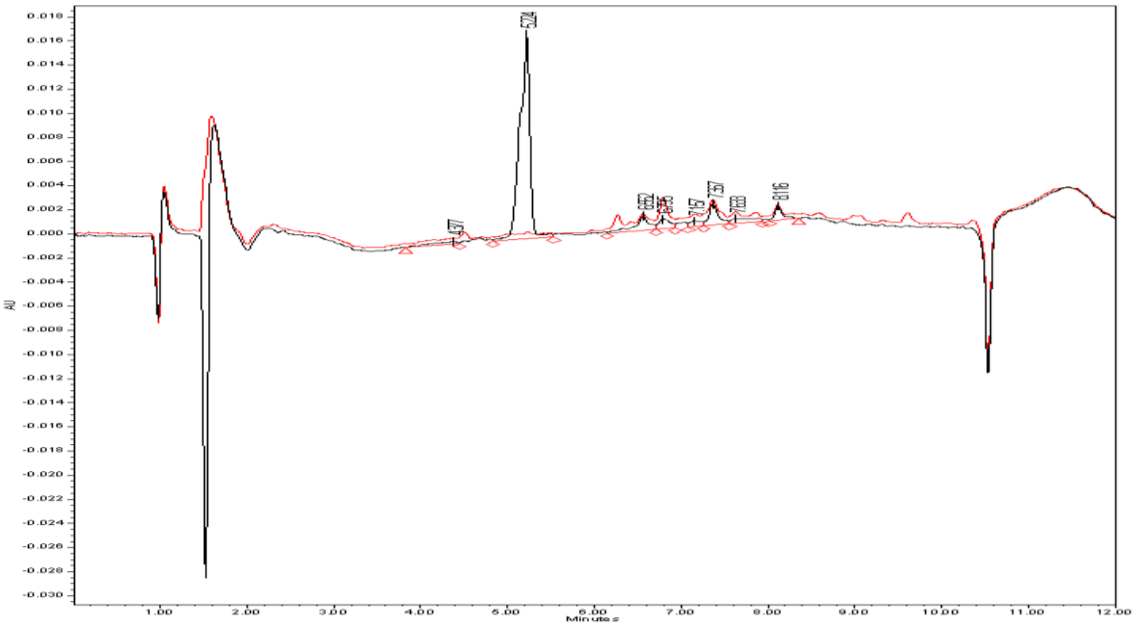
Calibration standard of 10 µM **226**
Cells treated with 10 µM **226**



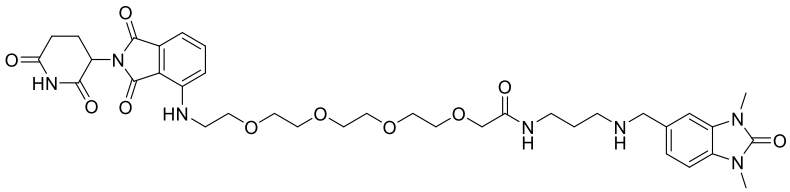


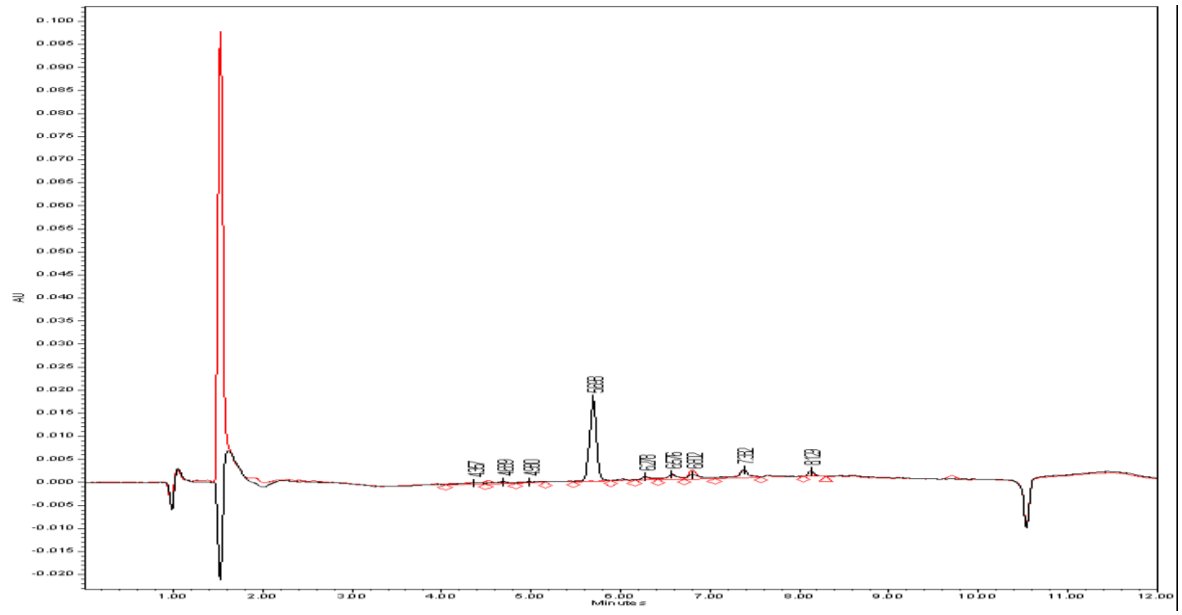
Calibration standard of 10 µM **225**
Cells treated with 10 µM **225**



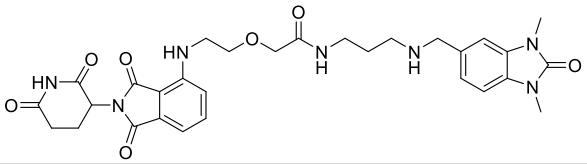


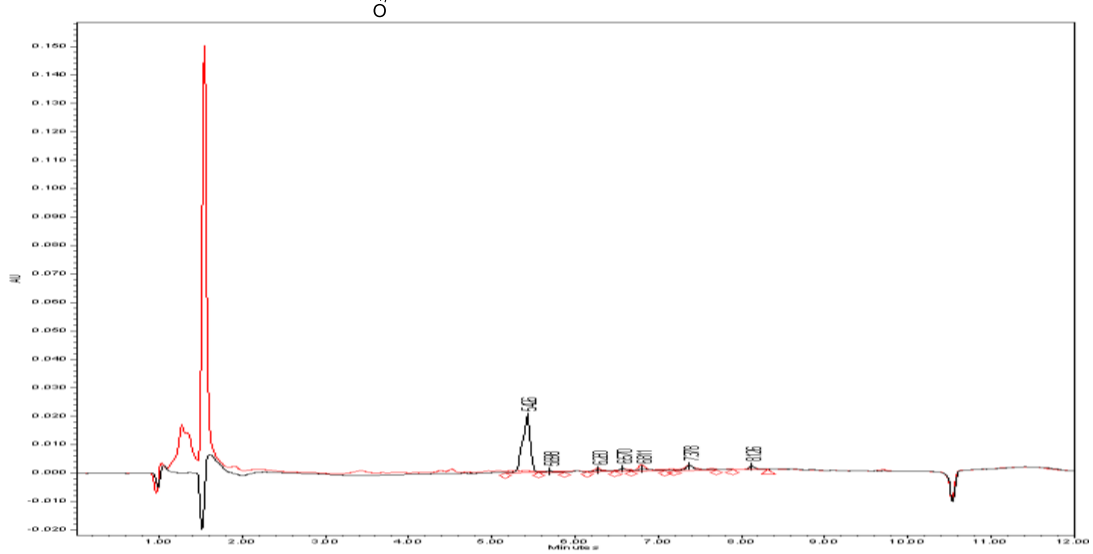
Calibration standard of 10 µM **227**
Cells treated with 10 µM **227**



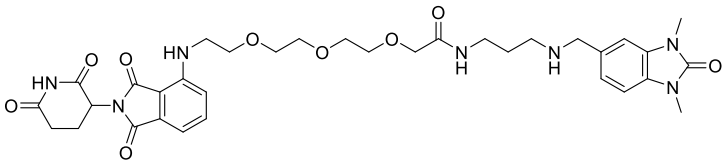


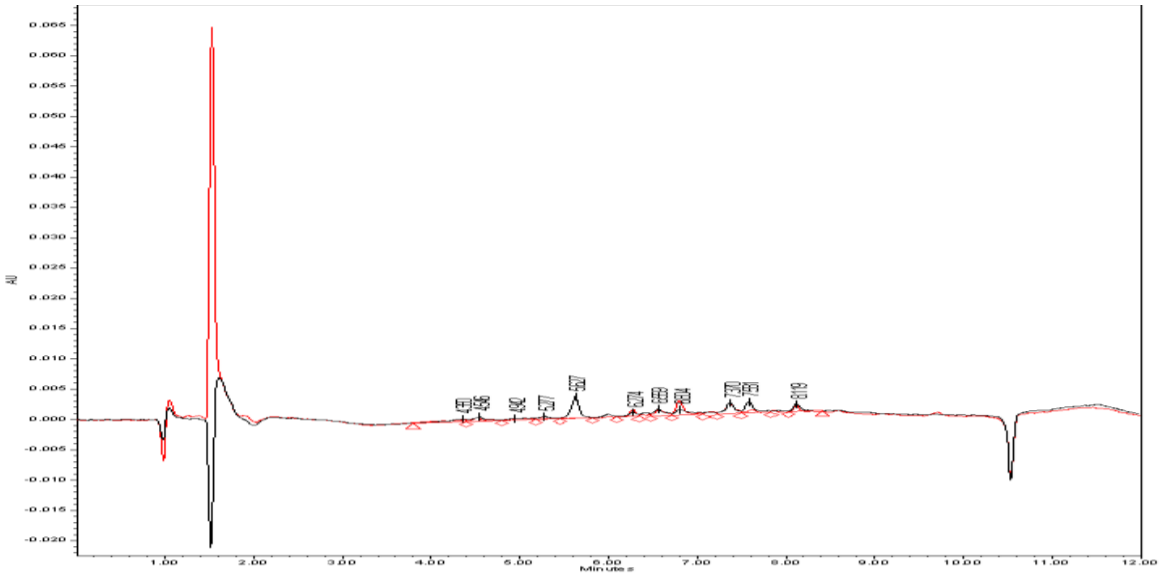
Calibration standard of 10 µM **230**
Cells treated with 10 µM **230**



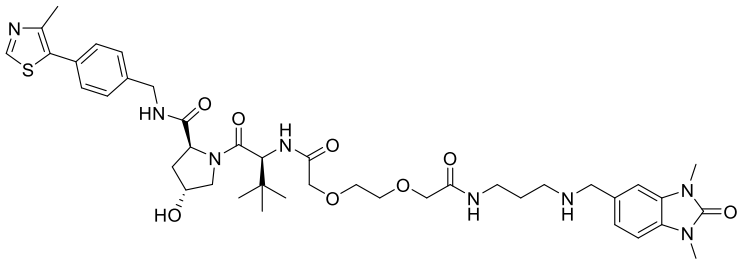


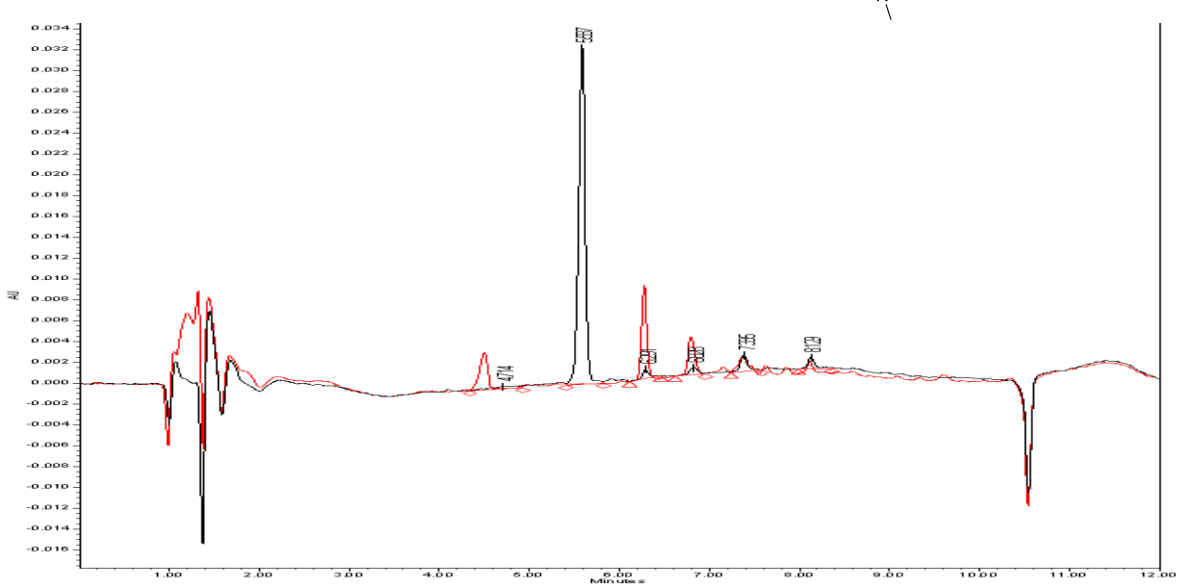
Calibration standard of 10 µM **228**
Cells treated with 10 µM **228**



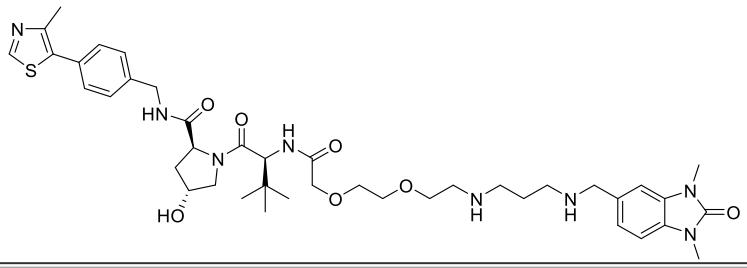


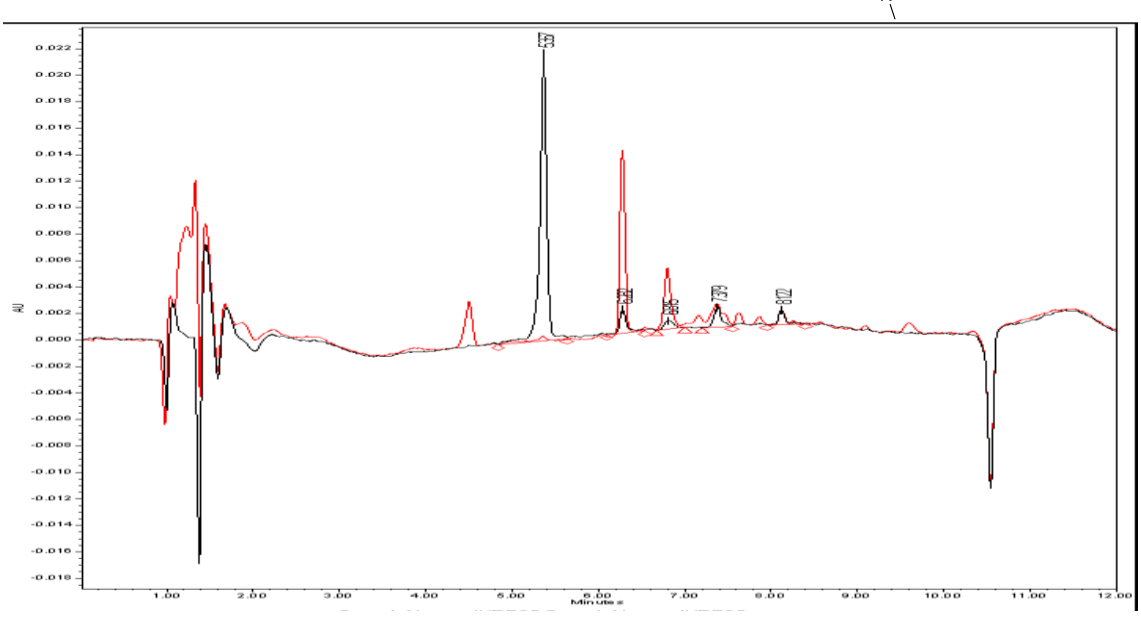
Calibration standard of 10 µM **229**
Cells treated with 10 µM **229**



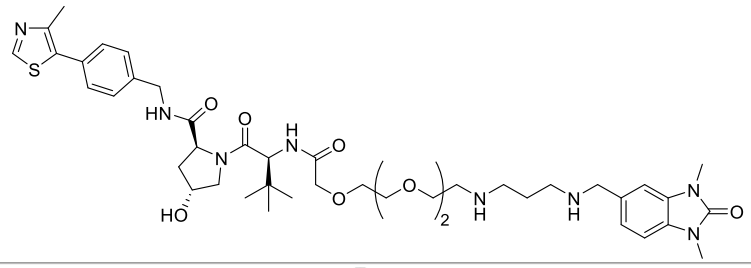


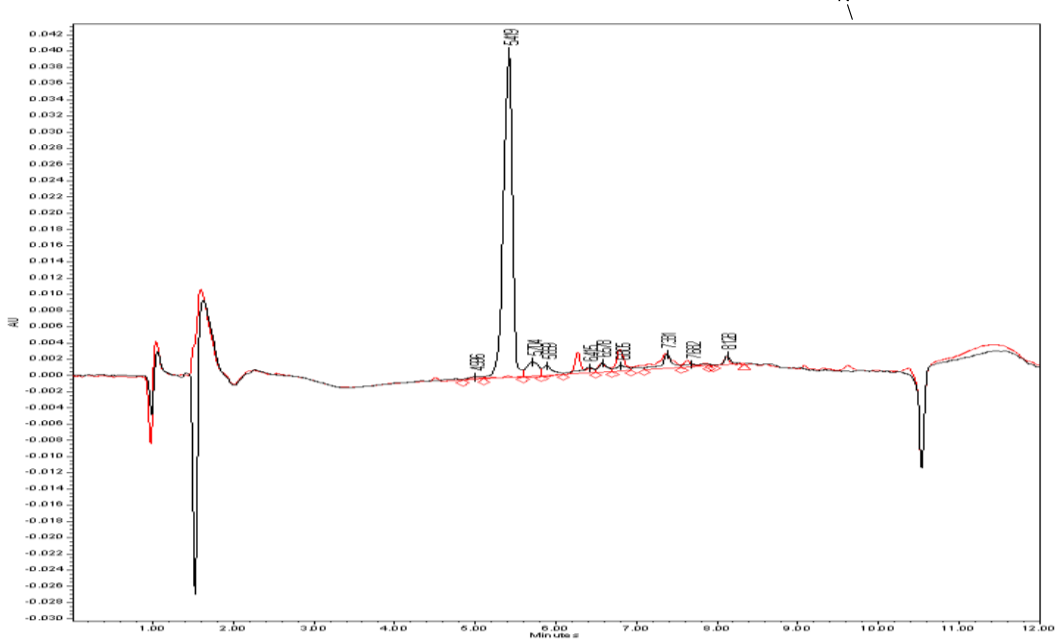
Calibration standard of 10 µM **144**
Cells treated with 10 µM **144**



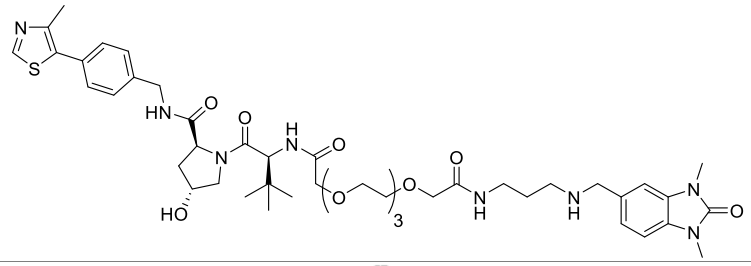


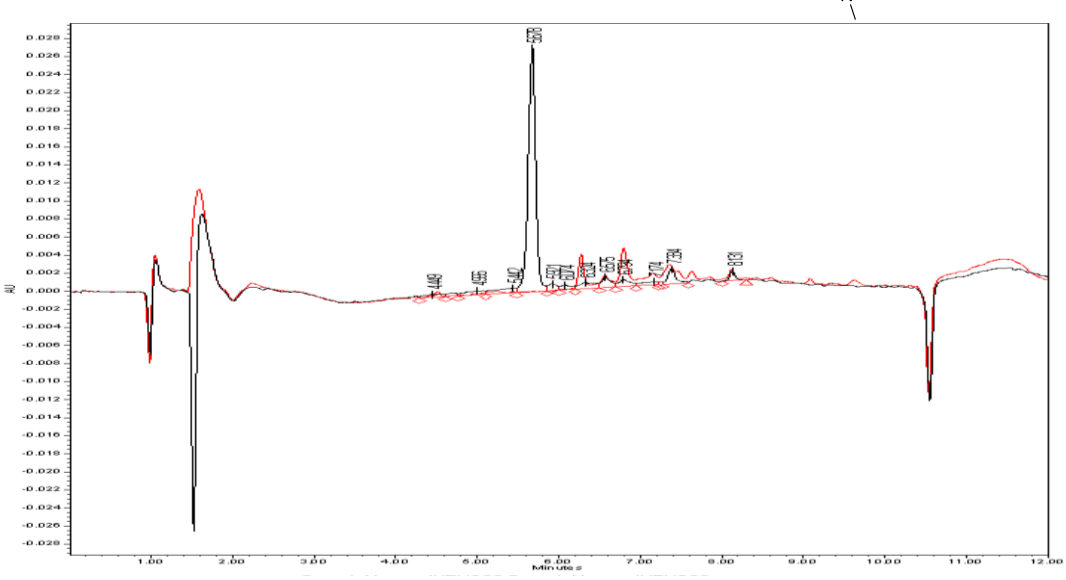
Calibration standard of 10 µM **139**
Cells treated with 10 µM **139**



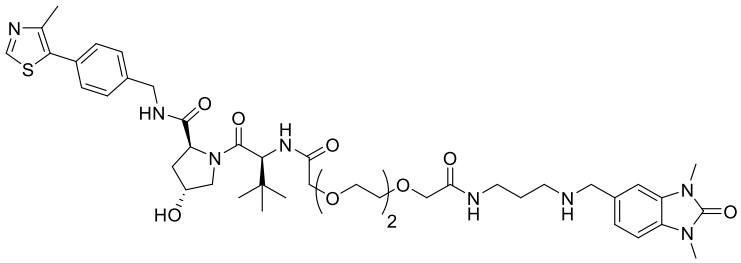


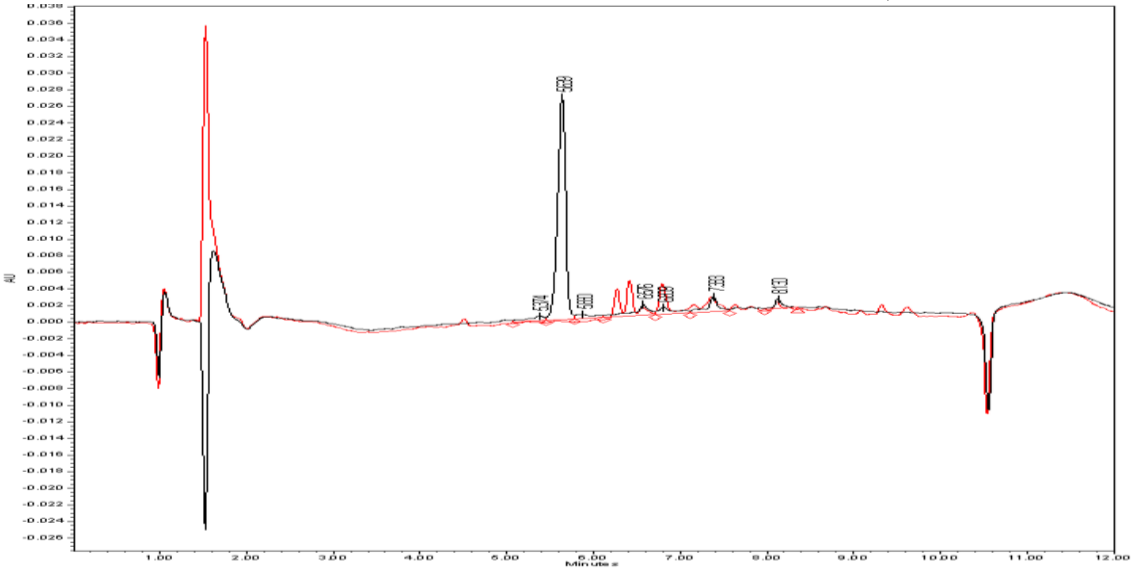
Calibration standard of 10 µM **140**
Cells treated with 10 µM **140**



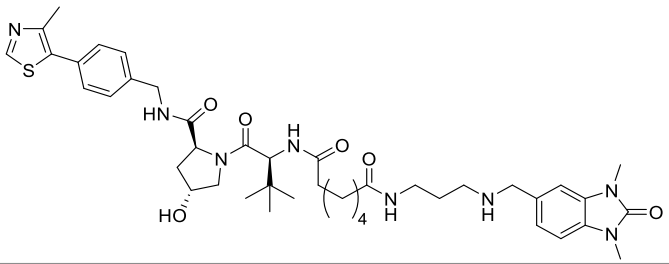


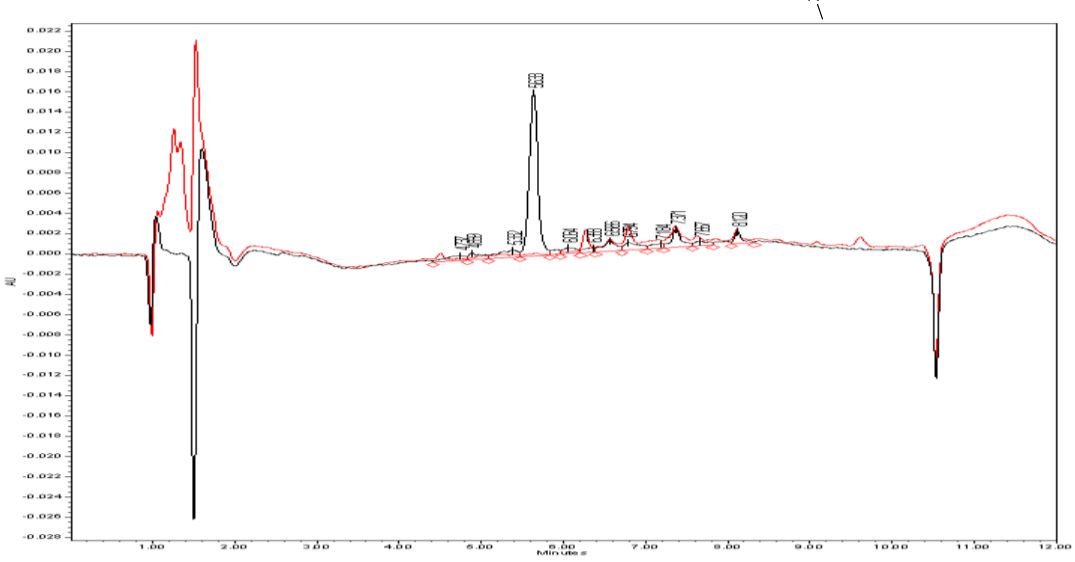
Calibration standard of 10 µM **146**
Cells treated with 10 µM **146**



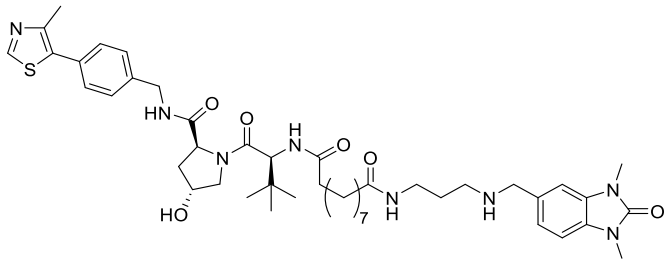


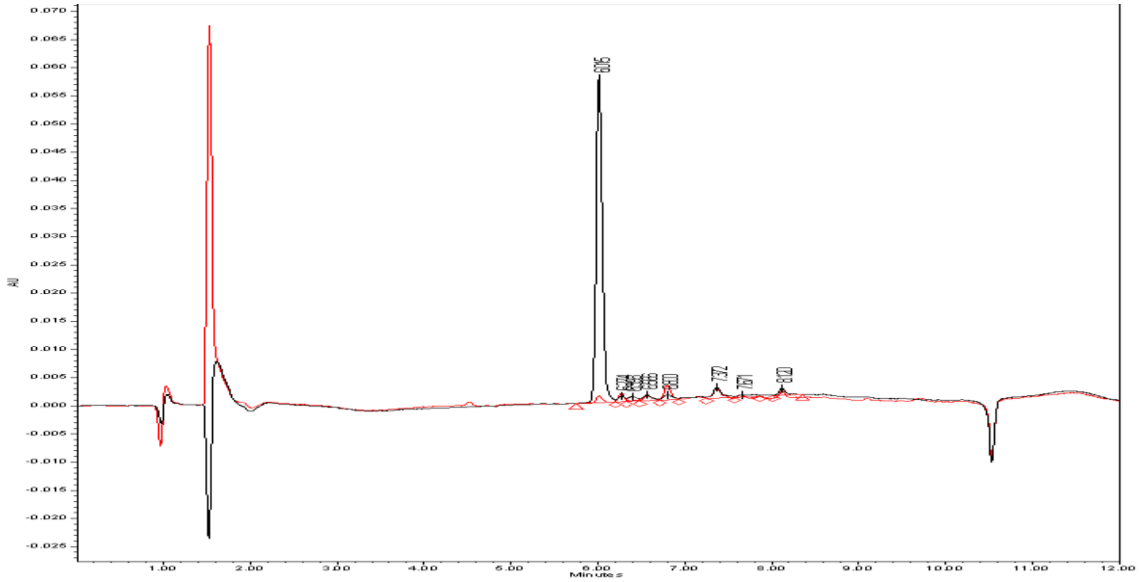
Calibration standard of 10 µM **145**
Cells treated with 10 µM **145**



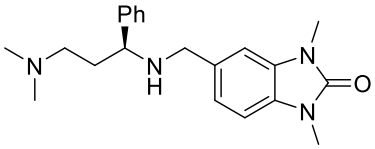


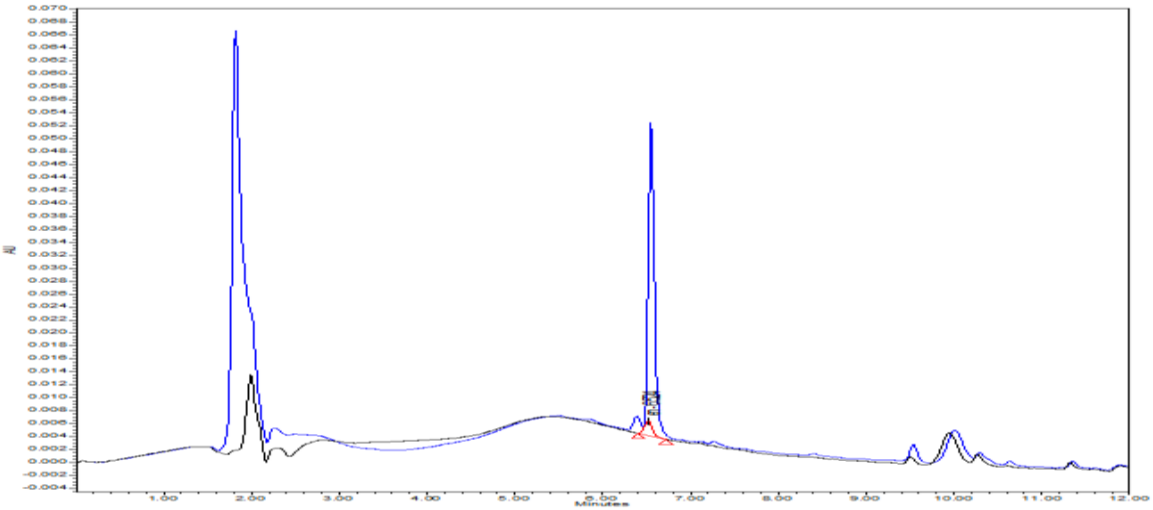
Calibration standard of 10 µM **147**
Cells treated with 10 µM **147**



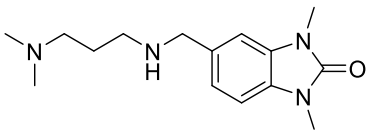


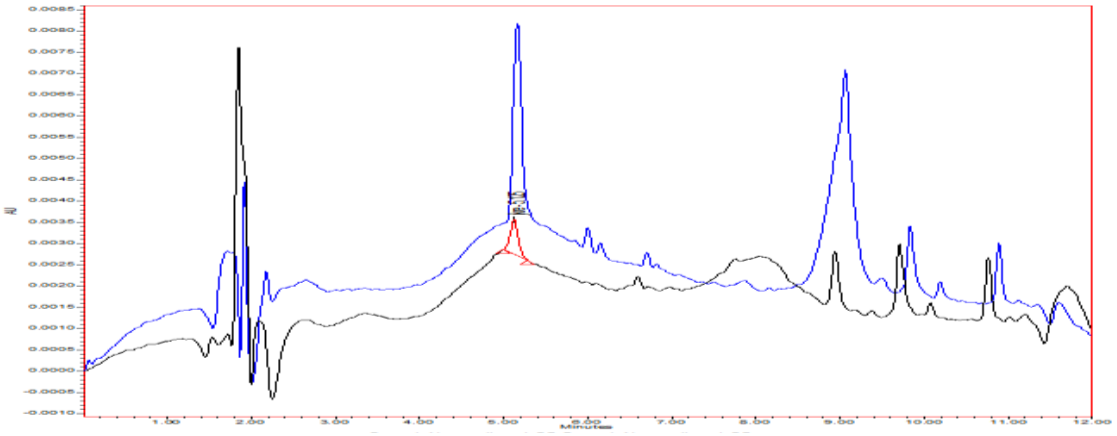
Calibration standard of 10 µM **148**
Cells treated with 10 µM **148**





Calibration standard of 10 µM **121**
Cells treated with 10 µM **121**

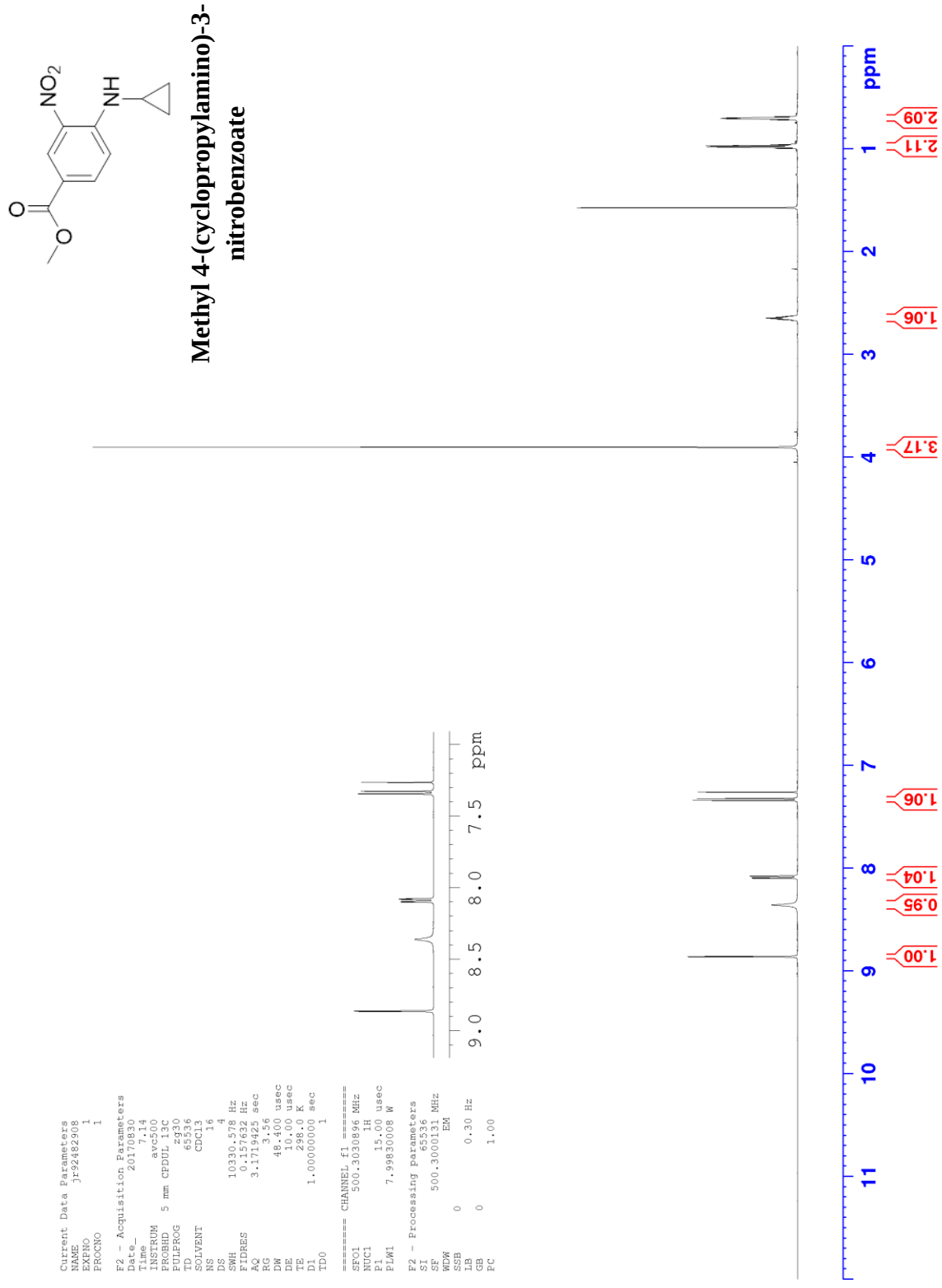




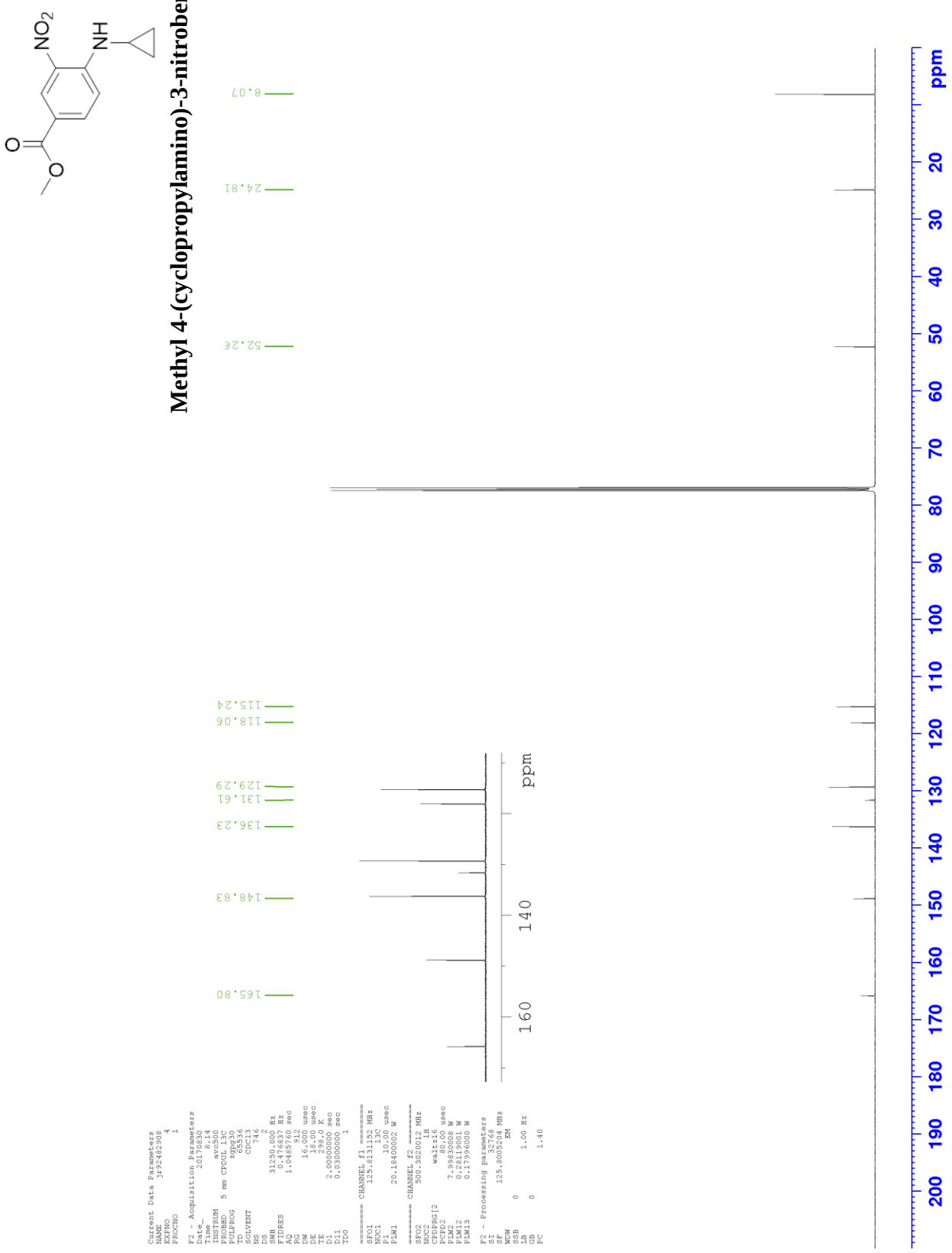
Calibration standard of 10 µM **25**
Cells treated with 10 µM **25**

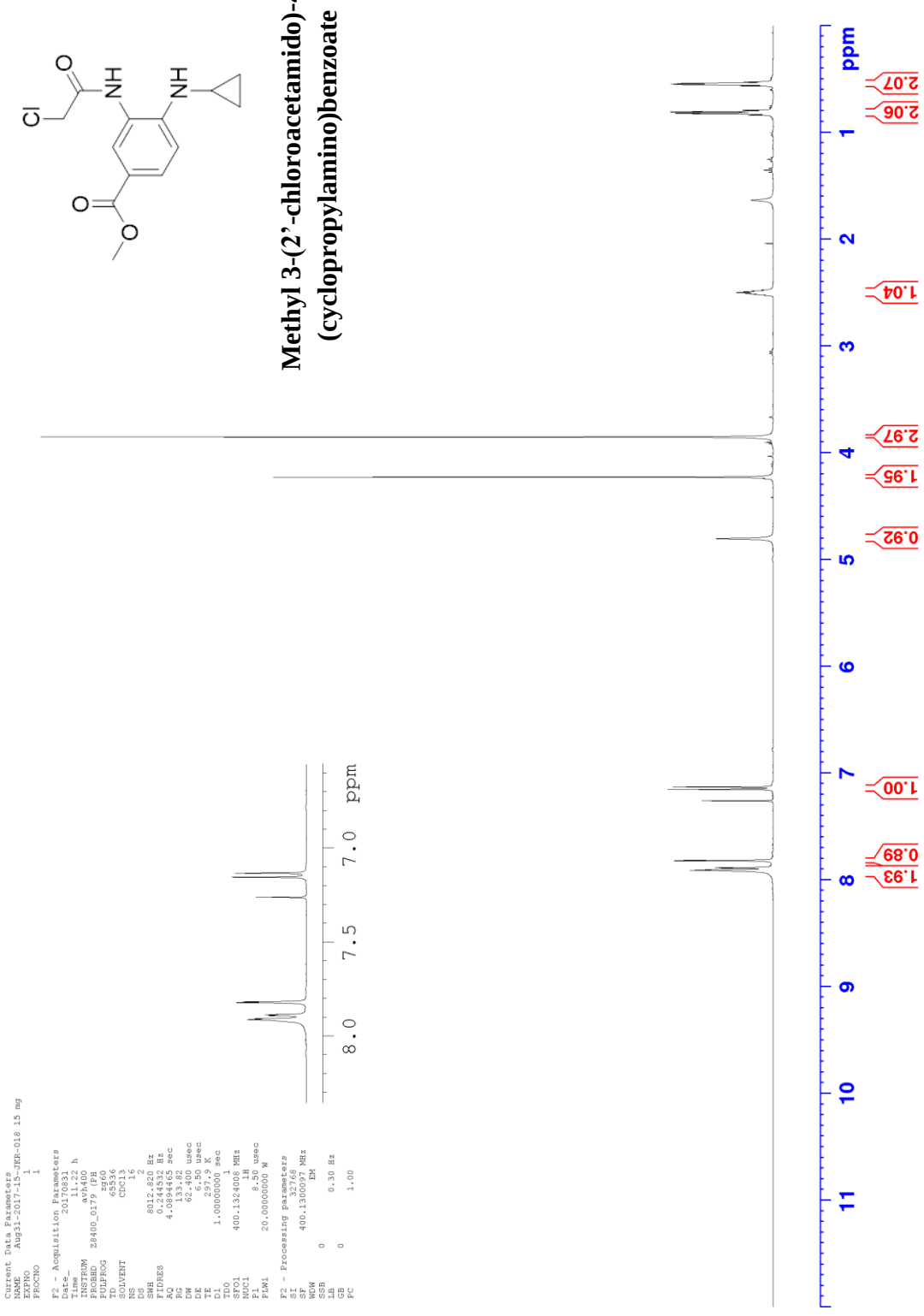
Appendix J: NMR Spectra of Novel Compounds

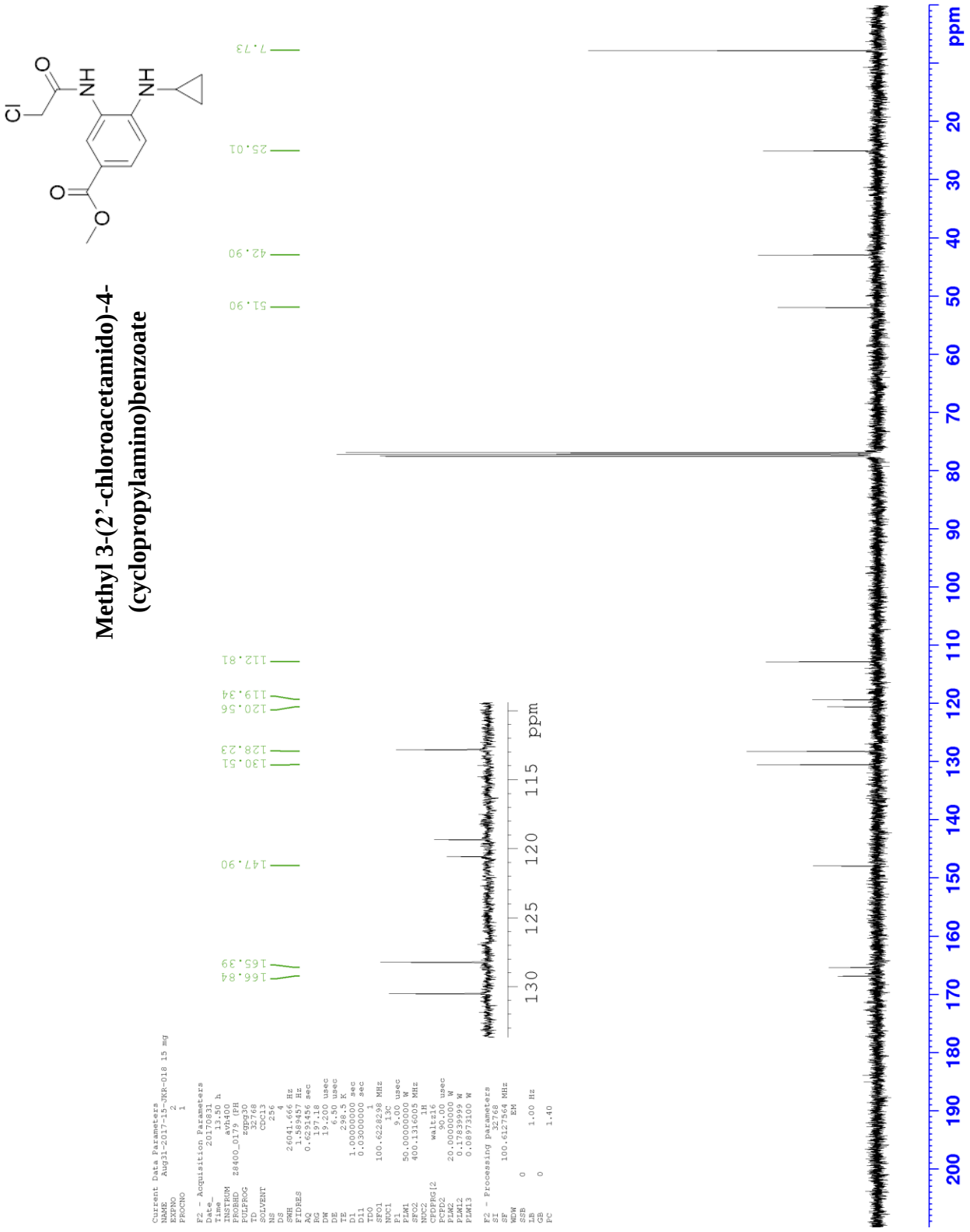
Chapter 2

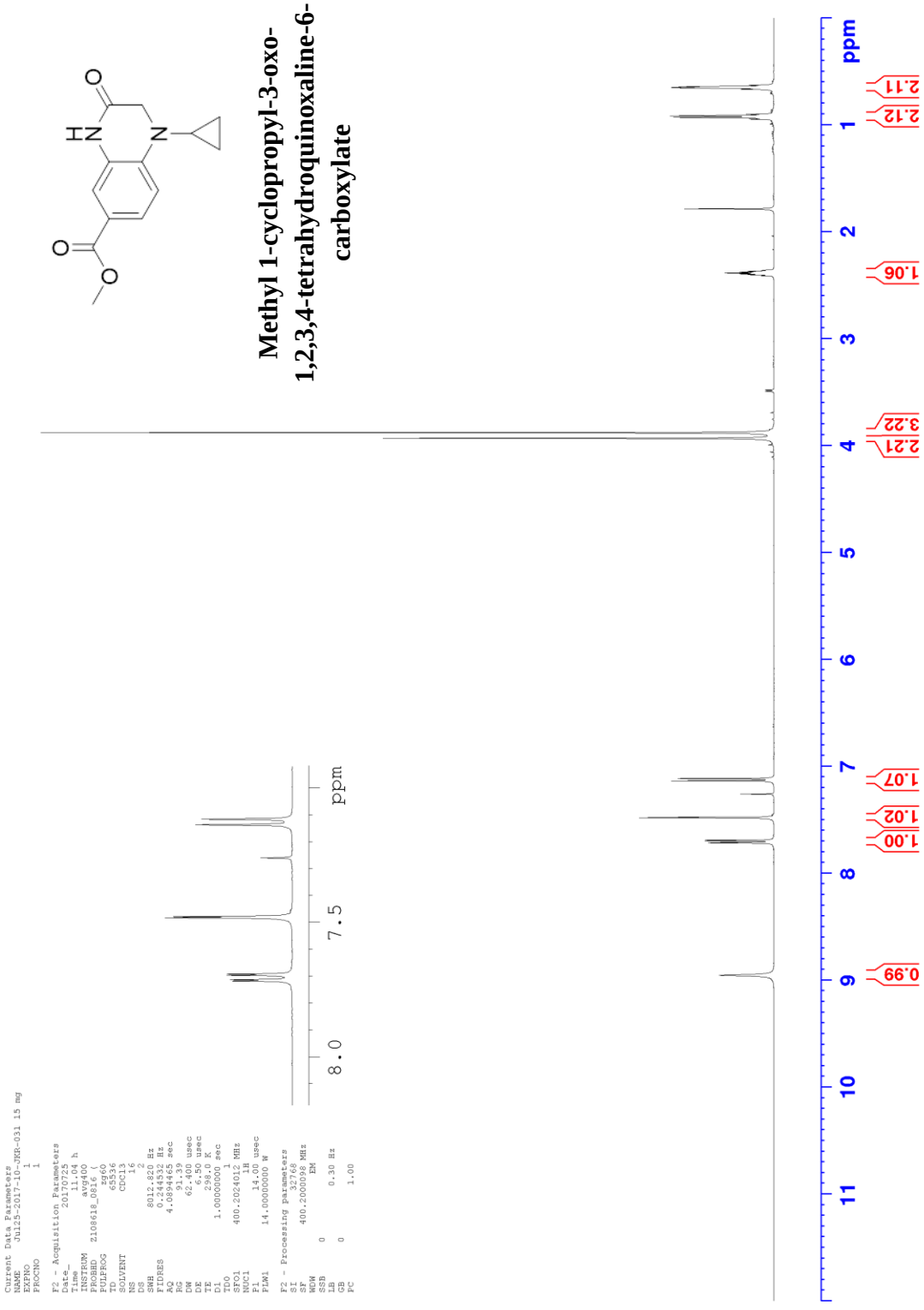

Methyl 4-(cyclopropylamino)-3-nitrobenzoate
NO2
NH
O
O
Current Data Parameters
NAME jr92482908
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170830
Time 7.14
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000131 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
9.0
8.5
8.0
7.5
ppm
1.00
0.95
1.04
1.06
1.06
3.17
2.11
2.09
ppm

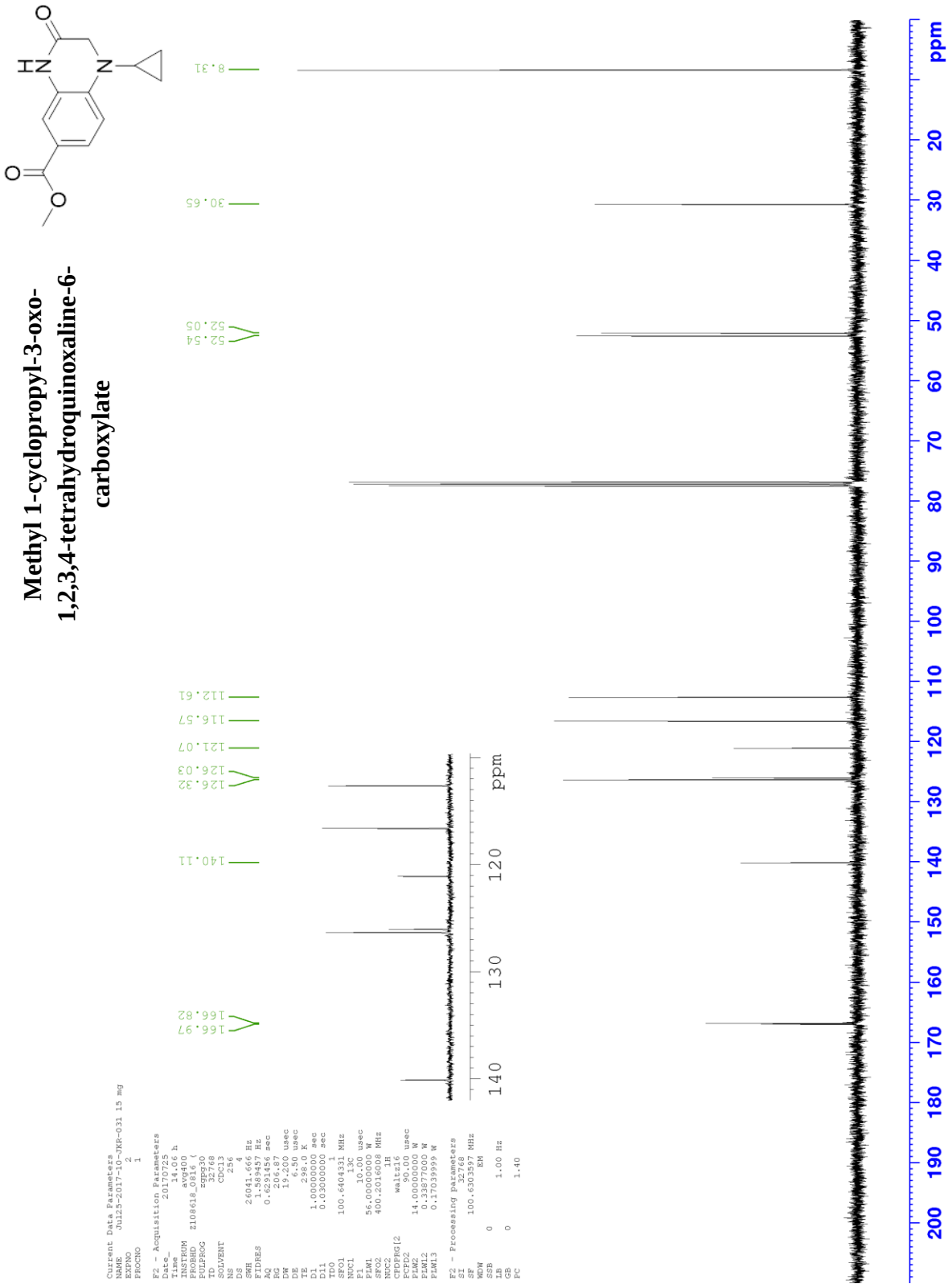
Methyl 4-(cyclopropylamino)-3-nitrobenzoate

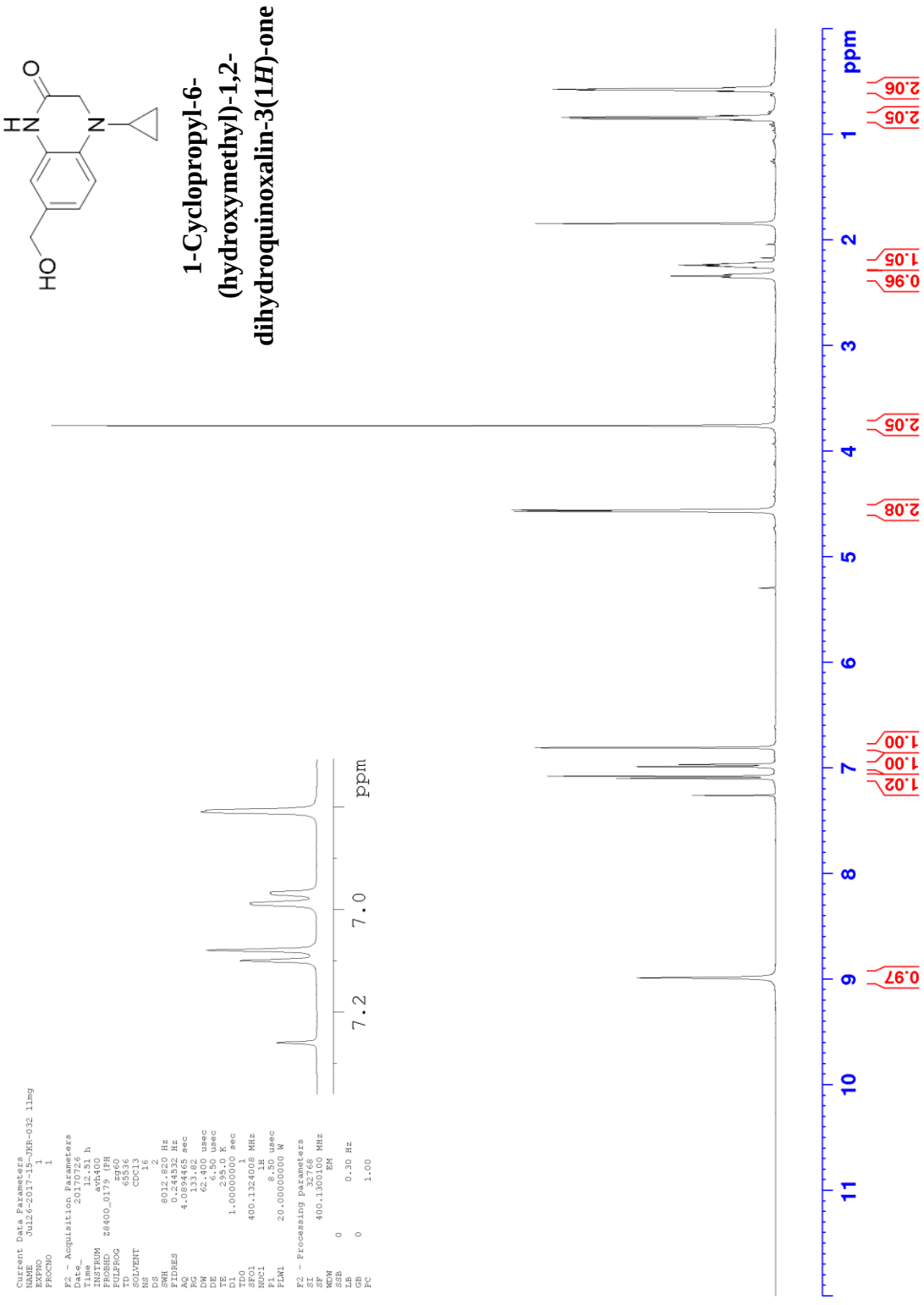



Methyl 3-(2'-chloroacetamido)-4-(cyclopropylamino)benzoate
Cl
O
NH
NH
O
O
ppm
2.07
2.06
1.04
2.97
1.95
0.92
1.00
1.93
0.89
8.0
7.5
7.0
Current Data Parameters
NAME 28400_0179
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170831
Time 11.22 h
INSTRUM avh400
PROBHD 28400_0179 (PH
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894464 sec
RG 133.82
DW 62.400 usec
DE 6.50 usec
TE 297.3 K
D1 1.00000001 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 8.65 usec
PLW1 20.00000000 W
F2 - Processing parameters
SI 32768
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

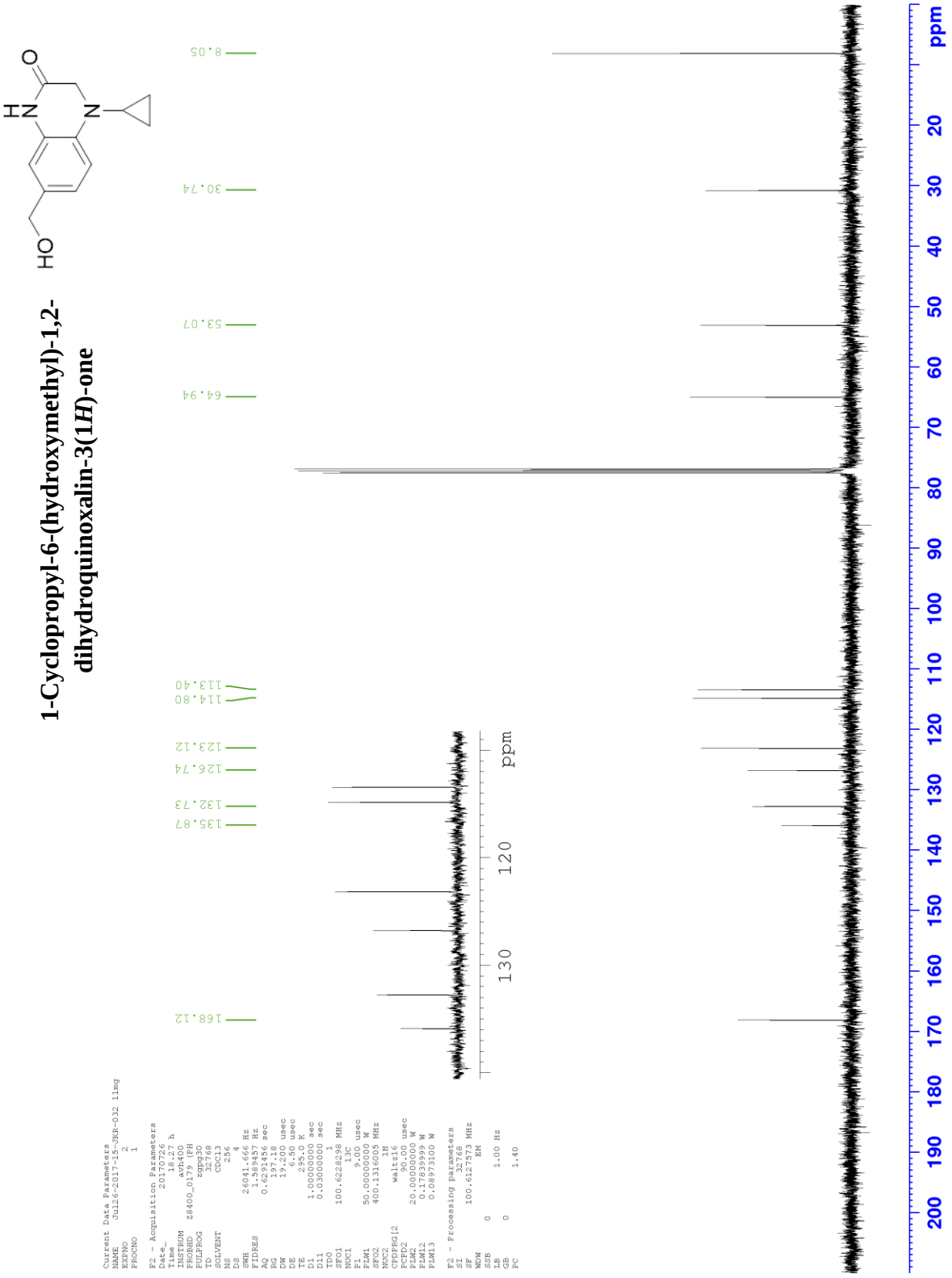

Methyl 3-(2'-chloroacetamido)-4-(cyclopropylamino)benzoate
Cl
O
NH
NH
O
O
Current Data Parameters
NAME Aug31-2017-15-JKR-018 15 mg
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170831
Time 13.30 h
INSTRUM spect
PROBHD Z8400_0179 (PH
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 256
DS 4
SWH 26041.666 Hz
FIDRES 1.589457 Hz
AQ 0.6291456 sec
RG 197.18
DW 19.200 usec
DE 6.50 usec
TE 298.5 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 100.6228298 MHz
NUC1 13C
P1 9.00 usec
PLW1 50.00000000 W
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 20.00000000 W
PLW12 0.17839999 W
PLW13 0.08973100 W
F2 - Processing parameters
SI 32768
SF 100.6127564 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
7.73
25.01
42.90
51.90
112.81
119.34
120.56
128.23
130.51
147.90
165.39
166.84
130
125
120
115
ppm
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


Methyl 1-cyclopropyl-3-oxo-1,2,3,4-tetrahydroquinoxaline-6-carboxylate
Current Data Parameters
NAME Ju125-2017-10-DER-031 15 mg
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170725
Time 11.04 h
INSTRUM avg400
PROBHD 2108618_0216 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244482 Hz
AQ 4.0864462 sec
RG 91.39
DW 62.400 usec
DE 6.50 usec
TE 298.1 K
D1 1.0000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.0
7.5
ppm
ppm
11
10
9
8
7
6
5
4
3
2
1
0.99
1.00
1.02
1.07
2.21
3.22
1.06
2.12
2.11

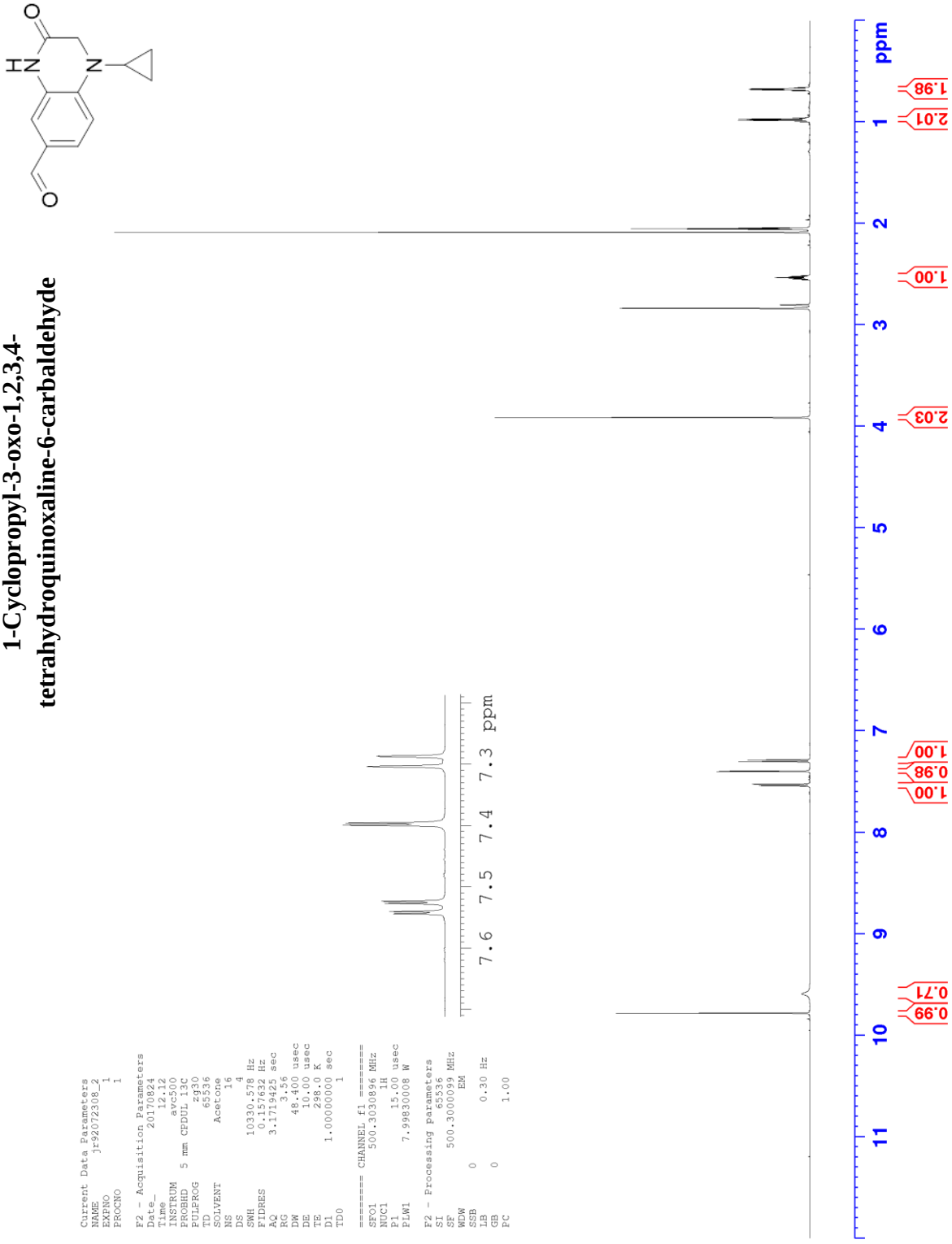

Methyl 1-cyclopropyl-3-oxo-1,2,3,4-tetrahydroquinoxaline-6-carboxylate
8.31
30.65
52.54
52.05
112.61
116.57
121.07
126.32
126.03
140.11
166.97
166.82
ppm


1-Cyclopropyl-6-(hydroxymethyl)-1,2-dihydroquinoxalin-3(1H)-one
Current Data Parameters
NAME Ju126-2017-15-ZKR-032 11mg
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170726
Time 12.51 h
INSTRUM spect
PROBHD Z8400_0179 (PH
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 13.02
DW 62.400 usec
DE 6.50 usec
TE 295.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 8.50 usec
PLW1 20.00000000 W
F2 - Processing parameters
SI 32768
SF 400.1300100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.2
7.0
ppm
0.97
1.02
1.00
1.00
2.08
2.05
0.96
1.05
2.05
2.06
ppm

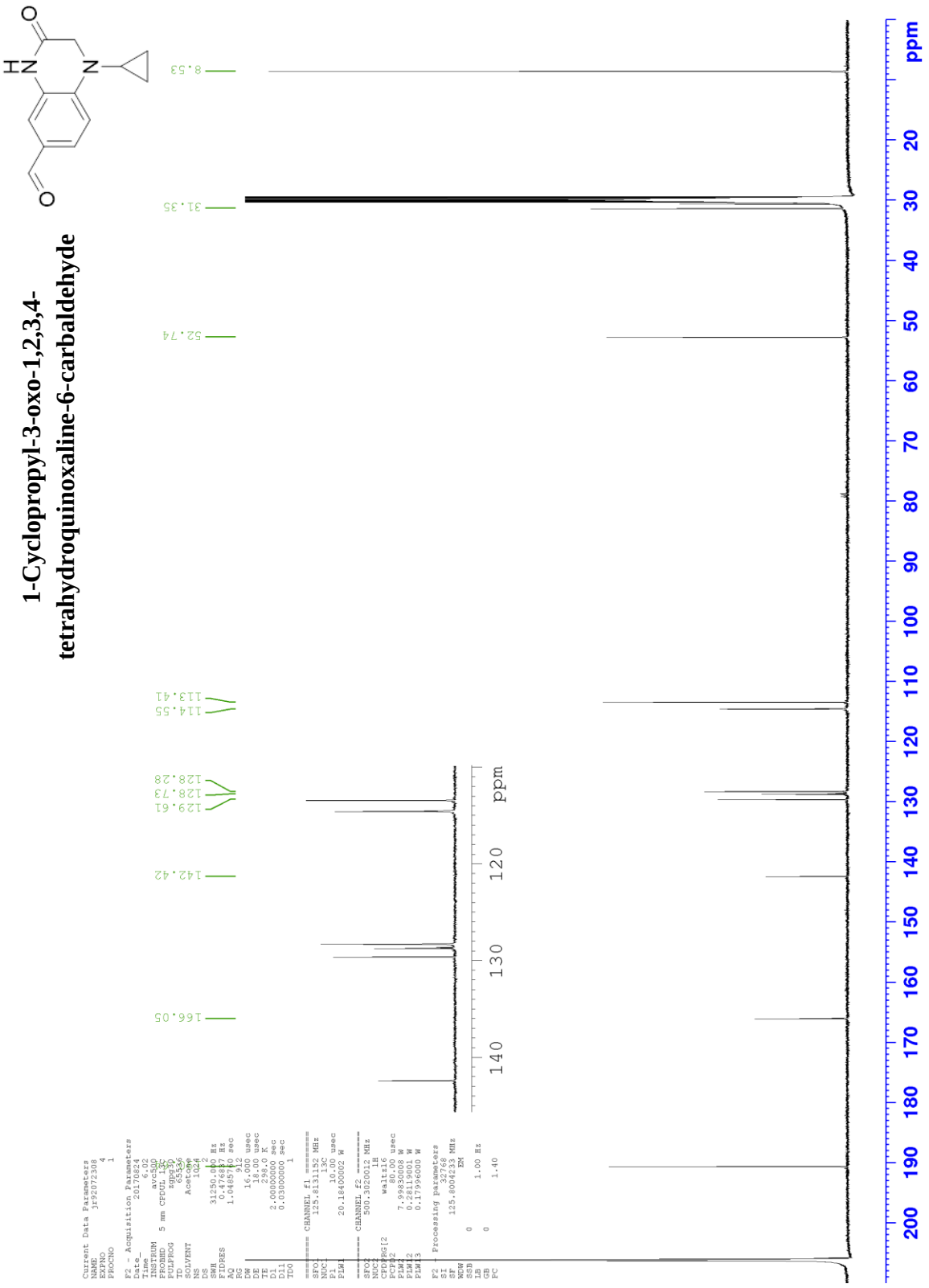
1-Cyclopropyl-6-(hydroxymethyl)-1,2-dihydroquinoxalin-3(1*H*)-one

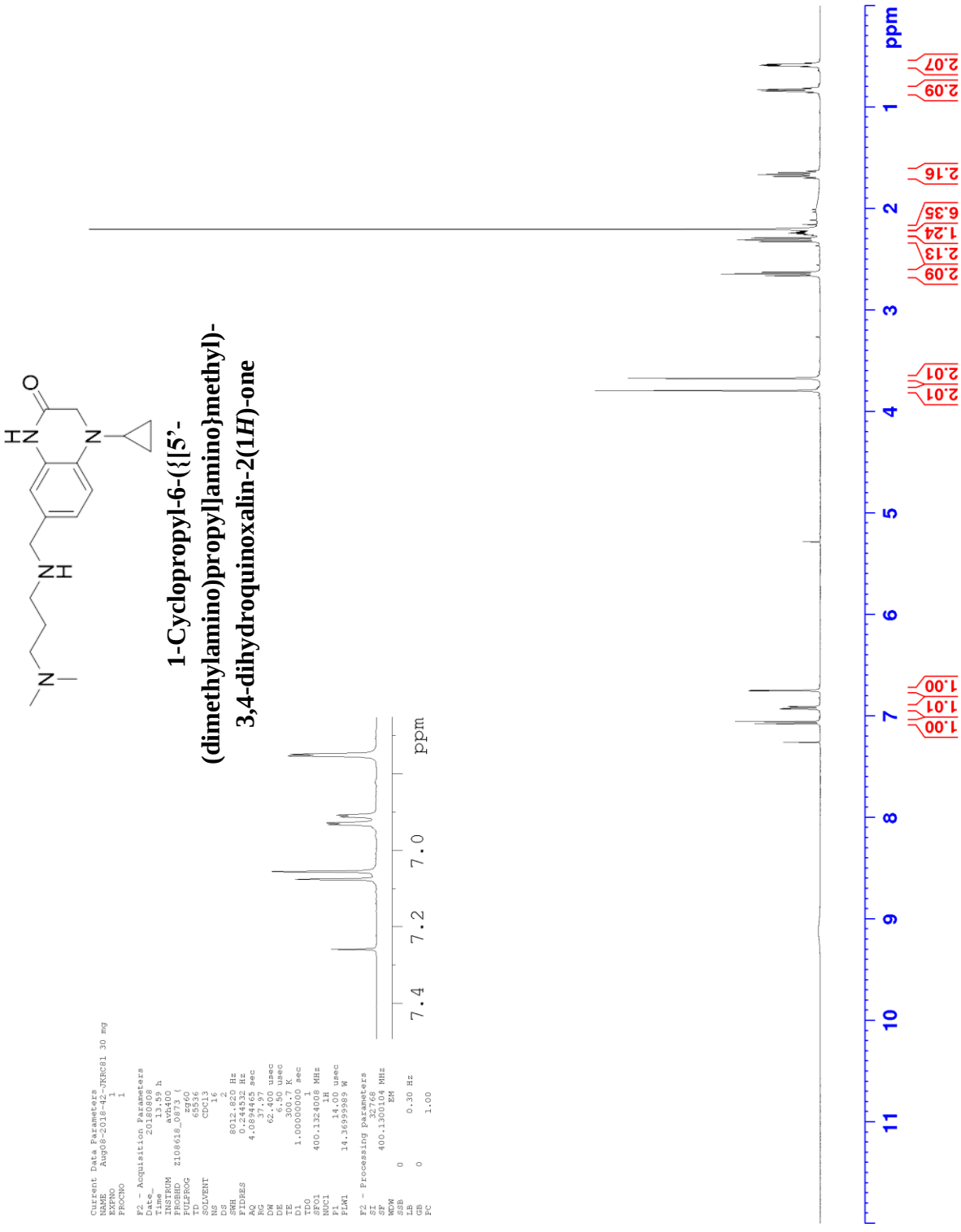


1-Cyclopropyl-3-oxo-1,2,3,4-tetrahydroquinoxaline-6-carbaldehyde

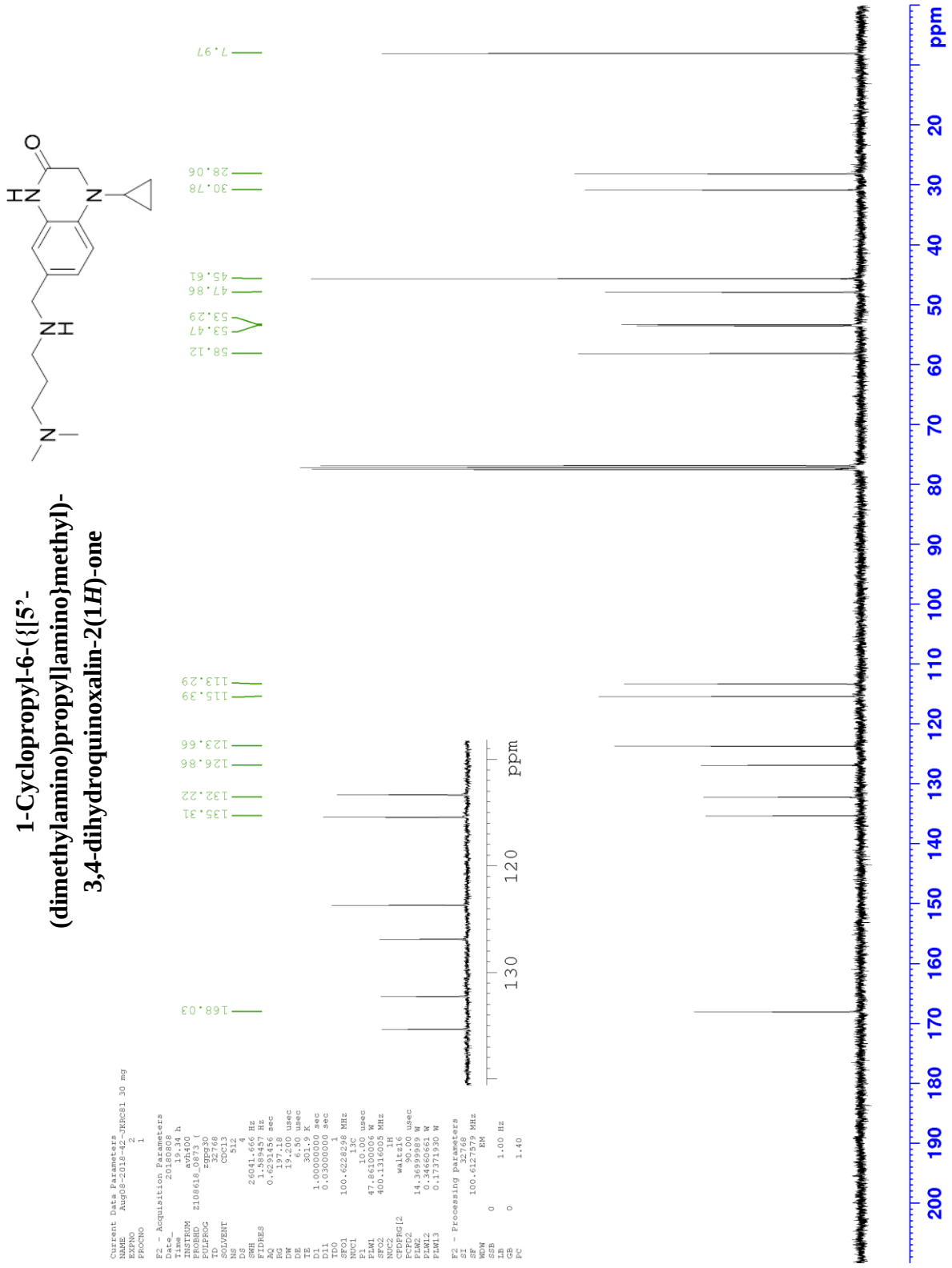


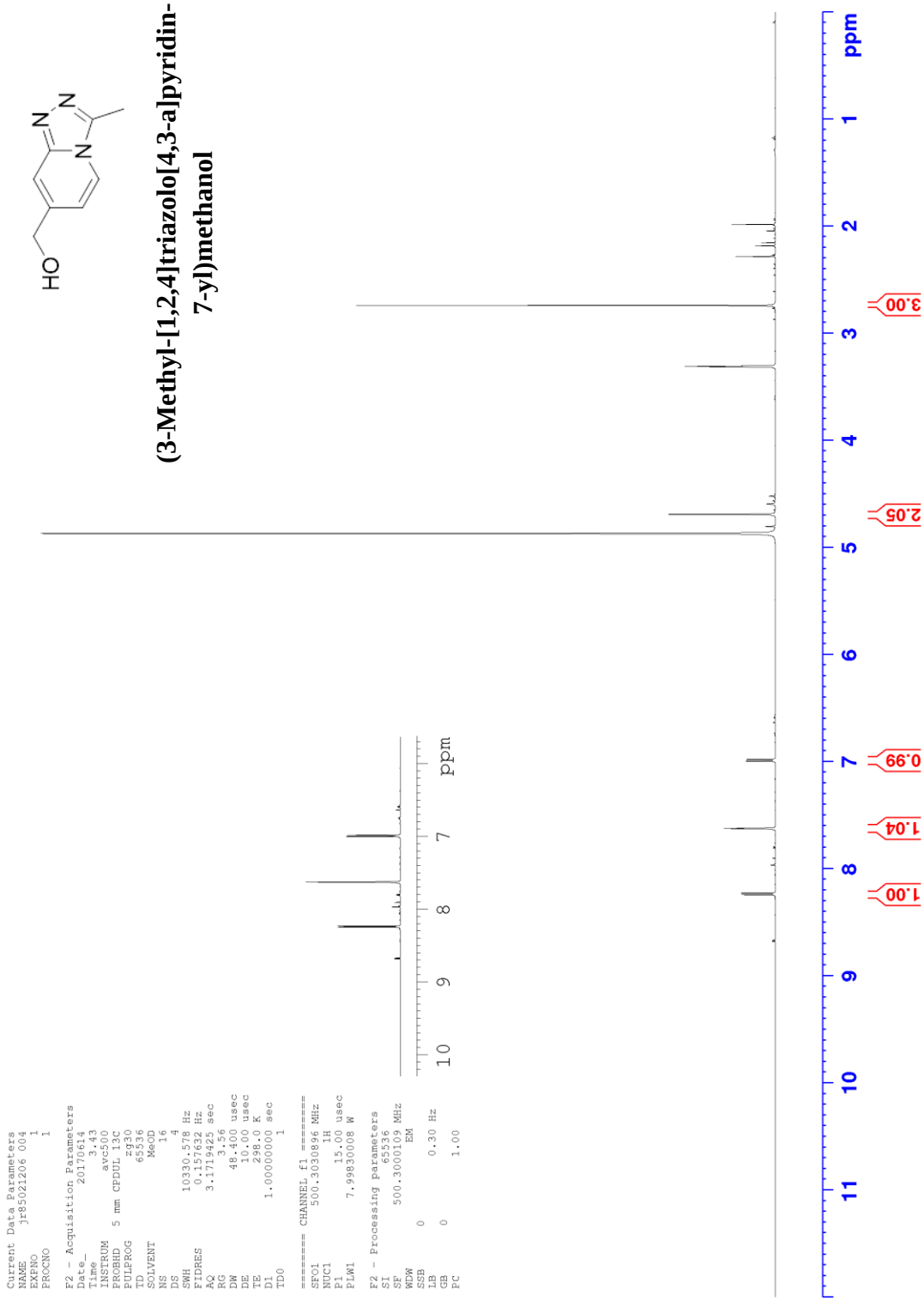
1-Cyclopropyl-3-oxo-1,2,3,4-tetrahydroquinoxaline-6-carbaldehyde



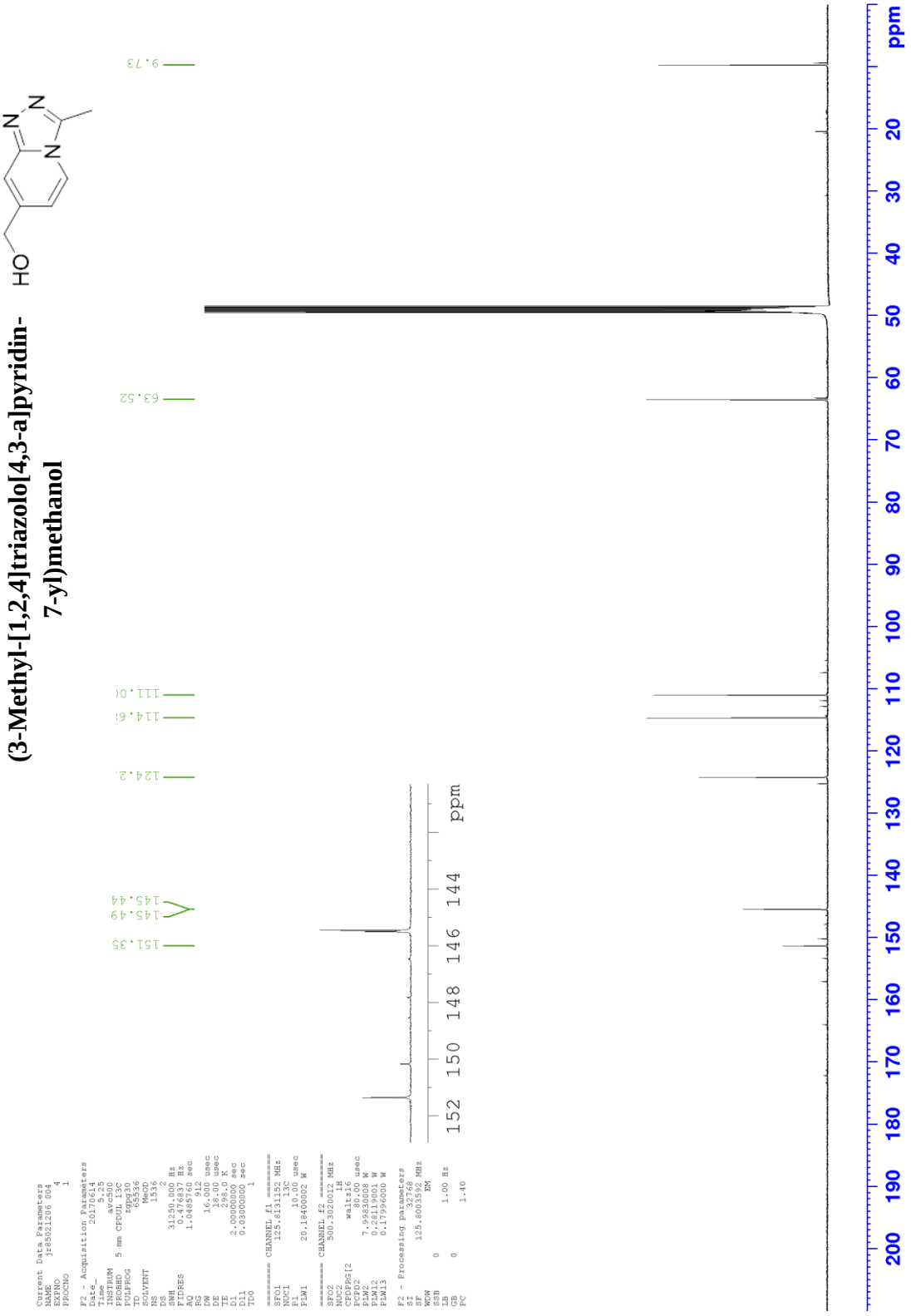

1-Cyclopropyl-6-({[5'-(dimethylamino)propyl]amino}methyl)-3,4-dihydroquinoxalin-2(1H)-one
Current Data Parameters
NAME Aug08-2018-42-ZKRC81 30 mg
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180808
Time 13.59 h
INSTRUM spect
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 37.97
DW 62.400 usec
DE 6.50 usec
TE 300.7 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300104 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.4
7.2
7.0
ppm
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
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2.16
2.09
2.07

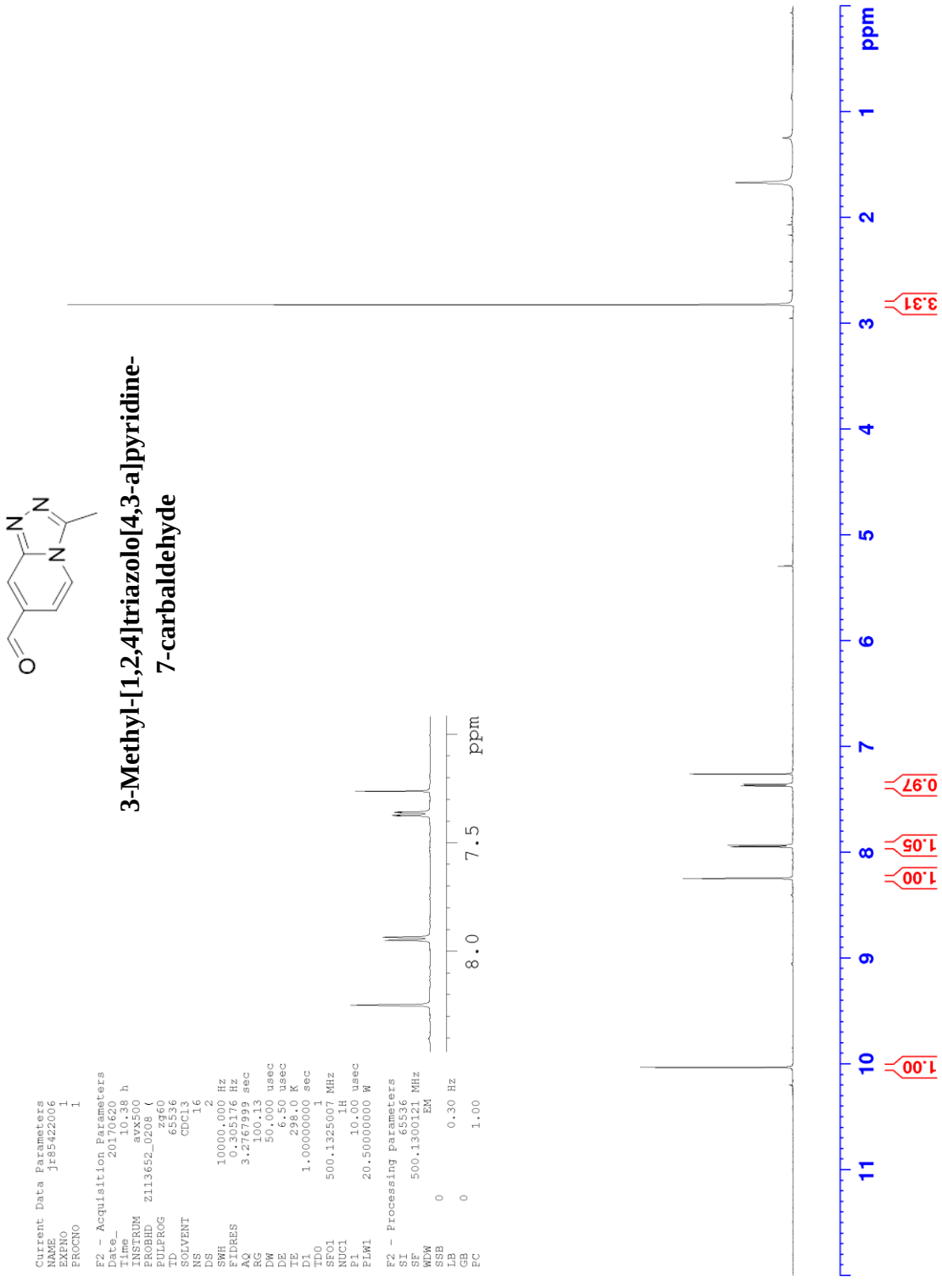
1-Cyclopropyl-6-({[5'-(dimethylamino)propyl]amino}methyl)-3,4-dihydroquinoxalin-2(1*H*)-one

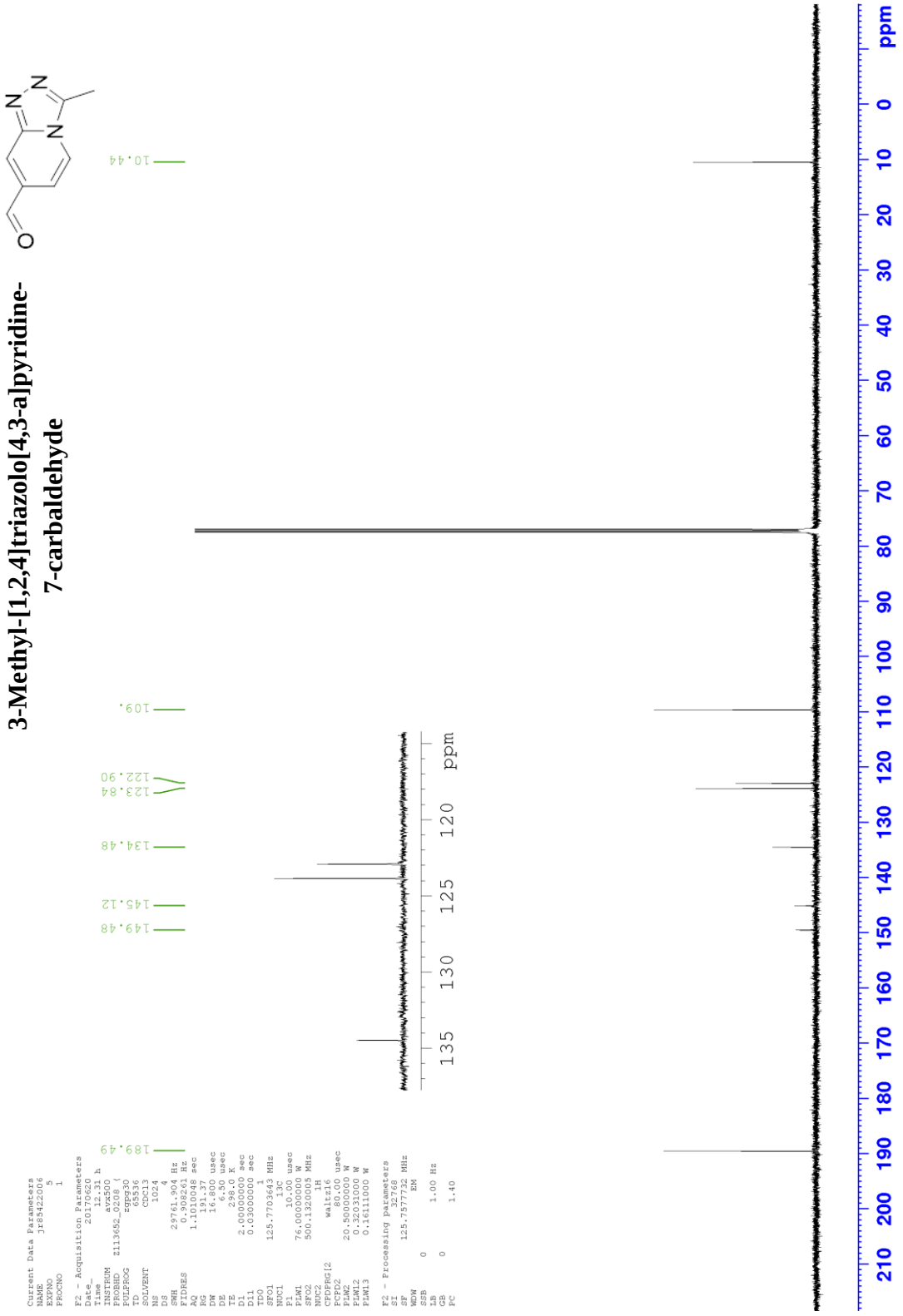


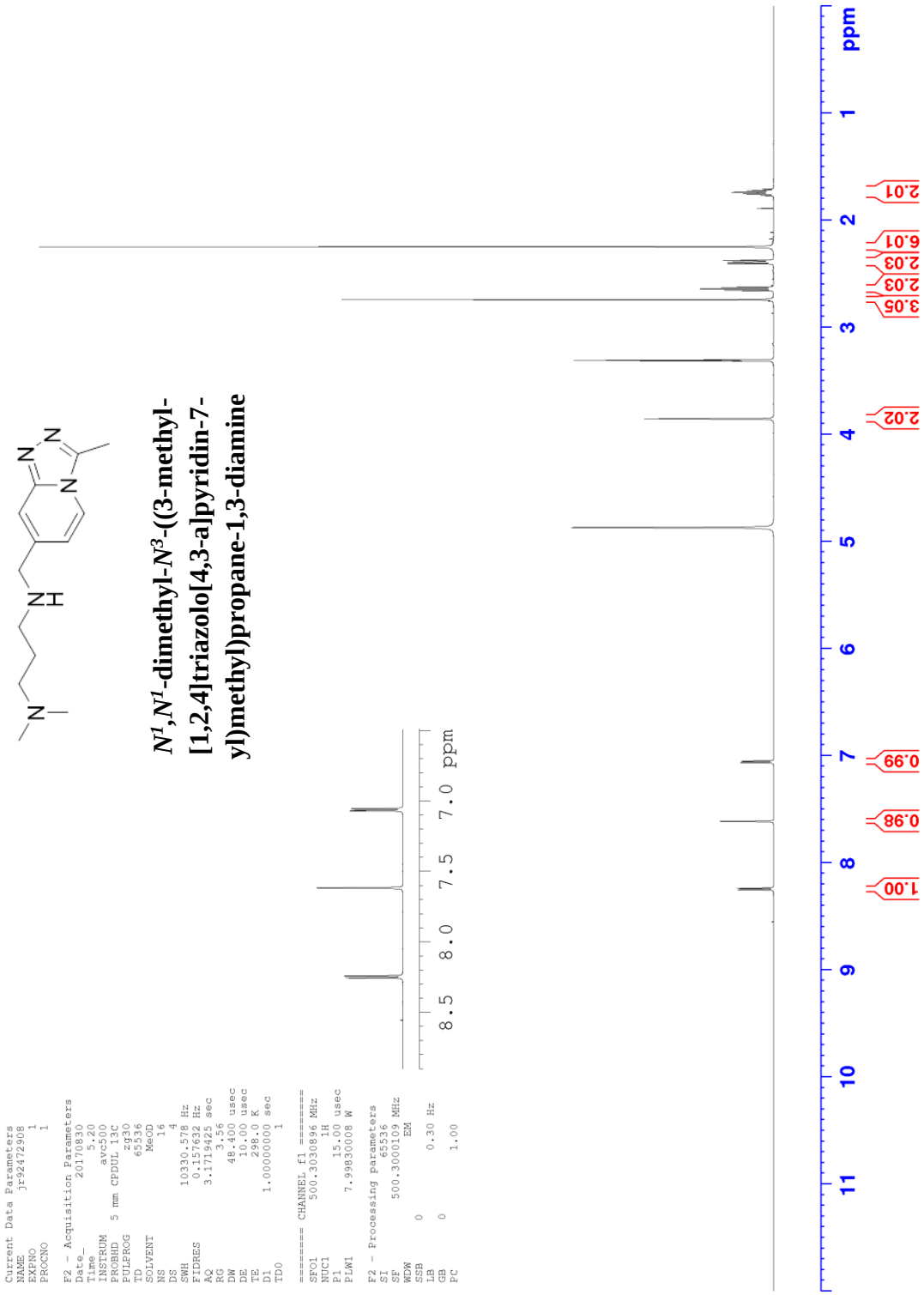

(3-Methyl-[1,2,4]triazolo[4,3-a]pyridin-7-yl)methanol
HO
N
N
N
Current Data Parameters
NAME jr85021206 004
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170614
Time 3.43
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000109 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
10
9
8
7
ppm
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.04
0.99
2.05
3.00

(3-Methyl-[1,2,4]triazolo[4,3-a]pyridin-7-yl)methanol



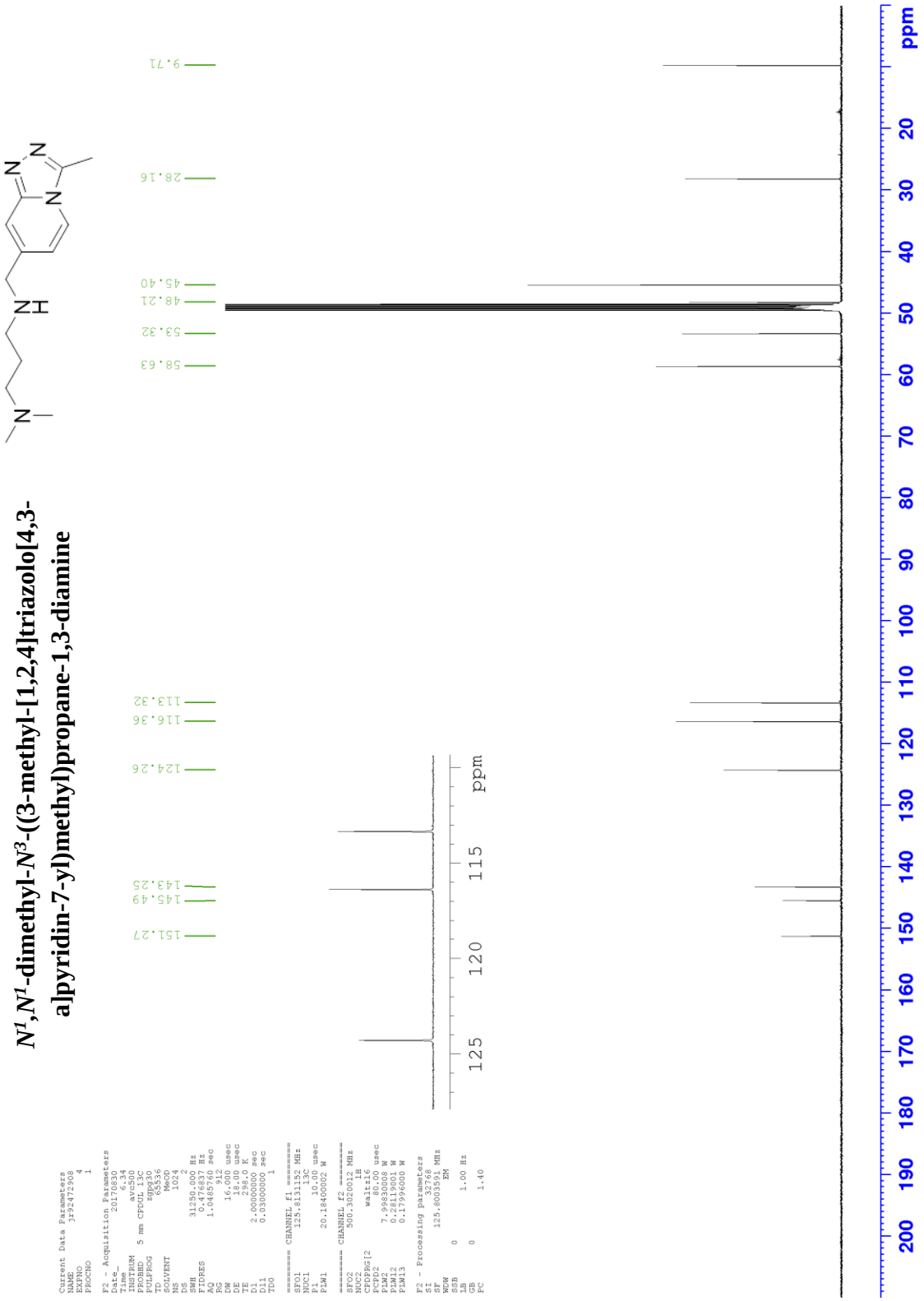

3-Methyl-[1,2,4]triazolo[4,3-a]pyridine-7-carbaldehyde
Current Data Parameters
NAME jr85422006
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170620
Time_ 10.38 h
INSTRUM avx500
PROBHD Z113652_0208 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 100.13
DW 50.000 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
SFO1 500.1325007 MHz
NUC1 1H
P1 10.00 usec
PLW1 20.50000000 W
F2 - Processing parameters
SI 65536
SF 500.1300121 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.0
7.5
ppm
1.00
1.00
1.05
0.97
3.31
11
10
9
8
7
6
5
4
3
2
1
ppm

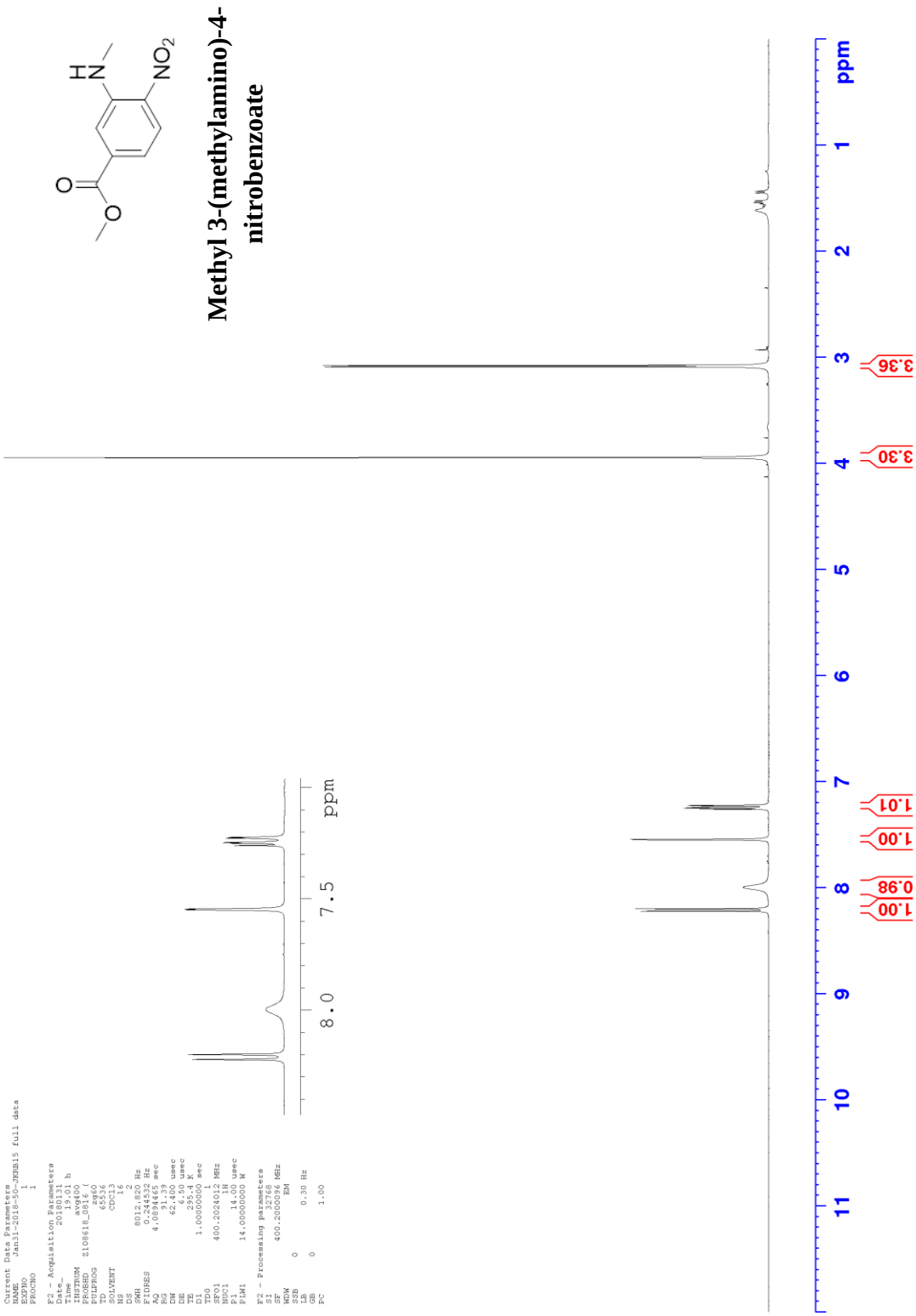

3-Methyl-[1,2,4]triazolo[4,3-a]pyridine-7-carbaldehyde
Current Data Parameters
NAME jr85422606
EXPNO 5
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170620
Time 12.31 h
INSTRUM spect
PROBHD Z113652_0208 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 4
SWH 29761.904 Hz
FIDRES 0.909261 Hz
AQ 1.1010048 sec
RG 181.37
DW 16.800 usec
DE 6.50 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7703643 MHz
NUC1 13C
P1 10.00 usec
PLW1 76.00000000 W
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 20.50000000 W
PLW12 0.32031000 W
PLW13 0.16111000 W
F2 - Processing parameters
SI 32768
SF 125.7577732 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
189.49
149.48
145.12
134.48
123.84
122.90
109.
10.44
135 130 125 120 ppm
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm



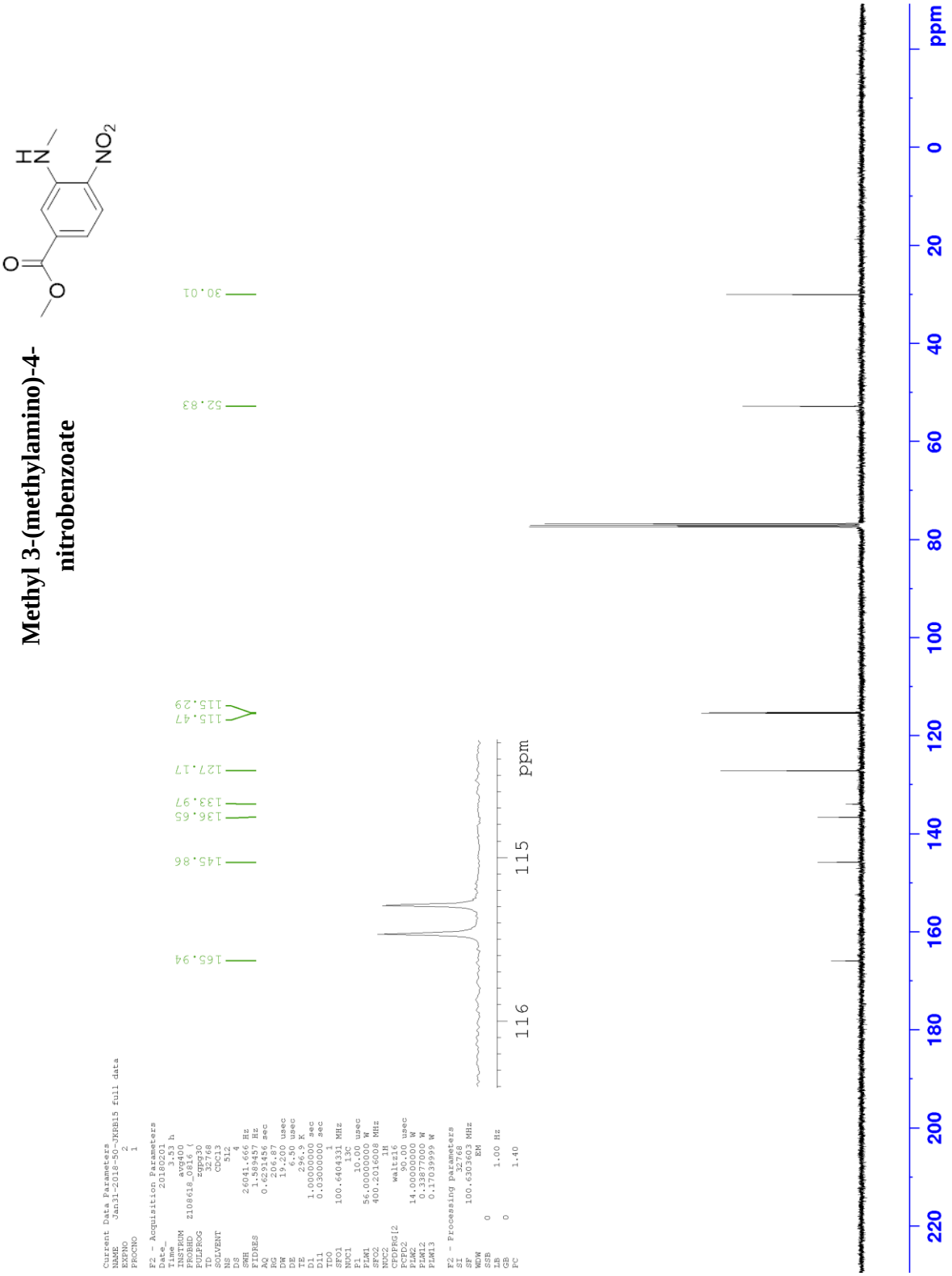
***N*^{1},*N*^{1}-dimethyl-*N*^{3}-((3-methyl-[1,2,4]triazolo[4,3-a]pyridin-7-yl)methyl)propane-1,3-diamine**

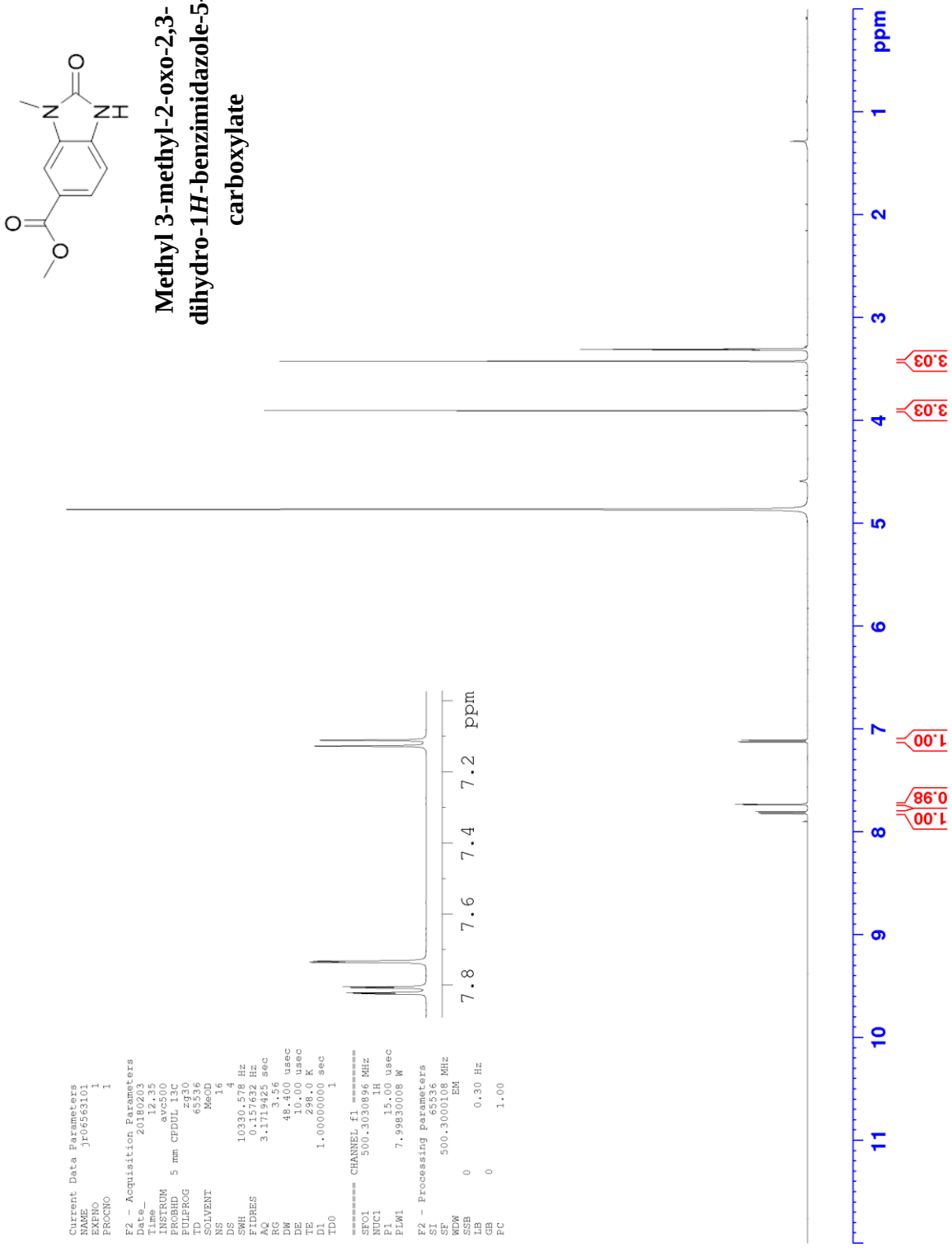
N^1,N^1-dimethyl-N^3-((3-methyl-[1,2,4]triazolo[4,3-a]pyridin-7-yl)methyl)propane-1,3-diamine

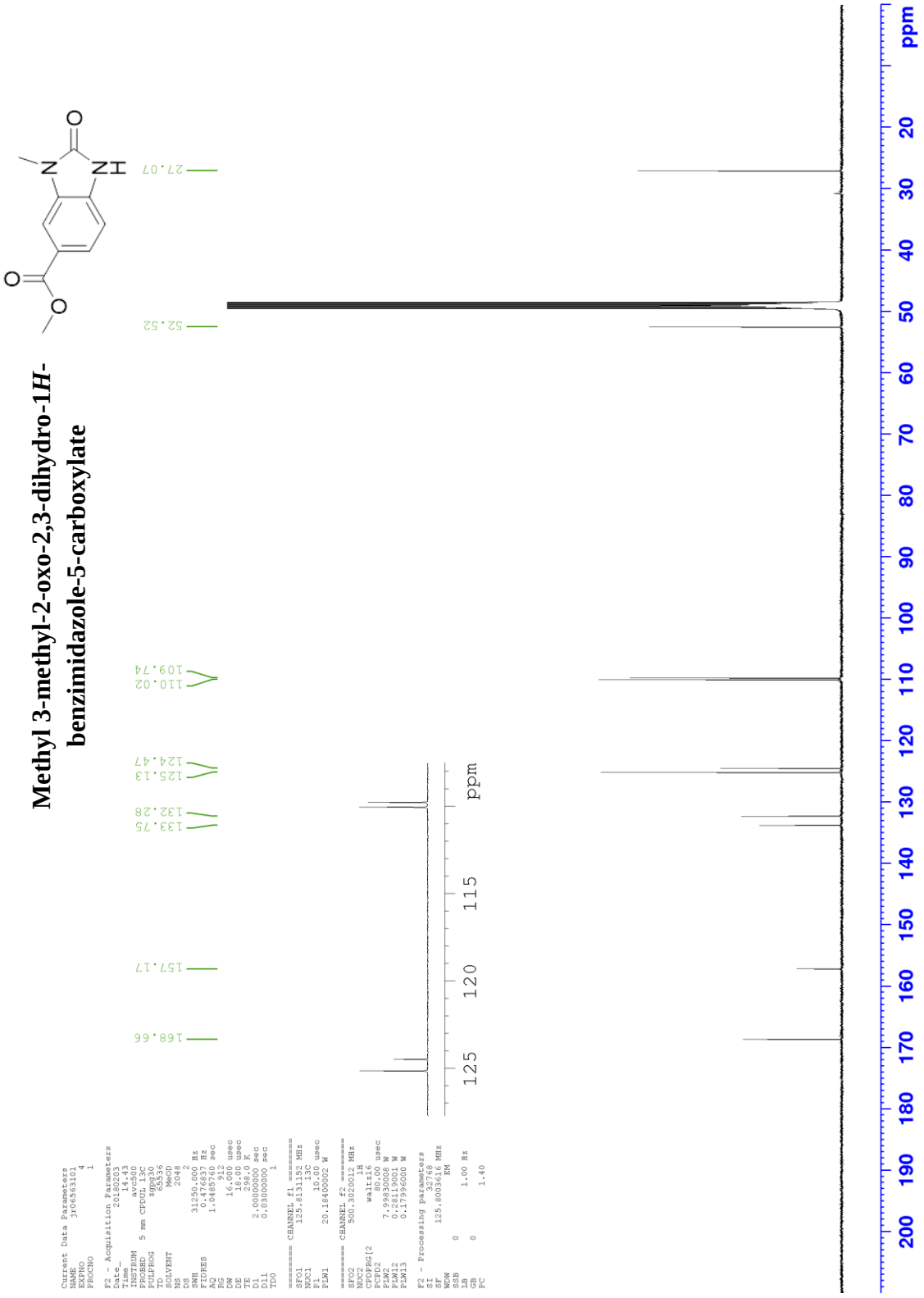


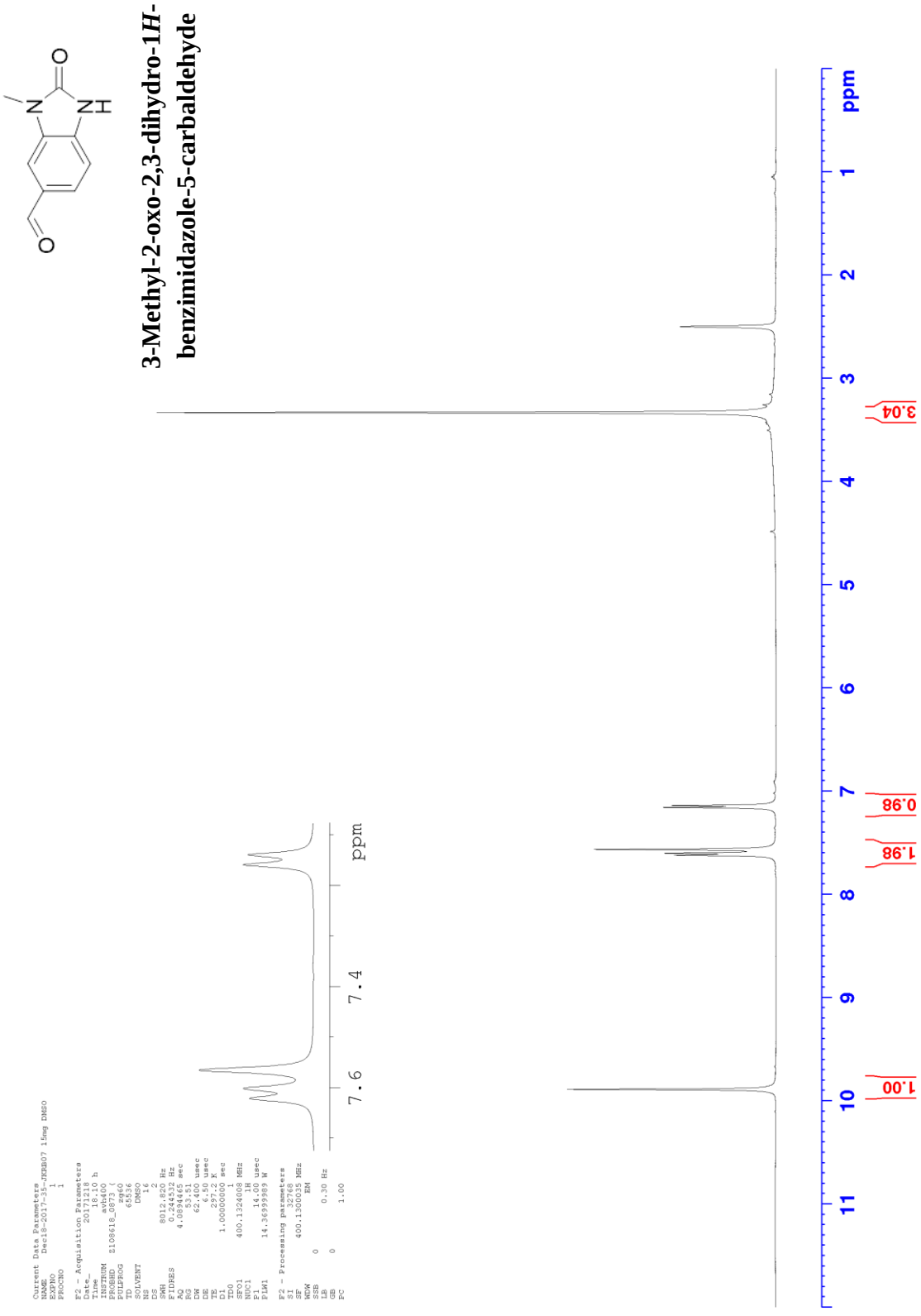

Methyl 3-(methylamino)-4-nitrobenzoate
Current Data Parameters
NAME Jan31-2018-SO_JKPS815 full data
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180131
Time 19.01 h
INSTRUM avg400
PROBHD 5 mm PABBO BB/
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244729 Hz
AQ 4.0894465 sec
RG 91.39
DW 62.400 usec
DE 6.50 usec
TE 295.4 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000056 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.0
7.5
ppm
O
O
H
N
NO2
3.36
3.30
1.01
1.00
0.98
1.00
ppm
1
2
3
4
5
6
7
8
9
10
11

Methyl 3-(methylamino)-4-nitrobenzoate

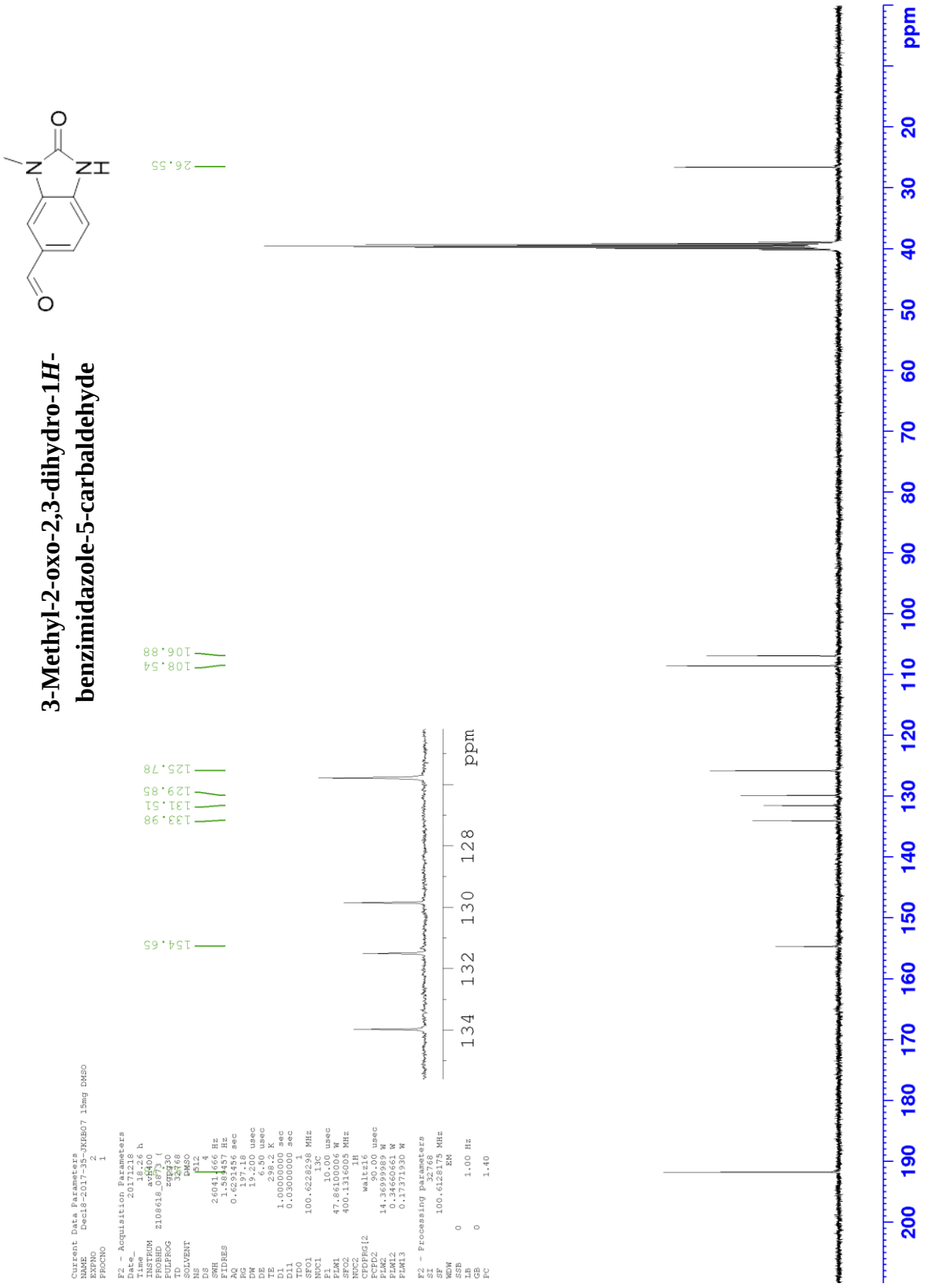


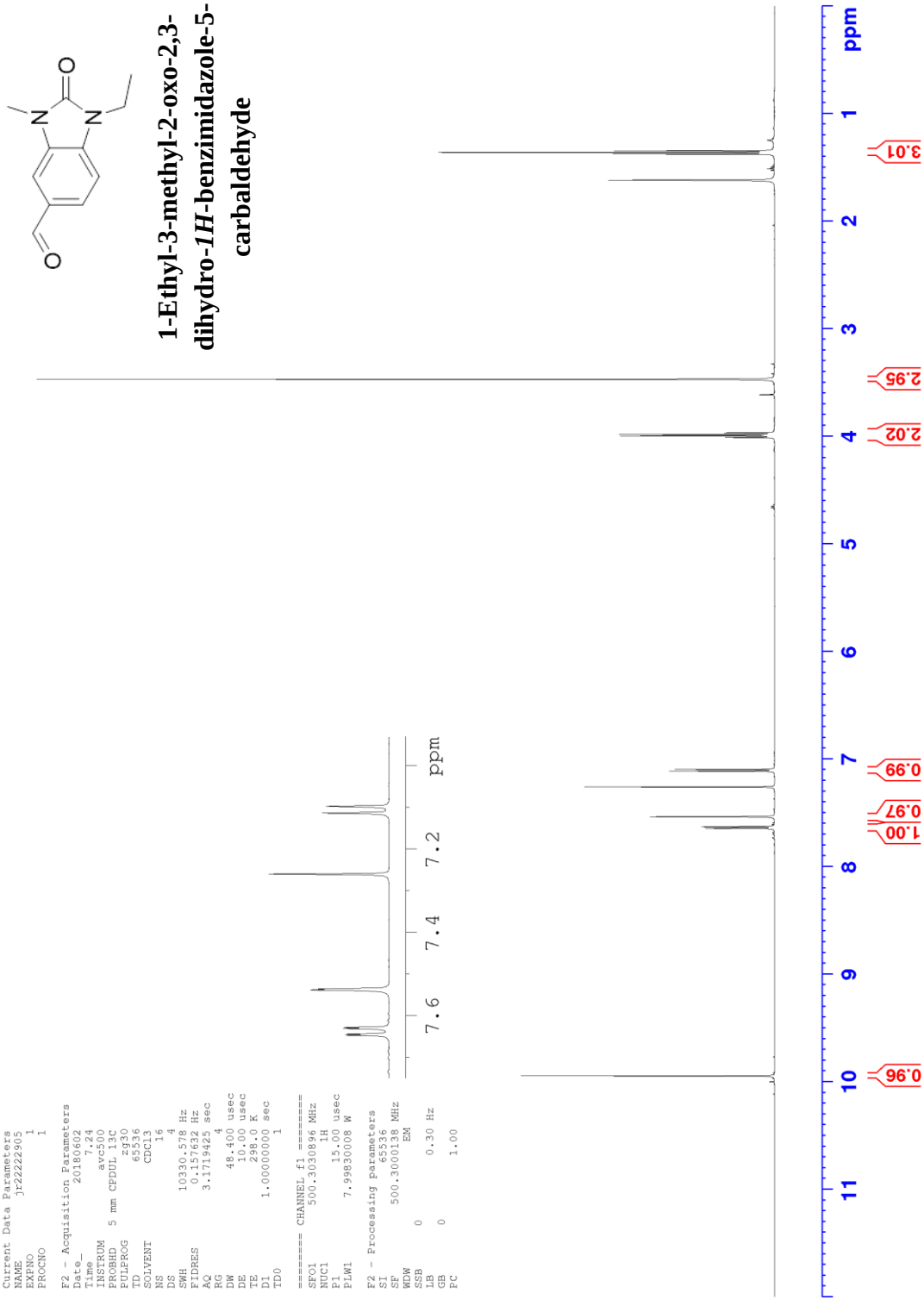

Methyl 3-methyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
Current Data Parameters
NAME jr06563101
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180203
Time 12.35
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000108 MHz
WDW EM
SSB 0 0.30 Hz
LB
GB 0
PC 1.00
7.8
7.6
7.4
7.2
ppm
3.03
3.03
1.00
0.98
1.00
11
10
9
8
7
6
5
4
3
2
1
ppm


Methyl 3-methyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
27.07
52.52
109.74
110.02
124.47
125.13
132.28
133.75
157.17
168.66
ppm

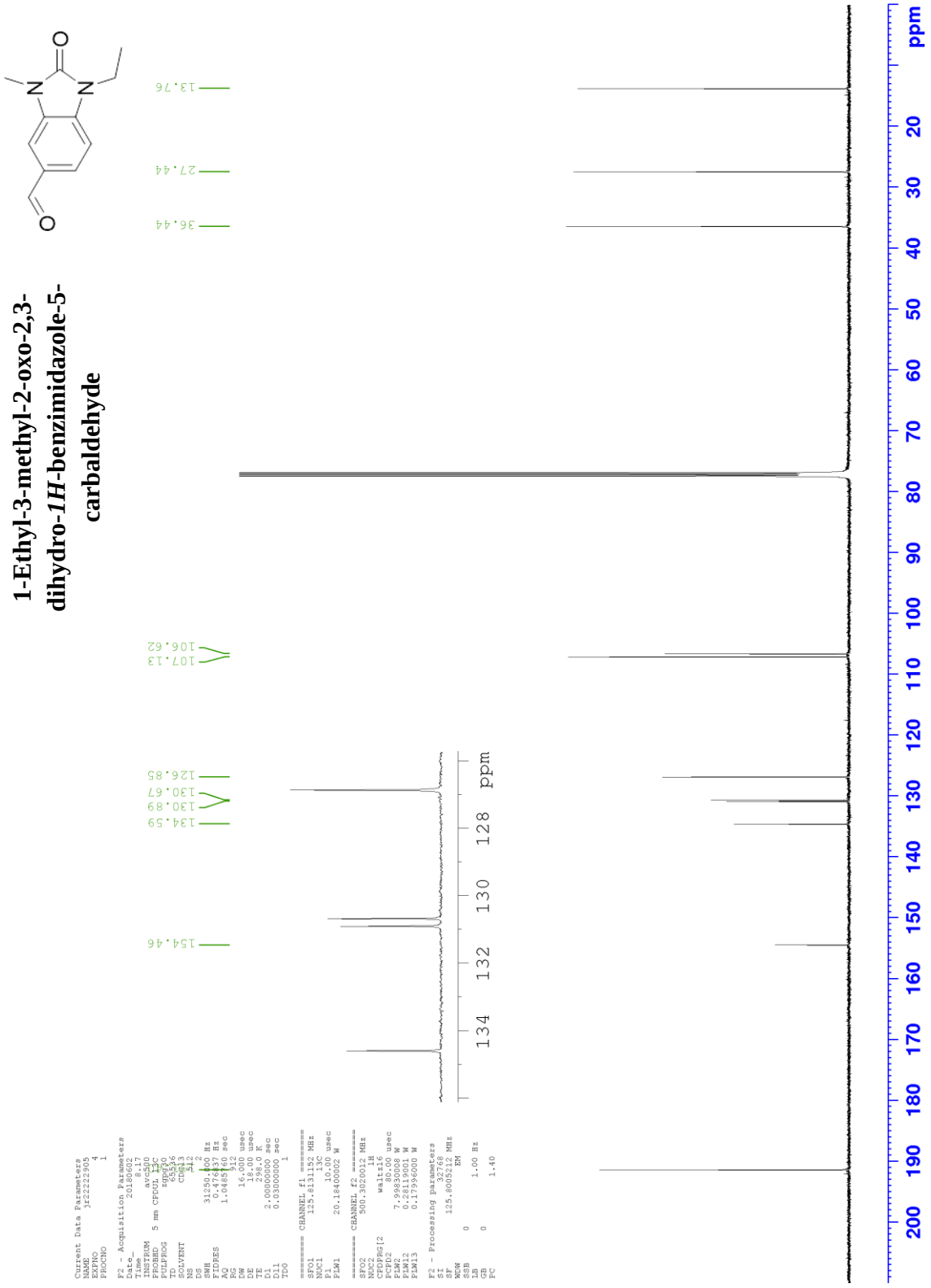

3-Methyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carbaldehyde
3.04
0.98
1.98
1.00
ppm

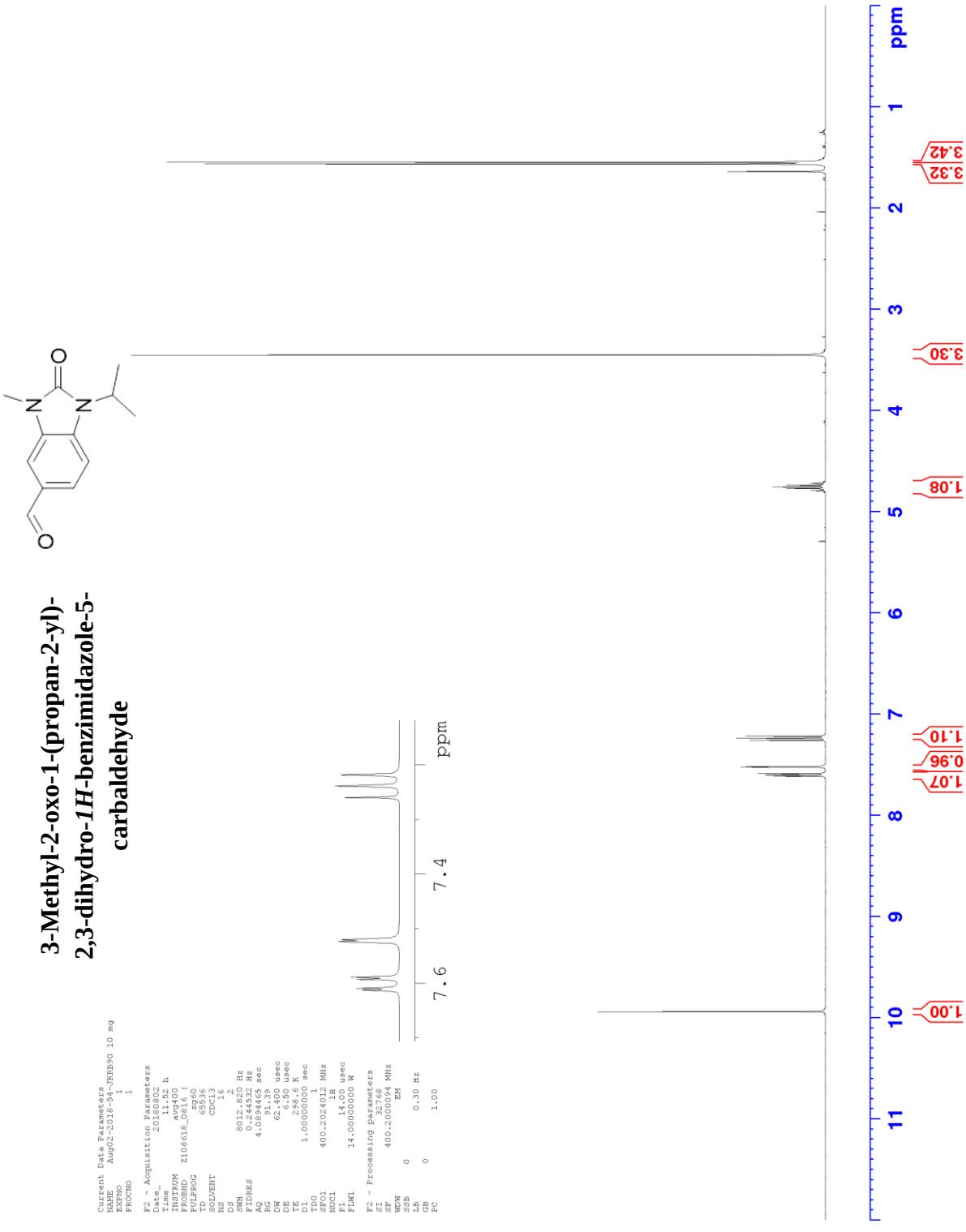
3-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde

