CHEMICAL TOOLS TO PROBE THE FUNCTION OF TRIM33

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Abstract

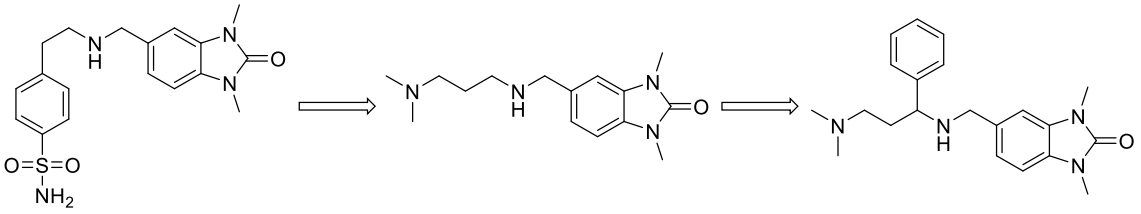
Epigenetic modifications, including chemical modification of DNA and post-translational modifications (PTMs) to histone proteins, are important in regulating gene expression. Patterns of PTMs recruit specific recognition modules, including bromodomains, which recognise acetylated lysine residues. Bromodomains play a central role in epigenetic gene regulation and, as aberrant access to genetic information is responsible for many diseases, they are considered attractive therapeutic targets.

TRIM33 belongs to the C-VI subfamily of TRIM proteins, possessing a C-terminal chromatin binding unit, which comprises a bromodomain adjacent to a PHD finger. In the PARP-dependent DNA damage repair pathway, TRIM33 is responsible for the timely dissociation of chromatin-remodelling enzyme ALC1 from chromatin. Regulation of ALC1 at chromatin is important as prolonged chromatin relaxation leaves DNA susceptible to further damage. It is hypothesised that TRIM33 bromodomain ligands will prevent TRIM33 binding to chromatin, resulting in the prolonged association of ALC1 at chromatin, inefficient repair of single-strand breaks, and further DNA damage.

This dissertation describes the development of chemical tools to validate TRIM33 as a therapeutic target. Bromodomain ligands and PROTACs, which enable the role of the bromodomain to be disconnected from the function of the whole protein, were designed and synthesised.

Previous work in the group had identified benzimidazolone **23** as a TRIM33 bromodomain ligand (TRIM33β IC_{50} = 11.9 μM) (Figure 1). The optimisation of the selectivity and affinity of **23** through iterative cycles of design, synthesis and testing is detailed. Compounds were first evaluated using an AlphaScreen™ assay, and hits were validated in waterLOGSY and ITC experiments. Initial work demonstrated the importance of the benzylic amine substituent for imparting selectivity over the closely related TRIM24 bromodomain. The selectivity results from interactions with a glutamic acid residue (E981) in TRIM33, which is switched to an alanine

residue (A923) in TRIM24. Compound **90** (TRIM33β IC_{50} = 13.7 μM) was developed as a selective ligand, which retains affinity for TRIM33, and was subsequently used as the basis for ligand design (Figure 1). The region 'above' the acetyl-lysine binding pocket was explored by extending off the headgroup of **90**. The amine chain and methylation state of the benzimidazolone core were also investigated. Despite an extensive investigation focused on benzimidazolone analogues, a relatively flat SAR was observed, with most ligands displaying low micromolar affinity. A structural water molecule was identified as preventing the amine chain residing in the ZA channel. Subsequent ligand design focused on a bidentate interaction of the amine chain with E981 which avoided displacing the tightly bound ZA channel water molecule. This approach resulted in **161** (TRIM33β IC_{50} = 4.83 μM), which has increased affinity for the TRIM33 bromodomain, potentially due to the phenyl ring enforcing a favourable conformation for a bidentate interaction with E981 (Figure 1).



23	**90**	**161**
TRIM24 IC_{50} = 40.3 μM	TRIM24 IC_{50} >250 μM	TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 3.88 μM	TRIM33α IC_{50} = 3.61 μM	TRIM33α IC_{50} = 1.56 μM
TRIM33β IC_{50} = 11.9 μM	TRIM33β IC_{50} = 13.7 μM	TRIM33β IC_{50} = 4.83 μM

Figure 1: Summary of the optimisation of the benzimidazolone series.

The knowledge gained from ligand optimisation was used to facilitate PROTAC design. The core structure of the bromodomain ligands was incorporated as the TRIM33β bromodomain binding moiety, and tethered *via* an appropriate attachment point to a ligand for the E3 ligase cereblon. A range of linker lengths and linking strategies were used to maximise the chance of achieving potent degradation of TRIM33. Some of the potential TRIM33 PROTACs were found to interfere with the AlphaScreen™ assay therefore an alternative assay is required to evaluate their binding to TRIM33.

Together the bromodomain ligands and PROTACs can be used to gain a deeper understanding of the physiological and pathological roles of TRIM33 and its bromodomain.

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Abbreviations

Å	Angstrom
A-^{ta}Phos	(4-(*N*,*N*-Dimethylamino)phenyl)di-*tert*-butyl phosphine
Ac	Acetyl
ACD	Advanced chemistry development
ADP	Adenosine diphosphate
ALC1	Amplified in liver cancer 1
AlphaScreen™	Amplified luminescent proximity homogeneous assay screen
APCI	Atmospheric pressure chemical ionisation
APS	Ammonium persulfate
aq.	Aqueous
AR	Androgen receptor
ATR	Attenuated total reflection
β-ME	β-Mercaptoethanol
B_2pin_2	Bis(pinacolato)diboron
BAZ2A	Bromodomain adjacent to zinc finger domain 2A
BAZ2B	Bromodomain adjacent to zinc finger domain 2B
BCP	Bromodomain-containing protein
BET	Bromodomain and extra-terminal
BLAST	Basic local alignment search tool
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
BRD2	Bromodomain-containing protein 2
BRD3	Bromodomain-containing protein 3
BRD4	Bromodomain-containing protein 4
BRDT	Bromodomain testis-specific protein

BRPF	Bromodomain and plant homeodomain finger-containing
BSA	Bovine serum albumin
Cbz	Carboxybenzyl
CC	Coiled-coil
CDI	1,1'-Carbonyldiimidazole
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
CI	Chemical ionisation
Conc.	Concentration
COSY	Correlation spectroscopy
CpG	5'–C–phosphate–G–3'
CRBN	Cereblon
CREBBP	CREB-binding protein
CRL4A	Cullin-RING ubiquitin E3 ligase 4A
CV	Column volumes
d.p.	Decomposition point
Da	Daltons
dba	Dibenzylideneacetone
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DCE	1,2-Dichloroethane
DDB1	Damage specific DNA binding protein 1
DIAD	Diisopropyl azodicarboxylate
DIBAL	Diisobutylaluminium hydride
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	*N*,*N*-Dimethyl-4-aminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

DNMTs	DNA methyltransferases
dppf	1,1'-Bis(diphenylphosphino)ferrocene
ε	Molar extinction coefficient
e.e.	Enantiomeric excess
E1	Ubiquitin-activating enzyme
E2	Ubiquitin-conjugating enzyme
E3	Ubiquitin-protein ligase
EI	Electron ionisation
eq.	Equivalents
ESI	Electrospray ionisation
Et	Ethyl
FAC	Final assay concentration
FPLC	Fast protein liquid chromatography
FRET	Förster resonance energy transfer
g	Gram(s)
GCI	Grand canonical integration
GCMC	Grand canonical Monte Carlo
GROMACS	GROningen MAchine for Chemical Simulations
h	Hour(s)
H3	Histone 3
HATs	Histone acetyltransferases
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate
HDACs	Histone deacetylases
HDMs	Histone demethylases
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HIF-1α	Hypoxia-inducible factor 1-alpha
HMBC	Heteronuclear multiple bond correlation

HMTs	Histone methyltransferases
HOBt	Hydroxybenzotriazole
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
IPTG	Isopropyl β-D-1-thiogalactopyranoside
ITC	Isothermal titration calorimetry
J	Coupling constant
K_a	Acid dissociation constant
KAc	Acetylated lysine
K_D	Dissociation constant
LE	Ligand efficiency
lit.	Literature value
LRMS	Low resolution mass spectrometry
M	Molar
m.p.	Melting point
MAPK	Mitogen-activated protein kinase
MD	Molecular dynamics
MDM2	Mouse double minute 2 homolog
Me	Methyl
mg	Milligram(s)
MHz	Megahertz
Min	Minute(s)
mL	Millilitre(s)
Ms	Methanesulfonyl

MW	Molecular weight
MWCO	Molecular weight cut-off
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide
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
PCy_3	Tricyclohexylphosphine
PD	Pharmacodynamic
PDB ID	Protein data bank identification
PEG	Polyethylene glycol
PG	Protecting group
Ph	Phenyl
PHD	Plant homeodomain
PHOTACs	Photochemically targeted chimeras
PK	Pharmacokinetic
PPI	Protein-protein interaction
ppm	Parts per million
PROTACs	Proteolysis targeting chimeras
PTMs	Post-translational modifications
rac	Racemic
R_f	Retention factor
RING	Really interesting new gene

RNA	Ribonucleic acid
rpm	Revolutions per minute
rt	Room temperature
SAR	Structure-activity relationship
SCF	Skp-Cullin-F box containing Hrt1
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
SFI	Solubility forecast index
shRNA	Short hairpin RNA
siRNA	Small interfering ribonucleic acid
Sirt2	Sirtuin 2
S_NAr	Nucleophilic aromatic substitution
SPR	Surface plasmon resonance
sRNA	Small ribonucleic acid
t-Bu	*tert*-Butyl
TBAI	Tetrabutylammonium iodide
TBDMS	*tert*-Butyldimethylsilyl
TEMED	Tetramethylethylenediamine
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TFA	Trifluoroacetic acid
TGF-β	Transforming growth factor-beta
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TMSI	Trimethylsilyl iodide
TRIM	Tripartite motif-containing
Troc	2,2,2-Trichloroethoxycarbonyl
UPLC-MS	Ultra performance liquid chromatography-mass spectrometry

UV	Ultraviolet
VHL	Von Hippel-Lindau
VT	Variable temperature
w/v	Weight per unit volume (10% = 10 g/100 mL)
w/w	Weight per unit weight (10% = 10 mg/100 mg)
WaterLOGSY	Water-ligand observed *via* gradient spectroscopy
XPhos	2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl

CHAPTER 1

Introduction

1.1 Epigenetics

Many phenomena, for example behaviour, susceptibility to disease and diversity of phenotypes within a population, cannot be explained by classical genetics alone. The term 'epigenetics' was introduced in the 1940s and bridges this gap between the genome and phenotype.[1,2]

Since its introduction, the understanding of the field has evolved rapidly. Initially, epigenetics was described as 'the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being'.[1,2] There is currently no universally accepted definition of epigenetics, but it is broadly used to describe 'heritable changes in gene expression that are not due to alterations in the DNA sequence'.[3]

1.1.1 Chromatin

In cells, DNA is organised into chromatin (Figure 1.1). The basic structural unit of chromatin is the nucleosome which comprises 146 base pairs of DNA wrapped around an octamer of histone proteins (two each of H2A, H2B, H3 and H4). The central domain of histone proteins is composed of three α-helices connected by two flexible loops and is referred to as the histone fold.[4] Histones in the core particle exist as heterodimers, heterodimerising through their histone fold in a 'handshake motif'. Histone proteins also have disordered N- and C- terminal tails which interact with DNA *via* the minor groove, and are required for nucleosome-nucleosome interactions. Adjacent nucleosomes are connected by linker histones and arrays of nucleosomes are compacted into chromatin fibres which are further condensed into poorly defined higher order structures.[5]

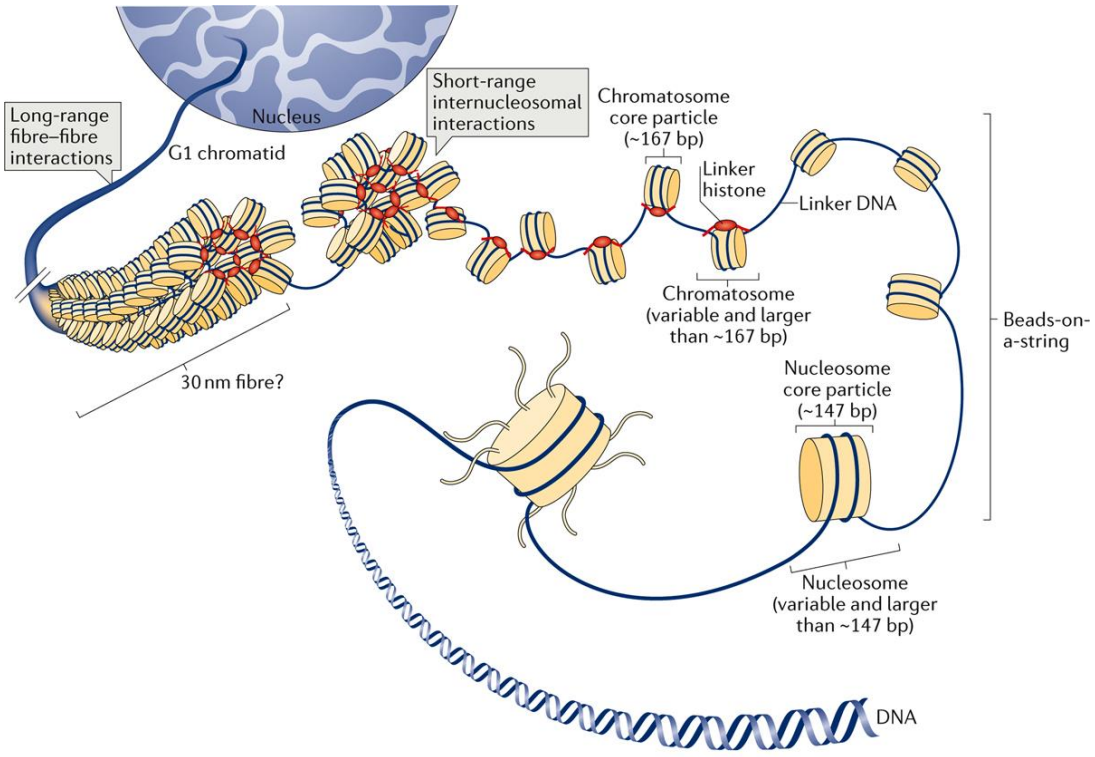


Figure 1.1: Packing of DNA into chromatin. Nucleosomes consist of DNA wrapped around an octamer of histone proteins. These nucleosomes coil to form chromatin, sometimes referred to as 'beads on a string', which condenses further into a chromatid of a chromosome. Figure reprinted from D. Fyodorov, B-R. Zhou, A. I. Skoultchi and Y. Bai, *Nat. Rev. Mol. Cell Biol.*, 2018, **19**, 192–206, doi: https://doi.org/10.1038/nrm.2017.94 with permission from Springer Nature, License No. 4640120411880.[6]

1.1.2 Epigenetic Modifications

There are two main types of epigenetic modifications: covalent DNA modifications and covalent histone modifications. Epigenetic modifications of RNA and small RNAs (sRNAs) functioning as epigenetic regulators have also been described.[7,8,9]

DNA methylation, catalysed by DNA methyltransferases (DNMTs), occurs on the 5-position of cytosine nucleotides which are predominantly proceeded by guanine residues (CpG). Promoters of protein-coding genes are rich in CpG sites, and DNA methylation regulates gene expression by preventing transcription factors binding to DNA, or by recruiting proteins involved in gene repression.[10]

There are more than 50 known post-translational modifications (PTMs) of histone proteins, these include methylation, acetylation, phosphorylation and ubiquitination. As PTMs modify the charge

of histone proteins, for example lysine acetylation neutralises positively charged lysine residues, the interactions between DNA and histone proteins are altered, and consequently the structure of chromatin is affected.[11] When chromatin is decondensed it is in its active conformation and described as euchromatin, when chromatin is condensed it is described as heterochromatin and is in an inactive state where DNA transcription is repressed.[12]

1.1.2.1 Ubiquitination

Ubiquitin is a highly conserved protein comprising 76 amino acids.[13] Ubiquitination is a PTM where ubiquitin is conjugated to lysine residues in the target protein, and is catalysed by the sequential activity of three enzymes: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin-protein ligases (E3) (Figure 1.2). Ubiquitin is attached *via* an isopeptide bond from its carboxy terminus to the ε-amino group of lysine and is successively added, leading to the formation of polyubiquitin chains. These polyubiquitin chains tag the protein for degradation by the 26S proteasome.[14]

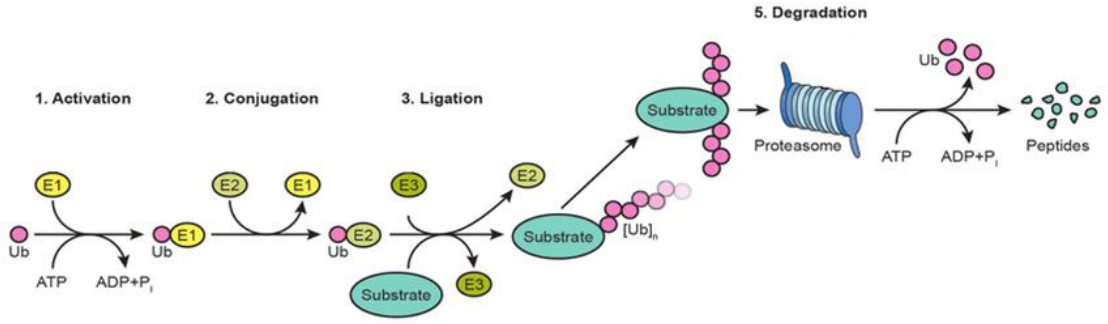


Figure 1.2: Summary of the ubiquitin system. 1) Ubiquitin-activating enzyme (E1) activates the C-terminal carboxyl group of ubiquitin. 2) The thioester formed between ubiquitin and the E1 enzyme is then transferred *via* a transacylation reaction to ubiquitin-conjugating enzyme (E2). 3) The E3 ubiquitin ligase specifically binds to the protein substrate and ubiquitin is transferred from the E2 enzyme to a lysine residue on the protein substrate. Successive repeats lead to the formation of polyubiquitin chains which mark the protein for degradation by the proteasome.[14] Figure reprinted from X. Huang and V. M. Dixit, *Cell Res.*, 2016, **26**, 484–498, doi: https://doi.org/10.1038/cr.2016.31 published under the terms of the Creative Commons Attributio-NonCommercial-NoDerivs 4.0 Unported License.[15]

1.1.2.2 The Histone Code

The N-terminal tails of histone proteins are the major sites for a diverse array of PTMs, but residues within the globular histone folds can also be modified. Patterns of PTMs recruit specific

proteins, resulting in a defined downstream phenotype, and are said to form the histone code.[16] The histone code characterises the transcriptional state of each gene and is 'read' and subsequently translated into a specific biological function, thus extending the information provided by the genetic code.[17]

1.1.3 Epigenetic Protein Domains

A protein domain is a structural and functional subunit of a protein.[18] There are three types of epigenetic protein domains: writers, erasers and readers, which add, remove and recognise epigenetic marks, respectively (Figure 1.3).

Lysine acetylation is a dynamic PTM which neutralises positively charged lysine residues and therefore reduces the affinity of the histone core for the negatively charged DNA molecule. Lysine acetylation is regulated by histone acetyl transferases (HATs) and histone deacetylases (HDACs).[19] Bromodomains are epigenetic readers that bind to acetylated lysine (KAc) residues, mediating specific downstream effects and regulating gene expression.

The methylation state of histone proteins is regulated by histone methyltransferases (HMTs) and histone demethylases (HDMs). Plant homeodomains (PHDs) are another family of epigenetic readers and recognise lysine residues in specific methylation states.[20]

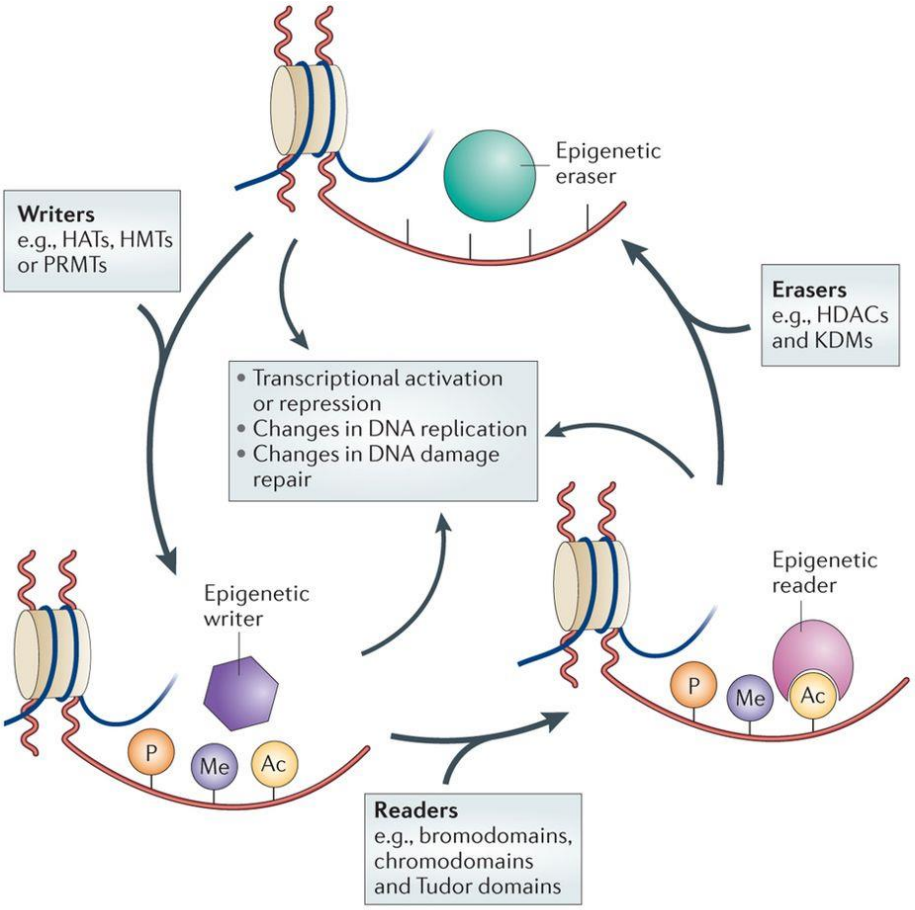


Figure 1.3: Epigenetic regulation is a dynamic process regulated by writers, erasers and readers, which add, remove and recognise epigenetic marks, respectively. Histone modifications regulate gene expression and affect transcription, DNA replication and DNA repair. Figure reprinted from K. J. Falkenberg and R. W. Johnstone, *Nat. Rev. Drug Discov.*, 2014, **13**, 673–691, doi: 10.1038/nrd4360 with permission from Springer Nature, License No. 4643110307642.[21]

1.1.3.1 Bromodomains

Bromodomains comprise approximately 110 amino acids and are transcriptional regulators that are recruited to chromatin *via* specific PTMs (i.e. acetylated lysines). There are 61 human bromodomains found in 46 different proteins, which are clustered into 8 families (Figure 1.4).

Bromodomains are characterised by a conserved left-handed bundle of four α-helices (αZ, αA, αB and αC) linked by loop regions (ZA and BC loops) which pack together to create the KAc-binding pocket (Figure 1.5). The binding pocket is largely hydrophobic in nature but is lined by a network of structural water molecules. KAc residues form hydrogen bonds to a conserved asparagine residue and to a water molecule at the base of the pocket.[22] The variable loop regions

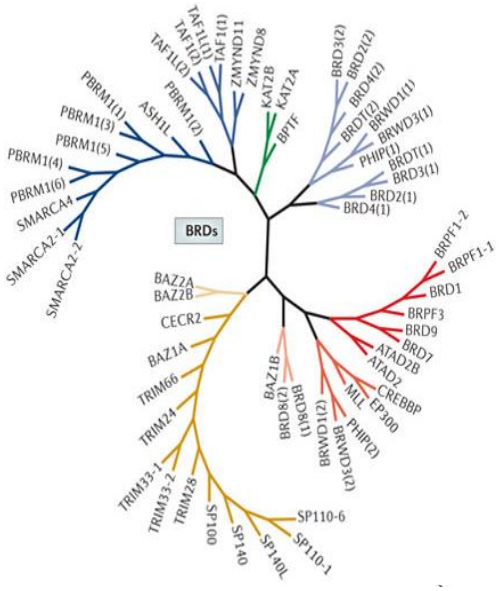


Figure 1.4: Phylogenetic tree of the 61 human bromodomains. Clustering of proteins on branches is based on the similarity of amino acid sequence and tends to represent structurally related proteins. Each family is highlighted in a different colour. Figure reprinted from D. Fyodorov, B-R. Zhou, A. I. Skoultchi and Y. Bai, *Nat. Rev. Mol. Cell Biol.*, 2018, **19**, 192–206, doi: https: //doi.org/10.1038/nrm.2017.94 with permission from Springer Nature, License No. 4640120411880.[6]

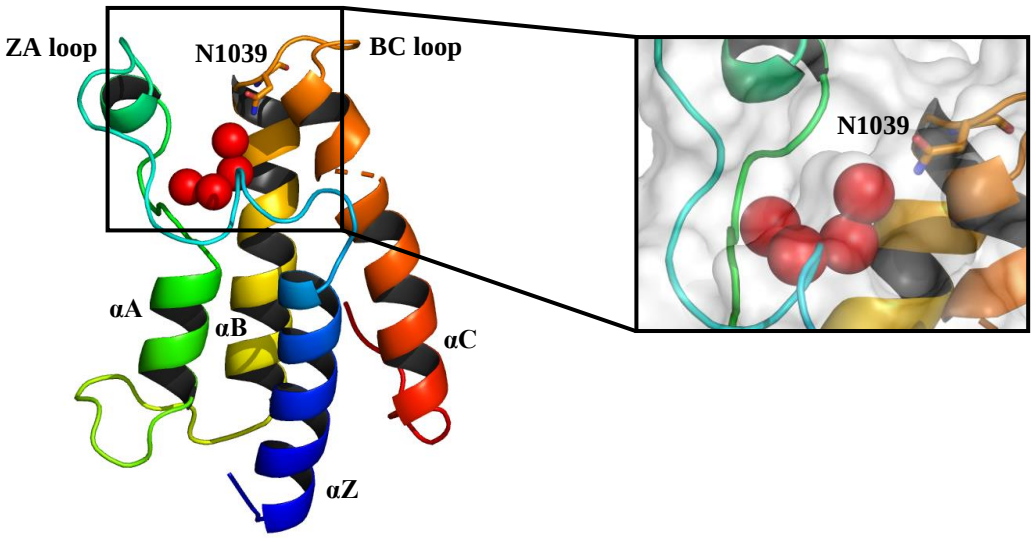


Figure 1.5: X-Ray crystal structure of the bromodomain of TRIM33β (PDB ID: 5MR8). Bromodomains are characterised by four α-helices: αZ (blue), αA (green), αB (yellow) and αC (red) and two loop regions (ZA loop and BC loop). The KAc-binding pocket is largely hydrophobic but four or five structural water molecules line the base. A conserved asparagine residue forms interactions with KAc residues.

form the entrance to the binding pocket and play a role in modulating substrate recognition.[23] Upon recognising KAc residues, bromodomains may recruit other nuclear proteins resulting in the formation of large chromatin remodelling and transcriptional regulatory complexes.[24] Bromodomains have diverse catalytic and scaffolding functions, mainly playing important roles in the regulation of gene expression.[25]

1.1.3.2 Plant Homeodomains (PHDs)

PHDs (50–80 amino acids) are found in a number of proteins and are composed of a zinc finger motif (Cys^4-His-Cys^3) which recognises lysine and arginine residues in specific methylation states (Figure 1.6).[26] This conserved fold is stabilised by two zinc atoms and consists of a two-strand anti-parallel β-sheet and a C-terminal α-helix.[26] The majority of PHDs read the N-terminal of histone H3, specifically the methylation state of H3K4. Binding of PHDs to H3K4Me_3 occurs through an aromatic cage. The composition of this cage varies between PHDs but typically contains two to four aromatic and hydrophobic residues. PHDs have a conserved tryptophan residue at the beginning of the β1 strand and an aromatic or hydrophobic residue at the start of the β2 strand. PHDs which lack an aromatic cage recognise unmodified H3K4.[27] For this group of PHDs, binding is prevented by methylation at H3K4. Binding of PHDs to chromatin results in a wide range of downstream processes including transcriptional activation or repression.[28,29]

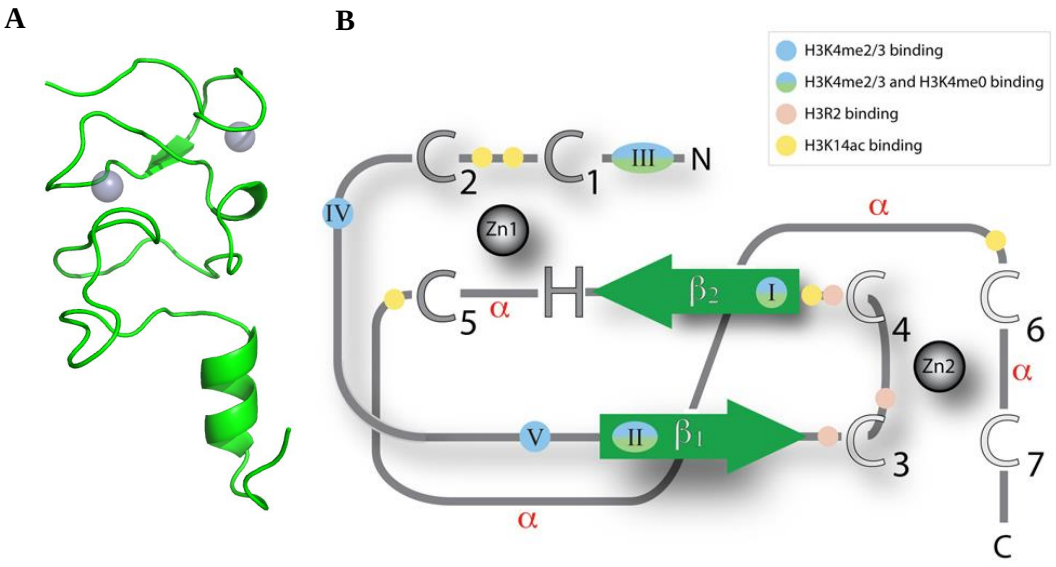


Figure 1.6: Structure of PHDs. (A) PHD of TRIM33β (green) (PDB ID: 5MR8). (B) Schematic of PHDs. PHDs have two core β-strands (green). The zinc atoms (grey spheres) and zinc-coordinating residues are shown (uppercase letters). Regions which are involved in recognition are also labelled (I-V). Figure reprinted from R. Sanchez and M-M. Zhou, *Trends Biochem. Sci.*, 2011, **36**, 364–372, doi: https://doi.org/10.1016/j.tibs.2011.03.005 with permission from Elsevier, License No. 4640250001896.[26]

1.1.4 Epigenetics and Disease

Aberrant access to genetic information is responsible for many diseases, ranging from cancer to neurological disorders. Genome-wide DNA hypomethylation and gene-specific methylation have been observed in human cancers,[30] including colon,[31] breast,[32] liver,[33] bladder[34] and ovarian.[35] Hypermethylation of promoter regions is associated with the abnormal silencing of genes and can provide a selective advantage to neoplastic cells.[36]

The methylation state of DNA is also critical for neuronal function and neurological behaviour. For example, schizophrenia is associated with altered DNA methylation patterns in blood cells and brain tissues.[37] In addition, alterations in DNA methylation have been implicated in Alzheimer's disease[38] and autism.[39]

Bromodomains play a central role in epigenetic gene regulation and abnormal regulation has been linked with the onset and progression of many diseases. Bromodomains have been found to be deregulated in cancer, both stimulating and supressing malignant phenotypes. This dual role implies bromodomains have context-dependent functions.[25] Bromodomains have also been

implicated in the initiation and development of neurological disorders,[40] inflammation-related pathologies[41] and viral replication.[42] As there are examples of bromodomain ligands being developed and because bromodomains have critical roles, bromodomains are considered attractive therapeutic targets.

PHDs are also recognised as contributing to a wide range of human diseases, including immunological disorders,[43] cancers[44] and neurological diseases.[45] Understanding the roles of epigenetic readers in the context of human disease will provide novel targets for a diverse range of diseases.

1.1.5 Small Molecule Inhibition of Bromodomains

As first demonstrated in 2010, the bromodomain-KAc protein-protein interaction (PPI) can be inhibited by small molecules.[46,47] Early bromodomain ligands targeted the bromodomain and extra-terminal (BET) family of bromodomains (Figure 1.7). The BET family of bromodomains are characterised by their conserved domain structure which contains two tandem bromodomains and an extra-terminal domain which mediates additional PPIs.[48] The BET family comprises BRD2, BRD3, BRD4 and testis-specific BRDT. Clinical trials have since been initiated to evaluate BET inhibitors for the treatment of human cancers,[49,50] atherosclerosis and associated cardiovascular disease.[51,52] The success of ligands developed for the BET family of bromodomain-containing proteins (BCPs) provided precedent for the druggability of bromodomains and paved the way for other classes of bromodomain inhibitors.

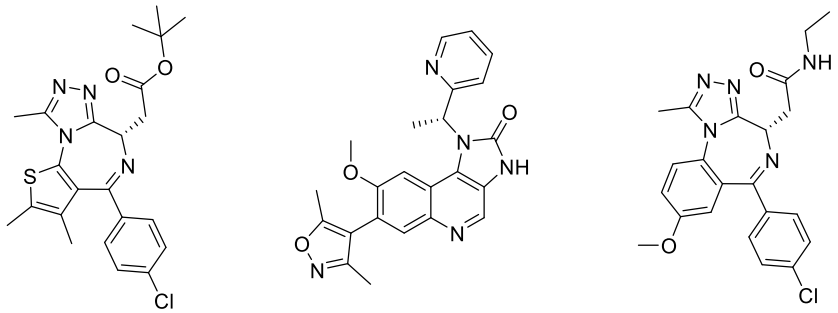


Figure 1.7: BET bromodomain inhibitors. (+)-JQ1 (**1**),[46] I-BET151 (**2**)[53] and I-BET762 (**3**)[54].

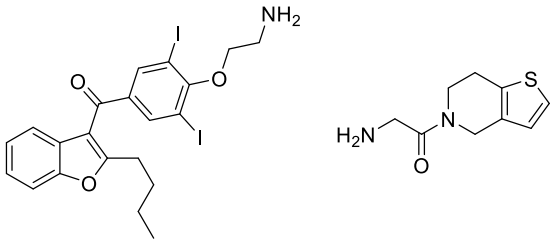
There are, however, challenges associated with developing bromodomain ligands. For example, limited chemotypes have been discovered that are able to act as KAc mimics and some bromodomains have shallow KAc-binding pockets which make developing high affinity ligands difficult. Bromodomains are viewed highly by many as desirable targets, however, the great prospects of bromodomains as drug targets remain to be seen. Initial clinical trials have shown that treatment with BET-inhibitors is associated with significant toxic side effects and the therapeutic benefit of BET-inhibitors compared to existing therapies remains to be realised.[55]

Druggability is defined as the ability of proteins to bind drug-like molecules.[56] The availability of bromodomain crystal structures led to the structure-based investigation of the druggability of bromodomains by Hoelder *et al*.[22] Proteins were submitted to SiteMap druggability assessment[57] and proteins with Dscores less than 0.75 were considered difficult to drug and proteins with Dscores greater than 0.85 were considered druggable. The study identified the BET family members as druggable, however, several other bromodomains showed lower predicted druggability, for example CREBBP and TRIM24 were classified as having intermediate and difficult druggability, respectively. Such bromodomains are likely to show lower hit rates in screening efforts and ligand optimisation may prove more difficult.

Following the identification of BET bromodomain ligands, other classes of bromodomain ligands have been developed. The overall bromodomain fold is highly conserved but the surface and loop regions are diverse, providing the opportunity to design inhibitors with high selectivity.[23] The availability of crystal structures also facilitates the rational design of high affinity and selective inhibitors.

1.1.6 Small Molecule Inhibition of PHDs

Small molecules have been identified which disrupt the binding of PHDs to chromatin (Figure 1.8). A HaloTag assay was used to identify ligands of the JARID1A PHD3[58] (**4**) and a fragment based approach identified ligands of the BAZ2A and BAZ2B PHDs (**5**).[59] Together these demonstrate the ligandability of PHDs and pave the way for future investigation of PHD inhibitors.



4 **5**

Figure 1.8: PHD ligands. Ligands **4**[58] and **5**[59] inhibit the JARID1A and BAZ2A/B PHDs, respectively.

1.2 Tripartite Motif-Containing (TRIM) Proteins

The tripartite motif-containing (TRIM) family of proteins are characterised by an RBCC motif at their N-terminus. This motif consists of a Really Interesting New Gene (RING) domain, one or two B-boxes (B1 and B2) and a coiled-coil (CC) region (Figure 1.9). Over 80 proteins belong to the TRIM family and they have various functions in cellular processes, including intracellular signalling, transcriptional regulation and tumour suppression.[60] The majority of TRIM proteins also have E3 ubiquitin ligase activity arising from the RING domain. The TRIM family is composed of 11 subfamilies, classified based on the nature of the C-terminal domain.[61] The C-VI subfamily (TRIM24, TRIM28 and TRIM33) have a chromatin binding unit comprising a C-terminal PHD adjacent to a bromodomain (Figure 1.9). Tandem PHD-bromodomain cassettes enable a cooperative association with epigenetically modified histone tails. Consequences of such cooperative association may include enhanced binding affinity, increased target specificity and new regulatory capabilities.[62]

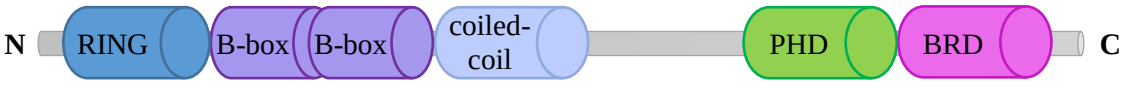


Figure 1.9: Schematic representing the C-VI TRIM subfamily. The TRIM family of proteins are characterised by an RBCC motif at their N-terminus and the C-VI subfamily have a tandem PHD-bromodomain cassette at their C-terminus.[63]

Due to their involvement in several pathways including cell proliferation, DNA repair and apoptosis, TRIM proteins are implicated in many diseases, including cancer,[60] neurological disorders[64] and inflammatory diseases.[65]

1.2.1 TRIM33

Possessing a tandem PHD-bromodomain cassette at its C-terminus, TRIM33 belongs to the C-VI subfamily of TRIM proteins (Figure 1.10). TRIM33 also has a RING E3 ligase, which has a zinc-binding domain and mediates the transfer of the ubiquitin-charged E2.[66] To prevent degradation of chromatin-remodelling complexes, TRIM33's ubiquitination activity requires tight regulation. The precise regulatory mechanism is unknown, but it has been linked to histone binding and consequently the C-terminal PHD and bromodomain.[67,68]

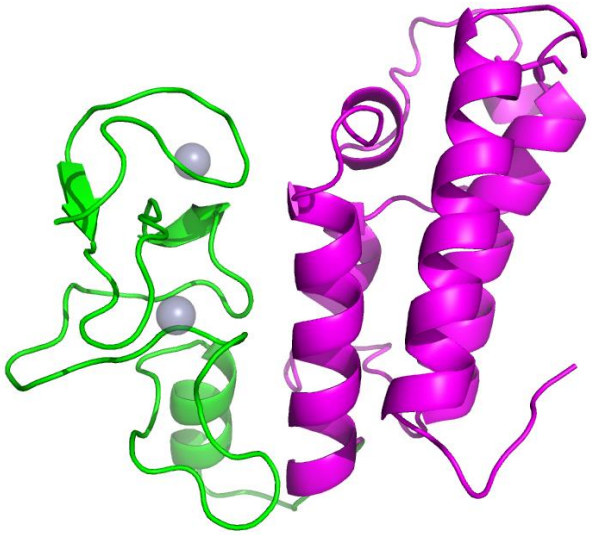


Figure 1.10: TRIM33β (PDB ID: 5MR8). The PHD is shown in green and the bromodomain in magenta.

TRIM33 exists as two isoforms, α and β (Figure 1.11). TRIM33β has a 17 amino acid deletion and possesses a canonical bromodomain. Due to its longer BC loop, TRIM33α has a non-canonical bromodomain where the conserved asparagine residue points out of the binding pocket (Figure 1.11). It may therefore be more difficult for TRIM33α to form interactions with small molecule binding partners and this key difference between the two isoforms may enable the design of structurally distinct ligands.

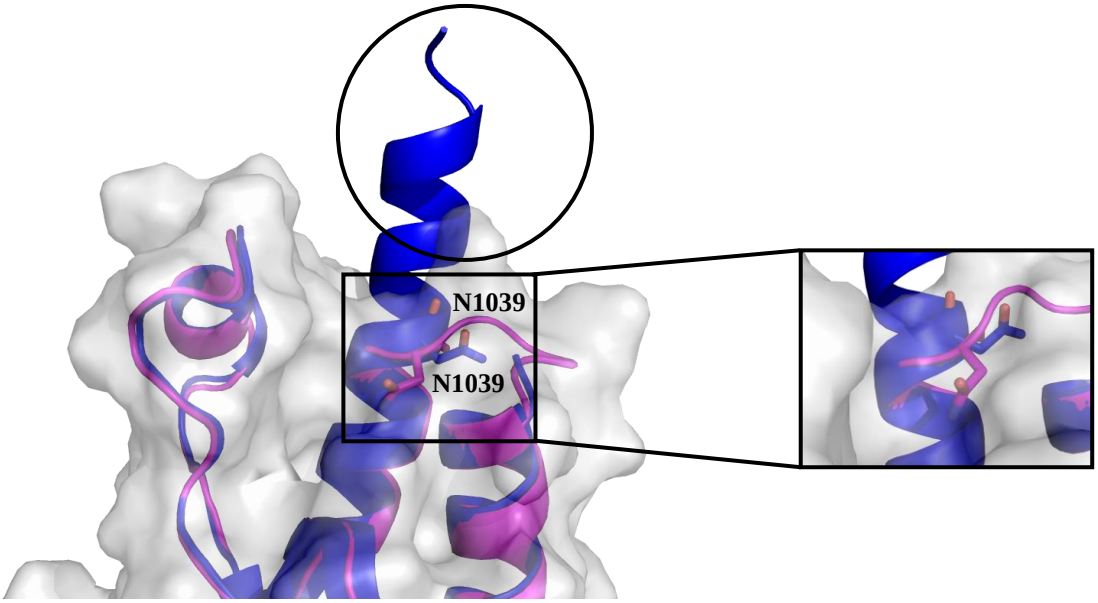


Figure 1.11: Overlay of TRIM33α (blue) (PDB ID: 3U5O) and TRIM33β (magenta) (PDB ID: 5MR8). The key residues are shown in the stick representation. The conserved asparagine residue (N1039) points into the binding site in TRIM33β but points out of the binding site in TRIM33α. The longer BC loop in TRIM33α (17 amino acids) is also highlighted.

TRIM33 is involved in the poly(ADP-ribose) polymerase (PARP)-dependent DNA damage repair pathway.[69] Following DNA damage, PARP1 is activated and DNA repair proteins are recruited, including Amplified in Liver Cancer 1 (ALC1). ACL1 helicase activity relaxes the chromatin structure to enable DNA repair. It is important that the association of ALC1 to chromatin is tightly regulated as prolonged chromatin relaxation leaves DNA susceptible to further damage. TRIM33 is dynamically recruited to sites of DNA damage in a PARP1- and ALC1-dependent manner.[69] TRIM33 associates with ALC1, regulating its timely removal from chromatin, and therefore playing an important role in the PARP-dependent DNA damage response.

TRIM33 knockdown has been shown to sensitise cells to DNA-damaging agents.[69] Moreover, deletion of the bromodomain of TRIM33 abrogated localisation of TRIM33 at sites of DNA damage.[69] This suggests there is the potential to use DNA-damaging agents in combination with TRIM33 bromodomain inhibitors to enhance their efficiency and delay repair of damage. It is proposed that inhibition of the TRIM33 bromodomain will prevent TRIM33 binding to chromatin and consequently the prolonged association of ALC1 at chromatin. This will result in inefficient

repair of single-strand breaks and ultimately replication fork stalling and DNA double-strand break equivalents.

It has also been shown that TRIM33 and ALC1 are more highly expressed in cancer cell lines than in normal cell lines (Professor Ester Hammond, unpublished) (Figure 1.12 (A)). It is hypothesised that TRIM33 inhibitors should be preferentially toxic to cancer cells due to ALC1 upregulation. The fact that TRIM33 is more highly expressed in cancer cell lines also indicates that the protein plays an important role in these disease states. In addition, siRNA mediated knockdown of TRIM33 led to an accumulation of DNA damage and loss of viability in hypoxia, demonstrating a role for TRIM33 in the hypoxic response (Professor Ester Hammond, unpublished) (Figure 1.12 (B)).

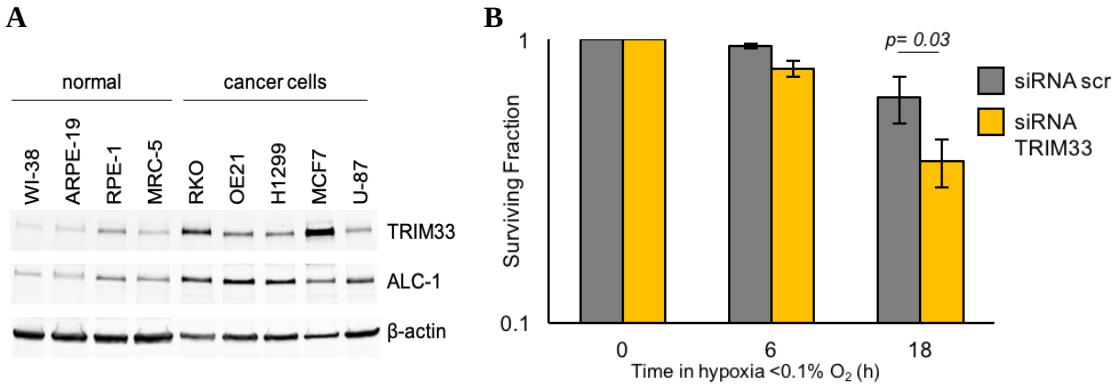


Figure 1.12: siRNA-mediated depletion of TRIM33 leads to the loss of viability in hypoxic conditions. (A) Expression of TRIM33 and ALC1 in normal and cancer cell lines. (B) siRNAs were used to compare the viability of RKO cells exposed to hypoxia in a colony survival assay (scr = scramble).

TRIM33 is also a negative regulator of transforming growth factor-beta (TGF-β) signalling[70] and acts as a tumour suppressor in hepatocellular cancer,[71] pancreatic cancer[72] and human chronic myelomonocytic leukaemia.[73] In addition, TRIM33 regulates mitosis,[74] controls transcription elongation[75] and regulates embryonic and adult haematopoiesis.[76]

1.2.2 Chemical Probes

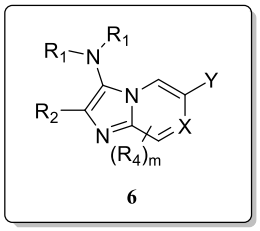
Chemical probes are used to explore the physiological function and therapeutic potential of targets. They can answer mechanistic and phenotypic questions and can prove invaluable tools in preclinical target validation.[77] Chemical probes complement genetic knockdown approaches, as

unlike shRNA- and siRNA-based approaches, chemical probes do not affect scaffolding functions of a protein, only the function of the specific domain they are targeting.

High quality ligands are essential to avoid misleading results, they should be selective, cell-permeable and have high affinity. They are distinctive to drug molecules in that they are not required to have specific pharmacokinetic (PK), pharmacodynamic (PD) and bioavailability profiles. Used correctly, high quality probes can have a major impact on the understanding of human biology and how we treat disease.[77]

1.2.2.1 TRIM33 Ligands

Upon commencing this work, there were no TRIM33 ligands reported in the literature. A patent claiming to have identified a series of compounds as binding to the bromodomain of TRIM33 has since been published (Figure 1.13).[78] Chapter 2 reports our evaluation of the binding of these compounds to TRIM24 and TRIM33.

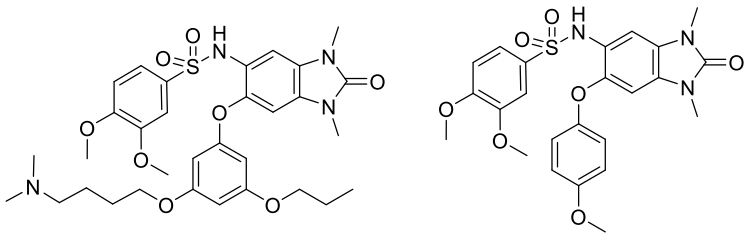


6

Figure 1.13: Reported TRIM33 inhibitors have general formula 6. Where m = 0–2.[78]

1.2.2.2 TRIM24 Ligands

Analogous to TRIM33, TRIM24 has a tandem PHD-bromodomain cassette at its C-terminus. Two ligands (**7** and **8**) have been reported as binding to the TRIM24 bromodomain (Figure 1.14).[79,80] These ligands (**7** and **8**) have a benzimidazolone core as the KAc mimic. X-Ray crystal structures (PDB ID: 4YC9 and 4ZQL) of these compounds in complex with TRIM24 show the carbonyl oxygen of the benzimidazolone moiety forming a hydrogen bond with the conserved asparagine residue (N980), and a water-mediated hydrogen bond with a tyrosine residue (Y935). A BROMOscan® recombinant protein binding assay used to determine the selectivity profile of **7** showed **7** to be selective over TRIM33. A thermal shift assay was used to examine the selectivity



IACS-9571 (7)
TRIM24 ITC K_D = 31 nM
BRPF1 ITC K_D = 14 nM

8
TRIM24 ITC K_D = 222 nM
BRPF1 ITC K_D = 137 nM

Figure 1.14: Published TRIM24 bromodomain inhibitors 7[79] and 8.[80]

of **8** against a panel of bromodomains including TRIM33 and BRPF1. The ΔT_m was less than 1 °C for TRIM33 and was not considered significant. Despite efforts to achieve selectivity, **7** and **8** were both TRIM24/BRPF1/2 dual inhibitors.

1.3 Proteolysis Targeting Chimeras (PROTACs)

First described by Craig Crews in 2001,[81] proteolysis targeting chimeras (PROTACs) hijack the cellular ubiquitin degradation system to mediate the degradation of target proteins. PROTACs therefore unveil the function of the whole protein, as opposed to small molecule ligands which provide information on the role of a specific domain.

PROTACs are heterobifunctional molecules consisting of a ligand for the target protein, a ligand for the recruitment of the E3 ubiquitin ligase and a linker connecting the two moieties (Figure 1.15).[15,82] PROTACs artificially target a protein for degradation by the proteasome by redirecting the substrate specificity of the ubiquitin-proteasome pathway. The proteasome refers to the protein degradation machinery found in cells. Formation of a stable ternary complex between the PROTAC, target protein and E3 ubiquitin ligase is required for efficient degradation.[83] Ternary complex formation is dependent on having the appropriate linker length and linker composition. To date polyethylene glycol (PEG) chains have been mainly employed due to the availability of a large range of lengths. Upon ternary complex formation, the target protein is in close proximity to the E3 ubiquitin ligase, this results in its ubiquitination and consequent degradation by the proteasome.

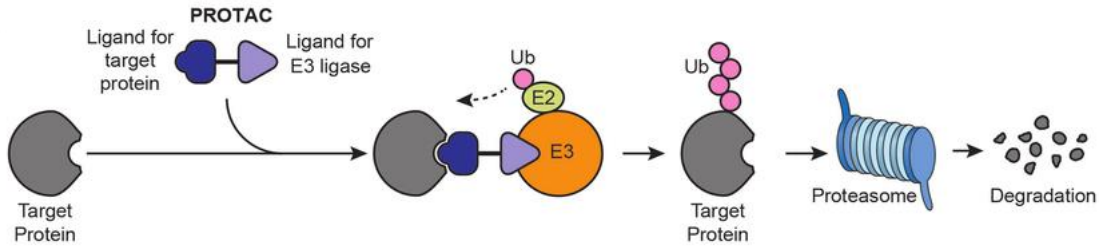


Figure 1.15: Heterobifunctional PROTAC molecules hijack the ubiquitin degradation system to selectivity degrade target proteins. A ligand for the target protein is tethered by a linker to a ligand for an E3 ligase. Formation of a ternary complex brings the E3 ligase in close proximity to the target protein. This results in the ubiquitination of the target protein, marking it for degradation by the proteasome. Figure reprinted from X. Huang and V. M. Dixit, *Cell Res.*, 2016, **26**, 484–498, doi: https://doi.org/10.1038/cr.2016.31 published under the terms of the Creative Commons Attributio-NonCommercial-NoDerivs 4.0 Unported License.[15]

1.3.1 E3 Ubiquitin Ligases

More than 600 E3 ligases are found in humans and are responsible for conferring selectivity to the target protein through substrate recognition. Each E3 ligase recognises a particular destruction sequence and therefore binds specifically to a limited number of protein targets. To date, the E3 ubiquitin ligases exploited by PROTACs have been limited. The first PROTAC described employed a phosphopeptide to target ubiquitin ligase complex Skp-Cullin-F box containing Hrt1 (SCF) as the E3 ubiquitin ligase.[81] PROTACs targeting the Von Hippel-Lindau (VHL) E3 ligase were first reported in 2004 and used a peptide derived from HIF-1α as the E3 recognition domain.[14] The first PROTACs to employ a small molecule as the E3 ubiquitin ligase ligand used Nutlin **9** to target MDM2 and trigger the degradation of the androgen receptor (AR) (Figure 1.16).[84] PROTACs targeting E3 ubiquitin ligase cereblon (CRBN) were first reported in 2015, using pomalidomide (**14**) as the E3 ligase recognising domain, to degrade BRD4.[85] The current most widely targeted E3 ubiquitin ligases are VHL and CRBN, this is in part due to the availability of small molecule ligands (Figure 1.16).

1.3.1.1 Small Molecule Ligands of E3 Ligases

The first small molecules targeting VHL were identified in 2012 (**10**, IC_{50} = 7.0 μM and K_D = 5.3 μM)[86] and have since been optimised (**11** and **12**, IC_{50} = 1.8 μM and K_D = 0.19 μM,

respectively).[87,88] Immunomodulatory drugs thalidomide (**13**), pomalidomide (**14**) and lenalidomide (**15**) were identified to bind to CRBN in 2010 and 2012.[89,90]

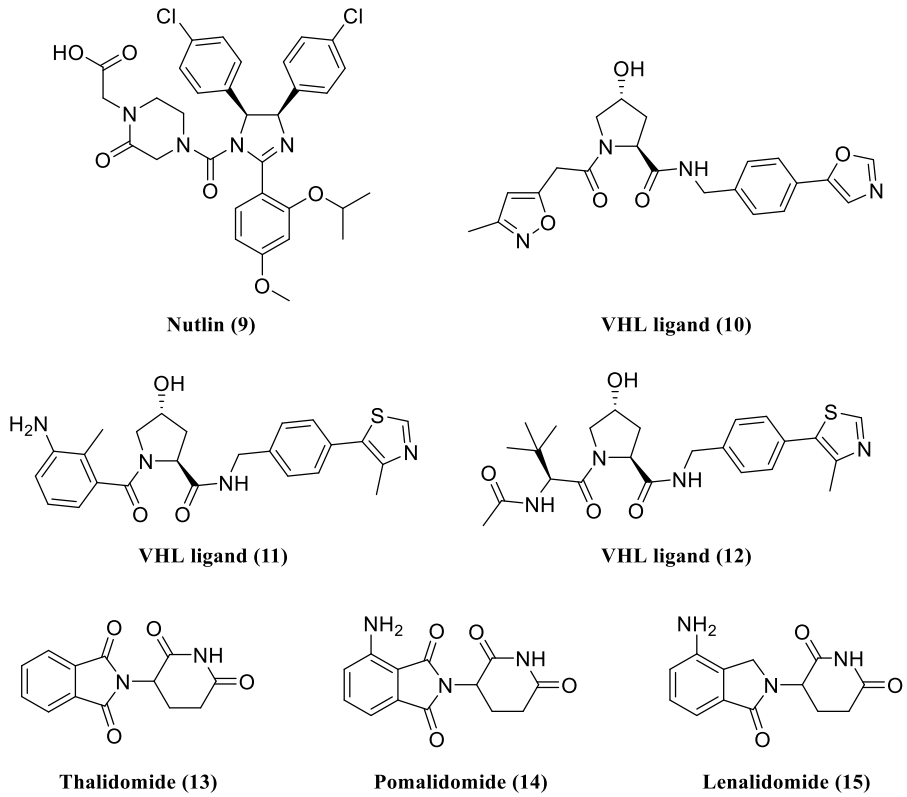


Figure 1.16: Small molecule binding partners for E3 ubiquitin ligases.[84,86,87,88,89,90]

1.3.2 Small Molecule Ligands *vs*. PROTACs

Despite frequently being the focus of drug development programmes, small molecule ligands have several limitations (Figure 1.17). For example, a pocket or active site is required for target engagement. However, only a small portion, approximately 25%, of the human proteome has an active site, therefore approximately 75% of the proteome is deemed undruggable using conventional strategies.[83,91] Furthermore, in order to sustain therapeutically relevant intracellular concentrations high dosages can be required, these are often associated with side and off-target effects.[83] In addition, small molecules only disrupt one domain of multidomain proteins, this may not be sufficient to obtain the desired therapeutic response as the remaining domains are still functional. Treatment with small molecules ligands can also lead to a compensatory increase of target proteins and thus the incomplete inhibition of downstream signalling pathways. Finally,

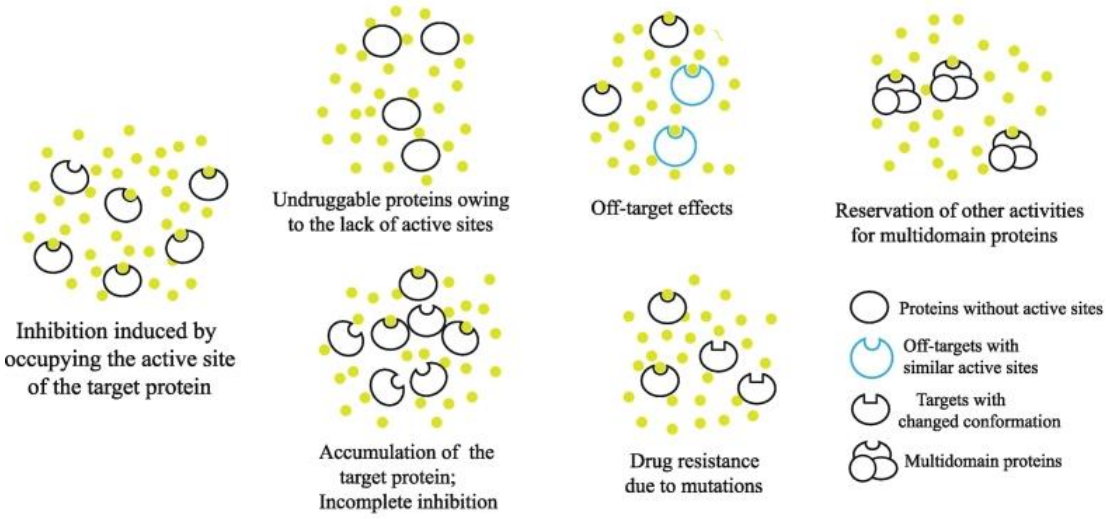


Figure 1.17: Limitations of small molecule ligands. Small molecule ligands competitively bind to active sites on target proteins. Several limitations are associated with this mechanism of inhibition and are illustrated in this figure. Figure reprinted from S. An and L. Fu, *EBioMedicine*, 2018, **36**, 553–562, doi: 10.1016/j.ebiom.2018.09.005 published under the terms of the Creative Commons Attribution-NonCommercial-No Derivatives License (CC BY NC ND).[83]

mutations in proteins produce conformational changes and loss of key interactions with ligands, providing a mechanism of resistance for small molecule ligands.[83]

PROTACs offer several advantages over small molecule ligands. For example, traditional drugs rely on occupancy-driven pharmacology and act stoichiometrically to modulate protein function. In contrast, each PROTAC can be involved in multiple degradation events, hence PROTAC induced degradation is sub-stoichiometric and PROTACs can be effective at lower doses. PROTACs also provide rapid and sustained depletion of target proteins and robust inhibition of downstream signalling. Indeed depletion of target proteins has been observed within 1 h *in vitro*.[92,93] In addition, PROTACs can evade drug resistance caused by mutations as they only rely on transient and reversible associations with their substrates.[94] It has also been shown that PROTACs can enhance the selectivity of the small molecule ligand employed. For example, a PROTAC tethering the small molecule bromodomain ligand (+)-JQ1 (**1**, Figure 1.7) to a VHL ligand, selectively degraded BRD4 over BRD2 and BRD3.[95] The improved selectivity *vs.* the parent ligand may arise through: direct interactions between the target protein and the E3 ubiquitin ligase; reduced steric constraints allowing more productive ternary complex formation or

preferential polyubiquitination of the target protein. Furthermore, selectivity can be modified through the linker length and composition. For example, isoform-selective (p38α or p38δ) PROTACs for the p38 MAPK family were developed by changing only the linker length and linker attachment of the PROTACs.[96] PROTACs also provide the opportunity to repurpose ligands that have no phenotypic effect as the ligand for the target protein does not need to occupy a binding site that affects protein function, it is only required to interact with the target protein.

There are, however, potential drawbacks with using a PROTAC strategy. PROTACs influence the cellular levels of target proteins. There are risks associated with disrupting all the domains of a protein and losing all its functions. For example, complete loss of BRD2 or BRD4 is lethal.[97,98] To circumvent this, targeting PROTACs to tissues and cells where their effects are desirable has begun to be investigated, for example PHOTACs (photochemically targeted chimeras) which are activated using light.[99]

1.3.3 TRIM24 PROTACs

TRIM24 bromodomain ligands **7** and **8** have high affinity for TRIM24 but do not exert effective antiproliferative effects, despite genetic knockdown of TRIM24 resulting in antiproliferative phenotypes and overexpression of TRIM24 correlating with poor patient prognosis.[79,80,100] This suggests that TRIM24 bromodomain inhibition alone is not sufficient as an anti-cancer strategy. PROTAC dTRIM24 (**16**) is reported to elicit potent and selective degradation of TRIM24 *via* conjugation of ineffective bromodomain ligand **7** to a VHL ligand (Figure 1.18).[101] An enhanced anti-proliferative response was observed for degradation *vs*. inhibition and using dTRIM24 (**16**), human acute leukaemia cells were found to be dependent on TRIM24. This work exemplifies how ineffective small molecule ligands can be incorporated into the design of PROTACs to achieve degradation of target proteins. TRIM24 and TRIM33β have high sequence identity (68%, BLAST),[102] dTRIM24 (**16**) therefore provides evidence that degradation of TRIM33 could be achieved using a PROTAC strategy. TRIM24 and TRIM33 PROTACs are especially interesting as both TRIM24 and TRIM33 possess their own E3 ligase activity.[67,68] It was shown that TRIM24

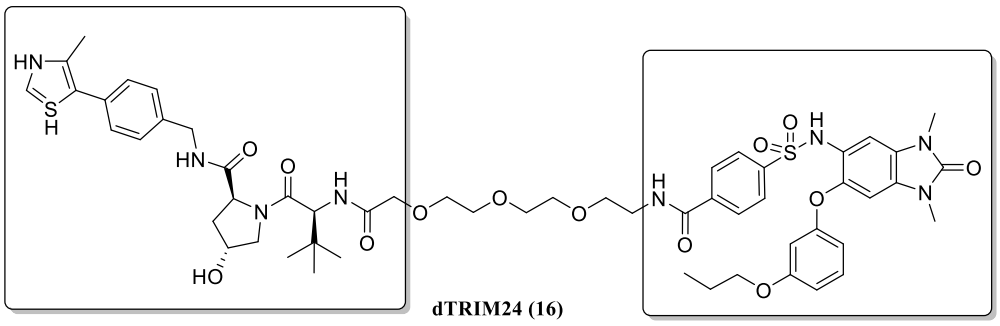


Figure 1.18: Structure of dTRIM24 (16). Ineffective TRIM24 ligand **7** was repurposed into the design of TRIM24 PROTAC **16**. dTRIM24 (**16**) elicits potent and selective degradation of TRIM24.[101]

degradation was not through autoubiquitination *via* its RING domain.[101] TRIM33 PROTACs will be explored in Chapter 4.

1.3.4 PROTAC Pharmacodynamics and Pharmacokinetics

PROTACs have particularly interesting pharmacodynamic (PD) and pharmacokinetic (PK) properties, where the desired PD profile need not match the PK profile.[103] This disconnect between PD and PK is dependent on the turnover rate of proteins. PROTACs are able to elicit prolonged PD effects as they can eliminate the existing pool of the target protein. PROTACs are therefore particularly effective against proteins with slow turnover rates as the protein pool takes longer to be resynthesised and following even brief exposure to the PROTAC a long-lasting effect can result. In contrast, if the protein turnover is high, continuous exposure of the PROTAC is required, similar to occupancy-driven small molecule ligands.[103] The event driven pharmacology demonstrated by PROTACs means the drug exposure need not be continuously above the efficacious level and consequently off-target toxicities are reduced.

1.3.5 Binding Kinetics

Understanding the kinetics of ternary complex formation is important as it is associated with degradation efficiency. The binding kinetics of PROTACs are, however, complicated due to a three-body binding equilibrium. Not only must each binding moiety interact with its target, these binding events must occur simultaneously to form a ternary complex. Cooperativity effects may also result from PPIs between the target proteins, whereby the binding of one ligand to its target

may be enhanced or decreased when the PROTAC is already bound to the second protein.[104] A surface plasmon resonance (SPR)-based assay was developed by Ciulli *et al.* to quantify the stability of PROTAC-induced ternary complexes.[104] The E3 ligase was immobilised onto a sensor chip and binding of the PROTAC was measured in either the absence (binary binding) or presence (ternary binding) of near-saturating concentrations of the target protein. It was observed that kinetic differences in ternary complex formation correlated to relative initial rates of target degradation. Despite the importance of ternary complex formation for degradation, no other methods to directly determine binding parameters for ternary complex kinetics have been reported.[105]

1.3.6 Challenges Associated with PROTACs

Compared to small molecule ligands, the relatively large size of PROTACs means they are inherently less drug-like. It may be that the permeability and oral bioavailability of PROTACs present the biggest challenges for translating PROTACs into drugs.[106,107]

Although enhanced selectivity has been observed with some PROTACs, off-target effects can still occur and achieving complete selectivity can be difficult. Off-target toxicities can result from the target protein ligand or E3 ubiquitin ligase ligand engaging with other proteins.

Despite an appropriate linker length and composition being essential for ternary complex formation, there are limited examples of rational linker design and current *in silico* modelling is insufficient. Until robust and reliable methods are developed to predict linker composition, the best way to determine the optimum linker is through the costly and time intensive synthesis of many analogues. Even the synthesis of large numbers of PROTACs does not guarantee degradation of the target protein.

1.3.7 Clinical Trials

The success of PROTACs is evidenced by them entering clinical trials. The first PROTAC set to enter phase I clinical trials targets the androgen receptor. This study will evaluate the safety, tolerability and pharmacokinetics of the PROTAC in patients.[108] The clinical outcome of

PROTAC technology remains uncertain and these initial studies will prove pivotal in the advancement of PROTAC technology from the bench to the clinic.

1.4 Assays to Evaluate Ligand Binding

1.4.1 AlphaScreen™

The primary assay used to quantify ligand binding will be AlphaScreen™ (Amplified Luminescent Proximity Homogeneous Assay Screen) (Figure 1.19). AlphaScreen™ is a fluorescence-based assay which relies on the interruption of a specific biological interaction by the compound being screened.[109]

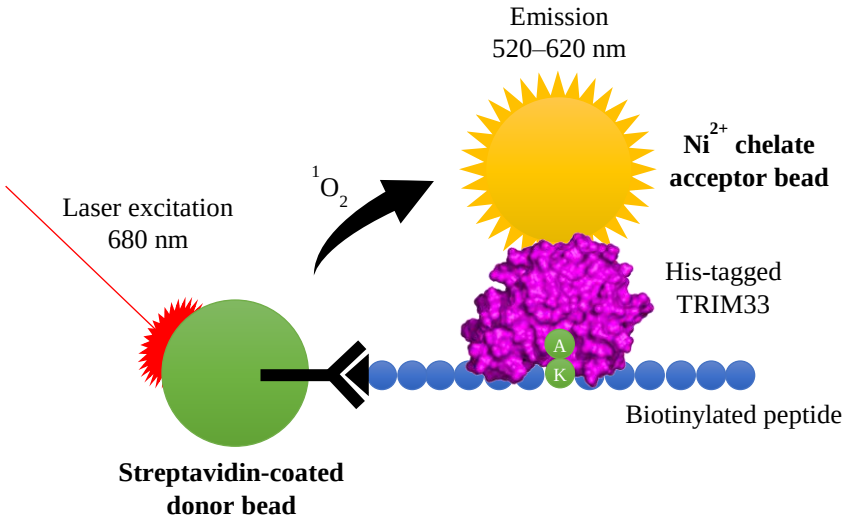


Figure 1.19: Schematic of the AlphaScreen™ assay.[110]

The AlphaScreen™ assay consists of two types of beads: donor beads and acceptor beads (Figure 1.20). The donor beads contain photosensitiser phthalocyanine (**17**), which upon illumination at 680 nm converts ambient oxygen to singlet oxygen, which can diffuse up to 200 nm in solution. If an acceptor bead is in close enough proximity, thioxene (**18**) reacts with the singlet oxygen and generates a chemiluminescence signal at 370 nm. This emission is transferred to anthracene (**21**) and then rubrene (**22**) *via* Förster resonance energy transfer (FRET), resulting in a shift in the emission wavelength to 520–620 nm. The intensity of this fluorescence is measured in the AlphaScreen™ assay and used to determine IC_{50} values.[111]

The donor and acceptor beads are coated with a hydrogel which minimises non-specific binding and aggregation and provides functional groups for bioconjugation. The AlphaScreen™ assays employed in this work use streptavidin-coated donor beads to bind biotinylated peptides and nickel chelate acceptor beads to bind His-tagged proteins. The proximity of the beads is therefore dependent on the interaction of the protein and peptide. If a compound binds to the protein, the protein-peptide interaction is disrupted, and a consequent reduction in emission is observed.

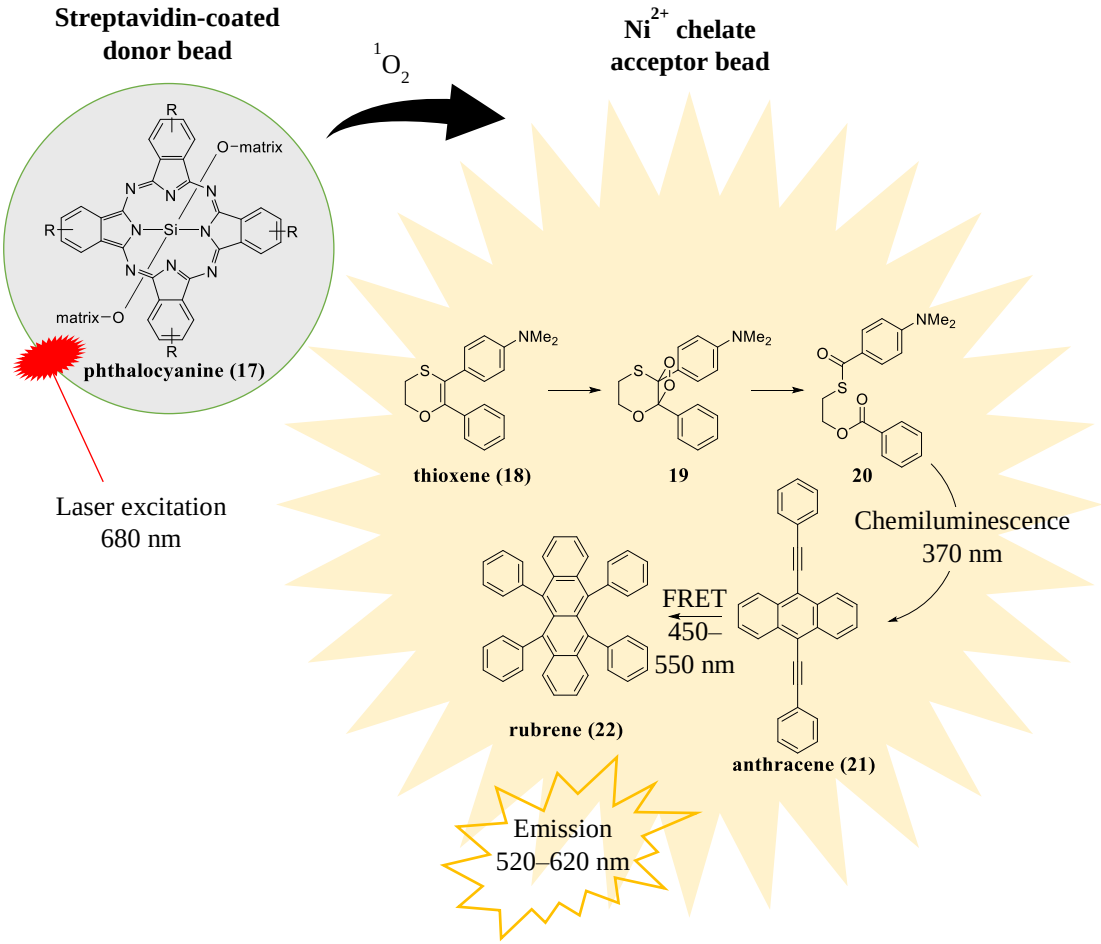


Figure 1.20: Reactions occurring in the AlphaScreen™ beads. The donor beads contain photosensitiser phthalocyanine (**17**) which generates singlet oxygen upon excitation. Following a cascade of reactions, rubrene (**22**) in the acceptor beads generates a fluorescence signal which is the readout in AlphaScreen™ assays. Figure adapted from M. Schiedel, M. Moroglu, D. M. H. Ascough, A. E. R. Chamberlain, J. J. A. A. G. Kamps, A. R. Sekirnik and S. J. Conway, *Angew. Chem. Int. Ed.*, in press, doi: https://doi.org/10.1002/anie.201812164.[112]

AlphaScreen™ assays were previously optimised for the bromodomain and PHD of TRIM24, TRIM33α and TRIM33β by Dr Angelina Sekirnik[113] and Dr Larissa See.[114] Three competitor histone peptides were identified as the protein binding partners, binding to the PHD ($H3_{1-27}K9Me_3$), the bromodomain ($H3_{1-27}K18Ac$) or both the PHD and bromodomain ($H3_{1-27}K9Me_3K18Ac$). The assay therefore also provides information on where the compounds bind based on which protein-peptide interaction they disrupt. The peptides were biotinylated on the side chain of the C-terminal lysine (K27) to interact with the streptavidin-coated donor beads, and the protein His-tagged to interact with the Ni^{2+} chelate acceptor beads. It must be noted that due to AlphaScreen™ being a peptide displacement assay, direct comparisons cannot be made between different protein-peptide combinations as each peptide has different affinity for each protein and is therefore harder or easier to displace.

1.4.2 WaterLOGSY NMR Experiments

Nuclear magnetic resonance (NMR)-based Water-Ligand Observed *via* Gradient SpectroscopY (waterLOGSY) experiments provide an orthogonal method to AlphaScreen™ assays for evaluating binding and were previously optimised for TRIM24, TRIM33α and TRIM33β by Dr Angelina Sekirnik[113] and Dr Larissa See.[114] WaterLOGSY is a ligand-observed NMR method used to identify weak binders, typically with K_D values in the μM to mM region. WaterLOGSY relies on the transfer of bulk water magnetisation to the ligand *via* the protein-ligand complex and does not require the labelling of compounds or proteins.[115] WaterLOGSY therefore provides the easy distinction between binders and non-binders, but unlike AlphaScreen™ assays, does not quantify binding events.

During the waterLOGSY experiment, the bulk water is excited, and the magnetisation transferred *via* the nuclear Overhauser effect (NOE) to molecules in solution (Figure 1.21 (A)). Magnetisation is then transferred from the surface of the protein to its core through spin diffusion, and subsequently to bound ligands *via* NOE.[116] Free ligands have a rapid tumbling regime and only receive positive NOEs directly from the water, whereas bound ligands have a slow tumbling regime and receive negative NOEs. The difference in sign of NOE is used to distinguish binders

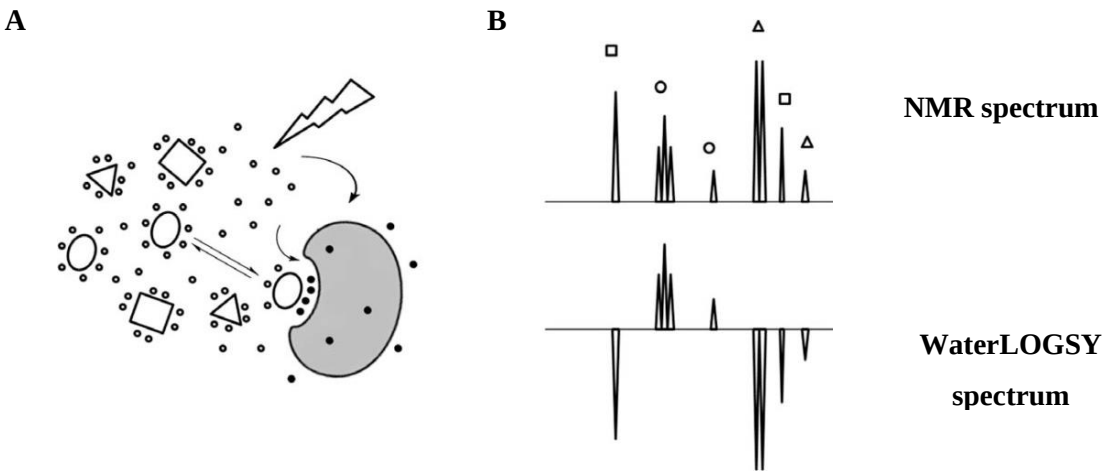


Figure 1.21: Schematic representation of the waterLOGSY experiment. (A) Bulk water molecules (small circles) are excited and the magnetisation transferred to ligands directly or *via* the protein-ligand complex. Ligands which bind (large circles) receive a negative NOE, whereas unbound compounds receive a positive NOE (squares and triangles). (B) Compound NMR spectrum (top) and waterLOGSY NMR spectrum (bottom). Binding is indicated by positive peaks in the waterLOGSY spectrum, negative peaks are observed for non-binders. Figure reprinted from M. d. C. Fernández-Alonso, D. Díaz, M. Á. Berbis, F. Marcelo, J. Cañada and J. Jiménez-Barbero, *Curr. Protein Pept. Sci.*, 2012, **13**, 816–830, doi: 10.2174/1389203711213080010. Copyright 2012 Bentham Science Publishers Ltd.[117]

from non-binders. WaterLOGSY is not a suitable technique for strong binders as slow dissociation rates mean the negative NOE signal is not carried to the free population prior to relaxation and they thus appear as false negatives in waterLOGSY experiments.