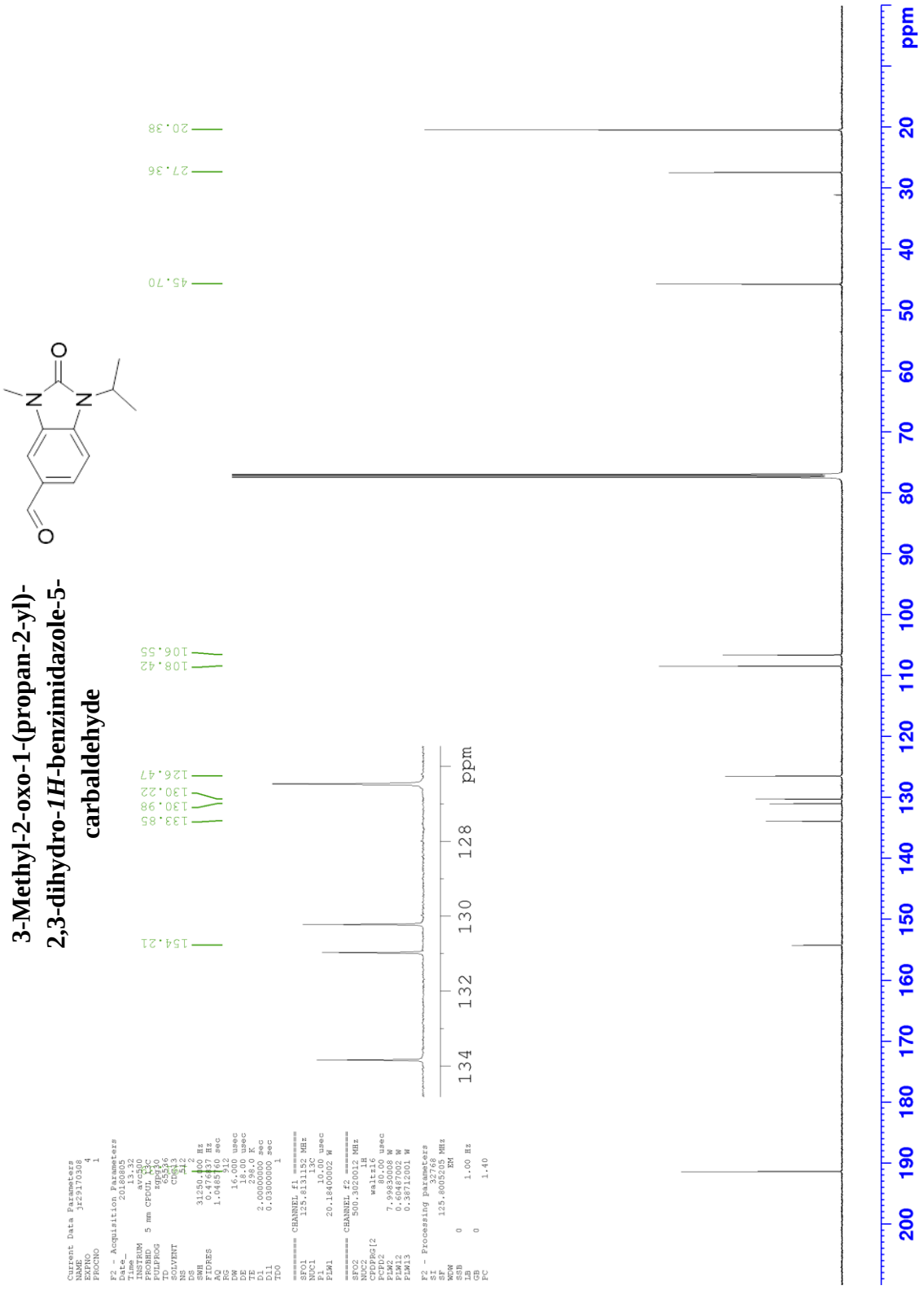



1-Ethyl-3-methyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carbaldehyde
Current Data Parameters
NAME jr22222905
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180602
Time 7.24
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000138 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.6
7.4
7.2
ppm
0.96
1.00
0.97
0.99
2.02
2.95
3.01
11
10
9
8
7
6
5
4
3
2
1
ppm

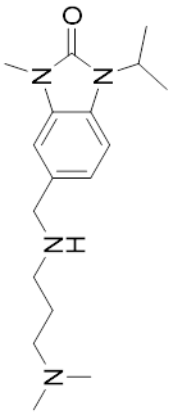
1-Ethyl-3-methyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carbaldehyde



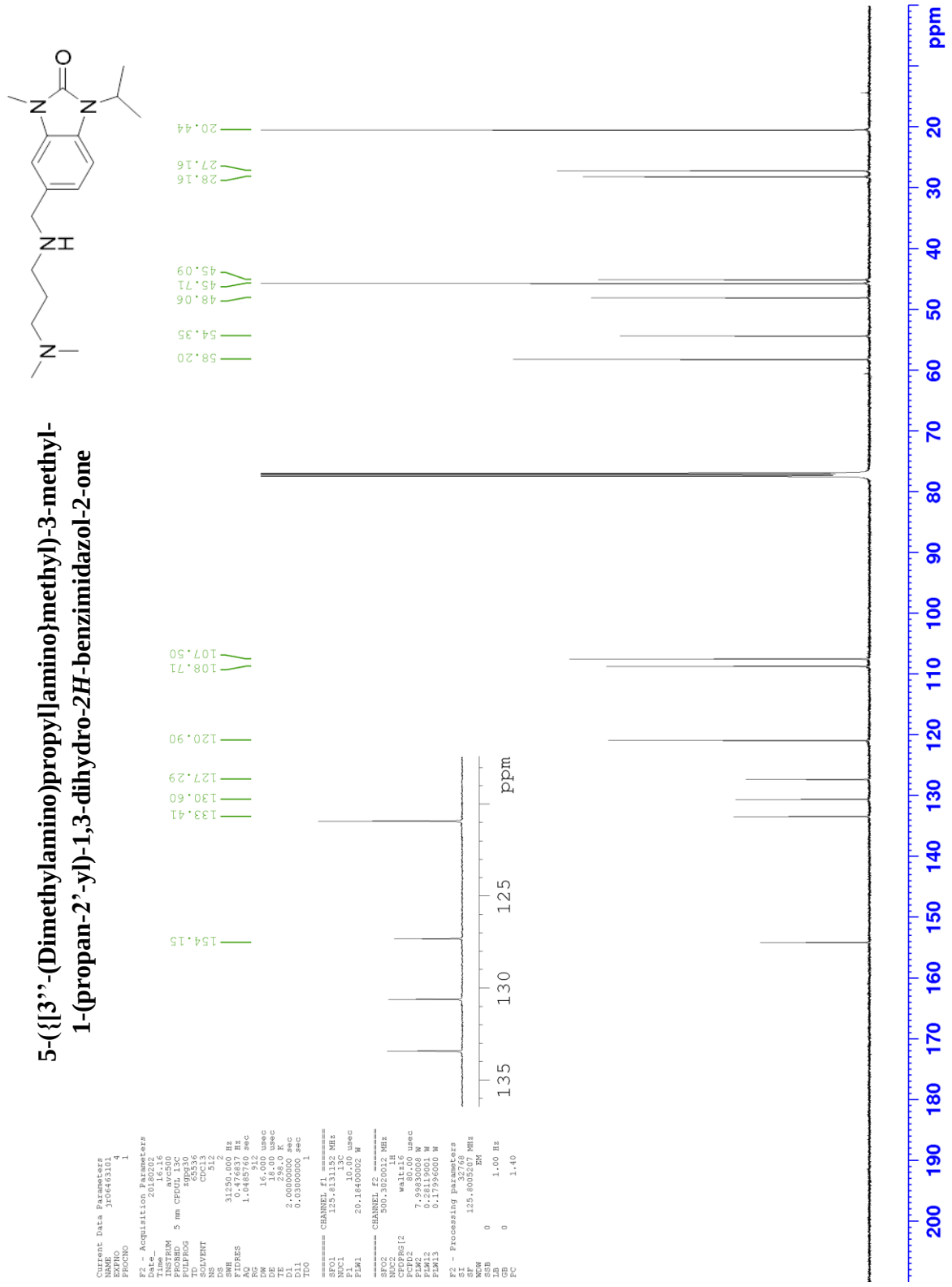

3-Methyl-2-oxo-1-(propan-2-yl)-2,3-dihydro-1H-benzimidazole-5-carbaldehyde
Current Data Parameters
NAME Aug02-2018-84-JKRB90 10 mg
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180802
Time 11.52 h
INSTRUM spect400
PROBHD Z108618_0316 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 91.19
DW 62.400 usec
DE 6.50 usec
TE 298.6 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000094 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.6
7.4
ppm
1.00
1.07
0.96
1.10
1.08
3.30
3.32
3.42
ppm

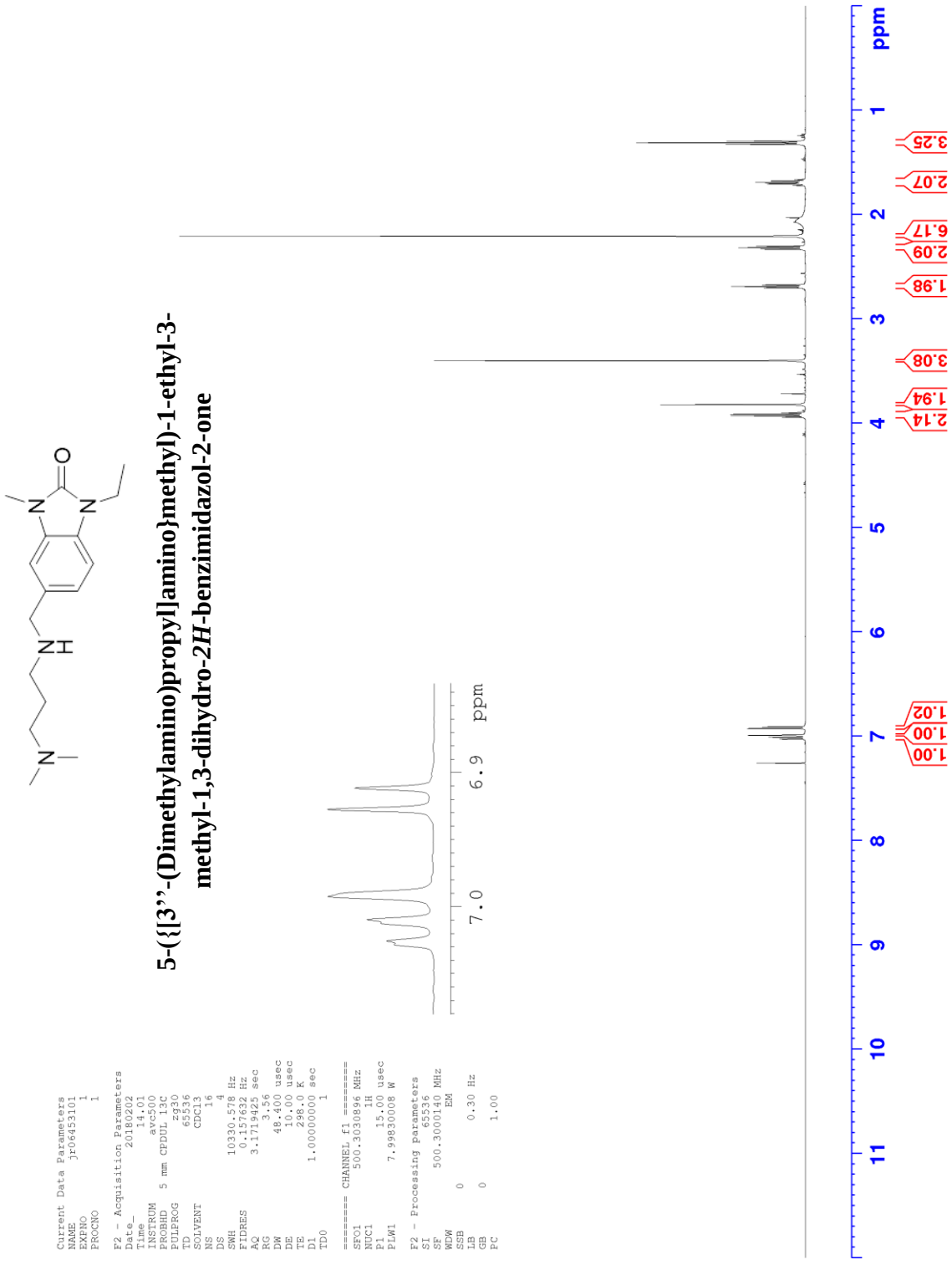

3-Methyl-2-oxo-1-(propan-2-yl)-2,3-dihydro-1H-benzimidazole-5-carbaldehyde
20.38
27.36
45.70
106.55
108.42
126.47
130.22
130.98
133.85
154.21
ppm

5-({[3''-(Dimethylamino)propyl]amino}methyl)-3-methyl-1-(propan-2'-yl)-1,3-dihydro-2*H*-benzimidazol-2-one

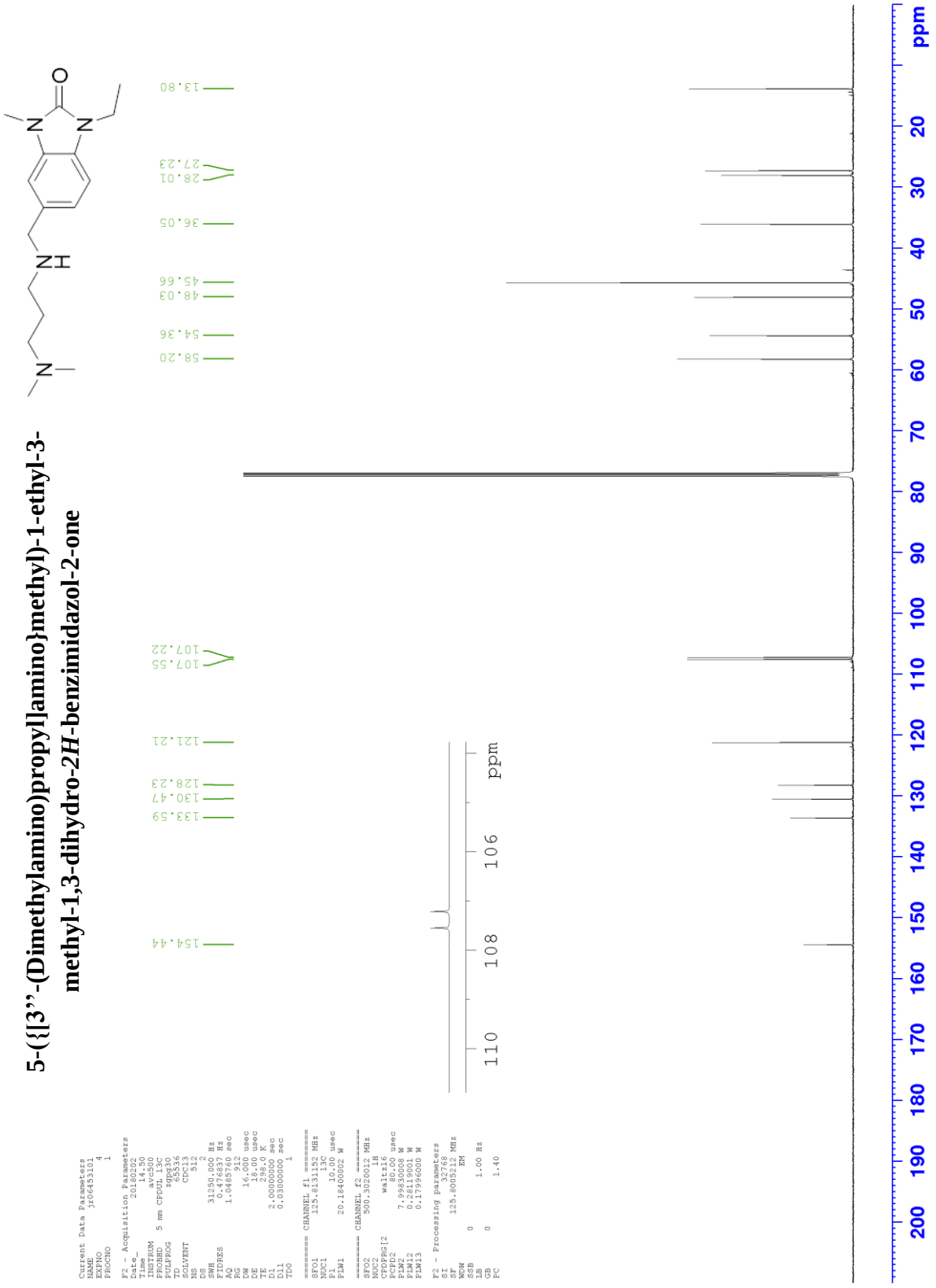


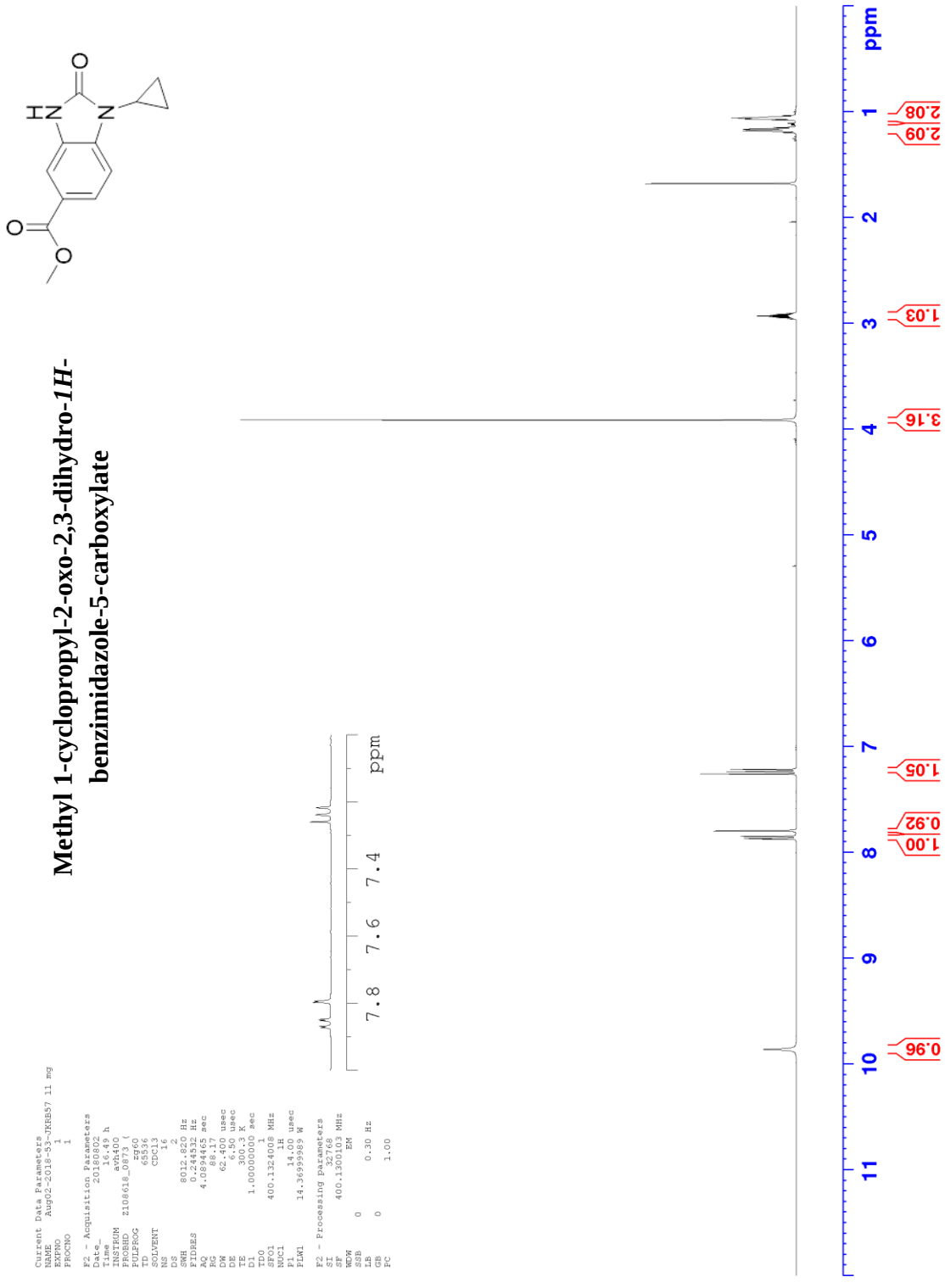
5-({[3''-(Dimethylamino)propyl]amino}methyl)-3-methyl-1-(propan-2'-yl)-1,3-dihydro-2*H*-benzimidazol-2-one

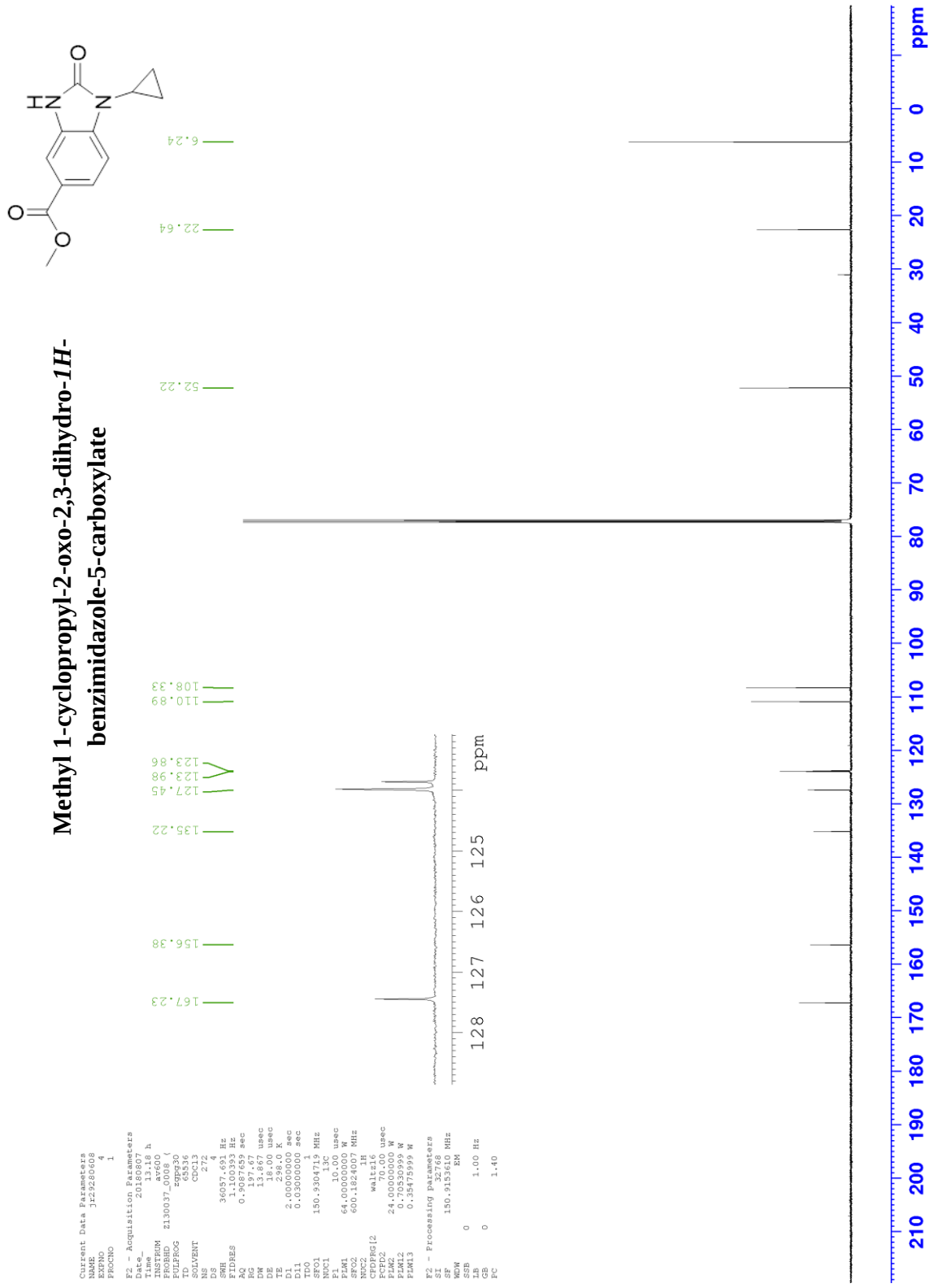



5-({[3''-(Dimethylamino)propyl]amino}methyl)-1-ethyl-3-methyl-1,3-dihydro-2H-benzimidazol-2-one
Current Data Parameters
NAME jr06453101
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180202
Time 14.01
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000140 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.0
6.9
ppm
1.00
1.00
1.02
2.14
1.94
3.08
1.98
2.09
6.17
2.07
3.25
ppm

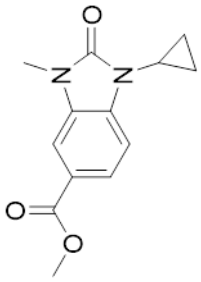
5-({[3''-(Dimethylamino)propyl]amino}methyl)-1-ethyl-3-methyl-1,3-dihydro-2*H*-benzimidazol-2-one

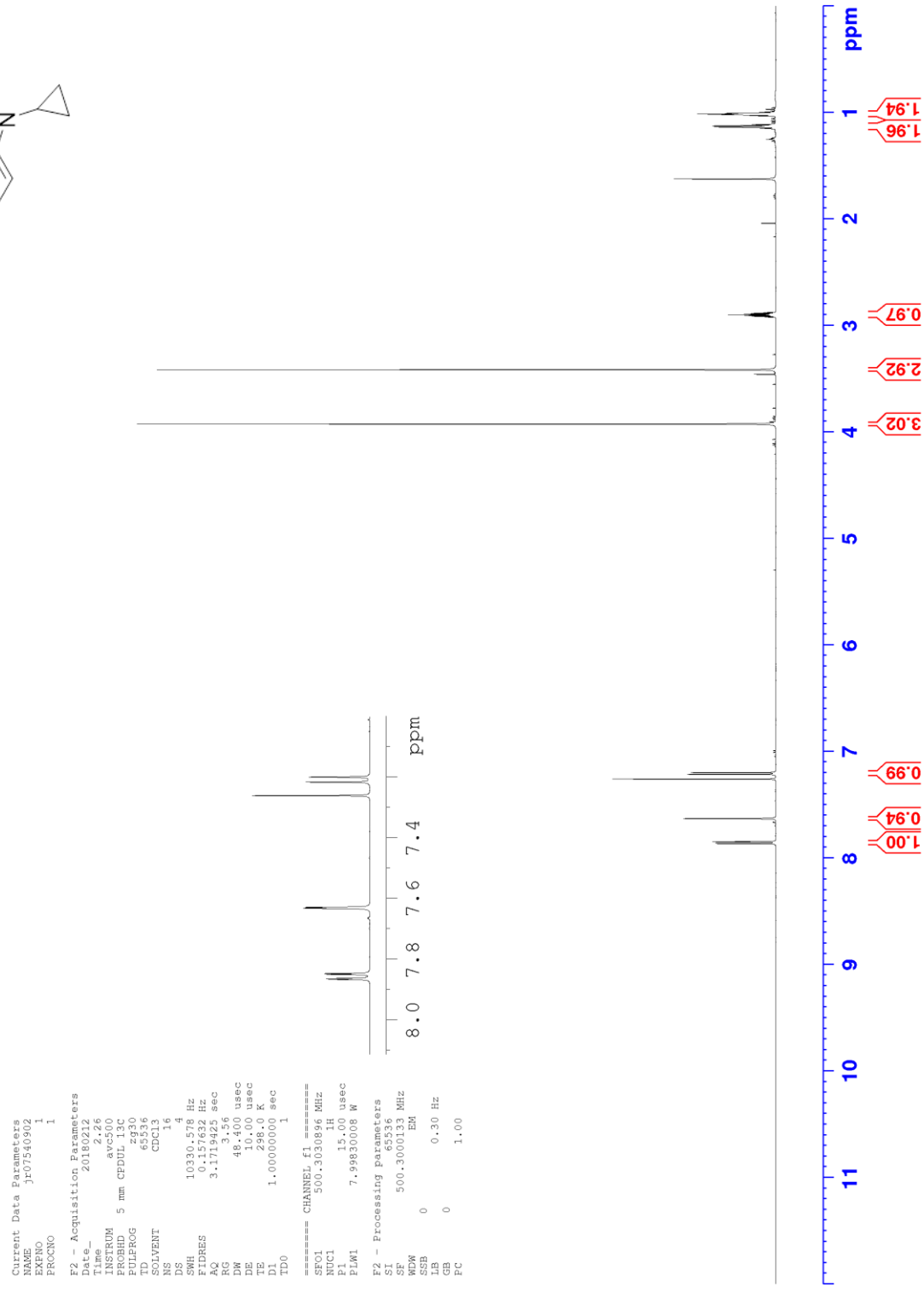



Methyl 1-cyclopropyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
Current Data Parameters
NAME Aug02-2018-53-JKRB57 11 mg
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180802
Time 16.49 h
INSTRUM spect400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 300.3 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300103 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.8
7.6
7.4
ppm
11
10
9
8
7
6
5
4
3
2
1
ppm
0.96
1.00
0.92
1.05
3.16
1.03
2.09
2.08

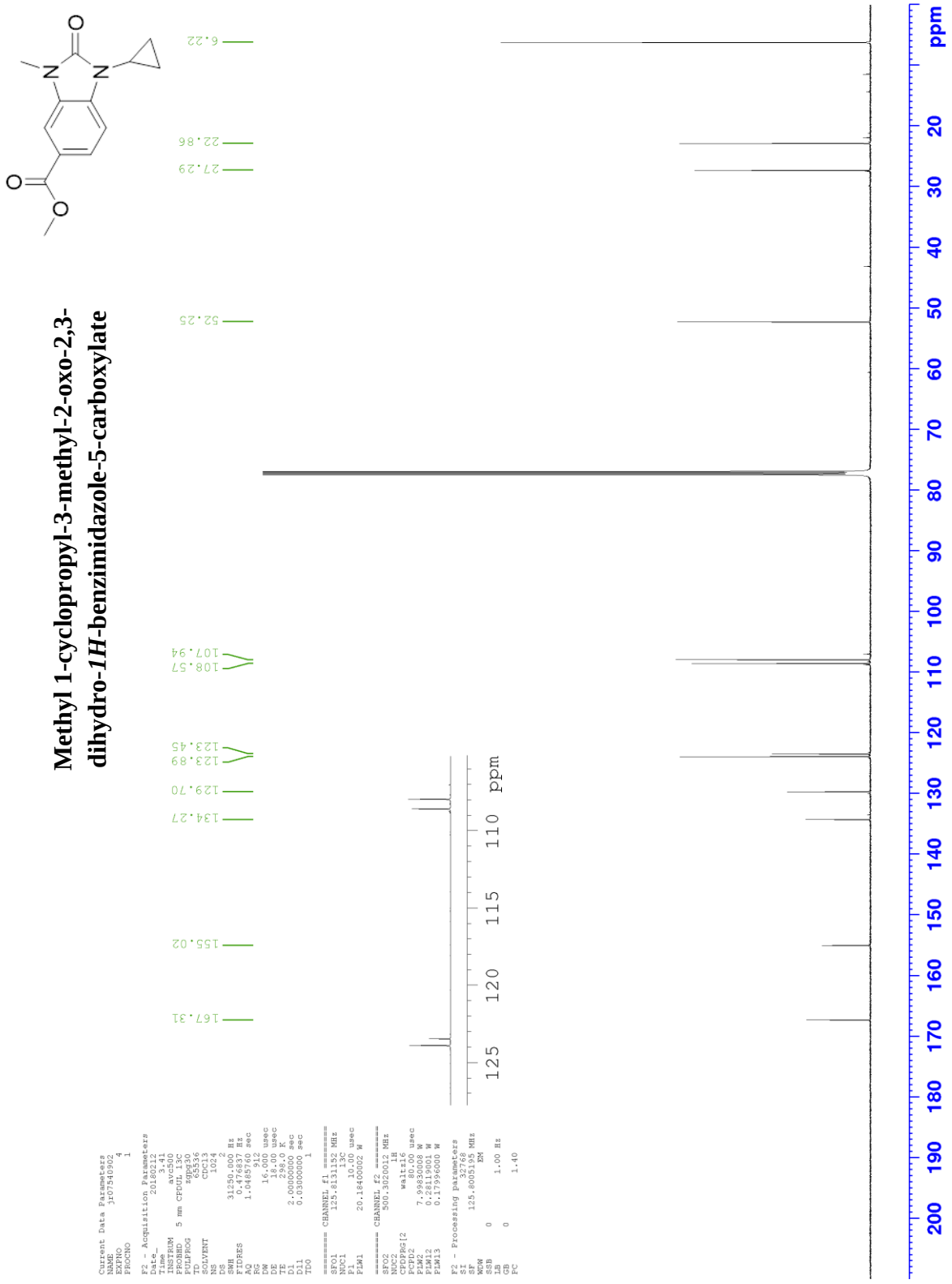

Methyl 1-cyclopropyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
167.23
156.38
135.22
127.45
123.98
123.86
110.89
108.33
52.22
22.64
6.24
ppm

Methyl 1-cyclopropyl-3-methyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carboxylate

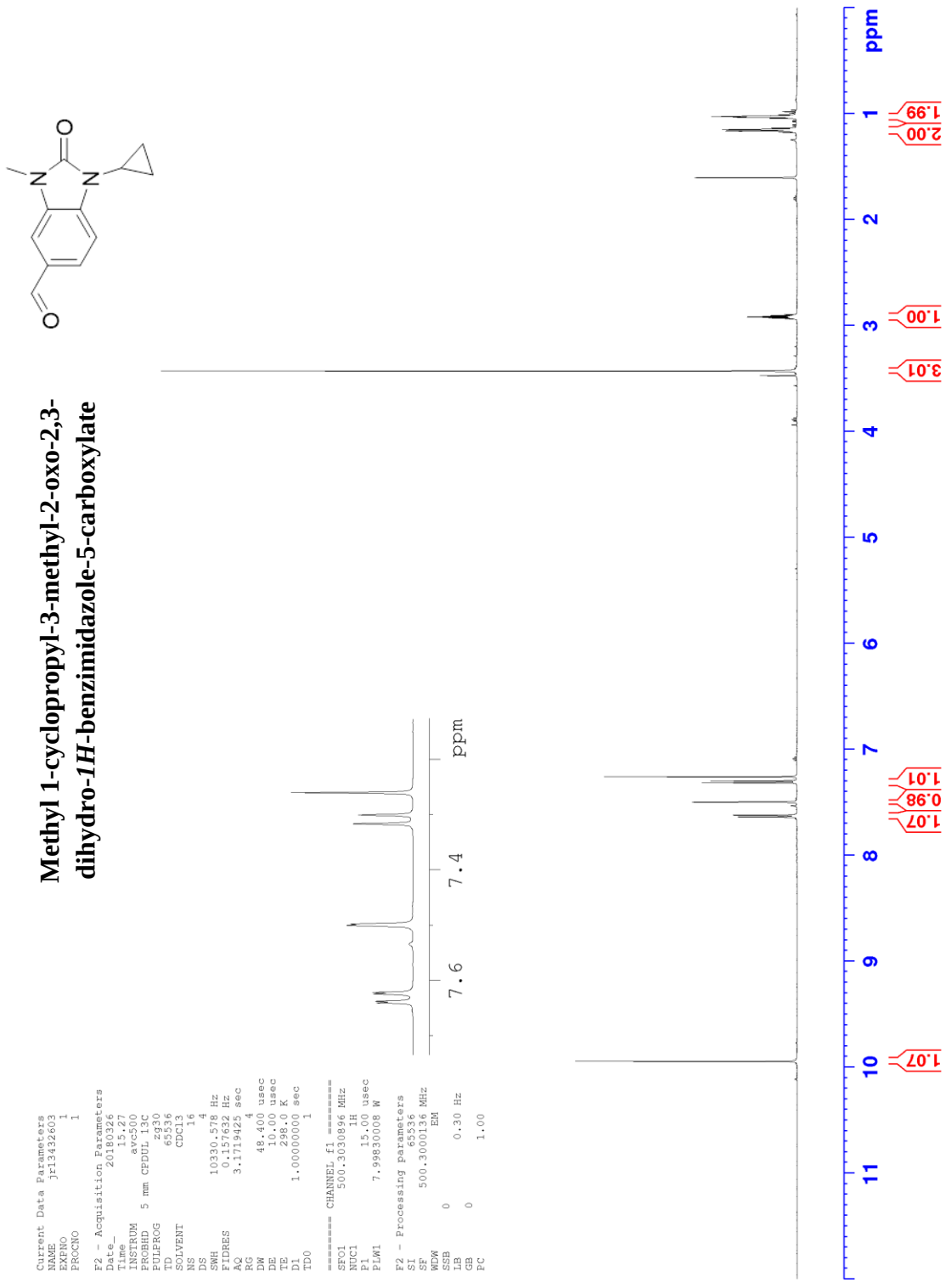




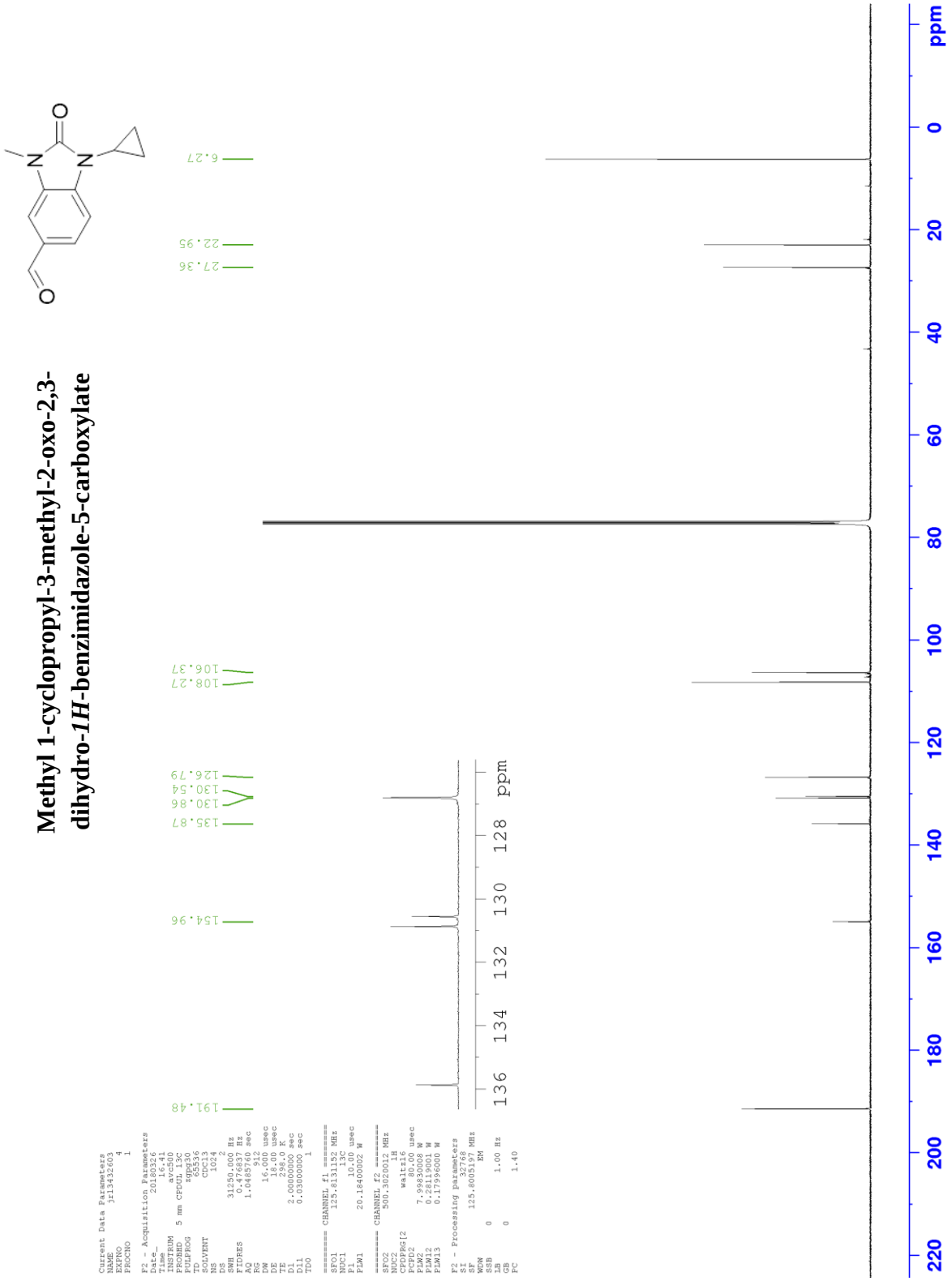
Methyl 1-cyclopropyl-3-methyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carboxylate

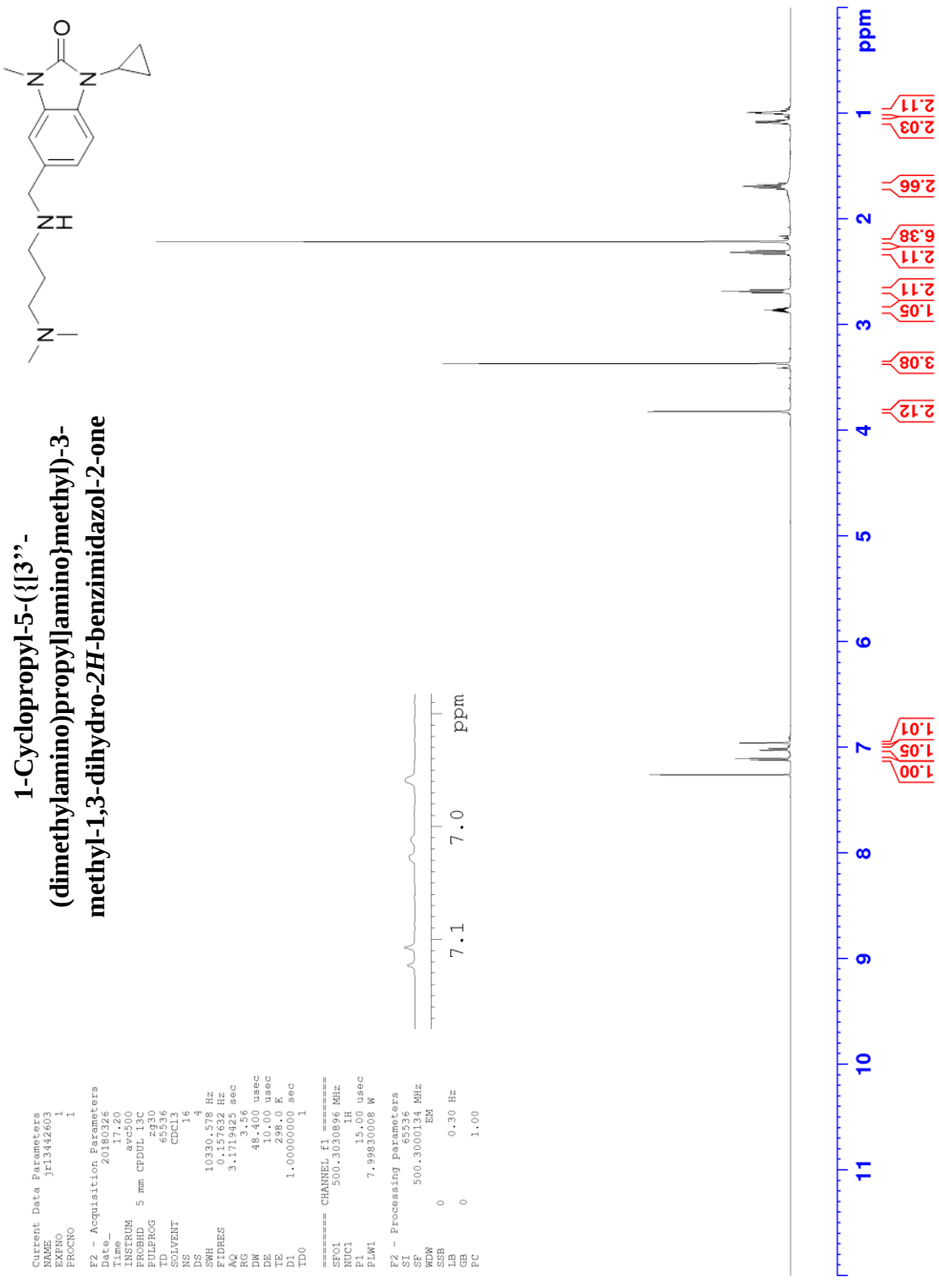


Methyl 1-cyclopropyl-3-methyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carboxylate

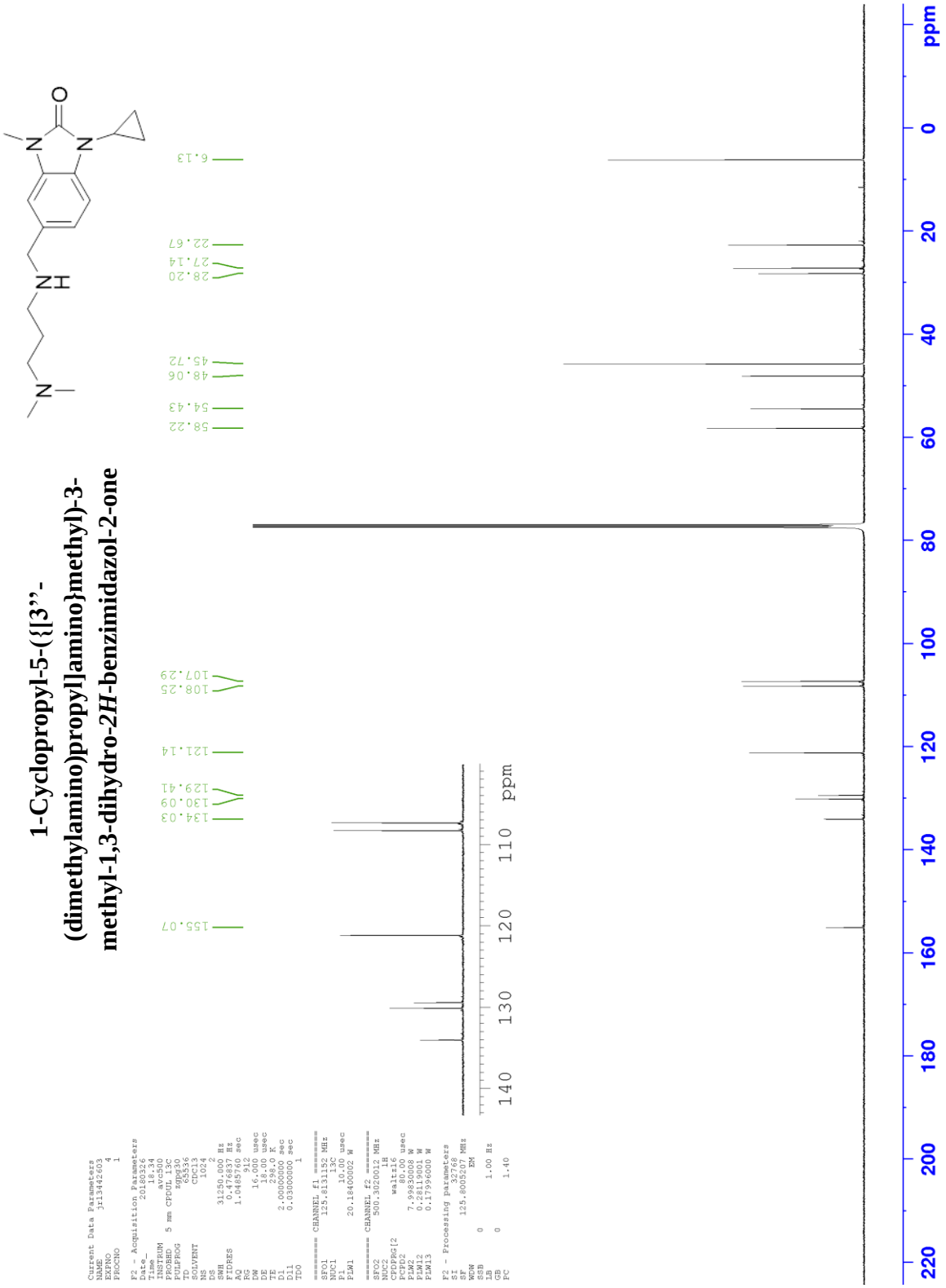


Methyl 1-cyclopropyl-3-methyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carboxylate

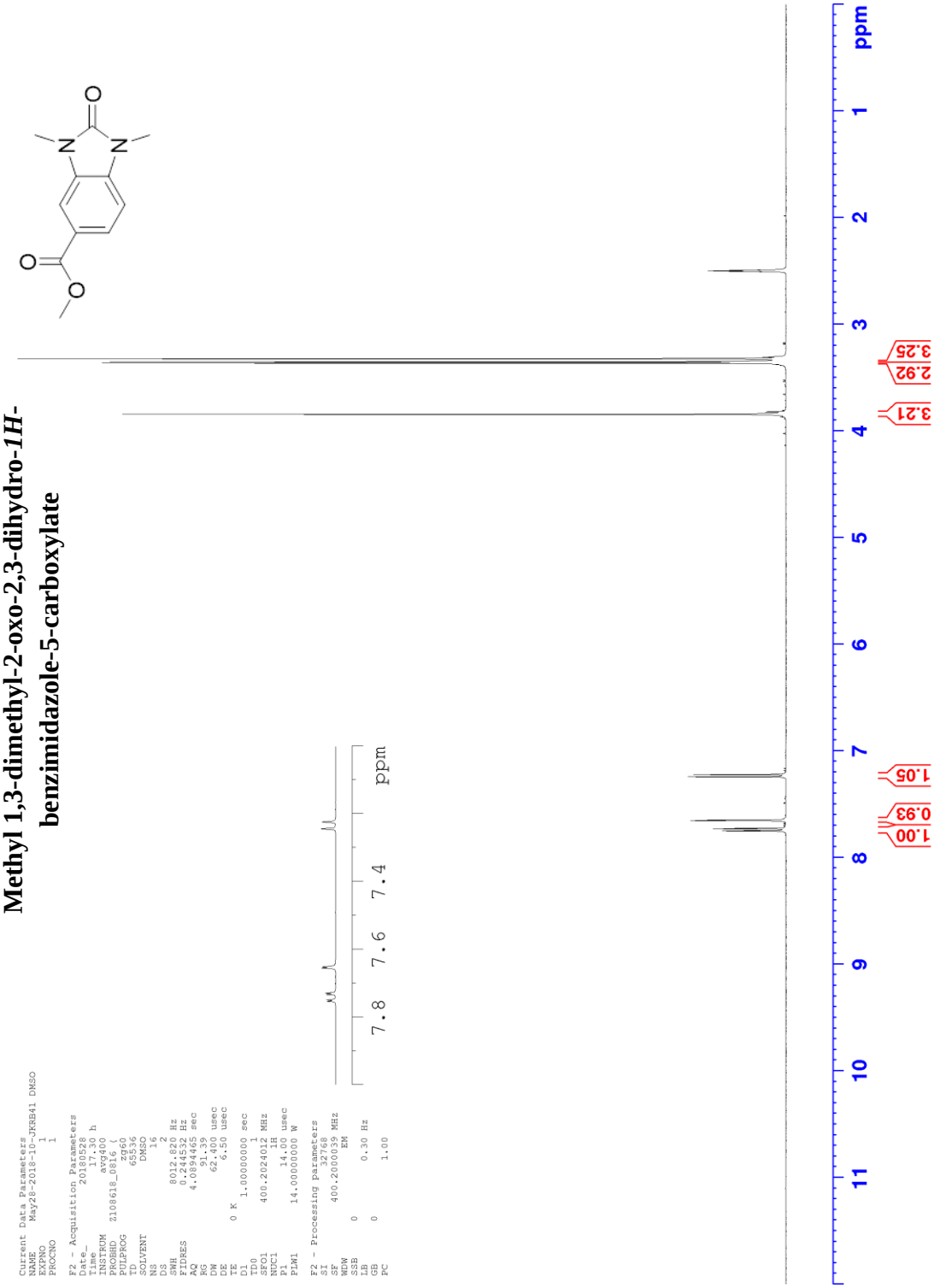



1-Cyclopropyl-5-({[3'-(dimethylamino)propyl]amino}methyl)-3-methyl-1,3-dihydro-2H-benzimidazol-2-one
Current Data Parameters
NAME jr13442603
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180326
Time 17.20
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000134 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.1
7.0
ppm
1.00
1.05
1.01
2.12
3.08
1.05
2.11
6.38
2.11
2.66
2.03
2.11
ppm

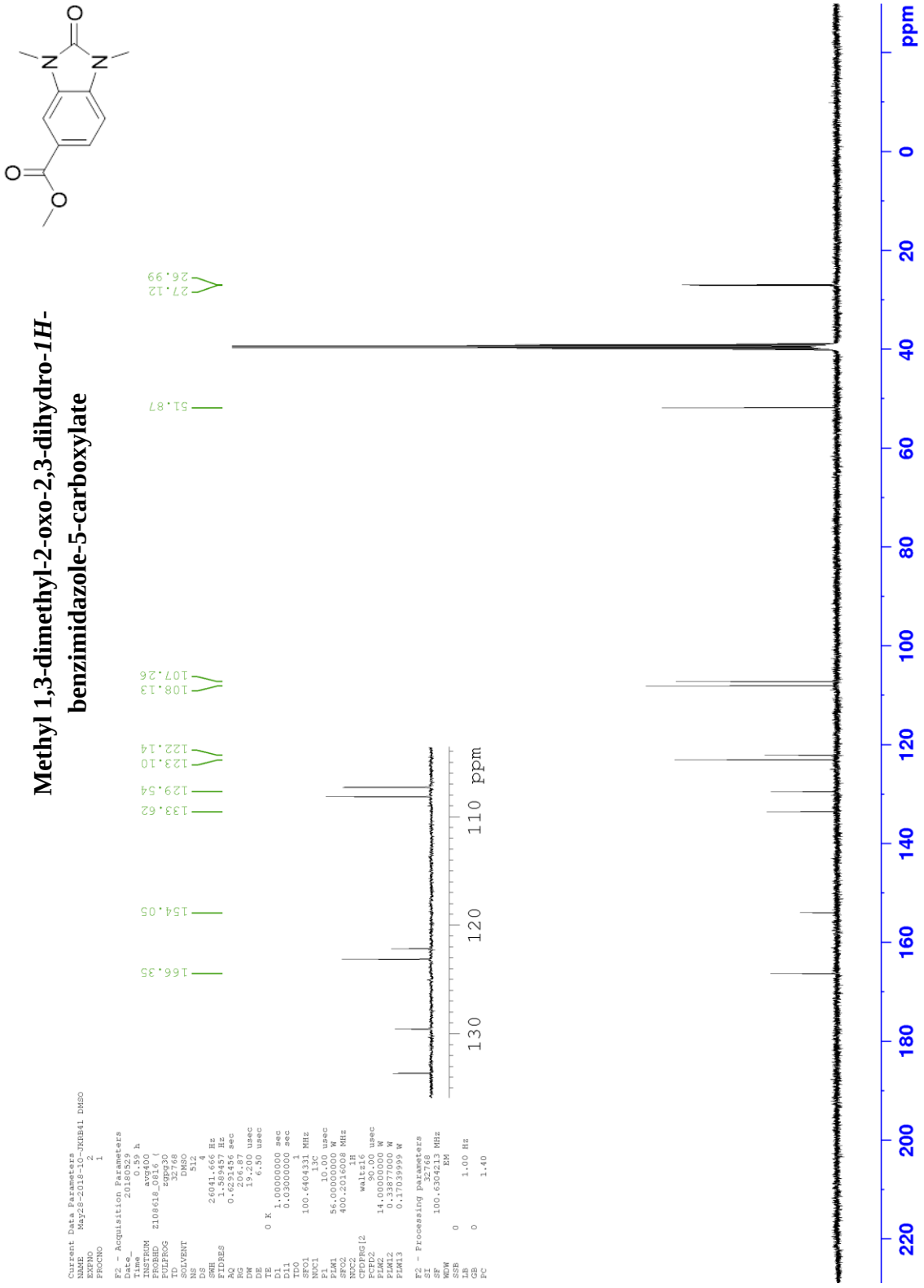
1-Cyclopropyl-5-({[3''-(dimethylamino)propyl]amino}methyl)-3-methyl-1,3-dihydro-2*H*-benzimidazol-2-one

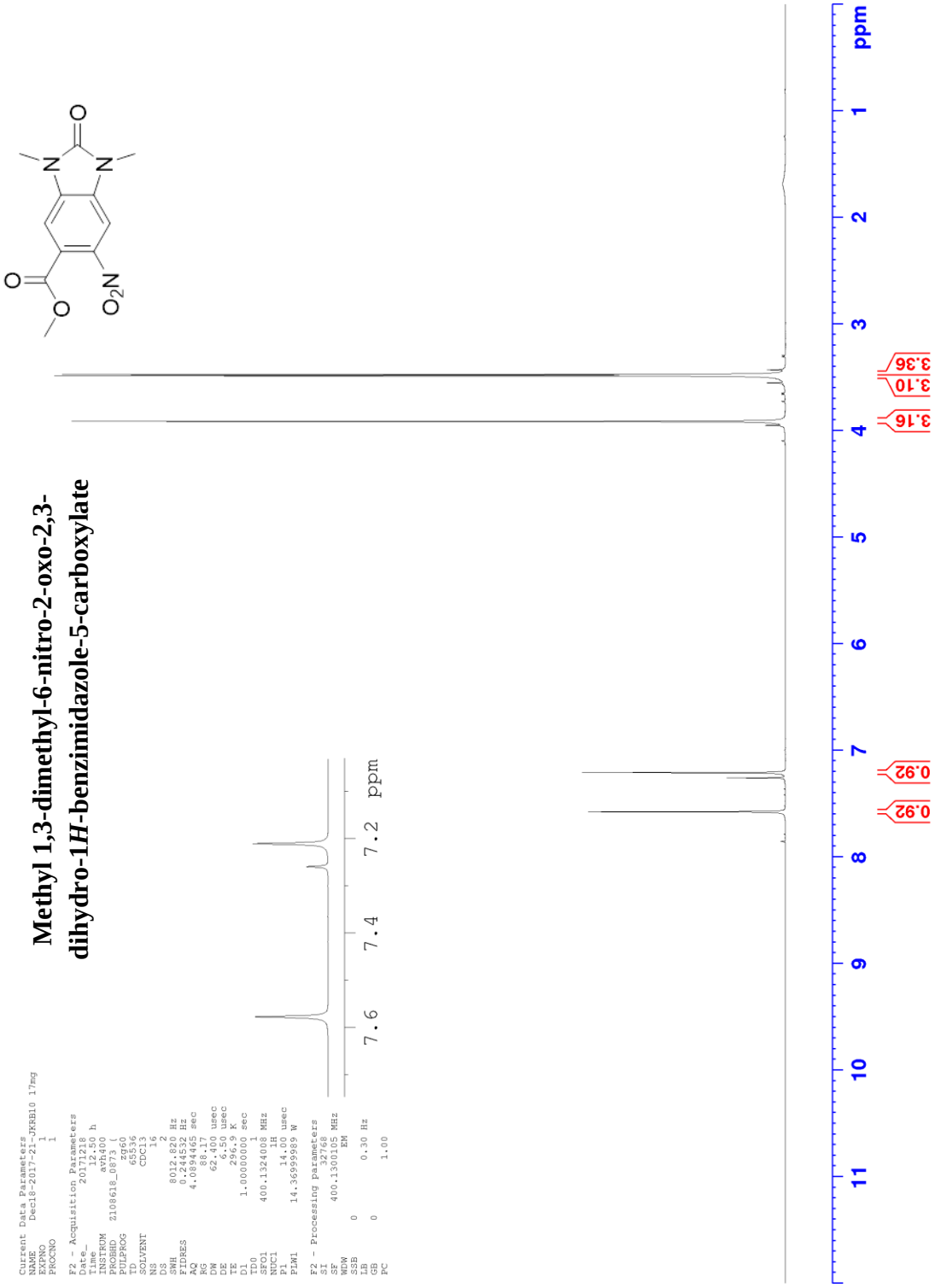


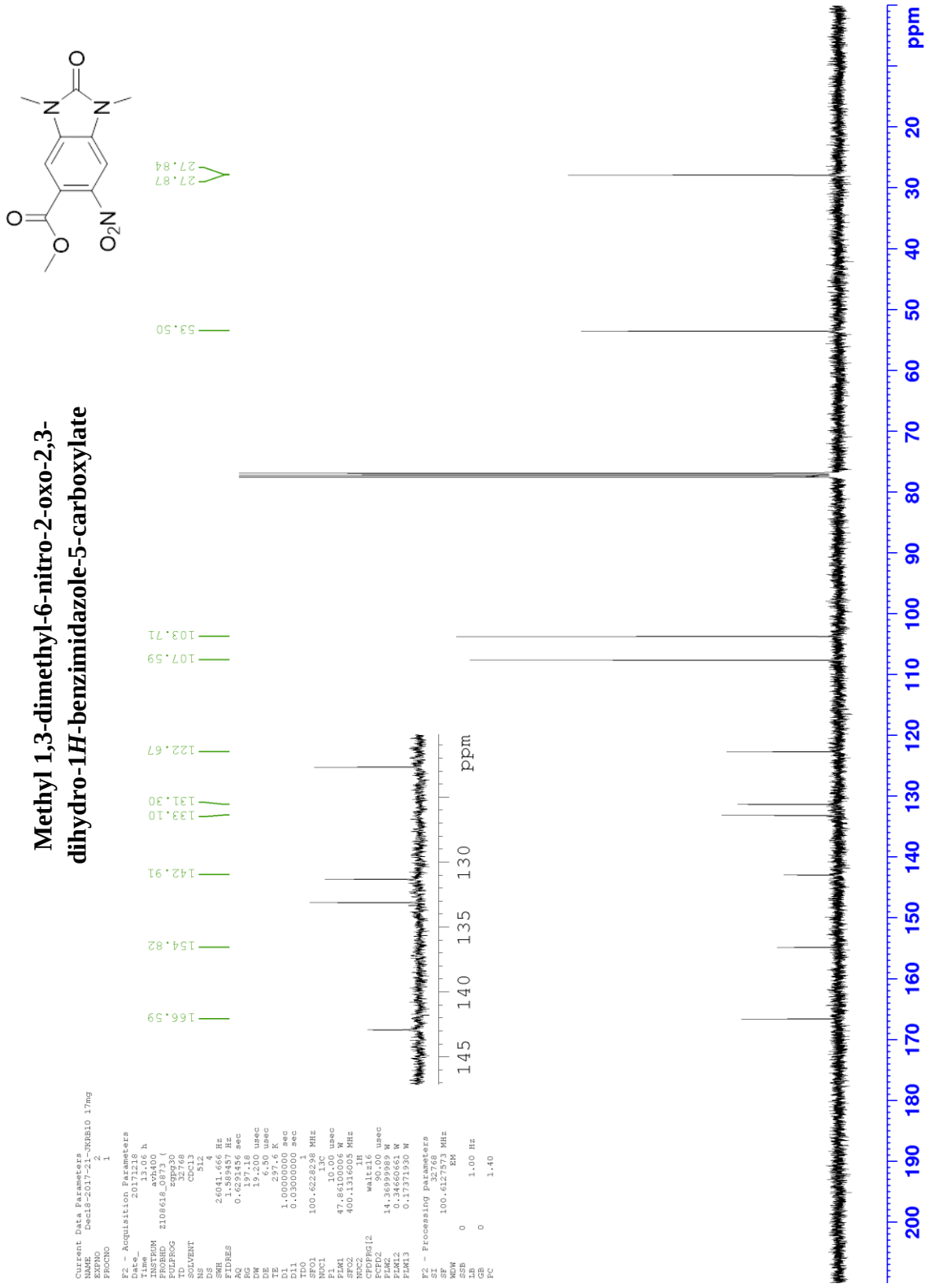
Methyl 1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carboxylate

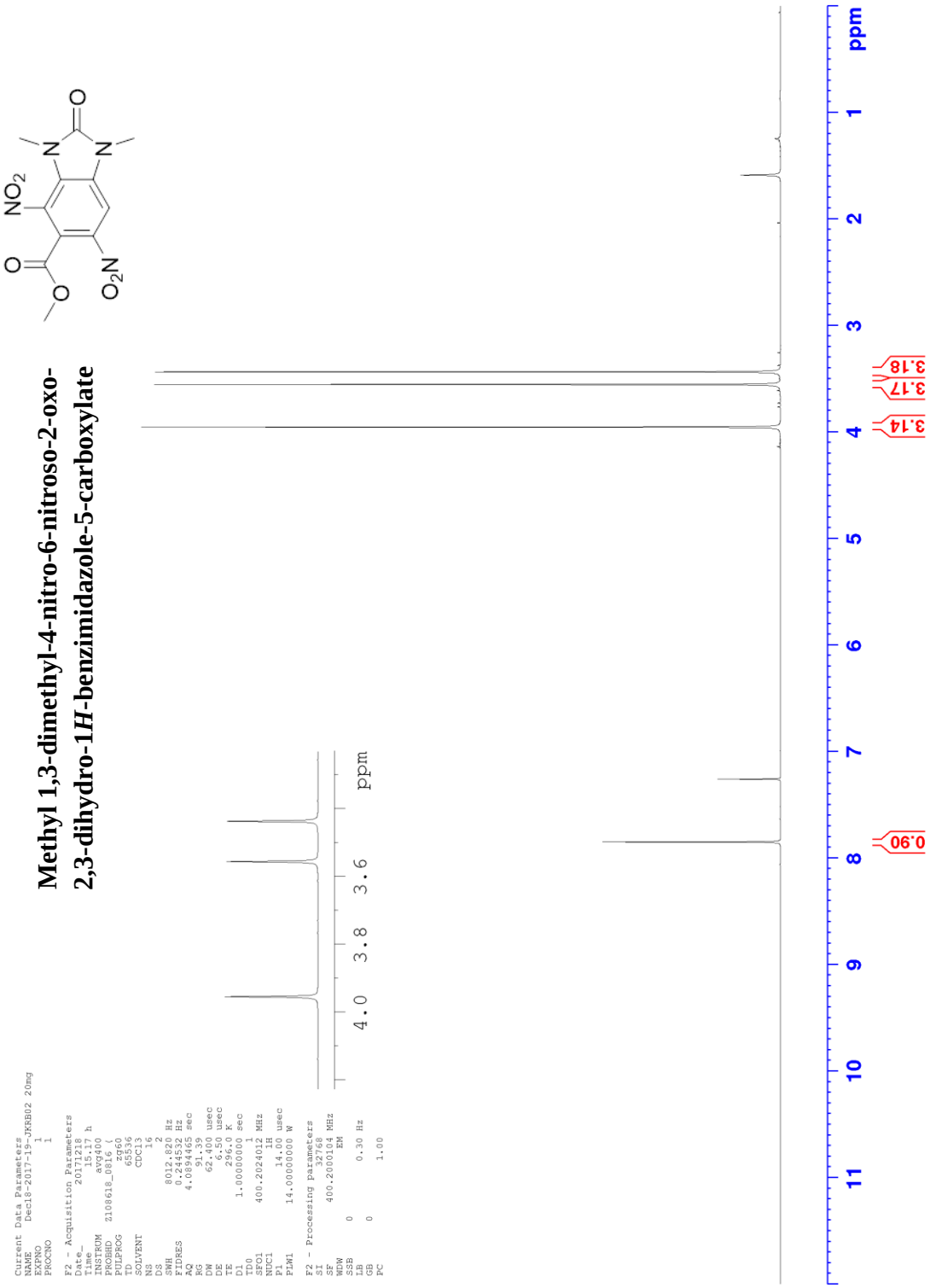


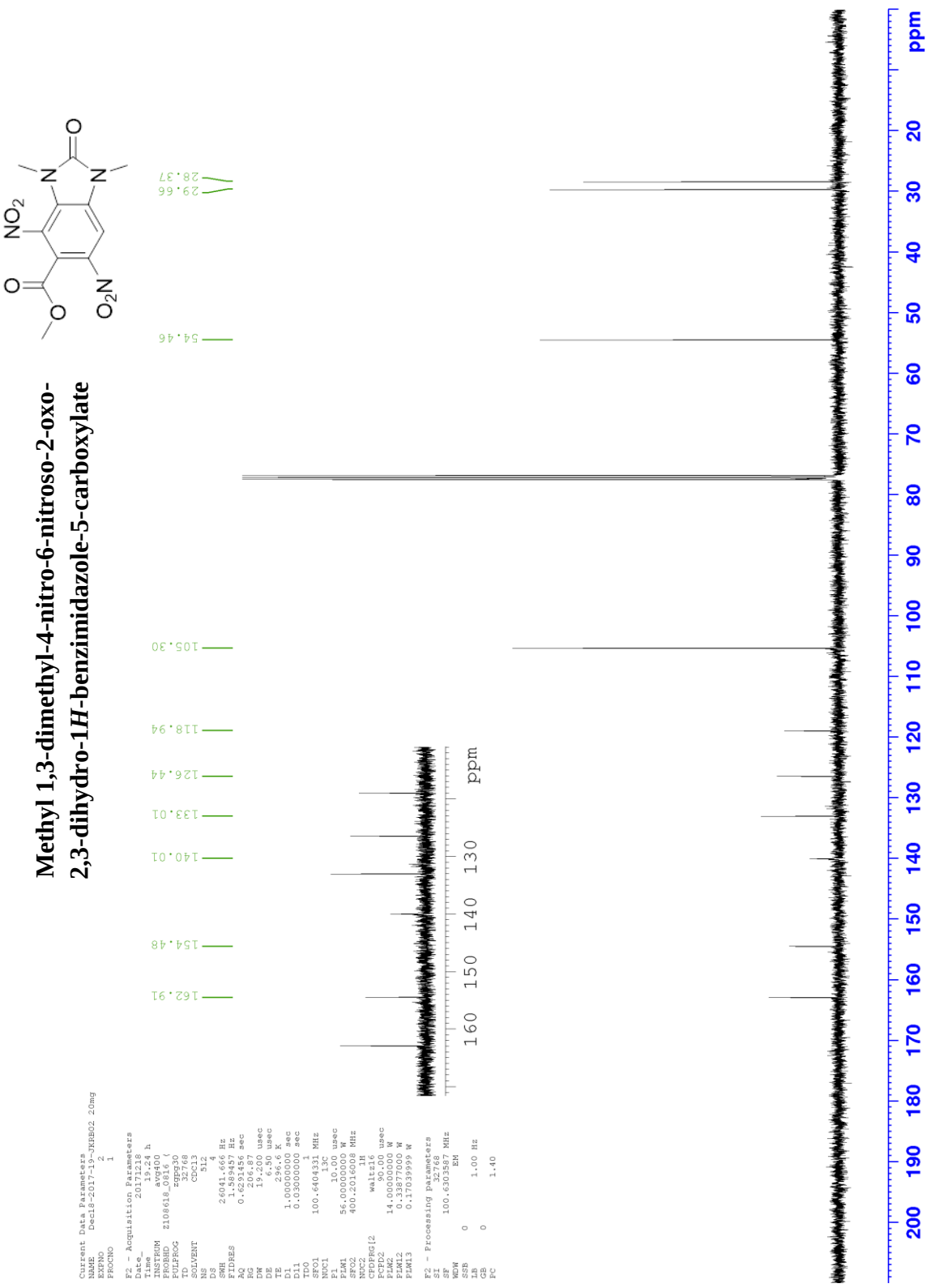
Methyl 1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carboxylate

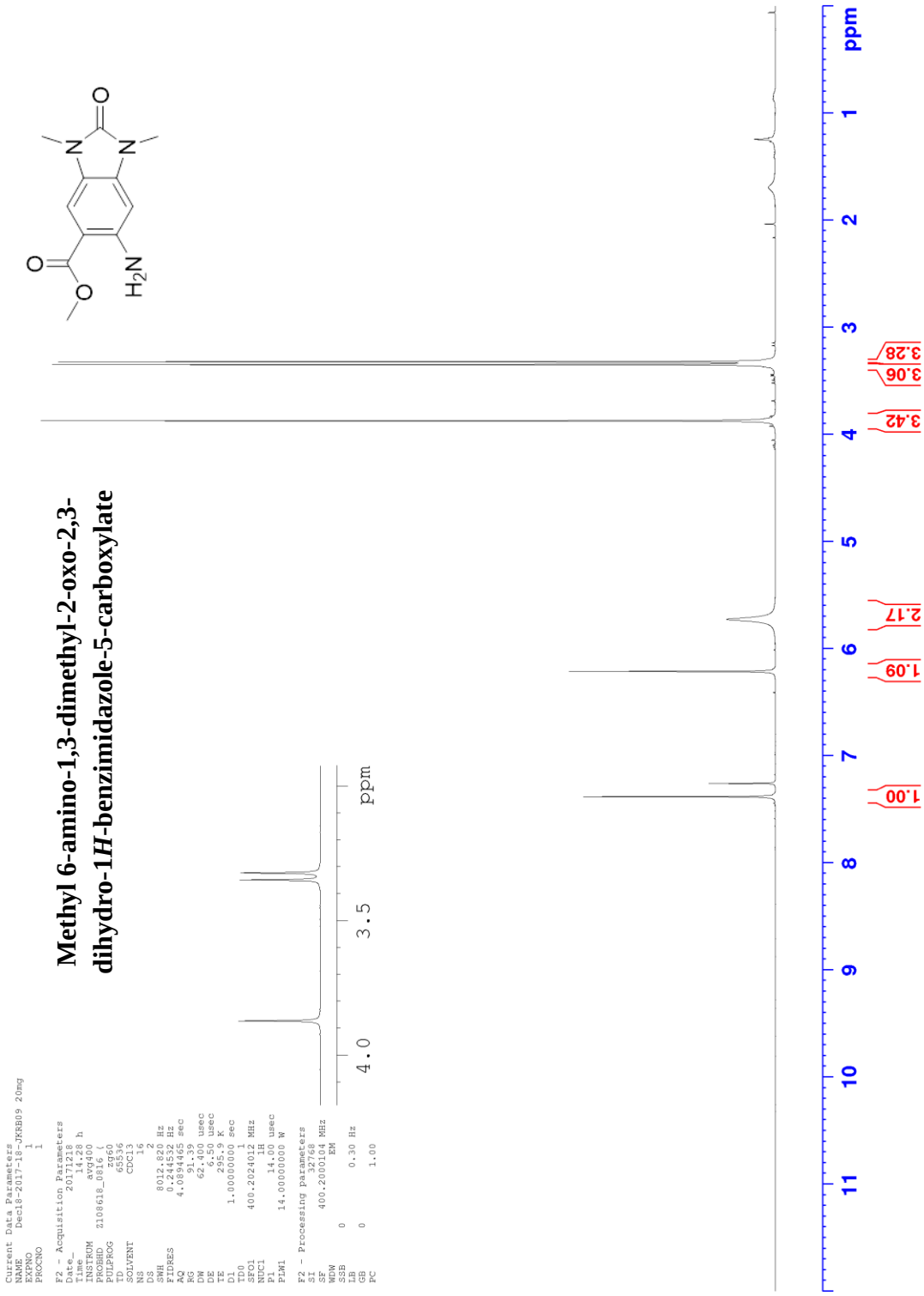


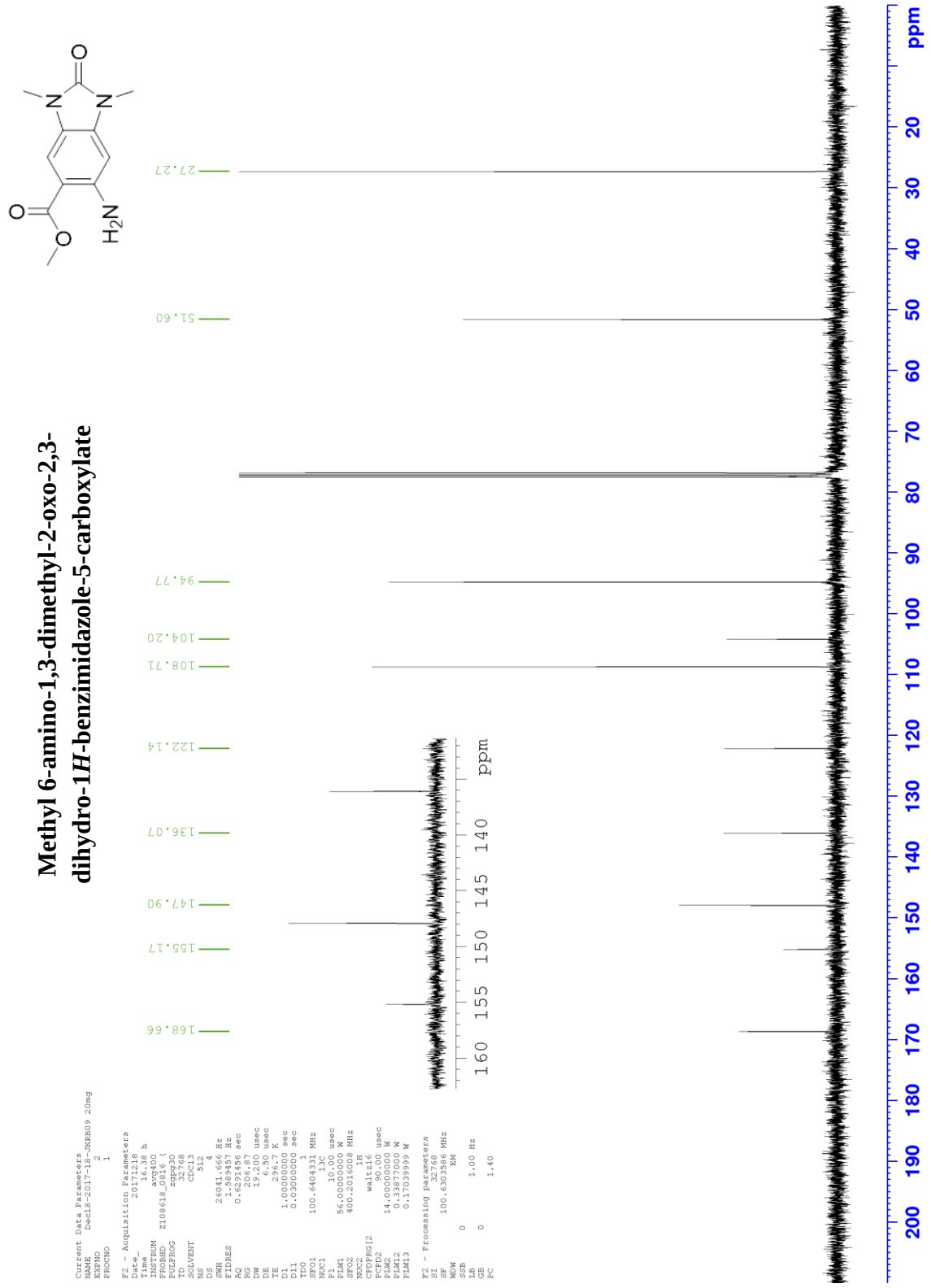

Methyl 1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
Current Data Parameters
NAME Dec18-2017-21-JKRBL0 17mg
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20171218
Time 12.50 h
INSTRUM av400
PROBHD 5 mm
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244552 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 296.9 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300105 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.6
7.4
7.2
ppm
0.92
0.92
3.16
3.10
3.36
ppm

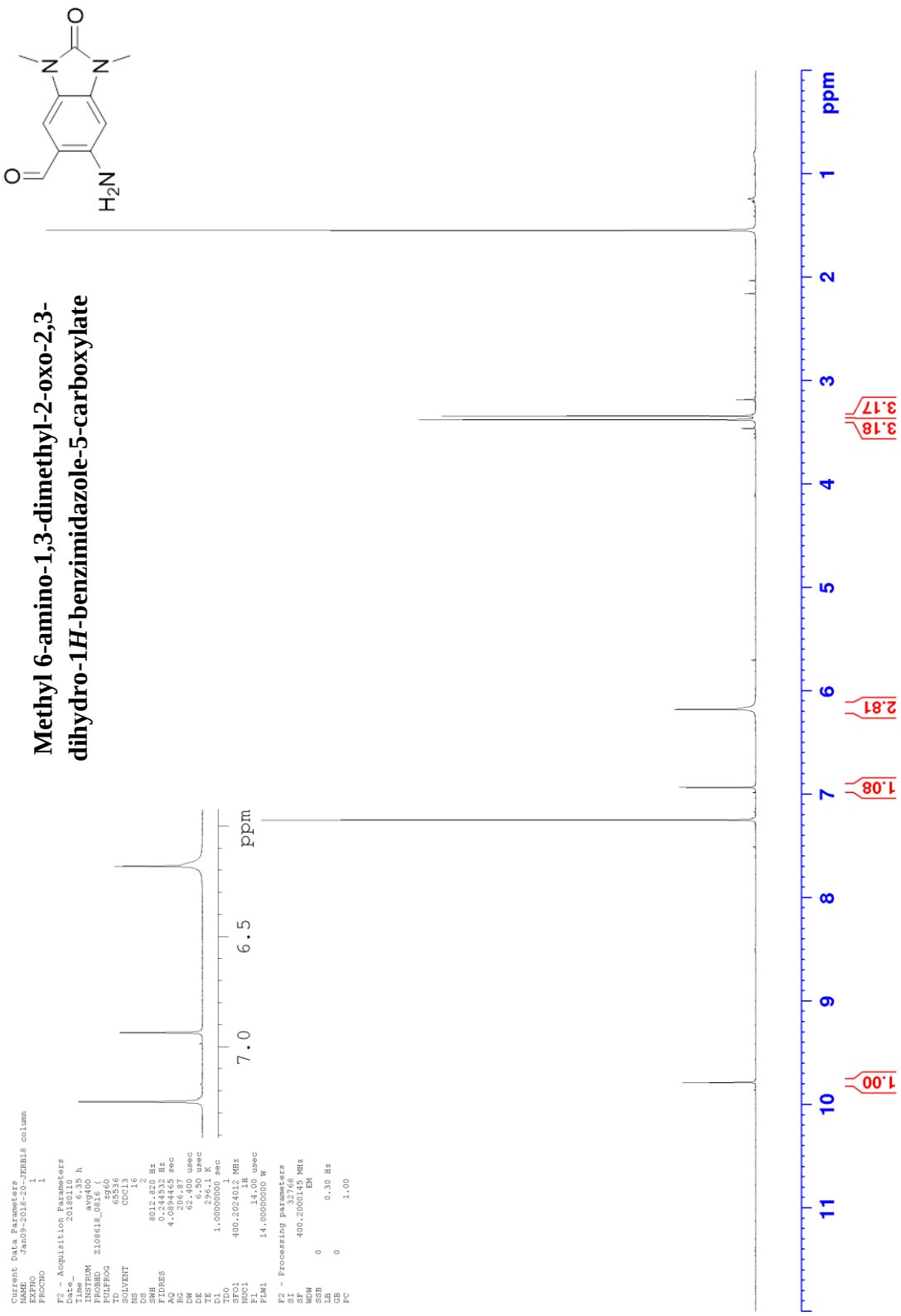

Methyl 1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
O2N
27.87
27.84
53.50
103.71
107.59
122.67
131.30
133.10
142.91
154.82
166.59
ppm
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


Methyl 1,3-dimethyl-4-nitro-6-nitroso-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
Current Data Parameters
NAME Dec18-2017-19-JKRB02 20mg
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20171218
Time 15.17 h
INSTRUM avg400
PROBHD 5 mm
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244552 Hz
AQ 4.0894465 sec
RG 91.39
DW 62.400 usec
DE 6.50 usec
TE 296.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000104 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
4.0
3.8
3.6
ppm
0.90
3.14
3.17
3.18
ppm

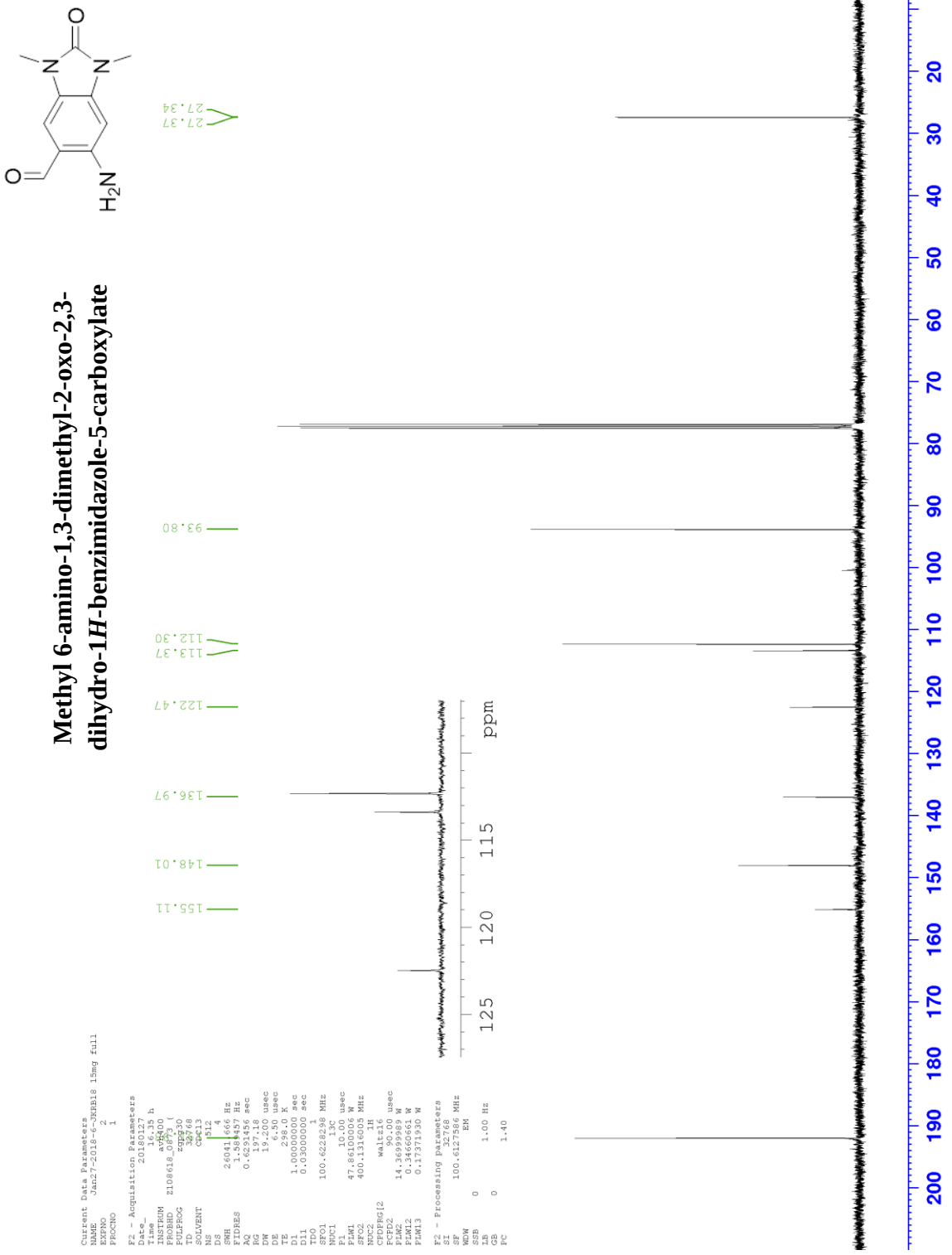

Methyl 1,3-dimethyl-4-nitro-6-nitroso-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
NO2
O2N
O
N
28.37
29.66
54.46
105.30
118.94
126.44
133.01
140.01
154.48
162.91
ppm

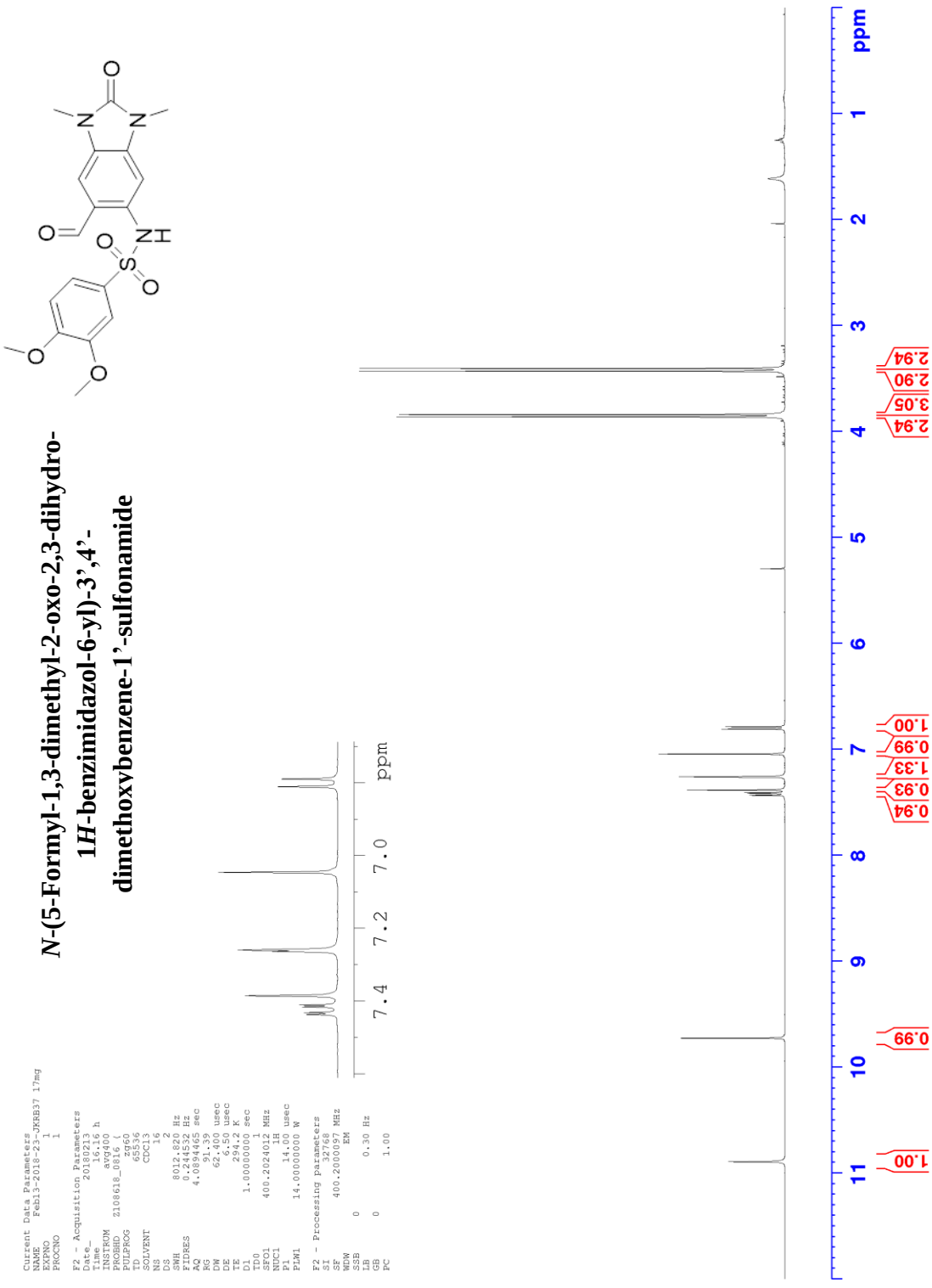

Methyl 6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
Current Data Parameters
NAME Dec18-2017-18-JKRH09 20mg
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20171218
Time 14.28 h
INSTRUM avg400
PROBHD 5108618_0816_0
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244552 Hz
AQ 4.0894465 sec
RG 91.39
DW 62.400 usec
DE 6.50 usec
TE 295.9 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000104 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
4.0
3.5
ppm
1.00
1.09
2.17
3.42
3.06
3.28
11
10
9
8
7
6
5
4
3
2
1
ppm


Methyl 6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
27.27
51.60
94.77
104.20
108.71
122.14
136.07
147.90
155.17
168.66
ppm


Methyl 6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
O
N
N
O
O
H2N
3.18
3.17
2.81
1.08
1.00
ppm
1
2
3
4
5
6
7
8
9
10
11
7.0
6.5

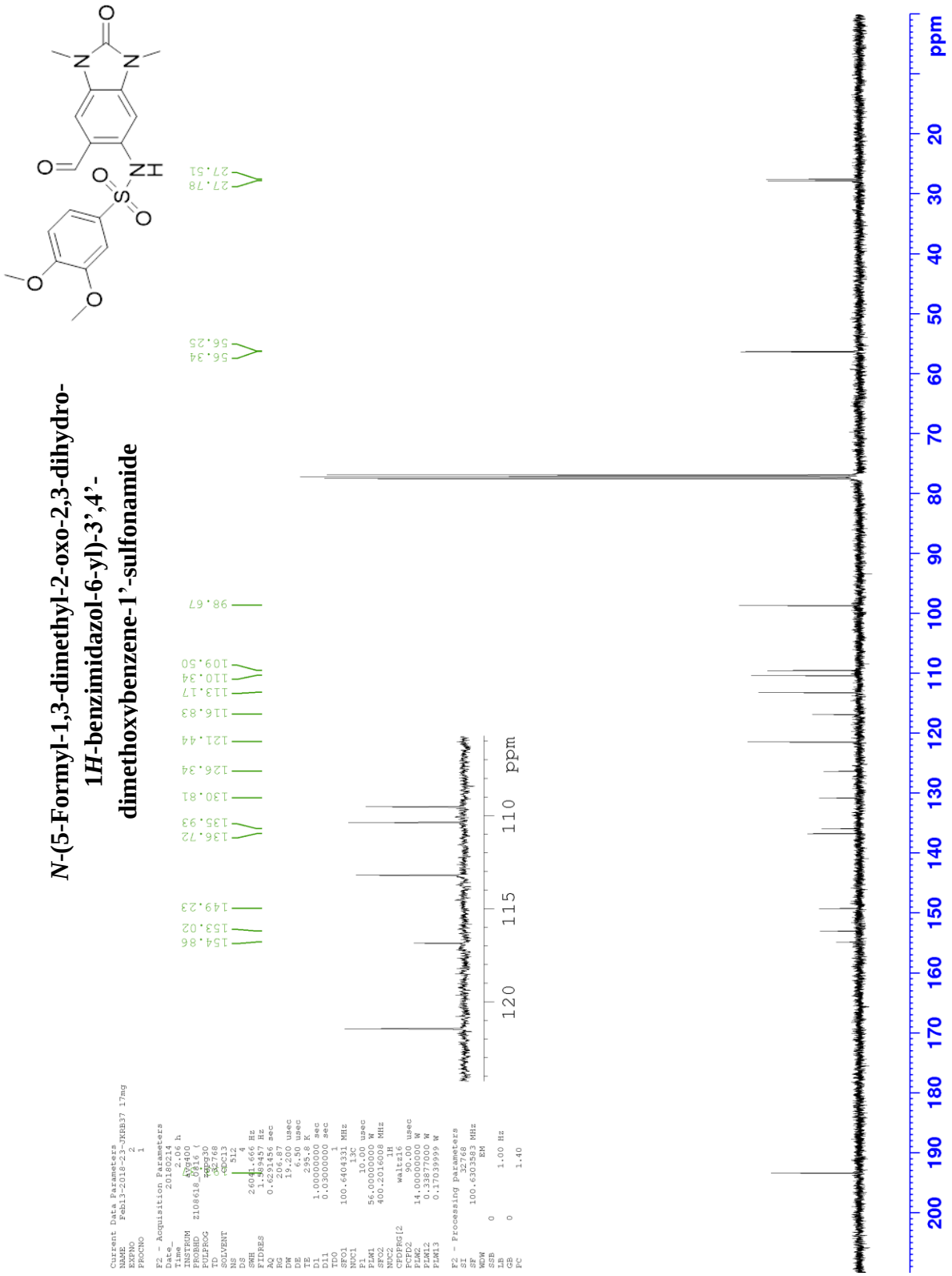
Methyl 6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate

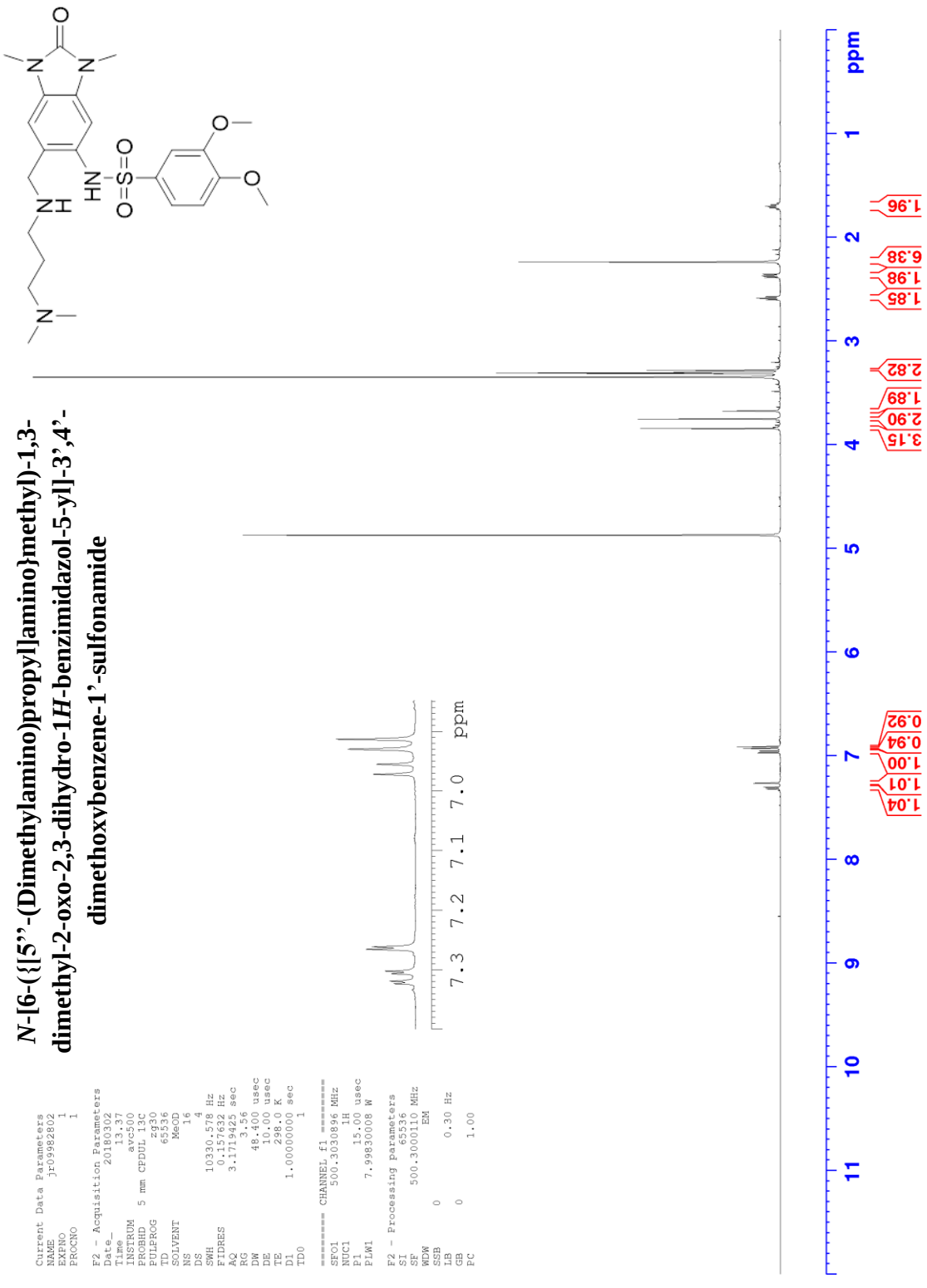




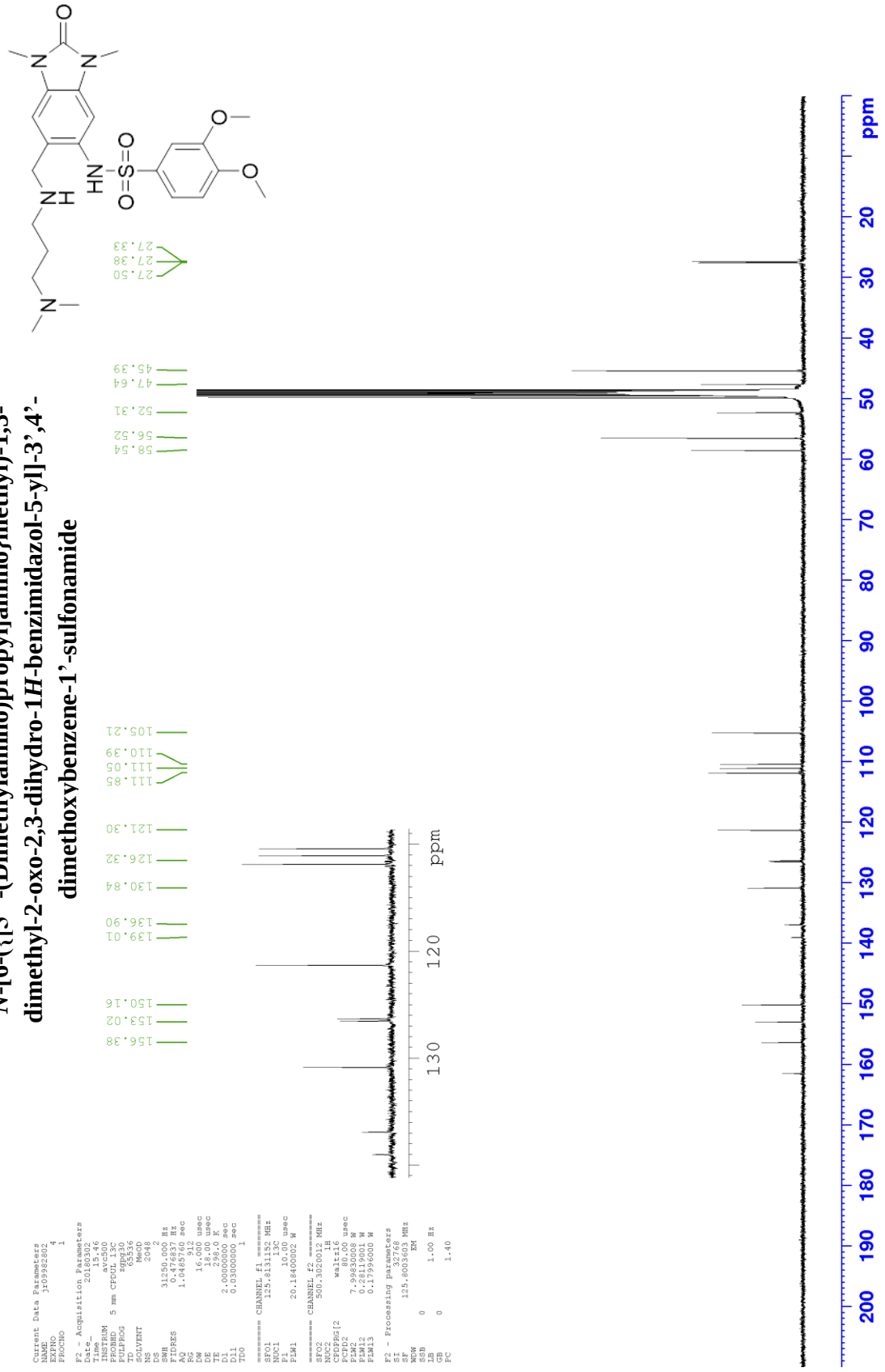
***N*-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-6-yl)-3',4'-dimethoxybenzene-1'-sulfonamide**

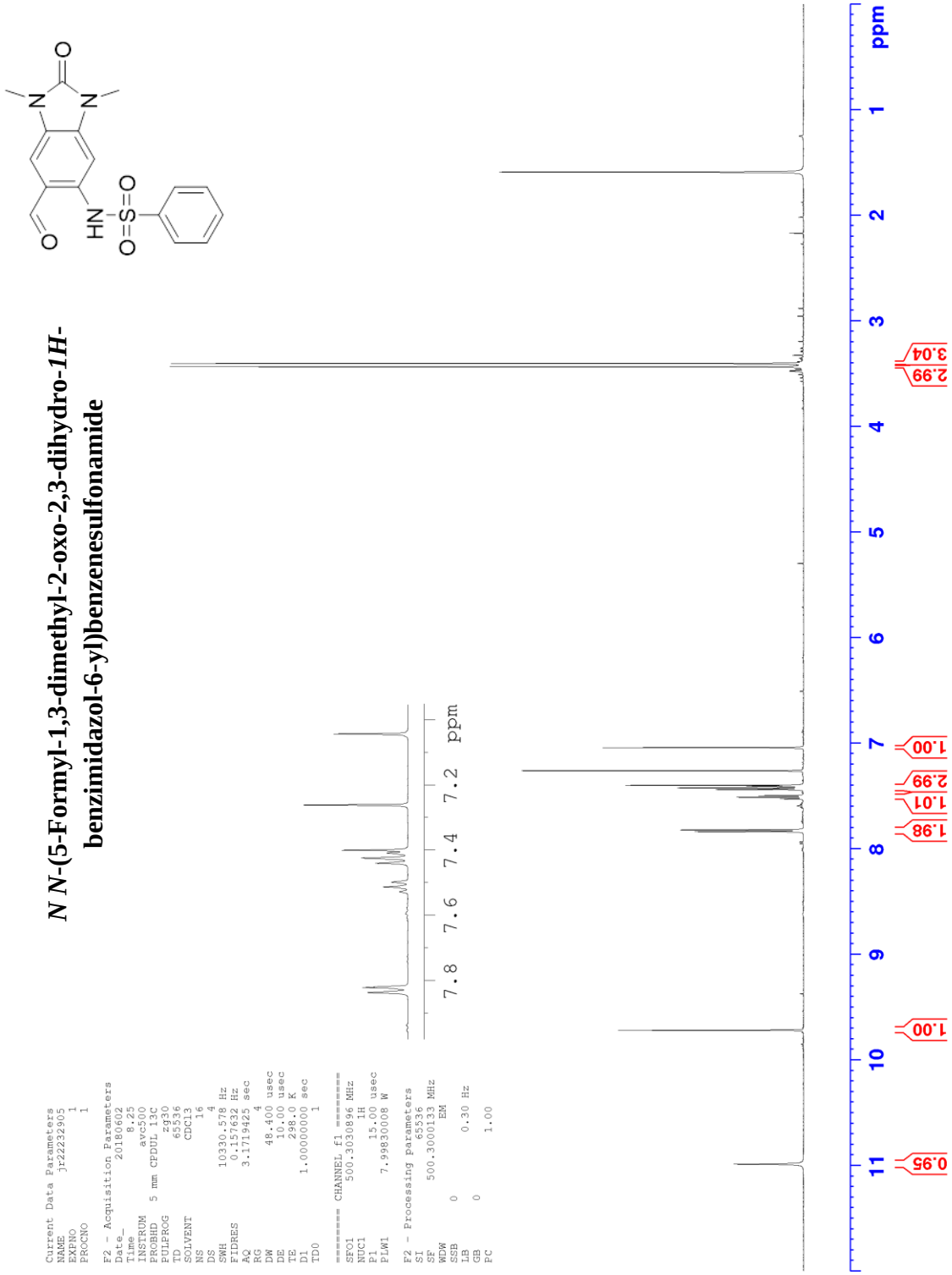
***N*-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-6-yl)-3',4'-dimethoxybenzene-1'-sulfonamide**



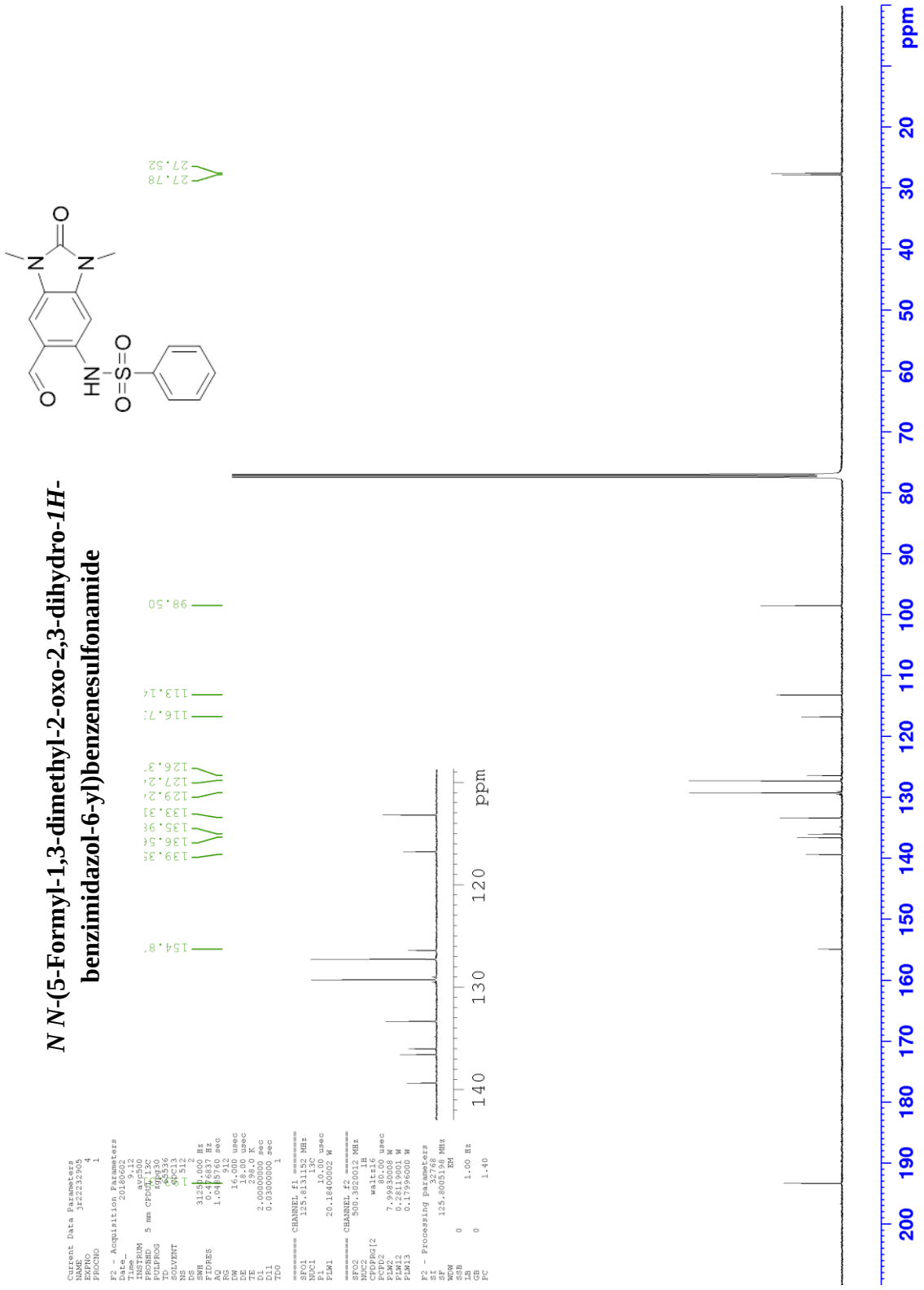

N-[6-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl]-3',4'-dimethoxybenzene-1'-sulfonamide
Current Data Parameters
NAME jr09982802
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180302
Time 13.37
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000110 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.3 7.2 7.1 7.0 ppm
1.04
1.01
1.00
0.94
0.92
3.15
2.90
1.89
2.82
1.85
1.98
6.38
1.96
11 10 9 8 7 6 5 4 3 2 1 ppm

_N_-[6-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl]-3',4'-dimethoxybenzene-1'-sulfonamide

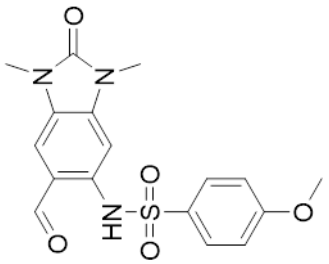



N N-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-6-yl)benzenesulfonamide
Current Data Parameters
NAME jr22232905
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180602
Time 8.25
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000133 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.8
7.6
7.4
7.2
ppm
11
10
9
8
7
6
5
4
3
2
1
ppm
0.95
1.00
1.00
1.98
1.01
2.99
3.04
2.99

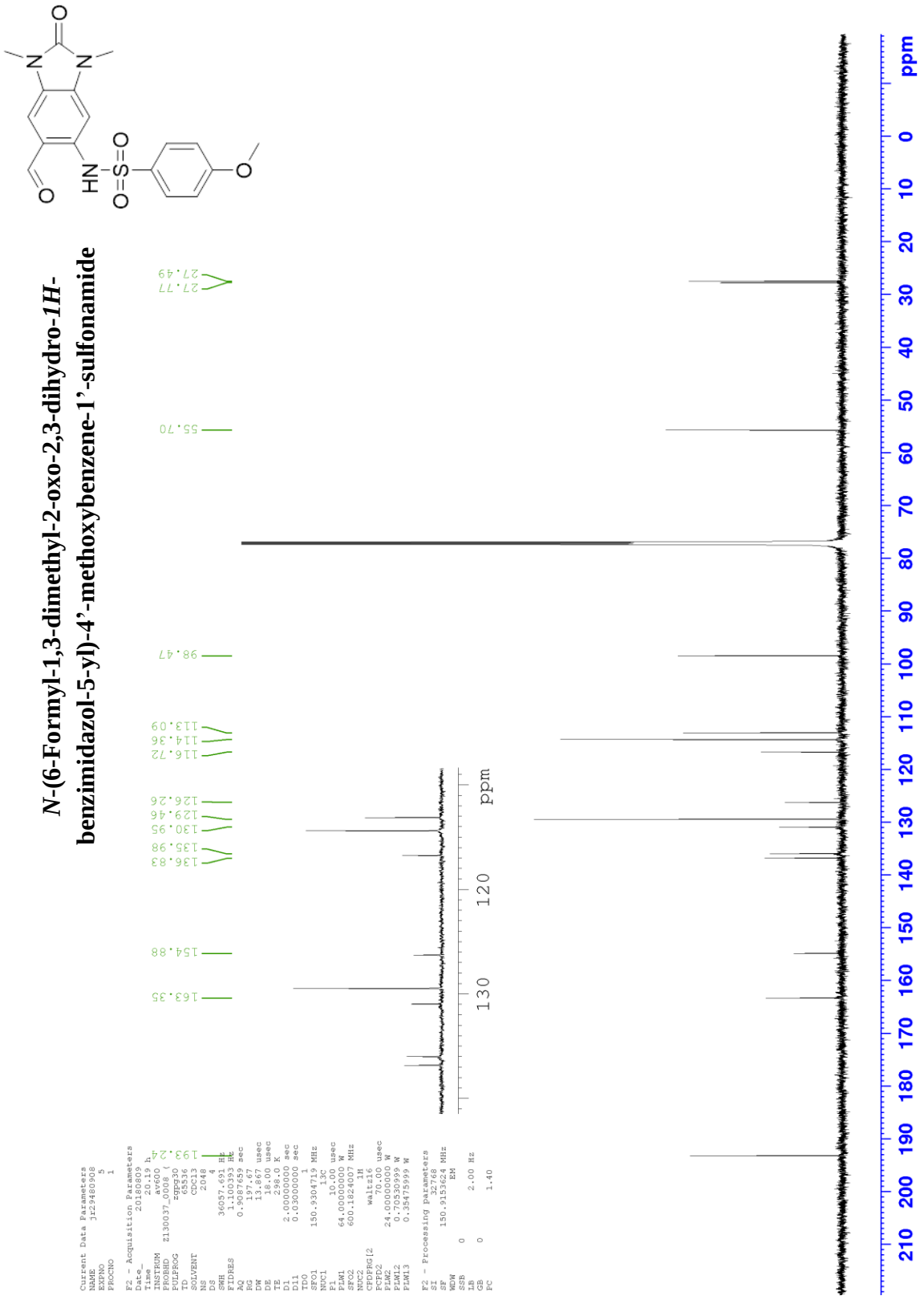
***N N*-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl)benzenesulfonamide**

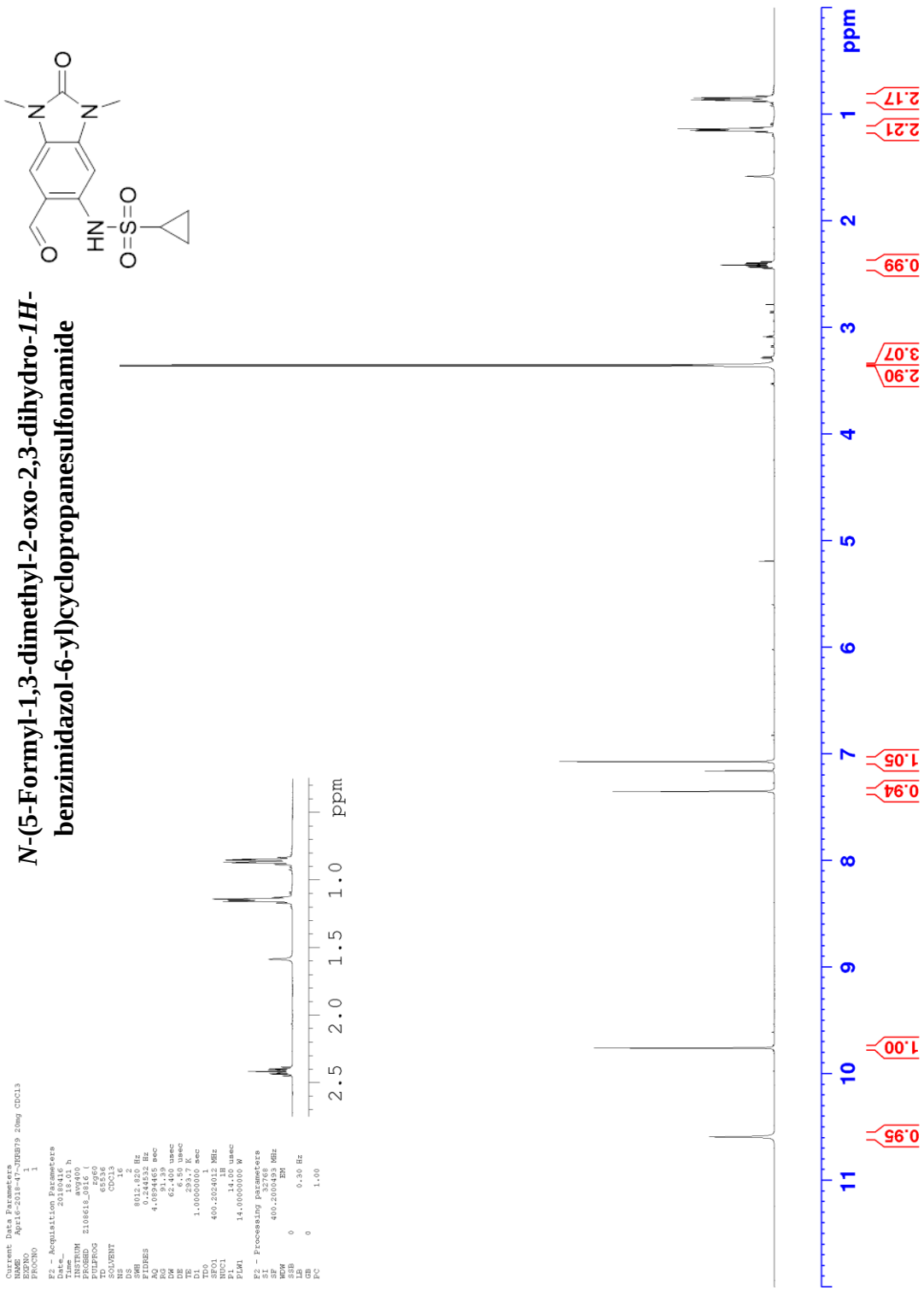


N-(6-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-5-yl)-4'-methoxybenzene-1'-sulfonamide

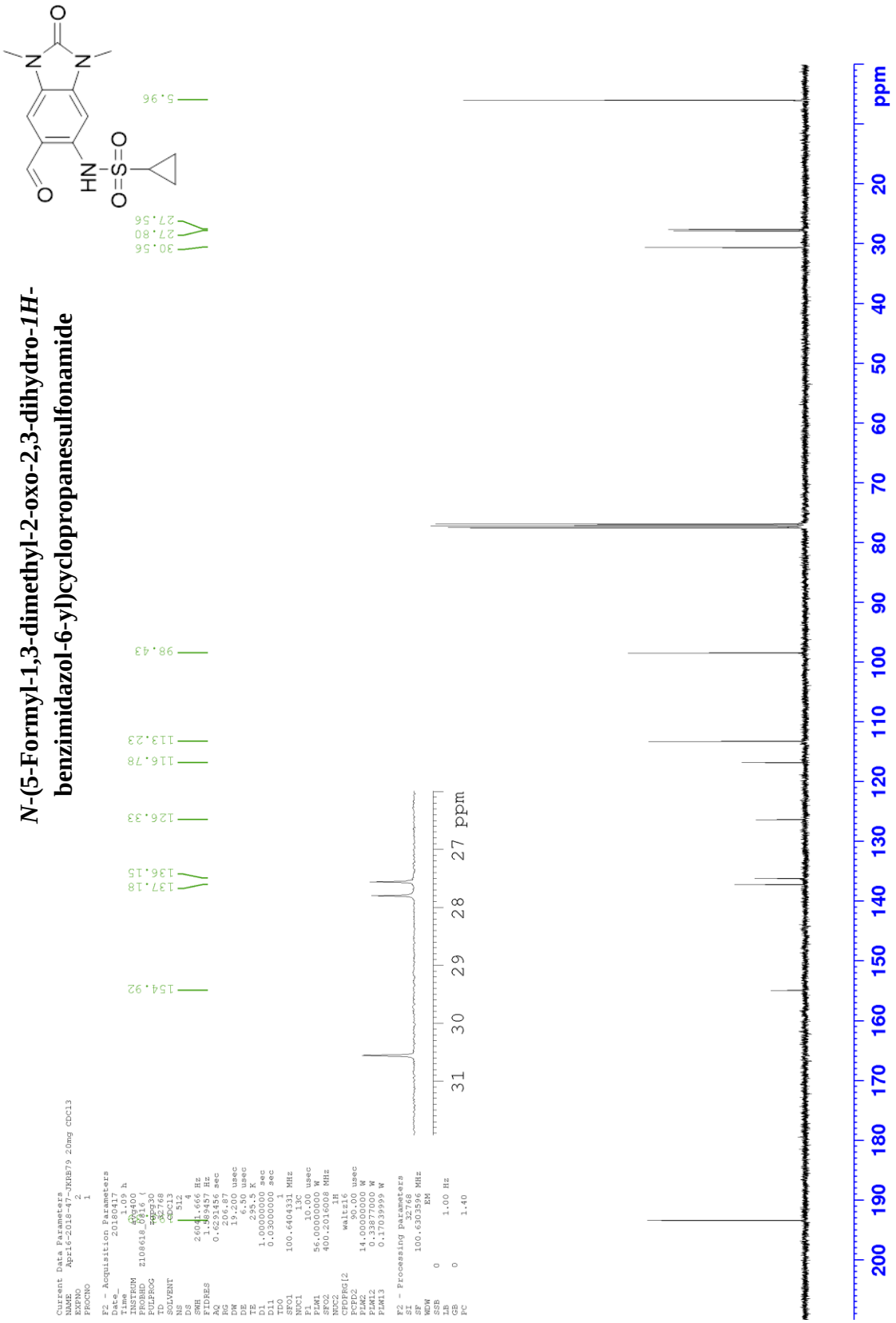


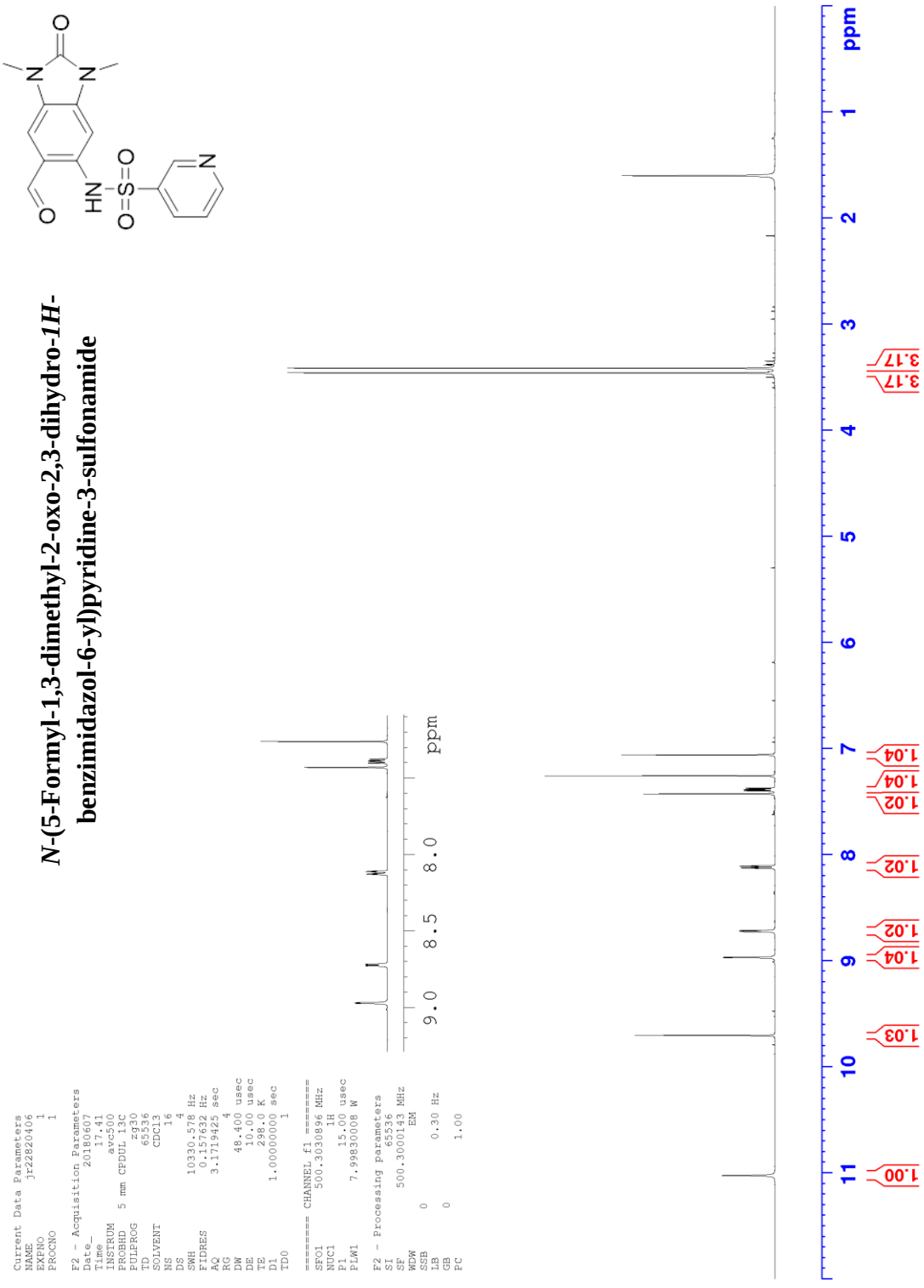
N-(6-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-5-yl)-4'-methoxybenzene-1'-sulfonamide



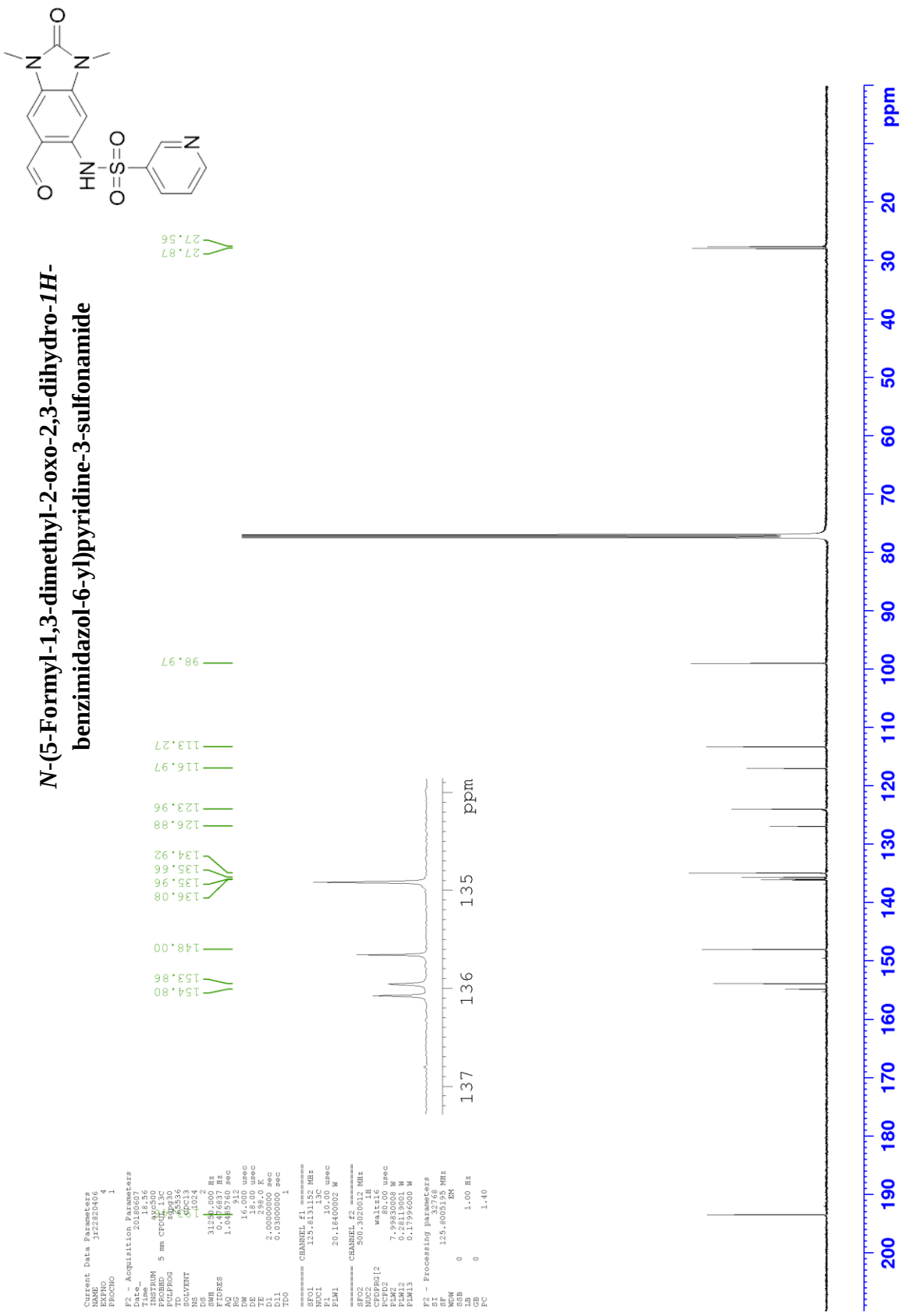

N-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-6-yl)cyclopropanesulfonamide
Current Data Parameters
NAME April-2018-41-TrzNDSB79 20mg CDCl3
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180416
Time 18.01 h
INSTRUM spect
PROBHD 2106618_0816 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8013.870 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 91.30
DW 62.400 usec
DE 6.50 usec
TE 293.7 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000493 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
2.5
2.0
1.5
1.0
ppm
0.95
1.00
1.05
0.94
7.30
7.28
7.26
7.24
7.22
3.32
2.90
3.07
2.21
2.17
0.99
11
10
9
8
7
6
5
4
3
2
1
ppm

_N_-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-_1H_-benzimidazol-6-yl)cyclopropanesulfonamide

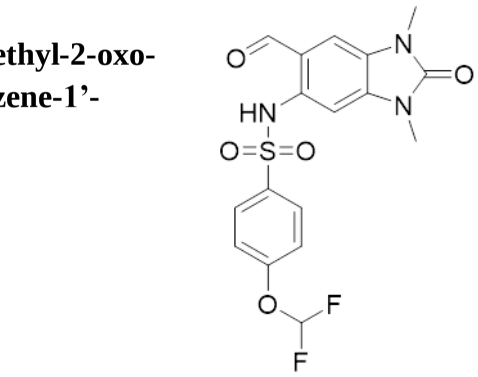



N-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-6-yl)pyridine-3-sulfonamide
Current Data Parameters
NAME jr22820406
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180607
Time 17.41
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000143 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
9.0
8.5
8.0
ppm
1.00
1.03
1.04
1.02
1.04
1.02
1.02
1.04
1.04
3.17
3.17
11
10
9
8
7
6
5
4
3
2
1
ppm

_N_-(5-Formyl-1,3-dimethyl-2-oxo-2,3-dihydro-_1H_-benzimidazol-6-yl)pyridine-3-sulfonamide



4’-(Difluoromethoxy)-*N*-(5-formyl-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl)benzene-1’-sulfonamide

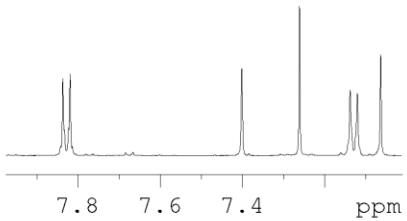


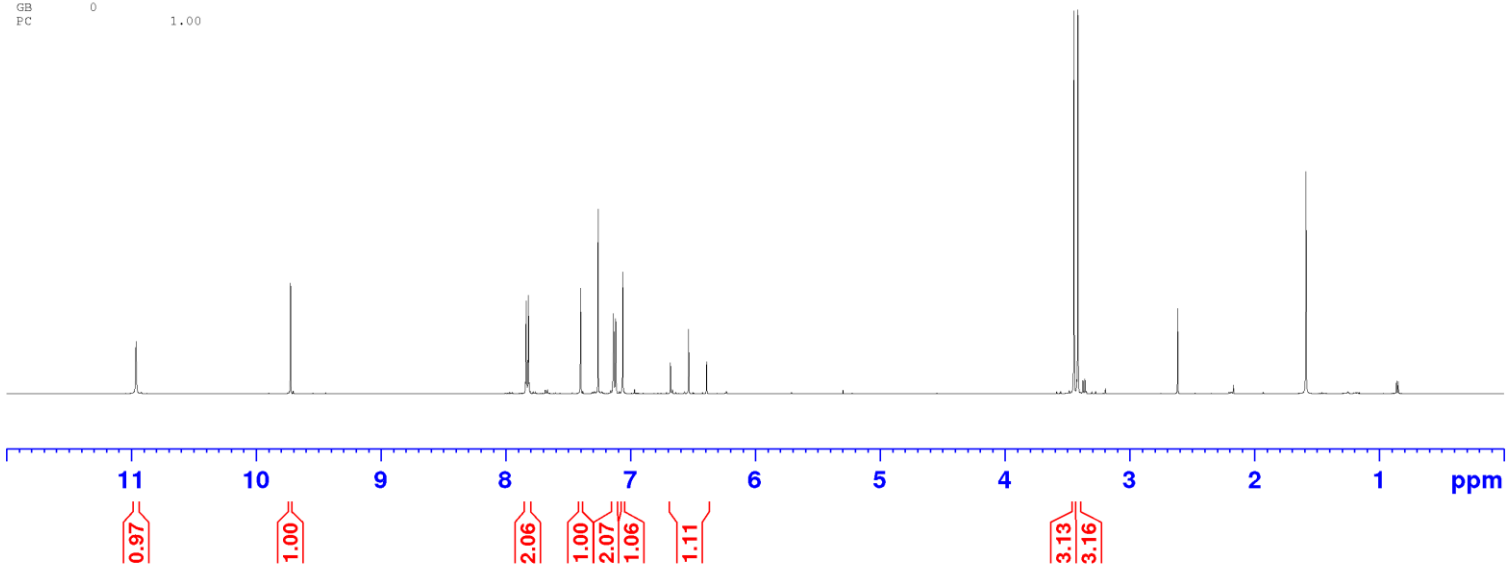

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Current Data Parameters
NAME          jr22390406
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20180605
Time               12.16
INSTRUM          avc500
PROBHD    5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719425 sec
RG                  3.56
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1            1.00000000 sec
TD0                    1

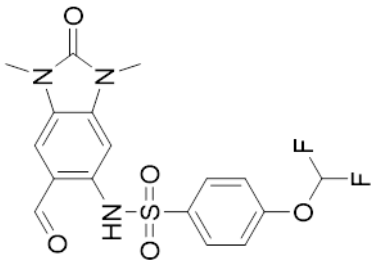
======== CHANNEL f1 ========
SFO1         500.3030896 MHz
NUC1                   1H
P1                  15.00 usec
PLW1          7.99830008 W

F2 - Processing parameters
SI                 65536
SF            500.3000137 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

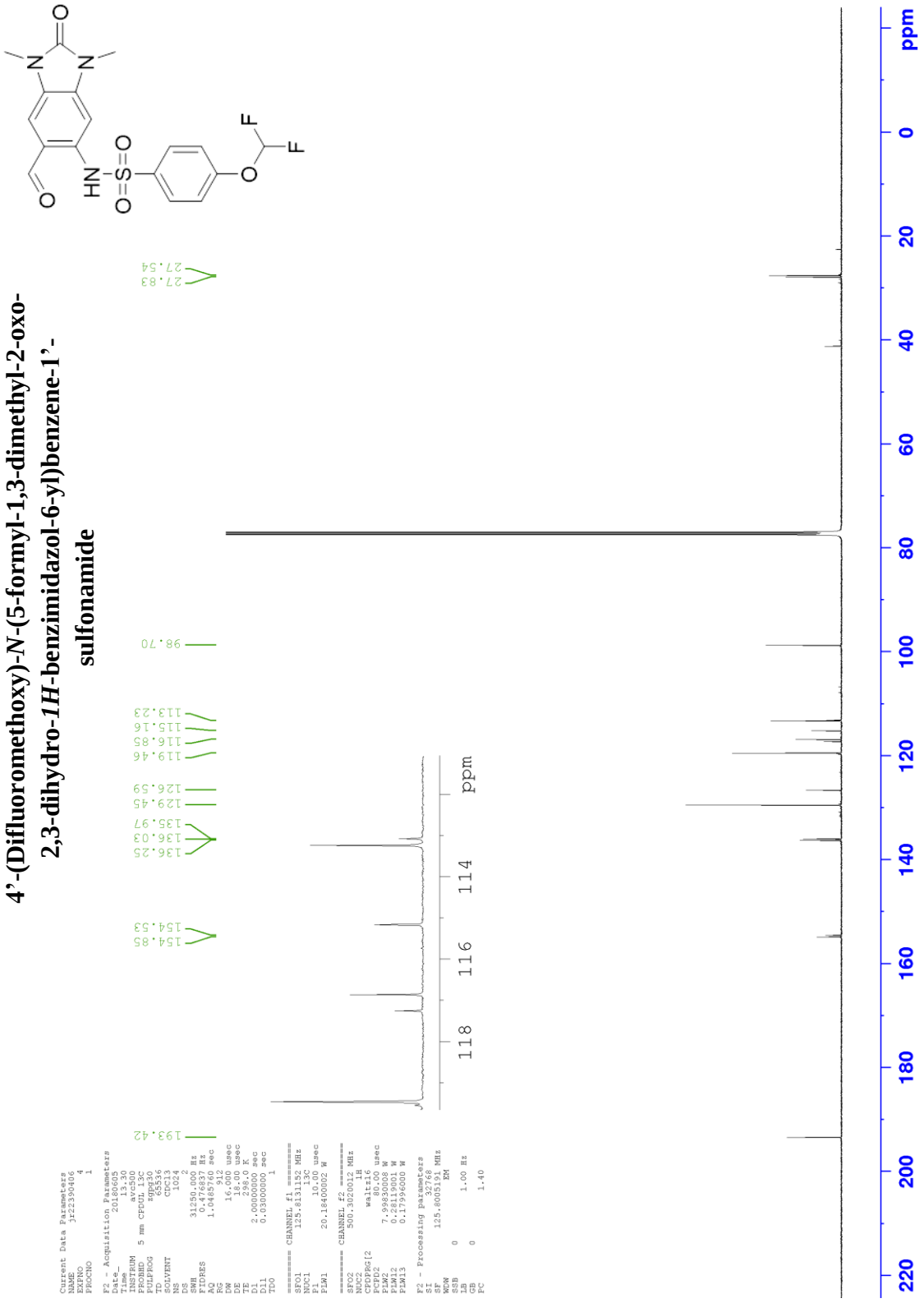




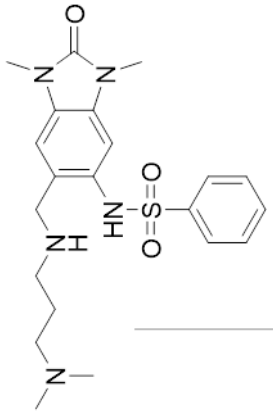
4'-(Difluoromethoxy)-*N*-(5-formyl-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl)benzene-1'-sulfonamide



4'-(Difluoromethoxy)-*N*-(5-formyl-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl)benzene-1'-sulfonamide



_N_-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-_1H_-benzimidazol-6-yl]benzenesulfonamide

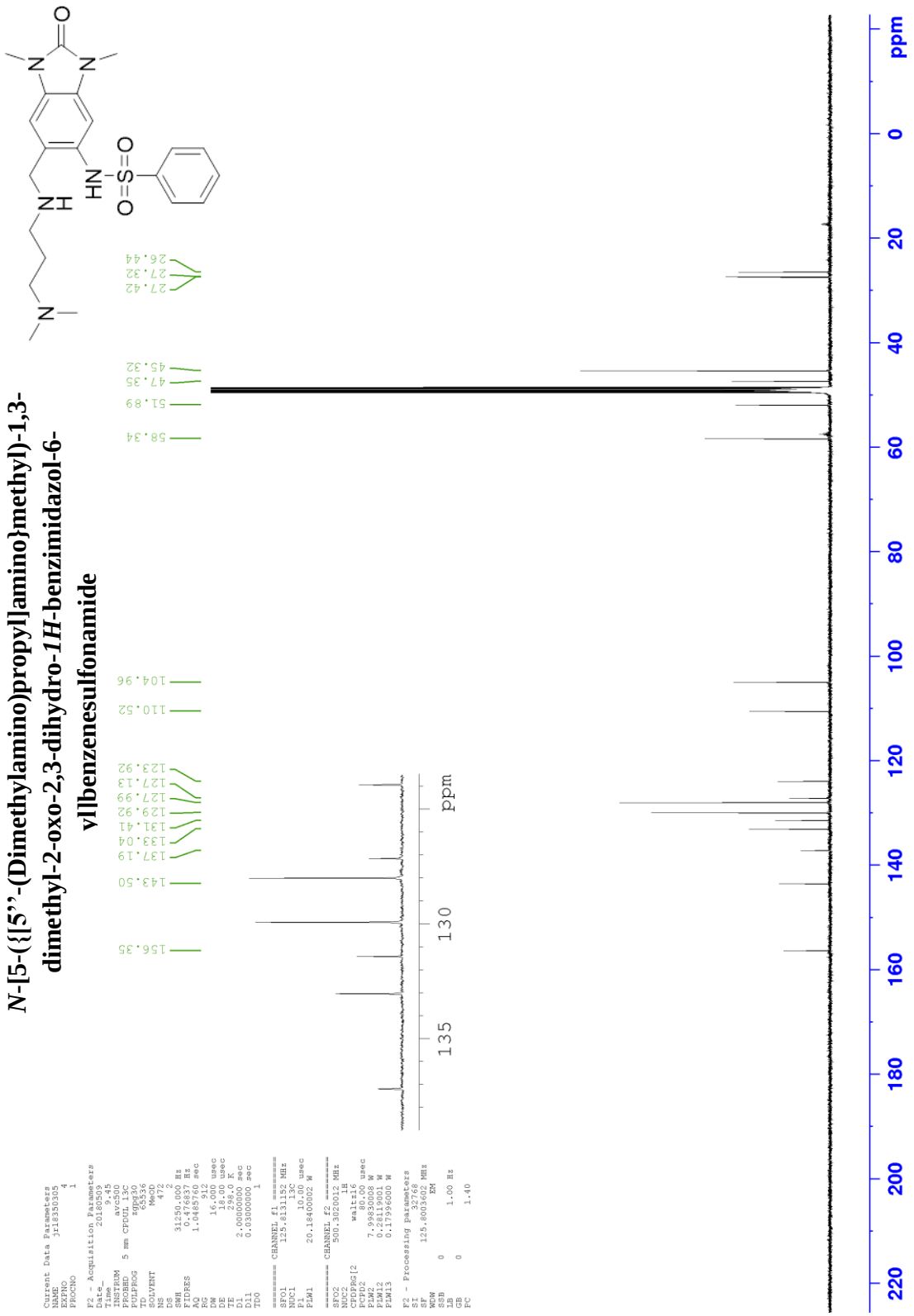



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Current Data Parameters
NAME          jr18350305
EXPNO                  1
PROCNO                 1