Ligand binding is indicated by positive signals in the waterLOGSY NMR spectrum, whereas peaks of non-binders remain negative (Figure 1.21 (B)). As the ligand signal in the waterLOGSY spectrum is an average of negative and positive NOE signals arising from the ligand in its bound and free states, respectively, peaks of binders may also appear as negative signals which are less intense than the reference spectrum. Differences in peak intensity can also be attributed to differences in binding mode affecting magnetisation transfer. Care must be taken with exchangeable protons as these always appear as positive signals due to chemical exchange with water molecules. Compound aggregation is responsible for false positives in waterLOGSY NMR experiments and is identified by positive signals in the absence of protein. A reference waterLOGSY spectrum is therefore first obtained in the absence of protein to identify compound

aggregation. Reducing the compound concentration in the NMR sample can prevent aggregation of compounds.

1.4.3 Isothermal Titration Calorimetry (ITC)

Isothermal titration calorimetry (ITC) provides a method for studying the thermodynamics of binding events and will be used to determine the equilibrium dissociation constant (K_D) of selected compounds. When a ligand binds to a protein there is an increase or decrease in enthalpy (ΔH), and a reduction in the degrees of freedom of both molecules (ΔS). ITC experiments enable the simultaneous determination of all binding parameters (K_D, ΔH, ΔS and n (number of binding sites)), providing a complete thermodynamic profile.

The ITC apparatus consists of two identical cells: the reference cell and the sample cell (Figure 1.22). In the ITC experiment, one binding partner is placed in a syringe and the other in the sample cell. During the experiment the syringe injects small amounts of binding partner 1 into the sample cell. It is important to have the binding partners in the same buffer as buffer mismatches can generate large heats of dilution and mask heat changes from ligand binding.[118] ITC experiments are performed at a constant temperature. To ensure ΔT between the two cells is 0, a small constant power is applied to the reference cell which activates the cell feedback circuit.[119] If the interaction between the binding partners is exothermic, less heat is required to heat the sample cell, if the interaction is endothermic, more energy is required to heat the sample cell. Once equilibrium has been reached, the system relaxes to reference power level and another injection occurs. As the experiment progresses, the number of binding events taking place decreases, therefore the difference in temperature between the two cells following injection becomes increasingly small, until only the heat of dilution of binding partner 1 is observed. The energy required to maintain the temperature of the sample cell is measured during the experiment and is used to calculate binding parameters.

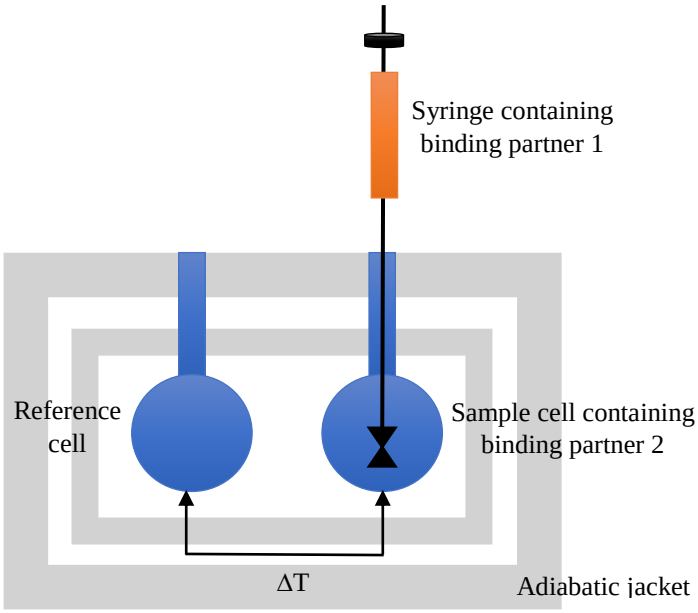
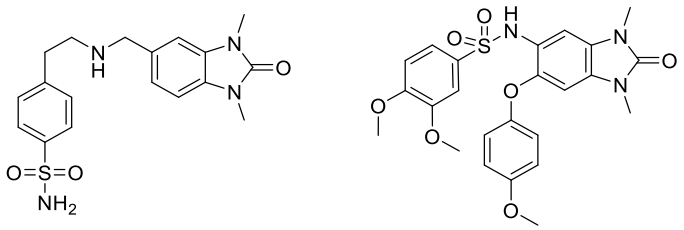


Figure 1.22: ITC apparatus. The reference cell and sample cell are contained in an adiabatic jacket. Binding partner 1 is injected into the sample cell containing binding partner 2. The energy required to maintain the cells at the same temperature during the experiment is measured. Figure adapted from K. Bouchemal and S. Mazzaferro, *Drug Discov. Today.*, 2012, **17**, 623–629, doi: https://doi.org/10.1016/j.drudis.2012.01.023 with permission from Elsevier, License No. 4642990069681.[120]

1.5 Previous Work and Aims

1.5.1 Previous Work

In previous work, Dr Angelina Sekirnik[113] screened the PPI-net[121] collection using an AlphaScreen™ assay and identified **23** as binding to TRIM33 (Figure 1.23). Follow up counter-screens, including waterLOGSY experiments, validated binding.[113,114] Consistent with the AlphaScreen™ results for the different cognate peptides, waterLOGSY experiments with mutant protein suggested **23** specifically binds to the bromodomain of TRIM33β. TRIM24 ligand **8** was also evaluated using an AlphaScreen™ assay and was found to be selective over TRIM33, consistent with the thermal shift assay previously reported.[80]



23 **8**

Figure 1.23: Compound 23 was identified as binding to the bromodomain of TRIM33. TRIM24 ligand **8** showed selectivity over TRIM33 in an AlphaScreen™ assay.[80]

1.5.2 Aims

The design of chemical probes for the bromodomain of TRIM33 will enable its physiological function to be explored, help elucidate its role in disease and demonstrate the therapeutic potential of targeting the epigenetic interactions in TRIM proteins.

The aim of this work is to develop benzimidazolone **23** into a high affinity and selective ligand for the TRIM33 bromodomain. Selectivity over closely related TRIM24 will be specifically considered. X-Ray crystal structures of TRIM24 (PDB ID: 4YC9), TRIM33α (PDB ID: 3U5O) and TRIM33β (PDB ID: 5MR8) are available and will be used in a computationally-led approach to compound design. Compounds will be initially evaluated using AlphaScreen™ assays and hits validated in orthogonal waterLOGSY and ITC experiments.

Following the development of a TRIM33 bromodomain ligand, TRIM33 PROTACs will be designed and synthesised employing this ligand as the TRIM33 binding moiety. The understanding of the TRIM33 bromodomain gained from ligand development will also be used to identify a suitable attachment point on the TRIM33 ligand for tethering to the E3 ligase ligand.

Together these two orthogonal approaches can highlight the differences between inhibition of a specific domain and degradation of the whole protein and can thus be employed in future work to reveal the physiological and pathological relevance of TRIM33 and its bromodomain.

In summary this work aims to achieve:

- The synthesis and evaluation of analogues of benzimidazolone **23** to identify key interactions with the TRIM33 bromodomain (Chapter 2).
- The development of a high affinity and selective ligand for the TRIM33 bromodomain through iterative cycles of design, synthesis and testing (Chapter 3).
- The synthesis and evaluation of TRIM33 PROTACs which afford the efficient degradation of TRIM33 (Chapter 4).

CHAPTER 2

Fragments

2.1 Introduction and Aims

Compound **23** (Figure 2.2 (A)) (PPI-net collection)[121] was identified by Dr Angelina Sekirnik[113] as binding to the bromodomain of TRIM33 using an AlphaScreen™ assay. WaterLOGSY NMR experiments performed by Dr Larissa See[114] confirmed binding of this compound to TRIM33. Both wild type and mutant proteins were used to show **23** is predicted to occupy the KAc-binding pocket of the bromodomain. The aim of the work in this chapter was to identify key interactions between **23** and the KAc-binding pocket of TRIM33. This information will be used to guide development of selective, high affinity ligands for the bromodomain of TRIM33 in Chapter 3.

To guide our ligand optimisation we considered ligand efficiency (LE), which is a measure of the binding free energy per atom of a ligand to its binding partner (Equation 1).[122] LE is an important parameter to consider when designing small fragments as fragments have inherently low affinity but LE can be used to identify useful protein-ligand interactions. Starting from a fragment with high LE is desirable as during compound optimisation LE often decreases as the molecular size increases.[123,124]

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

This work also aimed to identify a ligand efficient fragment for the TRIM33 bromodomain which can be developed into a high affinity ligand. Computational docking was carried out to facilitate the design of compounds. Compounds predicted to have low affinity for the TRIM33 bromodomain were also synthesised to validate hypotheses and to provide negative controls in future assays. In addition to increasing the affinity of ligands for the bromodomain of TRIM33, the selectivity of compounds was considered, especially over closely related TRIM24 due to TRIM24 ligands **7** and **8** also possessing a benzimidazolone core as the KAc mimic.

2.2 Preliminary Docking Studies

Preliminary docking was performed using CLC Drug Discovery Workbench 3.0. X-Ray crystal structures for TRIM33α (PDB ID: 3U5O, resolution: 2.7 Å), TRIM33β (PDB ID: 5MR8, resolution: 1.7 Å) and TRIM24 (PDB ID: 4YC9, resolution: 1.8 Å) have previously been solved and were used in these docking studies. In the crystal structure of TRIM33α, the amino acids in the BC loop are unresolved. Due to the importance of this region for understanding ligand binding, and the differences between the two isoforms of TRIM33, Joe Bluck modelled these residues into the structure using MODELLER. Compounds were docked in both TRIM33α and TRIM33β. The binding sites were defined as a 13 Å sphere centred around E981 and the docking was performed within this volume (Figure 2.1 (A)). A 13 Å radius was used to ensure the binding site volume was large enough to accommodate the ligands to be docked to the protein. For each binding site, the conserved water network at the bottom of the KAc-binding pocket was maintained. There are also three water molecules in the ZA channel. Initially the binding energies of these water molecules were not known, and binding sites were therefore set up maintaining different combinations of the ZA channel water molecules.

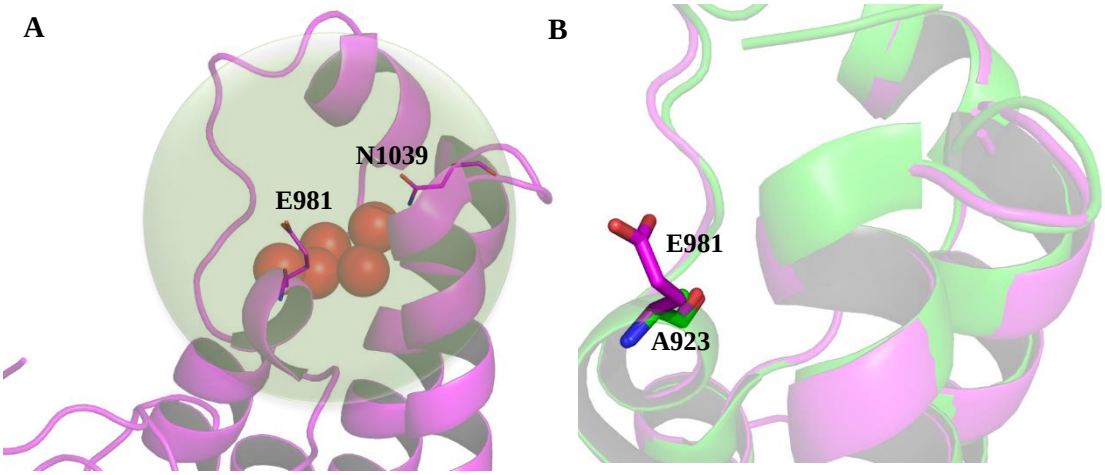


Figure 2.1: Initial docking studies. (A) Binding site set up. The docking site of TRIM33β (magenta) (PDB ID: 5MR8) is highlighted, and the conserved water network is shown. (B) Overlay of TRIM24 (green) (PDB ID: 4YC9) and TRIM33β (magenta) (PDB ID: 5MR8). Residue A923 in TRIM24 and residue E981 in TRIM33 are shown in the stick representation.

An ensemble of low-energy conformations for each ligand were generated (usually one to ten) and imported to a molecule table. The ligands in the molecule table were then docked in the protein. For each ligand in the molecule table, the docking simulation searched for the optimal binding mode to the binding site. 100 iterations were carried out in the search for the optimal binding mode for each ligand. During docking, the conformation of the ligand is changed *via* rotation around flexible bonds, but the protein remains rigid. The optimal binding modes together with a docking score were then returned, 10 docking results were presented for each ligand. The docking score used in the CLC Drug Discovery Workbench is the $PLANTS_{PLP}$ score, this mimics the potential energy change when the protein and ligand come together.[125] In the docking simulation the variables that are optimised are those that define a binding mode. Specifically, the rotation angle for all rotatable bonds in the ligand, the position of the ligand within the binding site and the overall rotation of the ligand with respect to the protein. CLC Drug Discovery Workbench was primarily used to generate ideas and hypotheses and following the simulations the results were manually inspected. Results which had the benzimidazolone core in a similar pose to that observed in the crystal structures of ligands **7** and **8** bound to TRIM24 (PDB IDs: 4YC9 and 4ZQL, respectively) were specifically considered.

Due to high structural conservation amongst bromodomains, achieving selectivity can be challenging. Differences in key residues in bromodomains enable them to interact with KAc residues in a sequence dependent manner.[27] TRIM24 and TRIM33β have 68% sequence identity (BLAST).[102] The lipophilic LAF shelf (residues 922–924) in TRIM24 and the IEF shelf (residues 980–982) in TRIM33 are equivalent to the WPF shelf found in the BET family of bromodomains. In TRIM33, the alanine residue (A923) found in the LAF shelf of TRIM24 is replaced with a glutamic acid residue (E981) (Figure 2.1 (B)). The replacement of hydrophobic A923 in TRIM24 with highly polar E981 in TRIM33 could be exploited to achieve selectivity between TRIM24 and TRIM33.

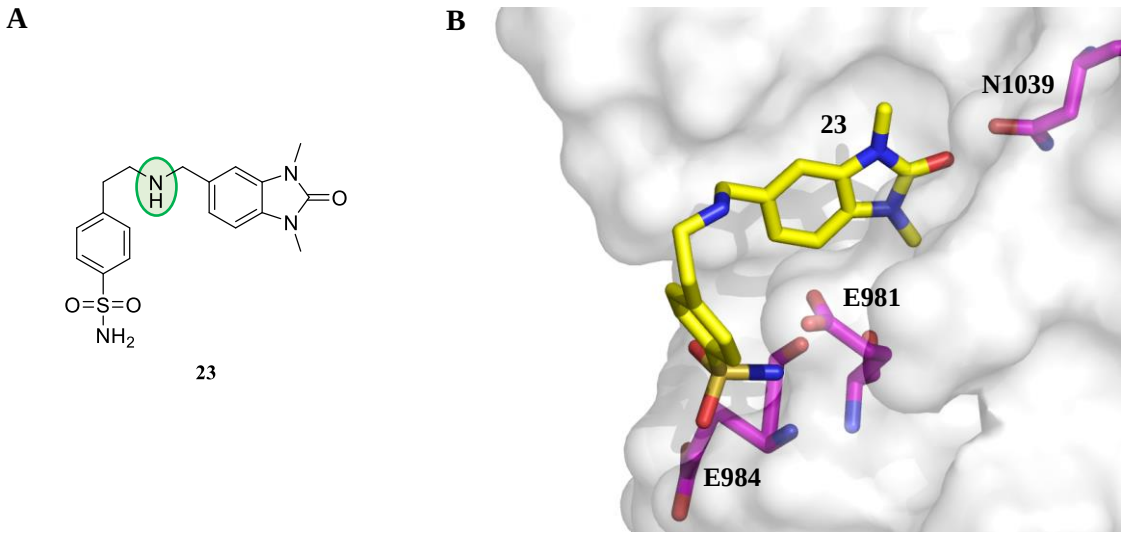


Figure 2.2: Compound 23 was previously identified as binding to the bromodomain of TRIM33. (A) Structure of **23** with the benzylic nitrogen which is predicted to interact with E981 highlighted. (B) Docking of **23** (yellow) in TRIM33β (PDB ID: 5MR8) using CLC Drug Discovery Workbench. The residues believed to be interacting with the ligand are displayed in the stick representation (magenta).

The initial hit (**23**) was docked into TRIM33β using CLC Drug Discovery Workbench 3.0 (Figure 2.2 (B)). As predicted, the docked pose suggests that the benzimidazolone core is acting as the KAc mimic, adopting a similar pose to TRIM24 ligands **7** and **8**.[79,80] The carbonyl oxygen of the benzimidazolone moiety forms a hydrogen bond with conserved asparagine N1039. From the docked pose it is predicted that an interaction is also being made between the benzylic nitrogen of **23** and E981 (Figure 2.2 (B)). As this glutamic acid residue (E981) is an alanine residue (A923) in TRIM24, the analogous interaction cannot be made and this additional interaction with TRIM33 is likely imparting selectivity over TRIM24. Glutamic acid E984 in TRIM33 (Figure 2.2 (B)) is further away from the conserved asparagine residue (N1039) than E981 but could also potentially form interactions with ligands. As this residue is an aspartic acid residue (D926) in TRIM24 it is, however, unlikely to contribute to selectivity.

2.3 Compound Design and Testing

During compound design it is important to filter out pan-assay interference compounds (PAINS).[126] Such compounds often appear as false positives in compound screens. It is not a

specific interaction between the molecule and protein which is providing the observed affect in the assay. PAINS tend to interact with many proteins, often possessing intrinsic activity.

Good solubility is required to allow reliable testing in assays and therefore the solubility forecast index (SFI) was calculated for each compound (Equation 2).[127] The SFI is a measure of the hydrophobicity of a molecule which takes into account the aromaticity of a compound as aromaticity is known to have a negative impact on solubility.[127,128] The SFI of ligands was kept below 6 as this predicts good compound solubility.

$$SFI = clogD_{pH7.4} + \#Ar\ , \tag{2}$$

where $clogD_{pH7.4}$ is the calculated logD at pH 7.4 and #Ar is number of aromatic rings

Compounds were initially screened in AlphaScreen™ assays and hits validated in orthogonal waterLOGSY NMR experiments. It is important to use orthogonal assays to identify false positives which may be interfering with an assay. The AlphaScreen™ TruHits™ kit is also designed to identify false positives in AlphaScreen™ assays and was performed alongside them.

To perform the AlphaScreen™ assays His-tagged proteins were required. The His-tagged protein is captured by the nickel chelate acceptor beads used in the AlphaScreen™ assay. The N-terminal His6 tag is also required for immobilised metal affinity chromatography (IMAC) purification. TRIM24 protein was provided by Dr Angelina Sekirnik.[113] TRIM33α and TRIM33β protein was produced using large scale recombinant protein expression in *Escherichia coli* BL21-Gold (DE3). Protein expression was induced by isopropyl β-D-1-thiogalactopyranoside (IPTG) and the growth media supplemented with zinc chloride to ensure proper folding of the PHD. The protein was purified using IMAC followed by size-exclusion chromatography (SEC) on an ÄKTA fast protein liquid chromatography (ÄKTAFPLC) system. The purity of the protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and protein mass spectrometry (Figure 2.3 and Figure 2.4).

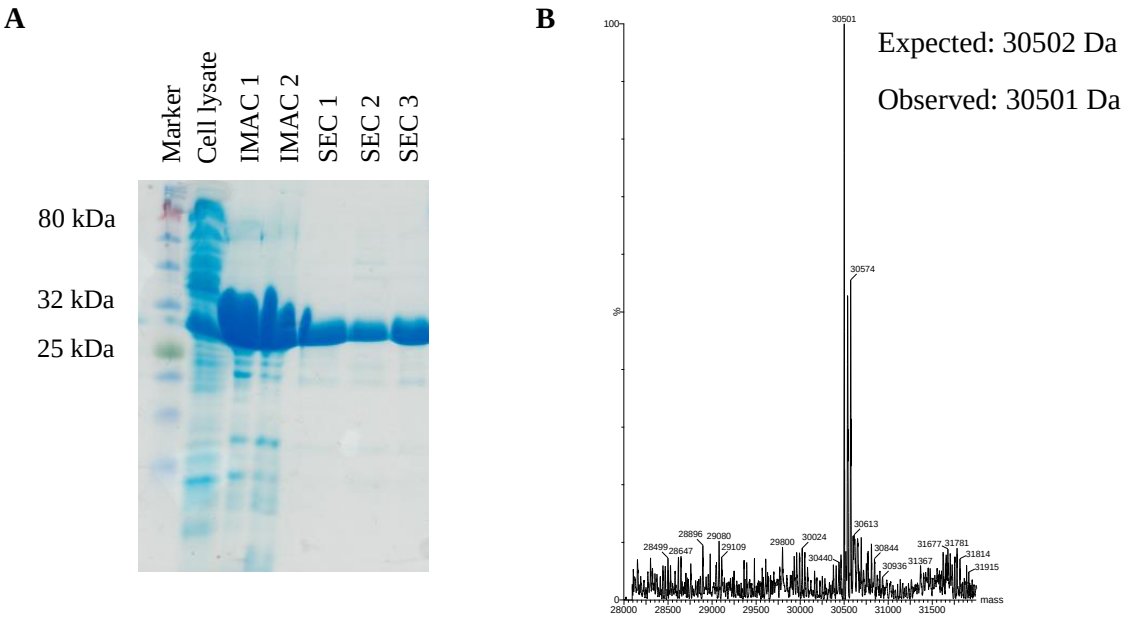


Figure 2.3: Purification of TRIM33α. (A) SDS-PAGE gel for TRIM33α. (B) Protein mass spectrum of TRIM33α.

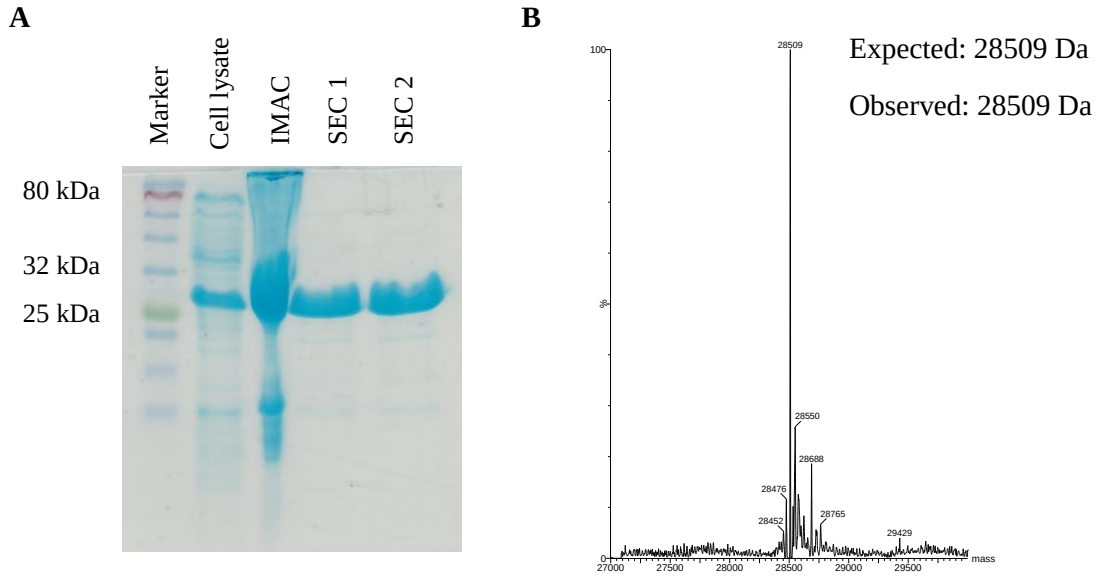


Figure 2.4: Purification of TRIM33β. (A) SDS-PAGE gel for TRIM33β. (B) Protein mass spectrum of TRIM33β.

Assays were previously optimised by Dr Angelina Sekirnik and Dr Larissa See for the evaluation of ligands binding to the bromodomain and/or PHD of TRIM24, TRIM33α and TRIM33β.[113,114] For the AlphaScreen™ assays, different biotinylated histone H3-mimicking peptides in combination with each protein were screened at a range of concentrations. Signal response curves were plotted which enabled the identification of suitable concentrations for use in the assay

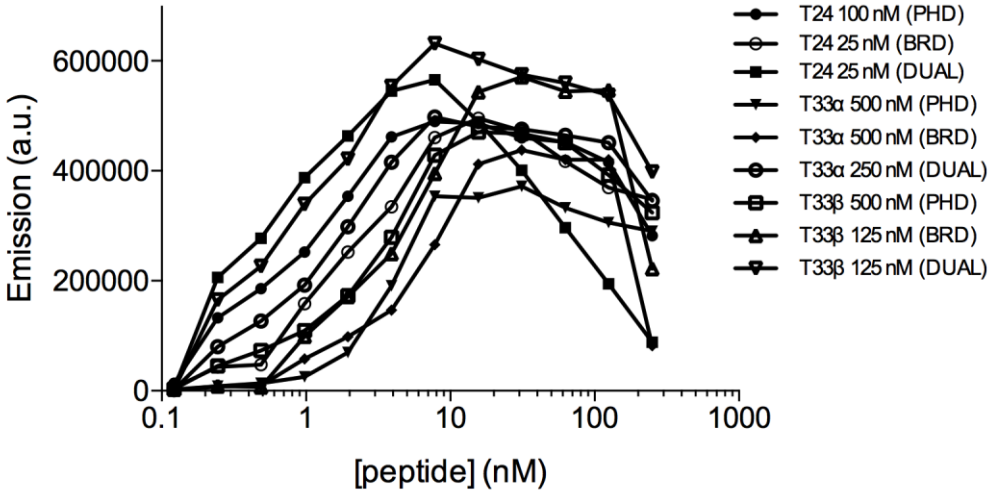


Figure 2.5: AlphaScreen™ signal response curves. Signal response experiments were performed on each new set of beads to verify appropriate protein-peptide concentrations were being used. Emission was plotted as a function of peptide concentration. Peptides (H3(PHD) – $H3_{1-27}K9Me_3$, H3(BRD) – $H3_{1-27}K18Ac$, H3(DUAL) – $H3_{1-27}K9Me_3K18Ac$) were serially diluted 1:2.

(Figure 2.5). These experiments were repeated for each new set of beads to verify appropriate concentrations were being used. Protein and peptide concentrations were chosen to provide a good assay window with high signal-to-noise ratio. Concentrations which produced emission intensities between 300000–500000 a.u. were used. At high emission intensities it becomes difficult to identify weaker affinity ligands, whereas at low emission intensities an increasing number of false positives are identified. The bell-shape of these curves is due to the 'hook effect'.[129] Following an initial concentration-dependent signal increase, a decrease in signal is observed with increasing peptide concentration. The hook point is the concentration where the signal begins to decrease. Below the hook point the donor and acceptor beads are not saturated, at the hook point one component becomes saturated and the signal is at its maximum. Above the hook point, excess peptide begins to inhibit the association of the beads and there is therefore a corresponding decrease in signal.

By using peptides which are methylated ($H3_{1-27}K9Me_3$), acetylated ($H3_{1-27}K18Ac$) or possess both modifications ($H3_{1-27}K9Me_3K18Ac$), the AlphaScreen™ assay provides information on where the compounds are binding based on which protein-peptide interaction they disrupt. Each peptide was tested in combination with TRIM24, TRIM33α and TRIM33β, resulting in a total of nine different

protein-peptide combinations. As AlphaScreen™ is a displacement assay, the IC_{50} values obtained are dependent on the strength of the interaction between the protein and peptide and hence IC_{50} values can only be compared for the same protein-peptide combination. ITC experiments performed by Dr Larissa See quantified the strength of the protein-peptide interactions (Table 2.1).[114] The data show the strongest interaction is between TRIM24 and the doubly modified peptide ($H3_{1–27}K9Me_3K18Ac$), this peptide will therefore be the hardest to displace out of the nine protein-peptide combinations used. The weakest interaction is between TRIM33α and the acetylated peptide ($H3_{1–27}K18Ac$), this peptide will therefore be the easiest to displace, which will be reflected in comparatively lower IC_{50} values.

Table 2.1: K_D values obtained using ITC for each protein-peptide combination (Dr Larissa See).[114]

	$H3_{1–27}K9Me_3$	**$H3_{1–27}K18Ac$**	**$H3_{1–27}K9Me_3K18Ac$**
TRIM24	3.90 µM	695 nM	309 nM
TRIM33α	9.46 µM	54.7 µM	5.58 µM
TRIM33β	9.25 µM	4.92 µM	1.11 µM

2.4 Small Benzimidazolone Fragments

2.4.1 Compound Design

Analogues of **23** were designed to identify key interactions with the bromodomain of TRIM33 (Figure 2.6). To confirm structural assignment and compound purity, initial hit **23** was also synthesised. Amide analogue **24** probes whether replacing the benzylic amine with an amide group is tolerated. The protonation state of the benzylic position is predicted to be important for interacting with E981. The amide group decreases the pK_{aH} of **23** from 8.73 to –1.25 (calculated using ACD I-Lab 2.0),[130] therefore at physiological pH (pH = 7.4) the molecule is no longer predominantly protonated and a weaker interaction with E981 is predicted. Analogues **25**, **26** and **27** (synthesised by Dr Larissa See[114]) were chosen to investigate the distance of the basic group from the benzimidazolone core and also the basicity (predicted pK_{aH}s = 5.86, 9.05 and 9.72, respectively, calculated using ACD I-Lab).[130] In addition, aniline **28** was designed to probe the position of the amine substituent on the benzimidazolone core. Analogues which replace the

benzylic functional group with an alcohol (**29**), acid (**30**) or ester (**31**) were also evaluated. It is predicted **29**, **30** and **31** will display lower affinity for TRIM33 as they are unable to form an ionic interaction with E981, the acid analogue **30** is instead expected to be repelled by E981.

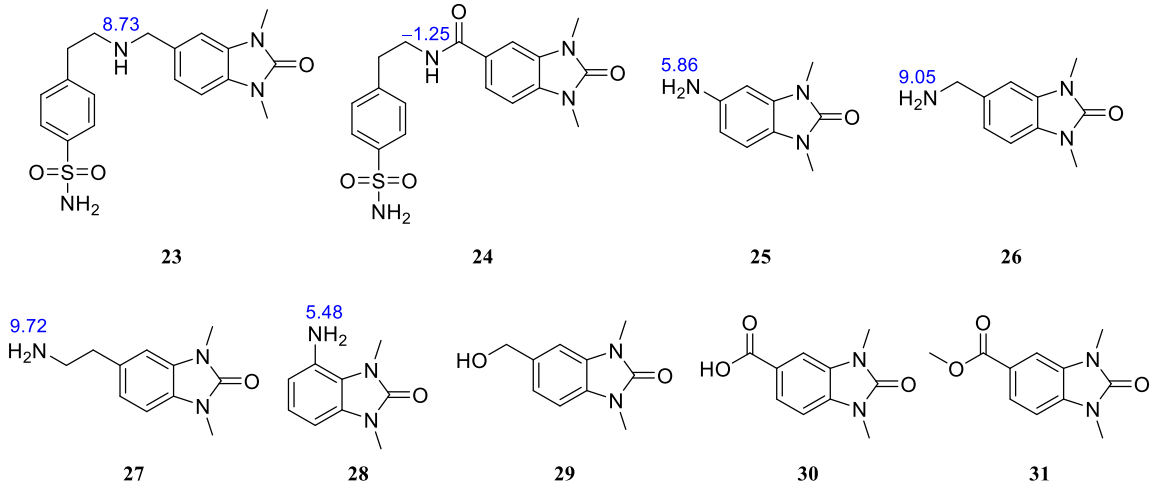
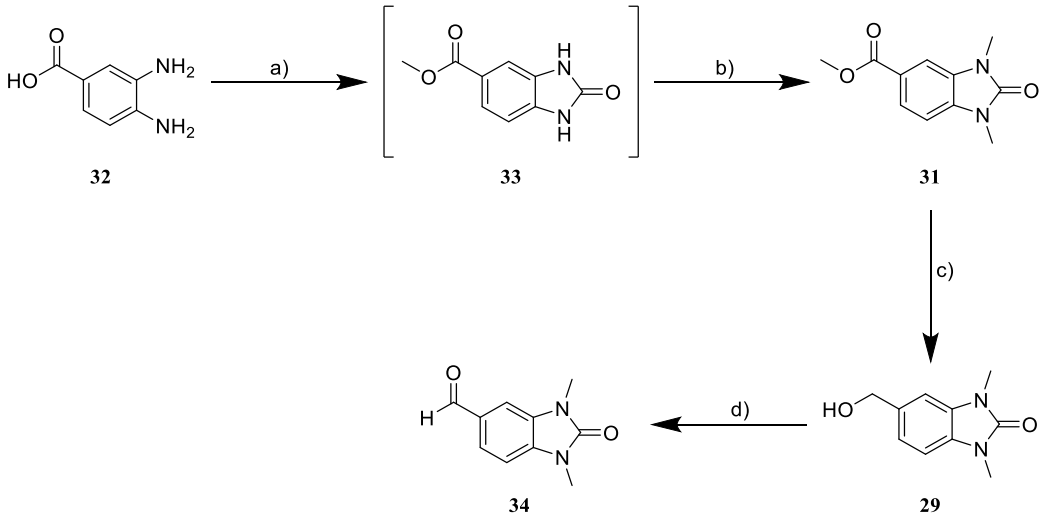


Figure 2.6: Analogues of 23 designed to probe its binding mode and key interactions with TRIM33. The pKa values of the conjugate acids calculated using ACD I-Lab 2.0 are shown in blue.[130]

2.4.2 Synthesis of Compounds

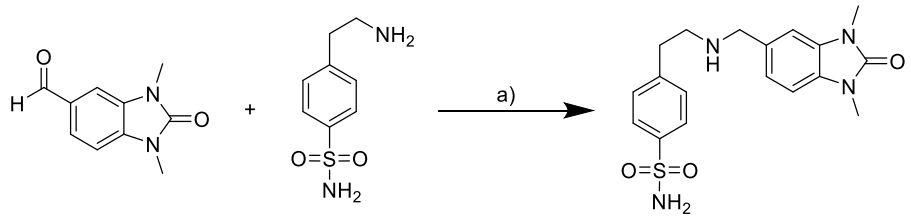
The aldehyde **34** was synthesised as an intermediate for the benzylic analogues (Scheme 2.1). The benzimidazolone ring was formed from 4-(dimethylamino)benzoic acid (**32**) using 1,1'-carbonyldiimidazole (CDI) and the activated acid quenched with methanol to give intermediate ester **33**, which was then methylated to yield ester **31**. The ester **31** was subsequently reduced to alcohol **29** using diisobutylaluminium hydride (DIBAL) or lithium borohydride. Reduction with lithium borohydride gave more consistent yields as problems during work-up (Rochelle salt or Fieser) were often encountered following reduction with DIBAL. The alcohol **29** was initially oxidised to aldehyde **34** using Dess-Martin periodinane (DMP). This reaction was performed on a 20 mg scale (0.09 mmol) with a yield of 63%. Efforts to scale-up the reaction were, however, unsuccessful, with purification proving difficult and therefore alternative oxidation conditions were required. Oxidation using (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and NaOCl only gave 6% conversion to aldehyde **34** (determined using ^{1}H NMR). Benzylic alcohols are readily oxidised using MnO_2[131] and oxidation of **29** using MnO_2 gave 100% conversion to aldehyde **34** (determined using ^{1}H NMR), which was isolated in a 97% yield on a

50 mg scale (0.23 mmol). Oxidation of **29** using MnO_2 was amenable to scale up, and a yield of 82% was achieved on a 940 mg scale (4.90 mmol).



Scheme 2.1: Synthesis of intermediate 34. *Reagents and conditions*: a) (i) CDI, CH_2Cl_2, rt; (ii) MeOH, rt; b) MeI, K_2CO_3, DMF, rt, 47–75% over 2 steps, n = 6; c) $LiBH_4$, THF, MeOH, 60 °C, 79–98%, n = 6; d) MnO_2, CH_2Cl_2, rt, 68–98%, n = 9.

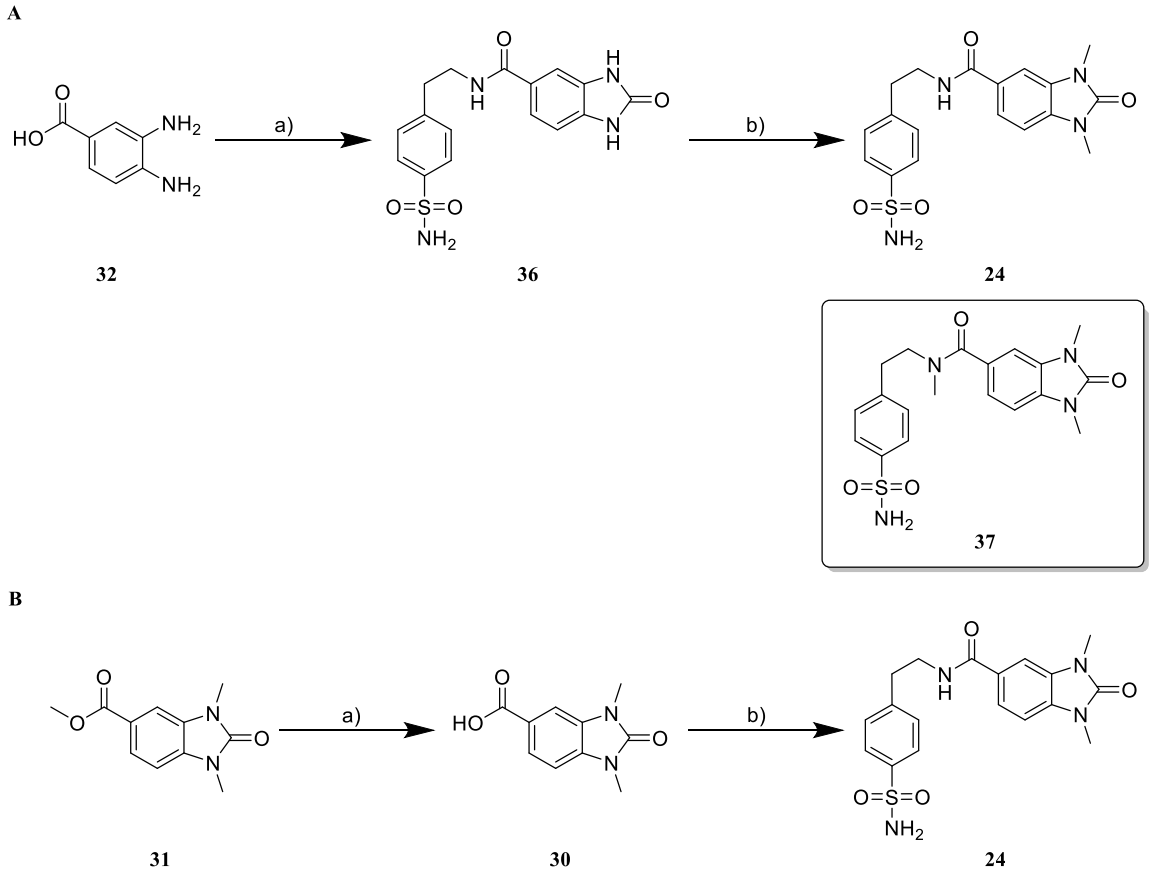
The aldehyde **34** was next reacted with amine **35** *via* a reductive amination to yield **23** (Scheme 2.2). Imine formation was monitored using both thin layer chromatography (TLC) and ^{1}H NMR analysis, in the ^{1}H NMR spectrum disappearance of the aldehyde peak was used to monitor imine formation. The imine was preformed by stirring amine **35** (2.0 eq.) and aldehyde **34** (1.0 eq.) in 1,2-dichloroethane (DCE) with the addition of acetic acid (2.0 eq.). The imine was subsequently reduced using $NaBH(OAc)_3$ (5.0 eq.). Compound **23** and its analogues are very polar, therefore purification sometimes proved challenging. Compound **23** was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) and subsequent analogues were purified using a combination of silica flash column chromatography and ISOLUTE® SCX-2 SPE columns.



34 **35** **23**

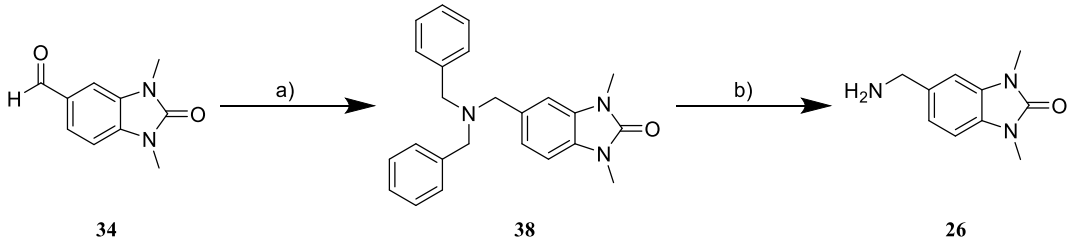
Scheme 2.2: Synthesis of the initial hit (23). *Reagents and conditions*: a) (i) AcOH, DCE, rt; (ii) $NaBH(OAc)_3$, rt, 25%, n = 1.

The amide analogue **24** was initially synthesised *via* the route shown in Scheme 2.3 (A). As predicted, methylation of **36** using sodium hydride and methyl iodide was unselective with the amide nitrogen also being methylated (**37**). The purity of **24** determined using analytical HPLC (254 nm) was only 82% and attempts to further purify **24** using semi-preparative HPLC were unsuccessful. Therefore, an alternative route was proposed which went *via* intermediate **31** (Scheme 2.3 (B)). This route avoids multiple methylation products as the benzimidazolone core is methylated prior to introduction of the amide. Intermediate **31** was hydrolysed to acid **30** using aqueous LiOH, and acid **30** was subsequently reacted in an amide coupling with amine **35**, using CDI as the coupling reagent. This route yielded **24** with 98% purity (analytical HPLC, 254 nm).



Scheme 2.3: Alternative routes to amide analogue 24. *Reagents and conditions*: (A) a) (i) CDI, THF, rt; (ii) **35**, rt, 24–29%, n = 2; b) (i) NaH, DMF, rt; (ii) MeI, rt, 11%, 82% purity, n = 1. (B) a) LiOH, H_2O, dioxane, 65 °C, 77%, n = 1; b) (i) CDI, CH_2Cl_2, rt; (ii) **35**, rt, 89%, n = 1.

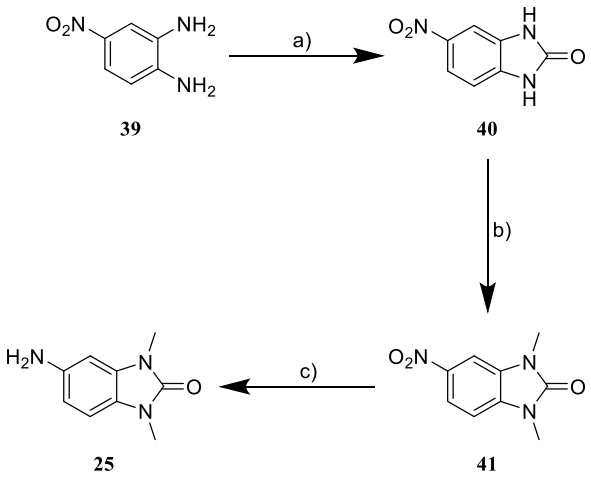
Compound **26** was synthesised *via* benzyl protected **38** which was obtained using a reductive amination between aldehyde **34** and dibenzylamine (Scheme 2.4). The benzyl groups were removed using H_2 and Pd/C in EtOH to yield **26** in 69% yield.



Scheme 2.4: Synthesis of primary benzylic amine 26. *Reagents and conditions*: a) (i) Dibenzylamine, AcOH, DCE, 3 Å molecular sieves, 0 °C; (ii) $NaBH(OAc)_3$, 0 °C → rt, 24–40%, n = 2; b) Pd/C (10% *w/w*), H_2, EtOH, rt, 69%, n = 1.

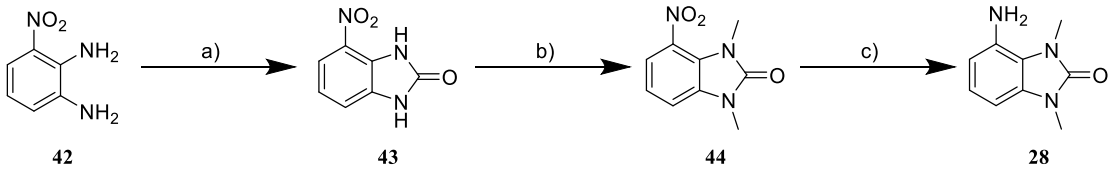
Compound **25** was synthesised from 4-nitrobenzene-1,2-diamine (**39**) (Scheme 2.5). The ring was formed using CDI and **40** methylated using methyl iodide to yield **41**. Reduction of the nitro group

was first attempted using H_2 and Pd/C in tetrahydrofuran (THF). TLC analysis, however, showed that this resulted in several products. Reduction using Zn and NH_4Cl also resulted in a number of products, and isolation using silica flash column chromatography was unsuccessful. Compound **25** was obtained in 54% yield using H_2 and Pd/C in EtOH with the addition of concentrated HCl (0.2 eq.). The HCl promotes hydrogenolysis if any of the compounds are poisoning the catalyst by coordinating to palladium, and under these conditions no side products were observed. Compound **27**, which also investigates the distance of the amine from the benzimidazolone core, was synthesised by Dr Larissa See.[114]



Scheme 2.5: Synthesis of amine 25. *Reagents and conditions*: a) CDI, CH_2Cl_2, rt, 54%, n = 1; b) MeI, K_2CO_3, DMF, rt, 75%, n = 1; c) Pd/C (10% *w/w*), H_2, concentrated HCl, EtOH, rt, 54%, n = 1.

Compound **28** was synthesised from 2,3-diamino-nitrobenzene (**42**) (Scheme 2.6). The ring was formed using CDI, and **43** methylated using methyl iodide to yield **44**. The nitro group was reduced using H_2 and Pd/C in EtOH with the addition of concentrated HCl (0.2 eq.) to yield amine analogue **28**.



Scheme 2.6: Synthesis of 28. *Reagents and conditions*: a) CDI, CH_2Cl_2, rt, 91%, n = 1; b) MeI, K_2CO_3, DMF, rt, 94%, n = 1; c) Pd/C (10% *w/w*), H_2, concentrated HCl, EtOH, rt, 10%, n = 1.

2.4.3 Evaluation of Compounds

2.4.3.1 AlphaScreen™ Assays

Synthesised compounds were initially evaluated in triplicate at two concentrations (30 μM and 150 μM) using an AlphaScreen™ assay. If the compound was shown to bind to TRIM33, it was tested in a dose-response AlphaScreen™ assay, and IC_{50} values were determined. The purity criteria for testing was >95% (determined using analytical HPLC (220 nm and 254 nm)). Each compound was tested with nine different protein-peptide combinations, the combinations used were TRIM24, TRIM33α and TRIM33β in combination with the previously described histone peptides.

When the protein-peptide interaction is competed by a ligand, the donor and acceptor beads in the AlphaScreen™ assay are no longer in close proximity. As there are no acceptor beads nearby, the singlet oxygen molecules produced by the donor beads relax back to the ground state as they have a short lifetime in aqueous solution (~4 μs). Binding in AlphaScreen™ assays is therefore indicated by a reduction in emission. In the single concentration (30 μM and 150 μM) AlphaScreen™ assay the percentage emission was calculated relative to the average dimethyl sulfoxide (DMSO) control. The results are summarised in Table 2.2.

Amide analogue **24** provided a small reduction in emission for TRIM33α, and no reduction in emission for TRIM33β. Amide analogue **24** (pK_{aH} = –1.25)[130] is less basic than amine analogue **23** (pK_{aH} = 8.73).[130] It is predicted that the lower affinity of **24** is due to a weaker interaction with E981 (pK_a = 4.25) as **24** is no longer predominately protonated under the assay conditions (pH = 7.6). Non-methylated analogue **36** was synthesised as an intermediate to **24**, and was also tested. Non-methylated **36** showed no reduction in emission for TRIM33α or TRIM33β suggesting that methylation of the benzimidazolone core is important for binding. This result is in agreement with published bromodomain benzimidazolone inhibitors which have demonstrated the dimethylated scaffold to be more potent than both the monomethylated and non-methylated scaffolds.[132]

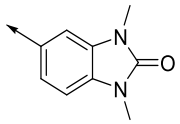
Another key comparison is between alcohol **29**, acid **30** and ester **31**. Acid **30** did not cause any reduction in emission for any of the protein-peptide combinations, this result agrees with the hypothesis that the acid is being repelled by E981, abrogating binding. Alcohol **29** gave reduction in emission for TRIM33α in combination with the acetylated peptide and the peptide possessing both modifications, when tested at 150 μM. Ester **31** caused a reduction in emission for TRIM24 in combination with the acetylated peptide and for TRIM33α in combination with both the acetylated peptide and the peptide possessing both modifications, when tested at 150 μM. This indicates the benzylic amine is important for imparting selectivity over TRIM24.

Despite aniline **26** being a small fragment, **26** gave reduction in emission for both TRIM33α and TRIM33β. The largest reduction in emission was observed for TRIM33α in combination with the acetylated peptide (>90% reduction in emission) and the dually modified peptide (>70% reduction in emission). A larger reduction in emission was observed compared to other benzimidazolone analogues, such as alcohol **29**, suggesting that in addition to the hydrogen bonding interaction between the carbonyl of the benzimidazolone core and N1039, additional interactions are being made with the KAc-binding pocket, likely with E981.

The AlphaScreen™ assay also showed that aniline analogue **25** has lower affinity for TRIM33 compared to benzylic analogue **26**. For **25** a reduction in emission was only observed for TRIM33α in combination with the acetylated or dually modified peptide, at a compound concentration of 150 μM. This demonstrates that an appropriate distance of the nitrogen from the benzimidazolone core is important for affinity to the bromodomain of TRIM33.

Compound **28** is a regioisomer of aniline **25**. A reduction in emission for aniline **28** is only observed for TRIM33α in combination with the acetylated peptide. The reduction in emission is smaller than the corresponding reduction in emission for **25**, suggesting that the amine vector of **25** is preferred.

Table 2.2: Single concentration AlphaScreen™ results. Percentage emission calculated relative to the average DMSO control. Compounds screened at 30 µM and 150 µM. Colour scale from low emission (red) to high emission (green). Quoted values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1-27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$.



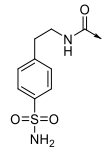
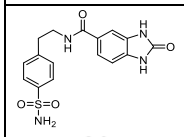
Compound	TRIM24 % Emission						TRIM33α % Emission						TRIM33β % Emission					
	H3(PHD)		H3(BRD)		H3(DUAL)		H3(PHD)		H3(BRD)		H3(DUAL)		H3(PHD)		H3(BRD)		H3(DUAL)	
	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM
 **24**	117	105	97	97	107	98	240	248	71	49	97	79	104	104	143	116	97	88
25	95	83	113	95	107	87	97	77	73	23	90	44	100	87	129	94	87	72
26	91	91	103	87	102	101	151	82	8	4	26	4	48	25	53	5	82	44

Table 2.2 continued.

Compound	TRIM24 % Emission						TRIM33α % Emission						TRIM33β % Emission					
	H3(PHD)		H3(BRD)		H3(DUAL)		H3(PHD)		H3(BRD)		H3(DUAL)		H3(PHD)		H3(BRD)		H3(DUAL)	
	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM
28	94	91	109	110	101	99	93	91	85	59	140	121	129	131	146	144	101	98
29	111	108	124	118	117	111	124	104	87	37	102	66	106	103	143	120	99	89
30	112	108	120	107	115	110	158	176	121	93	122	106	108	105	152	134	102	96
31	105	96	85	71	104	93	132	101	86	41	103	69	101	100	145	117	96	89
 **36**	110	98	91	84	113	103	148	133	123	105	121	113	107	96	156	145	100	96

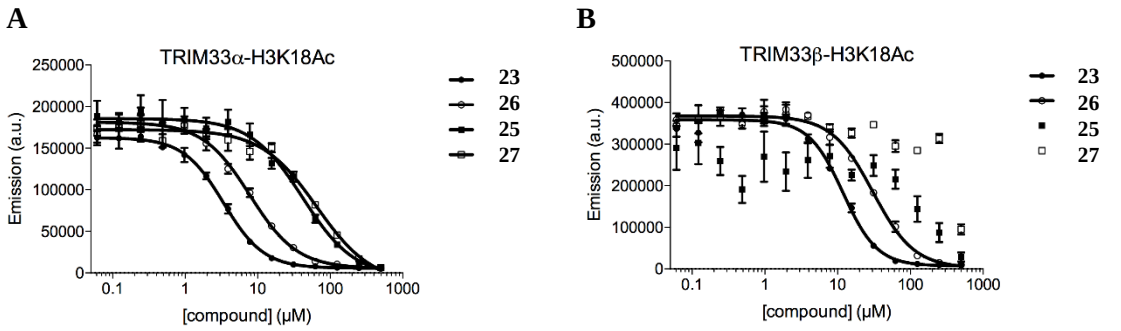
Compounds **25** and **26** provided a large reduction in emission in the single concentration AlphaScreen™ assay (Table 2.2). These compounds, together with **23** and **27**, were therefore tested in a dose-response AlphaScreen™ assay against all nine protein-peptide combinations to determine IC_{50} values (Figure 2.7, Table 2.3). As the compounds bind to the bromodomain, we are most interested in peptide $H3_{1–27}K18Ac$, the discussion of results will therefore be focused on this peptide and the data for the remaining peptides ($H3_{1–27}K9Me_3$ and $H3_{1–27}K9Me_3K18Ac$) can be found in Appendix B. GraphPad Prism 7 was used to fit data points to a sigmoidal four-parameter equation (Equation 3). Where an IC_{50} value was obtained, the LE was also calculated (Equation 1). Compounds with IC_{50} values <2× different were considered to have the same binding affinity.

$$Y = \text{Minimum} + \frac{(\text{Maximum}–\text{Minimum})}{1 + 10^{(\log IC_{50} – X)(\text{Hill Slope})}}, \quad (3)$$

where X is log[compound]

These results (Table 2.3) showed that primary benzylic amine analogue **26** (IC_{50} = 30.6 μM) has similar affinity to TRIM33β as **23** (IC_{50} = 11.9 μM). However, due to its smaller size, **26** has greater LE (0.45 *vs.* 0.27) which suggests that **26** makes good quality interactions with the protein. Unlike **23**, amine **26** was shown to be selective over TRIM24.

In contrast to **26**, aniline analogue **25** (IC_{50} = 168 μM) and the two carbon analogue **27** (>250 μM) were observed to have low affinity for TRIM33β. These results show that the distance of the amine substituent from the benzimidazolone core is critical for binding. Based on these results it was decided that **26** was a good starting point for the development of selective and high affinity ligands for TRIM33.



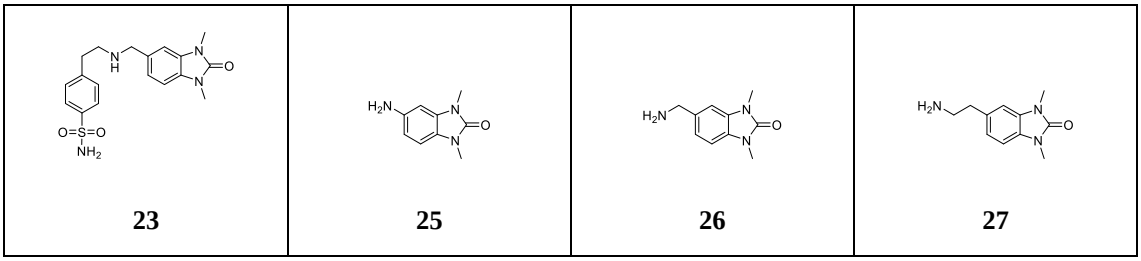
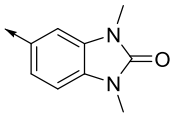
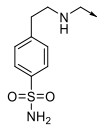


Figure 2.7: Dose-response curves obtained using an AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. (A) TRIM33α in combination with $H3_{1–27}$K18Ac. (B) TRIM33β in combination with $H3_{1–27}$K18Ac.

Table 2.3: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(BRD) – $H3_{1-27}$K18Ac. Ligand efficiencies are given in parentheses.



High affinity — Low affinity

Compound	Substituent	TRIM24 – H3(BRD) IC_{50} (μM)	TRIM33α – H3(BRD) IC_{50} (μM)	TRIM33β – H3(BRD) IC_{50} (μM)
23		40.3 ± 1.88 (0.24)	3.88 ± 1.03 (0.29)	11.9 ± 1.09 (0.27)
25		22.5 ± 1.45 (0.50)	41.8 ± 1.13 (0.47)	168 ± 1.96 (0.41)
26		>250	7.83 ± 1.10 (0.51)	30.6 ± 2.60 (0.45)
27		>250	65.1 ± 12.4 (0.39)	>250

2.4.3.2 WaterLOGSY Experiments

For compounds which were observed to bind to TRIM33 in AlphaScreen™ assays, binding was validated in orthogonal waterLOGSY NMR experiments. WaterLOGSY allows the detection of weakly binding ligands and typically works best for ligands with dissociation constants in the high µM to mM region.[133] Strongly bound ligands cannot be detected as the experiment relies on the fast dissociation of bound compounds to transfer the bulk water magnetisation into the free state *via* the protein-ligand complex. WaterLOGSY is therefore an ideal method for validating binding of weaker fragments, such as the benzimidazolone fragments. All waterLOGSY spectra can be found in Appendix D.

Binding of **23**, **25** and **26** to TRIM33 was validated in waterLOGSY NMR experiments. Initial hit **23** and aniline **25** showed binding to all three proteins, whilst benzylic analogue **26** only bound to TRIM33α and TRIM33β (Figure 2.8 and Table 2.4). This corroborates the results from the dose-response AlphaScreen™ assay.

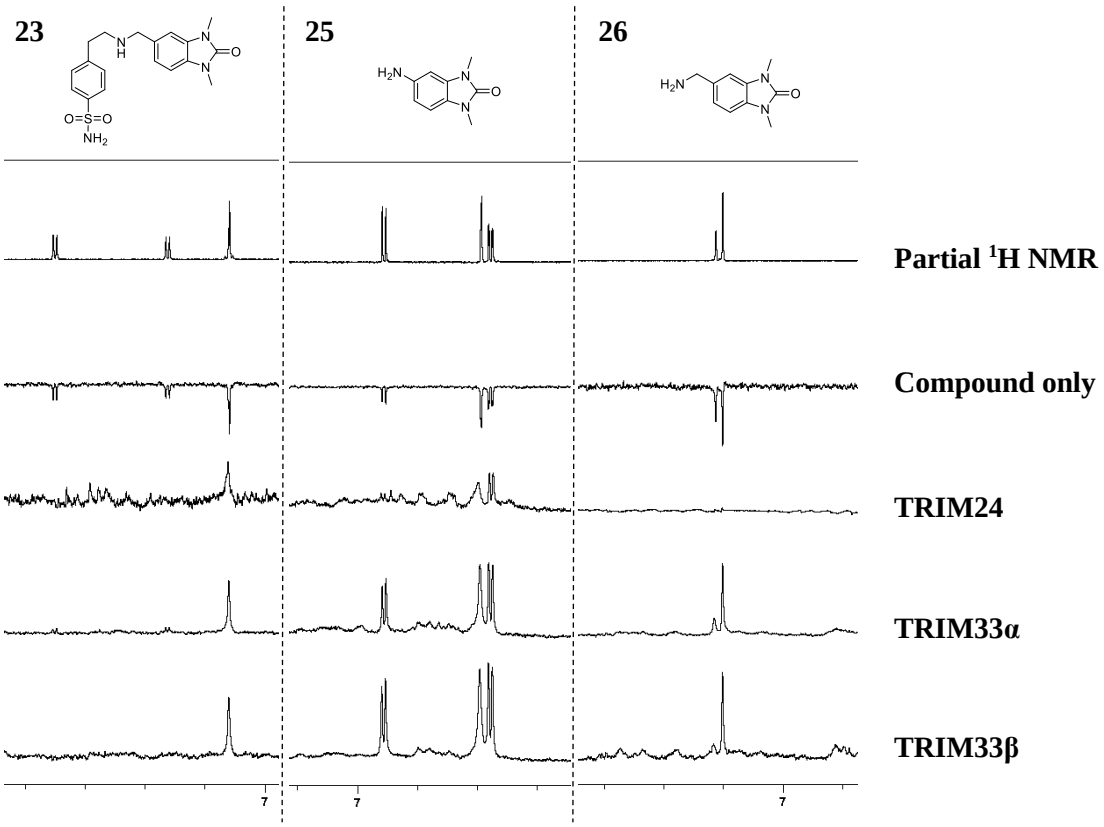


Figure 2.8: Partial waterLOGSY spectra for 23, 25 and 26. Stacked spectra (top to bottom): ^{1}H NMR, compound only waterLOGSY, TRIM24 waterLOGSY, TRIM33α waterLOGSY, TRIM33β waterLOGSY.

Table 2.4: Summary of waterLOGSY results for 23, 25 and 26. Compounds which were observed to bind are represented by ✓. Compounds which did not appear to bind are represented by ✖.

	TRIM24	TRIM33α	TRIM33β
23	✓	✓	✓
25	✓	✓	✓
26	✖	✓	✓

Higher affinity ligands are required for use in cellular studies and this affinity is likely to come from hydrophobic interactions with the protein. To understand where hydrophobic interactions can be made with the protein, the orientation of the benzimidazolone analogues in the bromodomain of TRIM33 was further explored and the key interactions identified using molecular dynamics (MD).

2.5 Computational Studies

MD studies (conducted by Joe Bluck) were used to explore the orientation of **23** in the bromodomain of TRIM33β (PDB ID: 5MR8). Ligands were docked by Joe Bluck using AutoDock 4 and simulated using GROMACS 2016.3. The binding site waters were retained during the simulations and the benzylic amine protonated. The benzimidazolone core was predicted to orientate in the binding pocket as expected, with the carbonyl in close proximity to the conserved asparagine residue (N1039) (Figure 2.9).

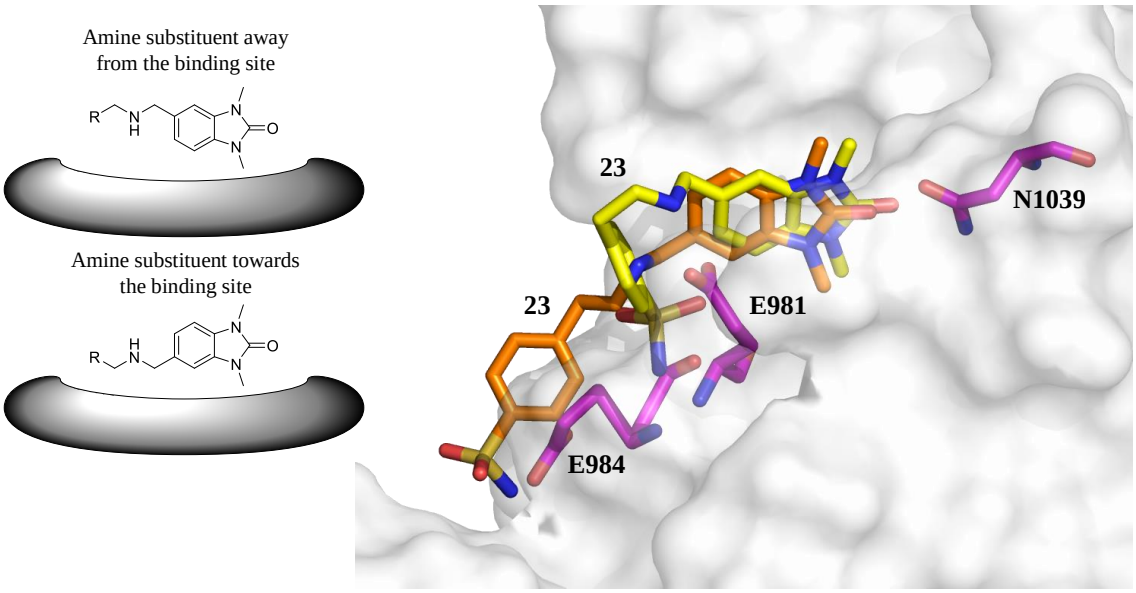


Figure 2.9: MD studies of 23 could not distinguish between the benzylic amine pointing away or towards the binding site (Joe Bluck). Benzylic amine pointing away from the binding site (yellow). Benzylic amine pointing towards the binding site (orange). TRIM33β (magenta) (PDB ID: 5MR8).

Barcode plots were generated to look at the proximity of the benzylic nitrogen to E981 during the simulations (Figure 2.10). Three MD simulations were performed with the benzylic amine pointing away from the KAc-binding site, and three with the benzylic amine pointing towards the KAc-binding site. The plots confirmed the hypothesis that the benzylic nitrogen is interacting with E981 as they remained in close proximity for the duration of the simulations. From these simulations it was, however, unclear whether the amine substituent points towards or away from the binding site and thus needs to be tested by structure-activity relationships (SAR).

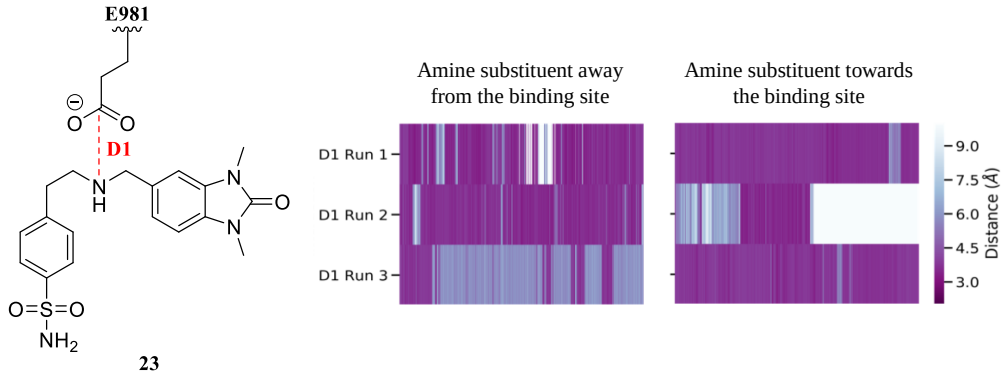


Figure 2.10: Barcode plots displaying the distance between E981 and the benzylic nitrogen of 23 (D1). The benzylic nitrogen remains in close proximity to E981 throughout the simulations suggesting a strong interaction. Simulations were performed using GROMACS 2016.3 using the AMBER99SB-ILDN forcefield. The ligands were parameterised using the General AMBER ForceField with AM1-BCC charges (Joe Bluck).

2.6 Hydrophobic Substituents

To identify areas for hydrophobic interactions, we looked at the binding of histone peptides. Overlay of an acetylated histone H3 peptide ($H3_{1–22}K9Me_3K14AcK18Ac$) (PDB ID: 3U5O) with **23** docked in the bromodomain of TRIM33β (PDB ID: 5MR8) suggested an unexploited hydrophobic region above the KAc-binding pocket (Figure 2.11). Residues I990, F1038 and I996 form this hydrophobic wall. It was hypothesised that hydrophobic interactions with this region would increase the affinity of ligands. Growing off the benzimidazolone core to expand into this space means the core is no longer symmetrical and hence the different vector combinations were explored.

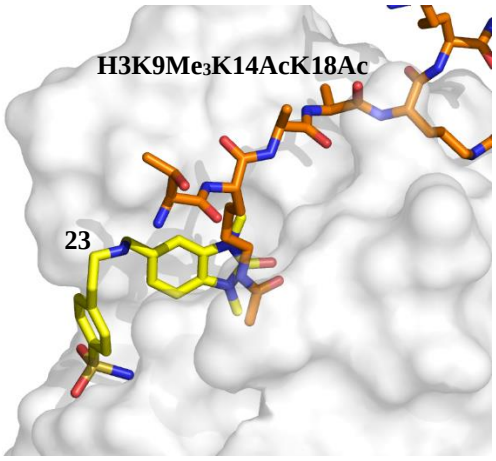


Figure 2.11: Overlay of a histone H3 peptide with 23 docked in TRIM33β. Compound **23** (yellow) docked in TRIM33β (PDB ID: 5MR8) using CLC Drug Discovery Workbench overlaid with $H3_{1–22}K9Me_3K14AcK18Ac$ (orange) (PDB ID: 3U5O).

Computational studies by Joe Bluck are also consistent with this hypothesis. These studies identified regions for potential hydrophobic interactions and are represented by the mesh displayed in Figure 2.12. This hydrophobic map overlaps with the region occupied by the histone peptide.

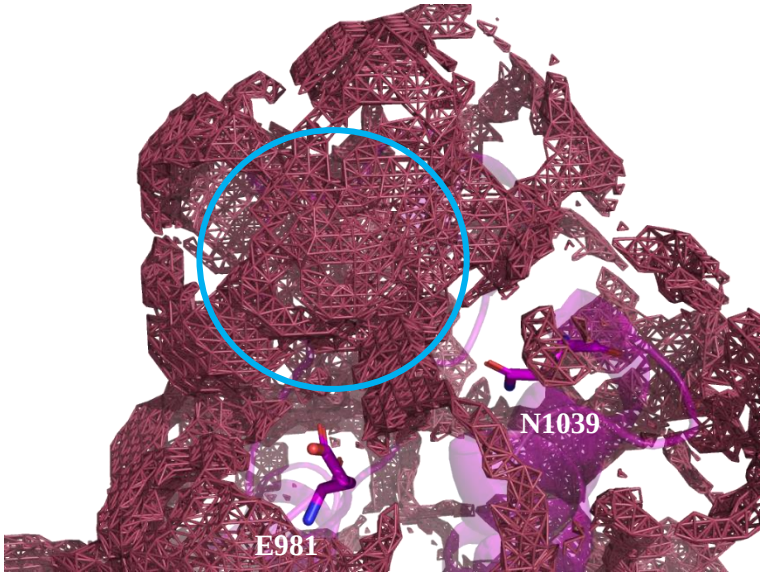


Figure 2.12: Mesh representing hydrophobic regions (Joe Bluck). Crystal structure of TRIM33β (magenta) (PDB ID: 5MR8) overlaid with a mesh representing regions for hydrophobic interactions. Area of interest is highlighted.

2.6.1 Compound Design

Docking of **23** in TRIM33β (PDB ID: 5MR8) suggests that a substituent on a headgroup nitrogen would project into the region above the binding pocket where the histone H3 peptide resides (Figure 2.11). Introducing a substituent on a headgroup nitrogen means the benzimidazolone core is no longer symmetrical. The four different vector combinations were therefore synthesised to experimentally determine which was optimal (**46**–**49**, Figure 2.13). The nomenclature shown in **45** is used. These compounds have a phenethyl substituent as it was predicted to be long enough to reach the hydrophobic peptide binding groove and make the desired hydrophobic interactions.

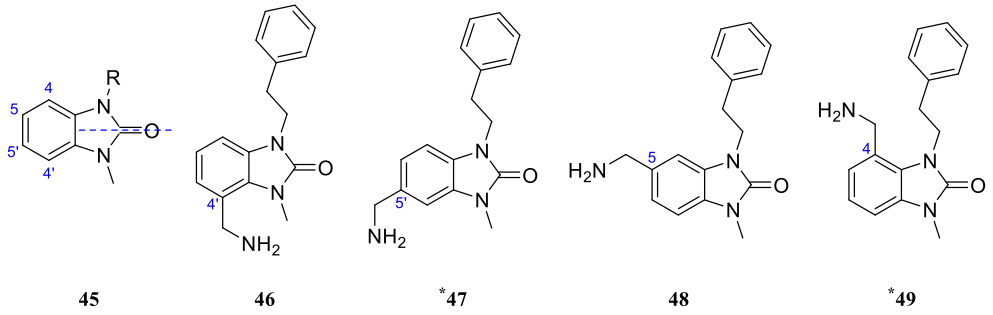
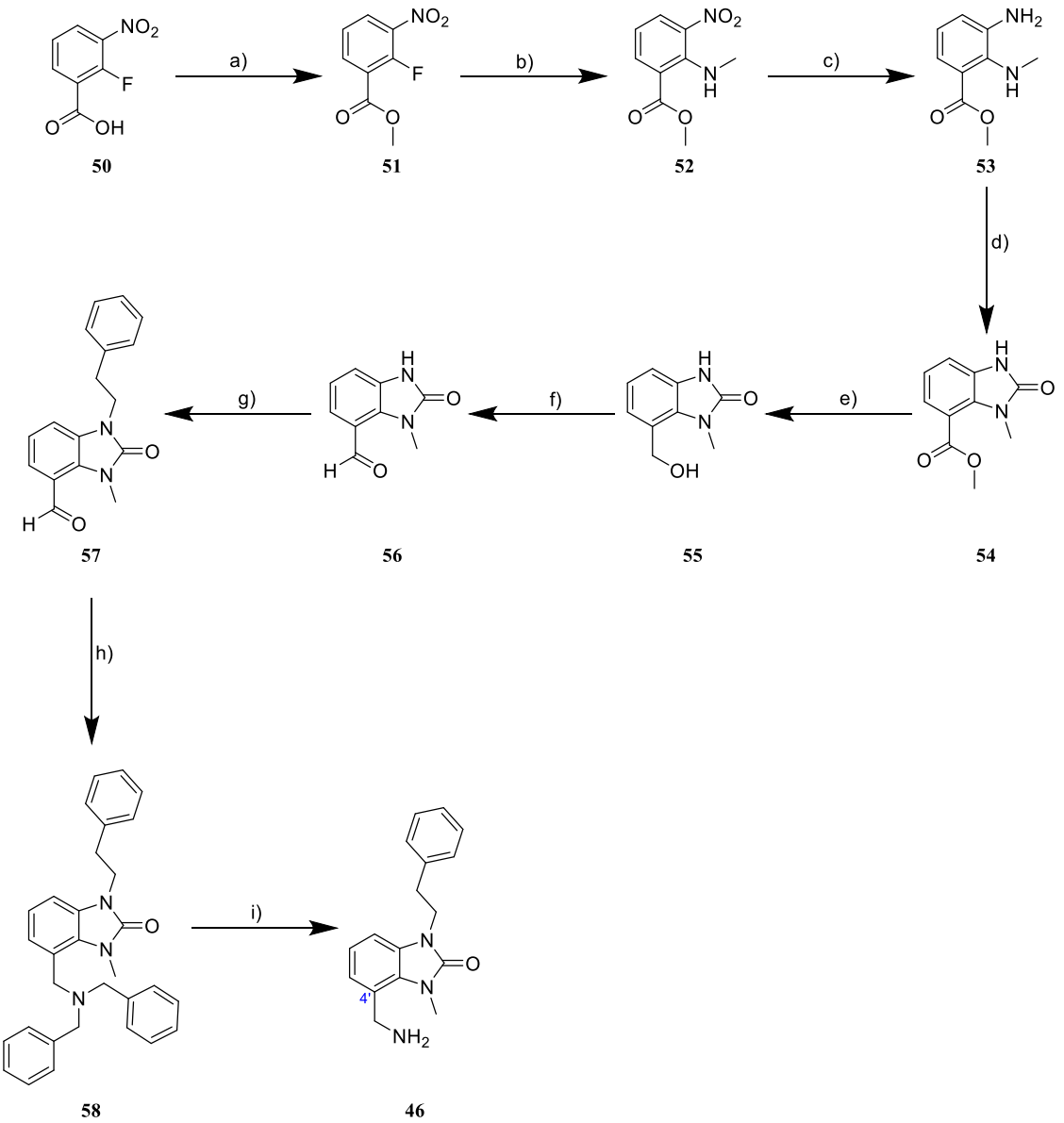


Figure 2.13: The line of symmetry is lost when introducing substituents on the benzimidazolone core. The different vector combinations associated with introducing a substituent on a headgroup nitrogen are shown. The nomenclature shown in **45** is used. *Analogues synthesised by Dr Larissa See.[114]

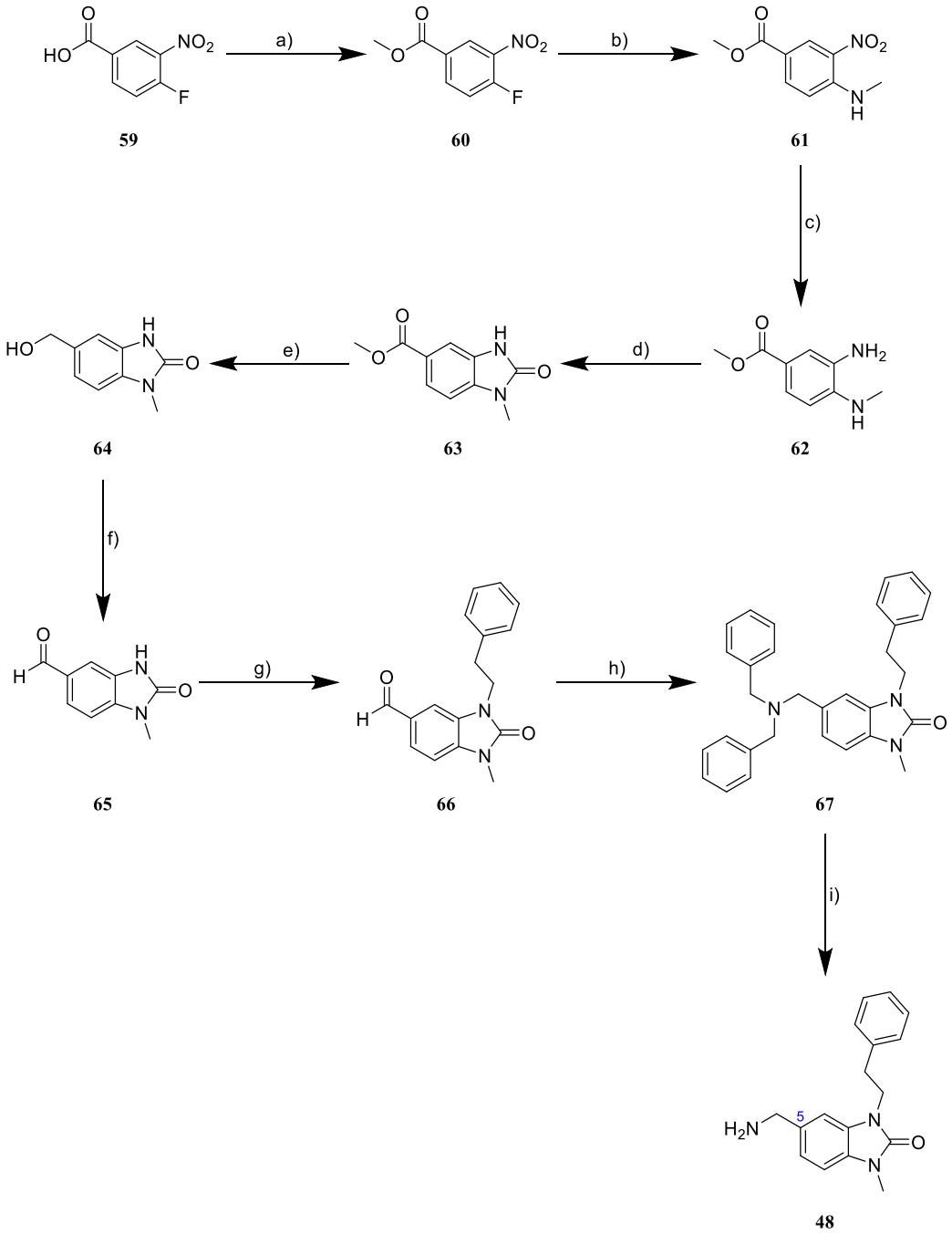
2.6.2 Synthesis of Compounds

The different vector combinations were synthesised as the primary benzylic amine analogues (Figure 2.13). Analogue **46**, with the benzylic substituent on C-4', was synthesised from 2-fluoro-3-nitrobenzoic acid (**50**), which was methylated to give methyl ester **51** (Scheme 2.7). Methylamine was subsequently introduced in an S_NAr reaction to yield **52** and hydrogenolysis used to reduce the nitro group to amine **53**. The benzimidazolone ring was then formed using CDI to give **54**. The ester **54** was next reduced to alcohol **55** using DIBAL and oxidised to aldehyde **56** using MnO_2. Aldehyde **56** was subsequently alkylated using 2-phenylethyl bromide to give **57** and protected **58** obtained *via* reductive amination with dibenzylamine. Finally, hydrogenolysis removed the benzyl groups and yielded **46** as the final compound.