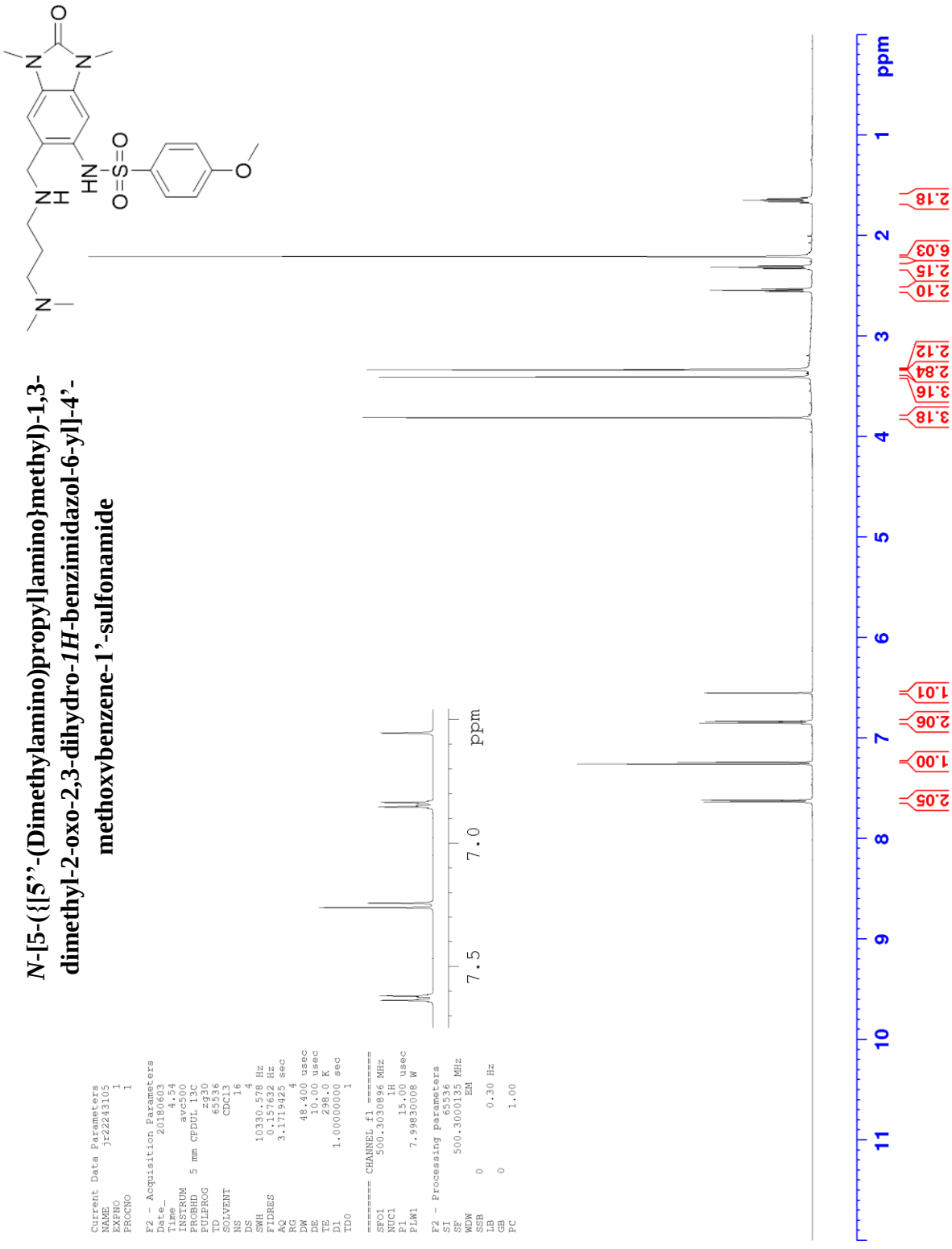
F2 - Acquisition Parameters
Date_           20180509
Time                8.59
INSTRUM           avc500
PROBHD  5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT            MeOD
NS                   16
DS                    4
SWH           10330.578 Hz
FIDRES        0.157632 Hz
AQ            3.1719425 sec
RG                 3.56
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         500.3030896 MHz
NUC1                 1H
P1                15.00 usec
PLW1         7.99830008 W

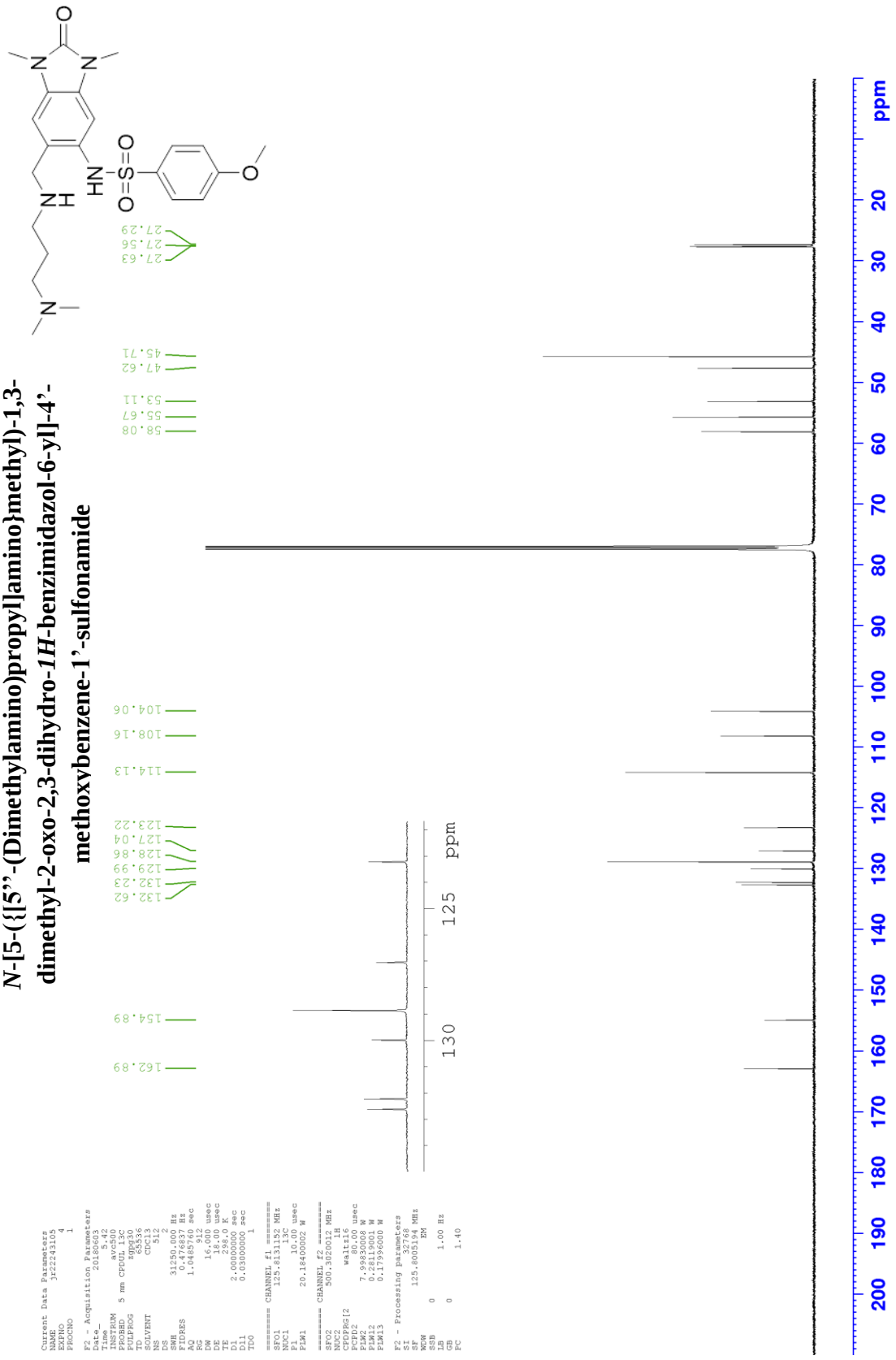
F2 - Processing parameters
SI                65536
SF           500.3000109 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```


N-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]benzenesulfonamide
156.35
143.50
137.19
133.04
131.41
129.92
127.99
127.13
123.92
110.52
104.96
58.34
51.89
47.35
45.32
27.42
27.32
26.44
135
130
ppm
220
200
180
160
140
120
100
80
60
40
20
0
ppm

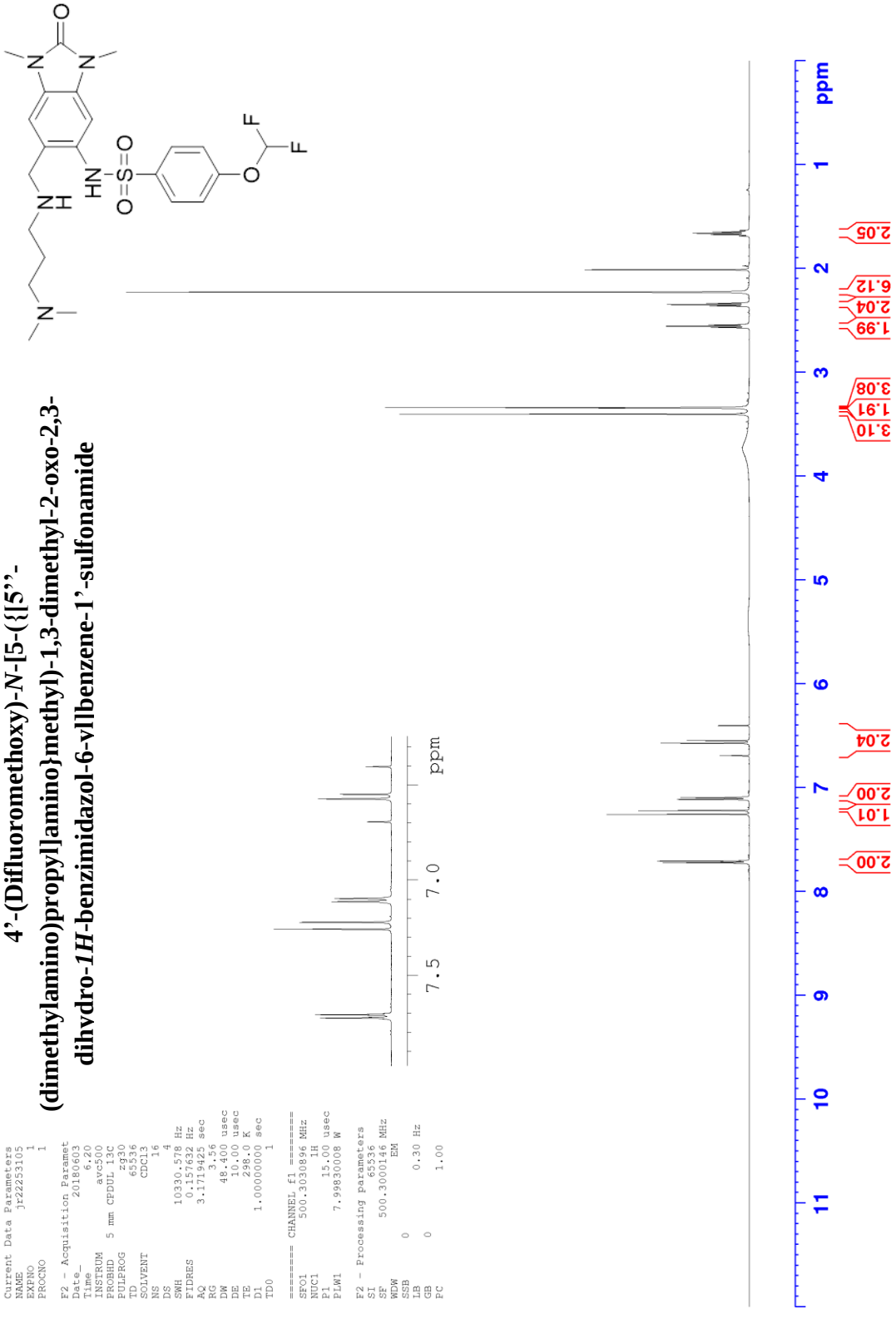
***N*-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]-4'-methoxybenzene-1'-sulfonamide**

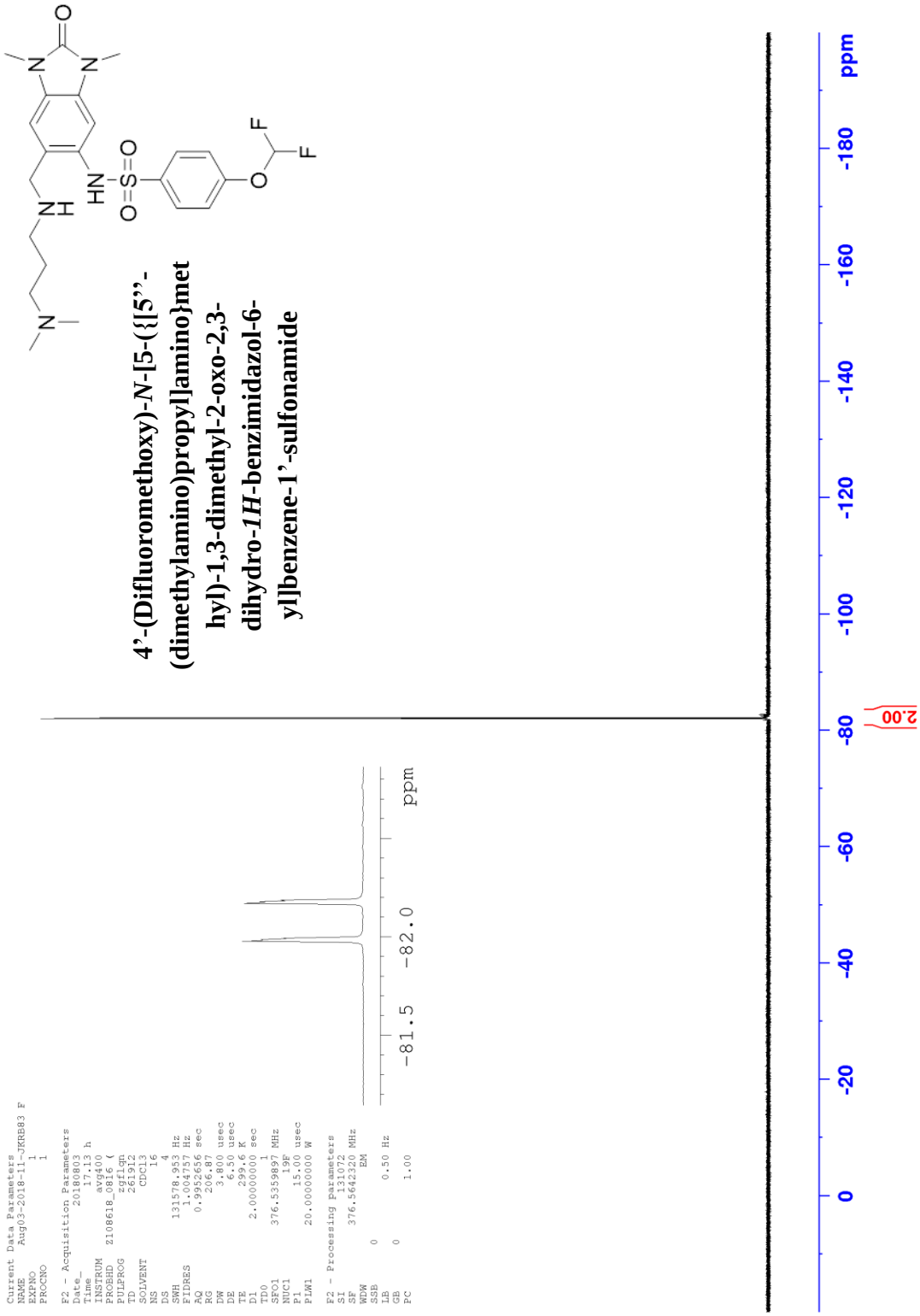


***N*-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]-4'-methoxybenzene-1'-sulfonamide**

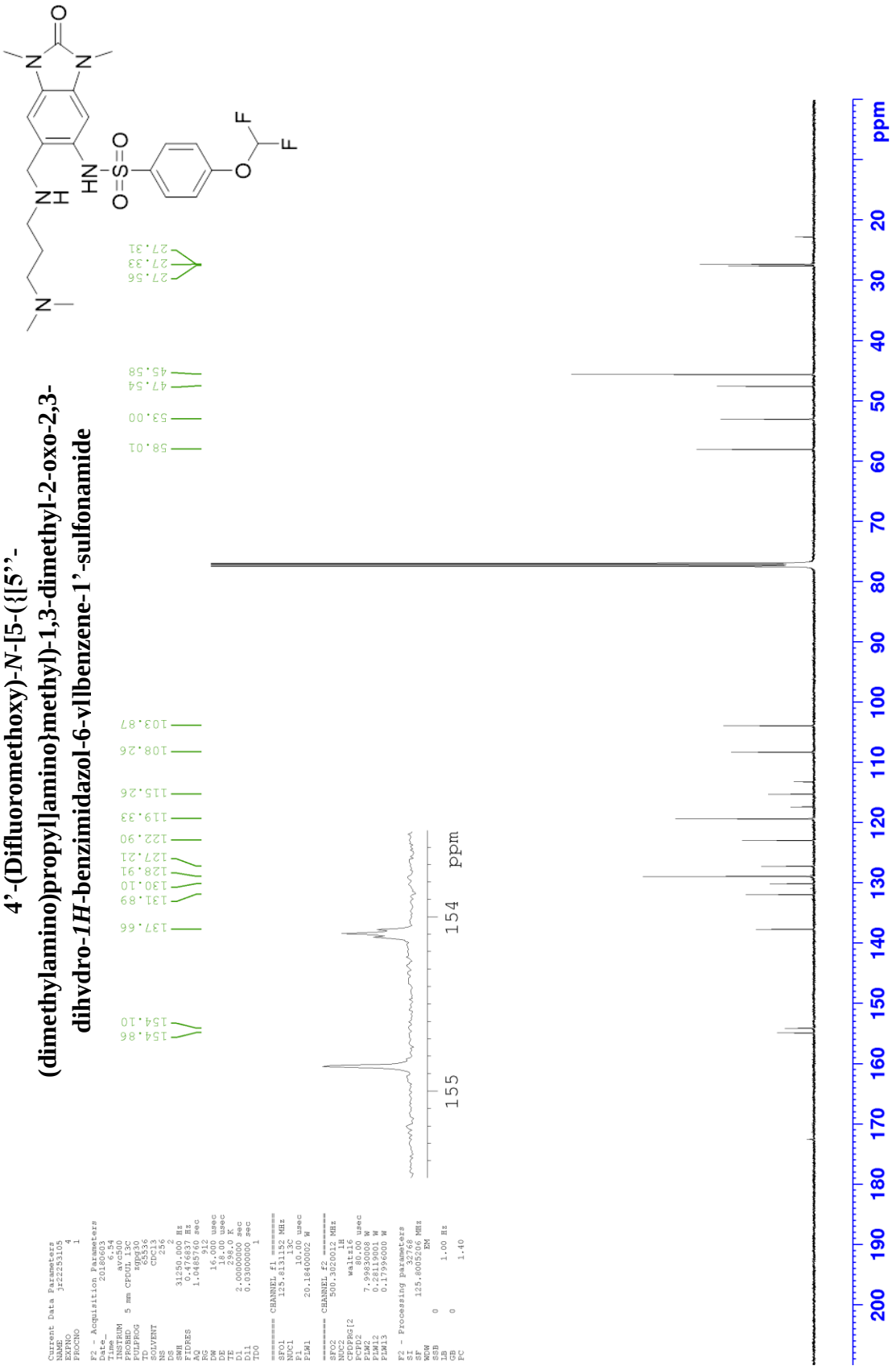


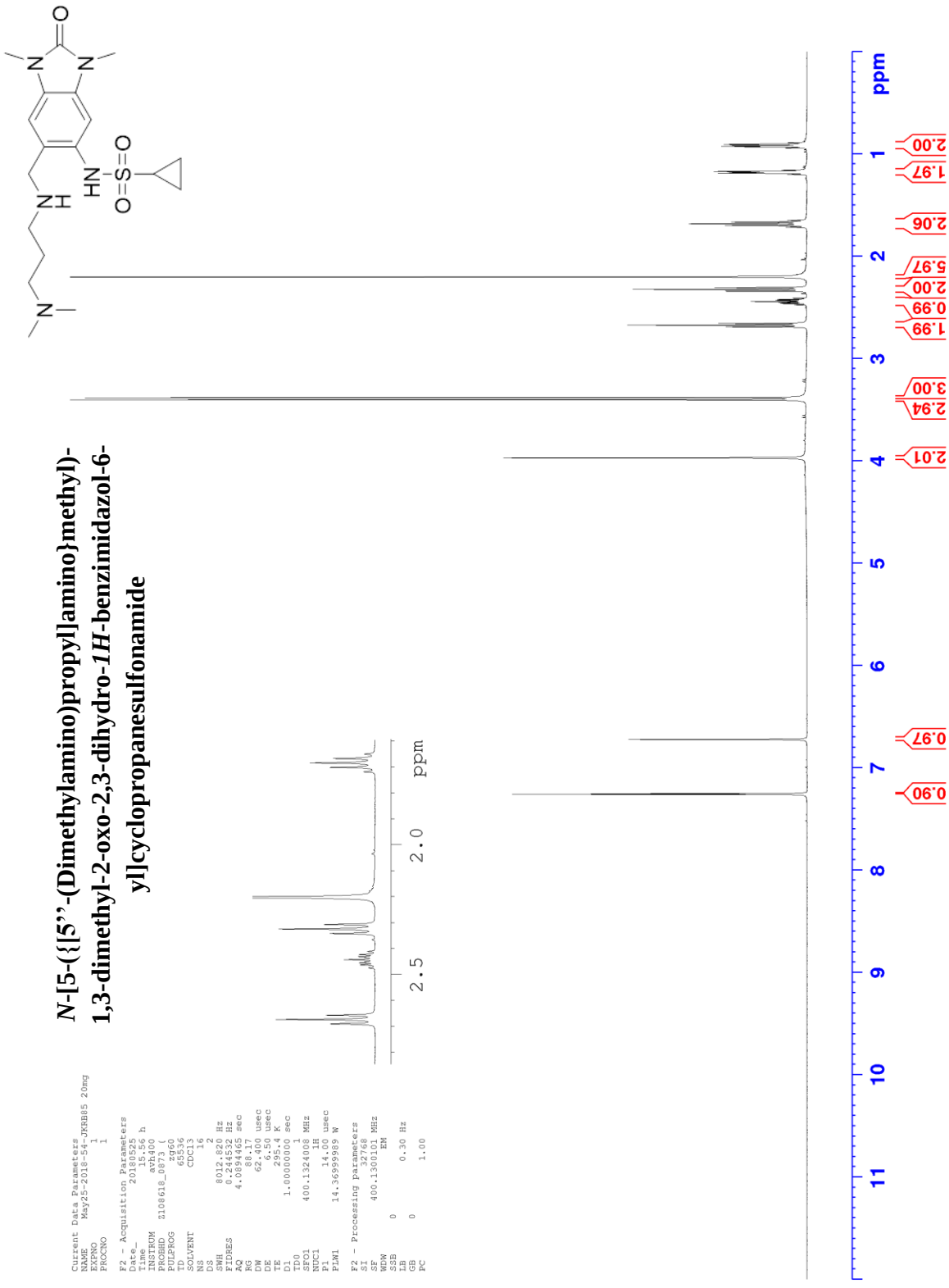
4'-(Difluoromethoxy)-*N*-[5-({[5''-(dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]benzene-1'-sulfonamide



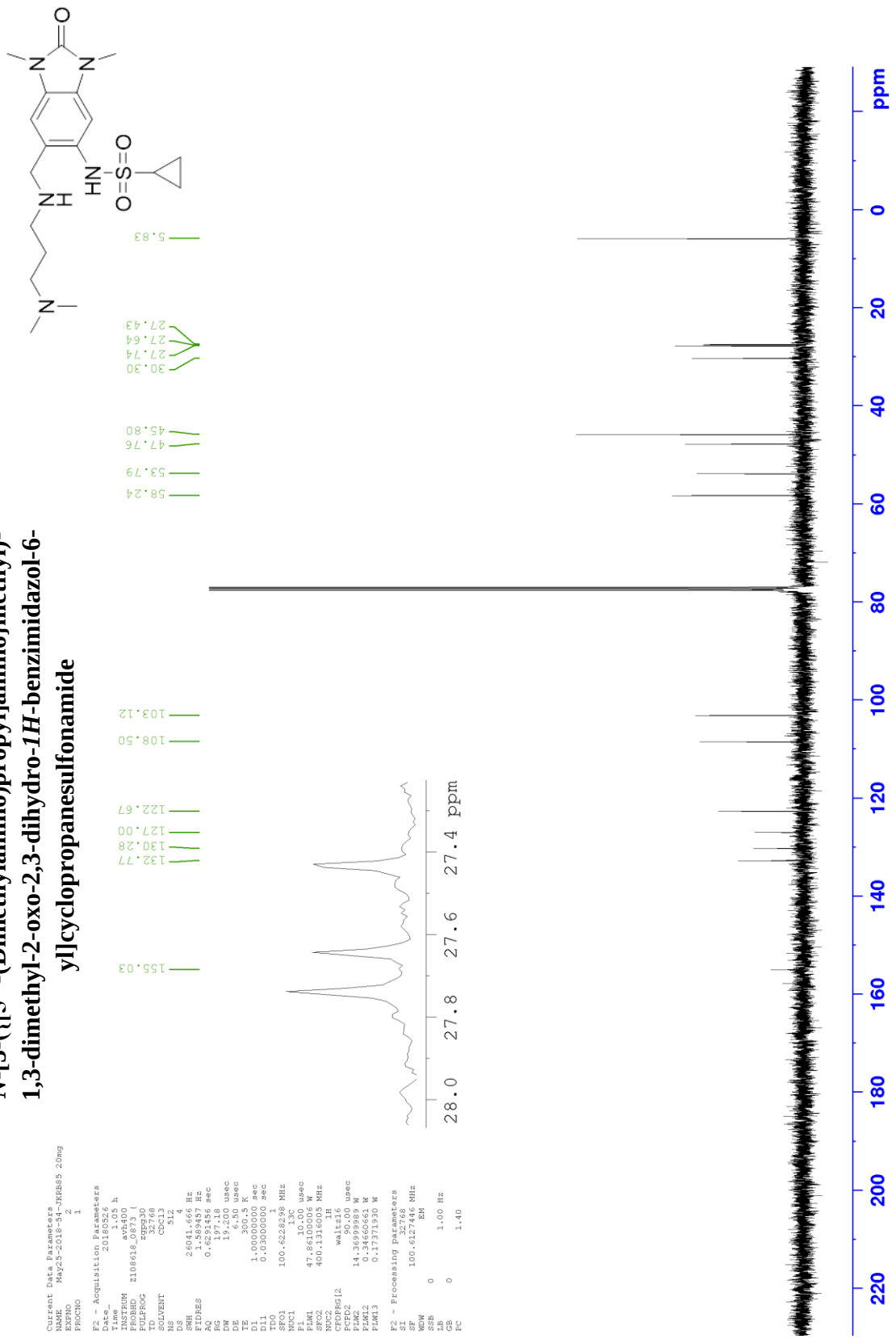

4'-(Difluoromethoxy)-*N*-[5-({[5''-(dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]benzene-1'-sulfonamide
2.00
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
ppm
-81.5
-82.0
ppm

4'-(Difluoromethoxy)-*N*-[5-({[5''-(dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]benzene-1'-sulfonamide

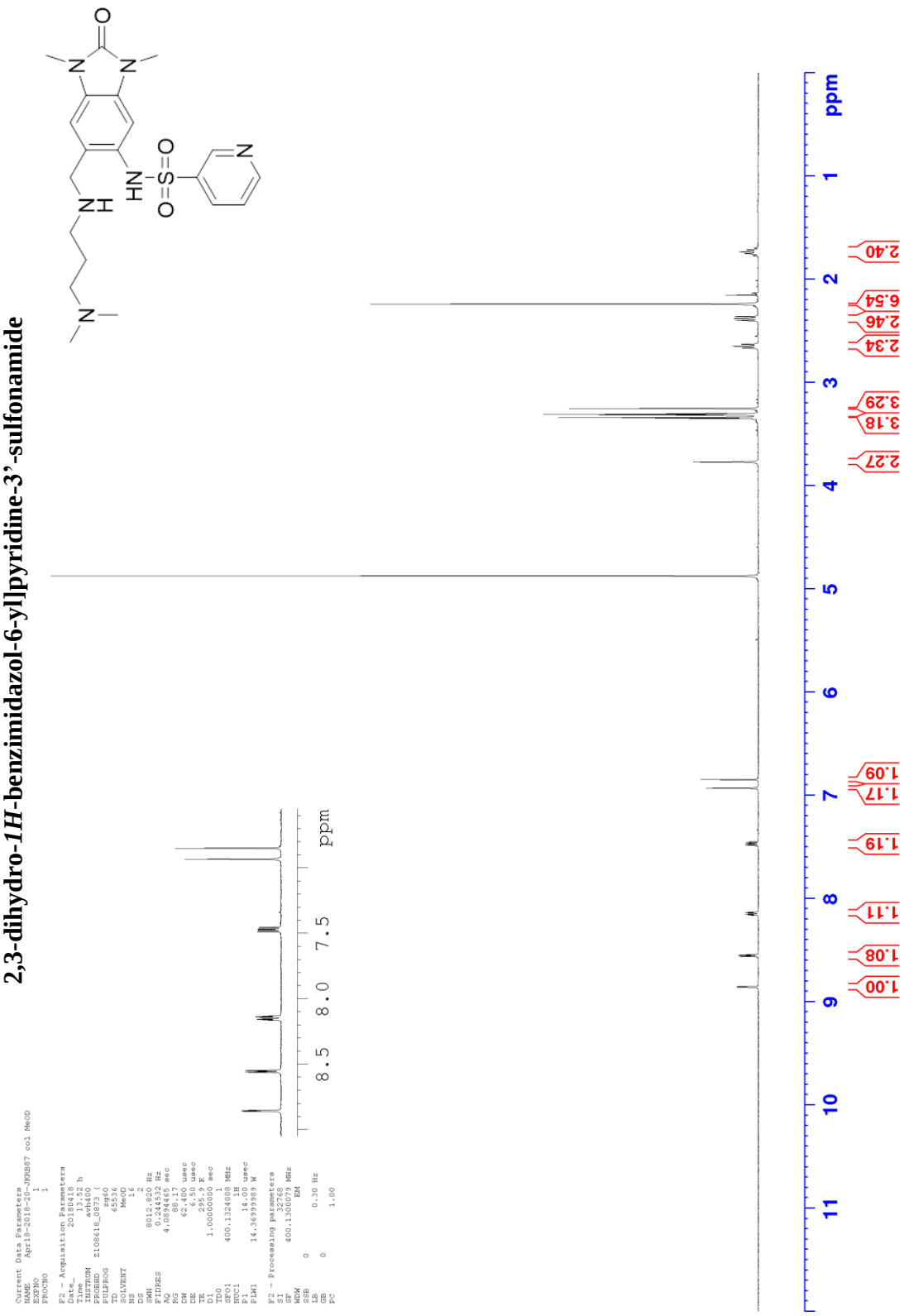


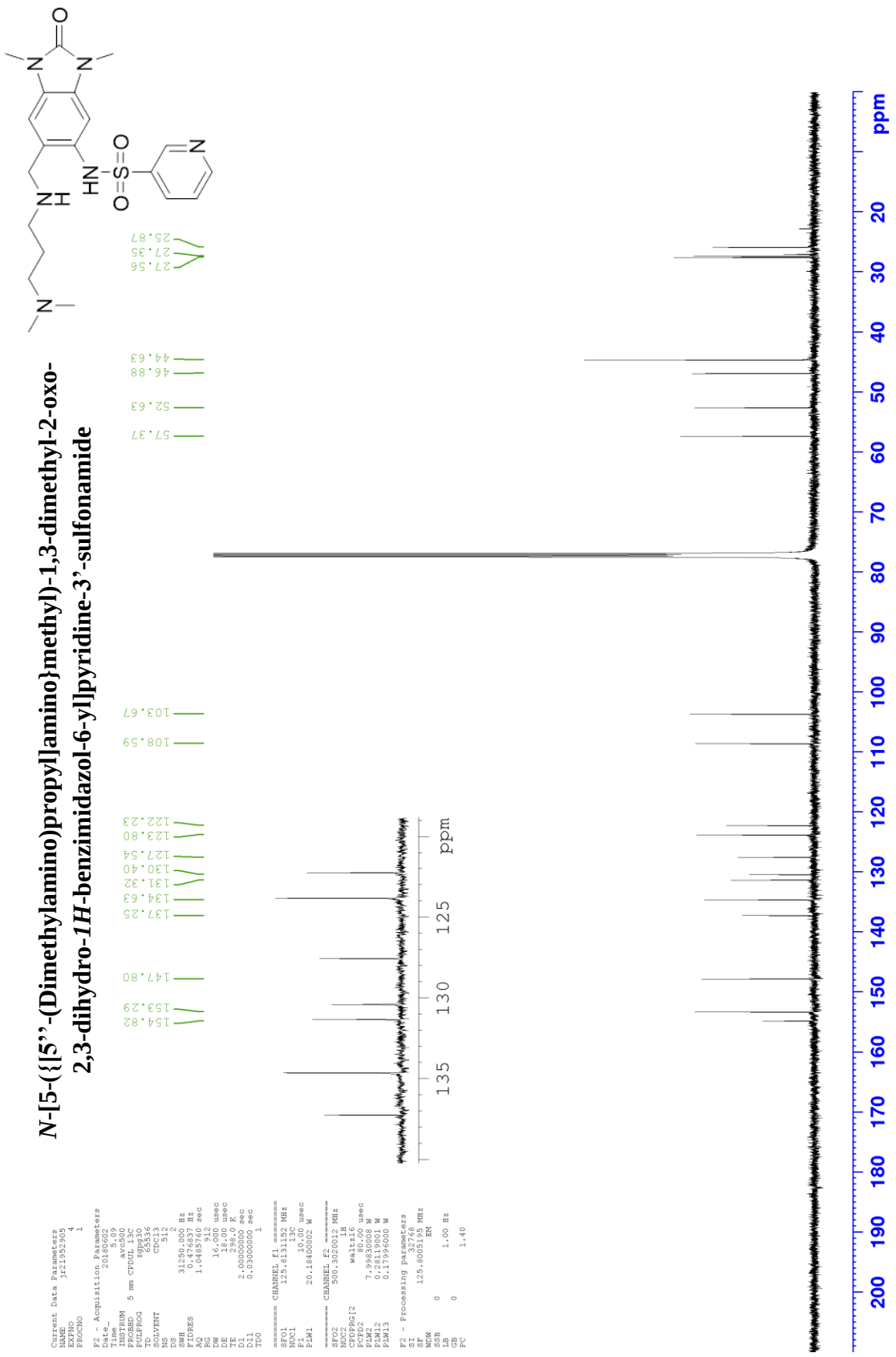

Current Data Parameters
NAME May25-2018-54-JKRB5 20mg
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180525
Time 15.56 h
INSTRUM av400
PROBHD 5mm I
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244552 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 295.4 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300101 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
N-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-
1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-6-
yl]cyclopropanesulfonamide
2.5
2.0
ppm
0.90
0.97
2.01
2.94
3.00
1.99
0.99
2.00
5.97
2.06
1.97
2.00
ppm

_N_-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]cyclopropanesulfonamide

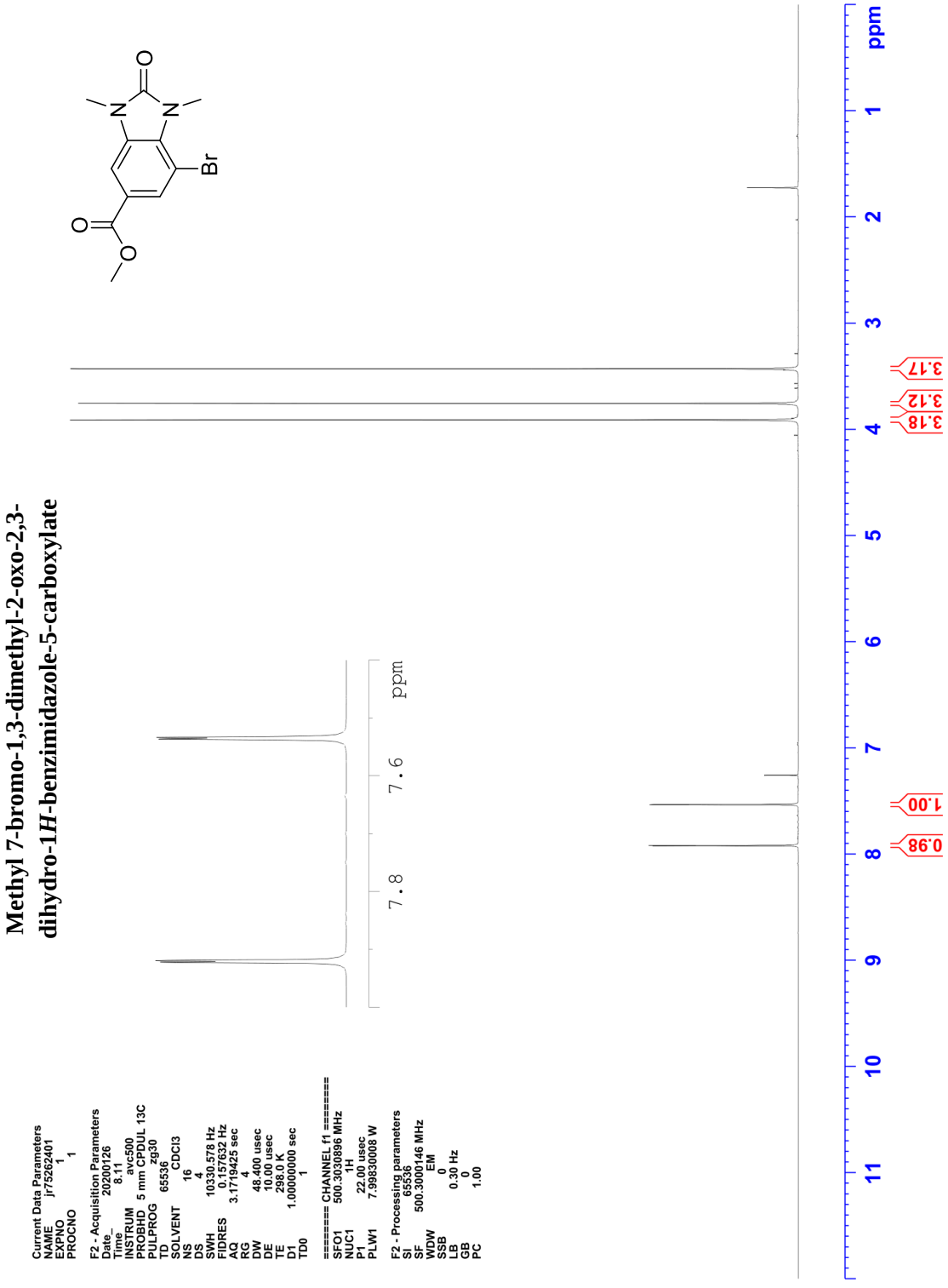


***N*-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazol-6-yl]pyridine-3'-sulfonamide**

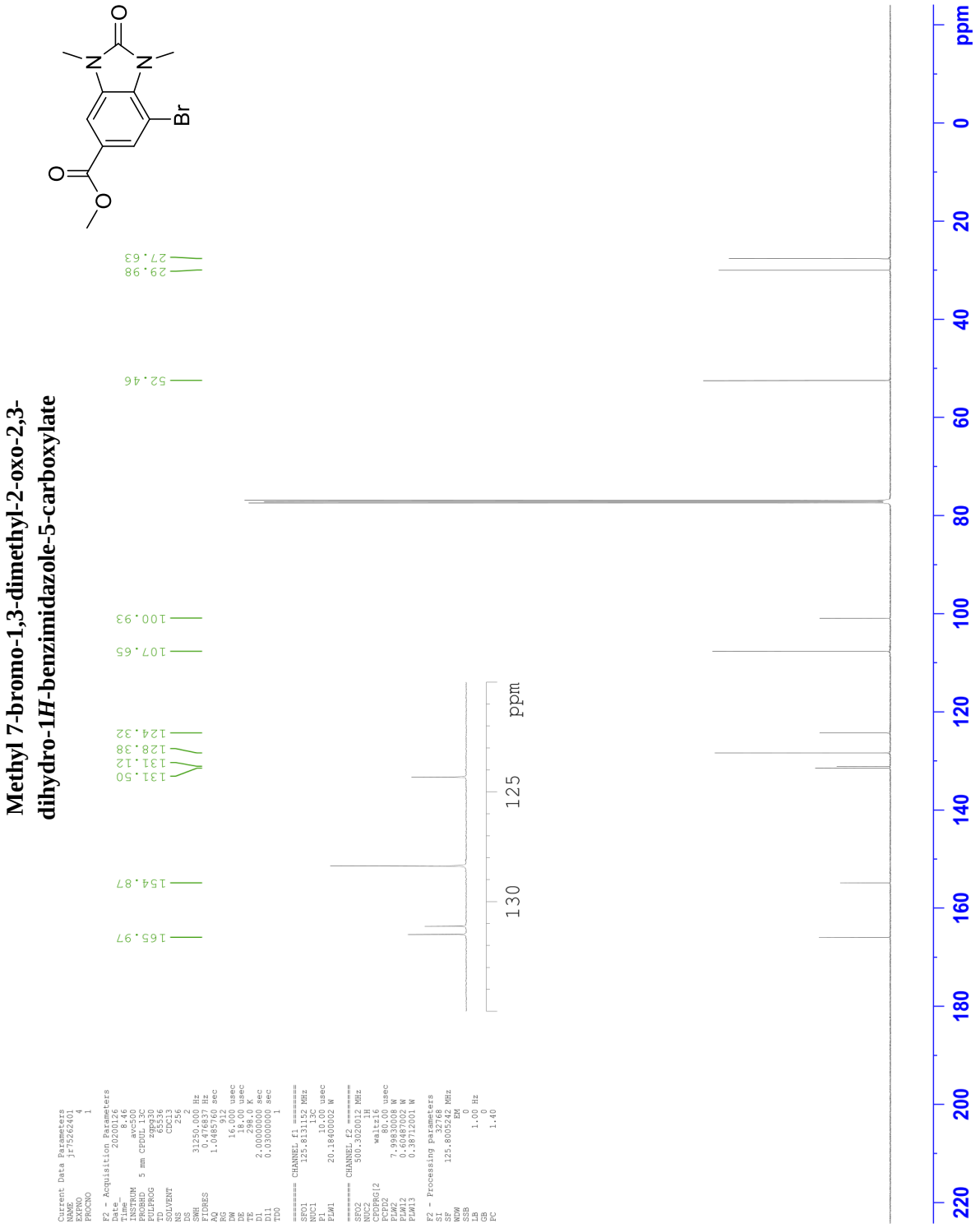


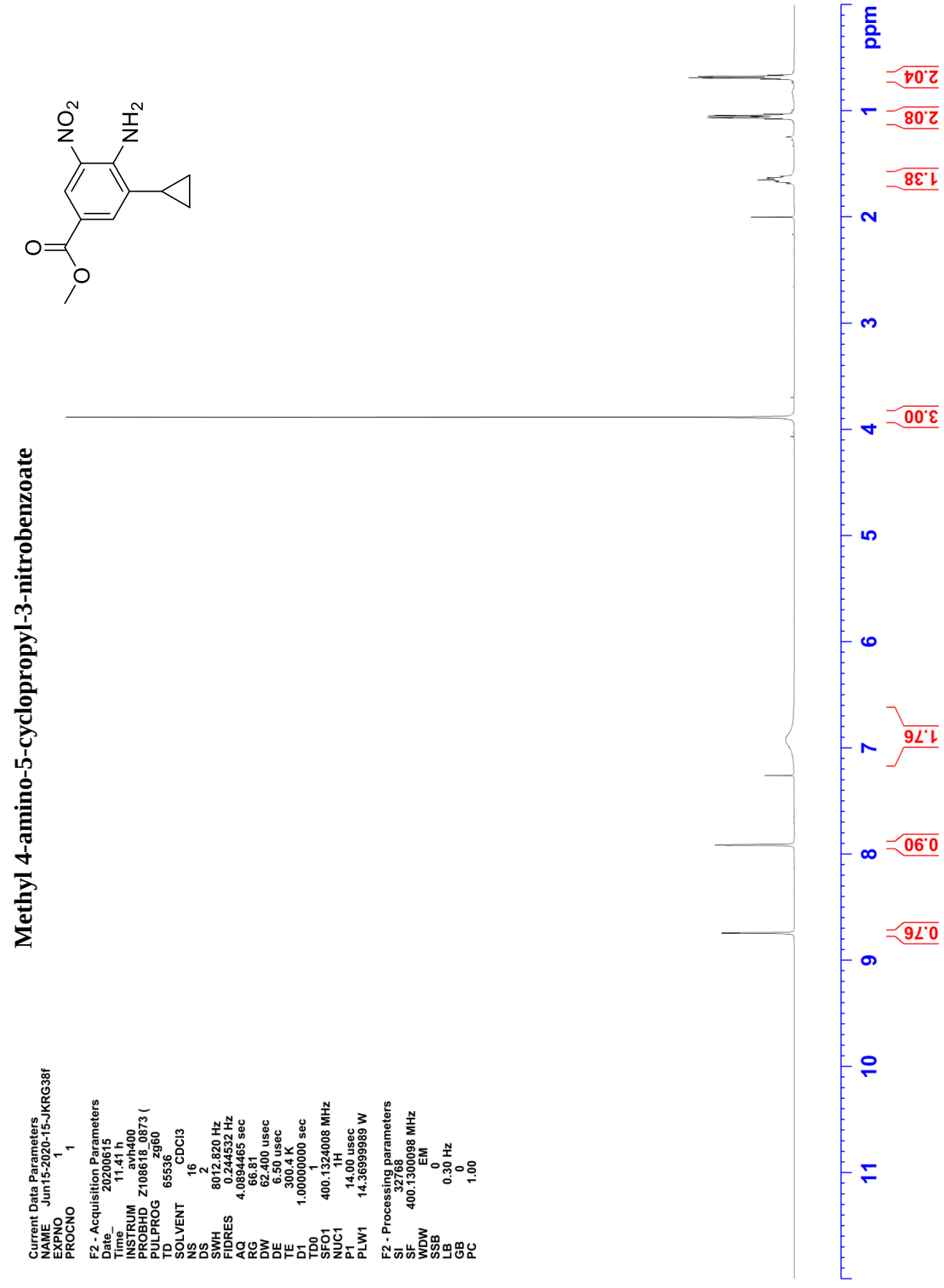

N-[5-({[5''-(Dimethylamino)propyl]amino}methyl)-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-6-yl]pyridine-3'-sulfonamide
Current Data Parameters
NAME jr21952905
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180602
Time_ 5.09
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG WURST40
TD 65536
SOLVENT CDCl3
NS 512
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 362
DW 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 7.99830008 W
PLW12 0.82800001 W
PLW13 0.17996000 W
F2 - Processing parameters
SI 32768
SF 125.8005195 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
154.82
153.29
147.80
137.25
134.63
131.32
130.40
127.54
123.80
122.23
108.59
103.67
57.37
52.63
46.88
44.63
27.56
27.35
25.87
135
130
125
ppm
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

Methyl 7-bromo-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate



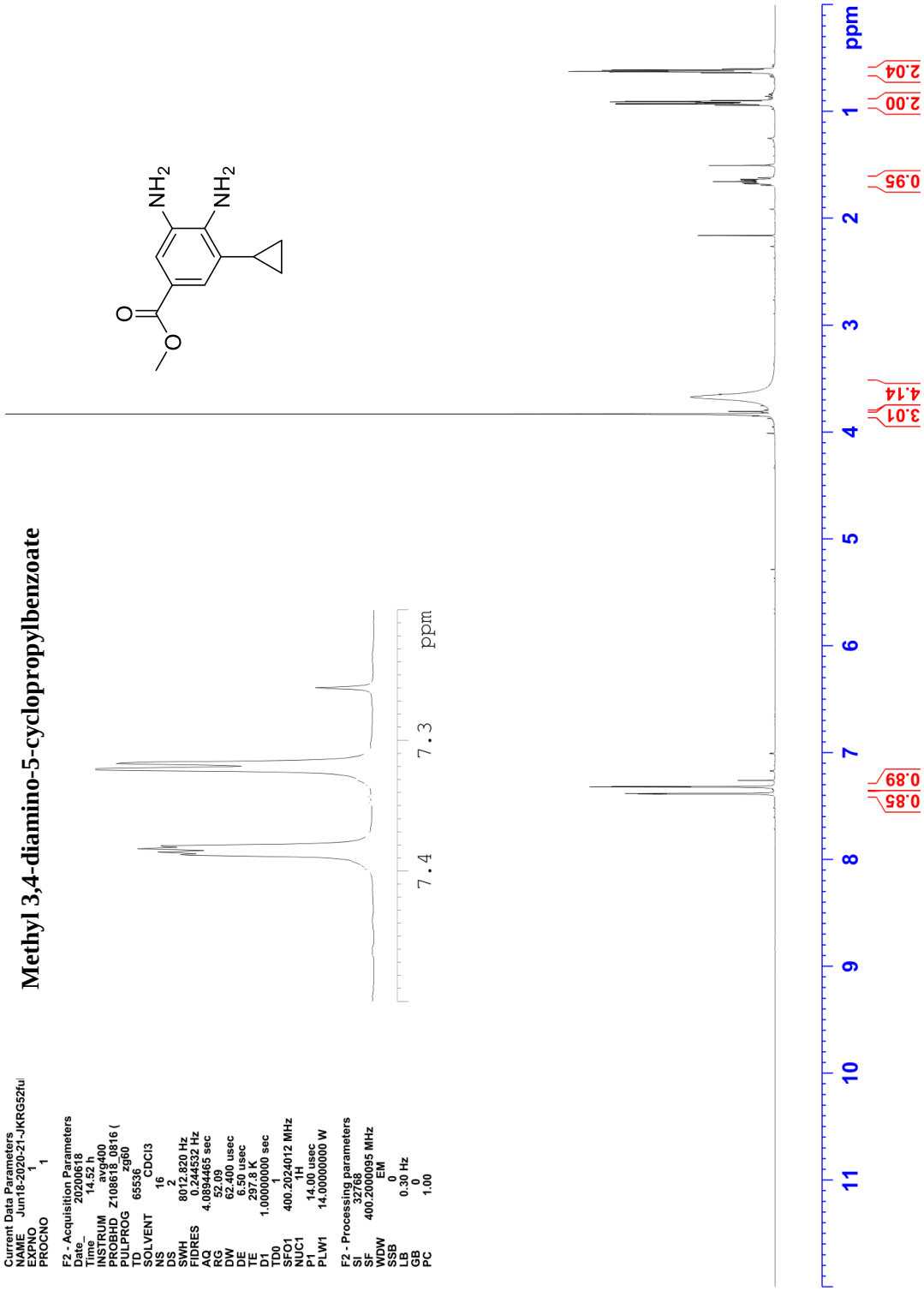
Methyl 7-bromo-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate

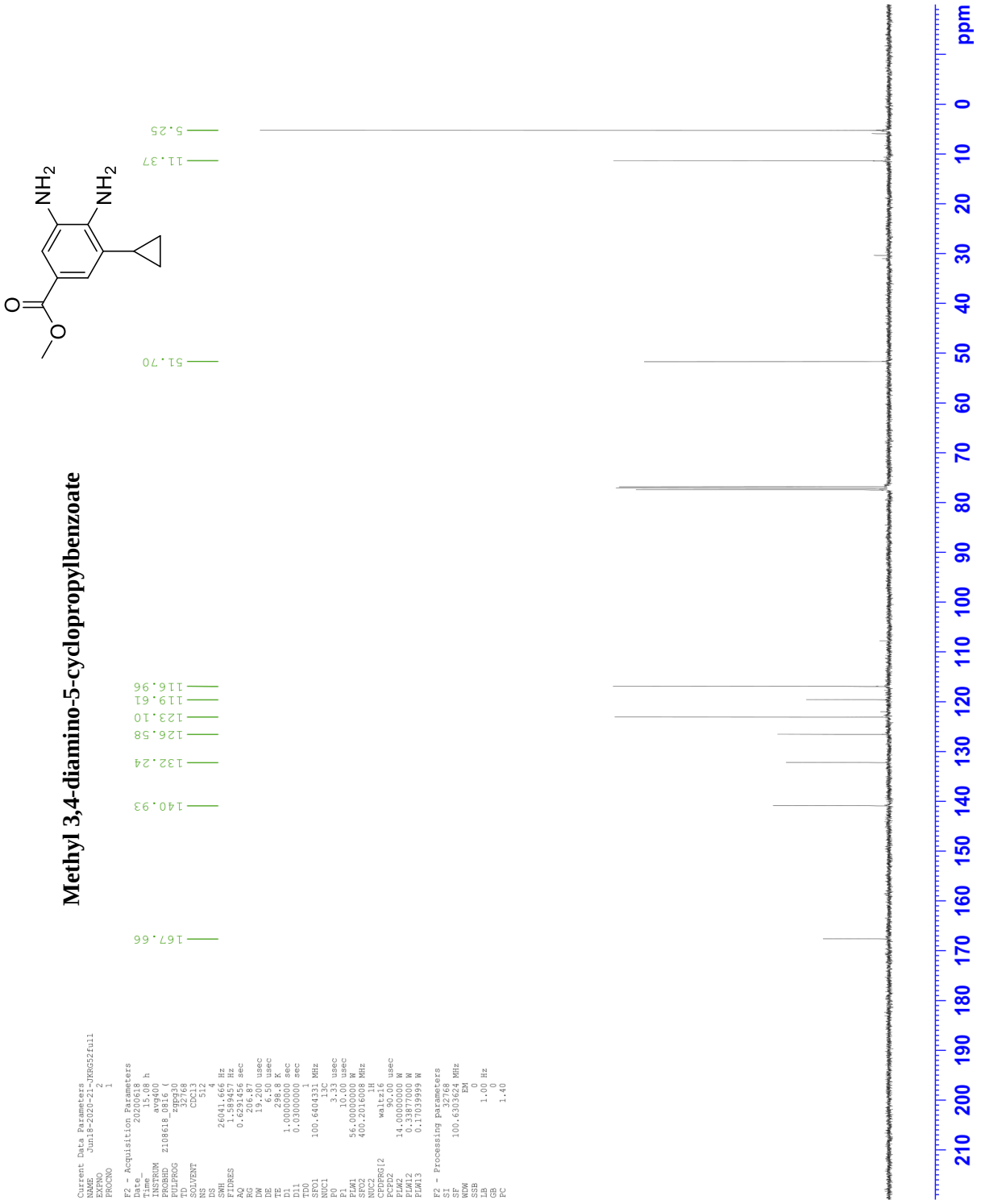


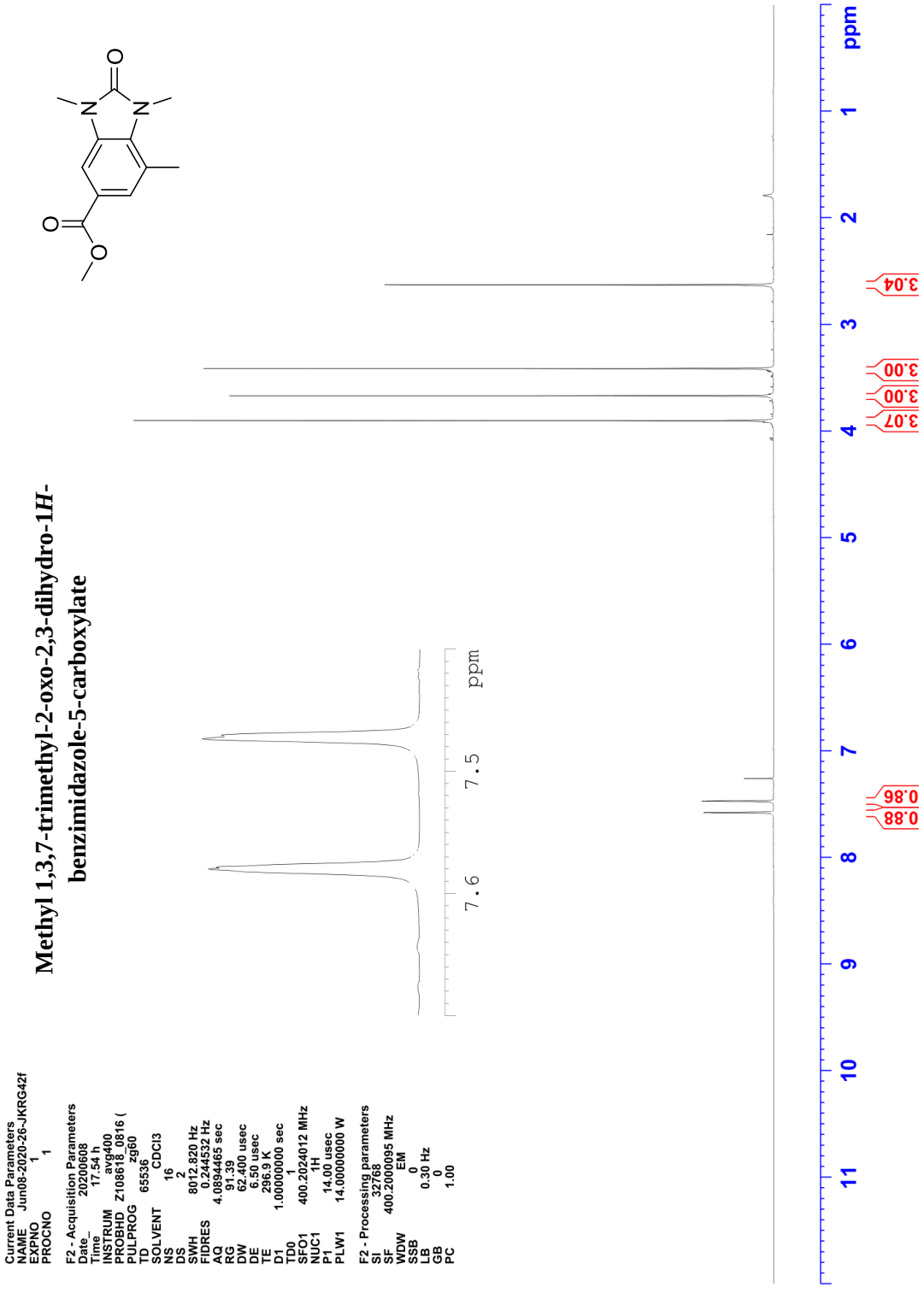

Methyl 4-amino-5-cyclopropyl-3-nitrobenzoate
Current Data Parameters
NAME Jun15-2020-15-JKRG38f
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200615
Time 11.41 h
INSTRUM avh400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 66.81
DW 62.400 usec
DE 6.50 usec
TE 300.4 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300098 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
NO2
NH2
O
O
ppm
11
10
9
8
7
6
5
4
3
2
1
0.76
0.90
1.76
3.00
1.38
2.08
2.04

Methyl 4-amino-5-cyclopropyl-3-nitrobenzoate

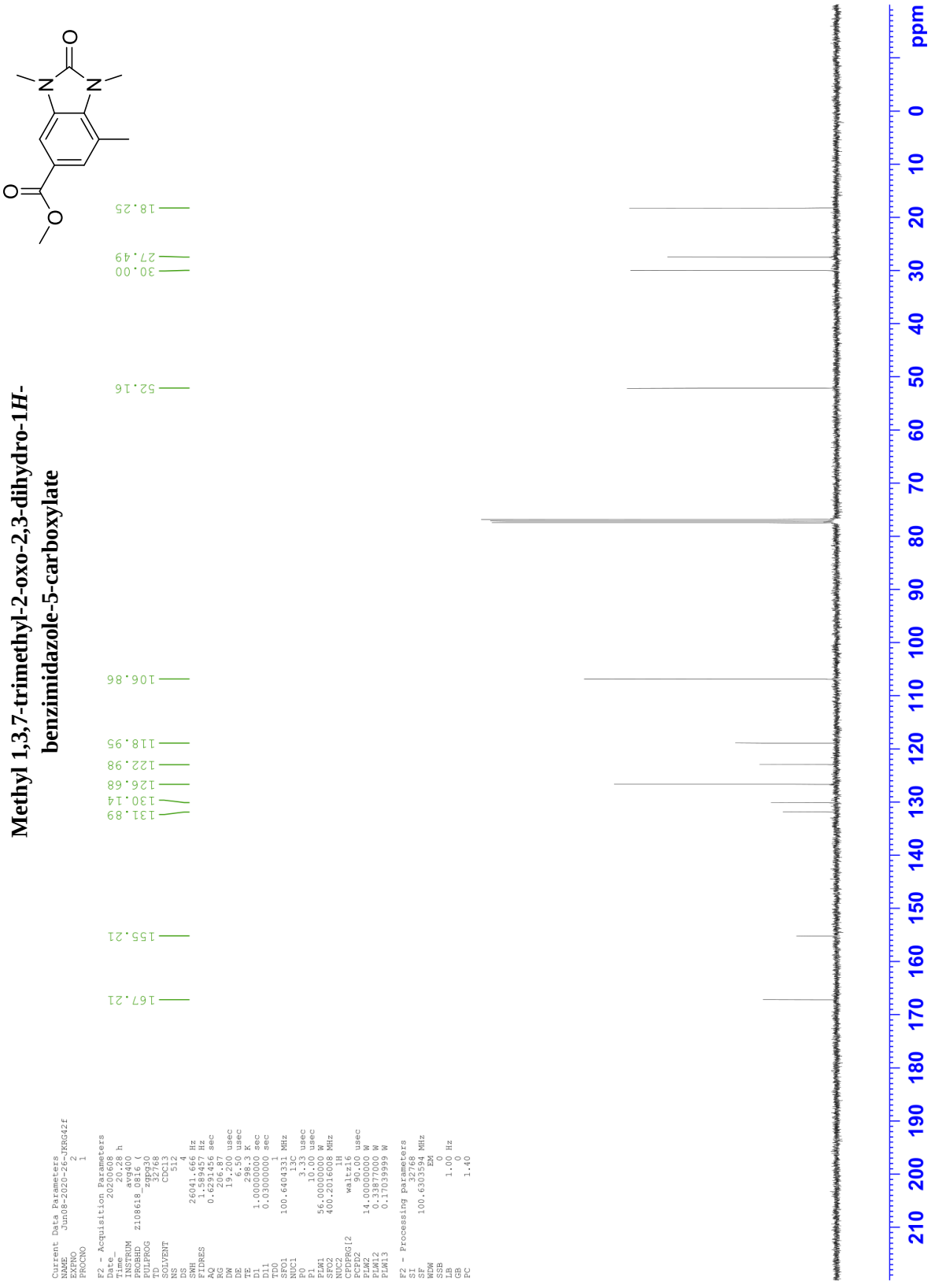


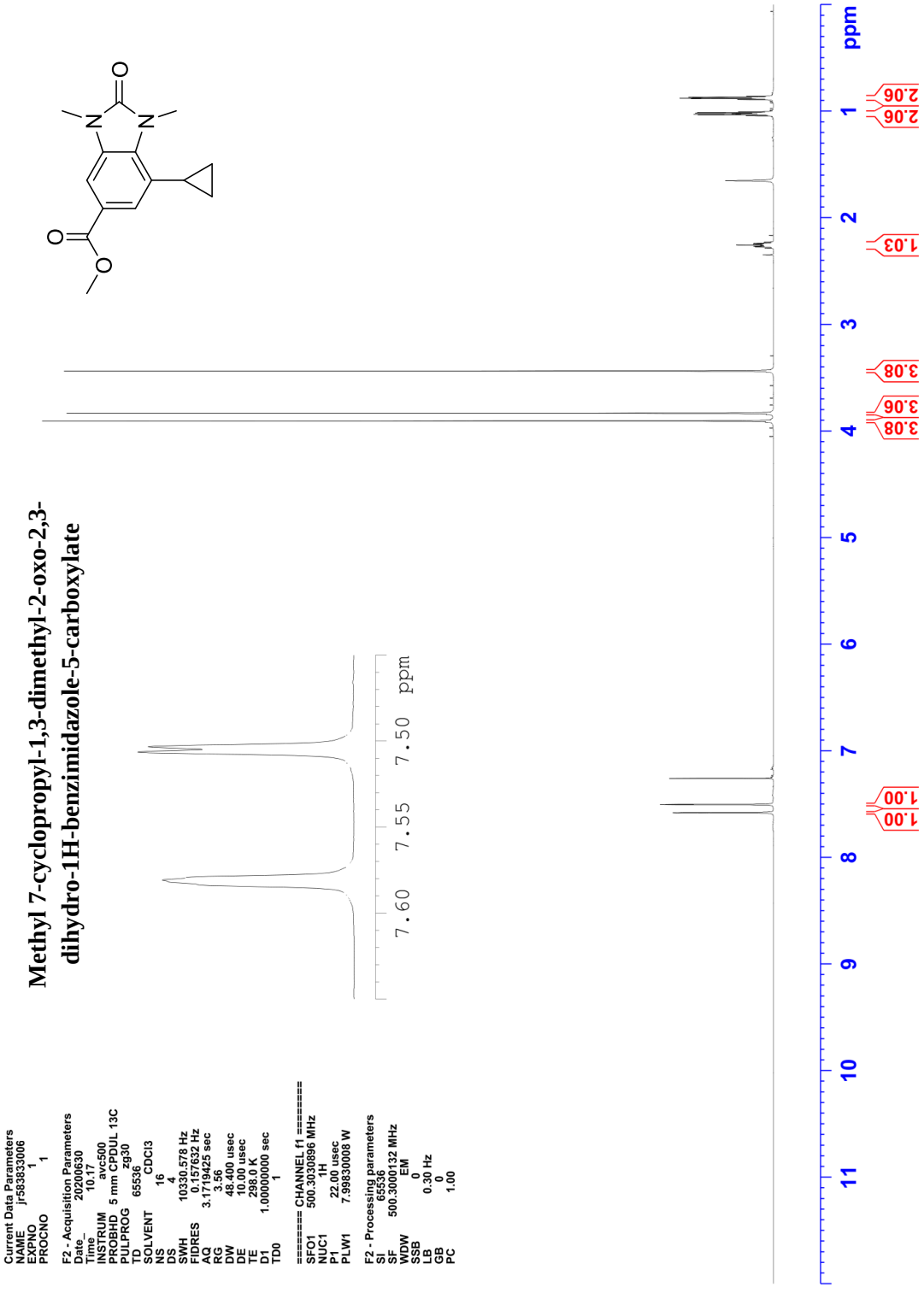

Methyl 3,4-diamino-5-cyclopropylbenzoate
Current Data Parameters
NAME Jun18-2020-21-JKRG52ful
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200618
Time_ 14.52 h
INSTRUM avg400
PROBHD Z108618_0816 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 52.09
DW 62.400 usec
DE 6.50 usec
TE 297.8 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000095 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
NH2
NH2
O
O
7.4
7.3
ppm
0.85
0.89
3.01
4.14
0.95
2.00
2.04
ppm


Methyl 3,4-diamino-5-cyclopropylbenzoate
NH2
NH2
O
O
5.25
11.37
51.70
116.96
119.61
123.10
126.58
132.24
140.93
167.66
ppm

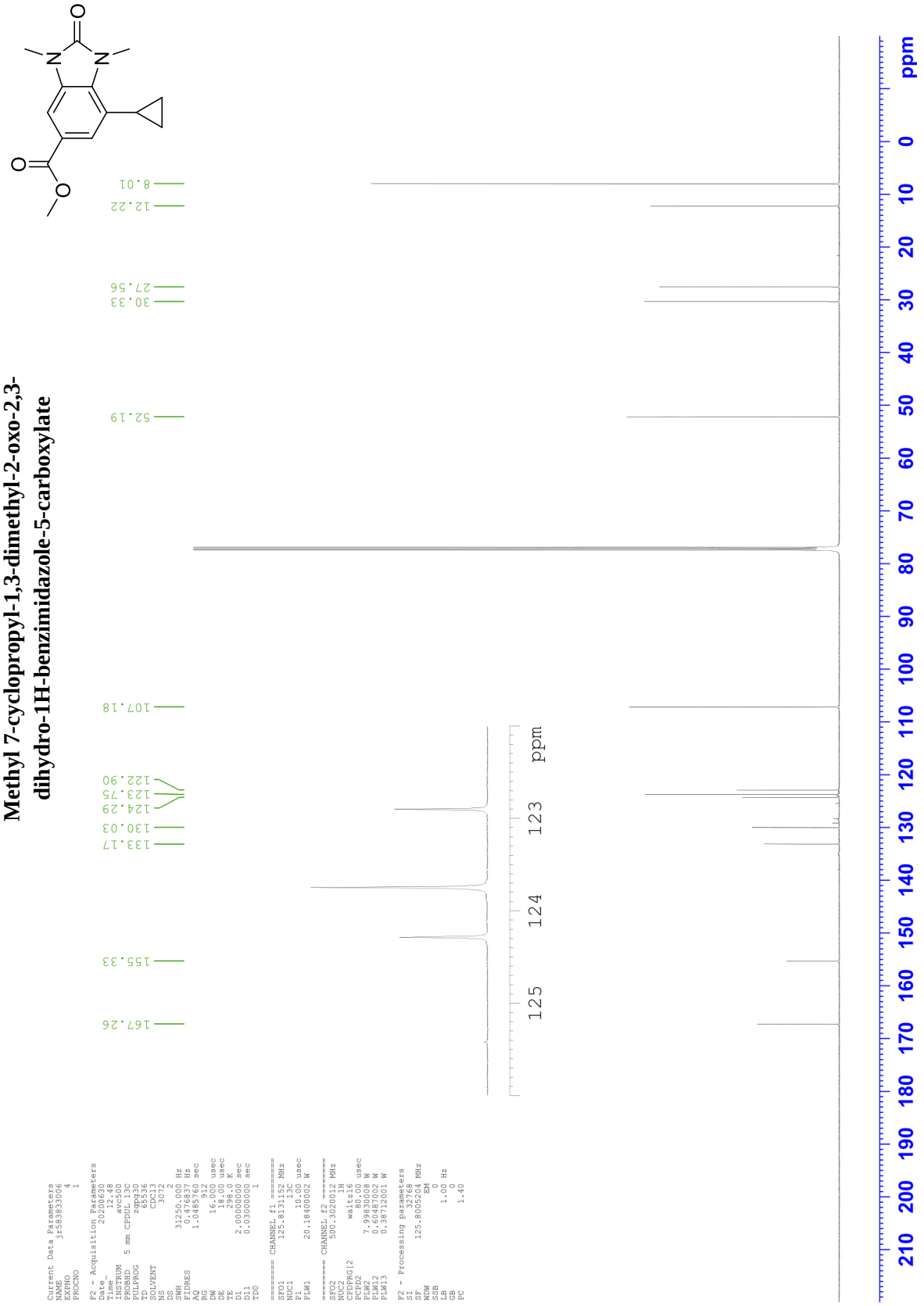

Methyl 1,3,7-trimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
Current Data Parameters
NAME Jun08-2020-26-JKRG42f
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200608
Time 17.54 h
INSTRUM avg400
PROBHD Z108618_0816 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 91.39
DW 62.400 usec
DE 6.50 usec
TE 296.9 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000095 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.6
7.5
ppm
3.04
3.00
3.00
3.07
0.88
0.86
ppm

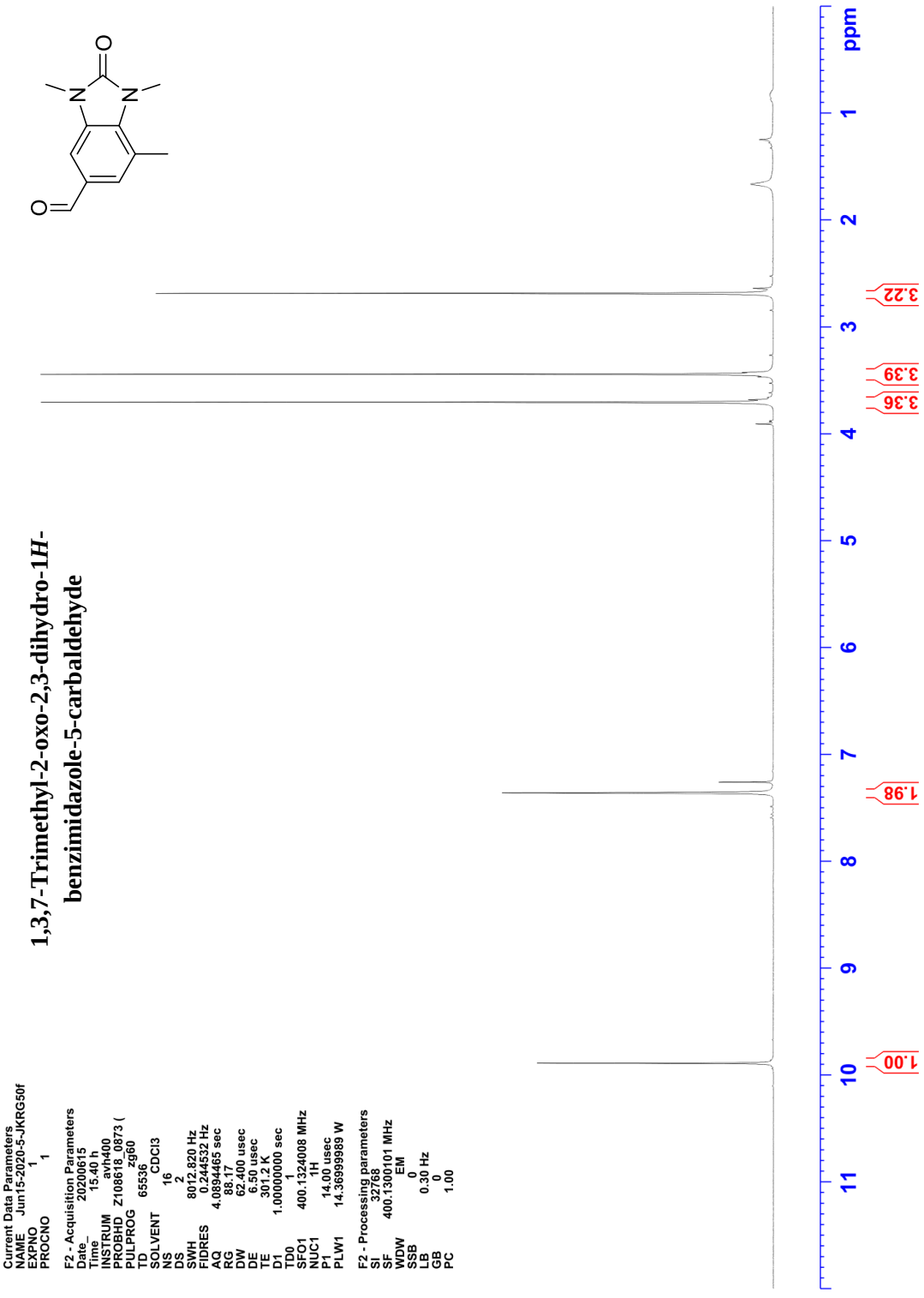
Methyl 1,3,7-trimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate



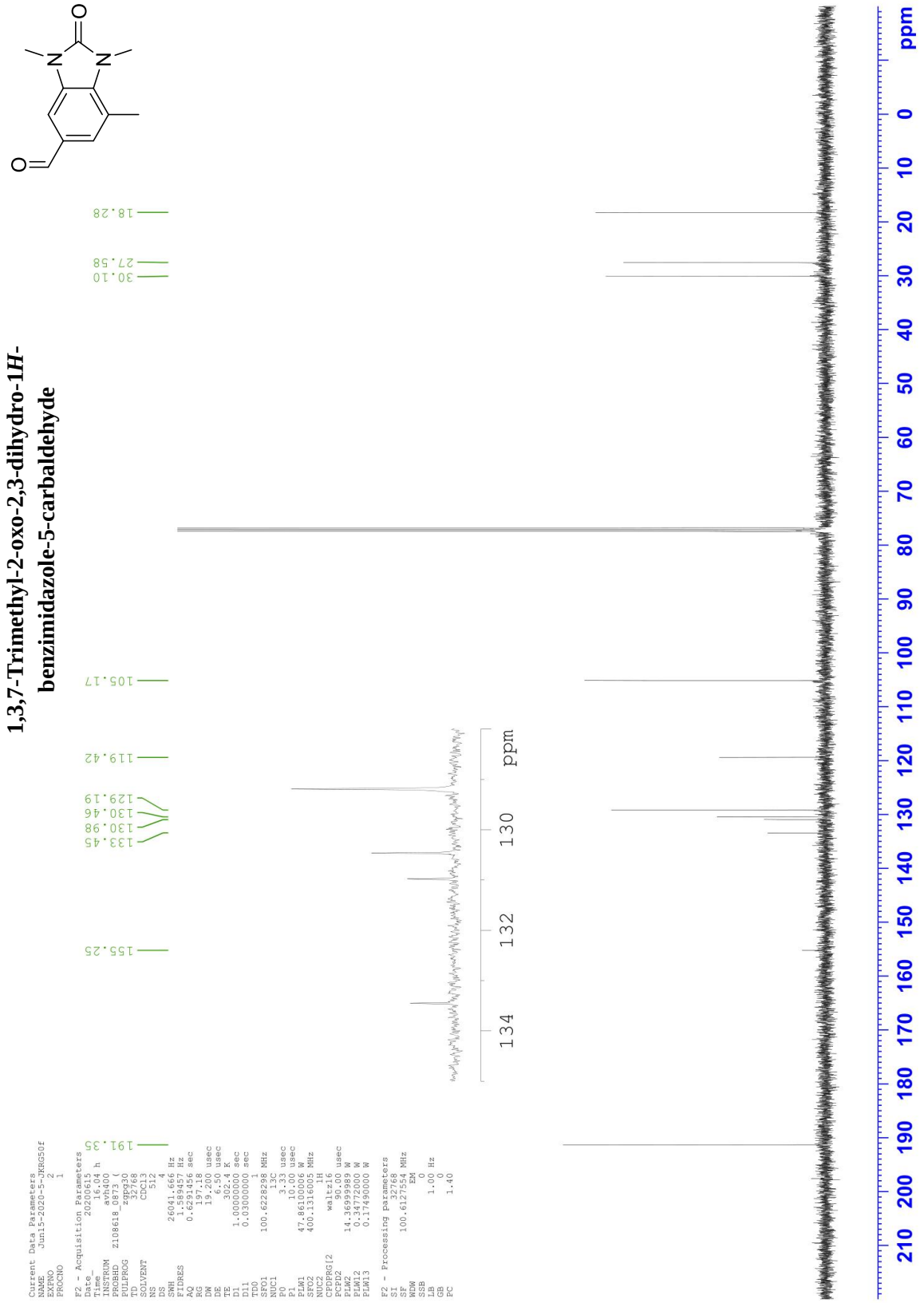

Current Data Parameters
NAME jr58383006
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200630
Time_ 10.17
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000132 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Methyl 7-cyclopropyl-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate
7.60
7.55
7.50
ppm
1.00
1.00
3.08
3.06
3.08
1.03
2.06
2.06
ppm

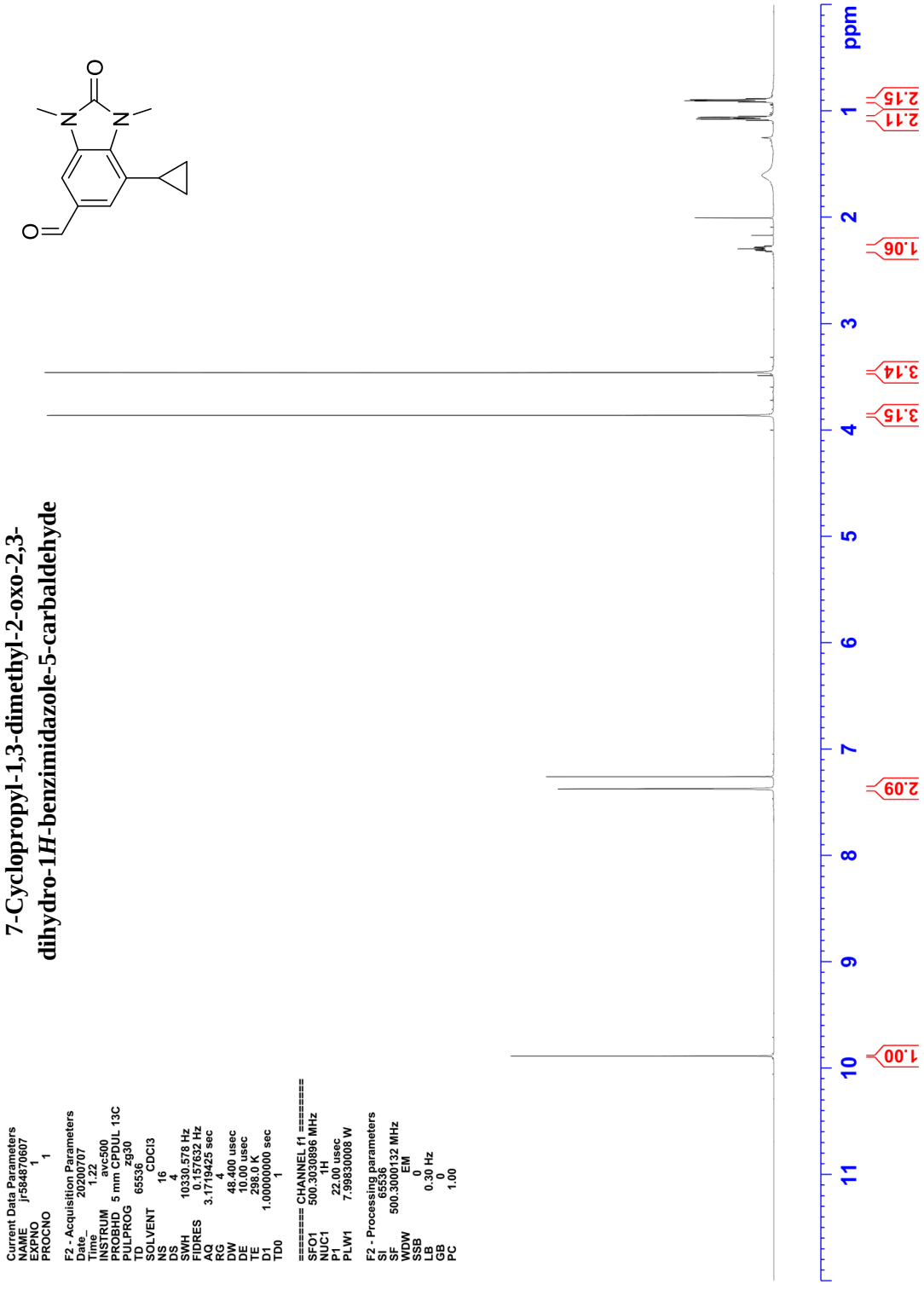
Methyl 7-cyclopropyl-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carboxylate

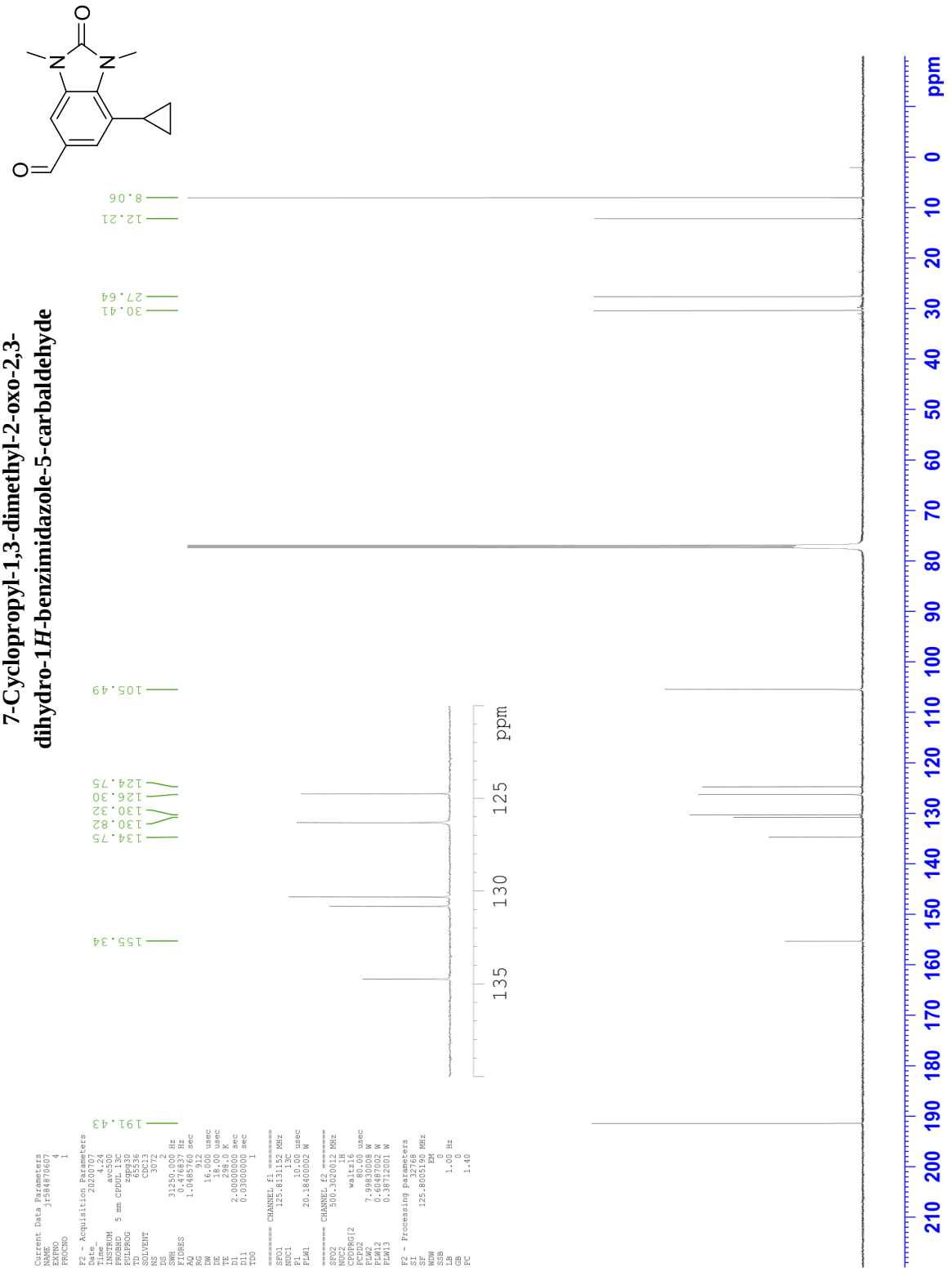


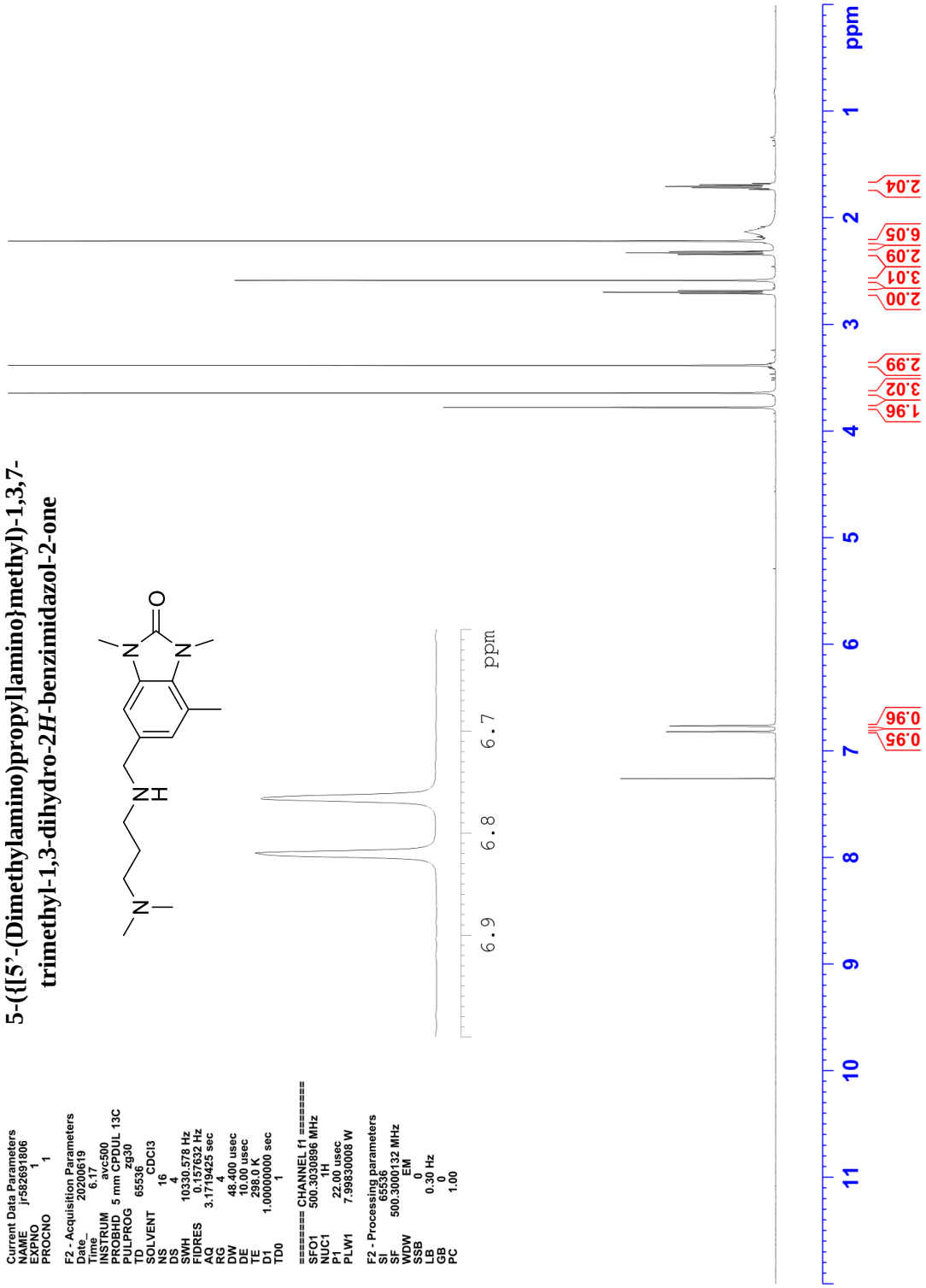

1,3,7-Trimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carbaldehyde
Current Data Parameters
NAME Jun15-2020-5-JKRG50f
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200615
Time 15.40 h
INSTRUM avh400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 88.17
DW 62.400 usec
DE 6.50 usec
TE 301.2 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300101 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1.00
1.98
3.36
3.39
3.22
ppm

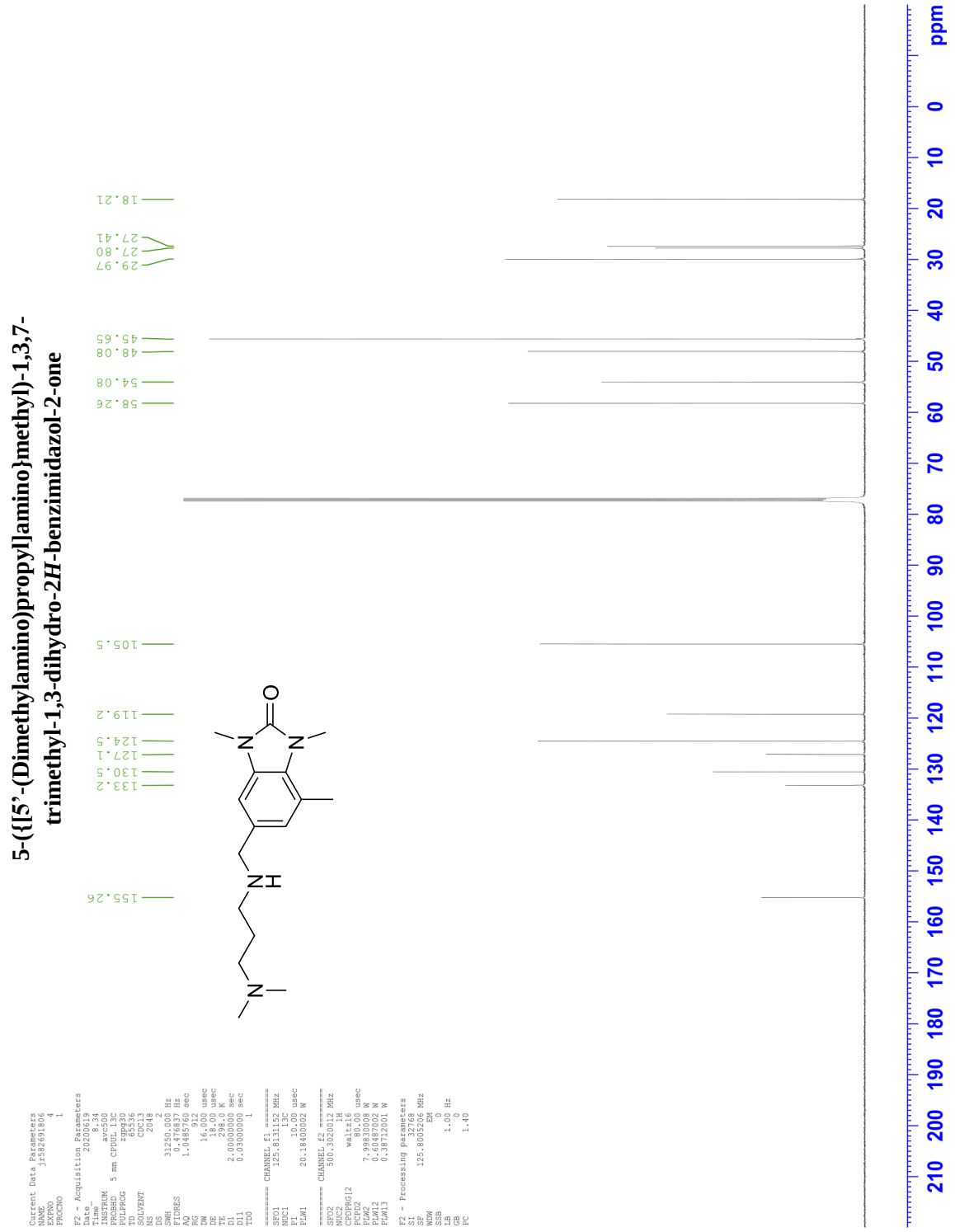
1,3,7-Trimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde

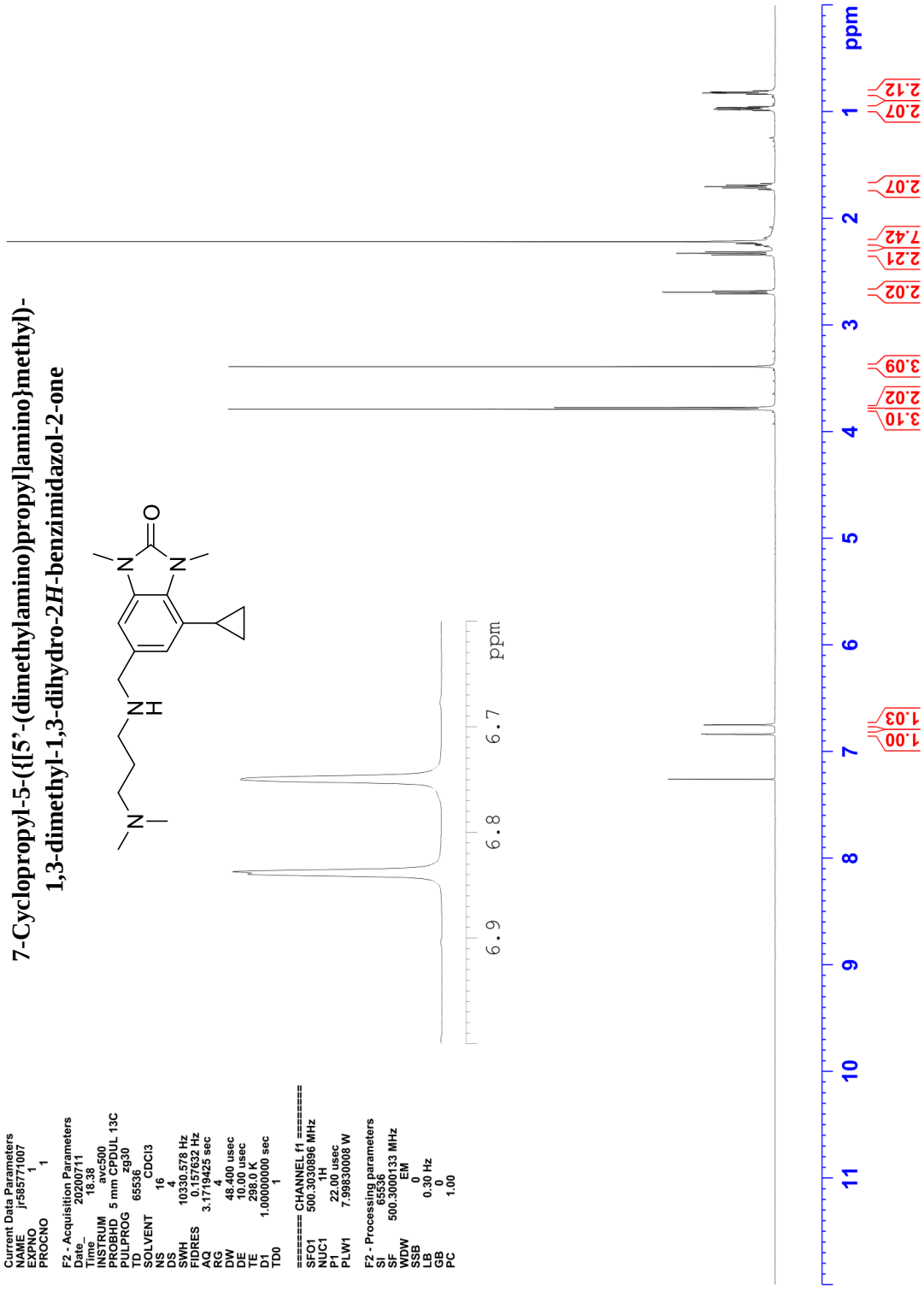



7-Cyclopropyl-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carbaldehyde
Current Data Parameters
NAME jr584870607
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200707
Time_ 1.22
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000132 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1.00
2.09
3.15
3.14
1.06
2.11
2.15
ppm

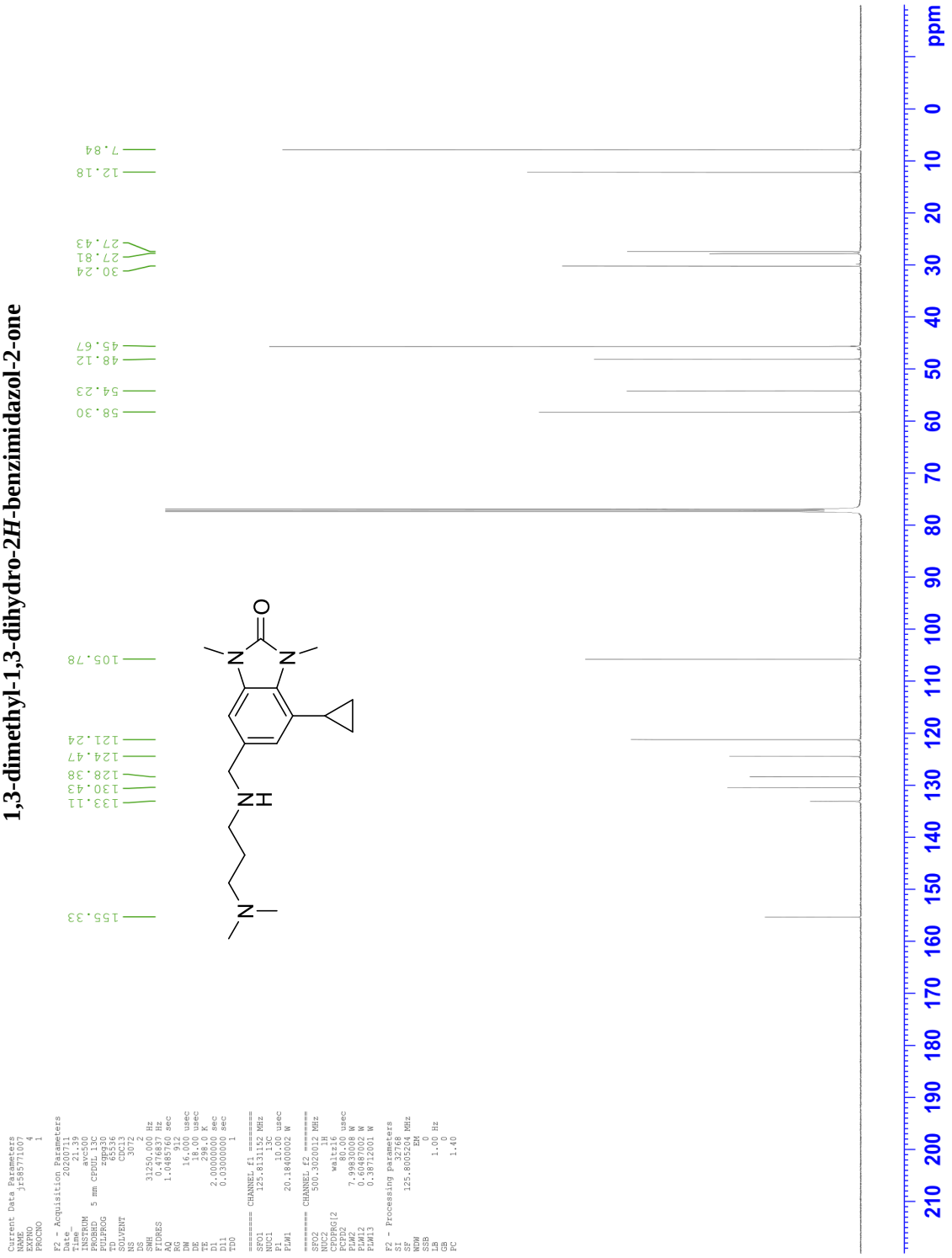

7-Cyclopropyl-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carbaldehyde
8.06
12.21
27.64
30.41
105.49
124.75
126.30
130.32
130.82
134.75
155.34
191.43
135
130
125
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
ppm

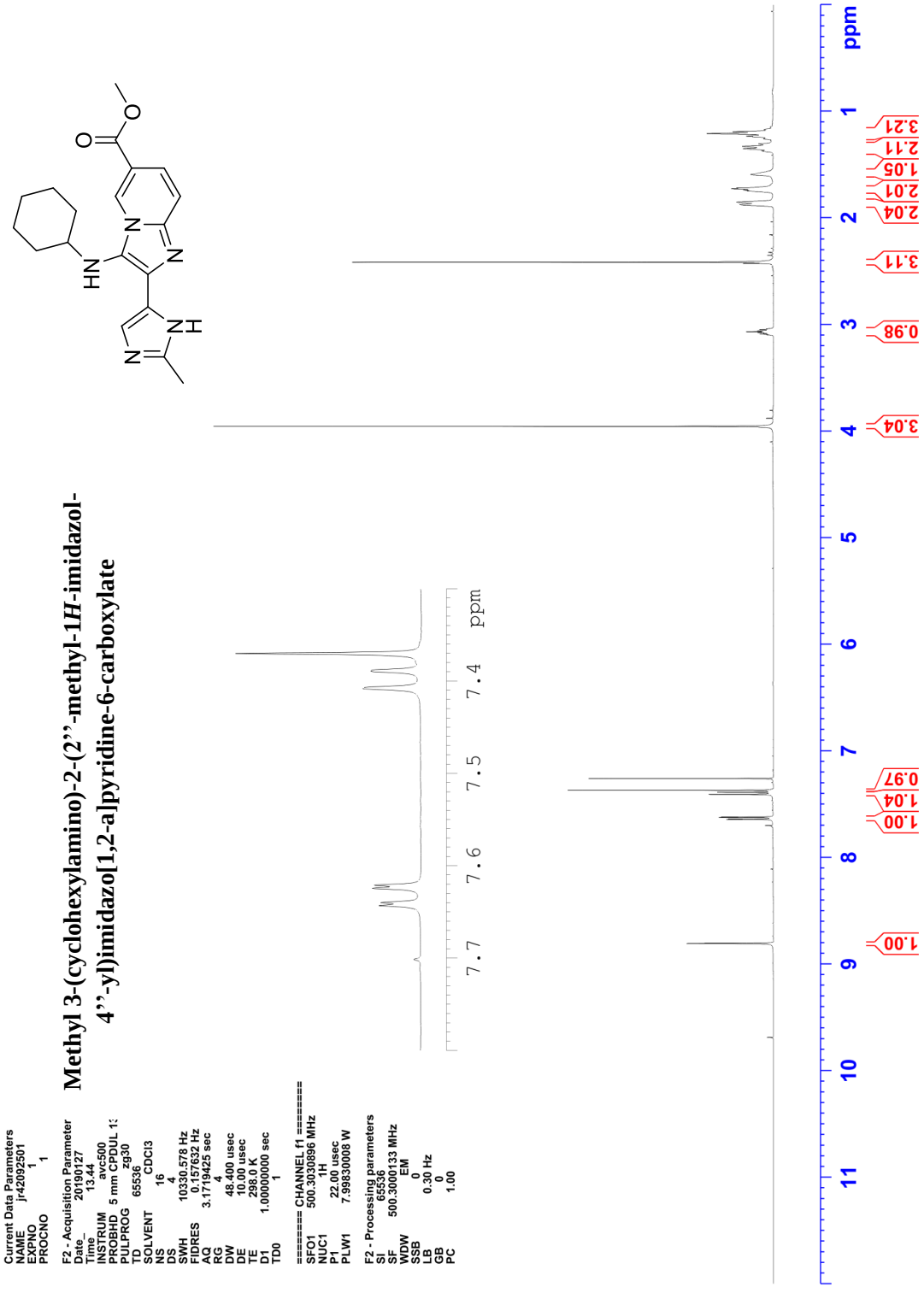

5-({[5'-(Dimethylamino)propyl]amino}methyl)-1,3,7-trimethyl-1,3-dihydro-2H-benzimidazol-2-one
Current Data Parameters
NAME js58289/606
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200619
Time_ 6.17
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000132 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
6.9
6.8
6.7
ppm
0.95
0.96
1.96
3.02
2.99
2.00
3.01
2.09
6.05
2.04
11
10
9
8
7
6
5
4
3
2
1
ppm

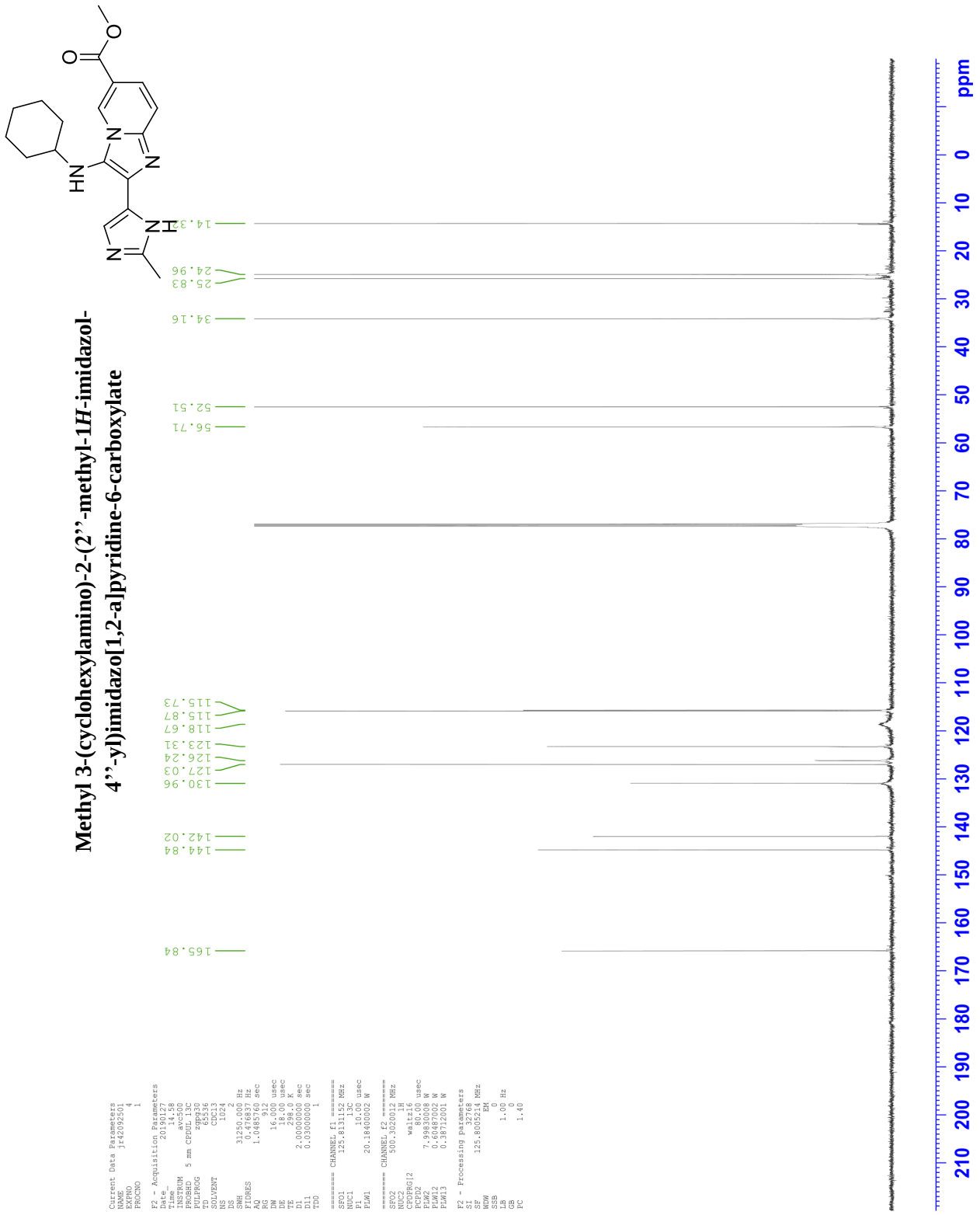

5-({[5'-(Dimethylamino)propyl]amino}methyl)-1,3,7-trimethyl-1,3-dihydro-2H-benzimidazol-2-one
18.21
27.41
27.80
29.97
45.65
48.08
54.08
58.26
105.5
119.2
124.5
127.1
130.5
133.2
155.26
ppm

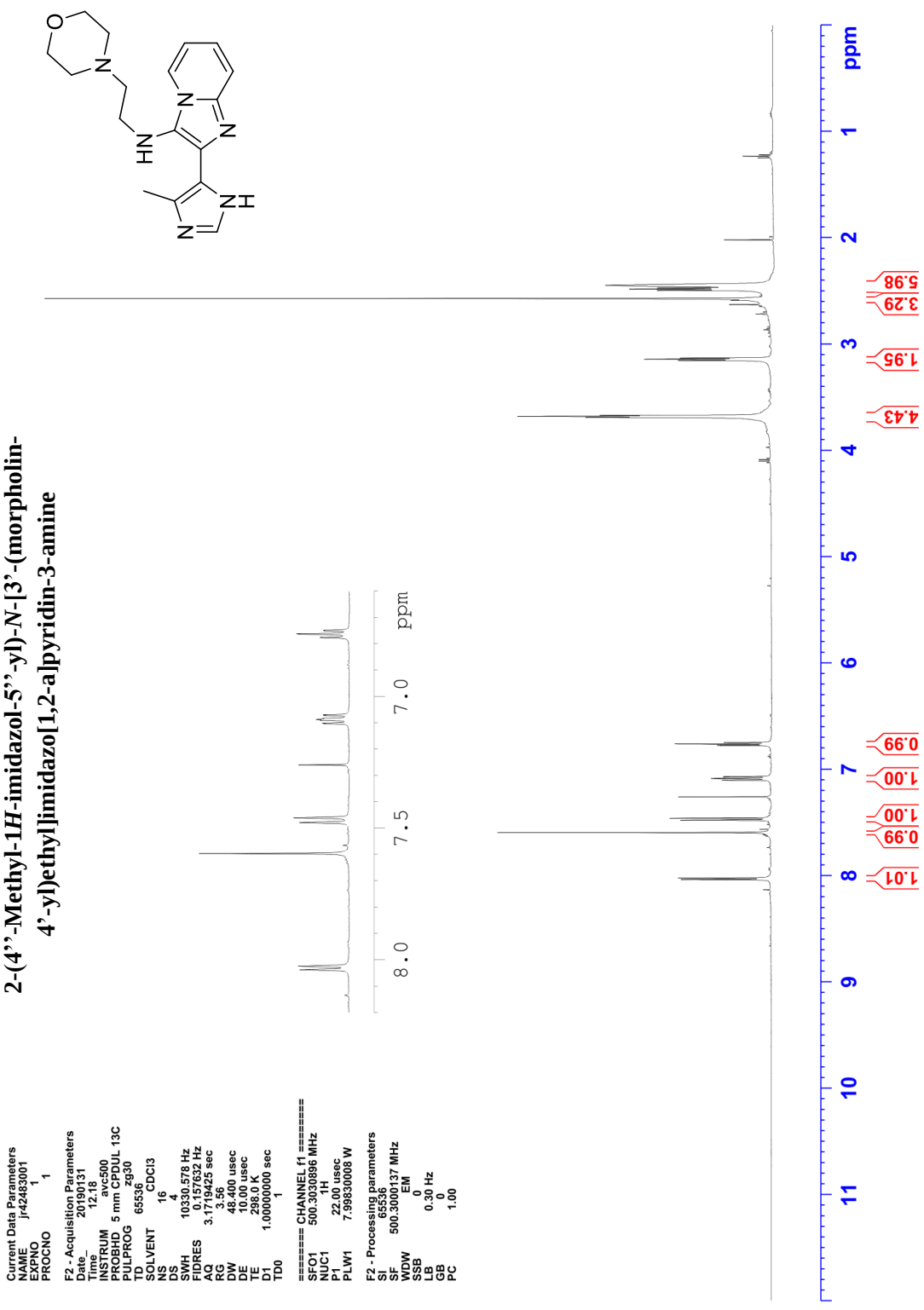

7-Cyclopropyl-5-({[5'-(dimethylamino)propyl]amino}methyl)-
1,3-dimethyl-1,3-dihydro-2H-benzimidazol-2-one
Current Data Parameters
NAME jr585771007
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200711
Time_ 18.38
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000133 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
6.9
6.8
6.7
ppm
1.00
1.03
3.10
2.02
3.09
2.02
2.21
7.42
2.07
2.07
2.12
11
10
9
8
7
6
5
4
3
2
1
ppm

7-Cyclopropyl-5-({[5'-(dimethylamino)propyl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one

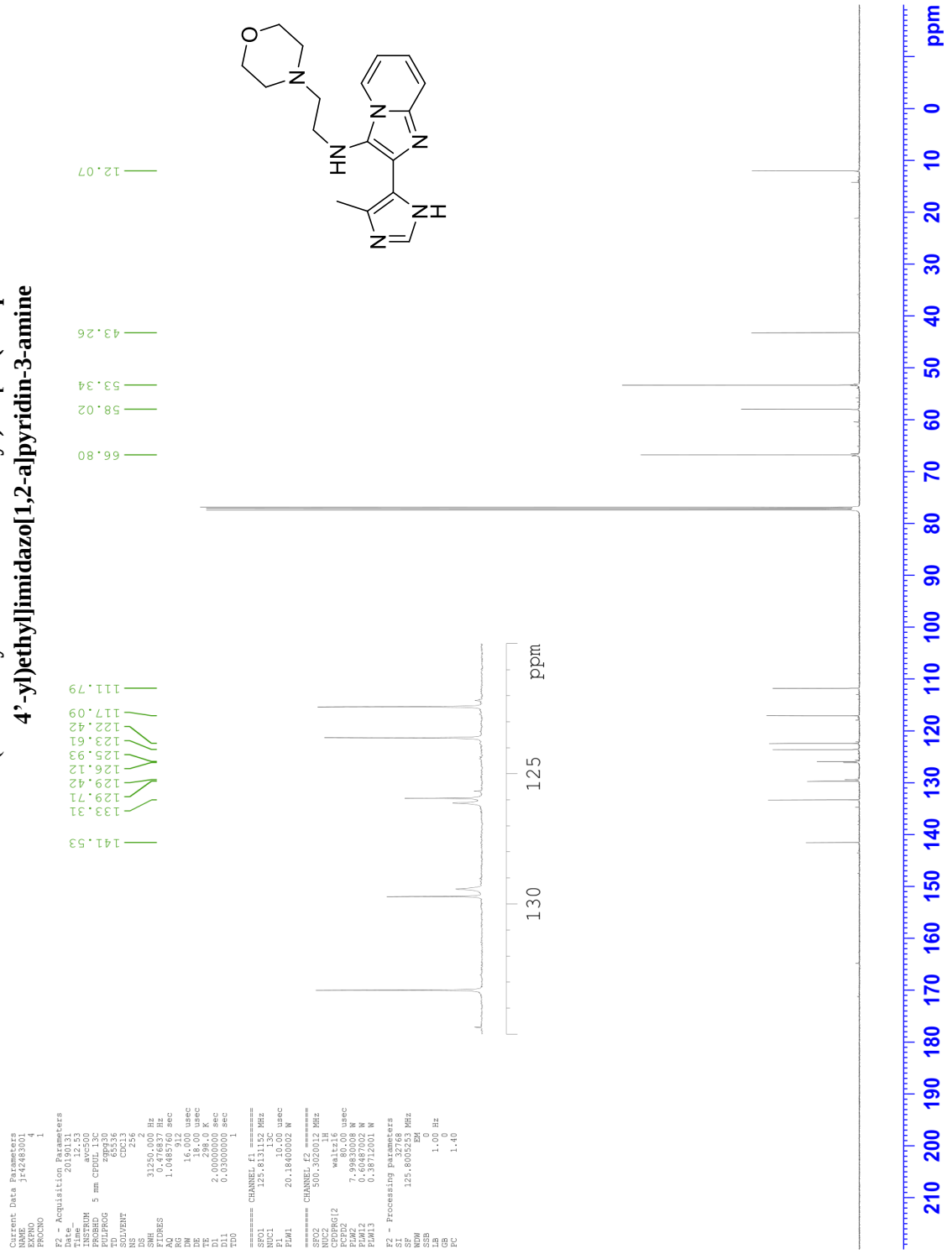



Current Data Parameters
NAME jr42092501
EXPNO 1
PROCNO 1
F2 - Acquisition Parameter
Date_ 20190127
Time_ 13.44
INSTRUM avc500
PROBHD 5 mm CPDUL 1
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000133 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Methyl 3-(cyclohexylamino)-2-(2''-methyl-1H-imidazol-4''-yl)imidazo[1,2-a]pyridine-6-carboxylate
HN
N
N
N
H
O
O
7.7
7.6
7.5
7.4
ppm
1.00
1.00
1.04
0.97
3.04
0.98
3.11
2.04
2.01
1.05
2.11
3.21
1.00
ppm

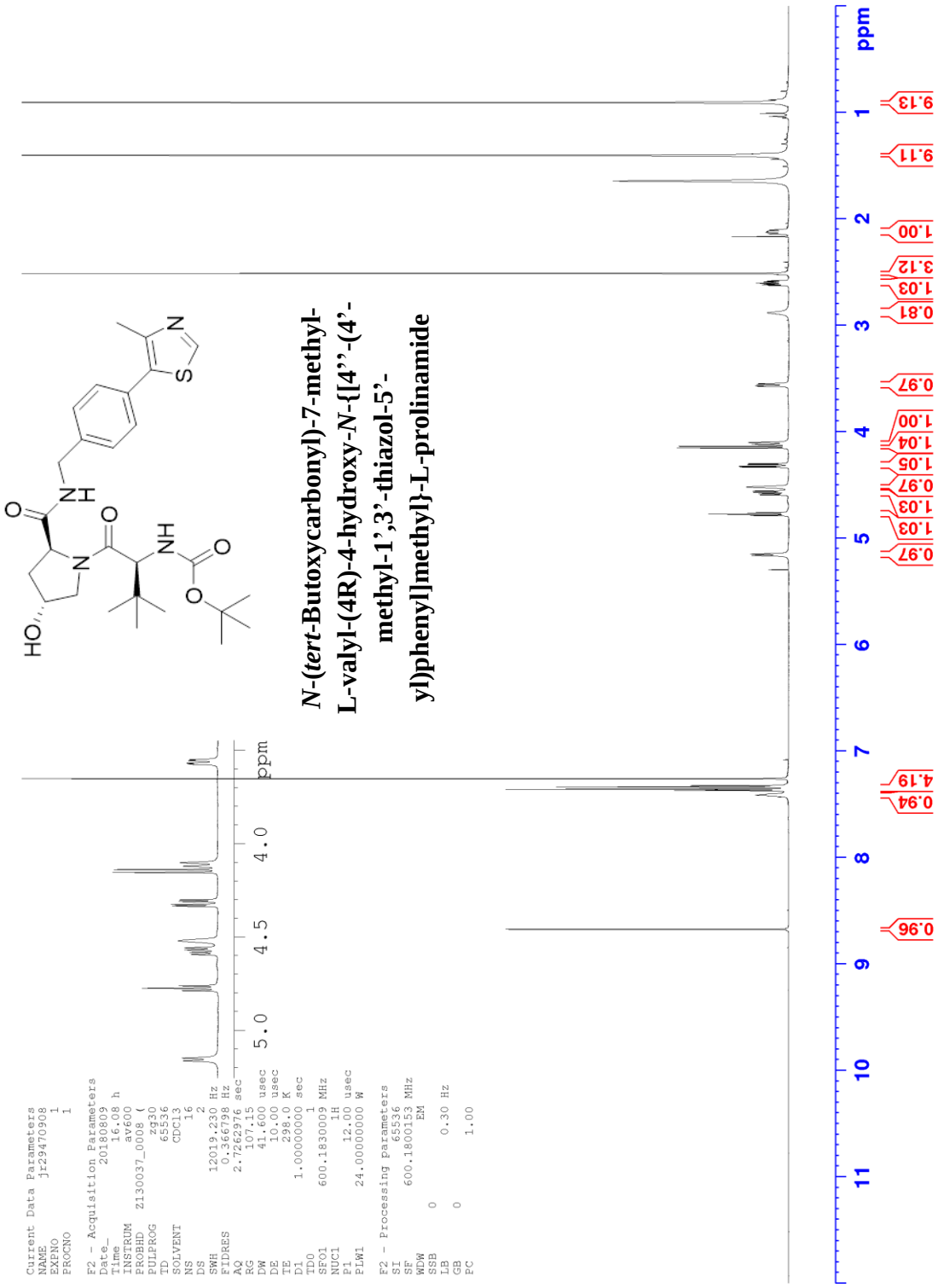

Methyl 3-(cyclohexylamino)-2-(2''-methyl-1H-imidazol-4''-yl)imidazo[1,2-a]pyridine-6-carboxylate
14.38
24.96
25.83
34.16
52.51
56.71
115.73
115.87
118.67
123.31
126.24
127.03
130.96
142.02
144.84
165.84
ppm


2-(4''-Methyl-1H-imidazol-5''-yl)-N-[3'-(morpholin-4'-yl)ethyl]imidazo[1,2-a]pyridin-3-amine
Current Data Parameters
NAME jr42483001
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20190131
Time_ 12.18
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000137 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.0
7.5
7.0
ppm
1.01
0.99
1.00
1.00
0.99
4.43
1.95
3.29
5.98
11
10
9
8
7
6
5
4
3
2
1
ppm

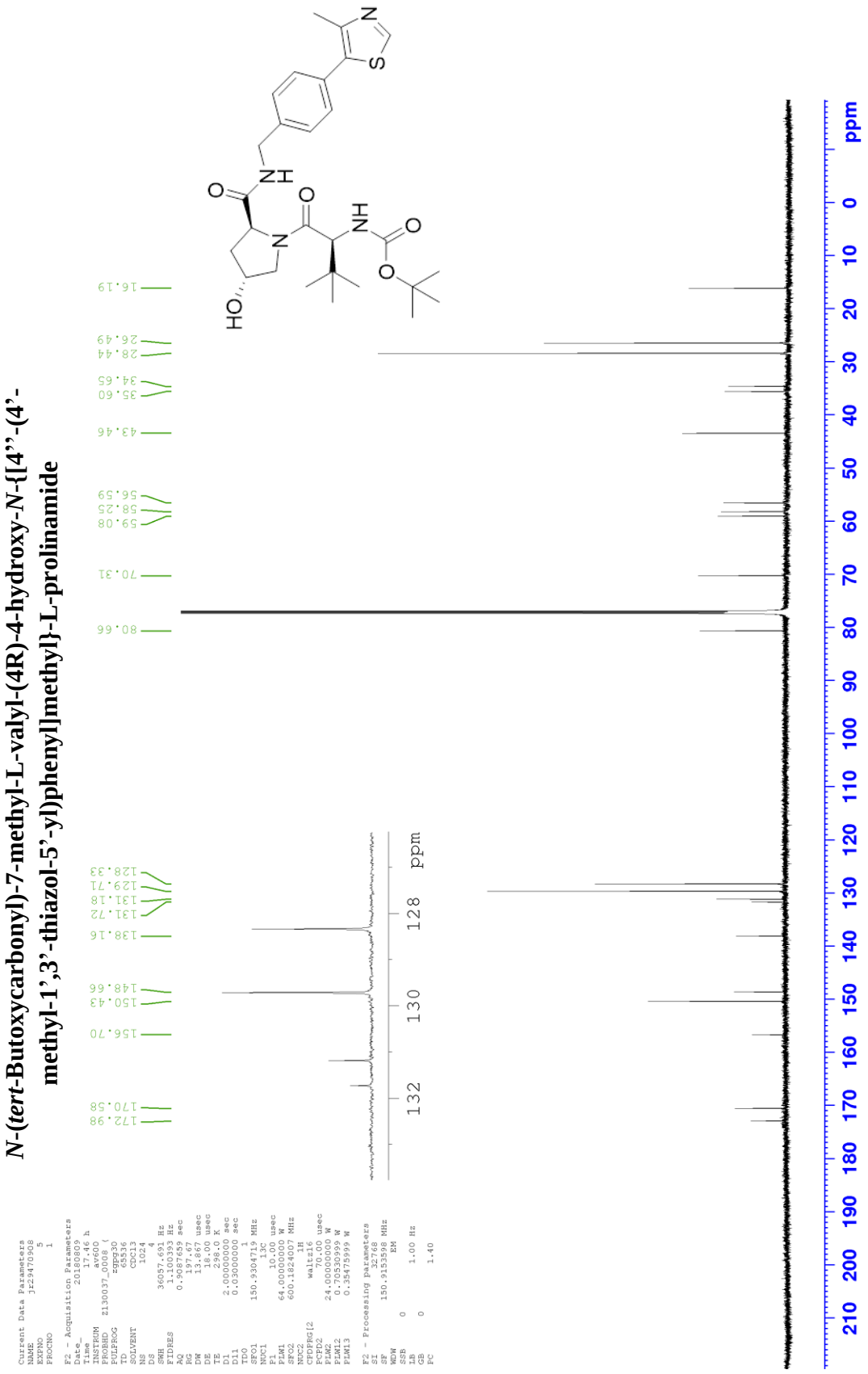
2-(4''-Methyl-1*H*-imidazol-5'' -yl)-*N*-[3'-(morpholin-4'-yl)ethyl]imidazo[1,2-a]pyridin-3-amine

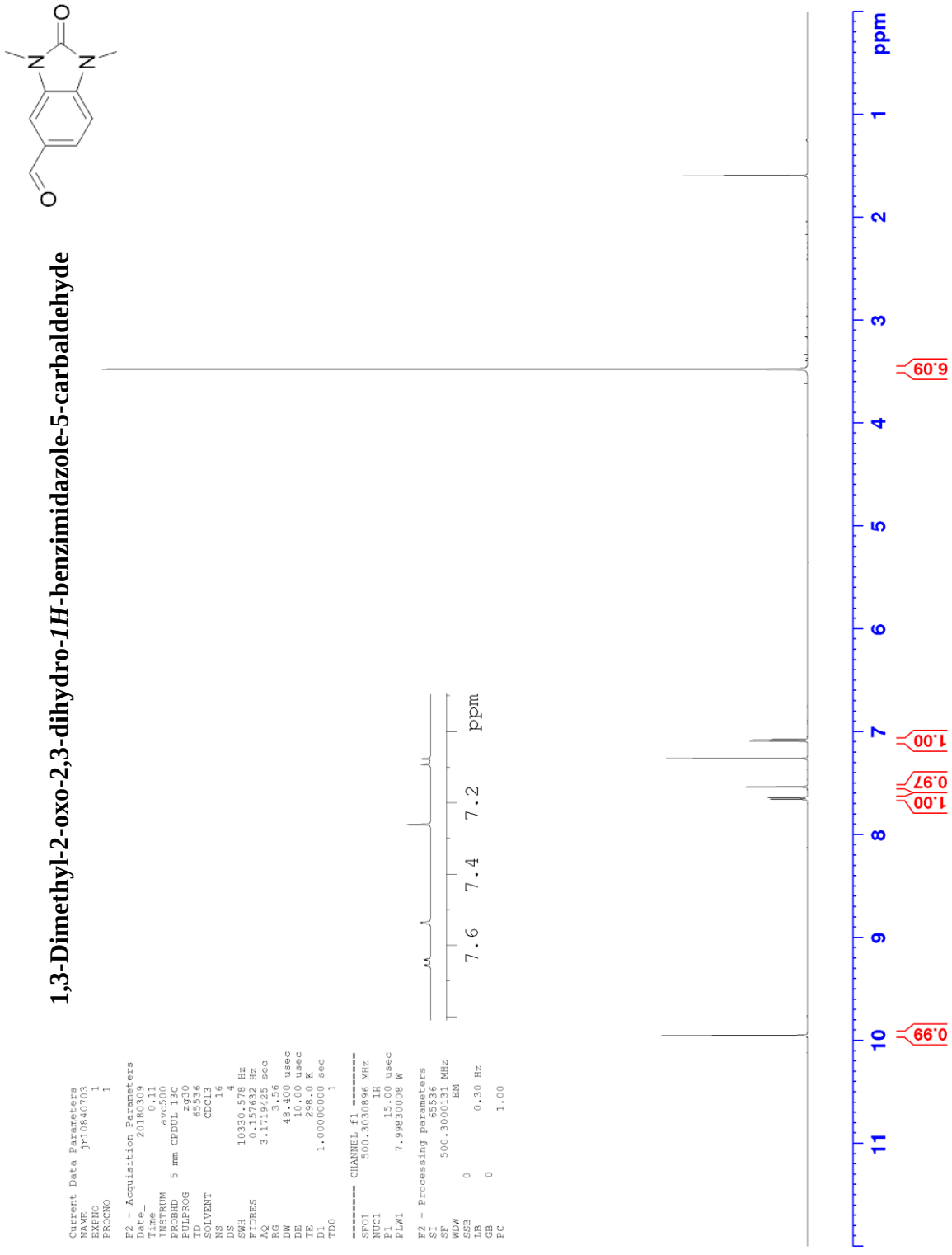


Chapter 3

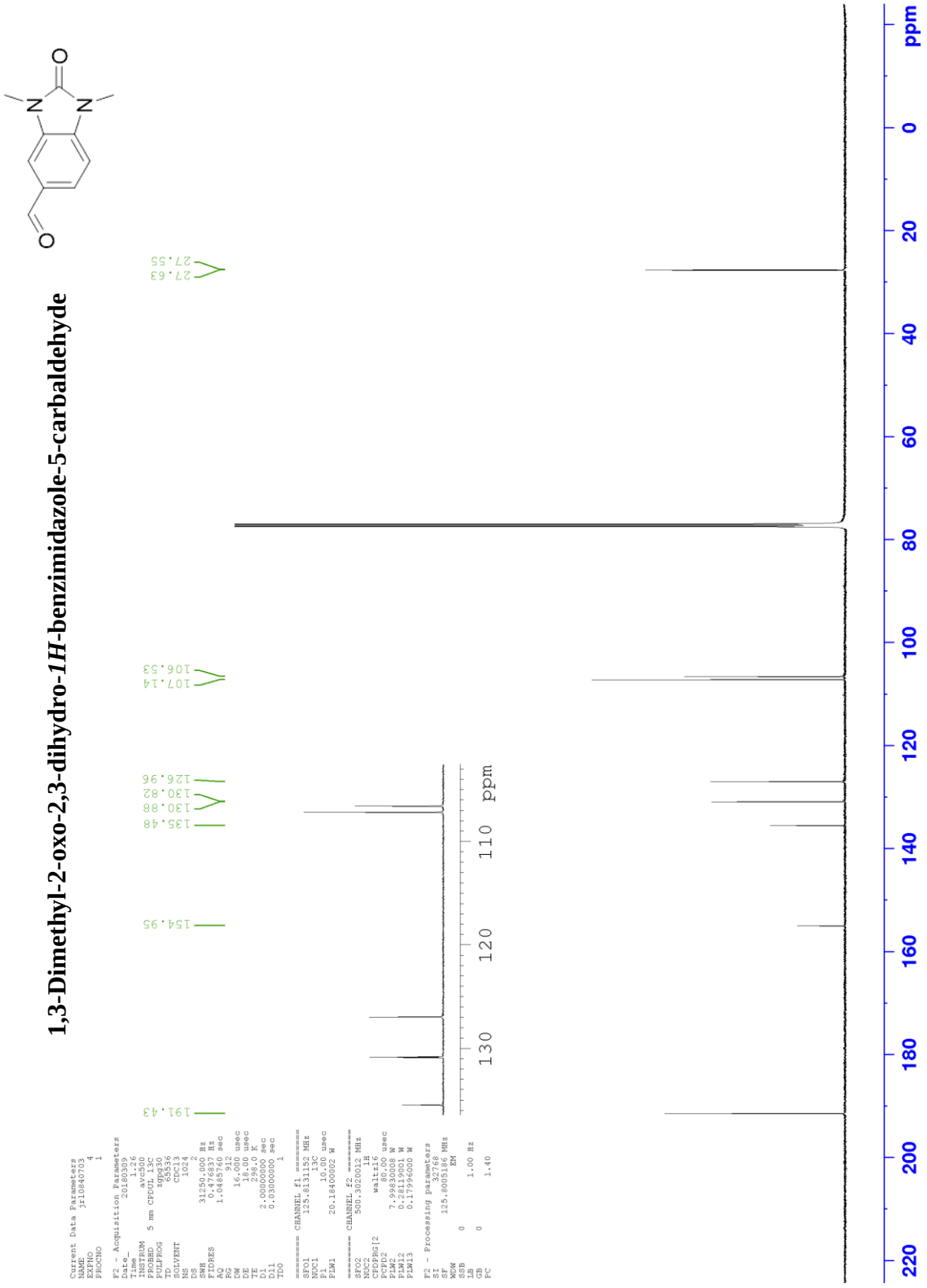


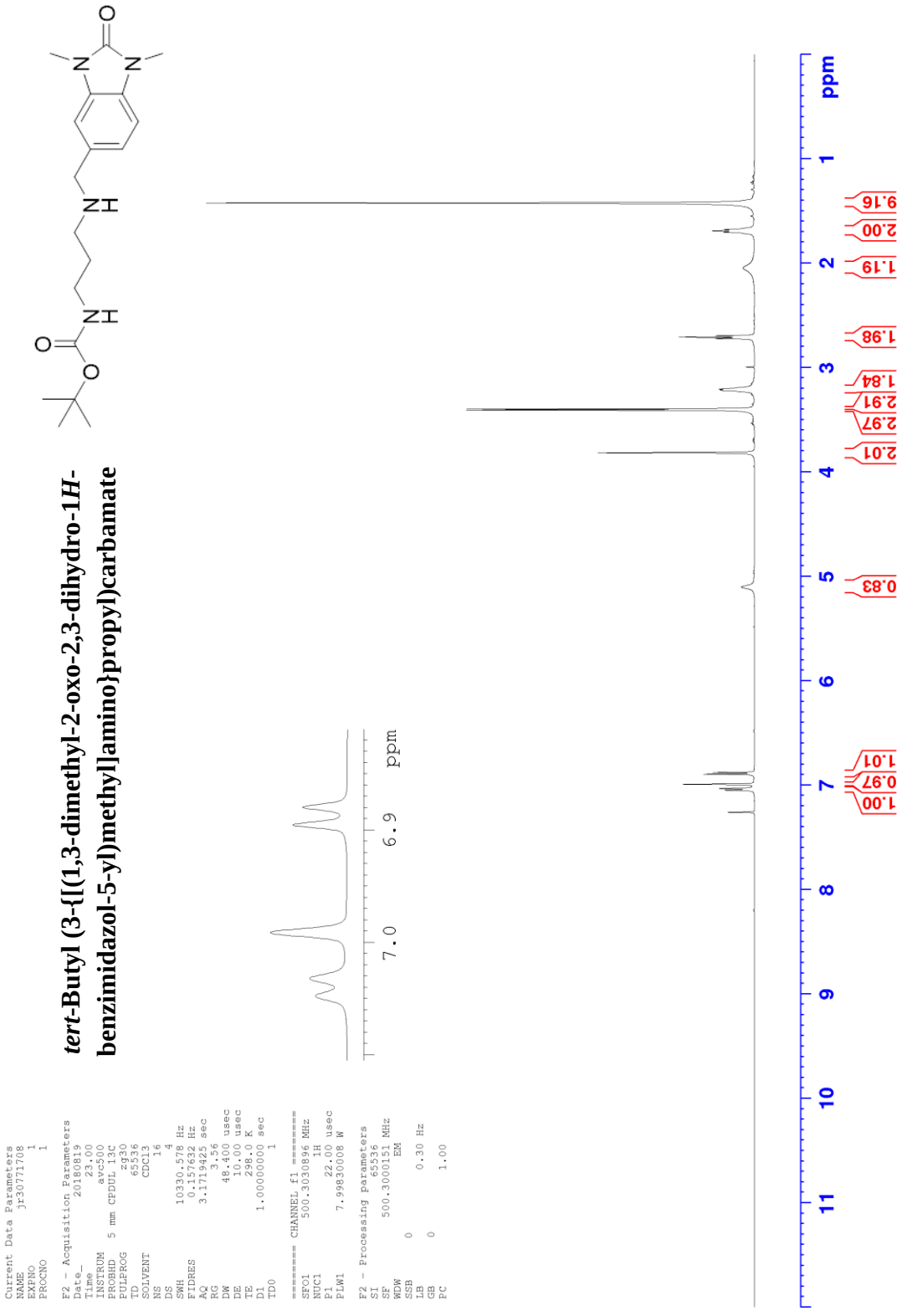
_N_-(*tert*-Butoxycarbonyl)-7-methyl-L-valyl-(4R)-4-hydroxy-_N_-{[4''-(4'-methyl-1',3'-thiazol-5'-yl)phenyl]methyl}-L-prolinamide

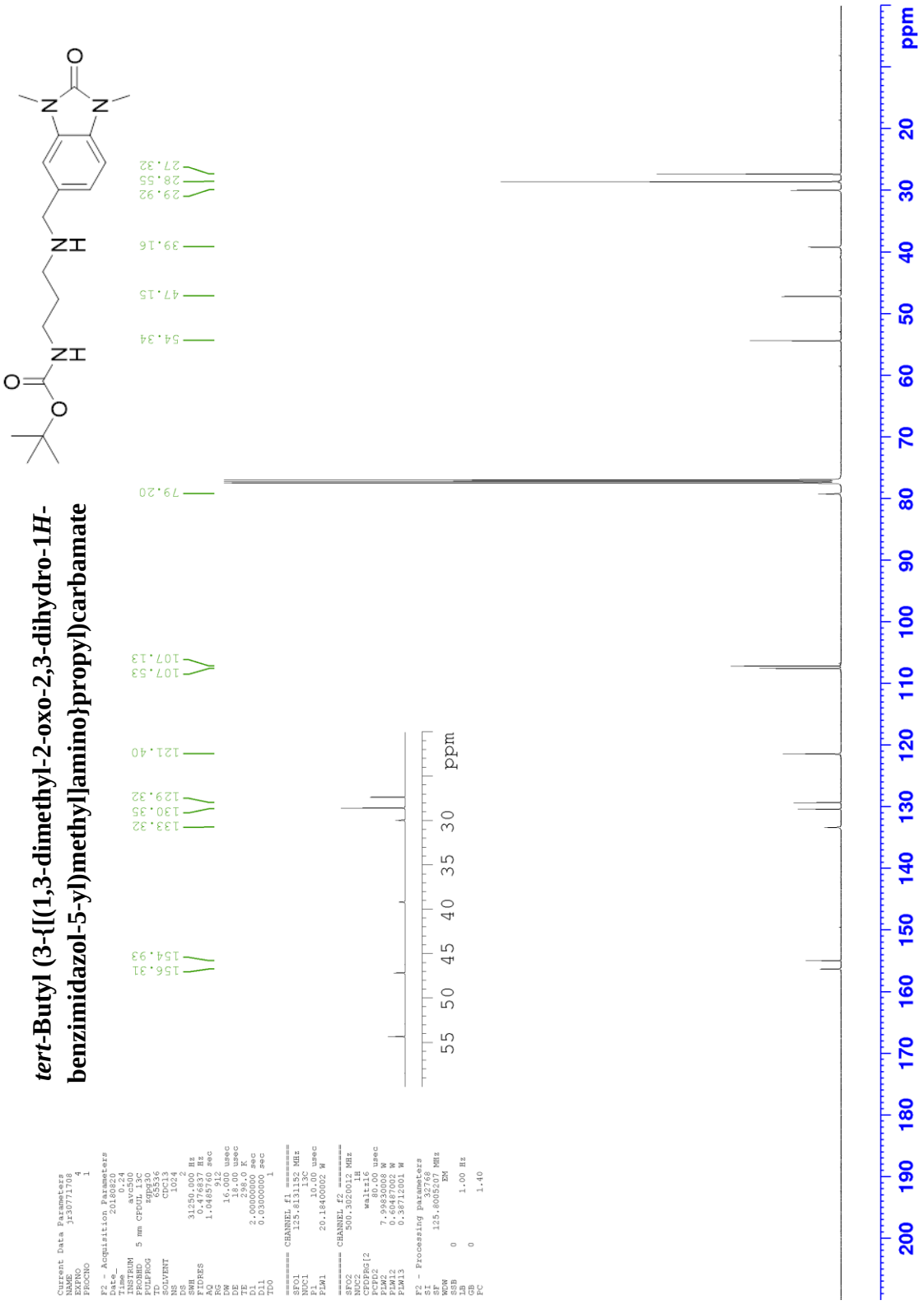

N-(tert-Butoxycarbonyl)-7-methyl-L-valyl-(4R)-4-hydroxy-N-{[4''-(4'-methyl-1',3'-thiazol-5'-yl)phenyl]methyl}-L-prolinamide
Current Data Parameters
NAME jr2947090
EXPNO 5
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180809
Time 17.46 h
INSTRUM spect
PROBHD Z130037_0008 (
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 4
SWH 36057.691 Hz
FIDRES 1.100393 Hz
AQ 0.9087659 sec
RG 197.67
DW 13.867 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
SFO1 150.9304719 MHz
NUC1 13C
P1 10.00 usec
PLW1 64.00000000 W
SFO2 600.1824007 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 70.00 usec
PLW2 24.00000000 W
PLW12 0.70530999 W
PLW13 0.35475999 W
F2 - Processing parameters
SI 32768
SF 150.9153598 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
172.98
170.58
156.70
150.43
148.66
138.16
131.72
131.18
129.71
128.33
80.66
70.31
59.08
58.25
56.59
43.46
35.60
34.65
28.44
26.49
16.19
HO
O
N
H
O
N
O
NH
O
O
N
S
132
130
128
ppm
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