Compound **48**, with the benzylic substituent on C-5, was synthesised from 4-fluoro-3-nitrobenzoic acid (**59**) *via* the analogous route (Scheme 2.8). The remaining two vectors (**47** and **49**) were synthesised by Dr Larissa See.[114]



Scheme 2.7: Synthesis of 46. *Reagents and conditions*: a) MeI, K_2CO_3, DMF, rt, 82%, n = 1; b) $NH_2Me{\cdot}HCl$, DIPEA, DMF, rt, 98%, n = 1; c) Pd/C (10% *w/w*), H_2, EtOAc, EtOH, rt, 99%, n = 1; d) CDI, CH_2Cl_2, rt, 48%, n = 1; e) DIBAL (1 M, hexanes), toluene, 0 °C → rt, 56%, n = 1; f) MnO_2, CH_2Cl_2, rt, 38%, n = 1; g) $BrCH_2CH_2C_6H_5$, K_2CO_3, DMF, 70 °C, 35%, n = 1; h) (i) Dibenzylamine, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, 64%, n = 1; i) Pd black, H_2, concentrated HCl, EtOH, rt, 87%, n = 1.



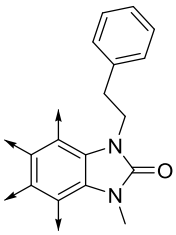
Scheme 2.8: Synthesis of 48. *Reagents and conditions*: a) MeI, K_2CO_3, DMF, rt, 67–97%, n = 2; b) $NH_2Me{\cdot}HCl$, DIPEA, DMF, rt, 95–99%, n = 2; c) Pd/C (10% *w/w*), H_2, EtOAc, EtOH, rt, 82–90%, n = 2; d) CDI, CH_2Cl_2, rt, 43–49%, n = 2; e) DIBAL (1 M, hexanes), toluene, 0 °C → rt, 39–56%, n = 2; f) MnO_2, CH_2Cl_2, rt, 35–48%, n = 2; g) $BrCH_2CH_2C_6H_5$, K_2CO_3, DMF, 70 °C, 70–72%, n = 2; h) (i) Dibenzylamine, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, 58%, n = 1; i) Pd black, H_2, concentrated HCl, EtOH, rt, 20%, n = 1.

2.6.3 Evaluation of Compounds

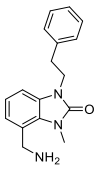
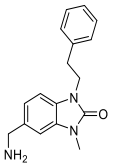
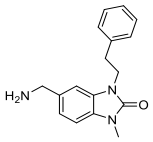
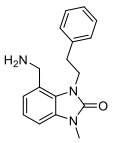
2.6.3.1 AlphaScreen™ Assays

The four different vector combinations (**46**, **47**, **48** and **49**) were evaluated using a dose-response AlphaScreen™ assay (Table 2.5). Low affinity was, however, observed across all nine protein-peptide combinations, for all four vectors. This low affinity observed is likely due to the compounds being small primary benzylic fragments which lack interactions with the KAc-binding pocket, compared to longer amine chain analogues. This is similar to the lower affinity observed for dimethyl analogue **26**. Compounds **46** and **49** were shown not to bind to TRIM33. Compounds **47** (IC_{50} = 12.1 µM) and **48** (IC_{50} = 23.3 µM) bound only to TRIM33α. Computational studies (Joe Bluck) showed that both **47** and **48** can be accommodated in the KAc-binding pocket, depending on whether the ligands bind with the benzylic substituent pointing towards or away from the binding pocket (Figure 2.9). The composition and steric bulk of the benzimidazolone nitrogen substituent and amine chain are likely to affect the optimum vector combination. Compounds **47** and **48** will be explored further in Chapter 3.

Table 2.5: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(BRD) – $H3_{1-27}$K18Ac. Ligand efficiencies are given in parentheses.



High affinity — Low affinity

Compound	Vector	TRIM24 – H3(BRD) IC_{50} (µM)	TRIM33α – H3(BRD) IC_{50} (µM)	TRIM33β – H3(BRD) IC_{50} (µM)
46		>250	>250	>250
47		>250	12.1 ± 1.12 (0.33)	>250
48		>250	23.3 ± 2.34 (0.31)	>250
49		>250	>250	>250

2.7 Alternative KAc Mimic

Replacing the benzimidazolone core with an alternative fused ring system as the KAc mimic was also explored. Changing the headgroup is likely to result in large changes to the physicochemical properties, as well as the binding affinity of compounds. Backup compounds are often considered in medicinal chemistry programmes to minimise risk as lead compounds may fail for a number of reasons during the later stages of drug development.[134,135] As backup compounds are

structurally distinct, they may be devoid, for example, of toxicity observed in lead compounds. In the case of chemical probes, having different series of compounds can be beneficial as it provides probes with distinct off-target pharmacology and different properties, such as permeability and solubility.[136]

2.7.1 Compound Design

Structures containing flat fused rings were docked in TRIM33β as potential replacements for the benzimidazolone core. Compound **68** has a benzisoxazole core and was chosen to evaluate the benzisoxazole structure as it was observed to dock well in TRIM33β and can be directly compared to **23** due to having the same benzylic amine substituent (Figure 2.14). Compound **68** contains a fused isoxazole. 3,5-Dimethylisoxazoles are known KAc mimics,[137] and docking of **68** into TRIM33β showed the benzisoxazole to be interacting with the conserved asparagine residue (N1039), therefore it was hypothesised that the benzisoxazole structure may also act as an KAc mimic.

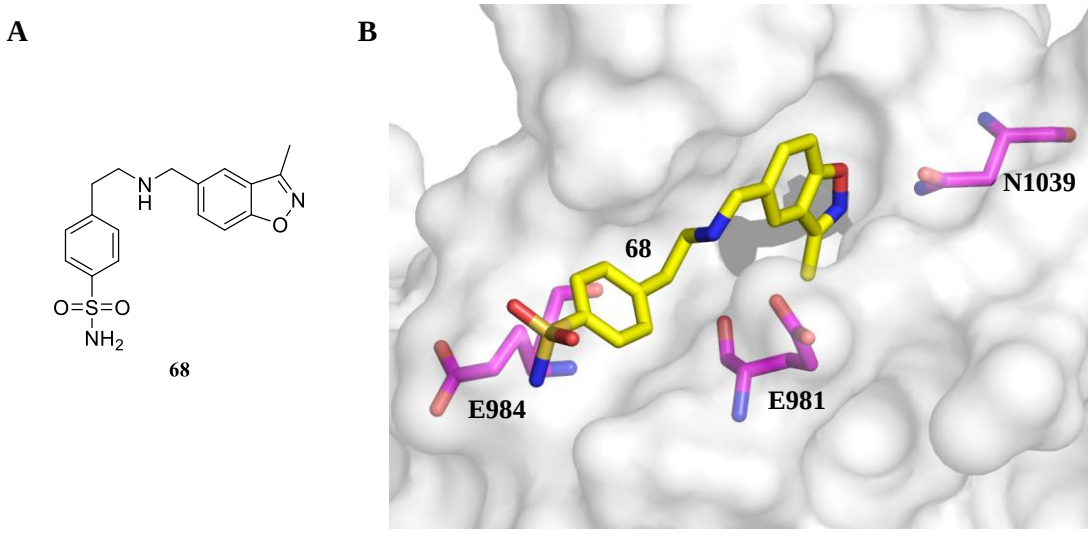
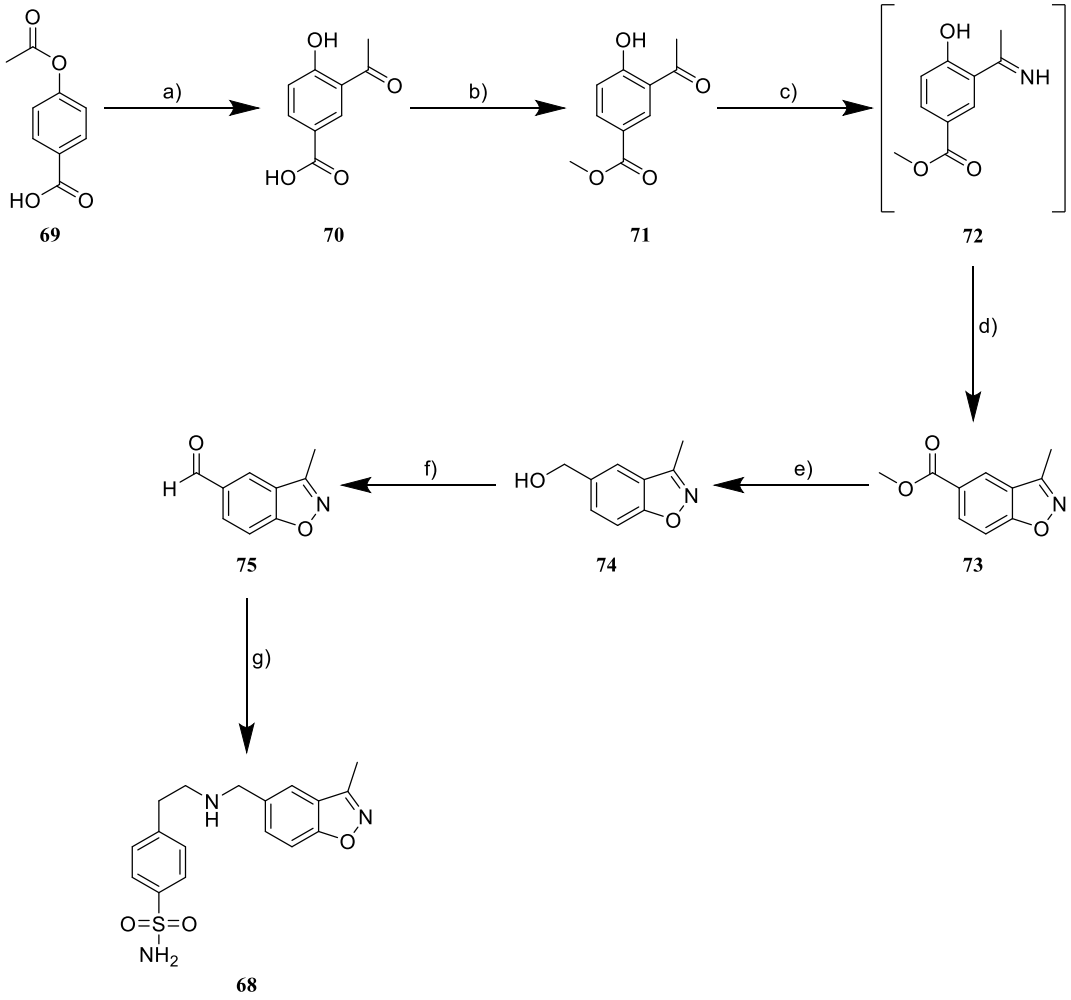


Figure 2.14: Compound 68 was chosen to investigate the benzisoxazole structure as an KAc mimic. (A) Structure of **68**. (B) Compound **68** (yellow) docked in TRIM33β (magenta) (PDB ID: 5MR8) using CLC Drug Discovery Workbench.

2.7.2 Synthesis of Compounds

Benzisoxazole **68** was synthesised *via* aldehyde **75**, similar to the benzimidazolone analogues (Scheme 2.9). Fries rearrangement of **69** yielded **70**[138] which was converted to methyl ester **71** by

stirring in methanol under acidic conditions. Imine **72** was obtained *via* reaction of **71** with 7 M NH_3 (MeOH) and the benzisoxazole ring formed using *N*-chlorosuccinimide (NCS) to yield **73**. The ester **73** was subsequently reduced to alcohol **74** using DIBAL, followed by oxidation to aldehyde **75** using MnO_2. Aldehyde **75** was then reacted *via* a reductive amination with amine **35** to yield **68**.



Scheme 2.9: Synthesis of 68. *Reagents and conditions*: a) $AlCl_3$, 155 °C, 36%, n = 1; b) H_2SO_4, MeOH, 65 °C, 62–92%, n = 2; c) 7 M NH_3 (MeOH), rt; d) NCS, K_2CO_3, THF, rt, 72% over 2 steps, n = 1; e) DIBAL (1 M, hexanes), toluene, –78 °C, 94%, n = 1; f) MnO_2, CH_2Cl_2, rt, 66%, n = 1; g) (i) **35**, AcOH, DCE, 3 Å molecular sieves, 0 °C; (ii) $NaBH(OAc)_3$, 0 °C → rt, 7%, n = 1.

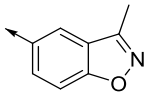
2.7.3 Evaluation of Compounds

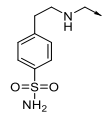
2.7.3.1 AlphaScreen™ Assays

Compound **68** was evaluated at both 30 µM and 150 µM using an AlphaScreen™ assay (Table 2.6). A reduction in emission, indicating binding, was only observed for TRIM33α in combination

with the acetylated peptide. This result suggests the benzisoxazole core is not a good replacement for the benzimidazolone core. The benzisoxazole series was therefore not pursued further.

Table 2.6: Single concentration AlphaScreen™ results. Percentage emission calculated relative to the average DMSO control. Compounds screened at 30 µM and 150 µM. Colour scale from low emission (red) to high emission (green). Quoted values are a mean of three technical repeats. H3(PHD) – $H3_{1-27}K9Me_3$, H3(BRD) – $H3_{1-27}K18Ac$, H3(DUAL) – $H3_{1-27}K9Me_3K18Ac$.



Compound	TRIM24 % Emission						TRIM33α % Emission						TRIM33β % Emission					
	H3(PHD)		H3(BRD)		H3(DUAL)		H3(PHD)		H3(BRD)		H3(DUAL)		H3(PHD)		H3(BRD)		H3(DUAL)	
	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM
 **68**	104	90	106	98	100	86	340	240	86	34	127	94	102	88	136	116	104	83

2.8 AlphaScreen™ TruHits™ Kit

When performing assays, it is important to check for assay interference and false positives. This is especially important for fluorescence-based assays where compounds can interfere with the observed fluorescence *via* a number of mechanisms. The AlphaScreen™ TruHits™ kit is used to identify false positives and interfering compounds in AlphaScreen™ assays (Figure 2.15). Biotinylated acceptor beads and streptavidin-coated donor beads are used to provide non-specific high emission. The effect of compounds on emission in the absence of protein and peptide can therefore be observed. There are several mechanisms by which compounds can interfere in the AlphaScreen™ assay.[111] Mechanisms include the quenching of singlet oxygen, acting as an inner filter and competing with the interaction between biotin and streptavidin. Two incubation methods are used to identify the different interference mechanisms. In the first method, the streptavidin-coated donor beads and the biotinylated acceptor beads are pre-incubated prior to the addition of compound, this enables the identification of quenchers and filters. In the second method, the compounds are pre-incubated with the streptavidin-coated donor beads prior to the addition of the biotinylated acceptor beads, this identifies compounds competing with the biotin-streptavidin interaction. For each compound tested, the difference from the DMSO average is calculated and normalised against the standard deviation of the DMSO control. Compounds which are more than two standard deviations greater than the mean DMSO control are considered to be interfering with the assay.

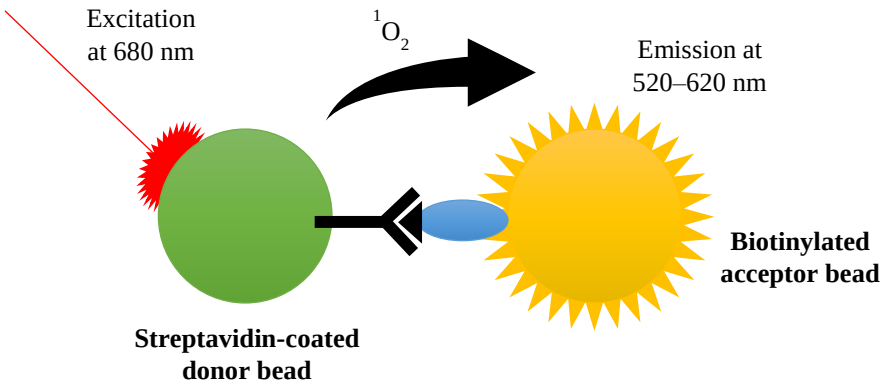
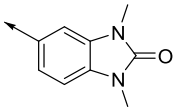
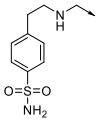


Figure 2.15: Schematic of the AlphaScreen™ TruHits™ kit. Streptavidin-coated donor beads and biotinylated acceptor beads are used to provide non-specific high emission. This assay allows the identification of compounds which are interfering in the AlphaScreen™ assay.

Selected compounds were tested with the AlphaScreen™ Truhits™ kit (Table 2.7). Aniline **25** demonstrated some assay interference which was dose-dependent. Anilines are known to be oxidised by singlet oxygen, therefore making them unsuitable for evaluating in AlphaScreen™ assays.[139–141] Initial hit **23** showed only low assay interference at the highest concentration (500 µM), and benzylic analogue **26** showed low assay interference which was not dose-dependent. Based on the results from the AlphaScreen™ Truhits™ kit and waterLOGSY experiments, it can be concluded that the benzylic benzimidazolone series does not interfere in the AlphaScreen™ assay and therefore AlphaScreen™ is an appropriate assay for initial compound screening.

Table 2.7: Results from the AlphaScreen™ TruHits™ kit. Compounds were screened at five concentrations and with two incubation formats. Results which are more than two standard deviations greater than the mean DMSO control are highlighted in blue.



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
 **23**	1.80	0.782	0.409	0.607	2.02	0.536	0.371	0.198	0.433	0.706
25	0.620	1.32	3.71	5.71	11.6	0.749	0.0950	0.246	0.221	1.67
26	3.45	2.79	2.83	3.38	2.25	3.70	2.74	3.25	3.71	4.03
Biotin	-	-	-	-	-	5.11	5.20	5.26	5.31	5.34

2.9 Published TRIM33 Inhibitors

During ligand optimisation a patent was published reporting TRIM33 inhibitors.[78] The reported inhibitors have general structure **6** (Figure 2.16). To validate binding of these structures to the bromodomain of TRIM33, five analogues were synthesised using a Groebke-Blackburn-Bienaymé multicomponent reaction (Figure 2.16, Schemes 2.10–2.12).[142–144] Yields between 31% and 60% were achieved, whereas comparably lower yields were achieved in the patent (6–15%). Analogues **79** and **80** were synthesised by Jessica Reynolds. The AlphaScreen™ assay performed in the patent used biotinylated $H3_{1–20}K9Me_3K18Ac$ peptide in combination with both TRIM24 and TRIM33. The patent does not state which isoform of TRIM33 was used. Selected compounds were also characterised by biophysical measurements and crystallography to establish binding mode, details of these are however not provided in the patent.[78]

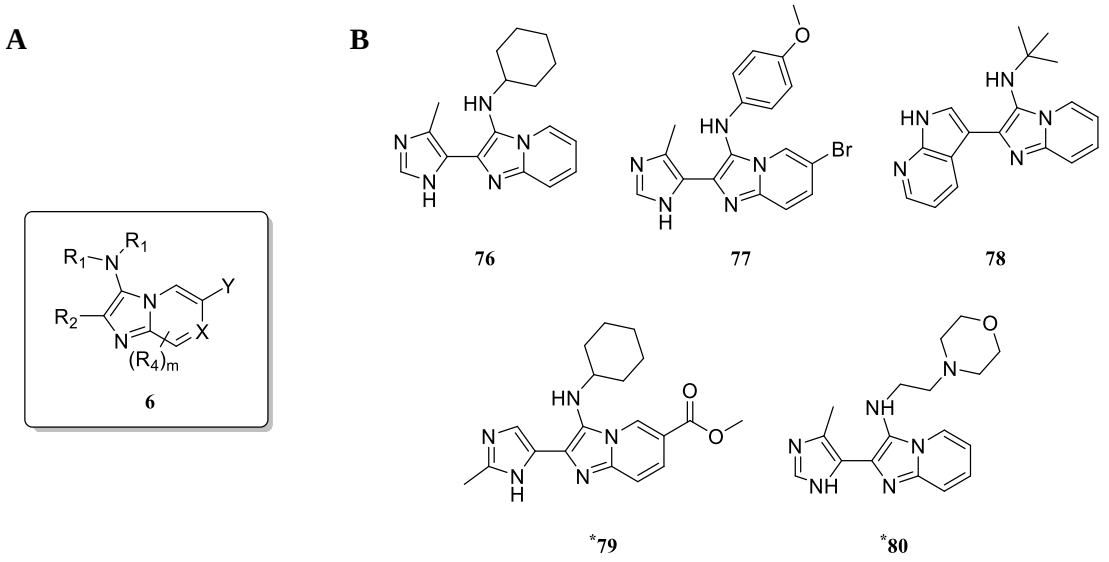
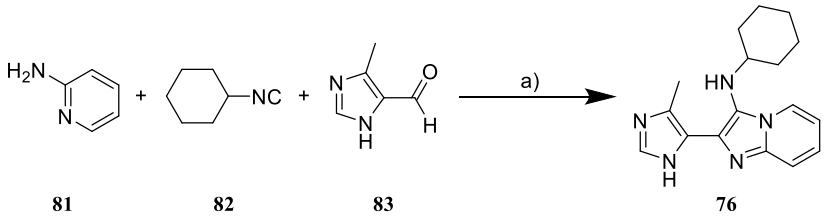
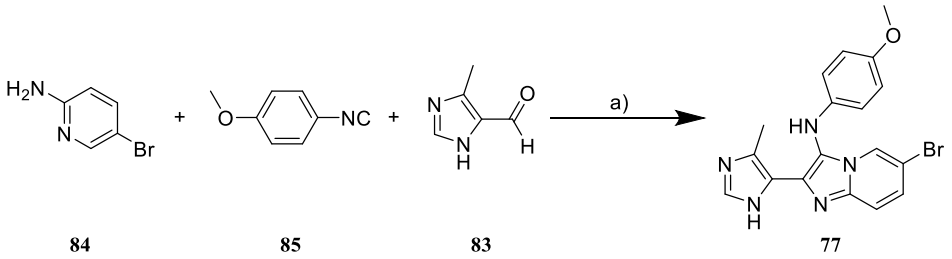


Figure 2.16: Published TRIM33 inhibitors.[78] (A) Reported TRIM33 inhibitors have general formula **6**. Where m = 0–2. (B) Analogues selected for synthesis. *Analogues synthesised by Jessica Reynolds.

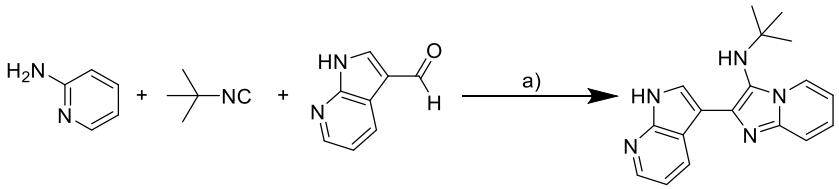


Scheme 2.10: Synthesis of 76. *Reagents and conditions*: a) $Sc(OTf)_3$, DMSO, 100 °C, 31%, n = 1.



84 **85** **83** **77**

Scheme 2.11: Synthesis of 77. *Reagents and conditions*: a) $Sc(OTf)_3$, DMSO, 100 °C, 60%, n = 1.



81 **86** **87** **78**

Scheme 2.12: Synthesis of 78. *Reagents and conditions*: a) $Sc(OTf)_3$, DMSO, 100 °C, 47%, n = 1.

The five analogues synthesised were evaluated using our previously optimised AlphaScreen™ assay conditions (Table 2.8). Compounds **76** and **77** bound to TRIM33α (**76** IC_{50} = 2.74 µM, **77** IC_{50} = 1.58 µM) and TRIM33β (**76** IC_{50} = 3.16 µM, **77** IC_{50} = 1.49 µM) with low micromolar IC_{50} values. They were however unselective, having similar affinity for TRIM24 (**76** IC_{50} = 4.21 µM, **77** IC_{50} = 1.76 µM), and comparable IC_{50} values across all nine protein-peptide combinations. Compound **78** was only observed to bind to TRIM24 (IC_{50} = 43.8 µM). Compound **79** showed similar affinity for TRIM33α (IC_{50} = 16.6 µM), TRIM33β (IC_{50} = 7.91 µM) and TRIM24 (IC_{50} = 20.0 µM). Compound **80** bound weakly to both TRIM33β (IC_{50} = 55.3 µM) and TRIM24 (IC_{50} = 18.6 µM) and did not bind to TRIM33α. The patent only reports IC_{50} values for **77** and **80** (Table 2.9).[78] For both compounds, the reported IC_{50} values are much lower than those determined in our AlphaScreen™ assay. IC_{50} values are dependent on the assay conditions, but a detailed description of the materials and concentrations used in the AlphaScreen™ assay in the patent is not available.

Table 2.8: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(BRD) – $H3_{1-27}$K18Ac. Ligand efficiencies are given in parentheses.

High affinity — Low affinity

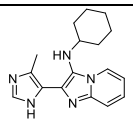
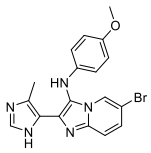
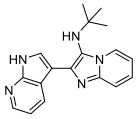
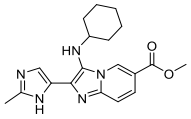
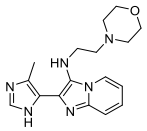
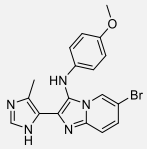
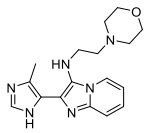
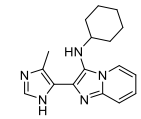
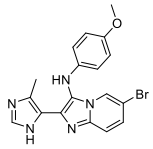
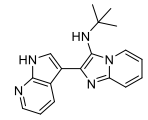
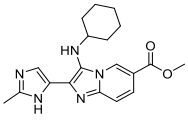
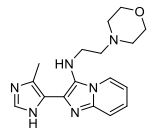
Compound	Structure	TRIM24 – H3(BRD) IC_{50} (µM)	TRIM33α – H3(BRD) IC_{50} (µM)	TRIM33β – H3(BRD) IC_{50} (µM)
76		4.21 ± 0.665 (0.34)	2.74 ± 0.529 (0.35)	3.16 ± 0.545 (0.35)
77		1.76 ± 0.100 (0.32)	1.58 ± 0.165 (0.32)	1.49 ± 0.218 (0.33)
78		43.8 ± 10.9 (0.27)	>250	>250
79		20.0 ± 2.95 (0.25)	16.6 ± 6.10 (0.26)	7.91 ± 1.84 (0.27)
80		18.6 ± 0.727 (0.28)	>250	55.3 ± 4.68 (0.25)

Table 2.9: IC_{50} values (µM) reported in the patent.[78] IC_{50} values reported for TRIM24 and TRIM33 in combination with $H3_{1–20}K9Me_3K18Ac$.

Compound	TRIM24 IC_{50} (µM)	TRIM33 IC_{50} (µM)
 **77**	0.234	0.136
 **80**	5.484	7.862

As the compounds did not display selectivity in the AlphaScreen™ assay, especially **76** and **77** which were active against all nine protein-peptide combinations, the AlphaScreen™ TruHits™ kit was performed (Table 2.10). A large amount of assay interference was found, this is indicated by the values highlighted in blue. Assay interference was greatest for **76**, **77** and **79** for which the lowest IC_{50} values were observed in the AlphaScreen™ assay. It was therefore concluded that AlphaScreen™ is an unsuitable assay for evaluating this series of compounds.

Table 2.10: Results from the AlphaScreen™ TruHits™ kit. Compounds were screened at five concentrations and with two incubation formats. Results which are more than two standard deviations greater than the mean DMSO control are highlighted in blue.

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
 **76**	40.9	42.3	43.0	43.4	43.6	3.84	3.94	4.03	4.04	4.06
 **77**	42.9	43.3	43.6	43.7	43.7	4.00	4.04	4.06	4.06	4.06
 **78**	2.87	8.92	17.8	25.3	32.7	0.792	1.27	1.65	2.28	3.23
 **79**	21.0	19.8	23.8	29.9	35.3	2.09	2.48	3.09	3.55	3.84
 **80**	1.97	2.58	3.96	2.64	4.39	2.07	2.89	3.16	3.00	3.66
Biotin	-	-	-	-	-	3.94	4.00	4.03	4.04	4.06

To confirm that these compounds were false positives, they were also evaluated in two orthogonal assays. Compounds **77** and **80** were chosen for further evaluation as IC_{50} values were reported in the patent (Table 2.9).[78]

WaterLOGSY experiments were performed on **77** and **80**. Compound **77** however formed soluble aggregates under the assay conditions, even at lower compound concentrations (125 µM) (Figure 2.17). This means waterLOGSY experiments are also an unsuitable assay for evaluating **77** as in the absence of protein there is inversion of the compound peaks to positive signal. Compound **80** did not form soluble aggregates under the assay conditions therefore waterLOGSY is a suitable assay to observe binding. For **80** the spectrum remained negative upon the addition of TRIM33β, indicating **80** is not binding (Figure 2.18).

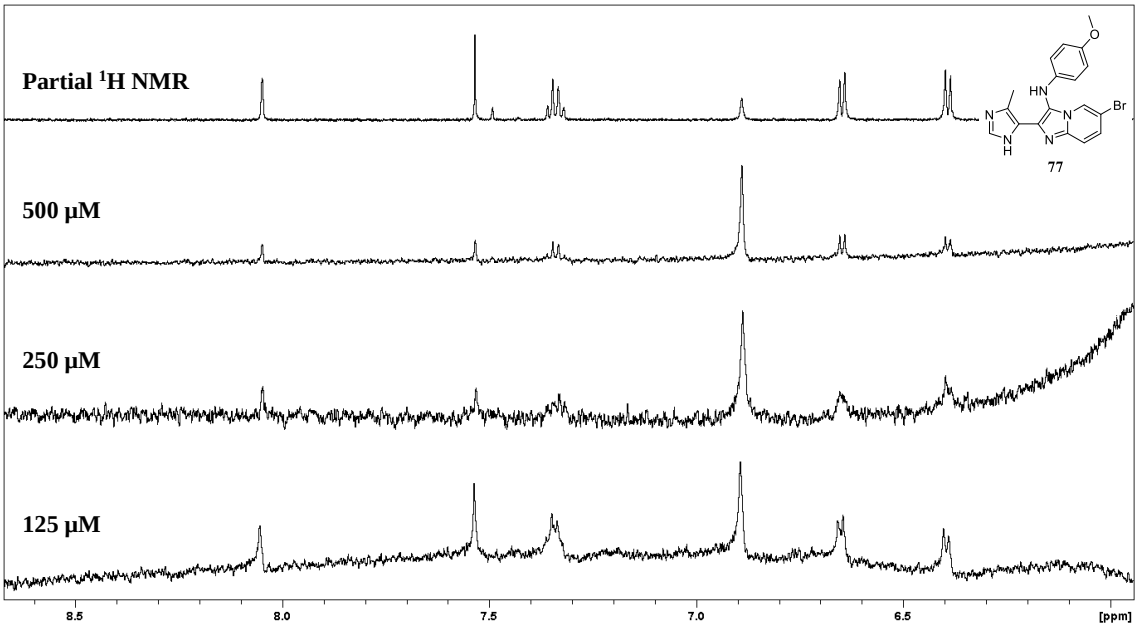


Figure 2.17: Partial waterLOGSY spectra for 77 in the absence of protein. **77** formed soluble aggregates even when its concentration was reduced. This is indicated by positive peaks in the absence of protein.

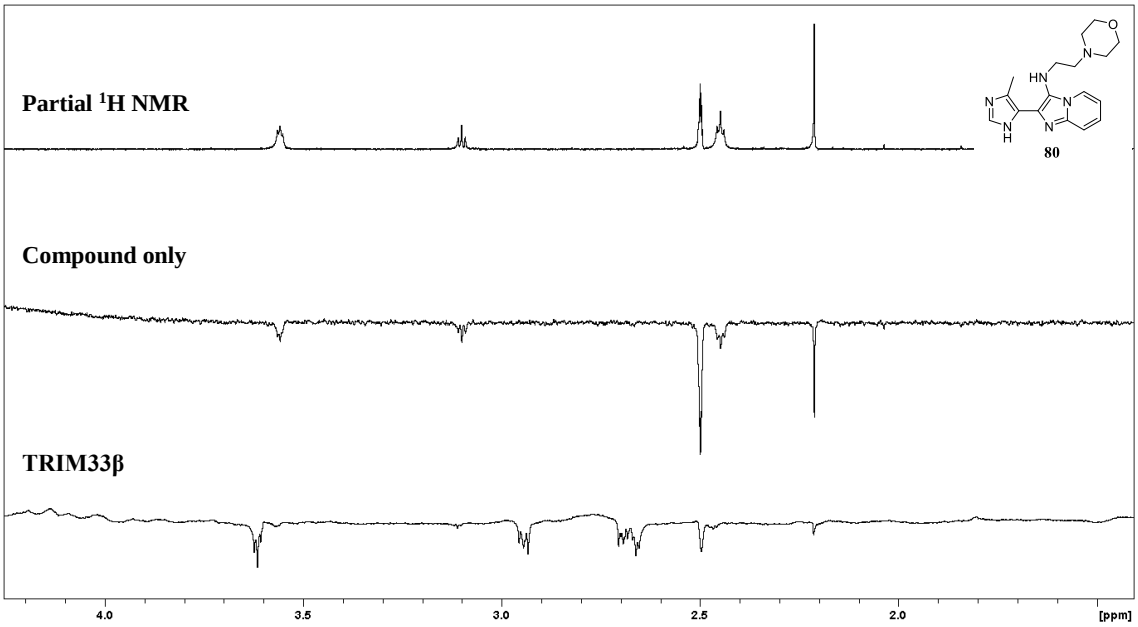


Figure 2.18: Partial waterLOGSY spectra for 80. Stacked spectra (top to bottom): ^{1}H NMR of **80**, compound only waterLOGSY, TRIM33β waterLOGSY.

ITC was next used to evaluate binding of **77** and **80** to TRIM33β. Before performing the experiment, the proteins were dialysed into HEPES buffer (50 mM, 150 mM NaCl) to ensure a good buffer match for the titration. The buffer was also supplemented with DMSO to match the DMSO concentration resulting from the compound DMSO stocks. The experiments were designed based on the reported IC_{50} values.[78] When designing ITC experiments, the unitless *c* value is used (Equation 4).[119] The *c* value refers to the shape of the binding isotherm and is dependent on the K_D value and the concentration of the component in the cell. *c* values between 10 and 500 are said to be optimal for an accurate determination of the binding constant, providing the best sigmoidal curves.[145] For high *c* values the binding isotherm becomes very steep and rectangular in shape, with too few points near equivalence. For low *c* values the equivalence point can be difficult to identify as the sigmoidal curve is lost and the transitions are very broad.[146]

$$c = K_D \times [M], \tag{4}$$

[M] = concentration of binding sites of the macromolecule in the cell

For both **77** and **80** a titration curve was not obtained (Figure 2.19). This suggests the compounds are not binding or are binding too weakly to be observed by ITC, under the experimental conditions used.

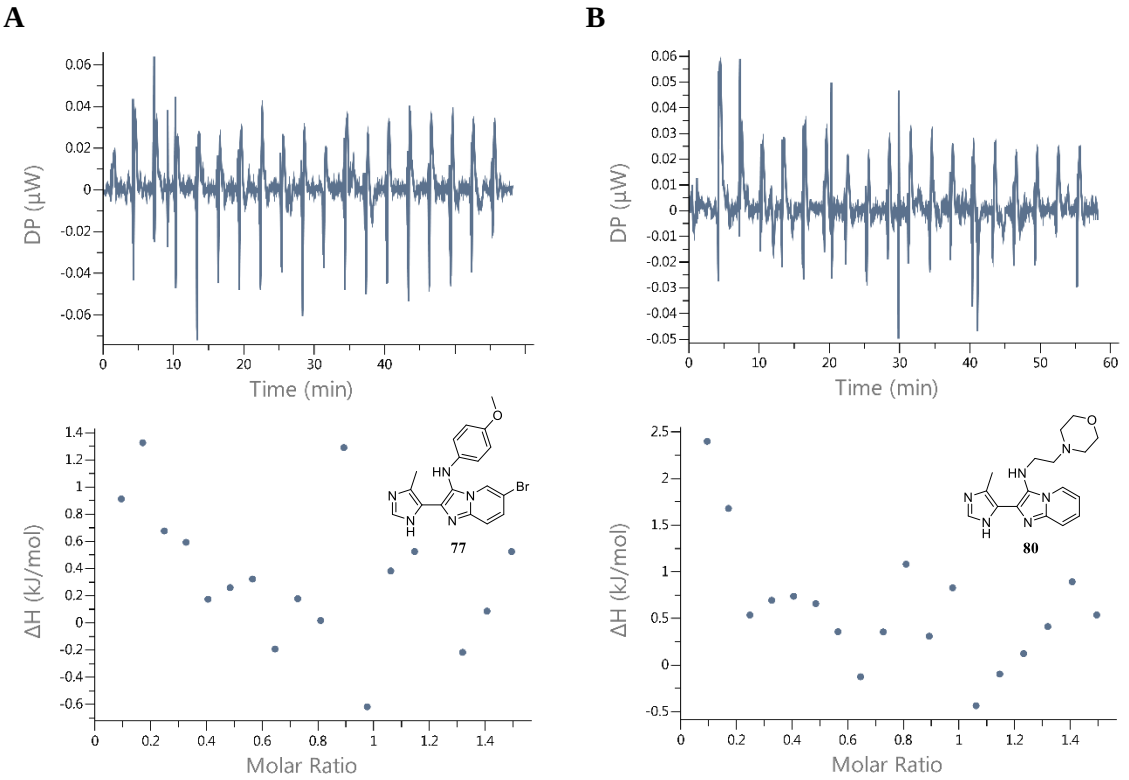


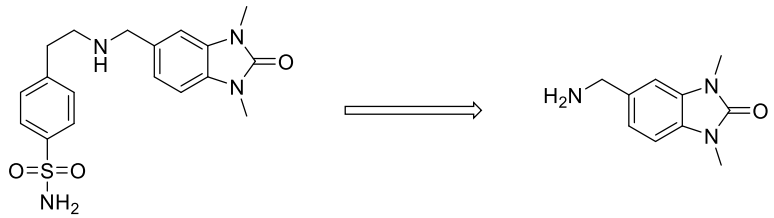
Figure 2.19: ITC data for 77 and 80 measured on TRIM33β. (A) ITC trace for **77**. (B) ITC trace for **80**. Shown are the heat effects for each injection (above) and the binding isotherms (below).

Together these results suggest that this series of compounds does not bind to TRIM33β and that the binding observed in the AlphaScreen™ assay is due to assay interference. This highlights the importance of checking for assay interference and validating hits in orthogonal assays before directing additional resources towards further evaluation and optimisation.

2.10 Conclusions

Analogues based on **23** were designed to gain a greater understanding of the TRIM33 KAc-binding pocket. The benzimidazolone core was identified as the KAc mimic and a diverse range of fragments possessing a benzimidazolone core were synthesised. Compounds were evaluated in AlphaScreen™ assays and binding confirmed in waterLOGSY NMR experiments.

Docking studies of **23** in TRIM33β identified E981 as interacting with the benzylic nitrogen. Primary benzylic fragment **26** was therefore synthesised to see if binding to TRIM33 was maintained with this smaller molecule. Compound **26** indeed proved to be a very ligand efficient fragment, suggesting it is forming good quality interactions with the protein. Compound **26** was also shown to be selective over TRIM24, in both AlphaScreen™ and waterLOGSY NMR experiments. In contrast, initial hit **23** was found to also bind to TRIM24. The benzylic core was therefore identified as a good starting point for the development of selective, high affinity ligands (Figure 2.20).

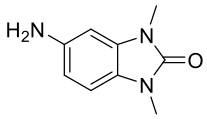


23
TRIM24 IC_{50} = 40.3 μM (0.24)
TRIM33α IC_{50} = 3.88 μM (0.29)
TRIM33β IC_{50} = 11.9 μM (0.27)

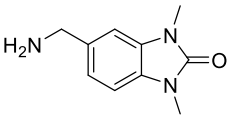
26
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 7.83 μM (0.51)
TRIM33β IC_{50} = 30.6 μM (0.45)

Figure 2.20: Benzylic fragment 26 was designed based on initial hit 23. The IC_{50} values were determined using a dose-response AlphaScreen™ assay with $H3_{1-27}$K18Ac peptide. Ligand efficiencies are given in parentheses.

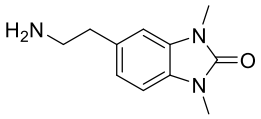
The distance of the amine substituent from the benzimidazolone core was also shown to be critical for binding (Figure 2.21). Analogues **25**, **26** and **27** differ only in the number of carbons between the amine substituent and the benzimidazolone core. The IC_{50} values determined for these compounds show that one carbon atom between the amine substituent and the benzimidazolone core is optimal (Figure 2.21).



25
TRIM24 IC_{50} = 22.5 μM (0.50)
TRIM33α IC_{50} = 41.8 μM (0.47)
TRIM33β IC_{50} = 168 μM (0.41)



26
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 7.83 μM (0.51)
TRIM33β IC_{50} = 30.6 μM (0.45)

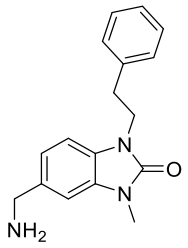


27
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 65.1 μM (0.39)
TRIM33β IC_{50} >250 μM

Figure 2.21: Analogues synthesised to investigate the distance of the amine substituent from the benzimidazolone core. The IC_{50} values were determined using a dose-response AlphaScreen™ assay with $H3_{1-27}$K18Ac peptide. Ligand efficiencies are given in parentheses.

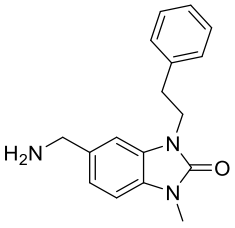
The work in this chapter has also led to the identification of areas of the ligand which can be explored further to increase affinity for TRIM33. Benzylic **26** is a small fragment, it has moderate affinity for TRIM33 but is very ligand efficient. Modifications to the amine chain are likely to interact with residues in the ZA channel, increasing both affinity and selectivity. The amine substituent will therefore be investigated in Chapter 3.

Docking of **23** in the crystal structure of TRIM33β (PDB ID: 5MR8) and overlaying with a histone peptide led to the observation that there is a hydrophobic region above the KAc-binding pocket. If this can be exploited, then an increase in binding affinity should be observed. A hydrophobic substituent on a headgroup nitrogen was therefore considered and initial work looked at the different vector combinations associated with this. Analogues **47** and **48** retained some affinity for TRIM33α, suggesting there is space to extend off the benzimidazolone core (Figure 2.22). Chapter 3 will look at investigating the SAR associated with this peptide binding groove, probing interactions with I990, F1038 and I996. These analogues should also provide a deeper understanding of how benzimidazolone analogues orientate in the TRIM33 bromodomain.



47

TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 12.1 μM (0.33)
TRIM33β IC_{50} >250 μM



48

TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 23.3 μM (0.31)
TRIM33β IC_{50} >250 μM

Figure 2.22: Analogues 47 and 48 have a hydrophobic substituent on a headgroup nitrogen. Some affinity was retained for TRIM33α, therefore analogues with different hydrophobic substituents will be explored in Chapter 3. The IC_{50} values were determined using a dose-response AlphaScreen™ assay with $H3_{1–27}$K18Ac peptide. Ligand efficiencies are given in parentheses.

The importance of validating hits using orthogonal assays and checking for assay interference was highlighted when investigating a recent patent.[78] Analogues **76**, **77** and **79** showed high affinity for TRIM33 in an AlphaScreen™ assay. The AlphaScreen™ TruHits™ kit, however, showed a large amount of assay interference from this series of compounds. To check if these compounds were false positives, waterLOGSY and ITC experiments were performed. Binding to TRIM33 was not observed, suggesting that these compounds were indeed false positives. These findings highlight the value of performing orthogonal assays and checking for assay interference. It will therefore be ensured that the relevant experiments are performed to validate hits during future optimisation work.

CHAPTER 3

Optimisation of the Benzimidazolone Series

3.1 Introduction and Aims

The work described in this chapter aimed to develop the fragment **26** into a selective and high affinity ligand for the TRIM33 bromodomain. Computational methods have been used to rationally design ligands and to help interpret binding affinities. The previously described AlphaScreen™ and waterLOGSY techniques have been used to evaluate synthesised compounds.

In Chapter 2 the different vector combinations associated with extending off the benzimidazolone core were considered, building on these studies the hydrophobic region above the KAc-binding site has been explored in more detail. Benzylic substitution on C-5 or C-5' was shown to be accommodated by the TRIM33α bromodomain and was therefore the focus of this work (Figure 3.1).

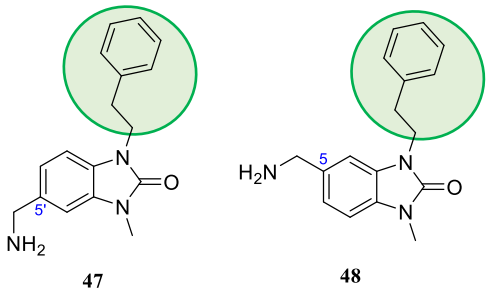


Figure 3.1: Benzylic substitution on C-5 or C-5' was shown to be accommodated in the TRIM33α KAc-binding pocket. Substitution on the benzimidazolone nitrogens has been explored further in this chapter.

Bromodomains typically have four or five structural water molecules at the base of the binding pocket.[147] Taking into account structural water molecules is critical when designing bromodomain ligands.[147,148] The water molecules present in the TRIM33 bromodomain were therefore considered in greater detail. Calculating the binding energies of these water molecules enabled the identification of those which can be more easily displaced and thus, provide a positive entropic contribution to ligand binding. Displacing tightly bound water molecules has a negative impact

on binding affinity. Consequent compound design will therefore avoid disrupting the water molecules predicted to have negative binding energies.

As selectivity is important for tool compounds, affinity for BRD4 was also evaluated.[136]

3.2 Compound Optimisation 1

3.2.1 Compound Design

The benzylic amine substituent was first explored as amine substituents could potentially increase ligand affinity through interactions with residues in the ZA channel (Figure 3.2 (A)). Commercially available amines which had a second basic functional group were chosen. It was predicted that this second group might interact with E984 (Figure 3.2 (B)).

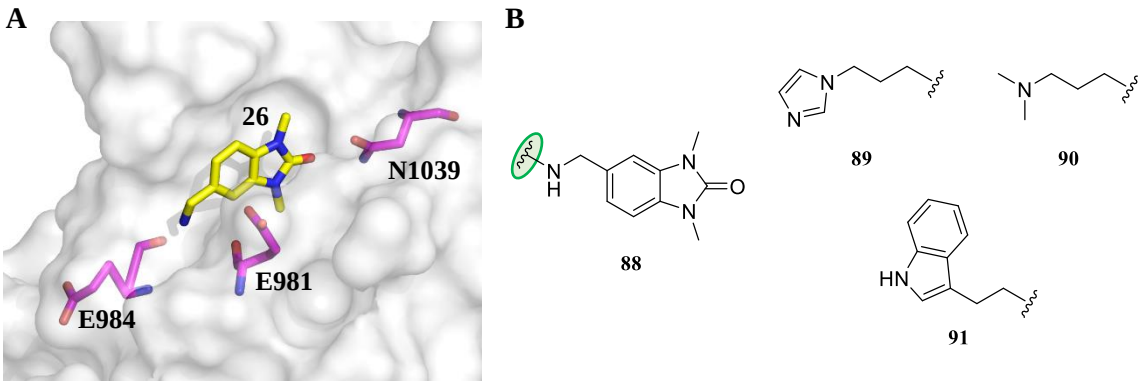
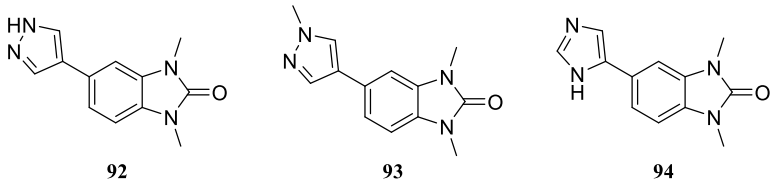


Figure 3.2: Benzylic amine substituents were first explored. (A) Compound **26** (yellow) docked in TRIM33β (PDB ID: 5MR8) using CLC Drug Discovery Workbench. Key residues are shown in the stick representation (magenta). (B) Commercially available amines, which had a second basic functional group to interact with E984, were chosen.

Replacing the benzylic substituent with a heterocycle was also considered as it was predicted that an interaction with E981 would be retained. Pyrazole analogues **92** and **93** and imidazole analogue **94** were synthesised so as to investigate this hypothesis (Figure 3.3). Methylpyrazole **93** was designed to investigate if substitution on the pyrazole would be tolerated or if the pyrazole NH is required for binding to the TRIM33 bromodomain.

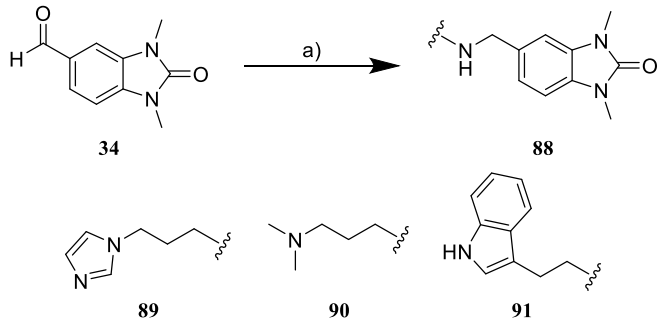


92 **93** **94**

Figure 3.3: Compounds designed to investigate replacing the benzylic substituent with a heterocycle. It was predicted that an interaction with E981 would be retained, providing affinity for TRIM33 and selectivity over TRIM24.

3.2.2 Synthesis of Compounds

The benzylic analogues were synthesised *via* reductive amination reactions between aldehyde **34** and the appropriate amine (Scheme 3.1). Yields between 10% and 64% were achieved. The lower yields were due to the products having poor organic solubility and high polarity making purification difficult.

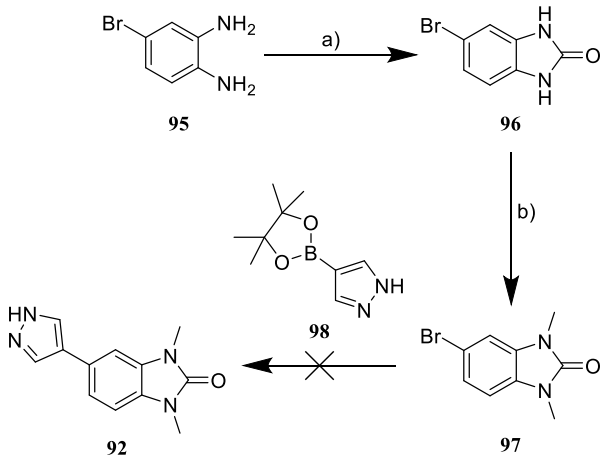


34 **88**

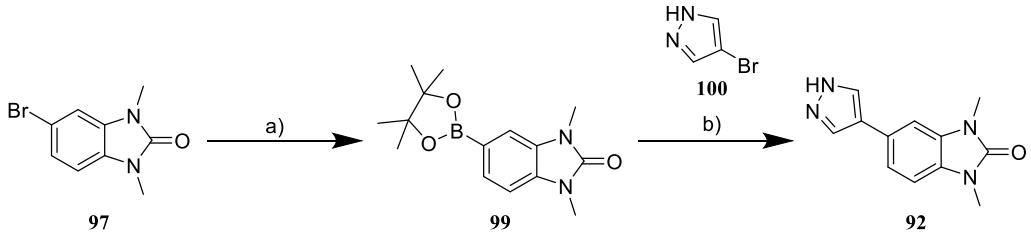
89 **90** **91**

Scheme 3.1: Synthesis of amine analogues. *Reagents and conditions*: a) (i) Amine, AcOH, DCE, 3 Å molecular sieves, 0 °C; (ii) $NaBH(OAc)_3$, 0 °C → rt, **89**: 64%, n = 1, **90**: 36%, n = 1, **91**: 10%, n = 1.

Pyrazole analogue **92** was synthesised *via* a Suzuki reaction.[149,150] Initially pyrazole was used as the boronic acid coupling partner (**98**) and the benzimidazolone as the organohalide (**97**) (Scheme 3.2). However, no reaction was observed, and so the coupling partners were reversed. Boronic acid pinacol ester **99** was synthesised from bromide **97** (Scheme 3.3). Three different sets of Suzuki conditions were investigated (Table 3.1). The third set of conditions afforded **92** in up to 58% yield.



Scheme 3.2: Attempted synthesis of 92. *Reagents and conditions*: a) CDI, DMF, rt, 69–81%, n = 2; b) MeI, K_2CO_3, DMF, rt, 80–83%, n = 2.



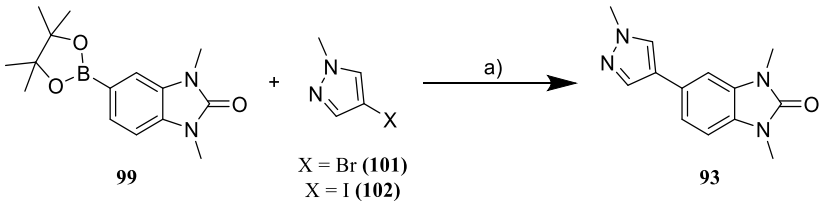
Scheme 3.3: Synthesis of 92. *Reagents and conditions*: a) B_2pin_2, $Pd_2(dba)_3$, XPhos, KOAc, 1,4-dioxane, 100 °C, 64–95%, n = 4; b) (A-^{ta}Phos)$_2PdCl_2$, K_2CO_3, 3:1 1,4-dioxane:H_2O, 115 °C, 18–58%, n = 5.

Table 3.1: Optimisation of the Suzuki coupling conditions between 99 and 100. 1.0 eq. of 4-bromopyrazole **100** was used. All reactions were heated to 115 °C and stirred overnight.

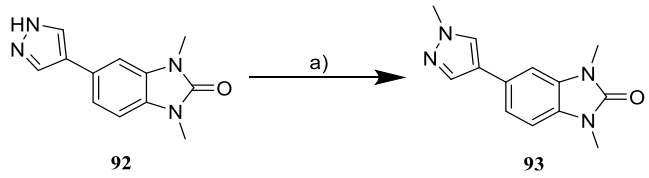
Condition	Boronic ester (99) eq.	Catalyst (eq.)	Ligand (eq.)	Base (eq.)	1,4-dioxane: H_2O	Conc. of 100 (mol dm^{-3})	Yield (%)
1	1.5	$Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (0.15)	-	K_3PO_4 (3.0)	10:1	0.04	0
2	1.1	$Pd_2(dba)_3$ (0.02)	PCy_3 (0.05)	K_3PO_4 (1.7)	1.4:1	0.18	0
3	1.3	(A-^{ta}Phos)$_2PdCl_2$ (0.03)	-	K_2CO_3 (3.0)	3:1	0.10	18–58

Synthesis of methylpyrazole **93** was first attempted *via* coupling between 4-halo-1-methyl-1*H*-pyrazole (**101** or **102**) and boronic acid pinacol ester **99**, using the conditions optimised for non-methylated analogue **92** (Scheme 3.4). Bromo- (**101**) and iodo- (**102**) pyrazoles were employed

in these reactions. Product isolation, however, proved difficult and using **101** the product was isolated with a purity of only 90% (analytical HPLC, 254 nm). Subsequently compound **93** was obtained with a purity >99% (analytical HPLC, 254 nm) *via* the direct methylation of **92** (Scheme 3.5).

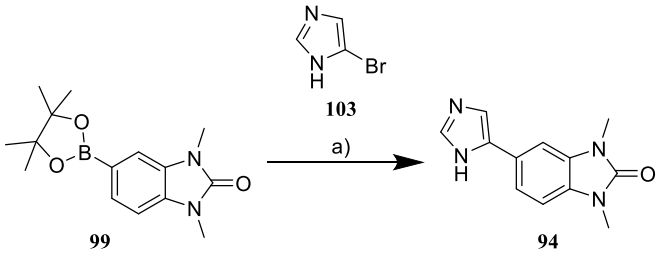


Scheme 3.4: Attempted synthesis of 93. *Reagents and conditions*: a) (A-^{ta}Phos)$_2$PdCl$_2$, K_2CO_3, 3:1 1,4-dioxane:H_2O, 110 °C, **101**: 31%, 90% purity, n = 1, **102**: 40%, 80% purity, n = 1.



Scheme 3.5: Synthesis of 93. *Reagents and conditions*: a) MeI, NaH, THF, rt, 97%, n = 1.

Imidazole analogue **94** was synthesised using the Suzuki conditions employed in the synthesis of **92** (Scheme 3.6).



Scheme 3.6: Synthesis of 94. *Reagents and conditions*: a) (A-^{ta}Phos)$_2$PdCl$_2$, K_2CO_3, 3:1 1,4-dioxane:H_2O, 115 °C, 18%, n = 1.

3.2.3 Evaluation of Compounds

3.2.3.1 AlphaScreen™ Assays

The compounds described above were tested in a single concentration AlphaScreen™ assay (30 μM and 150 μM) (Table 3.2). All compounds produced a large reduction in emission for TRIM33α in combination with both the acetylated and doubly modified peptides. Excluding **93**,

all compounds also resulted in a large reduction in emission for TRIM33β in combination with the acetylated peptide. Compound **90** additionally produced a large reduction in emission for TRIM33β in combination with the doubly modified peptide. Interestingly the compounds in which the benzylic substituent was replaced with a heterocycle (**92**, **93** and **94**) also showed some affinity for TRIM24, causing reduction in emission in combination with both the acetylated and doubly modified peptides.

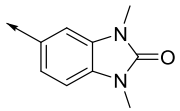
As only a small reduction in emission was observed for **93** with TRIM33β, **93** was not evaluated using a dose-response AlphaScreen™ assay. The results from the dose-response AlphaScreen™ assay for the remaining compounds are given in Table 3.3. Full AlphaScreen™ data can be found in Appendix B. Data points were fitted to a sigmoidal four-parameter equation (Equation 3). Where IC_{50} values had been determined, the ligand efficiencies were also calculated (Equation 1).

Amine chain analogues **89** (TRIM33α IC_{50} = 7.97 μM, TRIM33β IC_{50} = 11.9 μM) and **90** (TRIM33α IC_{50} = 3.61 μM, TRIM33β IC_{50} = 13.7 μM) demonstrated good affinity for both isoforms of TRIM33, similar to **23** (TRIM33α IC_{50} = 3.88 μM, TRIM33β IC_{50} = 11.9 μM). Unlike **23**, compounds **89** and **90** were selective over TRIM24. In contrast, **91** showed good affinity for TRIM33α (IC_{50} = 10.5 μM) but low affinity for TRIM33β (IC_{50} = 65.5 μM).

As observed in the single concentration AlphaScreen™ assay, pyrazole **92** and imidazole **94** showed some binding to TRIM24 with IC_{50} values of 92.1 μM and 52.4 μM, respectively. These values suggest that the benzylic nitrogen is important for imparting selectivity for TRIM33.[79,80] The heterocyclic rings of **92** and **94** might be stacking against the ZA loop, enhancing affinity for TRIM24, similar to TRIM24 inhibitors **7** and **8**.[79,80] Stronger binding was, however, observed with compounds **92** and **94** to TRIM33α (IC_{50} = 5.34 μM and 3.35 μM, respectively) and TRIM33β (IC_{50} = 23.3 μM and 26.5 μM, respectively), compared to TRIM24. This indicates that the benzylic amine is not essential for TRIM33 affinity, and alternative functional groups, including heterocycles, can provide affinity for TRIM33.

Compound **90** was subsequently used as a positive control in assays due to having high affinity for TRIM33 and selectivity over TRIM24.

Table 3.2: Single concentration AlphaScreen™ results. Percentage emission calculated relative to the average DMSO control. Compounds screened at 30 µM and 150 µM. Colour scale from low emission (red) to high emission (green). Quoted values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1-27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$.



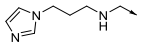
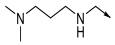
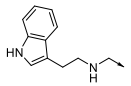
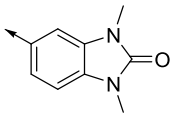
Compound	TRIM24 % Emission						TRIM33α % Emission						TRIM33β % Emission					
	H3(PHD)		H3(BRD)		H3(DUAL)		H3(PHD)		H3(BRD)		H3(DUAL)		H3(PHD)		H3(BRD)		H3(DUAL)	
	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM
 **89**	105	89	98	33	118	109	41	32	33	9	45	7	99	93	87	29	82	38
 **90**	108	101	106	97	107	105	51	37	4	3	8	3	77	57	18	4	51	5
 **91**	100	94	92	73	97	71	234	147	17	4	67	11	112	96	109	39	86	54
92	117	115	87	44	91	5	409	406	16	5	67	16	146	138	114	28	96	60

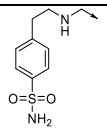
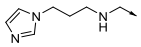
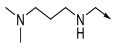
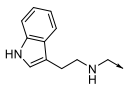
Table 3.2 continued.

Compound	TRIM24 % Emission						TRIM33α % Emission						TRIM33β % Emission					
	H3(PHD)		H3(BRD)		H3(DUAL)		H3(PHD)		H3(BRD)		H3(DUAL)		H3(PHD)		H3(BRD)		H3(DUAL)	
	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM	30 µM	150 µM
93	108	107	89	58	91	73	341	269	27	9	60	24	131	126	127	81	96	84
94	98	90	70	30	81	55	278	198	9	3	41	14	95	103	80	15	82	54

Table 3.3: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(BRD) – $H3_{1-27}$K18Ac. Ligand efficiencies are given in parentheses. *IC_{50} values are averaged over 14 repeats.



High affinity — Low affinity

Compound	Substituent	TRIM24 – H3(BRD) IC_{50} (µM)	TRIM33α – H3(BRD) IC_{50} (µM)	TRIM33β – H3(BRD) IC_{50} (µM)
23		40.3 ± 1.88 (0.24)	3.88 ± 1.03 (0.29)	11.9 ± 1.09 (0.27)
89		>250	7.97 ± 1.06 (0.32)	11.9 ± 1.12 (0.31)
***90**		>250	3.61 ± 0.352 (0.38)	13.7 ± 1.53 (0.35)
91		>250	10.5 ± 1.09 (0.28)	65.5 ± 1.18 (0.23)
92		92.1 ± 1.23 (0.33)	5.34 ± 1.10 (0.43)	23.3 ± 1.27 (0.38)
94		52.4 ± 1.21 (0.35)	3.35 ± 1.11 (0.45)	26.5 ± 1.10 (0.38)

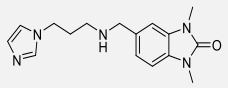
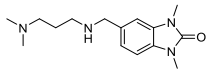
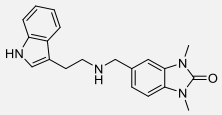
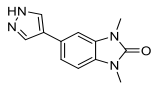
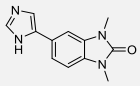
3.2.3.2 WaterLOGSY Experiments

WaterLOGSY NMR experiments were performed to validate binding (Table 3.4). All waterLOGSY spectra can be found in Appendix D. Compounds **89**, **90** and **91** bound to both isoforms of TRIM33 and were selective over TRIM24. Compounds **92** and **94** exhibited binding to all three proteins, which agrees with the results from the AlphaScreen™ assay where they were found to be unselective.

To confirm that it is specifically the bromodomain that these compounds bind to, waterLOGSY experiments were carried out with mutant proteins (provided by Dr Angelina Sekirnik[113] and Dr Larissa See[114]) (Table 3.5). The mutant TRIM24 and TRIM33 proteins possess a mutation of the

conserved asparagine residue (N980 or N1039, respectively) to phenylalanine (N980F or N1039F, respectively) (Figure 3.4). Unlike asparagine, phenylalanine is a bulky amino acid which is unable to form hydrogen bonds with ligands. Dr Angelina Sekirnik showed the phenylalanine mutation hindered recognition of peptide $H3_{1–27}K18Ac$.[113] Ligands which bind in the bromodomain and interact with the conserved asparagine residue should therefore have reduced affinity for the mutant proteins.

Table 3.4: Summary of waterLOGSY results for 89, 90, 91, 92, and 94. Compounds which were observed to bind are represented by ✓. Compounds which did not appear to bind are represented by ×.

Compound	TRIM24	TRIM33α	TRIM33β
 **89**	×	✓	✓
 **90**	×	✓	✓
 **91**	×	✓	✓
 **92**	✓	✓	✓
 **94**	✓	✓	✓

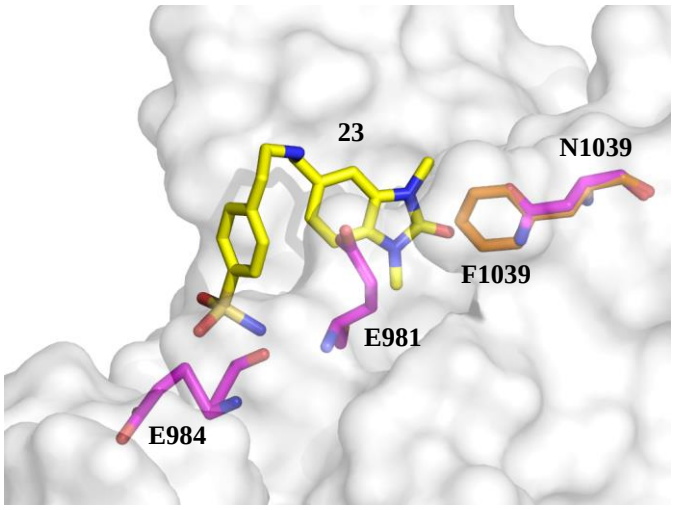
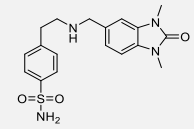
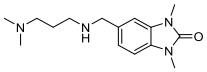
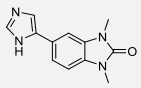


Figure 3.4: A representation of the mutation of the conserved asparagine residue (magenta) to phenylalanine (orange) generated using PyMOL mutagenesis wizard. Initial hit **23** (yellow) docked in TRIM33β (PDB ID: 5MR8) using CLC Drug Discovery Workbench. The key residues are shown in the stick representation.

Table 3.5: Summary of waterLOGSY results for 23, 90 and 94 with mutant proteins. Compounds which were observed to bind are represented by ✓. Compounds which did not appear to bind are represented by ×. Compounds not tested are represented by –.

Compound	TRIM24-N980F	TRIM33α-N1039F	TRIM33β-N1039F
 **23**	×	✓	✓
 **90**	–	✓	×
 **94**	–	✓	×

Dr Larissa See has previously shown that the initial hit **23** did not bind to TRIM24-N980F, and bound more weakly to TRIM33β-N1039F. Binding to TRIM33α-N1039F was retained.[114]

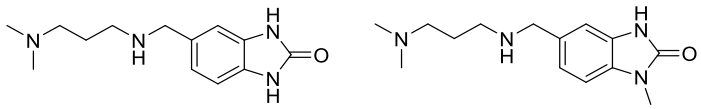
Compound **90** did not bind to mutant TRIM33β suggesting that N1039 is critical for binding, and that **90** is therefore binding in the bromodomain. Binding of **90** to TRIM33α-N1039F was, however, retained. Due to the longer BC loop in TRIM33α, N1039 does not reside in the

KAc-binding pocket and it is therefore unlikely that compounds binding in the bromodomain of TRIM33α are interacting with this residue. Consequently, mutating N1039 should not affect ligand binding, as is observed here for both **90** and **23**. Similar results were observed for **94**, where binding to TRIM33β-N1039F was abrogated, but binding to TRIM33α-N1039F was retained.

3.3 Compound Optimisation 2

3.3.1 Compound Design

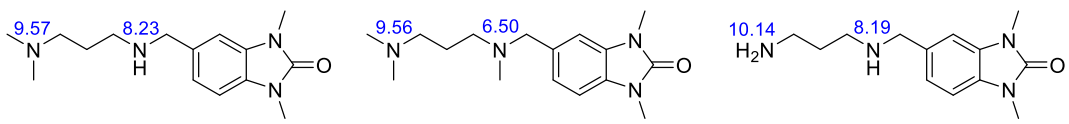
As compound **90** has high affinity for TRIM33 and is selective over TRIM24, compound **90** was investigated further. The methylation state of **90** was first considered as it has been shown in the literature that the methylation state of the benzimidazolone core is critical for binding to bromodomains.[132] In Chapter 2, it was found that the methylation state of the benzimidazolone core affected binding to the TRIM33 bromodomain. Non-methylated and mono-methylated analogues **104** and **105** were chosen to explore how the methylation state of **90** affects binding to the TRIM33 bromodomain (Figure 3.5).



104 **105**

Figure 3.5: Compounds designed to investigate the methylation state of the benzimidazolone core.

The methylation state of the amine chain was also investigated (Figure 3.6). Compound **106** is methylated at the benzylic position, this is predicted to reduce the pK_{aH} from 8.23 to 6.50.[130] An appropriate pK_{aH} of the benzylic position is predicted to be important for interacting with E981. Compound **107** has a primary terminal amine and similar pK_{aH} values to **90**.



90 **106** **107**

Figure 3.6: Analogues of 90 designed to probe the methylation state of the amine chain. The pK_a values of the conjugate acids calculated using ACD I-Lab 2.0 are shown in blue.[130]

A series of compounds (**108–112**) exploring the length of the amine chain and composition of the terminal amine was synthesised by Dr Larissa See (Figure 3.7).[114]

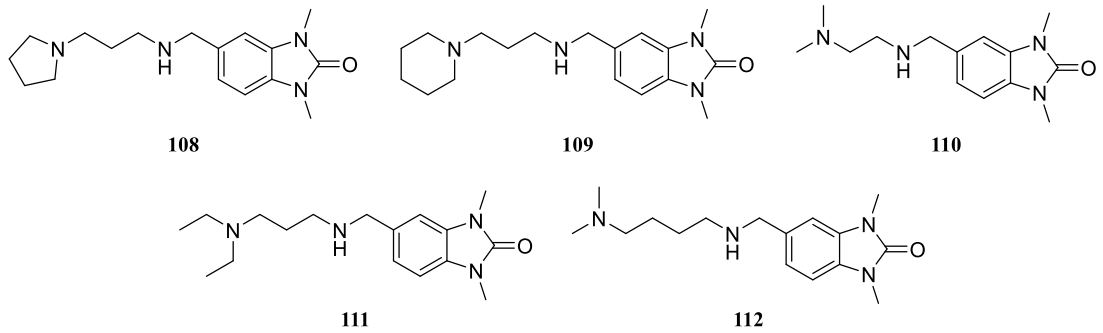


Figure 3.7: Compounds synthesised by Dr Larissa See designed to investigate the length of the amine chain of 90.[114]

To confirm that the benzimidazolone core is required for binding and not just the presence of a benzylic amine, **113** was synthesised (Dr Larissa See) (Figure 3.8).[114]

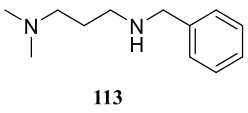
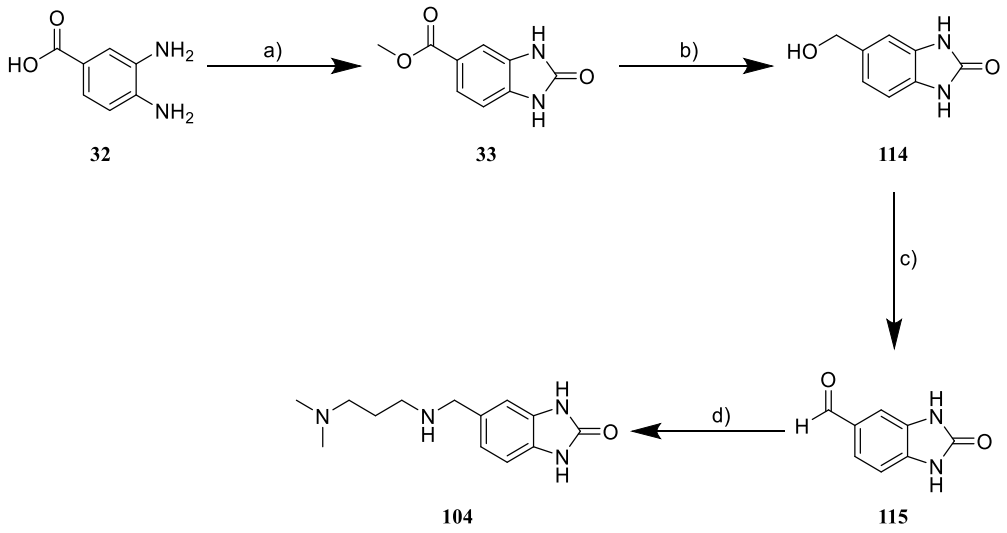


Figure 3.8: Compound 113 was synthesised to confirm the benzimidazolone core is required for binding to the bromodomain of TRIM33.[114]

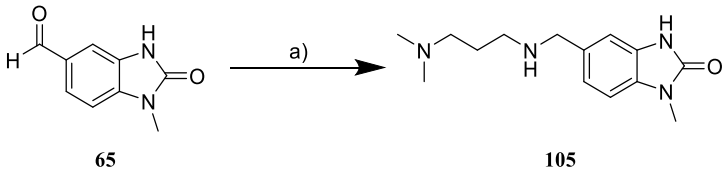
3.3.2 Synthesis of Compounds

Non-methylated analogue **104** was synthesised from 3,4-diaminobenzoic acid (**32**) (Scheme 3.7). The benzimidazolone ring was formed using CDI to yield **33**. Ester **33** was then reduced to alcohol **114** using DIBAL and oxidised to aldehyde **115** using MnO_2. A reductive amination reaction between aldehyde **115** and *N*,*N*-dimethyl-1,3-aminopropane afforded **104** in 45% yield.



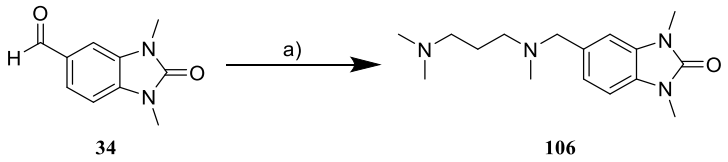
Scheme 3.7: Synthesis of non-methylated analogue 104. *Reagents and conditions*: a) (i) CDI, CH_2Cl_2, rt; (ii) MeOH, rt, 77–97%, n = 2; b) DIBAL (1 M, hexanes), toluene, –78 °C, 34%, n = 1; c) MnO_2, CH_2Cl_2, rt, 28%, n = 1; d) (i) *N*,*N*-Dimethyl-1,3-diaminopropane, AcOH, DCE, 3 Å molecular sieves, 0 °C → rt; (ii) $NaBH(OAc)_3$, rt, 45%, n = 1.

Intermediate **65** was reacted *via* a reductive amination with *N*,*N*-dimethyl-1,3-diaminopropane to yield mono-methylated analogue **105** (Scheme 3.8).



Scheme 3.8: Synthesis of mono-methylated analogue 105. *Reagents and conditions*: a) (i) *N*,*N*-Dimethyl-1,3-diaminopropane, AcOH, DCE, 3 Å molecular sieves, 0 °C → rt; (ii) $NaBH(OAc)_3$, rt, 49%, n = 1.

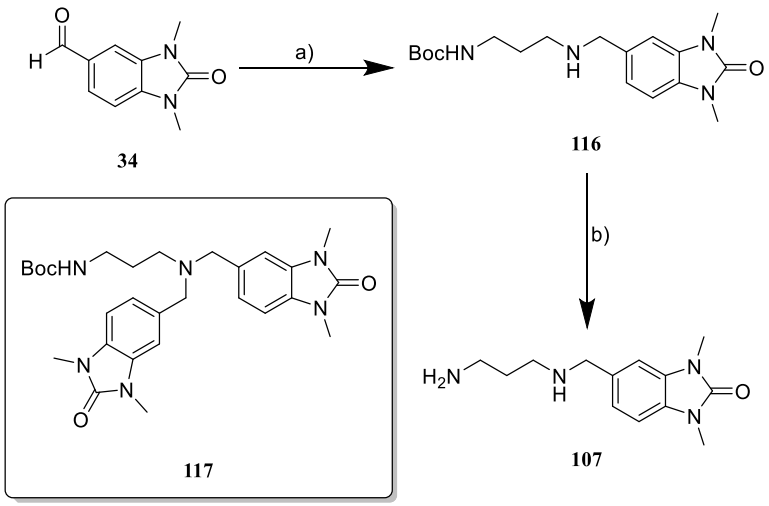
A reductive amination reaction between **34** and *N,N,N'*-trimethylpropane-1,3-diamine yielded compound **106** in 39% yield (Scheme 3.9).



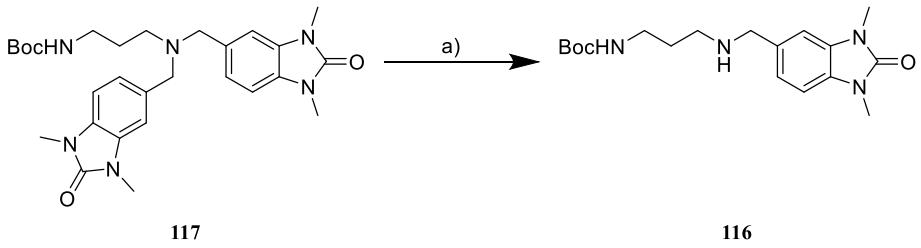
Scheme 3.9: Synthesis of 106. *Reagents and conditions*: a) (i) *N,N,N'*-Trimethylpropane-1,3-diamine, Et_3N, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, 39%, n = 1.

Compound **107** was synthesised from aldehyde **34** *via tert*-butyloxycarbonyl (Boc)-protected **116**. Compound **34** was reacted with *tert*-butyl *N*-(3-aminopropyl)carbamate in a reductive amination

to yield **116** (Scheme 3.10). However, the double reductive amination product **117** was also isolated (56% yield). A single benzyl group of **117** was removed to yield **116** by heating in DCE with 1-chloroethyl chloroformate, followed by heating under reflux in methanol (Scheme 3.11).[151–153] The debenzylation goes *via* a carbamate intermediate, which is removed when heated in methanol. The Boc group of **116** was removed under acidic conditions to yield **107**.



Scheme 3.10: Synthesis of 107. *Reagents and conditions*: a) (i) *tert*-Butyl *N*-(3-aminopropyl)carbamate, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, **116**: 44%, n = 1, **117**: 56%, n = 1; b) HCl (4 M, dioxane), 96%, n = 1.



Scheme 3.11: Removal of a single benzyl group from 117. *Reagents and conditions*: a) (i) 1-Chloroethyl chloroformate, DCE, 85 °C; (ii) MeOH, 70 °C, 82%, n = 1.

3.3.3 Evaluation of Compounds

3.3.3.1 AlphaScreen™ Assays

The above compounds were evaluated in a dose-response AlphaScreen™ assay against all nine protein-peptide combinations (Table 3.6). The results show that the methylation state of the

benzimidazolone core is critical for binding. Neither **104** nor **105** bound to TRIM24 or TRIM33, suggesting substitution on the benzimidazolone nitrogens is required for affinity. These compounds are, however, ideal candidates for negative controls in assays as, excluding TRIM33 affinity, they should display very similar properties to **90**.

Compound **106** remained selective over TRIM24 but had lower affinity for TRIM33β (IC_{50} = 23.5 μM), compared to **90** (IC_{50} = 13.7 μM). The predicted pK_{aH} of **106** (pK_{aH} = 6.50) is lower than the predicted pK_{aH} of **90** (pK_{aH} = 8.23).[130] Under the assay conditions (pH = 7.6), **90** will be mainly protonated and therefore able to form an ionic interaction with the side chain of E981 (pK_a = 4.25). In comparison, methylated analogue **106** is predicted to form a weaker interaction with E981 as it will only be partially protonated at pH 7.6, which may explain the reduction in affinity.

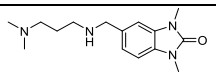
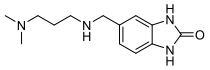
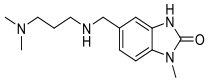
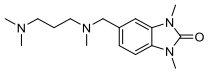
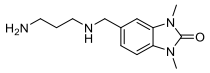
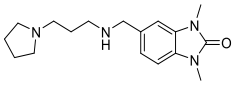
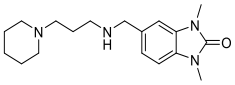
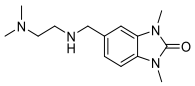
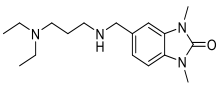
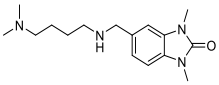
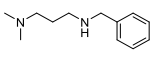
Compound **107** (TRIM33α IC_{50} = 3.73 μM, TRIM33β IC_{50} = 13.8 μM) showed very similar affinity for TRIM33 to **90**. This may be due to the terminal amines having similar pK_{aH} values meaning they are both protonated at pH 7.6, in addition to having similar steric bulk.

Compounds **108** (TRIM24 IC_{50} = 24.3 μM, TRIM33β IC_{50} = 9.30 μM), **109** (TRIM24 IC_{50} = 130 μM, TRIM33β IC_{50} = 9.24 μM) and **110** (TRIM24 IC_{50} = 71.9 μM, TRIM33β IC_{50} = 15.2 μM) were not selective over TRIM24 and had similar affinity for TRIM33β to **90** (IC_{50} = 13.7 μM). Compounds **111** and **112** were, however, selective over TRIM24 and had increased affinity for TRIM33β (**111** IC_{50} = 2.02 μM, **112** IC_{50} = 2.73 μM), as compared to **90** (IC_{50} = 13.7 μM). These results show that a longer amine chain is well accommodated in the KAc-binding pocket.

Compound **113** was synthesised to confirm the benzimidazolone core is required for binding to TRIM33, and not just the presence of a benzylic amine. Compound **113** did not bind to TRIM33 indicating the benzimidazolone core is essential for binding.

Table 3.6: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(BRD) – $H3_{1-27}$K18Ac. Ligand efficiencies are given in parentheses. *IC_{50} values are averaged over 14 repeats.

High affinity — Low affinity

Compound	Structure	TRIM24 – H3(BRD) IC_{50} (µM)	TRIM33α – H3(BRD) IC_{50} (µM)	TRIM33β – H3(BRD) IC_{50} (µM)
*90		>250	3.61 ± 0.352 (0.38)	13.7 ± 1.53 (0.35)
104		>250	>250	>250
105		>250	>250	>250
106		>250	5.69 ± 0.334 (0.35)	23.5 ± 2.80 (0.31)
107		>250	3.73 ± 0.383 (0.42)	13.8 ± 1.00 (0.38)
108		24.3 ± 1.36 (0.29)	4.11 ± 1.31 (0.34)	9.30 ± 1.47 (0.32)
109		130 ± 10.3 (0.24)	2.71 ± 1.08 (0.34)	9.24 ± 1.06 (0.31)
110		71.9 ± 1.30 (0.31)	5.89 ± 1.09 (0.39)	15.2 ± 1.07 (0.35)
111		>250	4.74 ± 1.14 (0.34)	2.02 ± 1.06 (0.36)
112		>250	7.96 ± 1.11 (0.34)	2.73 ± 1.14 (0.37)
113		>250	>250	>250

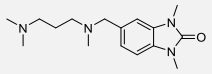
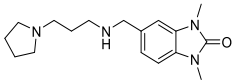
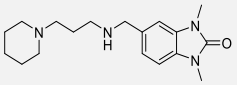
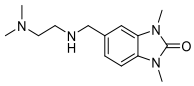
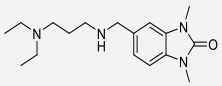
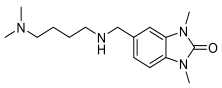
3.3.3.2 WaterLOGSY Experiments

Binding of **106**, **108**, **109**, **110**, **111** and **112** to TRIM24 and TRIM33 was evaluated in waterLOGSY experiments (Table 3.7). For TRIM33, positive peaks indicating binding were observed for all compounds. Whilst compounds **108**, **109** and **110** were observed to bind to

TRIM24 in the AlphaScreen™ assay, in the waterLOGSY experiments compound peaks were lost in the noise for TRIM24, suggesting the compounds are not binding or are only weakly binding. It may be that the observed reduction in emission in the AlphaScreen™ assay is due to assay interference, and if these compounds were to be pursued further, additional testing would be required.

Binding of **112** to mutant TRIM33 proteins was also evaluated in waterLOGSY experiments. Compound **112** bound to TRIM33α-N1039F but not TRIM33β-N1039F. This is similar to **90** and could potentially be attributed to differences in the BC loops, as previously described.

Table 3.7: Summary of waterLOGSY results for 106, 108, 109, 110, 111 and 112. Compounds which were observed to bind are represented by ✓. Compounds which did not appear to bind are represented by ×. Compounds where the result was unclear are represented by ~.

Compound	TRIM24	TRIM33α	TRIM33β
 **106**	×	✓	✓
 **108**	~	✓	✓
 **109**	~	✓	✓
 **110**	~	✓	✓
 **111**	×	✓	✓
 **112**	×	✓	✓

3.4 Compound Optimisation 3

3.4.1 Compound Design

Pyrazole analogue **93** was found to bind to TRIM33α but not TRIM33β (Table 3.2). This isoform-selectivity may be due to **93** being better accommodated by the longer BC loop in TRIM33α. Extending off the pyrazole ring could result in additional interactions with the protein and a consequent increase in binding affinity for either isoform of TRIM33. Therefore, we designed compound **118**, which is a hybrid of **89** and **92** (Figure 3.9 (A)). Docking of this compound in TRIM33β using CLC Drug Discovery Workbench suggests that the terminal imidazole may interact with E984 (Figure 3.9 (B)). Based on these observations we decided to synthesise the compound.

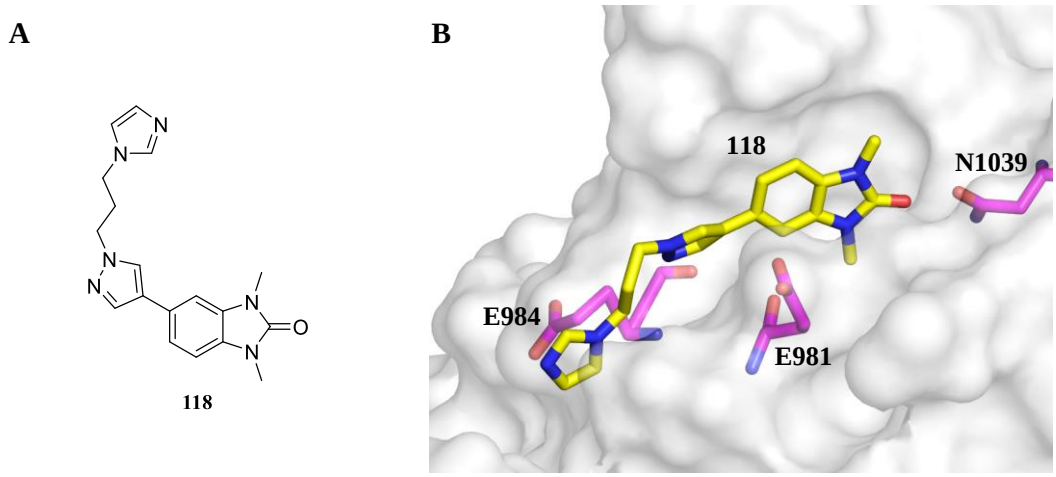
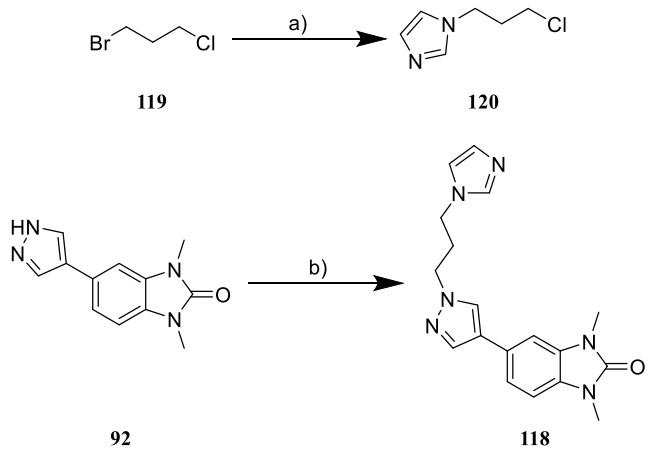


Figure 3.9: Compound 118 has an alkyl chain extending off the pyrazole ring. (A) Structure of **118**. (B) Docking of **118** (yellow) in TRIM33β (PDB ID: 5MR8) using CLC Drug Discovery Workbench**.** Key residues are shown in the stick representation (magenta).

3.4.2 Synthesis of Compounds

Compound **92** was alkylated with 1-(3-chloropropyl)-1*H*-imidazole (**120**) to yield **118** (Scheme 3.12).



Scheme 3.12: Synthesis of 118. *Reagents and conditions*: a) (i) NaH, THF, 0 °C → rt; (ii) Imidazole, THF, 0 °C → rt; (iii) 1-Bromo-3-chloropropane, 0 °C → rt, 29%, n = 1; b) (i) NaH, THF, 0 °C → rt; (ii) **120**, 0 °C → rt, 16%, n = 1.

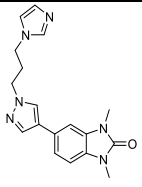
3.4.3 Evaluation of Compounds

3.4.3.1 AlphaScreen™ Assays

Compound **118** was evaluated in a dose-response AlphaScreen™ assay (Table 3.8). A small increase in affinity was observed for both TRIM33α (IC_{50} = 2.46 μM) and TRIM33β (IC_{50} = 15.3 μM), relative to pyrazole **92** (TRIM33α IC_{50} = 5.34 μM, TRIM33β IC_{50} = 23.3 μM). Compound **118** was, however, unselective for TRIM24, with an IC_{50} value of 34.5 μM.

Table 3.8: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 μM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(BRD) – $H3_{1-27}$K18Ac. Ligand efficiencies are given in parentheses.

High affinity — Low affinity

Compound	Structure	TRIM24 – H3(BRD) IC_{50} (μM)	TRIM33α – H3(BRD) IC_{50} (μM)	TRIM33β – H3(BRD) IC_{50} (μM)
118		34.5 ± 1.35 (0.25)	2.46 ± 1.07 (0.31)	15.3 ± 1.10 (0.27)

3.4.3.2 WaterLOGSY Experiments

WaterLOGSY NMR experiments validated binding of **118** to both isoforms of TRIM33. Weak binding to TRIM24 was also observed.

3.5 Compound Optimisation 4

3.5.1 Compound Design

In Chapter 2 a hydrophobic substituent on a benzimidazolone nitrogen was considered. Benzylic substitution on C-5 or C-5' was found to be preferred (**47** or **48**) (Figure 3.10), but the optimal vector may be substituent dependent. Benzimidazolone substituents were investigated further to see if affinity could be increased relative to the dimethyl analogues. As **90** had higher affinity than primary benzylic amine **26**, the amine chain of **90** was incorporated into the design of these compounds. Analogues of **47** and **48** with varying length alkyl phenyl chains (1–3 carbons) were initially chosen to explore substitution on the benzimidazolone nitrogens (Figure 3.10).

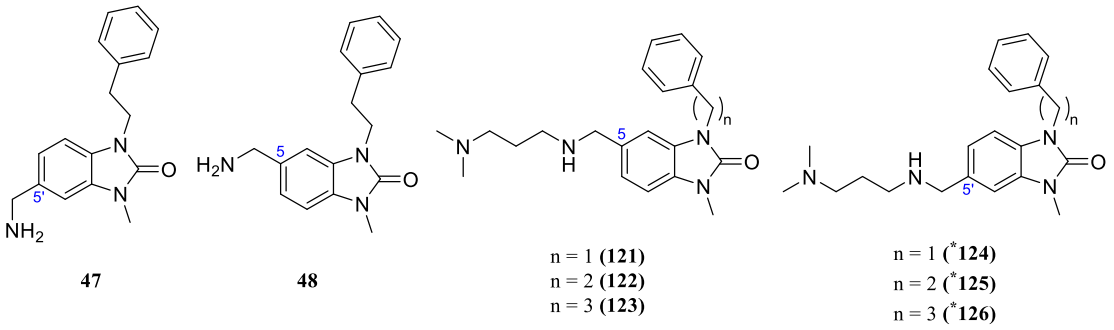
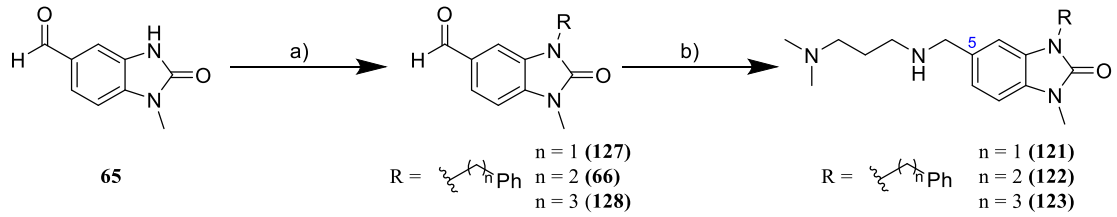


Figure 3.10: In Chapter 2, 47 (C-5') and 48 (C-5) were shown to have preferred vector combinations. Analogues of **47** and **48** were synthesised to probe the hydrophobic region above the KAc-binding pocket. *Analogues synthesised by Dr Larissa See.[114]

3.5.2 Synthesis of Compounds

Compounds **124**, **125** and **126** were synthesised by Dr Larissa See.[114] Analogues with benzylic substitution on C-5 were synthesised *via* intermediate aldehyde **65** (Scheme 3.13). The aldehyde was first alkylated with the appropriate alkyl bromide followed by reductive amination with *N*,*N*-dimethyl-1,3-diaminopropane.



Scheme 3.13: Synthesis of analogues of 48 (C-5). *Reagents and conditions*: a) Alkyl bromide, K_2CO_3, DMF, 70 °C, **127**: 99%, n = 1, **66**: 70%, n = 1, **128**: 95%, n = 1; b) (i) *N*,*N*-Dimethyl-1,3-diaminopropane, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, **121**: 46%, n = 1, **122**: 59%, n = 1, **123**: 51%, n = 1.

3.5.3 Evaluation of Compounds

3.5.3.1 AlphaScreen™ Assays

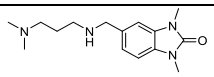
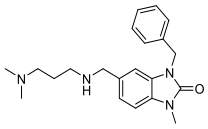
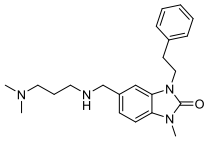
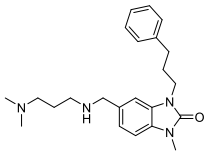
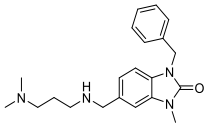
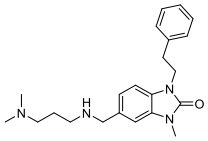
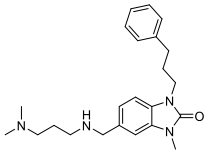
The phenyl chain analogues were evaluated in a dose-response AlphaScreen™ assay (Table 3.9).

Compounds **121**, **122** and **123** have C-5 benzylic substitution. These compounds showed binding to TRIM33α (**121** IC_{50} = 14.8 µM, **122** IC_{50} = 5.37 µM, **123** IC_{50} = 8.76 µM) but not TRIM33β.

Compounds **124**, **125** and **126** have C-5' benzylic substitution. These compounds showed similar affinity for TRIM33α (**124** IC_{50} = 3.64 µM, **125** IC_{50} = 4.34 µM, **126** IC_{50} = 2.47 µM) to dimethyl analogue **90** (IC_{50} = 3.61 µM). A large reduction in affinity for TRIM33β was, however, observed. The differences in affinity observed for the two isoforms are likely due to the differences in the loop regions. In TRIM33α the extended BC loop may better accommodate these larger substituents and hence the affinity of these compounds is higher for TRIM33α than TRIM33β. Together these results suggest that C-5' substitution is better accommodated in the bromodomain of TRIM33α than C-5 substitution.

Table 3.9: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(BRD) – $H3_{1-27}$K18Ac. Ligand efficiencies are given in parentheses. *IC_{50} values are averaged over 14 repeats.

High affinity — Low affinity

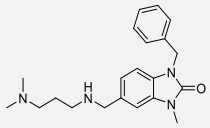
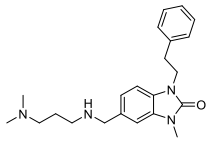
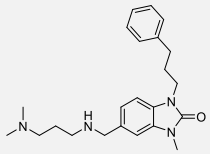
Compound	Structure	TRIM24 – H3(BRD) IC_{50} (µM)	TRIM33α – H3(BRD) IC_{50} (µM)	TRIM33β – H3(BRD) IC_{50} (µM)
*90		>250	3.61 ± 0.352 (0.38)	13.7 ± 1.53 (0.35)
121		>250	14.8 ± 1.12 (0.26)	>250
122		>250	5.37 ± 1.10 (0.27)	>250
123		>250	8.76 ± 1.15 (0.25)	>250
124		>250	3.64 ± 1.11 (0.29)	86.0 ± 1.15 (0.22)
125		>250	4.34 ± 1.08 (0.28)	59.7 ± 1.12 (0.22)
126		>250	2.47 ± 1.08 (0.28)	100 ± 1.10 (0.20)

3.5.3.2 WaterLOGSY Experiments

Compounds **124**, **125** and **126** were also evaluated in waterLOGSY NMR experiments (Table 3.10). Due to the formation of soluble aggregates under the assay conditions, compound concentrations were reduced (**124** 125 µM, **125** 250 µM and **126** 125 µM). Whilst compounds

124 and **125** were shown to bind to TRIM33α, the results were less clear for TRIM33β and TRIM24. Compound **126** was shown to bind selectively to TRIM33α and TRIM33β.

Table 3.10: Summary of waterLOGSY results for 124, 125 and 126. Compounds which were observed to bind are represented by ✓. Compounds which did not appear to bind are represented by ×. Compounds where the result was unclear are represented by ~.

Compound	TRIM24	TRIM33α	TRIM33β
 **124**	~	✓	~
 **125**	~	✓	~
 **126**	×	✓	✓

Binding of **125** to mutant TRIM33 protein was also evaluated in waterLOGSY experiments (Figure 3.11). Binding to TRIM33β-N1039F was observed. The result was a lot clearer for TRIM33β-N1039F than wildtype TRIM33β, indicating **125** has higher affinity for mutant TRIM33β than wildtype TRIM33β. An explanation for this could be that the phenyl substituent is forming hydrophobic interactions with the phenylalanine residue (F1039), compensating for the absence of the hydrogen bonding interaction with N1039.

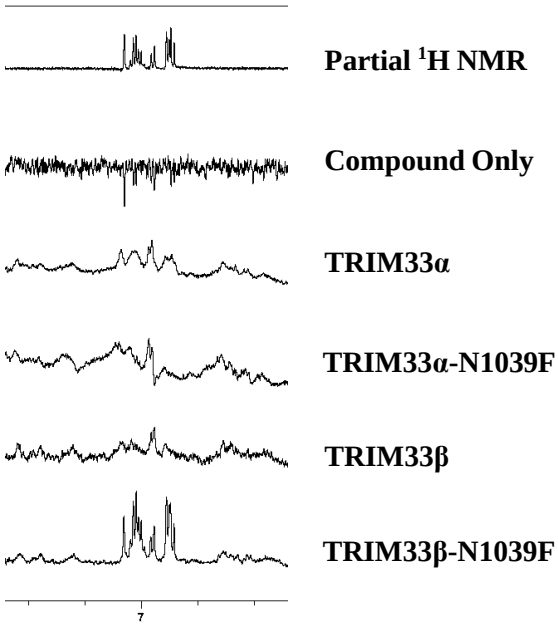


Figure 3.11: Partial waterLOGSY spectra for 125. Stacked spectra (top to bottom): ^{1}H NMR, compound only waterLOGSY, TRIM33α waterLOGSY, TRIM33α-N1039F waterLOGSY, TRIM33β waterLOGSY, TRIM33β-N1039F waterLOGSY.

3.6 Compound Optimisation 5

3.6.1 Compound Design

Despite hydrophobic analogues **124**, **125** and **126** maintaining affinity for TRIM33α, they had lower affinity for TRIM33β than dimethyl analogue **90**. It is possible that the alkyl phenyl chains are too flexible and lack interactions and are therefore free to move in the solvent exposed region above the KAc-binding pocket. It may also be that the hydrophobic substituents are not well accommodated by the smaller BC loop in TRIM33β, resulting in the ligands being forced out of the binding pocket, losing the hydrogen bonding interaction between the benzimidazolone carbonyl and N1039. The observed affinity for TRIM33α may mainly be due to interactions in the loop region. It is also possible that the chains are unable to reach into the lipophilic region and make the desired hydrophobic interactions. Consequently, a range of compounds were designed with different substituents on the headgroup to direct towards the hydrophobic region above the KAc-binding site (Figure 3.12).

Unsaturated analogues **129** and **142** and sulfones **131**, **140** and **144** were designed to restrict the conformation of the substituents. Compound **130** has a bulky adamantyl substituent and was

designed to maximise hydrophobic interactions with the protein and to also investigate if larger groups can be accommodated in this region. Computational studies by Joe Bluck predicted that a phenyl group or cyclopentyl ring directly attached to the benzimidazolone core would favour interactions with the hydrophobic region of interest, therefore **132** and **143** were synthesised. Smaller alkyl substituents were also chosen to investigate the effect minor modifications have on binding affinity, this led to the design of **134**, **135**, **136**, **137** and **138**.

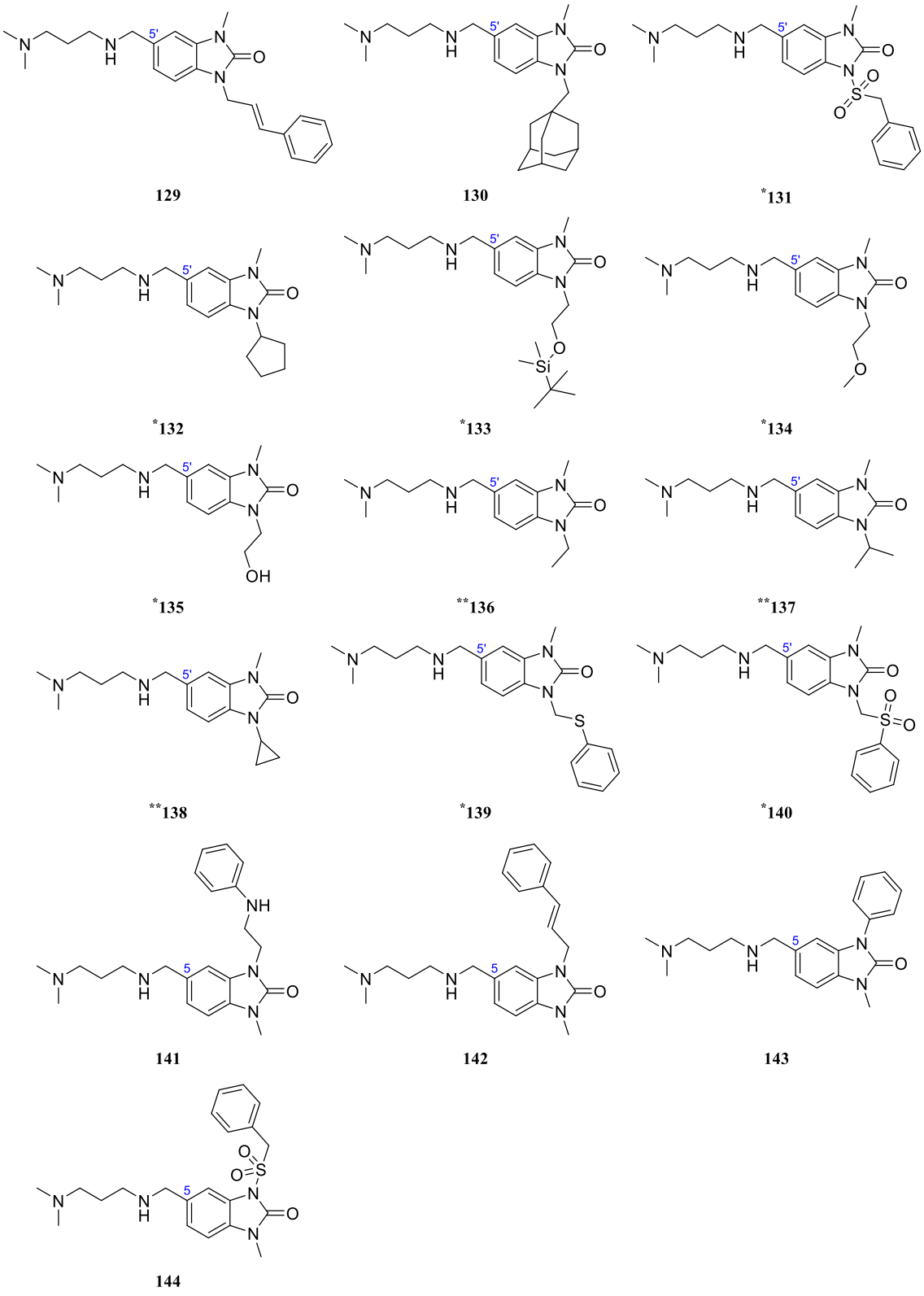
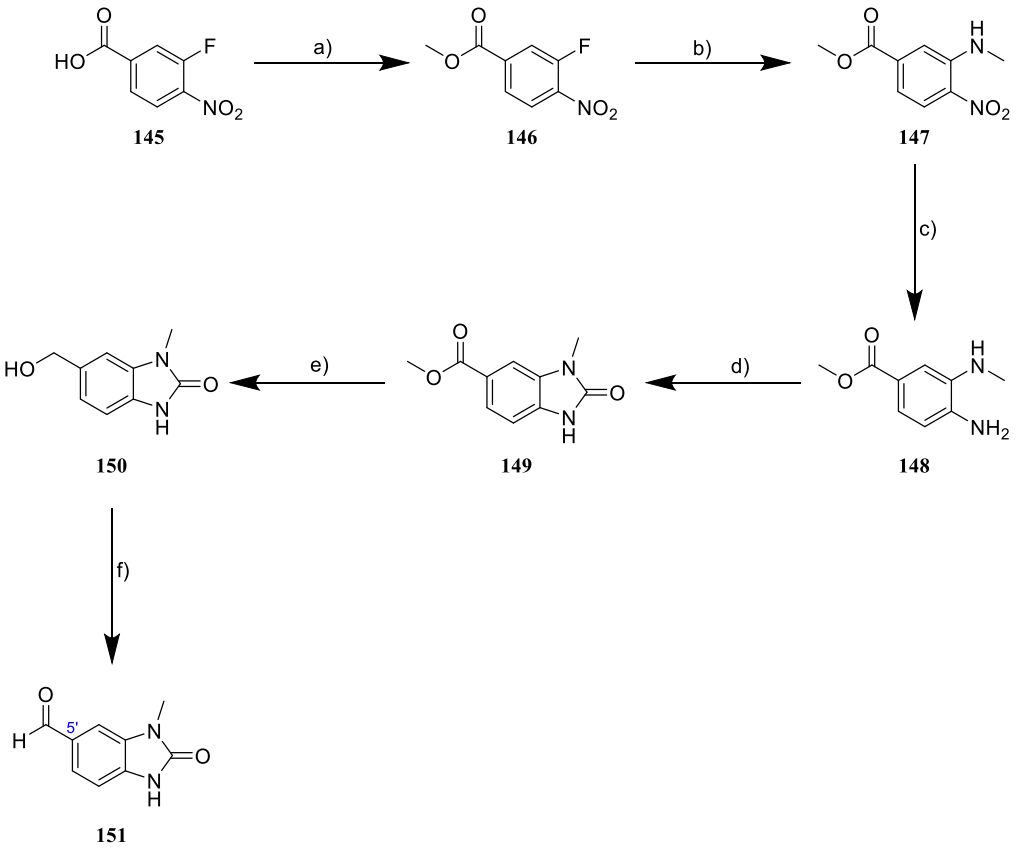


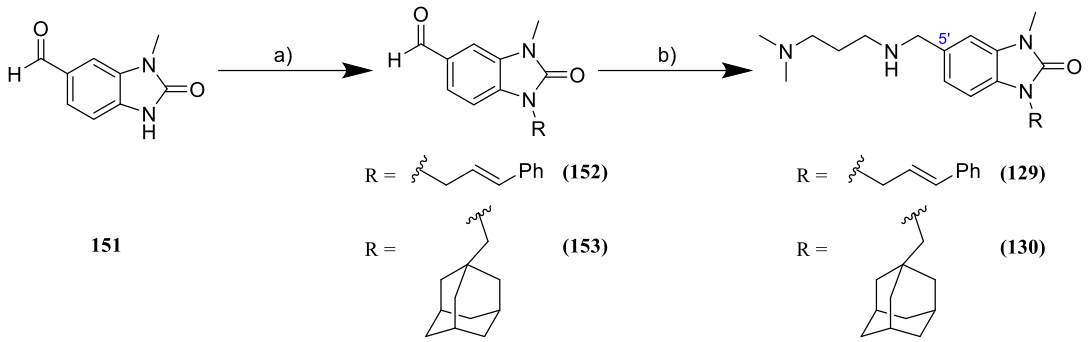
Figure 3.12: Analogues of 47 and 48 designed to probe the hydrophobic region above the KAc-binding pocket. *Analogues synthesised by Dr Larissa See.[114] **Analogues synthesised by Jessica Reynolds.

3.6.2 Synthesis of Compounds

Analogues with the benzylic substituent on C-5' were synthesised *via* intermediate aldehyde **151** (Scheme 3.14). Aldehyde **151** was synthesised from 3-fluoro-4-nitrobenzoic acid (**145**) *via* the equivalent route to **65**. Intermediate **151** was then alkylated with the relevant alkyl bromide and reacted in a reductive amination with *N*,*N*-dimethyl-1,3-diaminopropane to yield the final compounds (Scheme 3.15).

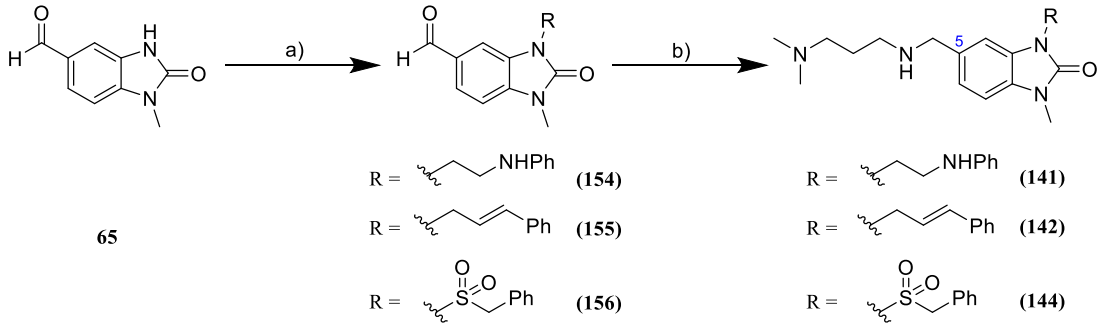


Scheme 3.14: Synthesis of intermediate aldehyde 151. *Reagents and conditions*: a) MeI, K_2CO_3, DMF, 0 °C → rt, 84%, n = 1; b) NH_2Me·HCl, DIPEA, DMF, rt, 95%, n = 1; c) Pd/C (10% *w*/*w*), H_2, EtOAc, EtOH, rt, 100%, n = 1; d) CDI, CH_2Cl_2, rt, 62%, n = 1; e) DIBAL (1 M, hexanes), toluene, 0 °C → rt, 59–99%, n = 2; f) MnO_2, CH_2Cl_2, rt, 47%, n = 1.

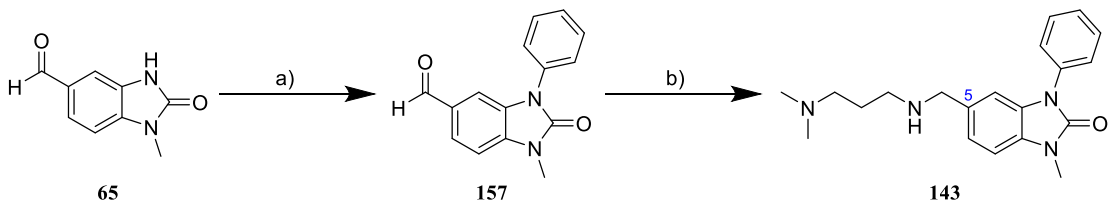


Scheme 3.15: Synthesis of C-5' substituted benzimidazolones. *Reagents and conditions*: a) Alkyl bromide, K_2CO_3, DMF, 70 °C, **152**: 11%, n = 1, **153**: 33%, n = 1; b) (i) *N*,*N*-Dimethyl-1,3-diaminopropane, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, **129**: 36%, n = 1, **130**: 45%, n = 1.

C-5 substituted analogues were synthesised *via* alkylation of **65** with the appropriate alkyl bromide followed by reductive amination with *N*,*N*-dimethyl-1,3-diaminopropane (Scheme 3.16). Analogue **143** was synthesised *via* a Chan-Lam coupling between aldehyde **65** and phenylboronic acid (Scheme 3.17).[154–156]



Scheme 3.16: Synthesis of C-5 substituted benzimidazolones. *Reagents and conditions*: a) Alkyl bromide, K_2CO_3, DMF, 70 °C, **154**: 40%, n = 1, **155**: 56%, n = 1, **156**: 44%, n = 1; b) (i) *N*,*N*-Dimethyl-1,3-diaminopropane, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, **141**: 17%, n = 1, **142**: 17%, n = 1, **144**: 66%, n = 1.



Scheme 3.17: Synthesis of 143. *Reagents and conditions*: a) $PhB(OH)_2$, $Cu(OAc)_2$, Et_3N, CH_2Cl_2, rt, 56%, n = 1; b) (i) *N*,*N*-Dimethyl-1,3-diaminopropane, AcOH, DCE, 3 Å molecular sieves, 0 °C → rt; (ii) $NaBH(OAc)_3$, rt, 64%, n = 1.

3.6.3 Evaluation of Compounds

3.6.3.1 AlphaScreen™ Assays

The compounds described above were evaluated in a dose-response AlphaScreen™ assay (Table 3.11). The strongest binding analogues were those possessing small alkyl substituents and C-5' benzylic substitution. Ethyl analogue **136** (IC_{50} = 14.7 μM) and isopropyl analogue **137** (IC_{50} = 19.6 μM) had the highest affinity for TRIM33β but had similar affinity as dimethyl analogue **90** (IC_{50} = 13.7 μM). Interestingly, the cyclopropyl analogue **138** had reduced affinity for TRIM33α (IC_{50} = 9.01 μM) and TRIM33β (IC_{50} = 96.0 μM), despite having a small alkyl substituent.

Cyclopentyl analogue **132** (IC_{50} = 60.2 μM) showed higher affinity for TRIM33β than cyclopropyl analogue **138** (IC_{50} = 96.0 μM), but lower affinity than ethyl analogue **136** (IC_{50} = 14.7 μM) and isopropyl analogue **137** (IC_{50} = 19.6 μM). MD studies conducted by Joe Bluck suggest that the cyclopentyl ring can form a favourable interaction with isoleucine I990 (Figure 3.13). It was also observed during the simulations that the cyclopentyl ring may pull the benzimidazolone core away from N1039. Consequently, the benzylic nitrogen is no longer in close proximity to E981 and the hydrogen bond to N1039 may be lost. It may therefore be necessary to optimise the distance between the basic substituent and the benzimidazolone core for each headgroup substituent. To test this, compound **158** (Figure 3.14) was synthesised by Dr Larissa See.[114] Cyclopentyl **158** has two carbon atoms between the benzimidazolone core and the basic group. Compound **158** was also evaluated using an AlphaScreen™ assay (Table 3.11). Cyclopentyl **158** showed higher affinity for TRIM33β (IC_{50} = 182 μM) than dimethyl analogue **27** (IC_{50} >250 μM). However, compound **158** still had lower affinity that primary benzylic dimethyl analogue **26** (TRIM33β IC_{50} = 30.6 μM). The optimal distance between the basic group and the benzimidazolone core remains to be optimised.

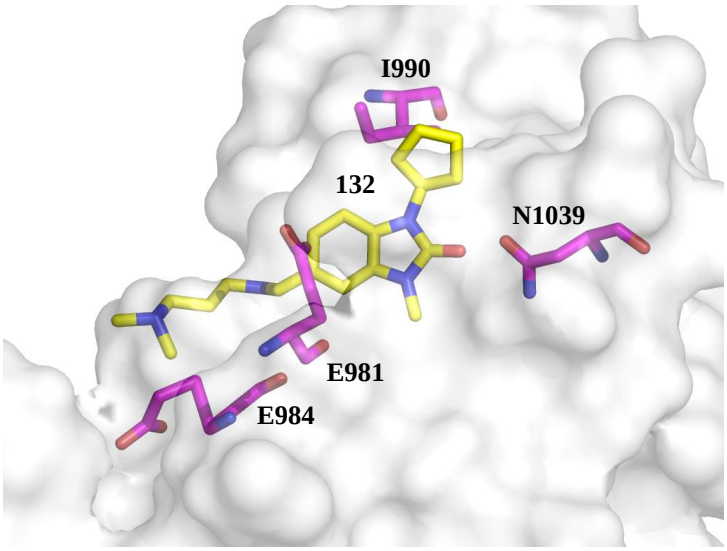
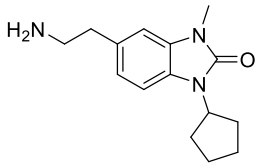
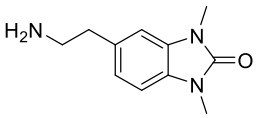


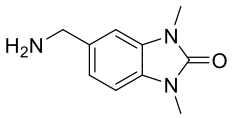
Figure 3.13: Compound 132 (yellow) docked in TRIM33β (PDB ID: 5MR8) using CLC Drug Discovery Workbench. It is predicted the cyclopentyl ring can form favourable interactions with I990 (magenta).



158
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 41.4 μM
TRIM33β IC_{50} = 182 μM



27
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 65.1 μM
TRIM33β IC_{50} >250 μM



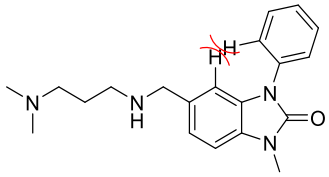
26
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 7.83 μM
TRIM33β IC_{50} = 30.6 μM

Figure 3.14: Compound 158 has two carbon atoms between the benzimidazolone core and the basic group. Compound **158** was synthesised to try and maximise interactions with N1039 and E981. The IC_{50} values were determined using a dose-response AlphaScreen™ assay with $H3_{1-27}$K18Ac peptide.

Alkenes **129** and **142** and sulfones **131**, **140** and **144** were designed to reduce the flexibility of the headgroup substituent. Compounds **131, 140**, **142** and **144** did not bind to TRIM33β. Alkene **129** (IC_{50} = 53.3 μM) had increased affinity for TRIM33β relative to alkyl **126** (IC_{50} = 100 μM). Compounds **129** and **142** have the same substituents but opposite vectors (C-5' *vs.* C-5). Compound **142** which has the benzylic substituent on C-5 did not bind to either isoform of TRIM33. This demonstrates the importance of having the correct vector between the benzylic amine and the headgroup nitrogen substituent.

The C-5' substituted hydroxyl **135** (IC_{50} = 20.6 μM) had higher affinity for TRIM33β than phenyl **125** (IC_{50} = 59.7 μM). The increase in affinity is possibly due to the hydroxyl group also interacting with N1039.

C-5 substituted phenyl **143** did not bind to TRIM33β and had poor affinity for TRIM33α (IC_{50} = 38.8 μM). It is probable that the phenyl ring does not lie flat relative to the benzimidazolone core due to steric clashes between aromatic protons (Figure 3.15). This conformation is unlikely to be accommodated in the KAc-binding pocket.



143

Figure 3.15: Compound 143 is unlikely to lie flat due to steric clashes between aromatic protons.

Compound **130**, which contains the bulky adamantyl group, was synthesised to see if large, bulky substituents on the benzimidazolone core are tolerated. Binding was abrogated for TRIM33 suggesting the binding pocket is not big enough to accommodate such large groups.

A possible explanation for the lack of affinity gained from introducing a substituent on the benzimidazolone core is that the region above the KAc-binding pocket is very solvent exposed. Any interactions with this region will therefore have a desolvation penalty associated with them and it may be that no binding event is sufficient to compensate for this desolvation penalty.

Table 3.11: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(BRD) – $H3_{1-27}$K18Ac. Ligand efficiencies are given in parentheses. *IC_{50} values are averaged over 14 repeats.

High affinity — Low affinity

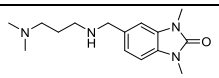
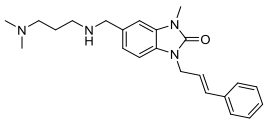
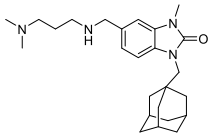
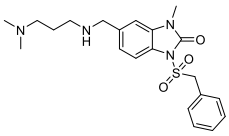
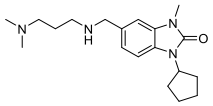
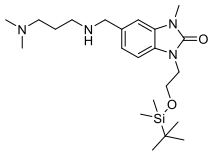
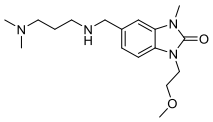
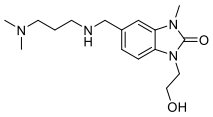
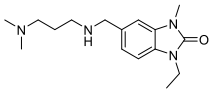
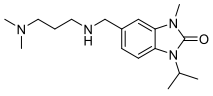
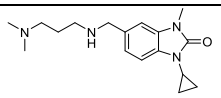
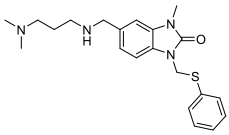
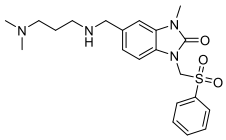
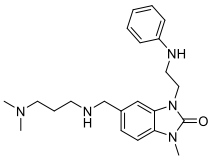
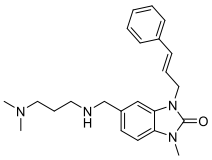
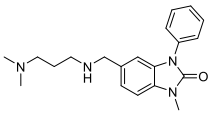
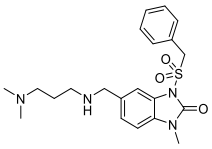
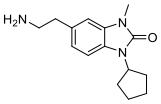
Compound	Structure	TRIM24 – H3(BRD) IC_{50} (µM)	TRIM33α – H3(BRD) IC_{50} (µM)	TRIM33β – H3(BRD) IC_{50} (µM)
*90		>250	3.61 ± 0.352 (0.38)	13.7 ± 1.53 (0.35)
129		>250	2.01 ± 0.240 (0.28)	53.3 ± 7.89 (0.21)
130		>250	>250	>250
131		>250	>250	>250
132		>250	4.32 ± 0.351 (0.31)	60.2 ± 7.42 (0.20)
133		>250	9.87 ± 2.16 (0.24)	>250
134		>250	16.4 ± 1.08 (0.29)	>250
135		>250	8.74 ± 0.819 (0.32)	20.6 ± 1.94 (0.30)
136		>250	2.07 ± 0.200 (0.38)	14.7 ± 1.47 (0.32)
137		>250	3.22 ± 0.246 (0.35)	19.6 ± 1.64 (0.30)

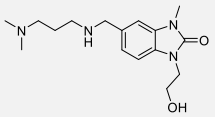
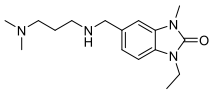
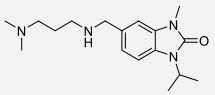
Table 3.11 continued.

Compound	Structure	TRIM24 – H3(BRD) IC_{50} (μM)	TRIM33α – H3(BRD) IC_{50} (μM)	TRIM33β – H3(BRD) IC_{50} (μM)
138		124 ± 32.3 (0.25)	9.01 ± 1.66 (0.32)	96.0 ± 24.9 (0.26)
139		>250	1.42 ± 0.131 (0.30)	>250
140		>250	>250	>250
141		>250	>250	>250
142		>250	>250	>250
143		>250	38.8 ± 1.15 (0.25)	>250
144		>250	>250	>250
158		>250	41.4 ± 0.317 (0.32)	182 ± 50.9 (0.28)

3.6.3.2 WaterLOGSY Experiments

WaterLOGSY experiments were performed to validate binding of **135**, **136** and **137** to TRIM33 (Table 3.12). The compounds were observed to bind to both isoforms of TRIM33 and showed selectivity over TRIM24.

Table 3.12: Summary of waterLOGSY results for 135, 136 and 137. Compounds which were observed to bind are represented by ✓. Compounds which did not appear to bind are represented by ✖.

Compound	TRIM24	TRIM33α	TRIM33β
 **135**	✖	✓	✓
 **136**	✖	✓	✓
 **137**	✖	✓	✓

3.7 Compound Optimisation 6

Joe Bluck performed MD studies on **90** and generated barcode plots depicting the proximities of the benzylic nitrogen and the terminal nitrogen of the amine chain to residues E981 and E984 (Figure 3.16). Joe Bluck docked the ligands using AutoDock4 and performed the simulations using GROMACS 2016.3. During the simulations only the terminal amine of **90** was protonated, despite multiple pK_a predictions indicating that both the benzylic amine and terminal amine should be protonated. Only one amine was protonated during the MD studies as problems were encountered if both amines were protonated and a solution could not be found. The binding site waters were retained. The top plot has the amine substituent pointing away from the binding site and the bottom plot has the amine substituent pointing towards the binding site. As observed for **23**, the benzylic nitrogen of **90** remains in close proximity to E981 throughout the simulations, especially when the amine substituent points towards the binding site, suggesting a strong interaction. During the simulations the terminal amine frequently interacts with E981, leaving E984 solvent exposed. As E984 is a largely solvent exposed residue, any interactions with E984 will have an associated desolvation penalty.

Based on these observations, compounds were designed to maximise interactions with E981. Interactions where both the benzylic and terminal amines are interacting with E981 will be

described as ‘pincer’ interactions. Strategies will include reducing the flexibility of the amine chain and biasing its conformation towards a ‘pincer’ interaction.

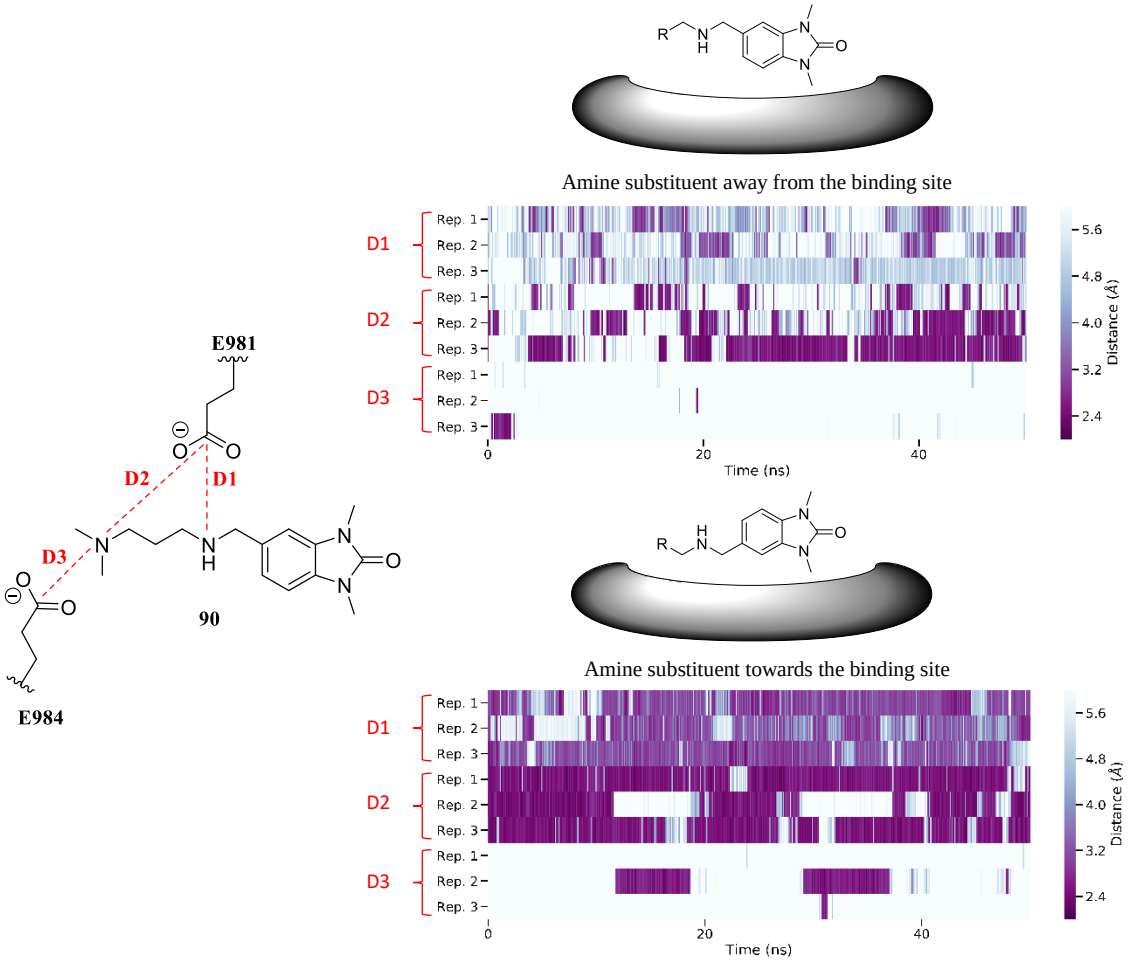


Figure 3.16: Barcode plots displaying distances between residues E981 and E984 and the benzylic and terminal nitrogens of 90 (D1, D2 and D3). Simulations were performed using GROMACS 2016.3 using the AMBER99SB-ILDN forcefield. The ligands were parameterised using the General AMBER ForceField with AM1-BCC charges (Joe Bluck).

3.7.1 Compound Design

Compounds were designed to restrict the conformation of the amine chain towards a ‘pincer’ interaction with E981 (Figure 3.17). It was hypothesised that the introduction of a gem-dimethyl group (**160**) or phenyl ring (**161** and **162**) on the amine chain would compress the chain and bias it towards a ‘pincer’ interaction. Compounds **163**, **164**, **169** and **170** have a pyrrolidine ring in the amine chain. Similarly, compounds **165** and **168** have a piperidine ring. The rings should reduce the flexibility of the amine chain and hopefully favour a conformation which allows a ‘pincer’ interaction with E981.

The AstraZeneca compound collection was searched for amines constrained in a ring. Compounds **166**, **(*R*)-167** and **(*S*)-167** were designed based on these amines. The fused rings should reduce the flexibility of the amine chain and hopefully favour interactions with E981.

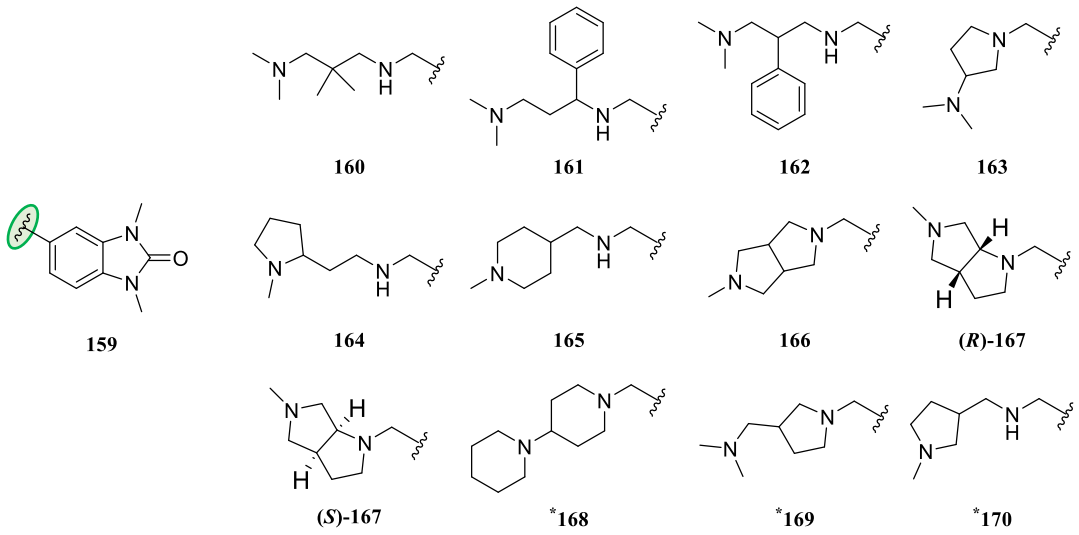
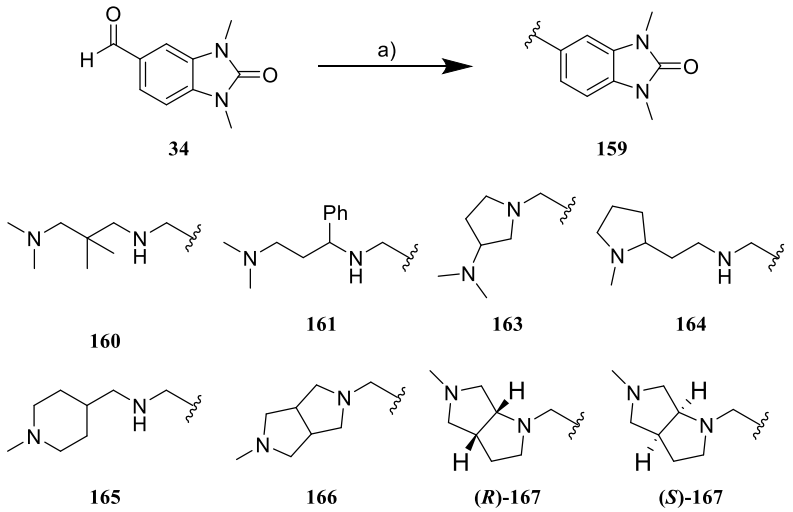


Figure 3.17: Compounds designed to favour a 'pincer' interaction with E981. *Analogues synthesised by Dr Larissa See.[114]

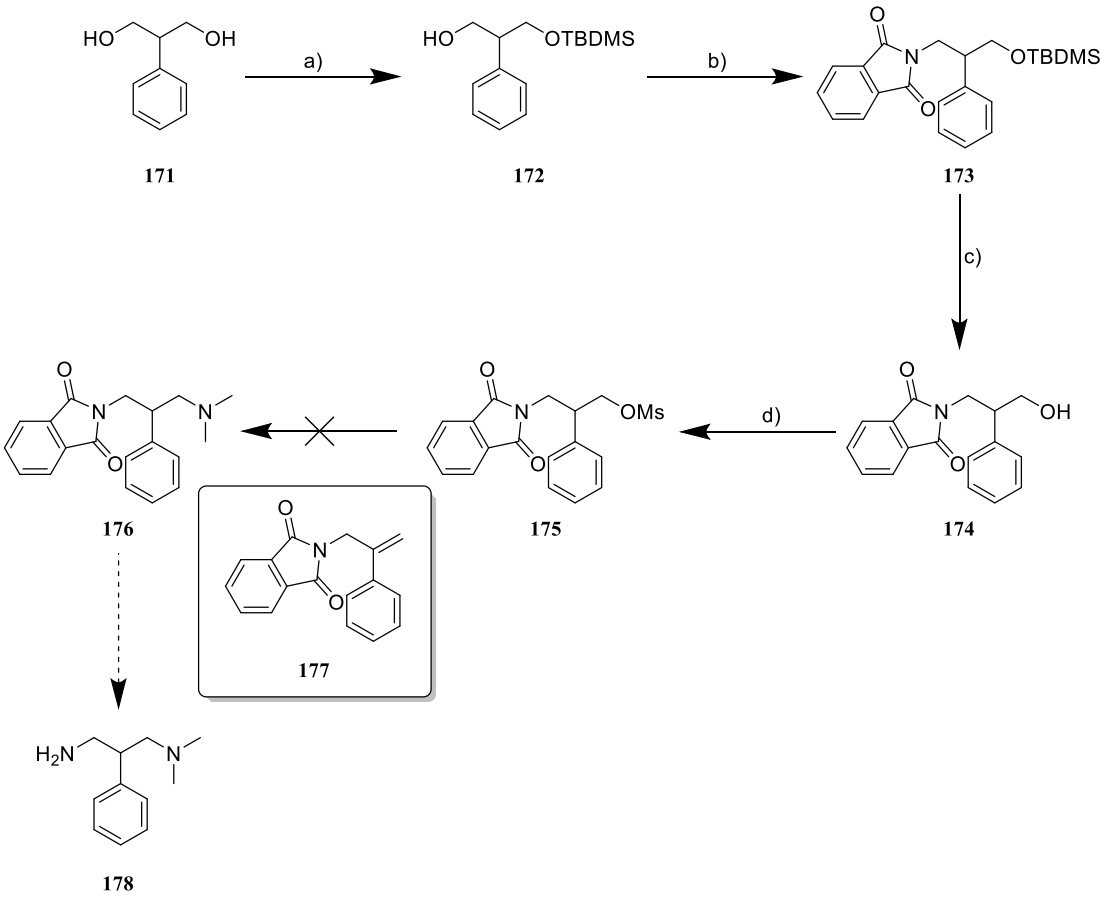
3.7.2 Synthesis of Compounds

Compounds were synthesised *via* a reductive amination between aldehyde **34** and the relevant amine (Scheme 3.18). Compounds **168**, **169** and **170** were synthesised by Dr Larissa See.[114]

To synthesise amine **178**, diol **171** was monoprotected with a *tert*-butyldimethylsilyl (TBDMS) group to yield **172** (Scheme 3.19). A Mitsunobu reaction converted the free alcohol to phthalimide **173**.[157] The TBDMS group was then removed using HCl to yield **174**, which was subsequently mesylated to afford **175**. Unfortunately, efforts to substitute the mesyl group with dimethylamine were unsuccessful, yielding a mixture of starting material and elimination product **177**.



Scheme 3.18: Synthesis of amine analogues. *Reagents and conditions*: a) (i) Amine, AcOH, DCE, 3 Å molecular sieves, 0 °C; (ii) $NaBH(OAc)_3$, 0 °C → rt, **160**: 51%, n = 1, **161**: 31%, n = 1, **163**: 47%, n = 1, **164**: 3%, n = 1, **165**: 18%, n = 1, **166**: 61%, n = 1, **(*R*)-167**: 54%, n = 1; **(*S*)-167**: 34%, n = 1.



Scheme 3.19: Attempted synthesis of amine 178. *Reagents and conditions*: a) TBDMSCl, NaH, THF, 0 °C → rt, 100%, n = 1; b) Phthalimide, DIAD, PPh_3, THF, 0 °C → rt, 84%, n = 1; c) HCl (aq.), THF, 66 °C, 80%, n = 1; d) MsCl, Et_3N, CH_2Cl_2, 0 °C → rt, 99%, n = 1.

3.7.3 Evaluation of Compounds

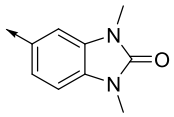
3.7.3.1 AlphaScreen™ Assays

Compounds were evaluated in a dose-response AlphaScreen™ assay (Table 3.13). Gem-dimethyl analogue **160** had lower affinity for both TRIM33α (IC_{50} = 19.8 μM) and TRIM33β (IC_{50} = 39.2 μM) than **90** (TRIM33α IC_{50} = 3.61 μM, TRIM33β IC_{50} = 13.7 μM). This indicates that the conformation of the amine chain enforced by the gem-dimethyl group or the increased steric bulk are not well accommodated in the binding pockets. In contrast, the phenyl derivative **161** had increased affinity for both TRIM33α (IC_{50} = 1.56 μM) and TRIM33β (IC_{50} = 4.83 μM). This suggests that the phenyl ring is enforcing a favourable conformation of the amine chain or is itself interacting with the protein, or both.

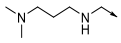
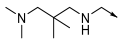
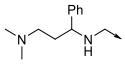
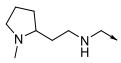
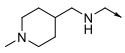
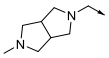
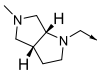
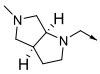
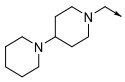
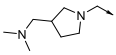
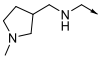
Compounds **163**, **165**, **169** and **170** showed similar affinity for TRIM33α (**163** IC_{50} = 6.61 μM, **165** IC_{50} = 5.49 μM, **169** IC_{50} = 5.81 μM, **170** IC_{50} = 1.60 μM) and TRIM33β (**163** IC_{50} = 12.8 μM, **165** IC_{50} = 12.0 μM, **169** IC_{50} = 15.2 μM, **170 =** 8.53 μM) to **90**, suggesting the conformation enforced by the pyrrolidine and piperidine rings is tolerated but is not improving interactions with the protein. In contrast, **164** was observed to have increased affinity for TRIM33α (IC_{50} = 1.52 μM) and TRIM33β (IC_{50} = 1.62 μM). The pyrrolidine ring is likely holding the amine chain in a conformation which favours a 'pincer' interaction with E981. Compound **168** has lower affinity for TRIM33β (IC_{50} = 40.7 μM), suggesting the conformation of the amine chain is not well accommodated in the binding pocket.

Relative to **90**, fused analogue **166** had lower affinity for both TRIM33α (IC_{50} = 25.6 μM) and TRIM33β (IC_{50} = 100 μM), suggesting the substituent is held in an unfavourable conformation. In contrast, fused analogue **(*R*)-167** maintained affinity for TRIM33α (IC_{50} = 3.97 μM) and TRIM33β (IC_{50} = 12.3 μM), but was no longer selective over TRIM24 (IC_{50} = 44.3 μM), enantiomer **(*S*)-167**, however, remained selective over TRIM24. It may be that **(*R*)-167** is able to make interactions with TRIM24 which **(*S*)-167** cannot make or enantiomer **(*S*)-167** forms unfavourable interactions with TRIM24 which are absent for **(*R*)-167**.

Table 3.13: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(BRD) – $H3_{1-27}$K18Ac. Ligand efficiencies are given in parentheses. *IC_{50} values are averaged over 14 repeats.



High affinity — Low affinity

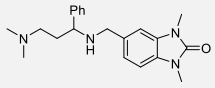
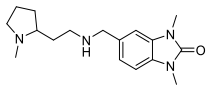
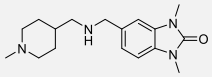
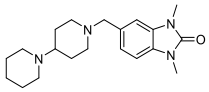
Compound	Substituent	TRIM24 – H3(BRD) IC_{50} (µM)	TRIM33α – H3(BRD) IC_{50} (µM)	TRIM33β – H3(BRD) IC_{50} (µM)
*90		>250	3.61 ± 0.352 (0.38)	13.7 ± 1.53 (0.35)
160		>250	19.8 ± 3.56 (0.30)	39.2 ± 8.41 (0.28)
161		>250	1.56 ± 0.100 (0.31)	4.83 ± 0.508 (0.29)
163		>250	6.61 ± 0.535 (0.35)	12.8 ± 2.09 (0.33)
164		>250	1.52 ± 0.0940 (0.37)	1.62 ± 0.140 (0.37)
165		>250	5.49 ± 1.12 (0.33)	12.0 ± 1.12 (0.31)
166		>250	25.6 ± 6.82 (0.29)	100 ± 20.7 (0.25)
(*R*)-167		44.3 ± 3.43 (0.28)	3.97 ± 0.858 (0.34)	12.3 ± 3.13 (0.31)
(*S*)-167		>250	5.79 ± 0.841 (0.33)	9.57 ± 1.74 (0.32)
168		>250	7.96 ± 1.11 (0.29)	40.7 ± 1.09 (0.25)
169		>250	5.81 ± 0.210 (0.33)	15.2 ± 0.787 (0.31)
170		>250	1.60 ± 0.137 (0.39)	8.53 ± 0.558 (0.34)

3.7.3.2 WaterLOGSY Experiments

Selected compounds were also evaluated in waterLOGSY NMR experiments (Table 3.14). Compounds **161**, **164** and **168** bound to TRIM33α and TRIM33β and demonstrated selectivity

over TRIM24. Piperidine **165** was observed to bind to all three proteins. The inversion of peaks to a positive signal for TRIM24 was, however, less definite, suggesting weaker binding.

Table 3.14: Summary of waterLOGSY results for 161, 164, 165 and 168. Compounds which were observed to bind are represented by ✓. Compounds which did not appear to bind are represented by ×.

Compound	TRIM24	TRIM33α	TRIM33β
 **161**	×	✓	✓
 **164**	×	✓	✓
 **165**	✓	✓	✓
 **168**	×	✓	✓

3.8 Compound Optimisation 7

3.8.1 Compound Design

Racemic phenyl analogue **161** had good affinity for TRIM33β (IC_{50} = 4.83 μM) and it may be that one enantiomer has higher affinity for the KAc-binding pocket than the other. The single enantiomers of **161** were therefore investigated to see if a further increase in binding affinity could be achieved (Figure 3.18).

Analogues **(*S*)-179** and **(*R*)-179** with the phenyl ring at the opposite end of the amine chain were also synthesised. These analogues provide information on whether extending off the amine chain is widely tolerated or limited to the carbon adjacent to the benzylic position (Figure 3.18).

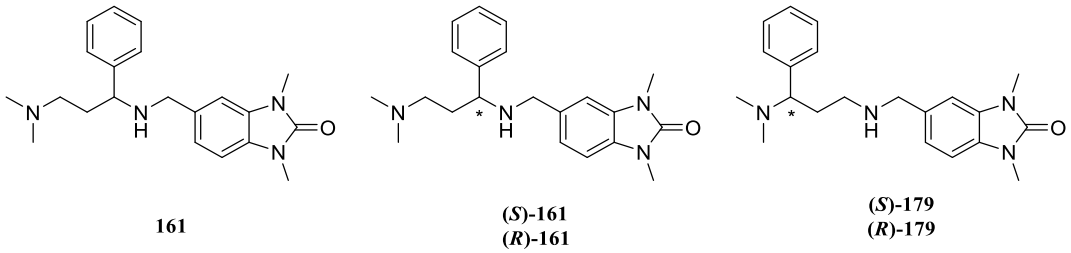
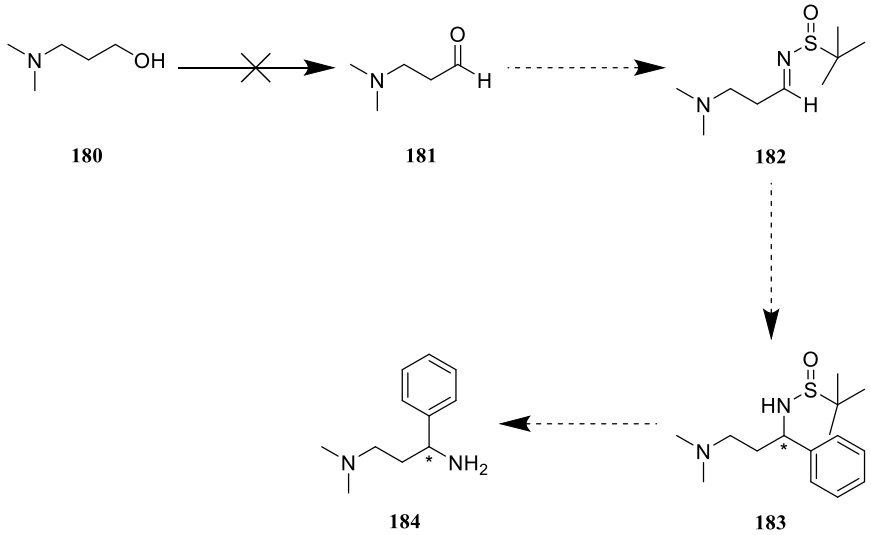


Figure 3.18: Compound 161 was synthesised as its single enantiomers to investigate whether one enantiomer has higher affinity than the other. Compounds **(*S*)-179** and **(*R*)-179** were synthesised to see if a substituent on the terminal amine chain carbon is tolerated.

3.8.2 Synthesis of Compounds

Several routes were attempted to obtain **(*S*)-161** and **(*R*)-161** (Scheme 3.20–Scheme 3.23).

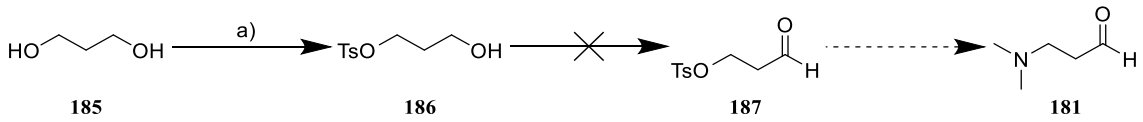
Initially it was proposed that the aldehyde **181** could be condensed with Ellman's chiral auxiliary to form chiral *tert*-butanesulfinyl imine **182** (Scheme 3.20).[158] Phenyl magnesium bromide could then be added to introduce the phenyl group (**183**) and the auxiliary removed to reveal the chiral primary amine **184**. There is, however, no literature precedent for the oxidation of alcohol **180** to aldehyde **181**, and attempts using DMP and MnO_2 failed to yield **181**.



Scheme 3.20: Proposed synthetic route to amine 184. Oxidation of alcohol **180** to aldehyde **181** was unsuccessful.

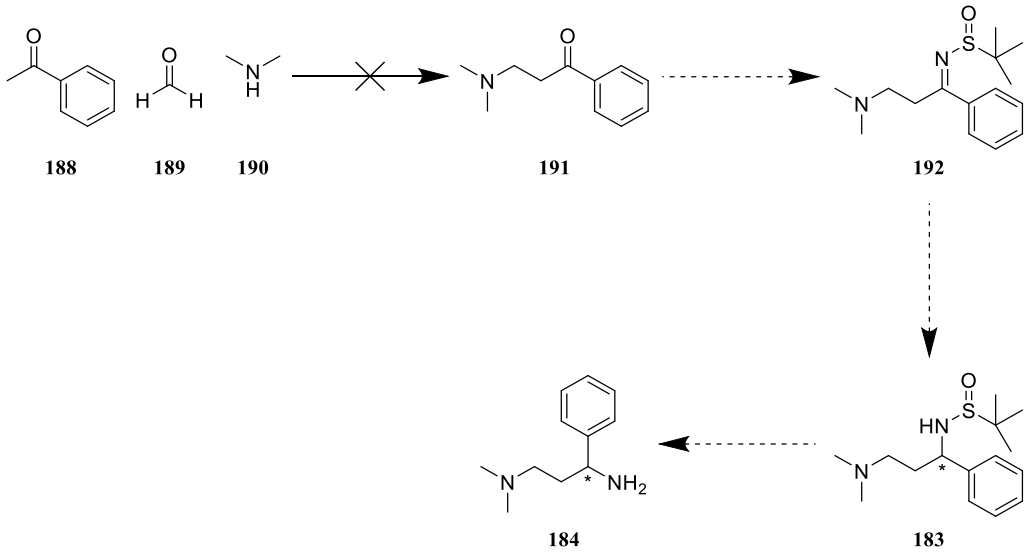
An alternative route to aldehyde **181** was attempted (Scheme 3.21). Diol **185** was tosylated to yield **186**. Oxidation using DMP initially looked promising as the crude ^{1}H NMR spectrum had a

large aldehyde peak. The compound was, however, unstable on silica, with *p*-toluenesulfonic acid being isolated following the presumed elimination of acrolein.



Scheme 3.21: Attempted synthesis of aldehyde 181. *Reagents and conditions*: a) TsCl, Et_3N, CH_2Cl_2, 0 °C → rt, 31–39%, n = 2.

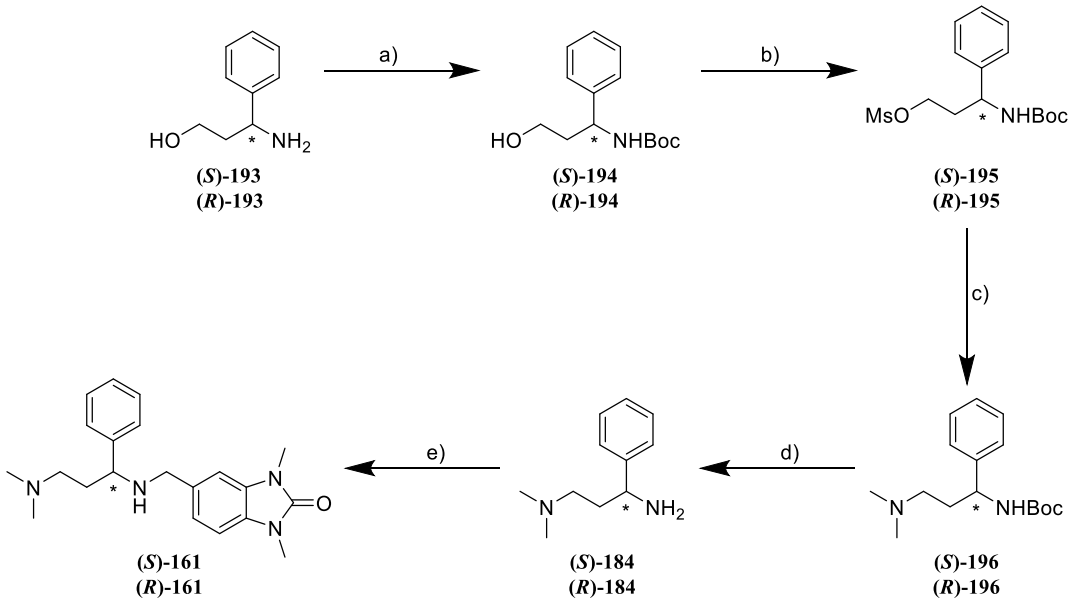
Another strategy to synthesise amine **184** was to use a Mannich reaction to obtain ketone **191** (Scheme 3.22).[159] Ketone **191** could then be reacted with Ellman's chiral auxiliary (**193**) and reduced to **183**.[158] Removal of the auxiliary would enable the chiral primary amine (**184**) to be obtained. The Mannich reaction between acetophenone (**188**), formaldehyde (**189**) and dimethylamine (**191**), however, failed to yield any product.



Scheme 3.22: Proposed synthetic route to amine 184. The Mannich reaction, however, proved unsuccessful.