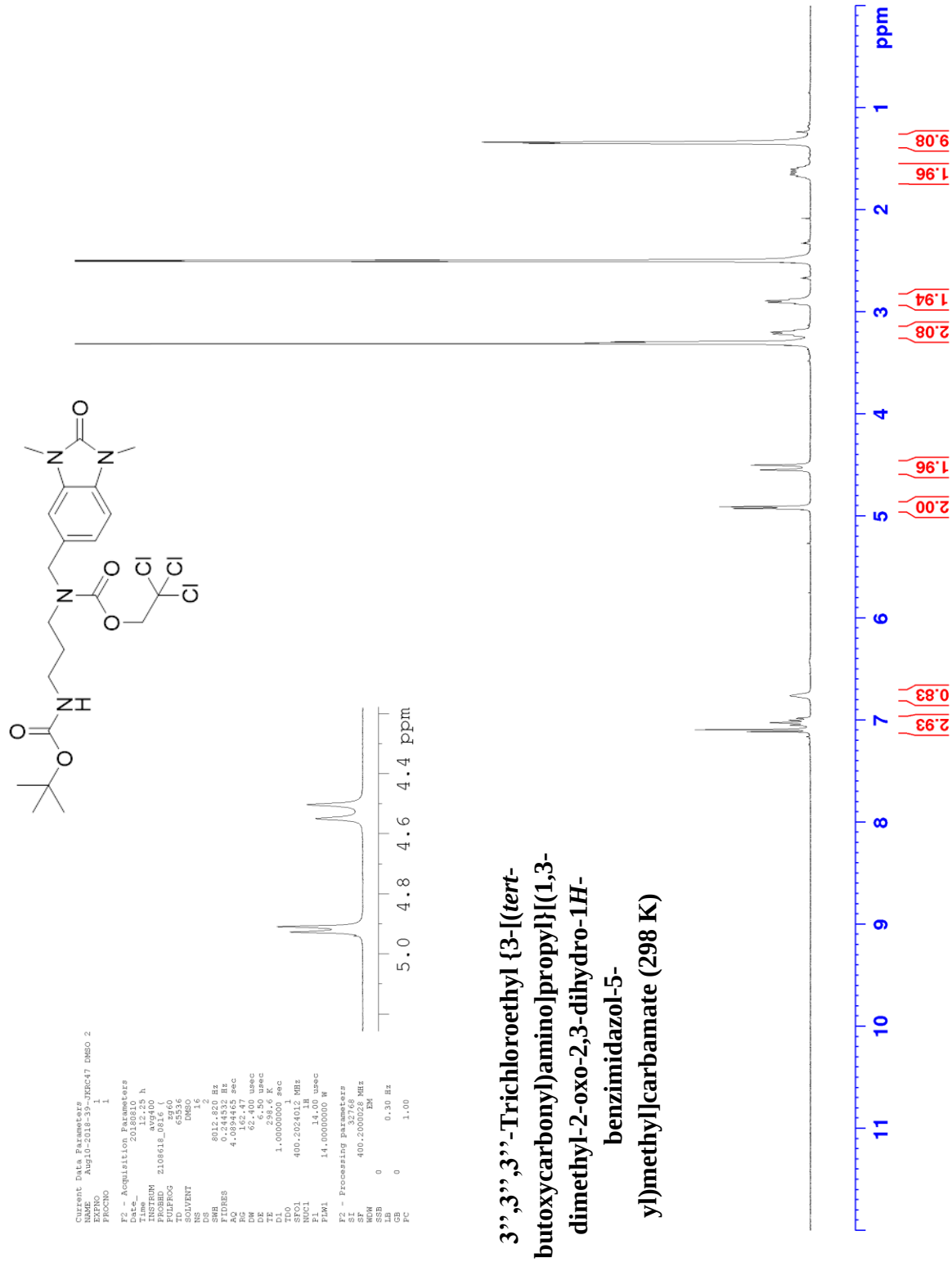

1,3-Dimethyl-2-oxo-2,3-dihydro-1H-benzimidazole-5-carbaldehyde
Current Data Parameters
NAME jr10840703
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180309
Time 0.11
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 15.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000131 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.6
7.4
7.2
ppm
0.99
1.00
0.97
1.00
6.09
11
10
9
8
7
6
5
4
3
2
1
ppm

1,3-Dimethyl-2-oxo-2,3-dihydro-*1H*-benzimidazole-5-carbaldehyde



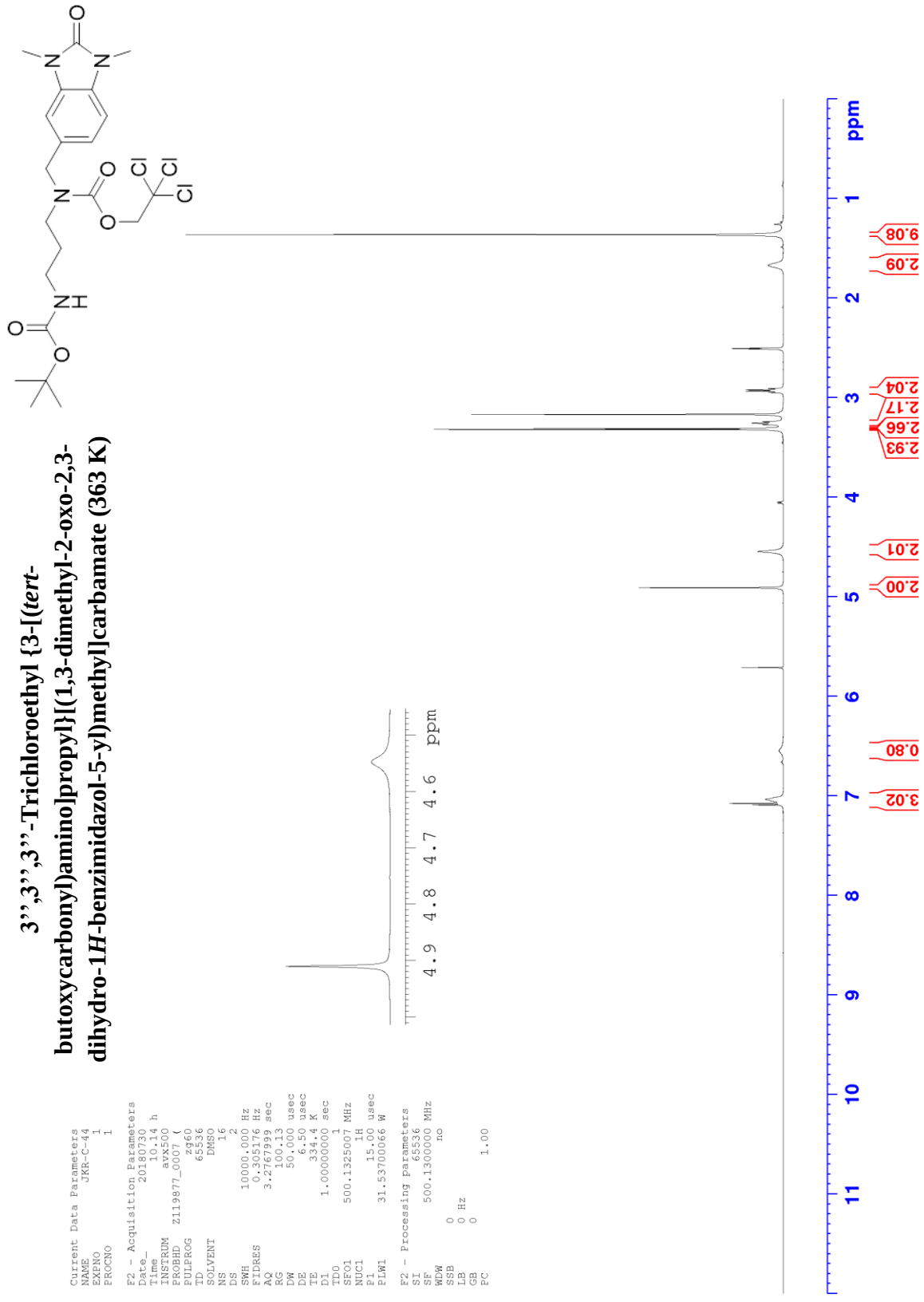

Current Data Parameters
NAME jr30771708
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180819
Time 23.00
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000151 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
tert-Butyl (3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl]amino}propyl)carbamate
7.0
6.9
ppm
1.00
0.97
1.01
0.83
2.01
2.97
2.91
1.84
1.98
1.19
2.00
9.16
ppm


tert-Butyl (3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl]amino}propyl)carbamate
79.20
107.53
107.13
121.40
129.32
130.35
133.32
154.93
156.31
27.32
28.55
29.92
39.16
47.15
54.34
ppm

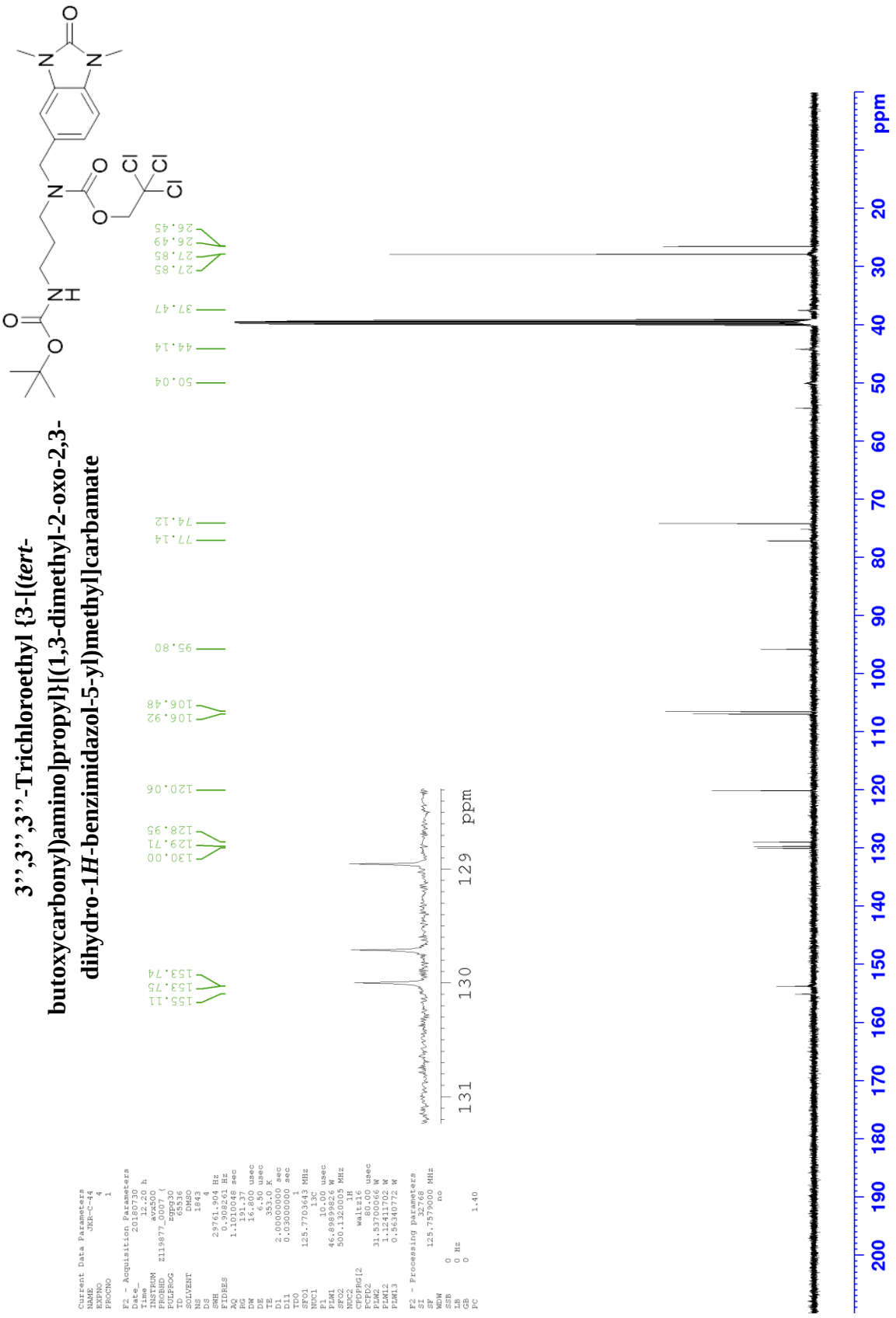


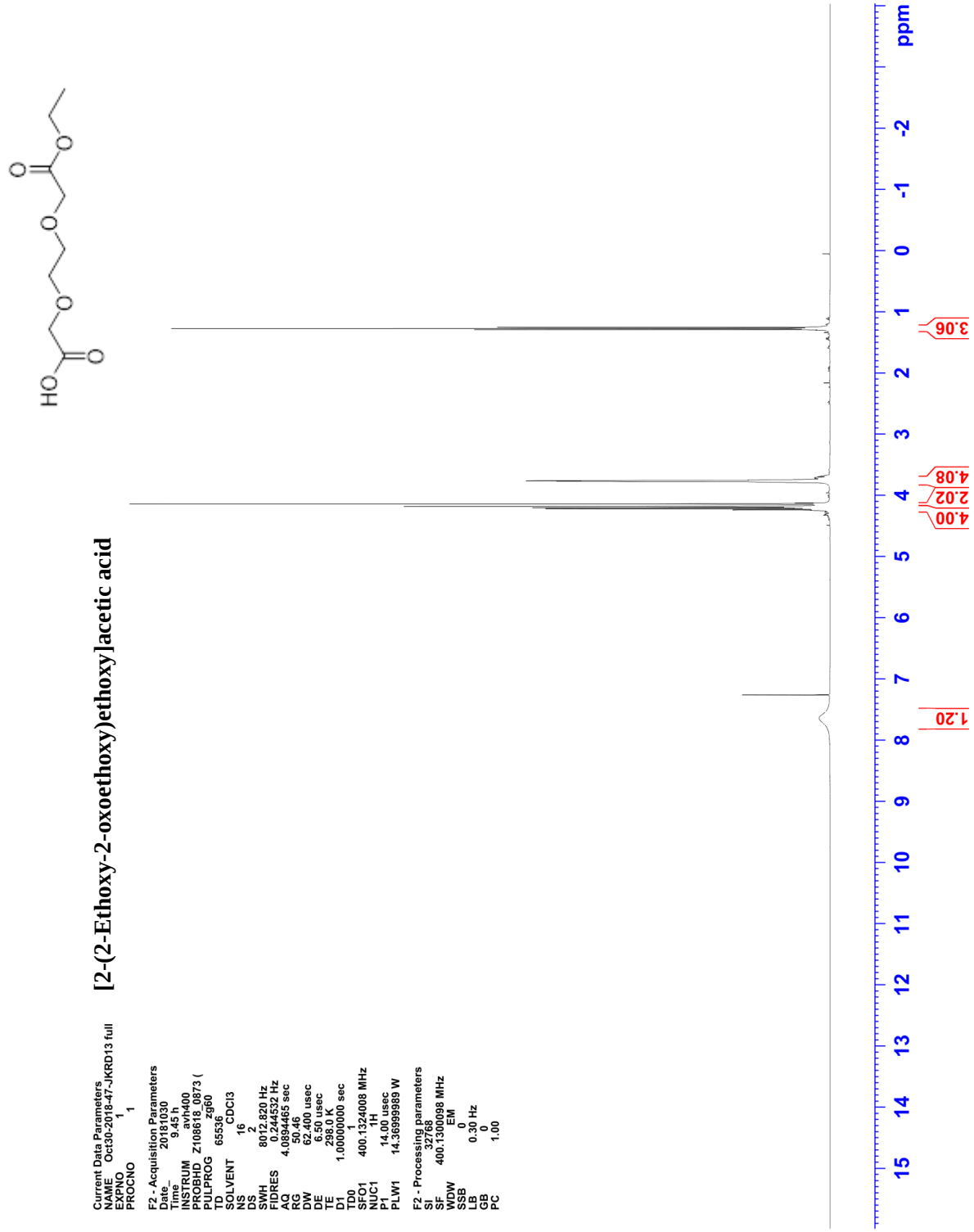
3'',3'',3''-Trichloroethyl {3-[(*tert*-butoxycarbonyl)amino]propyl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (298 K)

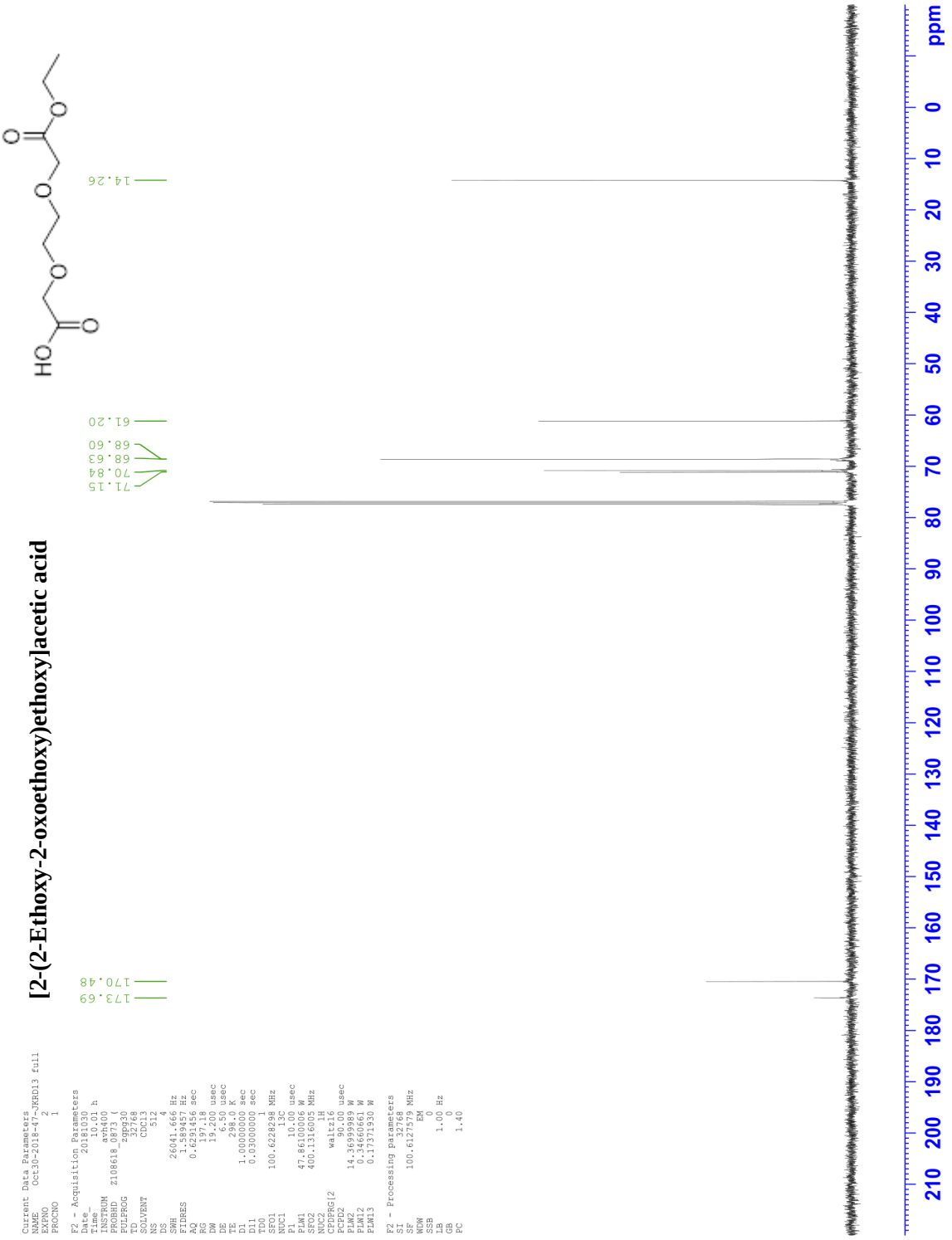
3'',3'',3''-Trichloroethyl {3-[(*tert*-butoxycarbonyl)amino]propyl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (363 K)

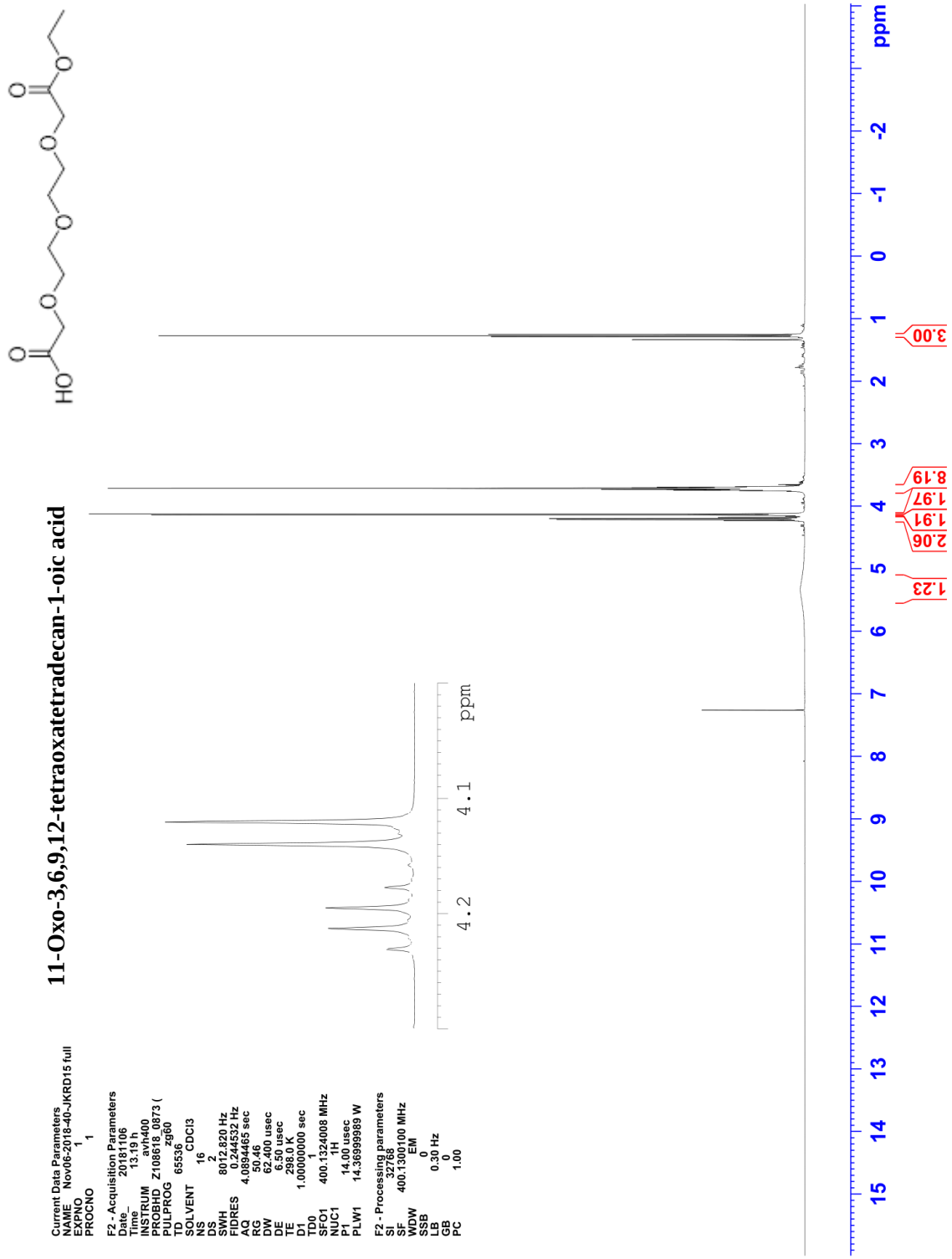


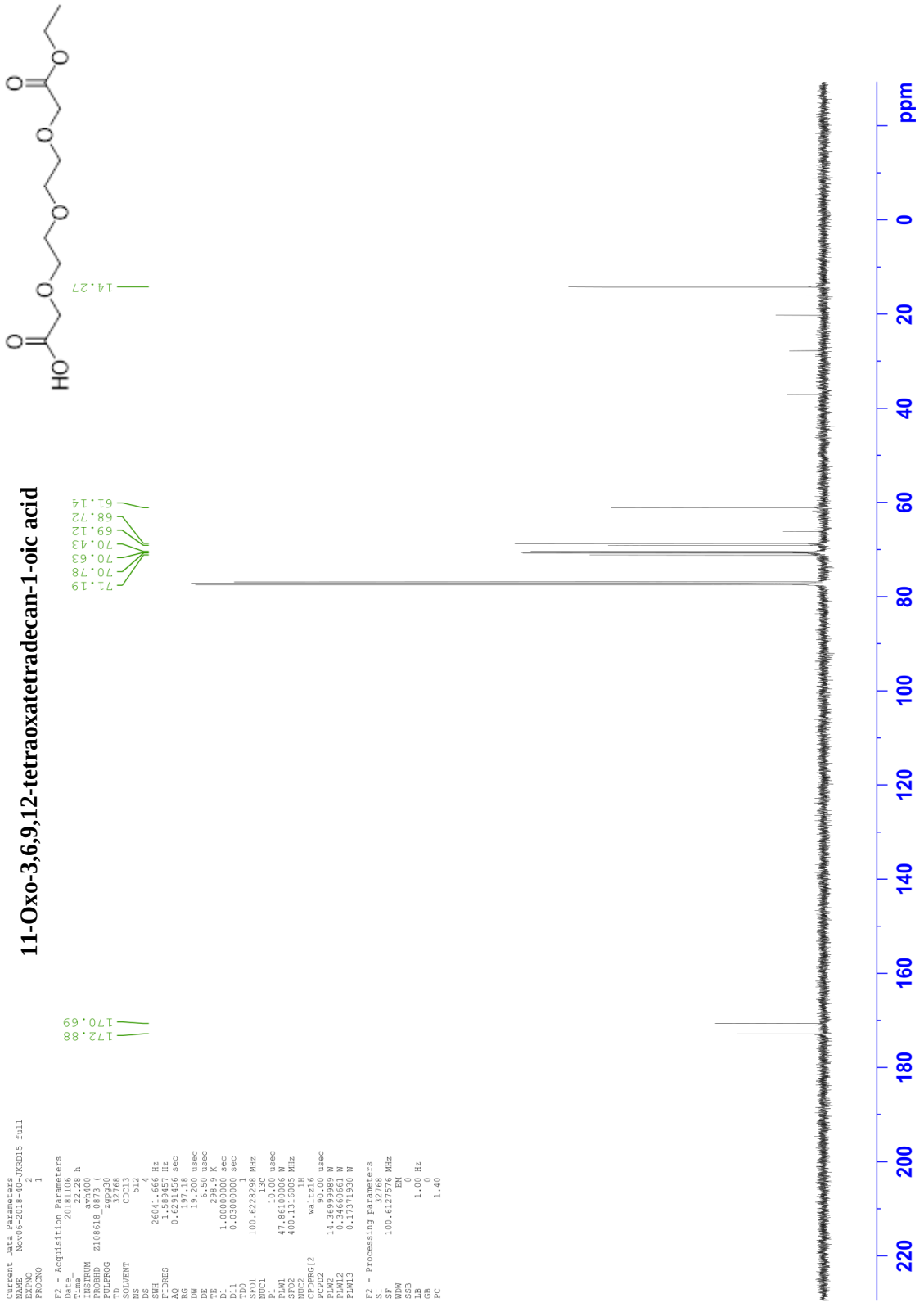
3'',3'',3''-Trichloroethyl {3-[(*tert*-butoxycarbonyl)amino]propyl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate

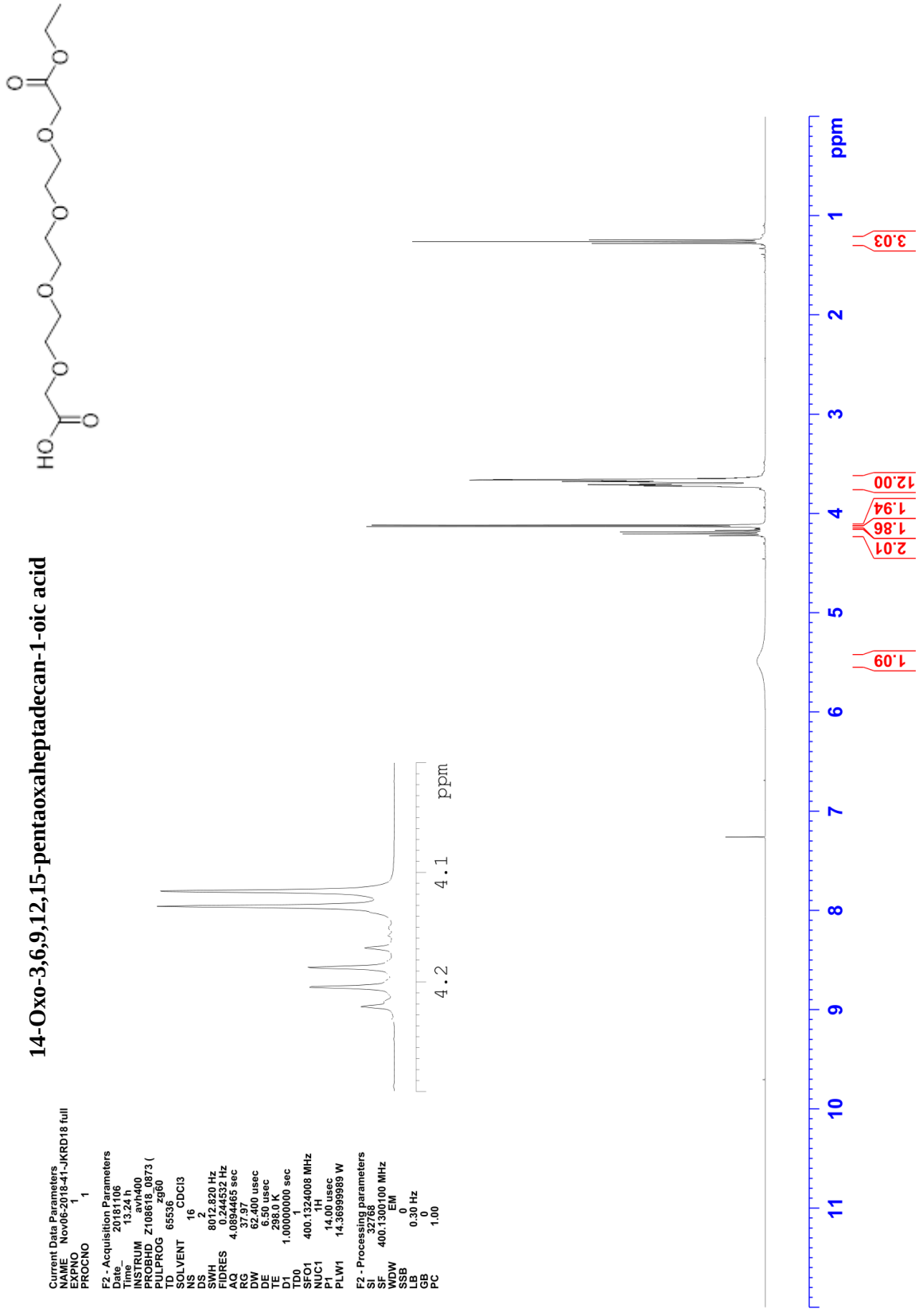


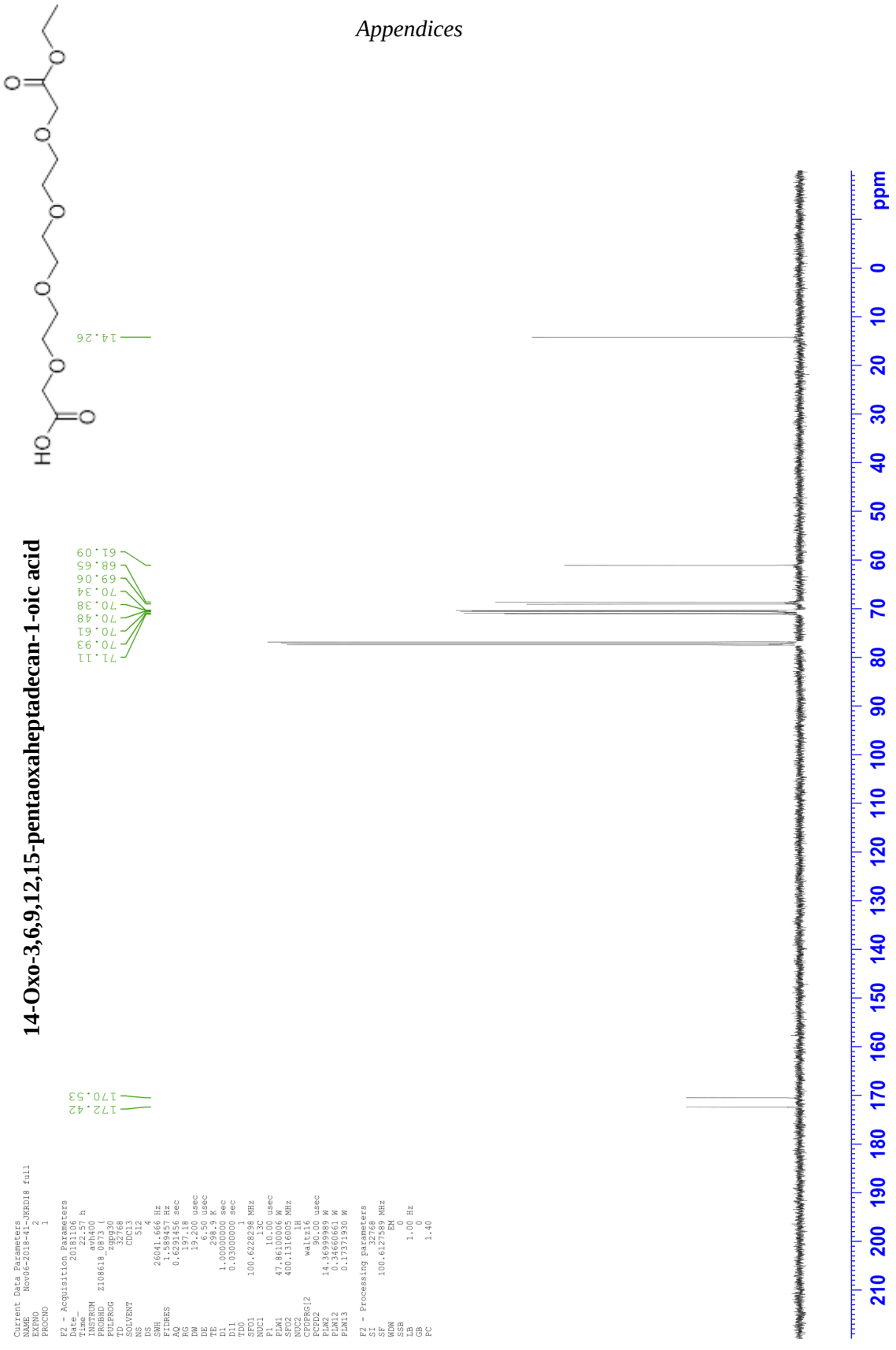

[2-(2-Ethoxy-2-oxoethoxy)ethoxy]acetic acid
Current Data Parameters
NAME Oct30-2015-47-JKRD13 full
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181030
Time_ 9.45 h
INSTRUM avh400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 50.46
DW 62.400 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999969 W
F2 - Processing parameters
SI 32768
SF 400.1300098 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
HO
O
O
O
O
O
O
3.06
4.00
2.02
4.08
1.20
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm


[2-(2-Ethoxy-2-oxoethoxy)ethoxy]acetic acid
170.48
173.69
61.20
68.60
68.63
70.84
71.15
14.26
ppm

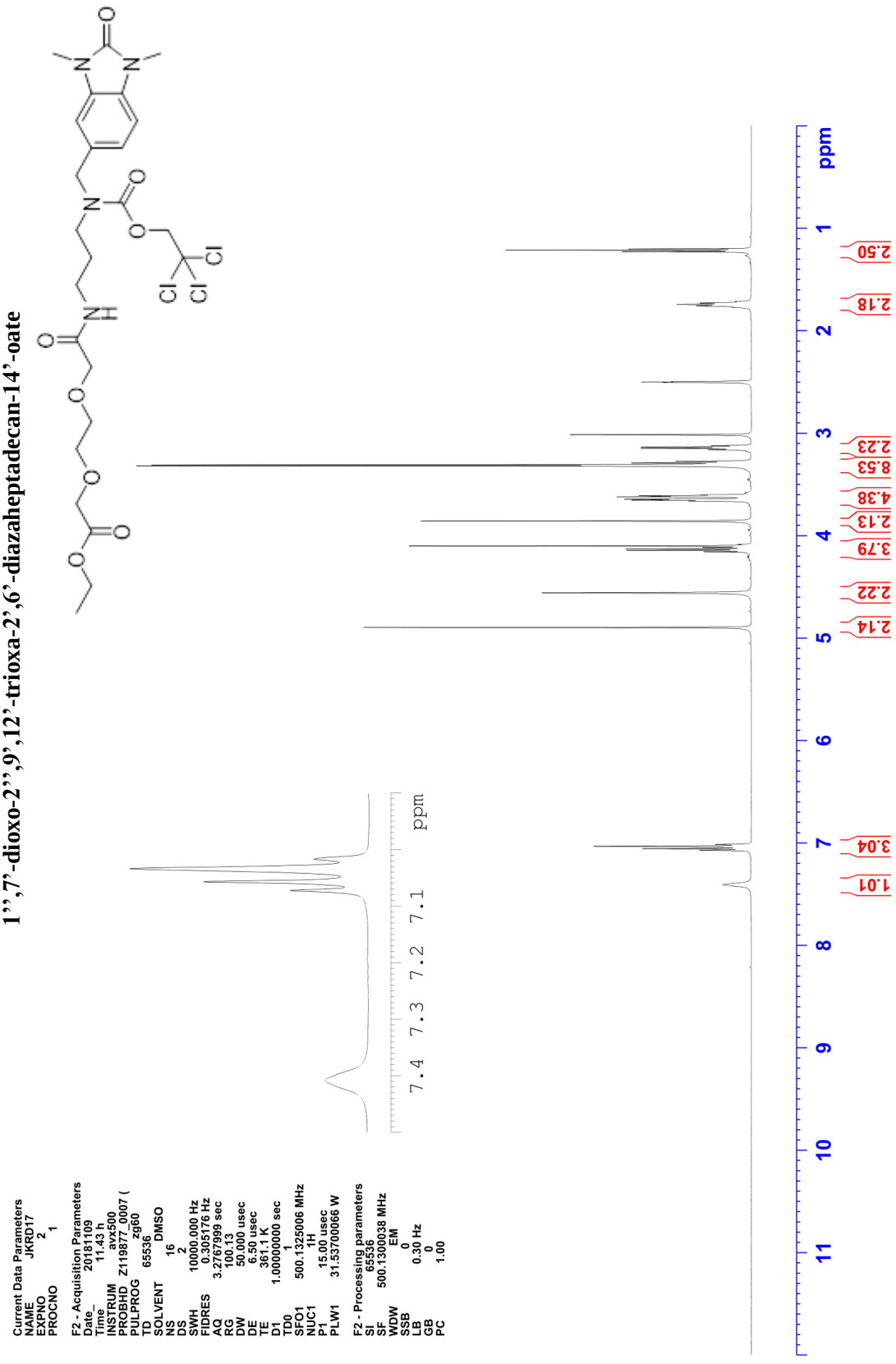

11-Oxo-3,6,9,12-tetraoxatetradecan-1-oic acid
HO
Current Data Parameters
NAME Nov06-2018-40-JKRD15 full
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181106
Time_ 13.19 h
INSTRUM avh400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 50.46
DW 62.400 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
4.2
4.1
ppm
3.00
8.19
1.97
1.91
2.06
1.23
ppm


11-Oxo-3,6,9,12-tetraoxatetradecan-1-oic acid
14.27
61.14
68.72
69.12
70.43
70.63
70.78
71.19
172.88
170.69
ppm
0
20
40
60
80
100
120
140
160
180
200
220

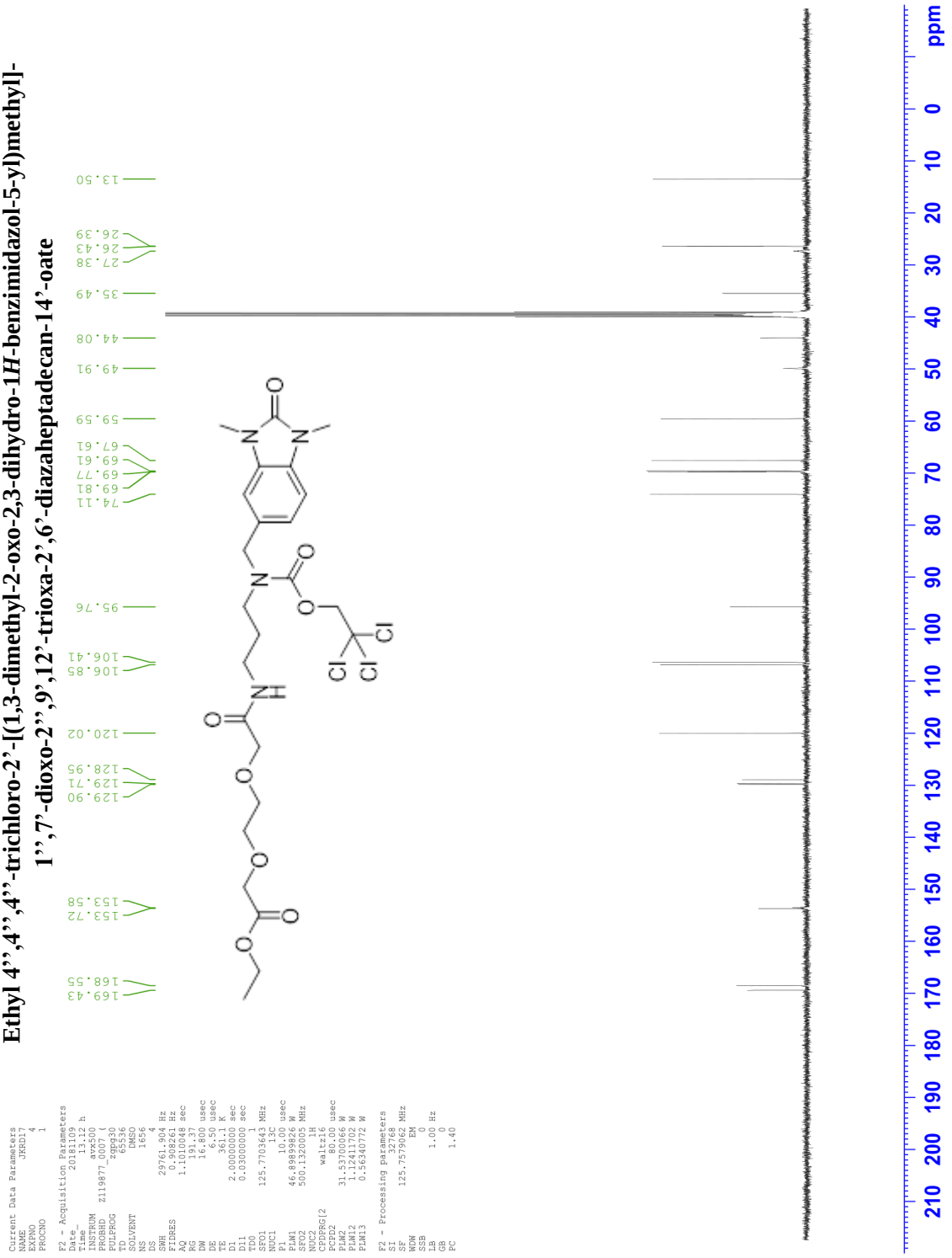

14-Oxo-3,6,9,12,15-pentaoxaheptadecan-1-oic acid
Current Data Parameters
NAME Nov06-2018-41-JKRD18 full
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181106
Time_ 13.24 h
INSTRUM avh400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 37.97
DW 62.400 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
HO
O
O
O
O
O
O
O
O
4.2
4.1
ppm
1
2
3
4
5
6
7
8
9
10
11
ppm
3.03
12.00
1.94
1.86
2.01
1.09


14-Oxo-3,6,9,12,15-pentaoxaheptadecan-1-oic acid
14.26
61.09
68.65
69.06
70.34
70.38
70.48
70.61
70.93
71.11
170.53
172.42
ppm

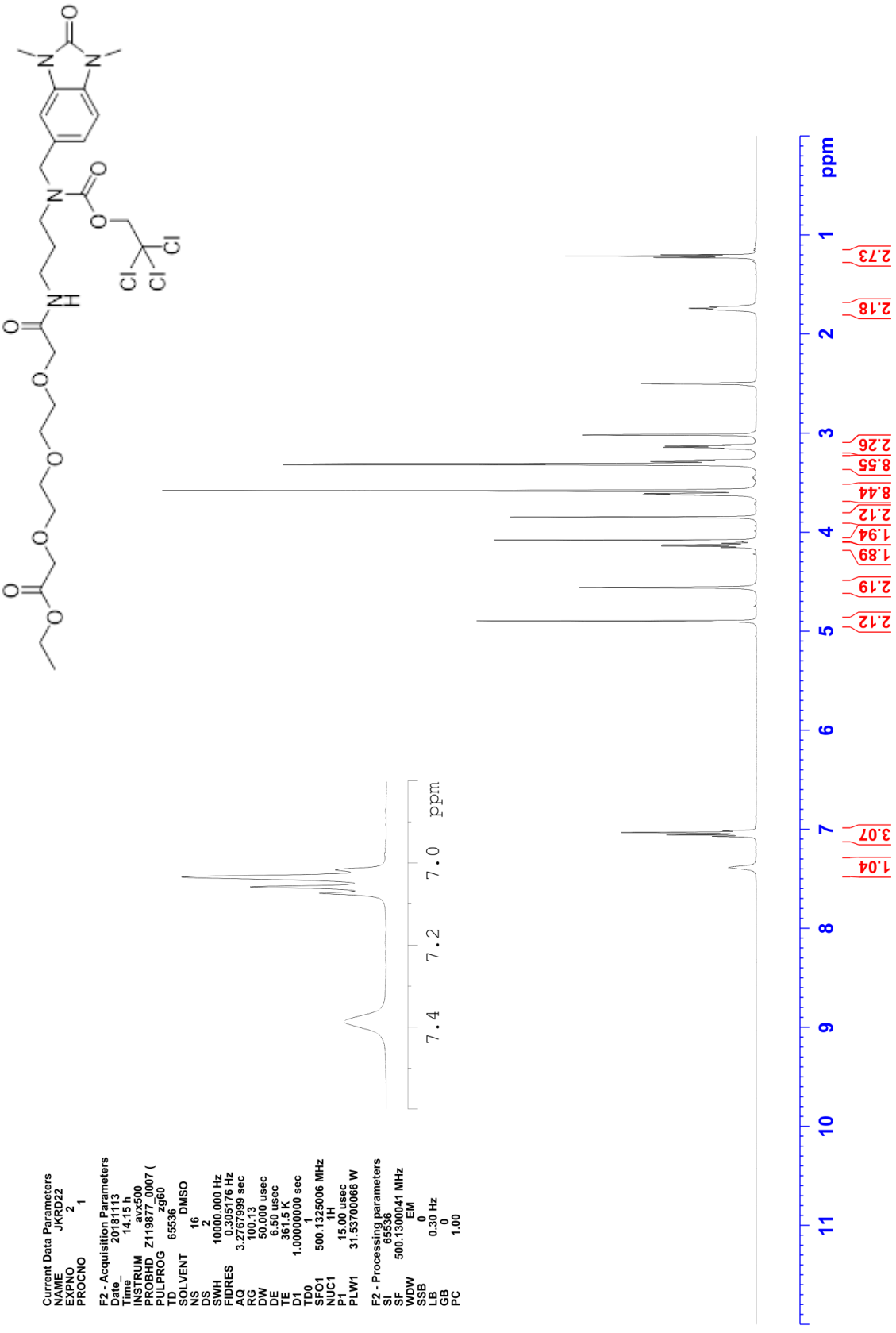
Ethyl 4’’,4’’,4’’-trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1’’,7’-dioxo-2’’,9’,12’-trioxa-2’,6’-diazaheptadecan-14’-oate



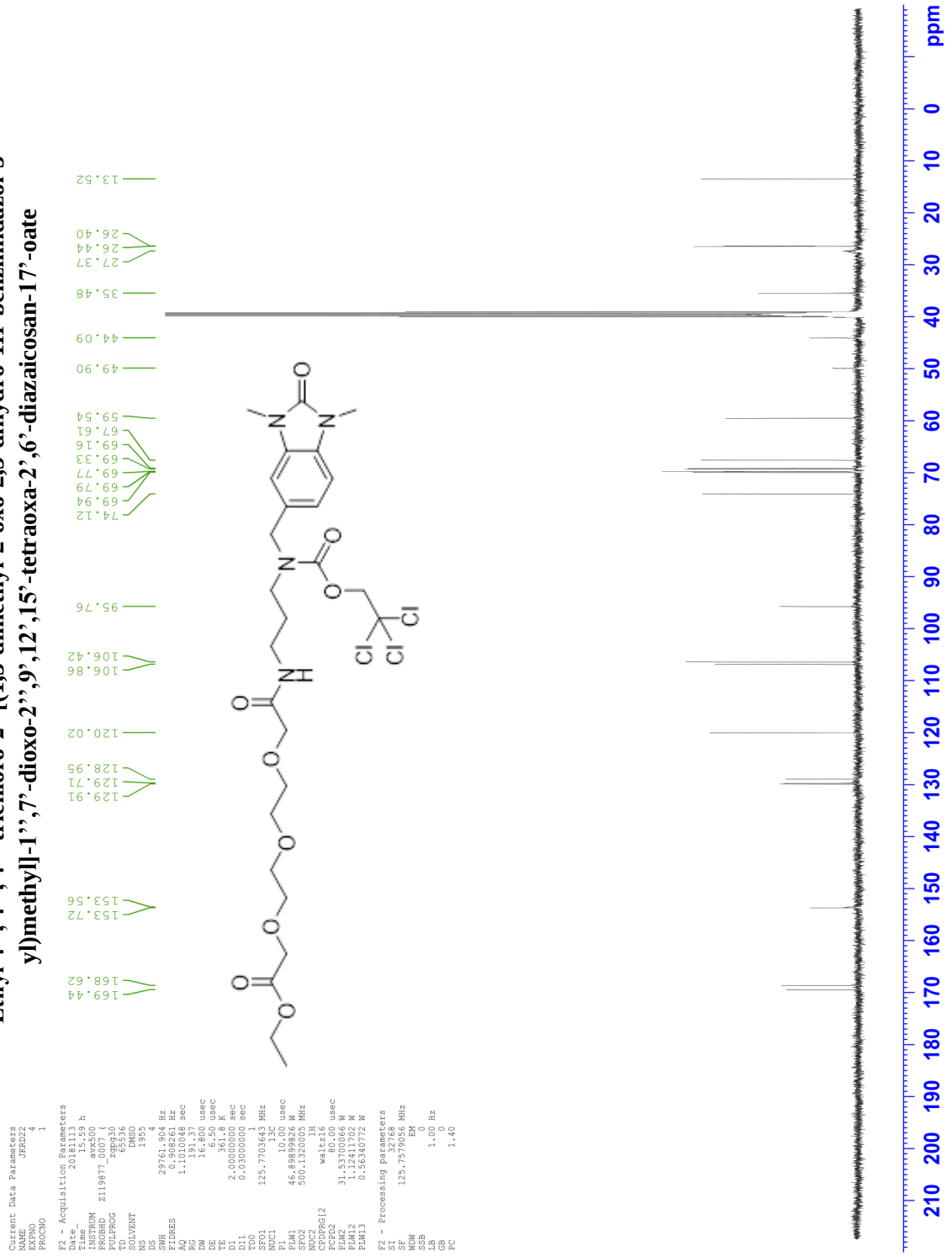
Ethyl 4’’,4’’,4’’-trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1’’,7’’-dioxo-2’’,9’,12’-trioxa-2’,6’-diazaheptadecan-14’-oate



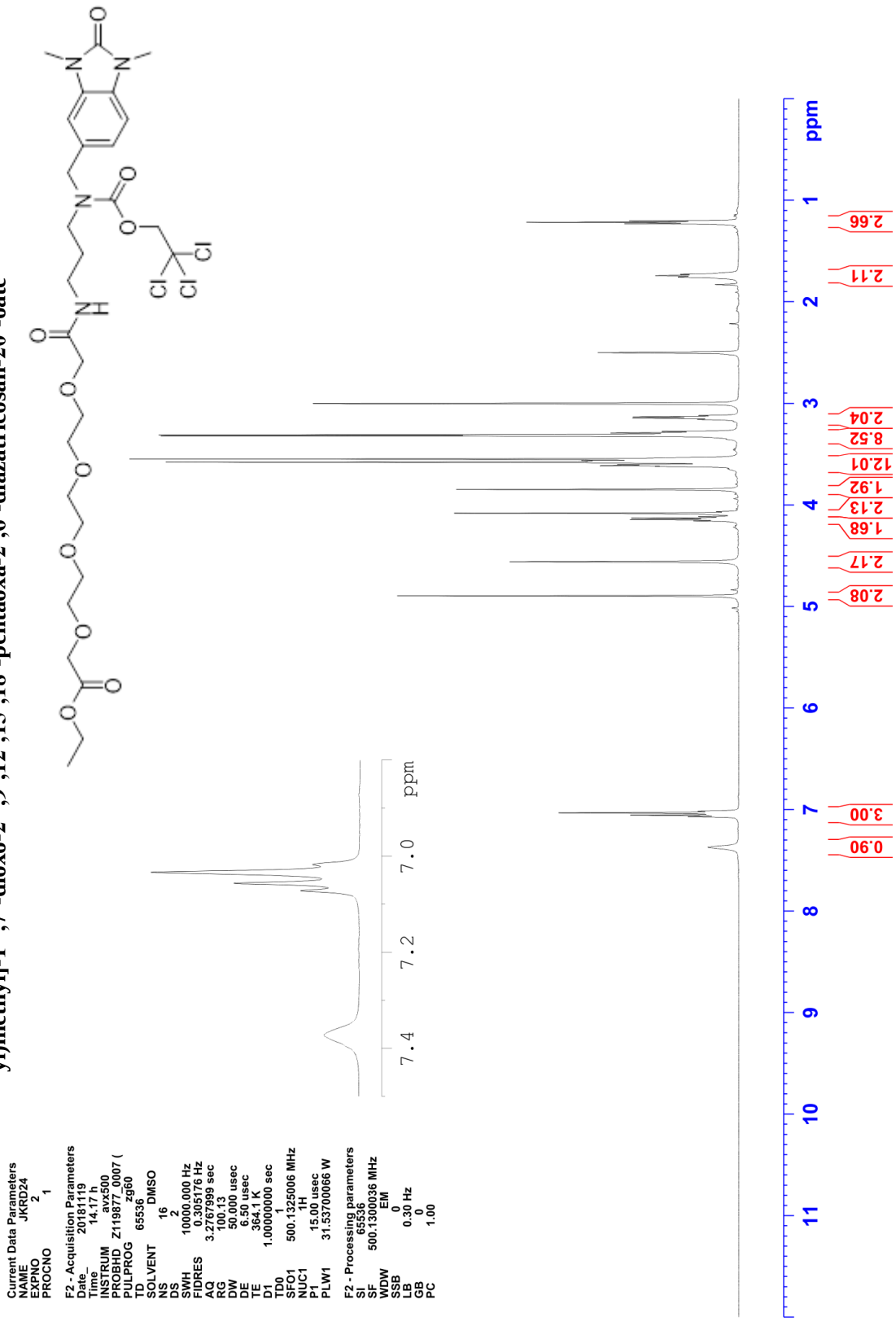
Ethyl 4'', 4'', 4''-trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7''-dioxo-2'',9'',12'',15''-tetraoxa-2'',6''-diazaicosan-17''-oate

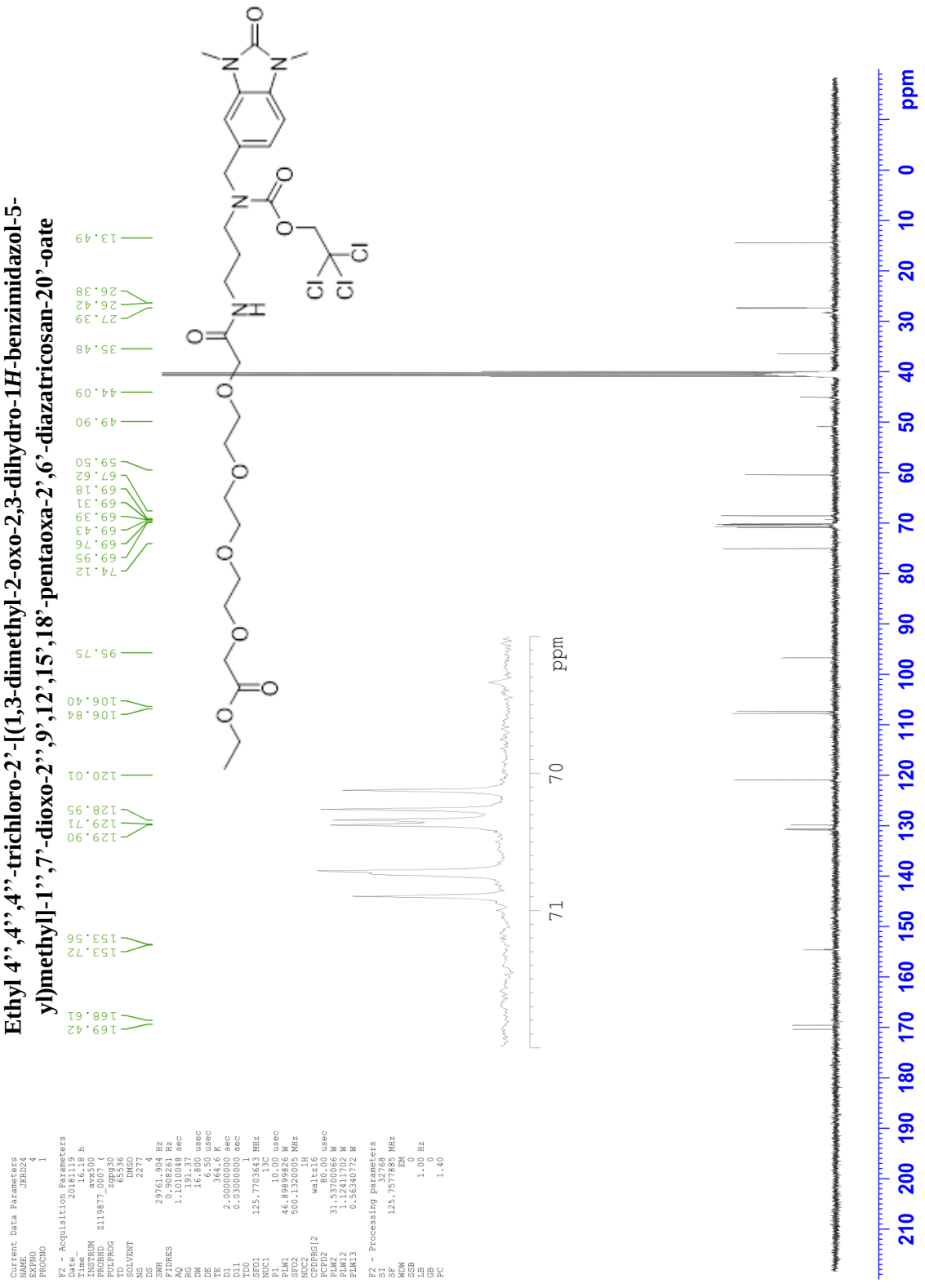


Ethyl 4'', 4'', 4''-trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7'-dioxo-2'',9',12',15'-tetraoxa-2',6'-diazaicosan-17'-oate

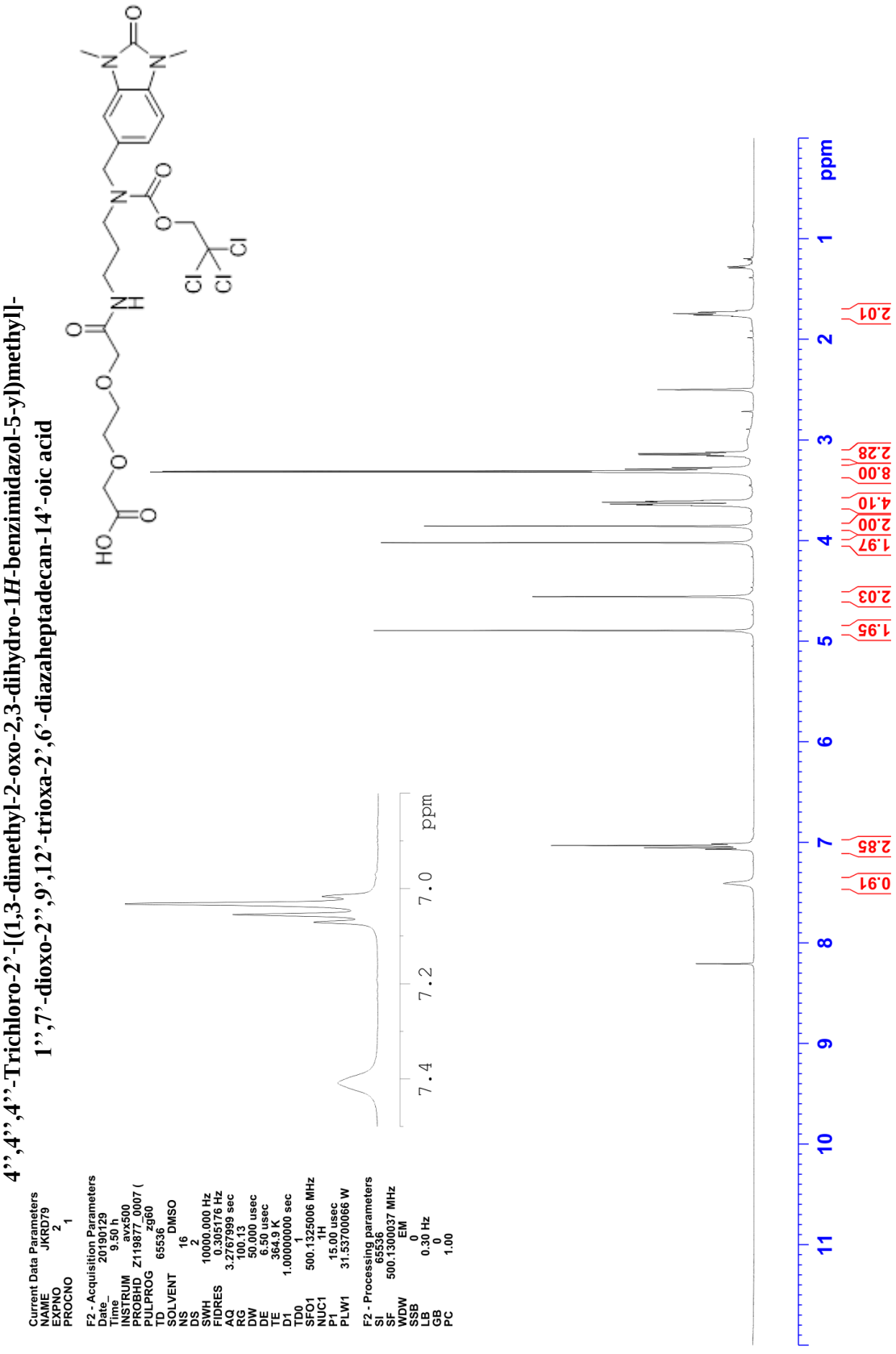


Ethyl 4'',4'',4''-trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7'-dioxo-2'',9',12',15',18'-pentaoxa-2',6'-diazatricosan-20'-oate

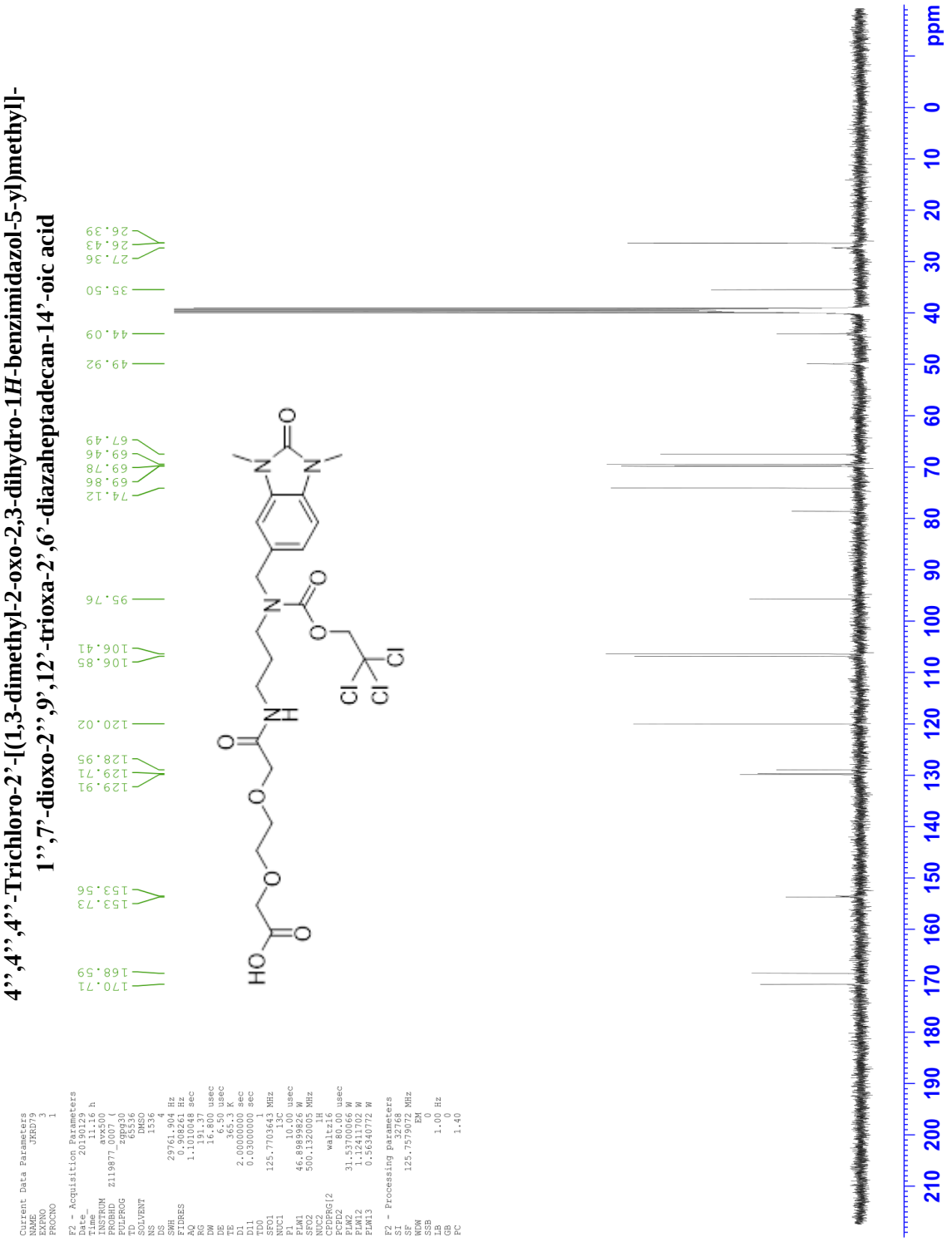



Ethyl 4'',4'',4''-trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl]-1'',7''-dioxo-2'',9',12',15',18'-pentaoxa-2',6'-diazatricosan-20'-oate
Current Data Parameters
NAME JKR024
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181119
Time 16.18 h
INSTRUM spect
PROBHD Z119877_0007 (
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 2277
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.10100048 sec
RG 191.37
DW 16.800 usec
DE 6.50 usec
TE 346.6 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 125.7703643 MHz
NUC1 13C
P1 10.00 usec
PLW1 46.89899826 W
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 31.53700066 W
PLW12 1.24117720 W
PLW13 0.56340772 W
F2 - Processing parameters
SI 32768
SF 125.7577885 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
169.42
168.61
153.72
153.56
129.90
129.71
128.95
120.01
106.84
106.40
95.75
74.12
69.95
69.76
69.43
69.39
69.31
69.18
67.62
59.50
49.90
44.09
35.48
27.39
26.42
26.38
13.49
71
70
ppm
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

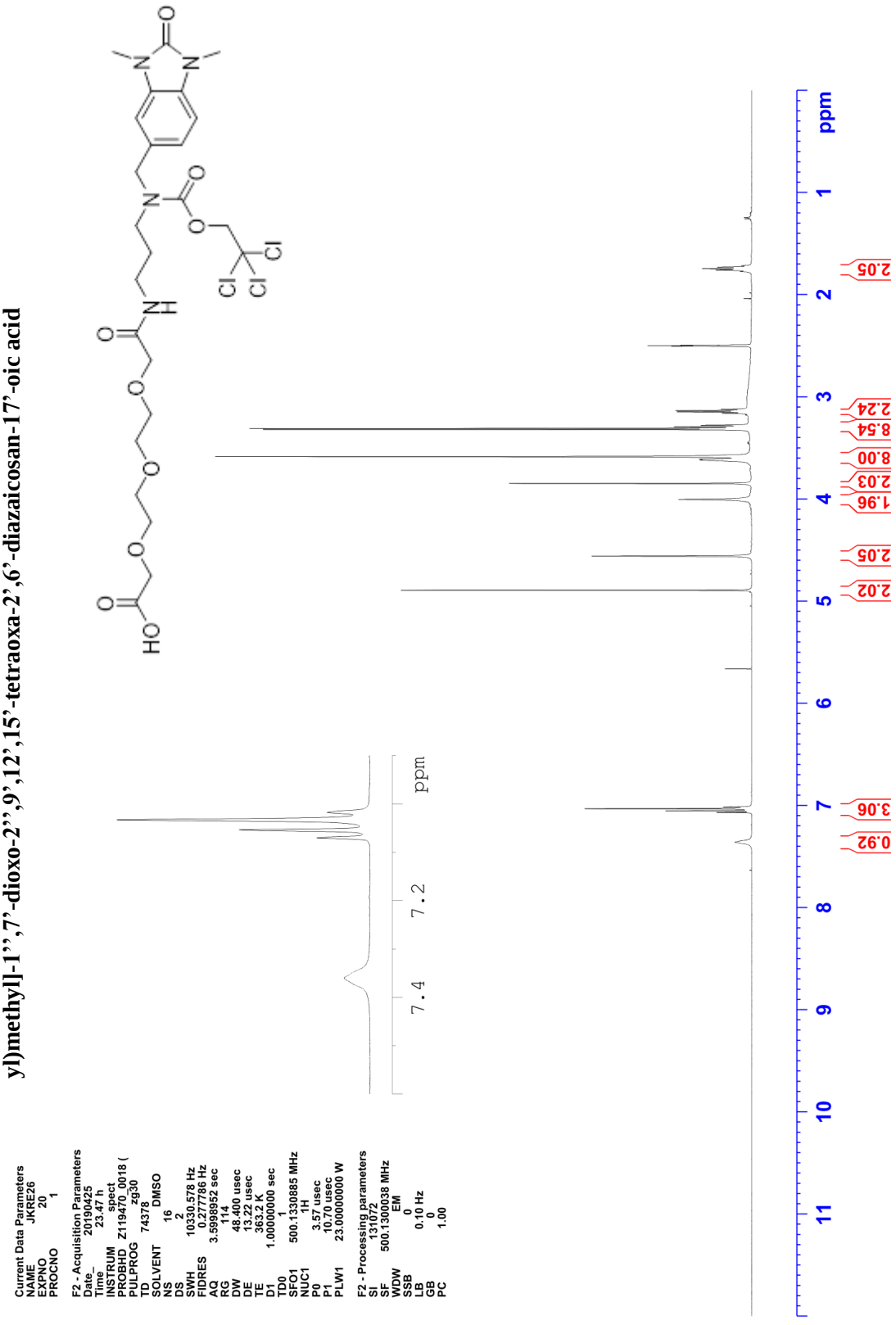
4'',4'',4''-Trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7'-dioxo-2'',9',12'-trioxa-2',6'-diazaheptadecan-14'-oic acid



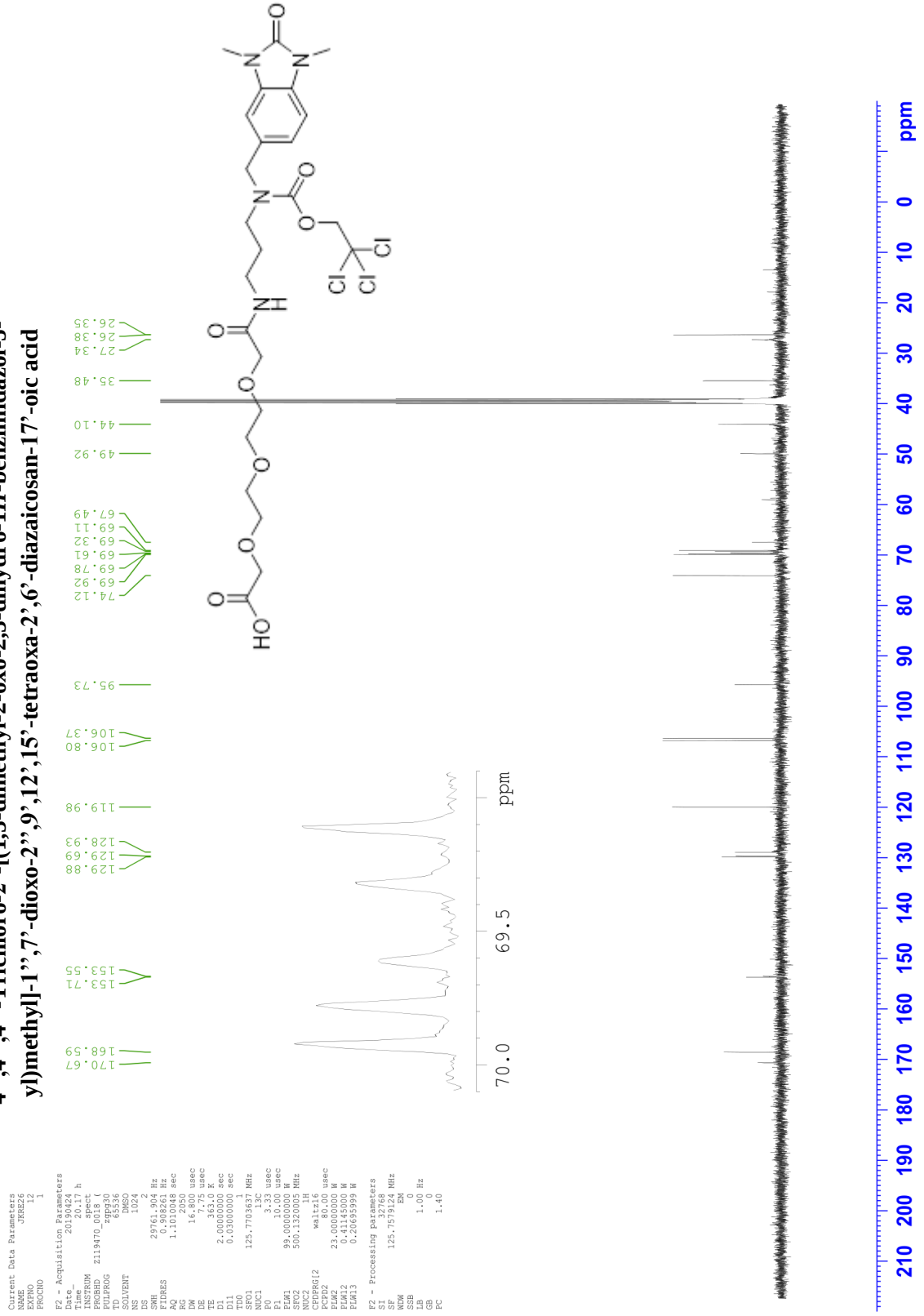
4'',4'',4''-Trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7'-dioxo-2'',9',12'-trioxa-2',6'-diazaheptadecan-14'-oic acid



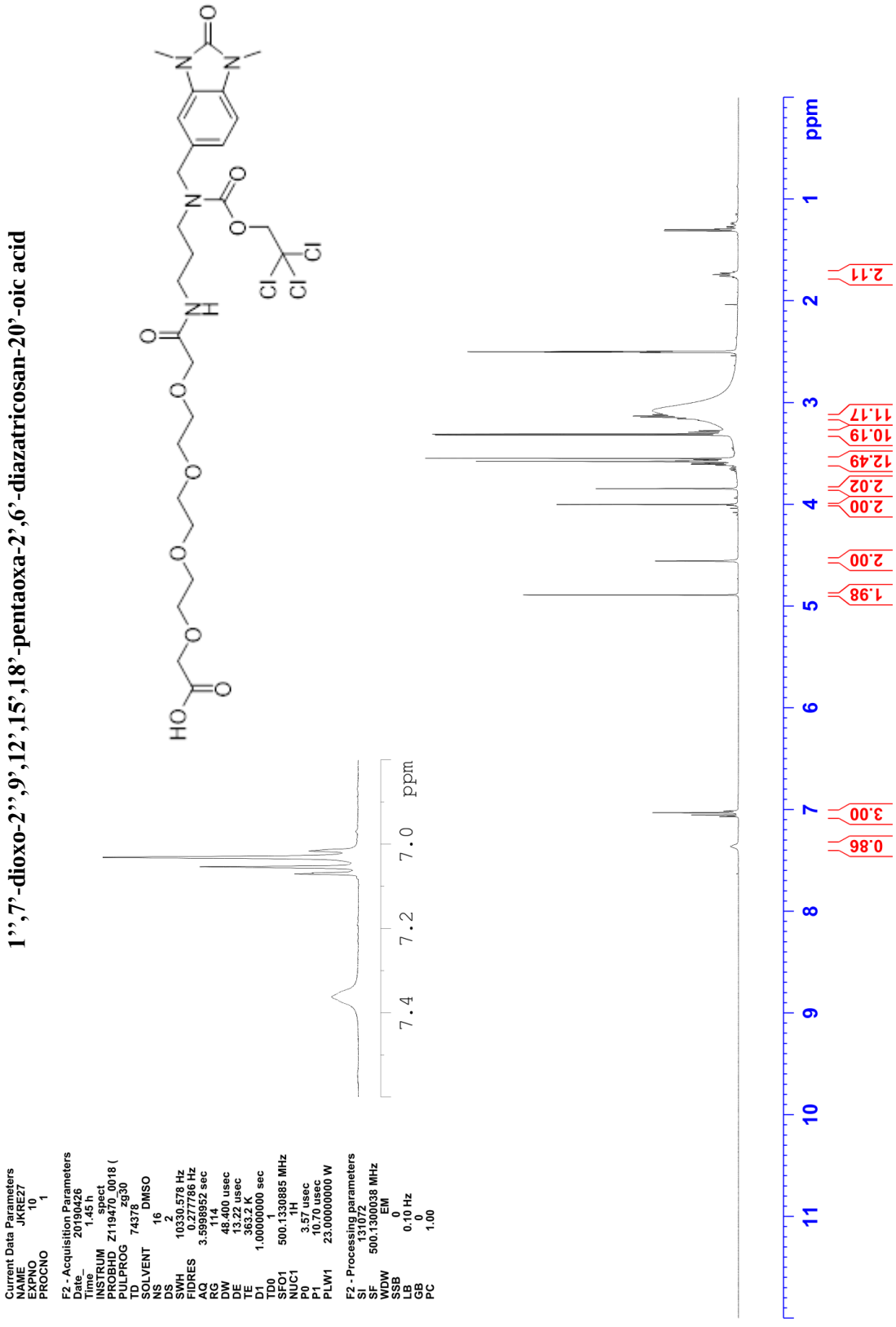
4’’,4’’,4’’-Trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1’’,7’-dioxo-2’’,9’,12’,15’-tetraoxa-2’,6’-diazaicosan-17’-oic acid



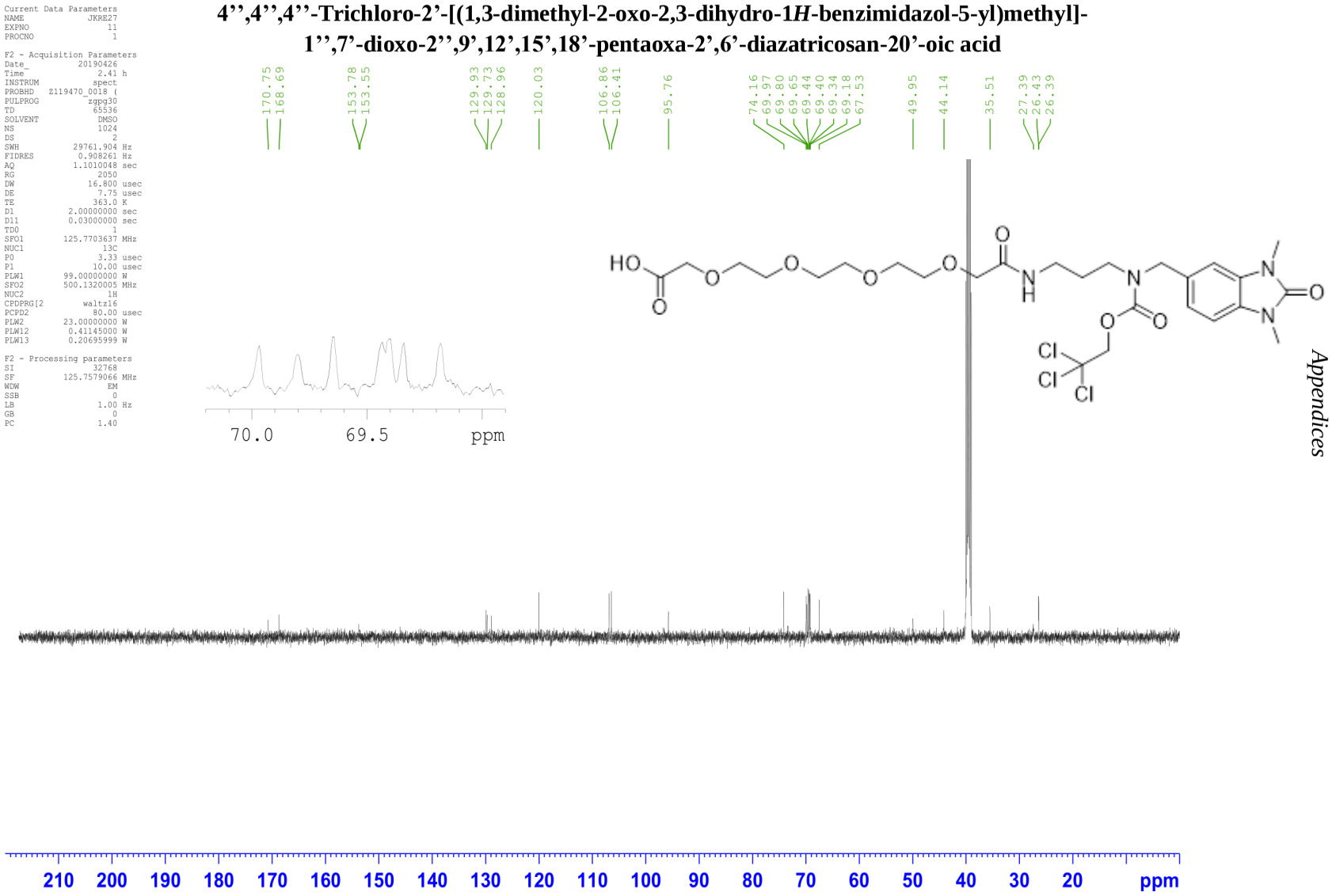
4'',4'',4''-Trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7'-dioxo-2'',9',12',15'-tetraoxa-2',6'-diazaicosan-17'-oic acid

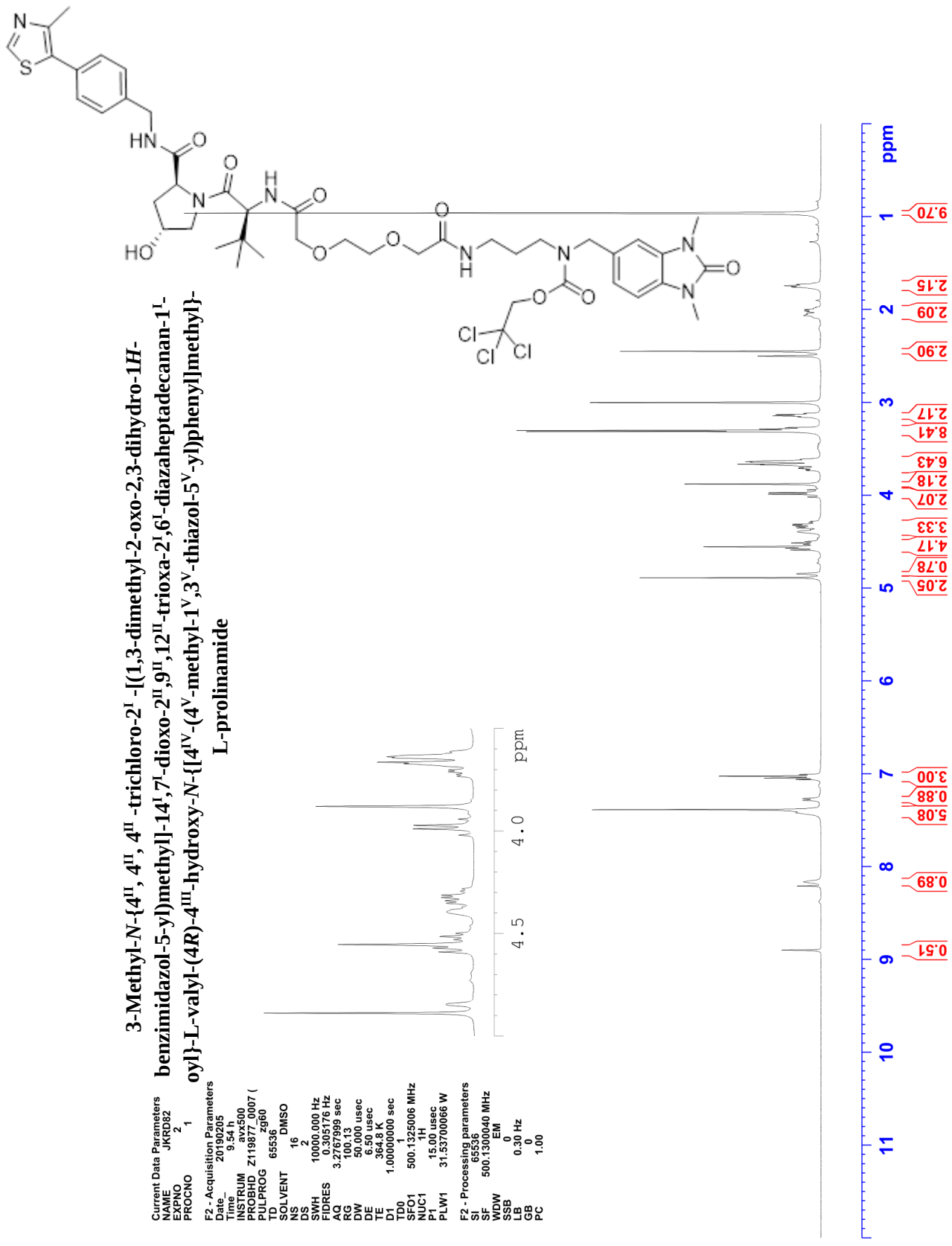


4'',4'',4''-Trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7'-dioxo-2'',9',12',15',18'-pentaoxa-2',6'-diazatricosan-20'-oic acid



4'',4'',4''-Trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-1'',7'-dioxo-2'',9',12',15',18'-pentaoxa-2',6'-diazatricosan-20'-oic acid





3-Methyl-*N*-{4^{II}, 4^{II}, 4^{II} -trichloro-2^{I} -[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-14^{I},7^{I}-dioxo-2^{II},9^{II},12^{II}-trioxa-2^{I},6^{I}-diazaheptadecanan-1^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide

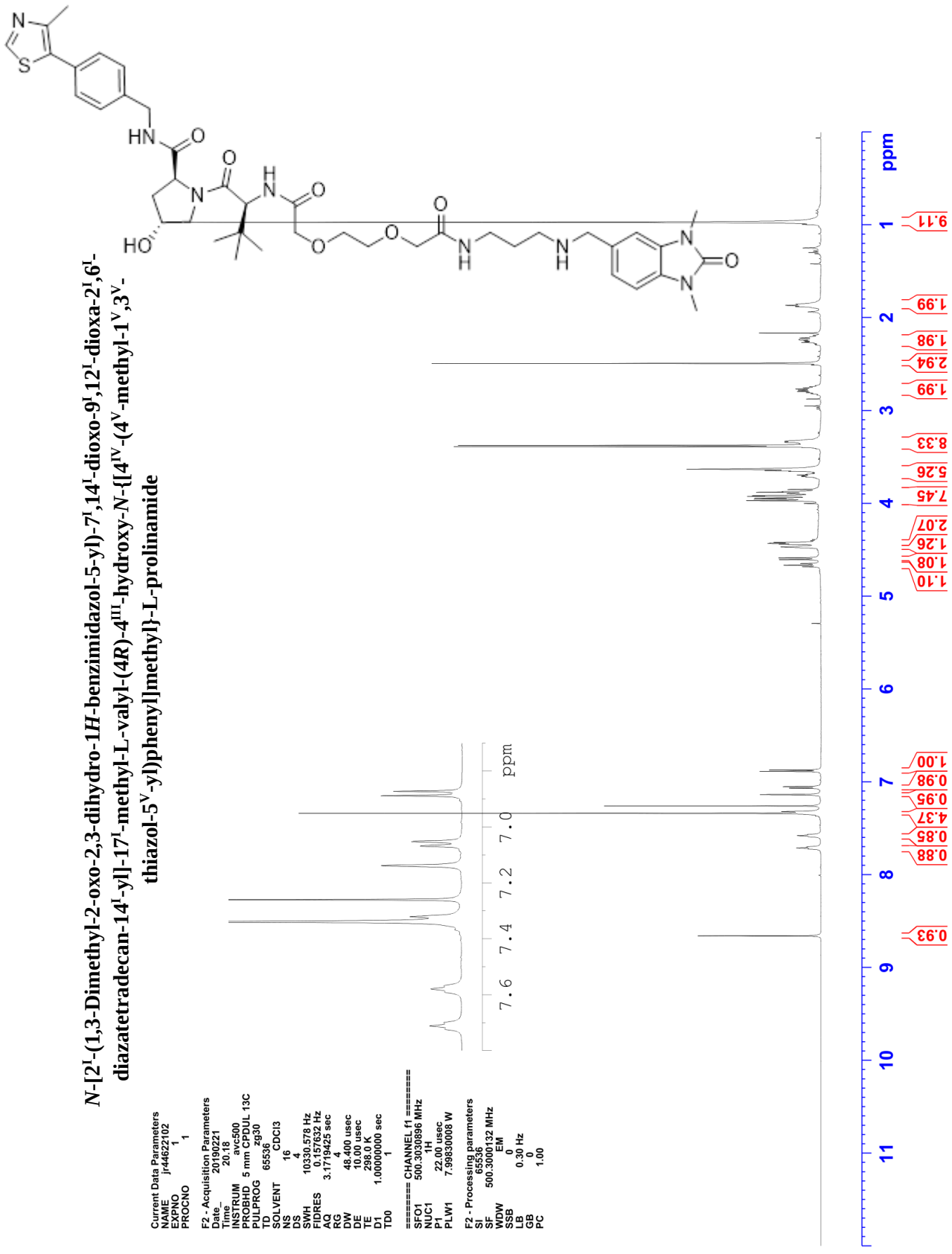
Current Data Parameters
NAME JKRD82
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190205
Time 9.54 h
INSTRUM avx500
PROBHD Z119877_0007 (
PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 100.13
DW 50.000 usec
DE 6.50 usec
TE 364.8 K
D1 1.00000000 sec
TD0 1
SFO1 500.1325006 MHz
NUC1 1H
P1 15.00 usec
PLW1 31.53700066 W

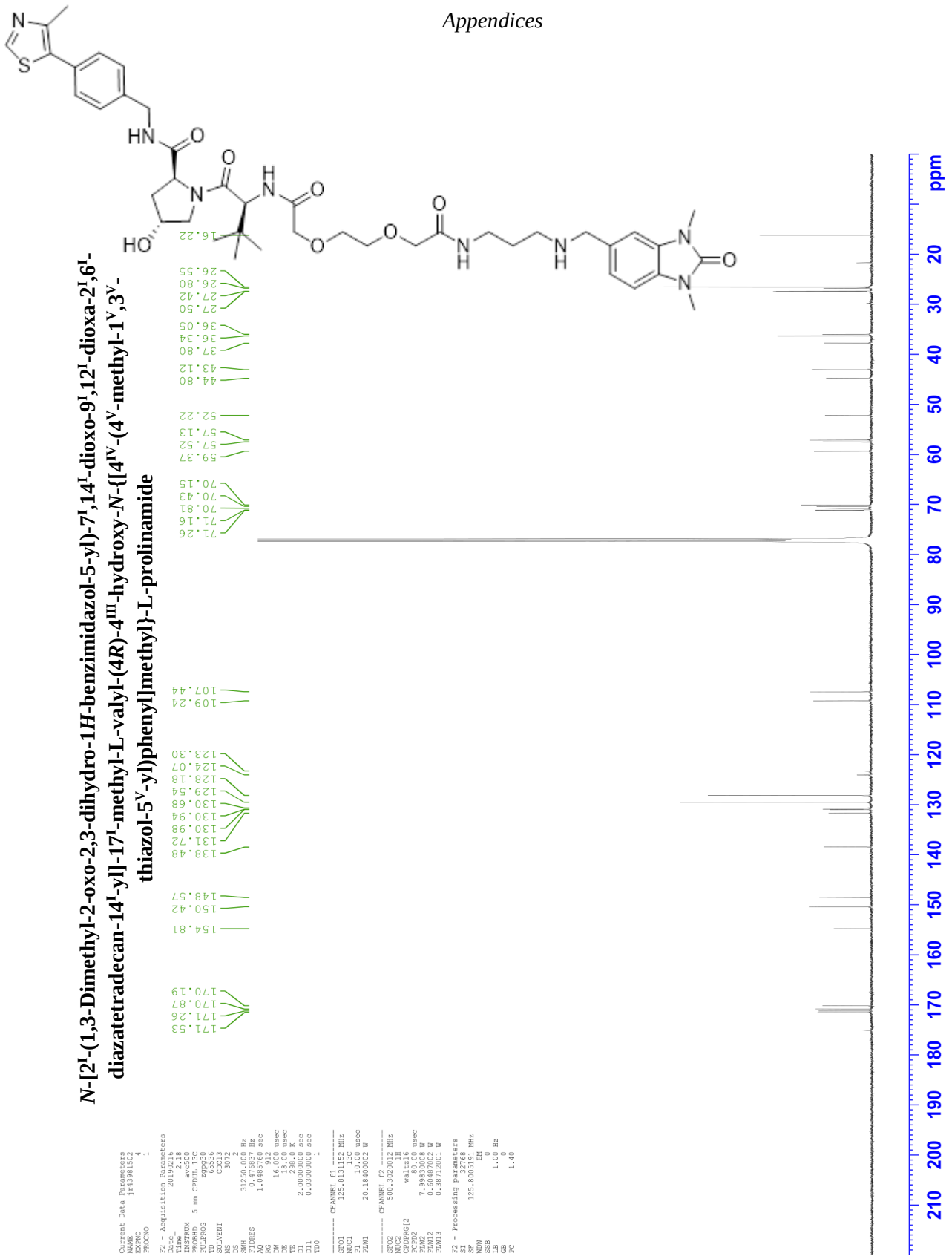
F2 - Processing parameters
SI 65536
SF 500.1300040 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



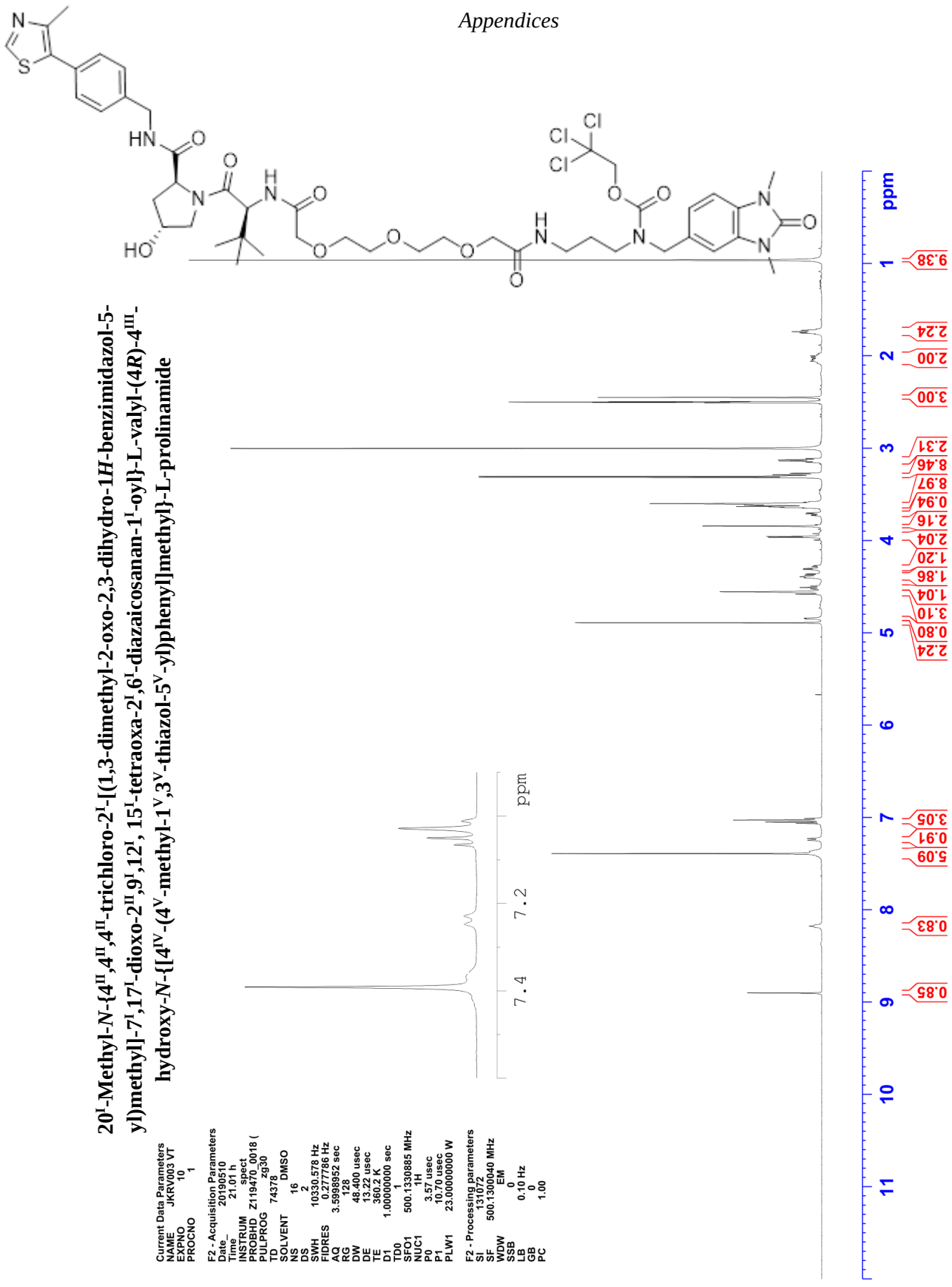
3-Methyl-*N*-{4^{II}, 4^{II} -trichloro-2^{I} -[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-14^{I},7^{I}-dioxo-2^{II},9^{II},12^{II}-trioxa-2^{I},6^{I}-diazaheptadecanan-1^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide



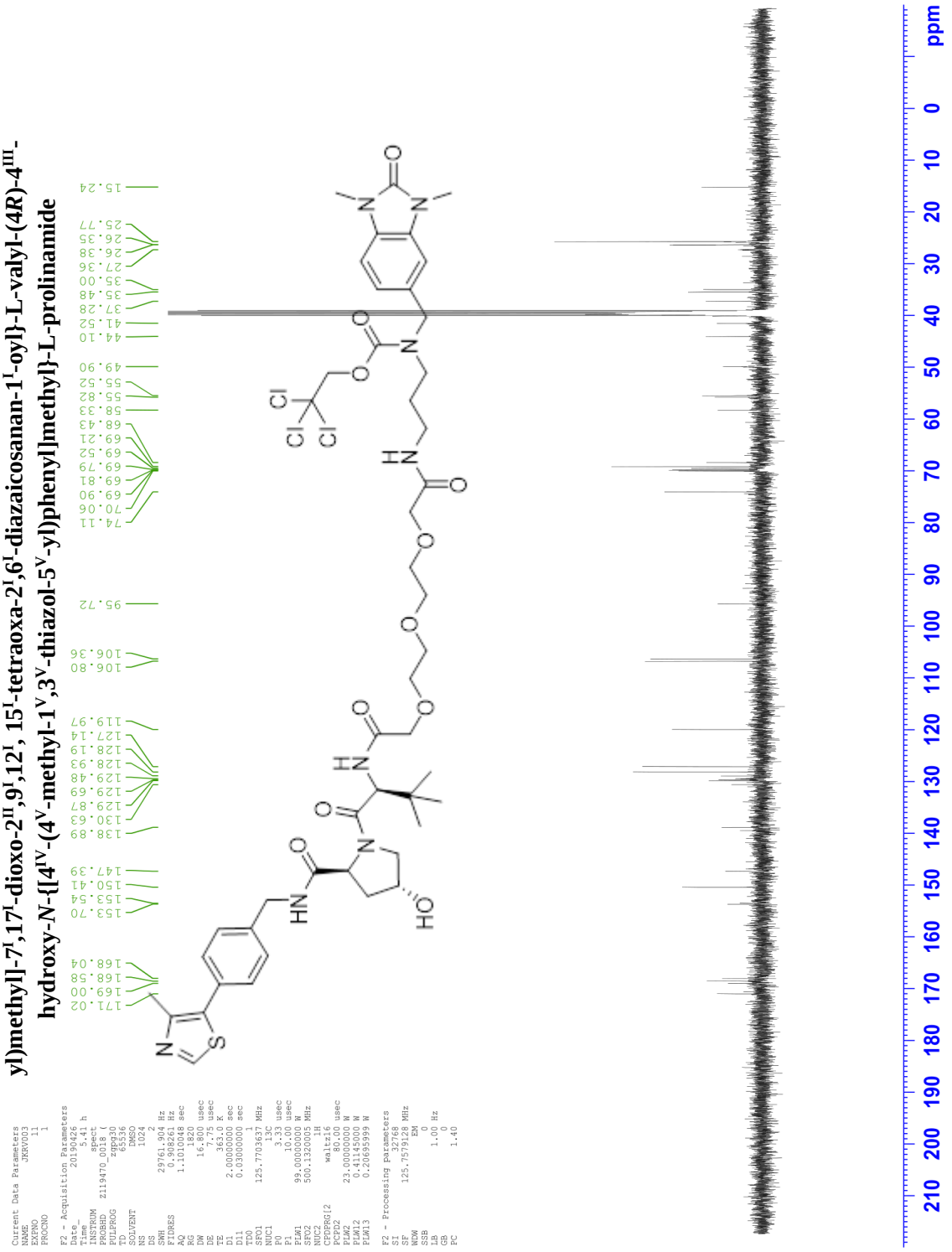
***N*-[2^{I}-(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-7^{I},14^{I}-dioxo-9^{I},12^{I}-dioxa-2^{I},6^{I}-diazatetradecan-14^{I}-yl]-17^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide**

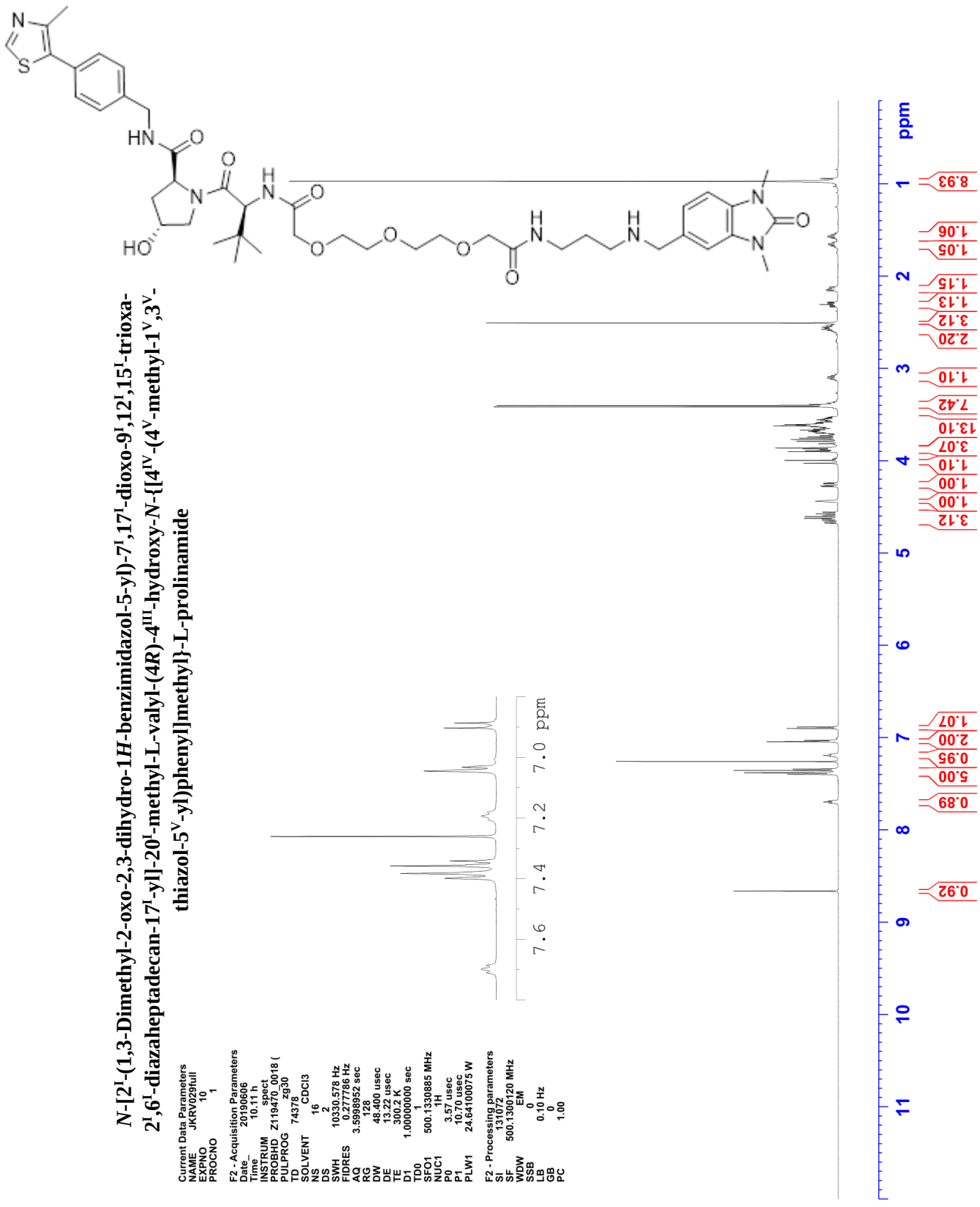

N-[2^I-(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)-7^I,14^I-dioxo-9^I,12^I-dioxa-2^I,6^I-diazatetradecan-14^I-yl]-17^I-methyl-L-valyl-(4R)-4^III-hydroxy-N-{[4^IV-(4^V-methyl-1^V,3^V-thiazol-5^V-yl)phenyl]methyl}-L-prolinamide
Current Data Parameters
NAME jt43981502
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20190216
Time_ 2.18
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 30/2
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 203
DW 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 7.99800006 W
PLW12 0.60487002 W
PLW13 0.38712001 W
F2 - Processing parameters
SI 32768
SF 125.8005191 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB
PC 1.40
171.53
171.26
170.87
170.19
154.81
150.42
148.57
138.48
131.72
130.98
130.94
130.68
129.54
128.18
124.07
123.30
109.24
107.44
71.26
71.16
70.81
70.43
70.15
59.37
57.52
57.13
52.22
44.80
43.12
37.80
36.34
36.05
27.50
27.42
26.80
26.55
16.22
ppm

20^{I}-Methyl-*N*-{4^{II},4^{II},4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-7^{I},17^{I}-dioxo-2^{II},9^{I},12^{I}, 15^{I}-tetraoxa-2^{I},6^{I}-diazaicosanan-1^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide



20^{1}-Methyl-*N*-{4^{II},4^{II},4^{II}-trichloro-2^{1}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-7^{1},17^{1}-dioxo-2^{II},9^{1},12^{1}, 15^{1}-tetraoxa-2^{1},6^{1}-diazaicosanan-1^{1}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide



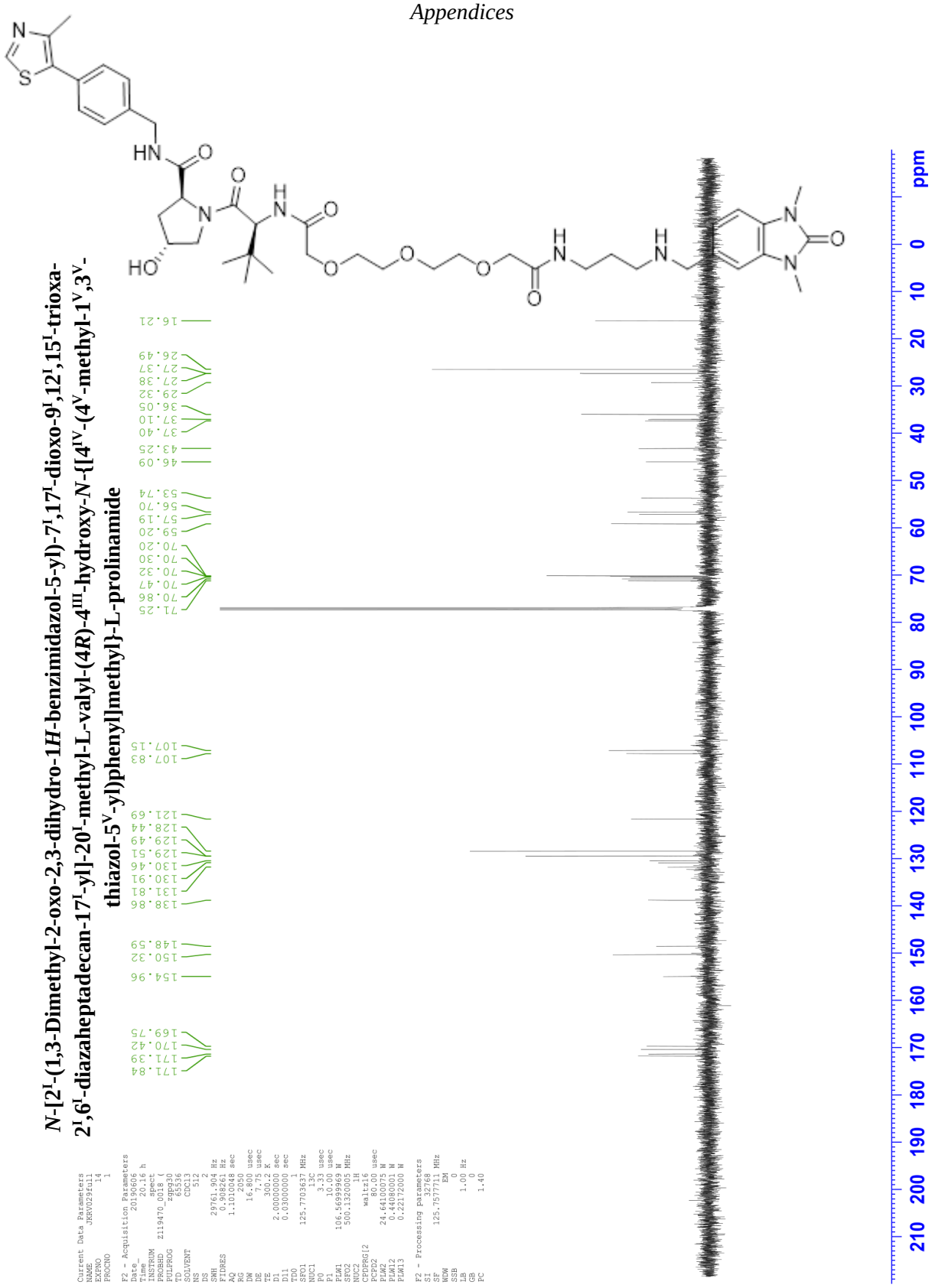


N-[2^{I}-(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-7^{I},17^{I}-dioxo-9^{I},12^{I},15^{I}-trioxa-2^{I},6^{I}-diazaheptadecan-17^{I}-yl]-20^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide

Current Data Parameters
NAME JKRV029full
EXPNO 10
PROCNO 1

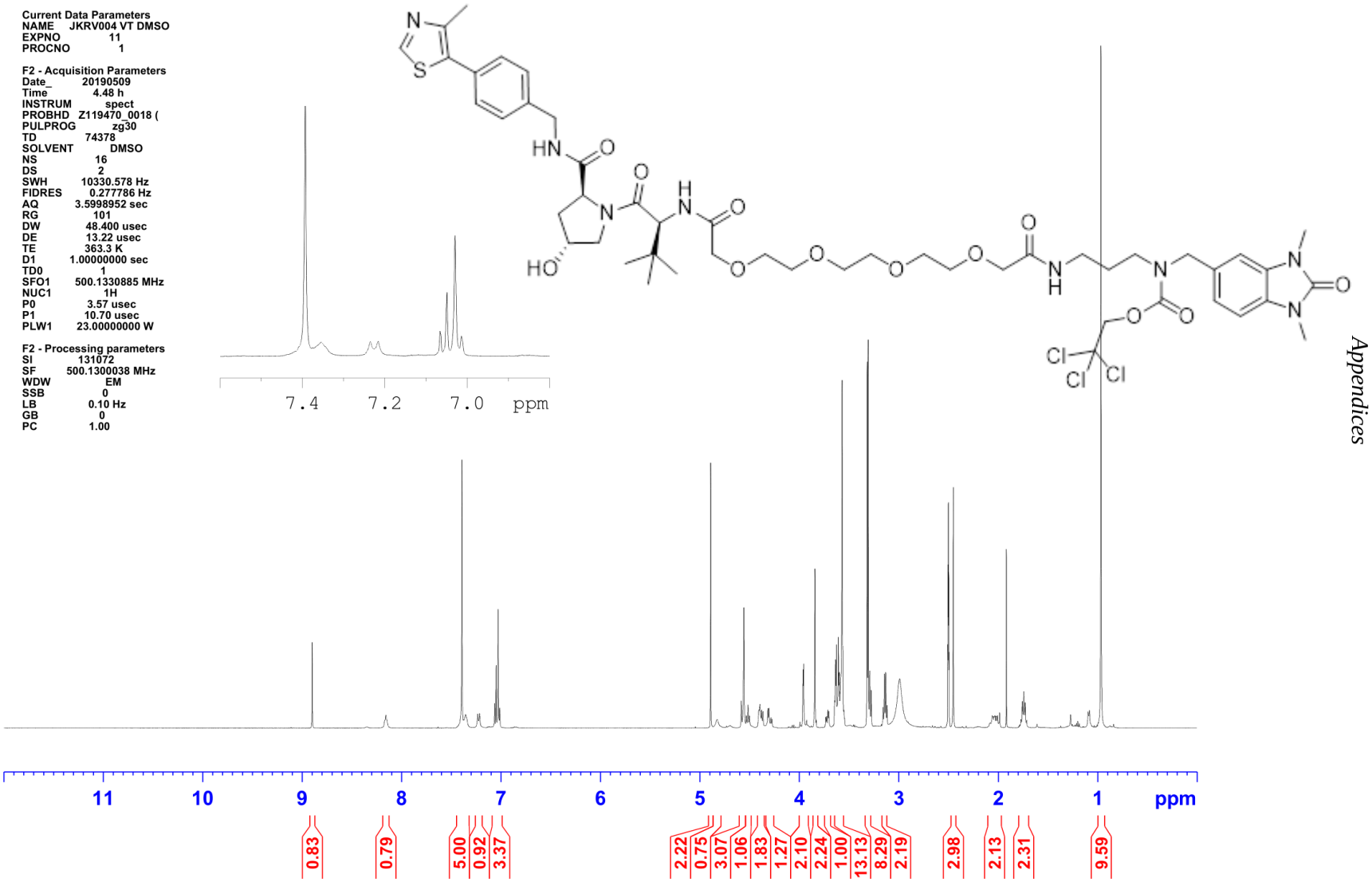
F2 - Acquisition Parameters
Date_ 20190606
Time 10.11 h
INSTRUM spect
PROBHD Z119470_0018 (
PULPROG zg30
TD 74378
SOLVENT CDCl3
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.277786 Hz
AQ 3.5998952 sec
RG 128
DW 48.400 usec
DE 13.22 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330885 MHz
NUC1 1H
P0 3.57 usec
P1 10.70 usec
PLW1 24.64100075 W

F2 - Processing parameters
SI 131072
SF 500.1300120 MHz
WDW EM
SSB 0
LB 0.10 Hz
GB 0
PC 1.00

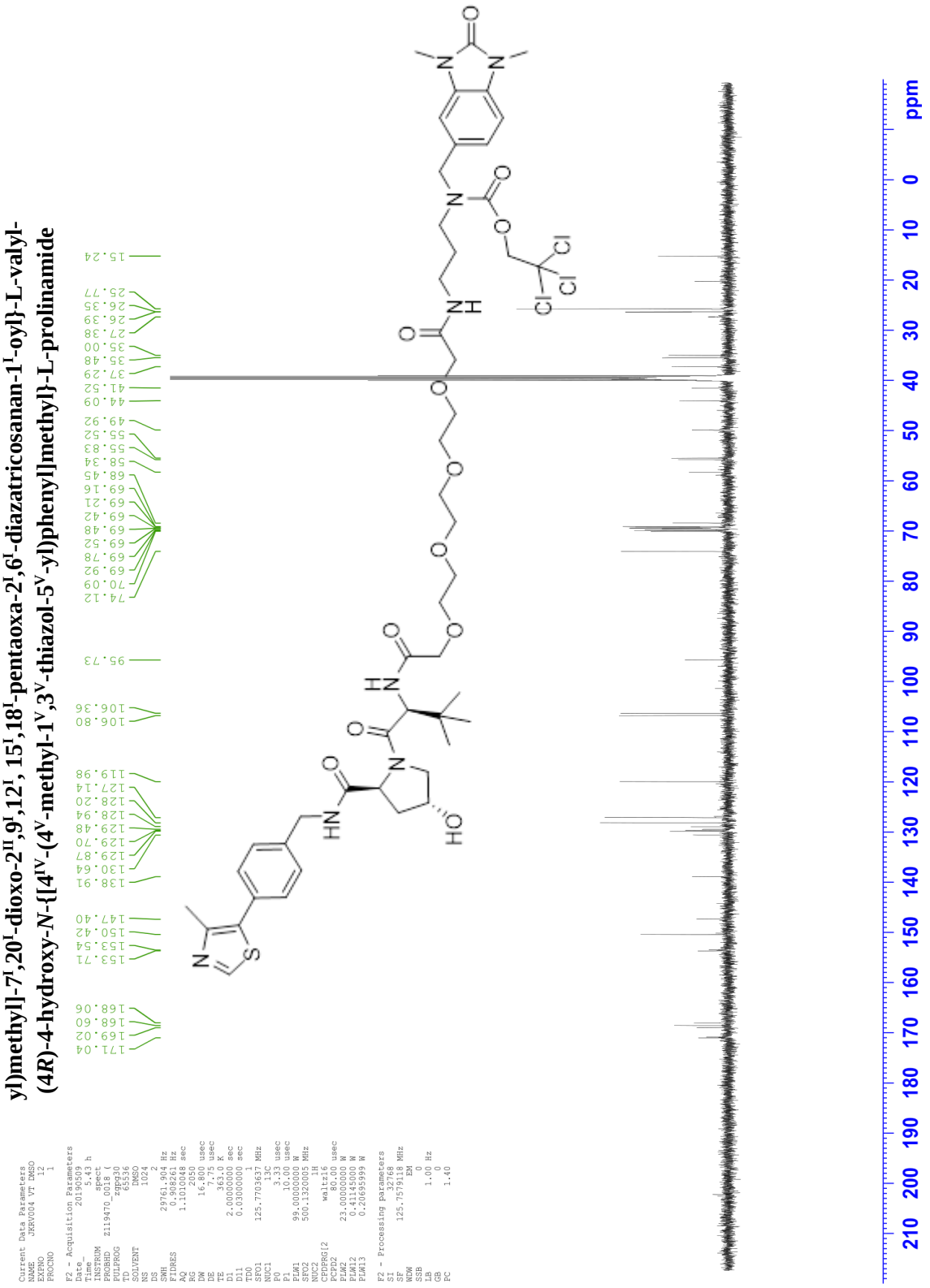


N-[2^{I}-(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-7^{I},17^{I}-dioxo-9^{I},12^{I},15^{I}-trioxa-2^{I},6^{I}-diazaheptadecan-17^{I}-yl]-20^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide

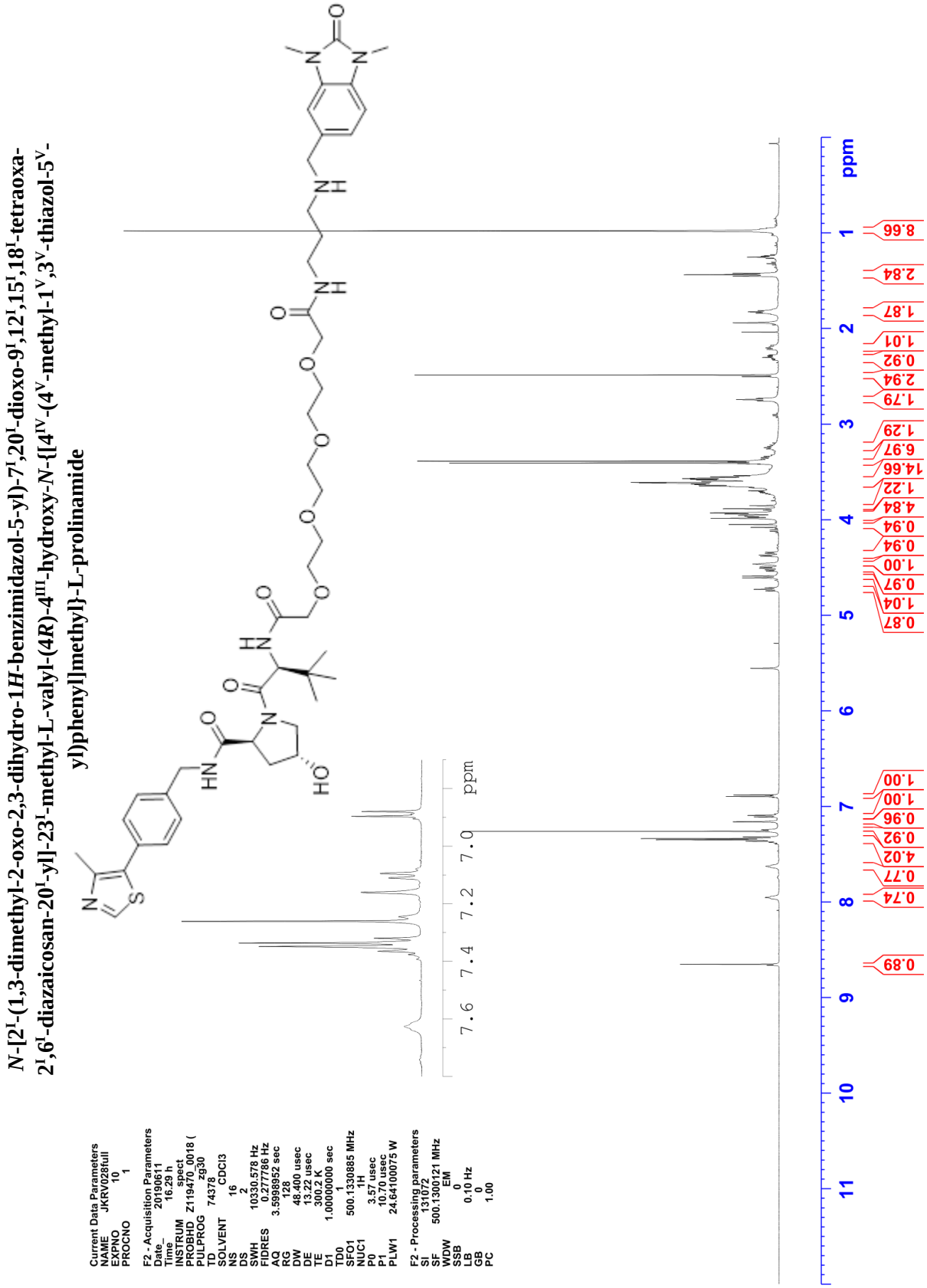
23^{I}-Methyl-*N*-{4^{II}, 4^{II}, 4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-7^{I},20^{I}-dioxo-2^{II},9^{I},12^{I}, 15^{I},18^{I}-pentaoxa-2^{I},6^{I}-diazatricosanan-1^{I}-oyl}-L-valyl-(4*R*)-4-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide



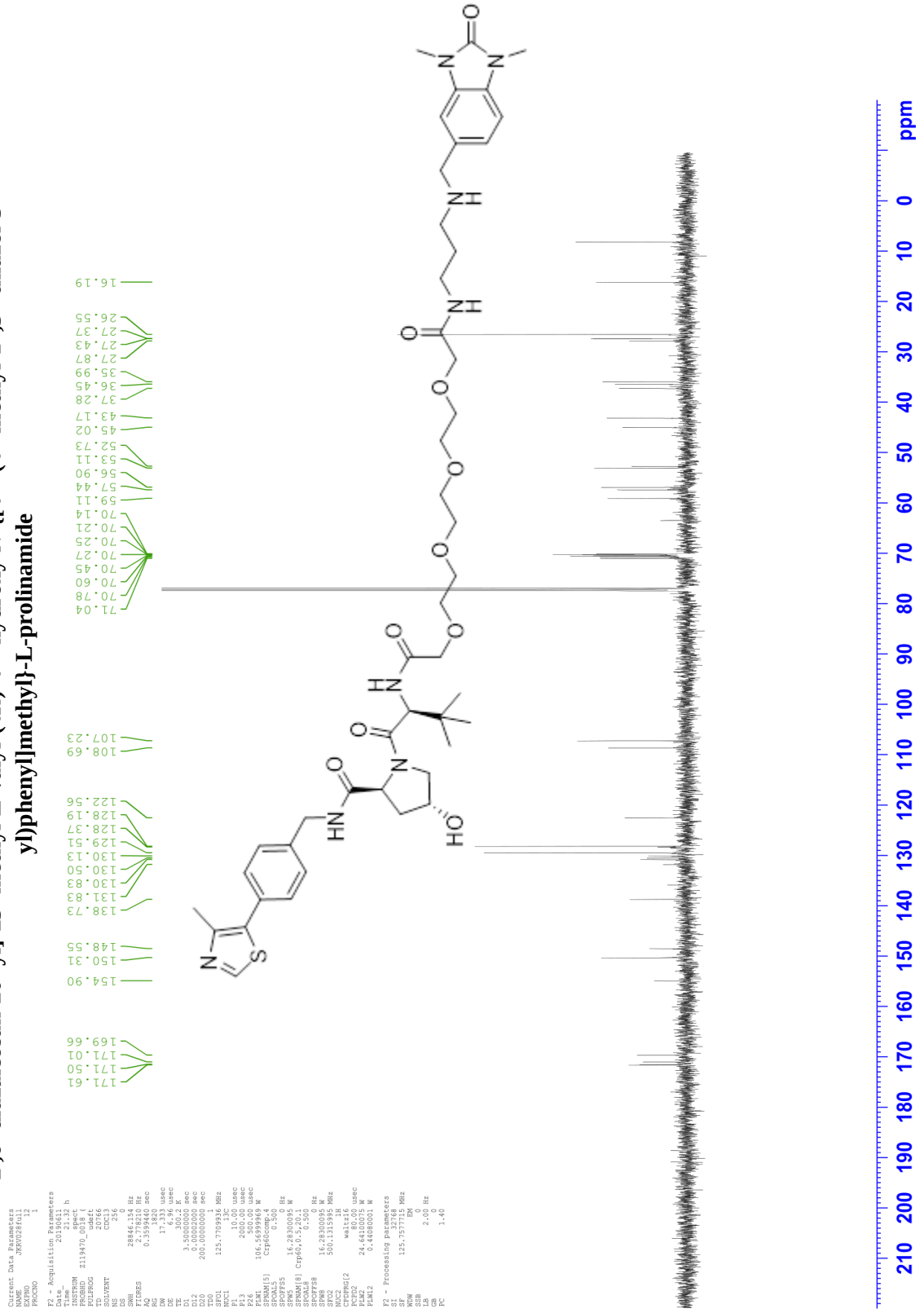
23^{I}-Methyl-*N*-{4^{II}, 4^{II}, 4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-7^{I},20^{I}-dioxo-2^{II},9^{I},12^{I}, 15^{I},18^{I}-pentaoxa-2^{I},6^{I}-diazatricosanan-1^{I}-oyl}-L-valyl-(4*R*)-4-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide

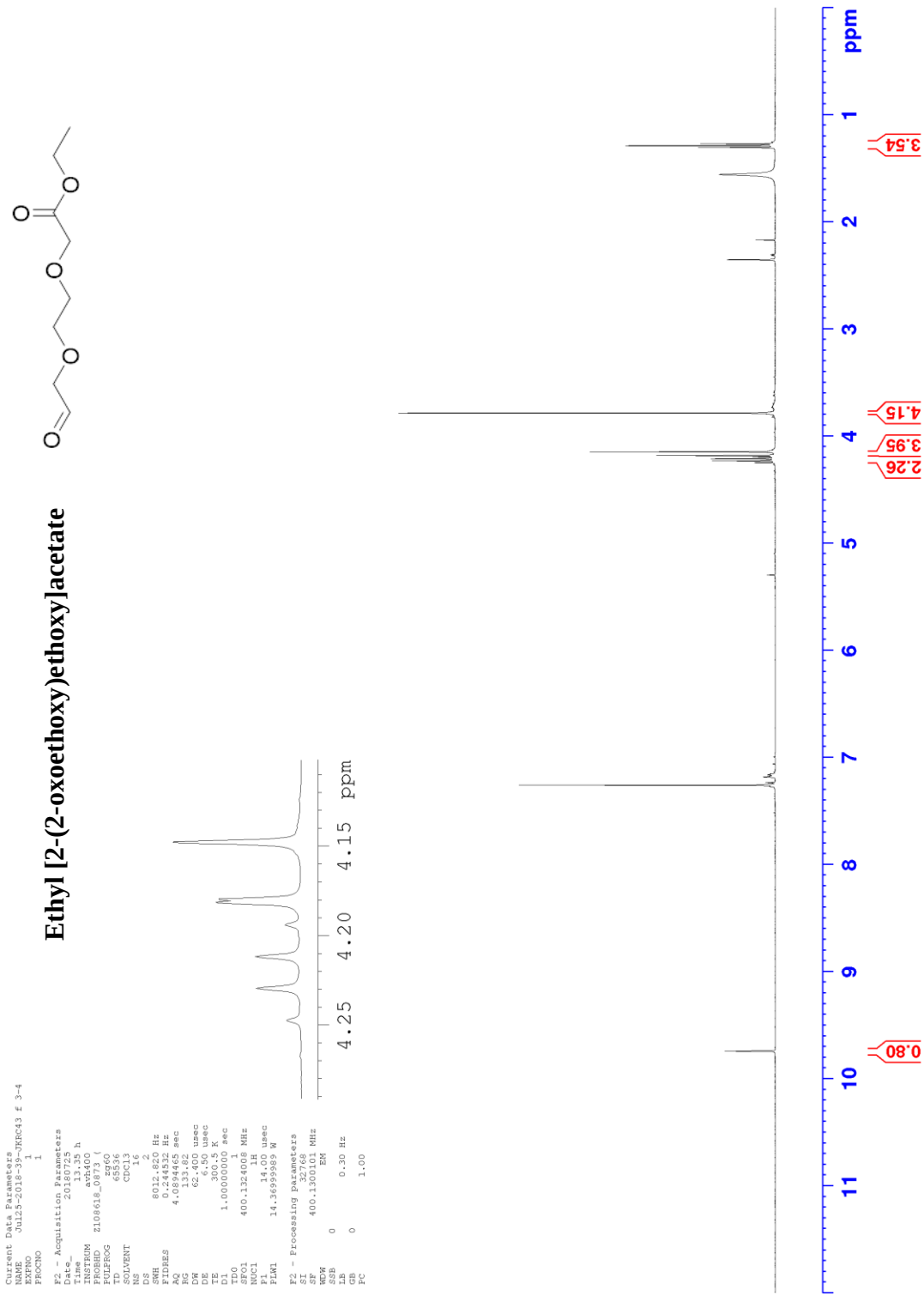


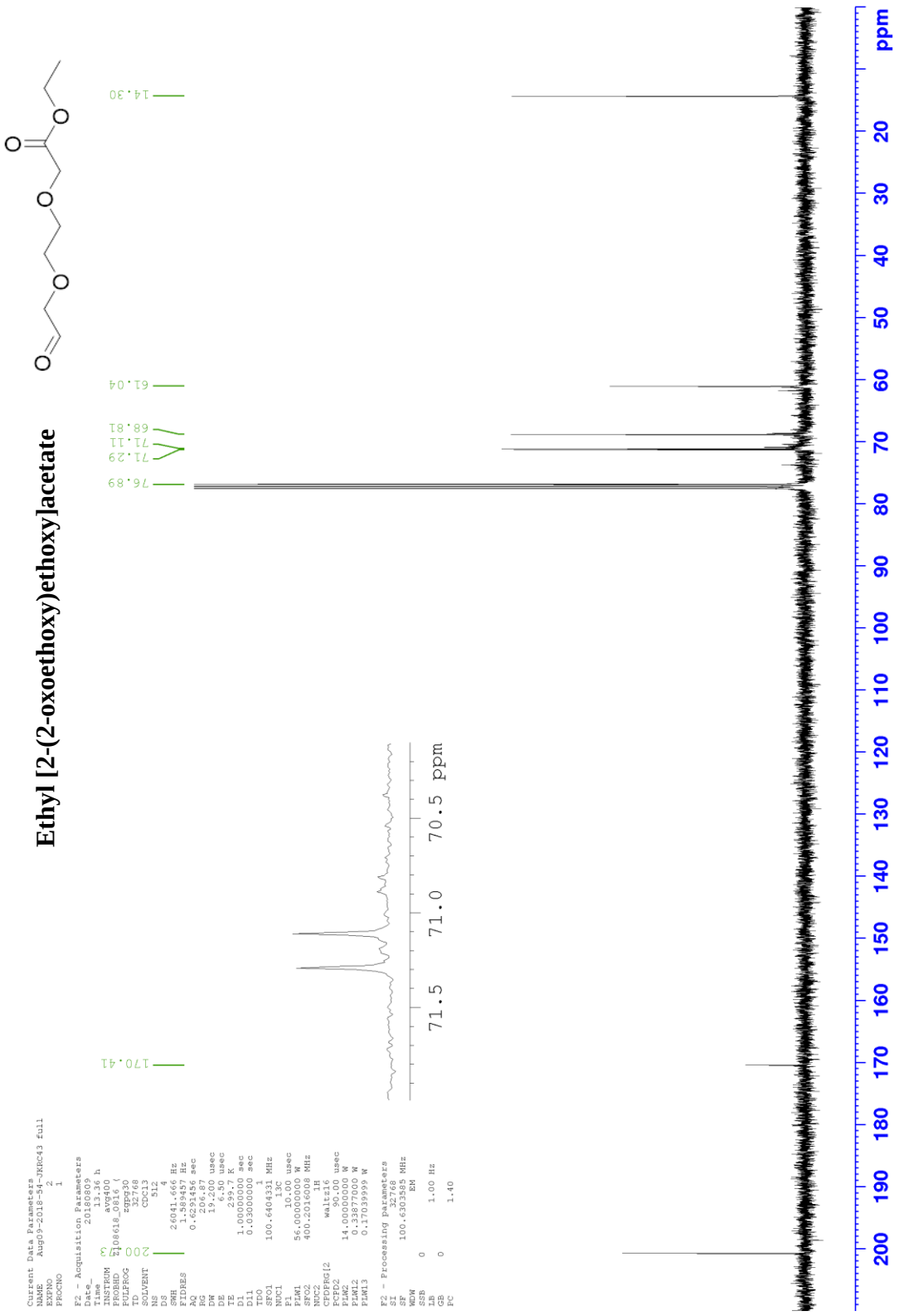
N-[2^{I}-(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-7^{I},20^{I}-dioxo-9^{I},12^{I},15^{I},18^{I}-tetraoxa-2^{I},6^{I}-diazaicosan-20^{I}-yl]-23^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide

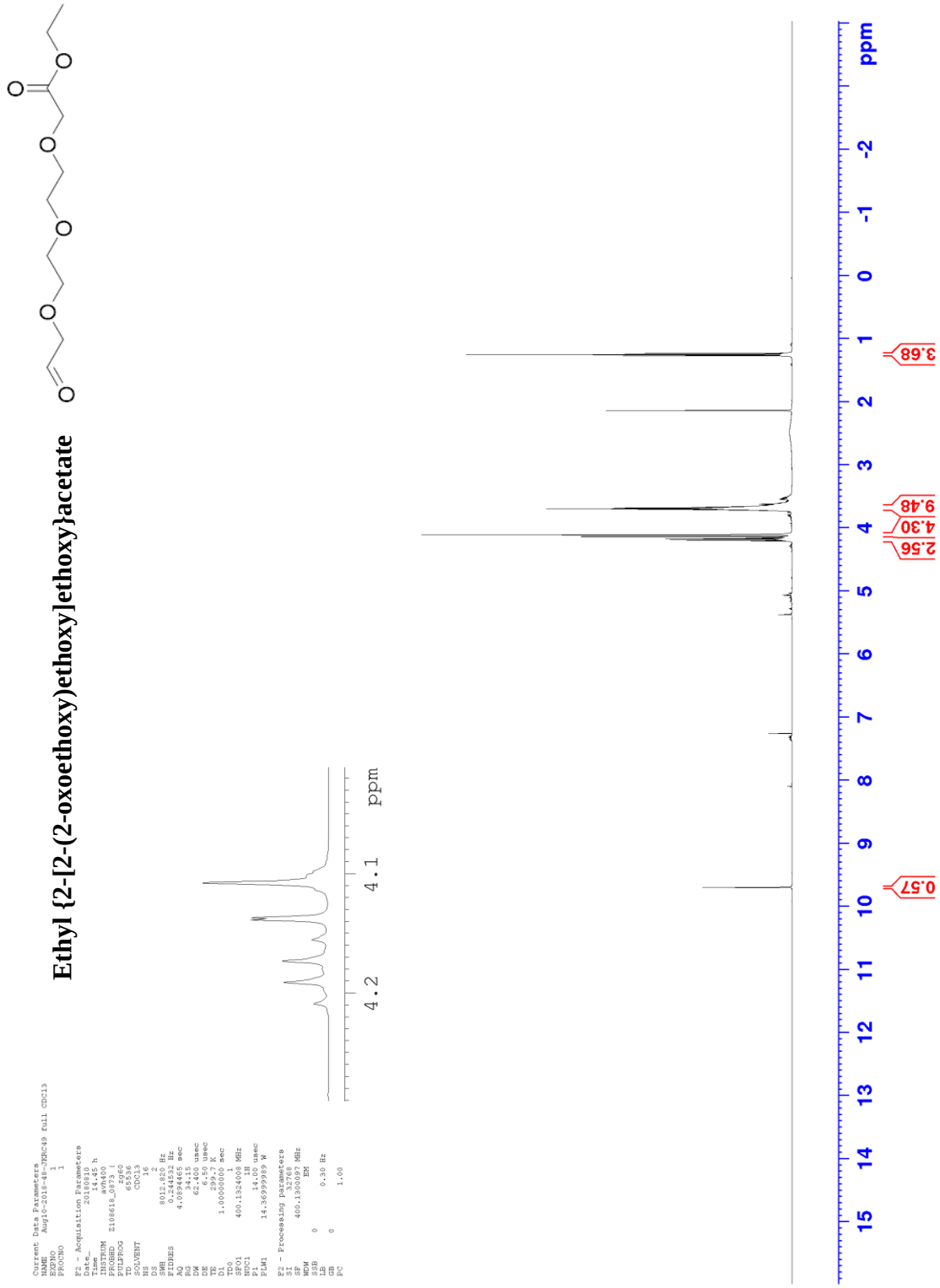


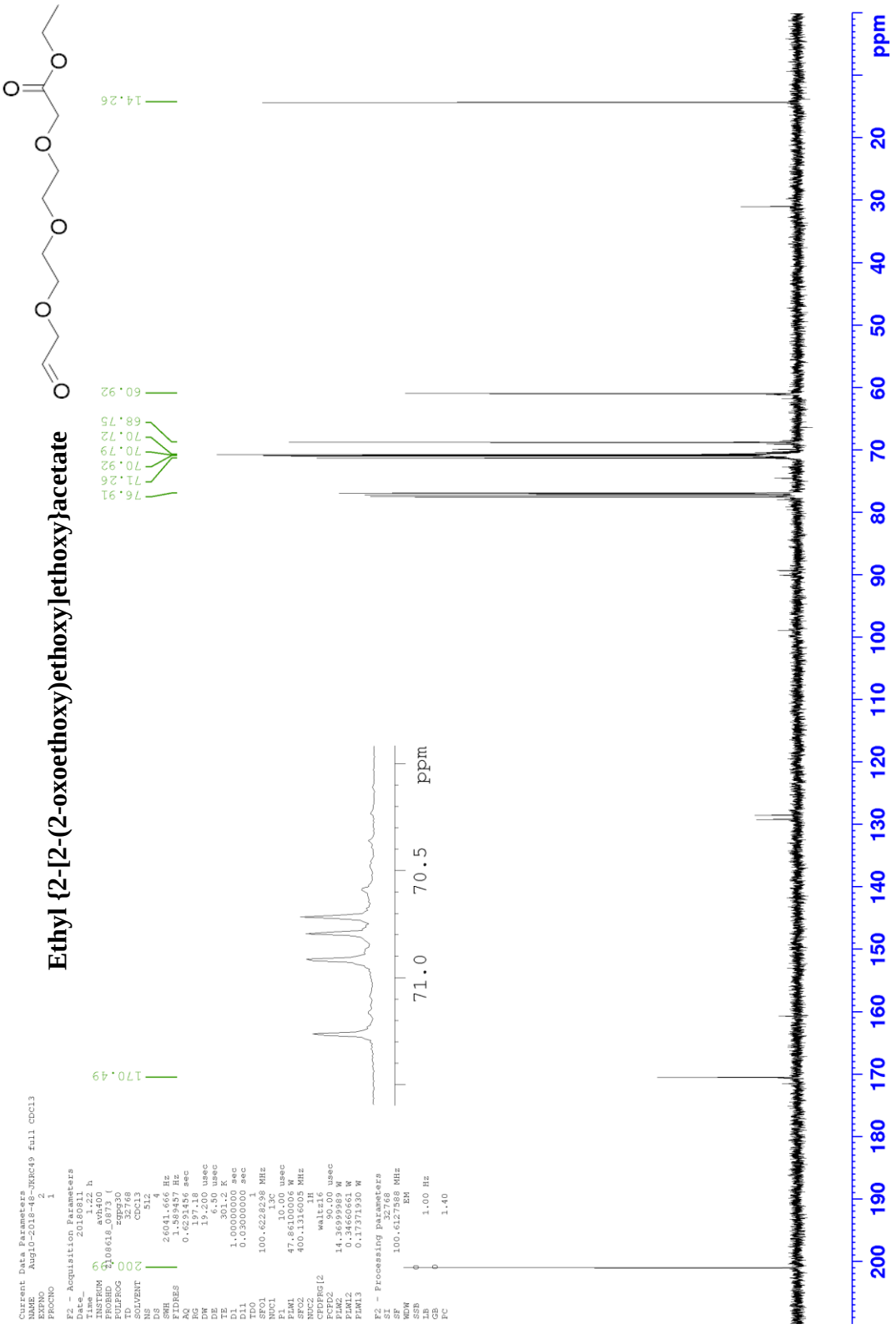
N-[2^{I}-(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-7^{I},20^{I}-dioxo-9^{I},12^{I},15^{I},18^{I}-tetraoxa-2^{I},6^{I}-diazaicosan-20^{I}-yl]-23^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide