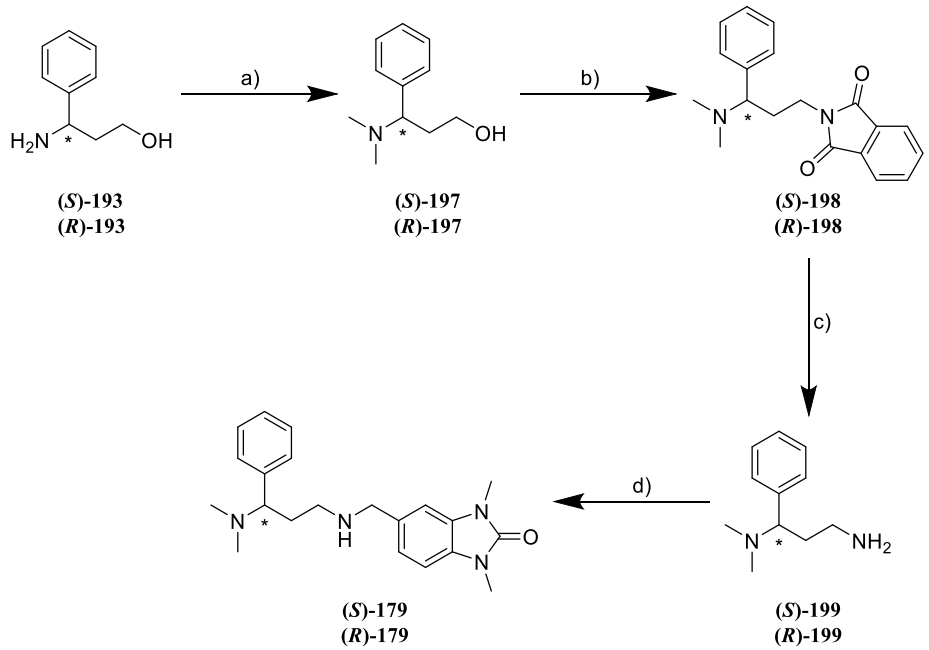
Compounds **(*S*)-161** and **(*R*)-161** were eventually synthesised from enantiomerically pure **(*S*)-193** and **(*R*)-193** (Scheme 3.23). Alkanolamines **(*S*)-193** and **(*R*)-193** were Boc protected to yield **(*S*)-194** and **(*R*)-194**, which were then mesylated to afford **(*S*)-195** and **(*R*)-195**, respectively. Substitution with dimethylamine and subsequent Boc deprotection yielded chiral amines **(*S*)-184**

and **(*R*)-184**, which were reacted with aldehyde **34** *via* a reductive amination reaction to yield enantiomerically pure **(*S*)-161** and **(*R*)-161**, respectively.



Scheme 3.23: Synthesis of (*S*)-161 and (*R*)-161. *Reagents and conditions*: a) Boc_2O, Et_3N, CH_2Cl_2, rt, **(*S*)-194**: 81–87%, n = 2, **(*R*)-194**: 72–75%, n = 2; b) MsCl, Et_3N, CH_2Cl_2, 0 °C → rt, **(*S*)-195**: 33–74%, n = 2, **(*R*)-195**: 72%, n = 1; c) $NHMe_2$ (THF), TBAI, 150 °C, mw, **(*S*)-196**: 36–60%, n = 3, **(*R*)-196**: 38–73%, n = 3; d) HCl (4 M, dioxane), rt, **(*S*)-184**: 67–89%, n = 2, **(*R*)-184**: 98%, n = 1; e) (i) **34**, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, **(*S*)-161**: 29–68%, n = 2, **(*R*)-161**: 34–79%, n = 2.

The amine chains required for the synthesis of analogues **(*S*)-179** and **(*R*)-179** were also synthesised from enantiomerically pure alkanolamines **(*S*)-193** and **(*R*)-193** (Scheme 3.24). Formaldehyde and formic acid were employed in an Eschweiler-Clarke reaction to dimethylate **(*S*)-193** and **(*R*)-193**.[160,161] A Mitsunobu reaction was next used to convert alcohols **(*S*)-197** and **(*R*)-197** to phthalimides **(*S*)-198** and **(*R*)-198**, respectively.[157] The amines were subsequently deprotected using hydrazine to yield primary amines **(*S*)-199** and **(*R*)-199**, which were reacted *via* a reductive amination with aldehyde **34** to yield final compounds **(*S*)-179** and **(*R*)-179**, respectively.



Scheme 3.24: Synthesis of (*S*)-179 and (*R*)-179. *Reagents and conditions*: a) Formic acid, formaldehyde, 100 °C, **(*S*)-198**: 51%, n = 1, **(*R*)-198**: 87%, n = 1; b) Phthalimide, DIAD, PPh_3, THF, 0 °C → rt, **(*S*)-199**: 67%, n = 1, **(*R*)-199**: 74%, n = 1; c) $NH_2NH_2 \cdot H_2O$, EtOH, 70 °C, **(*S*)-200**: 74%, n = 1, **(*R*)-200**: 64%, n = 1; d) (i) **34**, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, **(*S*)-179**: 25%, n = 1, **(*R*)-179**: 26%, n = 1.

3.8.3 Evaluation of Compounds

3.8.3.1 AlphaScreen™ Assays

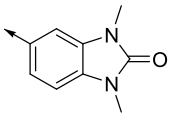
Compounds **(*S*)-161**, **(*R*)-161**, **(*S*)-179** and **(*R*)-179** were evaluated in a dose-response AlphaScreen™ assay (Table 3.15).

Single enantiomers **(*S*)-161** (IC_{50} = 1.32 µM) and **(*R*)-161** (IC_{50} = 2.45 µM) had similar affinities to racemic **161** (IC_{50} = 1.56 µM) for TRIM33α. There was also only a small difference in affinity of **(*S*)-161** (IC_{50} = 4.52 µM) and **(*R*)-161** (IC_{50} = 7.75 µM) for TRIM33β, suggesting the stereochemistry is unimportant for binding affinity.

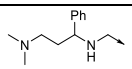
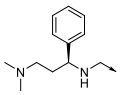
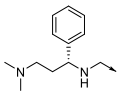
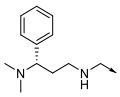
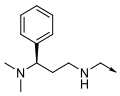
Compounds **(*S*)-179** (TRIM33α IC_{50} = 5.94 µM, TRIM33β IC_{50} = 9.63 µM) and **(*R*)-179** (TRIM33α IC_{50} = 5.56 µM, TRIM33β IC_{50} = 12.2 µM) had a small reduction in affinity for TRIM33α and TRIM33β, compared to regioisomers **(*S*)-161** and **(*R*)-161**. Compounds **(*S*)-179** and **(*R*)-179**, however, had similar affinities to **90** for TRIM33. These results suggest the phenyl

ring in **(*S*)-179** and **(*R*)-179** is accommodated in the KAc-binding pocket, but is neither making any additional interactions with the protein nor enforcing a favourable conformation.

Table 3.15: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(BRD) – $H3_{1-27}$K18Ac. Ligand efficiencies are given in parentheses.



High affinity — Low affinity

Compound	Substituent	TRIM24 – H3(BRD) IC_{50} (µM)	TRIM33α – H3(BRD) IC_{50} (µM)	TRIM33β – H3(BRD) IC_{50} (µM)
90		>250	1.56 ± 0.100 (0.31)	4.83 ± 0.508 (0.29)
(*S*)-161		>250	1.32 ± 0.0630 (0.32)	4.52 ± 0.596 (0.29)
(*R*)-161		>250	2.45 ± 0.241 (0.30)	7.75 ± 0.599 (0.28)
(*S*)-179		>250	5.94 ± 0.619 (0.28)	9.63 ± 2.00 (0.27)
(*R*)-179		>250	5.56 ± 0.559 (0.28)	12.2 ± 1.55 (0.26)

3.9 ITC Experiments

ITC is used to determine the thermodynamic parameters of a binding event. Determining K_D values allows ranking of binding affinities for different proteins. The IC_{50} values obtained using AlphaScreen™ assays for different protein-peptide combinations cannot be ranked as they are dependent on the assay conditions. ITC experiments for **90**, **111**, **112**, **164** and **170** were performed by Dr Larissa See[114] and Jessica Reynolds. Example traces are provided in Figure 3.19 and Figure 3.20. All ITC traces can be found in Appendix C.

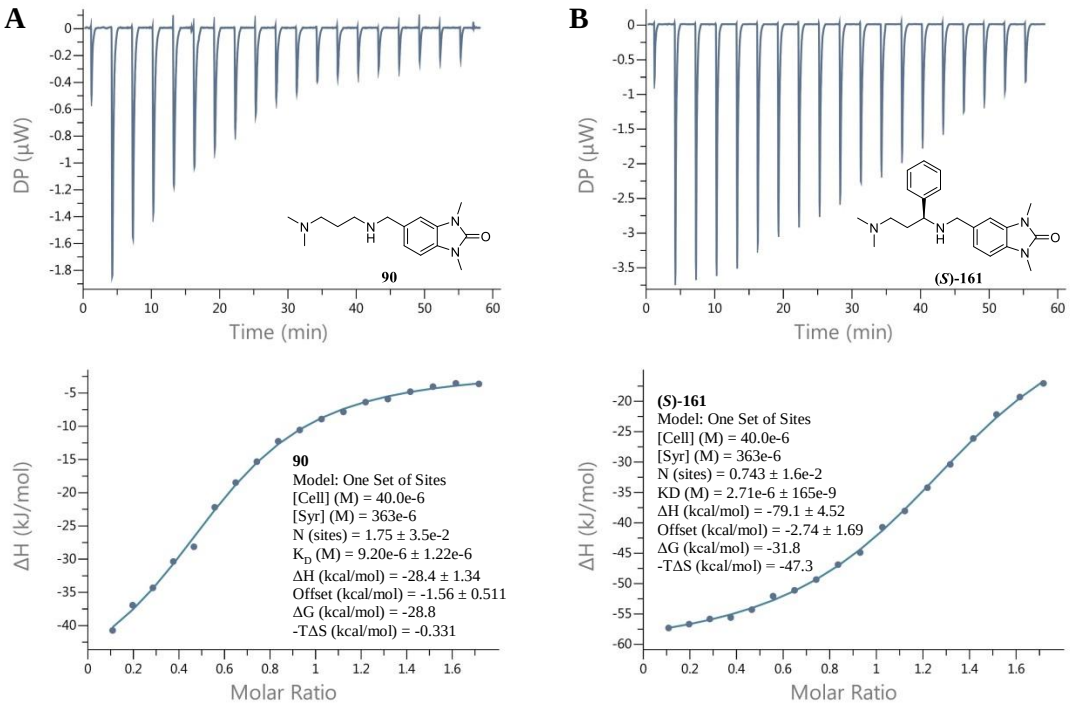


Figure 3.19: ITC data for 90 and (*S*)-161 measured with TRIM33α. (A) ITC trace for **90**. (B) ITC trace for **(*S*)-161**. Shown are the heat effects for each injection (above) and the normalised binding isotherms (below) including the fitted function (solid line).

The K_D values obtained from the ITC experiments are shown in Table 3.16. The IC_{50} values obtained from AlphaScreen™ assays for TRIM33 are also shown for comparison. Of the compounds evaluated by ITC, phenyl **(*S*)-161** has the highest affinity for TRIM33α and TRIM33β. Interestingly all six compounds have higher affinity for TRIM33α than TRIM33β, despite TRIM33α having a non-canonical bromodomain. Although the ranking of compounds by K_D value differs to the ranking of compounds by IC_{50} value, there is good agreement between the ITC data and AlphaScreen™ data. For example, the K_D and IC_{50} values show **(*S*)-161** has high affinity for both TRIM33α and TRIM33β. This data validates AlphaScreen™ as a good method for evaluating ligand binding to TRIM33.

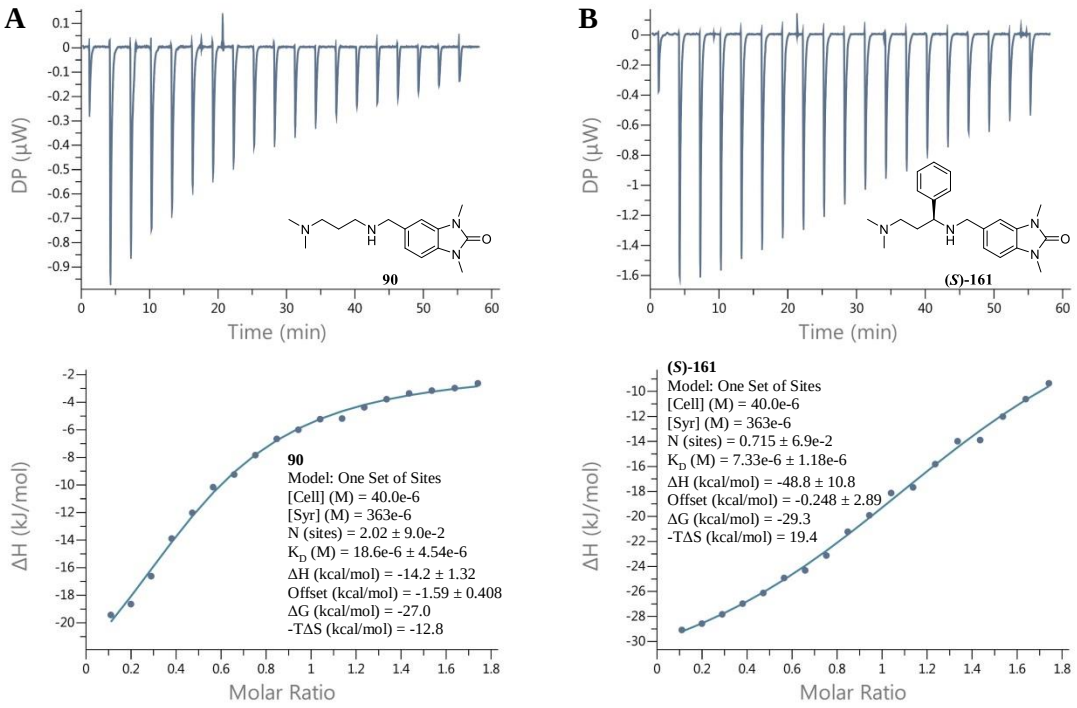
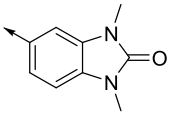
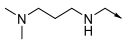
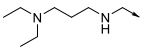
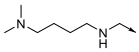
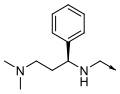
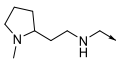
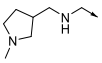


Figure 3.20: ITC data for 90 and (*S*)-161 measured on TRIM33β. (A) ITC trace for **90**. (B) ITC trace for **(*S*)-161**. Shown are the heat effects for each injection (above) and the normalised binding isotherms (below) including the fitted function (solid line).

Table 3.16: K_D values (μM) obtained using ITC for 90, 111, 112, (*S*)-161, 164 and 170. IC_{50} values (μM) determined using a dose-response AlphaScreen™ assay ($H3_{1–27}K18Ac$) are also shown for comparison. Colour scale from strongly binding (red) to weakly binding (green).



High affinity — Low affinity

Compound	TRIM33α (K_D)	TRIM33α (IC_{50})	TRIM33β (K_D)	TRIM33β (IC_{50})
 **90**	9.20 ± 1.22	3.61 ± 0.352	18.6 ± 4.54	13.7 ± 1.53
 **111**	5.46 ± 0.456	4.74 ± 1.14	11.3 ± 2.55	2.02 ± 1.04
 **112**	9.92 ± 0.819	7.96 ± 1.11	18.3 ± 2.71	2.73 ± 1.14
 **(*S*)-161**	2.71 ± 0.165	1.32 ± 0.0630	7.33 ± 1.18	4.52 ± 0.596
 **164**	5.23 ± 0.00530	1.52 ± 0.0940	7.82 ± 3.37	1.62 ± 0.140
 **170**	5.26 ± 0.274	1.60 ± 0.140	7.81 ± 1.10	8.53 ± 0.560

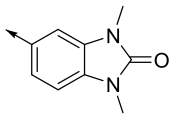
3.10 Validation of Binding

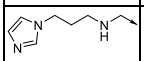
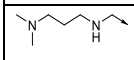
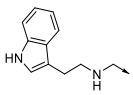
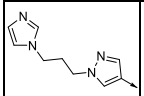
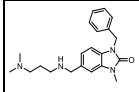
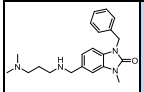
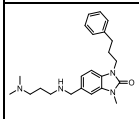
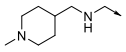
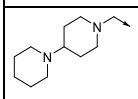
The AlphaScreen™ TruHits™ kit was used to identify false positives and compounds interfering in the AlphaScreen™ assay (Table 3.17).

Some dose-dependent assay interference was observed. The greatest interference was observed for tryptamine **91** and interference was also observed for pyrazole **92** and imidazoles **94** and **118**. Tryptamine,[162,163] pyrazole[164] and imidazole[165] are all known to quench reactive oxygen species. This explains the interference of these compounds in the AlphaScreen™ assay as they are quenching the singlet oxygen produced by the donor beads. Some assay interference was also observed for compounds **124**, **125** and **126**, which all have a phenyl substituent on a headgroup

nitrogen. The interference was only dose-dependent for **126**. WaterLOGSY experiments, however, confirmed binding of these compounds to TRIM33α and TRIM33β.

Table 3.17: Results from the AlphaScreen™ TruHits™ kit. Compounds were screened at five concentrations and with two incubation formats. Results which are more than two standard deviations greater than the mean DMSO control are highlighted in blue.



Structure	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
 **89**	1.23	0.109	0.827	1.59	4.18	1.03	0.454	0.129	0.222	0.600
 **90**	0.352	0.114	1.01	0.945	2.44	0.0100	0.160	0.0650	0.256	0.806
 **91**	0.602	2.26	5.53	8.84	15.0	0.294	0.0360	0.431	1.06	2.04
92	1.88	1.22	0.376	0.170	1.72	0.457	0.698	1.20	1.57	2.02
94	1.47	0.117	2.91	5.71	11.4	1.25	0.211	0.884	1.67	2.69
 **118**	0.715	2.35	3.93	5.71	9.88	0.245	0.306	0.574	1.30	2.18
 **124**	1.60	0.262	1.48	1.88	3.29	2.84	2.31	2.90	2.36	1.94
 **125**	2.60	0.675	0.988	1.50	3.46	4.72	3.16	3.47	3.40	3.20
 **126**	0.659	2.61	4.97	5.59	8.26	3.38	2.31	2.05	2.00	2.05
 **167**	1.37	0.389	0.119	0.491	1.80	0.749	0.0270	0.137	0.387	0.178
 **168**	0.876	0.00200	0.970	1.04	2.18	0.417	1.33	0.413	0.137	0.533
Biotin	-	-	-	-	-	5.86	5.96	6.14	6.22	6.27

3.11 Water Molecules

Despite an extensive investigation, a relatively flat SAR was observed across the benzimidazolone series. Bromodomains typically have four or five structural water molecules at the base of the binding pocket.[147] Bromodomains have also been crystallised with additional water molecules in the ZA channel. The importance of considering ZA channel water molecules is reported in the literature.[147,148] Joe Bluck therefore calculated the binding energies of the ZA water molecules by performing a Grand Canonical Monte Carlo (GCMC) simulation using ProtoMS (Figure 3.21 and Table 3.20).[166,167] Water 7 interacts with the backbones of I980 and E984 and has a negative binding energy (–3.33 kcal mol^{-1}). Water molecule 7 is therefore predicted to be difficult to displace. Water molecules 5 (+1.71 kcal mol^{-1}) and 6 (+0.82 kcal mol^{-1}) have positive binding energies and should therefore be displaceable.

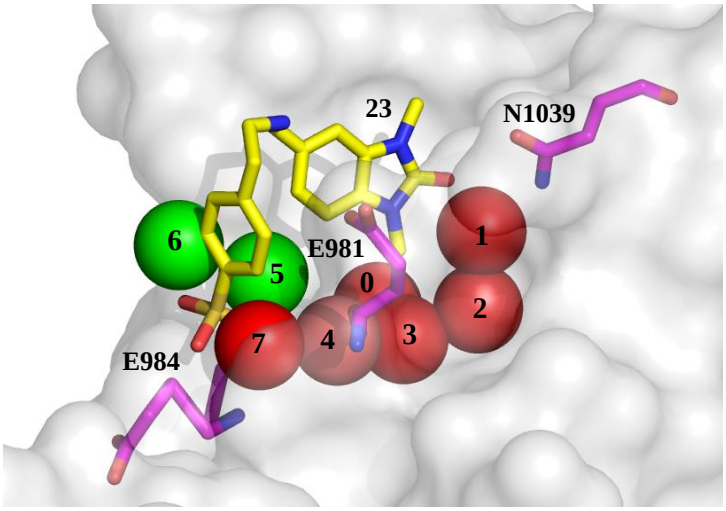


Figure 3.21: Compound 23 (yellow) docked in TRIM33β (PDB ID: 5MR8) using CLC Drug Discovery Workbench. The structural water molecules and water molecules in the ZA channel are shown. Water molecules in red have negative binding energies and water molecules in green have positive binding energies. Key residues are shown in the stick representation (magenta).

Table 3.20. Binding energies of the structural water molecules and water molecules in the ZA channel (Figure 3.21). The general protocol for water binding energy calculations was taken from Aldeghi.[166] The ZA channel was encompassed by a box shaped boundary, in which a GCMC simulation was performed using ProtoMS (version 3.3) that added and removed water molecules over 50M moves.[167] Using Grand Canonical Integration (GCI) it was possible to calculate the energy of moving waters from the ideal gas phase to the regions of the box where crystallographic waters are found, and hence estimate the binding energy of the crystallographic waters at the base of the binding pocket and in the ZA channel (Joe Bluck).

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

As water 7 has a negative binding energy, it is hypothesised that E984 is in fact a solvent exposed residue as it cannot reside in the ZA channel. To lie in the ZA channel E984 needs to be desolvated and displace water 7, there is therefore predicted to be a large energy penalty associated with this conformation. Residue E984 is consequently unlikely to interact with the amine chains of benzimidazolone analogues.

Water 7 also prevents the amine chain from being directed into the ZA channel and the chain is consequently predicted to be solvent exposed. Modifications to the amine chain are therefore unlikely to have any effect on binding affinity, possibly explaining the flat SAR observed. The presence of water 7 in the ZA channel further confirms that a 'pincer' interaction targeting E981, whilst avoiding the displacement of water 7, could provide a large increase in affinity.

Phenyl **(*S*)-161** has higher affinity for TRIM33 than alkyl **90**. Docking of **(*S*)-161** in TRIM33β using CLC Drug Discovery Workbench suggests that the phenyl ring displaces water molecule 6 (Figure 3.22). The corresponding increase in solvent entropy could explain the higher affinity of **(*S*)-161**.

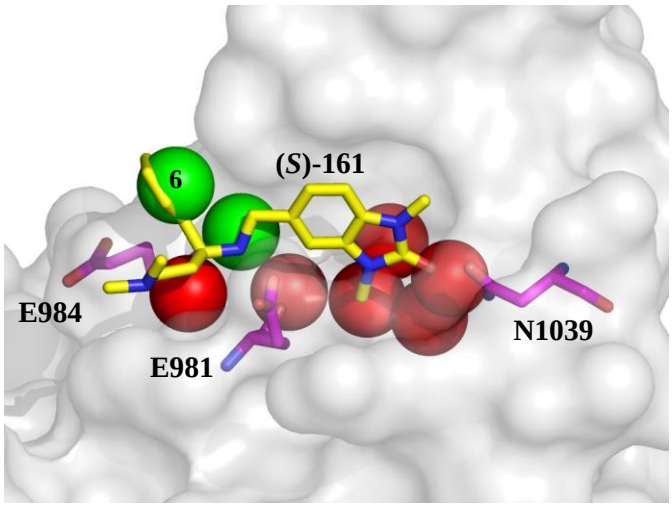


Figure 3.22: Phenyl (*S*)-161 docked in TRIM33β (PDB ID: 5MR8) using CLC Drug Discovery Workbench. Compound **(*S*)-161** (yellow) docked in TRIM33β with the water molecules overlaid. The phenyl ring is observed to occupy the same space as water 6. The key residues are shown in the stick representation (magenta).

3.12 Compound Optimisation 8

3.12.1 Compound Design

The identification of a structural water molecule in the ZA channel confirmed the hypothesis that a 'pincer' interaction with E981, avoiding displacing water 7, should increase affinity for TRIM33. Docking of urea analogue **200** in TRIM33β using CLC Drug Discovery Workbench showed the urea moiety forming a 'pincer' interaction with E981 (Figure 3.23). A benzyl urea was chosen as docking implied that the phenyl ring displaces water 6 and should therefore provide a positive entropic contribution, similar to **(*S*)-161**. The phenyl ring can also form hydrophobic interactions with I980.

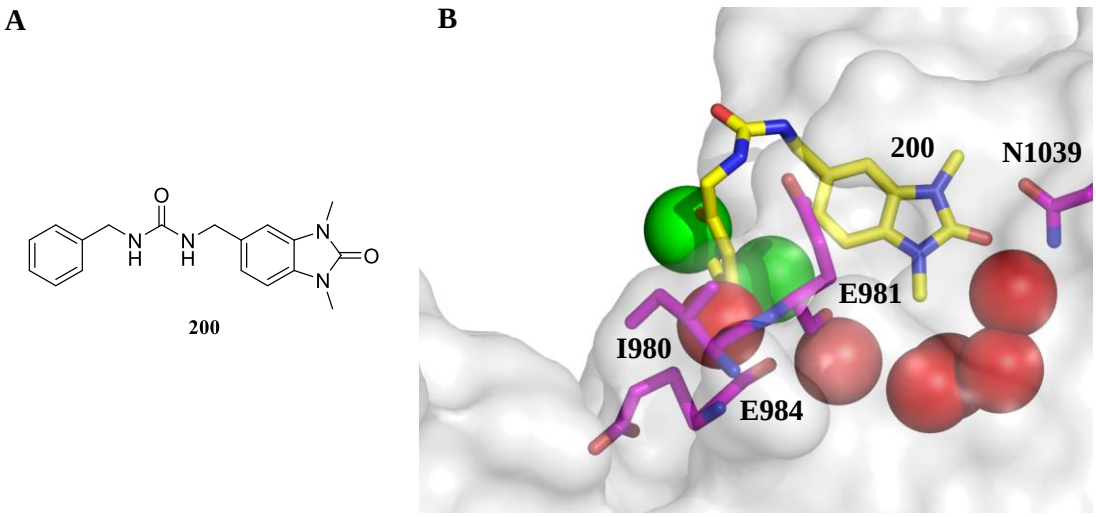
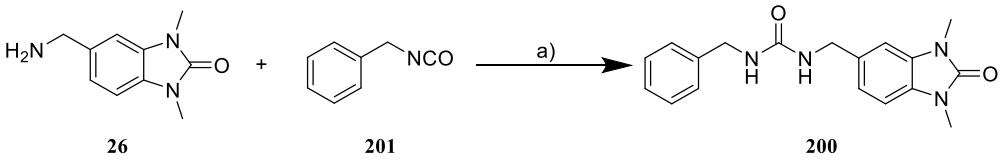


Figure 3.23: Urea 200 was designed to make a 'pincer' interaction with E981. (A) Structure of urea **200**. (B) Compound **200** (yellow) docked in TRIM33β (PDB ID: 5MR8) using CLC Drug Discovery Workbench with the water molecules overlaid. The phenyl ring is observed to occupy the same space as water 6. The key residues are shown in the stick representation (magenta).

3.12.2 Synthesis of Compounds

Urea **200** was synthesised by reacting amine **26** with benzyl isocyanate (**201**) (Scheme 3.25). This yielded **200** in 49% yield.



Scheme 3.25: Synthesis of urea substituted 200. *Reagents and conditions*: a) THF, rt, 49%, n = 1.

3.12.3 Evaluation of Compounds

3.12.3.1 AlphaScreen™ Assays

Compound **200** was evaluated in a dose-response AlphaScreen™ assay. Interestingly **200** did not bind to TRIM24 or TRIM33, this may be due to the benzyl substituent being held in an orientation which is not accommodated in the binding pockets. Future work will investigate synthesising urea analogues with a range of substituents to gain affinity for TRIM33.

3.13 Selectivity Against BRD4

High levels of selectivity are important for chemical probes. BRD4 belongs to the BET family of bromodomains, and due to its druggability, a large number of BRD4 inhibitors have been developed.[168,169] A broad phenotype is observed for BET inhibitors and consequently achieving selectivity over the BET family is highly desirable.[170] Selectivity against BRD4(1) was therefore considered. Compounds **23** and **90** were evaluated for binding to BRD4(1) using an AlphaScreen™ assay (Figure 3.24). Peptide $H4_{1–20}(KAc)_4$ was used as the binding partner. Pleasingly, selectivity over BRD4 was observed.

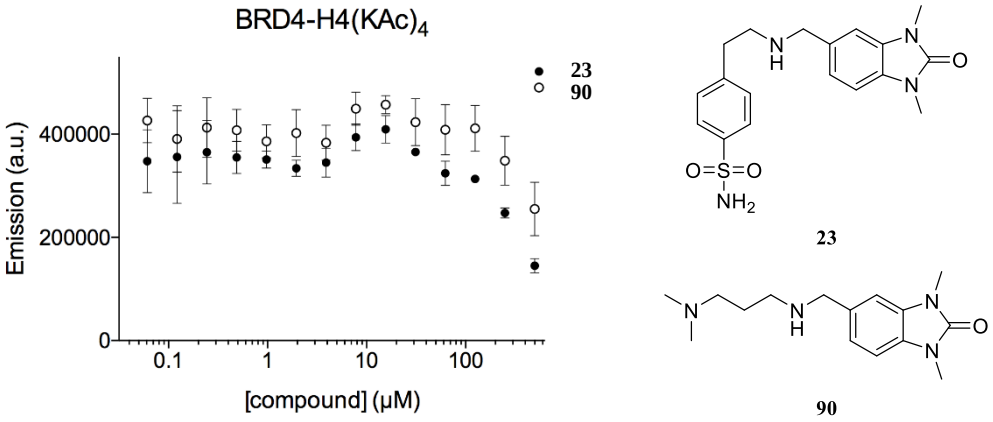
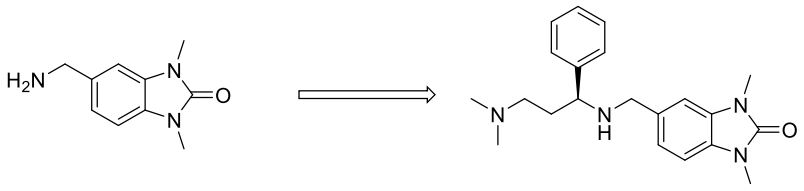


Figure 3.24: Dose-response curves for 23 and 90 obtained using an AlphaScreen™ assay. BRD4(1) in combination with $H4_{1–20}(KAc)_4$. Compounds were serially diluted 1:2 from 500 μM highest compound concentration.

3.14 Conclusions

In this chapter the amine chain of **26** was investigated extensively. The introduction of an amine chain increased affinity for TRIM33 without causing a large drop in ligand efficiency or loss of selectivity over TRIM24. Despite an extensive investigation, a relatively flat SAR was observed across the benzimidazolone series. A structural water molecule in the ZA channel was identified, indicating that E984 is largely solvent exposed. Interactions with residues in the ZA channel are therefore considered challenging and will have a desolvation penalty associated with them. The greatest success came when the amine chain substituents were directed towards the displaceable water molecules in the ZA channel, as in **161**, which was subsequently synthesised as its single

enantiomers (**(*S*)-161** and **(*R*)-161**). Unfortunately, the two enantiomers had very similar affinities for TRIM33 (Figure 3.25).



26
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 7.83 μM (0.51)
TRIM33β IC_{50} = 30.6 μM (0.45)

(*S*)-161
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 1.32 μM (0.32)
TRIM33β IC_{50} = 4.52 μM (0.29)

Figure 3.25: Phenyl (*S*)-161 can displace water 6 from the ZA channel. The IC_{50} values were determined using a dose-response AlphaScreen™ assay with $H3_{1–27}$K18Ac peptide. Ligand efficiencies are given in parentheses.

The potential of gaining affinity by extending off the benzimidazolone core into the hydrophobic region above the KAc-binding pocket was explored further. Only small alkyl substituents were tolerated, these, however, had similar affinity to dimethyl analogue **90** and larger groups had a negative effect on binding. It was hypothesised that due to the solvent exposed nature of this region, any interactions will have a large desolvation penalty associated with them.

ITC experiments enabled the comparison of binding between the two isoforms of TRIM33. The ITC data show that the AlphaScreen™ assay is a good method for initial compound screening and largely correctly predicts the ranking of ligands.

Selectivity is especially important when developing tool compounds so as to remove off-target effects and allow reliable target validation. Compounds **23** and **90** demonstrated selectivity over BRD4 in an AlphaScreen™ assay.

The current ligands do not have high enough affinity to be used as tools in mode of action studies. The Structural Genomics Consortium has the criterion for chemical probes to have an *in vitro* potency less than 100 nm. Future ligand development should take into careful consideration the water molecules in the ZA channel. Displacing water 7 should be avoided due to its negative binding energy, whilst displacing waters 5 and 6 could be favourable for binding. Maximising interactions with E981 should also be beneficial for both binding affinity and selectivity.

CHAPTER 4

TRIM33 Proteolysis Targeting Chimeras (PROTACs)

4.1 Introduction and Aims

Targeted degradation of TRIM33, using PROTACs, provides a complementary strategy to small molecule inhibition for understanding the role of TRIM33. To generate TRIM33-targeted PROTACs, the previously designed benzimidazolone ligands were linked to an E3 ubiquitin ligase ligand resulting in a heterobifunctional molecule, which should form a ternary complex with TRIM33 and the E3 ligase.

Work described in Chapters 2 and 3 provided an understanding of how the benzimidazolone analogues bind in the bromodomain of TRIM33. This information was used to guide PROTAC design by identifying linker attachment points which do not prevent TRIM33 binding.

To ensure the efficient degradation of targeted proteins, it is important to have a suitable linker. Potential PROTACs with varying linker lengths were therefore synthesised as linker design is not currently possible due to difficulties in accurately predicting protein-protein complexes computationally. The TRIM33 ligand was tethered *via* an amine or amide to the linker. Different linking strategies were explored as this can affect affinity for the target protein and degradation efficiency.

The synthesised compounds were evaluated using AlphaScreen™ assays to assess binding to TRIM33. However, the TRIM33 PROTACs were observed to interfere in the AlphaScreen™ assay and therefore an alternative assay is required to evaluate binding. If binding to TRIM33 is observed, the PROTACs will be further evaluated for their ability to degrade TRIM33 in human cancer cell lines.

4.2 E3 Ubiquitin Ligase

The PROTACs described will target CRBN. CRBN functions as a substrate receptor protein for the CRL4A E3 ubiquitin ligase complex and has been widely exploited by PROTACs as the target E3 ligase.[171] Thalidomide (**13**) and pomalidomide (**14**) were identified as CRBN ligands in 2010 and 2012, respectively (Figure 4.1),[89,90] and will be employed as CRBN ligands in the TRIM33 PROTACs.

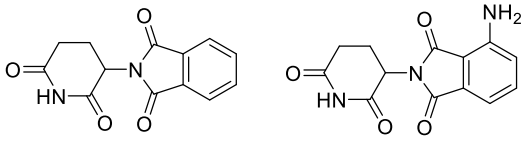


Figure 4.1: Thalidomide (13) and pomalidomide (14) will be employed as E3 ligase ligands.

Employing different E3 ligase ligands will yield PROTACs with different properties, binding affinities and degradation efficiencies. In addition to the PROTACs designed to hijack the E3 ubiquitin ligase CRBN, a series of PROTACs was synthesised by Jessica Reynolds to target the E3 ubiquitin ligase VHL (Figure 4.2).

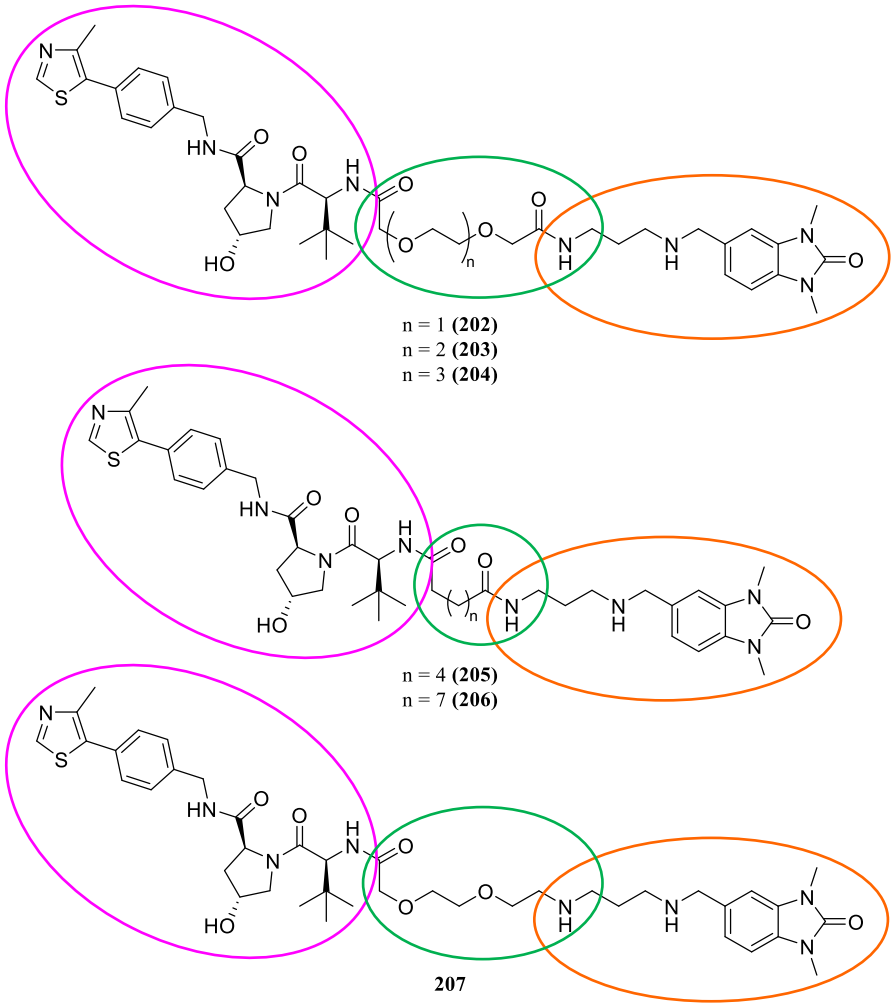


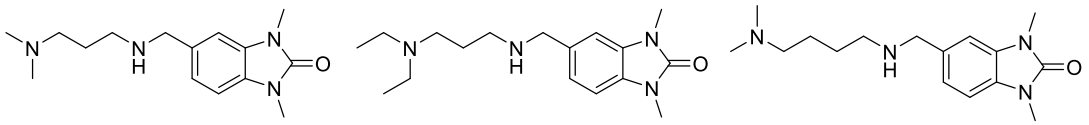
Figure 4.2: VHL-directed TRIM33 PROTACs synthesised by Jessica Reynolds. The VHL ligand is highlighted in magenta, the linker in green, and the TRIM33 ligand in orange.

4.3 PROTAC Design

In work described in Chapters 2 and 3, the SAR of the benzimidazolone series was extensively explored. This investigation led to the identification of ligands with low micromolar affinity for TRIM33 and a greater understanding of the bromodomain binding pocket. As demonstrated in the literature, the ligand for the target protein is not required to have very high affinity to afford a potent degrader.[172] This is because the PROTAC is only required to bring together the target protein and E3 ubiquitin ligase transiently for the first ubiquitin transfer to occur.[107] Subsequent ubiquitination is rapid.[173]

The information obtained in Chapters 2 and 3 can be used to identify possible linker tethering sites on **90**. The amine chain of **90** was predicted to be largely solvent exposed, and longer amine

chain analogues **111** and **112** were well tolerated (Figure 4.3). This suggests there is space to extend the linker from the terminal amine of **90**.



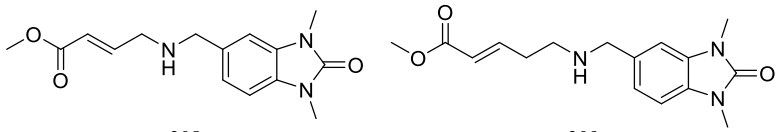
90
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 3.61 μM
TRIM33β IC_{50} = 13.7 μM

111
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 4.74 μM
TRIM33β IC_{50} = 2.02 μM

112
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 7.96 μM
TRIM33β IC_{50} = 2.73 μM

Figure 4.3: Analogues of 90. IC_{50} values were determined using an AlphaScreen™ assay with $H3_{1–27}$K18Ac peptide.

Covalent TRIM33 benzimidazolone ligands were developed by Dr Larissa See (Figure 4.4).[114] Compounds **208** and **209** lack a terminal amine substituent, yet in the case of **209**, affinity for TRIM33 is retained, suggesting a terminal amine is not essential for binding. These covalent ligands (**208** and **209**) provide further evidence that the terminal amine is a good tethering site for the linker which should not interfere with the ability of ligands to bind TRIM33.

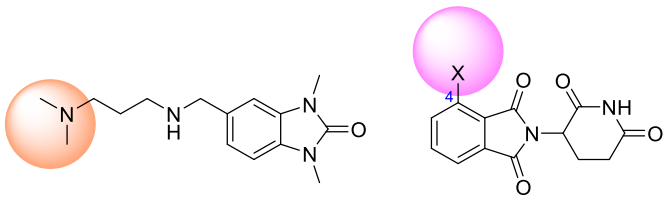


208
TRIM33α IC_{50} = 18.7 μM
TRIM33β IC_{50} = 85.2 μM

209
TRIM33α IC_{50} = 3.81 μM
TRIM33β IC_{50} = 12.6 μM

Figure 4.4: Covalent ligands synthesised by Dr Larissa See.[114] IC_{50} values were determined using an AlphaScreen™ assay with $H3_{1–27}$K18Ac peptide.

Crystal structures of DDB1–CRBN complex bound to thalidomide, lenalidomide and pomalidomide show that the C-4 position of these phthalimides is solvent exposed (Figure 4.5).[174] Tethering the linker off the C-4 position should therefore not affect binding to CRBN.



90 **210**

Figure 4.5: Proposed tethering sites on TRIM33 ligand 90 and phthalimide 210. These sites are largely solvent exposed therefore linking at these positions should not affect binding to target proteins.

The length and chemical composition of the linker have a major effect on the cellular potency of PROTACs.[172] For efficient ubiquitination to occur, the ubiquitin ligase machinery and the target protein need to have the appropriate orientation.[96,175] PPIs between the target protein and E3 ligase can also stabilise the ternary complex and lead to potent protein degradation.[172] As both the PPIs and orientation of the E3 ligase and target protein are affected by the linker, synthesising PROTACs with varying linkers is desirable. Even small changes to the linker (e.g. one ethylene glycol unit) can have a large effect on degradation efficiency.[172]

Despite the linker length being critical for the activity of PROTACs, there is yet to be a broadly applicable technique for predicting the optimal linker composition. Computationally predicting protein-protein complexes is difficult and may not be a true representation of the cellular setting. An example of *in silico* modelling applied to PROTAC design is the development of a Sirt2 PROTAC.[176] A model of Sirt2 complexed to CRBN was generated and used to dock PROTACs. The designed PROTAC did effectively induce Sirt2 degradation. This is, however, unusual and an appropriate linker is usually found through the synthesis of a large number of analogues of varying linker length and composition.

In this work, polyethylene glycol (PEG) chains will be used to link the CRBN ligand to the TRIM33 ligand. Two different linking strategies will be used. The first will have an amide linkage to the TRIM33 ligand (**211**–**218**), and the second will have an amine linkage (**219**–**222**) (Figure 4.6). Based on the reduced affinity of the covalent ligands (**208** and **209**) which lack a terminal amine (Figure 4.4), it is predicted that the amine-linked PROTACs **219**–**222** will have higher affinity for TRIM33 than the amide-linked PROTACs **211**–**218**.

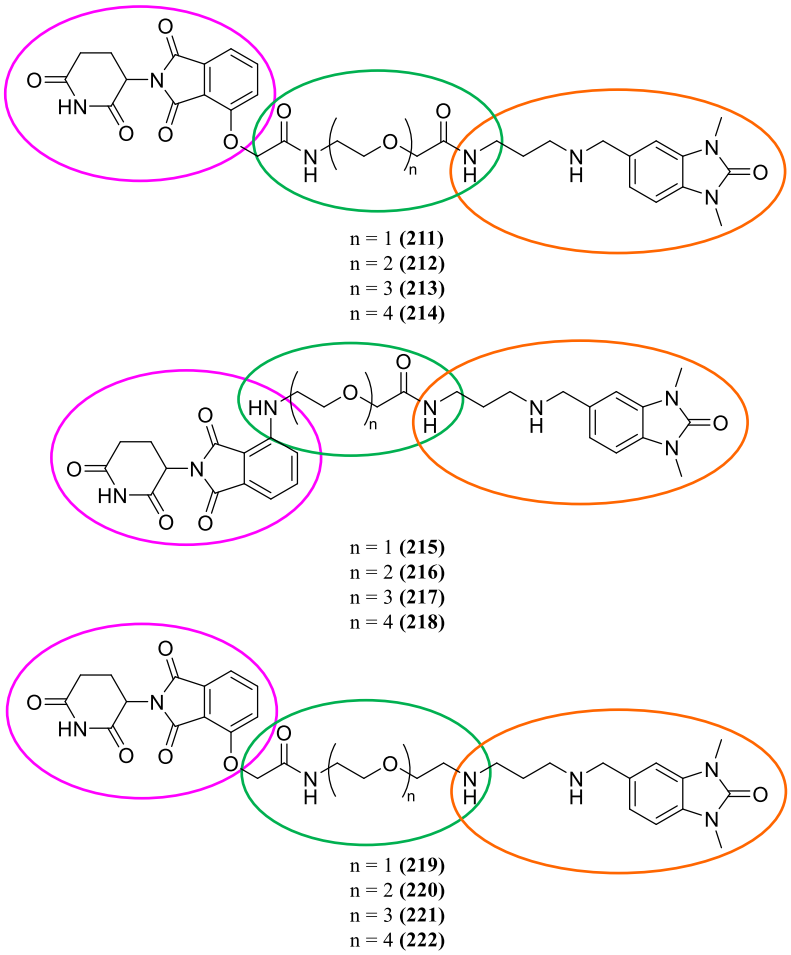


Figure 4.6: CRBN-directed TRIM33 PROTACs. PROTACs **211**–**218** are linked *via* an amide to the TRIM33 ligand and PROTACs **219**–**222** are linked *via* an amine to the TRIM33 ligand. The CRBN ligand is highlighted in magenta, the linker in green, and the TRIM33 ligand in orange.

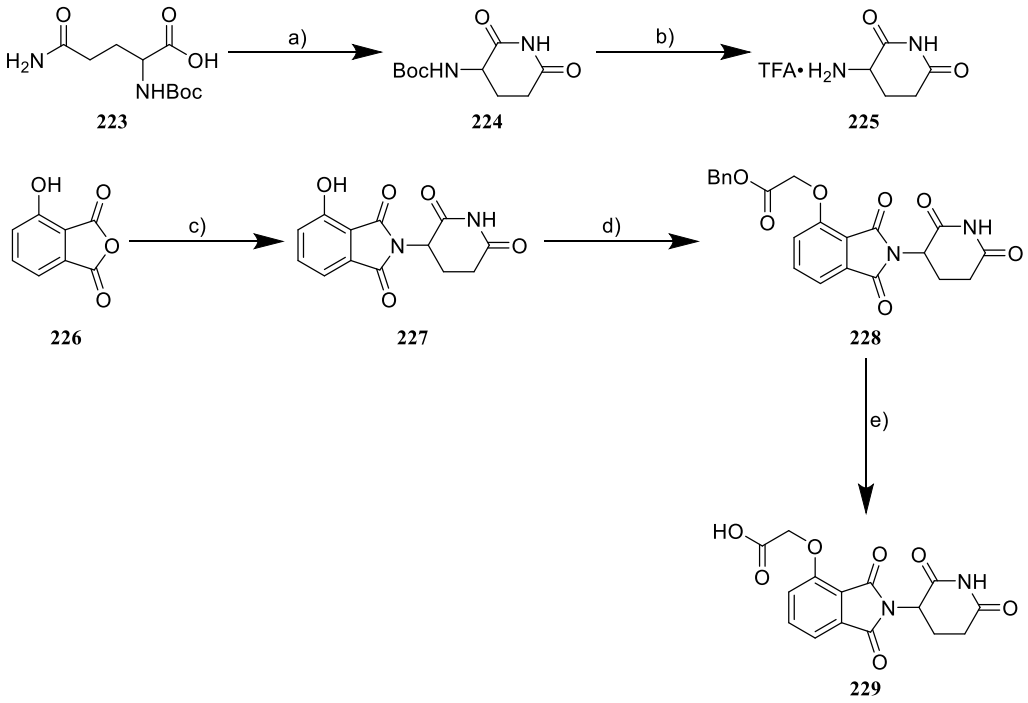
4.4 Synthetic Strategy

A synthetic strategy which allows facile diversification of the linker is desirable. An efficient strategy involves the synthesis of two functionalised ligands, one for TRIM33 and one for CRBN. The functional handles can then be reacted with a range of linkers, allowing rapid synthesis of a diverse range of PROTACs.

4.4.1 Synthesis of a Functionalised Thalidomide Derivative

Carboxylic acid-functionalised thalidomide **229** was synthesised following a route published by Miller (Scheme 4.1).[177] Boc-Gln-OH (**223**) was cyclised using CDI to yield Boc-protected **224**. The Boc group of **224** was then removed using trifluoroacetic acid (TFA) to yield primary amine **225** as the TFA salt. Amine **225** was next reacted with **226** to form the thalidomide core (**227**).

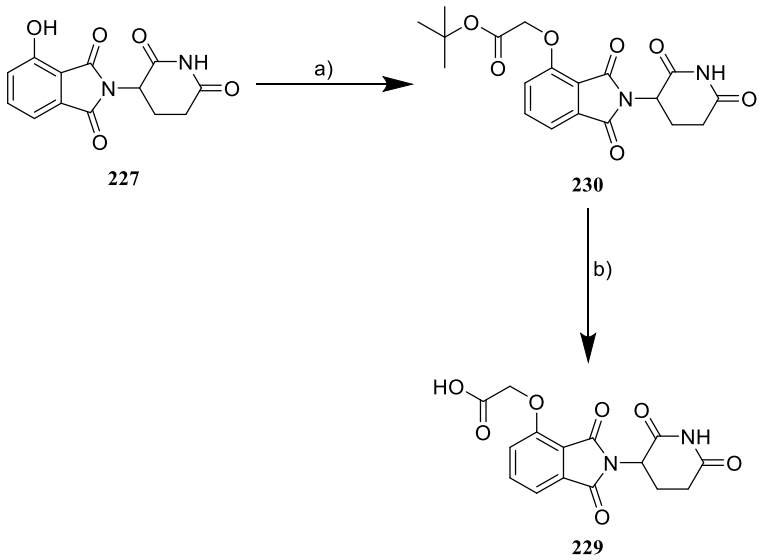
The benzyl protected acid was introduced using a Mitsunobu reaction (**228**),[157] and the benzyl group of **228** subsequently removed using hydrogenolysis to afford carboxylic acid **229** (33–84%, n = 14). In all cases, the reaction was observed to go to completion (TLC analysis). However, problems occurred during product isolation due to **229** having poor solubility in organic solvents. To maximise the amount of **229** isolated, once the reaction was observed to be complete (TLC analysis) it was heated to 60 °C. The hot reaction mixture was then filtered through a pad of Celite® to remove the palladium and the filter pad was washed with hot methanol. The product also required purification, and this was conducted using an ISOLUTE® PE-AX column. Product was likely lost during both filtration and purification steps, hence the range of yields observed.



Scheme 4.1: Synthesis of carboxylic acid-functionalised thalidomide 229. *Reagents and conditions*: a) CDI, DMAP, THF, 67 °C, 56–98%, n = 6; b) TFA, CH_2Cl_2, 0 °C → rt, 89–100%, n = 4; c) (i) **225**, Et_3N, THF, 70 °C; (ii) DCC, DMAP, 70 °C, 50–88%, n = 5; d) Benzyl glycolate, PPh_3, DIAD, THF, 0 °C → rt, 49–67%, n = 4; e) Pd/C, H_2, EtOAc, CH_2Cl_2, rt, 33–84%, n = 14.

Due to problems isolating **229**, an alternative route which avoided filtration was investigated (Scheme 4.2). t*ert*-Butyl ester **230** was synthesised *via* alkylation of **227** with *tert*-butyl bromoacetate. This reaction proceeded with poor yield (18–28%) and resulted in the isolation of both starting material and product. The *tert*-butyl group of **230** was then removed in acidic

conditions. Acid **229**, however, still required purification, this was achieved using an ISOLUTE® PE-AX column, to afford **229** in 54–66% yield. The low yield can be explained due to the poor solubility of **229** causing issues during purification. We decided that the initial synthesis of **229** was preferable due to higher overall yields.

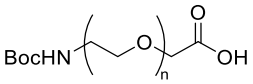


Scheme 4.2: Alternative synthesis of carboxylic acid-functionalised thalidomide 229. *Reagents and conditions*: a) *tert*-Butyl bromoacetate, K_2CO_3, DMF, rt, 18–28%, n = 2; b) TFA, CH_2Cl_2, rt, 54–66%, n = 2.

4.4.2 Synthesis of Amide-Linked PROTACs

4.4.2.1 Amide Coupling in the Absence of Protecting Groups

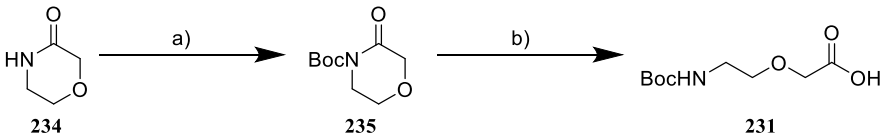
To synthesise the amide-linked PROTACs, linkers are required to have a carboxylic acid for amide coupling with the TRIM33 ligand, and an amine for amide coupling with the CRBN ligand. Linkers **231**–**233** have the amine Boc-protected to prevent homocoupling (Figure 4.7).



n = 1 **(231)**
n = 2 **(232)**
n = 3 **(233)**

Figure 4.7: PEG linkers synthesised with a Boc-protected amine on one end and a carboxylic acid on the other end.

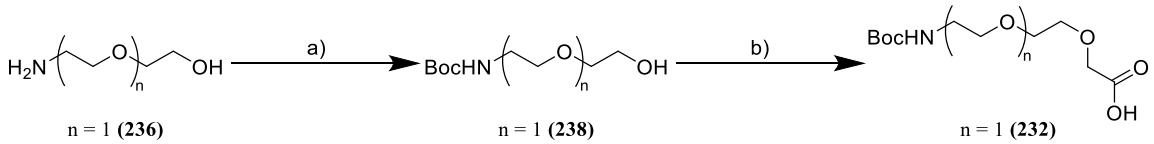
The linker **231** was synthesised from morpholinone **234** (Scheme 4.3). Morpholinone **234** was Boc protected (**235**) and the amide group hydrolysed using aqueous LiOH to yield linker **231**.



234 **235** **231**

Scheme 4.3: Synthesis of linker 231. *Reagents and conditions*: a) Boc_2O, DMAP, THF, rt, 53–75%, n = 2; b) LiOH, H_2O, THF, 0 °C → rt, 94–95%, n = 2.

Linkers **232** and **233** were synthesised from alkanolamines **236** and **237** (Scheme 4.4). The amines were first Boc protected to yield **238** and **239**. The alcohol was then deprotonated with sodium hydride, and reacted with bromoacetic acid to introduce the acid group (**232** and **233**).



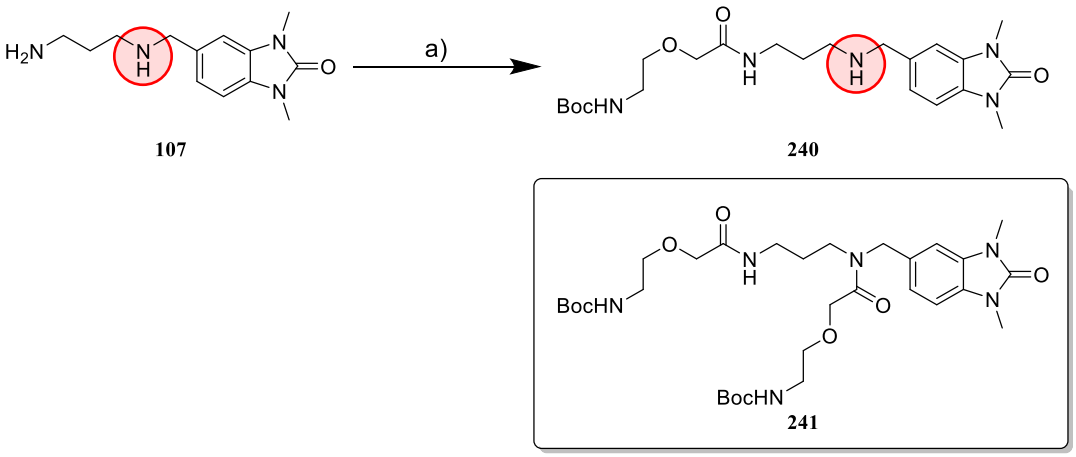
n = 1 **(236)**
n = 2 **(237)**

n = 1 **(238)**
n = 2 **(239)**

n = 1 **(232)**
n = 2 **(233)**

Scheme 4.4: Synthesis of linkers 232 and 233. *Reagents and conditions*: a) Boc_2O, CH_2Cl_2, rt, **238**: 91–97%, n = 2, **239**: 81–96%, n = 2; b) NaH, bromoacetic acid, THF, 0 °C → 60 °C, **232**: 35–55%, n = 3, **323**: 55%, n = 1.

Amide coupling of the TRIM33 ligand was initially attempted with the benzylic nitrogen unprotected (**107**) (Scheme 4.5). Avoiding the use of protecting groups is desirable to reduce the number of synthetic steps. As predicted, reaction of **107** with linker **231** yielded a mixture of products, with amide coupling also occurring at the benzylic position (**241**). Consequently, the desired product (**240**) was isolated in a very poor 9% yield (Scheme 4.5), and an alternative strategy was required.

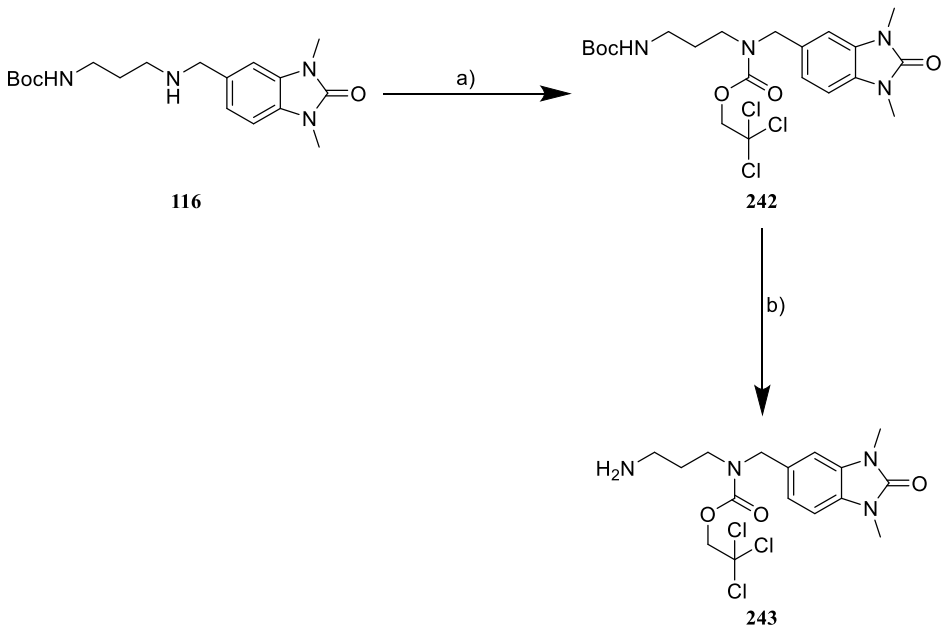


Scheme 4.5: Amide coupling in the absence of protecting groups. *Reagents and conditions*: a) **231**, HATU, DIPEA, DMF, rt, 9%, n = 1.

4.4.2.2 Troc Protecting Group

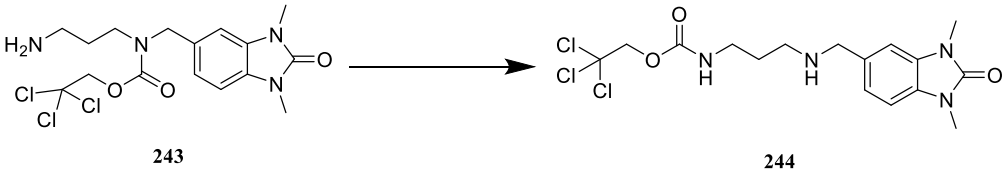
A 2,2,2-trichloroethoxycarbonyl (Troc) protecting group was chosen to protect the benzylic nitrogen as it is orthogonal to the Boc protecting group present on the linkers (**231**–**233**).

As Troc is stable under acidic conditions, **243** can be synthesised from Boc-protected **116** (Scheme 4.6). Compound **116** was treated with TrocCl to yield **242** and the Boc protecting group was subsequently removed under acidic conditions to yield **243**.



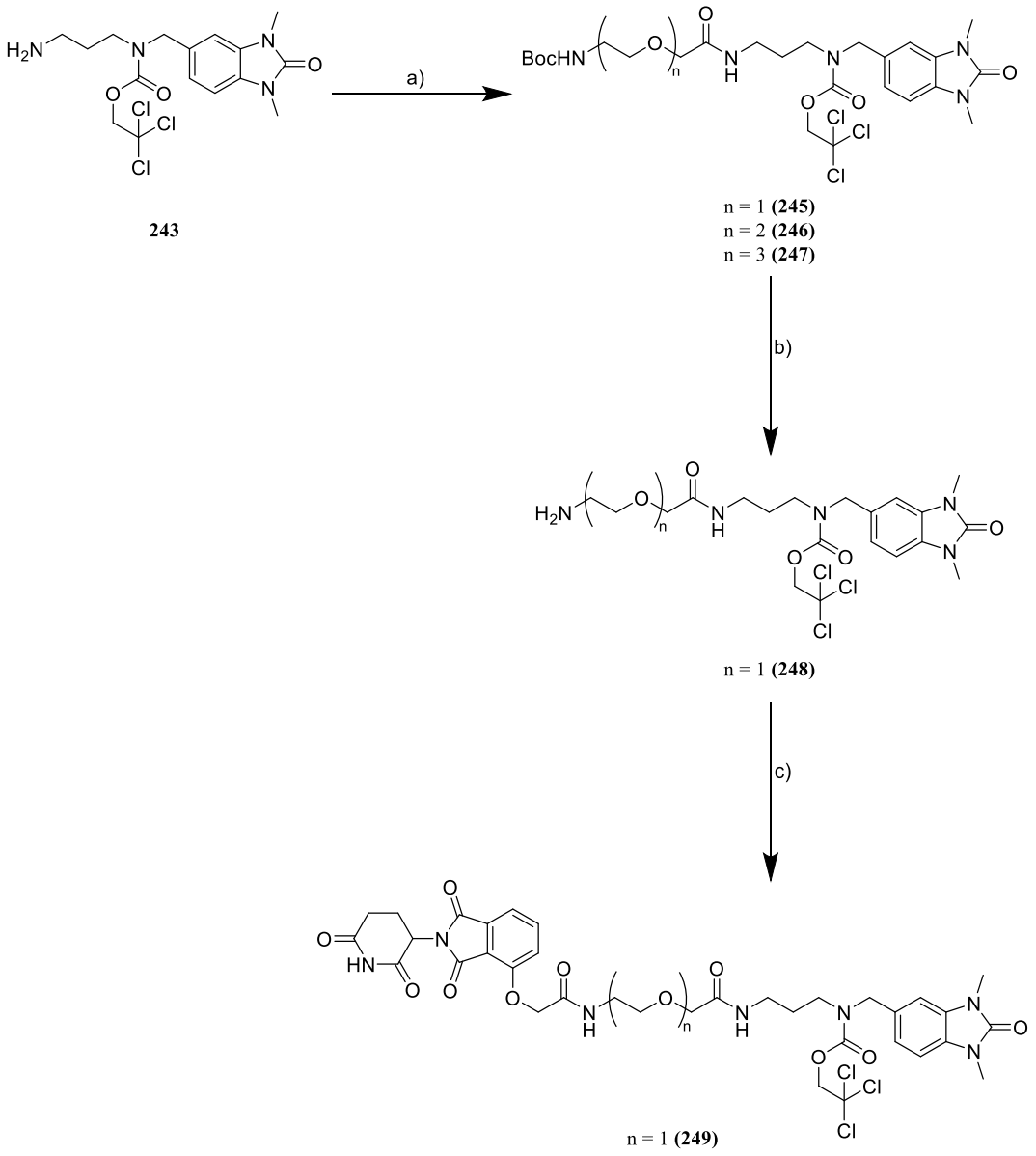
Scheme 4.6: Synthesis of Troc-protected 243. *Reagents and conditions*: a) TrocCl, DMAP, pyridine, CH_2Cl_2, 0 °C → rt, 65–78%, n = 3; b) HCl (4 M, dioxane), rt, 50–93%, n = 6.

As rotamers were observed in the ^{1}H NMR of **243**, variable temperature (VT) NMR experiments were performed to help resolve the spectrum. However, compound **243** was unstable at elevated temperatures (363 K). Following this observation, an older sample of **243** was reanalysed and this showed that even at rt **243** was unstable. The ^{1}H NMR spectrum indicated that **243** was degrading to a single product. From the ^{1}H NMR and mass spectrometry data, it is predicted that the Troc group migrates to the terminal nitrogen of the amine chain (**244**) (Scheme 4.7). The migration is likely facilitated by the Troc group being an electrophilic carbamate and the ability of **243** to form a 6-membered ring. Amine **243** was therefore reacted directly with the PEG linkers following Boc deprotection of **242**.

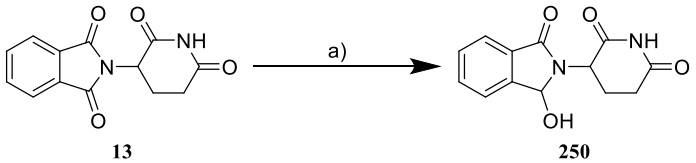


Scheme 4.7: Troc-protected 243 was unstable at rt. It is predicted that the Troc group migrates from the benzylic nitrogen to the terminal nitrogen.

The amine **243** was amide coupled, using HATU, to PEG linkers **231**, **232** and **233** (Scheme 4.8). The Boc group of **245** was removed under acidic conditions, and amine **248** was subsequently amide coupled to thalidomide **229** to yield **249**. Removal of the Troc group was initially attempted by heating **249** under reflux in acetic acid in the presence of zinc. This reaction goes *via* a reductive elimination process. These conditions, however, resulted in the degradation of **249**. A literature search showed that such reaction conditions reduce one of the carbonyl groups of thalidomide (Scheme 4.9).[178] Two alternative deprotection strategies were therefore evaluated for Troc-protected **243**, and thalidomide **229**, to see if the ligands were stable and if the Troc group was removed. Deprotection using zinc and acetic acid was repeated at 0 °C. Thalidomide **229** was still, however, partially reduced. Deprotection using 1-methylimidazole and zinc was also tried. The Troc group, however, was stable under these conditions, even when heated to 50 °C. It was therefore decided that the Troc group was an unsuitable protecting group in this synthesis.



Scheme 4.8: Attempted synthesis of amide-linked PROTACs using a Troc protecting group strategy. *Reagents and conditions*: a) Linker, HATU, DIPEA, DMF, rt, **245**: 63%, n = 1, **246**: 48%, n = 1, **247**: 45%, n = 1; b) TFA, CH_2Cl_2, 0 °C → rt, 86%, n = 1; c) **229**, HATU, DIPEA, DMF, rt, 78%, n = 1.



Scheme 4.9: Reduction of thalidomide (13). *Reagents and conditions*: a) AcOH, Zn, 90 °C, 87%.[178]

4.4.2.3 Building off the Thalidomide Derivative

An alternative approach to the amide-linked PROTACs involves coupling the linker to CRBN ligand **229** first (Figure 4.8). The acid **251** can then be amide coupled to the TRIM33 ligand to yield the final PROTAC (**252**). This strategy requires the linkers to have a primary amine on one end and a protected acid on the other end. To obtain PROTAC **211**, the methyl ester **254** was synthesised as the linker (Scheme 4.10). The Boc protecting group of **231** was removed in acidic conditions to yield **253**, which was then converted to methyl ester **254** using thionyl chloride and methanol.

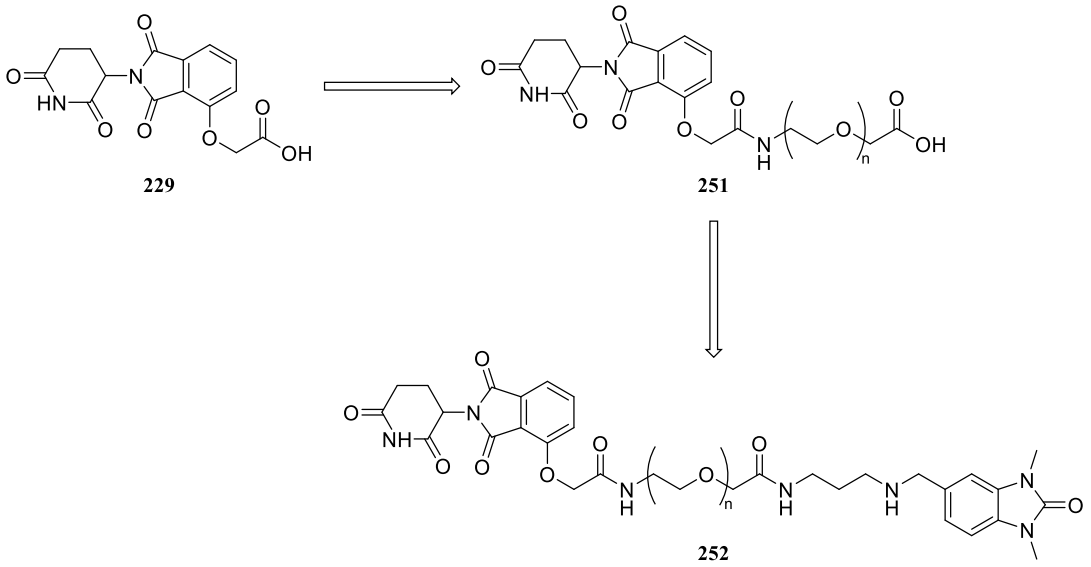
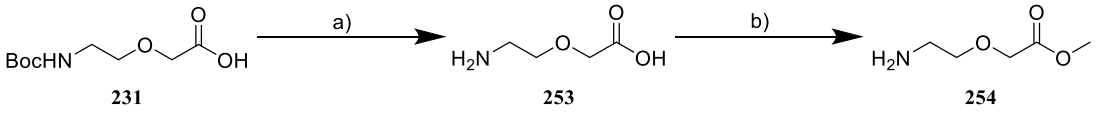
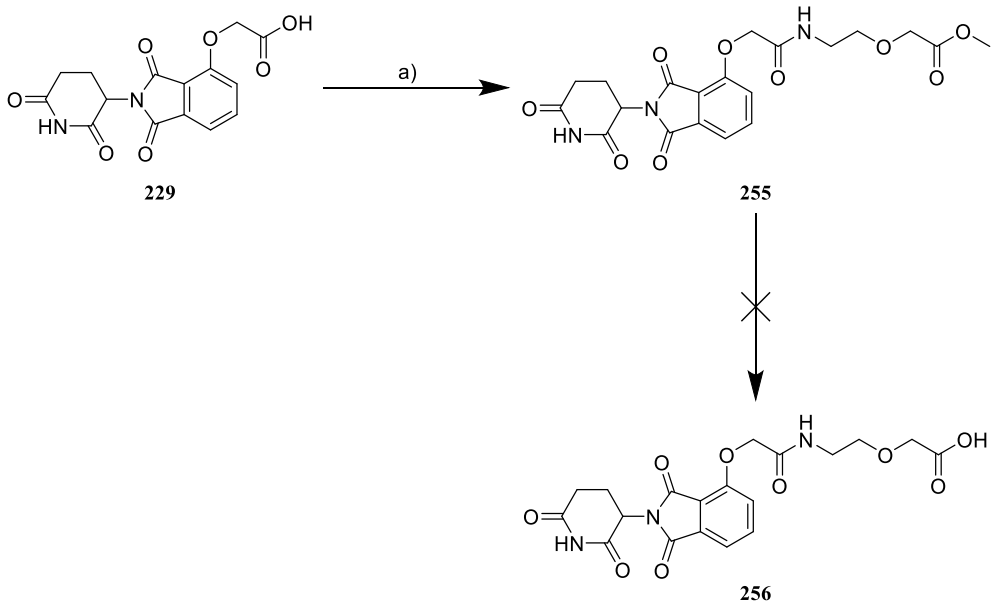


Figure 4.8: Alternative route to amide-linked PROTACs. The linker is first coupled to thalidomide derivative **229** prior to amide coupling to the TRIM33 ligand.



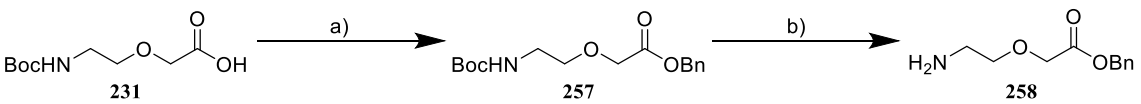
Scheme 4.10: Synthesis of linker 254. *Reagents and conditions*: a) TFA, CH_2Cl_2, 0 °C → rt, 79%, n = 1; b) $SOCl_2$, MeOH, 0 °C → rt, 99%, n = 1.

The linker **254** was amide coupled to thalidomide **229** to yield **255** (Scheme 4.11). Aqueous LiOH was used to hydrolyse methyl ester **255** to acid **256**, however, following aqueous work up acid **256** could not be isolated, likely due to high levels of aqueous solubility.

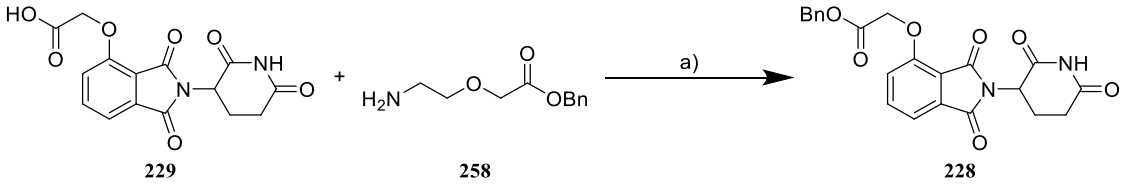


Scheme 4.11: Acid 256 could not be isolated following the hydrolysis of 255. *Reagents and conditions*: a) **254**, HATU, DIPEA, DMF, rt, 49%, n = 1.

The methyl ester of **254** was subsequently replaced with a benzyl ester (**258**). A benzyl ester was chosen as it can be removed using hydrogenolysis and the product isolated by filtration, avoiding the requirement of an aqueous work up. The acid **231** was converted to benzyl ester **257** using benzyl alcohol and *N*,*N*'-dicyclohexylcarbodiimide (DCC) or CDI, and the Boc group was then removed using acidic conditions to yield **258** (Scheme 4.12). Unexpectedly, amide coupling of **258** with thalidomide **229** resulted in the formation of benzyl ester derivative **228** (Scheme 4.13). This product was confirmed using ^{1}H NMR as compound **228** had previously been synthesised as an intermediate to **229**; the ^{1}H NMR spectra were identical. It is hypothesised that **258** cyclised under basic conditions, resulting in the elimination of benzyl alcohol which then reacted in an esterification with acid **229**.



Scheme 4.12: Synthesis of linker 258. *Reagents and conditions*: a) Benzyl alcohol, DCC, DMAP, CH_2Cl_2, rt, 84%, n = 1 or benzyl alcohol, CDI, CH_2Cl_2, rt, 80%, n = 1; b) HCl (4 M, dioxane), rt, 90%, n = 1.



Scheme 4.13: Amide coupling of acid 229 with linker 258 unexpectedly yielded ester 228. *Reagents and conditions*: a) HATU, DIPEA, DMF, rt, 73%, n = 1.

To avoid the problem of cyclisation of **258**, and subsequent esterification of **229**, it was envisaged that linker **258** could be first amide coupled to the amine chain of the TRIM33 ligand (**259**) to yield **260** (Figure 4.9). The final step in this strategy is a reductive amination with aldehyde **34** to introduce the benzylic nitrogen on the benzimidazolone core. This route therefore removes the requirement of a protecting group on the benzylic nitrogen.

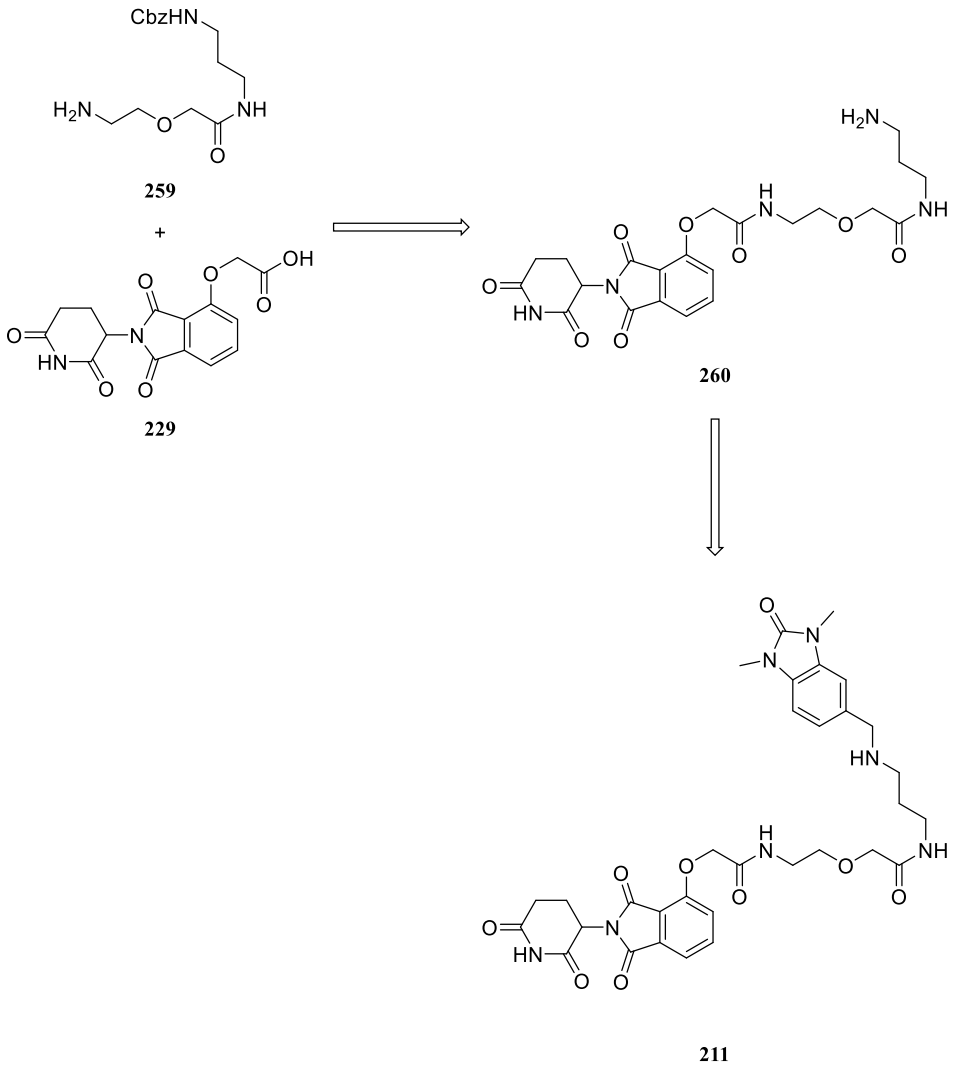
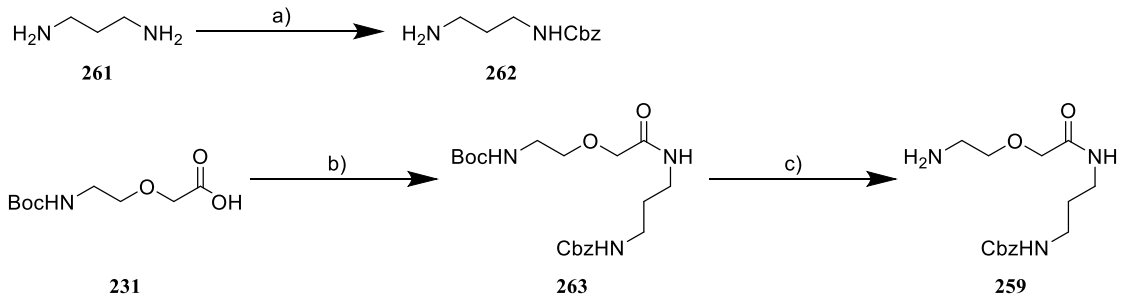


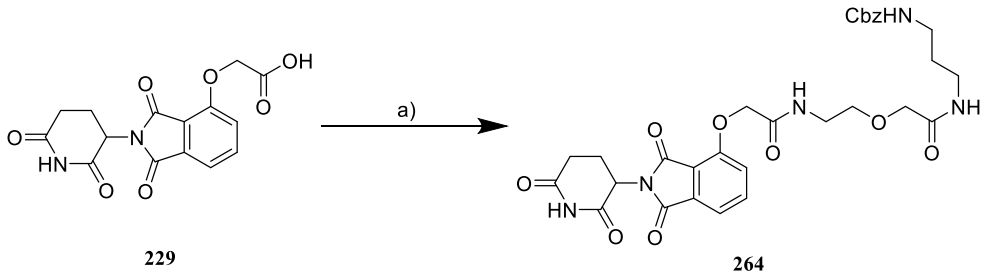
Figure 4.9: Alternative route to amide-linked PROTACs. The CRBN ligand is first amide coupled to linker **259**. The final step in this strategy is a reductive amination with aldehyde **34** which removes the requirement of a protecting group on the benzylic nitrogen.

Linker **259** was synthesised from acid **231** (Scheme 4.14). Acid **231** was amide coupled with carboxybenzyl (Cbz)-protected **262** to yield **263**. The Boc group of **263** was removed under acidic conditions to yield **259**.



Scheme 4.14: Synthesis of linker 259. *Reagents and conditions*: a) Benzyl chloroformate, $CHCl_3$, 0 °C → rt, 34–57%, n = 3; b) **262**, HATU, DIPEA, DMF, rt, 62–72%, n = 2; c) HCl (4 M, dioxane), rt, 64–68%, n = 2.

Amide coupling of the linker with **229** should no longer be problematic due to the longer linker length of **259**. Indeed, amide coupling of **229** and **259**, using HATU, yielded **264** in 66% yield (Scheme 4.15).



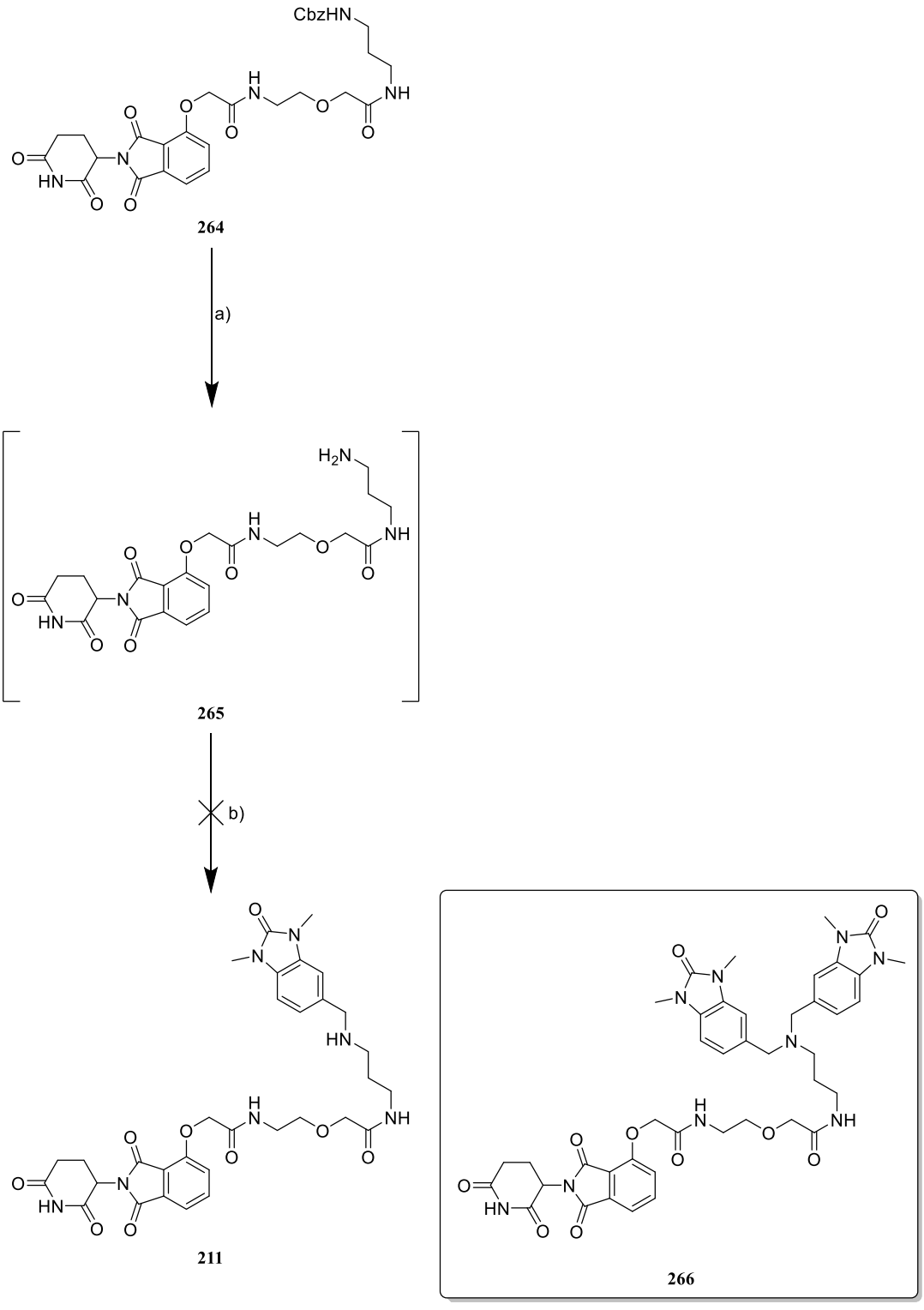
Scheme 4.15: Amide coupling of 229 and 259. *Reagents and conditions*: a) **259**, HATU, DIPEA, DMF, rt, 66%, n = 1.

Removal of the Cbz group, however, proved challenging (Scheme 4.16). Initially H_2 and Pd/C in methanol were used. TLC analysis indicated no reaction had occurred and correspondingly starting material **264** was isolated. The reaction was subsequently repeated with the addition of acetic acid, this should promote hydrogenolysis if **264** is coordinating to palladium and poisoning the catalyst, however, once again no reaction was observed. It was hypothesised that the lack of reactivity was due to the poor solubility of **264** in methanol. The reaction solvent was therefore changed to DMF as **264** was observed to be fully soluble. TLC analysis of the reaction mixture showed the slow formation of another species. The reaction was stopped by purging with nitrogen, and the palladium removed by filtration. ^{1}H NMR analysis of the crude material showed a complex mixture of starting material **264** and related compounds. Purification using an ISOLUTE® SCX-2 SPE column was attempted but this failed to isolate **265**.

Trimethylsilyl iodide (TMSI) can also be used to cleave carbamates. An intermediate trimethyl silyl carbamate is formed which decomposes upon the addition of methanol or water.[179,180] TLC analysis of the reaction between **264** and TMSI indicated consumption of starting material. ^{1}H NMR analysis of the crude material, however, showed a complex mixture resulted from the reaction.

Heating **264** under reflux in 6 M HCl led to the consumption of starting material. In addition, one of the products observed by TLC stained with ninhydrin solution, suggesting the presence of an amine. The HCl was removed by concentration *in vacuo* and the resulting material purified using an ISOLUTE® SCX-2 SPE column. However, the material eluted from the column was not **265**.

Replacing Pd/C with $Pd(OH)_2$ and performing the hydrogenolysis in DMF with acetic acid resulted in a faster reaction. The results from previous reactions suggested the product might be unstable. Therefore, once the reaction was observed to go to completion (TLC analysis), the palladium was removed by filtration and the DMF removed by concentration *in vacuo*. The crude material was then reacted directly *via* a reductive amination with aldehyde **34**. Initially 1.2 eq. of aldehyde **34** were used in the reaction. Amine **265**, however, reacted with two molecules of **34** to yield **266** and desired product **211** was not isolated. It was predicted that **211** was more soluble than starting material **265** and therefore reacted faster. To try and minimise **211** reacting with a second equivalent of **34**, only 0.8 eq. of aldehyde **34** were used. A small amount of PROTAC **211** was observed in the ^{1}H NMR spectrum but could not be isolated and purified from the complex reaction mixture.



Scheme 4.16: Attempted synthesis of PROTAC 211. *Reagents and conditions*: a) $Pd(OH)_2$, H_2, AcOH, DMF, rt, not isolated; b) (i) **34**, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, not isolated.

Due to problems with the final reductive amination step, an alternative route was proposed to the amide-linked PROTACs (Figure 4.10). Having a reliable and clean reaction as the final step is desirable. We decided that a Boc deprotection would be favourable as the acid used for the deprotection can simply be removed by concentration *in vacuo* to yield the final compound.

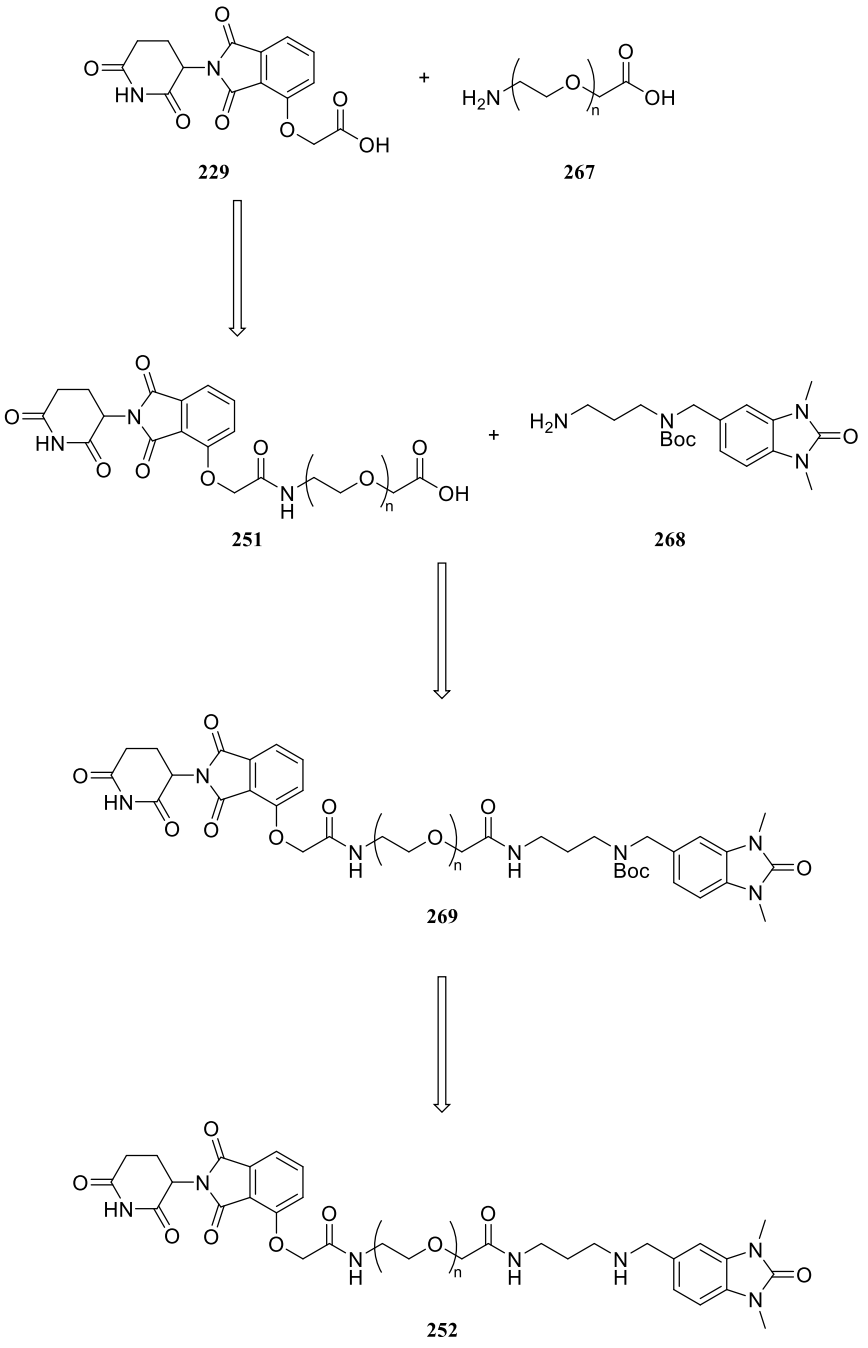
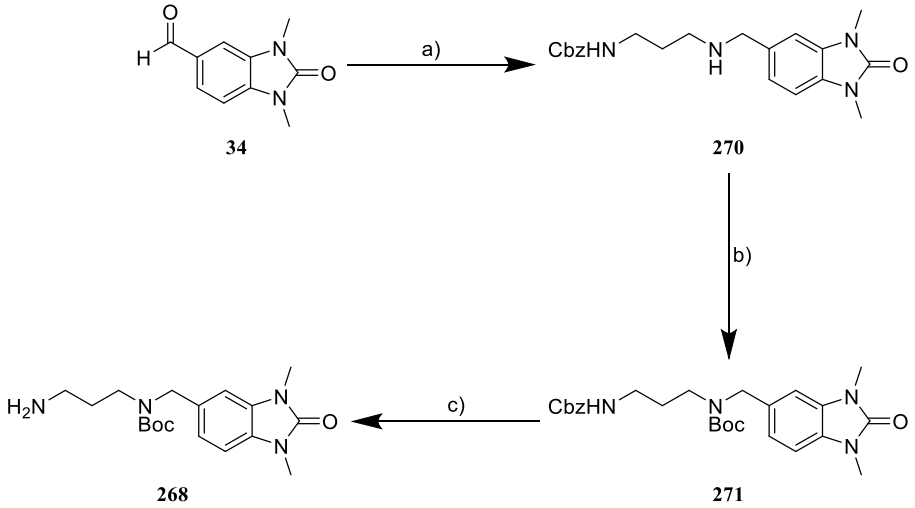


Figure 4.10: Proposed synthetic route to amide-linked PROTACs. A Boc deprotection as the final step is desirable as the product can be easily isolated by concentration *in vacuo*.

The TRIM33 ligand was synthesised with the benzylic nitrogen Boc-protected (**268**) (Scheme 4.17). Reductive amination of the aldehyde **34** with amine **262** yielded **270**. The benzylic nitrogen was then Boc protected to afford **271** and the Cbz group subsequently removed using hydrogenolysis to yield primary amine **268**.



Scheme 4.17: Synthesis of Boc-protected 268. *Reagents and conditions*: a) (i) **262**, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, 58–60%, n = 3; b) Boc_2O, Et_3N, CH_2Cl_2, 0 °C → rt, 80–85%, n = 3; c) Pd/C, H_2, MeOH, rt, 74–99%, n = 2.

To first link the linker to the CRBN ligand **229** and then to the TRIM33 ligand **268**, linkers were required to have a primary amine and a protected acid (Figure 4.11). Synthesis of the benzyl ester **272** was first attempted, however, linker **272** was observed to be unstable. After concentrating the NMR sample of **272** and redissolving in $CDCl_3$, relative peak intensities had changed in the ^1H NMR spectrum, and new peaks were observed. The benzyl ester was therefore replaced with a *tert*-butyl ester as there was literature precedent for their syntheses (Figure 4.11).[181,182]

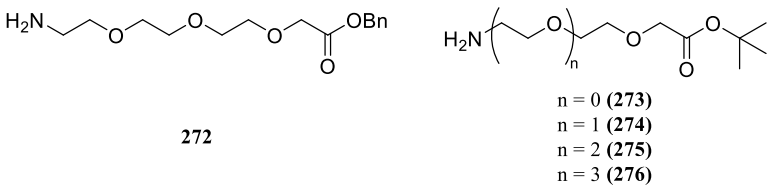
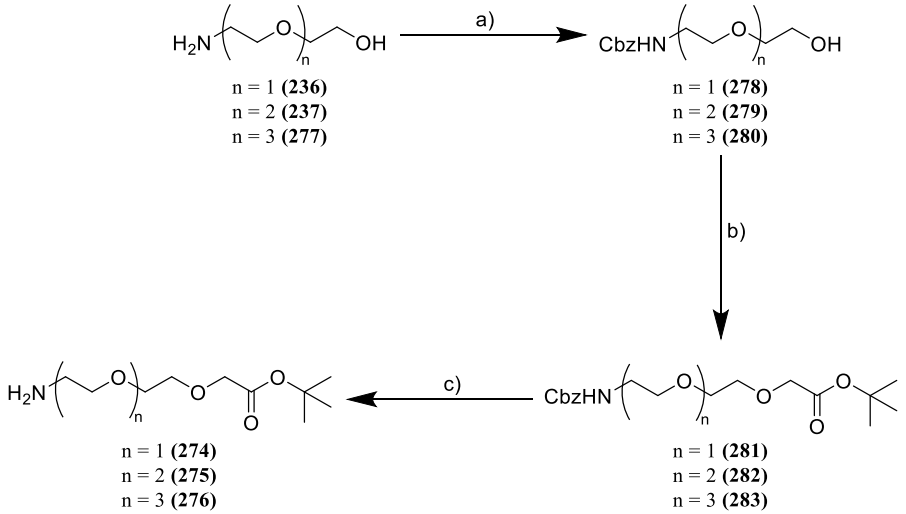


Figure 4.11: Benzyl ester 272 was observed to be unstable. Linkers were therefore synthesised with a *tert*-butyl ester.

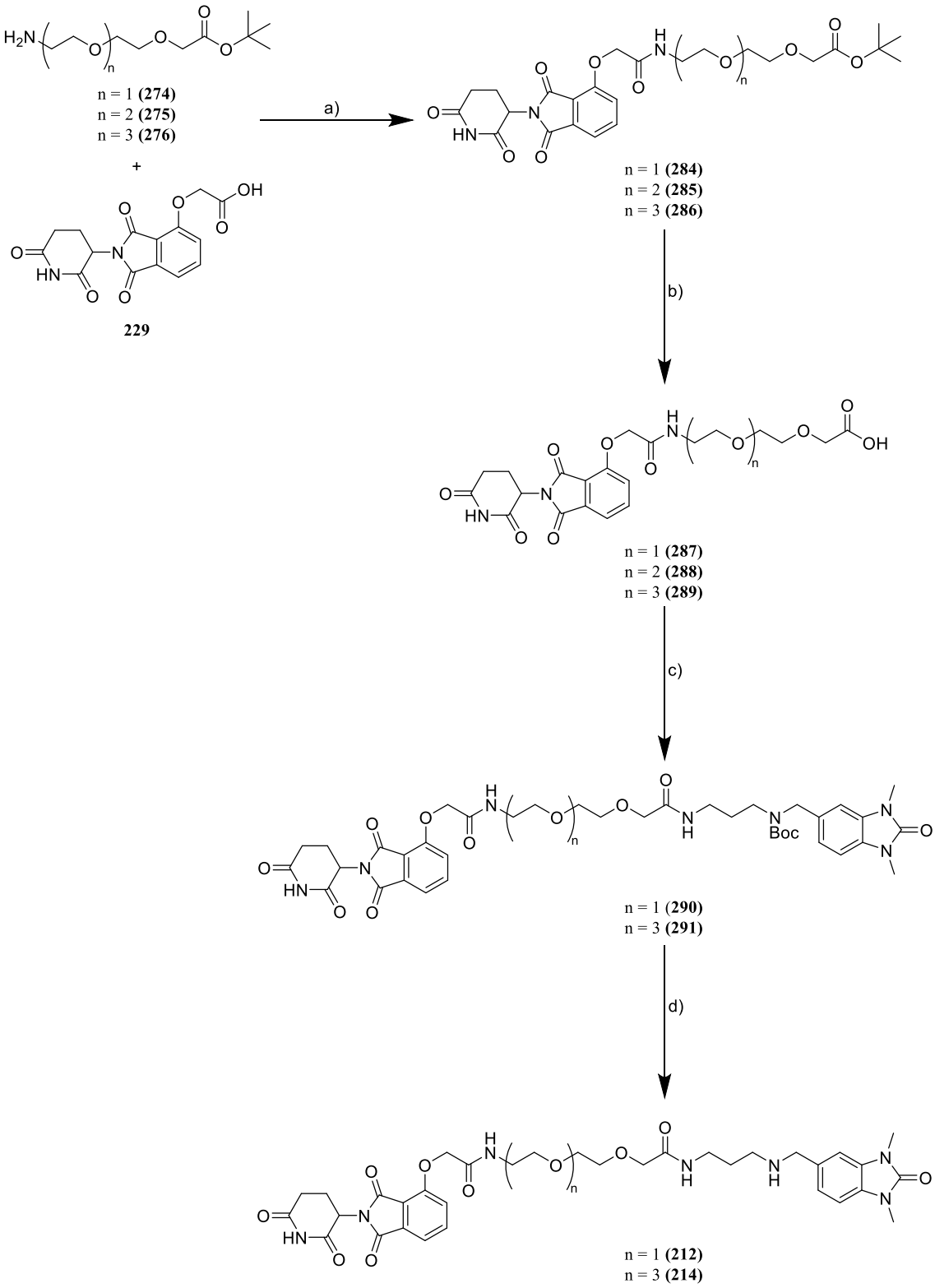
Linkers **274**, **275** and **276** were synthesised with a *tert*-butyl ester (Scheme 4.18). However, problems were encountered during the synthesis of **273**, likely due to its ability to form

6-membered rings. Alkanolamines **236**, **237** and **277** were protected with a Cbz group to yield **278**, **279** and **280** and then reacted with *tert*-butyl bromoacetate to afford *tert*-butyl esters **281**, **282** and **283**. Finally, the Cbz group was removed using hydrogenolysis to yield primary amines **274**, **275** and **276**.



Scheme 4.18: Synthesis of linkers 274, 275 and 276. *Reagents and conditions*: a) Benzyl chloroformate, Et_3N, CH_2Cl_2, 0 °C → rt, **278**: 97%, n = 1, **279**: 54–73%, n = 2, **280**: 71–74%, n = 2; b) *tert*-Butyl bromoacetate, *t*-BuOK, THF, 0 °C → rt, **281**: 53%, n = 1, **282**: 32–37%, n = 3, **283**: 31–40%, n = 2; c) Pd/C, H_2, MeOH, rt, **274**: 94%, n = 1, **275**: 76–94%, n = 3, **276**: 85%, n = 1.

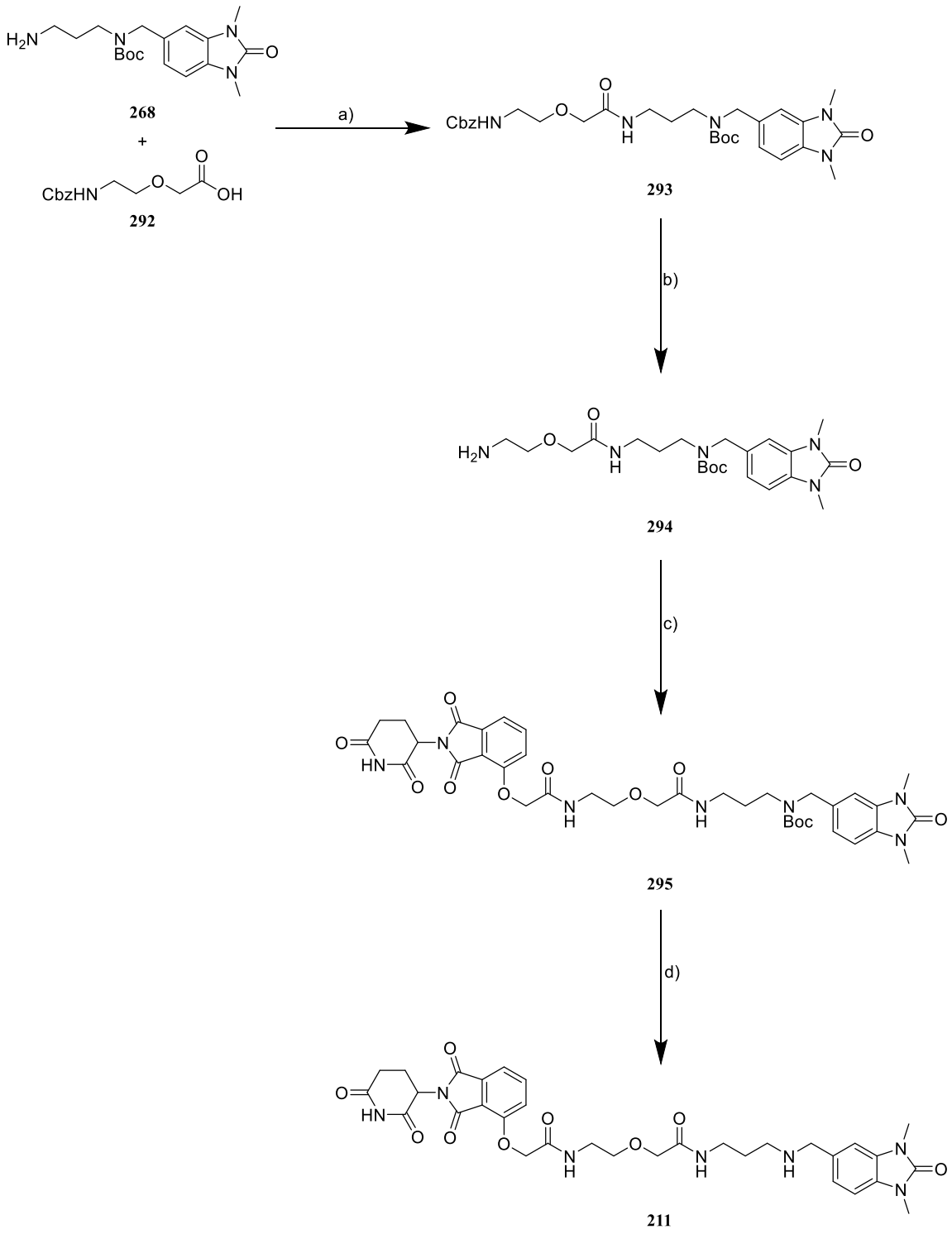
Linkers **274**, **275** and **276** were employed in amide couplings with **229** to yield **284**, **285** and **286**, and the *tert*-butyl esters removed under acidic conditions to afford **287**, **288** and **289** (Scheme 4.19). The acids **287** and **289** were then amide coupled with **268** to yield **290** and **291**. The Boc groups were removed in the final step yielding PROTACs **212** and **214**. The Boc deprotections proceeded with high yields and proved clean reactions as the TFA was removed simply by concentration *in vacuo* and purification was not required. PROTAC **213** remains to be synthesised and for this scale up of **288** is required for amide coupling to TRIM33 ligand **268**. The final Boc deprotection to yield PROTAC **213** is predicted to be a clean reaction, similar to **212** and **214**.



Scheme 4.19: Synthesis of PROTACs 212 and 214. *Reagents and conditions*: a) HATU, DIPEA, DMF, rt, **284**: 60%, n = 1, **285**: 20–33%, n = 3, **286**: 20%, n = 1; b) TFA, CH_2Cl_2, 0 °C → rt, **287**: 96%, n = 1, **288**: 100%, n = 1, **289**: 91%, n = 1; c) **268**, HATU, DIPEA, DMF, rt, **290**: 51%, n = 1, **291**: 38%, n = 1; d) TFA, CH_2Cl_2, rt, **212**: 100%, n = 1, **214**: 100%, n = 1.

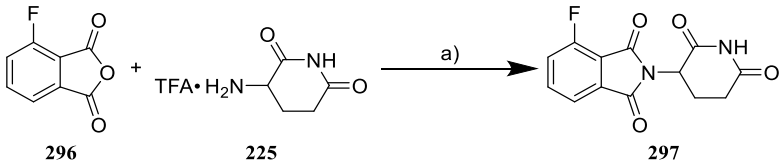
As problems were encountered synthesising linker **273**, an alternative route to PROTAC **211** was required. PROTAC **211** was synthesised using the commercially-available **292** as the linker (Scheme 4.20).

The acid **292** and amine **268** were coupled together to afford amide **293**. The Cbz group was subsequently removed using hydrogenolysis to yield **294**, which was then amide coupled with acid **229** to afford Boc-protected PROTAC **295**. As predicted, removal of the Boc group proved a very clean reaction and concentration *in vacuo* yielded PROTAC **211** in >95% purity (analytical HPLC, 254 nm).



Scheme 4.20: Synthesise of PROTAC 211. *Reagents and conditions*: a) HATU, DIPEA, DMF, rt, 58–77%, n = 2; b) Pd/C, H_2, MeOH, rt, 75–88%, n = 2; c) **229**, HATU, DIPEA, DMF, rt, 20%, n = 1; d) TFA, CH_2Cl_2, rt, 92%, n = 1.

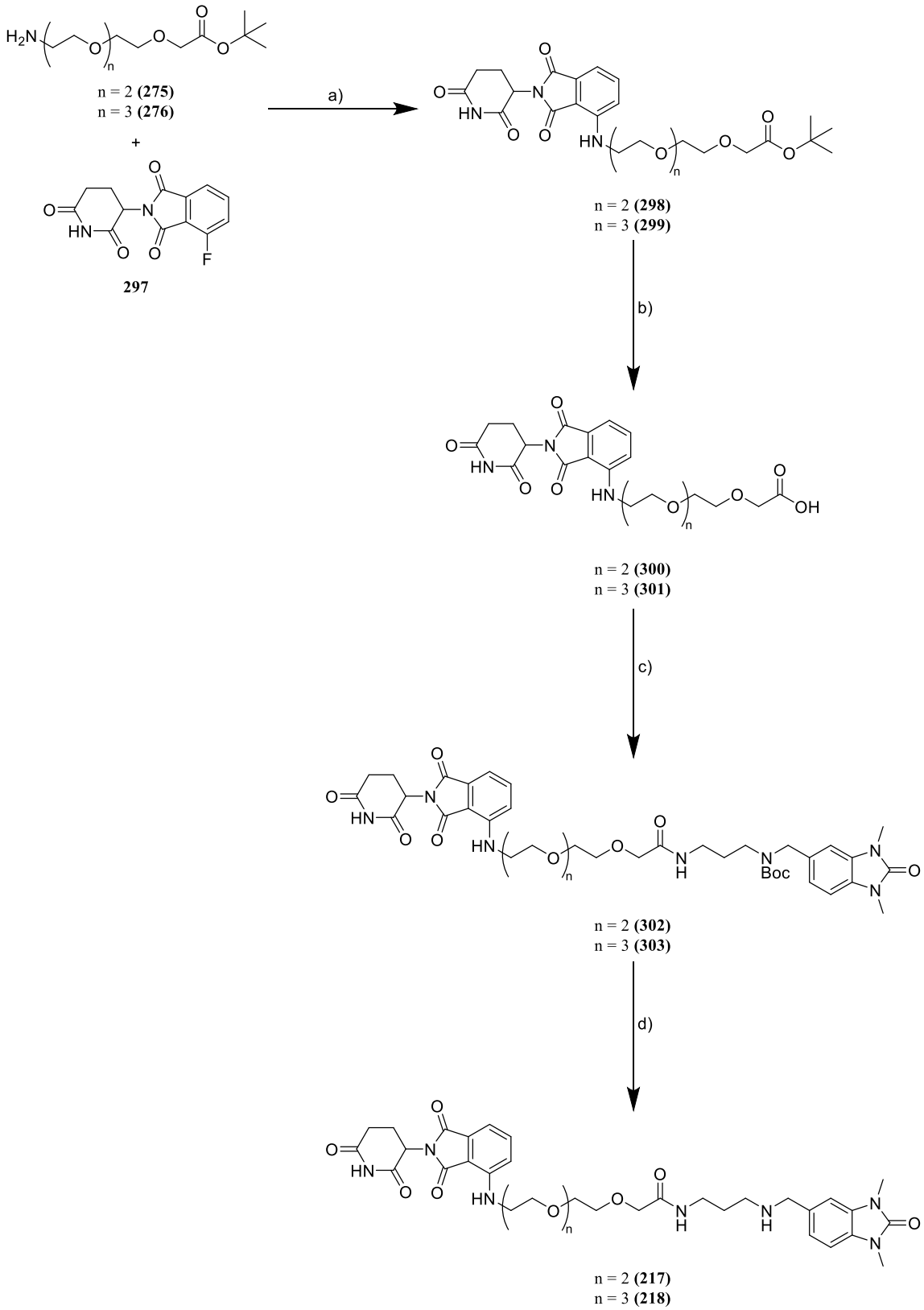
Three PROTACs (**215**, **217** and **218**) were synthesised using pomalidomide as the CRBN ligand. Fluorinated **297** was synthesised by Margarida Espadinha (Scheme 4.21).[183]



296 **225** **297**

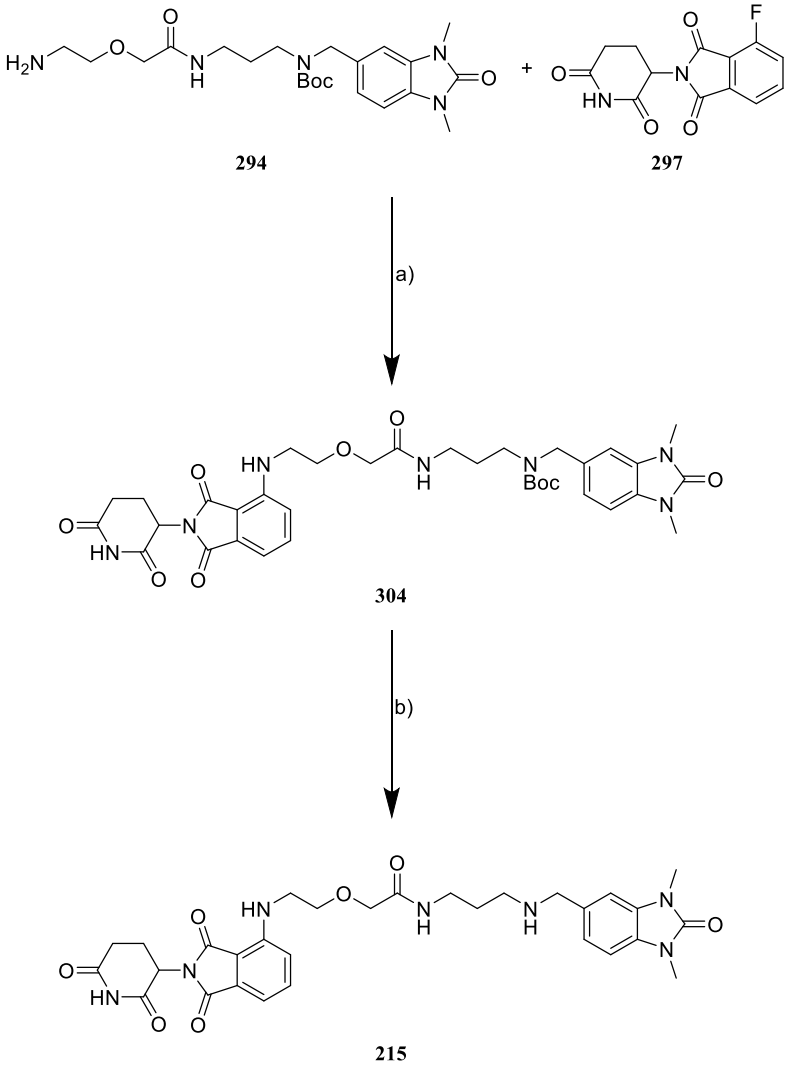
Scheme 4.21: Fluorinated pomalidomide derivative 297 was synthesised by Margarida Espadinha. *Reagents and conditions*: a) NaOAc, AcOH, 140 °C, 76%, n = 1.

To synthesise PROTACs **217** and **218**, amines **275** and **276** were reacted in nucleophilic aromatic substitution (S_NAr) reactions with **297** to yield **298** and **299** (Scheme 4.22). The yields obtained from the S_NAr reactions were poor (26–38%). All the starting materials were consumed (TLC analysis), but more than one product resulted from the reactions. One of the by-products was extremely polar (TLC analysis), suggesting the *tert*-butyl ester had been removed revealing the acid. The *tert*-butyl esters of **298** and **299** were removed in the next step under acidic conditions and acids **300** and **301** were subsequently amide coupled to **268** to yield **302** and **303**. Finally, removal of the Boc protecting groups using TFA afforded PROTACs **217** and **218**.



Scheme 4.22: Synthesis of PROTACs 217 and 218. *Reagents and conditions*: a) DIPEA, NMP, 90 °C, **298**: 38%, n = 1, **299**: 26%, n = 1; b) TFA, CH_2Cl_2, rt, **300**: 100%, n = 1, **301**: 71%, n = 1; c) **268**, HATU, DIPEA, HOBt, DMF, rt, **302**: 20%, n = 1, **303**: 36%, n = 1; d) TFA, CH_2Cl_2, rt, **217**: 100%, n = 1, **218**: 100%, n = 1.

The pomalidomide-PROTAC **215** was synthesised from **294**, which had previously been used as an intermediate in the synthesis of **211**. The amine **294** was reacted in an S_NAr reaction with **297** to yield **304** and the Boc group was subsequently removed in acidic conditions to yield PROTAC **215** (Scheme 4.23).



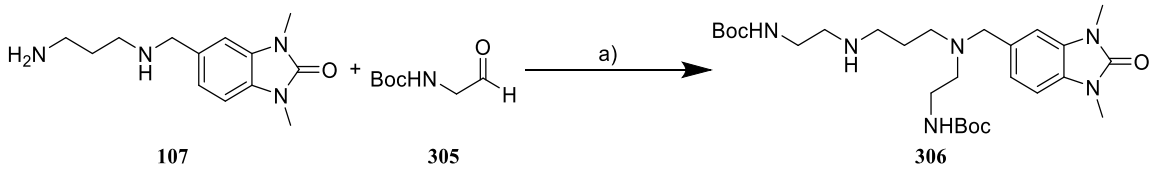
Scheme 4.23: Synthesis of PROTAC 215. *Reagents and conditions*: a) DIPEA, NMP, 90 °C, 23%, n = 1; b) TFA, CH_2Cl_2, rt, 100%, n = 1.

4.4.3 Synthesis of Amine-Linked PROTACs

The amine series of PROTACs was synthesised in parallel to the amide series.

4.4.3.1 Reductive Amination Linking Strategy

To introduce the amine linkage, reductive amination onto the terminal nitrogen of the TRIM33 amine chain was proposed. A model reductive amination between unprotected **107** and *N*-Boc-2-aminoacetaldehyde (**305**) was performed to determine if protection of the benzylic nitrogen was required (Scheme 4.24). It was observed that **305** reacted at both the terminal amine and the benzylic amine, yielding **306**. Therefore, similar to the amide series of PROTACs, the benzylic amine required protection.



107 **305** **306**

Scheme 4.24: Model reductive amination between 107 and 305. *Reagents and conditions*: a) (i) AcOH, DCE, rt; (ii) $NaBH(OAc)_3$, rt.

Troc-protected **243** was synthesised for the amide-linked series. As the amide and amine series were synthesised in parallel, problems with removal of the Troc group were unknown at this time. Troc-protected **243** was therefore also employed in the synthesis of the amine-linked series. It was proposed that alkanolamines could be amide coupled to CRBN ligand **229**, and the resulting alcohol oxidised to an aldehyde for reaction *via* a reductive amination with **243** (Figure 4.12).

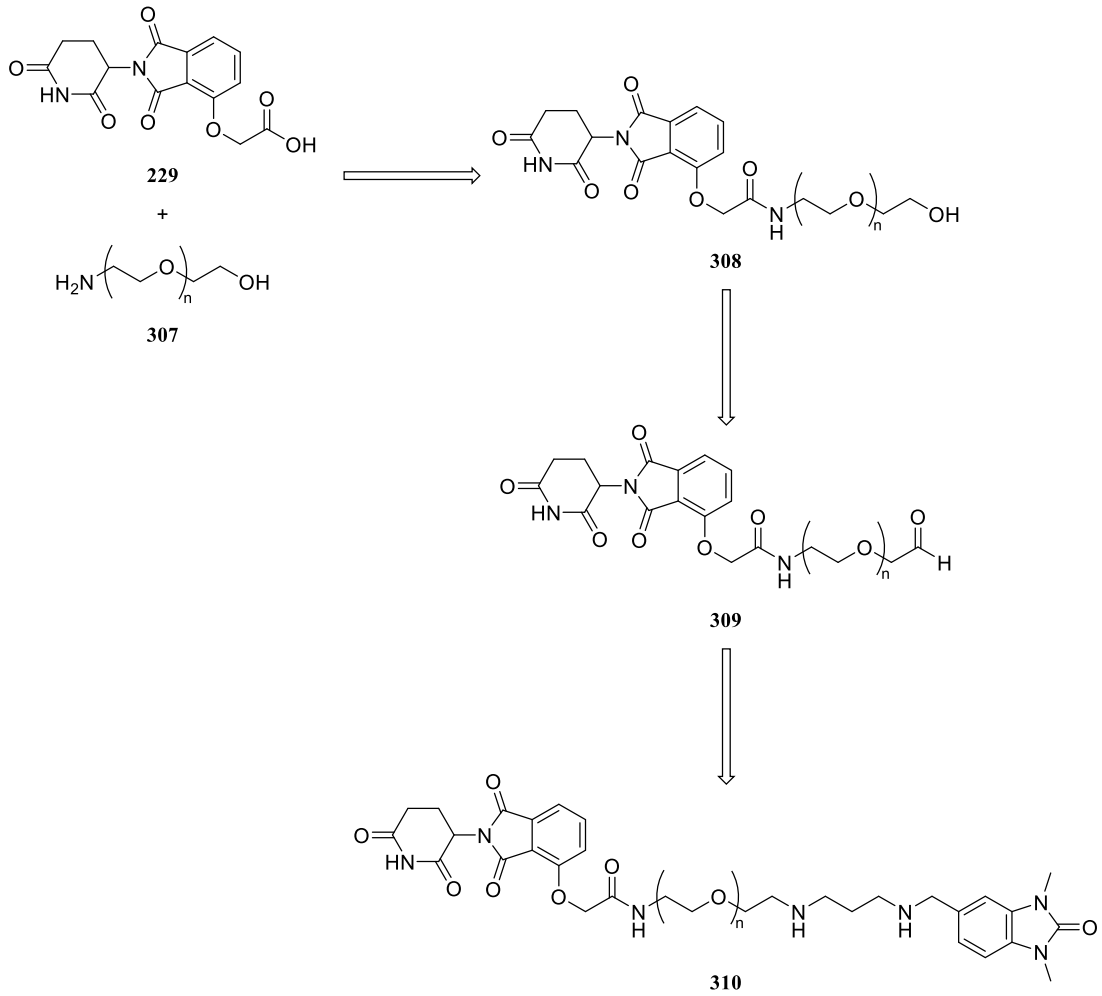
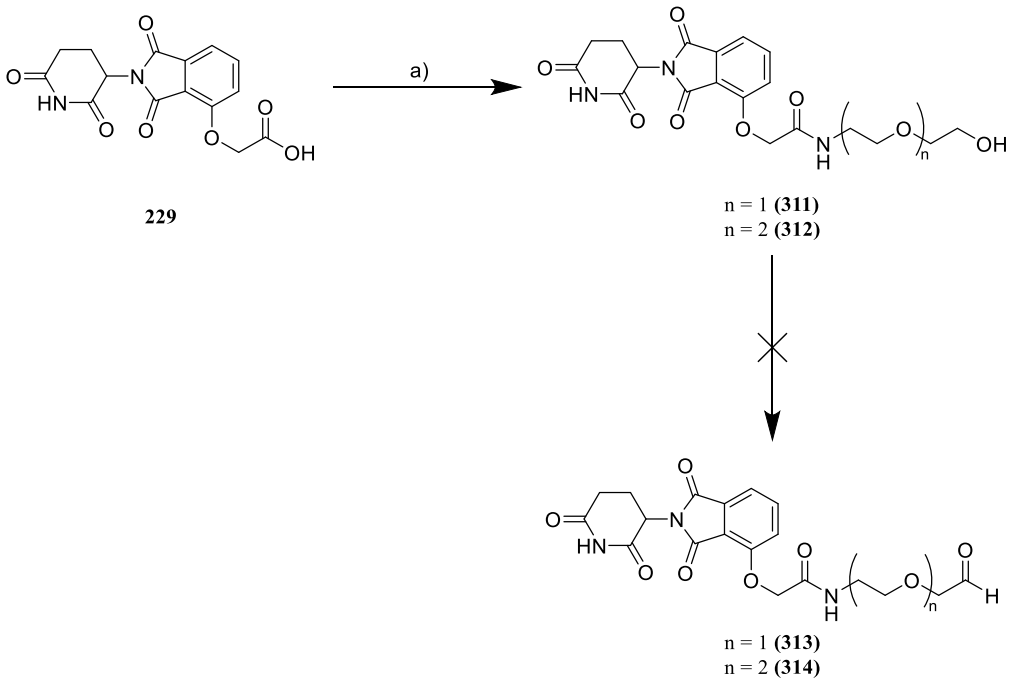


Figure 4.12: Proposed synthetic route to amine-linked PROTACs. A reductive amination is used to introduce the amine linkage.

Alkanolamines were amide coupled with **229** to yield alcohols **311** and **312** in moderately low yields (29–33%) (Scheme 4.25). Oxidation of alcohols **311** and **312** to aldehydes **313** and **314** using Dess-Martin periodinane (DMP) however proved challenging. Despite consumption of starting material by TLC analysis, no aldehyde peaks were observed in the ^{1}H NMR spectra.



Scheme 4.25: Attempted synthesis of aldehydes 313 and 314. *Reagents and conditions*: a) Alkanolamine, HATU, DIPEA, DMF, rt, **311**: 29%, n = 1, **312**: 33%, n = 1.

Reductive amination of the linker to TRIM33 ligand **243** prior to amide coupling to CRBN ligand **229** was next attempted (Figure 4.13). This route, however, requires additional protection and deprotection steps to ensure amide coupling with thalidomide **229** only occurs at the terminal amine.

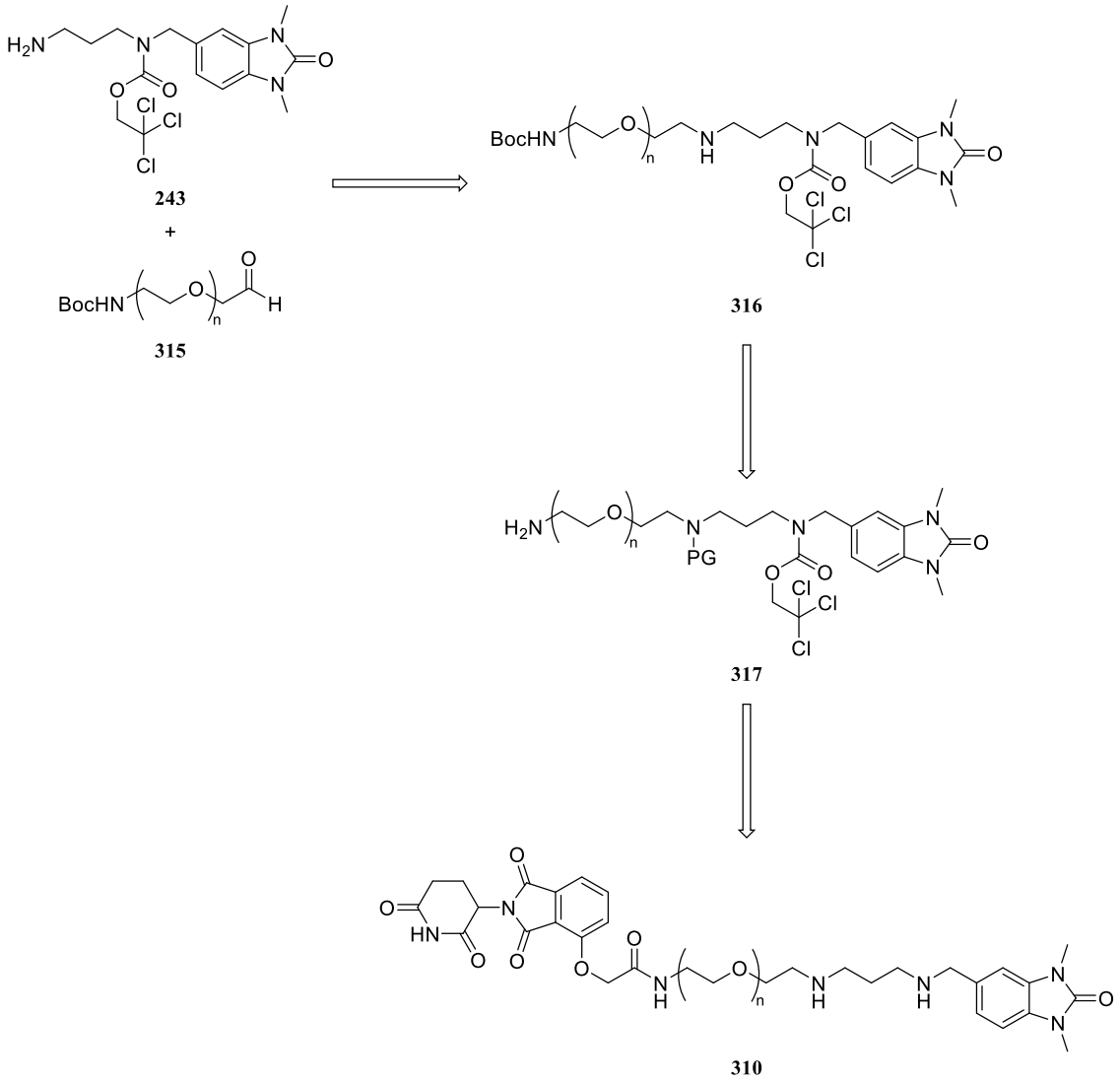
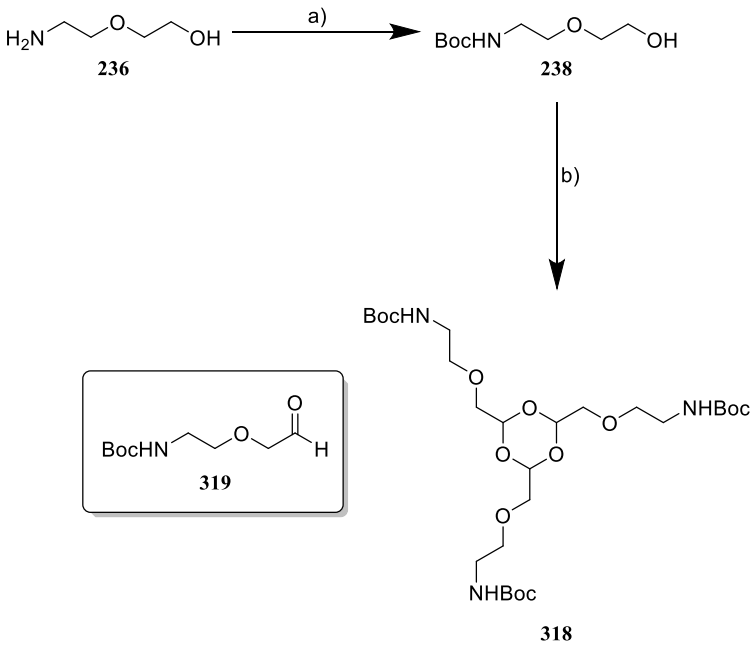
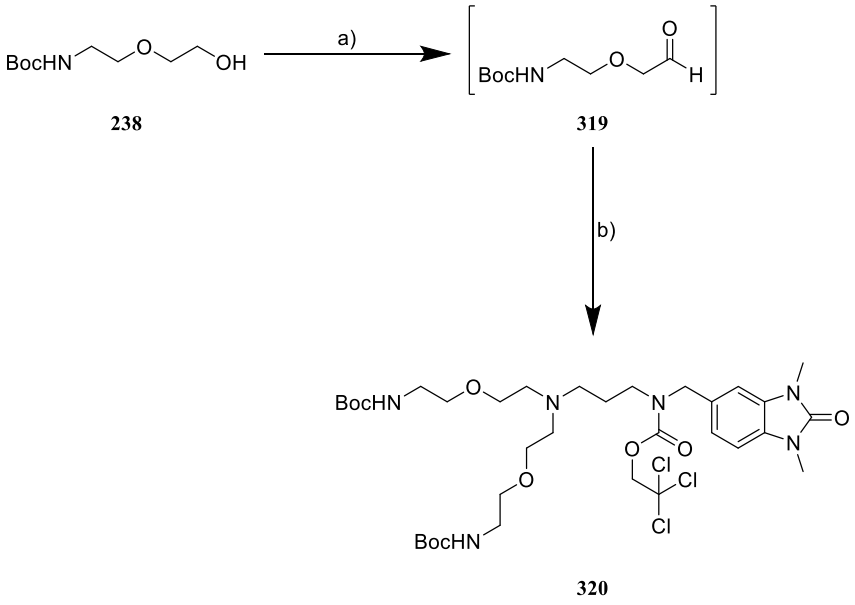


Figure 4.13: Proposed route to amine-linked PROTACs. Reductive amination between **243** and linker **315** introduces the amine linkage.

Oxidation of alkanolamine **238** to give aldehyde **319** proved challenging. The amino group of alkanolamine **236** was first Boc protected (**238**) (Scheme 4.26). Initially DMP was used for the oxidation of **238**, but aldehyde peaks were not observed in the ^{1}H NMR spectrum. Swern oxidation of **238** was next attempted (Scheme 4.26).[184,185] Literature shows that aldehyde **319** is unstable;[186] trimerisation to **318** was indeed observed and **319** was not isolated. To try and reduce the amount of trimerisation product, concentration *in vacuo* was conducted at <4 °C and the resulting residue was directly subjected to the reductive amination with **243** without purification (Scheme 4.27). Unfortunately, the reductive amination reaction yielded **320** resulting from the aldehyde reacting twice at the terminal amine of **243**.



Scheme 4.26: Attempted synthesis of aldehyde 319. *Reagents and conditions*: a) Boc_2O, CH_2Cl_2, rt, 100%, n = 1; b) (i) Oxalyl chloride, CH_2Cl_2, –78 °C → rt; (ii) DMSO, CH_2Cl_2, –78 °C; (iii) **238**, CH_2Cl_2, –78 °C; (iv) Et_3N, –78 °C → rt, 67%, n = 1.



Scheme 4.27: Reductive amination between aldehyde 319 and TRIM33 ligand 243. *Reagents and conditions*: a) (i) Oxalyl chloride, CH_2Cl_2, –78 °C → rt; (ii) DMSO, CH_2Cl_2, –78 °C; (iii) **238**, CH_2Cl_2, –78 °C; (iv) Et_3N, –78 °C → rt; b) (i) **243**, Et_3N, DCE, rt; (ii) $NaBH(OAc)_3$, rt, 23%, n = 1.

4.4.3.2 Substitution Linking Strategy

An alternative strategy to obtain the amine-linked series was to use a substitution reaction (Figure 4.14). It was envisaged that the alcohol **308** could be converted to the bromide **321**, which could then be substituted with the amine chain of the TRIM33 ligand to introduce the amine linkage (**322**).

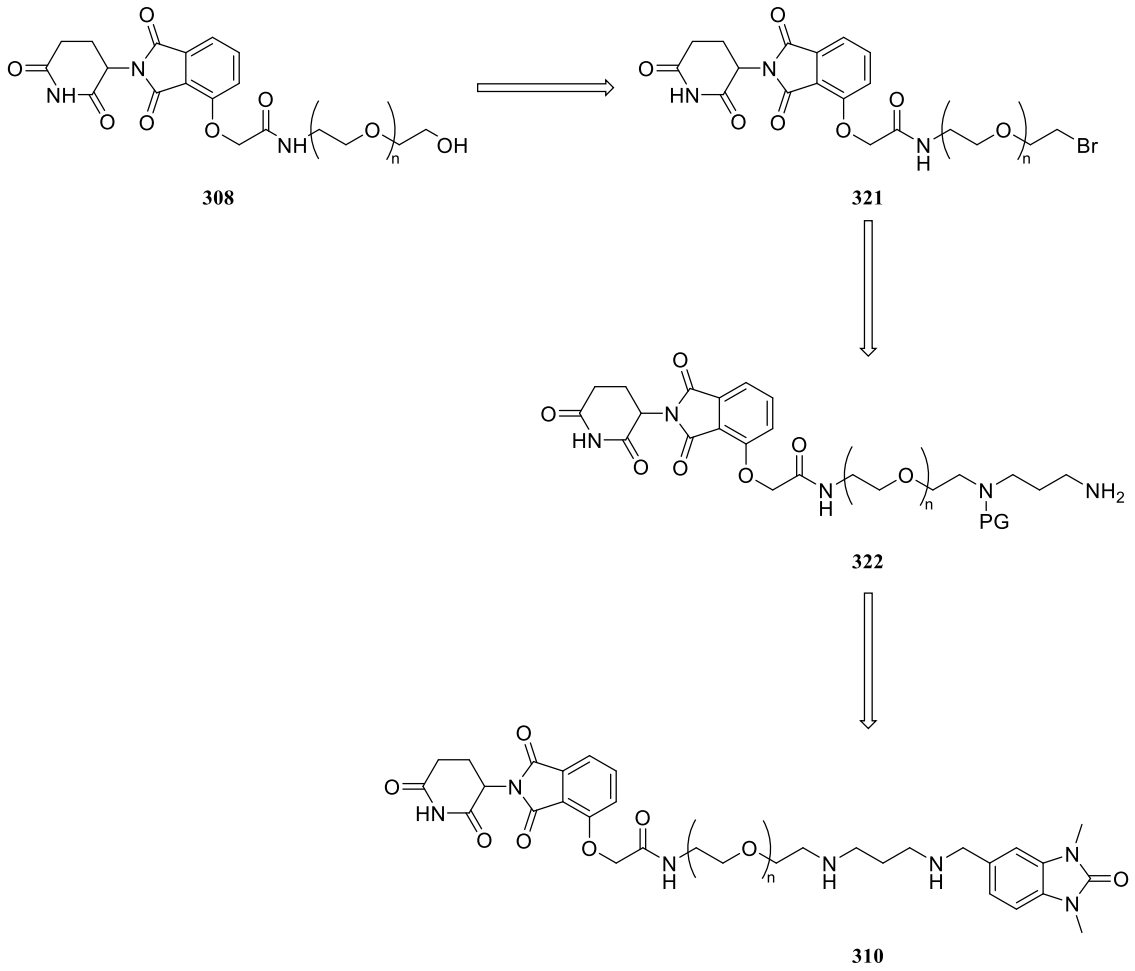
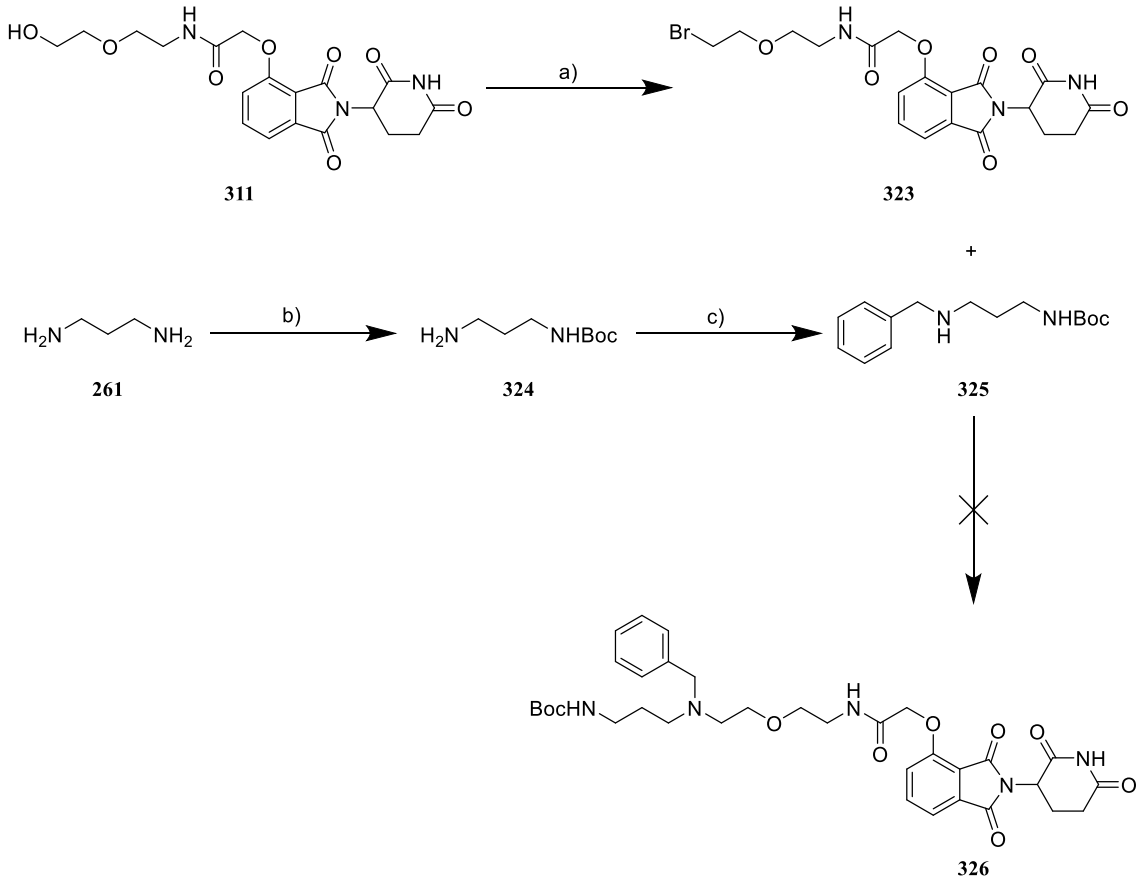


Figure 4.14: Proposed synthetic route to amine-linked PROTACs using a substitution reaction. A substitution reaction is used to introduce the amine linkage.

Conversion of the alcohol **311** to the bromide **323** was achieved using an Appel reaction (Scheme 4.28).[187] Literature precedent showed diamine **325** to undergo substitution reactions at the benzylic nitrogen,[188,189] and a substitution reaction was attempted with bromide **323**. The reagents were dissolved in DMF and DIPEA was added. The reaction was performed at both rt and 60 °C. Unfortunately, **326** was not observed and both starting materials were recovered.



Scheme 4.28: Attempted synthesis of amine-linked PROTACs using a substitution reaction. *Reagents and conditions*: a) PPh_3, NBS, CH_2Cl_2, 0 °C → rt, 84%, n = 1; b) Boc_2O, $CHCl_3$, 0 °C → rt, 83%, n = 1; c) (i) Et_3N, benzaldehyde, MeOH, $MgSO_4$, rt; (ii) $NaBH_4$, –5 °C → 0 °C, 57%, n = 1.

4.4.3.3 Mitsunobu Linking Strategy

An alternative linking strategy using a Mitsunobu reaction was proposed to form the amine linkage (Figure 4.15).[157] Careful consideration was given to the choice of protecting groups, especially considering the stability of the TRIM33 ligand and the CRBN ligand to the deprotection conditions.

A nitrobenzenesulfonyl (nosyl) group was chosen to protect the terminal amine of **107**. The acidity of the nucleophile is important in the Mitsunobu reaction, as the betaine formed from the reaction between the azodicarboxylate and PPh_3, which removes the acidic proton of the nucleophile, has a pK_a around 13.[190] The pK_a of nucleophile **327** used in this strategy is predicted to be 10.5 (calculated using ACD I-Lab 2.0).[130]

A Cbz group was chosen to protect the benzylic nitrogen of the TRIM33 ligand as both the CRBN ligand and TRIM33 ligand are known to be stable under hydrogenolysis conditions.

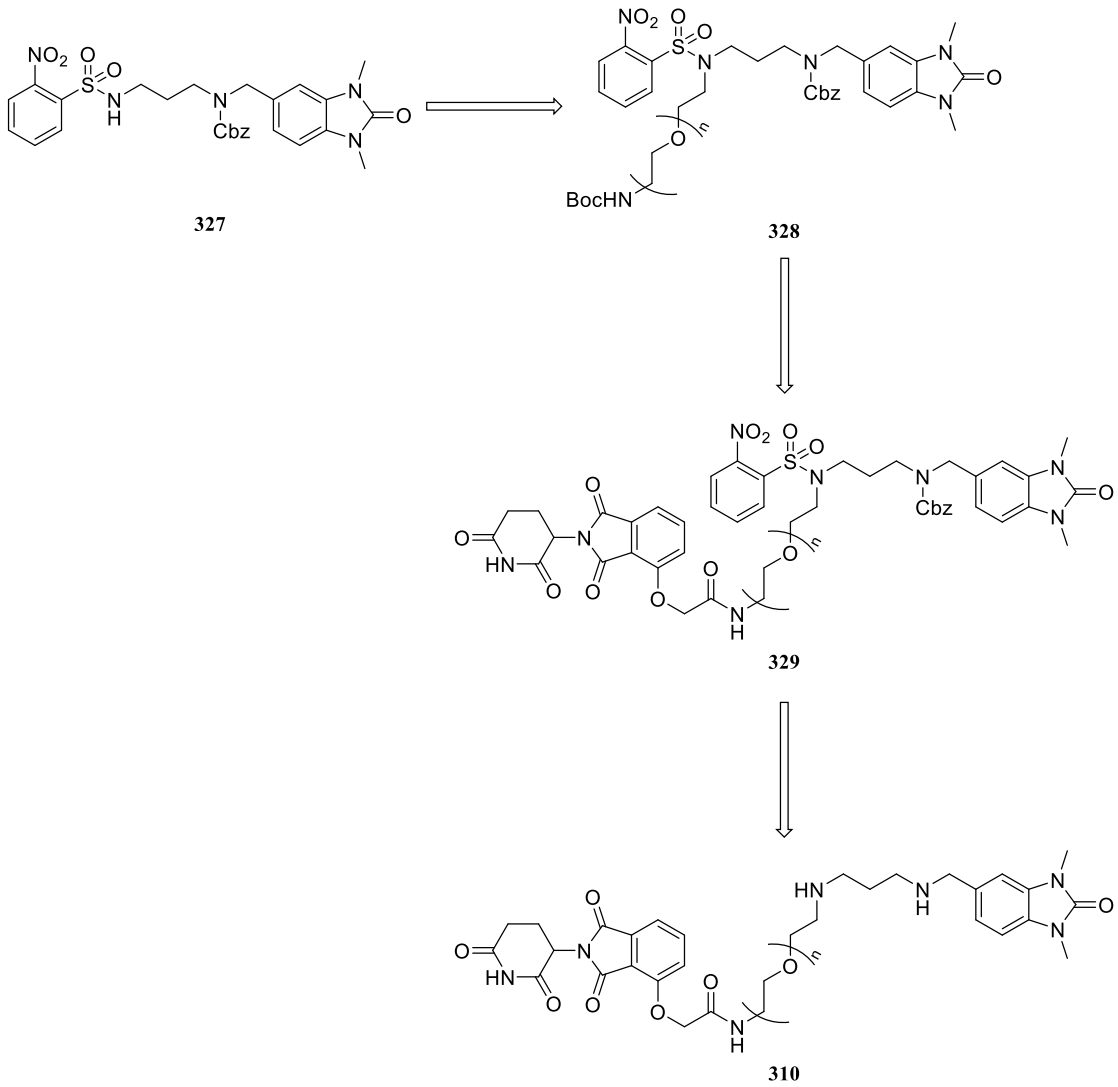
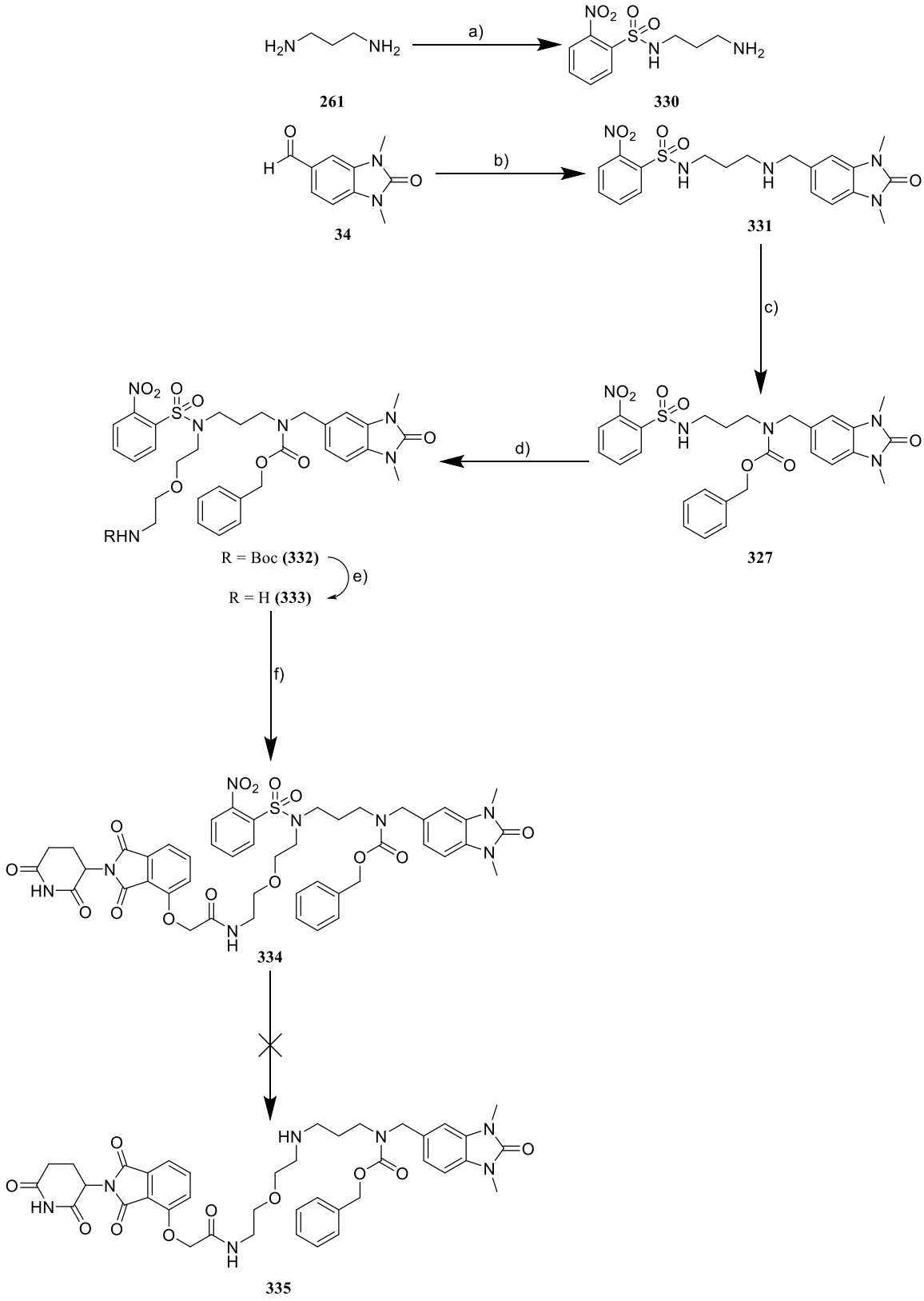


Figure 4.15: Proposed route to amine-linked PROTACs using a Mitsunobu reaction.

The route to the protected PROTAC **334** is shown in Scheme 4.29. The diamine **261** was monoprotected with a nosyl group to afford **330**. The amine **330** was then reacted with the aldehyde **34** in a reductive amination to yield **331**, and the benzylic nitrogen protected with a Cbz group to yield **327**. Nosyl-protected **327** was next reacted in a Mitsunobu reaction with **238** to yield **332**. Removal of the Boc group under acidic conditions afforded **333** which was then amide coupled with acid **229** to yield protected **334**. Removal of the nosyl group was attempted using thioglycolic acid, which goes *via* an S_NAr reaction. The reaction mixture turned bright yellow, suggesting the nitroaromatic had been removed. However, only a small amount of product was

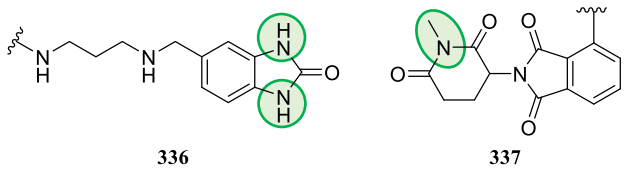
observed in the ^{1}H NMR spectrum, and could not be isolated. Alternative deprotection strategies are currently being investigated to complete the synthesis of the amine-linked series.



Scheme 4.29: Attempted synthesis of amine-linked PROTAC 219. *Reagents and conditions*: a) 2-Nitrobenzenesulfonyl chloride, CH_2Cl_2, –5 °C → rt, 51%, n = 1; b) (i) **330**, AcOH, DCE, 3 Å molecular sieves, rt; (ii) $NaBH(OAc)_3$, rt, 48%, n = 1; c) Benzyl chloroformate, Et_3N, CH_2Cl_2, 0 °C → rt, 77%, n = 1; d) **238**, DIAD, PPh_3, THF, 0 °C → rt, 58–69%, n = 2; e) HCl (4 M, dioxane), rt, 86%, n = 1; f) **229**, HATU, DIPEA, DMF, rt, 50–79%, n = 2.

4.5 Control Compounds

To confirm that the PROTACs are acting *via* the predicted mechanism it is important to have negative controls (Figure 4.16). The methylation states of the TRIM33 ligands and CRBN ligands are critical for binding. In Chapter 3 it was shown that substitution on the benzimidazolone nitrogens is required for TRIM33 affinity. If compound **336** is used as the TRIM33 ligand, binding to TRIM33 should be abrogated. Crystal structures of thalidomide bound to CRBN show the amino group forms hydrogen bonds to His380 and Ser382. Methylation of this nitrogen (**337**) removes affinity for CRBN as the methyl group causes steric hindrance and removes the key hydrogen bonding interactions.[191] Having a negative control for the E3 ligase ligand will help to identify proteins whose levels are observed to decrease due to transcriptional changes or destabilisation by the TRIM33 inhibitor alone, not due to PROTAC-mediated degradation.[172]



336 **337**

Figure 4.16: Negative controls for the TRIM33 ligand and the CRBN ligand. In both cases the methylation state affects binding.

The importance of the linker portion of the PROTAC can also be demonstrated by directly coupling the TRIM33 ligand and the CRBN ligand. Future work will include synthesising these control compounds to validate degradation mechanisms.

4.6 Evaluation of PROTACs

4.6.1 AlphaScreen™ Assays

The synthesised compounds were evaluated using an AlphaScreen™ assay to assess binding to TRIM33 (Table 4.1 and Appendix B). The CRBN ligand **227** and the VHL ligand **338** were also evaluated to ensure they do not independently bind to TRIM33.

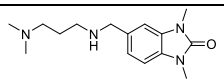
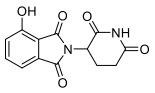
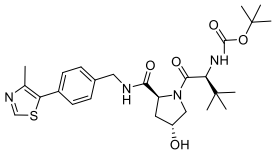
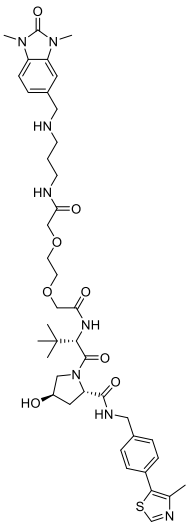
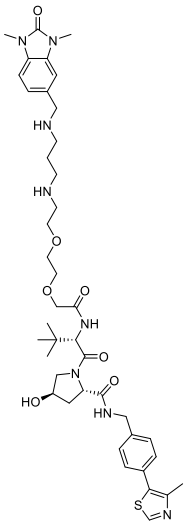
Unfortunately, problems were encountered with the AlphaScreen™ assay, and IC_{50} values could only be obtained for VHL-PROTACs **202** and **207** (Table 4.1). Pleasingly the CRBN ligand **227** and the VHL ligand **338** did not bind to TRIM33, indicating any affinity demonstrated by the

PROTACs is occurring *via* the TRIM33 ligand portion. VHL-PROTACs **202** and **207** were observed to bind to TRIM33.

Compound **90** is routinely used as a positive control in our AlphaScreen™ assays, however, upon testing the remaining PROTACs, binding of **90** to TRIM33α and TRIM33β was not observed. Signal-response experiments were performed, and concentrations of proteins and peptides adjusted accordingly, yet still no binding of **90** was detected. The data obtained from these assays are therefore unreliable and do not give an accurate description of binding affinities. Work to resolve this problem is ongoing.