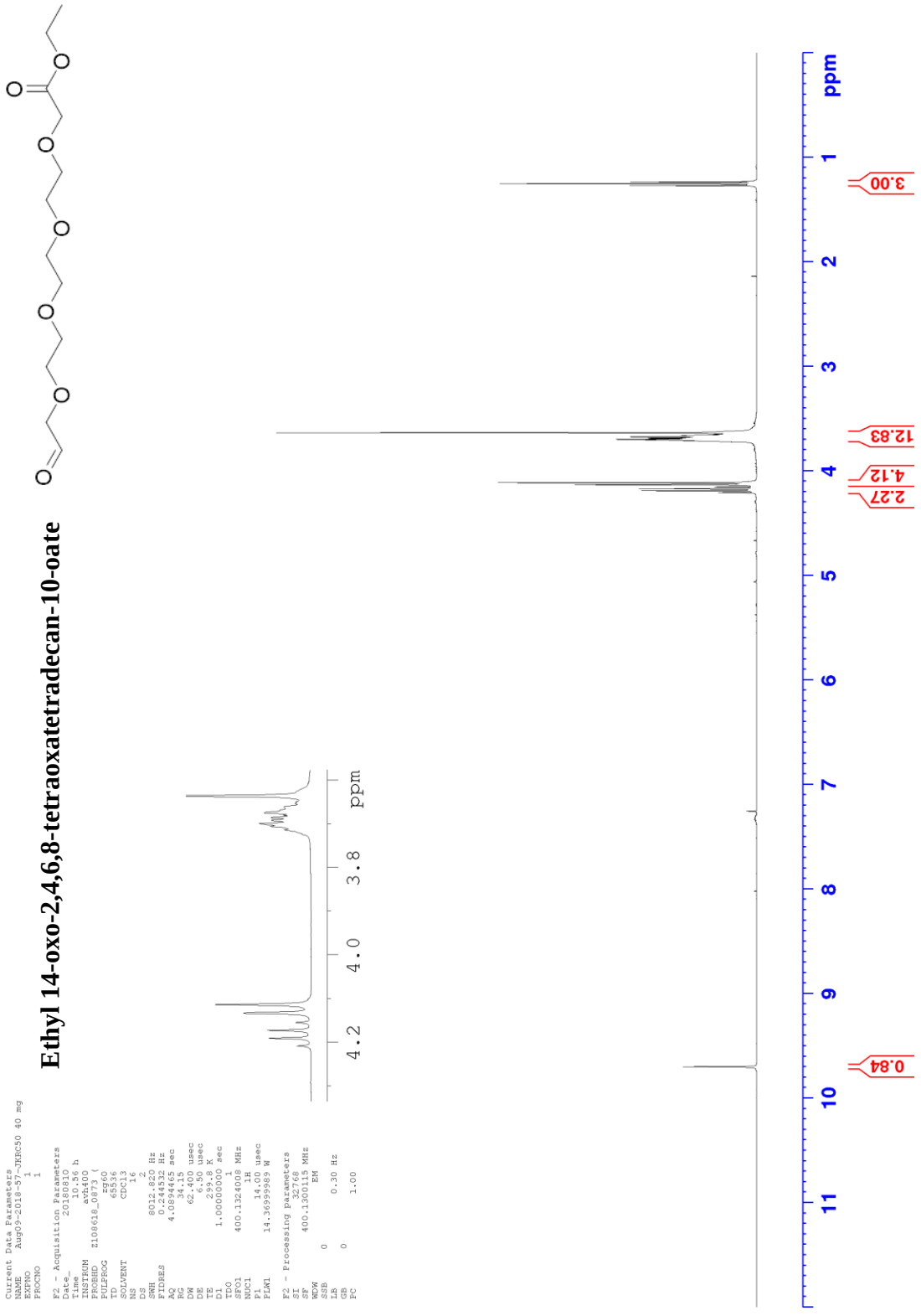


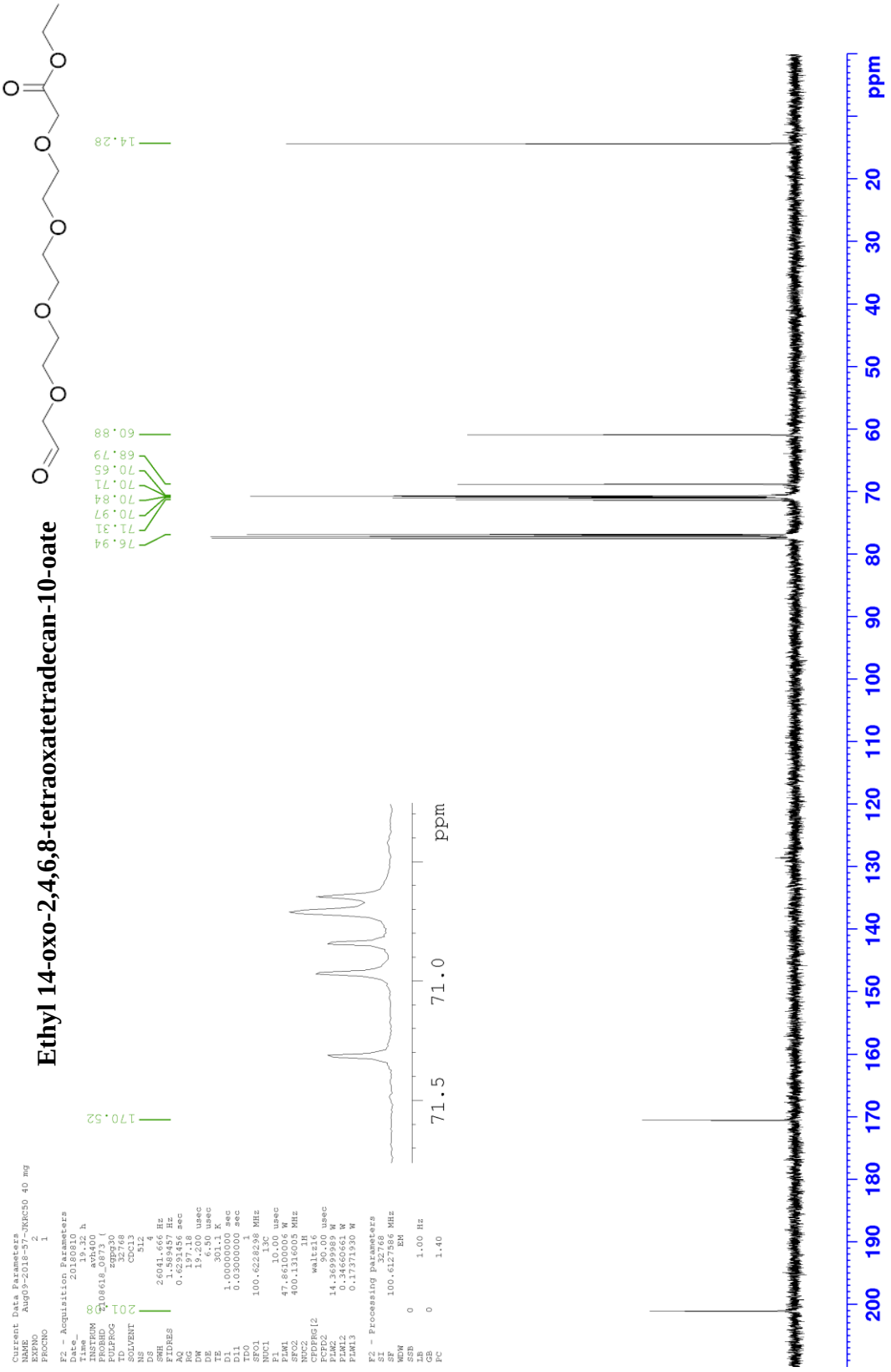

Current Data Parameters
NAME Ju125-2018-39-ZKRC43 f 3-4
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180725
Time 13.35 h
INSTRUM spect
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 13.02
DW 62.400 usec
DE 6.50 usec
TE 300.5 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300101 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Ethyl [2-(2-oxoethoxy)ethoxy]acetate
4.25
4.20
4.15 ppm
0.80
2.26
3.95
4.15
3.54
ppm

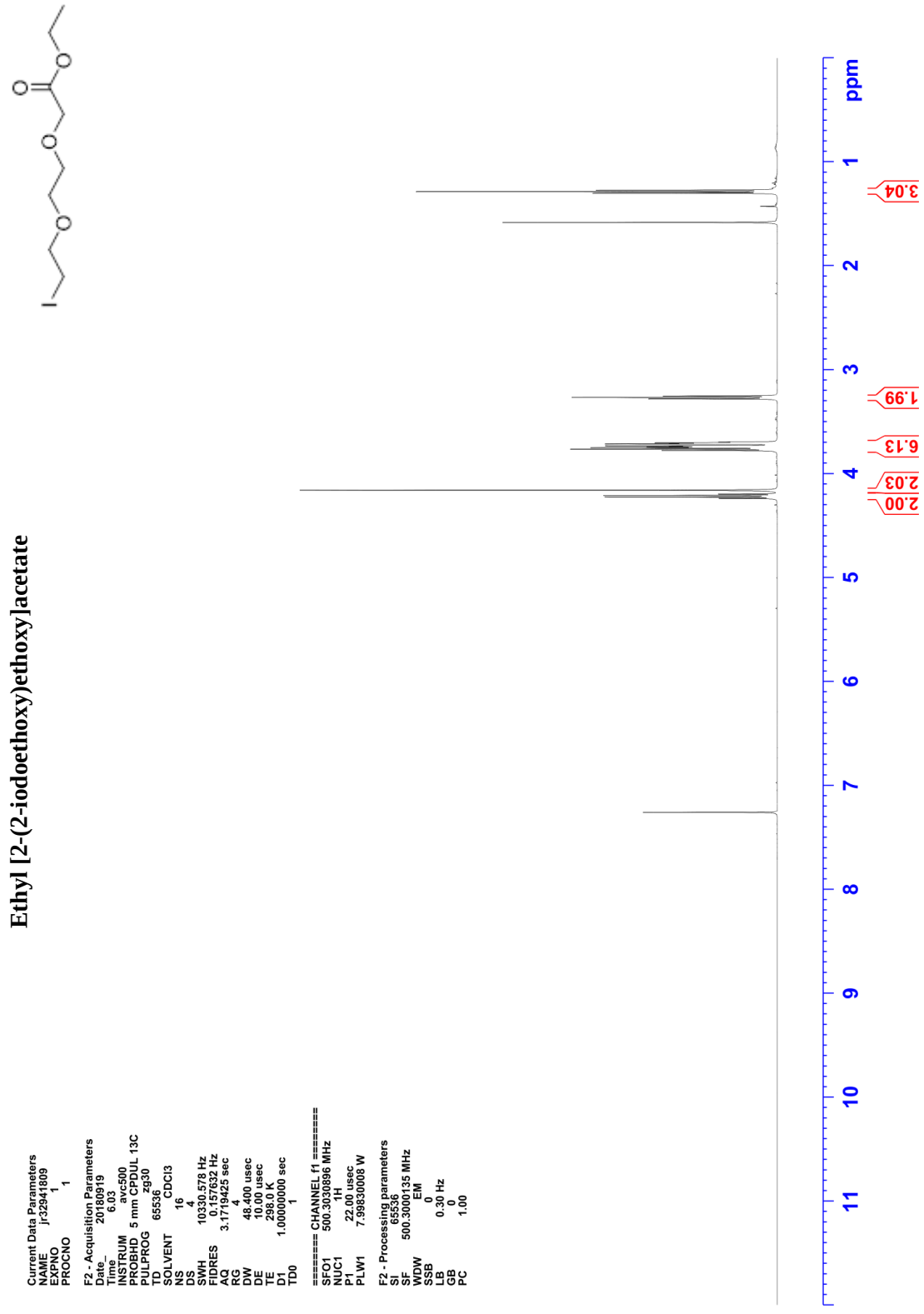

Ethyl [2-(2-oxoethoxy)ethoxy]acetate
Current Data Parameters
NAME Aug09-2018-54-JRC443 full
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180809
Time 13.136 h
INSTRUM spect
PROBHD Z108618_0816 (
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 512
DS 4
SWH 26041.666 Hz
FIDRES 1.589457 Hz
AQ 0.629456 sec
RG 206.87
DW 19.200 usec
DE 6.50 usec
TE 299.7 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 100.6404331 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.00000000 W
SFO2 400.2016008 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 14.00000000 W
PLW12 0.33877000 W
PLW13 0.17039999 W
F2 - Processing parameters
SI 32768
SF 100.6303585 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200.13
170.41
76.89
71.29
71.11
68.81
61.04
14.30
71.5
71.0
70.5 ppm
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

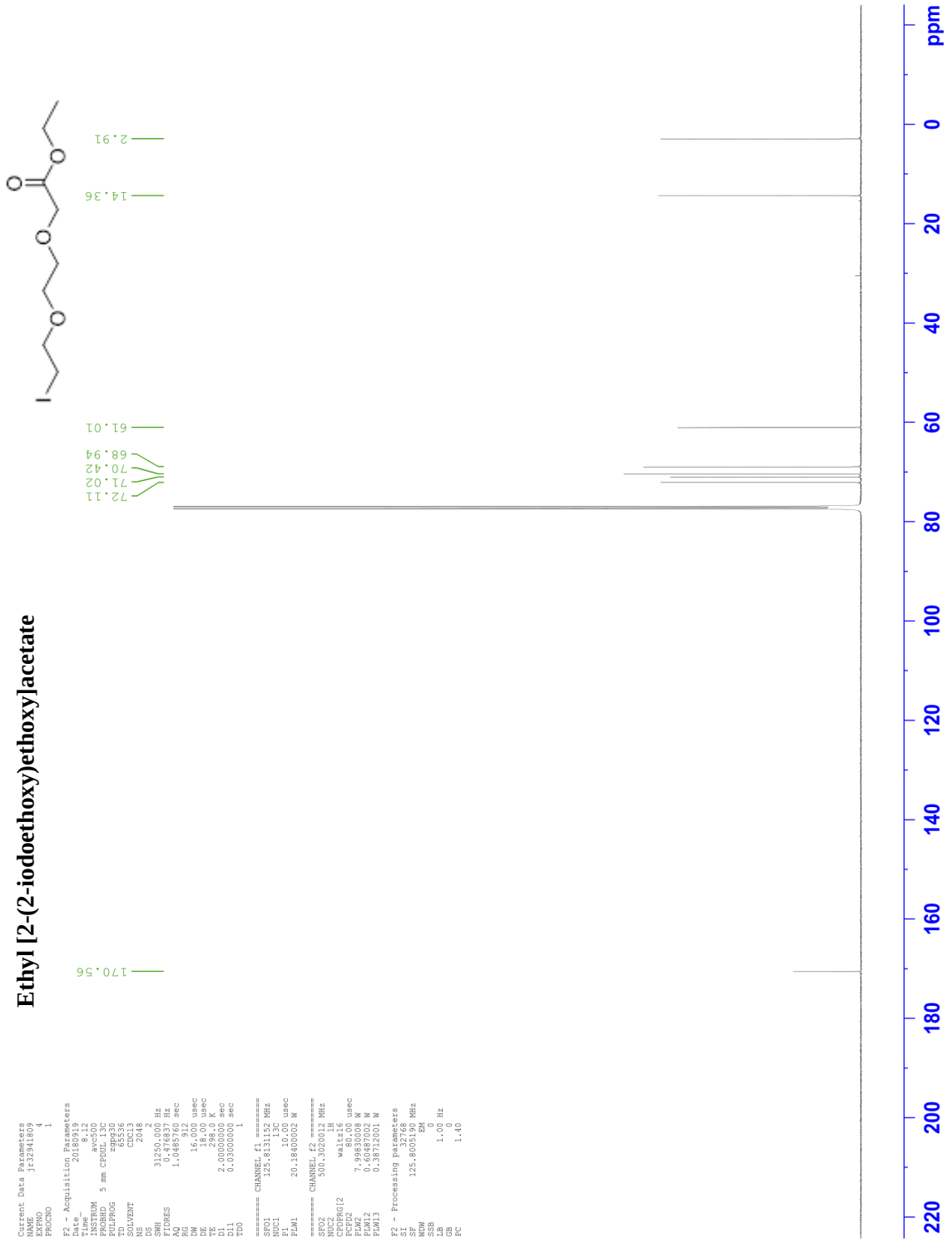

Ethyl {2-[2-(2-oxoethoxy)ethoxy]ethoxy}acetate
Current Data Parameters
NAME Augia-2018-4TheZMBC49 full CDCl3
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180810
Time 14.45 h
INSTRUM spect
PROBHD 2106618_0973 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8013.870 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 34.15
DW 62.400 usec
DE 6.50 usec
TE 299.7 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300097 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
4.2
4.1
ppm
0.57
3.68
2.56
4.30
9.48
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm


Ethyl {2-[2-(2-oxoethoxy)ethoxy]ethoxy}acetate
Current Data Parameters
NAME Aug10-2018-48-JRK049 full CDCl3
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180811
Time 23.22 h
INSTRUM av3400
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 512
DS 4
SWH 26041.666 Hz
FIDRES 1.589457 Hz
AQ 0.6291456 sec
RG 197.18
DW 19.200 usec
DE 6.50 usec
TE 301.2 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 100.6228298 MHz
NUC1 13C
P1 10.00 usec
PLW1 47.86100006 W
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 14.36999989 W
PLW12 0.34660661 W
PLW13 0.17371930 W
F2 - Processing parameters
SI 32768
SF 100.6127588 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170.49
76.91
71.26
70.92
70.79
70.72
68.75
60.92
14.26
71.0
70.5
ppm
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

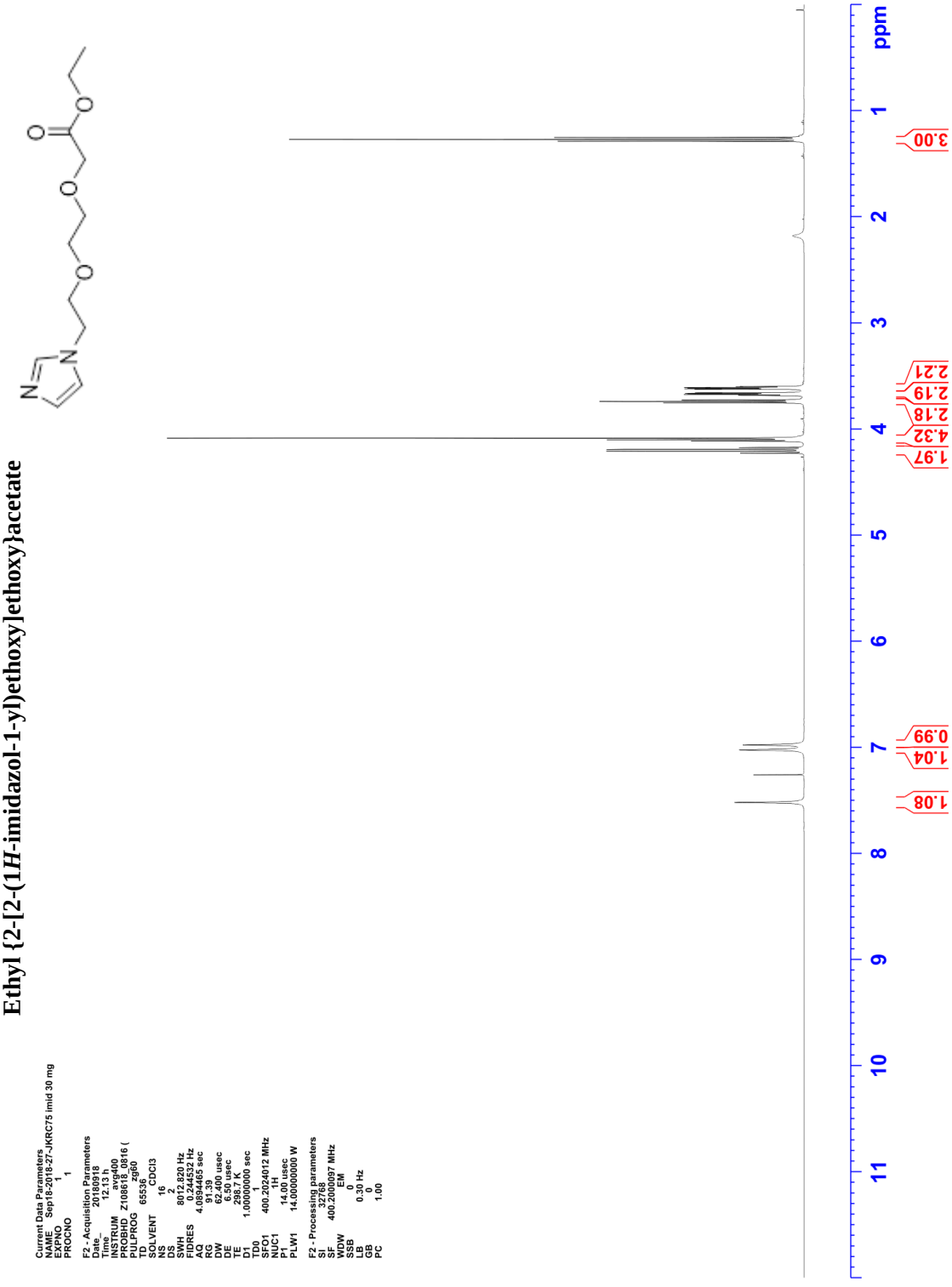

Ethyl 14-oxo-2,4,6,8-tetraoxatetradecan-10-oate
Current Data Parameters
NAME Aug09-2018-57-JKRC50 40 mg
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180810
Time 10.56 h
INSTRUM spect400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 34.15
DW 62.400 usec
DE 6.50 usec
TE 299.8 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300115 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
4.2
4.0
3.8
ppm
0.84
3.00
12.83
4.12
2.27
ppm


Ethyl 14-oxo-2,4,6,8-tetraoxatetradecan-10-oate
170.52
76.94
71.31
70.97
70.84
70.71
70.65
68.79
60.88
14.28
201.18
71.5
71.0
ppm
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

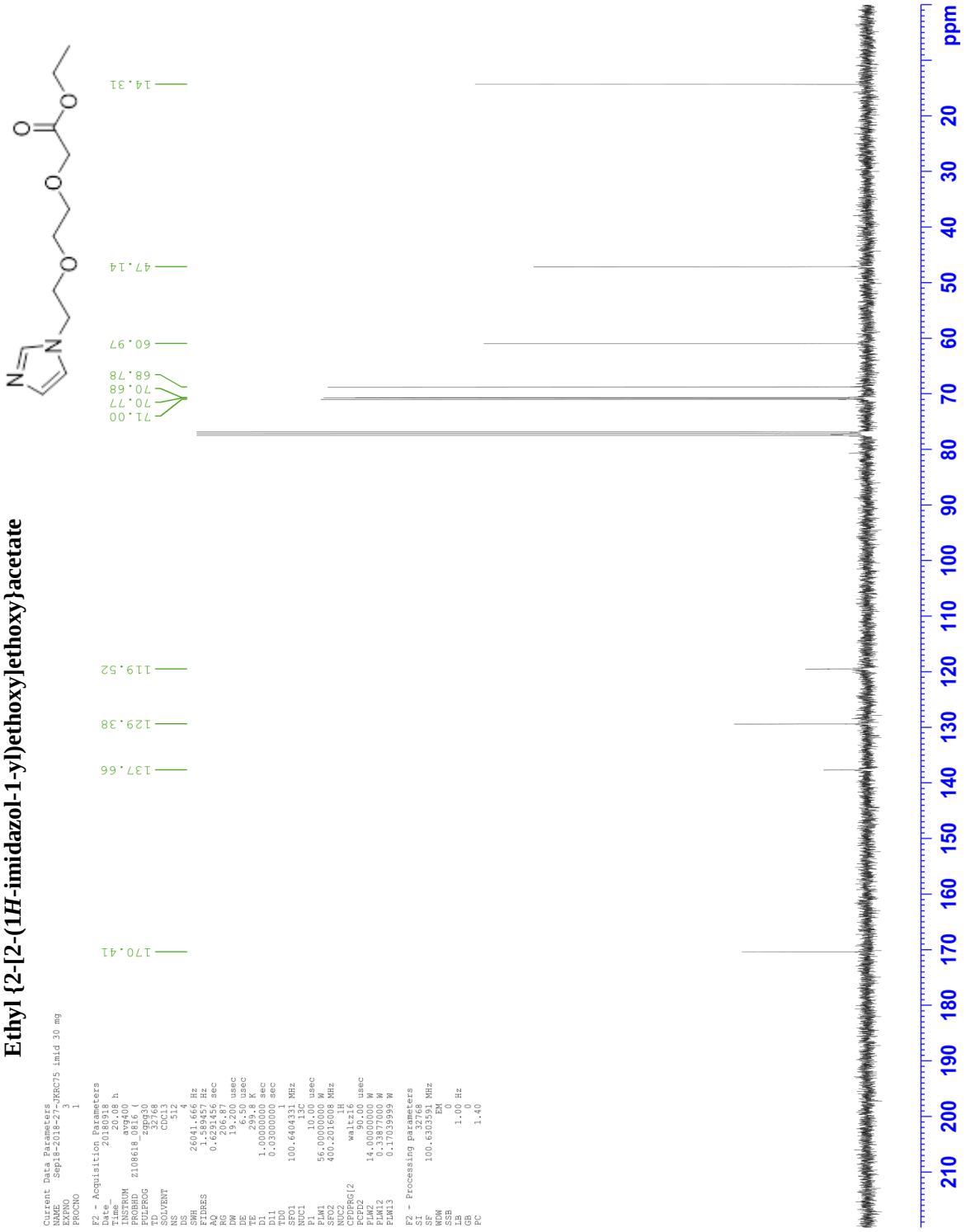

Ethyl [2-(2-iodoethoxy)ethoxy]acetate
Current Data Parameters
NAME jr32941609
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180919
Time_ 6.03
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000135 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
3.04
1.99
6.13
2.03
2.00
ppm
1
2
3
4
5
6
7
8
9
10
11


Ethyl [2-(2-iodoethoxy)ethoxy]acetate
Current Data Parameters
NAME j132941809
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180919
Time_ 8.12
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 2048
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 203
DW 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 2.0000000 sec
D11 0.0300000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 7.99800006 W
PLW12 0.60487002 W
PLW13 0.38712001 W
F2 - Processing parameters
SI 32768
SF 125.8005190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
170.56
72.11
71.02
70.42
68.94
61.01
14.36
2.91
ppm
220
200
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140
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100
80
60
40
20
0

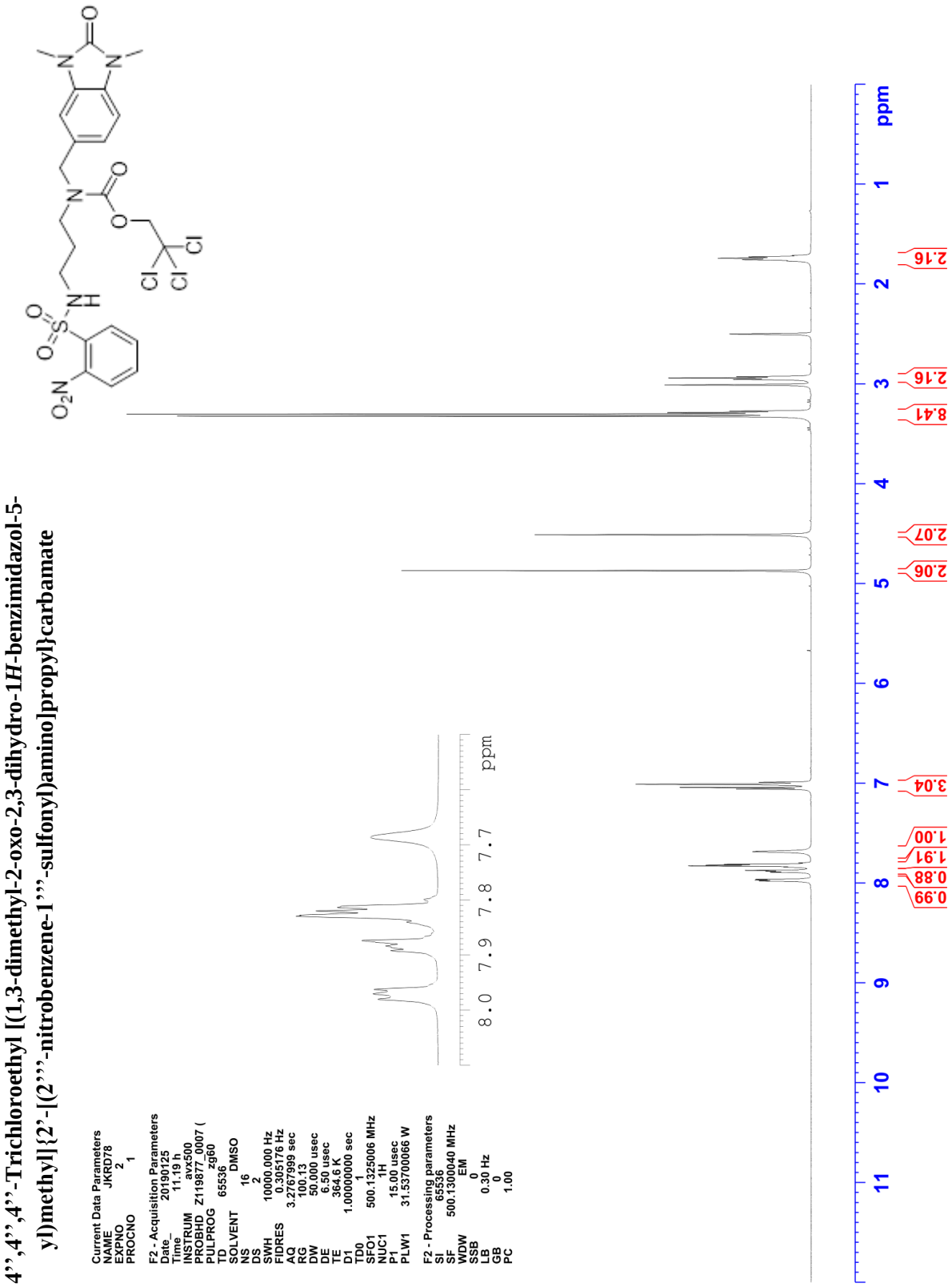
Ethyl {2-[2-(1*H*-imidazol-1-yl)ethoxy]ethoxy}acetate



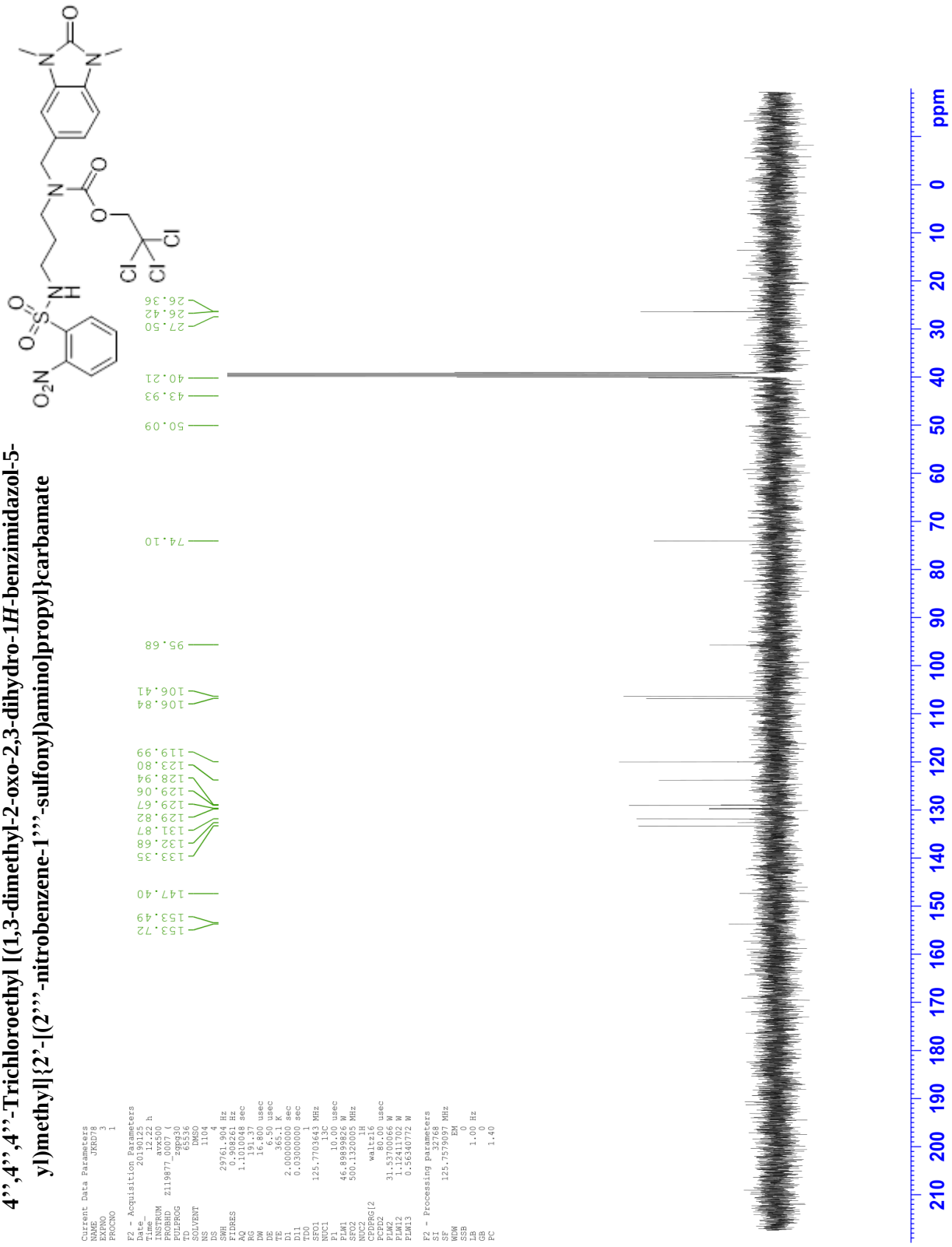
Ethyl {2-[2-(1*H*-imidazol-1-yl)ethoxy]ethoxy}acetate



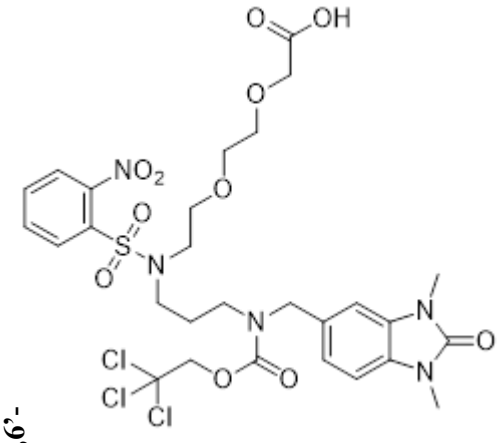
4'', 4'', 4''-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{2'-[(2'''-nitrobenzene-1'''-sulfonyl)amino]propyl}carbamate



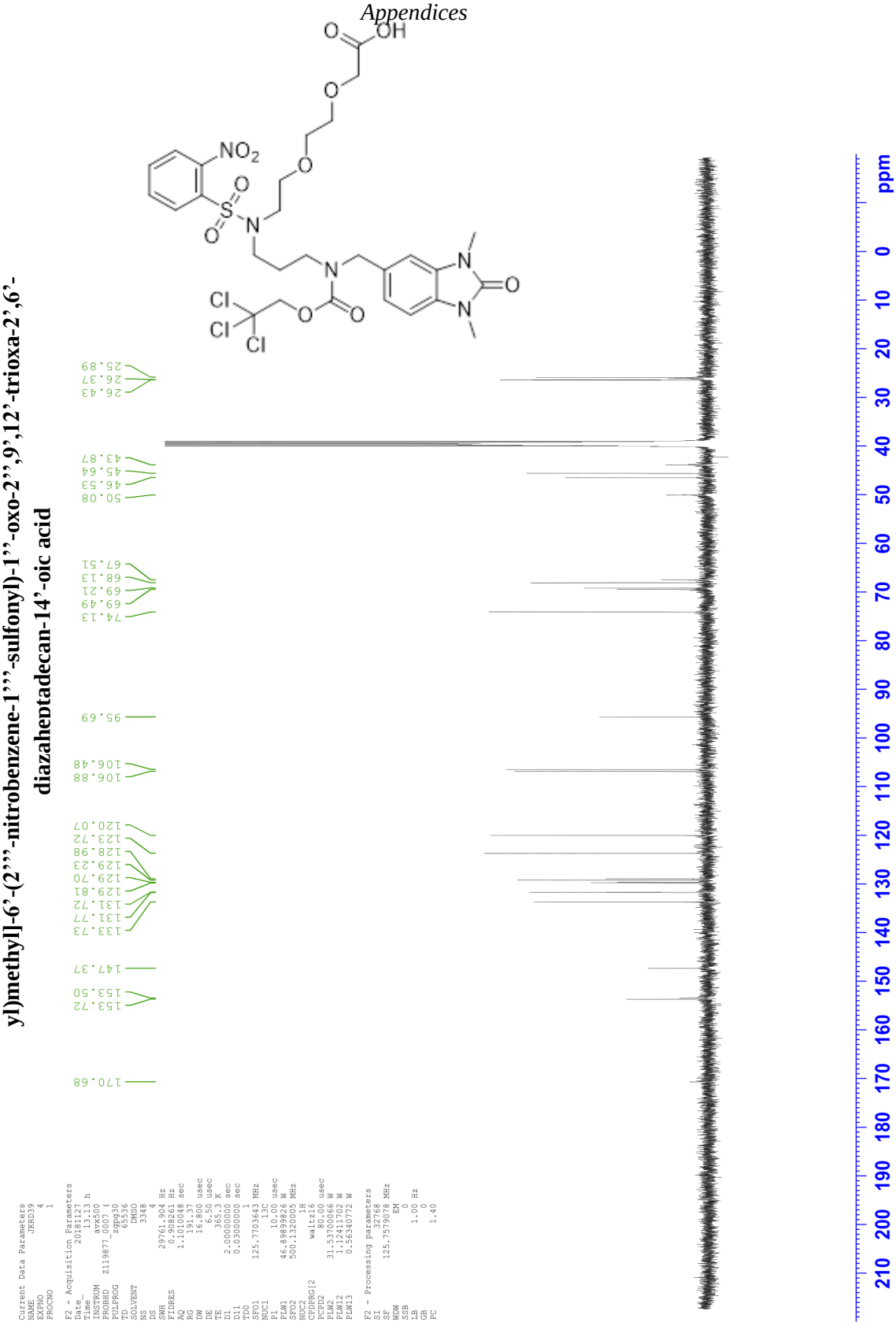
4'',4'',4''-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{2'-[(2''-nitrobenzene-1''-sulfonyl)amino]propyl}carbamate



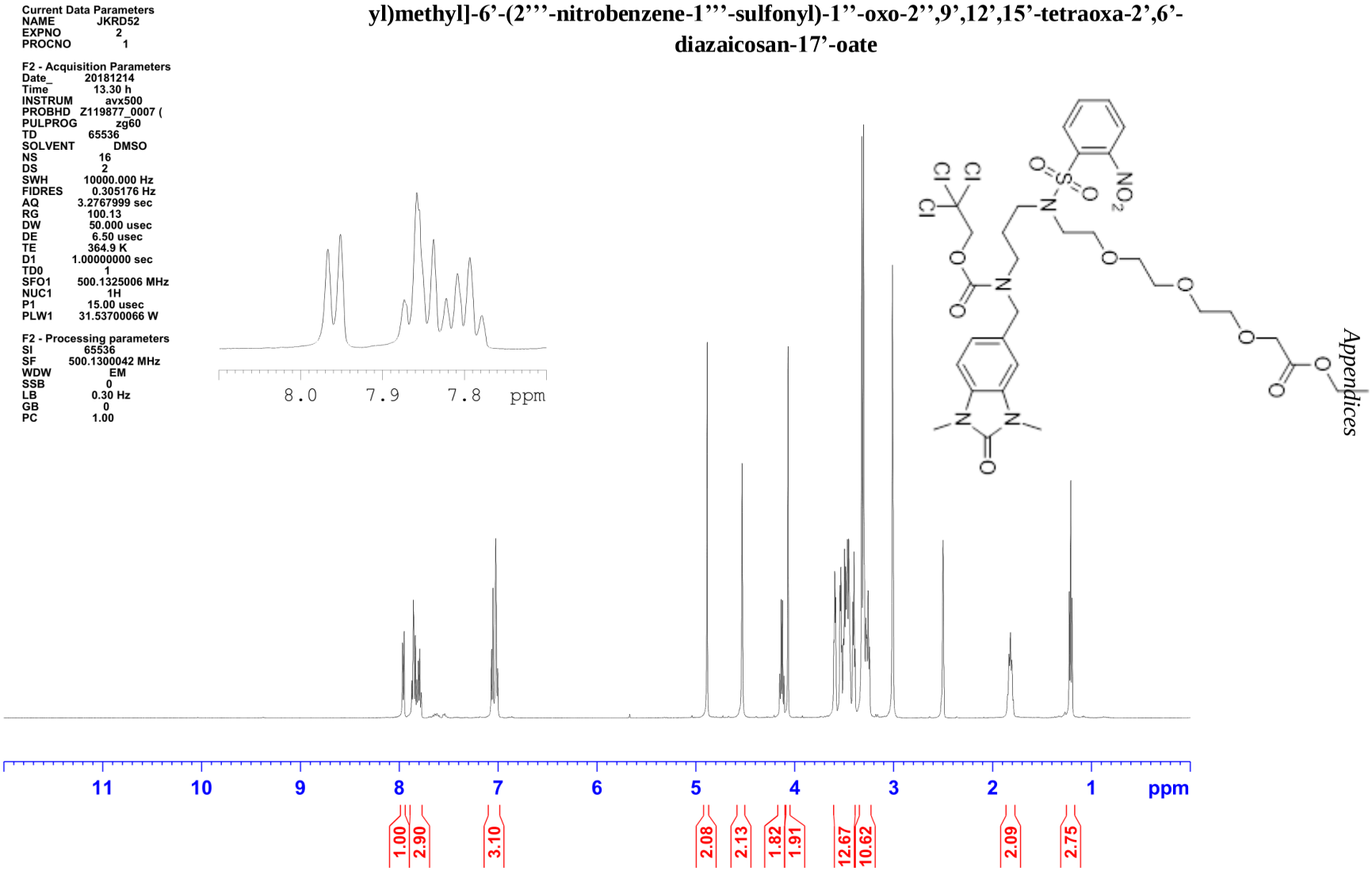
4'',4'',4''-Trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6'-(2'''-nitrobenzene-1'''-sulfonyl)-1''-oxo-2'',9',12'-trioxa-2',6'-diazaheptadecan-14'-oic acid

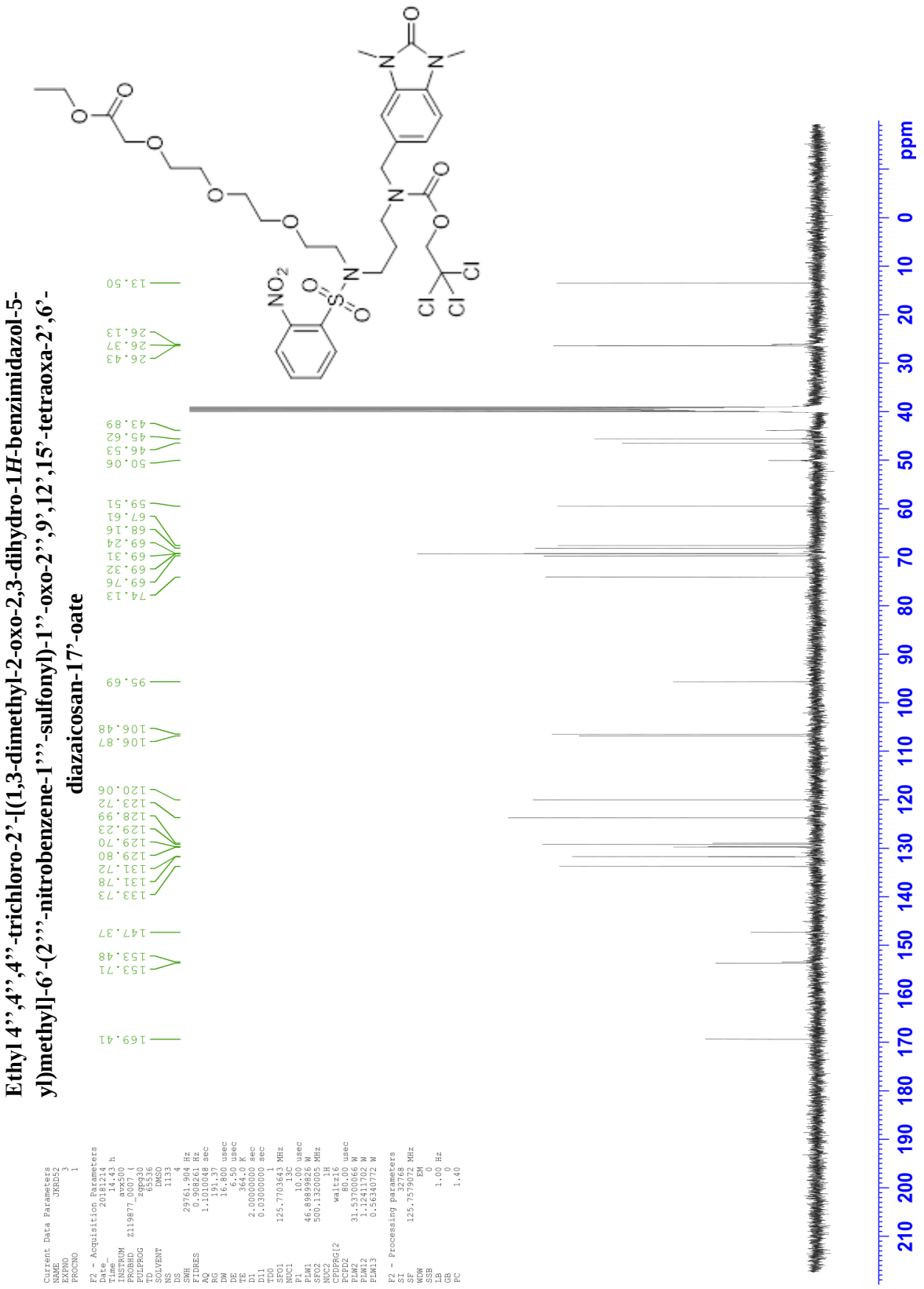


4’’,4’’,4’’-Trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6’-(2’’’-nitrobenzene-1’’’-sulfonyl)-1’’-oxo-2’’,9’,12’-trioxa-2’,6’-diazaheptadecan-14’-oic acid

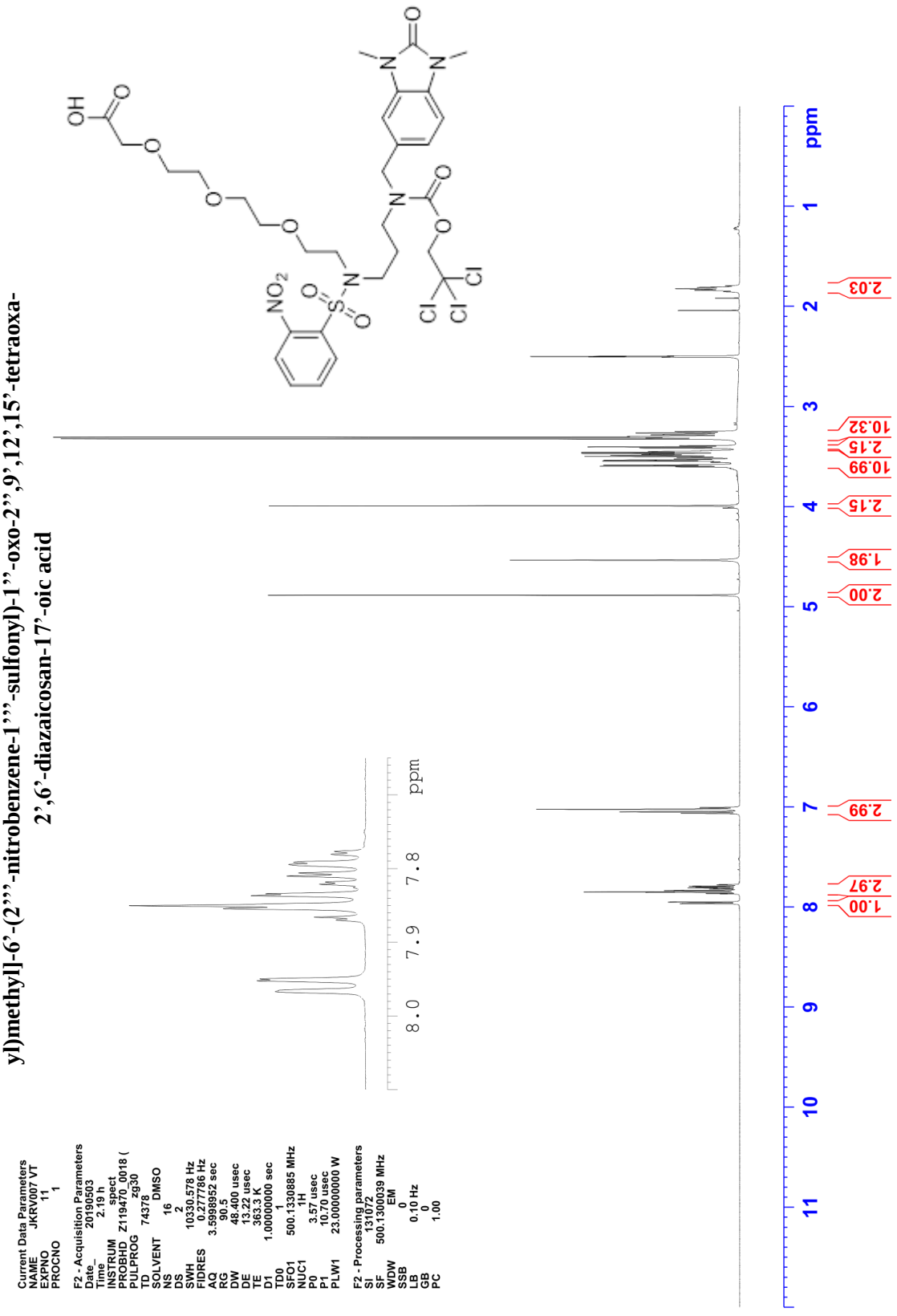


Ethyl 4’’,4’’,4’’-trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6’-(2’’’-nitrobenzene-1’’’-sulfonyl)-1’’-oxo-2’’,9’,12’,15’-tetraoxa-2’,6’-diazaicosan-17’-oate

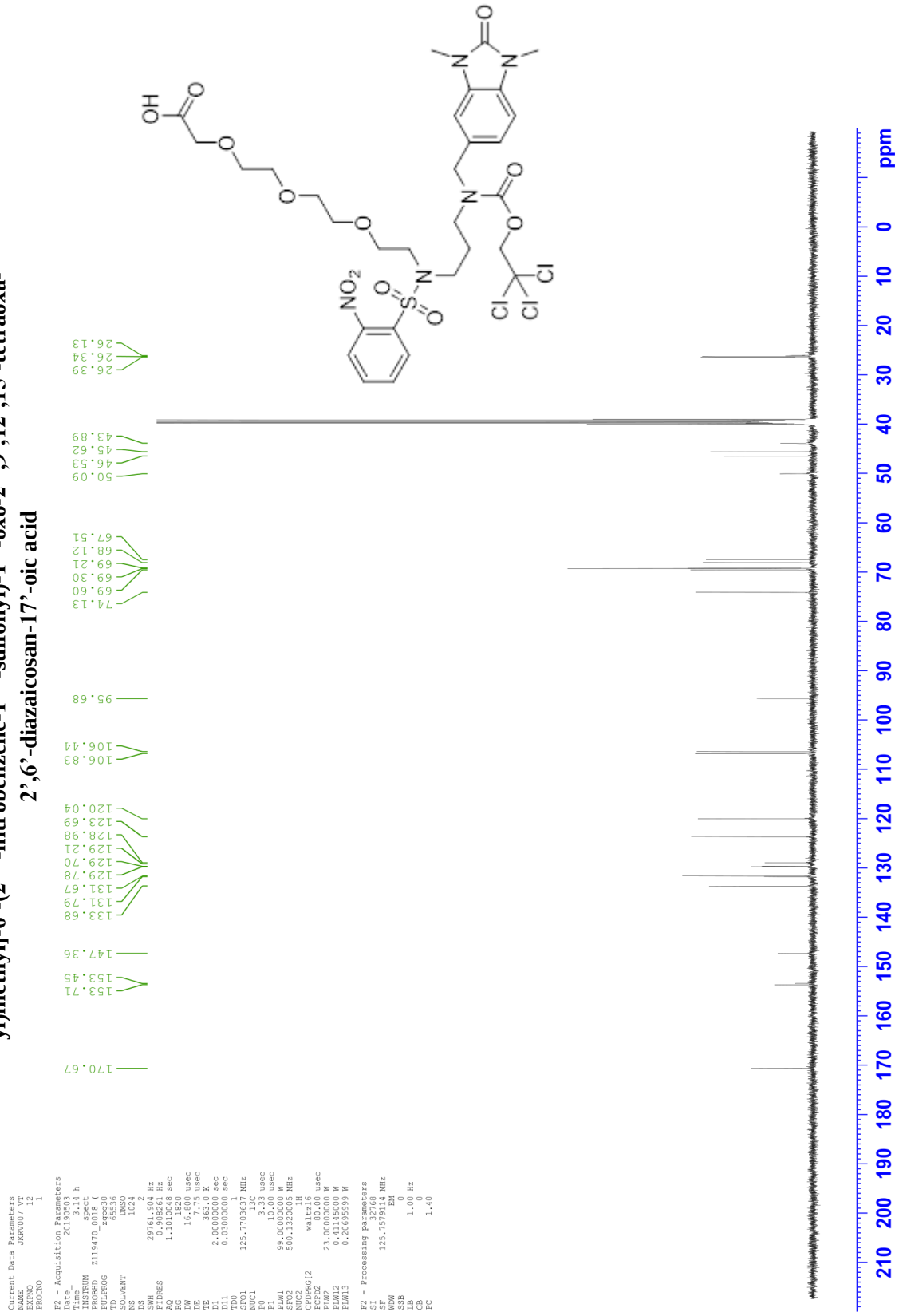


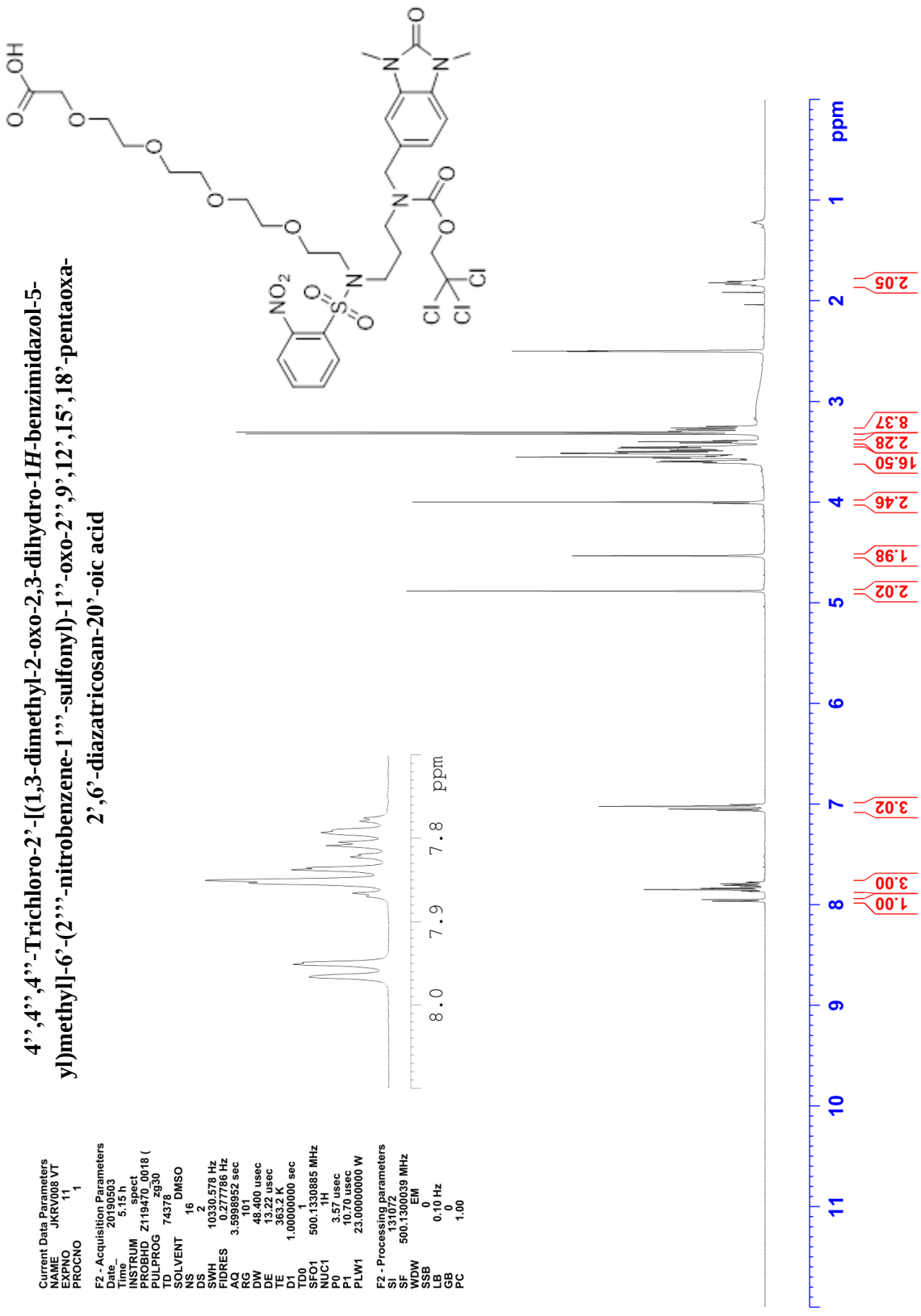

Ethyl 4'',4'',4''-trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl]-6'-(2'''-nitrobenzene-1'''-sulfonyl)-1''-oxo-2'',9',12',15'-tetraoxa-2',6'-diazaicosan-17'-oate
ppm

4’’,4’’,4’’-Trichloro-2’-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6’-(2’’’-nitrobenzene-1’’’-sulfonyl)-1’’-oxo-2’’,9’,12’,15’-tetraoxa-2’,6’-diazaicosan-17’-oic acid

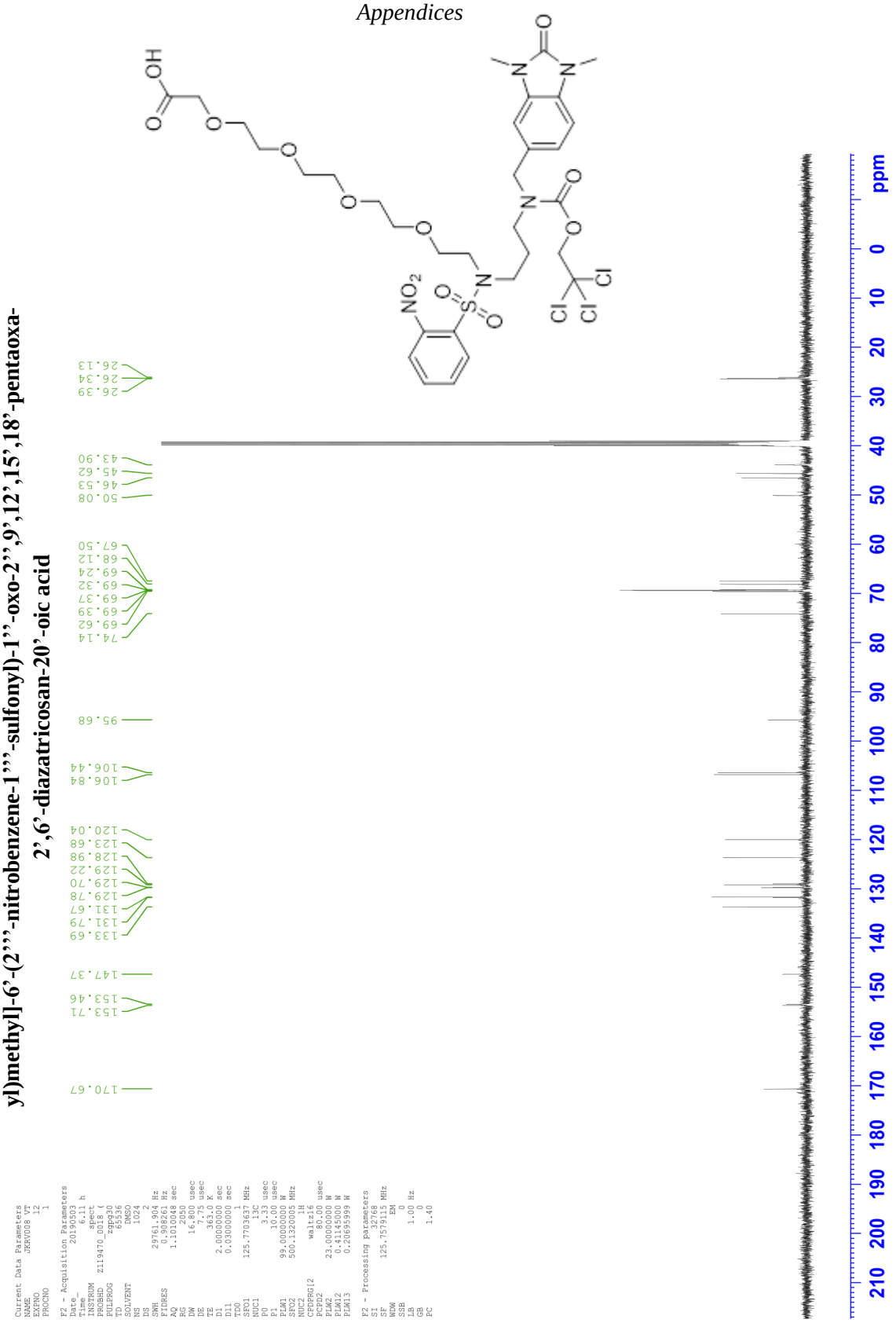


4'',4'',4''-Trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6'-(2'''-nitrobenzene-1'''-sulfonyl)-1''-oxo-2'',9',12',15'-tetraoxa-2',6'-diazaicosan-17'-oic acid

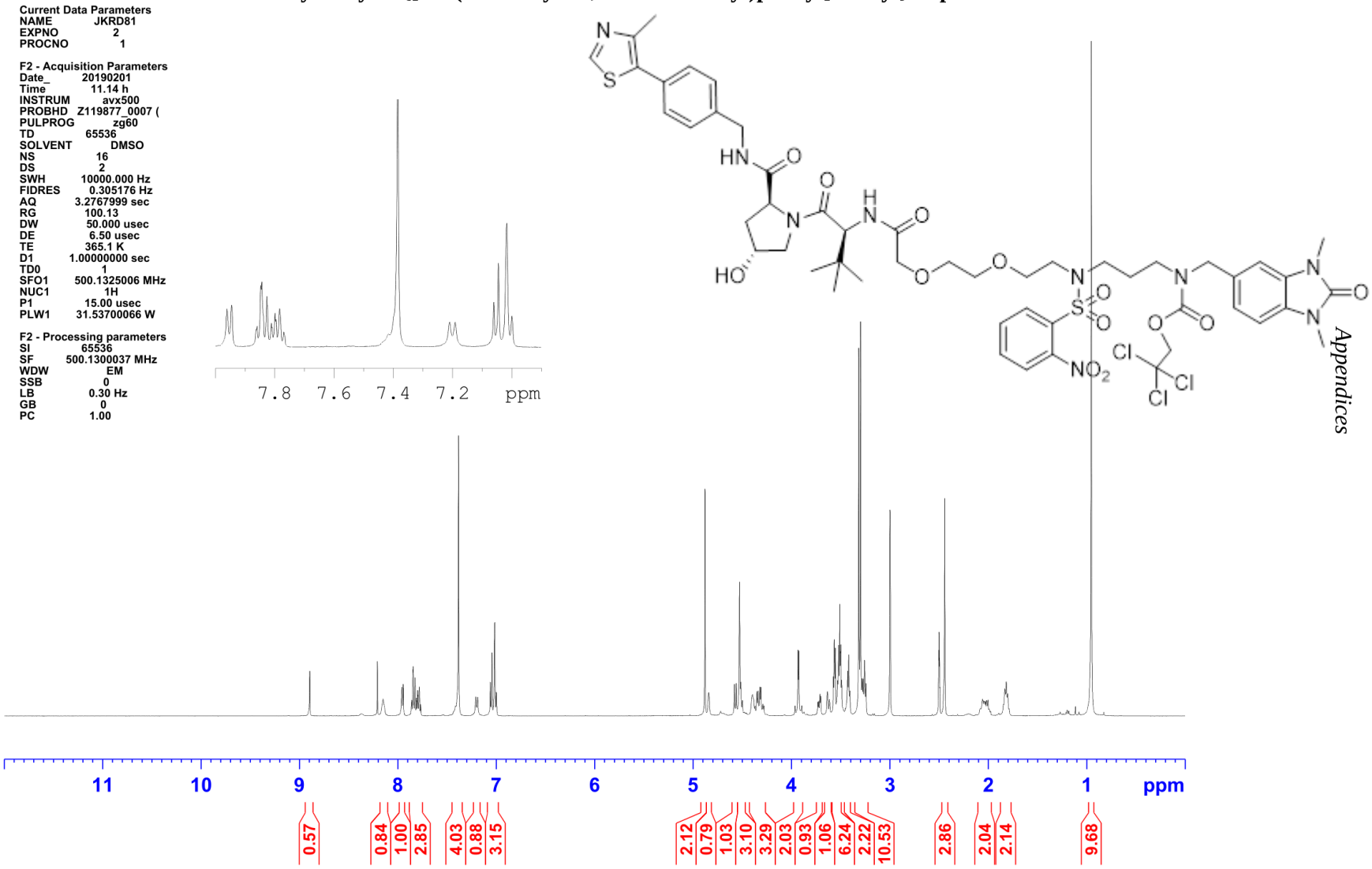



4'',4'',4''-Trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl]-6'-(2'''-nitrobenzene-1'''-sulfonyl)-1''-oxo-2'',9',12',15',18'-pentaoxa-2',6'-diazatricosan-20'-oic acid
Current Data Parameters
NAME JKRV008 VT
EXPNO 11
PROCNO 1
F2 - Acquisition Parameters
Date_ 20190503
Time 5.15 h
INSTRUM spect
PROBHD Z119470_0018 (
PULPROG zg30
TD 74378
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.277786 Hz
AQ 3.5969952 sec
RG 101
DW 48.400 usec
DE 13.22 usec
TE 363.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330885 MHz
NUC1 1H
P0 3.57 usec
P1 10.70 usec
PLW1 23.00000000 W
F2 - Processing parameters
SI 131072
SF 500.1300039 MHz
WDW EM
SSB 0
LB 0.10 Hz
GB 0
PC 1.00
8.0
7.9
7.8
ppm
2.05
8.37
2.28
16.50
2.46
1.98
2.02
3.02
1.00
3.00
ppm

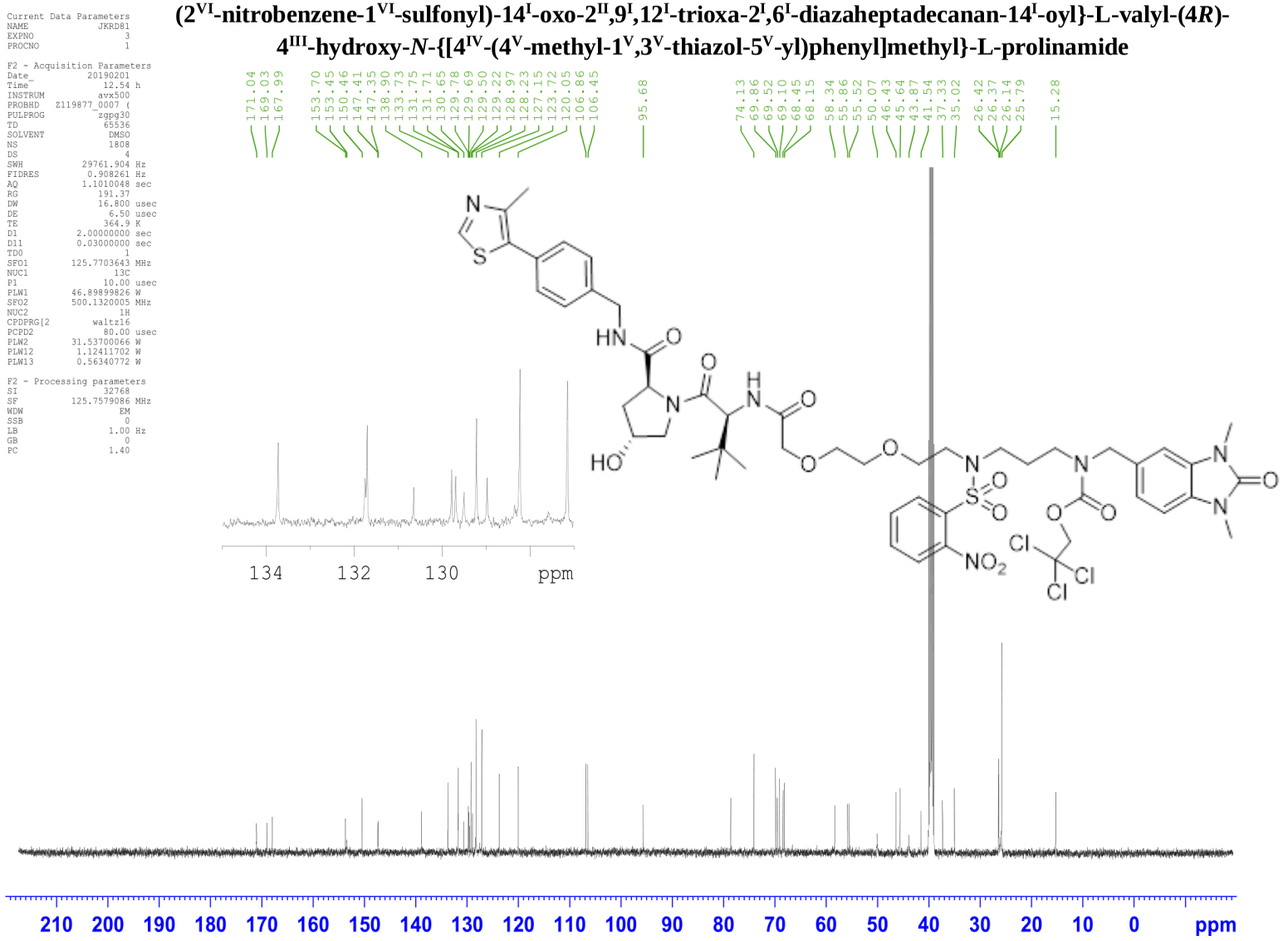
4'',4'',4''-Trichloro-2'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6'-(2'''-nitrobenzene-1'''-sulfonyl)-1''-oxo-2'',9',12',15',18'-pentaoxa-2',6'-diazatricosan-20'-oic acid



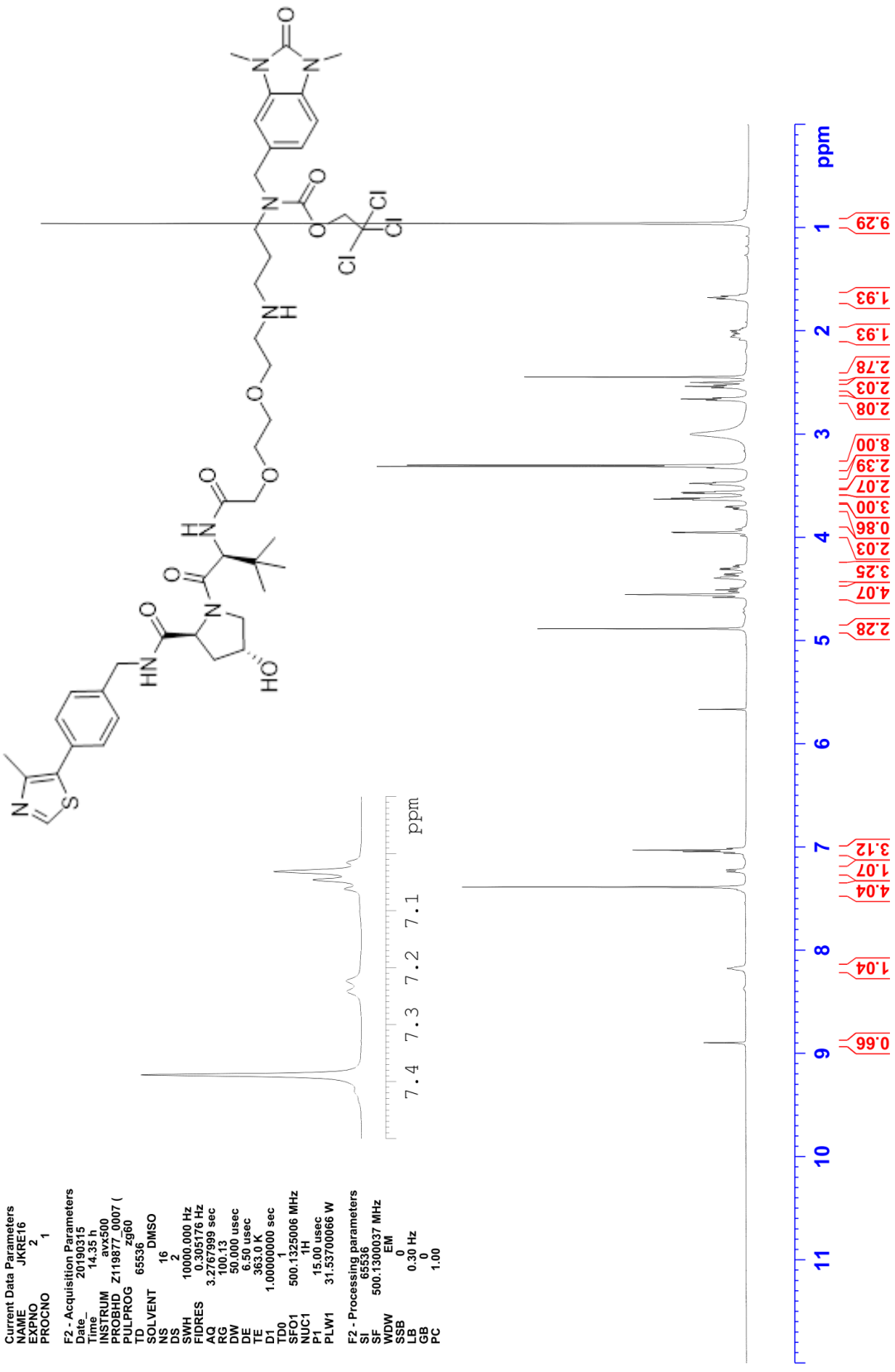
17^{I}-Methyl-*N*-{4^{II},4^{II},4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6^{I}-(2^{VI}-nitrobenzene-1^{VI}-sulfonyl)-14^{I}-oxo-2^{II},9^{I},12^{I}-trioxa-2^{I},6^{I}-diazaheptadecanan-14^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide



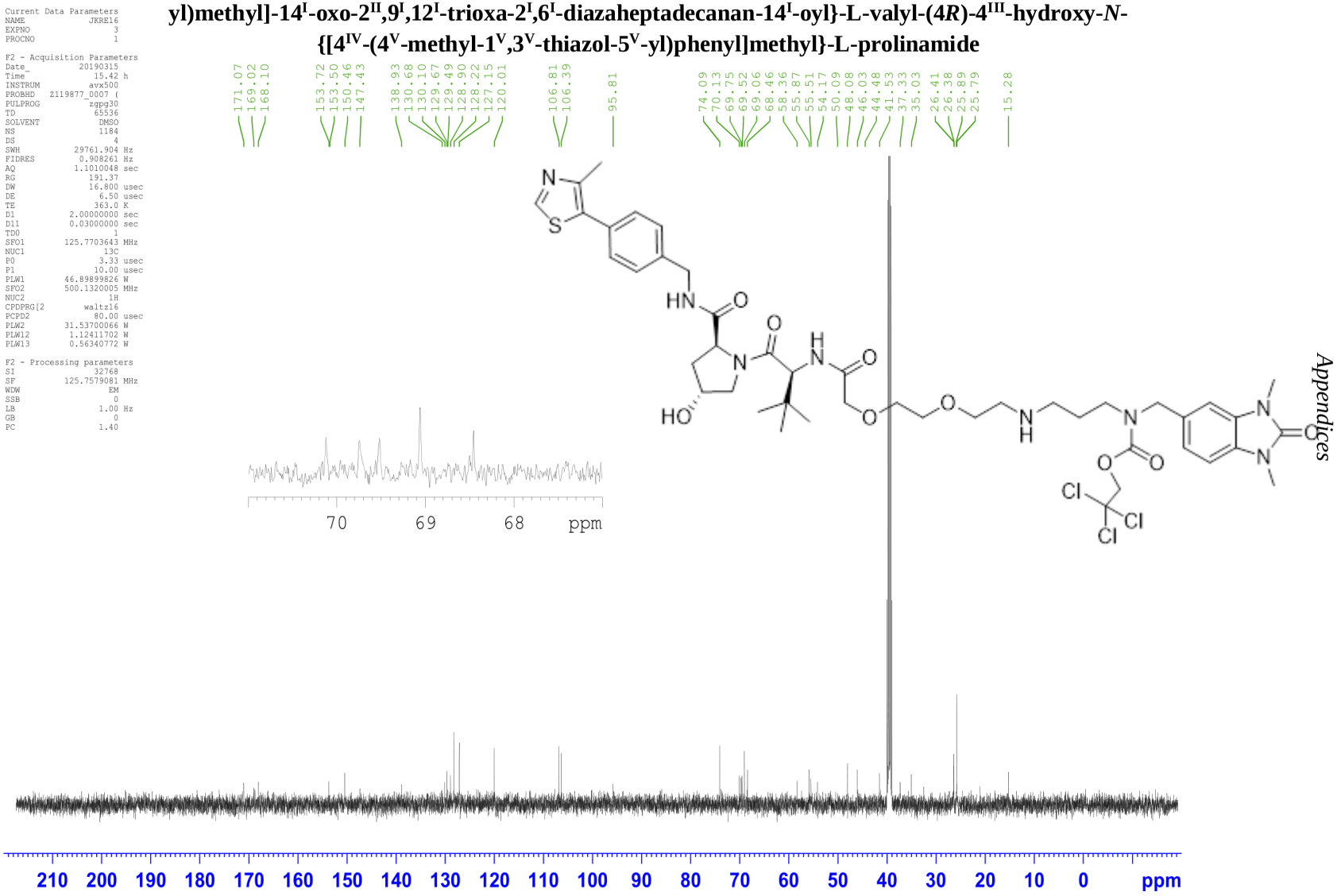
17^{I}-Methyl-*N*-{4^{II},4^{II},4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6^{I}-(2^{VI}-nitrobenzene-1^{VI}-sulfonyl)-14^{I}-oxo-2^{II},9^{I},12^{I}-trioxa-2^{I},6^{I}-diazaheptadecanan-14^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide

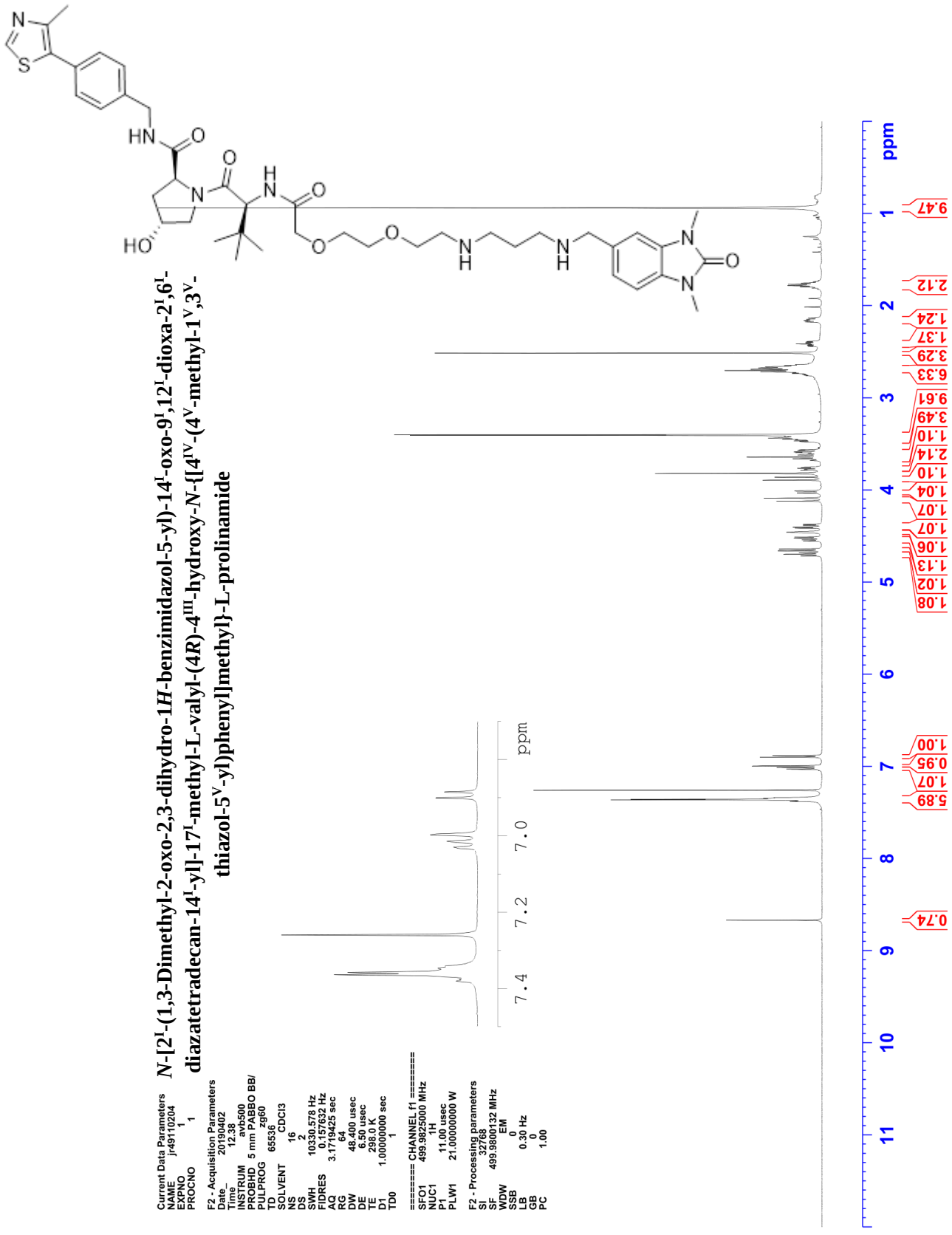


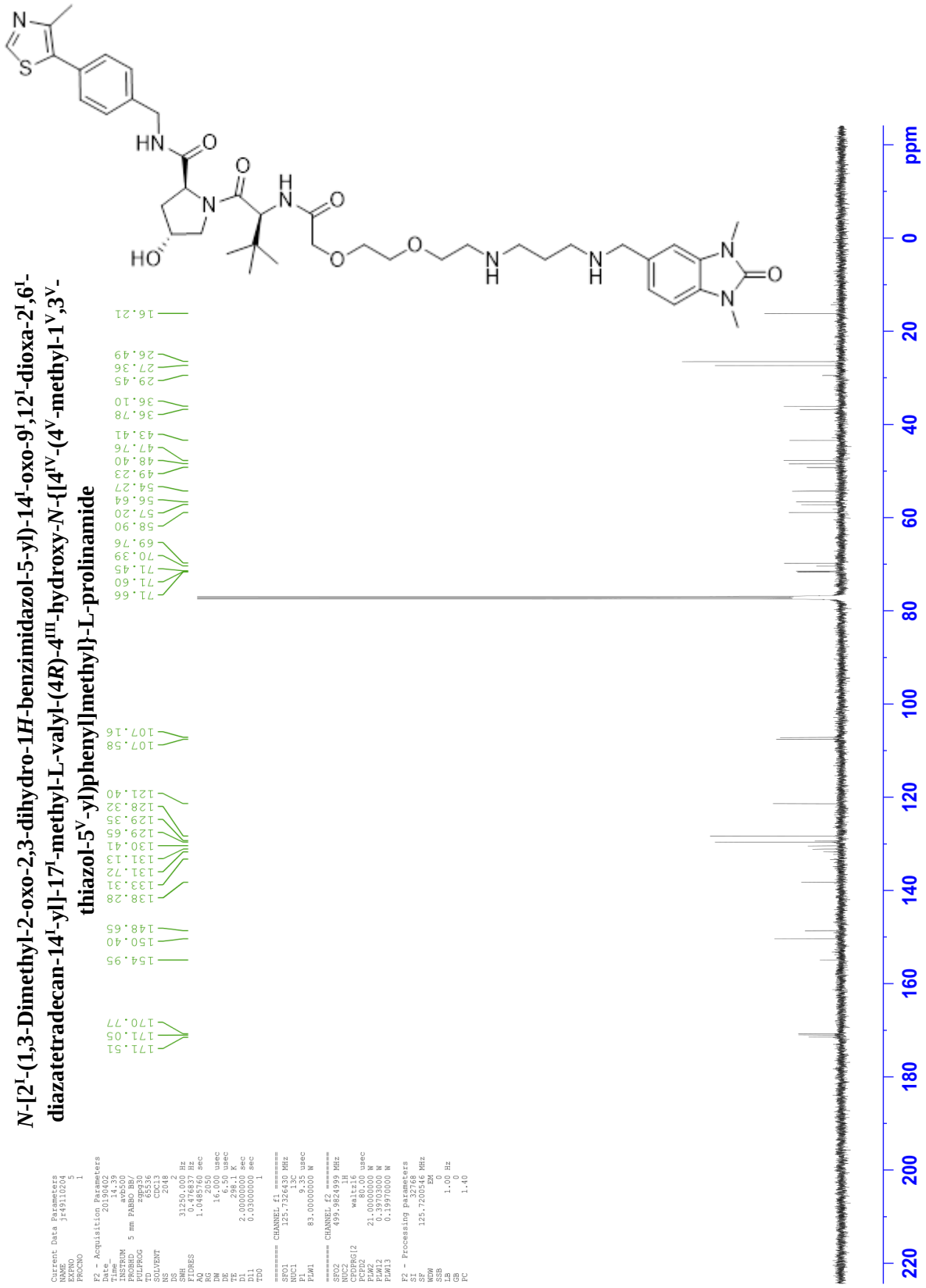
17^{I}-Methyl-*N*-{4^{II},4^{II},4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-14^{I}-oxo-2^{II},9^{I},12^{I}-trioxa-2^{I},6^{I}-diazaheptadecanan-14^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide



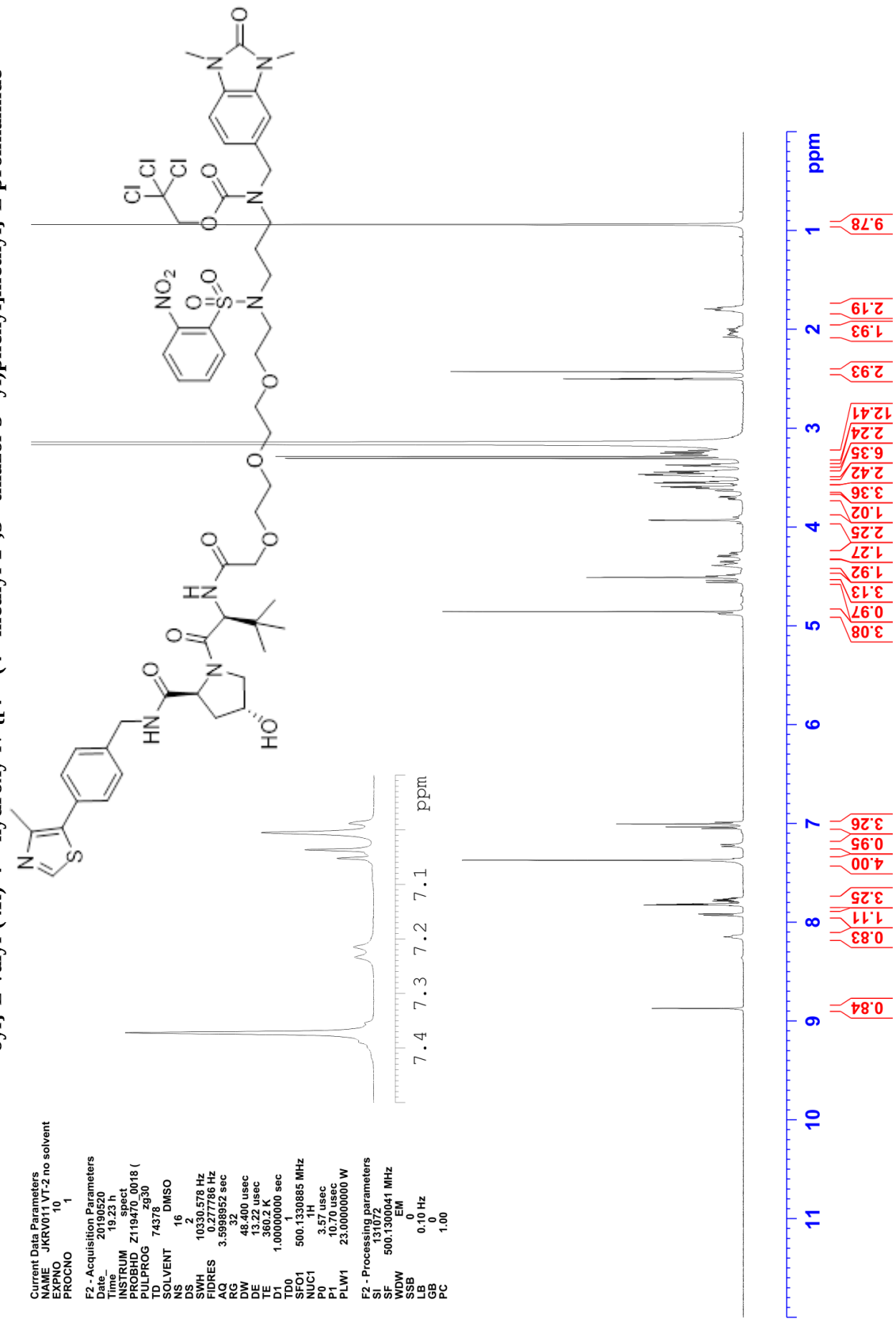
17^{I}-Methyl-*N*-{4^{II},4^{II},4^{II}-trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-14^{I}-oxo-2^{II},9^{I},12^{I}-trioxa-2^{I},6^{I}-diazaheptadecanan-14^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide



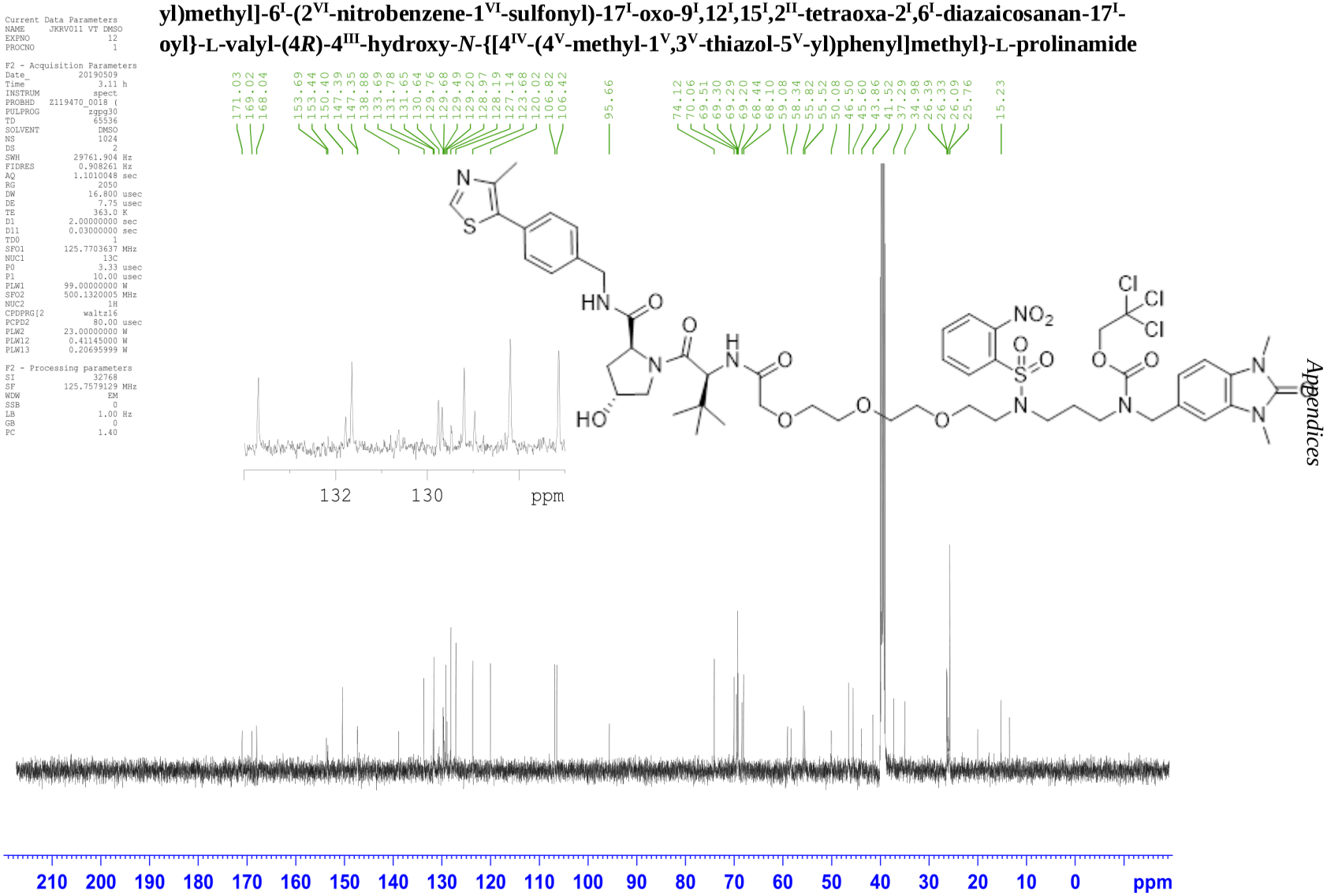

N-[2^I-(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)-14^I-oxo-9^I,12^I-dioxa-2^I,6^I-diazatetradecan-14^I-yl]-17^I-methyl-L-valyl-(4R)-4^III-hydroxy-N-{[4^IV-(4^V-methyl-1^V,3^V-thiazol-5^V-yl)phenyl]methyl}-L-prolinamide
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EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
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Time_ 12.38
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PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 64
DW 48.400 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 499.9825000 MHz
NUC1 1H
P1 11.00 usec
PLW1 21.00000000 W
F2 - Processing parameters
SI 32768
SF 499.9800132 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.4
7.2
7.0
ppm
0.74
5.89
1.07
0.95
1.00
1.08
1.02
1.13
1.06
1.07
1.07
1.04
1.10
2.14
1.10
3.49
9.61
6.33
3.29
1.37
1.24
2.12
9.47
ppm


N-[2^L-(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)-14^L-oxo-9^L,12^L-dioxa-2^L,6^L-diazatetradecan-14^L-yl]-17^L-methyl-L-valyl-(4R)-4^III-hydroxy-N-{[4^IV-(4^V-methyl-1^V,3^V-thiazol-5^V-yl)phenyl]methyl}-L-prolinamide
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PROCNO 1
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PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 2048
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 2050
DW 16.000 usec
DE 6.50 usec
TE 298.1 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.7326430 MHz
NUC1 13C
P1 9.35 usec
PLW1 83.00000000 W
======== CHANNEL f2 ========
SFO2 499.9824999 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 21.00000000 W
PLW12 0.39703000 W
PLW13 0.19970000 W
F2 - Processing parameters
SI 32768
SF 125.7200346 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
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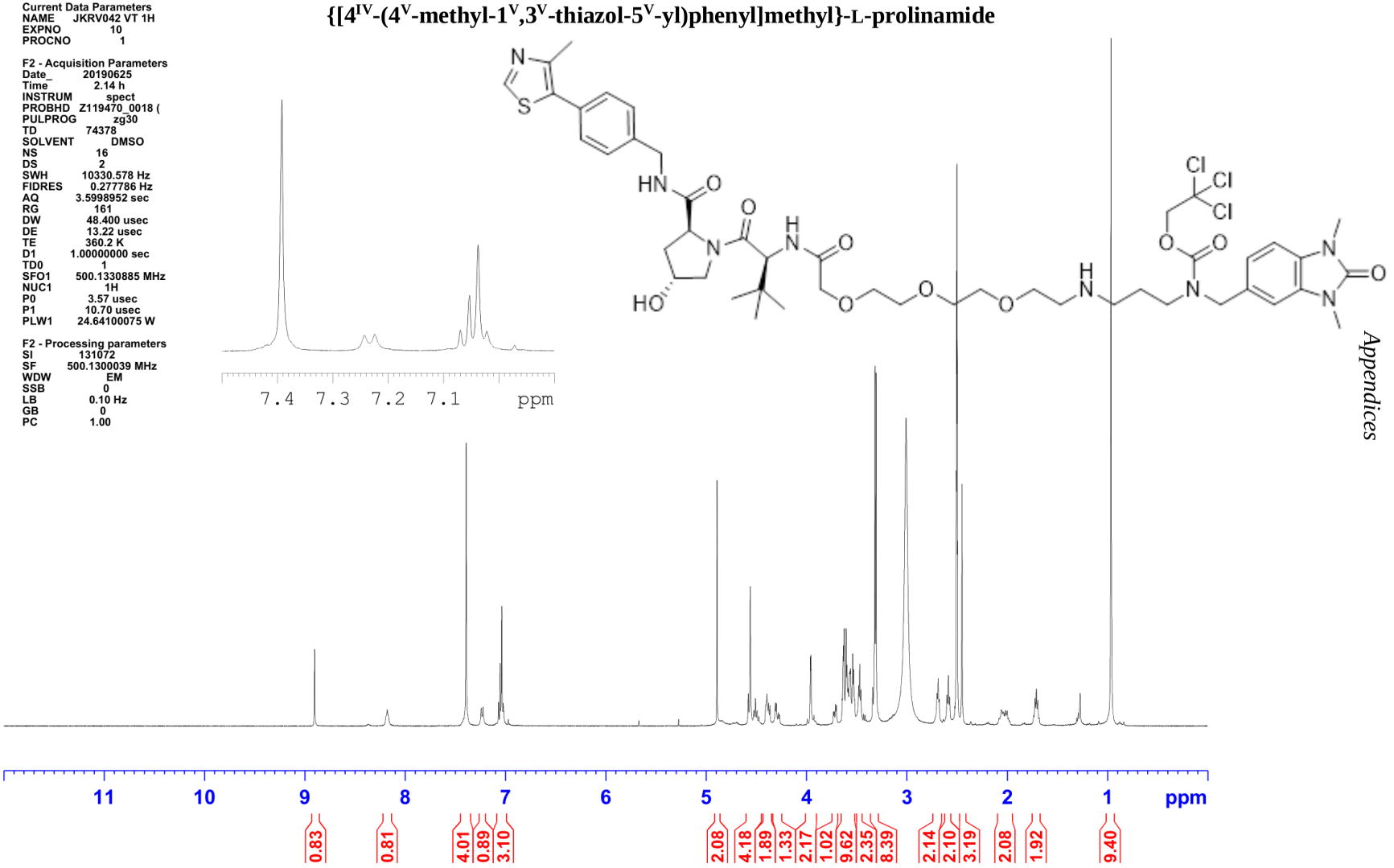
20^{I}-Methyl-*N*-{4^{II},4^{II},4^{II} -trichloro-2^{I}-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-6^{I}-(2^{VI}-nitrobenzene-1^{VI}-sulfonyl)-17^{I}-oxo-9^{I},12^{I},15^{I},2^{II}-tetraoxa-2^{I},6^{I}-diazaicosanan-17^{I}-oyl}-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-[[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide



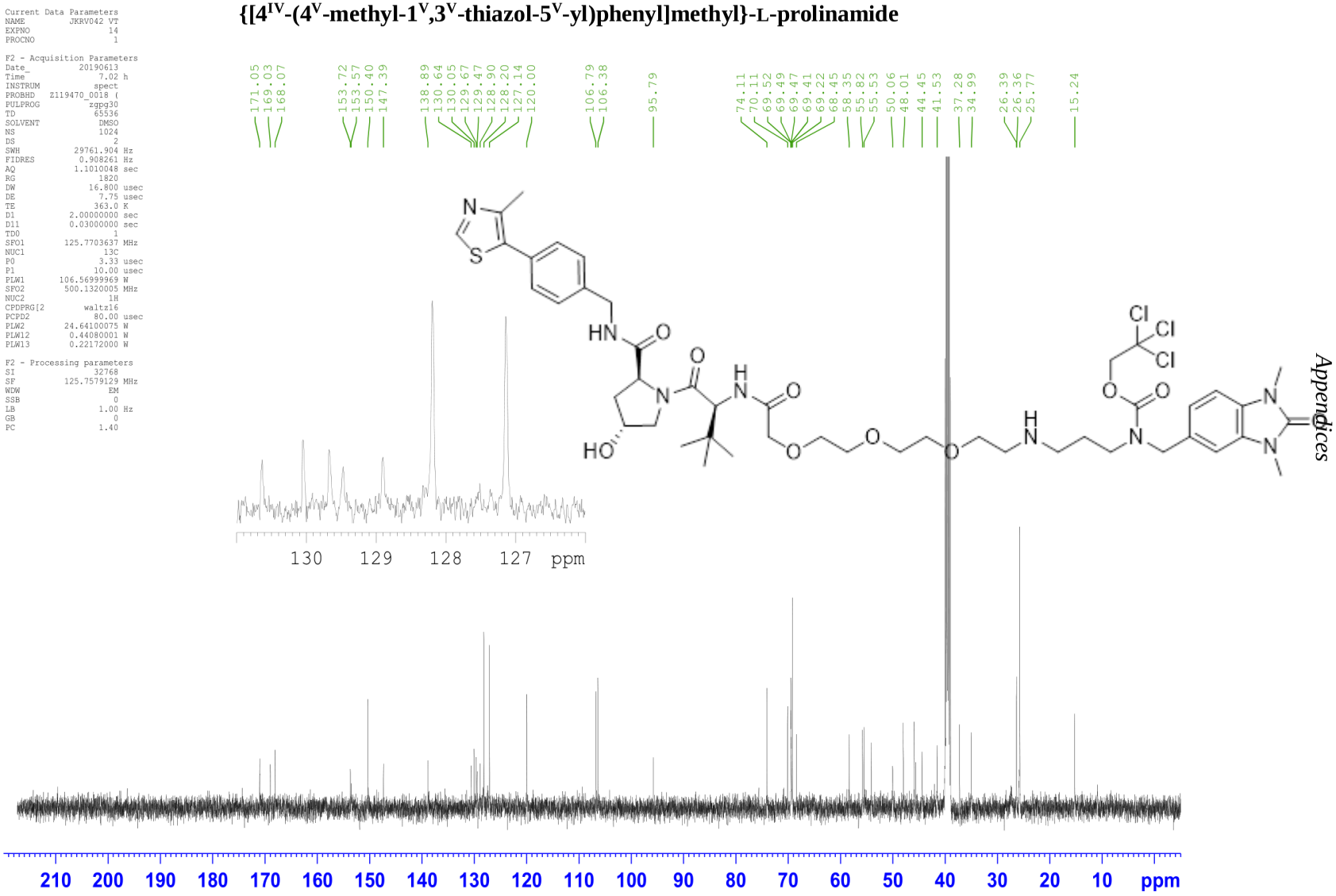
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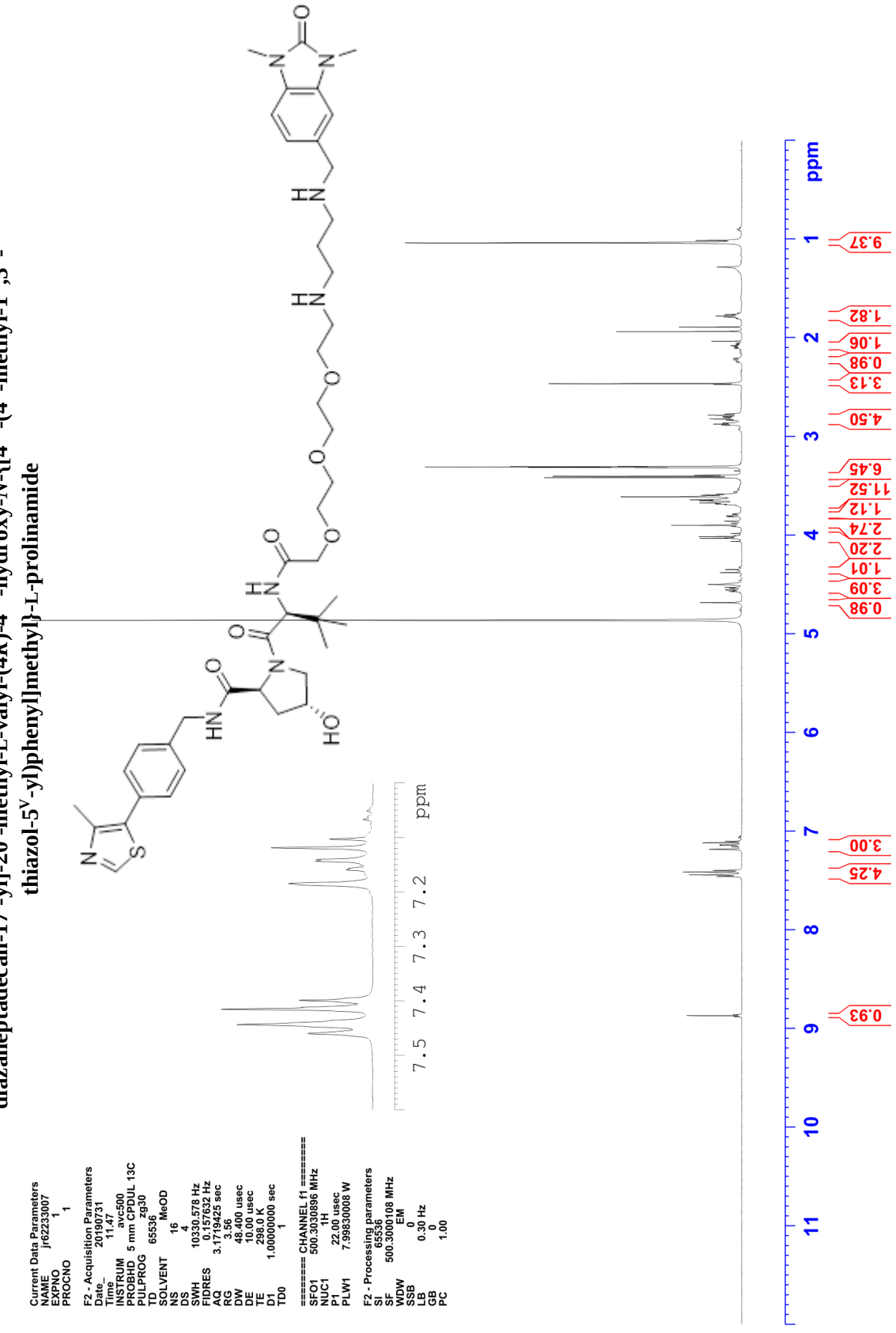
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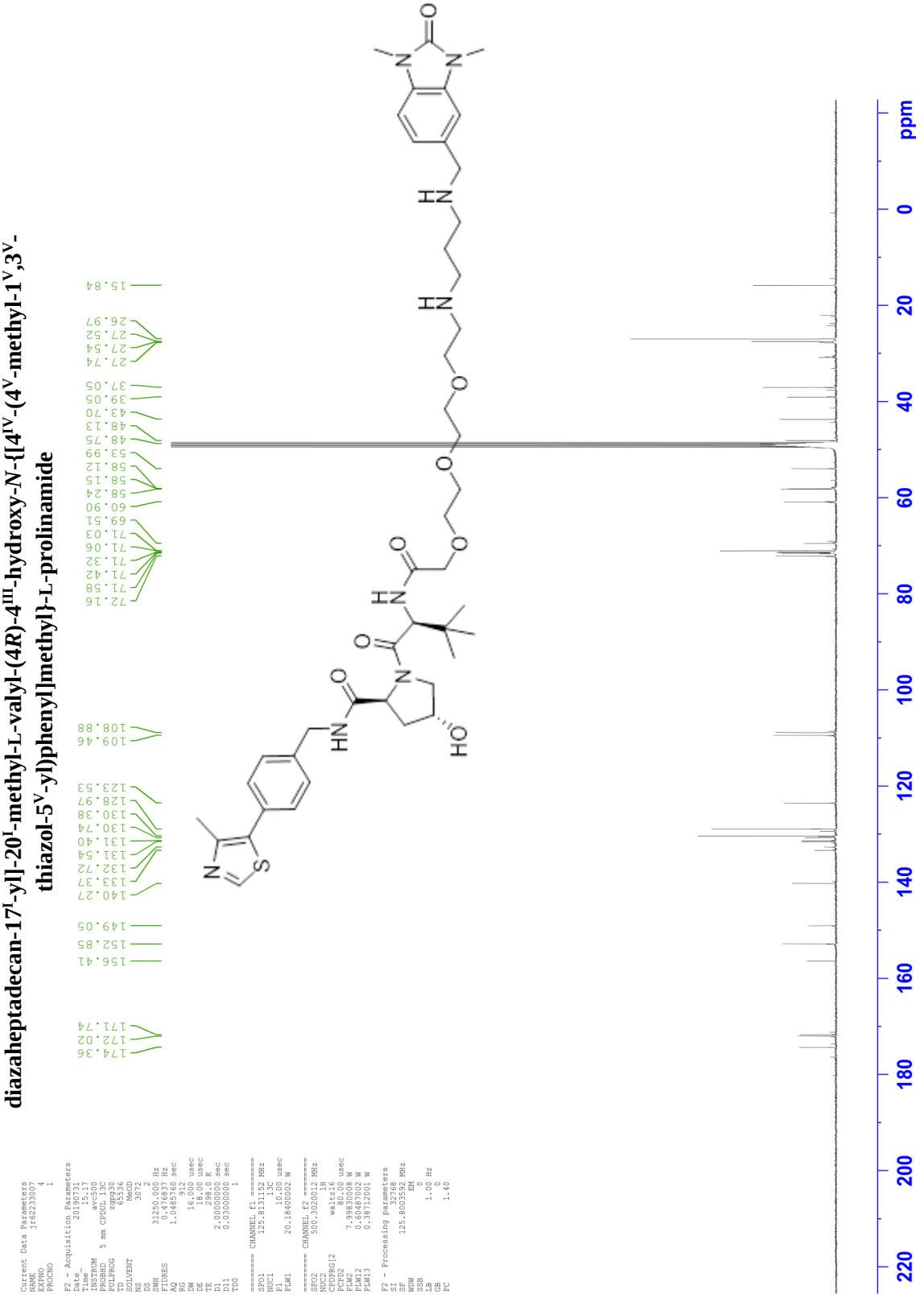
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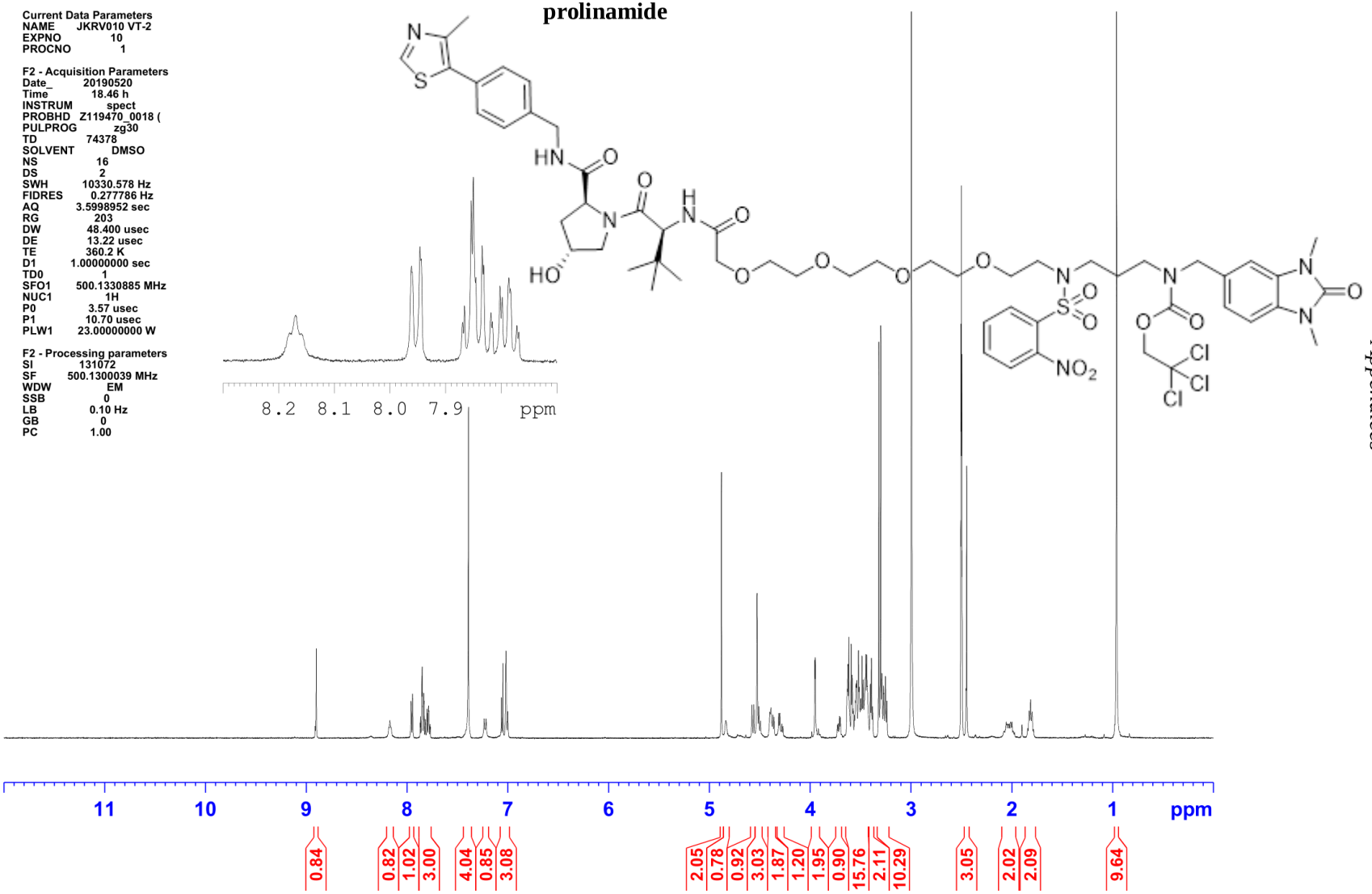
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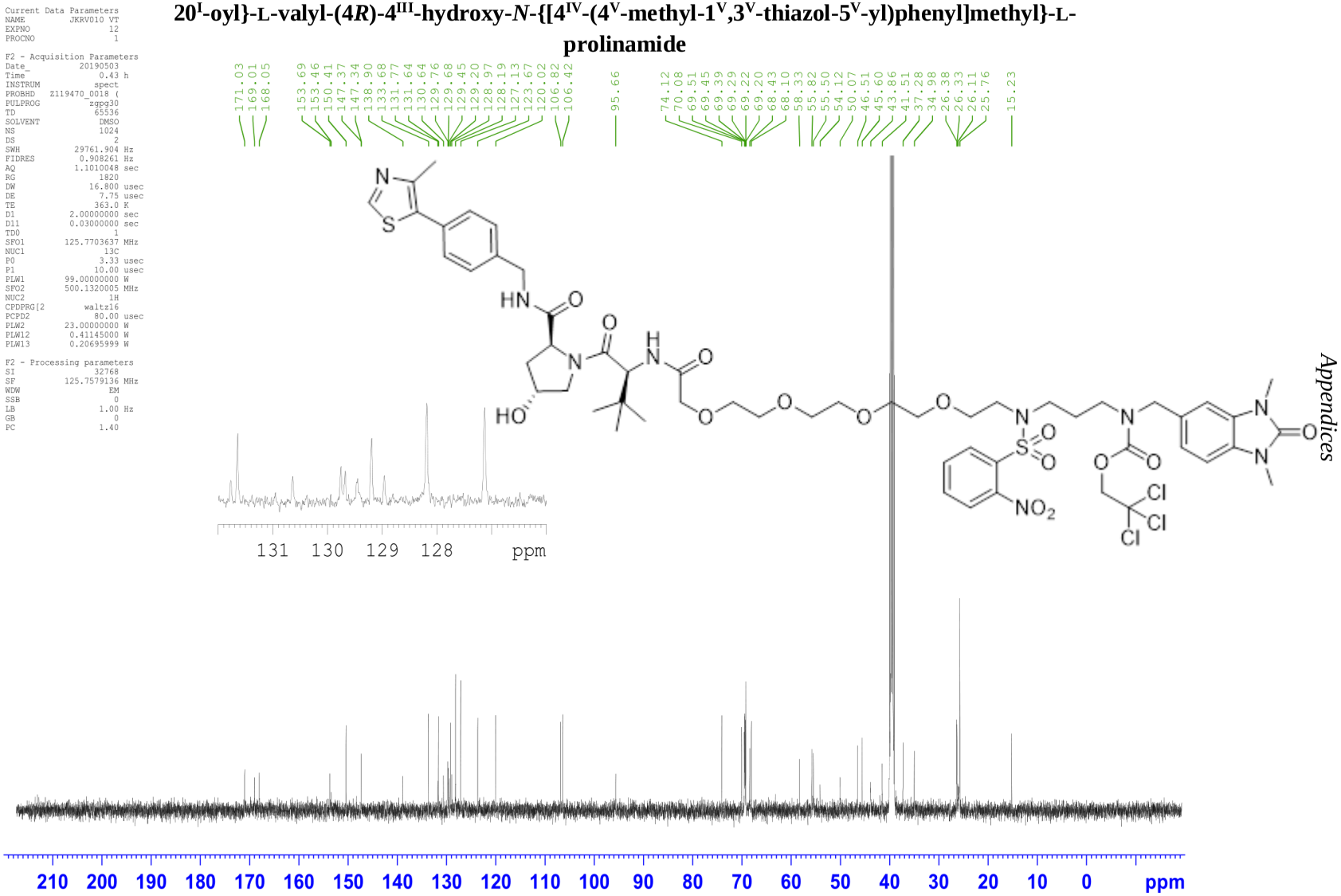
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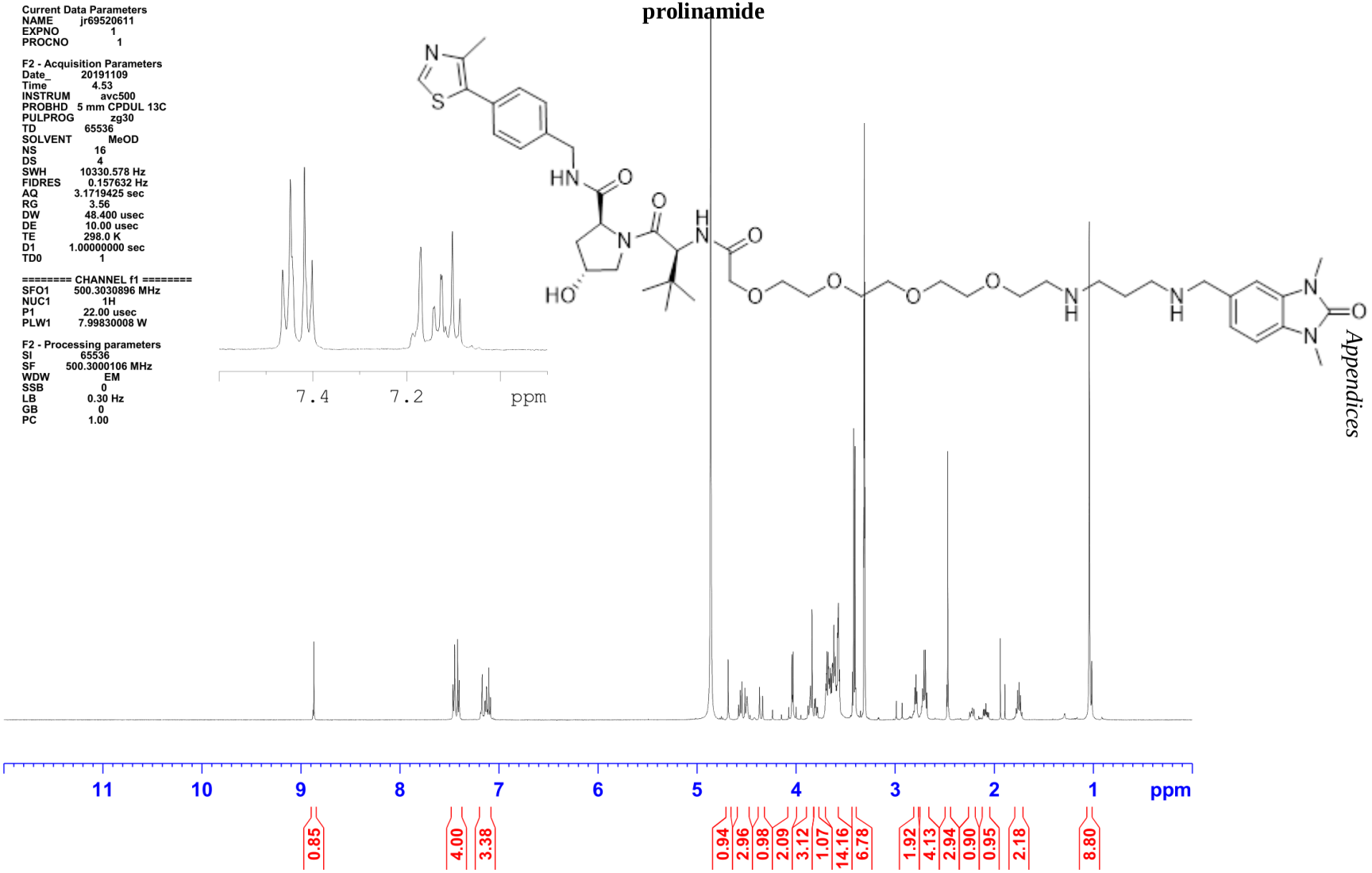
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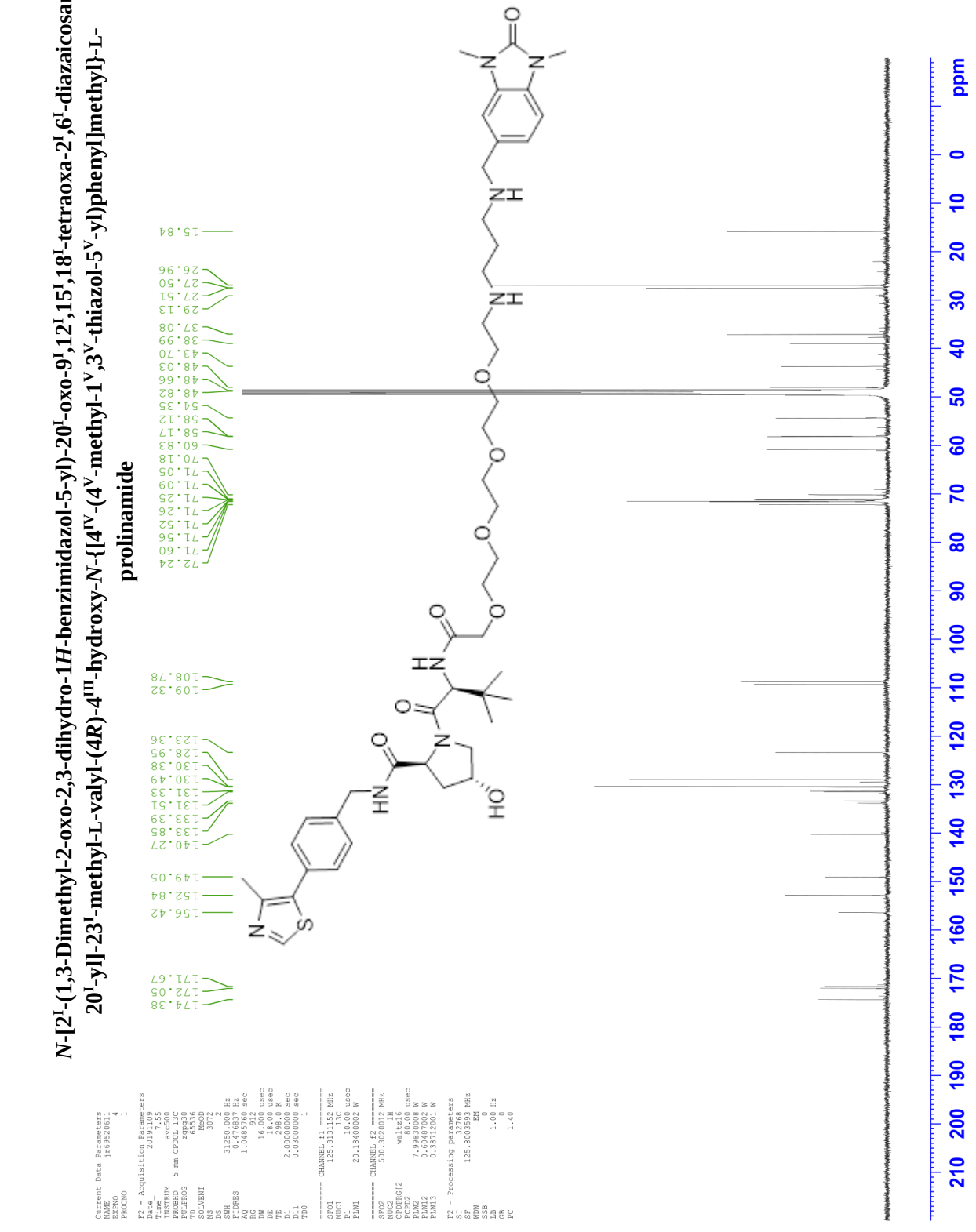
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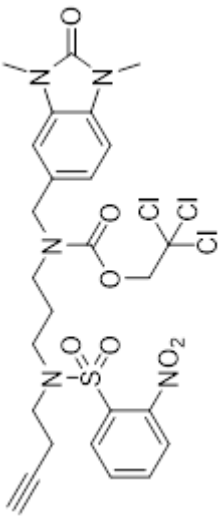
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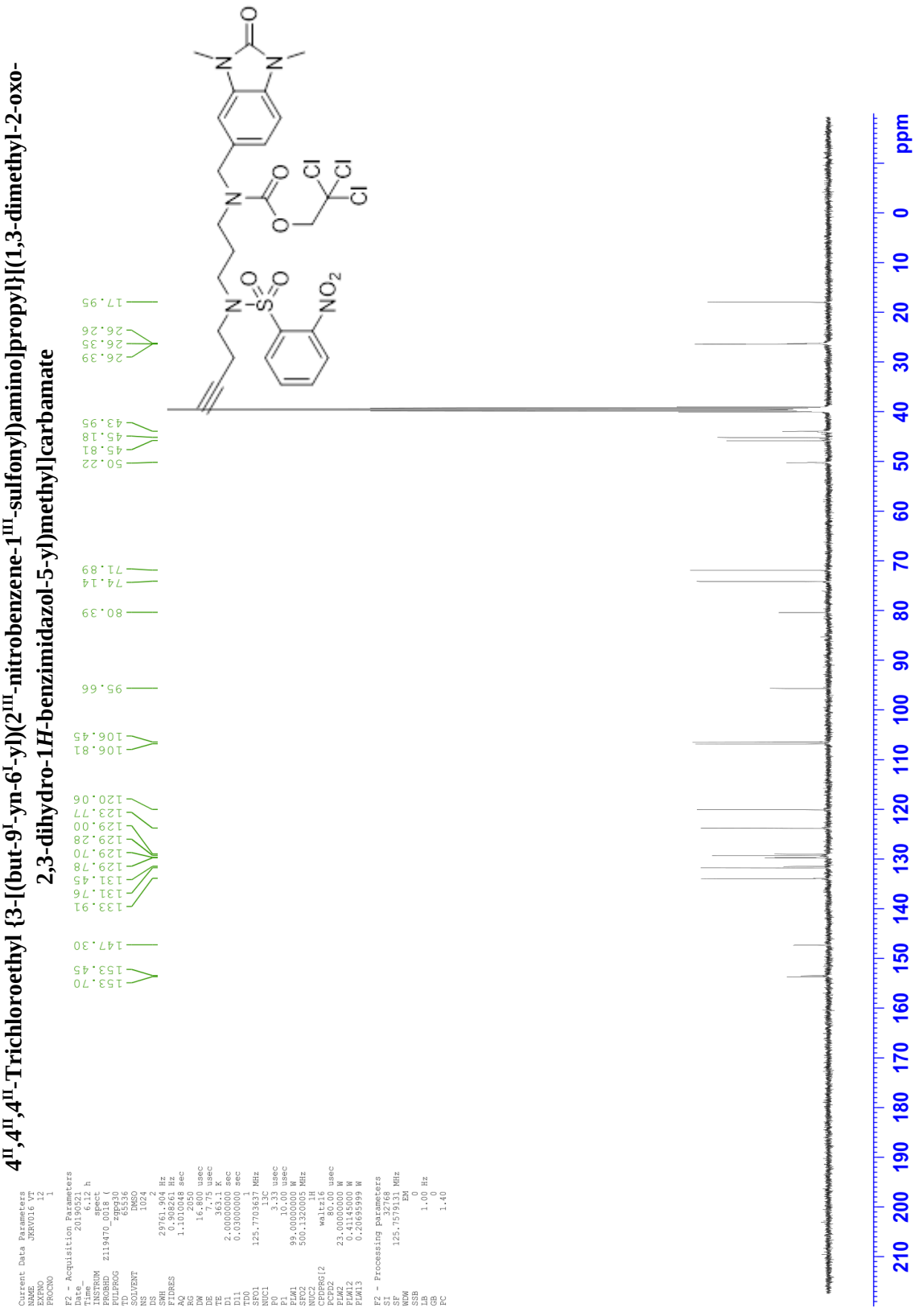
N-[2^{1}-(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-20^{1}-oxo-9^{1},12^{1},15^{1},18^{1}-tetraoxa-2^{1},6^{1}-diazaicosan-20^{1}-yl]-23^{1}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide



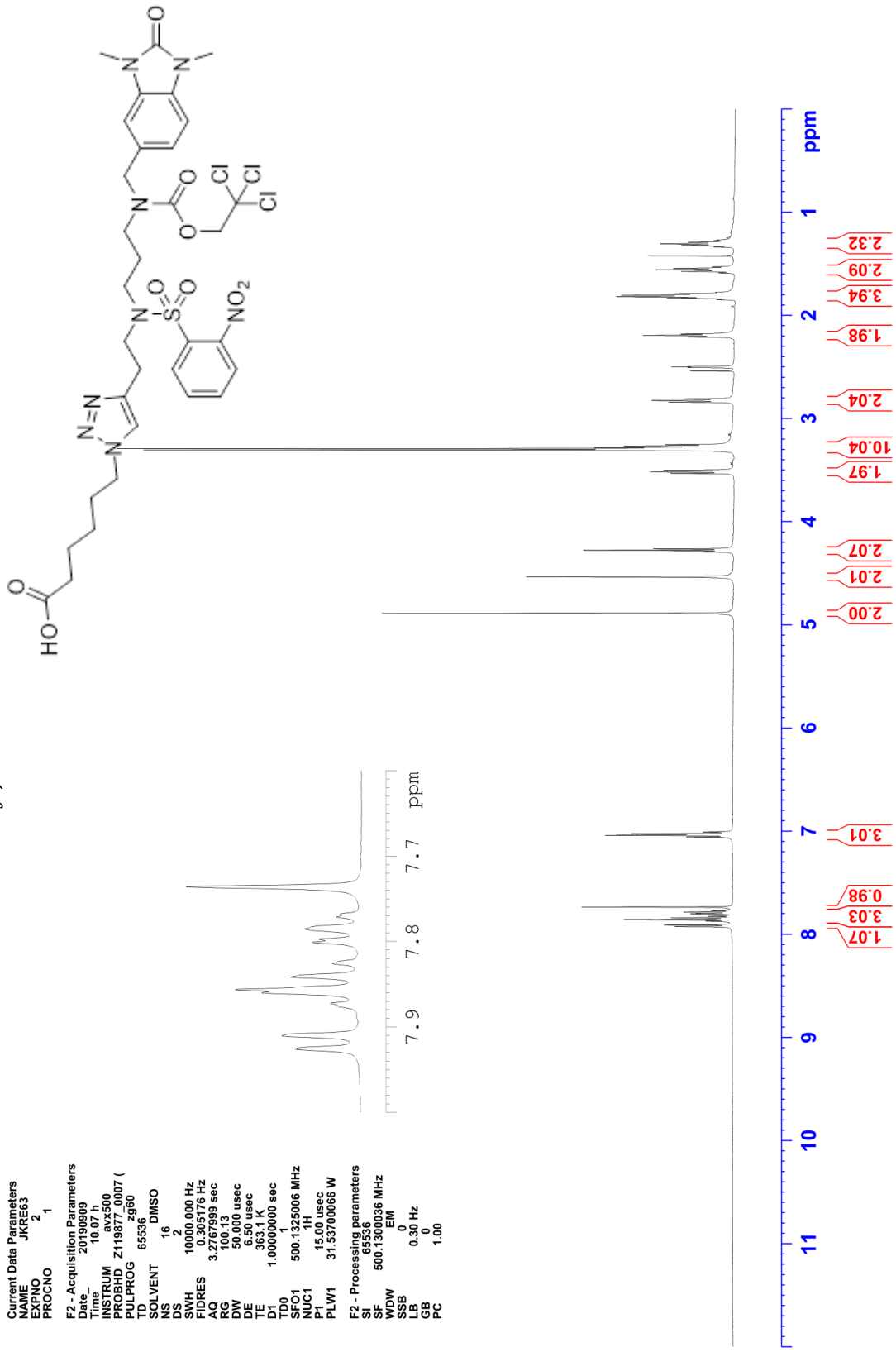
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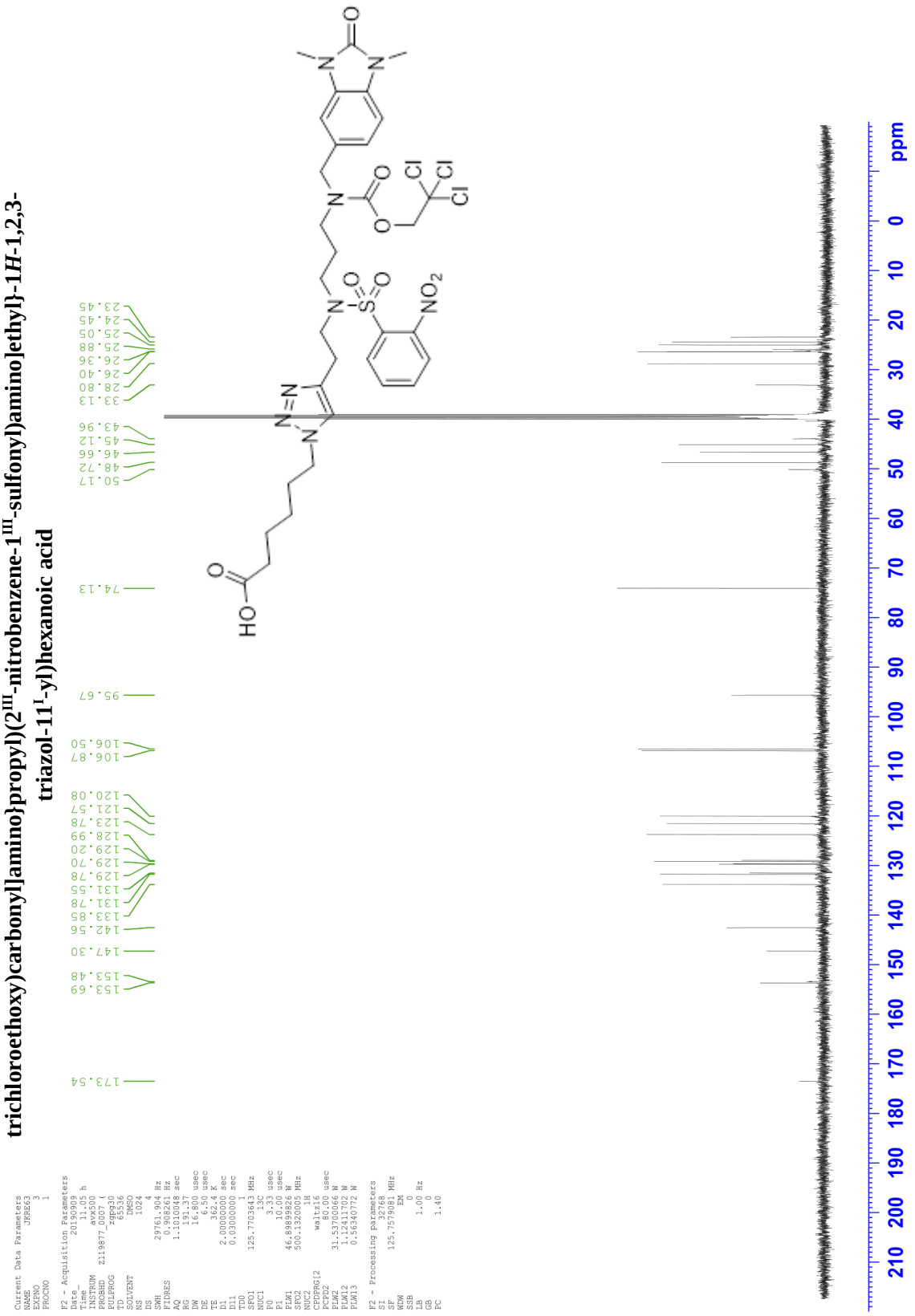
4^{II},4^{II},4^{II}-Trichloroethyl {3-[(but-9^{I}-yn-6^{I}-yl)(2^{III}-nitrobenzene-1^{III}-sulfonyl)amino]propyl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate



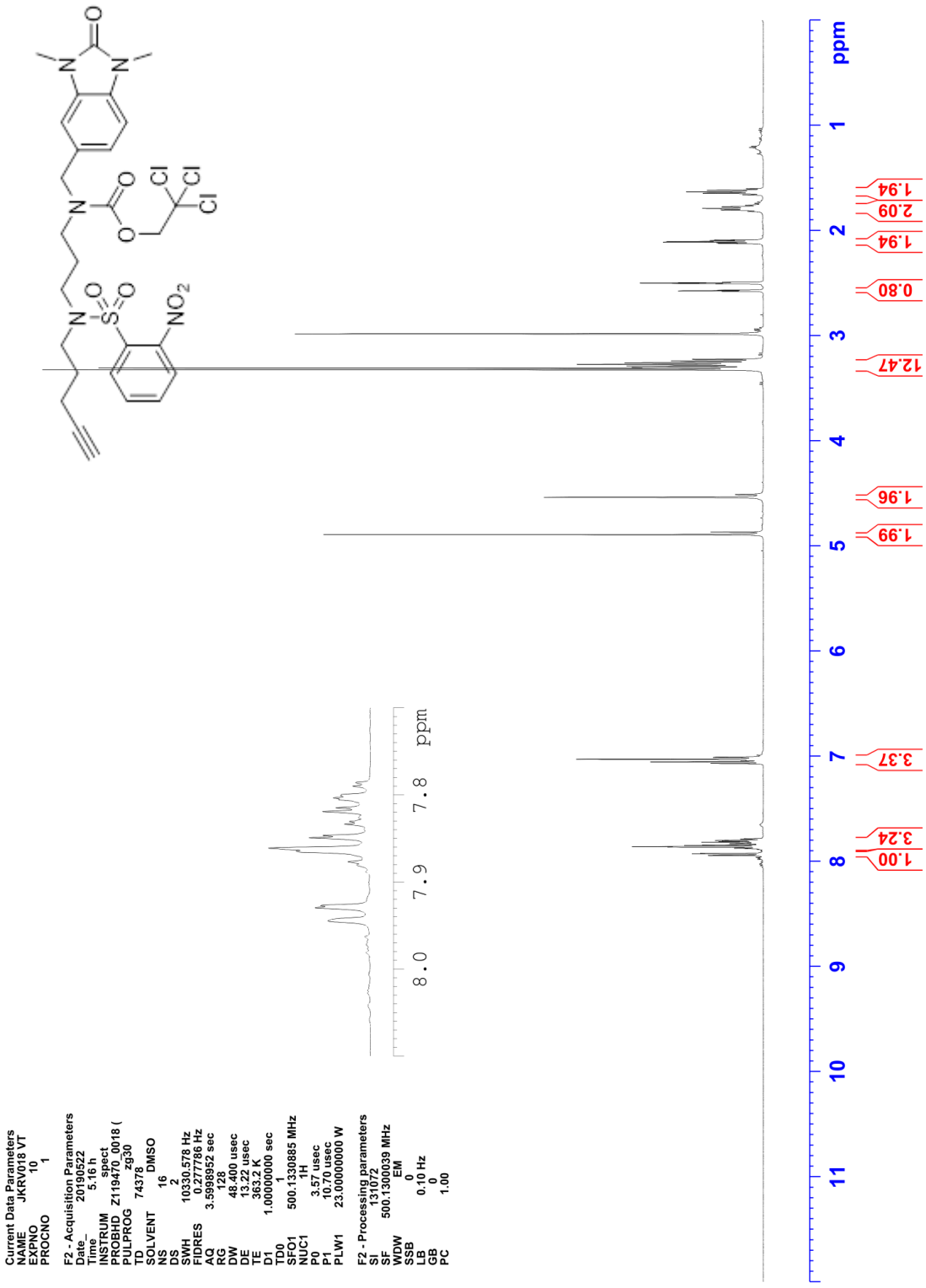
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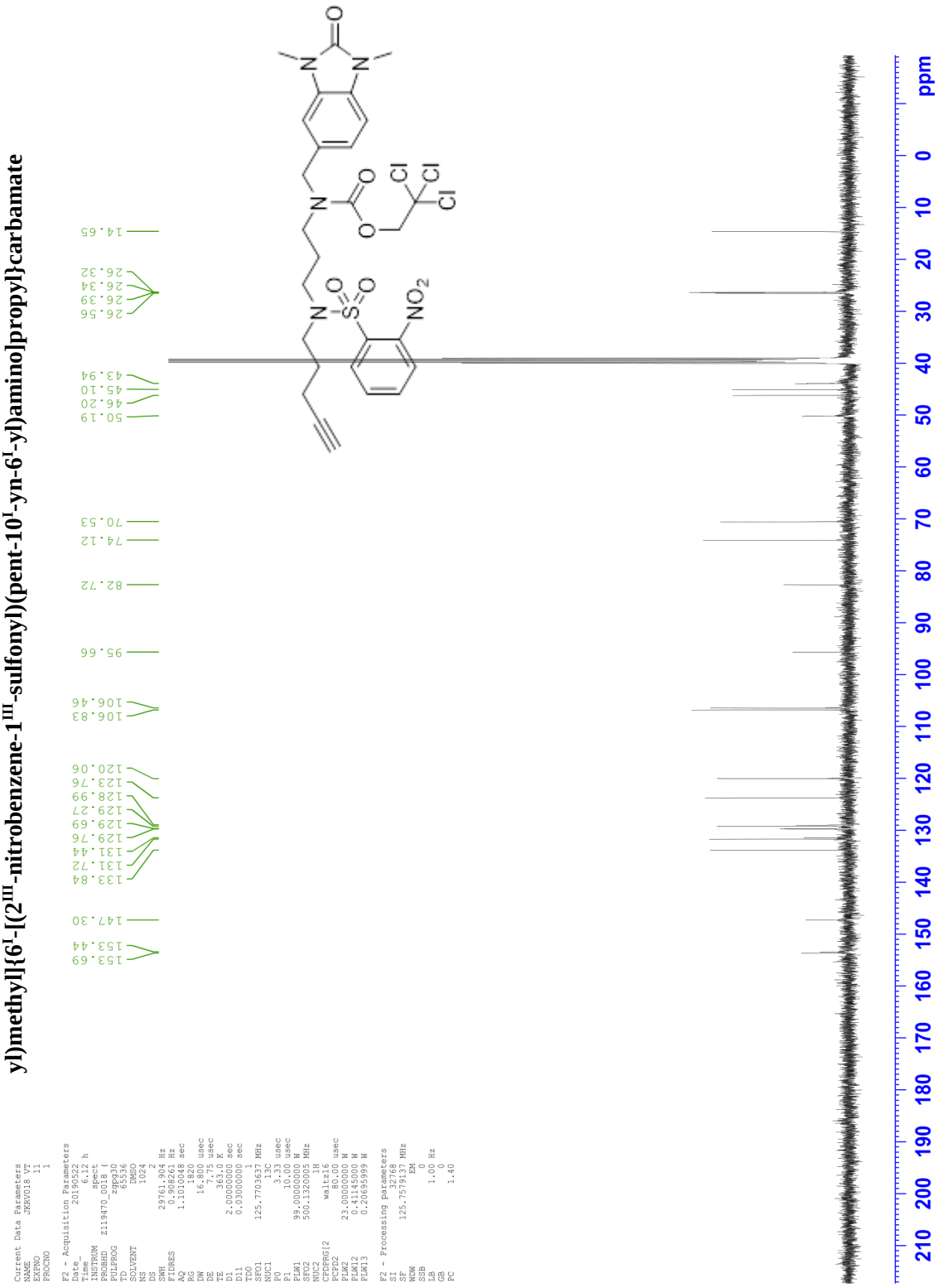
17^{l}-(9^{l}-{6^{l}-[(2^{l}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)(2^{III}-nitrobenzene-1^{III}-sulfonyl)amino]ethyl}-1*H*-1,2,3-triazol-11^{I}-yl)hexanoic acid