Table 4.1: IC_{50} values determined using a dose-response AlphaScreen™ assay. Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). H3(BRD) – $H3_{1-27}$K18Ac.

High affinity — Low affinity

Compound	Structure	TRIM24 – H3(BRD) IC_{50} (µM)	TRIM33α – H3(BRD) IC_{50} (µM)	TRIM33β – H3(BRD) IC_{50} (µM)
90		>250	0.597 ± 0.161	1.82 ± 0.286
227		>250	>250	>250
338		>250	>250	>250
202		>250	2.81 ± 0.452	6.26 ± 1.75
207		>250	0.913 ± 0.179	6.15 ± 1.28

As problems were encountered with the AlphaScreen™ assay, the AlphaScreen™ TruHits™ kit was performed to check that the PROTACs are not interfering (Table 4.2). Unfortunately, dose-dependent assay interference was observed, this was particularly prominent for PROTACs containing the VHL ligand. These compounds either quench the singlet oxygen produced by the donor beads or act as inner filters, thus reducing the observed fluorescence. Orthogonal assays, such as waterLOGSY and ITC experiments, are required to validate the PROTACs as binding to TRIM33. This assay interference, however, does not necessarily account for the positive control (**90**) not being observed to bind in the assays previously performed.

Table 4.2: Results from the AlphaScreen™ TruHits™ kit. Compounds were screened at five concentrations and with two incubation formats. Results which are more than two standard deviations greater than the mean DMSO control are highlighted in blue.

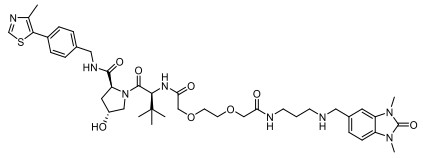
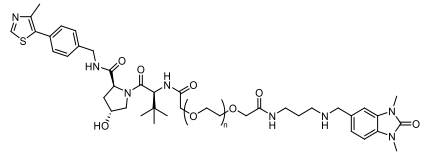
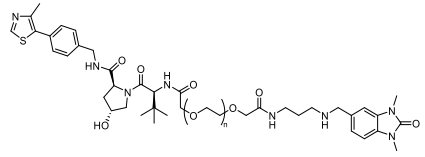
Compound	Difference from DMSO average, normalised against standard deviation of DMSO control									
	Pre-incubate beads					Pre-incubate donor beads with compound				
	31.25 µM	62.5 µM	125 µM	250 µM	500 µM	31.25 µM	62.5 µM	125 µM	250 µM	500 µM
 **202**	0.649	1.11	1.87	3.10	6.34	1.14	1.04	0.708	1.08	1.04
 **n = 2** **203**	3.16	4.30	5.88	10.0	15.8	0.775	0.931	0.728	1.22	1.61
 **n = 3** **204**	0.435	1.02	1.60	2.67	7.34	0.655	0.438	0.461	0.539	1.11

Table 4.2 continued.

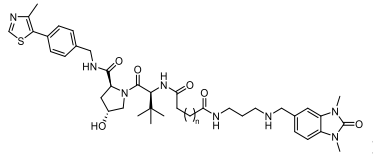
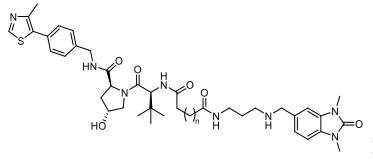
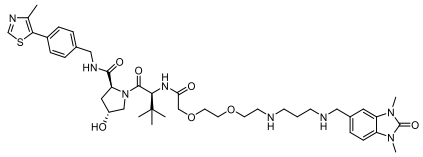
Compound	Difference from DMSO average, normalised against standard deviation of DMSO control									
	Pre-incubate beads					Pre-incubate donor beads with compound				
	31.25 µM	62.5 µM	125 µM	250 µM	500 µM	31.25 µM	62.5 µM	125 µM	250 µM	500 µM
 **n = 4** **205**	0.531	1.07	2.68	4.95	10.6	0.0490	0.0210	0.123	0.601	1.25
 **n = 7** **206**	0.0798	0.566	1.51	2.70	7.21	0.469	0.254	0.190	0.620	1.10
 **207**	3.22	4.09	5.45	7.30	10.8	1.58	1.62	1.07	1.73	1.59

Table 4.2 continued.

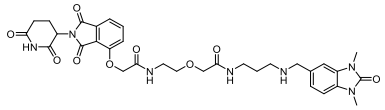
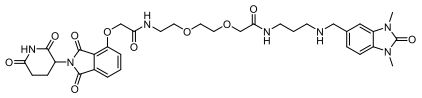
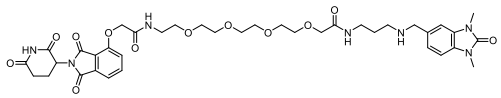
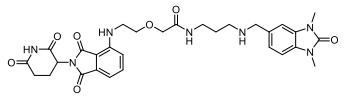
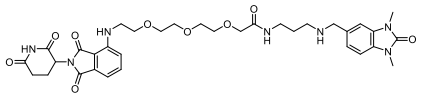
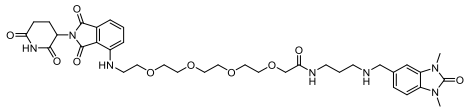
Compound	Difference from DMSO average, normalised against standard deviation of DMSO control									
	Pre-incubate beads					Pre-incubate donor beads with compound				
	31.25 µM	**62.5 µM**	**125 µM**	**250 µM**	**500 µM**	**31.25 µM**	**62.5 µM**	**125 µM**	**250 µM**	**500 µM**
 **211**	2.09	0.942	0.0610	1.14	4.58	0.665	1.50	0.786	0.165	0.0760
 **212**	1.62	0.920	0.0850	1.12	3.82	1.31	1.77	1.35	0.619	0.141
 **214**	1.44	0.100	0.0420	1.60	5.93	0.814	1.29	0.710	0.242	0.781

Table 4.2 continued.

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
 **215**	0.967	0.789	1.51	1.36	2.00	0.863	0.846	0.426	1.06	1.41
 **217**	0.828	1.04	1.78	1.74	1.22	0.690	0.432	0.124	0.421	0.501
 **218**	3.55	3.78	3.25	3.64	7.37	0.0520	0.576	0.0610	0.622	0.681
Biotin	-	-	-	-	-	1.44	2.72	3.62	3.99	4.08

4.7 Conclusions

Six potential CRBN-directed PROTACs and six potential VHL-directed PROTACs (Jessica Reynolds) targeting TRIM33 have been synthesised. Due to their complex structures, their syntheses were challenging and the correct choice of protecting groups proved key.

To maximise the chance of identifying an efficient degrader of TRIM33, PROTACs were synthesised with varying linker lengths, linking strategies and E3 ubiquitin ligase ligands. For the amide-linked series the benzylic nitrogen was Boc protected. This proved a desirable protecting group as its removal was clean and the final PROTAC was easily isolated. Three PROTACs were also synthesised with pomalidomide, as the CRBN ligand, as changing the E3 ligase ligand can have a large effect on degradation efficiency. For the amine-linked series a Mitsunobu reaction was employed as the key linking step. A nosyl protecting group was used for the Mitsunobu reaction to ensure an appropriate pK_a of the pronucleophile. Conditions for its removal remain to be identified; however, test reactions looked promising and a similar strategy was employed for the synthesis of amine-linked VHL-PROTAC **207**.

An AlphaScreen™ assay confirmed that both the CRBN ligand **227** and the VHL ligand **338** do not bind to TRIM33 meaning any affinity observed is coming from the TRIM33 ligand portion of the PROTAC.

PROTACs **202** and **207** were observed to retain affinity for TRIM33 using an AlphaScreen™ assay. Unfortunately, the AlphaScreen™ TruHits™ kit detected a large amount of assay interference, thus PROTACs **202** and **207** may in fact be false positives. To confirm binding to TRIM33, evaluation using an orthogonal assay, such as an NMR-based assay or ITC experiments, is required and is the current focus of this work. If binding to TRIM33 is observed, the effect of the compounds on TRIM33 protein levels will be evaluated to see if the compounds are indeed behaving as PROTACs.

The identification of reliable synthetic routes to potential TRIM33 PROTACs aids future linker optimisation. Different linker compositions will be explored, including alkyl chains and more

rigid variants, for example a triazole could be introduced, which also provides additional linking strategies using ‘click’ chemistry.[176] Future work will also focus on completing the synthesis of the amine-linked series, specifically finding clean and reliable conditions for removal of the nosyl group.

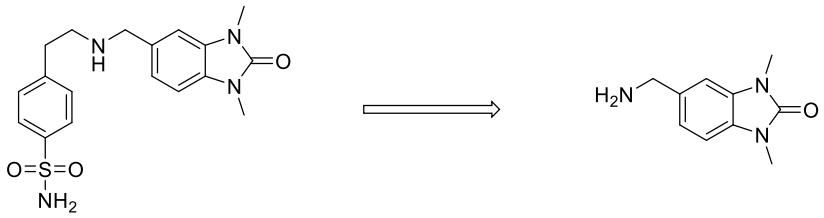
CHAPTER 5

Conclusions

5.1 Conclusions

The aim of the work described here was to develop chemical tools for TRIM33 that can be used to study its physiological and pathological roles. Two orthogonal approaches were employed, bromodomain ligands and PROTACs, to enable the role of the bromodomain to be disconnected from the function of the whole protein.

The work described in Chapter 2 aimed to increase the understanding of the KAc-binding pocket to aid future ligand design. Building on previous work in the group, benzimidazolone analogue **23** was reduced to primary benzylic amine fragment **26** (Figure 5.1). Removal of the sulfonamide chain improved selectivity over TRIM24 and resulted in a fragment with a ligand efficiency of 0.45. Compound **26** was, therefore, used as the starting point for ligand development in Chapter 3.



23
TRIM24 IC_{50} = 40.3 μM (0.24)
TRIM33α IC_{50} = 3.88 μM (0.29)
TRIM33β IC_{50} = 11.9 μM (0.27)

26
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 7.83 μM (0.51)
TRIM33β IC_{50} = 30.6 μM (0.45)

Figure 5.1: Initial hit 23 was reduced to primary benzylic fragment 26. This resulted in improved selectivity and increased ligand efficiency. The IC_{50} values were determined using a dose-response AlphaScreen™ assay with $H3_{1-27}$K18Ac peptide. Ligand efficiencies are given in parentheses.

The benzylic nitrogen on the benzimidazolone core was found to be essential for imparting selectivity over TRIM24, and docking studies suggested that this was due to interactions with E981. Molecular dynamic studies corroborated this, as the benzylic nitrogen remained in close proximity to E981 for the duration of the simulations. The methylation state of the headgroup was

critical for binding to TRIM33, as was the distance of the basic substituent from the benzimidazolone core. A space of one methylene group between the benzimidazolone core and the basic group, as in **26** (Figure 5.1), was found to be optimal, likely due to maximising interactions with E981.

Overlaying a histone H3 peptide with **23** docked into the TRIM33β bromodomain revealed a hydrophobic region above the KAc-binding pocket (Figure 5.2 (A)). One of the methyl groups on the benzimidazolone core of **26** was therefore replaced with a hydrophobic substituent, in an attempt to increase affinity through hydrophobic interactions. As this meant that the benzimidazolone core was no longer symmetrical, initial work focused on identifying the optimal vector combination (Figure 5.2 (B)). A phenethyl substituent was used for this preliminary work, in which benzylic substitution on C-5 or C-5' was shown to be most tolerated.

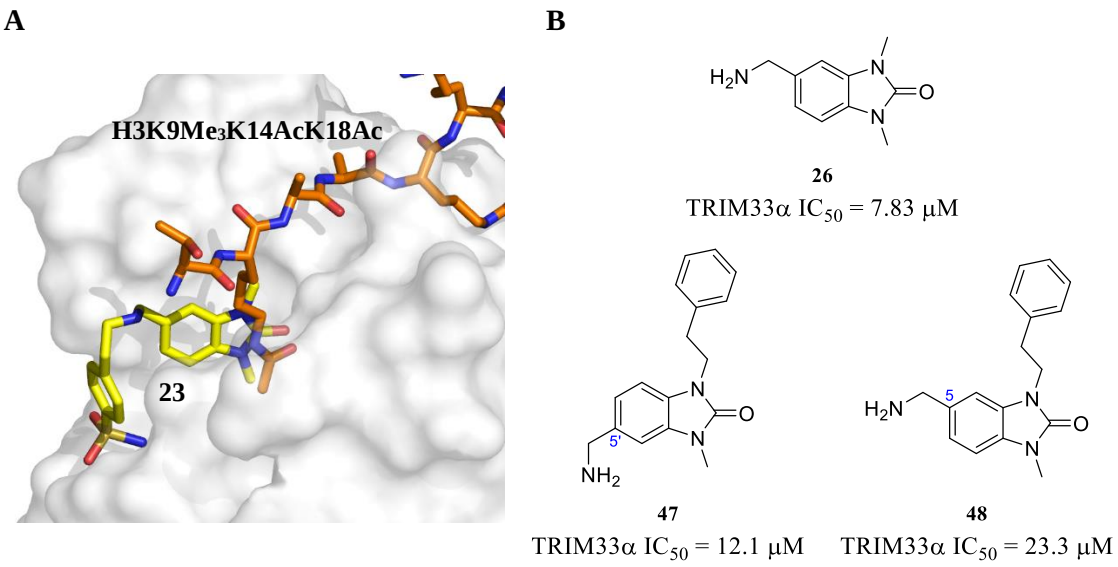
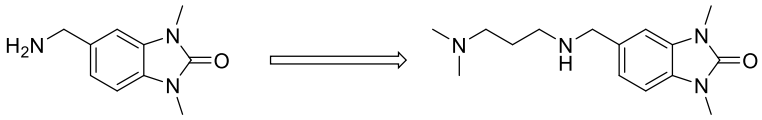


Figure 5.2: A hydrophobic region above the KAc-binding pocket was identified. (A) Compound **23** (yellow) docked in TRIM33β (PDB ID: 5MR8) using CLC Drug Discovery Workbench overlaid with $H3_{1–22}K9Me_3K14AcK18Ac$ (orange) (PDB ID: 3U5O). (B) Benzylic substitution on C-5 or C-5' was found to be tolerated for TRIM33α and was the focus of further exploration in this hydrophobic pocket. The IC_{50} values were determined using a dose-response AlphaScreen™ assay with $H3_{1–27}K18Ac$ peptide.

The AlphaScreen™ TruHits™ kit confirmed that the benzimidazolone compounds were not interfering with the assay and thus AlphaScreen™ was deemed an appropriate assay for primary screening. In contrast, the reported TRIM33 bromodomain ligands[78] demonstrated a large amount

of assay interference, and subsequent waterLOGSY and ITC experiments showed that the compounds were false positives in the AlphaScreen™ assay. This work highlights the importance of validating hits in orthogonal assays and checking for assay interference before proceeding with further ligand optimisation.

Having identified key interactions between benzimidazolone analogues and the TRIM33 bromodomain, the selectivity and affinity of **23** was optimised, which is described in Chapter 3. A robust synthetic route was developed which enabled facile diversification of the amine chain. This work led to the identification of amine **90**, which was selective over the TRIM24 bromodomain (IC_{50} >250 μM) and retained affinity for TRIM33β (IC_{50} = 13.7 μM) (Figure 5.3). WaterLOGSY experiments performed using a mutant TRIM33β protein (TRIM33β-N1039F) confirmed **90** was binding in the bromodomain. Consequently, compound **90** was the basis of subsequent ligand development.



26
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 7.83 μM
TRIM33β IC_{50} = 30.6 μM

90
TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 3.61 μM
TRIM33β IC_{50} = 13.7 μM

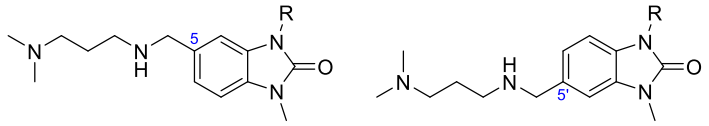
Figure 5.3: Investigation of the amine chain led to the identification of 90. Compound **90** was selective over TRIM24 and had similar affinity as **23** (IC_{50} = 11.9 μM) for TRIM33β. The IC_{50} values were determined using a dose-response AlphaScreen™ assay with $H3_{1-27}$K18Ac peptide.

It is important that chemical probes are selective to ensure that the biological activity is due to an on-target effect. A BRD4 AlphaScreen™ showed **90** is selective over BRD4. BRD4 was specifically considered due to the BET family of bromodomains having a significant phenotype.

The necessity of the benzylic amine for selectivity was further investigated by replacement with a pyrazole or imidazole moiety. These heterocyclic analogues showed that the benzylic amine was required for selectivity over the TRIM24 bromodomain but was not essential for affinity for the TRIM33 bromodomain. In addition, methylation of the benzylic amine reduced affinity for the TRIM33 bromodomain. Methylation lowers the pK_{aH} of this amine from 8.23 to 6.50 and thus

the compound is only partially protonated at physiological pH. Interactions with E981 are consequently reduced, which is reflected in the higher IC_{50} values. In addition, the increased steric bulk could contribute to the reduced affinity. Consequently, all future compounds were unmethylated at this position.

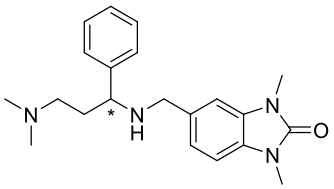
Building on the knowledge that a hydrophobic substituent on the benzimidazolone core is tolerated, the hydrophobic peptide binding groove was explored further in Chapter 3 (Figure 5.4). Alkyl phenyl chains of various lengths were first chosen to explore benzylic substitution at C-5 and C-5'. Compounds with C-5 benzylic substitution did not bind to TRIM33β and compounds with C-5' benzylic substitution had reduced affinity for TRIM33β. Based on this result, C-5' substituted compounds were synthesised with a wide range of substituents on the benzimidazolone nitrogen. Analogues possessing small alkyl substituents had the highest affinity for TRIM33β but had similar affinity to dimethyl analogue **90**. Further investigation is required to determine whether substituting on the benzimidazolone headgroup can increase affinity.



339 **340**

Figure 5.4: A range of substituents were introduced onto the benzimidazolone headgroup. C-5' substitution was found to be better tolerated than C-5, however all analogues had weaker affinity for TRIM33β than dimethyl analogue **90**.

Molecular dynamic studies of **90** showed that its terminal amine frequently interacted with E981, while E984 was solvent-exposed. Any interaction with E984 will therefore have an associated desolvation penalty. Based on this result, compounds were synthesised to maximise interactions with E981 by favouring a 'pincer' interaction. Compound **161** (IC_{50} = 4.83 µM) has a phenyl ring on the amine chain and was designed to try and bias a bidentate interaction with E981 (Figure 5.5). Pleasingly, an increase in affinity for TRIM33β relative to **90** was observed, which could be due to the phenyl ring enforcing a favourable conformation. Compound **161** was subsequently synthesised as its single enantiomers (Figure 5.5), but this showed that the stereochemistry was unimportant for binding affinity (**(*S*)-161** IC_{50} = 4.52 µM, **(*R*)-161** IC_{50} = 7.75 µM).



***rac*-161**	**(*S*)-161**	**(*R*)-161**
TRIM24 IC_{50} >250 μM	TRIM24 IC_{50} >250 μM	TRIM24 IC_{50} >250 μM
TRIM33α IC_{50} = 1.56 μM	TRIM33α IC_{50} = 1.32 μM	TRIM33α IC_{50} = 2.45 μM
TRIM33β IC_{50} = 4.84 μM	TRIM33β IC_{50} = 4.52 μM	TRIM33β IC_{50} = 7.75 μM

Figure 5.5: Compound 161 was designed to bias a ligand conformation which favours a 'pincer' interaction with E981. 161 was synthesised as its single enantiomers, however, the stereochemistry was found to have little effect on binding affinity. The IC_{50} values were determined using a dose-response AlphaScreen™ assay with $H3_{1–27}$K18Ac peptide.

Despite an extensive investigation of the benzimidazolone series, a relatively flat SAR was observed. In an attempt to understand the lack of affinity gained, Joe Bluck performed a Grand Canonical Monte Carlo (GCMC) simulation to calculate the binding energies of the water molecules in the KAc-binding pocket and ZA channel. As expected, the water molecules at the base of the binding pocket had negative binding energies. A water molecule in the ZA channel was also found to have a negative binding energy. This water molecule is predicted to be difficult to displace and prevents the amine chain from residing in the ZA channel. Modifications to the amine chain are therefore unlikely to interact with residues in this channel, which could explain the flat SAR. This finding also supports the hypothesis that maximising interactions with E981 could prove an effective strategy.

Although the affinity for the TRIM33β bromodomain was increased during ligand optimisation, the affinity of the compounds is not yet high for them to be used in mode of action studies. The knowledge gained about the TRIM33 bromodomain will be essential for future ligand development.

Chapter 4 describes the synthesis of a series of potential TRIM33 PROTACs, which incorporate the core structure of the TRIM33 bromodomain ligands as the TRIM33 binding moiety (Figure 5.6). A suitable attachment point for the E3 ligase ligand was identified using the knowledge gained from ligand development. To maximise the chance of effectively degrading TRIM33,

VHL and CRBN were both employed as the target E3 ligases. A range of linker lengths and linking strategies were also employed to tether the two ligands together.

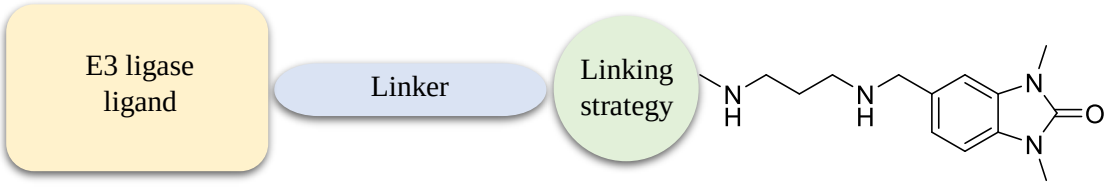


Figure 5.6: Schematic of the TRIM33 PROTACs synthesised.

Our AlphaScreen™ assay proved unsuitable for evaluating the potential TRIM33 PROTACs as a large amount of assay interference was observed in the AlphaScreen™ TruHits™ kit. Alternative assays, such as NMR-based assays or ITC experiments, are therefore required to evaluate the binding of the compounds to TRIM33. The waterLOGSY experiments previously used to validate hits are relatively low throughput and only provide qualitative information on binding, and so they are not ideal for initial screening of these compounds.

In summary, a multidisciplinary project, employing chemical, computational and biological methods was pursued. This led to the identification of novel TRIM33 bromodomain ligands and the synthesis of a diverse series of compounds which could potentially degrade TRIM33.

5.2 Future Work

Further optimisation of the TRIM33 bromodomain ligands is required to develop them into high affinity chemical probes for use in cellular studies. These *in vivo* experiments are necessary to validate TRIM33 as a therapeutic target. Future optimisation should carefully consider the water molecules in the ZA channel and maximise interactions with E981. Replacing the flexible chain of **90** with more rigid moieties that can form optimised bidentate interactions with E981 should result in compounds of higher affinity for the TRIM33 bromodomain.

The hydrophobic region above the KAc-binding pocket was explored but the strategy employed led to compounds with reduced affinity. A thorough investigation into this region could result in an increase in affinity for the TRIM33 bromodomain. As this region was identified by overlaying

a histone H3 peptide with initial hit **23** docked in TRIM33β (Figure 5.2 (A)), a peptidomimetic strategy could be used to replicate the interactions that this histone peptide makes with the protein.

Following optimisation of the TRIM33 bromodomain ligands, functional modifications could be made to provide the ligands with additional capabilities. For example, photoaffinity labels containing a photoactivatable functional group could be used to covalently cross-link the ligand to residues in the target proteins. A reporter tag, such as biotin, could then be used to identify proteins interacting with the ligand. The ligands could also be fluorescently labelled to allow determination of the cellular/subcellular compound location.

The priority for the PROTAC development is to find a suitable assay to evaluate the compounds that have presently been synthesised as potential TRIM33 degraders. Whilst the AlphaScreen™ assay was effective for evaluating small molecule ligands, this assay was found to be unsuitable for the PROTACs due to significant assay interference. Future work should therefore focus on identifying an assay for evaluating the PROTACs which is both high throughput and quantifies binding events.

To support the PROTAC development described in Chapter 4, negative controls need to be synthesised to validate degradation mechanisms. The methylation states of both the TRIM33 ligand and CRBN ligand have been shown to be critical for binding and will be used to abrogate binding to the target proteins.

Employing different linking strategies maximises the probability of identifying a PROTAC which efficiently degrades TRIM33. CRBN targeting PROTACs were successfully synthesised with an amide linkage to the TRIM33 ligand, however suitable deprotection conditions are still required for the amine-linked series.

Collectively this work has led to the development of both TRIM33 bromodomain ligands and potential TRIM33 degraders. Following further optimisation, the TRIM33 bromodomain ligands can be used as chemical probes to unravel the complex mechanisms involved in epigenetic control. If the synthesised PROTACs are observed to bind and degrade TRIM33, they can be used

to reveal the functions of the whole protein. Ultimately, it is envisaged that these tools will demonstrate that TRIM33 bromodomain inhibition can cause increased and sustained DNA damage, and can therefore be effective against cancer, particularly in combination with current radiation treatments.

CHAPTER 6

Experimental

6.1 General Chemistry Methods

Reagents and solvents used were of commercially available reagent grade and were used without further purification, unless stated. All non-aqueous reactions were carried out under an argon atmosphere in a flame-dried flask. Anhydrous solvents were obtained from an MBRAUN Solvent Purification System 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).

Analytical thin layer chromatography (TLC) was carried out on Merck Silica gel 60 F_{254} aluminium-supported thin layer chromatography sheets. Visualisation was by absorption of UV light (λ_{max} 254 nm and 365 nm), or thermal development after staining in one of: aqueous basic solution of potassium permanganate, ethanolic solution of phosphomolybdic acid, aqueous acidic solution of 2,4-dinitrophenylhydrazine or ethanolic solution of ninhydrin. Reverse phase TLC was carried out on Merck Silica gel 60 RP-18 F_{254S} aluminium-supported thin layer chromatography sheets.

Silica gel flash column chromatography was performed under a positive pressure of nitrogen using Merck Silica gel 60 (240–400 mesh).

Melting points were obtained using a Gallenkamp MF-370 capillary tube melting point apparatus (Registered Design No. 889339) and are uncorrected. For samples which decomposed, the decomposition point (d.p.) is reported.

Infrared spectra were recorded from neat samples using a Bruker Tensor 27 spectrometer with a diamond ATR Spectrometer. Absorption maxima ($\tilde{\nu}_{max}$) are reported in wavenumbers (cm^{-1}).

The strength of each band is reported according to the following convention: s = strong, m = medium, w = weak, br = broad.

^{1}H NMR spectra were measured on a Bruker AVIII HD 400 (400 MHz), Bruker AVII 500 (500 MHz) or Bruker AVIII HD 500 (500 MHz) spectrometer in the solvent stated 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*.[192] The number of protons (n) for a given resonance signal is indicated by nH. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); q (quartet); m (multiplet); or combinations thereof. 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 and MestReNova v12.0.4 software. Identical coupling constants were averaged. Spectra were assigned using COSY, HSQC, HMBC and NOE experiments, as appropriate. Where the assignment is uncertain, the possible assignments are separated by (/). Spectra recorded at 363 K are indicated.

^{13}C NMR spectra were measured on a Bruker AVII 500 (126 MHz) spectrometer in the solvent stated 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*.[192] Signals are quoted to 1 decimal place unless peaks are indistinguishable, in which case 2 decimal places are used. The multiplicity of each signal is singlet unless otherwise stated. Spectra were assigned using HSQC and HMBC experiments, as appropriate. Where the assignment is uncertain, the possible assignments are separated by (/). Spectra recorded at 363 K are indicated.

^{19}F NMR spectra were recorded on a Bruker AVIII HD 400 (376 MHz) spectrometer in the solvent stated. 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 singlet unless otherwise stated.

Mass spectra were acquired on either an Agilent 6120 spectrometer (low resolution) or Bruker micrOToF spectrometer (high resolution) using electrospray ionisation (ESI) from solutions of methanol or water. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses. For compounds which did not ionise with ESI, spectra were acquired on a Waters GCT classic spectrometer using electron ionisation (EI), atmospheric pressure chemical ionisation (APCI) or chemical ionisation (CI).

Specific optical rotations were measured using a Schmidt + Haensch UniPol L2000 polarimeter, in cells with a path length of 1 dm at 25 °C, using a sodium lamp at 589 nm. The concentration is expressed in g/100 mL. Specific rotations are denoted $[\alpha]_D^{25}$ and values are reported in implied units of 10^{-1} deg cm^2 g^{-1}.

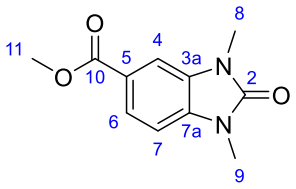
Analytical high-performance liquid chromatography (HPLC) was performed on a PerkinElmer Flexar system with a Binary LC Pump and UV/VIS LC Detector set at 254 nm (unless otherwise stated). For determination of purity, a Dionex Acclaim® 120 C18 reverse phase column [5 µm, 12 Å, 150 mm × 4.6 mm] was used with a constant flow rate of 1.5 mL min^{-1} and gradient method (10 min [95:5 H_2O:acetonitrile (0.1% TFA) to 5:95 H_2O:acetonitrile (0.1% TFA)]; 5 min hold). Samples injected were prepared by dissolving in methanol, water or acetonitrile, and filtered. Samples run on an Atlantis™ C18 column are indicated. Atlantis™ reverse phase column [3 µM, 100 Å, 150 mm × 4.6 mm] was used with a constant flow rate of 1.0 mL min^{-1} and gradient method (2 min hold; 13 min [100:0 H_2O:acetonitrile (0.1% TFA) to 5:95 H_2O:acetonitrile (0.1% TFA)]; 5 min hold). Samples injected were prepared by dissolving in H_2O, and filtered.

Ultra performance liquid chromatography-mass spectrometry (UPLC-MS) was performed on an ACQUITY UPLC System, wavelength range 220–320 nm. A CSH C18 column [1.7 µm, 50 mm × 2.1 mm] was used with a constant flow rate of 1.0 mL min^{-1} and gradient method (1.3 min [97:3 H_2O:acetronitrile to 3:97 H_2O:acetonitrile]). Samples injected were prepared by dissolving in methanol. Samples were run with either 0.1% NH_3 or 0.1% formic acid.

6.2 Chemistry Experimental Procedures

Chapter 2 Compounds

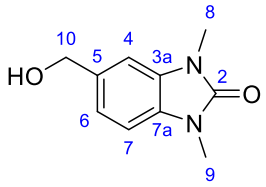
Methyl 1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (31)



3,4-Diaminobenzoic acid (5.00 g, 32.9 mmol, 1.0 eq.) was suspended in anhydrous CH_2Cl_2 (40.0 mL) and 1,1'-carbonyldiimidazole (11.6 g, 71.5 mmol, 2.2 eq.) was added. The reaction mixture was stirred at rt for 1 h. After this time TLC analysis indicated complete consumption of starting material (3:7 EtOH/CH_2Cl_2). MeOH (30.0 mL) was added and the reaction mixture stirred at rt for 2 h. After this time TLC analysis indicated complete consumption of the intermediate (1:9 MeOH/CH_2Cl_2). The reaction mixture was cooled on ice and the precipitate collected *via* filtration. The filter pad was washed with H_2O. The precipitate was suspended in MeOH (30.0 mL) and the solvent removed *in vacuo*. The solid was suspended in *N*,*N*-dimethylformamide (60.0 mL) and K_2CO_3 (18.1 g, 132 mmol, 4.0 eq.) was added. The reaction mixture was cooled to 0 °C and methyl iodide (28.7 mL, 461 mmol, 14.0 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 17 h. After this time TLC analysis indicated complete consumption of the intermediate (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (50 mL) and extracted with EtOAc (50 mL). The organic fraction was washed sequentially with an aqueous solution of LiCl (0.5 M, 4 × 50 mL), and brine (50 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the title compound as a light brown solid (4.69 g, 21.3 mmol, 65%): R_f 0.24 (2:3 petroleum ether/EtOAc); m.p. 145–147 °C (from EtOAc); $\bar{\nu}_{max}$/cm^{-1} 3063 (br), 2917 (br), 1698 (C=O, s), 1622 (m), 1509 (m), 1463 (m), 1445 (m), 1225 (s), 1103 (s), 977 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (1H, dd, *J* 8.2, 1.6, C^6-H), 7.66 (1H, d, *J* 1.6, C^4-H), 6.99 (1H, d, *J* 8.2, C^7-H), 3.93 (3H, s, C^{11}-H$_3$), 3.46 (3H, s, C^8-H$_3$), 3.45 (3H,

s, C^{9}-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2 (C^{10}), 154.9 (C^{2}), 133.8 (C^{3a}), 129.9 (C^{7a}), 124.0 (C^{6}), 123.3 (C^{5}), 108.6 (C^{4}), 106.7 (C^{7}), 52.2 (C^{2}), 27.41 ($C^{8/9}$), 27.35 ($C^{8/9}$); HRMS *m/z* (CI^+) [Found: 221.0920, $C_{11}H_{12}N_2O_3$ requires $[M+H]^+$ 221.0921]; unable to detect by LRMS; HPLC retention time 7.3 min, 98.2%.

5-(Hydroxymethyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (29)



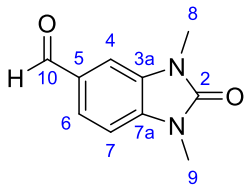
Method 1

Methyl 1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (**31**) (473 mg, 2.15 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (10.0 mL). Lithium borohydride (2 M, THF) (2.15 mL, 4.30 mmol, 2.0 eq.) and MeOH (250 μL) were added dropwise. The reaction mixture was heated to 80 °C and stirred for 19 h. After this time TLC analysis indicated complete consumption of starting material (2:3 EtOAc/petroleum ether). The reaction was cooled to rt, quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The organic fractions were combined and washed sequentially with H_2O (20 mL), and brine (20 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the title compound as a light brown solid (325 mg, 1.69 mmol, 79%): R_f 0.65 (4:1 CH_2Cl_2/EtOH); m.p. 142–144 °C (from EtOAc); $\tilde{\nu}_{max}$/cm^{-1} 3368 (br), 2862 (br), 1675 (C=O, s), 1508 (m), 1459 (m), 1398 (m), 1272 (w), 1194 (w), 1139 (w), 1027 (m), 988 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (1H, dd, *J* 7.9, 1.5, C^{6}-H), 6.97 (1H, d, *J* 1.5, C^{4}-H), 6.86 (1H, d, *J* 7.9, C^{7}-H), 4.70 (2H, s, C^{10}-H_2), 3.35 (3H, s, $C^{8/9}$-H_3), 3.34 (3H, s, $C^{8/9}$-H_3), 1.85 (1H, br s, O-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^{2}), 134.8 (C^{5}), 130.1 (C^{3a}), 129.5 (C^{7a}), 120.4 (C^{6}), 107.1 (C^{7}), 106.5 (C^{4}), 65.6 (C^{10}), 27.32 ($C^{8/9}$), 27.27 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 193.09728, $C_{10}H_{12}N_2O_2$ requires $[M+H]^+$ 193.09715]; LRMS *m/z* (ESI^+) 193.0 ($[M+H]^+$, 100%); HPLC retention time 5.4 min, 98.9%.

Method 2

Methyl 1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (**31**) (1.00 g, 4.54 mmol, 1.0 eq.) was dissolved in anhydrous toluene (40.0 mL) and cooled to –78 °C. Diisobutylaluminium hydride (1 M, hexanes) (45.4 mL, 45.4 mmol, 10 eq.) was added dropwise and the reaction mixture stirred at –78 °C for 5.5 h. After this time TLC analysis indicated complete consumption of starting material (3:2 EtOAc/petroleum ether). The reaction was quenched at –78 °C with aqueous HCl (1 M, 30 mL) and warmed to rt. The reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the title compound as a light brown solid (780 mg, 4.06 mmol, 89%). The spectroscopic data are in good agreement with those obtained for method 1.

1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (34)



Method 1

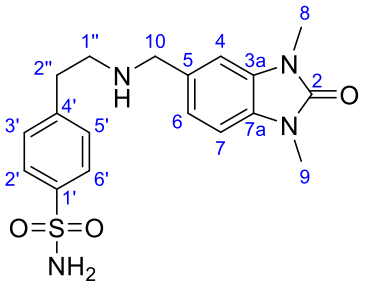
5-(Hydroxymethyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**29**) (20 mg, 0.10 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (0.80 mL) and Dess-Martin periodinane (88 mg, 0.21 mmol, 2.0 eq.) was added. The reaction mixture was stirred at rt for 3 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with an aqueous solution of Na_2SO_3 (2 M, 10 mL) and extracted with EtOAc (10 mL). The organic fraction was washed sequentially with a saturated aqueous solution of $NaHCO_3$ (10 mL), and brine (10 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–10% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (13 mg, 0.066 mmol, 63%): R_f 0.37 (19:1 CH_2Cl_2/EtOH); m.p. 142–144 °C (from CH_2Cl_2);

$\tilde{\nu}_{max}$/cm^{-1} 3044 (br), 2941 (br), 1712 (C=O, s), 1670 (C=O, s), 1620 (m), 1600 (m), 1509 (m), 1481 (s), 1448 (s), 1397 (m), 1353 (m), 1270 (m), 1190 (s), 914 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.95 (1H, s, C^{10}-H), 7.65 (1H, dd, *J* 8.0, 1.5, C^{6}-H), 7.54 (1H, d, *J* 1.5, C^{4}-H), 7.09 (1H, d, *J* 8.0, C^{7}-H), 3.48 (6H, s, C^{8}-H_3, C^{9}-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.3 (C^{10}), 154.7 (C^{2}), 135.3 (C^{7a}), 130.7 (C^{5}), 130.6 (C^{3a}), 126.7 (C^{6}), 107.0 (C^{4}), 106.4 (C^{7}), 27.5 ($C^{8/9}$), 27.4 ($C^{8/9}$); HRMS *m/z* (EI$^+$) [Found: 190.0731, $C_{10}H_{10}N_2O_2$ requires $[M]^+$ 190.0737]; unable to detect by LRMS; HPLC retention time 6.3 min, 99.2%.

Method 2

5-(Hydroxymethyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**29**) (942 mg, 4.90 mmol, 1.0 eq.) was suspended in CH_2Cl_2 (100 mL) and MnO_2 (12.8 g, 147 mmol, 30.0 eq.) was added. The reaction mixture was stirred at rt for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was filtered through a pad of Celite® and the filtrate concentrated *in vacuo* to yield the title compound as a colourless solid (763 mg, 3.99 mmol, 81%). The spectroscopic data are in good agreement with those obtained for method 1.

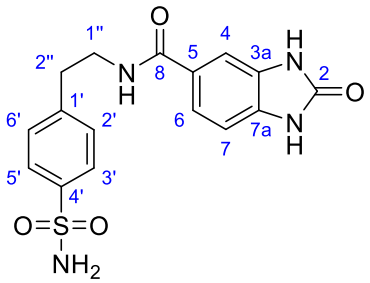
4'-(2''-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}ethyl)benzene-1'-sulfonamide (23)



1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**34**) (100 mg, 0.526 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (13.0 mL) and the reaction mixture cooled to 0 °C. 4-(2-Aminoethyl)benzenesulfonamide (211 mg, 1.05 mmol, 2.0 eq.) and acetic acid (60.0 μL, 1.05 mmol, 2.0 eq.) were added and the reaction mixture stirred at 0 °C for 6 h. After this time TLC analysis indicated partial imine formation (1:9 MeOH/CH_2Cl_2). $NaBH(OAc)_3$

(557 mg, 2.63 mmol, 5.0 eq.) was added at 0 °C. The reaction mixture was warmed to rt and stirred for 15 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL) and extracted with CH_2Cl_2 (2 × 15 mL). The aqueous fractions were extracted with EtOAc (15 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 100% EtOH isocratic) to yield the title compound as a colourless solid (50.0 mg, 0.134 mmol, 25%): R_f 0.12 (EtOH); d.p. >160 °C (from EtOH); $\tilde{\nu}_{max}$/cm^{-1} 3280 (N-H, br), 2852 (br), 1679 (C=O, s), 1513 (w), 1464 (w), 1334 (m), 1161 (S=O, s), 1121 (m), 1097 (m), 1019 (w), 990 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.71 (2H, d, *J* 8.3, C$^{2'}$-H, C$^{6'}$-H), 7.39 (2H, d, *J* 8.3, C$^{3'}$-H, C$^{5'}$-H), 7.27 (2H, br s, N-H$_2$), 7.09–7.02 (2H, m, C^4-H, C^7-H), 6.99 (1H, dd, *J* 7.9, 1.5, C^6-H), 3.73 (2H, s, C^{10}-H$_2$), 3.30 (3H, s, C$^{8/9}$-H$_3$), 3.29 (3H, s, C$^{8/9}$-H$_3$), 2.83–2.76 (2H, m, C$^{2''}$-H$_2$), 2.77–2.69 (2H, m, C$^{1''}$-H$_2$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.0 (C^2), 145.0 (C$^{4'}$), 141.7 (C$^{1'}$), 133.9 (C^5), 129.6 (C^{3a}), 129.1 (C$^{3'}$, C$^{5'}$), 128.4 (C^{7a}), 125.6 (C$^{2'}$, C$^{6'}$), 120.6 (C^6), 107.2 (C^4), 107.1 (C^7), 53.0 (C^{10}), 49.8 (C$^{1''}$), 35.6 (C$^{2''}$), 26.9 (C^8, C^9); HRMS *m/z* (ESI$^+$) [Found: 375.14846, $C_{18}H_{22}N_4O_3S$ requires $[M+H]^+$ 375.14854]; LRMS *m/z* (ESI$^-$) 373.1 ($[M–H]^-$, 100%); unable to detect by LRMS; HPLC retention time 5.5 min, 97.7%.

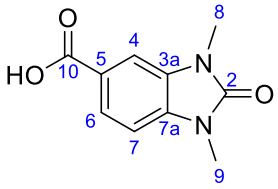
2-Oxo-*N*-[2''-(4'-sulfamoylphenyl)ethyl]-2,3-dihydro-1*H*-benzimidazole-5-carboxamide (36)



3,4-Diaminobenzoic acid (1.00 g, 6.57 mmol, 1.0 eq.) was suspended in anhydrous tetrahydrofuran (10.0 mL) and 1,1'-carbonyldiimidazole (2.24 g, 13.8 mmol, 2.1 eq.) was added. The reaction mixture was stirred at rt for 30 min. After this time TLC analysis indicated complete consumption of starting material (3:7 EtOH/CH_2Cl_2). 4-(2-Aminoethyl)benzenesulfonamide

(1.32 g, 6.57 mmol, 1.0 eq.) was added and the reaction mixture stirred at rt for 3.5 h. After this time TLC analysis indicated complete consumption of the intermediate (3:7 EtOH/CH_2Cl_2). The reaction mixture was diluted with EtOAc (5 mL) and the precipitate collected *via* filtration and washed with H_2O. The solid was crystallised from EtOH:H_2O (2:3) to yield the title compound as a light brown solid (675 mg, 1.87 mmol, 28%): R_f 0.30 (30:19:1 EtOH/$CHCl_3$/Et_3N); m.p. >300 °C (from EtOH/H_2O); $\tilde{\nu}_{max}$/cm^{-1} 3257 (br), 1752 (C=O, s), 1632 (s), 1604 (m), 1547 (m), 1337 (s), 1151 (s), 1098 (w), 1059 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.84 (2H, br s, N-H, N-H), 8.45 (1H, t, *J* 5.6, N-H), 7.74 (2H, d, *J* 8.3, C$^{3'}$-H, C$^{5'}$-H), 7.48 (1H, dd, *J* 8.2, 1.7, C^6-H), 7.45–7.39 (3H, m, C^4-H, C$^{2'}$-H, C$^{6'}$-H), 7.28 (2H, br s, N-H_2), 6.95 (1H, d, *J* 8.2, C^7-H), 3.53–3.45 (2H, m, C$^{1''}$-H_2), 2.91 (2H, t, *J* 7.2, C$^{2''}$-H_2); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.7 (C^8), 156.0 (C^2), 144.4 (C$^{1'}$), 142.5 (C$^{4'}$), 132.6 (C^{7a}), 129.9 (C^5), 129.6 (C$^{2'}$, C$^{6'}$), 127.4 (C^{3a}), 126.2 (C$^{3'}$, C$^{5'}$), 120.8 (C^6), 108.2 (C^7), 108.0 (C^4), 40.9 (C$^{1''}$), 35.3 (C$^{2''}$); HRMS *m/z* (ESI$^-$) [Found: 359.08185, $C_{16}H_{16}N_4O_4S$ requires [M–H]$^-$ 359.08195]; LRMS *m/z* (ESI$^-$) 359.0 ([M–H]$^-$, 100%); HPLC retention time 5.6 min, >99.9%.

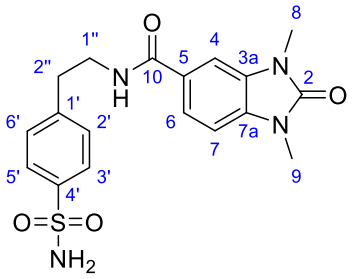
1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylic acid (30)



Methyl 1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (**31**) (300 mg, 1.36 mmol, 1.0 eq.) was dissolved in 1,4-dioxane (6.00 mL) and LiOH (326 mg, 13.6 mmol, 10 eq.) dissolved in H_2O (25.0 mL) was added. The reaction mixture was heated to 65 °C and stirred for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was cooled to rt, concentrated *in vacuo* and aqueous HCl (1 M, 5 mL) was added. The resulting precipitate was collected *via* filtration. The precipitate was washed with H_2O to yield the title compound as a light brown solid (215 mg, 1.04 mmol, 77%): R_f 0.41 (4:1 CH_2Cl_2/EtOH); m.p. 263–265 °C (from H_2O); $\tilde{\nu}_{max}$/cm^{-1} 2917 (br), 1678 (C=O, s), 1626 (s), 1451 (m), 1397 (m), 1220 (m), 1106 (m); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.71

(1H, br s, O-H), 7.73 (1H, dd, *J* 8.2, 1.6, C^6-H), 7.66 (1H, d, *J* 1.6, C^4-H), 7.22 (1H, d, *J* 8.2, C^7-H), 3.37 (3H, s, C^8-H_3), 3.35 (3H, s, C^9-H_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.5 (C^{10}), 154.1 (C^2), 133.4 (C^{7a}), 129.5 (C^{3a}), 123.4 (C^6), 123.2 (C^5), 108.4 (C^4), 107.2 (C^7), 27.2 (C^8), 27.0 (C^9); HRMS *m/z* (ESI^+) [Found: 207.07650, $C_{10}H_{10}N_2O_3$ requires $[M+H]^+$ 207.07642]; LRMS *m/z* (ESI^-) 205.1 ($[M–H]^-$, 100%); HPLC retention time 5.8 min, >99.9%.

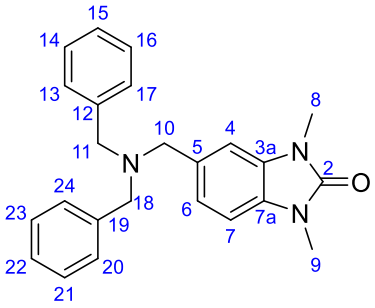
1,3-Dimethyl-2-oxo-*N*-[2''-(4'-sulfamoylphenyl)ethyl]-2,3-dihydro-1*H*-benzimidazole-5-carboxamide (24)



1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylic acid (**30**) (70 mg, 0.34 mmol, 1.0 eq.) was suspended in anhydrous CH_2Cl_2 (7.0 mL) and 1,1'-carbonyldiimidazole (66 mg, 0.41 mmol, 1.2 eq.) was added. The reaction mixture was stirred at rt for 1 h. 4-(2-Aminoethyl)benzenesulfonamide (75 mg, 0.37 mmol, 1.1 eq.) was added and the reaction mixture stirred at rt for 2.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 EtOH/$CHCl_3$). The reaction mixture was concentrated *in vacuo* and the solid was crystallised from EtOH:H_2O (1:1) to yield the title compound as an off-white solid (48 mg, 0.12 mmol, 37%): R_f 0.33 (4:1 $CHCl_3$/EtOH); m.p. 154–156 °C (from EtOH/H_2O); $\bar{\nu}_{max}$/cm^{-1} 3306 (br), 1697 (C=O, s), 1633 (C=O, s), 1541 (m), 1508 (m), 1452 (w), 1394 (m), 1341 (m), 1269 (m), 1157 (s), 1096; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.49 (1H, t, *J* 5.6, N-H), 7.75 (2H, d, *J* 8.3, $C^{3'}$-H, $C^{5'}$-H), 7.66–7.59 (2H, m, C^4-H, C^6-H), 7.43 (2H, d, *J* 8.3, $C^{2'}$-H, $C^{6'}$-H), 7.29 (2H, br s, N-H_2), 7.19 (1H, d, *J* 8.7, C^7-H), 3.57–3.49 (2H, m, $C^{1''}$-H_2), 3.36 (3H, s, $C^{8/9}$-H_3), 3.35 (3H, s, $C^{8/9}$-H_3), 2.94 (2H, t, *J* 7.2, $C^{2''}$-H_2); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.1 (C^{10}), 154.1 (C^2), 143.9 ($C^{1'}$), 142.0 ($C^{4'}$), 132.0 (C^{3a}), 129.4 (C^{7a}), 129.2 ($C^{2'}$, $C^{6'}$), 127.3 (C^5), 125.7 ($C^{3'}$, $C^{5'}$), 120.6 (C^6), 107.0 (C^7), 106.6 (C^4), 40.5 ($C^{1''}$), 34.9 ($C^{2''}$), 27.2 ($C^{8/9}$), 27.0 ($C^{8/9}$); HRMS *m/z*

(ESI$^+$) [Found: 389.12782, $C_{18}H_{20}N_4O_4S$ requires $[M+H]^+$ 389.12780]; LRMS *m/z* (ESI$^+$) 389.1 ($[M+H]^+$, 100%); HPLC retention time 6.0 min, 98.0%.

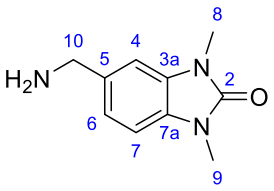
5-[(Dibenzylamino)methyl]-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (38)



1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**34**) (50 mg, 0.26 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (6.5 mL) and 3 Å molecular sieves were added. Dibenzylamine (52 µL, 0.53 mmol, 2.0 eq.) and acetic acid (30 µL, 0.53 mmol, 2.0 eq.) were added at 0 °C and the reaction mixture stirred at 0 °C for 5 h. After this time TLC analysis indicated partial imine formation (1:9 MeOH/CH_2Cl_2). $NaBH(OAc)_3$ (0.28 g, 1.3 mmol, 5.0 eq.) was added at 0 °C and the reaction mixture warmed to rt and stirred for 17 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (25 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (3 × 25 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 20–60% EtOAc/petroleum ether + 1% Et_3N gradient) to yield the title compound as a colourless oil (24 mg, 0.064 mmol, 24%): R_f 0.42 (3:2 EtOAc/petroleum ether); $\tilde{\nu}_{max}$/cm^{-1} 2930 (br), 2796 (br), 2358 (br), 1709 (s), 1630 (w), 1507 (w), 1451 (w), 1395 (w), 1364 (w), 1269 (w), 1189 (w), 1124 (w), 1077 (w), 1028 (w), 974 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44–7.38 (4H, m, C^{13}-H, C^{17}-H, C^{20}-H, C^{24}-H), 7.38–7.29 (4H, m, C^{14}-H, C^{16}-H, C^{21}-H, C^{23}-H), 7.24 (2H, dd, *J* 7.5, 1.3, C^{15}-H, C^{22}-H), 7.13 (1H, dd, *J* 7.9, 1.5, C^6-H), 7.02 (1H, d, *J* 1.3, C^4-H), 6.90 (1H, d, *J* 7.9, C^7-H), 3.61 (2H, s, C^{10}-H_2), 3.58 (4H, s, C^{11}-H_2, C^{18}-H_2), 3.43 (3H, s, C^8-H_3), 3.40 (3H, s, C^9-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 139.7 (C^{12}, C^{19}), 133.1 (C^5), 130.2 (C^{3a}), 129.2 (C^{7a}), 128.9 (C^{13}, C^{17}, C^{20}, C^{24}), 128.4 (C^{14}, C^{16}, C^{21}, C^{23}), 127.0 (C^{15}, C^{22}), 121.8 (C^6), 107.9 (C^4), 107.0 (C^7), 58.2 (C^{10}), 58.0 (C^{11}, C^{18}), 27.32

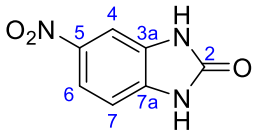
(C^{9}), 27.31 (C^{8}); HRMS *m/z* (ESI^{+}) [Found: 372.20631, $C_{24}H_{25}N_3O$ requires $[M+H]^{+}$ 372.20704]; LRMS *m/z* (ESI^{+}) 372.2 ($[M+H]^{+}$, 100%); HPLC retention time 7.5 min, 99.6%.

5-(Aminomethyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (26)



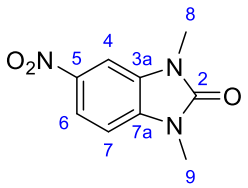
Pd on carbon (10% *w/w*, 21 mg, 0.019 mmol, 0.06 eq.) was added to a solution of 5-[(dibenzylamino)methyl]-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**38**) (0.12 g, 0.32 mmol, 1.0 eq.) in degassed EtOH (12 mL). The reaction mixture was purged with H_2 and stirred at rt under an atmosphere of H_2 for 3 h. After this time TLC analysis indicated partial consumption of starting material (1:9 $MeOH/CH_2Cl_2$). The reaction mixture was purged with N_2 and additional Pd on carbon (10% *w/w*, 25 mg, 0.024 mmol, 0.07 eq.) was added. The reaction mixture was purged with H_2 and stirred at rt under an atmosphere of H_2 for 20 h. After this time TLC analysis indicated complete consumption of starting material (1:9 $MeOH/CH_2Cl_2$). The reaction mixture was purged with N_2, filtered through a pad of Celite®, and the filter pad washed with EtOH. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless solid (41 mg, 0.22 mmol, 69%): R_f 0.24 (EtOH + 1% Et_3N); m.p 108–110 °C (from EtOH); $\tilde{\nu}_{max}/cm^{-1}$ 3370 (N-H, w), 3056 (w), 3022 (w), 2938 (br), 1684 (C=O, s), 1606 (w), 1507 (w), 1461 (m), 1395 (w), 1338 (w), 1267 (w), 1243 (w), 1188 (w), 1137 (w), 1083 (w), 1027 (w), 909 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01 (1H, dd, *J* 8.0, 1.5, C^{6}-H), 6.96 (1H, d, *J* 1.5, C^{4}-H), 6.89 (1H, d, *J* 8.0, C^{7}-H), 3.90 (2H, s, C^{10}-H_2), 3.394 (3H, s, $C^{8/9}$-H_3), 3.386 (3H, s, $C^{8/9}$-H_3), 1.79 (2H, br s, N-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^{2}), 136.7 (C^{5}), 130.4 (C^{3a}), 129.1 (C^{7a}), 120.2 (C^{6}), 107.2 (C^{7}), 106.5 (C^{4}), 46.7 (C^{10}), 27.3 ($C^{8/9}$), 27.2 ($C^{8/9}$); HRMS *m/z* (ESI^{+}) [Found: 192.11336, $C_{10}H_{14}N_3O$ requires $[M+H]^{+}$ 192.11314]; LRMS *m/z* (ESI^{+}) 175.0 ($[M–NH_2]^{+}$, 100%); unable to obtain a HPLC trace due to multiple protonation states.

5-Nitro-1,3-dihydro-2*H*-benzimidazol-2-one (40)[193,194,195]



4-Nitro-*o*-phenylenediamine (1.00 g, 6.53 mmol, 1.0 eq.) was dissolved in anhydrous CH_2Cl_2 (8.00 mL) and 1,1'-carbonyldiimidazole (1.77 g, 10.9 mmol, 1.7 eq.) was added. The reaction mixture was stirred at rt for 72 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (10 mL). The resulting precipitate was collected *via* filtration and washed with H_2O. The solid was crystallised from EtOH:H_2O (1:1) to yield the title compound as a light yellow solid (650 mg, 3.63 mmol, 54%): R_f 0.23 (1:9 $CHCl_3$/EtOH); d.p. >310 °C (from EtOH/H_2O) [lit.[193] 308–309 °C, lit.[196] 307 °C, lit.[197] 306 °C]; $\tilde{\nu}_{max}$/cm^{-1} 3091 (br), 1684 (C=O, s), 1516 (N-O, s), 1487 (s), 1332 (N-O, s), 1070 (m), 1019 (m); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.29 (2H, br s, N-H, N-H), 7.94 (1H, dd, *J* 8.7, 2.3, C^6-H), 7.71 (1H, d, *J* 2.3, C^4-H), 7.10 (1H, d, *J* 8.7, C^7-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.5 (C^2), 141.3 (C^5), 135.7 (C^{7a}), 129.7 (C^{3a}), 117.8 (C^6), 108.1 (C^7), 103.7 (C^4); HRMS *m/z* (ESI$^-$) [Found: 178.02556, $C_7H_5N_3O_3$ requires $[M–H]^-$ 178.02472]; LRMS *m/z* (ESI$^-$) 178.0 ($[M–H]^-$, 100%); HPLC retention time 5.8 min, >99.9%. The spectroscopic data are in good agreement with literature values.[193,194,195]

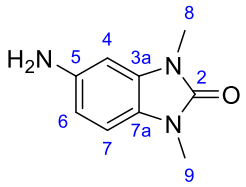
1,3-Dimethyl-5-nitro-1,3-dihydro-2*H*-benzimidazol-2-one (41)[198]



5-Nitro-1,3-dihydro-2*H*-benzimidazol-2-one (**40**) (300 mg, 1.67 mmol, 1.0 eq.) was dissolved in *N*,*N*-dimethylformamide (3.00 mL) and K_2CO_3 (1.39 g, 10.0 mmol, 6.0 eq.) and methyl iodide (620 μL, 10.0 mmol, 6.0 eq.) were added. The reaction mixture was stirred at rt for 15 h. After this time TLC analysis indicated complete consumption of starting material (3:2

EtOAc/petroleum ether). The reaction was quenched with H_2O (15 mL) and extracted with EtOAc (2 × 15 mL). The organic fractions were combined and washed sequentially with an aqueous solution of LiCl (0.5 M, 3 × 15 mL), and brine (15 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the title compound as a yellow solid (257 mg, 1.24 mmol, 75%): R_f 0.35 (3:2 EtOAc/petroleum ether); m.p. 202–204 °C (from EtOAc) [lit.[199] 202–204 °C]; $\bar{\nu}_{max}$/cm^{-1} 3075 (br), 1707 (C=O, s), 1620 (m), 1511 (N-O, s), 1396 (m), 1329 (N-O, s), 1269 (m), 1133 (w), 1062 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (1H, dd, *J* 8.7, 2.2, C^6-H), 7.89 (1H, d, *J* 2.2, C^4-H), 7.02 (1H, d, *J* 8.7, C^7-H), 3.50 (3H, s, C^8-H$_3$), 3.49 (3H, s, C^9-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.0 (C^2) 142.7 (C^5), 135.1 (C^{7a}), 130.0 (C^{3a}), 118.6 (C^6), 106.6 (C^7), 103.3 (C^4), 27.8 (C$^{8/9}$), 27.7 (C$^{8/9}$); HRMS *m/z* (EI) [Found: 207.0635, $C_9H_9N_3O_3$ requires $[M]^+$ 207.0638]; unable to detect by LRMS; HPLC retention time 7.8 min, 99.8%. The spectroscopic data are in good agreement with literature values.[198]

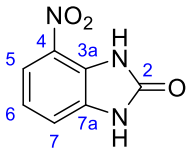
5-Amino-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (25)[200]



Pd on carbon (10% *w*/*w*, 13 mg, 0.013 mmol, 0.05 eq.) was added to a round-bottom flask and the flask purged with N_2. 1,3-Dimethyl-5-nitro-1,3-dihydro-2*H*-benzimidazole-2-one (**41**) (50 mg, 0.24 mmol, 1.0 eq.) dissolved in degassed EtOH (2.5 mL), and concentrated HCl (3.6 µL, 0.043 mmol, 0.2 eq.) were added. The reaction mixture was purged with H_2 and the reaction mixture stirred at rt under an atmosphere of H_2 for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was purged with N_2, filtered through a pad of Celite®, and the filter pad washed with EtOH. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 5–10% EtOH/CH_2Cl_2 gradient) to yield the title compound as an off-white solid (23 mg, 0.13 mmol, 54%): R_f 0.63 (1:9 MeOH/CH_2Cl_2); m.p. 150–152 °C (from CH_2Cl_2) [lit.[200] 160 °C, lit.[199] 135–136 °C]; $\bar{\nu}_{max}$/cm^{-1} 3433 (N-H, br), 3323 (N-H, br), 3214 (br), 1680 (C=O, s),

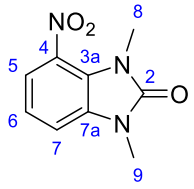
1634 (s), 1612 (s), 1503 (s), 1472 (m), 1445 (m), 1395 (m), 1383 (m), 1271 (m), 1248 (m), 1225 (w), 1137 (w), 1085 (w), 1027 (w), 957 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.78 (1H, d, *J* 8.2, C^{7}-H), 6.35 (1H, d, *J* 2.1, C^{4}-H), 6.31 (1H, dd, *J* 8.2, 2.1, C^{6}-H), 4.78 (2H, br s, N-H_2), 3.21 (3H, s, C^{9}-H_3), 3.20 (3H, s, C^{8}-H_3); HRMS *m/z* (ESI$^+$) [Found: 178.09755, $C_9H_{11}N_3O$ requires $[M+H]^+$ 178.09749]; LRMS *m/z* (ESI$^+$) 178.2 ($[M+H]^+$, 100%); HPLC retention time 5.2 min, 99.0%. The spectroscopic data are in good agreement with literature values.[200]

4-Nitro-1,3-dihydro-2*H*-benzimidazol-2-one (43)[193,194]



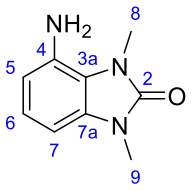
3-Nitro-1,2-phenylenediamine (800 mg, 5.22 mmol, 1.0 eq.) was dissolved in anhydrous CH_2Cl_2 (6.00 mL) and 1,1'-carbonyldiimidazole (1.02 g, 6.27 mmol, 1.2 eq.) was added. The reaction mixture was stirred at rt for 17 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* and H_2O (20 mL) was added. The reaction mixture was stirred at rt for 2.5 h. The product was collected *via* filtration and washed with H_2O to yield the title compound as a yellow solid (848 mg, 4.73 mmol, 91%): R_f 0.30 (1:9 EtOH/$CHCl_3$); m.p. >300 °C (from H_2O) [lit.[193] 308–309 °C, lit.[196] 307 °C, lit.[197] 306 °C]; $\bar{\nu}_{max}$/cm^{-1} 3145 (br), 1742 (C=O, s), 1688 (m), 1642 (w), 1610 (w), 1529 (N=O, s), 1489 (w), 1340 (s), 1317 (N=O, s), 1265 (m), 1211 (m), 1187 (m), 1161 (w), 1065 (w), 1013 (w), 991 (s); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.59 (1H, br s, N-H), 11.33 (1H, br s, N-H), 7.74 (1H, dd, *J* 8.6, 1.0, C^{5}-H), 7.31 (1H, dd, *J* 7.7, 1.0, C^{7}-H), 7.12 (1H, dd, *J* 8.6, 7.7, C^{6}-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.1 (C^{2}), 132.4 (C^{4}), 130.2 (C^{7a}), 125.9 (C^{3a}), 120.4 (C^{6}), 115.3 (C^{5}), 114.4 (C^{7}); HRMS *m/z* (ESI$^+$) [Found: 178.02545, $C_7H_5N_3O_3$ requires $[M+H]^+$ 178.02581]; LRMS *m/z* (ESI$^+$) 178.1 ($[M+H]^+$, 100%); HPLC retention time 5.9 min, 95.1%. The spectroscopic data are in good agreement with literature values.[193,194]

1,3-Dimethyl-4-nitro-1,3-dihydro-2*H*-benzimidazol-2-one (44)[200]



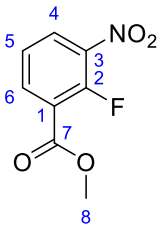
4-Nitro-1,3-dihydro-2*H*-benzimidazol-2-one (**43**) (700 mg, 3.91 mmol, 1.0 eq.) was dissolved in *N*,*N*-dimethylformamide (7.00 mL) and K_2CO_3 (3.24 g, 25.5 mmol, 6.0 eq.) and methyl iodide (1.46 mL, 23.5 mmol, 6.0 eq.) were added. The reaction mixture was stirred at rt for 16 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (20 mL) and extracted with EtOAc (2 × 20 mL). The organic fractions were combined and washed with an aqueous solution of LiCl (0.5 M, 3 × 20 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the title compound as an orange solid (761 mg, 3.67 mmol, 94%): R_f 0.35 (3:2 EtOAc/petroleum ether); m.p. 174–176 °C (from EtOAc) [lit.[200] 220 °C, lit.[201] 208–209 °C]; $\tilde{\nu}_{max}$/cm^{-1} 3072 (br), 2956 (br), 1699 (C=O, s), 1626 (w), 1592 (m), 1524 (N=O, s), 1493 (m), 1460 (m), 1443 (m), 1414 (w), 1382 (m), 1353 (m), 1327 (m), 1292 (m), 1249 (m), 1199 (w), 1136 (w), 1101 (m), 1067 (m), 964 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (1H, dd, *J* 8.0, 1.4, C^5-H), 7.19 (1H, dd, *J* 8.0, 1.4, C^7-H), 7.16 (1H, dd, *J* 8.0, 8.0, C^6-H), 3.56 (3H, s, C^8-H$_3$), 3.48 (3H, s, C^9-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.0 (C^2), 134.3 (C^4), 133.0 (C^{7a}), 124.1 (C^{3a}), 120.7 (C^6), 117.9 (C^5), 111.8 (C^7), 31.4 (C^8), 27.9 (C^9); HRMS *m/z* (CI) [Found: 208.0721, $C_9H_{10}N_3O$ requires $[M+H]^+$ 208.0717]; unable to detect by LRMS; HPLC retention time 7.7 min, 92.2%. The spectroscopic data are in good agreement with literature values.[200]

4-Amino-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (28)[202]



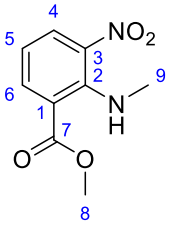
1,3-Dimethyl-4-nitro-1,3-dihydro-2*H*-benzimidazol-2-one (**44**) (0.10 g, 0.48 mmol, 1.0 eq.) dissolved in degassed EtOH (6.0 mL) and concentrated HCl (7.2 μL, 0.086 mmol, 0.2 eq.) was added to a flask containing Pd on carbon (10% *w*/*w*, 27 mg, 0.024 mmol, 0.05 eq.). The reaction mixture was purged with H_2 and the reaction mixture stirred at rt under an atmosphere of H_2 for 1.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was purged with N_2, filtered through a pad of Celite®, and the filter pad washed with EtOAc. The reaction mixture was concentrated *in vacuo* and purified using silica flash column chromatography (elution with 0–5% EtOH/CH_2Cl_2 gradient). The product was further purified using silica flash column chromatography (elution with 80–100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (8.7 mg, 0.049 mmol, 10%): R_f 0.33 (1:9 EtOH/$CHCl_3$); m.p. 212–214 °C (from EtOAc); $\bar{\nu}_{max}$/cm^{-1} 3449 (N-H, w), 3343 (N-H, w), 3233 (w), 2950 (br), 1681 (C=O, s), 1625 (w), 1508 (s), 1469 (s), 1445 (s), 1417 (w), 1388 (m), 1343 (w), 1320 (w), 1293 (m), 1269 (m), 1157 (w), 1106 (w), 1068 (m), 1030 (w), 982 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.88 (1H, dd, *J* 7.9, 7.9, C^6-H), 6.48 (1H, dd, *J* 7.9, 1.0, C^7-H), 6.43 (1H, dd, *J* 7.9, 1.0, C^5-H), 3.79–3.71 (2H, m, N-H_2), 3.71 (3H, s, C^9-H_3), 3.35 (3H, s, C^8-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.8 (C^2), 131.4 (C^4), 130.9 (C^{7a}), 122.1 (C^6), 117.8 (C^{3a}), 111.3 (C^5), 99.9 (C^7), 29.6 (C^9), 27.5 (C^8); HRMS *m/z* (ESI^+) [Found: 178.09753, $C_9H_{11}N_3O$ requires $[M+H]^+$ 178.09749]; unable to detect by LRMS; HPLC retention time 5.6 min, 95.7%. The spectroscopic data are in good agreement with literature values.[202]

Methyl 2-fluoro-3-nitrobenzoate (51)[203]



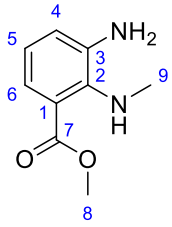
2-Fluoro-3-nitrobenzoic acid (3.00 g, 16.2 mmol, 1.0 eq.) was suspended in anhydrous *N*,*N*-dimethylformamide (50.0 mL) and K_2CO_3 (11.2 g, 81.0 mmol, 5.0 eq.) was added. The reaction mixture was cooled to 0 °C and methyl iodide (2.04 mL, 32.4 mmol, 2.0 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 19.5 h. After this time TLC analysis indicated complete consumption of starting material (3:2 EtOAc/petroleum ether). The reaction was quenched with H_2O (300 mL) and extracted with EtOAc (300 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 3 × 300 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a pale yellow solid (2.63 g, 13.2 mmol, 82%): R_f 0.34 (3:7 EtOAc/petroleum ether); m.p. 68–70 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 1721 (C=O, s), 1613 (m), 1589 (m), 1528 (N-O, s), 1450 (w), 1438 (m), 1349 (N-O, s), 1271 (C-F, s), 1219 (w), 1192 (w), 1133 (s), 1090 (w), 984 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26–8.14 (2H, m, C^4-H, C^6-H), 7.37 (1H, ddd, *J* 8.0, 8.0, 1.3, C^5-H), 3.98 (3H, s, C^8-H$_3$); ^{19}F NMR (376 MHz, $CDCl_3$) δ −116.6; HRMS *m/z* (APCI) [Found: 199.02807, $C_8H_6FNO_4$ requires $[M]^-$ 199.02863]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[203]

Methyl 2-(methylamino)-3-nitrobenzoate (52)[204]



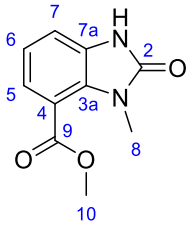
Methyl 2-fluoro-3-nitrobenzoate (**51**) (2.58 g, 12.9 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (14.0 mL). Methylamine hydrochloride (1.75 g, 25.9 mmol, 2.0 eq.) and *N*,*N*-diisopropylethylamine (6.76 mL, 38.8 mmol, 3.0 eq.) were added. The reaction mixture was stirred at rt for 2 h. After this time TLC analysis indicated complete consumption of starting material (95:5 CH_2Cl_2/petroleum ether). The reaction was quenched with H_2O (100 mL) and extracted with EtOAc (100 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 3 × 100 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a bright orange solid (2.67 g, 12.7 mmol, 98%): R_f 0.12 (1:24 CH_2Cl_2/petroleum ether); m.p. 66–67 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 1690 (C=O, s), 1605 (w), 1575 (w), 1535 (N-O, s), 1500 (m), 1461 (w), 1436 (m), 1421 (w), 1403 (w), 1343 (N-O, m), 1253 (s), 1218 (m), 1186 (w), 1164 (w), 1139 (w), 1109 (s), 977 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (1H, br s, N-H), 8.06 (1H, dd, *J* 7.8, 1.7, C^4-H), 7.98 (1H, dd, *J* 7.8, 1.7, C^6-H), 6.67 (1H, dd, *J* 7.8, 7.8, C^5-H), 3.92 (3H, s, C^8-H$_3$), 2.83 (3H, d, *J* 5.5, C^9-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.9 (C^7), 146.8 (C^2), 136.9 (C^4), 131.7 (C^6, C^3), 116.5 (C^1), 114.1 (C^5), 52.4 (C^8), 33.1 (C^9); LRMS *m/z* (ESI$^+$) 211.0 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[204]

Methyl 3-amino-2-(methylamino)benzoate (53)[205]



Methyl 2-(methylamino)-3-nitrobenzoate (**52**) (2.63 g, 12.5 mmol, 1.0 eq.) was dissolved in EtOAc (42.0 mL) and EtOH (20.0 mL). The reaction mixture was purged with N_2. Pd on carbon (10% *w/w*, 665 mg, 0.625 mmol, 0.05 eq.) was added and the reaction mixture purged with H_2. The reaction mixture was stirred at rt under an atmosphere of H_2 for 3 h. After this time TLC analysis indicated complete consumption of starting material (1:19 CH_2Cl_2/petroleum ether). The reaction mixture was purged with N_2, filtered through a pad of Celite®, and the filter pad washed with EtOH. The filtrate was concentrated *in vacuo* to yield the title compound as a yellow oil (2.23 g, 12.4 mmol, 99%): R_f 0.41 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (1H, dd, *J* 6.4, 3.0, C^6-H), 6.86–6.78 (2H, m, C^4-H, C^5-H), 3.85 (3H, s, C^8-H_3), 2.73 (3H, s, C^9-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.8 (C^7), 141.41 ($C^{2/3}$), 141.35 ($C^{2/3}$), 121.8 (C^4), 120.8 (C^6), 120.1 (C^1), 119.8 (C^5), 51.8 (C^8), 33.5 (C^9); HRMS *m/z* (APCI) [Found: 181.09705, $C_9H_{13}N_2O_2$ requires $[M+H]^+$ 181.09715]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[205]

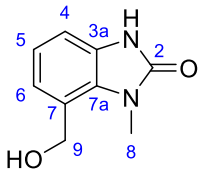
Methyl 3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate (54)



Methyl 3-amino-2-(methylamino)benzoate (**53**) (2.06 g, 11.4 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (15.0 mL) and 1,1'-carbonyldiimidazole (4.07 g, 25.1 mmol, 2.2 eq.) was added. The reaction mixture was stirred at rt for 5.5 h. After this time TLC analysis indicated complete

consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (50 mL). The precipitate was collected *via* filtration and washed with H_2O to yield the title compound as a colourless solid (1.12 g, 5.44 mmol, 48%): R_f 0.37 (1:19 MeOH/CH_2Cl_2); m.p. 178–179 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 1721 (C=O, s), 1687 (C=O, s), 1627 (w), 1497 (w), 1460 (w), 1439 (w), 1415 (w), 1384 (w), 1351 (w), 1289 (w), 1246 (m), 1200 (m), 1148 (w), 1100 (m), 1063 (m), 941 (w), 919 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.39 (1H, br s, N-H), 7.52 (1H, dd, *J* 8.0, 1.2, C^5-H), 7.26 (1H, dd, *J* 8.0, 1.2, C^7-H), 7.08 (1H, dd, *J* 8.0, 8.0, C^6-H), 3.95 (3H, s, C^{10}-H$_3$), 3.59 (3H, s, C^8-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6 (C^9), 156.6 (C^2), 130.0 (C^{3a}), 129.4 (C^{7a}), 123.7 (C^5), 121.0 (C^6), 114.8 (C^4), 113.2 (C^7), 52.5 (C^{10}), 31.0 (C^8); HRMS *m/z* (ESI$^+$) [Found: 207.07656, $C_{10}H_{11}N_2O_3$ requires [M+H]$^+$ 207.07642]; LRMS *m/z* (ESI$^-$) 205.1 ([M−H]$^-$, 100%); HPLC retention time 6.7 min, 99.4%.

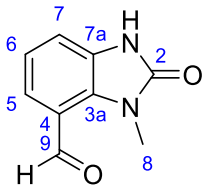
7-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (55)



Methyl 3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate (**54**) (0.16 g, 0.83 mmol, 1.0 eq.) was dissolved in anhydrous toluene (5.7 mL). The reaction mixture was cooled to 0 °C and diisobutylaluminium hydride (1 M, hexanes) (5.8 mL, 5.8 mmol, 7.0 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 16 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was cooled to 0 °C and diluted with diethyl ether (20 mL). H_2O (33 μL) was added followed by an aqueous solution of NaOH (2 M, 69 μL) and H_2O (52 μL). The reaction mixture was warmed to rt and stirred for 15 min. Na_2SO_4 was added and the reaction mixture stirred for 15 min. The reaction mixture was filtered, and concentrated *in vacuo* to yield the title compound as a pale pink solid (83 mg, 0.46 mmol, 56%): R_f 0.37 (1:9 MeOH/CH_2Cl_2); m.p. 230–232 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3365 (br), 3180 (br), 1678 (C=O, s), 1623 (m), 1503 (w), 1473 (m), 1435 (w), 1391 (m), 1299 (w), 1275 (w), 1246 (w), 1162 (w), 1108 (w), 1071 (w), 1048 (m), 1009 (m), 946 (w); ^{1}H

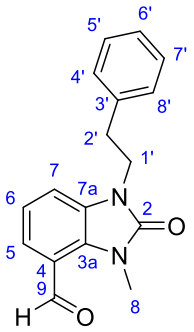
NMR (500 MHz, DMSO-d_6) δ 10.88 (1H, br s, N-H), 6.99–6.88 (3H, m, C^4-H, C^5-H, C^6-H), 5.30 (1H, br s, O-H), 4.71 (2H, s, C^9-H_2), 3.55 (3H, s, C^8-H_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.6 (C^2), 128.6 (C^{7a}), 128.4 (C^{3a}), 123.4 (C^7), 122.2 (C^6), 120.3 (C^5), 108.2 (C^4), 60.1 (C^9), 28.2 (C^8); HRMS *m/z* (ESI^-) [Found: 177.06682, $C_9H_9N_2O_2$ requires $[M–H]^-$ 177.06695]; LRMS *m/z* (ESI^-) 177.1 ($[M–H]^-$, 100%); HPLC retention time 5.0 min, 95.1%.