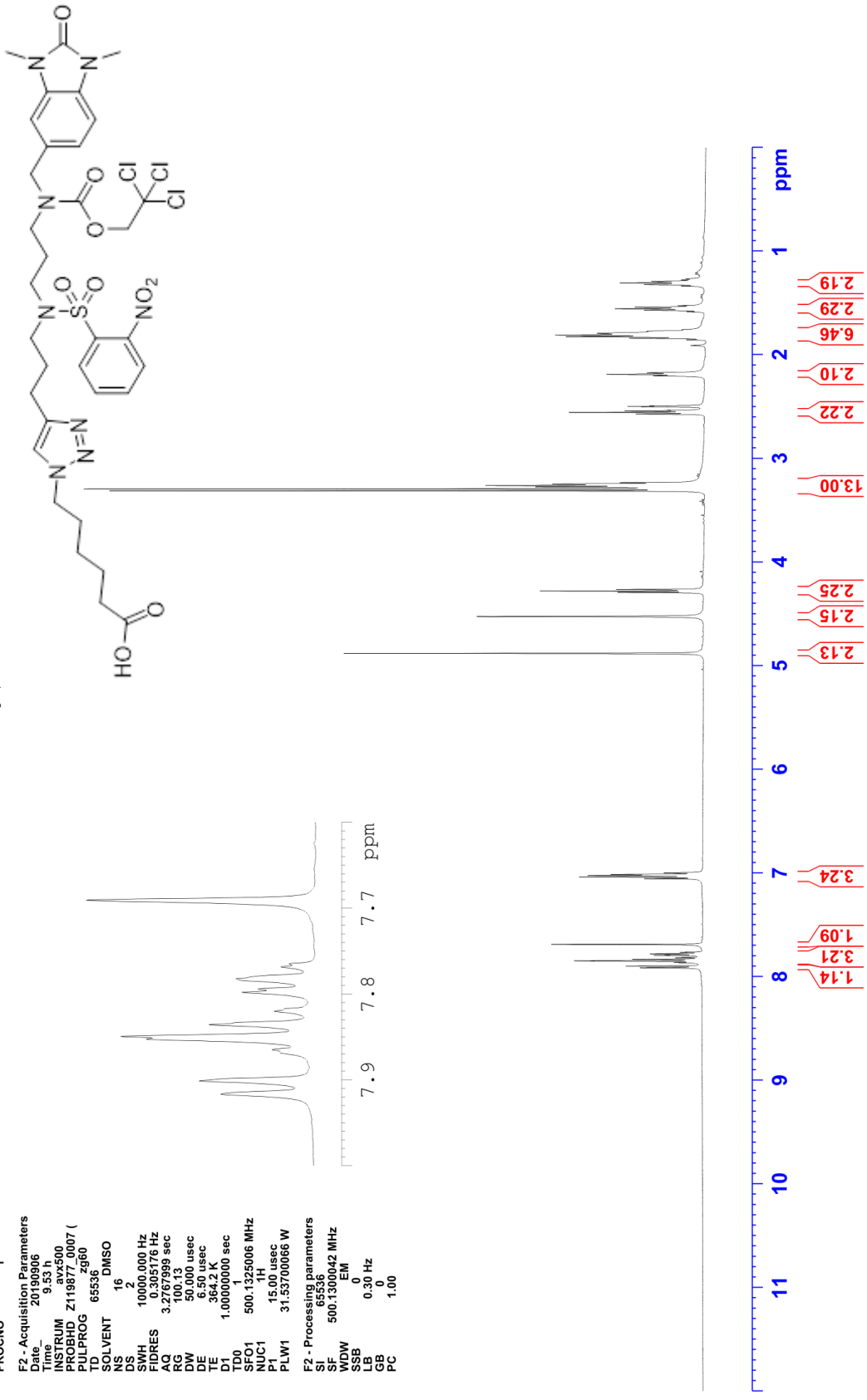
$4^{II},4^{II},4^{II}$-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{6^{I}-[(2^{III}-nitrobenzene-1^{III}-sulfonyl)(pent-10^{I}-yn-6^{I}-yl)amino]propyl}carbamate



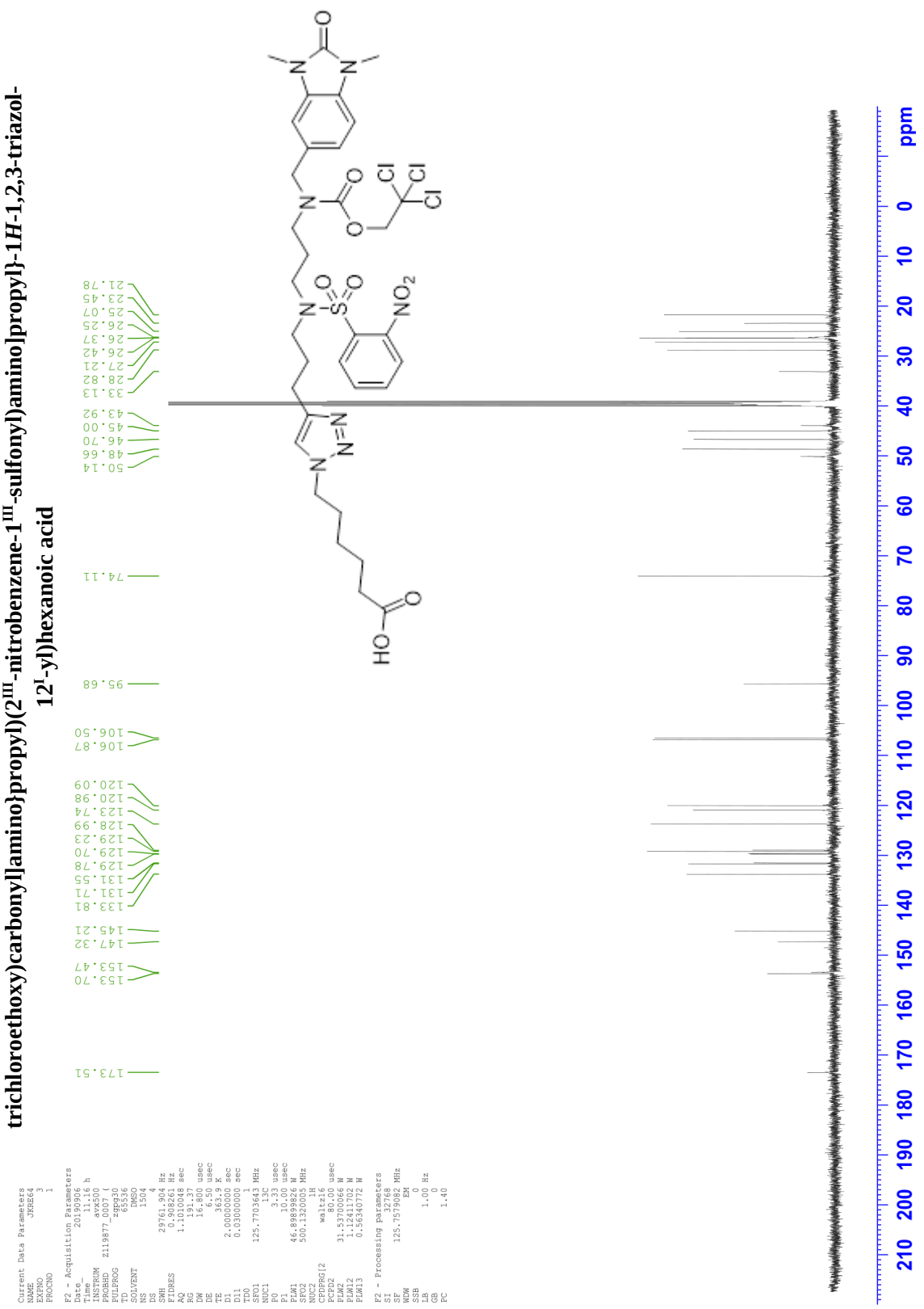
4^{II},4^{II},4^{II}-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{6^{I}-[(2^{III}-nitrobenzene-1^{III}-sulfonyl)(pent-10^{I}-yn-6^{I}-yl)amino]propyl}carbamate



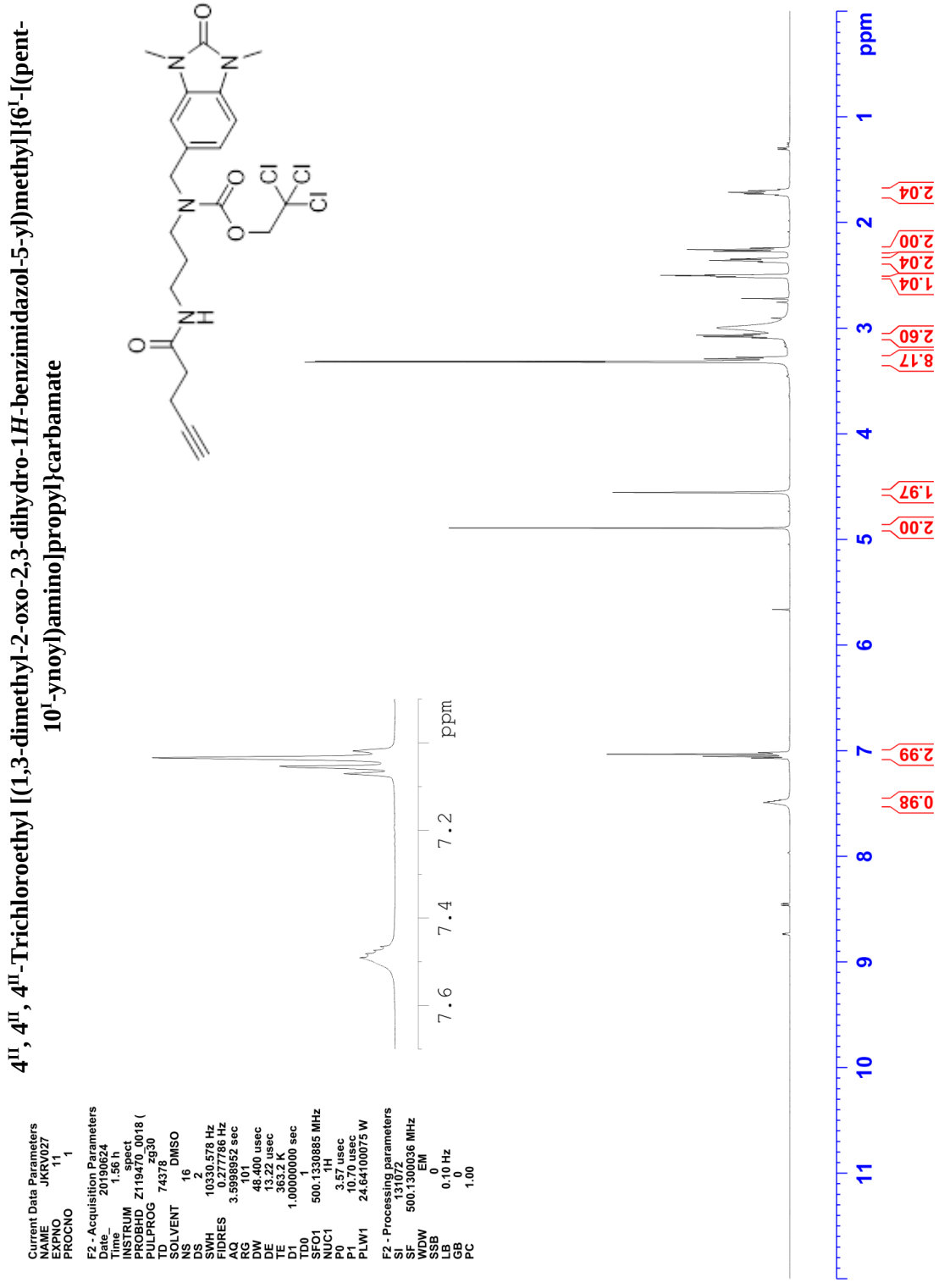
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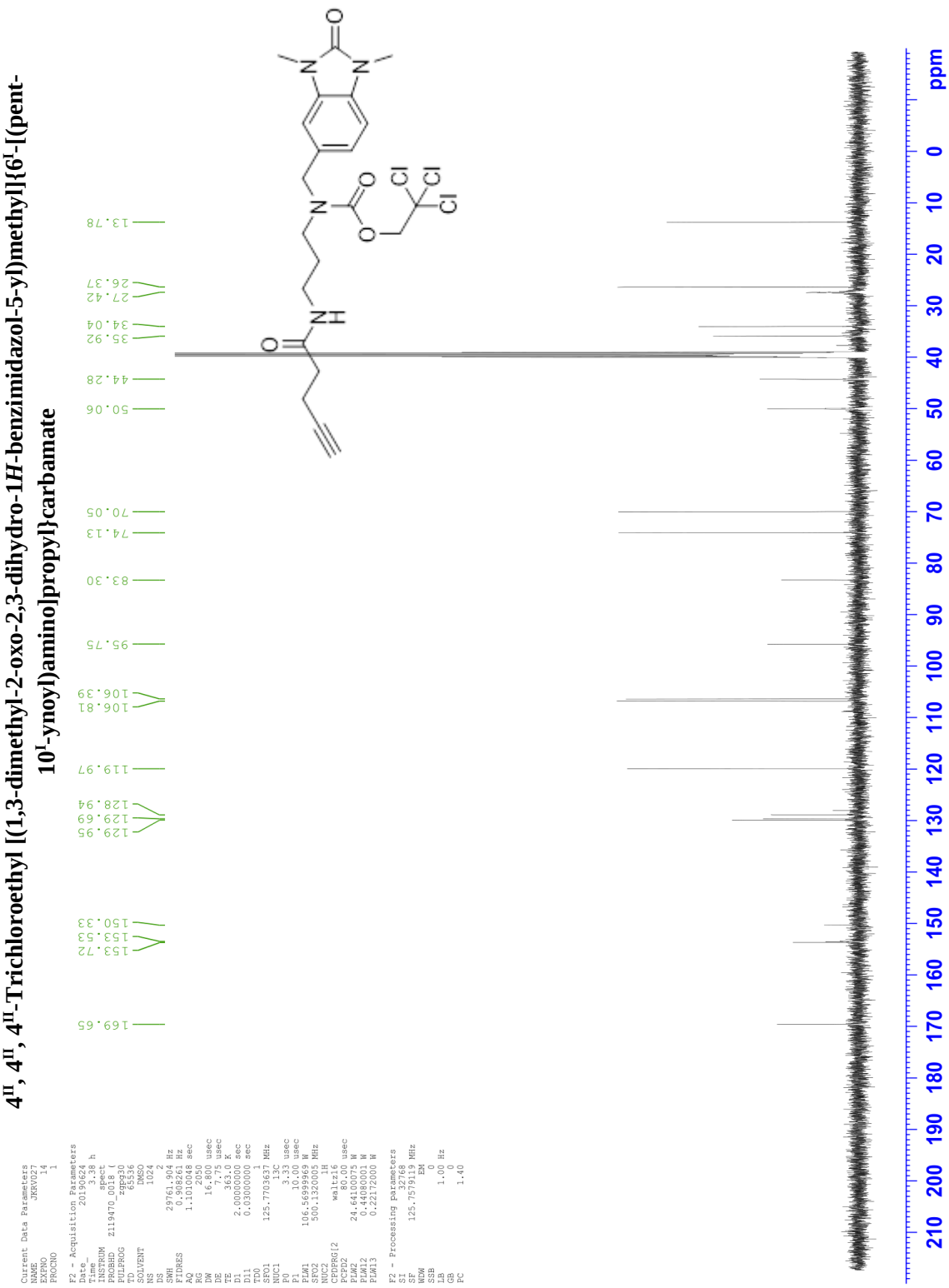
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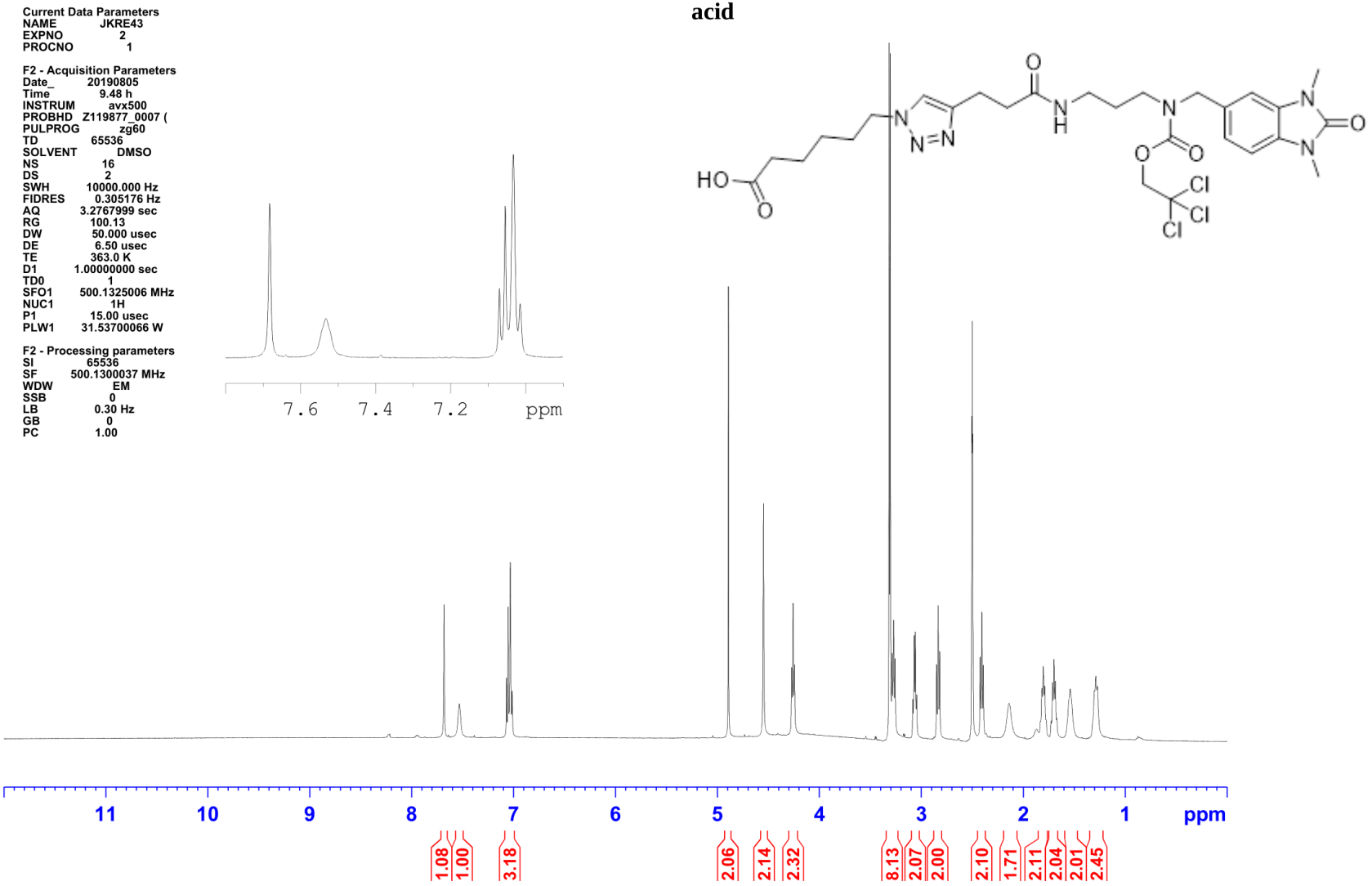
4^{II}, 4^{II}, 4^{II}-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{6^{I}-[(pent-10^{I}-ynoyl)amino]propyl}carbamate



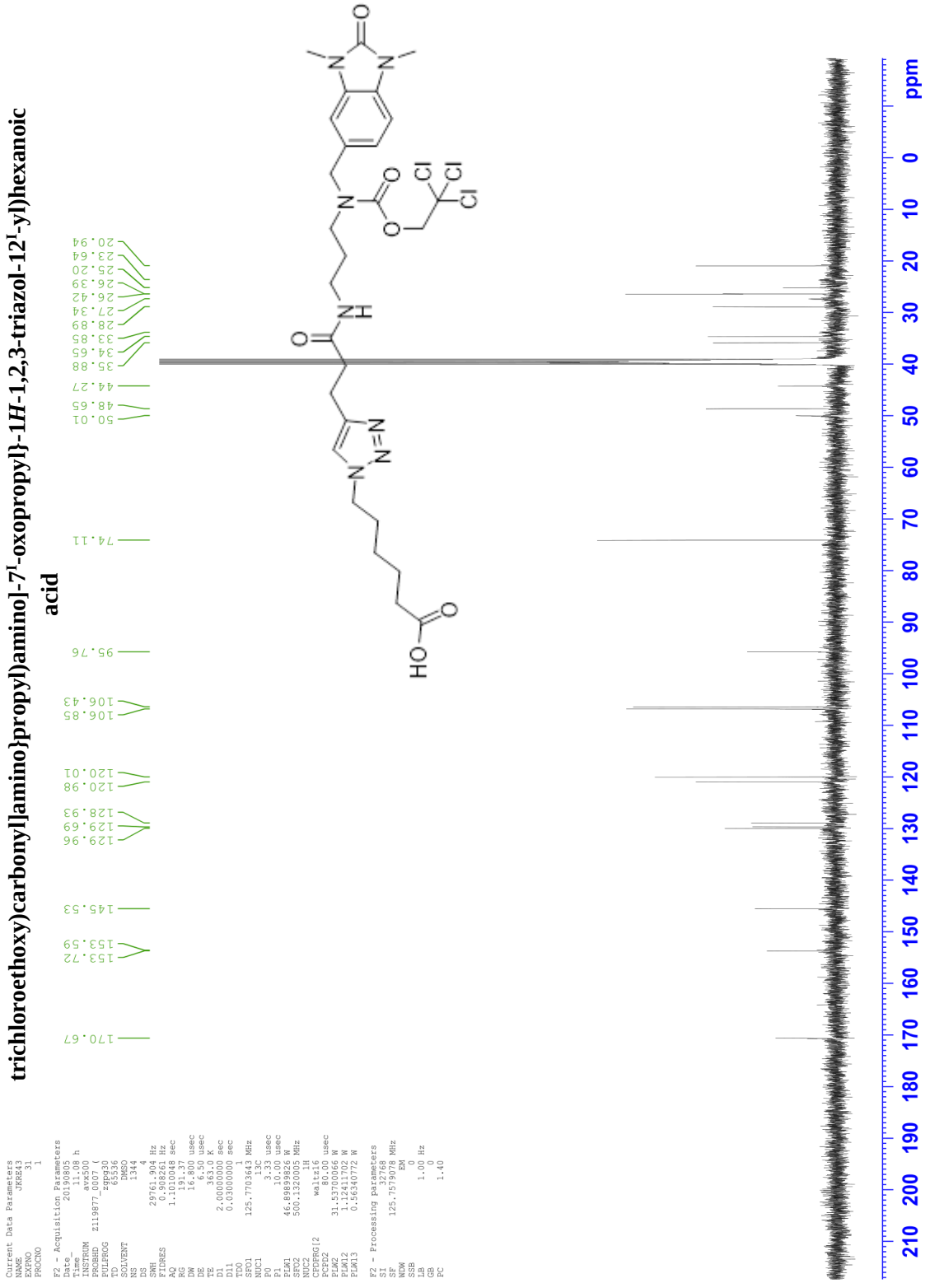
4^{II}, 4^{II}, 4^{II}-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{6^{I}-[(pent-10^{I}-ynoyl)amino]propyl}carbamate



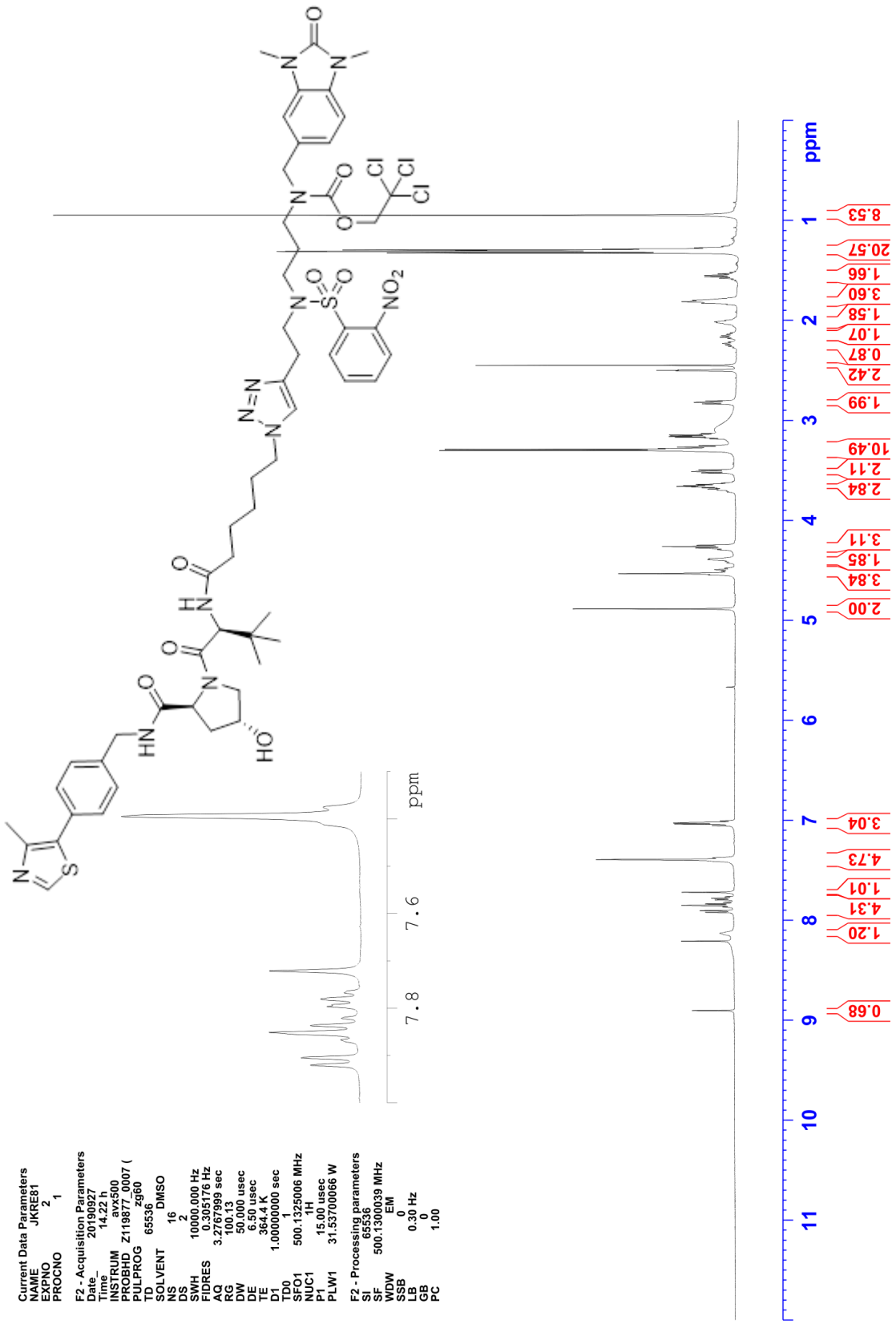
18^{I}-(10^{I}-{6^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-7^{I}-oxopropyl}-1*H*-1,2,3-triazol-12^{I}-yl)hexanoic acid



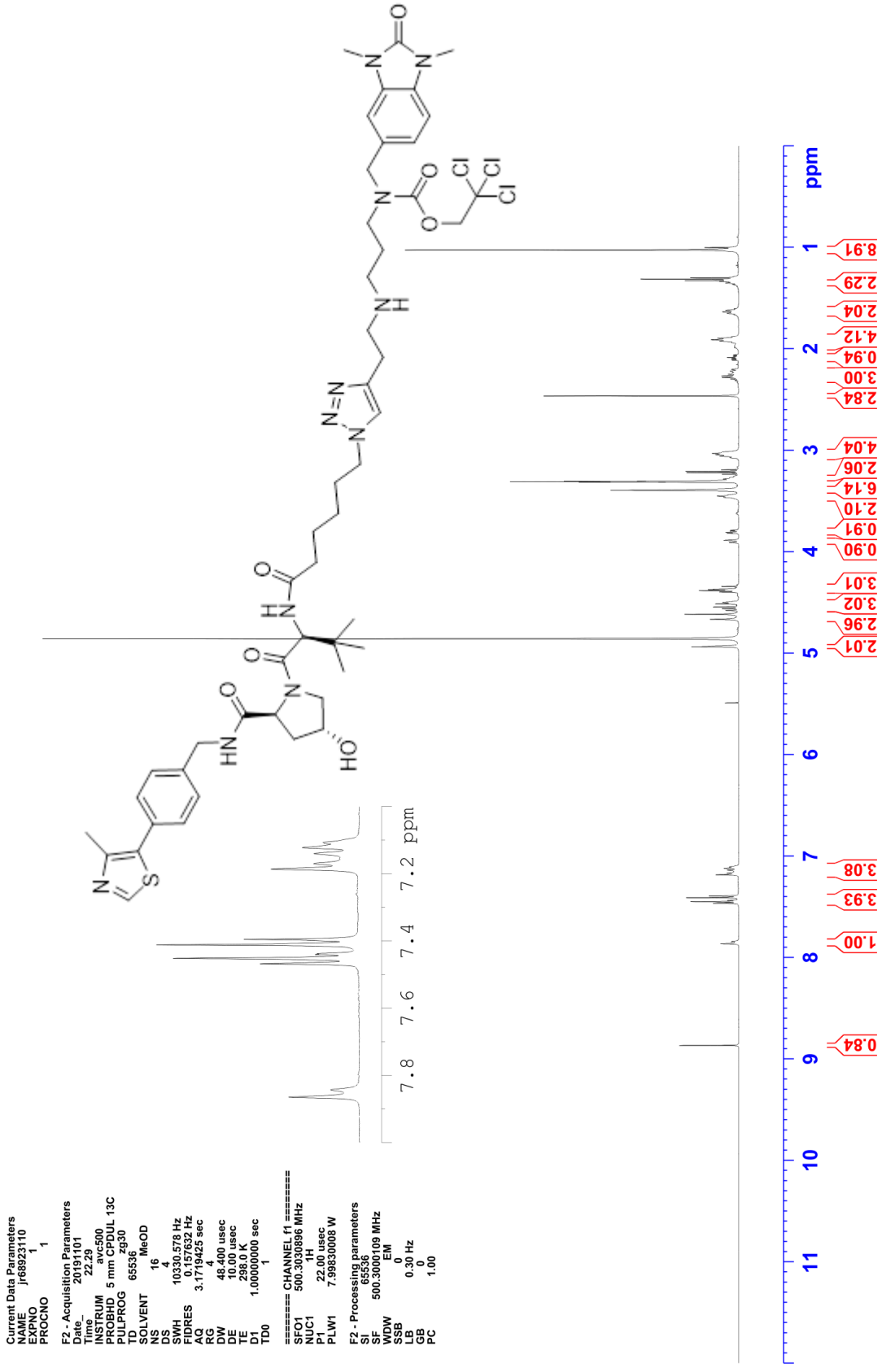
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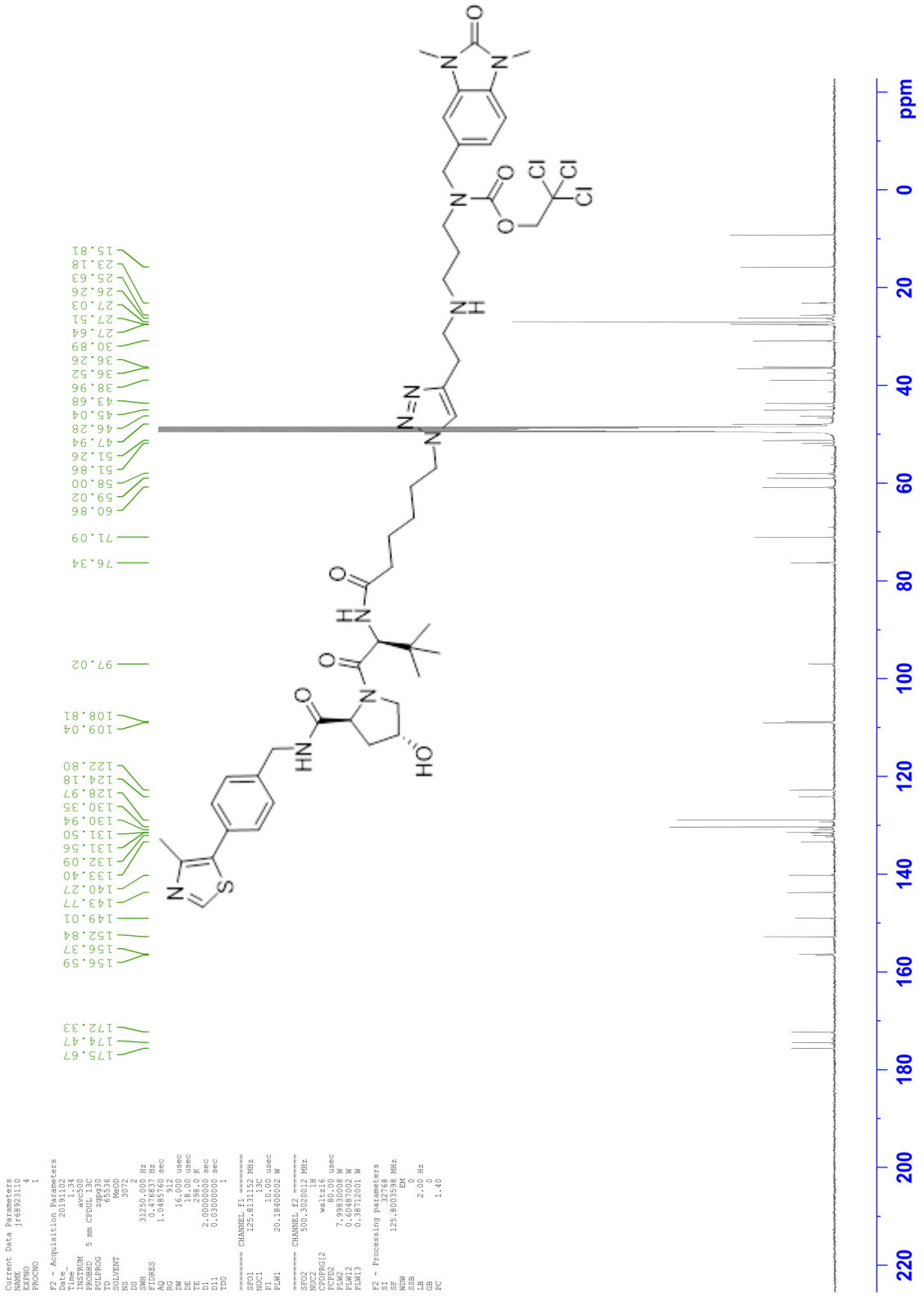
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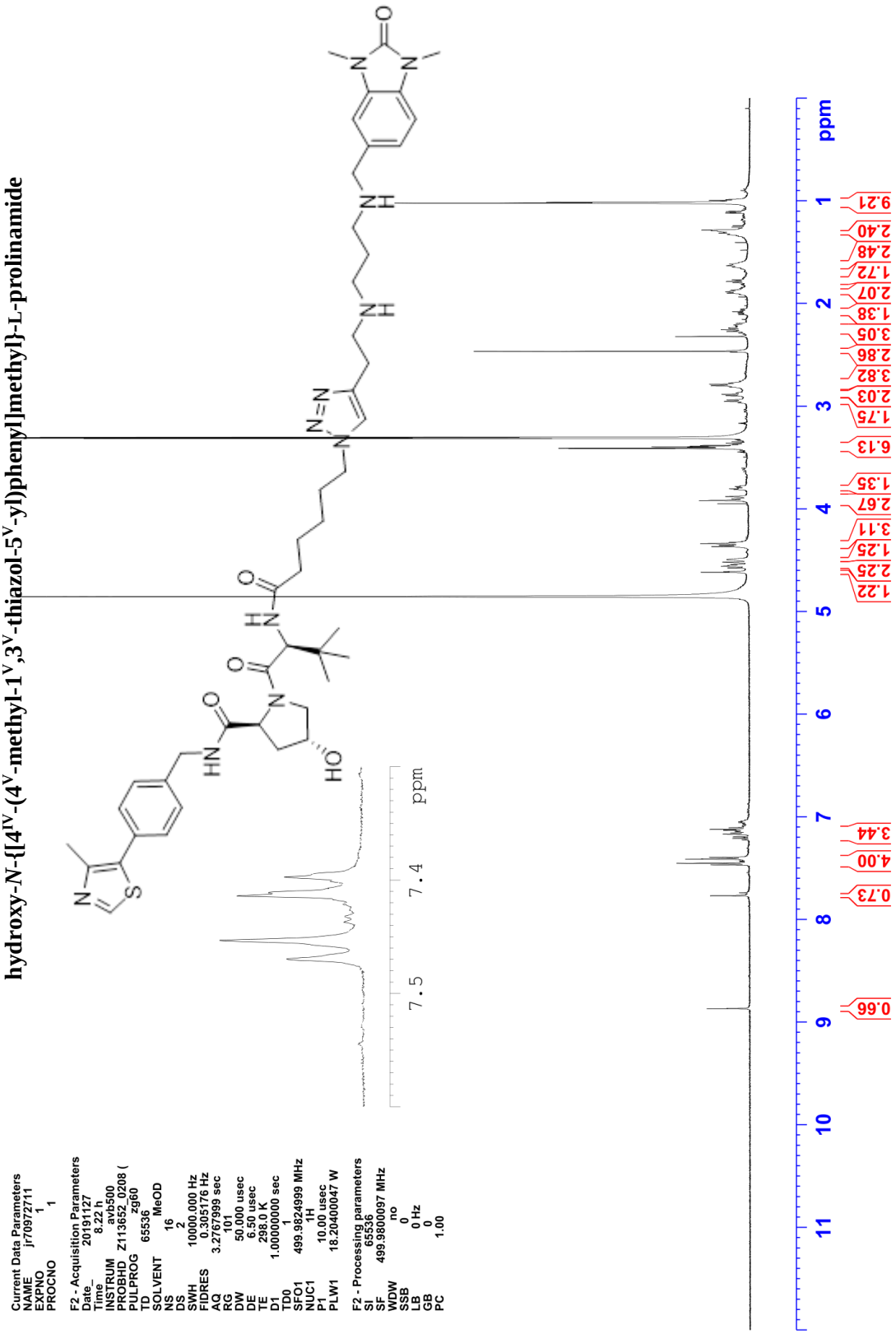
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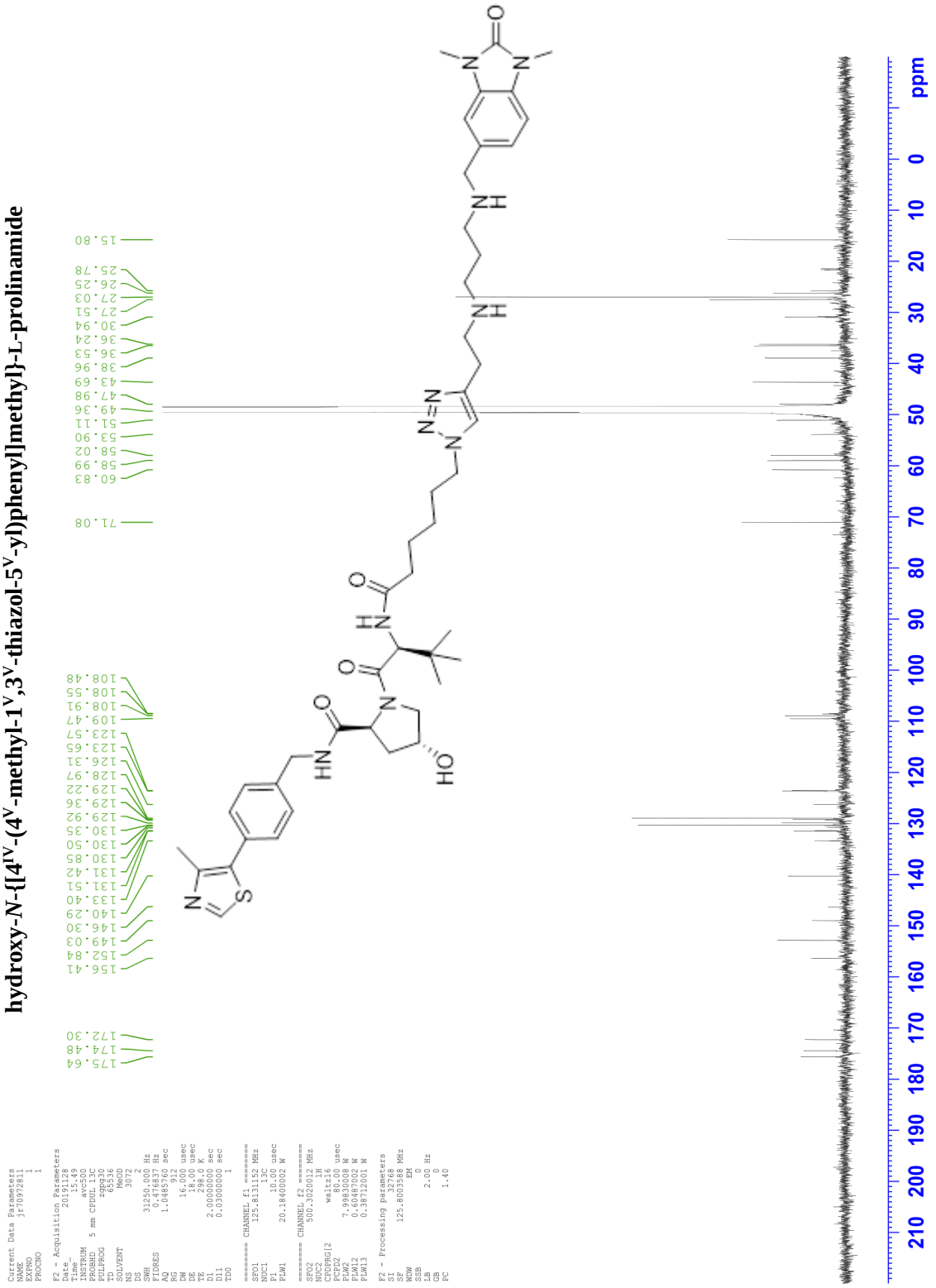
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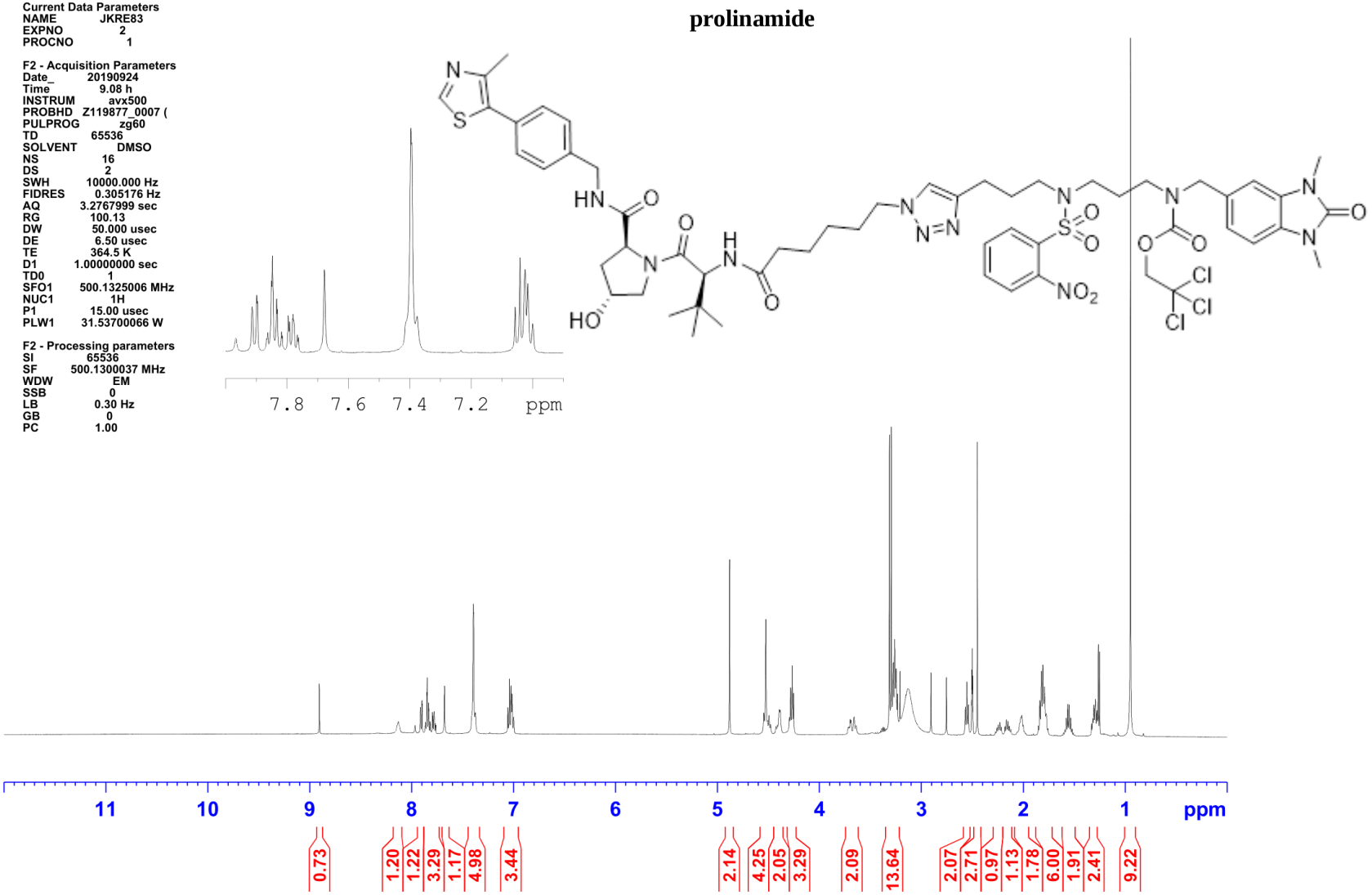
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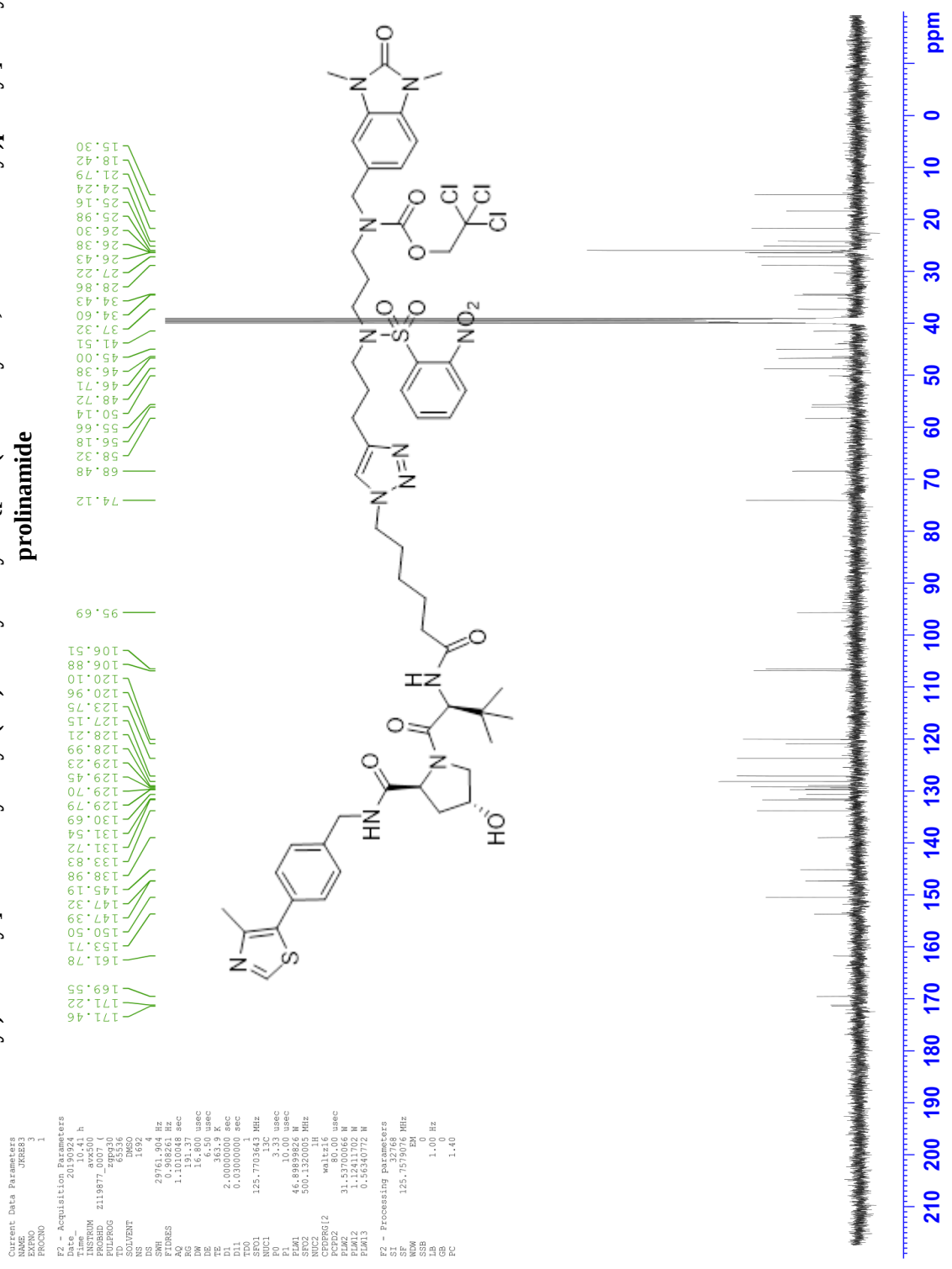
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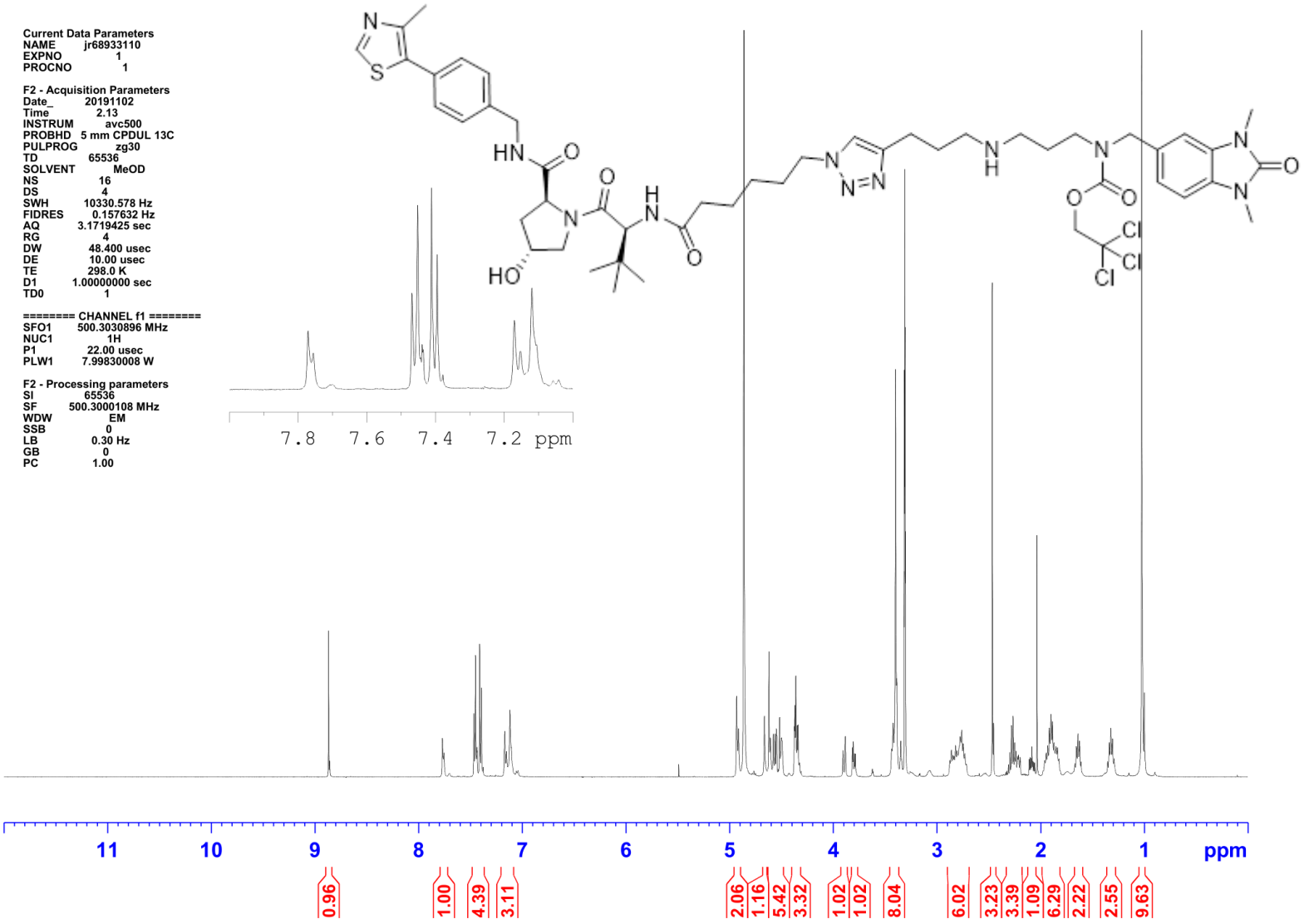
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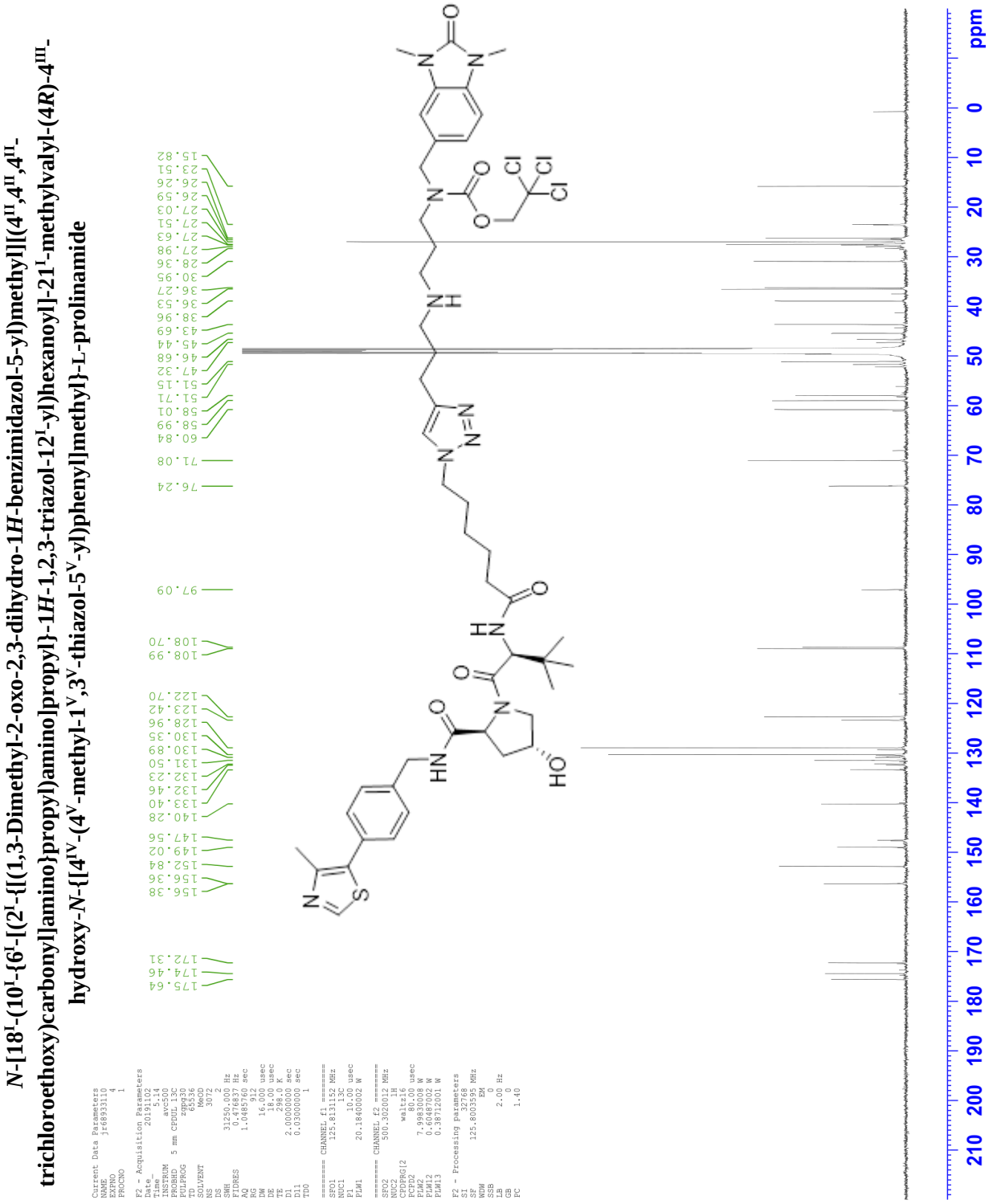
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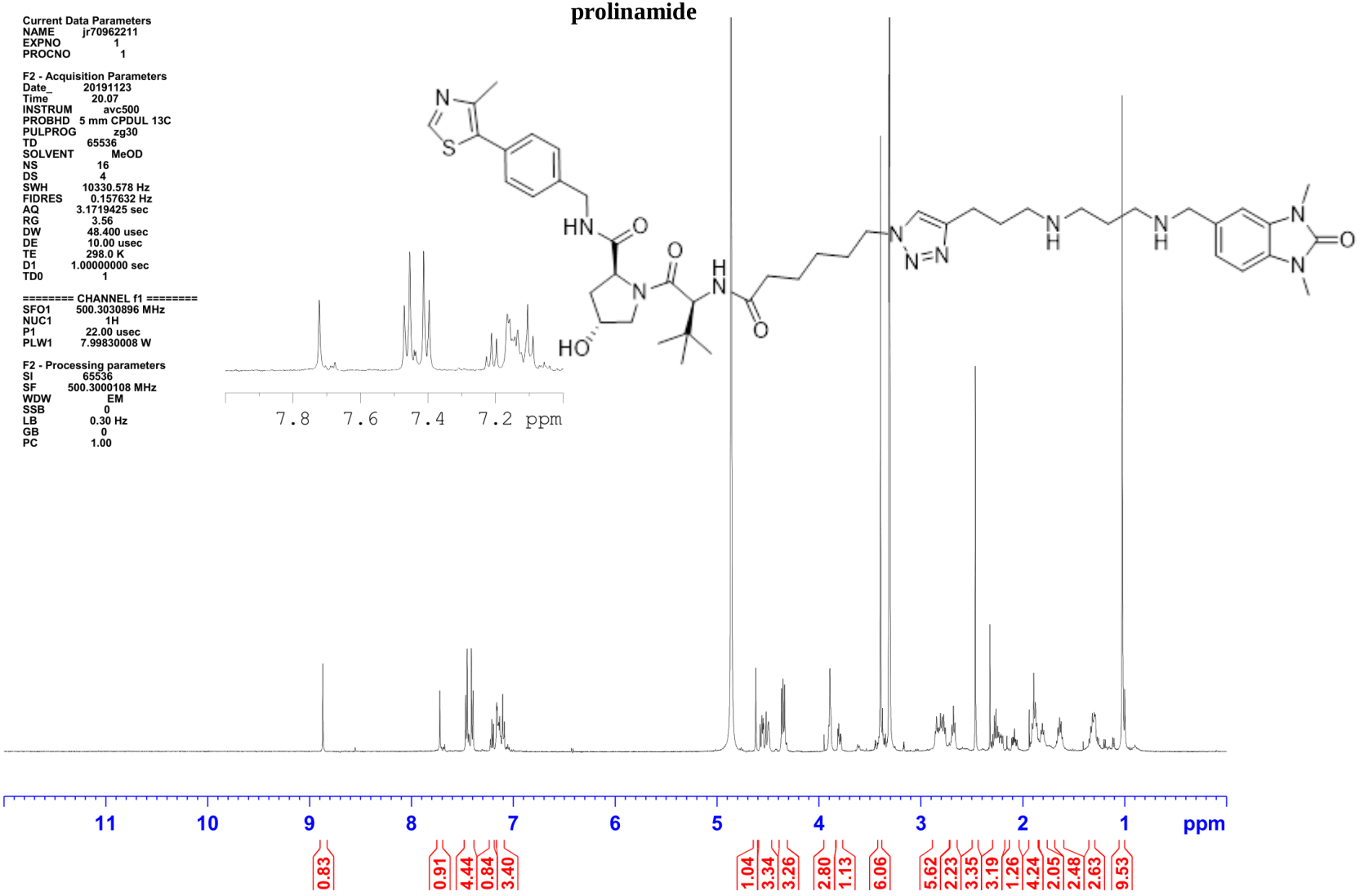
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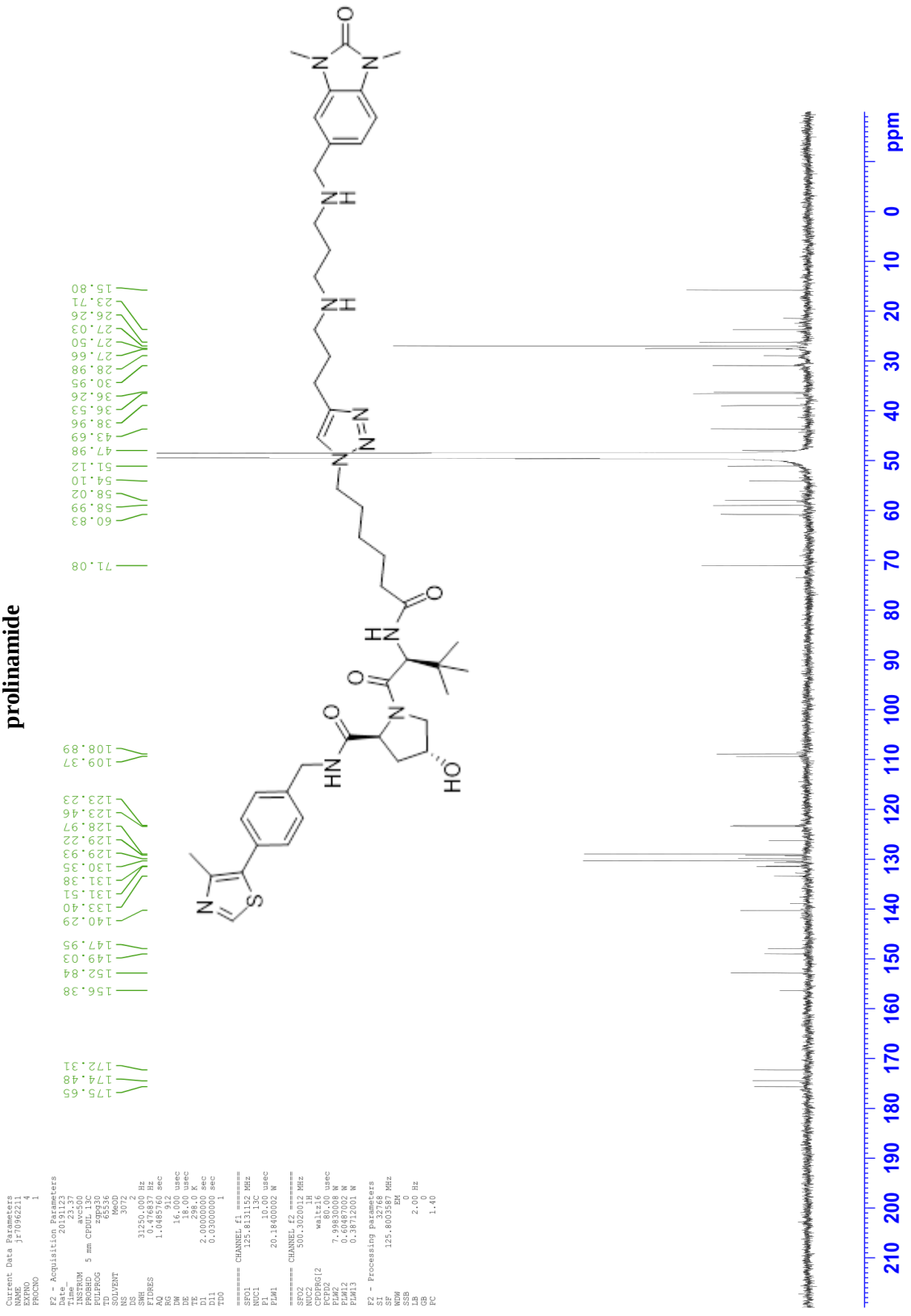
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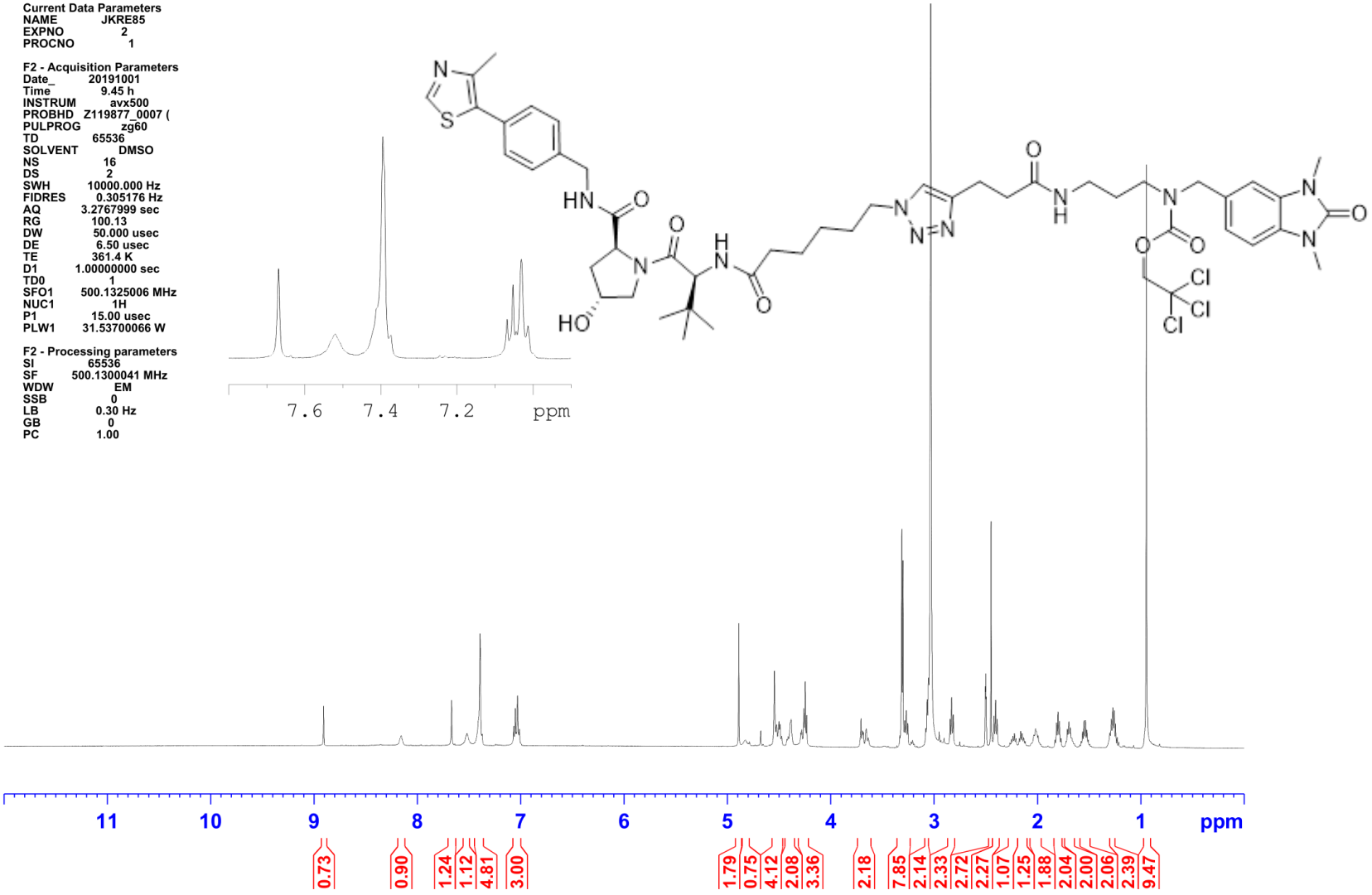
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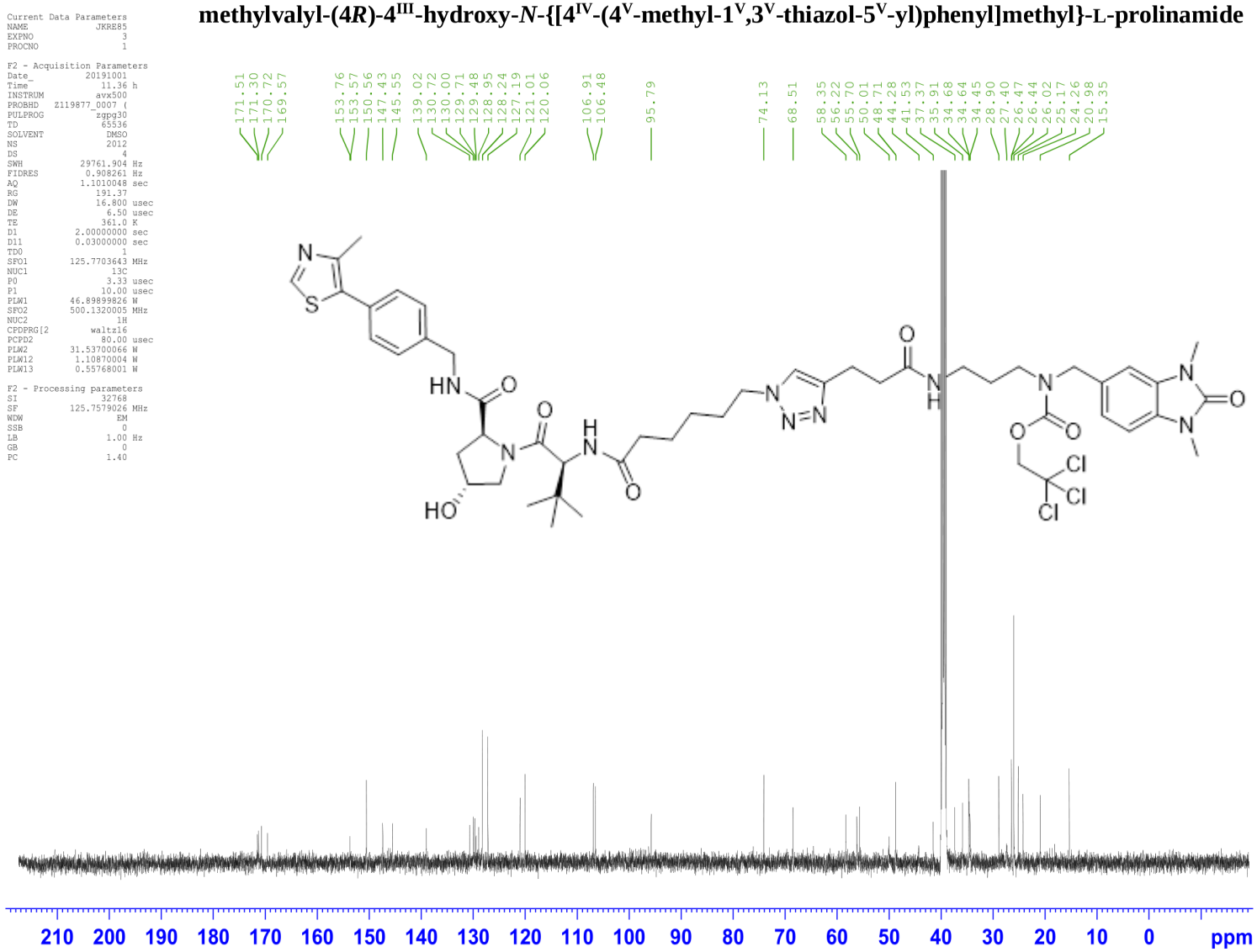
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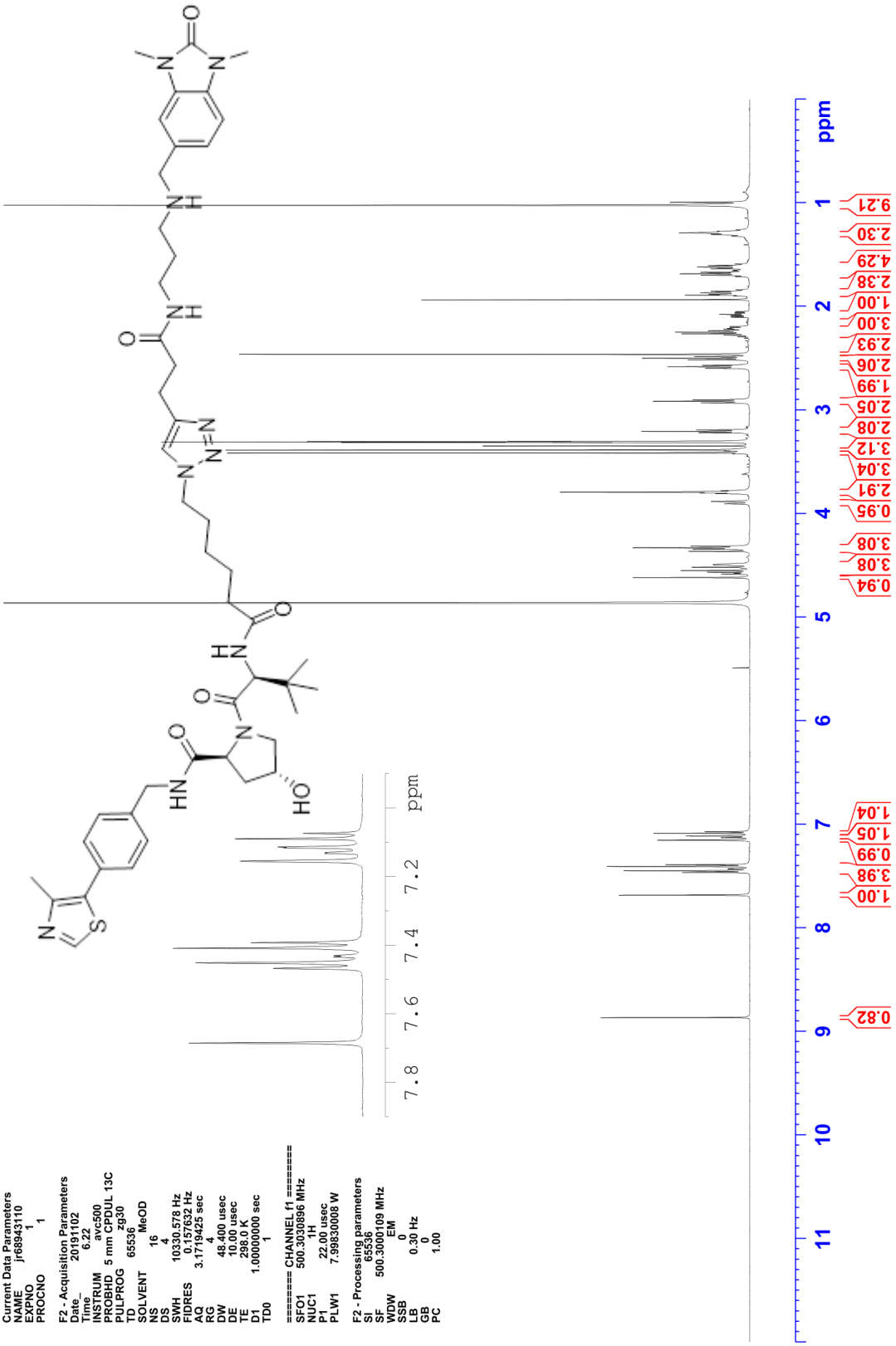
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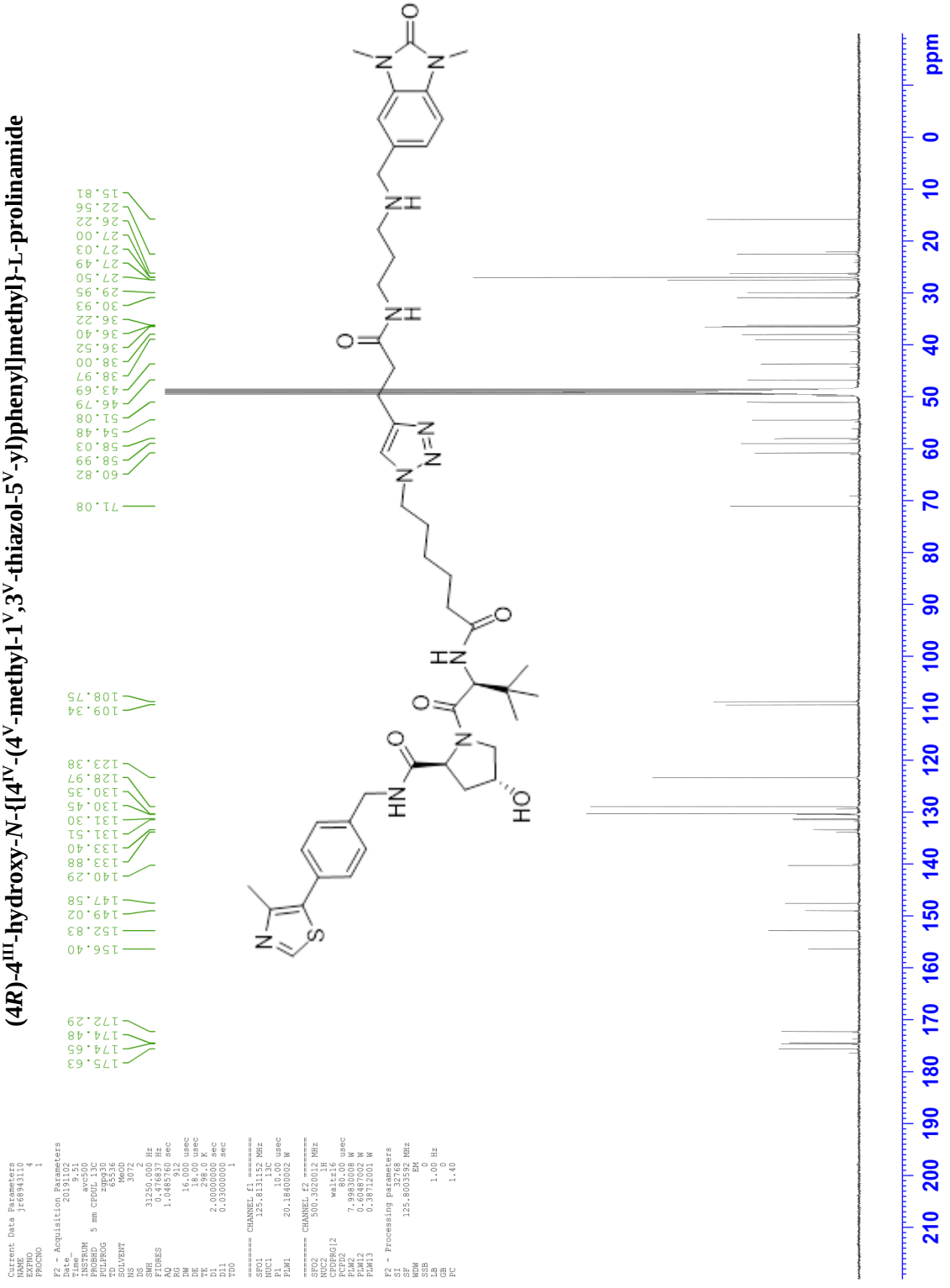
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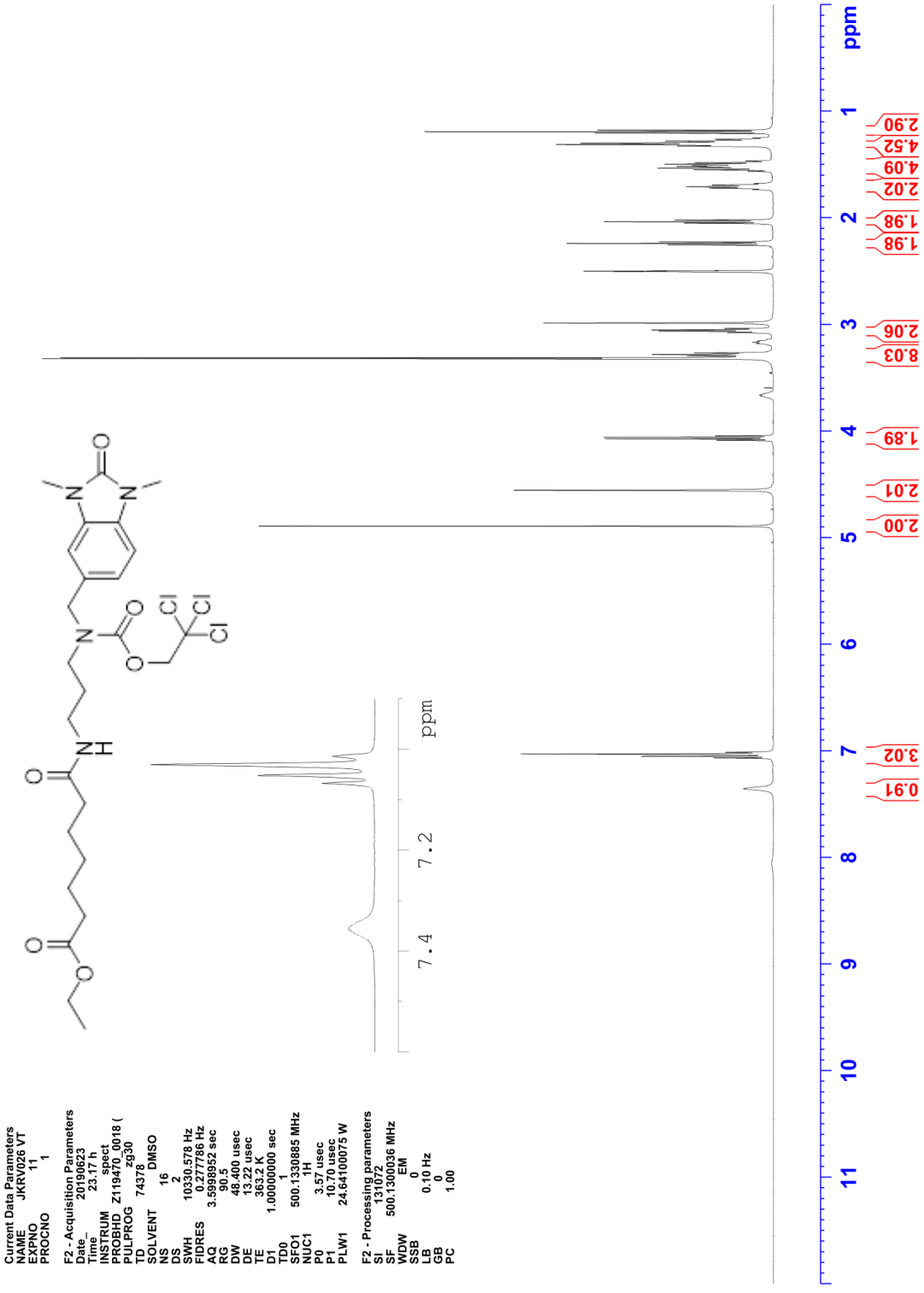
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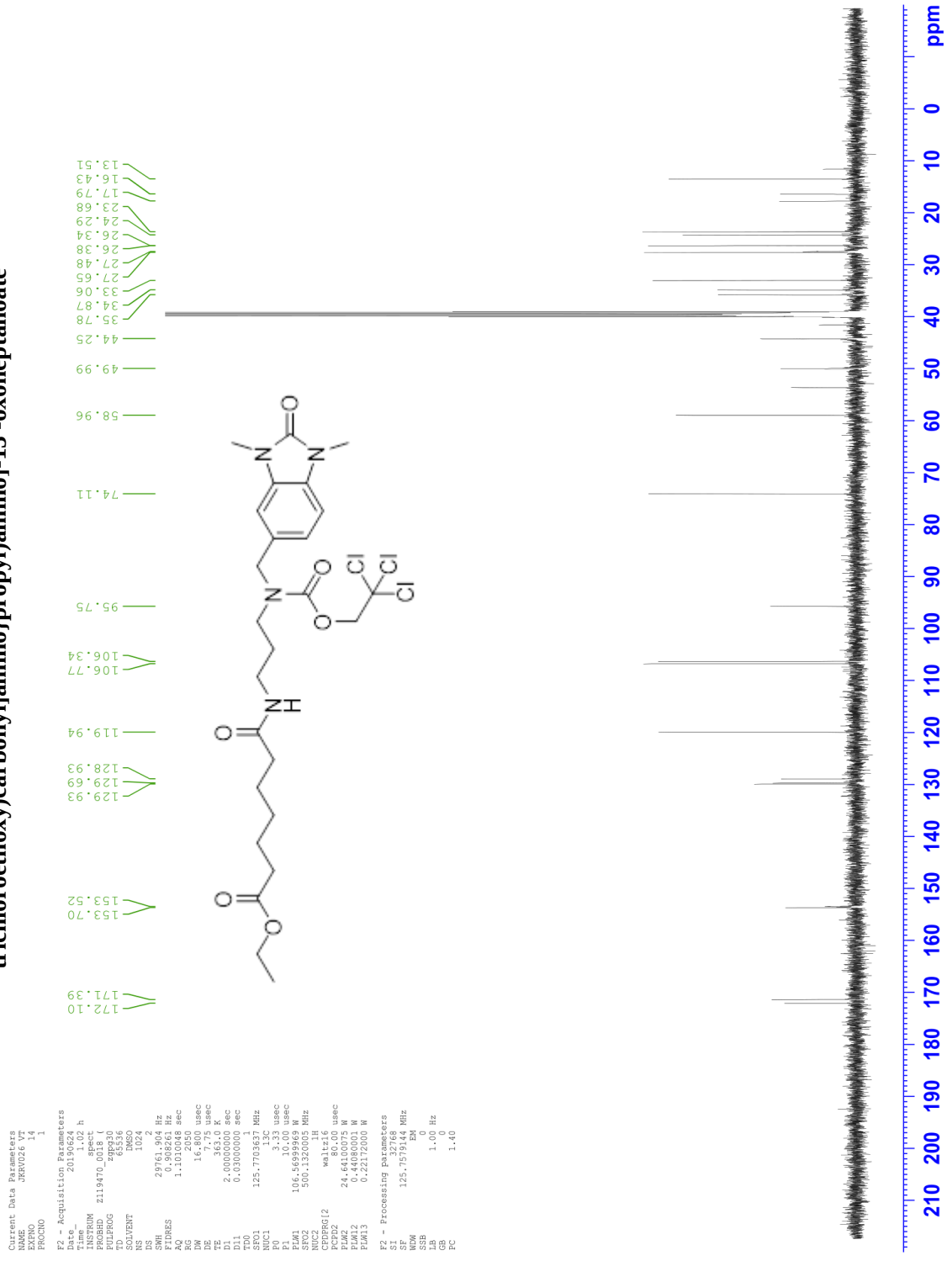
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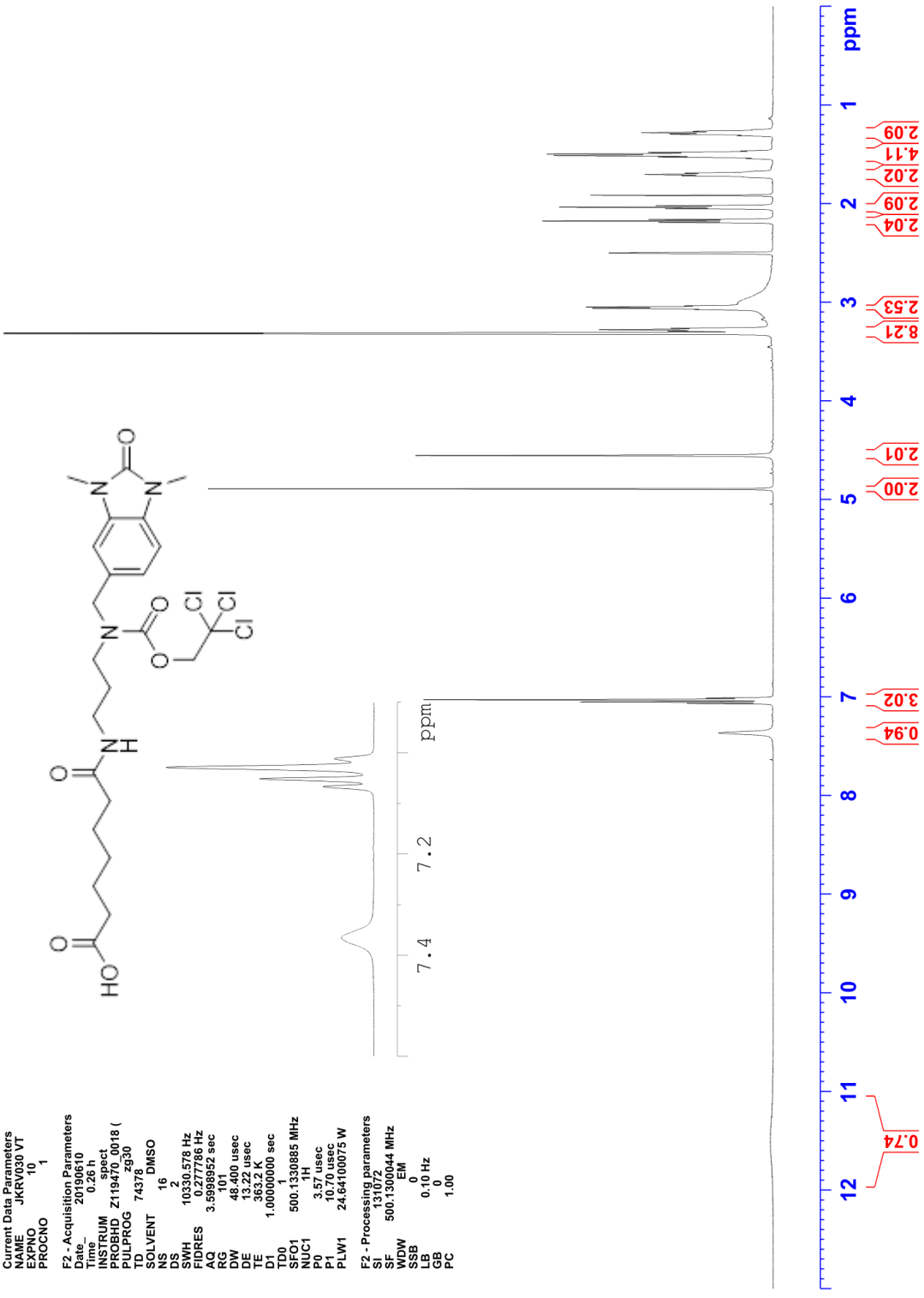
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Ethyl 7^{1}-[(2^{1}-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-13^{1}-oxoheptanoate



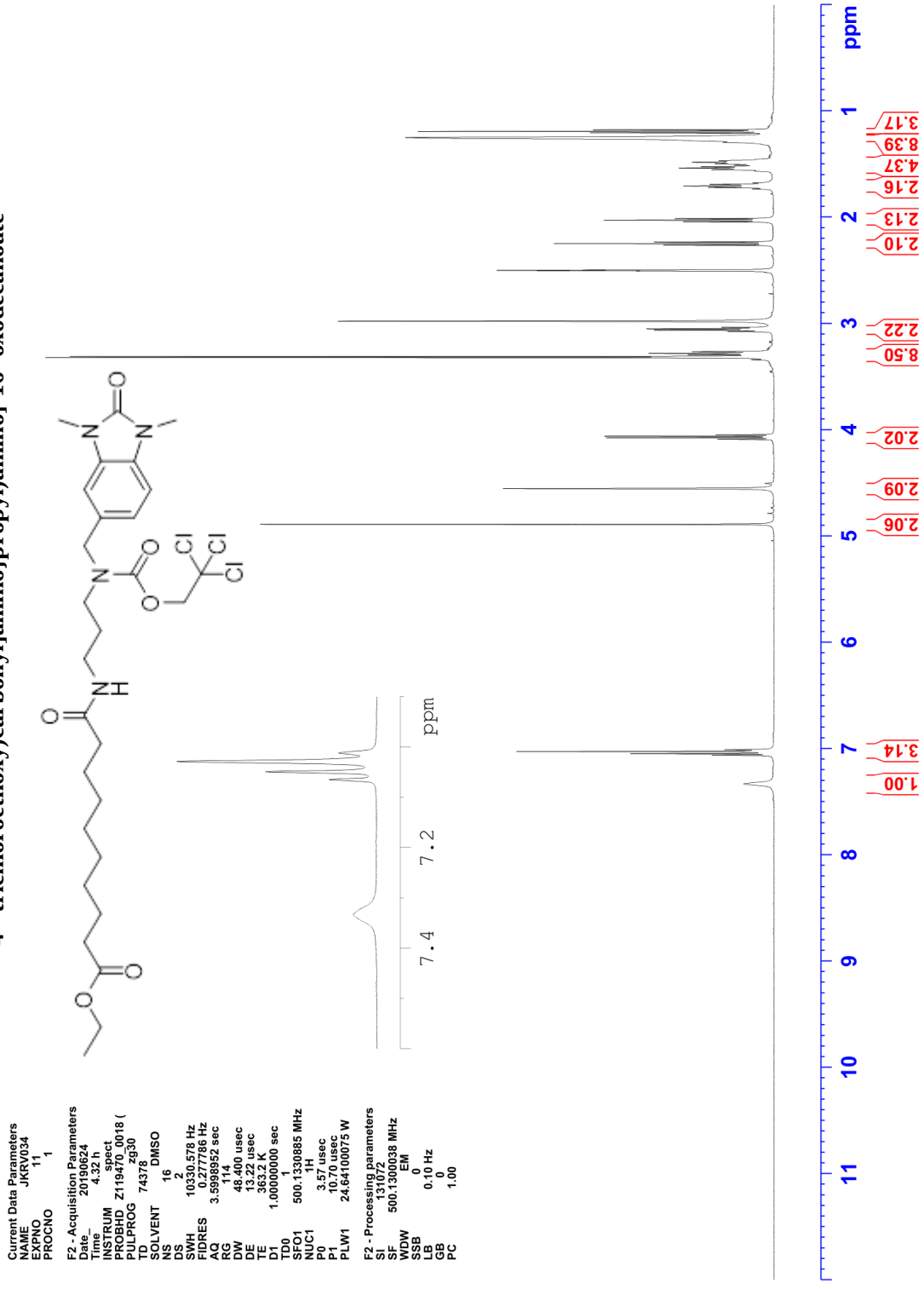
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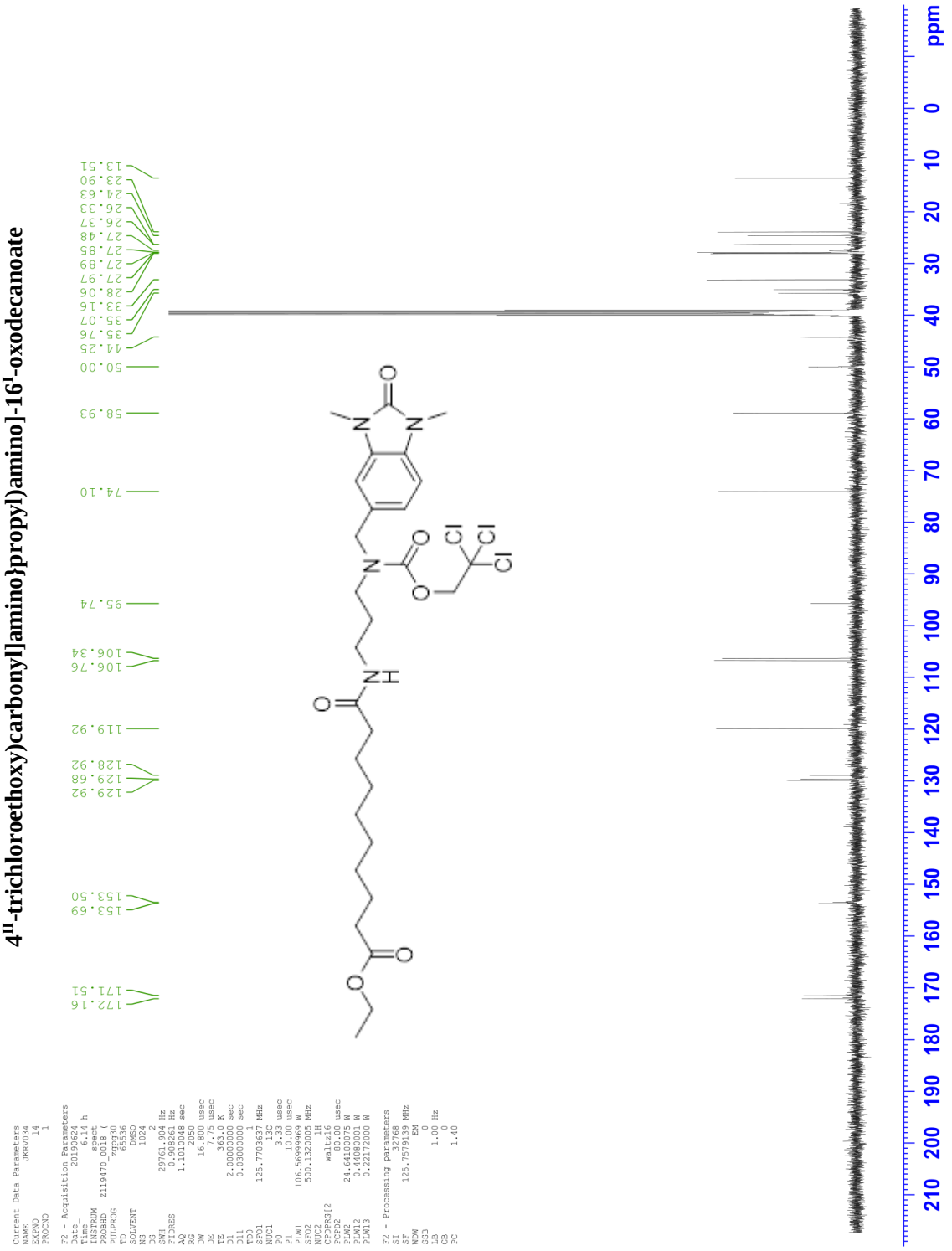
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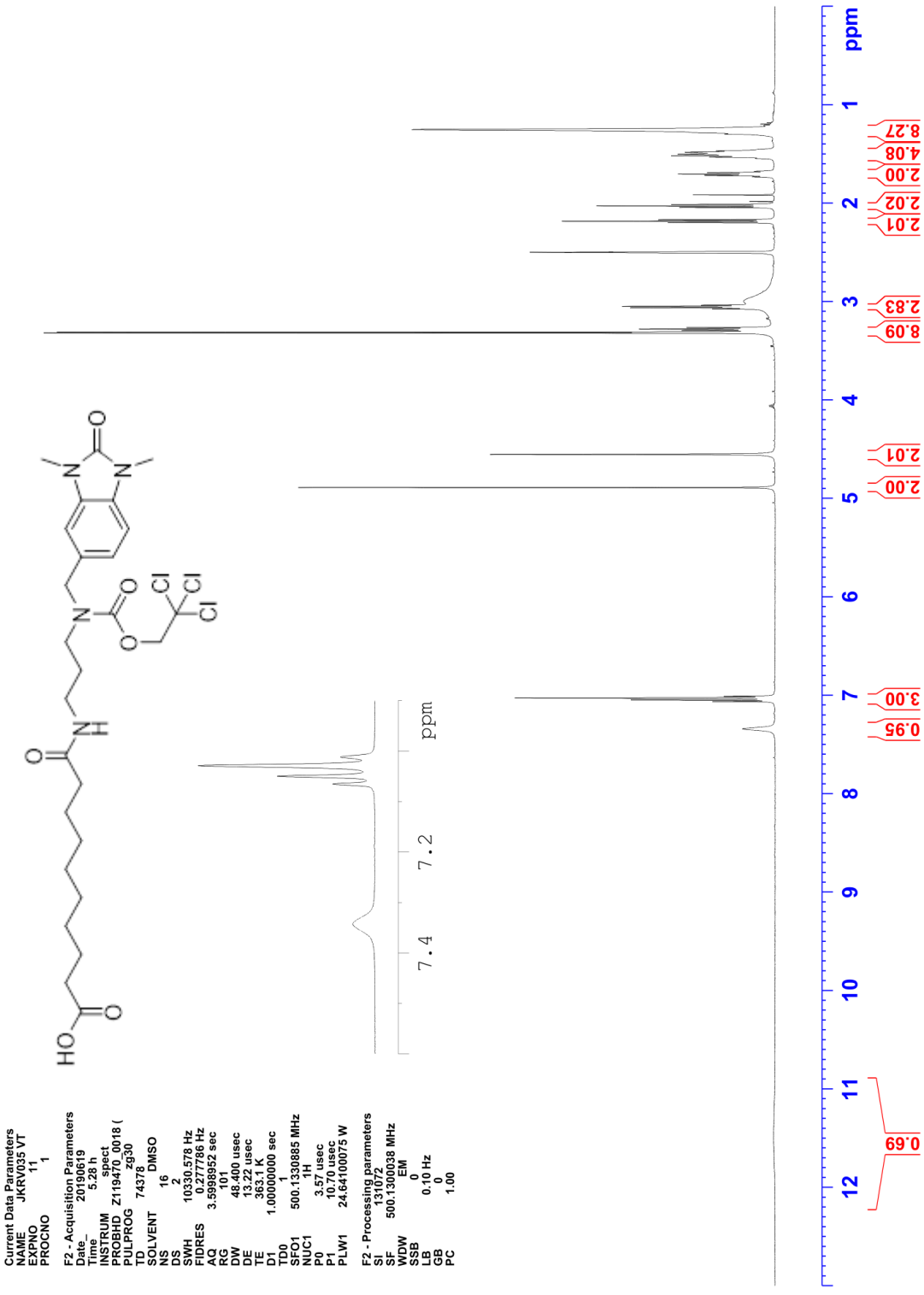
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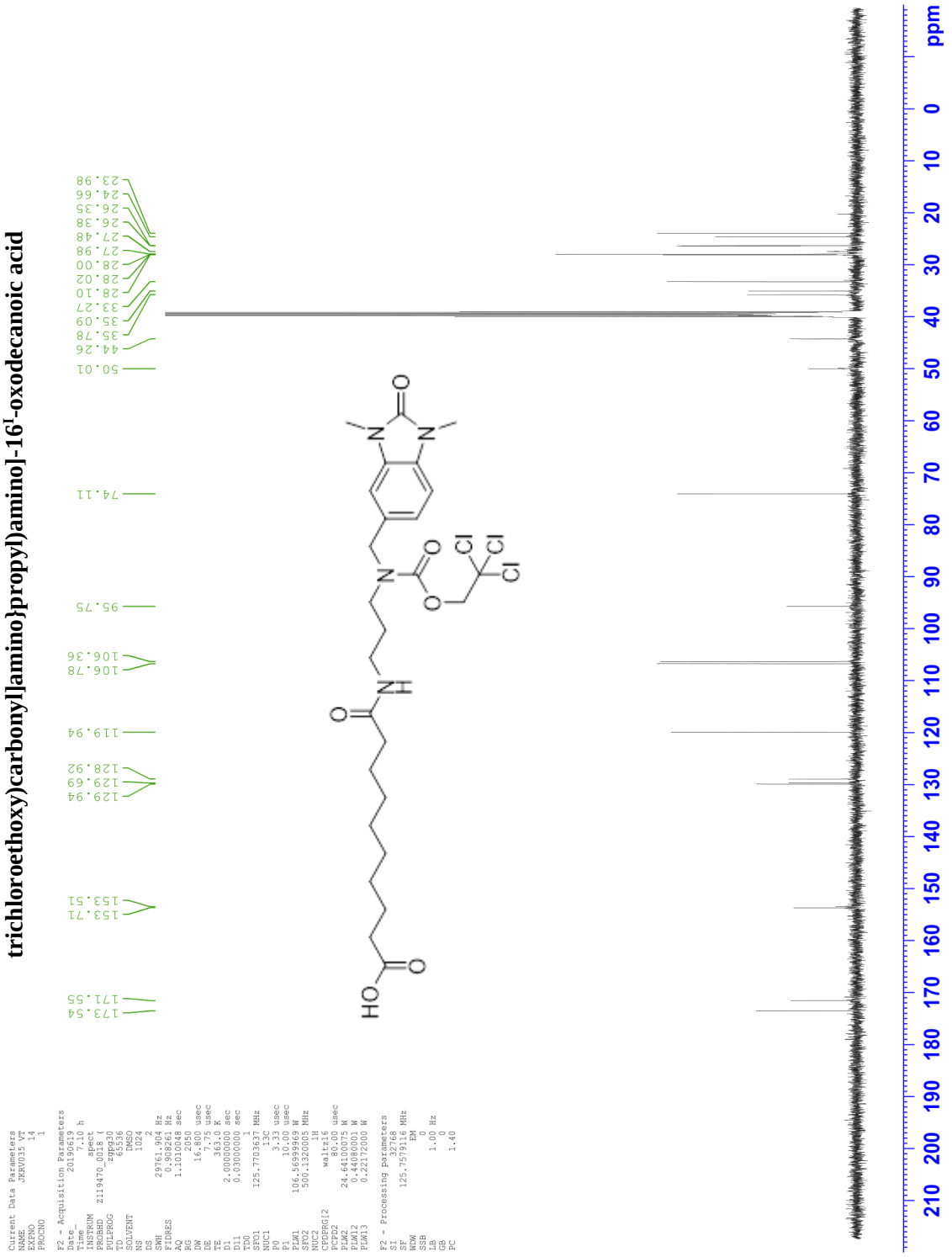
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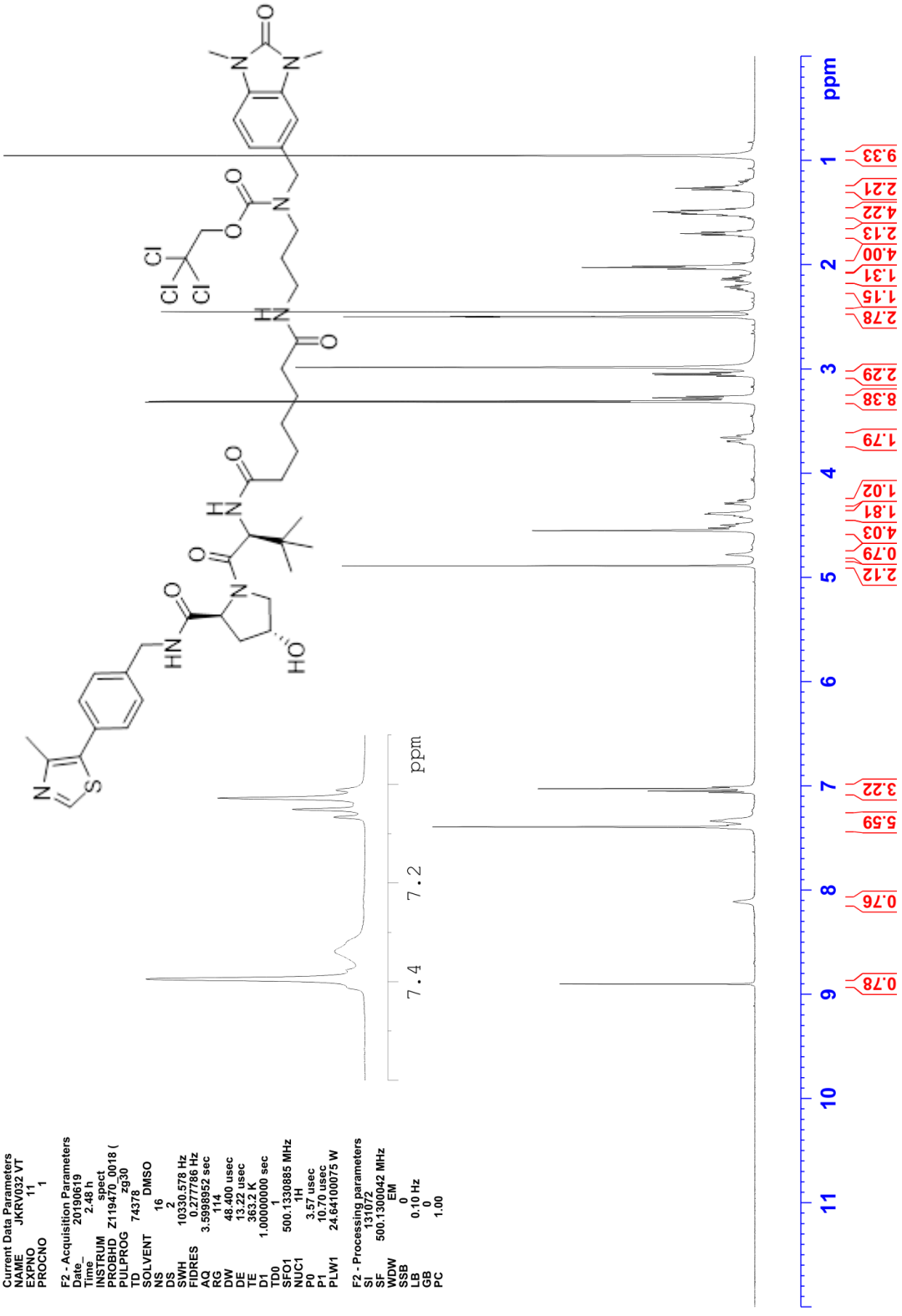
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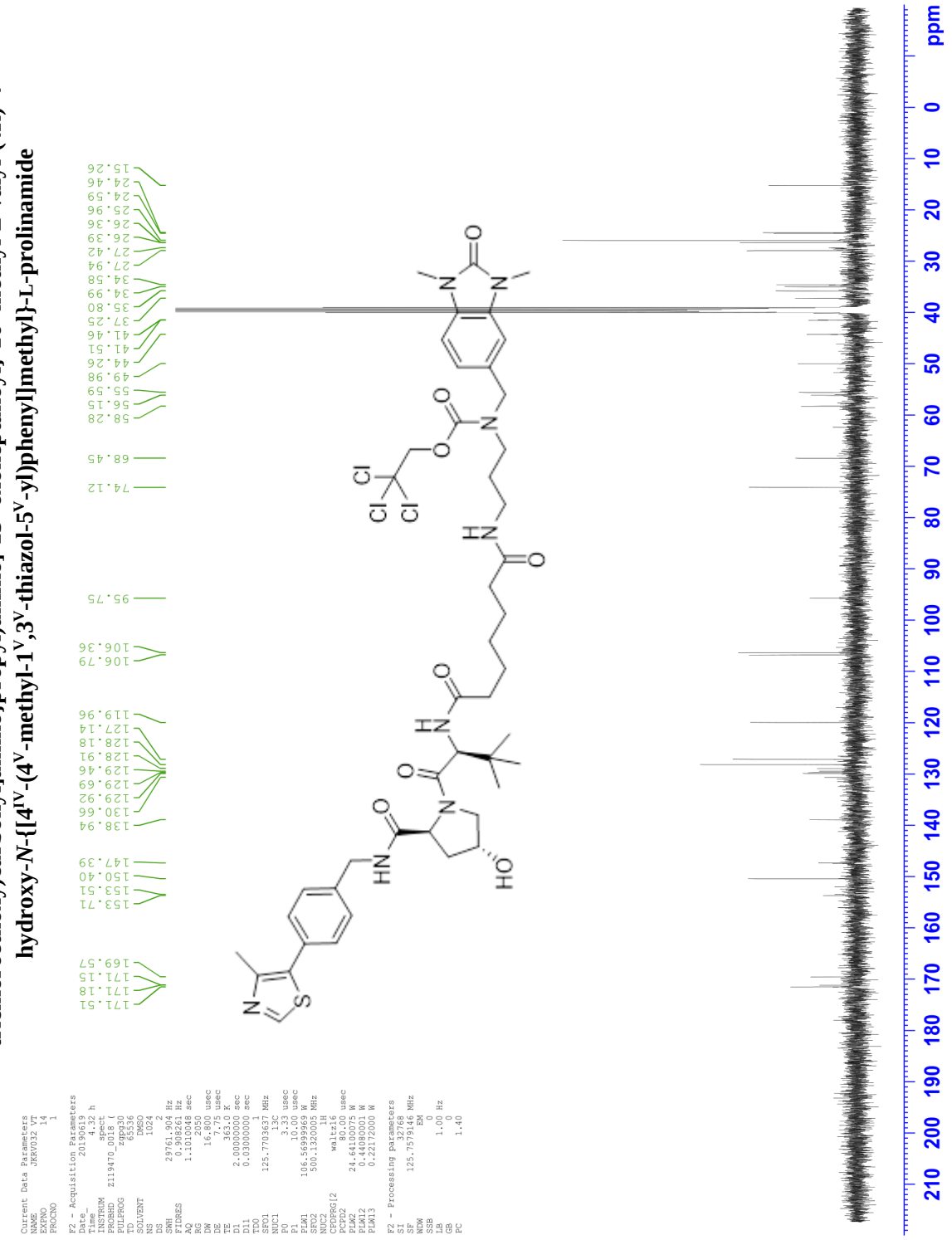


7^{l}-[(2^{l}-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II}, 4^{II}, 4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-16^{l}-oxodecanoic acid

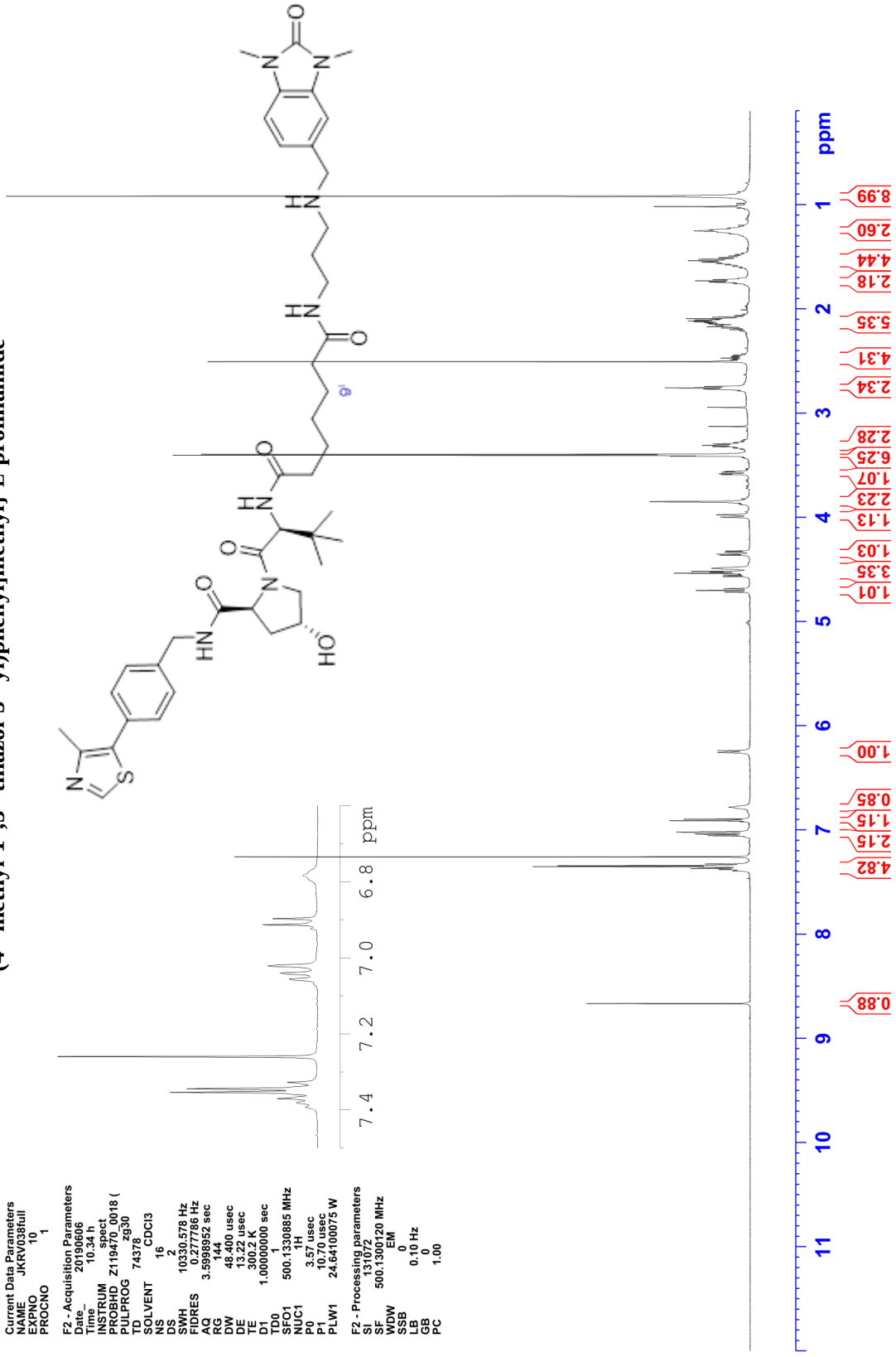


***N*-{7^{I}-[(2^{I}-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-13^{I}-oxoheptanoyl}-16^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide**

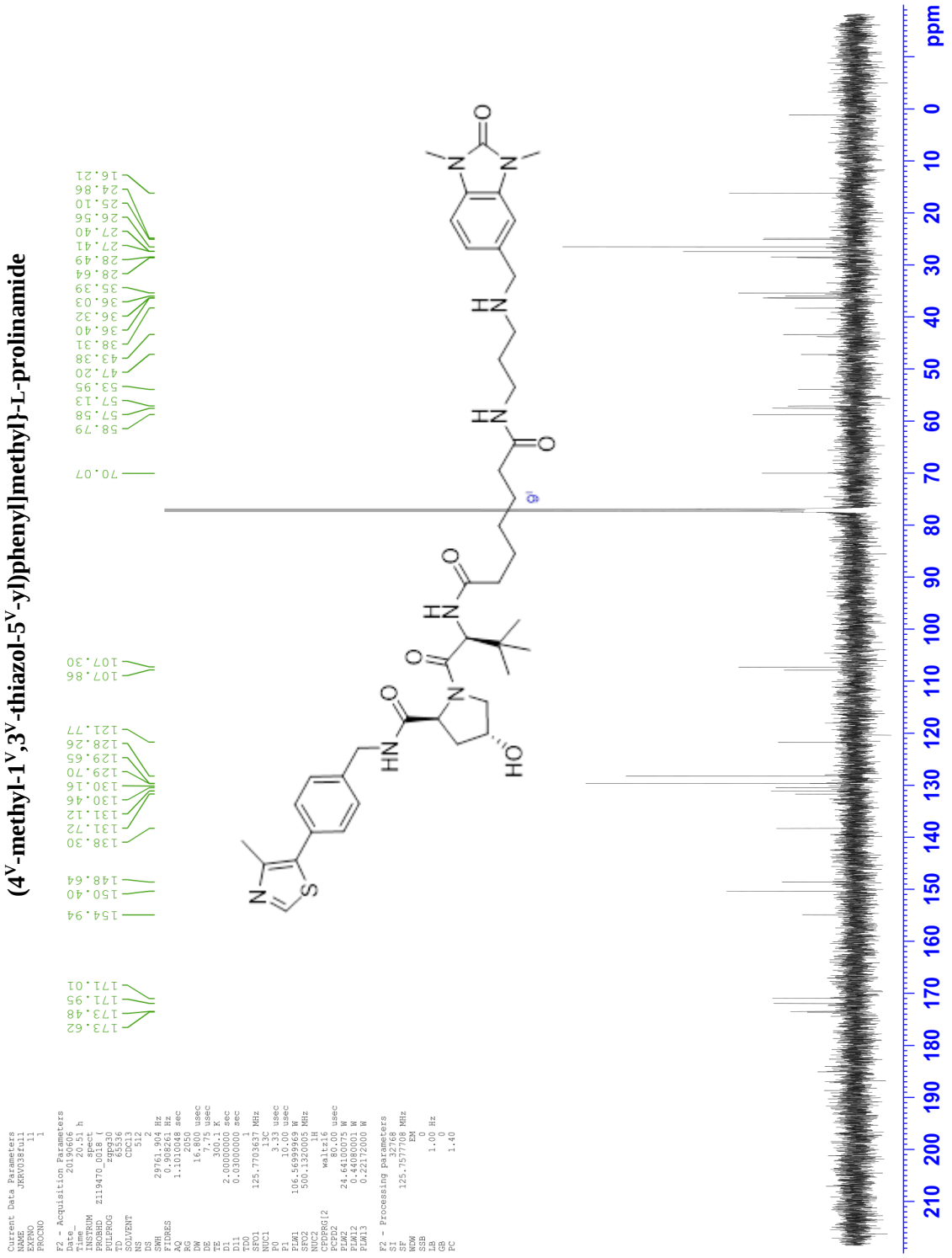



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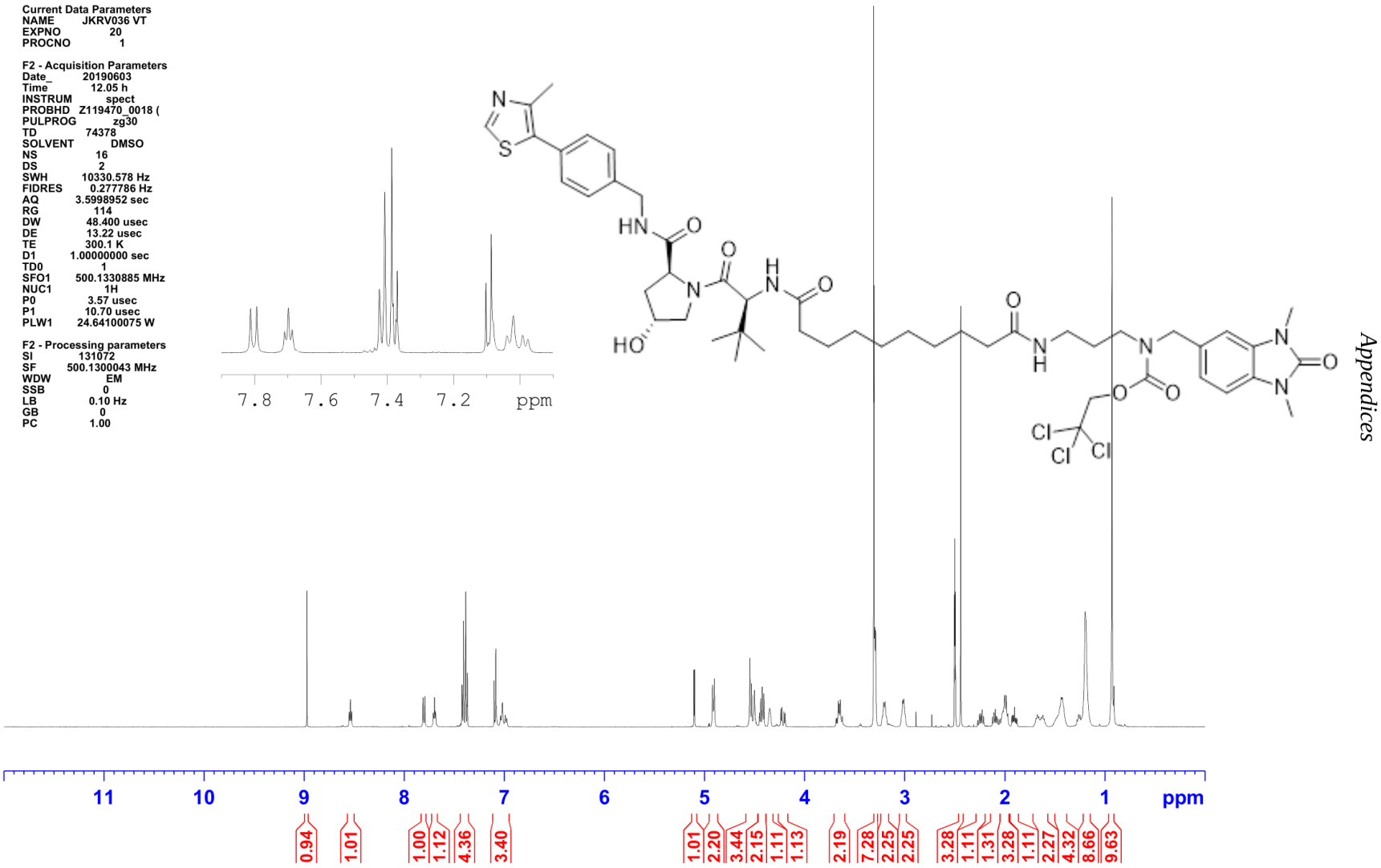
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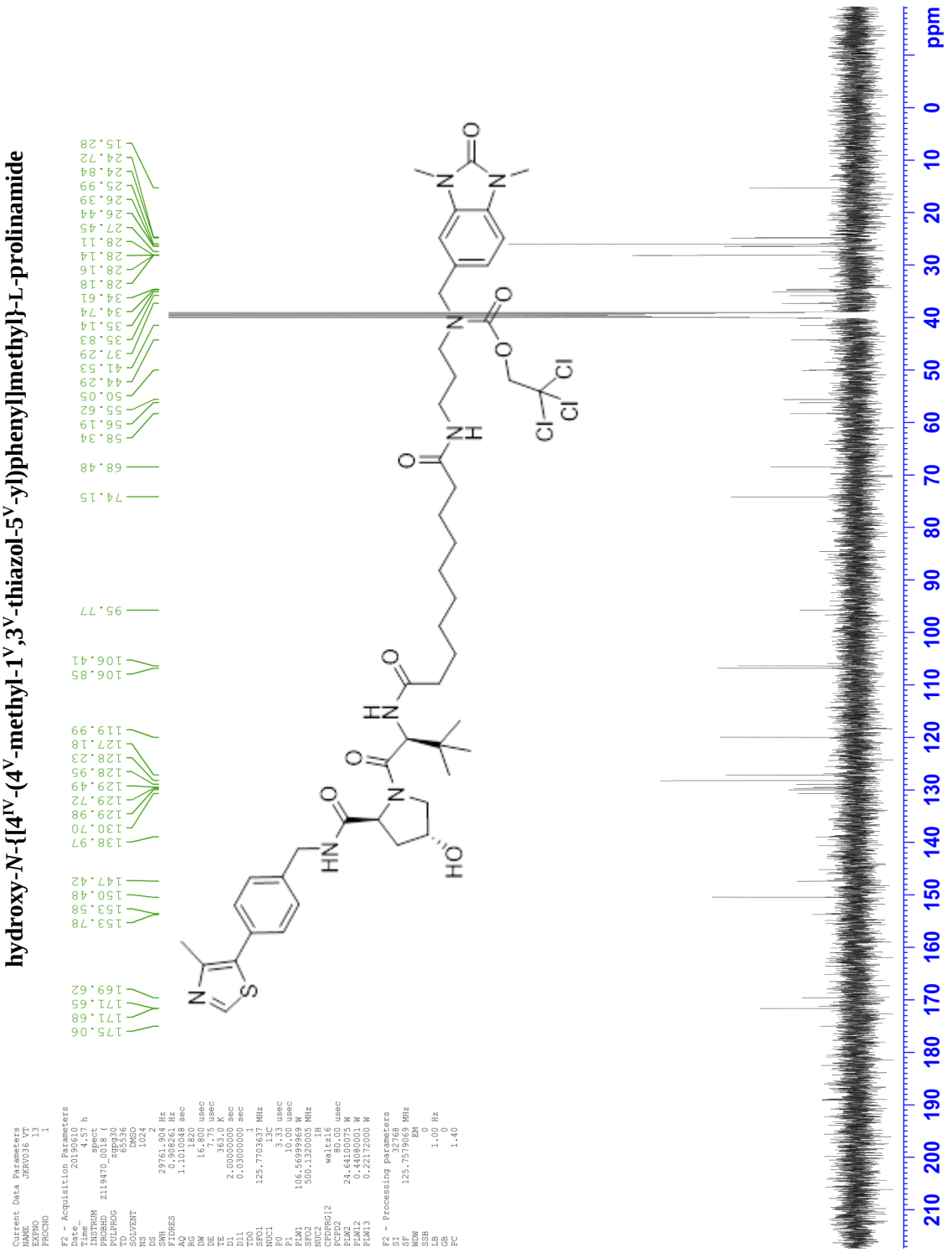
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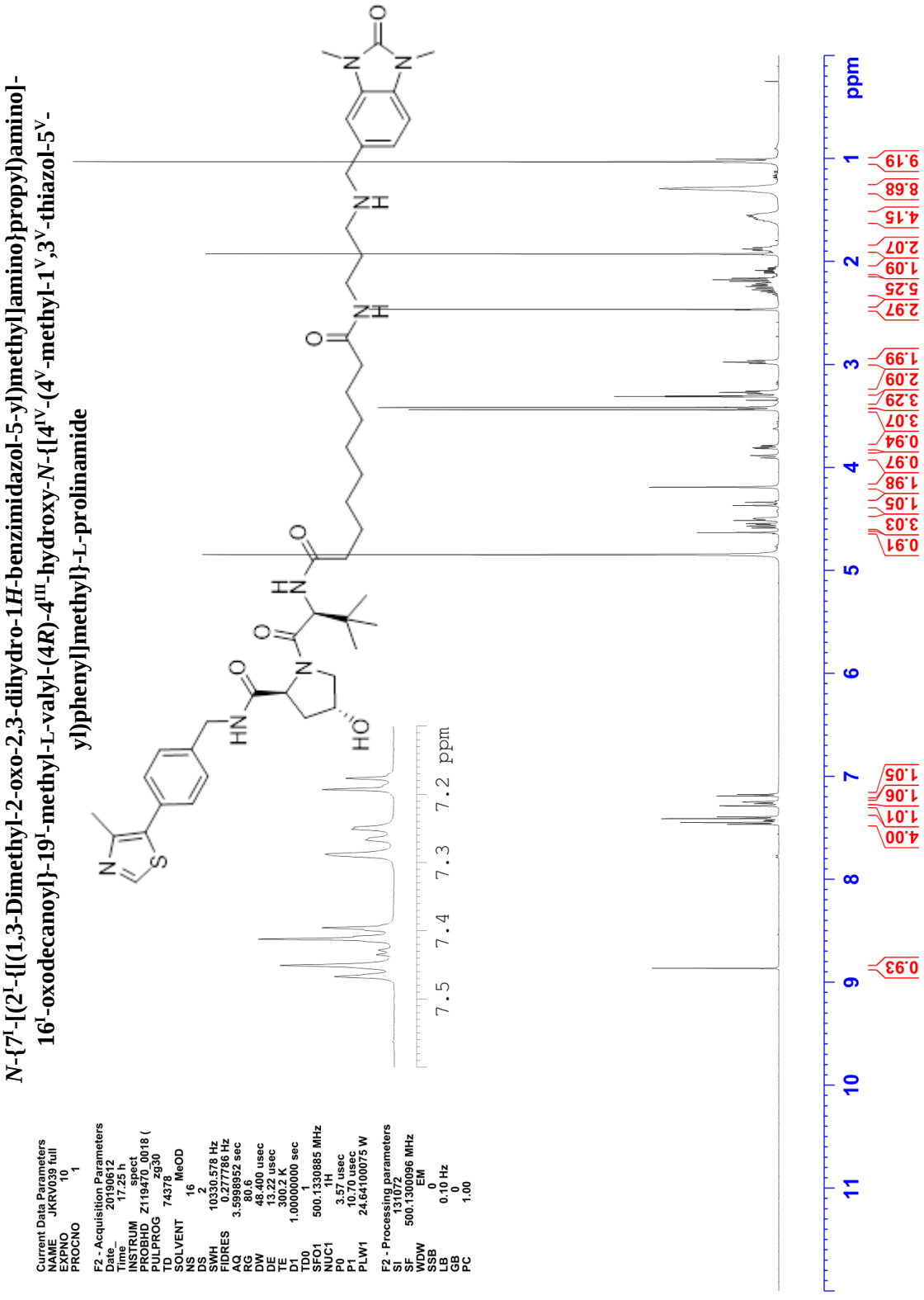


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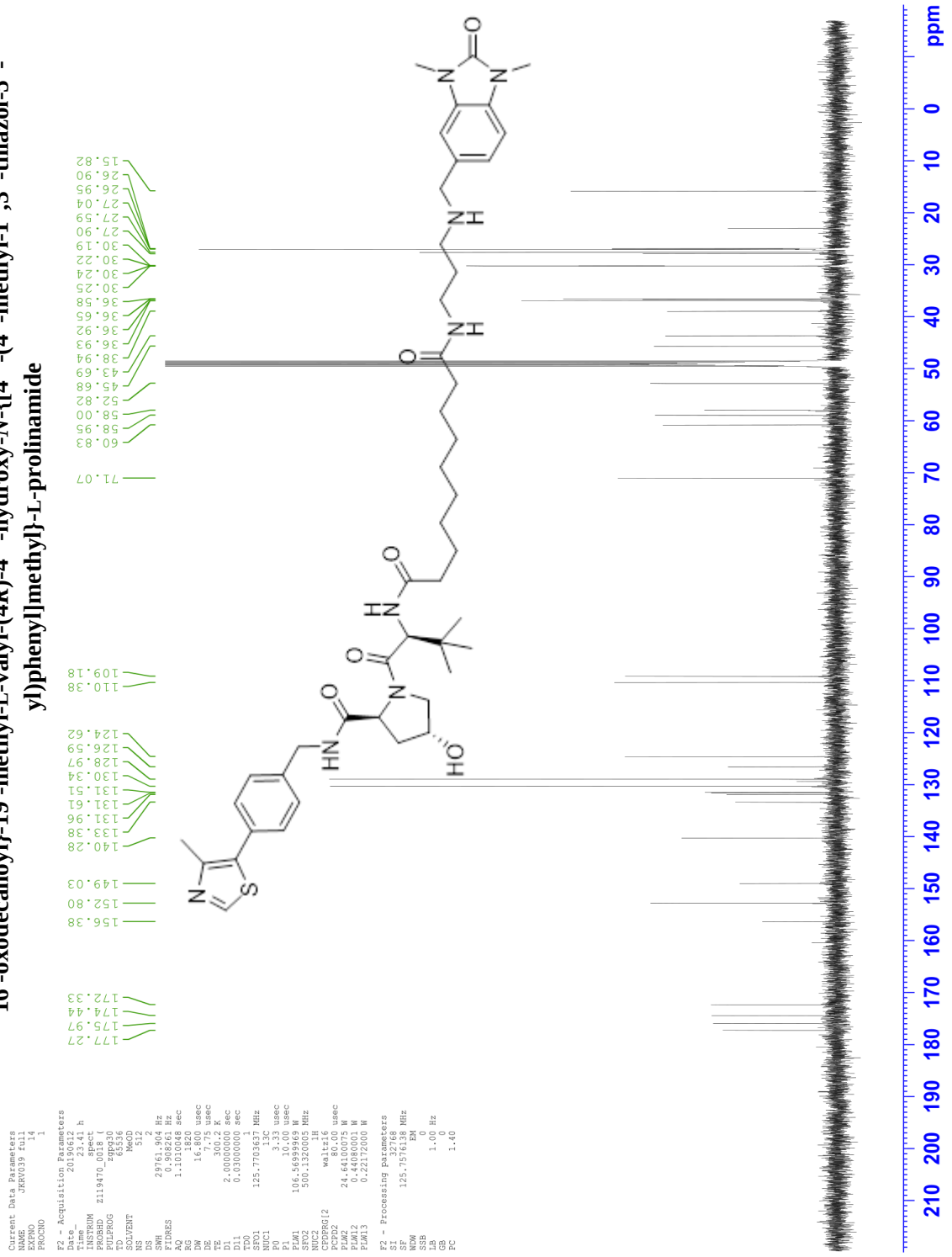


N-{7^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]}(4^{II},4^{II},4^{II}-trichloroethoxy)carbonyl]amino}propyl)amino]-16^{I}-oxodecanoyl}-19^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-N-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide

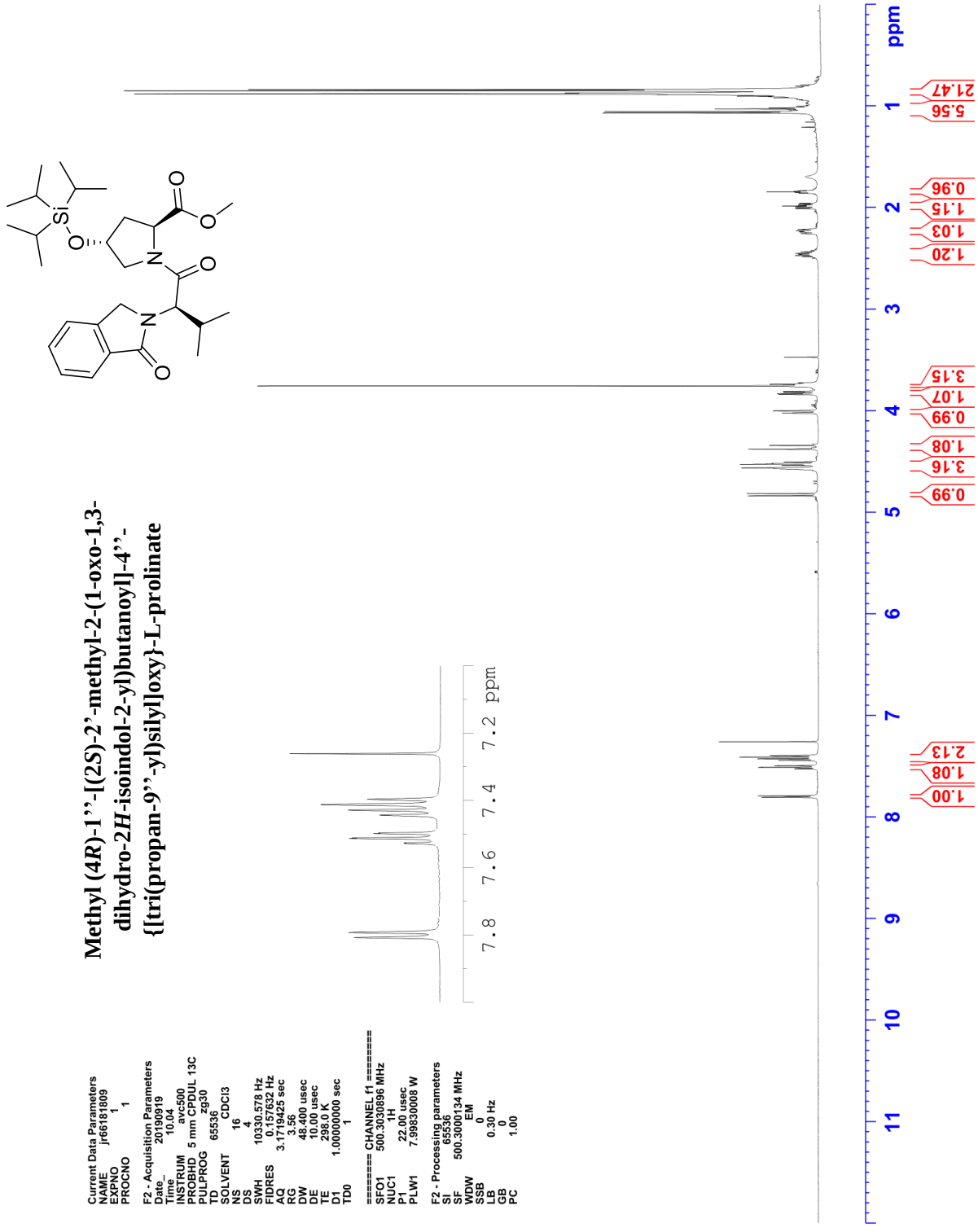



N-{7[1]-[(2[1]-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl]amino}propyl)amino]-16[1]-oxodecanoyl}-19[1]-methyl-L-valyl-(4R)-4[III]-hydroxy-N-{[4[IV]-(4[V]-methyl-1[V],3[V]-thiazol-5[V]-yl)phenyl]methyl}-L-prolinamide
Current Data Parameters
NAME JKRV039 full
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20190612
Time 17.25 h
INSTRUM spect
PROBHD Z119470_0018 (
PULPROG zg30
TD 74378
SOLVENT MeOD
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.277786 Hz
AQ 3.5998952 sec
RG 90.5
DW 48.400 usec
DE 13.22 usec
TE 302.2 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330885 MHz
NUC1 1H
P0 3.57 usec
P1 10.70 usec
PLW1 24.64100075 W
F2 - Processing parameters
SI 131072
SF 500.1300096 MHz
WDW EM
SSB 0
LB 0.10 Hz
GB 0
PC 1.00
7.5 7.4 7.3 7.2 ppm
ppm
0.93
4.00
1.01
1.06
1.05
0.91
3.03
1.05
1.98
0.97
0.94
3.07
3.29
2.09
1.99
2.97
5.25
1.09
2.07
4.15
8.68
9.19

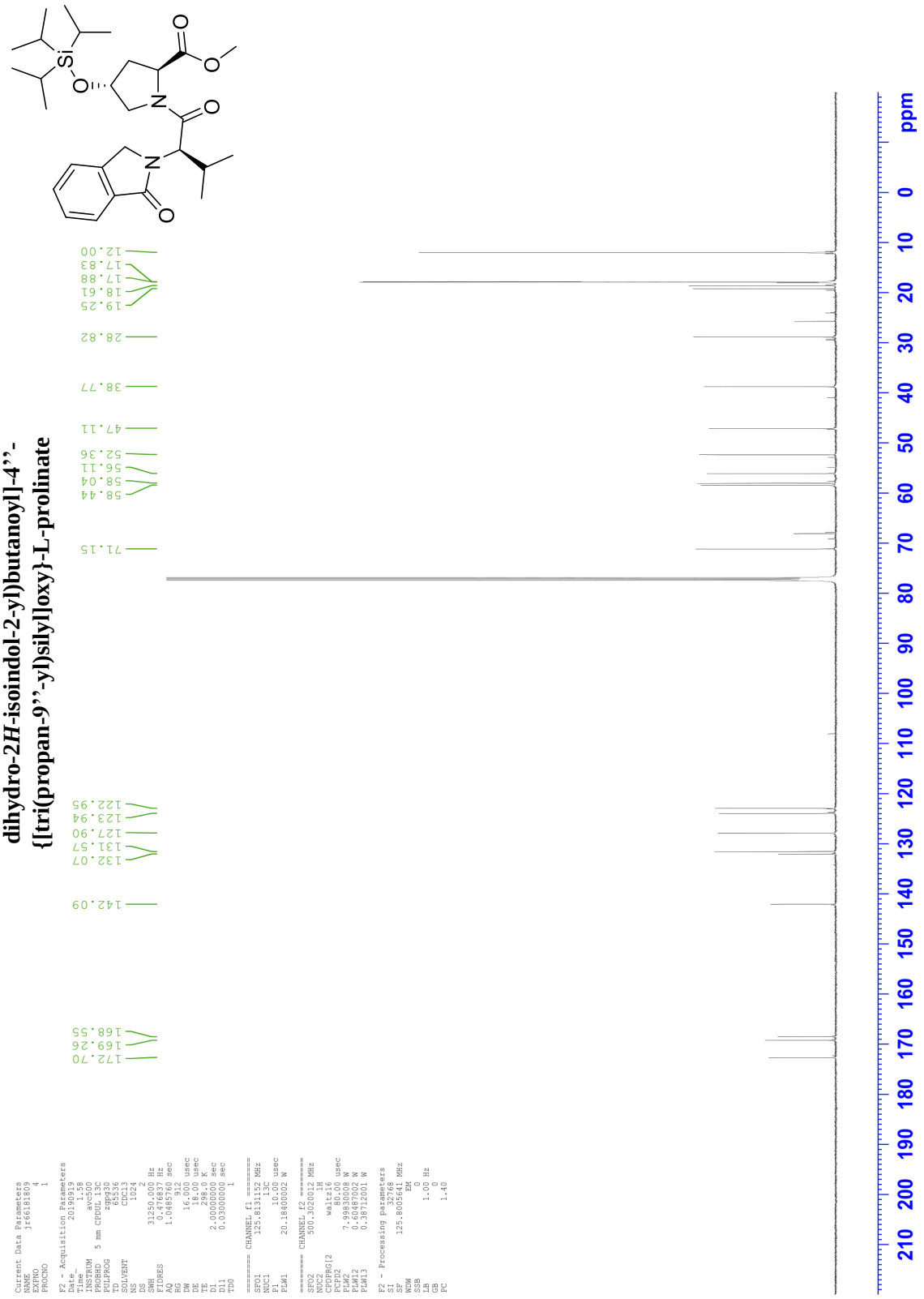
N-{7^{I}-[(2^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)amino]-16^{I}-oxodecanoyl}-19^{I}-methyl-L-valyl-(4*R*)-4^{III}-hydroxy-*N*-{[4^{IV}-(4^{V}-methyl-1^{V},3^{V}-thiazol-5^{V}-yl)phenyl]methyl}-L-prolinamide

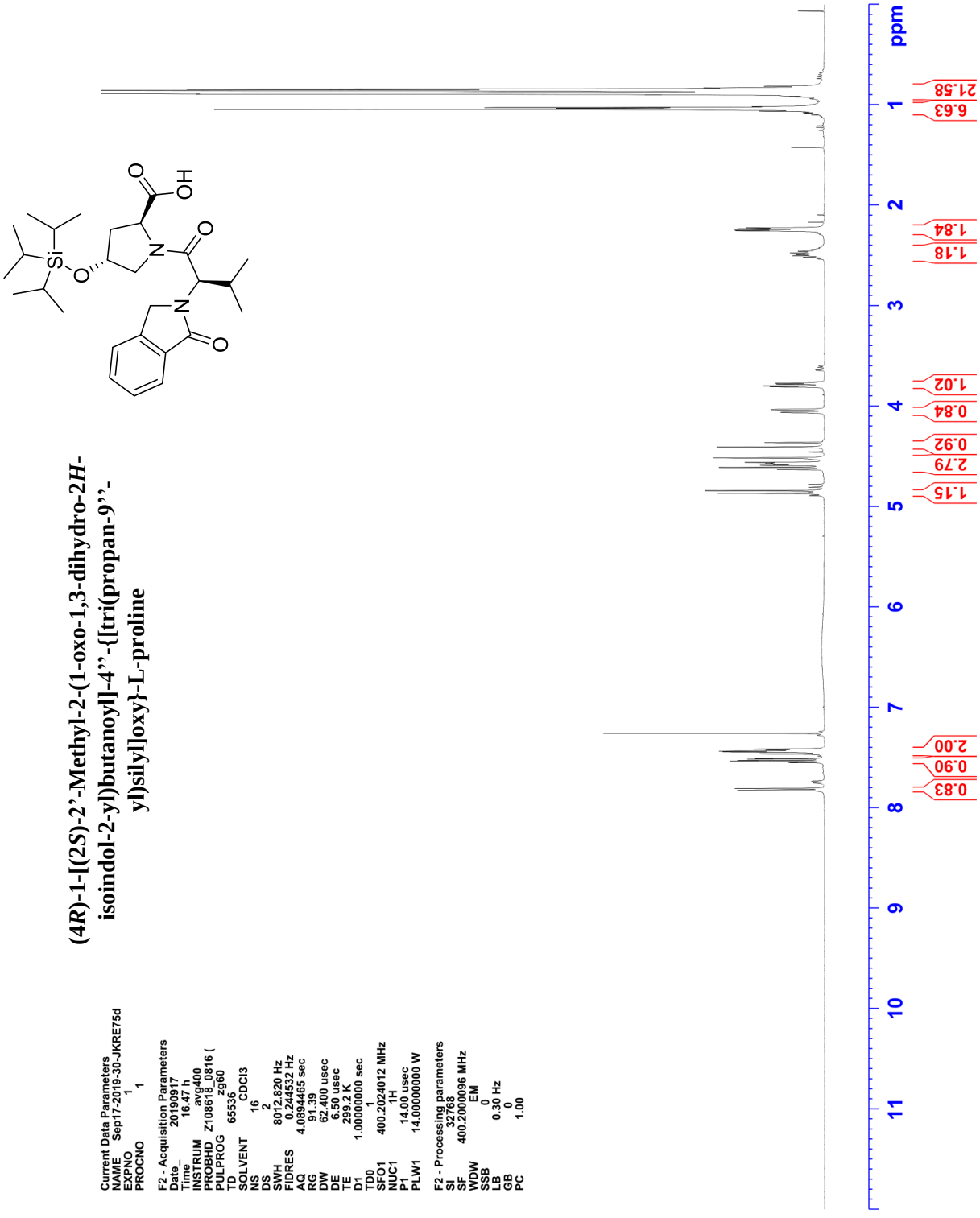


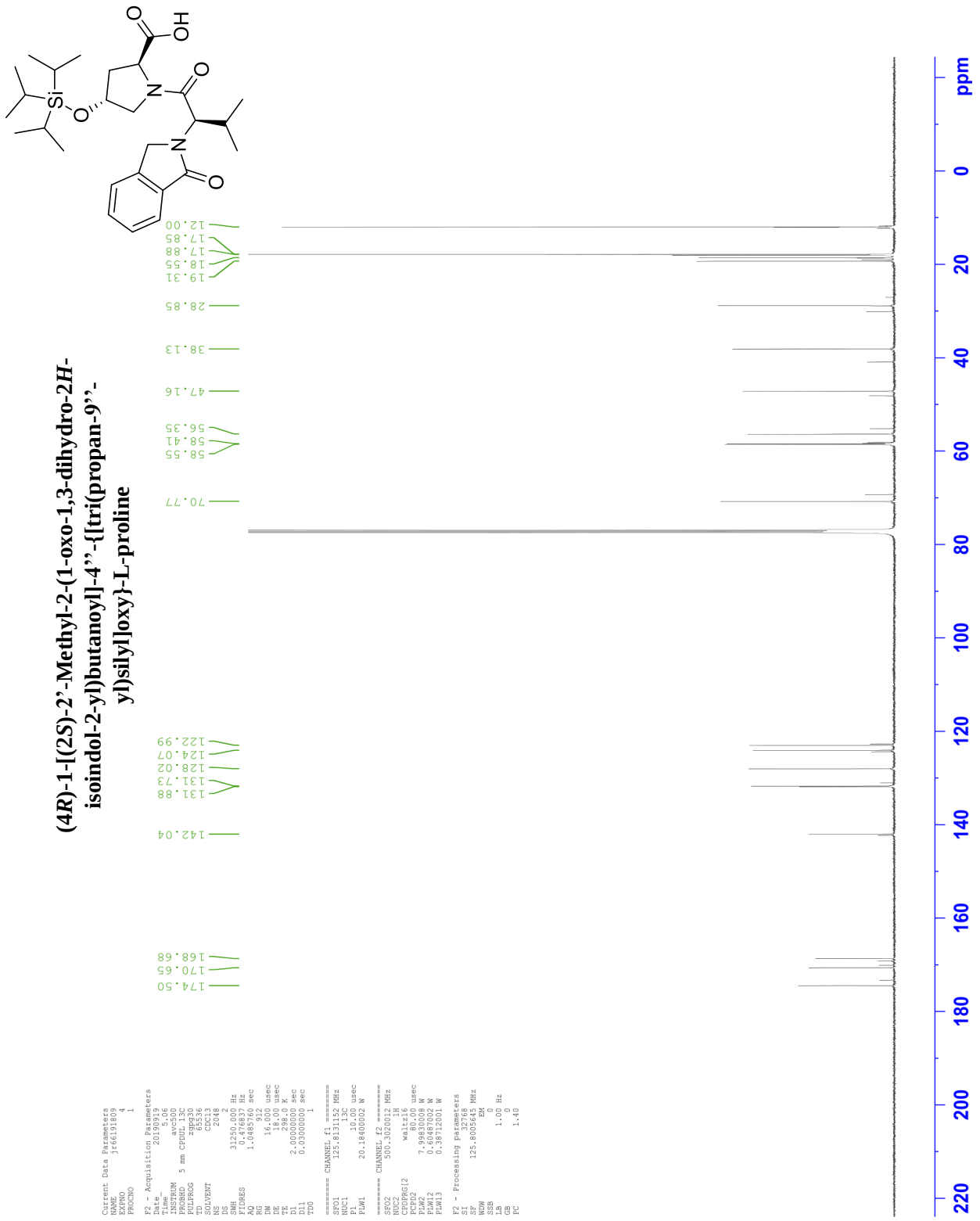
Chapter 4


Methyl (4R)-1''-[(2S)-2'-methyl-2-(1-oxo-1,3-dihydro-2H-isoindol-2-yl)butanoyl]-4''-{[tri(propan-9''-yl)silyl]oxy}-L-prolinate
Current Data Parameters
NAME jr66181609
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20190919
Time_ 10.04
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 3.56
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000134 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.8
7.6
7.4
7.2 ppm
ppm
1.00
1.08
2.13
0.99
3.16
1.08
0.99
1.07
3.15
1.20
1.03
1.15
0.96
5.56
21.47

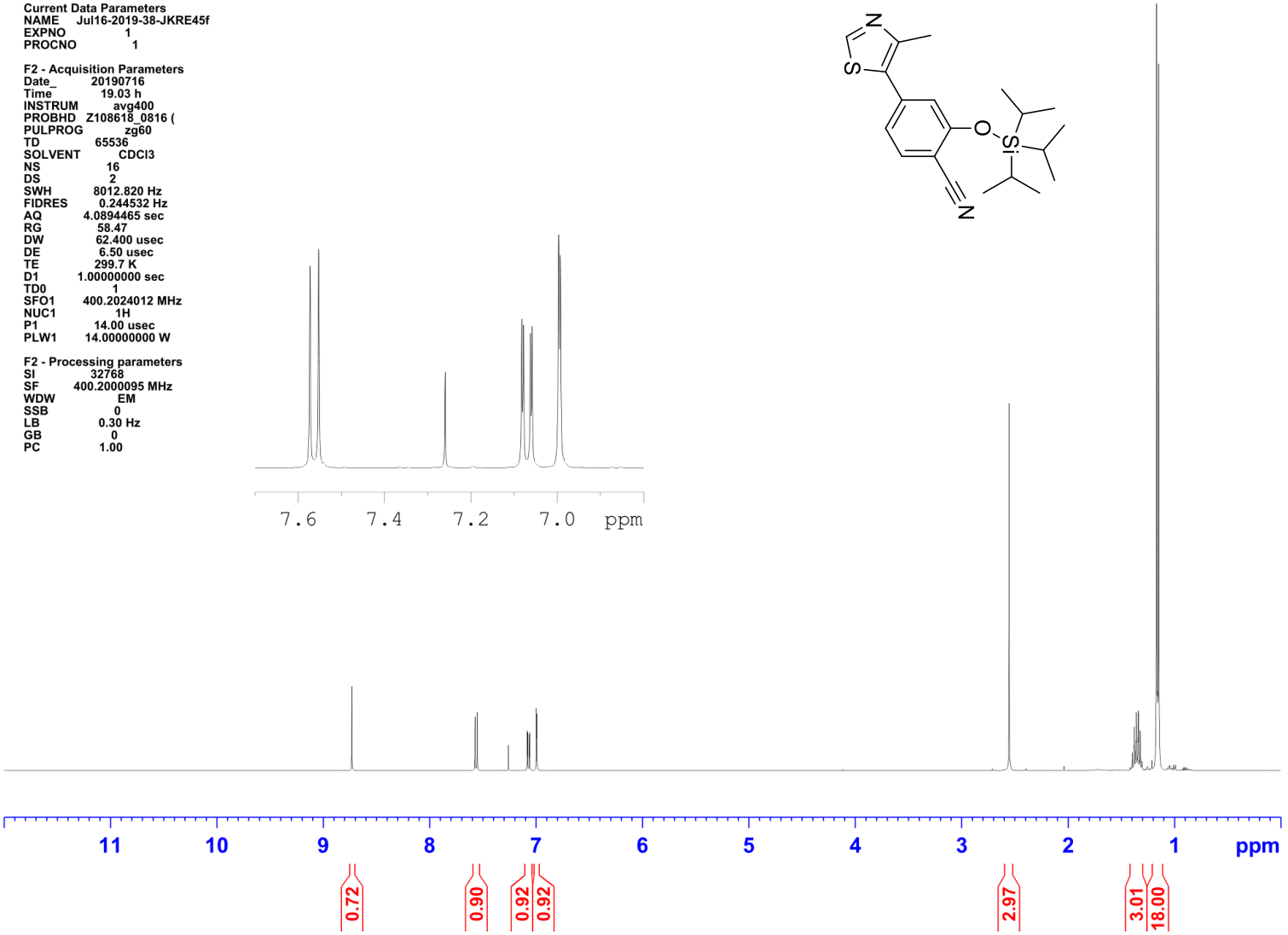
Methyl (4*R*)-1''-[(2*S*)-2'-methyl-2-(1-oxo-1,3-dihydro-2*H*-isoindol-2-yl)butanoyl]-4''-{[tri(propan-9''-yl)silyl]oxy}-L-prolinate

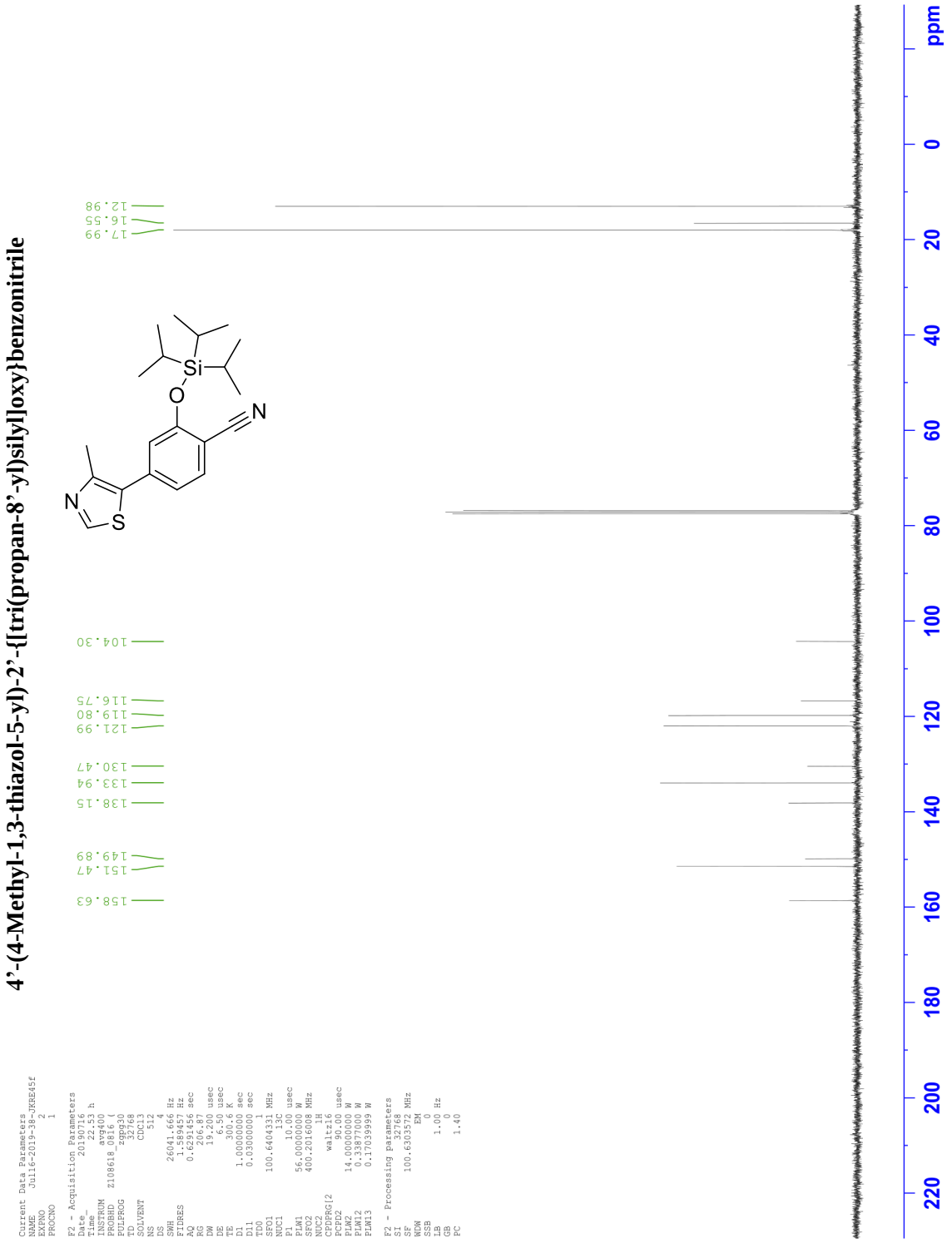



(4R)-1-[(2S)-2'-Methyl-2-(1-oxo-1,3-dihydro-2H-isoindol-2-yl)butanoyl]-4''-{[tri(propan-9''-yl)silyl]oxy}-L-proline
Current Data Parameters
NAME Sep17-2019-30-JKRE75d
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20190917
Time 16.47 h
INSTRUM avg400
PROBHD Z108618_0816 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 91.39
DW 62.400 usec
DE 6.50 usec
TE 299.2 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000096 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
21.58
6.63
1.84
1.18
1.02
0.84
0.92
2.79
1.15
2.00
0.90
0.83

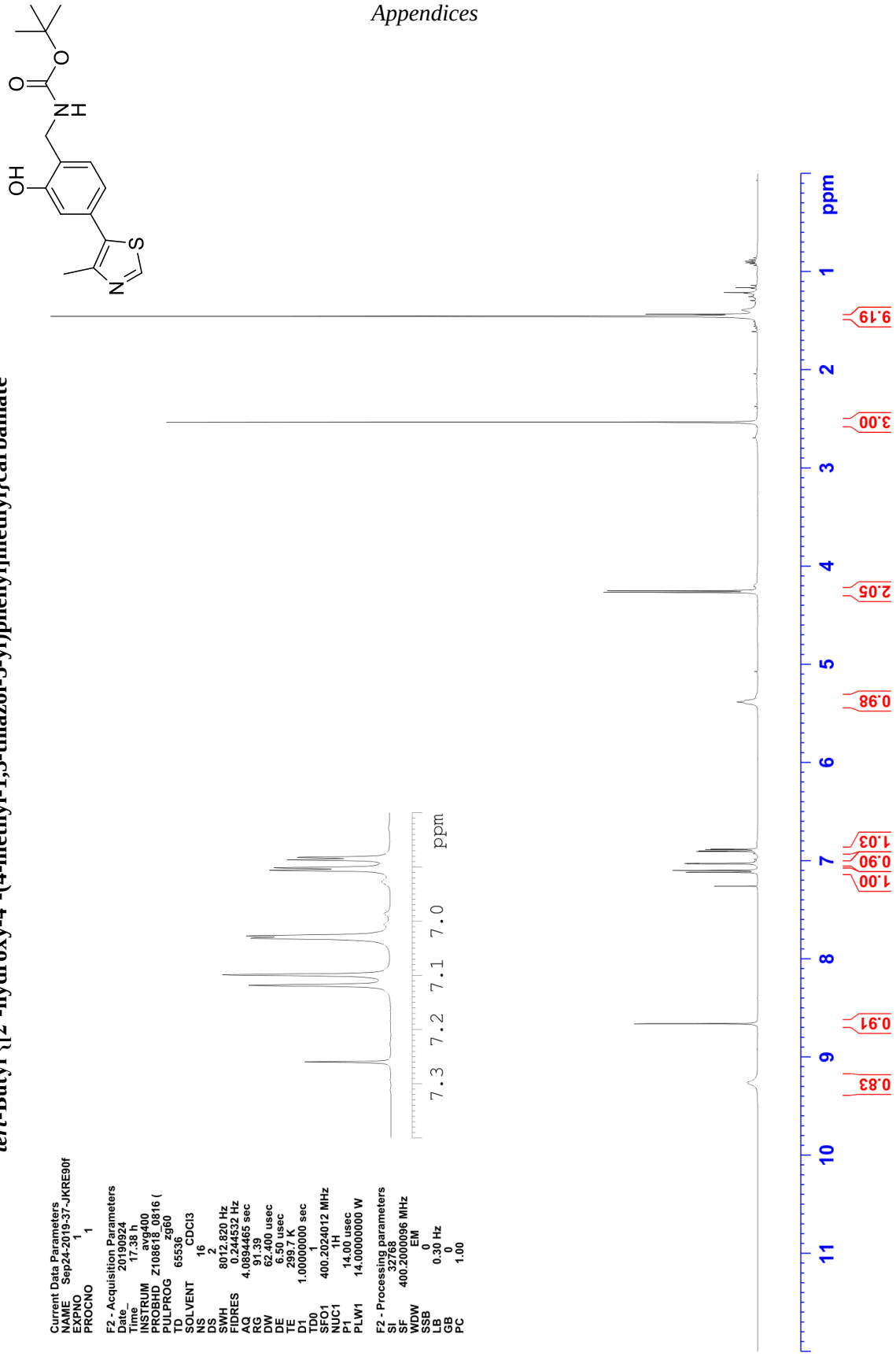

(4R)-1-[(2S)-2'-Methyl-2-(1-oxo-1,3-dihydro-2H-isoindol-2-yl)butanoyl]-4'' -{[tri(propan-9'' -yl)silyl]oxy}-L-proline
12.00
17.85
17.88
18.55
19.31
28.85
38.13
47.16
56.35
58.41
58.55
70.77
122.99
124.07
128.02
131.73
131.88
142.04
168.68
170.65
174.50
ppm
0
20
40
60
80
100
120
140
160
180
200
220

4’-(4-Methyl-1,3-thiazol-5-yl)-2’-{[tri(propan-8’-yl)silyl]oxy}benzonitrile

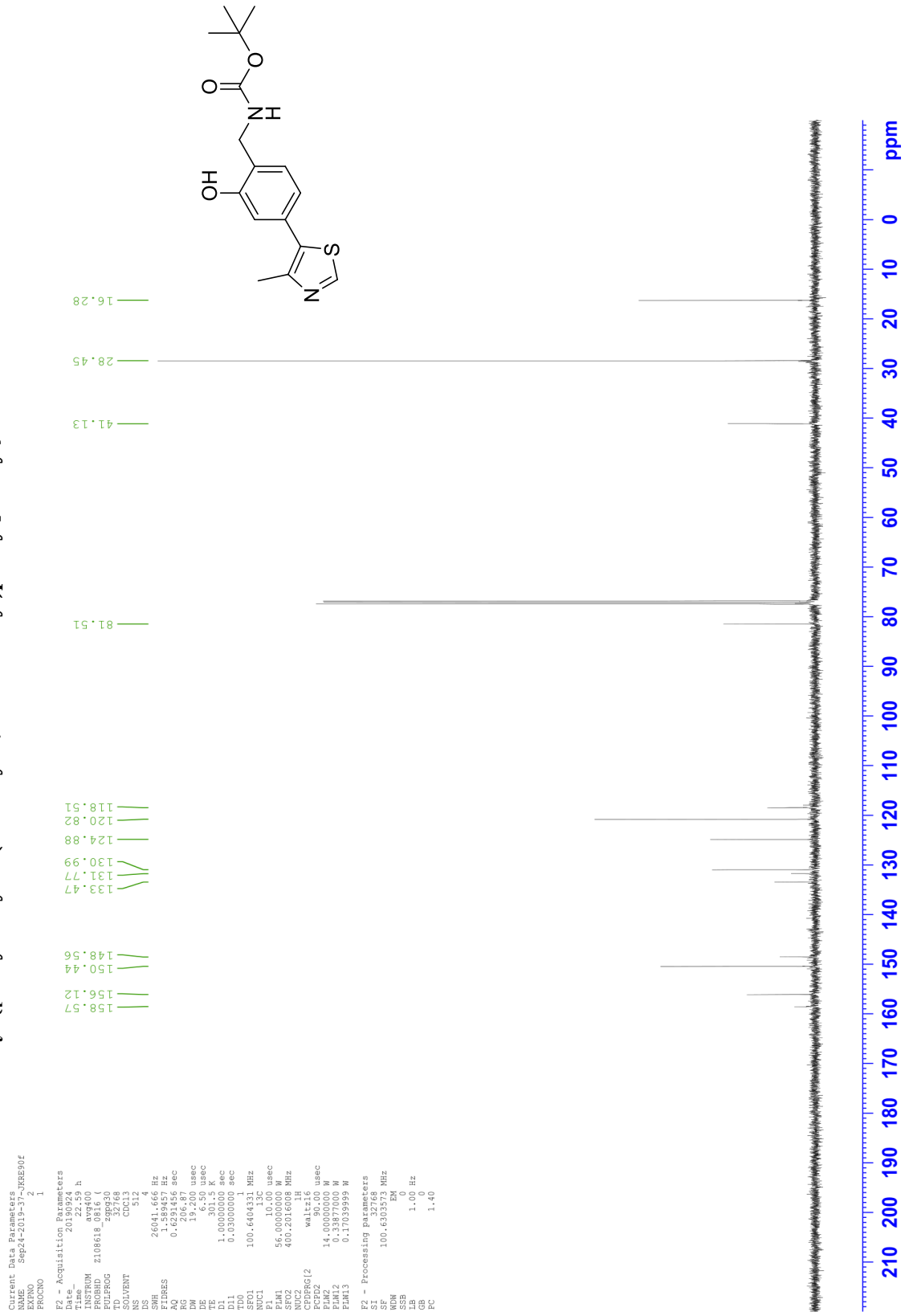


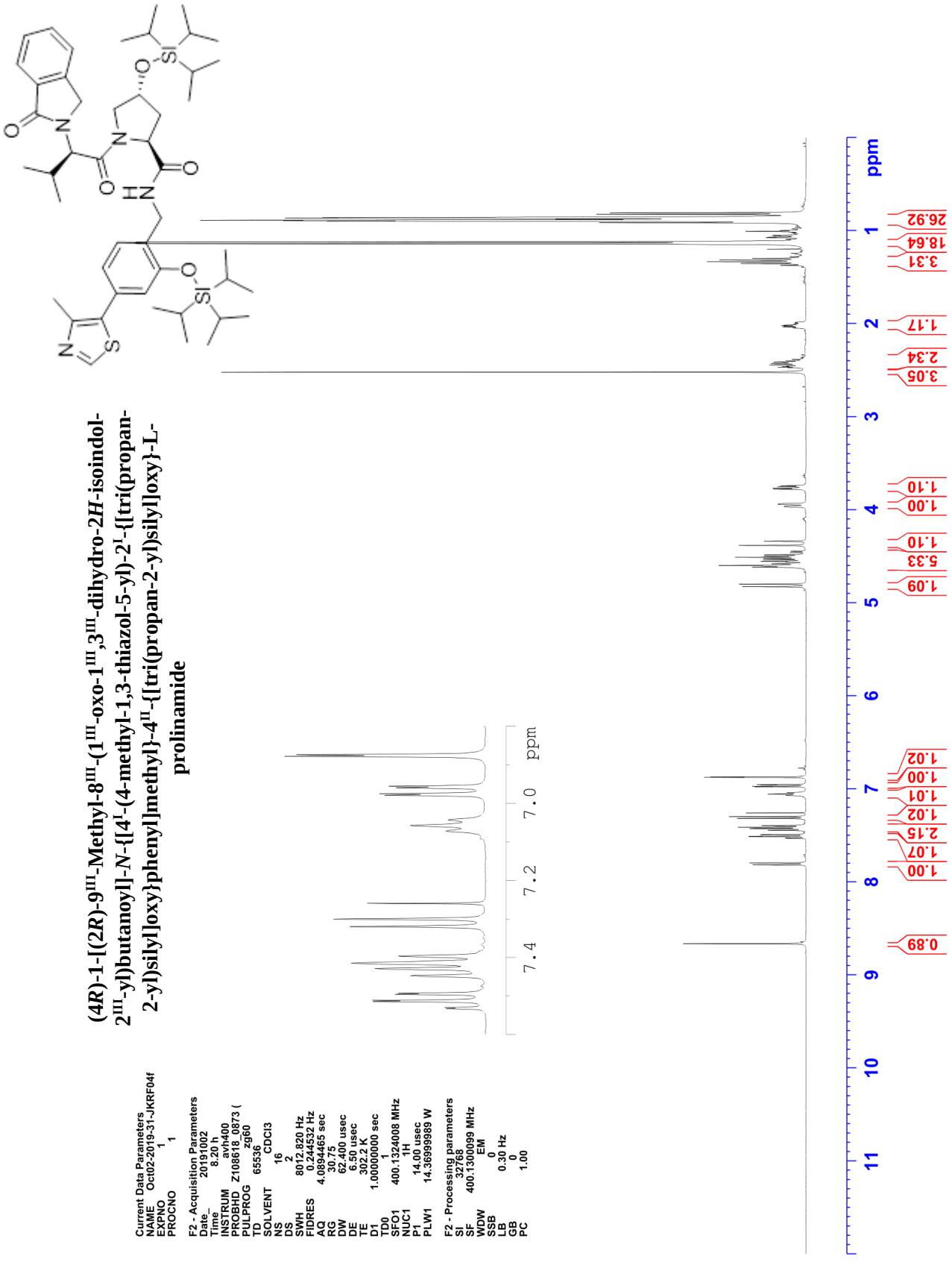

4'-(4-Methyl-1,3-thiazol-5-yl)-2'-{[tri(propan-8'-yl)silyl]oxy}benzonitrile
Current Data Parameters
NAME JU16-2019-3c-JKRE15f
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20190716
Time 13.53 h
INSTRUM av400
PROBHD Z108618_0816 (
PULPROG zgpg30
TD 32768
SOLVENT CDCl3
NS 512
DS 4
SWH 26041.666 Hz
FIDRES 1.589457 Hz
AQ 0.6291456 sec
RG 206.87
DW 19.200 usec
DE 6.50 usec
TE 300.6 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1
SFO1 100.6404331 MHz
NUC1 13C
P1 10.00 usec
PLW1 56.00000000 W
SFO2 400.2016008 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 14.00000000 W
PLW12 0.13700000 W
PLW13 0.17039999 W
F2 - Processing parameters
SI 32768
SF 100.6303572 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
158.63
151.47
149.89
138.15
133.94
130.47
121.99
119.80
116.75
104.30
17.99
16.55
12.98
Si
O
N
N
S
220
200
180
160
140
120
100
80
60
40
20
0
ppm

***tert*-Butyl {[2'-hydroxy-4'-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}carbamate**

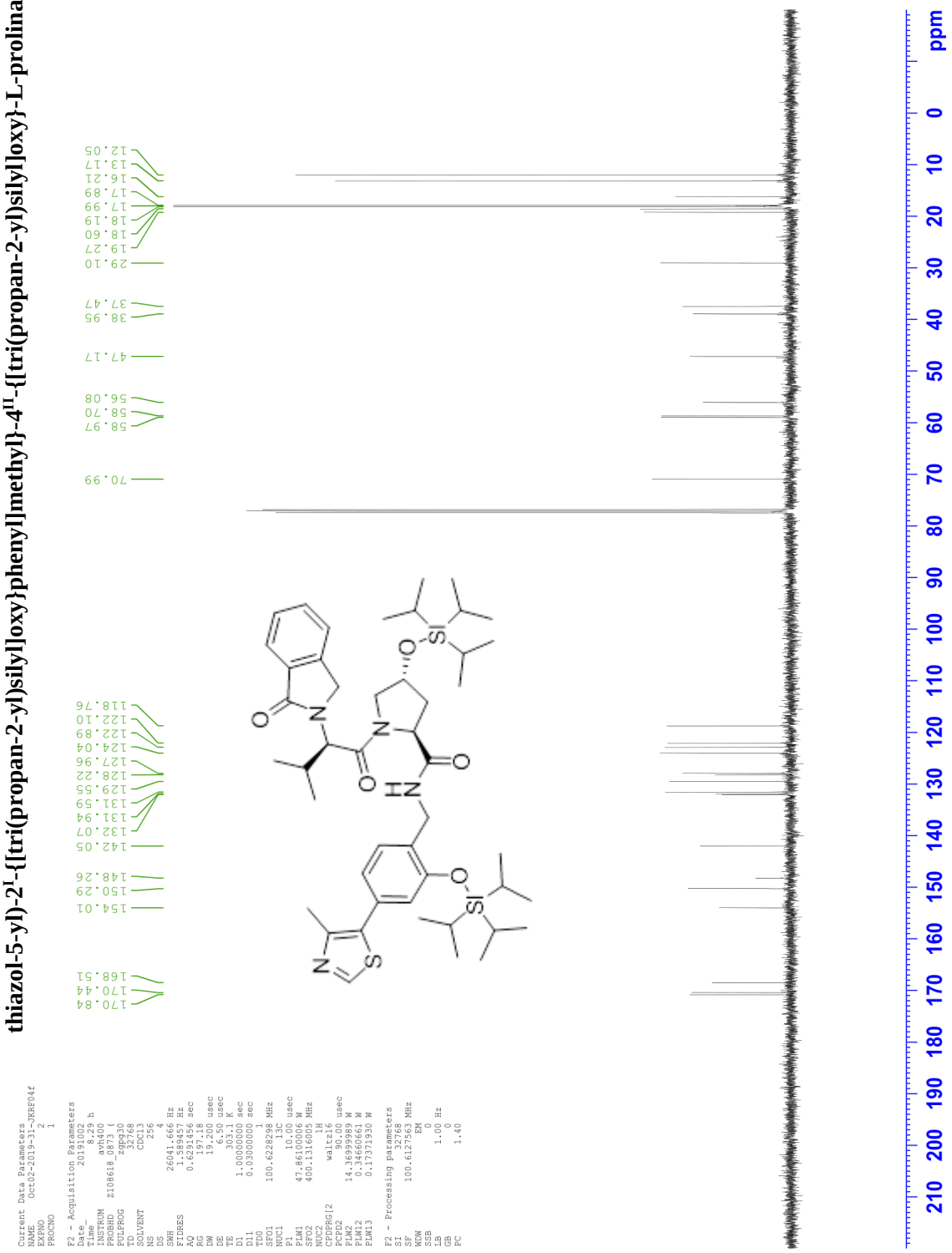


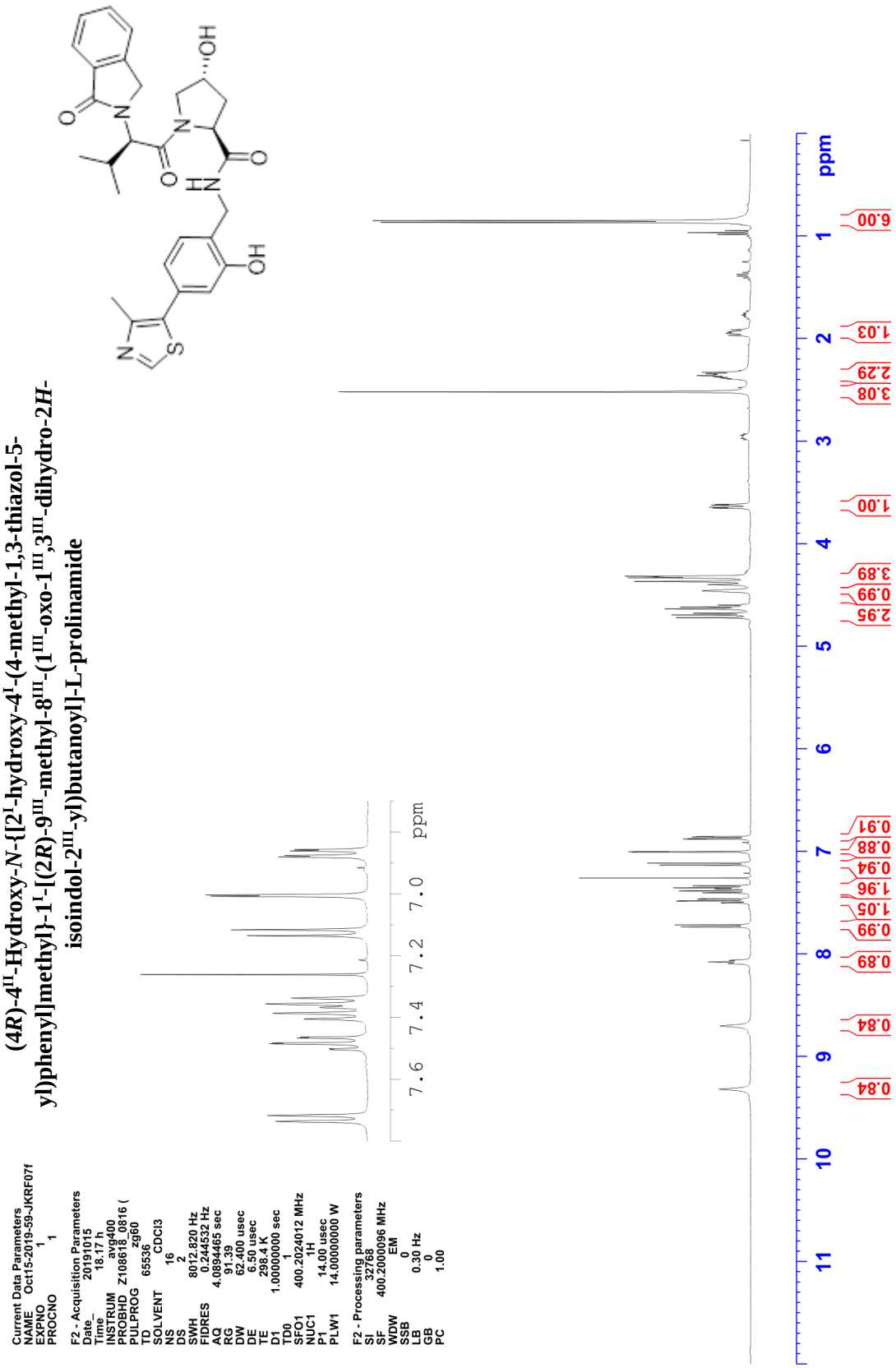
***tert*-Butyl {[2'-hydroxy-4'-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}carbamate**



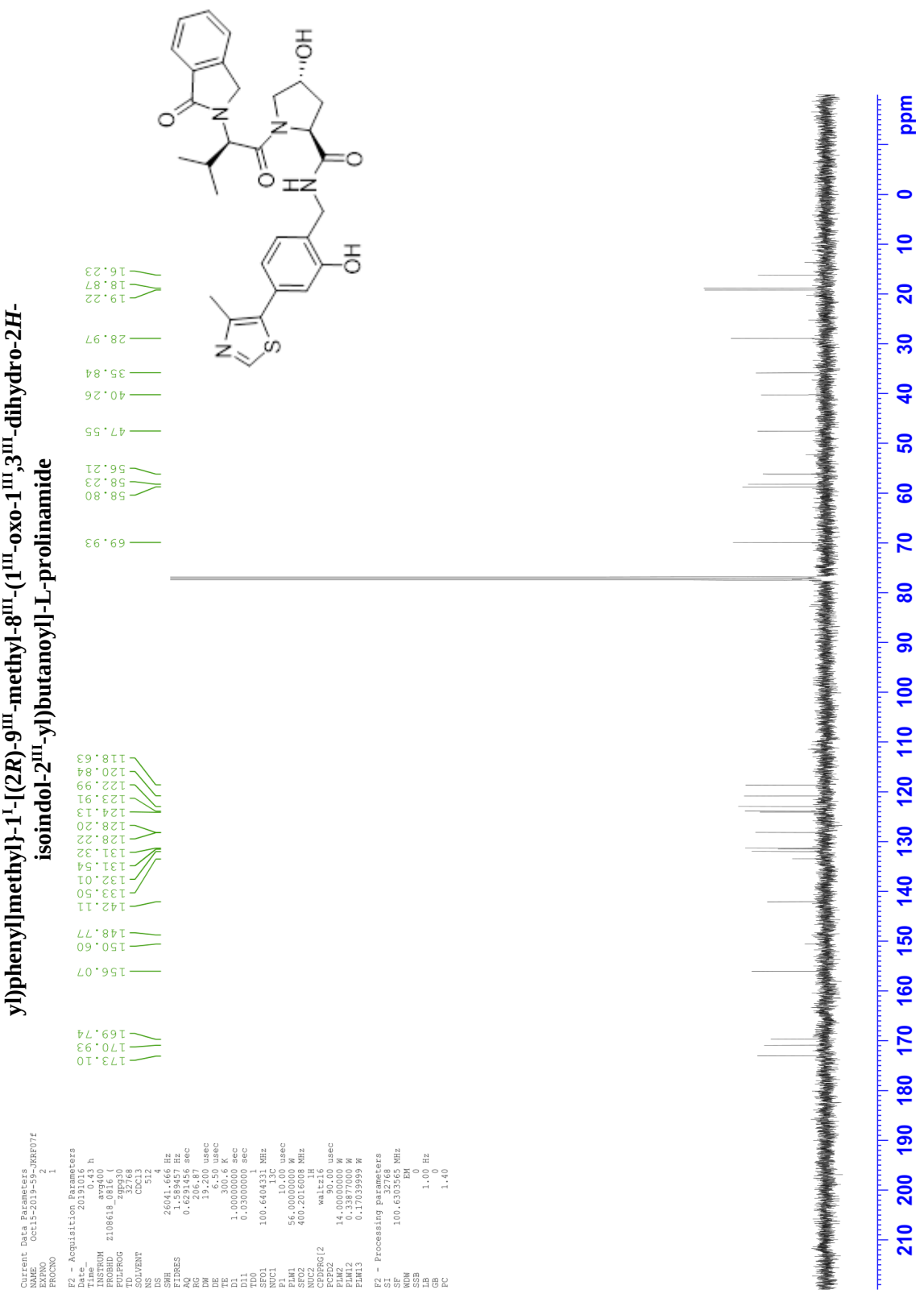

(4R)-1-[(2R)-9III-Methyl-8III-(1III-oxo-1III,3III-dihydro-2H-isoindol-2III-yl)butanoyl]-N-{[4I-(4-methyl-1,3-thiazol-5-yl)-2I-{[tri(propan-2-yl)silyl]oxy}phenyl]methyl}-4II-{[tri(propan-2-yl)silyl]oxy}-L-prolinamide
Current Data Parameters
NAME Oct02-2019-31-JKRF04f
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191002
Time 8.20 h
INSTRUM avh400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 30.75
DW 62.400 usec
DE 6.50 usec
TE 302.2 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300099 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.4
7.2
7.0
ppm
0.89
1.00
1.07
2.15
1.02
1.01
1.00
1.02
1.09
5.33
1.10
1.00
1.10
3.05
2.34
1.17
3.31
18.64
26.92
11
10
9
8
7
6
5
4
3
2
1
ppm

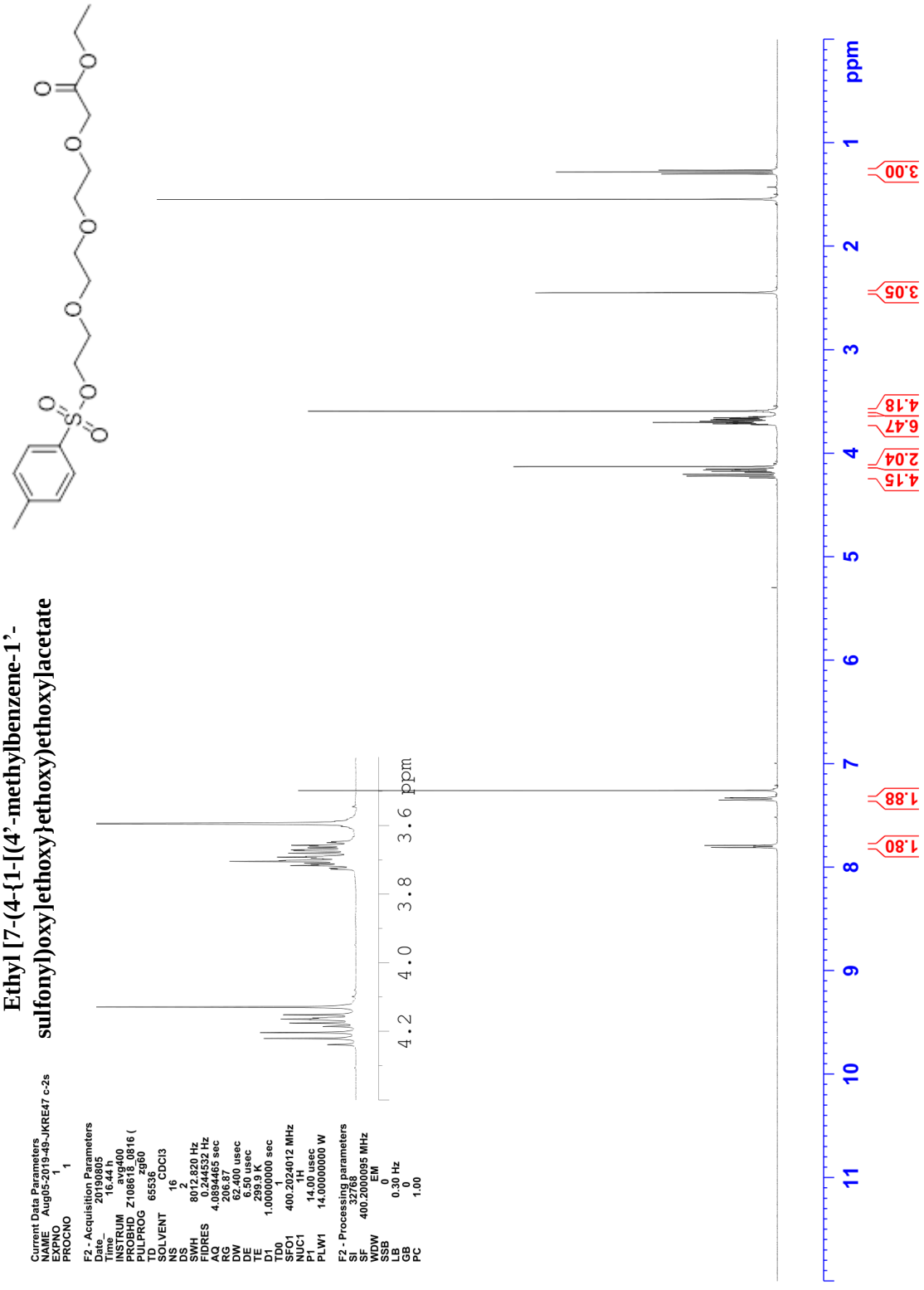
(4*R*)-1-[(2*R*)-9^{III}-Methyl-8^{III}-(1^{III}-oxo-1^{III},3^{III}-dihydro-2*H*-isoindol-2^{III}-yl)butanoyl]-*N*-{[4^{I}-(4-methyl-1,3-thiazol-5-yl)-2^{I}-{[tri(propan-2-yl)silyl]oxy}phenyl]methyl}-4^{II}-{[tri(propan-2-yl)silyl]oxy}-L-prolinamide

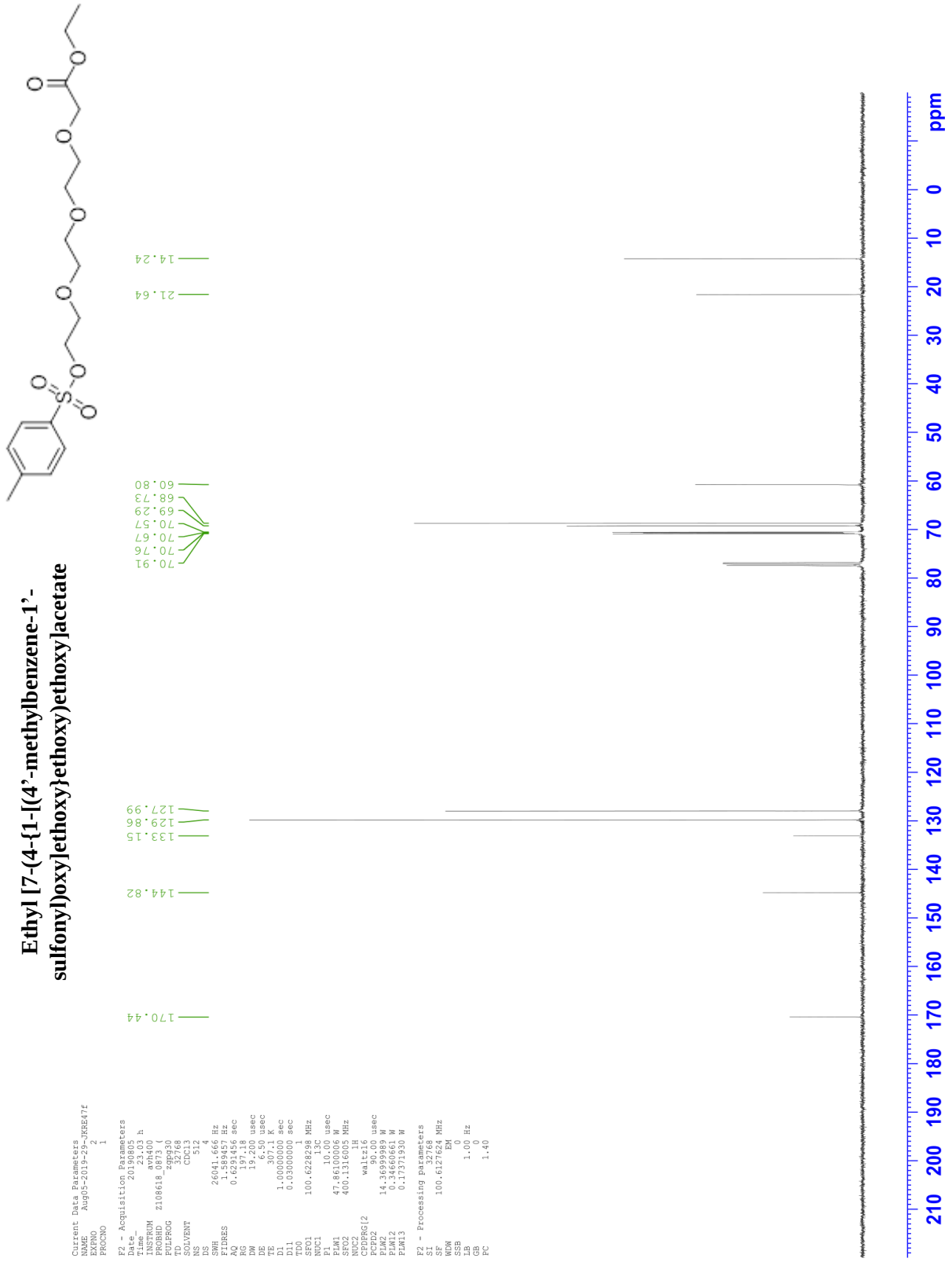


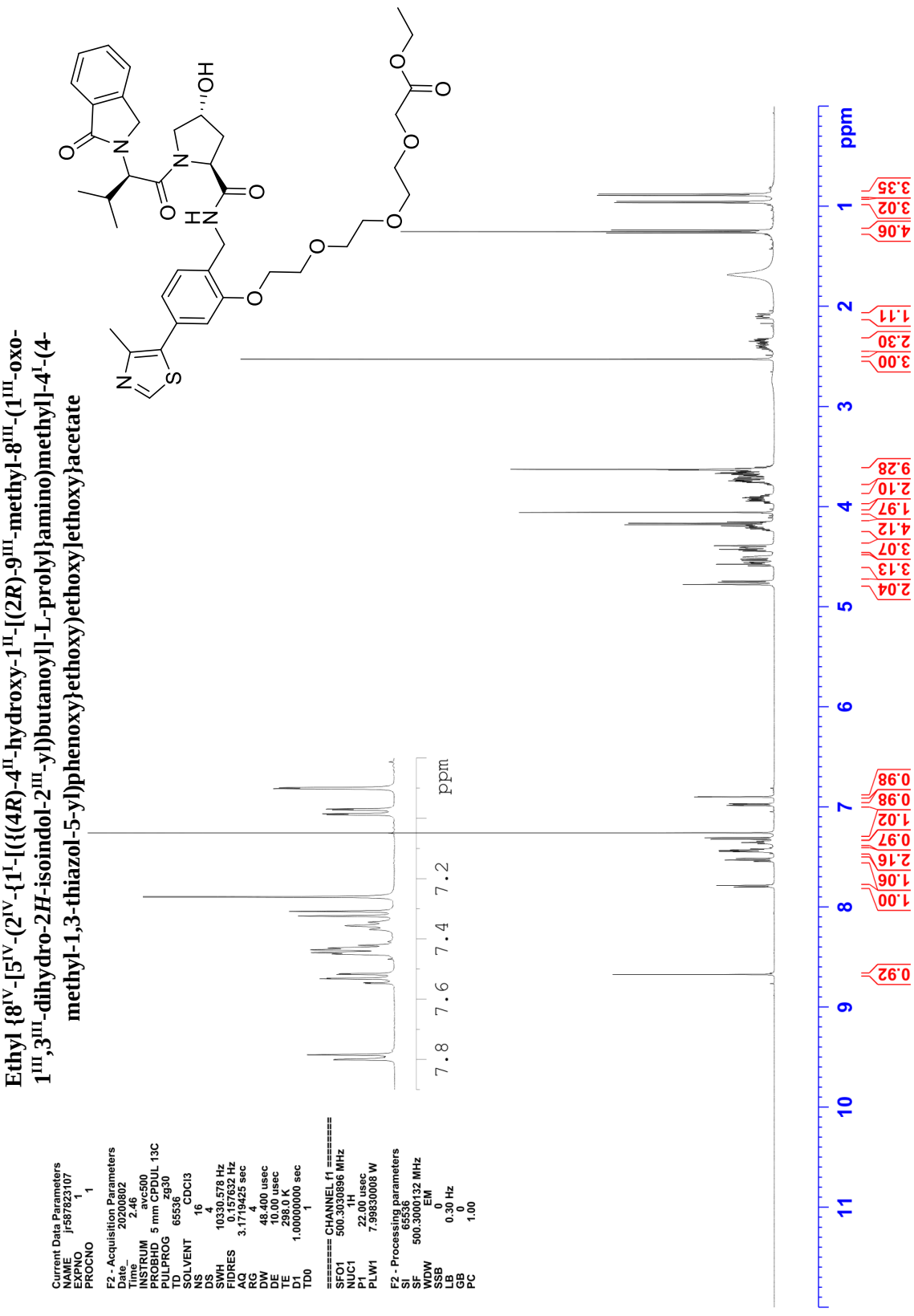

(4R)-4II-Hydroxy-N-{[2I-hydroxy-4I-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}-1I-[(2R)-9III-methyl-8III-(1III-oxo-1III,3III-dihydro-2H-isoindol-2III-yl)butanoyl]-L-prolinamide
Current Data Parameters
NAME Oct15-2019-59-JKRF07f
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191015
Time 18.17 h
INSTRUM avg400
PROBHD Z108618_0816 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 91.39
DW 62.400 usec
DE 6.50 usec
TE 298.4 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000096 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.6
7.4
7.2
7.0
ppm
0.84
0.84
0.89
0.99
1.05
1.96
0.94
0.88
0.91
1.00
3.89
0.99
2.95
1.03
2.29
3.08
6.00
11
10
9
8
7
6
5
4
3
2
1
ppm

(4*R*)-4^{II}-Hydroxy-*N*-{[2^{I}-hydroxy-4^{I}-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}-1^{I}-[(2*R*)-9^{III}-methyl-8^{III}-(1^{III}-oxo-1^{III},3^{III}-dihydro-2*H*-isoindol-2^{III}-yl)butanoyl]-L-prolinamide

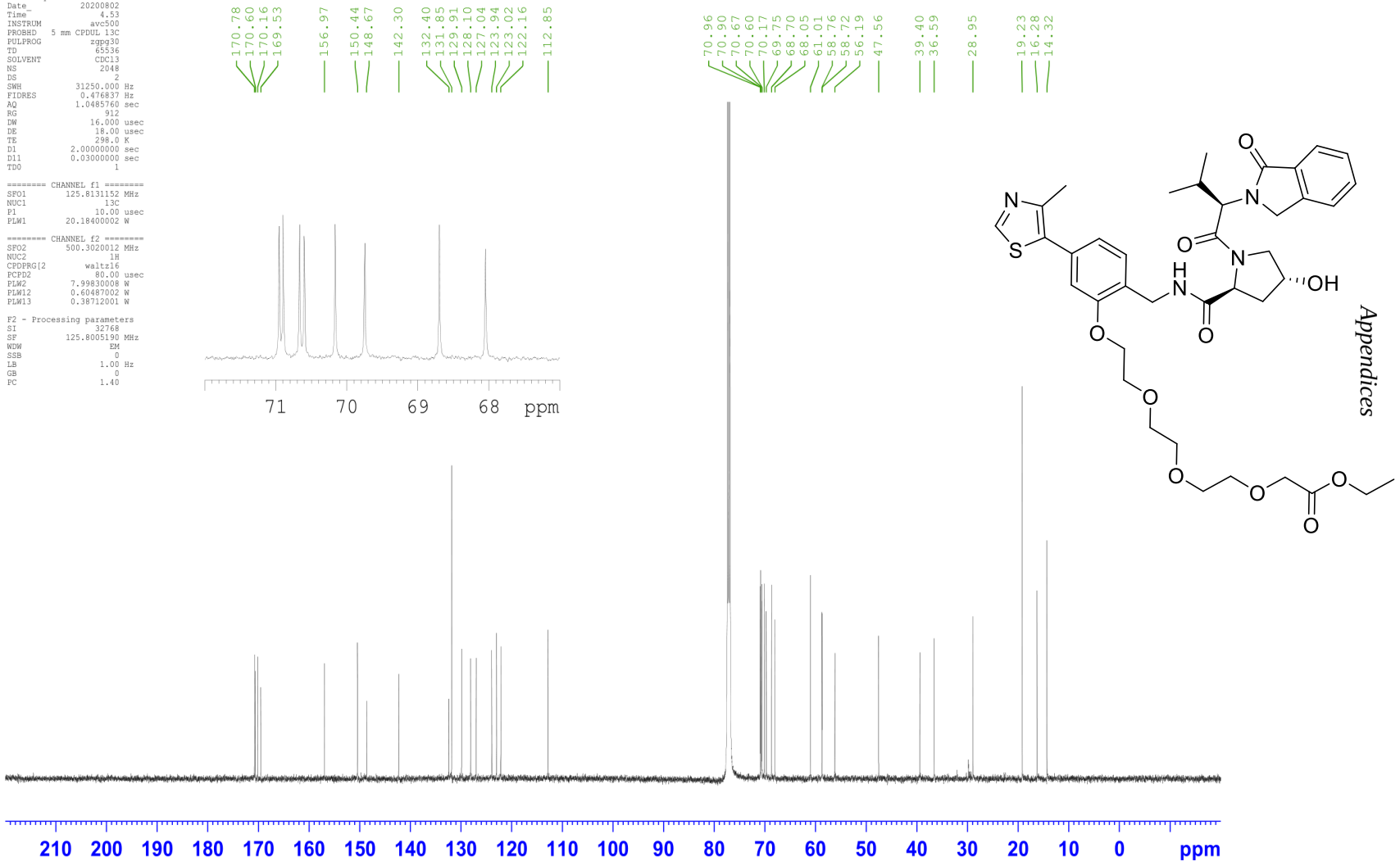


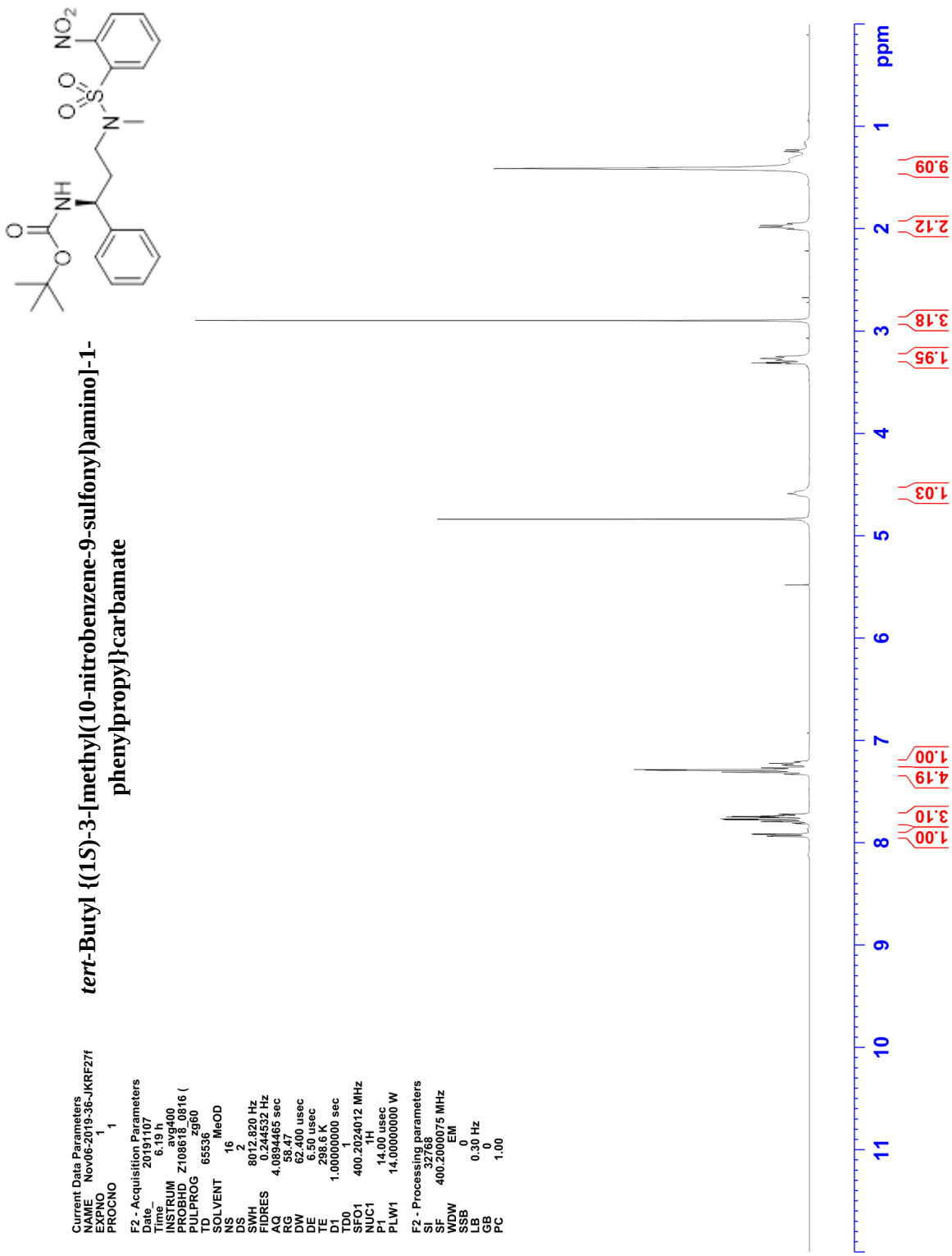

Ethyl [7-(4-{1-[(4'-methylbenzene-1'-sulfonyl)oxy]ethoxy}ethoxy)ethoxy]acetate
Current Data Parameters
NAME Aug05-2019-49-JKRE47 c-2s
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20190805
Time 16.44 h
INSTRUM avg400
PROBHD Z108618_0816 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 206.87
DW 62.400 usec
DE 6.50 usec
TE 299.9 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000095 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
4.2
4.0
3.8
3.6
ppm
3.00
3.05
4.18
6.47
2.04
4.15
1.88
1.80
ppm


Ethyl [7-(4-{1-[(4'-methylbenzene-1'-sulfonyl)oxy]ethoxy}ethoxy)ethoxy]acetate
14.24
21.64
60.80
68.73
69.29
70.57
70.67
70.76
70.91
127.99
129.86
133.15
144.82
170.44
ppm

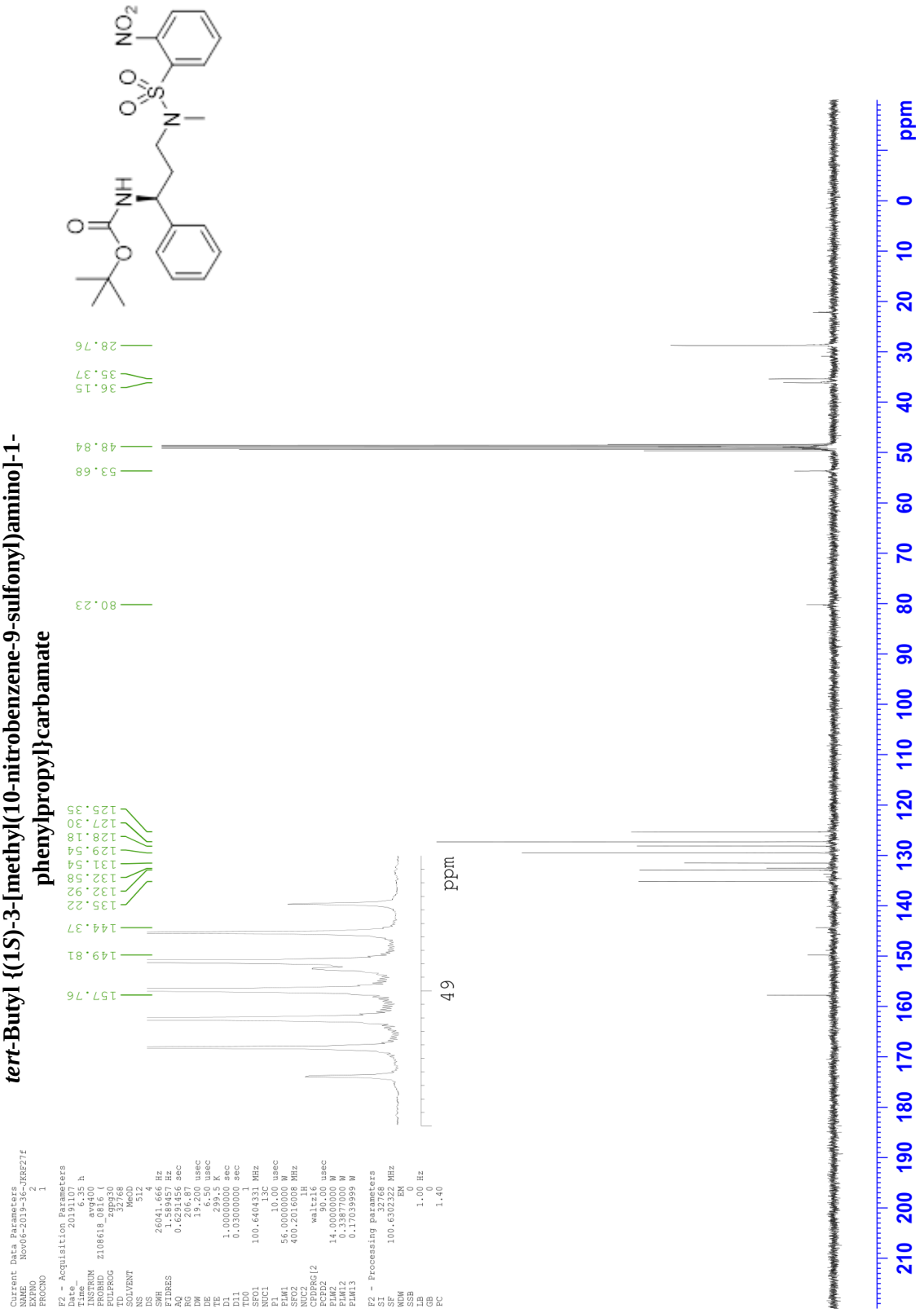

Ethyl {8IV-[5IV-(2IV-{1I-[({(4R)-4II-hydroxy-1II-[(2R)-9III-methyl-8III-(1III-oxo-1III,3III-dihydro-2H-isoindol-2III-yl)butanoyl]-L-prolyl}amino)methyl]-4I-(4-methyl-1,3-thiazol-5-yl)phenoxy}ethoxy)ethoxy]ethoxy}acetate
Current Data Parameters
NAME jr587823107
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200802
Time_ 2.46
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000132 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.8
7.6
7.4
7.2
ppm
0.92
1.00
1.06
2.16
0.97
1.02
0.98
0.98
2.04
3.13
3.07
4.12
1.97
2.10
9.28
3.00
2.30
1.11
4.06
3.02
3.35
11
10
9
8
7
6
5
4
3
2
1
ppm

Ethyl {8^{IV}-[5^{IV}-(2^{IV}-{1^{I}-[({(4*R*)-4^{II}-hydroxy-1^{II}-[(2*R*)-9^{III}-methyl-8^{III}-(1^{III}-oxo-1^{III},3^{III}-dihydro-2*H*-isoindol-2^{III}-yl)butanoyl]-L-prolyl}amino)methyl]-4^{I}-(4-methyl-1,3-thiazol-5-yl)phenoxy}ethoxy)ethoxy]ethoxy}acetate

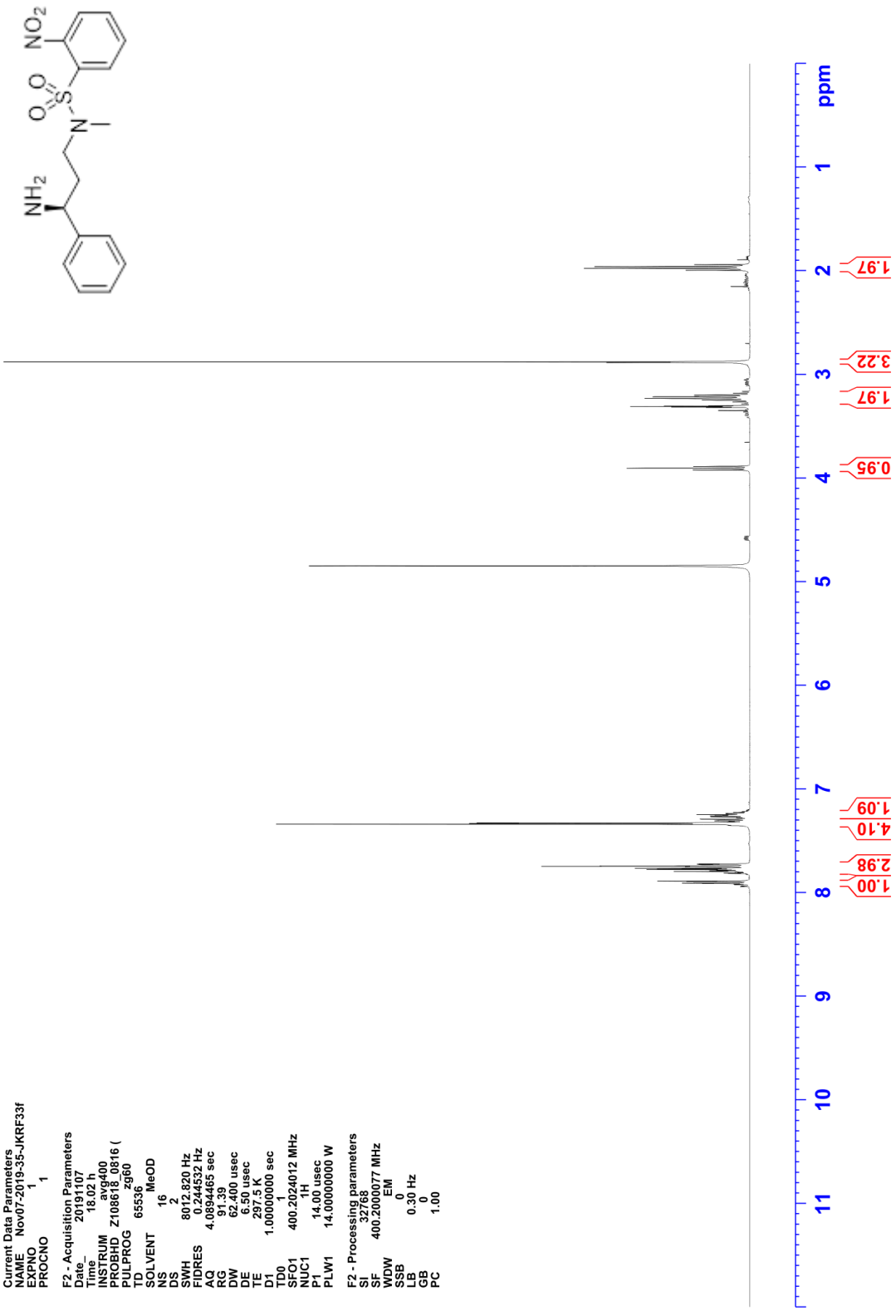



Current Data Parameters
NAME Nov06-2019-36-JKRF27f
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191107
Time 6.19 h
INSTRUM avg400
PROBHD Z108618_0816 (
PULPROG zg60
TD 65536
SOLVENT MeOD
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 58.47
DW 62.400 usec
DE 6.50 usec
TE 298.6 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000075 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
tert-Butyl {(1S)-3-[methyl(10-nitrobenzene-9-sulfonyl)amino]-1-phenylpropyl}carbamate
NO2
O
O
S
N
NH
O
O
ppm
11
10
9
8
7
6
5
4
3
2
1
1.00
3.10
1.00
4.19
1.03
1.95
3.18
2.12
9.09

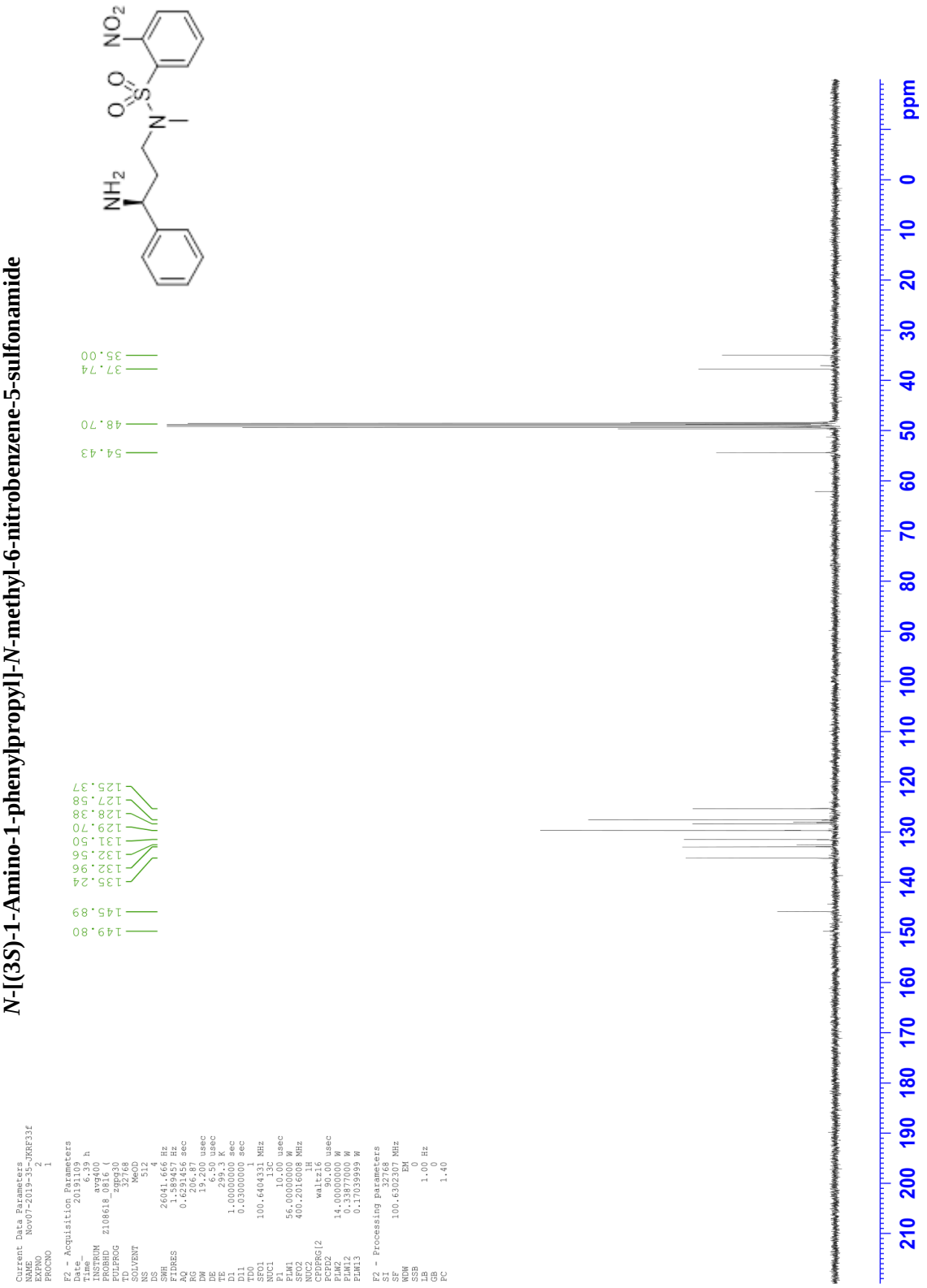
***tert*-Butyl {(1*S*)-3-[methyl(10-nitrobenzene-9-sulfonyl)amino]-1-phenylpropyl}carbamate**

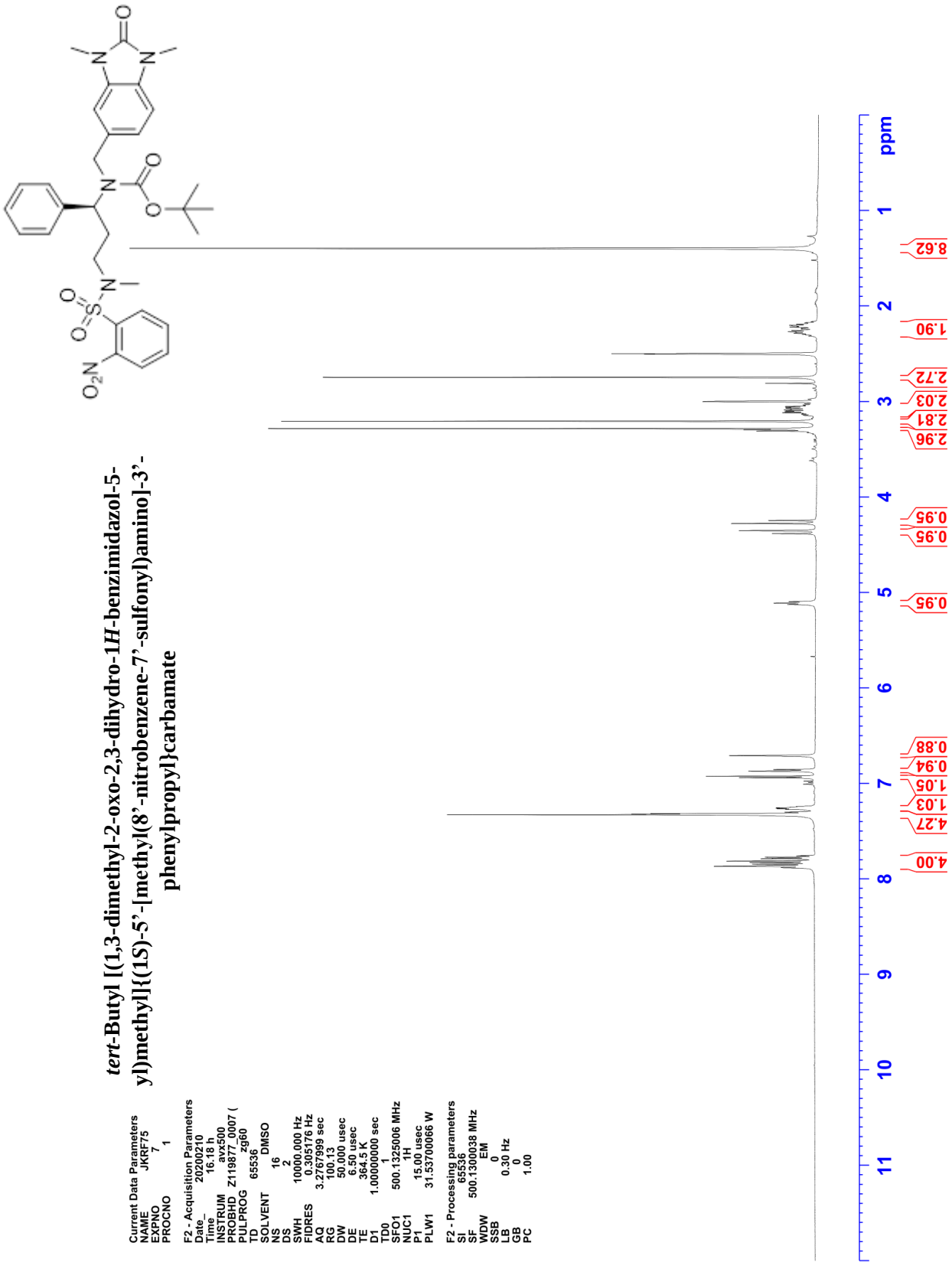


_N_-[(3S)-1-Amino-1-phenylpropyl]-_N_-methyl-6-nitrobenzene-5-sulfonamide

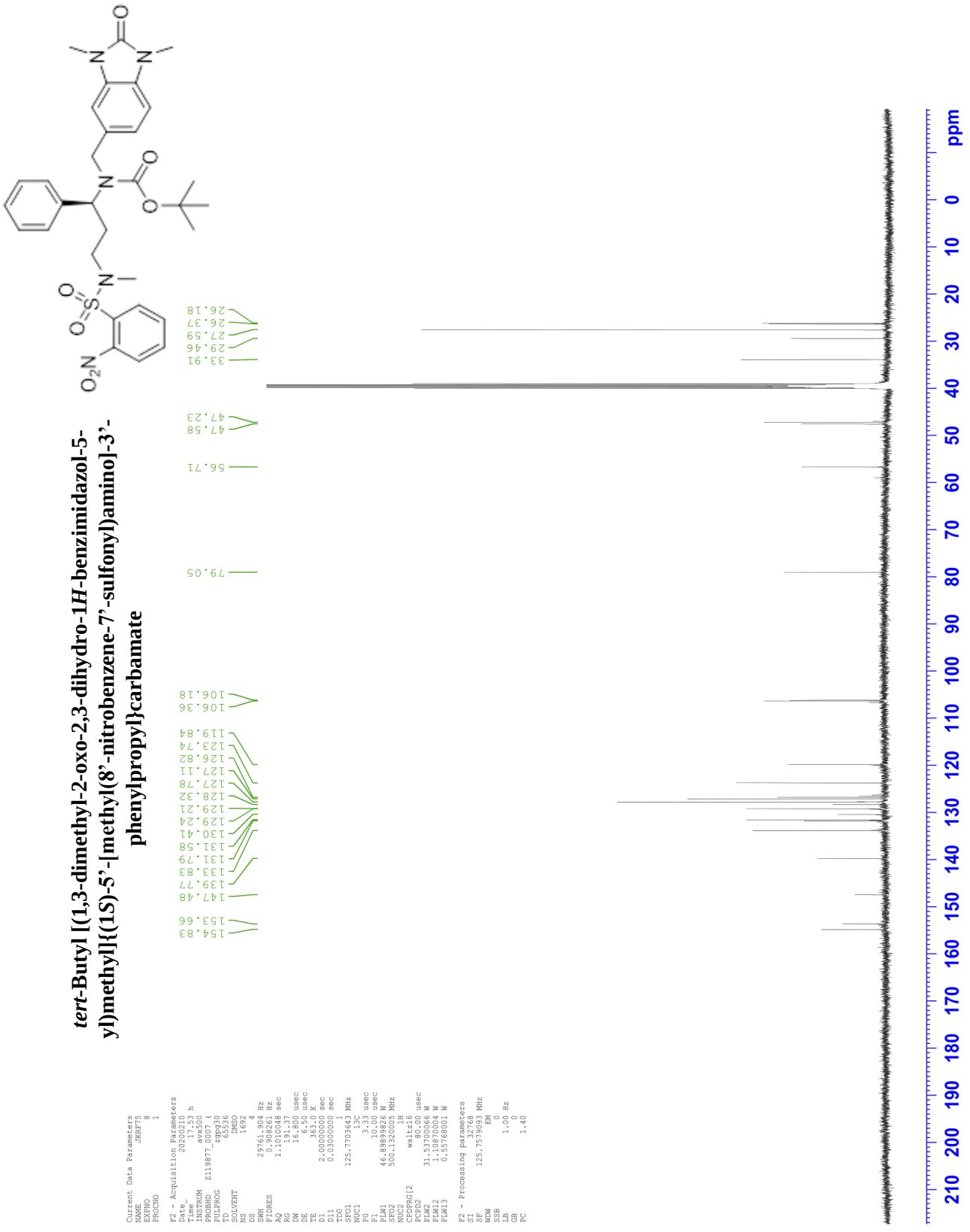


***N*-[(3S)-1-Amino-1-phenylpropyl]-*N*-methyl-6-nitrobenzene-5-sulfonamide**




tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl]{(1S)-5'-[methyl(8'-nitrobenzene-7'-sulfonyl)amino]-3'-phenylpropyl}carbamate
Current Data Parameters
NAME JKRF75
EXPNO 7
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200210
Time 16.18 h
INSTRUM avx500
PROBHD Z119877_0007 (
PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 100.13
DW 50.000 usec
DE 6.50 usec
TE 364.5 K
D1 1.00000000 sec
TD0 1
SFO1 500.1325006 MHz
NUC1 1H
P1 15.00 usec
PLW1 31.53700066 W
F2 - Processing parameters
SI 65536
SF 500.1300038 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.62
1.90
2.72
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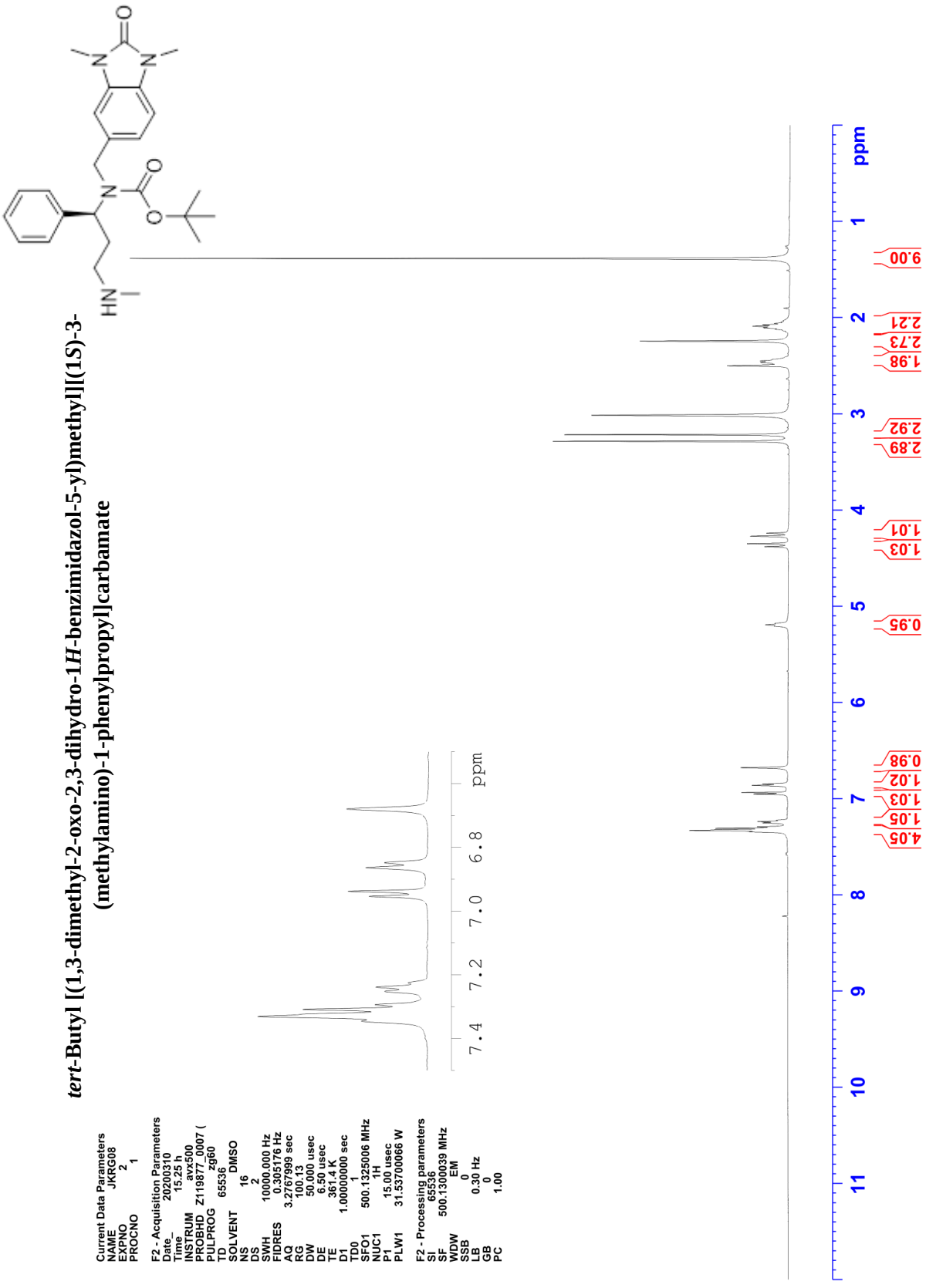
***tert*-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{(1*S*)-5'-[methyl(8'-nitrobenzene-7'-sulfonyl)amino]-3'-phenylpropyl}carbamate**



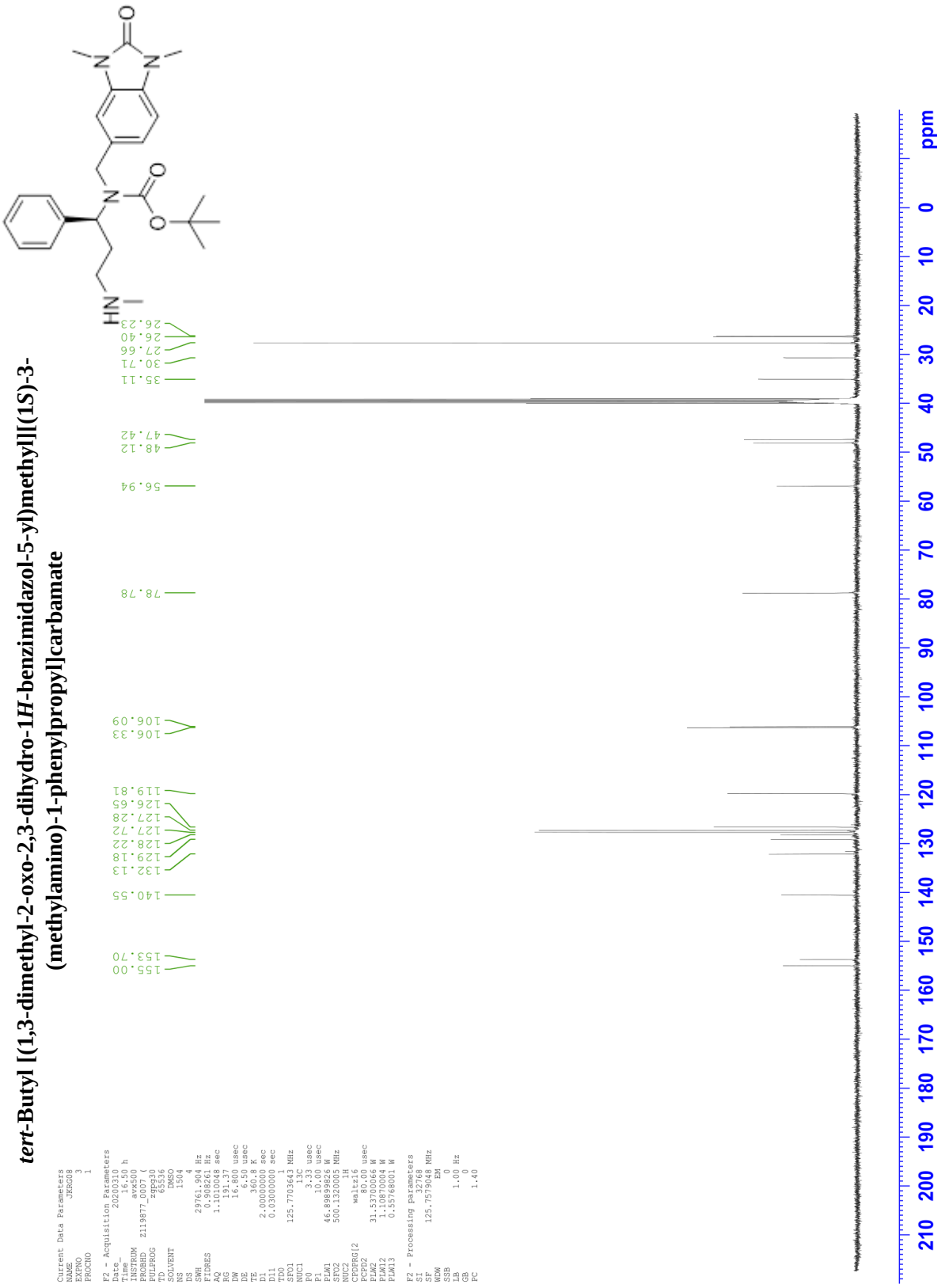

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EXPNO              8
PROCNO             1

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Time         11.13 h
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PULPROG        zgpg30
TD             65536
SOLVENT          DMSO
NS                1692
DS                   4
SWH          29761.904 Hz
FIDRES        0.908261 Hz
AQ            1.1010048 sec
RG               18.37
DW              16.800 usec
DE               6.50 usec
TE              363.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                  1
SFO1        125.7703643 MHz
NUC1                13C
P0                 3.33 usec
P1                10.00 usec
PLW1        46.89899826 W
SFO2        500.1320005 MHz
NUC2                1H
CPDPRG[2        waltz16
PCPD2             80.00 usec
PLW2        31.53700066 W
PLW12        1.10870004 W
PLW13        0.55768001 W

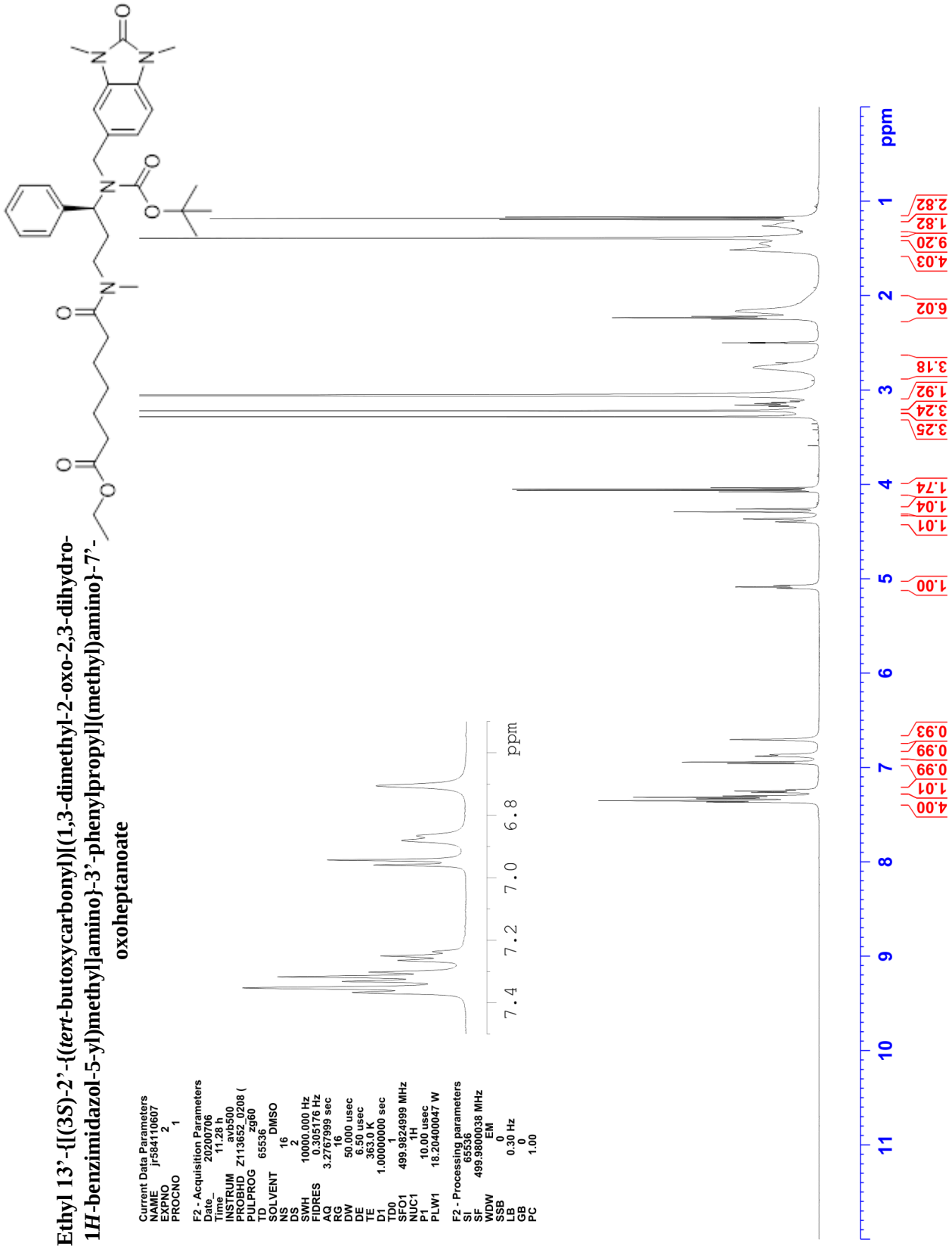
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tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzimidazol-5-yl)methyl][(1S)-3-(methylamino)-1-phenylpropyl]carbamate
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NAME JKRG08
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200310
Time 15.25 h
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PULPROG zg60
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 100.13
DW 50.000 usec
DE 6.50 usec
TE 361.4 K
D1 1.00000000 sec
TD0 1
SFO1 500.1325006 MHz
NUC1 1H
P1 15.00 usec
PLW1 31.53700066 W
F2 - Processing parameters
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SF 500.1300039 MHz
WDW EM
SSB 0
LB 0.30 Hz
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PC 1.00
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ppm
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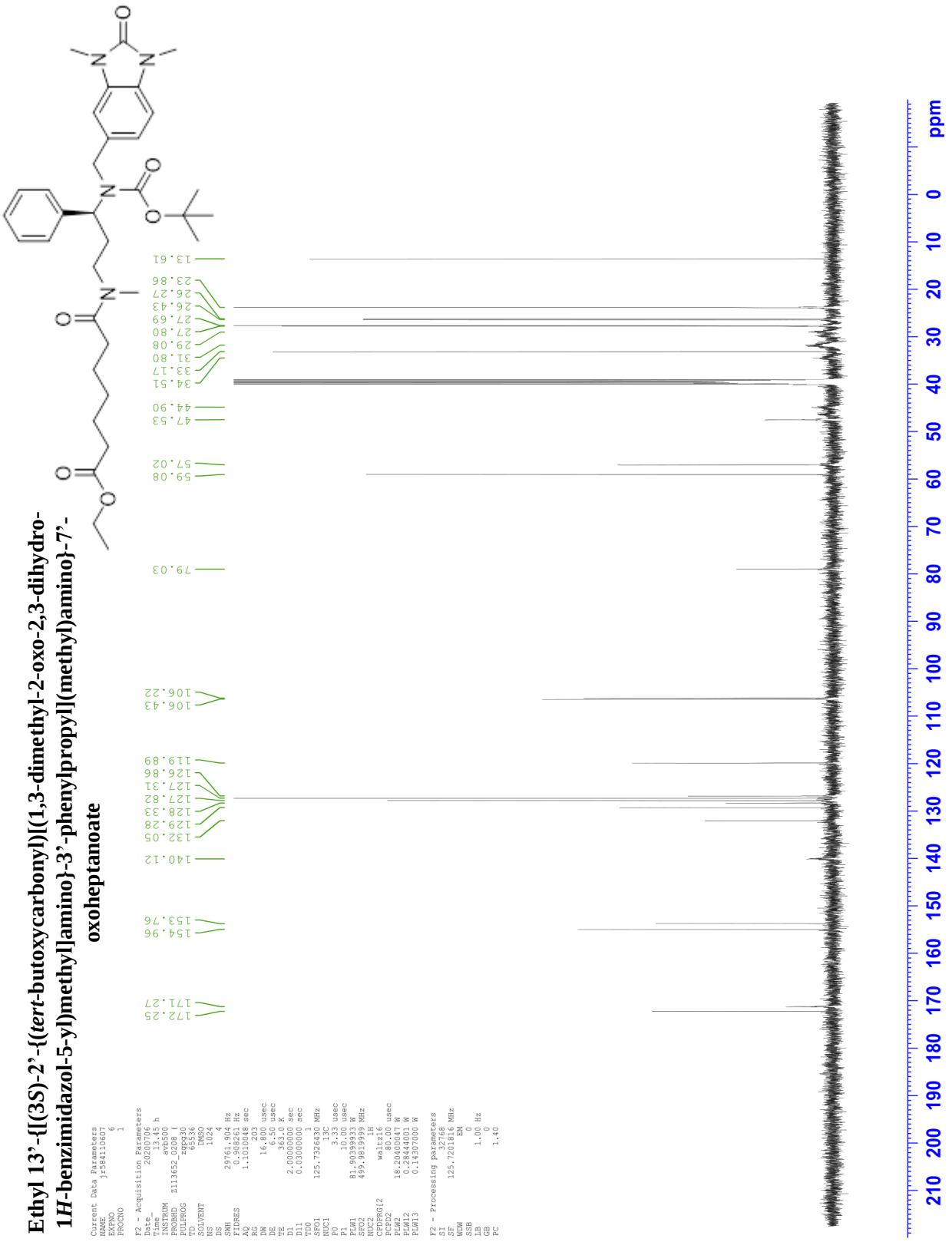
***tert*-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl][(1*S*)-3-(methylamino)-1-phenylpropyl]carbamate**



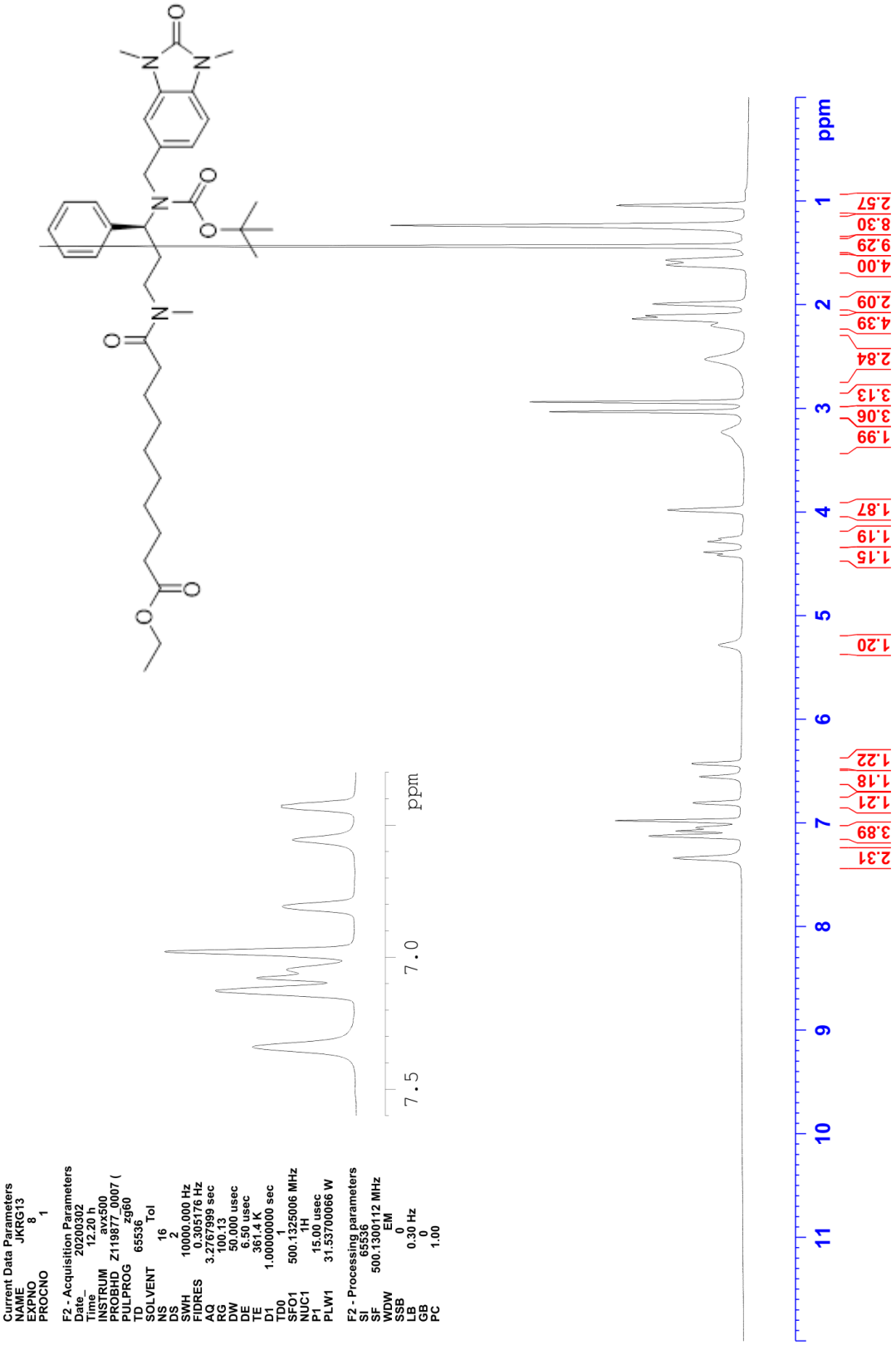
Ethyl 13'-{[(3*S*)-2'-{(*tert*-butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3'-phenylpropyl](methyl)amino}-7'-oxoheptanoate



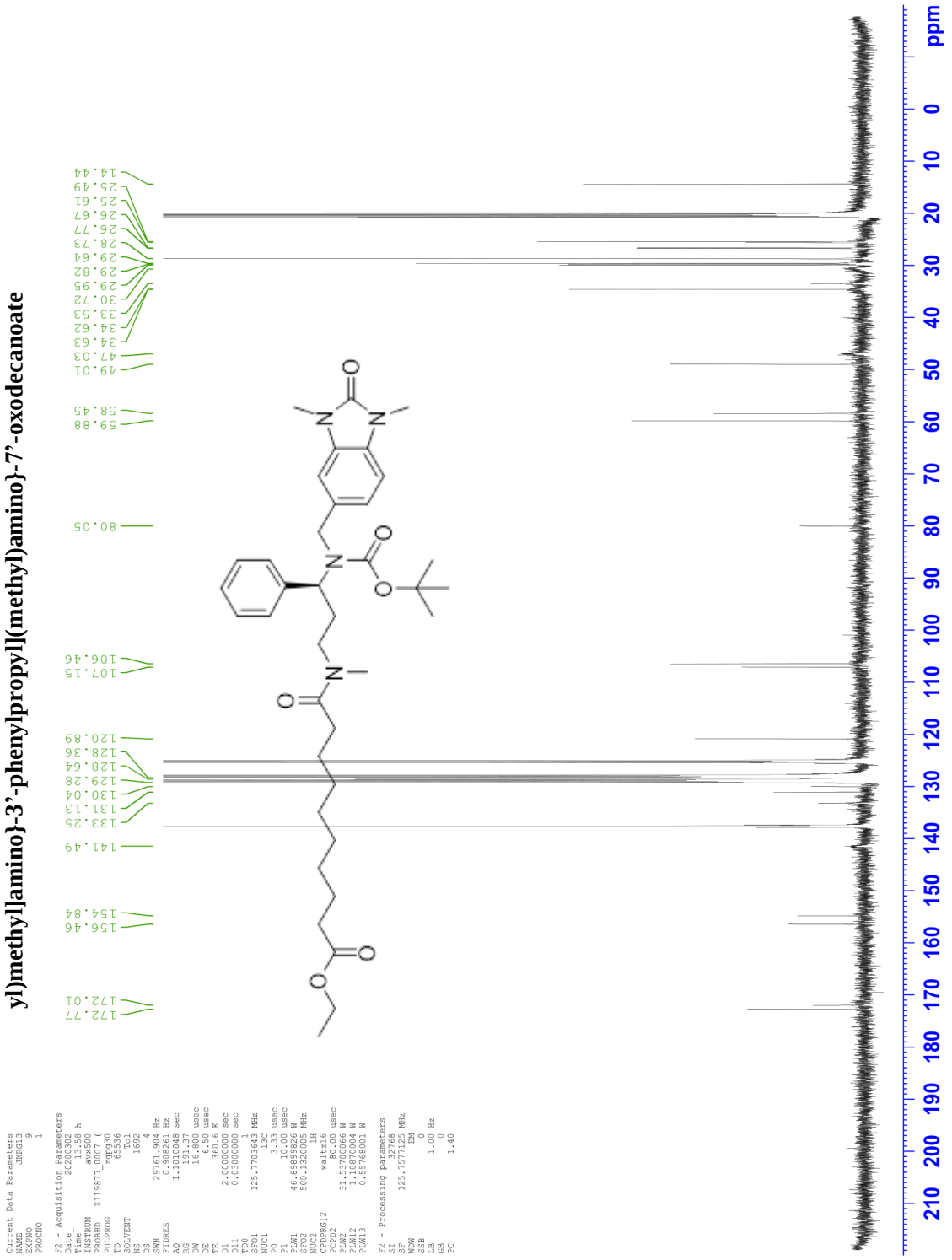
Ethyl 13'-{[(3S)-2'-{(*tert*-butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3'-phenylpropyl](methyl)amino}-7'-oxoheptanoate



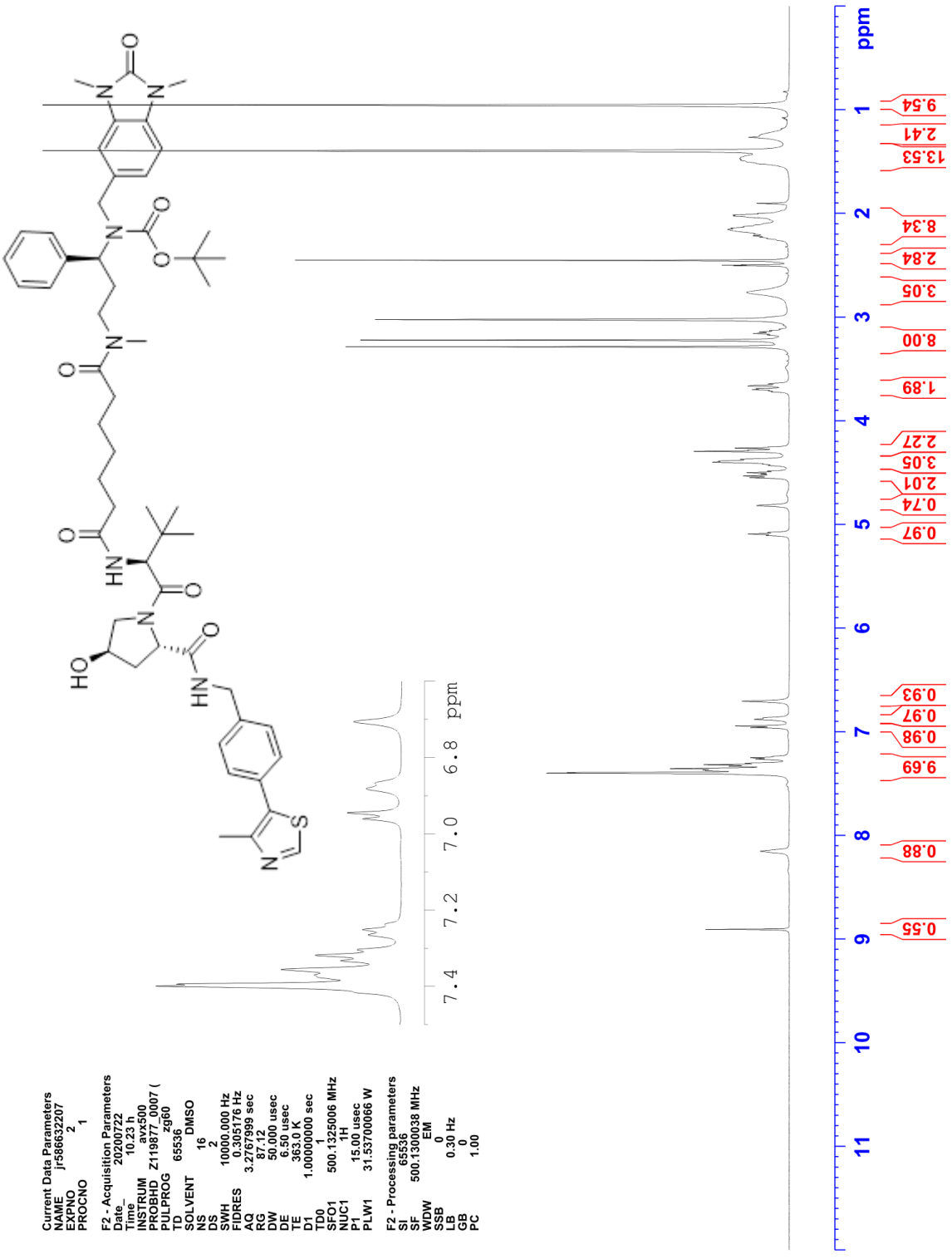
Ethyl 16'-{[(3*S*)-2'-{(*tert*-butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3'-phenylpropyl](methyl)amino}-7'-oxodecanoate



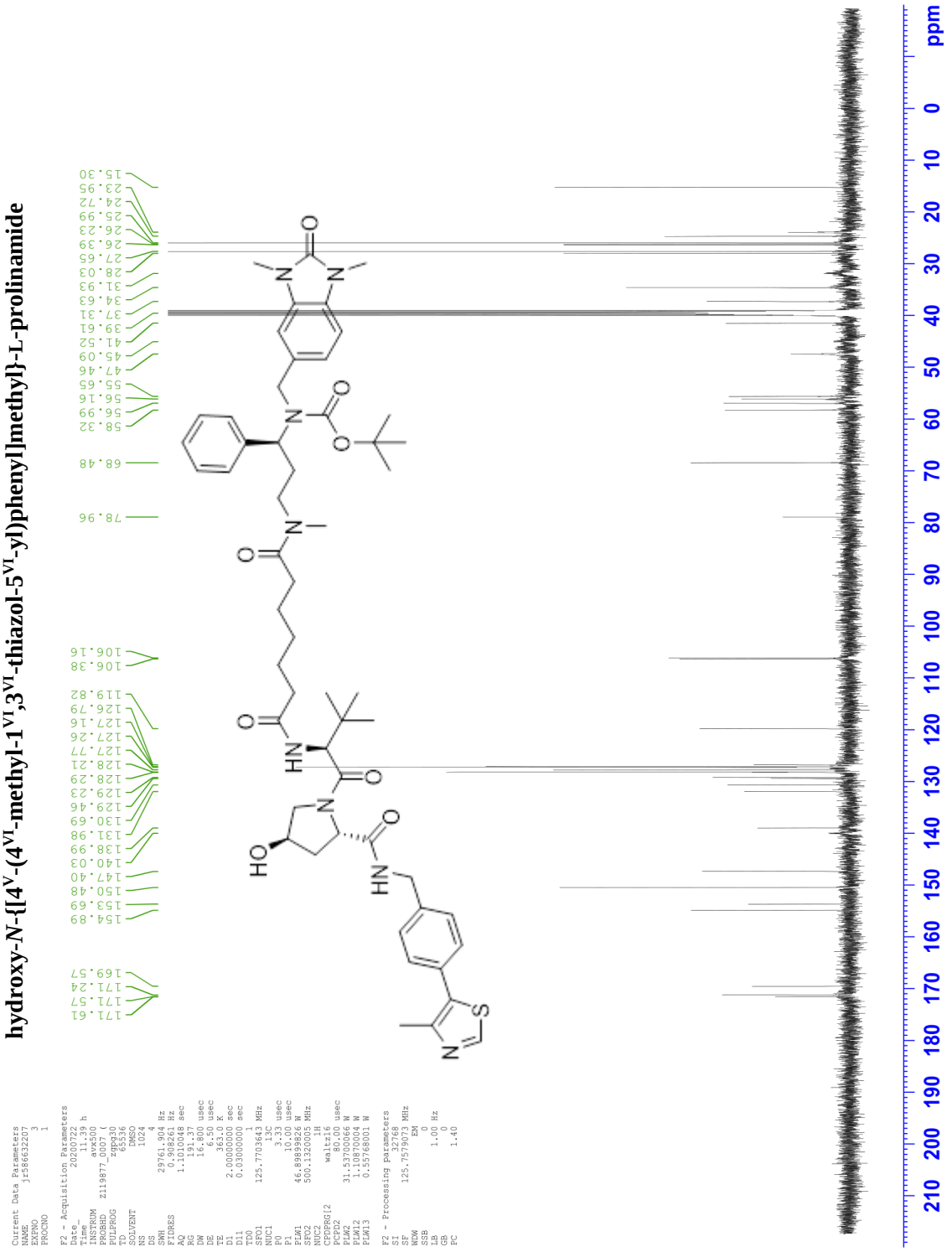
Ethyl 16'-{[(3*S*)-2'-{(*tert*-butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3'-phenylpropyl](methyl)amino}-7'-oxodecanoate



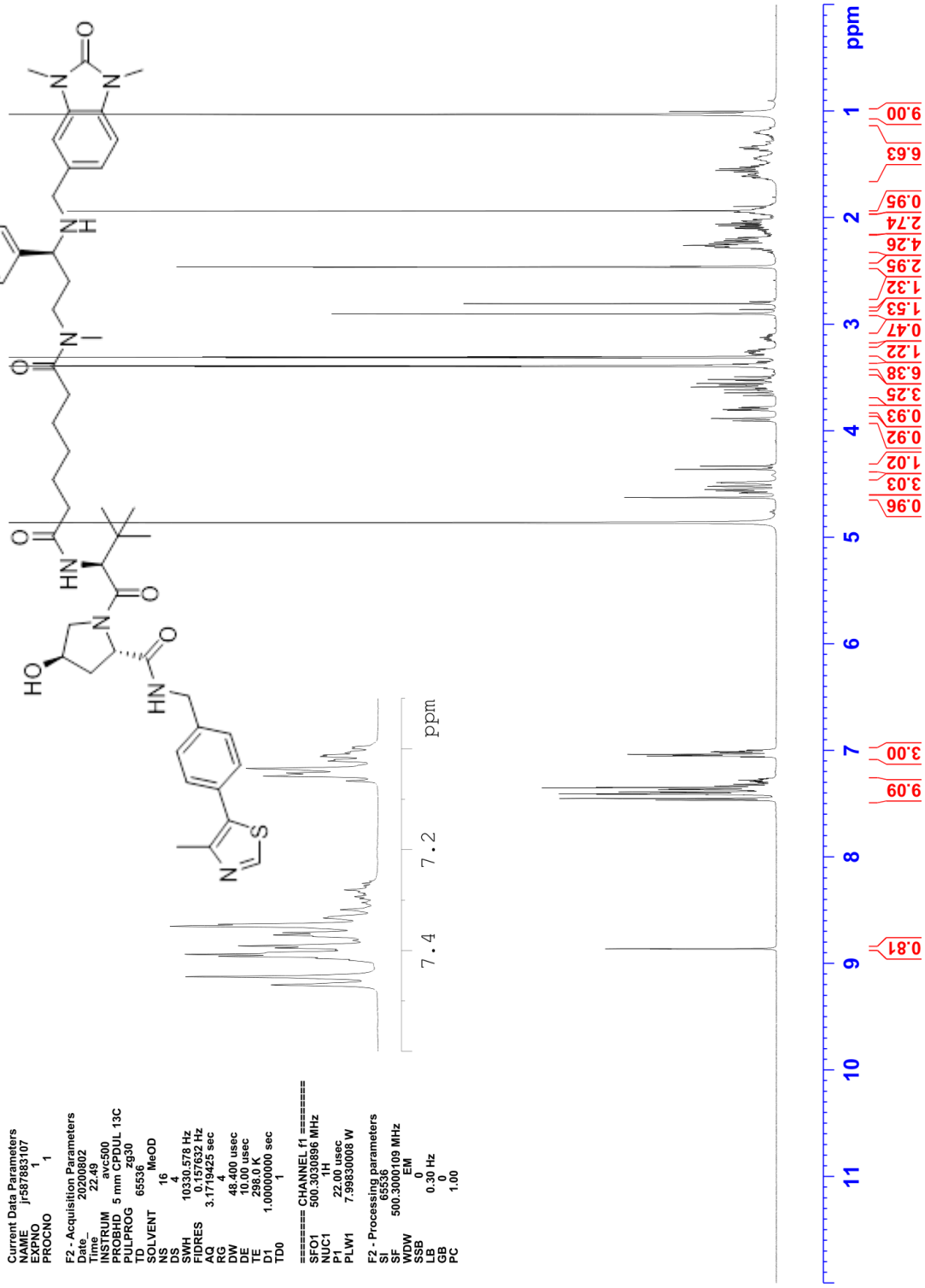
_N_-(14^I-{[(3_S_)-2^I-{(*tert*-Butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}-3^I-phenylpropyl](methyl)amino}-7^I-oxoheptanoyl)-16^I-methyl-L-valyl-(4_R_)-4^IV-hydroxy-_N_-{[4^V-(4^VI-methyl-1^VI,3^VI-thiazol-5^VI-yl)phenyl]methyl}-L-prolinamide



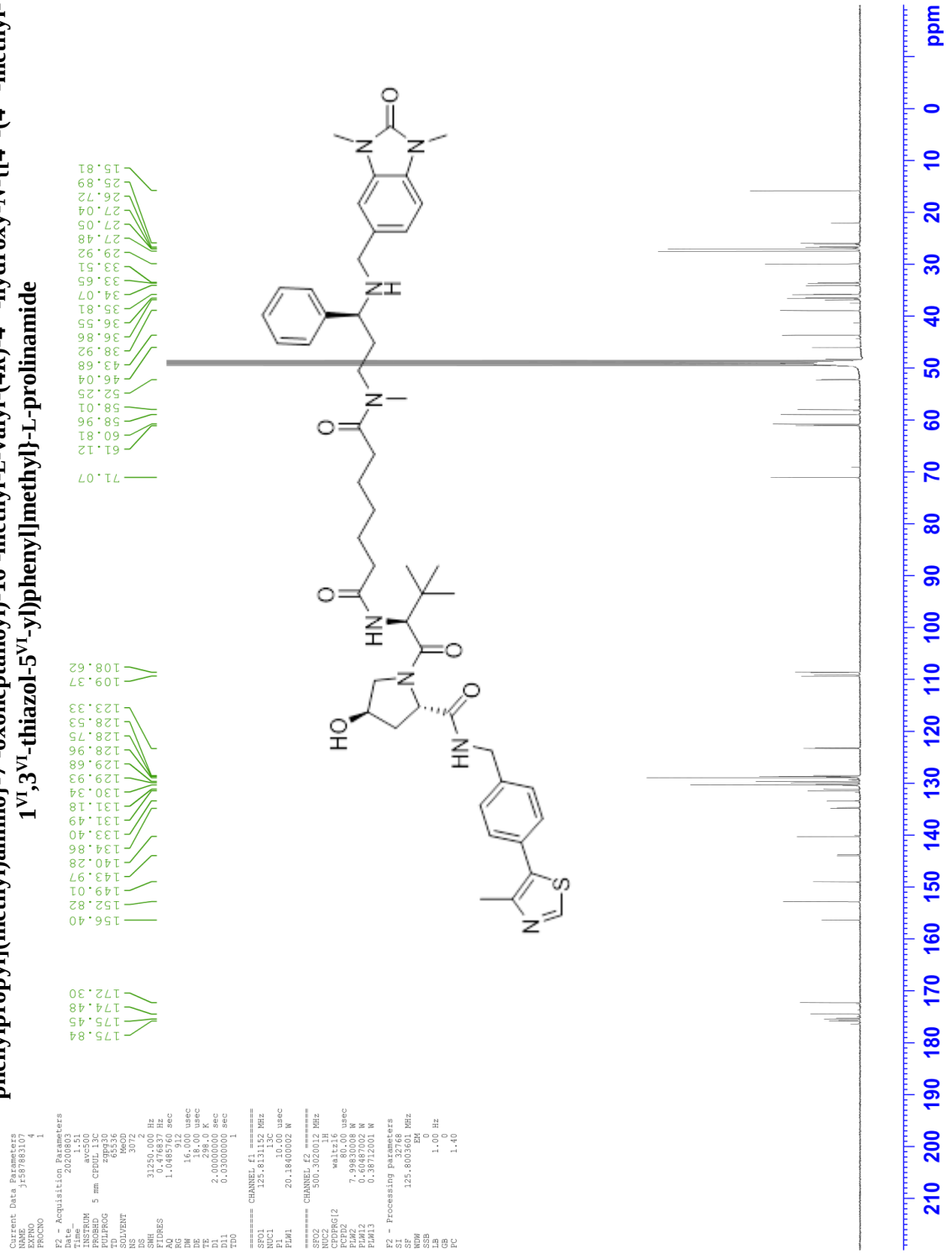
***N*-(14^{I}-{[(3*S*)-2^{I}-{(*tert*-Butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl](methyl)amino}-7^{I}-oxoheptanoyl)-16^{I}-methyl-L-valyl-(4*R*)-4^{IV}-hydroxy-*N*-{[4^{V}-(4^{VI}-methyl-1^{VI},3^{VI}-thiazol-5^{VI}-yl)phenyl]methyl}-L-prolinamide**



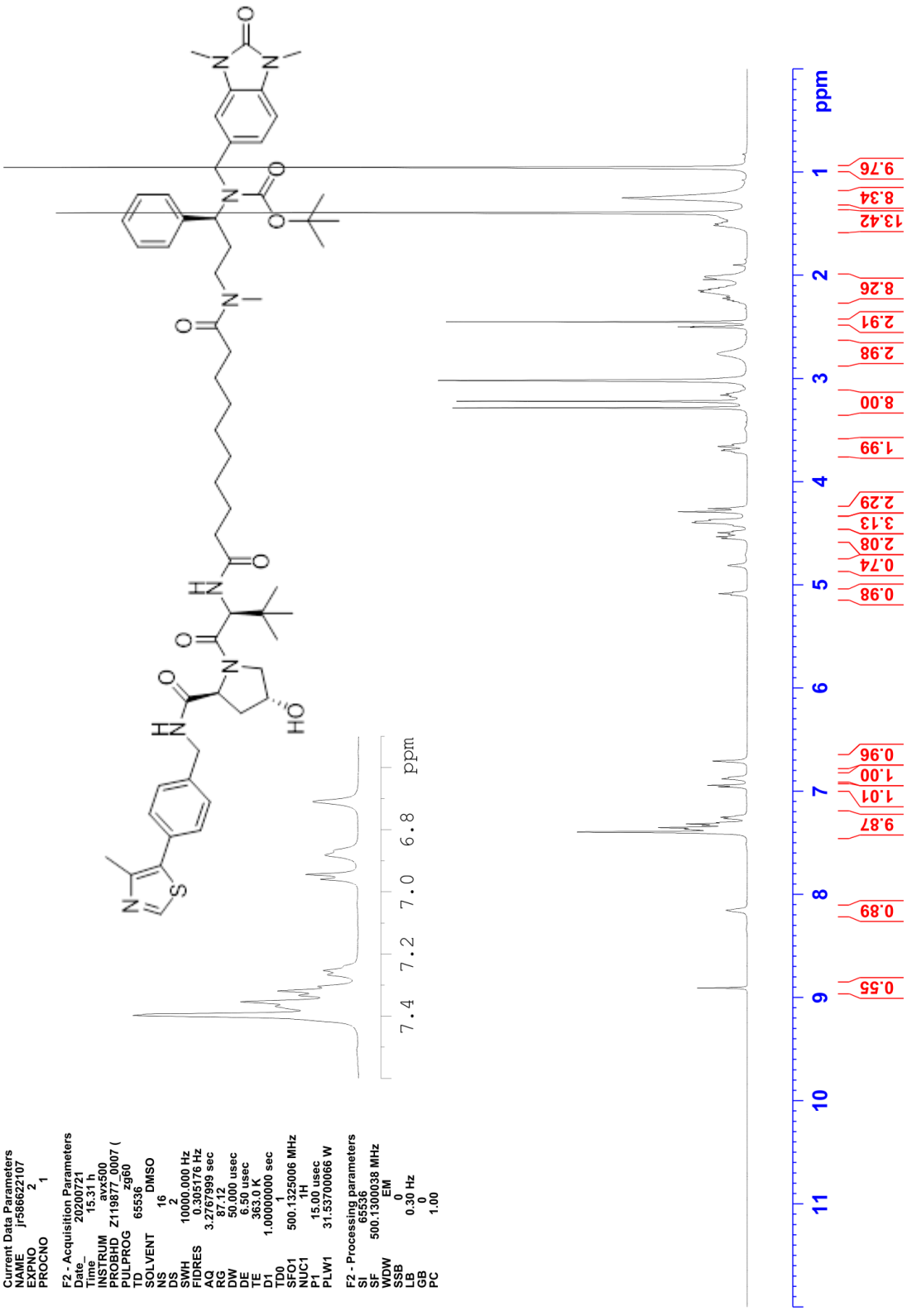
N-(14^{I}-{[(3*S*)-1^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl](methyl)amino}-7^{I}-oxoheptanoyl)-16^{I}-methyl-L-valyl-(4*R*)-4^{IV}-hydroxy-*N*-{[4^{V}-(4^{VI}-methyl-1^{VI},3^{VI}-thiazol-5^{VI}-yl)phenyl]methyl}-L-prolinamide



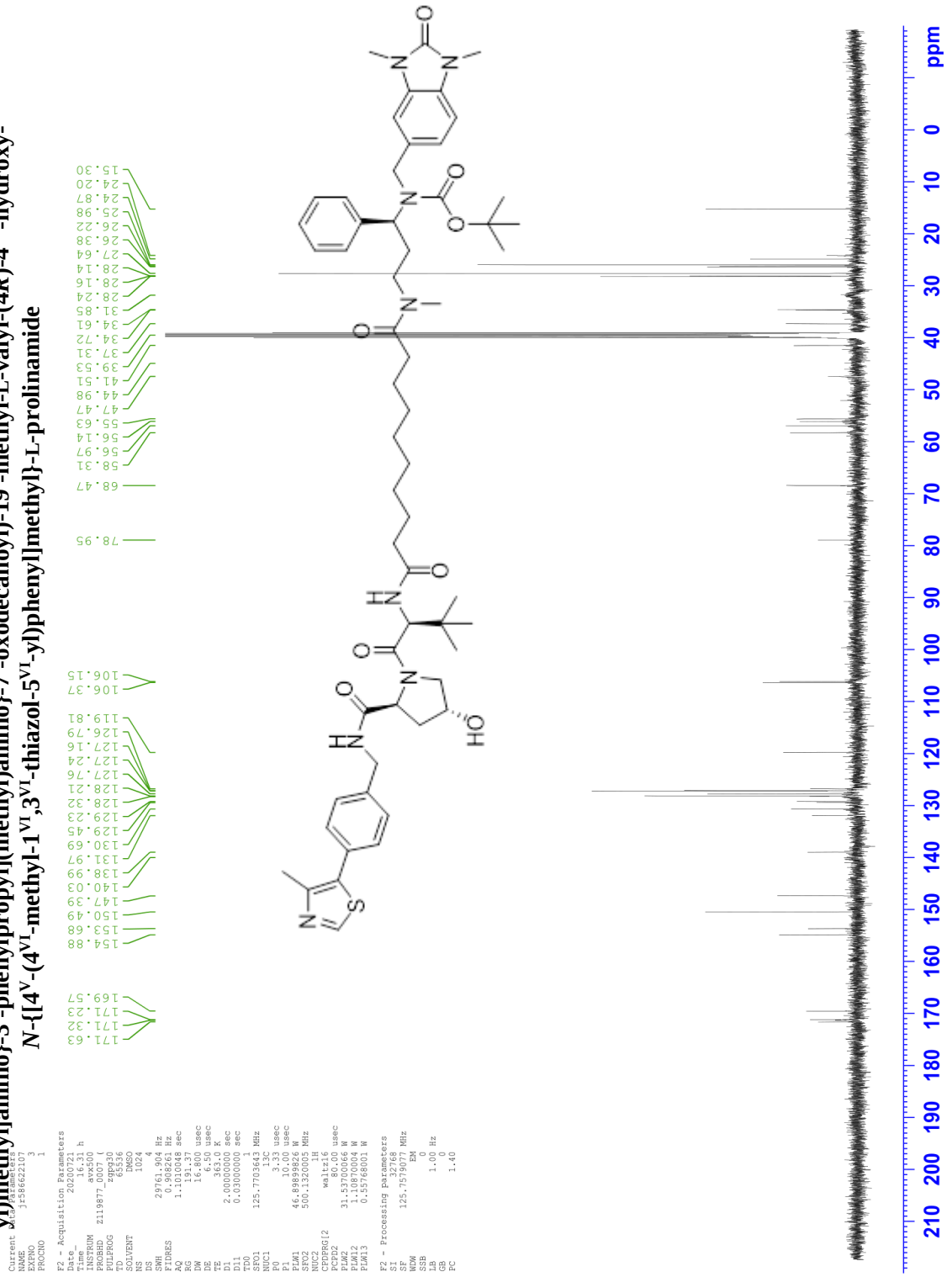
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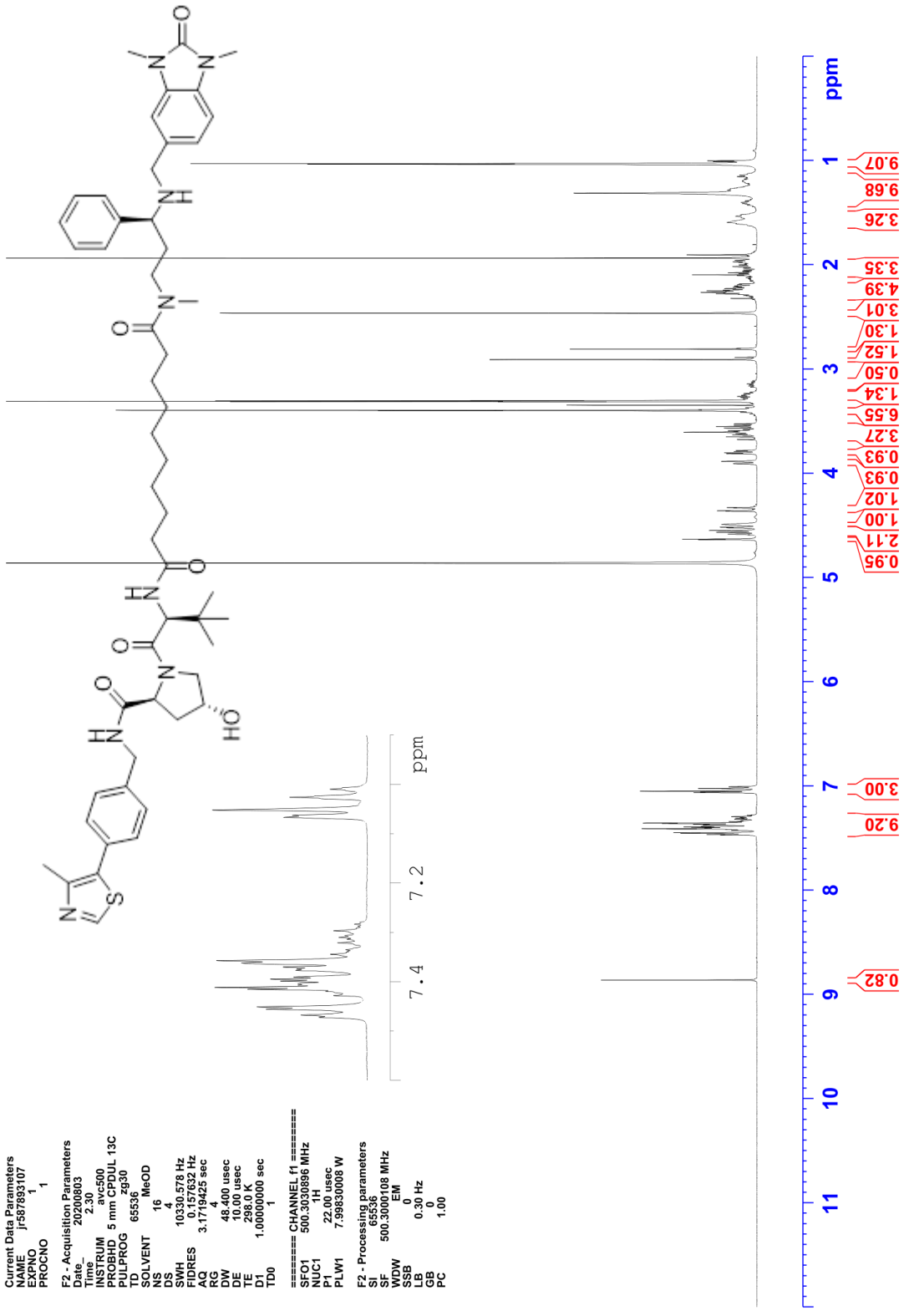
***N*-(17^{I}-{[(3S)-2^{I}-{(*tert*-Butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl](methyl)amino}-7^{I}-oxodecanoyl)-19^{I}-methyl-L-valyl-(4*R*)-4^{IV}-hydroxy-*N*-{[4^{V}-(4^{VI}-methyl-1^{VI},3^{VI}-thiazol-5^{VI}-yl)phenyl]methyl}-L-prolinamide**



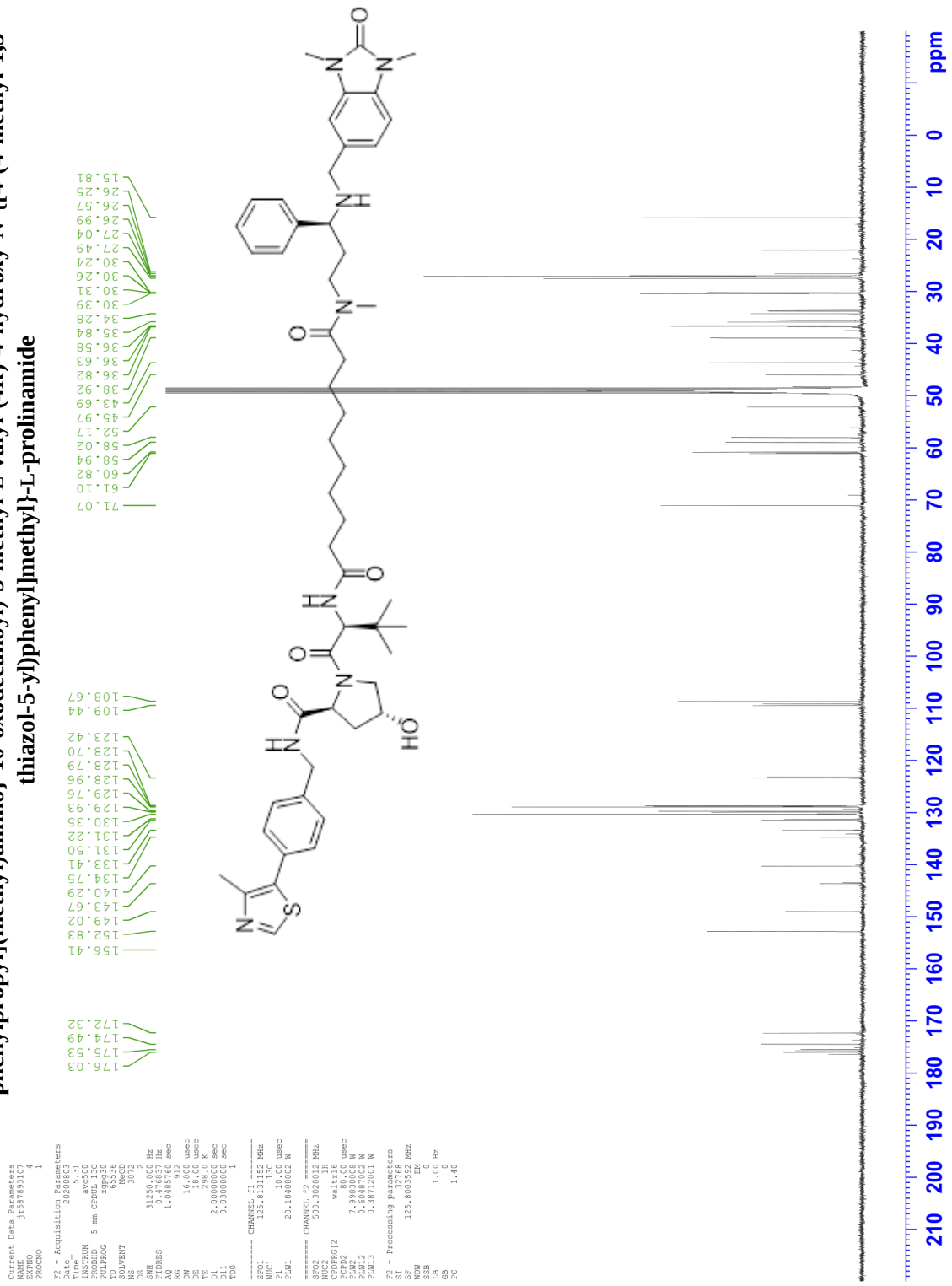
***N*-(17^{I}-{[(3*S*)-2^{I}-{(*tert*-Butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl](methyl)amino}-7^{I}-oxodecanoyl)-19^{I}-methyl-L-valyl-(4*R*)-4^{IV}-hydroxy-*N*-{[4^{V}-(4^{VI}-methyl-1^{VI},3^{VI}-thiazol-5^{VI}-yl)phenyl]methyl}-L-prolinamide**



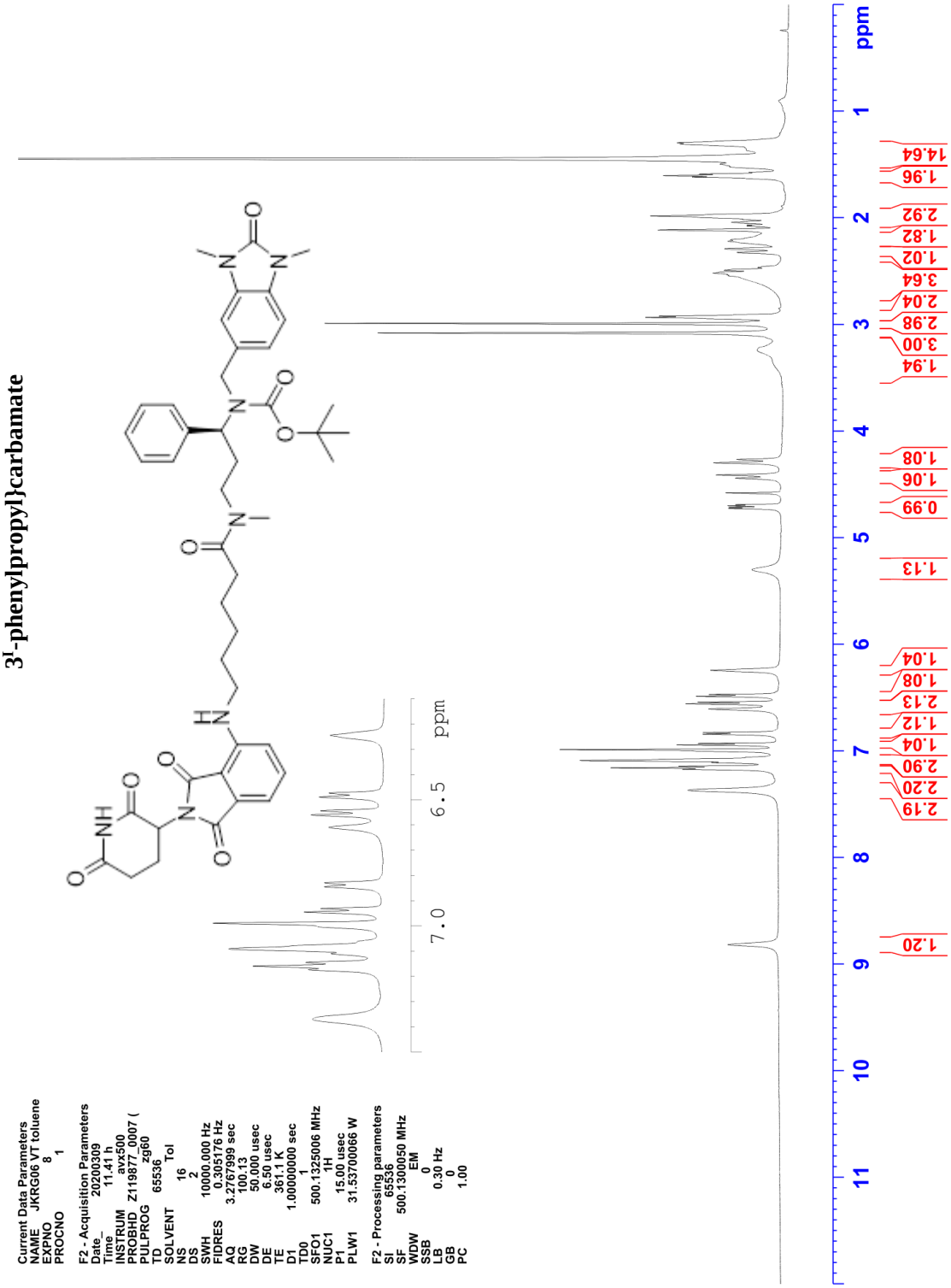
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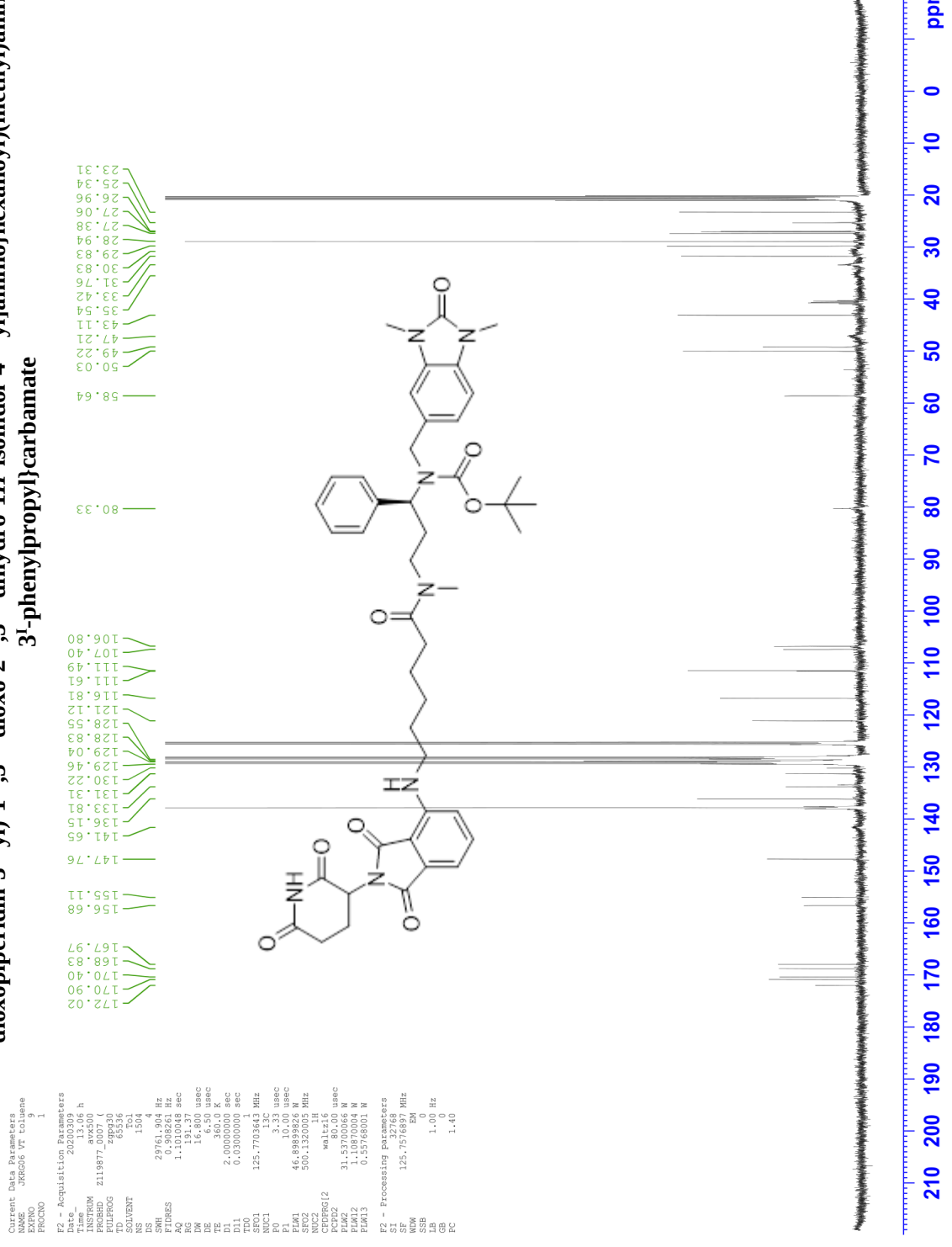
_N_-(10-{[(3_S_)-3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}-3-phenylpropyl](methyl)amino}-10-oxodecanoyl)-3-methyl-L-valyl-(4_R_)-4-hydroxy-_N_-{[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl}-L-prolinamide



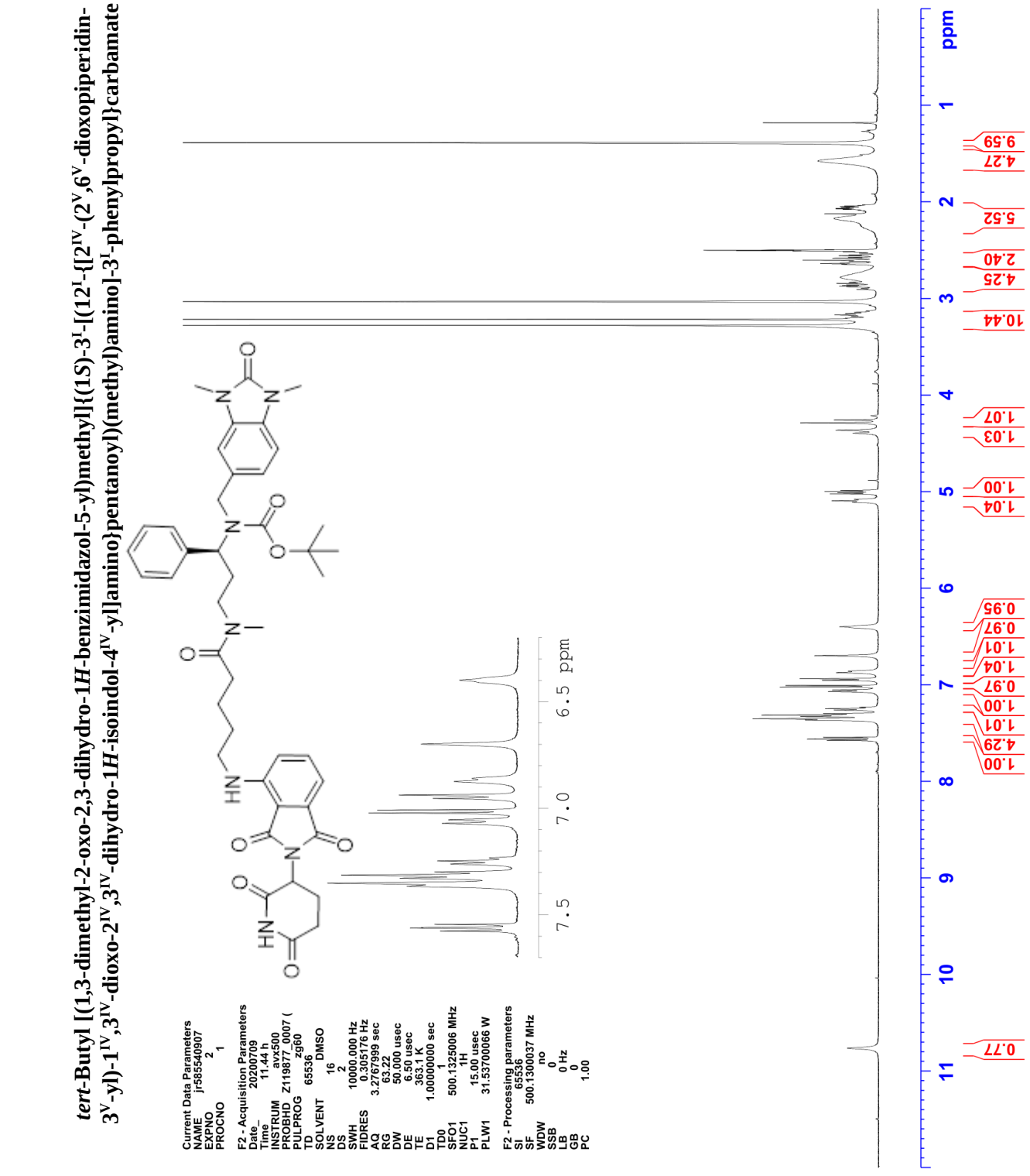
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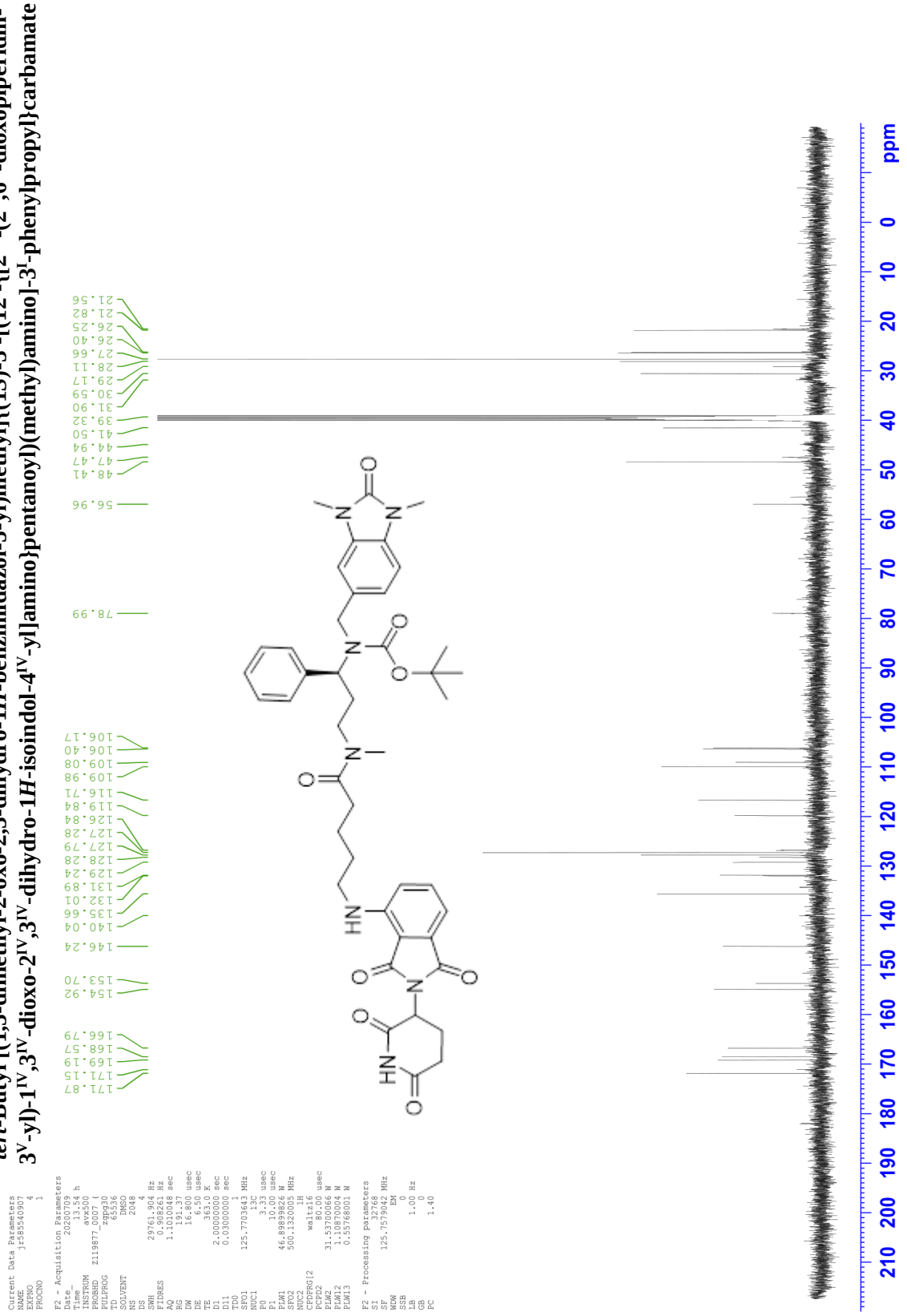
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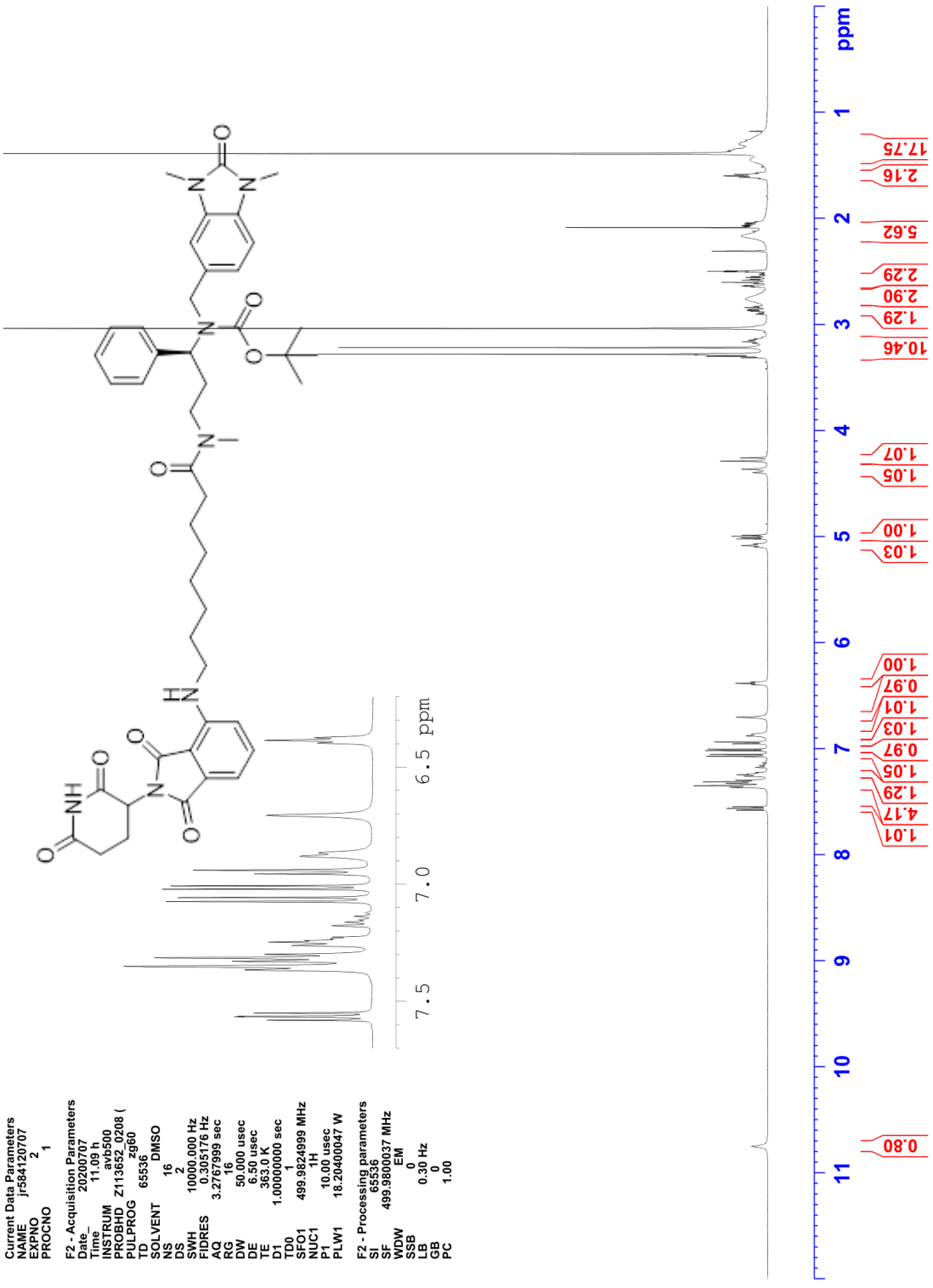
***tert*-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{(1*S*)-3^{I}-[(12^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}pentanoyl)(methyl)amino]-3^{I}-phenylpropyl}carbamate**



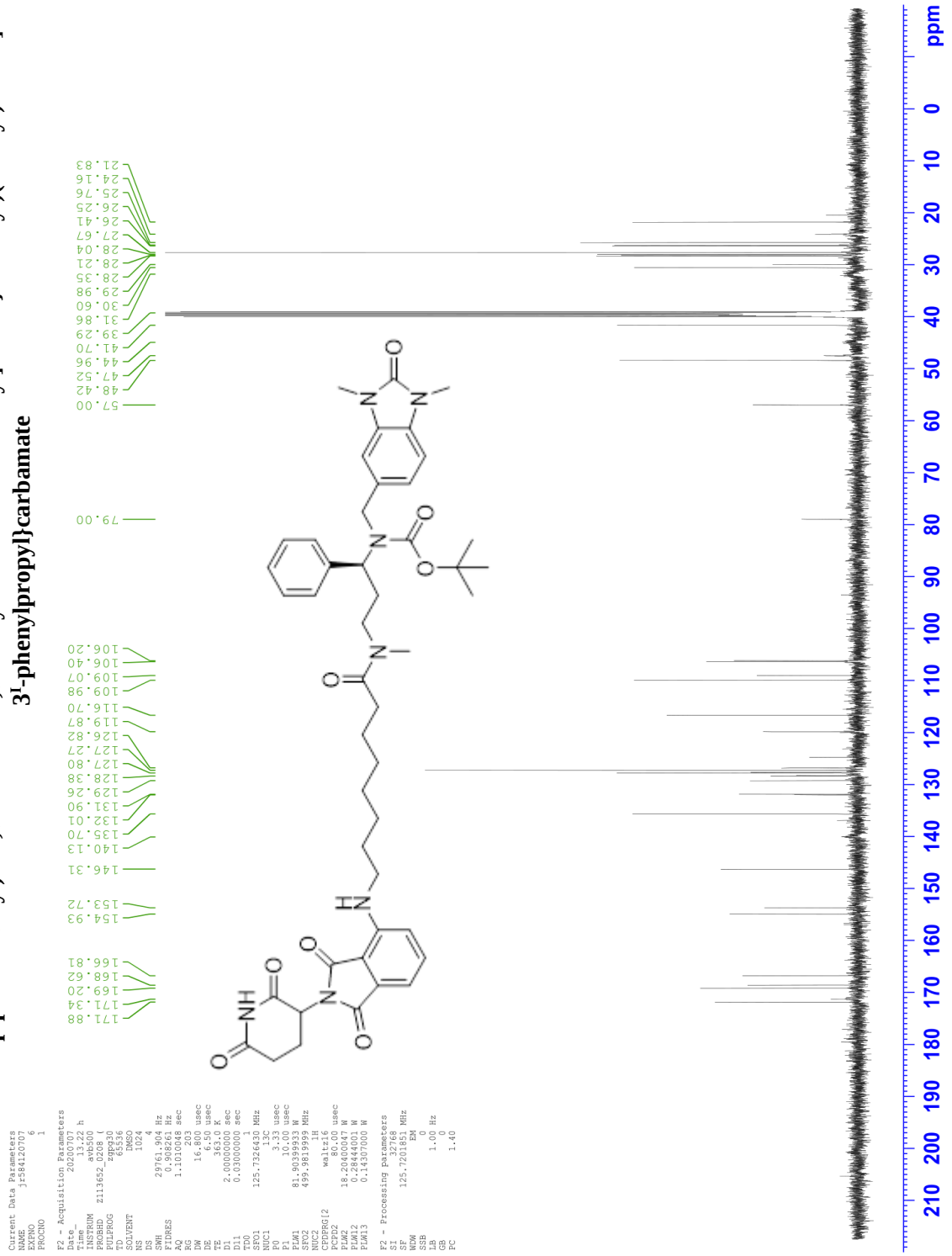
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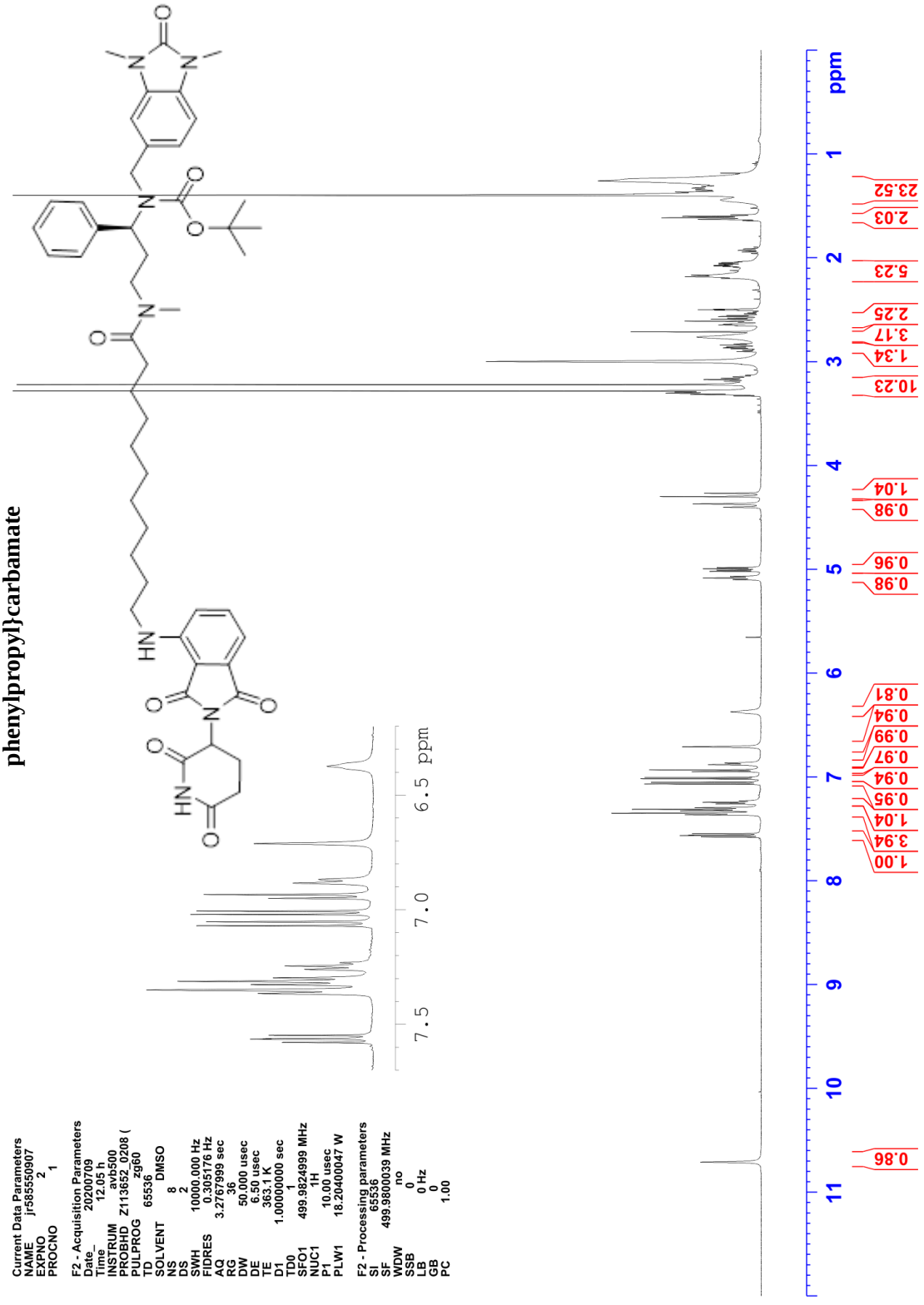
***tert*-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{(1*S*)-3^{1}-[(15^{1}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}octanoyl)(methyl)amino]-3^{1}-phenylpropyl}carbamate**



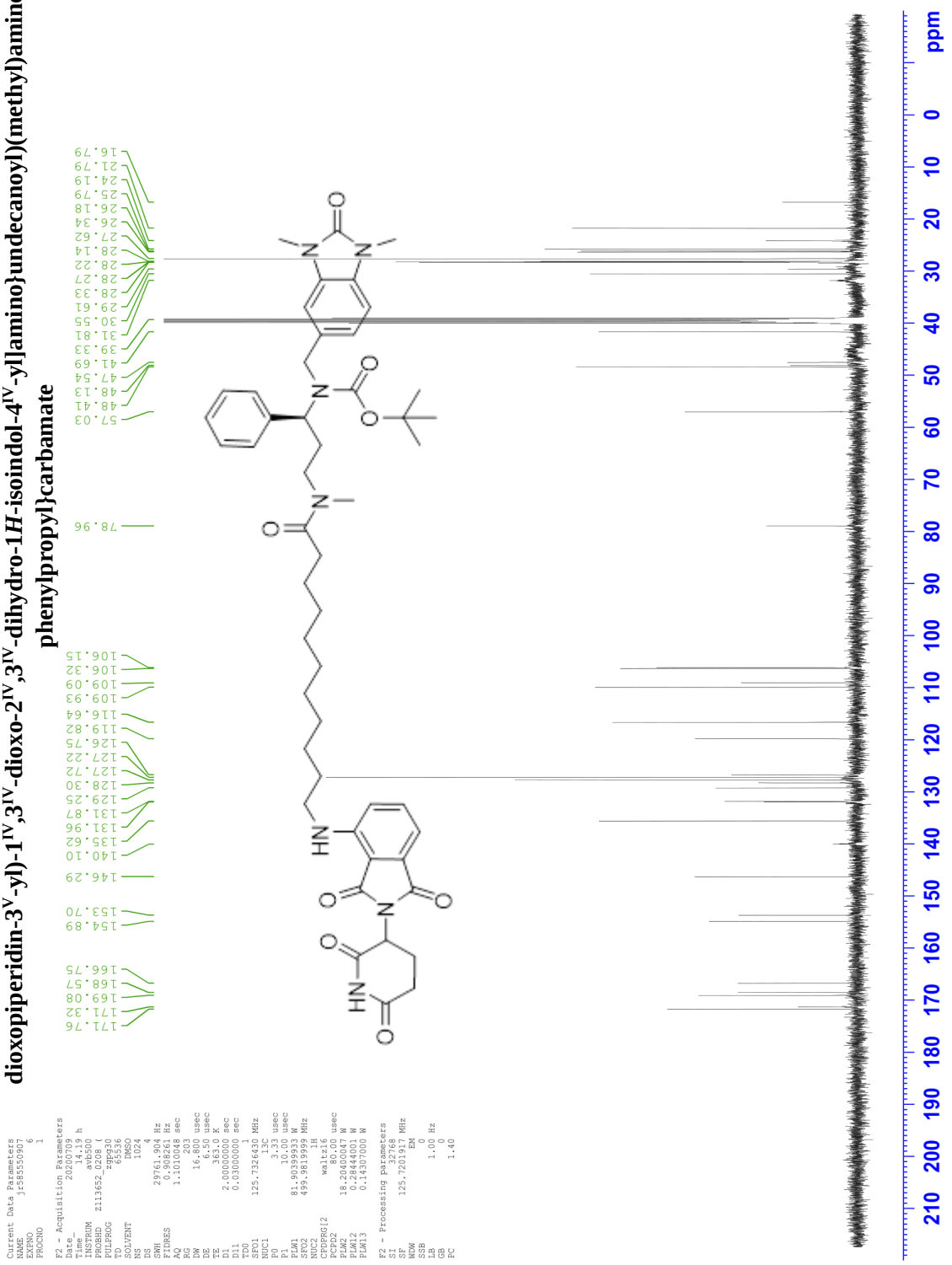
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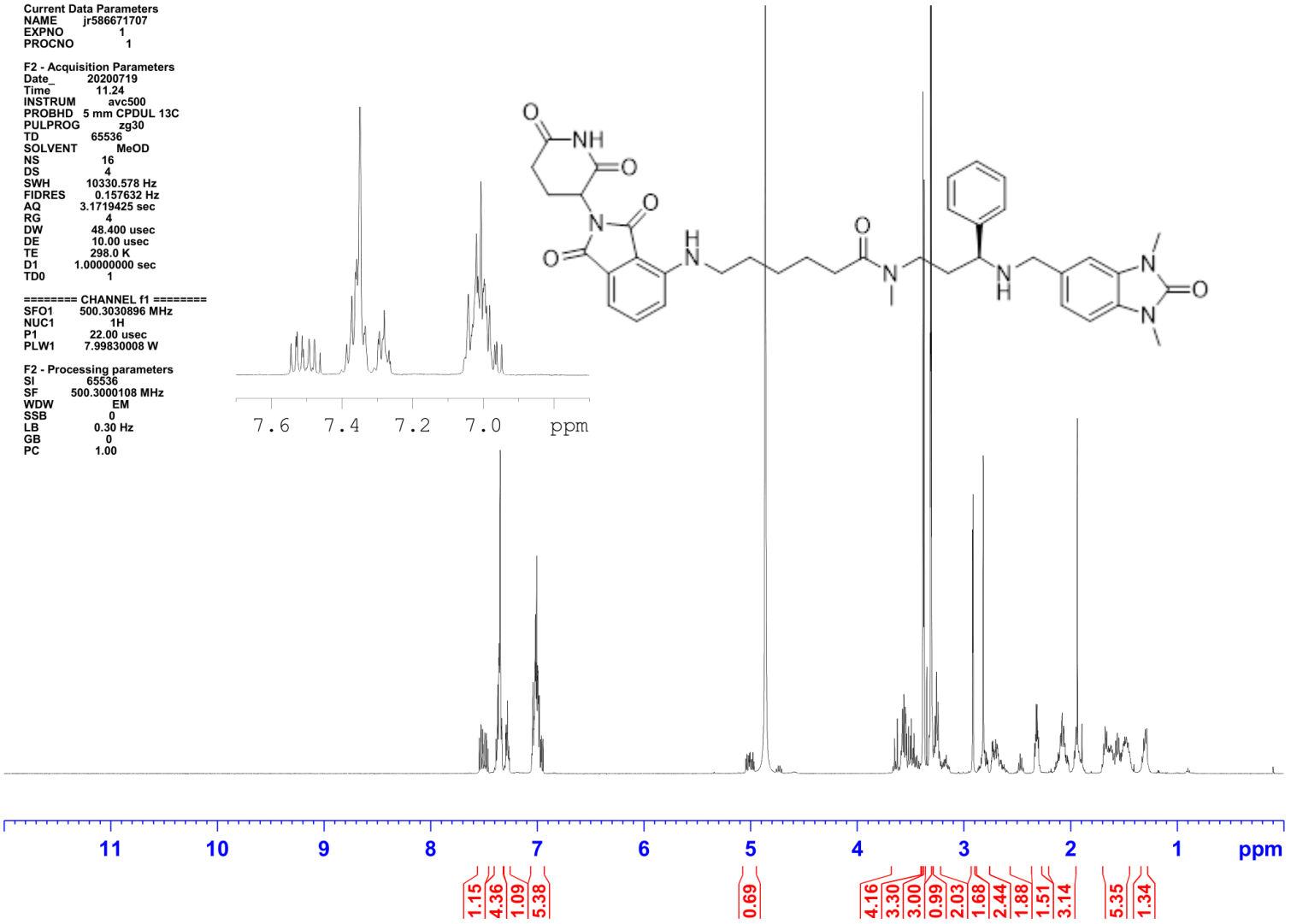
***tert*-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{(1*S*)-3^{I}-[(18^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}undecanoyl)(methyl)amino]-3^{I}-phenylpropyl}carbamate**



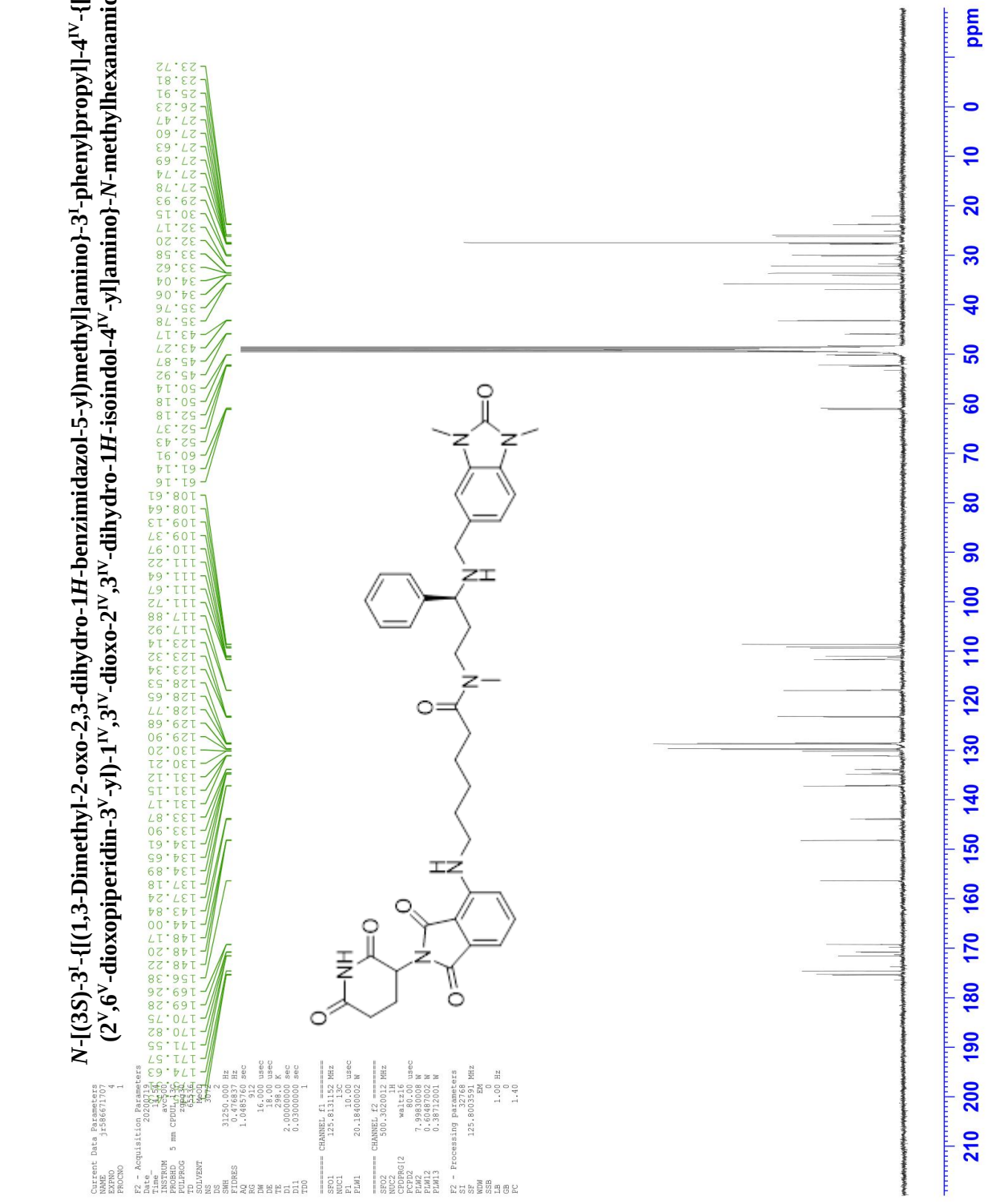
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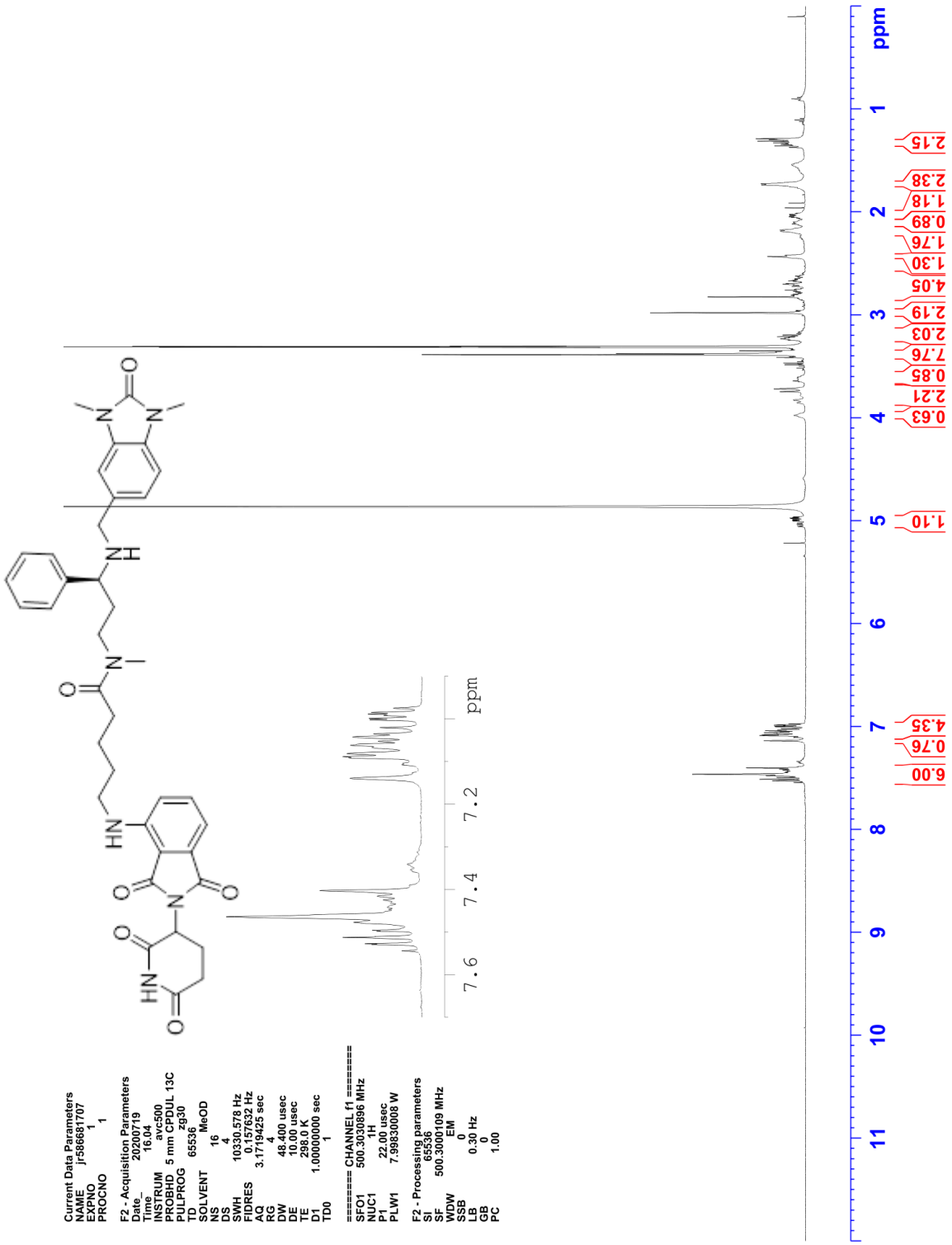
N-[(3*S*)-3^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-4^{IV}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}-*N*-methylhexanamide



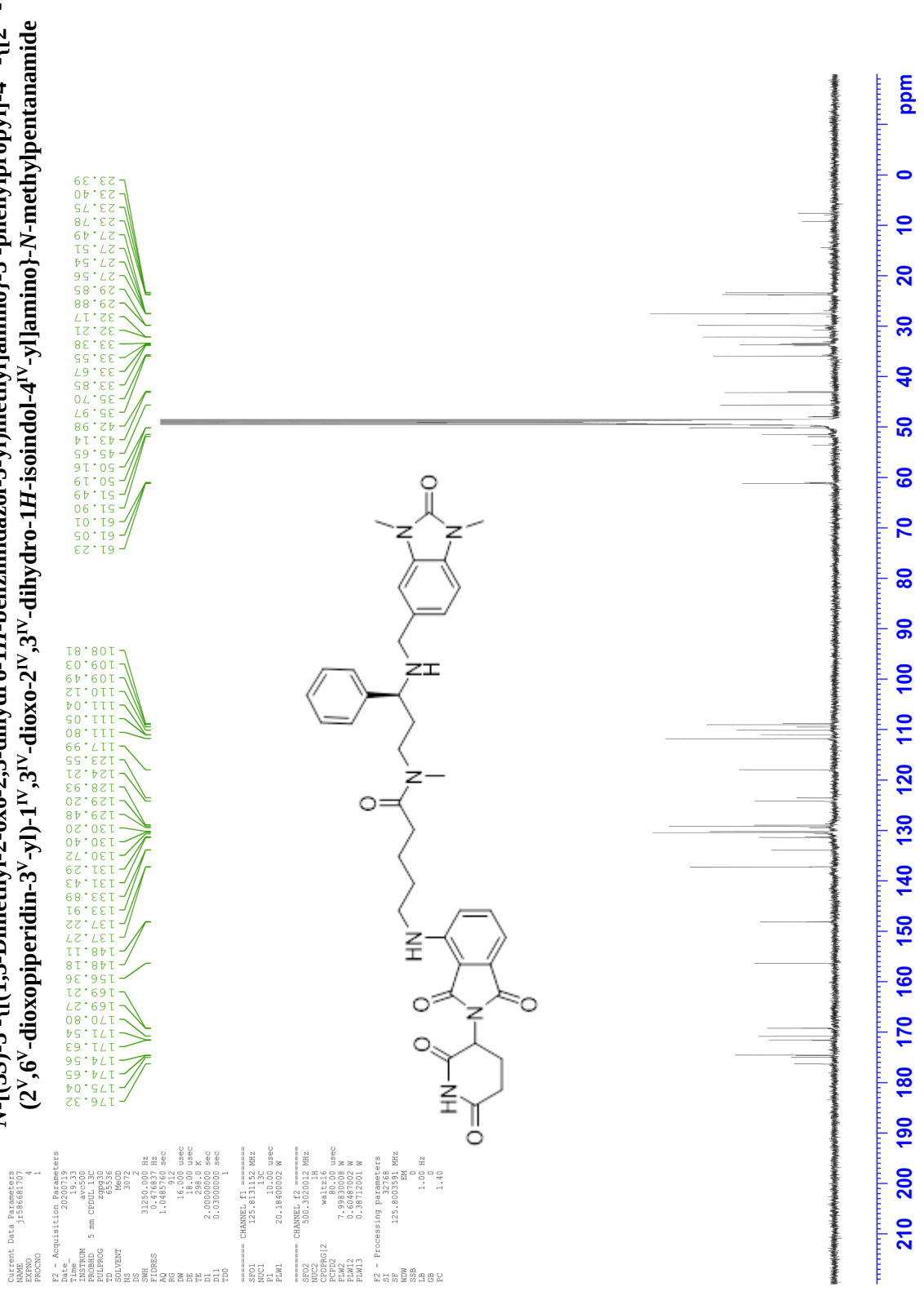
***N*-[(3S)-3^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-4^{IV}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}-*N*-methylhexanamide**



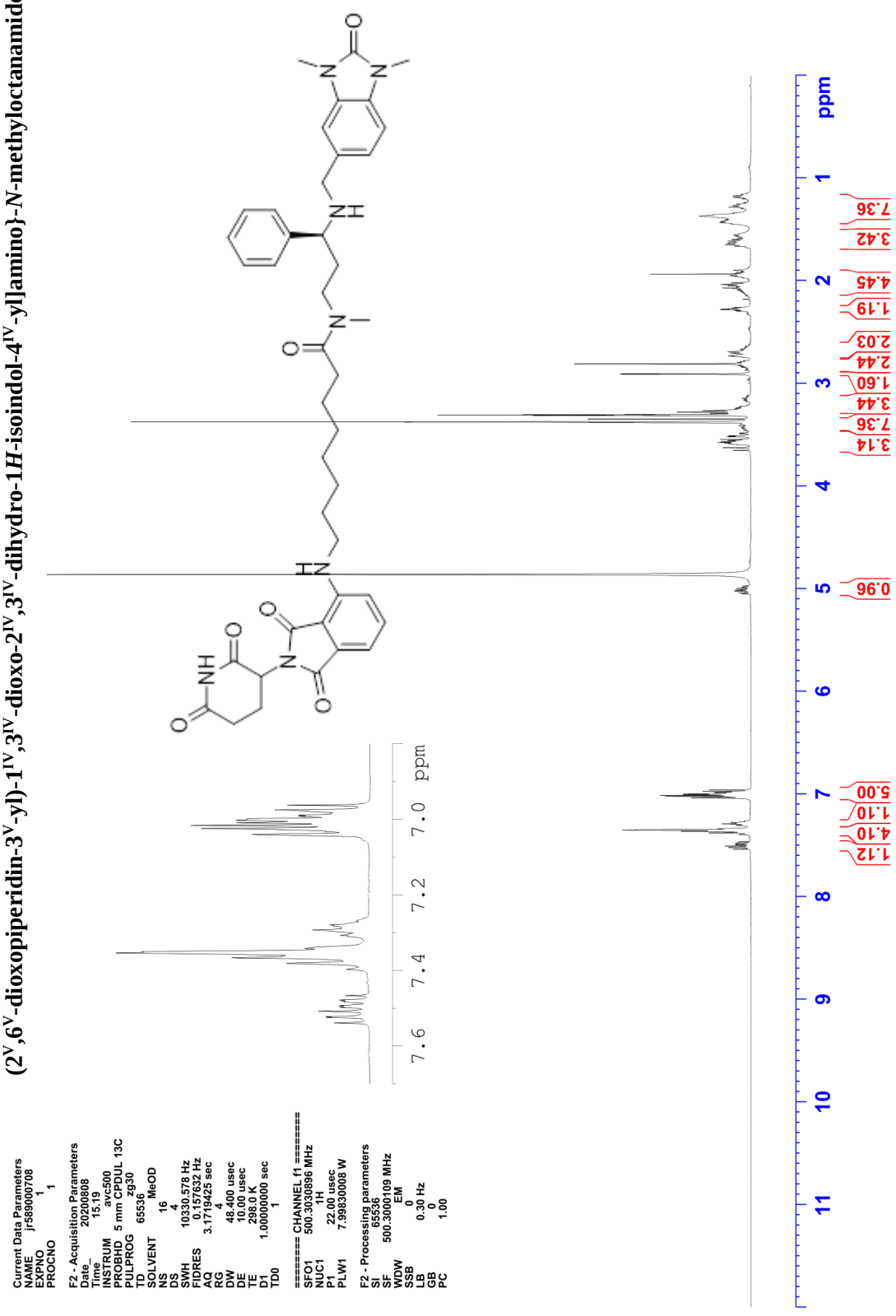
N-[(3S)-3^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-4^{IV}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}-*N*-methylpentanamide



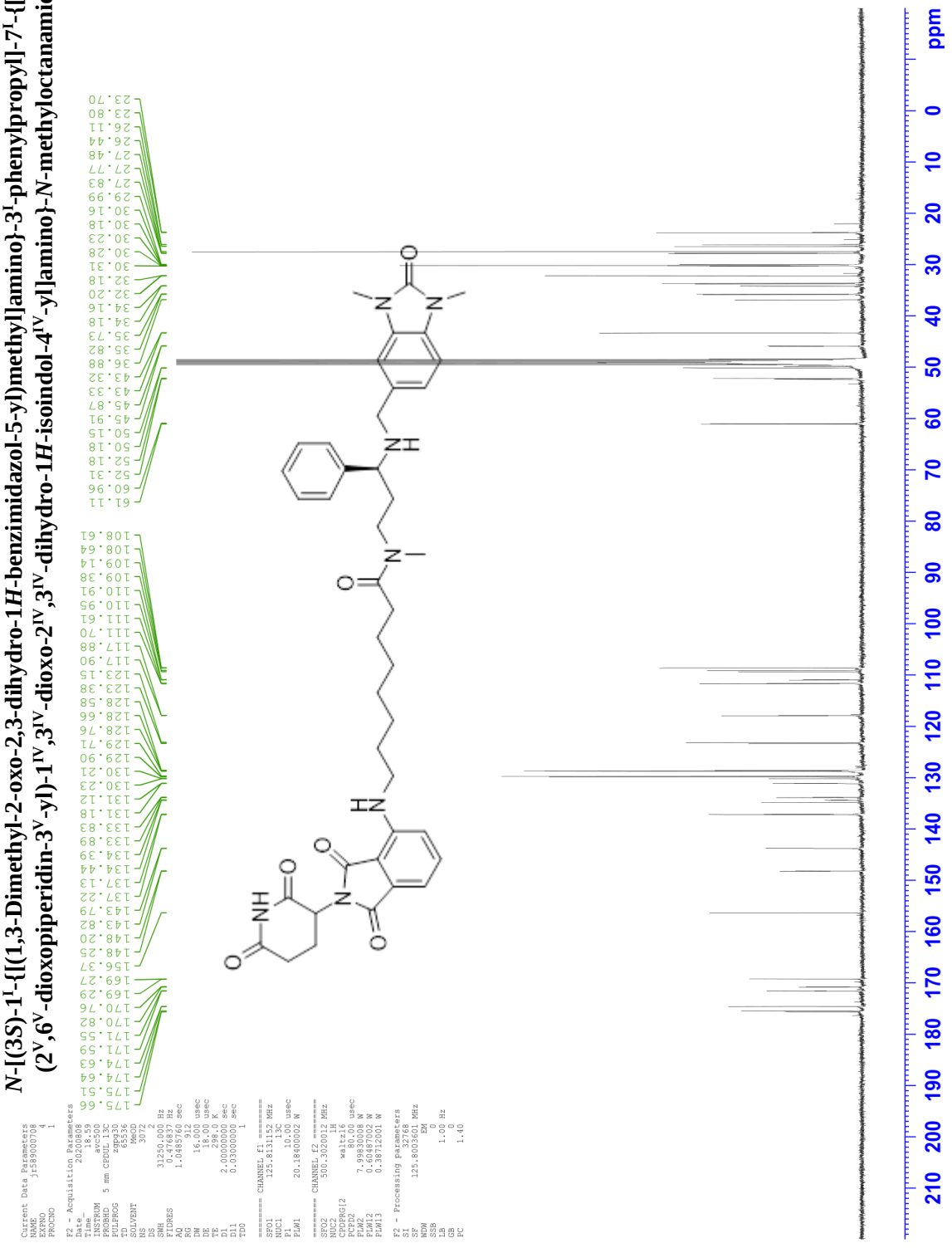
***N*-[(3S)-3^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-4^{IV}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}-*N*-methylpentanamide**



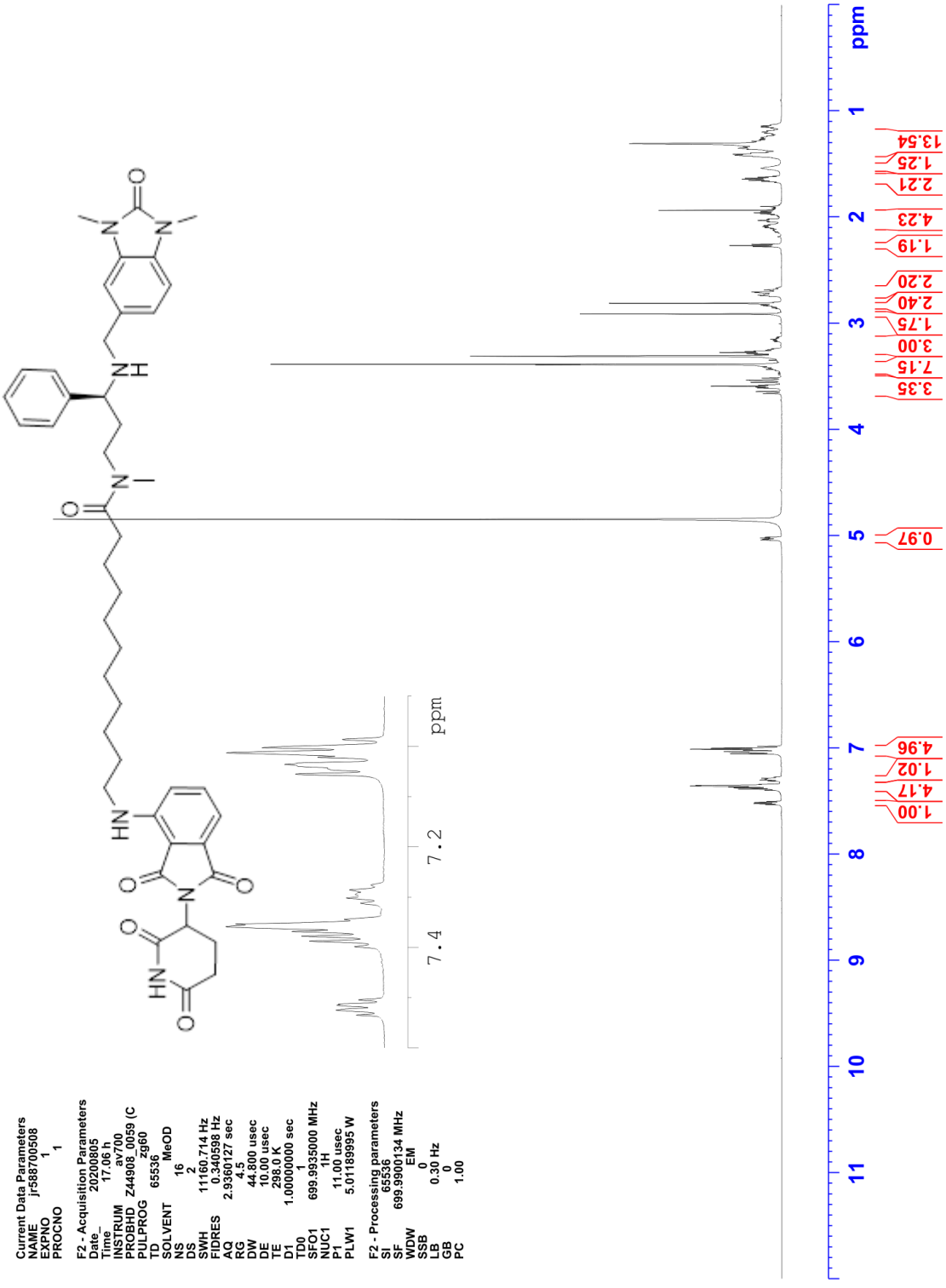
_N_-[(3S)-1^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-7^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1_H_-isoindol-4^{IV}-yl]amino}-_N_-methyloctanamide



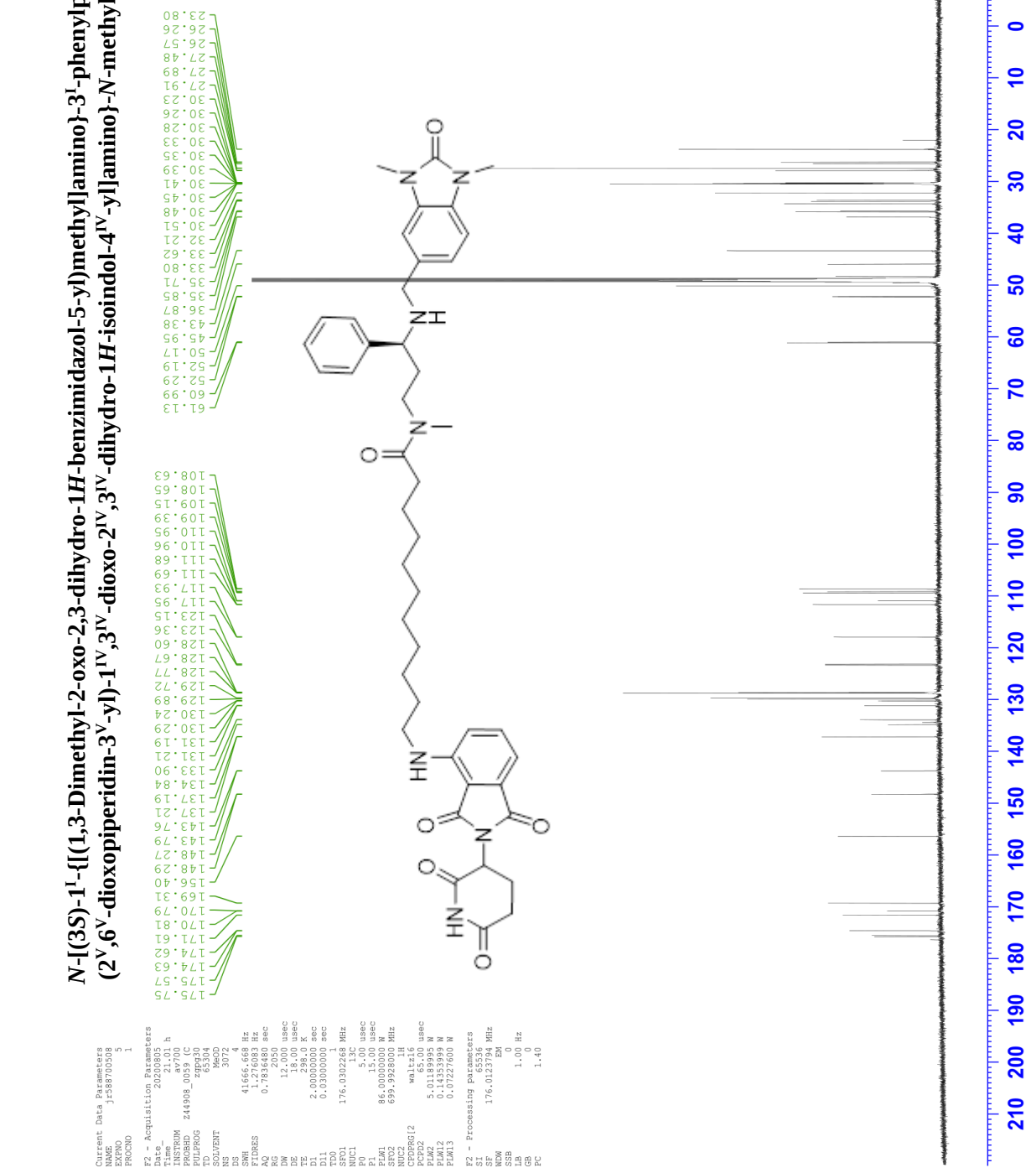
N-[(3S)-1^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-7^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1*H*-isoindol-4^{IV}-yl]amino}-*N*-methyloctanamide



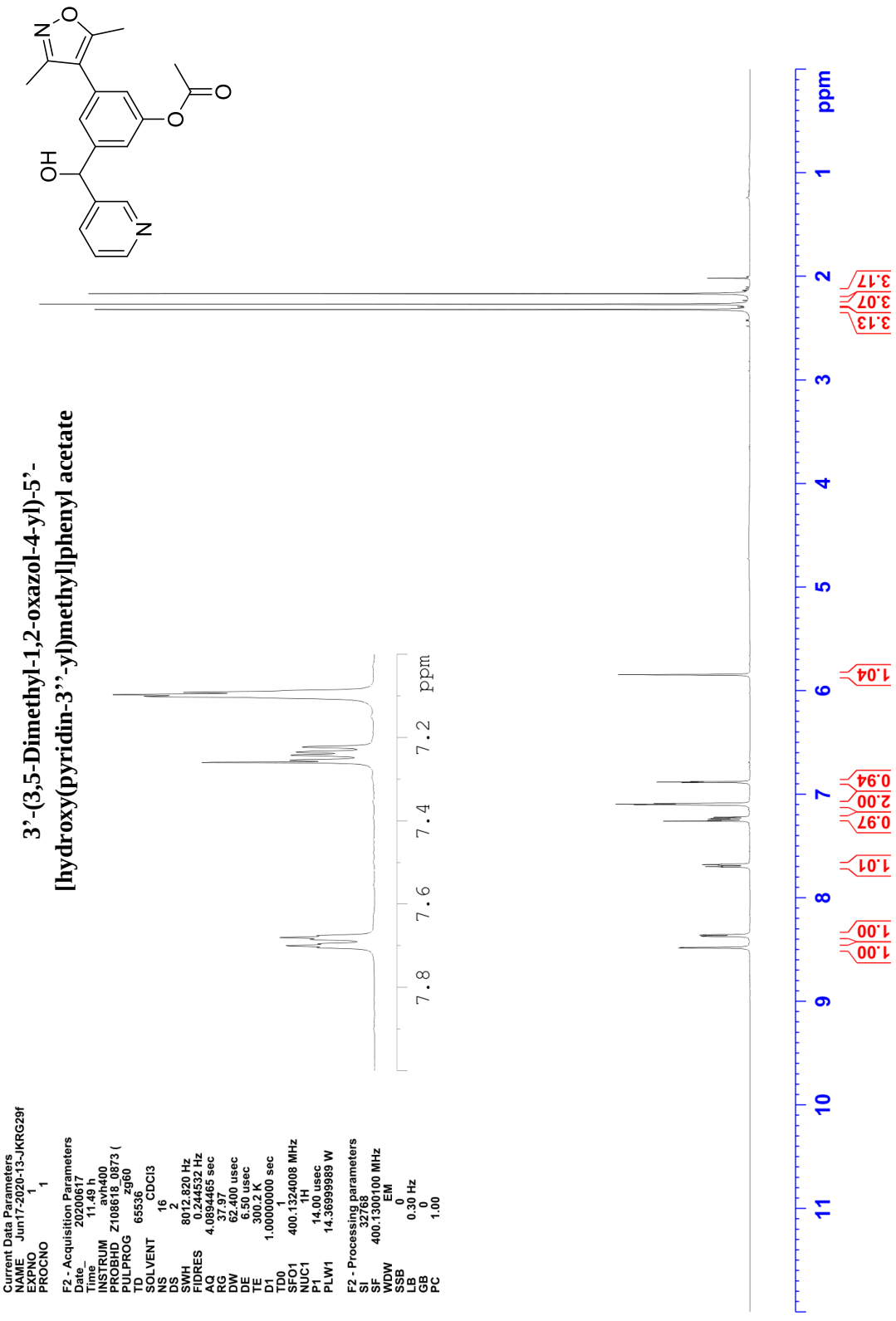
_N_-[(3S)-1^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-7^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1_H_-isoindol-4^{IV}-yl]amino}-_N_-methylundecanamide



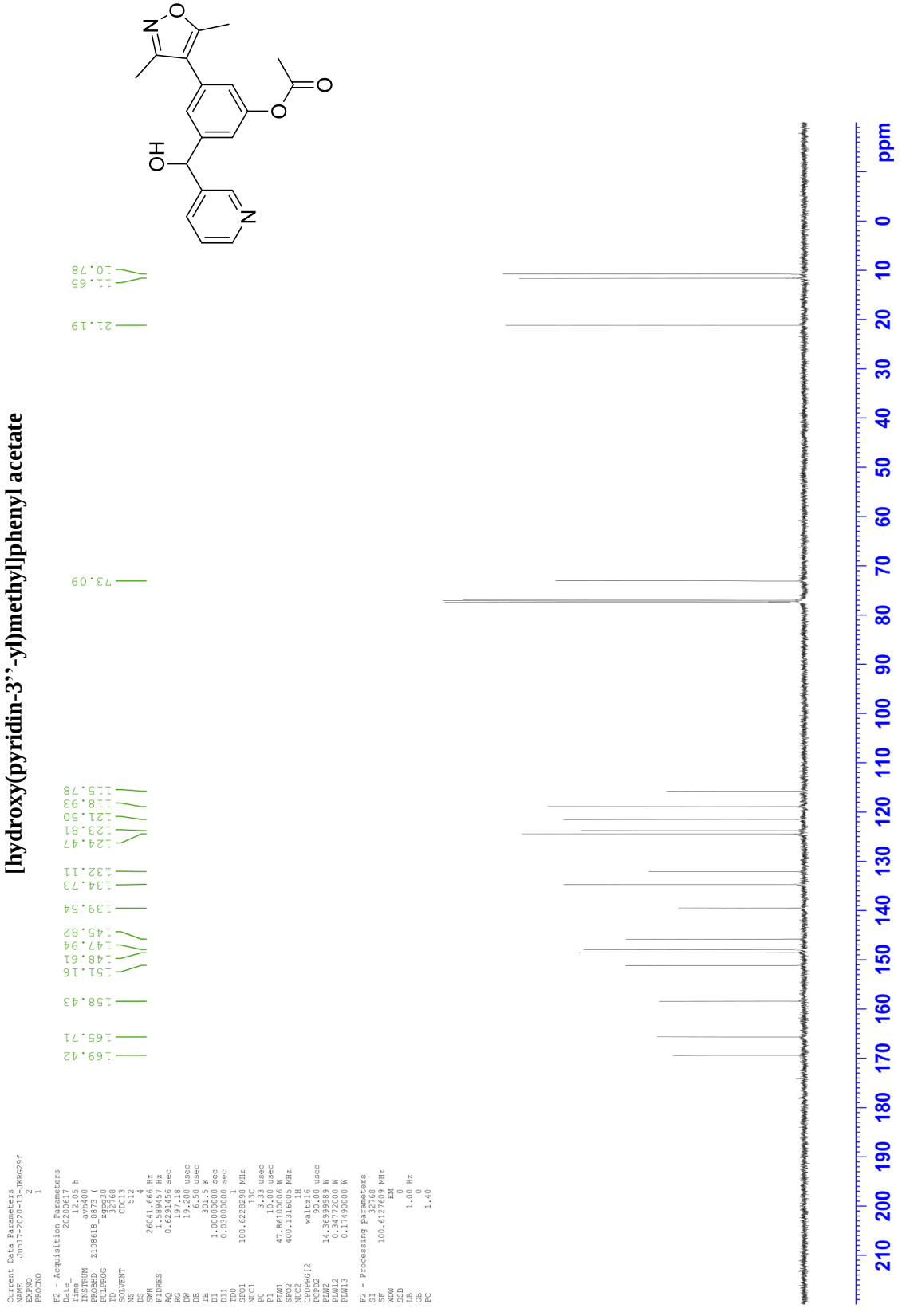
_N_-[(3S)-1^{I}-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}-3^{I}-phenylpropyl]-7^{I}-{[2^{IV}-(2^{V},6^{V}-dioxopiperidin-3^{V}-yl)-1^{IV},3^{IV}-dioxo-2^{IV},3^{IV}-dihydro-1_H_-isoindol-4^{IV}-yl]amino}-_N_-methylundecanamide

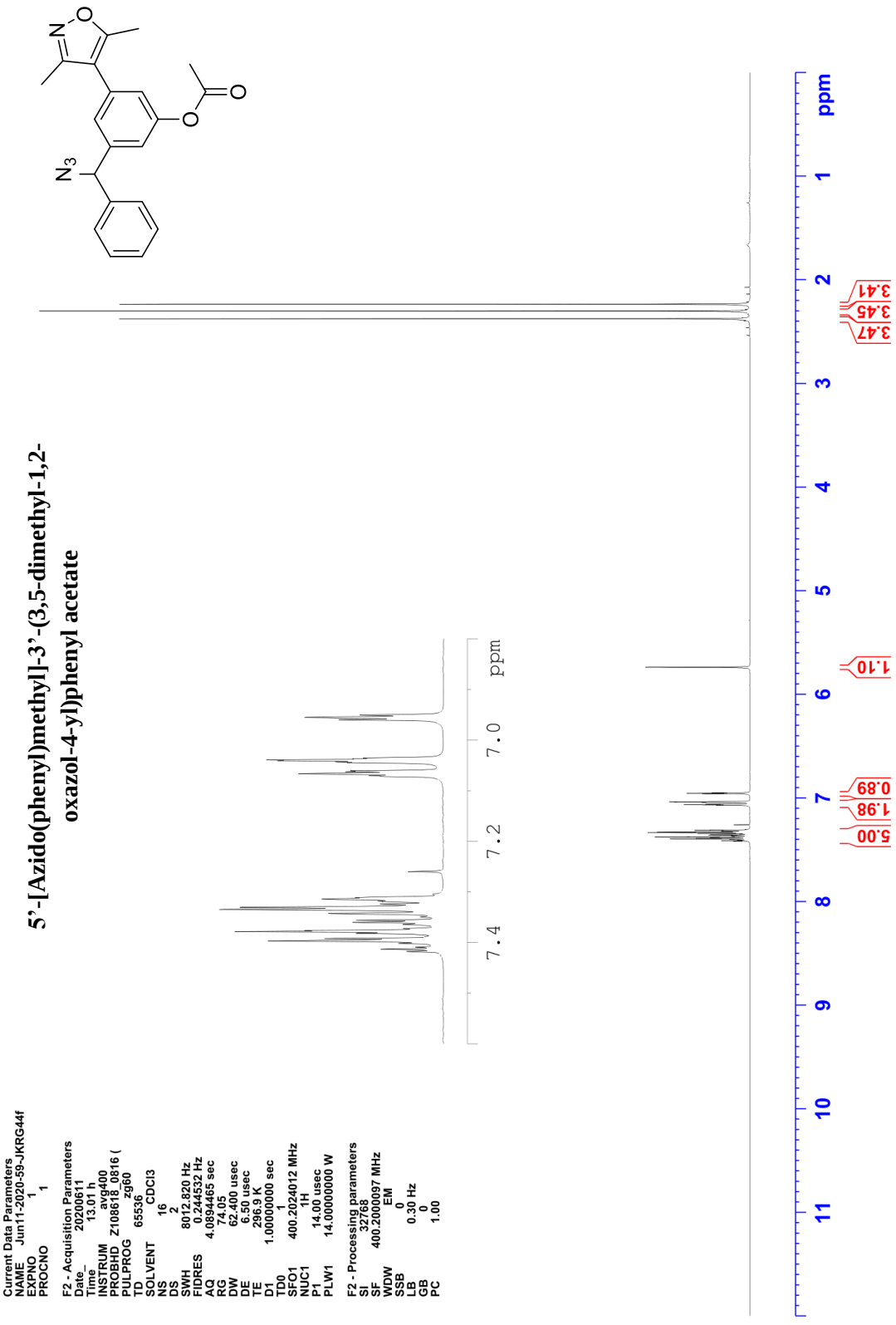


Chapter 5

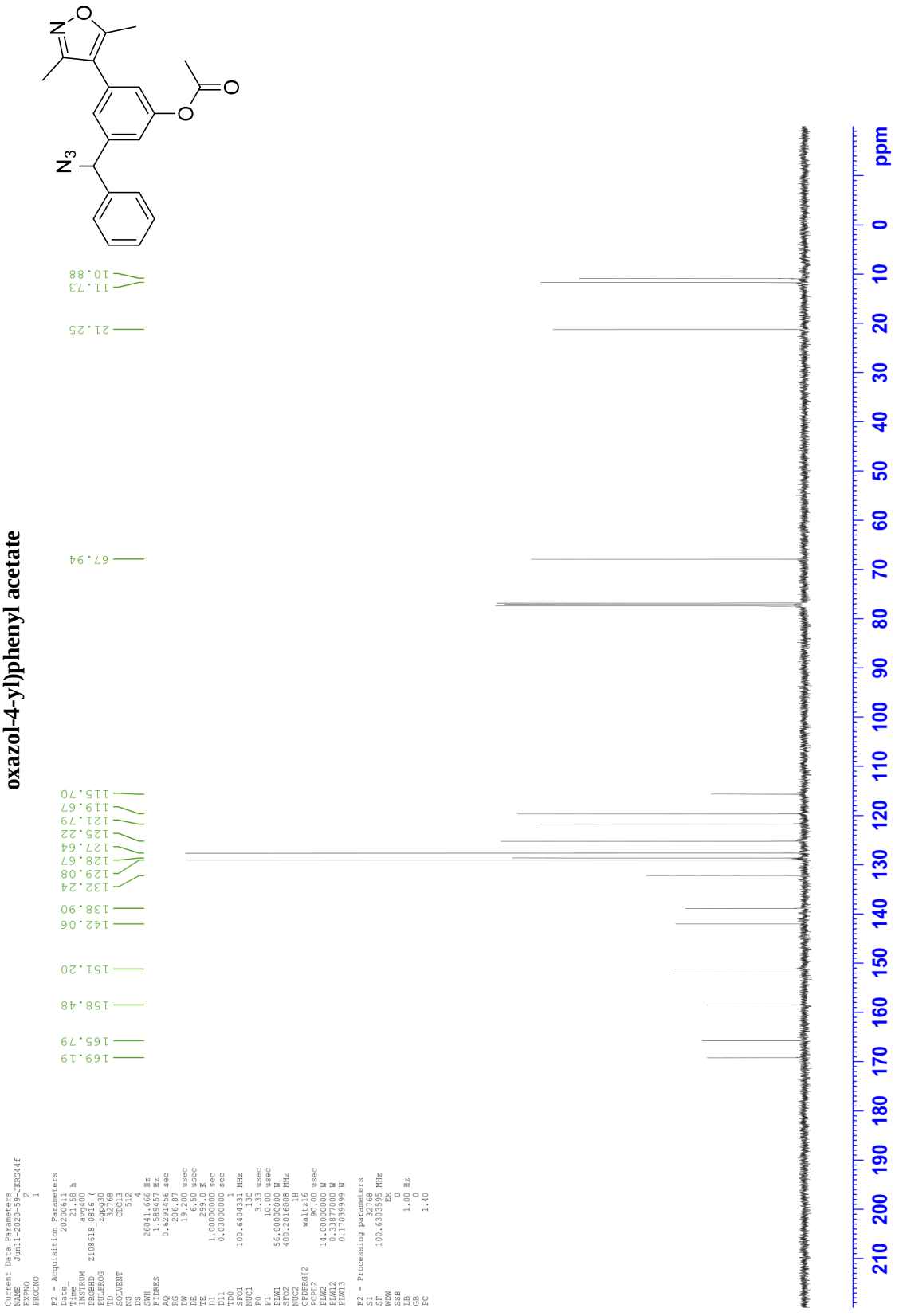

3'-(3,5-Dimethyl-1,2-oxazol-4-yl)-5'-[hydroxy(pyridin-3''-yl)methyl]phenyl acetate
Current Data Parameters
NAME Jun17-2020-13-JKRG29f
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200617
Time 11.49 h
INSTRUM avh400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 37.97
DW 62.400 usec
DE 6.50 usec
TE 300.2 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.8
7.6
7.4
7.2
ppm
1.00
1.00
1.01
0.97
2.00
0.94
1.04
3.13
3.07
3.17
11
10
9
8
7
6
5
4
3
2
1
ppm

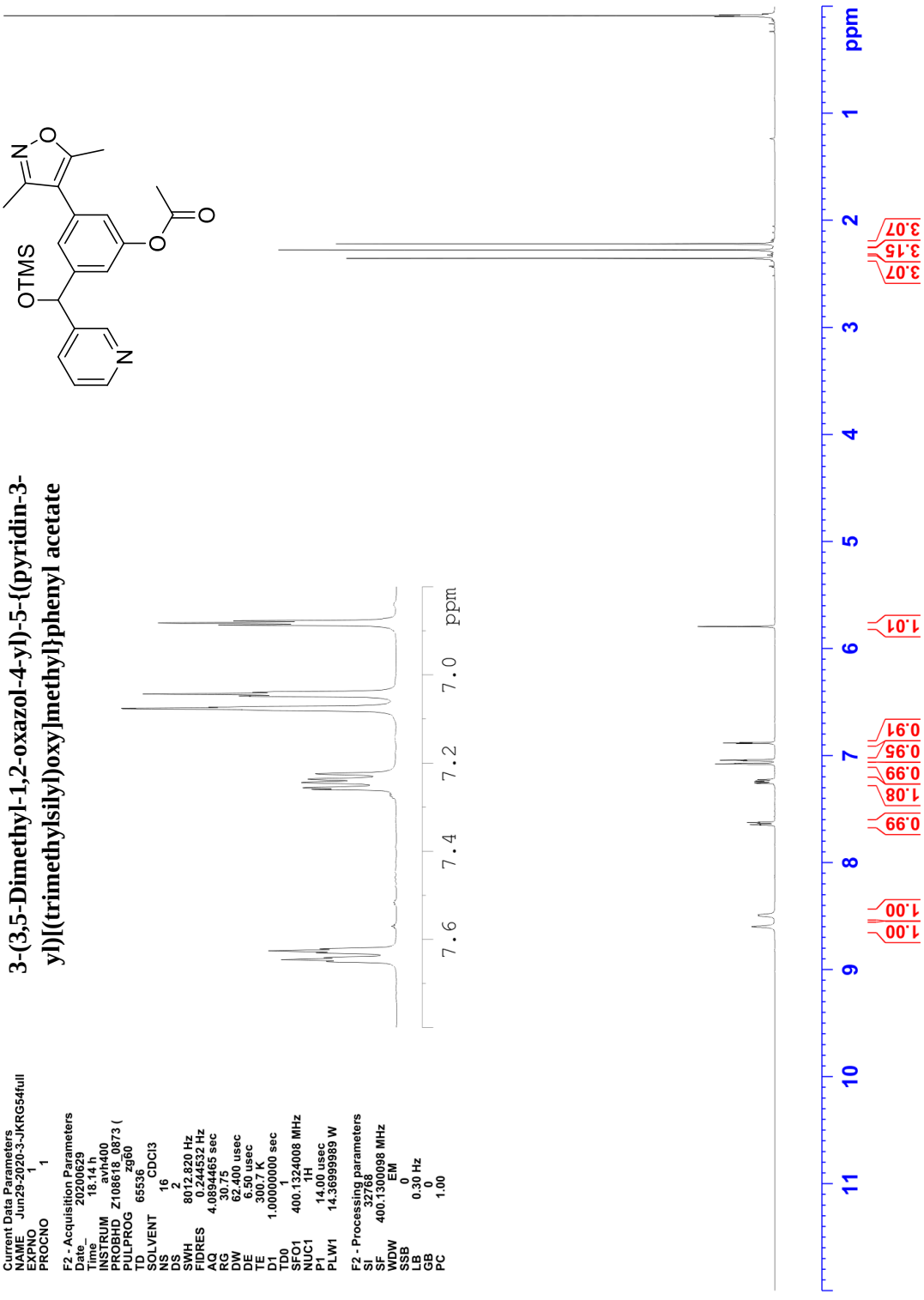
3'-(3,5-Dimethyl-1,2-oxazol-4-yl)-5'-[hydroxy(pyridin-3''-yl)methyl]phenyl acetate

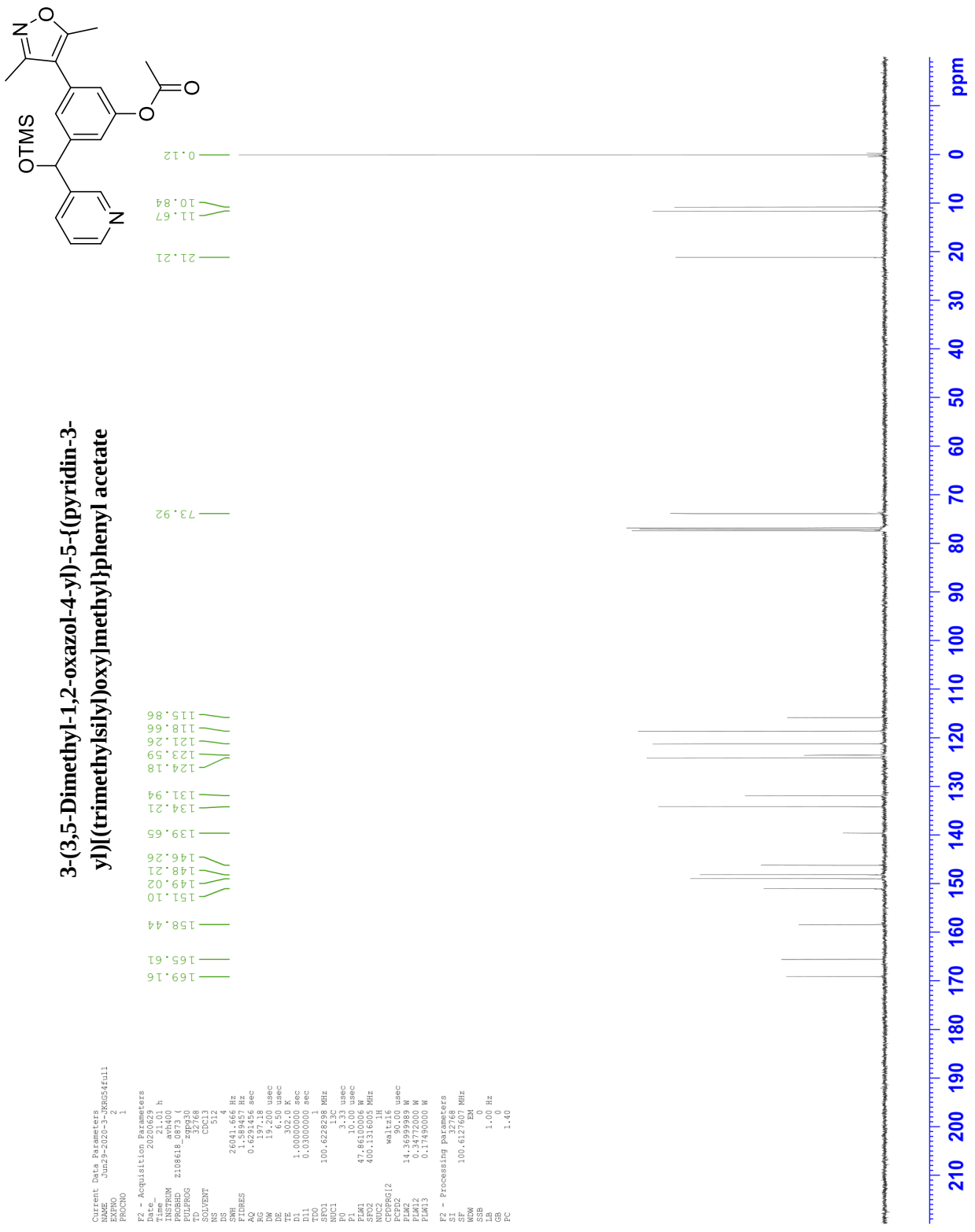


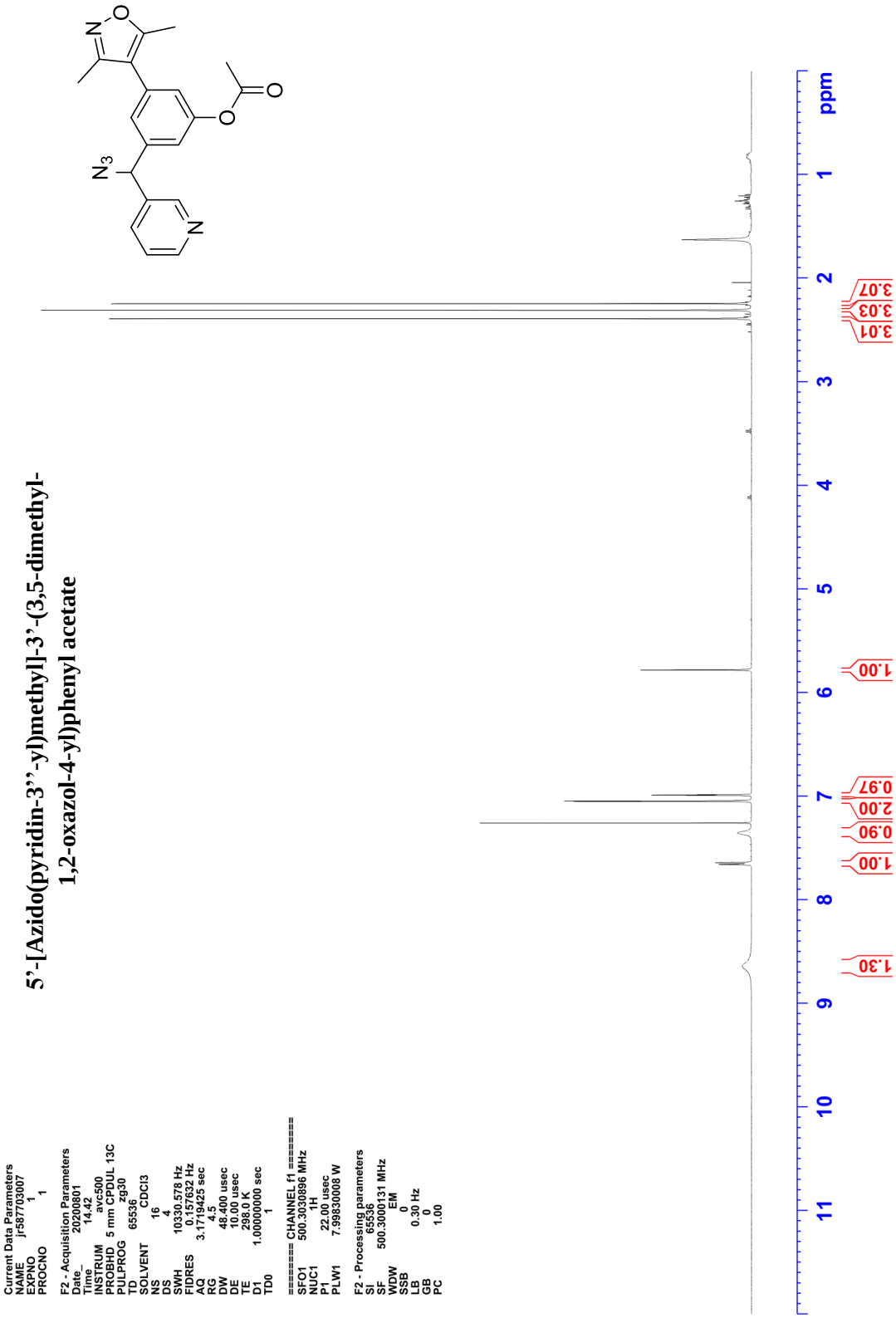

5'-[Azido(phenyl)methyl]-3'-(3,5-dimethyl-1,2-oxazol-4-yl)phenyl acetate
Current Data Parameters
NAME Jun11-2020-59-JKRG44f
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200611
Time 13.01 h
INSTRUM avg400
PROBHD Z108618_0816 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 74.05
DW 62.400 usec
DE 6.50 usec
TE 296.9 K
D1 1.00000000 sec
TD0 1
SFO1 400.2024012 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 32768
SF 400.2000097 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.4
7.2
7.0
ppm
1.10
5.00
1.98
0.89
3.47
3.45
3.41
ppm

5'-[Azido(phenyl)methyl]-3'-(3,5-dimethyl-1,2-oxazol-4-yl)phenyl acetate

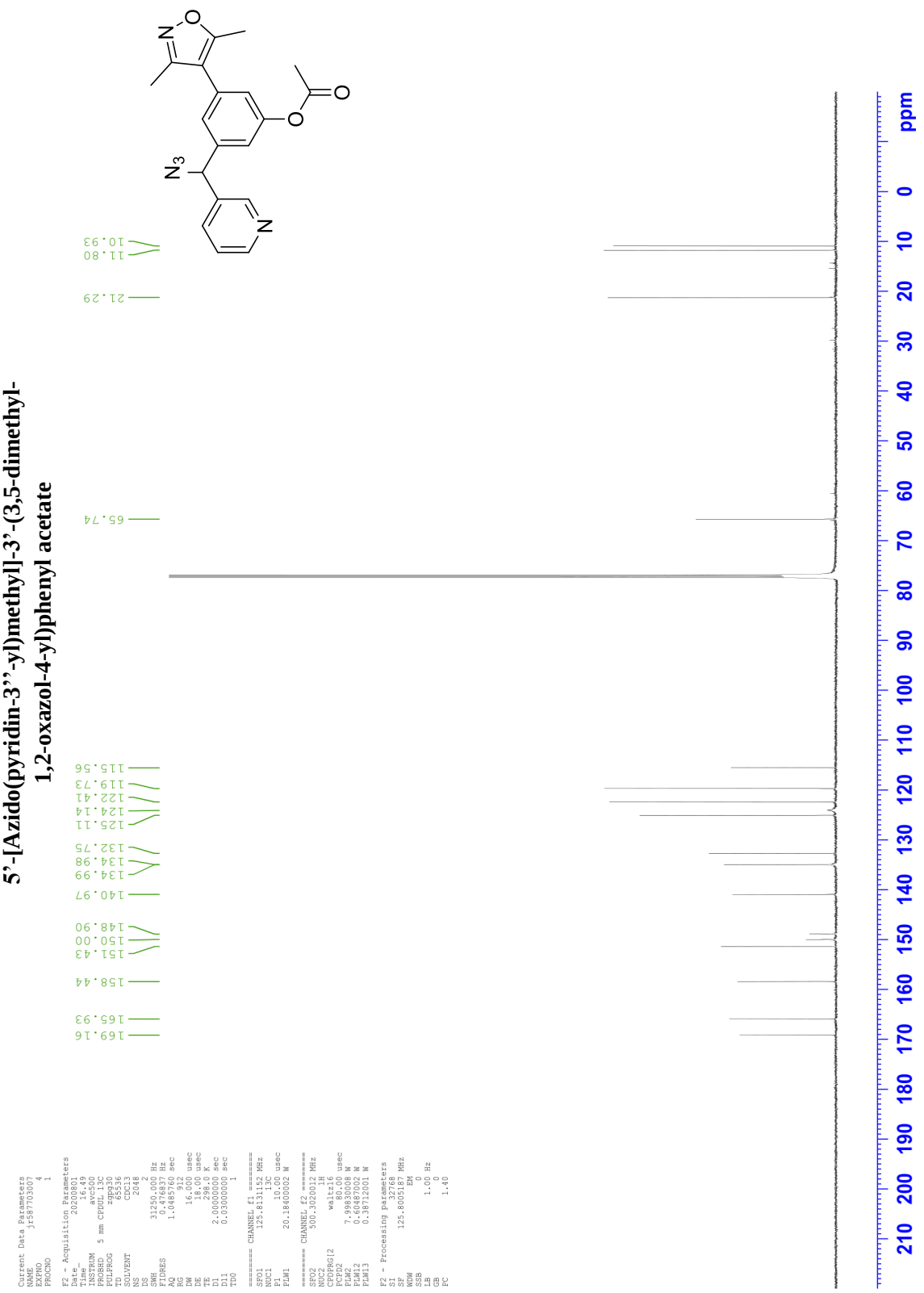



3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-{(pyridin-3-yl)[(trimethylsilyl)oxy]methyl}phenyl acetate
OTMS
N
O
O
O
N
Current Data Parameters
NAME Jun29-2020-3-JKRG54full
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200629
Time_ 18.14 h
INSTRUM avh400
PROBHD Z108618_0873 (
PULPROG zg60
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 30.75
DW 62.400 usec
DE 6.50 usec
TE 300.7 K
D1 1.00000000 sec
TD0 1
SFO1 400.1324008 MHz
NUC1 1H
P1 14.00 usec
PLW1 14.36999989 W
F2 - Processing parameters
SI 32768
SF 400.1300098 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.6
7.4
7.2
7.0
ppm
3.07
3.15
3.07
1.01
0.91
0.95
0.99
1.08
0.99
1.00
1.00
ppm

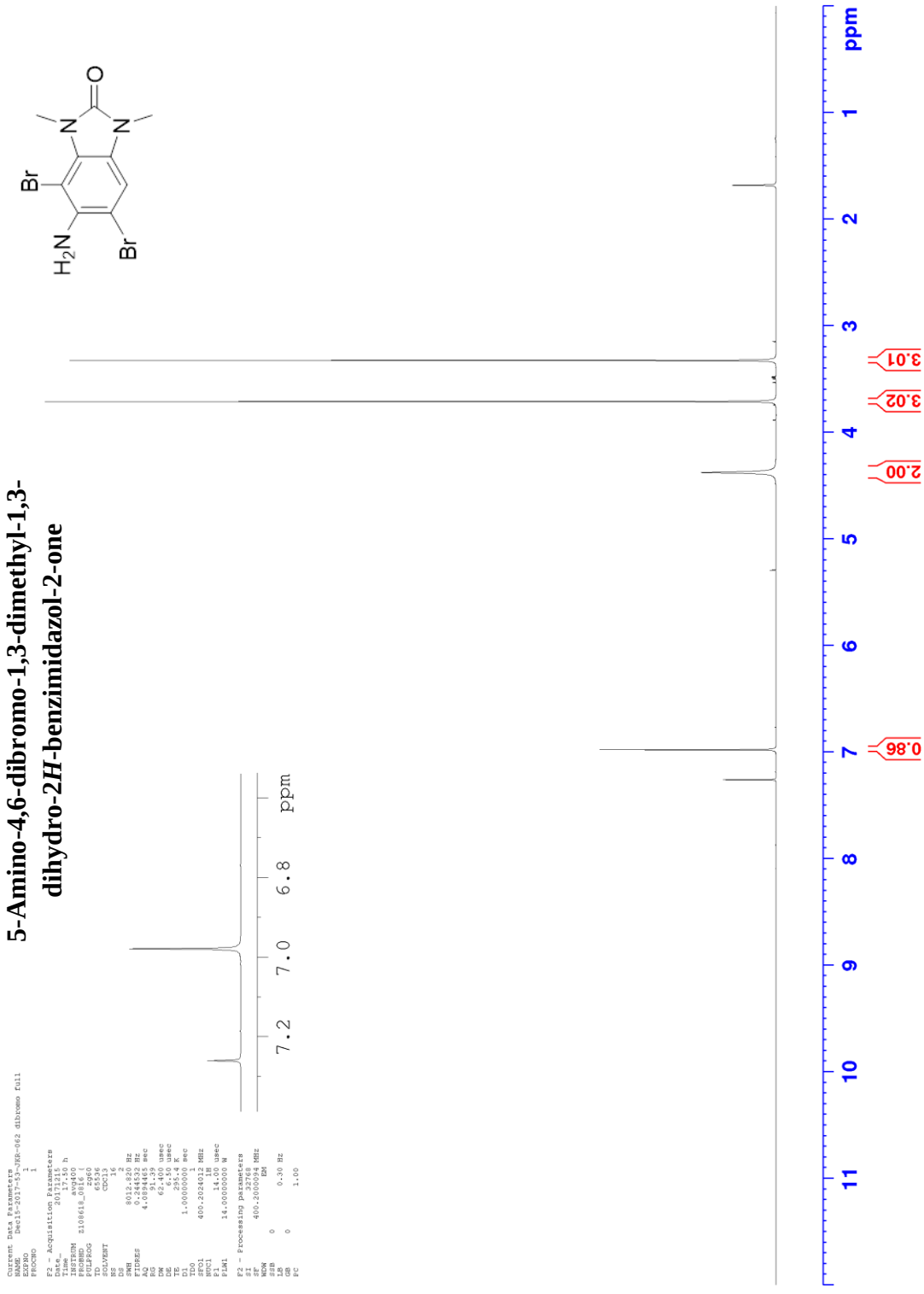

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-{(pyridin-3-yl)[(trimethylsilyl)oxy]methyl}phenyl acetate
OTMS
N
O
N
O
O
0.12
10.84
11.67
21.21
73.92
115.86
118.66
121.26
123.59
124.18
131.94
134.21
139.65
146.26
148.21
149.02
151.10
158.44
165.61
169.16
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210

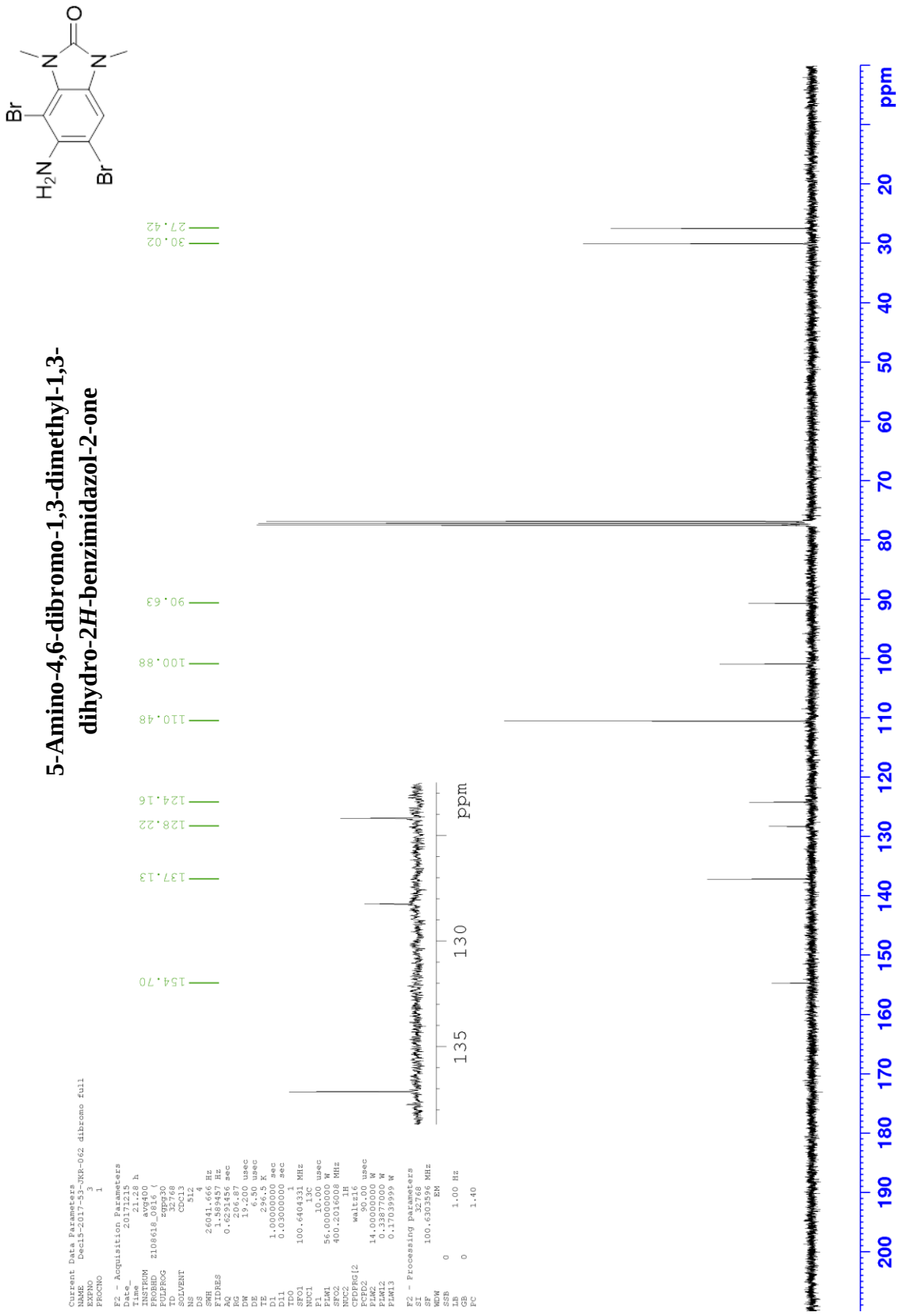

5'-[Azido(pyridin-3''-yl)methyl]-3'-(3,5-dimethyl-1,2-oxazol-4-yl)phenyl acetate
Current Data Parameters
NAME jr58773007
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200801
Time_ 14.42
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4.5
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000131 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
N3
O
N
ppm
1.30
1.00
0.90
2.00
0.97
1.00
3.01
3.03
3.07

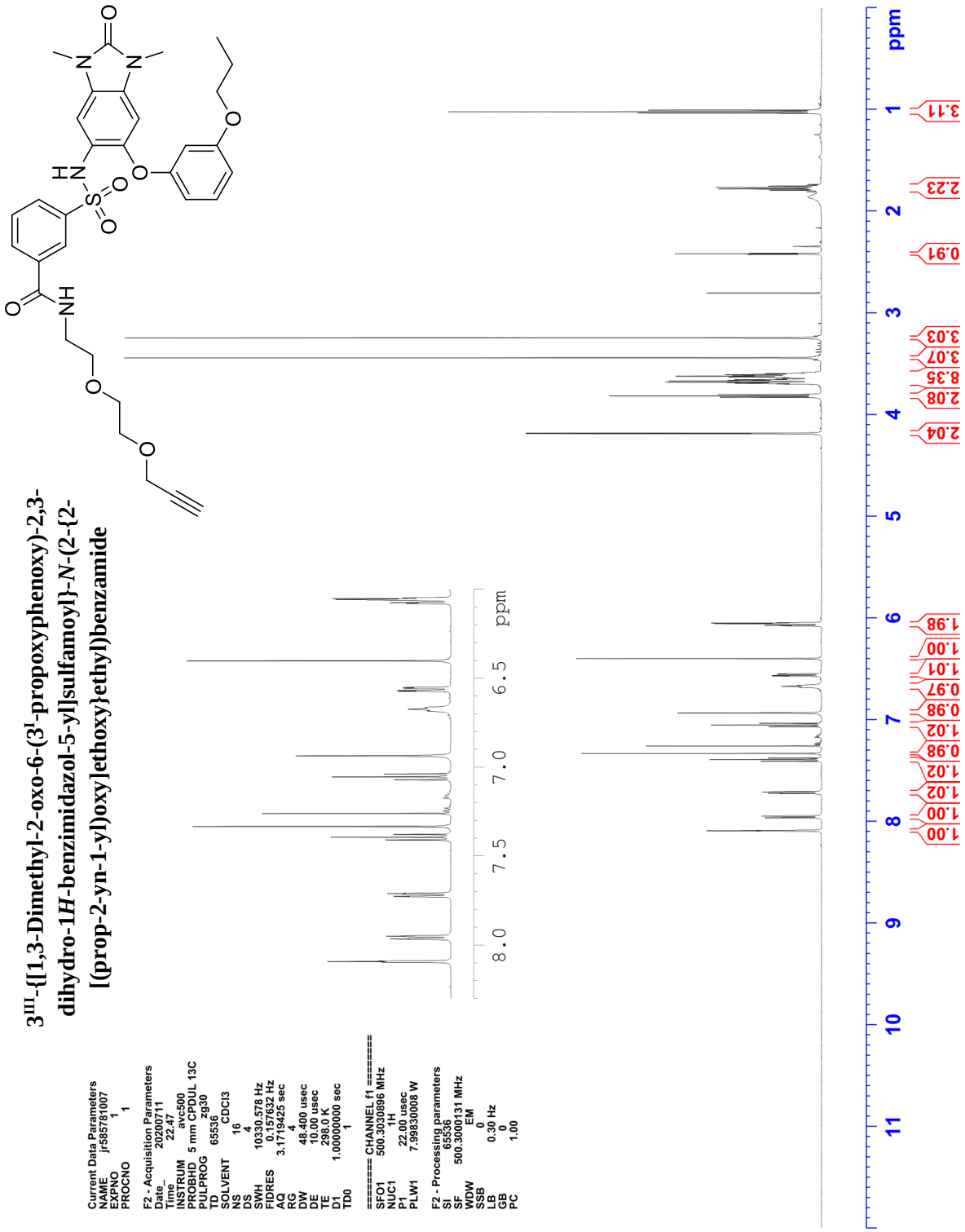
5'-[Azido(pyridin-3''-yl)methyl]-3'-(3,5-dimethyl-1,2-oxazol-4-yl)phenyl acetate

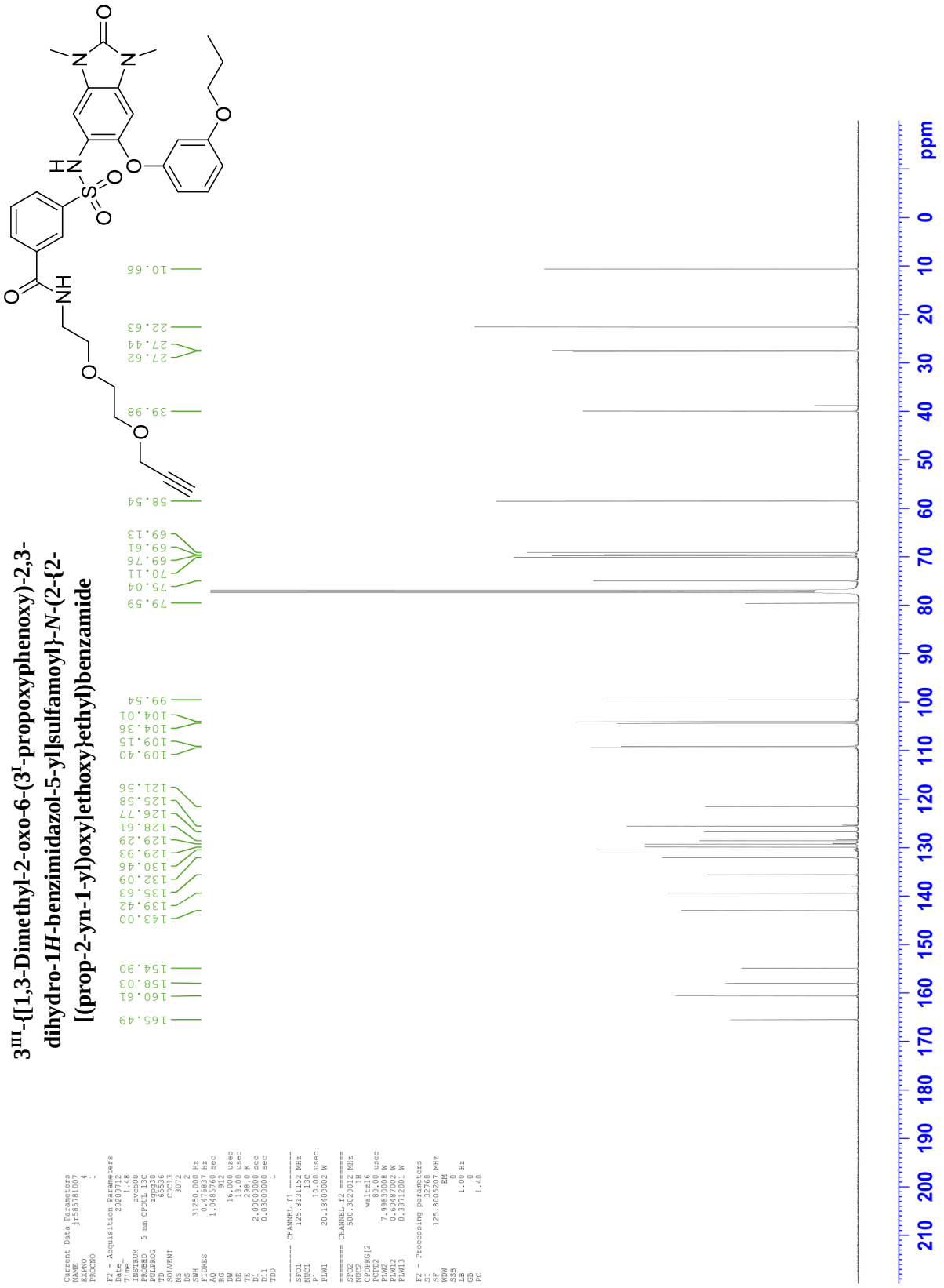


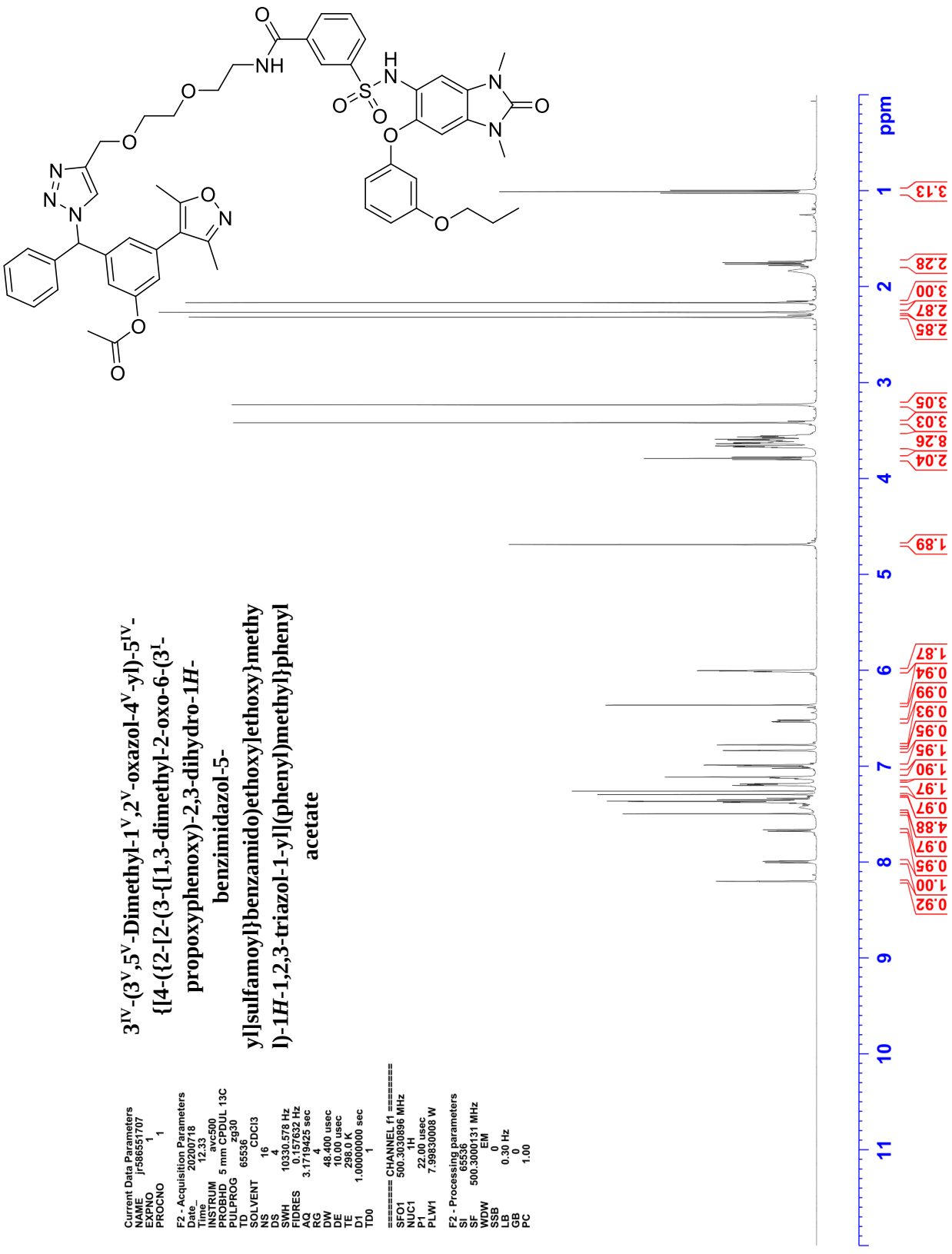
5-Amino-4,6-dibromo-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one

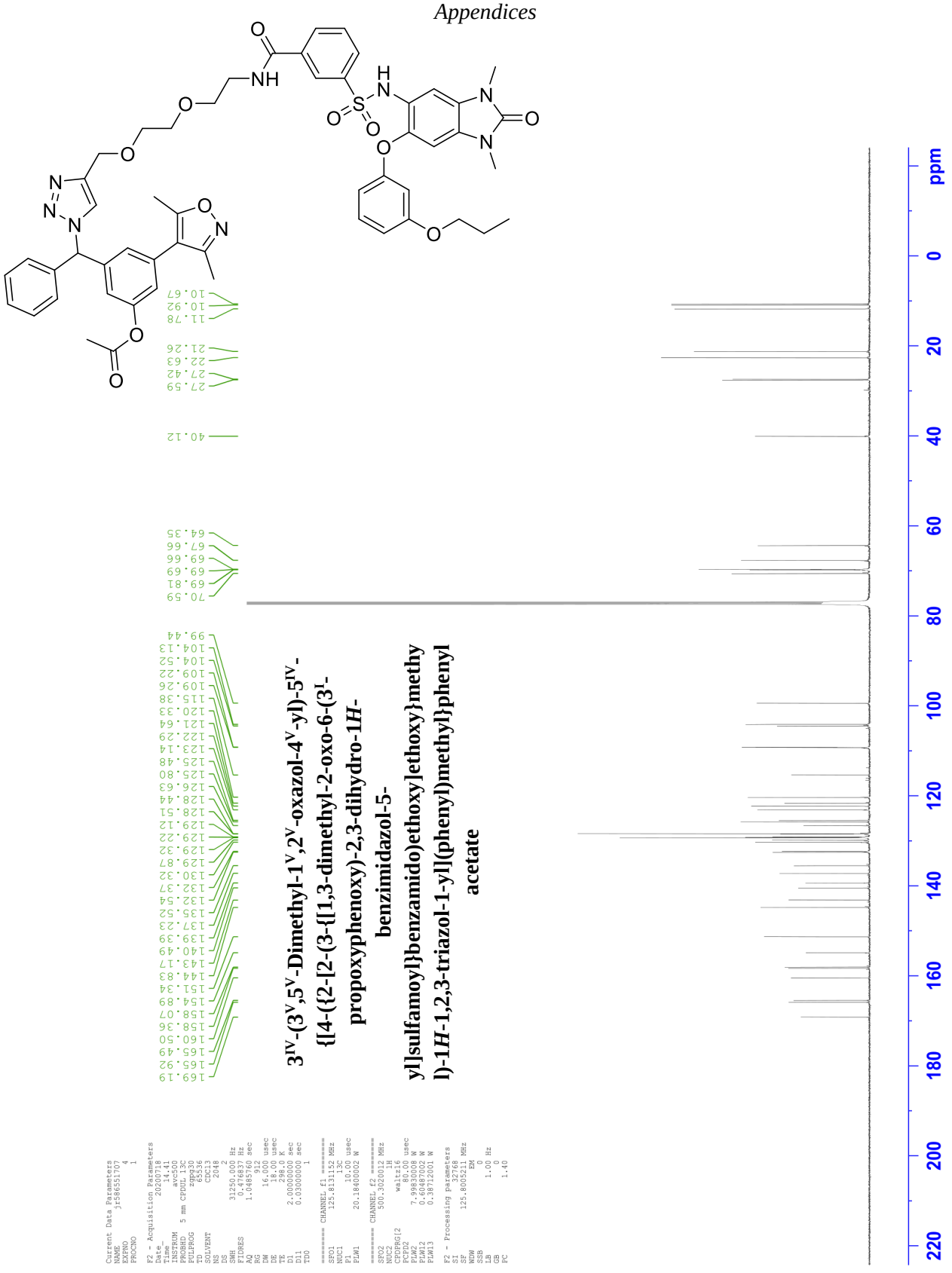


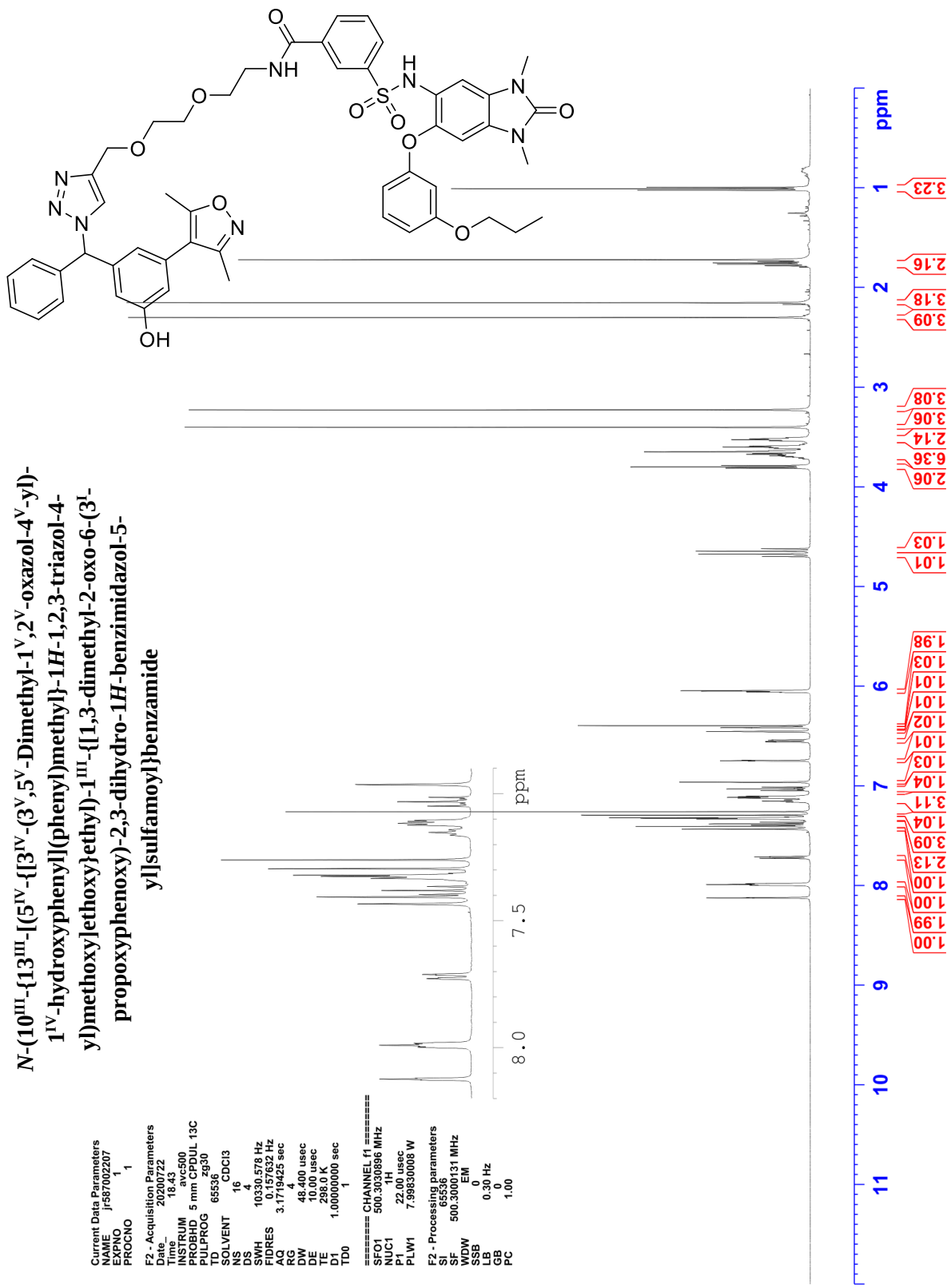

5-Amino-4,6-dibromo-1,3-dimethyl-1,3-dihydro-2H-benzimidazol-2-one
Br
H2N
Br
O
N
N
27.42
30.02
90.63
100.88
110.48
124.16
128.22
137.13
154.70
135
130
ppm
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


3III-{[1,3-Dimethyl-2-oxo-6-(3I-propoxyphenoxy)-2,3-dihydro-1H-benzimidazol-5-yl]sulfamoyl}-N-(2-{2-[(prop-2-yn-1-yl)oxy]ethoxy}ethyl)benzamide
Current Data Parameters
NAME jr58576/1007
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200711
Time_ 22.47
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000131 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.0
7.5
7.0
6.5
ppm
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.00
1.02
1.02
0.98
1.02
0.98
0.97
1.01
1.00
1.98
2.04
2.08
8.35
3.07
3.03
0.91
2.23
3.11

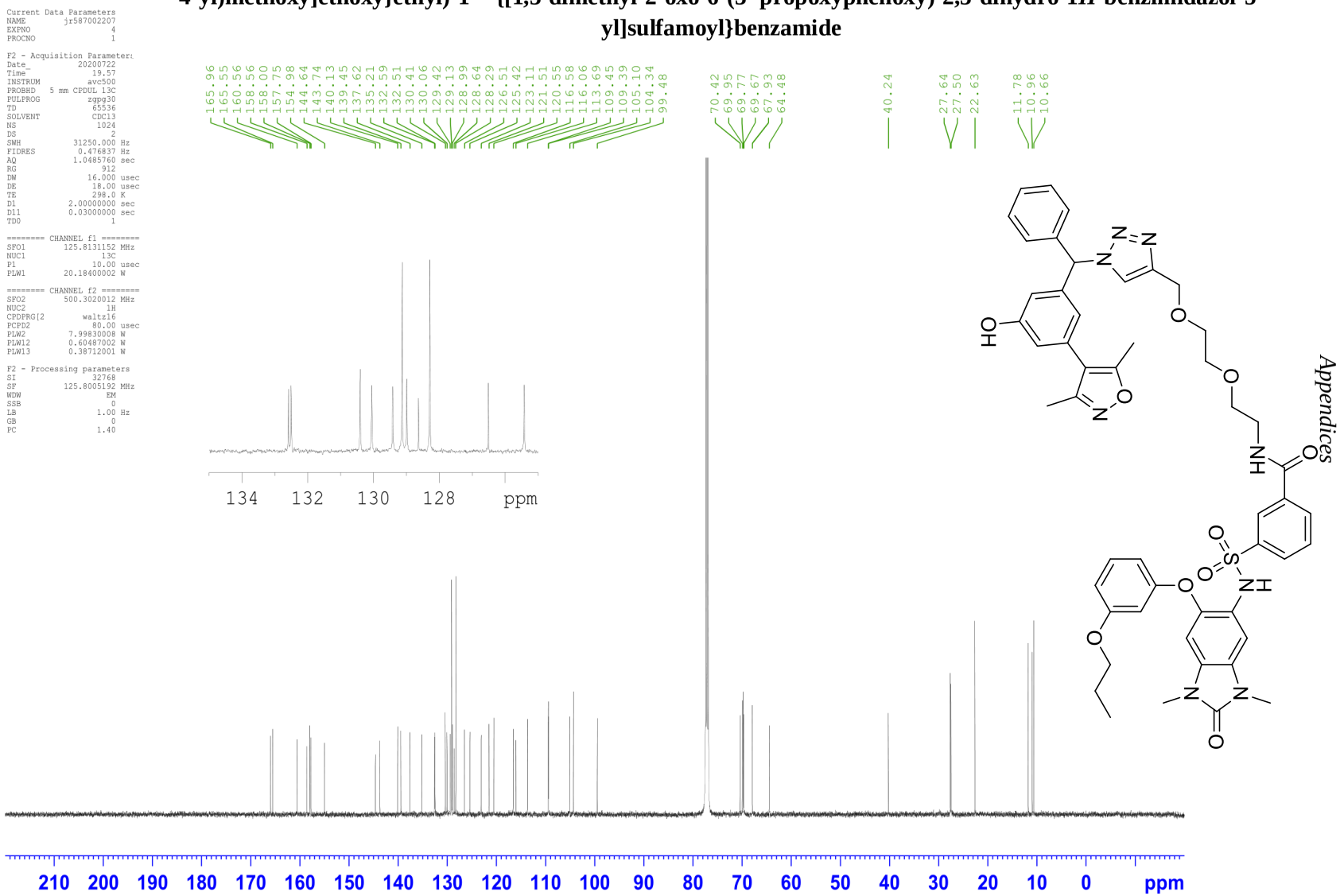

3III-{[1,3-Dimethyl-2-oxo-6-(3I-propoxyphenoxy)-2,3-dihydro-1H-benzimidazol-5-yl]sulfamoyl}-N-(2-{2-[(prop-2-yn-1-yl)oxy]ethoxy}ethyl)benzamide
Current Data Parameters
NAME j158578107
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200712
Time_ 1.48
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 3072
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 203
DW 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W
======== CHANNEL f2 ========
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 7.99800006 W
PLW12 0.60487002 W
PLW13 0.38712001 W
F2 - Processing parameters
SI 32768
SF 125.8005207 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
10.66
22.63
27.44
27.62
39.98
58.54
69.13
69.61
69.76
70.11
75.04
79.59
99.54
104.01
104.36
109.15
109.40
121.56
125.58
126.77
128.61
129.29
129.93
130.46
132.09
135.63
139.42
143.00
154.90
158.03
160.61
165.49
ppm


3IV-(3V,5V-Dimethyl-1V,2V-oxazol-4V-yl)-5IV-{[4-({2-[2-(3-{[1,3-dimethyl-2-oxo-6-(3I-propoxyphenoxy)-2,3-dihydro-1H-benzimidazol-5-yl]sulfamoyl}benzamido)ethoxy]ethoxy}methyl)-1H-1,2,3-triazol-1-yl](phenyl)methyl}phenyl acetate
Current Data Parameters
NAME jr586551707
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200718
Time_ 12.33
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000131 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
1
2
3
4
5
6
7
8
9
10
11
3.13
2.28
3.00
2.87
2.85
3.05
3.03
8.26
2.04
1.89
1.87
0.94
0.99
0.93
0.95
1.95
1.90
1.97
0.97
4.88
0.97
0.95
1.00
0.92

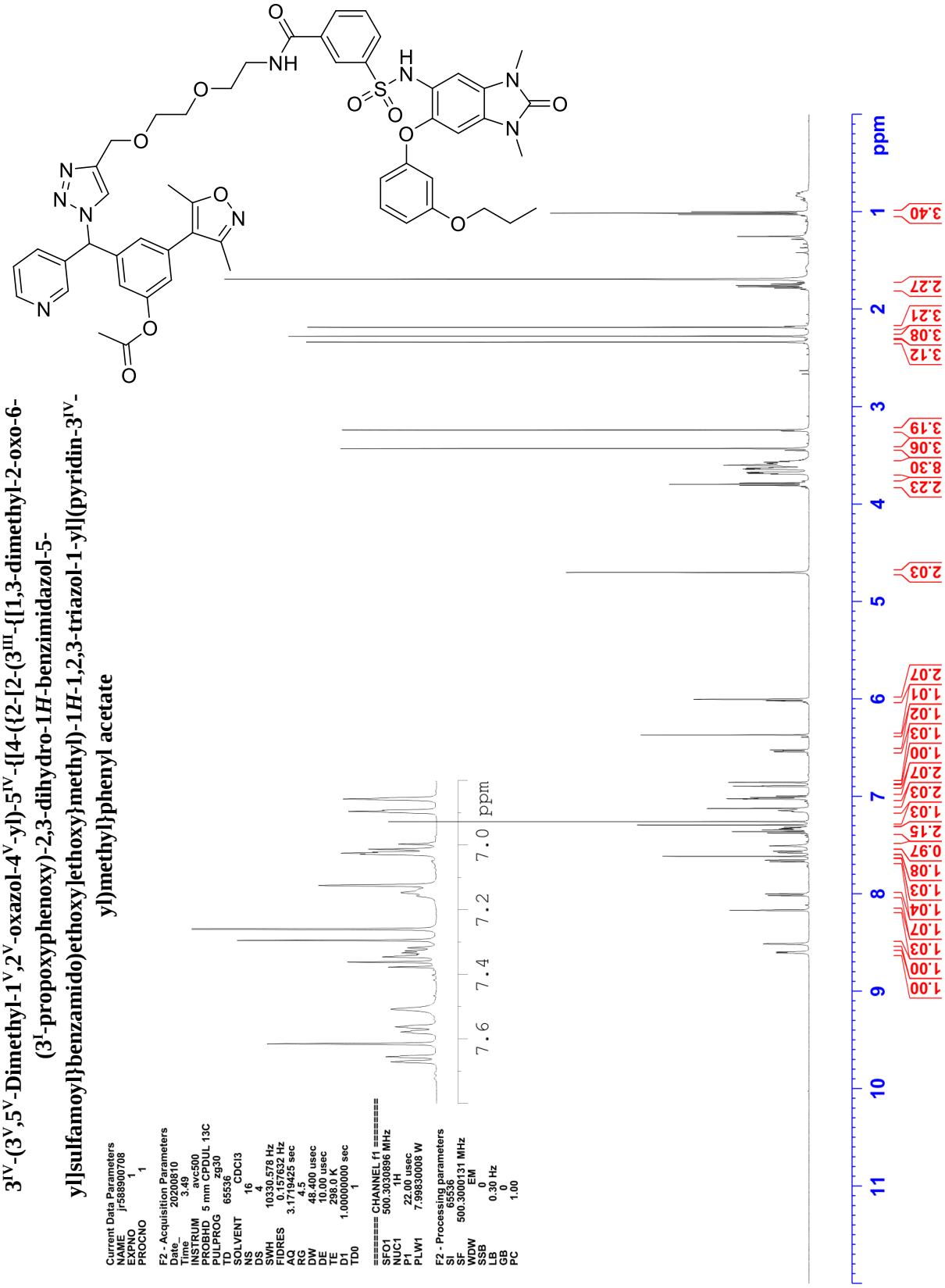

3IV-(3V,5V-Dimethyl-1V,2V-oxazol-4V-yl)-5IV-{[4-({2-[2-(3-{[1,3-dimethyl-2-oxo-6-(3I-propoxyphenoxy)-2,3-dihydro-1H-benzimidazol-5-yl]sulfamoyl}benzamido)ethoxy]ethoxy}methyl)-1H-1,2,3-triazol-1-yl](phenyl)methyl}phenyl acetate
ppm


N-(10III-{13III-I[(5IV-{[3IV-(3V,5V-Dimethyl-1V,2V-oxazol-4V-yl)-1IV-hydroxyphenyl](phenyl)methyl}-1H-1,2,3-triazol-4-yl)methoxy]ethoxy}ethyl)-1III-{[1,3-dimethyl-2-oxo-6-(3I-propoxyphenoxy)-2,3-dihydro-1H-benzimidazol-5-yl]sulfamoyl}benzamide
Current Data Parameters
NAME jr587002207
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20200722
Time_ 18.43
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.3000131 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
OH
ppm
8.0
7.5
3.23
2.16
3.18
3.09
3.08
3.06
2.14
6.36
2.06
1.03
1.01
1.98
1.03
1.01
1.01
1.02
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1.00
1.00
1.99
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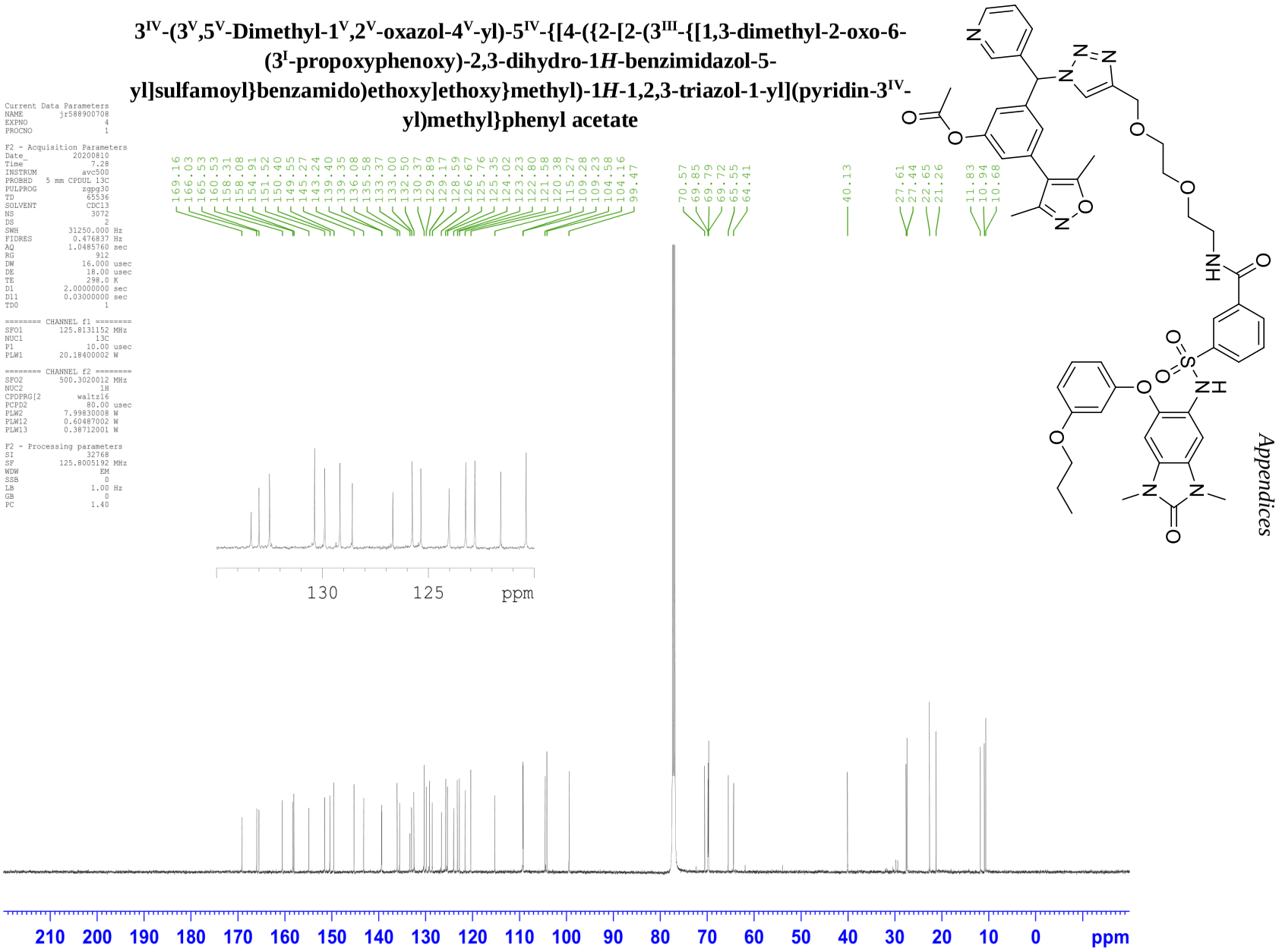
N-(10^{III}-{13^{III}-[(5^{IV}-{[3^{IV}-(3^{V},5^{V}-Dimethyl-1^{V},2^{V}-oxazol-4^{V}-yl)-1^{IV}-hydroxyphenyl](phenyl)methyl}-1*H*-1,2,3-triazol-4-yl)methoxy]ethoxy}ethyl)-1^{III}-{[1,3-dimethyl-2-oxo-6-(3^{I}-propoxyphenoxy)-2,3-dihydro-1*H*-benzimidazol-5-yl]sulfamoyl}benzamide

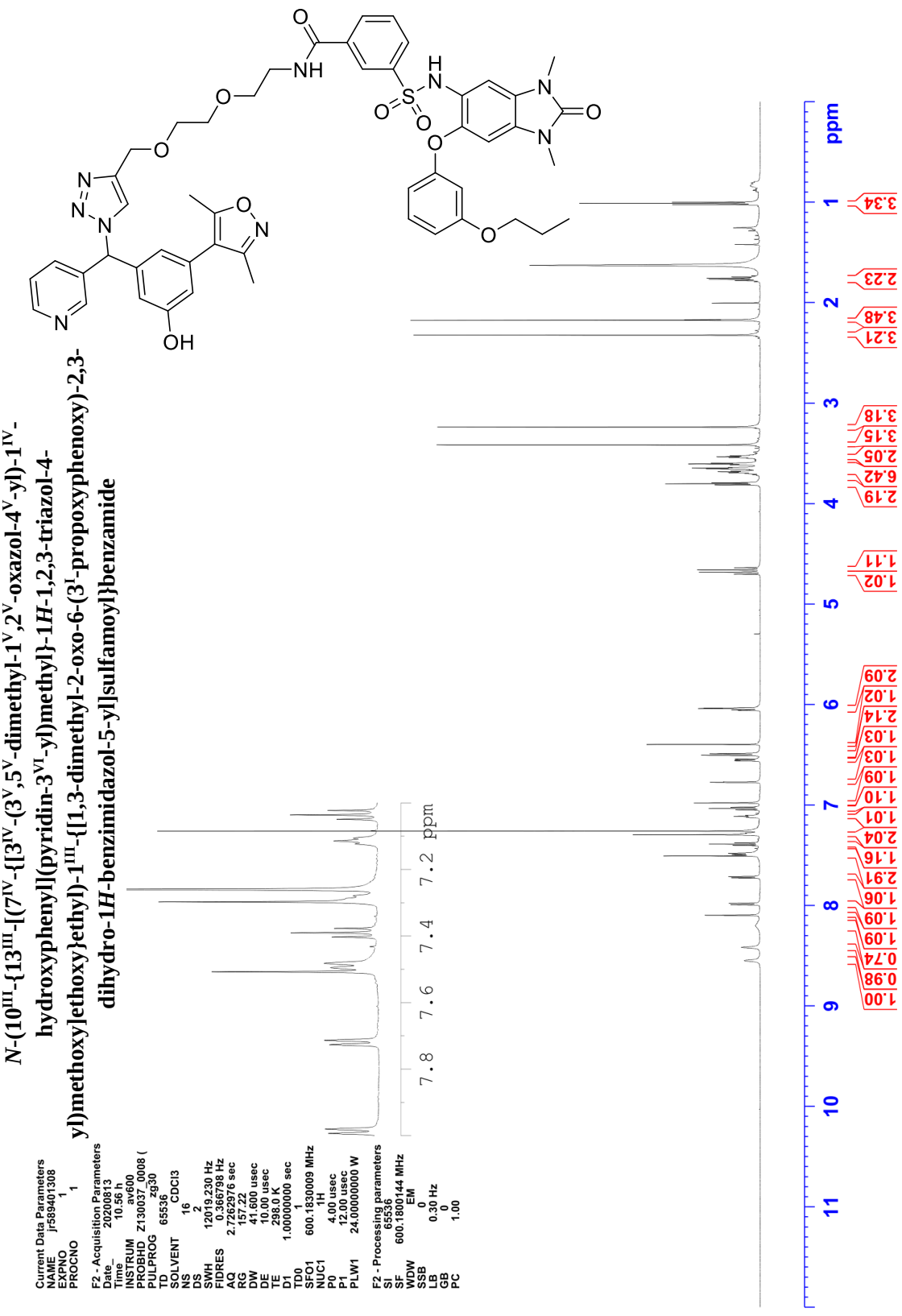


3^{IV}-(3^{V},5^{V}-Dimethyl-1^{V},2^{V}-oxazol-4^{V}-yl)-5^{IV}-{[4-({2-[2-(3^{III}-{[1,3-dimethyl-2-oxo-6-(3^{I}-propoxyphenoxy)-2,3-dihydro-1*H*-benzimidazol-5-yl]sulfamoyl}benzamido)ethoxy]ethoxy}methyl)-1*H*-1,2,3-triazol-1-yl](pyridin-3^{IV}-yl)methyl]phenyl acetate

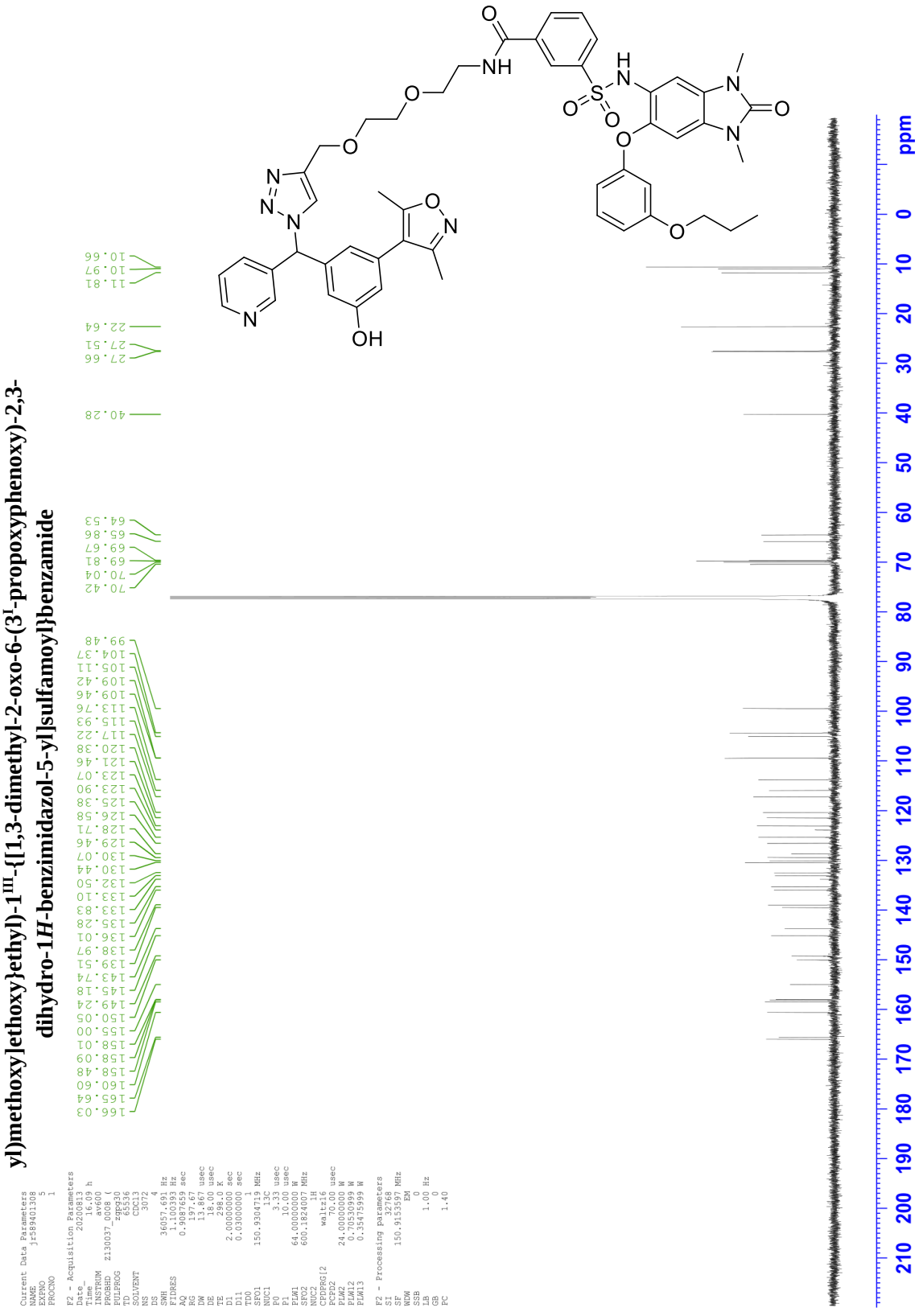


3^{IV}-(3^{V},5^{V}-Dimethyl-1^{V},2^{V}-oxazol-4^{V}-yl)-5^{IV}-{[4-({2-[2-(3^{III}-{[1,3-dimethyl-2-oxo-6-(3^{I}-propoxyphenoxy)-2,3-dihydro-1*H*-benzimidazol-5-yl]sulfamoyl}benzamido)ethoxy]ethoxy}methyl)-1*H*-1,2,3-triazol-1-yl](pyridin-3^{IV}-yl)methyl}phenyl acetate




N-(10III-{13III-[(7IV-{[3IV-(3V,5V-dimethyl-1V,2V-oxazol-4V-yl)-1IV-hydroxyphenyl](pyridin-3VI-yl)methyl}-1H-1,2,3-triazol-4-yl)methoxy]ethoxy}ethyl)-1III-{[1,3-dimethyl-2-oxo-6-(3I-propoxyphenoxy)-2,3-dihydro-1H-benzimidazol-5-yl]sulfamoyl}benzamide
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PROCNO 1
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DS 2
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FIDRES 0.366798 Hz
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0.98
1.00

N-(10^{III}-{13^{III}-[(7^{IV}-{[3^{IV}-(3^{V},5^{V}-dimethyl-1^{V},2^{V}-oxazol-4^{V}-yl)-1^{IV}-hydroxyphenyl](pyridin-3^{VI}-yl)methyl}-1H-1,2,3-triazol-4-yl)methoxy}ethoxy}ethyl)-1^{III}-{[1,3-dimethyl-2-oxo-6-(3^{I}-propoxyphenoxy)-2,3-dihydro-1H-benzimidazol-5-yl]sulfamoyl}benzamide



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Author: David S. Hewings, Timothy P. C. Rooney, Laura E. Jennings, et al
Publication: Journal of Medicinal Chemistry
Publisher: American Chemical Society
Date: Nov 1, 2012

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Publication: Nature
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33(Suppl. 1), S253–S262. doi: 10.3233/JAD-2012-129005

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Reviewed by:
Fred J. Helmstetter, University of Wisconsin—Milwaukee, USA
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