3-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carbaldehyde (56)



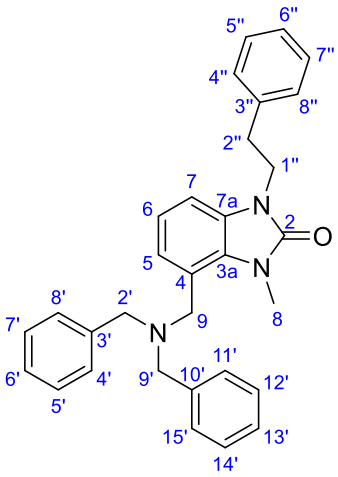
7-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (**55**) (76 mg, 0.46 mmol, 1.0 eq.) was suspended in CH_2Cl_2 (12 mL) and MnO_2 (0.40 g, 4.6 mmol, 10 eq.) was added. The reaction mixture was stirred at rt for 28 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was filtered through a glass microfibre filter and the filter washed with EtOH. The filtrate was concentrated *in vacuo* and the product purified using silica flash column chromatography (elution with 0–5% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless solid (31 mg, 0.18 mmol, 38%): R_f 0.16 (1:49 MeOH/CH_2Cl_2); m.p. 194–196 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3020 (br), 2917 (br), 1706 (C=O, s), 1675 (C=O, s), 1625 (m), 1585 (w), 1475 (m), 1406 (w), 1379 (w), 1353 (w), 1261 (w), 1241 (w), 1199 (w), 1168 (w), 1104 (w), 1082 (m), 1055 (m), 1029 (s), 1009 (s), 927 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (1H, br s, N-H), 10.36 (1H, s, C^9-H), 7.53 (1H, dd, *J* 7.8, 1.2, C^5-H), 7.26 (1H, dd, *J* 7.8, 1.2, C^7-H), 7.13 (1H, dd, *J* 7.8, 7.8, C^6-H), 3.60 (3H, s, C^8-H_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 189.5 (C^9), 154.5 (C^2), 131.4 (C^{3a}), 129.8 (C^{7a}), 121.6 (C^5), 120.6 (C^6), 120.1 (C^4), 113.7 (C^7), 31.1 (C^8); HRMS *m/z* (ESI^+) [Found: 177.06606, $C_9H_9N_2O_2$ requires $[M+H]^+$ 177.06565]; LRMS *m/z* (ESI^-) 175.1 ($[M–H]^-$, 100%); HPLC retention time 6.0 min, >99.9%.

3-Methyl-2-oxo-1-(2'-phenylethyl)-2,3-dihydro-1*H*-benzimidazole-4-carbaldehyde (57)



3-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carbaldehyde (**56**) (31 mg, 0.18 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (2.0 mL). (2-Bromoethyl)benzene (29 μL, 0.21 mmol, 1.2 eq.) and K_2CO_3 (36 mg, 0.26 mmol, 1.5 eq.) were added. The reaction mixture was heated to 70 °C and stirred for 16 h. After this time TLC analysis indicated complete consumption of starting material (4:1 EtOAc/petroleum ether). The reaction mixture was cooled, quenched with H_2O (15 mL), and extracted with EtOAc (15 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 3 × 15 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–60% EtOAc/petroleum ether gradient). The product was dissolved in acetonitrile (15 mL) and washed with hexane (3 × 15 mL). The acetonitrile fraction was concentrated *in vacuo* to yield the title compound as a colourless oil (21 mg, 0.074 mmol, 35%): R_f 0.46 (3:2 EtOAc/petroleum ether); $\bar{\nu}_{max}$/cm^{-1} 1704 (C=O, s), 1677 (C=O, s), 1615 (m), 1480 (m), 1454 (w), 1429 (w), 1413 (w), 1388 (w), 1364 (w), 1249 (w), 1188 (w), 1166 (w), 1115 (w), 1062 (w), 980 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.31 (1H, s, C^9-H), 7.57 (1H, dd, *J* 7.9, 1.2, C^5-H), 7.36–7.19 (5H, m, $C^{4'}$-H, $C^{5'}$-H, $C^{6'}$-H, $C^{7'}$-H, $C^{8'}$-H), 7.14 (1H, dd, *J* 7.9, 7.9, C^6-H), 7.01 (1H, dd, *J* 7.9, 1.2, C^7-H), 4.16 (2H, t, *J* 7.5, $C^{1'}$-H_2), 3.78 (3H, s, C^8-H_3), 3.06 (2H, t, *J* 7.5, $C^{2'}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 189.3 (C^9), 154.6 (C^2), 138.0 ($C^{3'}$), 130.8 (C^{7a}), 130.1 (C^{3a}), 128.9 ($C^{6'}$), 128.8 ($C^{4'/5'/7'/8'}$) 126.9 ($C^{4'/5'/7'/8'}$), 124.8 (C^5), 120.80 (C^6), 120.76 (C^4), 112.6 (C^7), 43.3 ($C^{1'}$), 34.8 ($C^{2'}$), 32.6 (C^8); HRMS *m/z* (ESI$^+$) [Found: 281.12853, $C_{17}H_{17}N_2O_2$ requires $[M+H]^+$ 281.12845]; LRMS *m/z* (ESI$^+$) 281.0 ($[M+H]^+$, 50%); HPLC retention time 9.4 min, 98.8%.

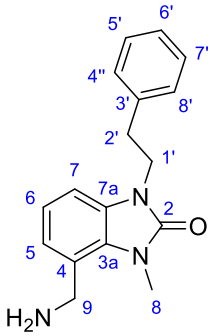
4-[(Dibenzylamino)methyl]-3-methyl-1-(2''-phenethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (58)



3-Methyl-2-oxo-1-(2-phenylethyl)-2,3-dihydro-1*H*-benzimidazole-4-carbaldehyde (**57**) (10 mg, 0.037 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (1.5 mL) and 3 Å molecular sieves were added. Dibenzylamine (14 µL, 0.074 mmol, 2.0 eq.) and acetic acid (4.2 µL, 0.074 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated partial imine formation. $NaBH(OAc)_3$ (39 mg, 0.18 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 17 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL) and filtered through a pad of Celite®. The filter pad was washed with a saturated aqueous solution of $NaHCO_3$ and CH_2Cl_2. The filtrate was extracted, and the aqueous fraction extracted with CH_2Cl_2 (2 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–10% EtOH/petroleum ether gradient) and further purified using silica flash column chromatography (elution with 0–10% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (11 mg, 0.023 mmol, 64%): R_f 0.30 (3:7 EtOAc/petroleum ether); $\bar{\nu}_{max}$/cm^{-1} 1700 (C=O, s), 1655 (w), 1620 (w), 1605 (w), 1582 (w), 1506 (m), 1472 (w), 1445 (w), 1393 (w), 1266 (w), 1240 (w), 1134 (w), 1087 (w), 1055 (w), 1026 (w), 942 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33–7.16 (15H, m, C^{Ar}-H_{15}), 7.03 (1H, dd, *J* 7.9, 1.1, C^5-H), 6.93 (1H, dd, *J* 7.9, 7.9, C^6-H), 6.76 (1H, dd, *J* 7.9, 1.1, C^7-H), 4.09–4.02 (2H, m, $C^{1''}$-H_2), 3.83 (2H, s, C^9-H_2), 3.54 (4H, s, $C^{2'}$-H_2, $C^{10'}$-H_2), 3.49 (3H, s,

C^{8}-H_3), 3.02–2.95 (2H, m, $C^{2''}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.6 (C^{2}), 138.7 ($C^{3'}$, $C^{10'}$), 138.4 ($C^{3''}$), 129.8 (C^{7a}), 129.7 (C^{3a}), 129.4 ($C^{4'}$, $C^{8'}$, $C^{11'}$, $C^{15'}$), 129.0 ($C^{4''}$, $C^{8''}$), 128.7 ($C^{5''}$, $C^{7''}$), 128.3 ($C^{5'}$, $C^{7'}$, $C^{12'}$, $C^{14'}$), 127.2 ($C^{6''}$), 126.7 ($C^{6'}$, $C^{13'}$), 125.2 (C^{5}), 120.8 (C^{4}), 120.6 (C^{6}), 107.0 (C^{7}), 58.2 ($C^{2'}$, $C^{9'}$), 55.8 (C^{9}), 42.9 ($C^{1''}$), 34.8 ($C^{2''}$), 29.7 (C^{8}); HRMS *m/z* (ESI^+) [Found: 462.25369, $C_{31}H_{32}N_3O$ requires $[M+H]^+$ 462.25399]; LRMS *m/z* (ESI^+) 484.2 ($[M+Na]^+$, 12%); HPLC retention time 9.4 min, >99.9%.

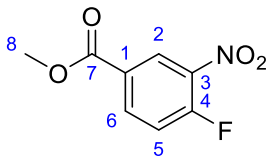
4-(Aminomethyl)-3-methyl-1-(2'-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (46)



To EtOH (7.5 mL) purged with N_2 was added Pd black (25 mg, 0.23 mmol, 2.0 eq.). The reaction mixture was purged with H_2. A degassed solution of 4-((dibenzylamino)methyl)-3-methyl-1-phenethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**58**) (54 mg, 0.12 mmol, 1.0 eq.) in EtOH (7.5 mL) and HCl (10% EtOH, 0.75 mL) was added. The reaction mixture was stirred at rt under an atmosphere of H_2 for 18 h. After this time TLC analysis indicated complete consumption of starting material (EtOAc). The reaction mixture was purged with N_2, filtered through a pad of Celite® and the filter pad washed with EtOH. The filtrate was concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) and further purified using silica flash column chromatography (elution with EtOAc isocratic then EtOH isocratic) to yield the title compound as a colourless oil (29 mg, 0.10 mmol, 87%): R_f 0.18 (EtOH + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 1692 (C=O, s), 1603 (w), 1496 (w), 1472 (m), 1455 (w), 1429 (w), 1399 (w), 1385 (w), 1363 (w), 1332 (w), 1251 (w), 1192 (w), 1164 (w), 1116 (w), 1068 (w), 1032 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28–7.14 (5H, m, $C^{4'}$-H, $C^{5'}$-H, $C^{6'}$-H, $C^{7'}$-H, $C^{8'}$-H), 6.99–6.89 (2H, m, C^{5}-H, C^{6}-H), 6.77 (1H, dd, *J* 7.4, 1.6, C^{7}-H), 4.09 (2H, s, C^{9}-H_2), 4.07–4.00 (2H, m,

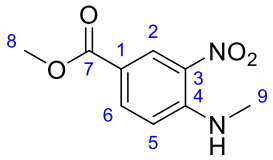
$C^{1'}$-H_2), 3.70 (3H, s, C^8-H_3), 3.00–2.93 (2H, m, $C^{2'}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.6 (C^2), 138.4 ($C^{3'}$), 129.8 (C^{7a}), 128.9 ($C^{4'/5'/7'/8'}$), 128.7 ($C^{4'/5'/7'/8'}$), 127.6 (C^{3a}), 126.7 ($C^{6'}$), 124.8 (C^4), 122.5 (C^5), 121.2 (C^6), 106.8 (C^7), 42.9 ($C^{1'}$), 42.8 (C^9), 34.8 ($C^{2'}$), 29.7 (C^8); HRMS *m/z* (ESI^+) [Found: 282.16041, $C_{17}H_{20}N_3O$ requires $[M+H]^+$ 282.16009]; LRMS *m/z* (ESI^+) 282.1 ($[M+H]^+$, 93%); HPLC retention time 6.7 min, 98.1%.

Methyl 4-fluoro-3-nitrobenzoate (60)[206–208]



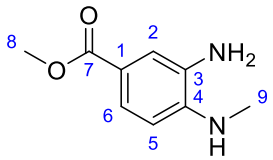
4-Fluoro-3-nitrobenzoic acid (5.00 g, 27.0 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (50.0 mL) and K_2CO_3 (18.7 mg, 135 mmol, 5.0 eq.) was added. Methyl iodide (3.37 mL, 54.1 mmol, 2.0 eq.) was added dropwise and the reaction mixture stirred at rt for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (200 mL) and extracted with EtOAc (3 × 200 mL). Each organic fraction was washed sequentially with an aqueous solution of LiCl (0.5 M, 3 × 200 mL), and brine (200 mL). The organic fractions were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield the title compound as a yellow solid (5.19 g, 26.1 mmol, 97%): R_f 0.57 (1:1 EtOAc/petroleum ether); m.p. 48–50 °C (from CH_2Cl_2) [lit.[207] 62–65 °C, lit.[208] 58–59 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (1H, dd, *J* 7.2, 2.2, C^2-H), 8.31 (1H, ddd, *J* 8.7, 4.3, 2.2, C^6-H), 7.38 (1H, dd, *J* 10.3, 8.7, C^5-H), 3.96 (3H, s, C^8-H_3); ^{19}F NMR (376 MHz, $CDCl_3$) δ –110.5; HRMS *m/z* (CI) [Found: 200.0356, $C_8H_7FNO_4$ requires $[M+H]^+$ 200.0354]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[206–208]

Methyl 4-(methylamino)-3-nitrobenzoate (61)[209]



Methylamine hydrochloride (339 mg, 5.02 mmol, 2.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (5.00 mL). Methyl 4-fluoro-3-nitrobenzoate (**60**) (500 mg, 2.51 mmol, 1.0 eq.) and *N*,*N*-diisopropylethylamine (1.31 mL, 7.53 mmol, 3.0 eq.) were added. The reaction mixture was stirred at rt for 4 h. After this time TLC analysis indicated complete consumption of starting material (1:4 EtOAc/petroleum ether). The reaction was quenched with H_2O (100 mL) and extracted with EtOAc (3 × 60 mL). The organic fractions were combined and washed sequentially with an aqueous solution of LiCl (0.5 M, 3 × 100 mL), and brine (100 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the title compound as a yellow solid (524 mg, 2.49 mmol, 99%): R_f 0.55 (1:1 EtOAc/petroleum ether); m.p. 136–138 °C (from CH_2Cl_2) [lit.[209] 145–147 °C]; $\tilde{\nu}_{max}$/cm^{-1} 3363 (w), 2919 (br), 1707 (C=O, s), 1621 (s), 1562 (N-O, m), 1522 (w), 1471 (w), 1423 (m), 1403 (w), 1362 (w), 1321 (m), 1289 (N-O, s), 1224 (s), 1174 (s), 1131 (m), 1103 (m), 1044 (w), 966 (w), 922 (w), 911 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (1H, d, *J* 2.1, C^2-H), 8.36 (1H, br s, N-H), 8.08 (1H, dd, *J* 9.0, 2.1, C^6-H), 6.86 (1H, d, *J* 9.0, C^5-H), 3.90 (3H, s, C^8-H$_3$), 3.09 (3H, d, *J* 5.1, C^9-H$_3$); LRMS *m/z* (ESI$^+$) 211.0 ([M+H]$^+$, 80%). The spectroscopic data are in good agreement with literature values.[209]

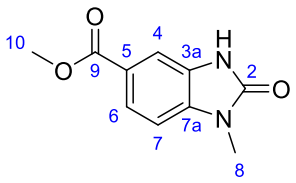
Methyl 3-amino-4-(methylamino)benzoate (62)[210]



Methyl 4-(methylamino)-3-nitrobenzoate (**61**) (518 mg, 2.46 mmol, 1.0 eq.) was suspended in EtOAc (12.0 mL) and EtOH (6.00 mL). The reaction mixture was purged with N_2. Pd on carbon (10% *w*/*w*, 131 mg, 0.123 mmol, 0.05 eq.) was added and the reaction mixture purged with H_2.

The reaction mixture was stirred at rt under an atmosphere of H_2 for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:1 EtOAc/petroleum ether). The reaction mixture was purged with N_2, filtered through a pad of Celite® and the filter pad washed with EtOAc. The filtrate was concentrated *in vacuo* to yield the title compound as a dark brown oil (362 mg, 2.01 mmol, 82%): R_f 0.49 (1:1 EtOAc/petroleum ether); $\bar{\nu}_{max}$/cm^{-1} 3406 (br), 3325 (w), 2947 (w), 1677 (C=O, s), 1633 (w), 1592 (s), 1532 (m), 1482 (w), 1436 (m), 1357 (w), 1304 (s), 1272 (m), 1220 (s), 1166 (s), 1110 (m), 1073 (w), 1055 (w), 986 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (1H, dd, *J* 8.3, 2.0, C^6-H), 7.40 (1H, d, *J* 2.0, C^2-H), 6.58 (1H, d, *J* 8.3, C^5-H), 3.85 (3H, s, C^8-H$_3$), 3.25 (2H, br s, N-H$_2$), 2.91 (3H, s, C^9-H$_3$); LRMS *m/z* (ESI$^+$) 181.0 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[210]

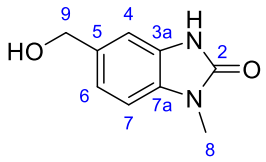
Methyl 1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (63)



Methyl 3-amino-4-(methylamino)benzoate (**62**) (362 mg, 2.01 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (4.50 mL) and 1,1'-carbonyldiimidazole (423 mg, 2.61 mmol, 1.3 eq.) was added. The reaction mixture was stirred at rt for 3 h. After this time TLC analysis indicated complete consumption of starting material (1:1 EtOAc/petroleum ether). The reaction mixture was cooled on ice and the resulting precipitate collected *via* filtration. The precipitate was washed with cold diethyl ether to yield the title compound as a colourless solid (177 mg, 0.858 mmol, 43%): R_f 0.16 (1:1 EtOAc/petroleum ether); m.p. 242–244 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3022 (br), 1703 (C=O, s), 1631 (w), 1607 (w), 1511 (w), 1492 (w), 1465 (w), 1434 (m), 1394 (w), 1321 (w), 1285 (s), 1222 (s), 1139 (w), 1103 (m), 1081 (w), 990 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.12 (1H, br s, N-H), 7.70 (1H, dd, *J* 8.2, 1.6, C^6-H), 7.50 (1H, d, *J* 1.6, C^4-H), 7.19 (1H, d, *J* 8.2, C^7-H), 3.82 (3H, s, C^{10}-H$_3$), 3.31 (3H, s, C^8-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.4 (C^9), 154.6 (C^2), 135.0 (C^{7a}), 128.1 (C^{3a}), 122.8 (C^6), 122.0 (C^5), 109.1 (C^4), 107.4 (C^7), 51.9 (C^{10}), 26.7 (C^8);

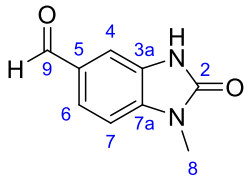
HRMS *m/z* (ESI$^+$) [Found: 207.07645, $C_{10}H_{10}N_2O_3$ requires $[M+H]^+$ 207.07642]; LRMS *m/z* (ESI$^-$) 205.0 ($[M–H]^-$, 100%); unable to detect by LRMS; HPLC retention time 6.5 min, 99.3%.

5-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (64)



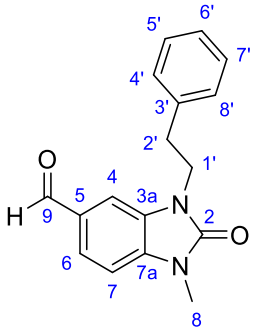
Methyl 1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (**63**) (500 mg, 2.43 mmol, 1.0 eq.) was suspended in anhydrous toluene (18.0 mL). The reaction mixture was cooled to –78 °C and diisobutylaluminium hydride (1 M, hexanes) (24.3 mL, 24.3 mmol, 10 eq.) was added dropwise. The reaction mixture was stirred at –78 °C for 3 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched at –78 °C with a saturated aqueous solution of potassium sodium tartrate (100 mL), warmed to rt and stirred for 1 h. The reaction mixture was extracted with EtOAc (2 × 150 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–10% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless solid (169 mg, 0.948 mmol, 39%): R_f 0.23 (1:9 MeOH/CH_2Cl_2); m.p. 218–220 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3414 (w), 2957 (m), 2923 (s), 2852 (m), 2361 (w), 2341 (w), 1678 (C=O, s), 1486 (w), 1463 (w), 1395 (w), 1291 (w), 1261 (m), 1136 (w), 1090 (m), 1044 (m); ^{1}H NMR (400 MHz, CD_3OD) δ 7.14–7.02 (3H, C^4-H, C^6-H, C^7-H), 4.61 (2H, s, C^9-H_2), 3.38 (3H, s, C^8-H_3); ^{13}C NMR (126 MHz, CD_3OD) δ 157.2 (C^2), 136.6 (C^5), 131.6 (C^{7a}), 129.6 (C^{3a}), 121.7 (C^6), 109.4 (C^4), 108.7 (C^7), 65.5 (C^9), 27.0 (C^8); HRMS *m/z* (ESI$^+$) [Found: 179.08157, $C_9H_{10}N_2O_2$ requires $[M+H]^+$ 179.08150]; LRMS *m/z* (ESI$^-$) 177.1 ($[M–H]^-$, 100%); HPLC retention time 5.0 min, 96.7%.

1-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (65)



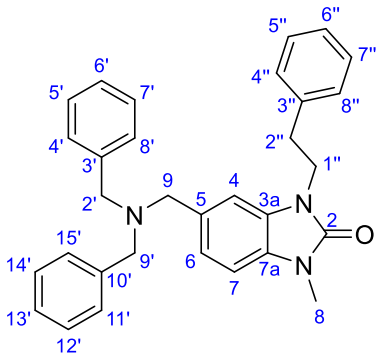
5-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (**64**) (0.17 g, 0.93 mmol, 1.0 eq.) was suspended in CH_2Cl_2 (25 mL) and MnO_2 (2.4 g, 28 mmol, 30 eq.) was added. The reaction mixture was stirred at rt for 1.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was filtered through a pad of Celite® and the filter pad washed with CH_2Cl_2. The filtrate was concentrated *in vacuo* to yield the title compound as a colourless solid (14 mg, 0.081 mmol, 9%): R_f 0.38 (1:19 EtOH/CH_2Cl_2); m.p. 224–226 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3105 (br), 2847 (br), 1723 (C=O, s), 1673 (C=O, s), 1625 (m), 1602 (m), 1487 (m), 1438 (w), 1395 (w), 1363 (w), 1320 (m), 1259 (w), 1228 (w), 1199 (w), 1142 (w), 1089 (w), 1048 (w), 930 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (1H, br s, N-H), 9.89 (1H, s, C^9-H), 7.66 (1H, dd, *J* 8.1, 1.5, C^6-H), 7.44 (1H, d, *J* 1.5, C^4-H), 7.30 (1H, d, *J* 8.1, C^7-H), 3.34 (3H, s, C^8-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 192.0 (C^9), 154.7 (C^2), 136.3 (C^{7a}), 130.1 (C^5), 128.7 (C^{3a}), 125.3 (C^6), 107.9 (C^4), 107.7 (C^7), 26.8 (C^8); HRMS *m/z* (ESI$^+$) [Found: 177.06607, $C_9H_8N_2O_2$ requires [M+H]$^+$ 177.06585]; LRMS *m/z* (ESI$^-$) 175.1 ([M–H]$^-$, 100%); HPLC retention time 5.7 min, 95.8%.

1-Methyl-2-oxo-3-(2'-phenylethyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (66)



1-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**65**) (50 mg, 0.28 mmol, 1.0 eq.) was dissolved in *N*,*N*-dimethylformamide (3.5 mL) and (2-bromoethyl)benzene (47 µL, 0.34 mmol, 1.2 eq.) and K_2CO_3 (59 mg, 0.43 mmol, 1.5 eq.) were added. The reaction mixture was heated to 70 °C and stirred for 7 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (50 mL) and extracted with EtOAc (2 × 50 mL). The organic fractions were combined and washed with an aqueous solution of LiCl (0.5 M, 3 × 100 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–60% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (56 mg, 0.20 mmol, 70%): R_f 0.45 (3:2 EtOAc/petroleum ether); m.p. 112–114 °C (from EtOAc); $\tilde{\nu}_{max}$/cm^{-1} 2931 (br), 1709 (C=O, s), 1680 (C=O, s), 1616 (m), 1507 (w), 1476 (w), 1455 (w), 1399 (w), 1375 (w), 1348 (w), 1255 (w), 1193 (m), 1136 (w), 1103 (w), 1080 (w), 1033 (w), 918 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.91 (1H, s, C^9-H), 7.64 (1H, dd, *J* 8.0, 1.4, C^6-H), 7.36 (1H, d, *J* 1.4, C^4-H), 7.35–7.20 (5H, m, $C^{4'}$-H, $C^{5'}$-H, $C^{6'}$-H, $C^{7'}$-H, $C^{8'}$-H), 7.10 (1H, d, *J* 8.0, C^7-H), 4.18 (2H, dd, *J* 8.4, 6.8, $C^{1'}$-H_2), 3.50 (3H, s, C^8-H_3), 3.09 (2H, dd, *J* 8.4, 6.8, $C^{2'}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.3 (C^9), 154.5 (C^2), 137.9 ($C^{3'}$), 135.3 (C^{7a}), 130.7 (C^5), 130.0 (C^{3a}), 129.0 ($C^{4'}$, $C^{8'}$), 128.8 ($C^{5'}$, $C^{7'}$), 127.0 (C^6), 126.5 ($C^{6'}$), 107.2, (C^7), 106.9 (C^4), 43.1 ($C^{1'}$), 34.9 ($C^{2'}$), 27.6 (C^8); HRMS *m/z* (APCI) [Found: 281.12847, $C_{17}H_{17}N_2O_2$ requires $[M+H]^+$ 281.12845]; unable to detect by LRMS; HPLC retention time 8.9 min, 98.7%.

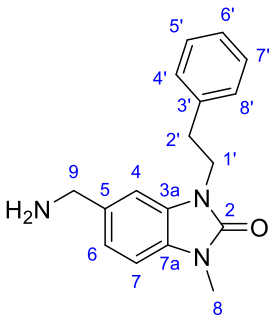
5-[(Dibenzylamino)methyl]-1-methyl-3-(2''-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (67)



3-(2-Anilinoethyl)-1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**66**) (83 mg, 0.30 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (1.5 mL) and 3 Å molecular sieves were added. Dibenzylamine (0.11 mL, 0.59 mmol, 2.0 eq.) and acetic acid (34 µL, 0.59 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 6 h. $NaBH(OAc)_3$ (0.31 g, 1.5 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 22 h. The reaction was quenched with H_2O (10 mL) and filtered through a pad of Celite®. The filter pad was washed with EtOH and the filtrate volume reduced by concentration *in vacuo*. The reaction mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–50% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (84 mg, 0.17 mmol, 58%): R_f 0.33 (2:3 EtOAc/petroleum ether); $\tilde{\nu}_{max}$/cm^{-1} 3027 (br), 2797 (br), 2360 (w), 1707 (C=O, s), 1606 (w), 1504 (m), 1454 (w), 1403 (w), 1364 (w), 1192 (w), 1119 (w), 1029 (w); ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ 7.49–7.43 (4H, m, C$^{4'}$-H, C$^{8'}$-H, C$^{11'}$-H, C$^{15'}$-H), 7.37–7.30 (4H, m, C$^{5'}$-H, C$^{7'}$-H, C$^{12'}$-H, C$^{14'}$-H), 7.28–7.22 (6H, m, C$^{6'}$-H, C$^{13'}$-H, C$^{4''}$-H, C$^{5''}$-H, C$^{7''}$-H, C$^{8''}$-H), 7.21–7.12 (1H, m, C$^{6''}$-H), 7.10 (1H, dd, *J* 7.9, 1.4, C^6-H), 7.07 (1H, d, *J* 1.4, C^4-H), 7.00 (1H, d, *J* 7.9, C^7-H), 4.10 (2H, d, *J* 7.7, C$^{1''}$-H$_2$), 3.57–3.53 (6H, m, C^9-H$_2$, C$^{2'}$-H$_2$, C$^{9'}$-H$_2$), 3.34 (3H, s, C^8-H$_3$), 3.03 (2H, d, *J* 7.7, C$^{2''}$-H$_2$); ^{13}C NMR (126 MHz, $(CD_3)_2CO$) δ 154.8 (C^2), 140.7 (C$^{3'}$, C$^{10'}$), 139.6 (C$^{3''}$), 133.2 (C^5), 130.4 (C^{3a}), 130.2 (C^{7a}), 129.7 (C$^{4''}$, C$^{8''}$), 129.6 (C$^{4'}$, C$^{8'}$, C$^{11'}$, C$^{15'}$), 129.3 (C$^{5''}$, C$^{7''}$), 129.1 (C$^{5'}$, C$^{7'}$, C$^{12'}$, C$^{14'}$),

127.8 ($C^{6'}$, $C^{13'}$), 127.3 ($C^{6''}$), 122.1 (C^{6}), 108.8 (C^{4}), 107.8 (C^{7}), 58.5 (C^{9}), 58.4 ($C^{2'}$, $C^{9'}$), 43.1 ($C^{1''}$), 35.3 ($C^{2''}$), 27.1 (C^{8}); HRMS *m/z* (ESI^+) [Found: 462.25321, $C_{31}H_{32}N_2O$ requires $[M–H]^–$ 462.25399]; LRMS *m/z* (ESI^+) 177.1 ($[M–C_{14}H_{14}N]^+$, 23%); HPLC retention time 7.6 min, 99.2%.

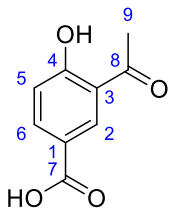
5-(Aminomethyl)-1-methyl-3-(2'-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (48)



5-[(Dibenzylamino)methyl]-1-methyl-3-(2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (**67**) (73 mg, 0.16 mmol, 1.0 eq.) was dissolved in EtOH (6.0 mL) and the reaction mixture purged with N_2. Pd on carbon (10% *w/w*, 8.5 mg, 0.0080 mmol, 0.05 eq.) was added and the reaction mixture purged with H_2. The reaction mixture was stirred at rt under an atmosphere of H_2 for 6 h. After this time TLC analysis indicated partial consumption of starting material (1:1 EtOAc/petroleum ether). The reaction mixture was purged with N_2, additional Pd on carbon (10% *w/w*, 8.5 mg, 0.0080 mmol, 0.05 eq.) was added and the reaction mixture purged with H_2. The reaction mixture was stirred at rt under an atmosphere of H_2 for 29 h. After this time TLC analysis indicated complete consumption of starting material (1:1 EtOAc/petroleum ether). The reaction mixture was purged with N_2, filtered through a pad of Celite®, and the filter pad washed with EtOH. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic), further purified using silica flash column chromatography (elution with 50–60% EtOAc/petroleum ether gradient) and further purified using silica flash column chromatography (elution with 0–40% EtOAc/EtOH + 1% Et_3N gradient) to yield the title compound as a colourless oil (8.9 mg, 0.032 mmol, 20%): R_f 0.16 (EtOH + 1% Et_3N); $\bar{\nu}_{max}/cm^{-1}$ 3027 (br), 1701 (C=O, s), 1506 (w), 1455 (w), 1404 (w), 1260 (w), 1197 (w), 1036 (w); ^1H NMR (500 MHz, CD_3OD) δ 7.26–7.14 (5H, m, $C^{4'}$-H, $C^{5'}$-H, $C^{6'}$-H, $C^{7'}$-H, $C^{8'}$-H), 7.13–7.05 (2H, m, C^{6}-H, C^{7}-H), 6.97 (1H, s, C^{4}-H), 4.13 (2H, t, *J* 7.3, $C^{1'}$-H_2), 3.80

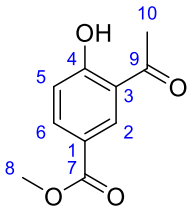
(2H, s, C^{9}-H_2), 3.38 (3H, s, C^{8}-H_3), 3.03 (2H, t, *J* 7.3, $C^{2'}$-H_2); ^{13}C NMR (126 MHz, CD_3OD) δ 156.1 (C^{2}), 139.8 ($C^{3'}$), 136.5 (C^{5}), 130.6 (C^{3a}), 130.2 (C^{7a}), 130.1 ($C^{4'}$, $C^{8'}$), 130.0 ($C^{5'}$, $C^{7'}$), 127.6 ($C^{6'}$), 122.1 (C^{6}), 108.9 (C^{7}), 108.8 (C^{4}), 46.6 (C^{9}), 43.8 ($C^{1'}$), 35.6 ($C^{2'}$), 27.4 (C^{8}); HRMS *m/z* (ESI^+) [Found: 304.14198, $C_{10}H_{14}N_3ONa$ requires $[M+Na]^+$ 304.14203]; LRMS *m/z* (ESI^+) 265.1 ($[M–NH_2]^+$, 96%); HPLC retention time 5.7 min, 97.9%.

3-Acetyl-4-hydroxybenzoic acid (70)[211]



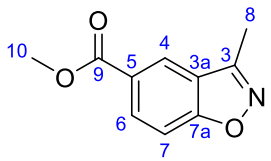
4-Acetoxybenzoic acid (2.50 g, 23.1 mmol, 1.0 eq.) and $AlCl_3$ (9.25 g, 69.3 mmol, 3.0 eq.) were heated to 155 °C and stirred for 7 h. After this time TLC analysis indicated partial consumption of starting material (1:9 $MeOH/CH_2Cl_2$). The reaction mixture was cooled to 0 °C and an aqueous solution of HCl (2 M, 100 mL) was added. The reaction mixture was warmed to rt and extracted with EtOAc (2 × 150 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was crystallised from $CHCl_3$ to yield the title compound as a colourless solid (1.51 g, 8.39 mmol, 36%): R_f 0.31 (1:9 $MeOH/CH_2Cl_2$); d.p. >220 °C [lit.[212] 227–230 °C, lit.[213] 241–242 °C]; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.93 (1H, br s, O-H), 12.22 (1H, br s, O-H), 8.38 (1H, d, *J* 2.2, C^{2}-H), 8.03 (1H, dd, *J* 8.6, 2.2, C^{6}-H), 7.05 (1H, d, *J* 8.6, C^{5}-H), 2.68 (3H, s, C^{9}-H_3); LRMS *m/z* (ESI^-) 179.1 ($[M–H]^-$, 100%). The spectroscopic data are in good agreement with literature values.[211]

Methyl 3-acetyl-4-hydroxybenzoate (71)[214,215]



3-Acetyl-4-hydroxybenzoic acid (**70**) (525 mg, 8.11 mmol, 1.0 eq.) was dissolved in MeOH (25.0 mL) and 2 drops of H_2SO_4 were added. The reaction mixture was heated to 65 °C and stirred for 25 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was cooled to rt and concentrated *in vacuo*. The residue was dissolved in EtOAc (15 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (15 mL). The aqueous fraction was extracted with EtOAc (15 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the title compound as a light yellow solid (348 mg, 1.79 mmol, 62%): R_f 0.35 (1:9 MeOH/CH_2Cl_2); m.p. 88–90 °C (from H_2O) [lit.[214] 101–102 °C, lit.[216] 92–93 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.67 (1H, br s, O-H), 8.48 (1H, d, *J* 2.1, C^2-H), 8.13 (1H, dd, *J* 8.9, 2.1, C^6-H), 7.01 (1H, d, *J* 8.9, C^5-H), 3.92 (3H, s, C^8-H_3), 2.70 (3H, s, C^{10}-H_3); LRMS *m/z* (ESI^-) 193.0 ($[M–H]^-$, 100%). The spectroscopic data are in good agreement with literature values.[214,215]

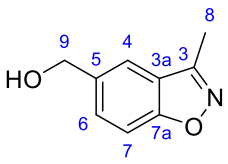
Methyl 3-methyl-1,2-benzoxazole-5-carboxylate (73)[217]



Methyl 3-acetyl-4-hydroxybenzoate (**71**) (348 mg, 1.79 mmol, 1.0 eq.) was dissolved in NH_3 (7 M, MeOH) (25.0 mL) and the reaction mixture stirred at rt for 14.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. The residue was dissolved in anhydrous tetrahydrofuran (11.0 mL). *N*-Chlorosuccinimide (304 mg, 2.28 mmol, 1.5 eq.) and K_2CO_3 (420 mg, 3.04 mmol, 2.0 eq.) were added and the reaction mixture stirred at rt for 5 h. After this time TLC analysis

indicated complete consumption of the intermediate (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. H_2O (15 mL) was added to the residue and extracted with EtOAc (2 × 15 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–10% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (209 mg, 1.09 mmol, 61%): R_f 0.38 (1:9 EtOAc/petroleum ether); m.p. 94–96 °C (from EtOAc); $\tilde{\nu}_{max}$/cm^{-1} 3088 (w), 3038 (w), 2964 (w), 2850 (w), 1723 (C=O, s), 1614 (m), 1535 (w), 1469 (w), 1437 (m), 1391 (w), 1359 (w), 1337 (m), 1284 (s), 1258 (s), 1239 (s), 1193 (m), 1144 (w), 1115 (s), 1062 (w), 1039 (w), 1017 (w), 961 (m), 912 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (1H, dd, *J* 1.7, 0.8, C^4-H), 8.27 (1H, dd, *J* 8.8, 1.7, C^6-H), 7.60 (1H, dd, *J* 8.8, 0.8, C^7-H), 3.99 (3H, s, C^{10}-H$_3$), 2.65 (3H, s, C^8-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5 (C^9), 165.1 (C^{7a}), 155.8 (C^3), 131.3 (C^6), 126.0 (C^5), 124.3 (C^4) 122.8 (C^{3a}), 109.9 (C^7), 52.6 (C^{10}), 10.2 (C^8); HRMS *m/z* (CI) [Found: 192.0657, $C_{10}H_{10}NO_3$ requires $[M+H]^+$ 192.0655]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[217]

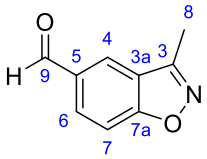
(3-Methyl-1,2-benzoxazol-5-yl)methanol (74)



Methyl 3-methyl-1,2-benzoxazole-5-carboxylate (**73**) (0.10 g, 0.52 mmol, 1.0 eq.) was dissolved in anhydrous toluene (5.0 mL). The reaction mixture was cooled to –78 °C and diisobutylaluminium hydride (1 M, hexanes) (5.2 mL, 5.2 mmol, 10 eq.) was added dropwise. The reaction mixture was stirred at –78 °C for 2.5 h. After this time TLC analysis indicated complete consumption of starting material (3:7 EtOAc/petroleum ether). The reaction was quenched at –78 °C with a saturated aqueous solution of potassium sodium tartrate (50 mL) and warmed to rt. The reaction mixture was extracted with EtOAc (3 × 50 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the title compound as a colourless solid (80 mg, 0.49 mmol, 94%): R_f 0.63 (1:9 MeOH/CH_2Cl_2); m.p. 32–34 °C (from

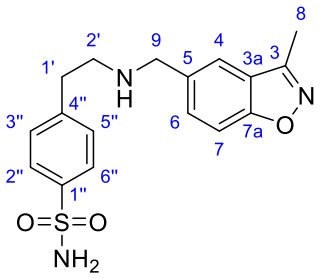
EtOAc); $\bar{\nu}_{max}$/cm^{-1} 3380 (O-H, br), 2918 (w), 2849 (w), 2360 (w), 2341 (w), 1695 (w), 1619 (w), 1531 (w), 1508 (w), 1452 (w), 1390 (w), 1335 (w), 1307 (w), 1264 (w), 1237 (w), 1198 (w), 1049 (s), 1026 (s), 1007 (s); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.76 (1H, d, *J* 0.9, C^{4}-H), 7.68–7.54 (2H, m, C^{6}-H, C^{7}-H), 5.36 (1H, t, *J* 5.4, O-H), 4.62 (2H, d, *J* 5.4, C^{9}-H$_2$), 2.54 (3H, s, C^{8}-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.4 (C^{7a}), 155.1 (C^{3}), 138.2 (C^{5}), 129.4 (C^{6}), 121.8 (C^{3a}), 119.1 (C^{4}), 109.2 (C^{7}), 62.5 (C^{9}), 9.7 (C^{8}); HRMS *m/z* (ESI^{+}) [Found: 164.07082, $C_9H_9NO_2$ requires [M+H]$^{+}$ 164.07061]; LRMS *m/z* (ESI^{+}) 164.0 ([M+H]$^{+}$, 92%); HPLC retention time 6.4 min, 98.7%.

3-Methyl-1,2-benzoxazole-5-carbaldehyde (75)



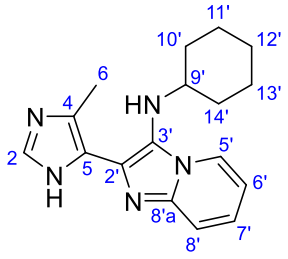
(3-Methyl-1,2-benzoxazol-5-yl)methanol (**74**) (66 mg, 0.41 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (10 mL) and MnO_2 (1.2 g, 14 mmol, 34 eq.) was added. The reaction mixture was stirred at rt for 30 min. After this time TLC analysis indicated complete consumption of starting material (3:7 EtOAc/petroleum ether). The reaction mixture was filtered through a pad of Celite® and the filtrate concentrated *in vacuo* to yield the title compound as a colourless solid (43 mg, 0.27 mmol, 66%): R_f 0.35 (3:7 EtOAc/petroleum ether); m.p. 78–80 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3094 (w), 3026 (w), 2918 (w), 2849 (w), 2771 (w), 1691 (s), 1607 (m), 1533 (w), 1474 (w), 1447 (w), 1431 (w), 1393 (w), 1362 (w), 1335 (w), 1323 (w), 1276 (w), 1248 (m), 1189 (m), 1132 (w), 1061 (w), 1014 (w), 941 (w), 925 (w), 900 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.11 (1H, s, C^{9}-H), 8.52 (1H, d, *J* 1.5, C^{4}-H), 8.16 (1H, dd, *J* 8.7, 1.5, C^{6}-H), 7.89 (1H, d, *J* 8.7, C^{7}-H), 2.64 (3H, s, C^{8}-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 191.8 (C^{9}), 164.8 (C^{7a}), 156.4 (C^{3}), 132.7 (C^{5}), 130.5 (C^{6}), 126.3 (C^{4}), 122.7 (C^{3a}), 110.6 (C^{7}), 9.6 (C^{8}); LRMS *m/z* (ESI^{+}) 179.0 ([M+NH$_4$]$^{+}$, 52%). The spectroscopic data are in good agreement with literature values.[217]

4''-(2'-{[(3-Methyl-1,2-benzoxazol-5-yl)methyl]amino}ethyl)benzene-1-sulfonamide (68)



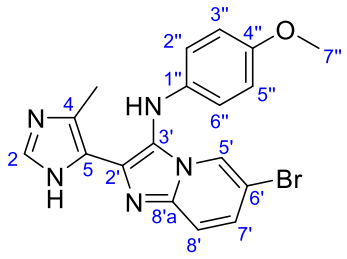
3-Methyl-1,2-benzoxazole-5-carbaldehyde (**75**) (40 mg, 0.25 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (5.0 mL) and 3 Å molecular sieves were added. The reaction mixture was cooled to 0 °C and 4-(2-aminoethyl)benzenesulfonamide (0.10 g, 0.50 mmol, 2.0 eq.) and acetic acid (28 μL, 0.50 mmol, 2.0 eq.) were added. The reaction mixture was warmed to rt and stirred for 3.5 h. $NaBH(OAc)_3$ (0.26 g, 1.2 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 18 h. The reaction was quenched with H_2O (10 mL) and filtered through a pad of Celite®. The filter pad was washed with H_2O (5 mL) and the filtrate volume reduced by concentration *in vacuo*. The residue was dissolved in CH_2Cl_2 (15 mL) and washed with saturated aqueous $NaHCO_3$ (15 mL). The aqueous fraction was extracted with CH_2Cl_2 (15 mL). The organic fractions were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless solid (5.6 mg, 0.016 mmol, 7%): R_f 0.38 (EtOH + 2% Et_3N); m.p. 136–138 °C (from EtOH); $\tilde{\nu}_{max}$/cm^{-1} 3289 (br), 2919 (br), 2849 (br), 1621 (w), 1600 (w), 1533 (w), 1452 (m), 1429 (w), 1412 (w), 1389 (w), 1316 (s), 1301 (s), 1236 (m), 1157 (s), 1122 (w), 1093 (s), 1016 (w), 983 (w), 917 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (2H, d, *J* 8.3, $C^{2''}$-H, $C^{6''}$-H), 7.54 (1H, s, C^4-H), 7.51–7.44 (2H, m, C^6-H, C^7-H), 7.35 (2H, d, *J* 8.3, $C^{3''}$-H, $C^{5''}$-H), 3.92 (2H, s, C^9-H_2), 2.99–2.86 (4H, m, $C^{1'}$-H_2, $C^{2'}$-H_2), 2.56 (3H, s, C^8-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.4 (C^{7a}), 155.1 (C^3), 145.8 ($C^{4''}$), 140.0 ($C^{1''}$), 135.5 (C^5), 130.5 (C^6), 129.6 ($C^{3''}$, $C^{5''}$), 126.8 ($C^{2''}$, $C^{6''}$), 122.6 (C^{3a}), 120.3 (C^4), 109.9 (C^7), 53.6 (C^9), 50.2 ($C^{2'}$), 36.4 ($C^{1'}$), 10.3 (C^8); HRMS *m/z* (ESI$^+$) [Found: 346.12203, $C_{17}H_{19}N_3O_3S$ requires $[M+H]^+$ 346.12199]; LRMS *m/z* (ESI$^-$) 344.1 ($[M–H]^-$, 100%); HPLC retention time 6.1 min, 95.3% (220 nm).

_N_-Cyclohexyl-2'-(4-methyl-1_H_-imidazol-5-yl)imidazo[1,2-_a_]pyridin-3'-amine (76)



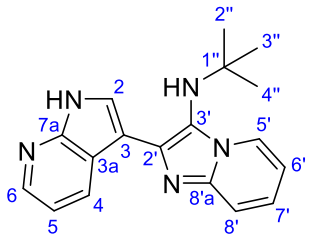
2-Aminopyridine (0.10 g, 1.1 mmol, 1.0 eq.) was dissolved in anhydrous dimethyl sulfoxide (3.0 mL). Cyclohexyl isocyanide (0.16 mL, 1.3 mmol, 1.2 eq.), 4-methyl-5-imidazole carboxaldehyde (0.18 g, 1.6 mmol, 1.5 eq.) and scandium triflate (52 mg, 0.11 mmol, 0.1 eq.) were added. The reaction mixture was heated to 100 °C and stirred for 17 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was cooled to rt and quenched with H_2O (20 mL). The reaction mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, washed sequentially with a saturated aqueous solution of $NaHCO_3$ (30 mL), and brine (30 ml), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 1:6 EtOH/EtOAc isocratic then 1:2:12 NH_4OH/EtOH/EtOAc isocratic) to yield the title compound as a yellow oil (98 mg, 0.33 mmol, 31%): R_f 0.63 (1:2:12 NH_4OH/EtOH/EtOAc); $\tilde{\nu}_{max}$/cm^{-1} 2981 (br), 2462 (br), 2160 (s), 2030 (s), 1978 (s), 1694 (w), 1613 (w), 1541 (w), 1445 (w), 1339 (w), 1238 (w), 1164 (w), 1053 (m), 1026 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (1H, dd, *J* 6.8, 1.3, C$^{5'}$-H), 7.56 (1H, s, C^2-H), 7.47 (1H, dd, *J* 9.0, 1.1, C$^{8'}$-H), 7.07 (1H, ddd, *J* 9.0, 6.8, 1.3, C$^{7'}$-H), 6.76 (1H, ddd, *J* 6.8, 6.8, 1.1, C$^{6'}$-H), 2.95–2.85 (1H, m, C$^{9'}$-H), 2.60 (3H, s, C^6-H$_3$), 1.85–1.78 (2H, m, C$^{10'}$-H$_a$, C$^{14'}$-H$_a$), 1.72–1.63 (2H, m, C$^{11'}$-H$_a$, C$^{13'}$-H$_a$), 1.58–1.51 (1H, m, C$^{12'}$-H$_a$), 1.28–1.10 (5H, m, C$^{10'}$-H$_b$, C$^{11'}$-H$_b$, C$^{12'}$-H$_b$, C$^{13'}$-H$_b$, C$^{14'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 141.3 (C$^{3'}$), 133.2 (C^2), 130.1 (C^4), 127.9 (C$^{2'}$), 127.4 (C^5), 126.2 (C$^{8'a}$), 123.3 (C$^{7'}$), 122.7 (C$^{5'}$), 116.9 (C$^{8'}$), 111.6 (C$^{6'}$), 56.4 (C$^{9'}$), 34.0 (C$^{10'}$, C$^{14'}$), 25.9 (C$^{12'}$), 25.1 (C$^{11'}$, C$^{13'}$), 11.9 (C^6); HRMS *m/z* (ESI$^+$) [Found: 296.18649, $C_{17}H_{22}N_5$ requires [M+H]$^+$ 296.18697]; LRMS *m/z* (ESI$^+$) 296.2 ([M+H]$^+$, 100%); HPLC retention time 5.5 min, 99.7%.

6'-Bromo-*N*-(4''-methoxyphenyl)-2'-(4-methyl-1*H*-imidazol-5-yl)imidazo[1,2-*a*]pyridin-3'-amine (77)



2-Amino-5-bromopyridine (100 mg, 0.578 mmol, 1.0 eq.) was dissolved in anhydrous dimethyl sulfoxide (1.60 mL). 4-Methoxyphenyl isocyanide (92.4 mg, 0.694 mmol, 1.2 eq.), 4-methyl-5-imidazolecarboxaldehyde (95.5 mg, 0.867 mmol, 1.5 eq.) and scandium triflate (28.4 mg, 0.0878 mmol, 0.1 eq.) were added. The reaction mixture was heated to 100 °C and stirred for 16.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was cooled to rt and quenched with H_2O (20 mL). The reaction mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, washed sequentially with a saturated aqueous solution of $NaHCO_3$ (30 mL), and brine (30 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−10% MeOH/CH_2Cl_2 gradient) to yield the title compound as a yellow oil (138 mg, 0.347 mmol, 60%): R_f 0.45 (1:2:12 NH_4OH/EtOH/EtOAc); $\bar{\nu}_{max}$/cm^{-1} 2537 (br), 2160 (s), 2033 (s), 1977 (s), 1602 (w), 1509 (m), 1460 (w), 1408 (w), 1325 (w), 1281 (w), 1246 (m), 1179 (w), 1128 (w), 1038 (w), 948 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (1H, dd, *J* 2.0, 0.9, $C^{5'}$-H), 7.60 (1H, s, C^2-H), 7.44 (1H, d, *J* 9.4, $C^{8'}$-H), 7.30 (1H, dd, *J* 9.5, 1.9, $C^{7'}$-H), 6.71–6.64 (2H, m, $C^{2''}$-H, $C^{6''}$-H), 6.46–6.39 (2H, m, $C^{3''}$-H, $C^{5''}$-H), 3.63 (3H, s, $C^{7''}$-H_3), 2.34 (3H, s, C^6-H_3); ^{13}C NMR (126 MHz, CD_3OD) δ 154.8 ($C^{4''}$), 141.9 ($C^{8'a}$), 139.6 ($C^{1''}$), 135.7 (C^2), 134.0 (C^5), 131.2 (C^4), 129.4 ($C^{7'}$), 126.0 ($C^{3'}$), 124.4 ($C^{2'}$), 122.4 ($C^{5'}$), 118.4 ($C^{8'}$), 116.0 ($C^{2''}$, $C^{6''}$), 115.8 ($C^{3''}$, $C^{5''}$), 108.0 ($C^{6'}$), 56.0 ($C^{7''}$), 12.0 (C^6); HRMS *m/z* (ESI^+) [Found: 398.06078, $C_{18}H_{17}{}^{79}BrN_5O$ requires $[M+H]^+$ 398.06110; Found: 400.05859, $C_{18}H_{17}{}^{81}BrN_5O$ requires $[M+H]^+$ 400.05905]; LRMS *m/z* (ESI^+) 400.0 ($[M+H]^+$, 100%); HPLC retention time 5.9 min, 95.6%.

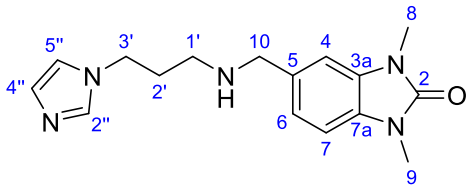
_N_-*tert*-Butyl-2'-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)imidazo[1,2-*a*]pyridin-3'-amine (78)



2-Aminopyridine (100 mg, 1.06 mmol, 1.5 eq.) was dissolved in anhydrous dimethyl sulfoxide (3.00 mL). *tert*-Butyl isocyanide (80.0 µL, 0.706 mmol, 1.0 eq.), 7-azaindole-3-carboxaldehyde (232 mg, 1.59 mmol, 2.3 eq.) and scandium triflate (52.2 mg, 0.106 mmol, 0.2 eq.) were added. The reaction mixture was heated to 100 °C and stirred for 17 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was cooled to rt and quenched with H_2O (20 mL). The reaction mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, washed sequentially with a saturated aqueous solution of $NaHCO_3$ (30 mL), and brine (30 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−9% MeOH/CH_2Cl_2 gradient) to yield the title compound as a pale green solid (102 mg, 0.334 mmol, 47%): R_f 0.52 (1:2:12 NH_4OH/EtOH/EtOAc); $\bar{\nu}_{max}$/cm^{-1} 3068 (br), 2963 (br), 2568 (br), 2160 (m), 2031 (m), 1978 (m), 1629 (w), 1574 (w), 1527 (w), 1501 (w), 1453 (w), 1423 (w), 1390 (w), 1363 (w), 1339 (w), 1320 (w), 1284 (w), 1269 (w), 1221 (w), 1191 (w), 1141 (w), 1120 (w), 1015 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.14 (1H, br s, N-H), 8.55 (1H, d, *J* 7.9, C^4-H), 8.38 (1H, d, *J* 4.3, C^6-H), 8.23 (1H, d, *J* 6.8, C$^{5'}$-H), 7.84 (1H, s, C^2-H), 7.57 (1H, d, *J* 9.1, C$^{8'}$-H), 7.19 (1H, dd, *J* 7.9, 4.3, C^5-H), 7.14 (1H, ddd, *J* 9.1, 6.8, 1.3, C$^{7'}$-H), 6.79 (1H, ddd, *J* 6.8, 6.8, 1.1, C$^{6'}$-H), 3.06 (1H, br s, N-H), 1.05 (9H, s, C$^{2''}$-H$_3$, C$^{3''}$-H$_3$, C$^{4''}$-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 148.8 (C^{7a}), 143.4 (C^6), 142.4 (C$^{8'a}$), 135.4 (C$^{3'}$), 130.4 (C^4), 123.92 (C$^{7'}$), 123.85 (C^2), 123.3 (C$^{5'}$), 123.1 (C$^{2'}$), 119.4 (C^{3a}), 117.1 (C$^{8'}$), 116.5 (C^5), 111.5 (C$^{6'}$), 110.1 (C^3), 56.6 (C$^{1''}$), 30.5 (C$^{2''}$, C$^{3''}$, C$^{4''}$); HRMS *m/z* (ESI$^+$) [Found: 306.17127, $C_{18}H_{20}N_5$ requires [M+H]$^+$ 306.17132]; LRMS *m/z* (ESI$^+$) 306.2 ([M+H]$^+$, 100%); HPLC retention time 5.6 min, 99.8%.

Chapter 3 Compounds

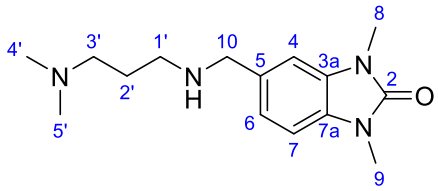
5-({[3'-(1*H*-Imidazol-1''-yl)prop-1'-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (89)



1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**34**) (100 mg, 0.526 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (13.0 mL) and 3 Å molecular sieves were added. 3-(1*H*-Imidazol-1-yl)propan-1-amine (125 µL, 1.05 mmol, 2.0 eq.) and acetic acid (60.0 µL, 1.05 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was stirred at 0 °C for 4 h. After this time TLC analysis indicated partial imine formation (1:9 MeOH/CH_2Cl_2). $NaBH(OAc)_3$ (557 mg, 2.63 mmol, 5.0 eq.) was added at 0 °C. The reaction mixture was warmed to rt and stirred for 16.5 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL), filtered through a pad of Celite®, and the filter pad washed with EtOH. The reaction volume was reduced by concentration *in vacuo*. The reaction mixture was extracted with CH_2Cl_2 (2 × 20 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 2% Et_3N isocratic) to yield the title compound as a colourless oil (101 mg, 0.338 mmol, 64%): R_f 0.21 (EtOH + 2% Et_3N); $\tilde{\nu}_{max}$/cm^{-1} 3294 (N-H, br), 2938 (br), 1692 (C=O, s), 1629 (w), 1508 (m), 1447 (m), 1396 (m), 1342 (w), 1268 (w), 1232 (w), 1190 (w), 1139 (w), 1010 (w), 1081 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (1H, s, $C^{2''}$-H), 7.05–7.01 (1H, m, $C^{4''}$-H), 7.00 (1H, dd, *J* 7.7, 1.5, C^6-H), 6.92 (1H, d, *J* 1.5, C^4-H), 6.90–6.86 (2H, m, C^7-H, $C^{5''}$-H), 4.04 (2H, t, *J* 6.8, $C^{3'}$-H_2), 3.78 (2H, s, C^{10}-H_2), 3.40 (3H, s, $C^{8/9}$-H_3), 3.39 (3H, s, $C^{8/9}$-H_3), 2.61 (2H, t, *J* 6.8, $C^{1'}$-H_2), 1.94 (2H, tt, *J* 6.8, 6.8, $C^{2'}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 137.3 ($C^{2''}$), 133.4 (C^5), 130.3 (C^{3a}), 129.4 ($C^{4''}$), 129.3 (C^{7a}), 121.2 (C^6), 118.9 ($C^{5''}$), 107.3 (C^4),

107.1 (C^{7}), 54.3 (C^{10}), 45.8 ($C^{1'}$), 44.8 ($C^{3'}$), 31.3 ($C^{2'}$), 27.27 ($C^{8/9}$), 27.26 ($C^{8/9}$); HRMS *m/z* (ESI^{+}) [Found: 300.18173, $C_{16}H_{21}N_5O$ requires $[M+H]^{+}$ 300.18189]; unable to detect by LRMS; HPLC retention time 4.8 min, 98.4%.

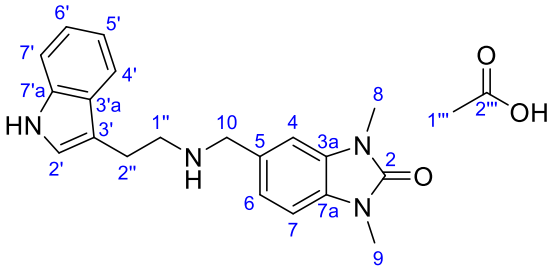
5-({[3'-(Dimethylamino)prop-1'-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (90)



1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**34**) (70 mg, 0.37 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (10 mL) and 3 Å molecular sieves were added. *N',N'*-Dimethylpropane-1,3-diamine (86 mg, 0.84 mmol, 2.3 eq.) and acetic acid (48 μL, 0.84 mmol, 2.3 eq.) were added at 0 °C and the reaction mixture stirred at 0 °C for 4 h. After this time TLC analysis indicated partial imine formation (1:9 MeOH/CH_2Cl_2). $NaBH_4$ (0.45 g, 2.1 mmol, 5.7 eq.) was added at 0 °C. The reaction mixture was warmed to rt and stirred for 16.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (7 × 15 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 10–20% EtOH/$CHCl_3$ + 2% Et_3N gradient) to yield the title compound as a colourless oil (37 mg, 0.13 mmol, 36%): R_f 0.68 (1:9 MeOH/CH_2Cl_2); $\tilde{\nu}_{max}/cm^{-1}$ 2940 (br), 2815 (br), 1699 (C=O, s), 1508 (m), 1461 (m), 1395 (w), 1268 (w), 1138 (w), 1081 (w), 1028 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01 (1H, dd, *J* 7.9, 1.5, C^{6}-H), 6.97 (1H, d, *J* 1.5, C^{4}-H), 6.87 (1H, d, *J* 7.9, C^{7}-H), 3.81 (2H, s, C^{10}-H_2), 3.39 (3H, s, C^{9}-H_3), 3.38 (3H, s, C^{8}-H_3), 2.67 (2H, t, *J* 7.1, $C^{1'}$-H_2), 2.30 (2H, t, *J* 7.1, $C^{3'}$-H_2), 2.19 (6H, s, $C^{4'}$-H_3, $C^{5'}$-H_3), 1.67 (2H, tt, *J* 7.1, 7.1, $C^{2'}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^{2}), 134.0 (C^{5}), 130.3 (C^{3a}), 129.1 (C^{7a}), 121.2 (C^{6}), 107.4 (C^{4}), 107.0 (C^{7}), 58.2 ($C^{3'}$), 54.4 (C^{10}), 48.0 ($C^{1'}$), 45.7 ($C^{4'}$, $C^{5'}$), 28.1 ($C^{2'}$), 27.2 (C^{8}, C^{9}); HRMS *m/z* (ESI^{+}) [Found:

277.20198, $C_{15}H_{24}N_4O$ requires $[M+H]^+$ 277.20229]; unable to detect by LRMS; HPLC retention time 4.5 min, 99.0%.

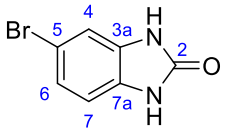
5-({[2''-(1'*H*-Indol-3'-yl)ethyl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one acetate (91)



1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**34**) (0.10 g, 0.53 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (13 mL) and 3 Å molecular sieves were added. Tryptamine (0.17 g, 1.1 mmol, 2.0 eq.) and acetic acid (60 µL, 1.1 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was warmed to rt and stirred for 6 h. After this time ^{1}H NMR analysis of the reaction mixture indicated partial imine formation. $NaBH(OAc)_3$ (0.56 g, 2.6 mmol, 5.0 eq.) was added at 0 °C. The reaction mixture was warmed to rt and stirred for 15 h. After this time TLC analysis indicated partial consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (50 mL), filtered through a pad of Celite®, and the filter pad washed with H_2O. The reaction mixture was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 2% Et_3N isocratic) to yield the title compound as a colourless solid (21 mg, 0.053 mmol, 10%) (removal of the acetate was attempted by heating under vacuum): R_f 0.21 (EtOH + 2% Et_3N); m.p. 196–198 °C (from EtOH); $\tilde{\nu}_{max}$/cm^{-1} 2980 (w), 2360 (w), 2323 (w), 2162 (w), 1718 (C=O, s), 1561 (s), 1512 (m), 1484 (m), 1440 (s), 1409 (s), 1343 (w), 1268 (w), 1243 (w), 1228 (w), 1201 (w), 1130 (w), 1080 (w), 1042 (w), 1011 (w), 924 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.85 (1H, br s, N-H), 7.48 (1H, dd, *J* 7.9, 1.0, C$^{4'}$-H), 7.31 (1H, dd, *J* 8.0, 1.0, C$^{7'}$-H), 7.11 (1H, d, *J* 2.3, C$^{2'}$-H), 7.09 (1H, d, *J* 1.3, C^4-H), 7.07–6.98 (3H, m, C^6-H, C^7-H, C$^{6'}$-H), 6.93 (1H, ddd, *J* 7.9, 7.0, 1.0, C$^{5'}$-H), 3.76 (2H, s, C^{10}-H$_2$), 3.30 (3H, s, C^9-H$_3$), 3.28 (3H, s, C^8-H$_3$), 2.89–2.81 (2H, m, C$^{2''}$-H$_2$),

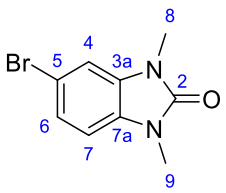
2.82–2.75 (2H, m, $C^{1'''}$-H_2), 1.72 (3H, s, $C^{1'''}$-H_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.0 ($C^{2'''}$), 154.0 (C^{2}), 136.2 ($C^{7'a}$), 134.0 (C^{5}), 129.6 (C^{3a}), 128.4 (C^{7a}), 127.3 ($C^{3'a}$), 122.6 ($C^{2'}$), 120.8 ($C^{6'}$), 120.6 (C^{6}), 118.3 ($C^{4'}$), 118.1 ($C^{5'}$), 112.6 ($C^{3'}$), 111.3 ($C^{7'}$), 107.3 (C^{4}), 107.1 (C^{7}), 53.1 (C^{10}), 49.4 ($C^{1''}$), 26.93 (C^{8}), 26.85 (C^{9}), 25.5 ($C^{2''}$), 23.9 ($C^{1'''}$); HRMS *m/z* (ESI^+) [Found: 335.18663, $C_{20}H_{23}N_4O$ requires $[M+H]^+$ 335.18664]; unable to detect by LRMS; HPLC retention time 6.6 min, >99.9%.

5-Bromo-1,3-dihydro-2*H*-benzimidazol-2-one (96)[218,219,220]



4-Bromobenzene-1,2-diamine (2.00 g, 10.7 mmol, 1.0 eq.) was dissolved in *N*,*N*-dimethylformamide (40.0 mL) and 1,1'-carbonyldiimidazole (1.91 g, 11.8 mmol, 1.1 eq.) was added. The reaction mixture was stirred at rt for 16 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was poured onto H_2O (150 mL). The resulting precipitate was collected *via* filtration and the precipitate washed with H_2O to yield the title compound as a yellow/brown solid (1.84 g, 8.64 mmol, 81%): R_f 0.44 (1:9 MeOH/CH_2Cl_2); m.p. >300 °C (from H_2O) [lit.[201] 336–337 °C]; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.77 (2H, br s, N-H, N-H), 7.11–7.02 (2H, m, C^4-H, C^6-H), 6.87 (1H, d, *J* 8.2, C^7-H); LRMS *m/z* (ESI^-) 212.9 ($[M-H]^-$, 50%). The spectroscopic data are in good agreement with literature values.[218,219,220]

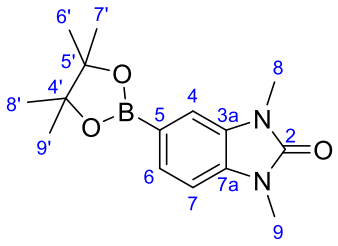
5-Bromo-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (97)[221]



5-Bromo-1,3-dihydro-2*H*-benzimidazol-2-one (**96**) (773 mg, 3.65 mmol, 1.0 eq.) was dissolved in *N*,*N*-dimethylformamide (8.50 mL) and K_2CO_3 (3.02 g, 21.9 mmol, 6.0 eq.) was added. Methyl

iodide (680 μL, 11.0 mmol, 3.0 eq.) was added dropwise and the reaction mixture stirred at rt for 6.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was diluted with EtOAc (30 mL) and washed with an aqueous solution of LiCl (0.5 M, 3 × 30 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the title compound as a brown solid (733 mg, 3.04 mmol, 83%): R_f 0.59 (3:7 petroleum ether/EtOAc); m.p. 150–152 °C (from EtOAc) [lit.[201] 166–167 °C]; $\bar{\nu}_{max}$/cm^{-1} 1700 (C=O, s), 1506 (s), 1445 (s), 1393 (s), 1266 (s), 1240 (s), 1134 (s), 1087 (s), 1055 (s), 1026 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (1H, dd, *J* 8.3, 1.8, C^6-H), 7.11 (1H, d, *J* 1.8, C^4-H), 6.83 (1H, d, *J* 8.3, C^7-H), 3.41 (3H, s, C^8-H_3), 3.40 (3H, s, C^9-H_3); HRMS *m/z* (CI) [Found: 240.9973, $C_9H_9{}^{79}BrN_2O$ requires $[M+H]^+$ 240.9971; Found: 242.9954, $C_9H_9{}^{81}BrN_2O$ requires $[M+H]^+$ 242.9951]; unable to detect by LRMS; HPLC retention time 8.3 min, 96.9%. The spectroscopic data are in good agreement with literature values.[221]

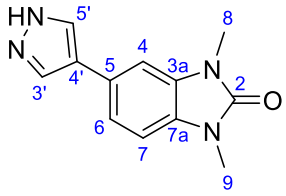
1,3-Dimethyl-5-(4',4',5',5'-tetramethyl-1',3'-dioxaborolan-2'-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (99)



5-Bromo-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**97**) (355 mg, 1.47 mmol, 1.0 eq.), bis(pinacolato)diboron (564 mg, 2.21 mmol, 1.5 eq.), $Pd_2(dba)_3$ (27.0 mg, 0.0294 mmol, 0.02 eq.), XPhos (28.0 mg, 0.0589 mmol, 0.04 eq.) and KOAc (433 mg, 4.41 mmol, 3.0 eq.) were placed in a microwave vial. Degassed anhydrous 1,4-dioxane (3.00 mL) was added and the reaction mixture heated to 110 °C and stirred for 19.5 h. After this time TLC analysis indicated partial consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was cooled to rt, diluted with EtOAc (25 mL) and washed with H_2O (25 mL). The organic fraction was washed with brine (25 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 40–60% EtOAc/petroleum ether gradient). The

product was dissolved in H_2O (20 mL) and concentrated *in vacuo*. The product was redissolved in H_2O (20 mL) and concentrated *in vacuo* to yield the title compound as an off-white solid (339 mg, 1.17 mmol, 80%): R_f 0.26 (2:3 EtOAc/petroleum ether); m.p. 112–114 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 2977 (br), 1706 (C=O, s), 1621 (w), 1510 (m), 1476 (m), 1371 (m), 1346 (B-O, s), 1310 (m), 1260 (m), 1214 (m), 1142 (s), 1111 (m), 1090 (m), 965 (m), 942 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (1H, dd, *J* 7.8, 1.3, C^6-H), 7.41 (1H, d, *J* 1.3, C^4-H), 6.98 (1H, d, *J* 7.8, C^7-H), 3.44 (3H, s, C^8-H$_3$), 3.43 (3H, s, C^9-H$_3$), 1.36 (12H, s, C$^{6'}$-H$_3$, C$^{7'}$-H$_3$, C$^{8'}$-H$_3$, C$^{9'}$-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 132.8 (C^{7a}), 129.8 (C^{3a}), 128.7 (C^6), 121.2 (C^5), 113.3 (C^4), 106.9 (C^7), 84.0 (C$^{4'}$, C$^{5'}$), 27.4 (C^8, C^9), 25.0 (C$^{6'}$, C$^{7'}$, C$^{8'}$, C$^{9'}$); HRMS *m/z* (ESI$^+$) [Found: 289.17184, $C_{15}H_{21}BN_2O_3$ requires $[M+H]^+$ 289.17180]; LRMS *m/z* (ESI$^+$) 289.2 ($[M+H]^+$, 100%); HPLC retention time 9.4 min, 98.3%.

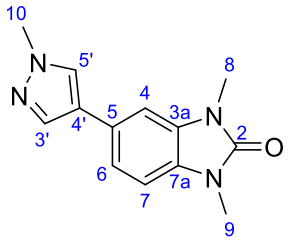
1,3-Dimethyl-5-(1'*H*-pyrazol-4'-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (92)



1,3-Dimethyl-5-(4,4,5,5-tetramethyl-1,3-dioxaborolan-2-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (**99**) (0.10 g, 0.35 mmol, 1.3 eq.), 4-bromopyrazole (41 mg, 0.28 mmol, 1.0 eq.) and (A-^{ta}Phos)$_2$PdCl$_2$ (4.9 mg, 0.0070 mmol, 0.03 eq.) were placed in a microwave vial. Degassed 1,4-dioxane (2.0 mL) and a degassed solution of K_2CO_3 (0.12 g, 0.83 mmol, 3.0 eq.) in H_2O (0.70 mL) were added. The reaction mixture was heated to 110 °C and stirred for 18 h. After this time TLC analysis indicated partial consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was filtered through a pad of Celite®, and the filter pad washed with EtOAc. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–5% MeOH/CH_2Cl_2 gradient). The product was dissolved in acetonitrile (15 mL) and washed with hexane (3 × 15 mL). The acetonitrile fraction was concentrated *in vacuo* to yield the title compound as a colourless solid (12 mg, 0.051 mmol, 18%):

R_f 0.49 (1:9 EtOH/CH_2Cl_2); m.p. 206–208 °C (from acetonitrile); $\bar{\nu}_{max}$/cm^{-1} 3206 (br), 2945 (br), 1680 (C=O, s), 1635 (w), 1609 (w), 1576 (w), 1506 (m), 1460 (m), 1396 (w), 1345 (w), 1262 (w), 1137 (w), 1078 (w), 1036 (w), 933 (w), 910 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.86 (1H, br s, N-H), 8.03 (2H, s, C$^{3'}$-H, C$^{5'}$-H), 7.40 (1H, d, *J* 1.6, C^4-H), 7.32 (1H, dd, *J* 8.1, 1.6, C^6-H), 7.10 (1H, d, *J* 8.1, C^7-H), 3.35 (3H, s, C$^{8/9}$-H$_3$), 3.32 (3H, s, C$^{8/9}$-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.0 (C^2), 131.2 (C$^{3'}$, C$^{5'}$), 130.2 (C^{3a}), 128.0 (C^{7a}), 126.3 (C^5), 121.7 (C$^{4'}$), 118.0 (C^6), 107.8 (C^7), 104.6 (C^4), 26.98 (C$^{8/9}$), 26.95 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 229.10842, $C_{12}H_{13}N_4O$ requires [M+H]$^+$ 229.10839]; LRMS *m/z* (ESI$^-$) 227.1 ([M–H]$^-$, 100%); HPLC retention time 5.9 min, 99.1%.

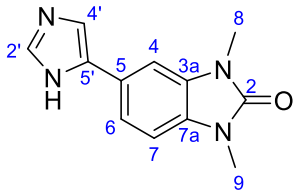
1,3-Dimethyl-5-(1'-methyl-1'*H*-pyrazol-4'-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (93)



1,3-Dimethyl-5-(1*H*-pyrazol-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (**92**) (13 mg, 0.055 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.50 mL) and sodium hydride (60% dispersion in mineral oil, 4.4 mg, 0.11 mmol, 2.0 eq.) and methyl iodide (6.8 μL, 0.11 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 4.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). MeOH (0.5 mL) was added and the reaction mixture concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–3% MeOH/CH_2Cl_2 gradient) and further purified using silica flash column chromatography (elution with EtOAc + 2% Et_3N isocratic) to yield the title compound as a colourless solid (13 mg, 0.053 mmol, 97%): R_f 0.46 (1:19 MeOH/CH_2Cl_2); m.p. 152–154 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 2919 (br), 1707 (C=O, s), 1687 (C=O, s), 1633 (w), 1609 (w), 1569 (m), 1508 (w), 1477 (w), 1439 (w), 1420 (w), 1392 (w), 1367 (w), 1261 (w), 1243 (w), 1210 (w), 1191 (w), 1143 (w), 1129 (w), 1088 (w), 1064 (w), 1026 (w), 986 (w), 933 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (1H, d, *J* 0.9, C$^{3'}$-H), 7.59 (1H, d, *J* 0.9, C$^{5'}$-H), 7.21 (1H, dd,

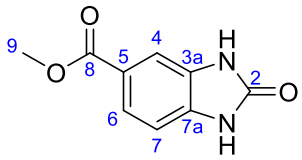
J 8.0, 1.6, C^6-H), 7.04 (1H, d, *J* 1.6, C^4-H), 6.95 (1H, d, *J* 8.0, C^7-H), 3.95 (3H, s, C^{10}-H_3), 3.44 (3H, s, C^8-H_3), 3.43 (3H, s, C^9-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 136.7 ($C^{3'}$), 130.7 (C^{3a}), 128.9 (C^{7a}), 126.7 ($C^{5'}$), 126.5 (C^5), 123.7 ($C^{4'}$), 119.0 (C^6), 107.7 (C^7), 104.9 (C^4), 39.3 (C^{10}), 27.41 ($C^{8/9}$), 27.37 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 243.12407, $C_{13}H_{15}N_4O$ requires $[M+H]^+$ 243.12404]; unable to detect by LRMS; HPLC retention time 6.7 min, 99.5%.

5-(1*H*-Imidazol-4-yl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (94)



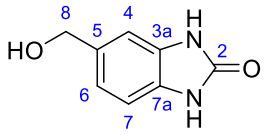
1,3-Dimethyl-5-(4,4,5,5-tetramethyl-1,3-dioxaborolan-2-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (**99**) (0.10 g, 0.35 mmol, 1.3 eq.), 4-bromo-1*H*-imidazole (41 mg, 0.28 mmol, 1.0 eq.), (A-^{ta}Phos)$_2PdCl_2$ (4.9 mg, 0.0070 mmol, 0.025 eq.) and K_2CO_3 (0.12 g, 0.83 mmol, 3.0 eq.) were placed in a microwave vial. Degassed 1,4-dioxane (2.0 mL) and H_2O (0.70 mL) were added. The reaction mixture was heated to 115 °C and stirred for 26 h. The reaction mixture was cooled to rt, filtered through a pad of Celite® and the filter pad washed with CH_2Cl_2. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 5–10% EtOH/$CHCl_3$ gradient). The product was dissolved in acetonitrile (15 mL) and washed with hexane (3 × 15 mL). The acetonitrile fraction was concentrated *in vacuo* to yield the title compound as a colourless solid (11 mg, 0.049 mmol, 18%): R_f 0.28 (1:9 EtOH/CH_2Cl_2); m.p. 138–140 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3187 (br), 3052 (br), 2928 (br), 1673 (C=O, s), 1609 (w), 1506 (w), 1460 (w), 1420 (w), 1396 (w), 1340 (w), 1322 (w), 1264 (w), 1234 (w), 1219 (w), 1141 (w), 1092 (w), 1056 (w), 1025 (w), 931;); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (1H, d, *J* 1.2, $C^{2'}$-H), 7.49–7.42 (2H, m, C^4-H, C^7-H), 7.32 (1H, d, *J* 1.2, $C^{4'}$-H), 6.97 (1H, d, *J* 8.7, C^6-H), 3.45 (3H, s, $C^{8/9}$-H_3), 3.43 (3H, s, $C^{8/9}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 140.4 ($C^{5'}$), 135.3 ($C^{2'}$), 130.6 (C^{7a}), 129.3 (C^{3a}), 127.4 (C^5), 118.3 (C^7), 112.7 ($C^{4'}$), 107.5 (C^6), 104.5 (C^4), 27.4 (C^8, C^9); HRMS *m/z* (ESI^+) [Found: 229.10833, $C_{12}H_{12}N_5O$ requires $[M+H]^+$ 229.10839]; LRMS *m/z* (ESI^-) 227.1 ($[M–H]^-$, 100%); HPLC retention time 5.1 min, >99.9%.

Methyl 2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (33)[222,223]



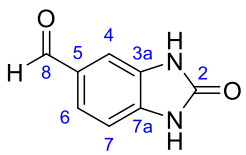
3,4-Diaminobenzoic acid (1.00 g, 6.57 mmol, 1.0 eq.) was suspended in CH_2Cl_2 (8.00 mL), and 1,1'-carbonyldiimidazole (2.34 g, 14.5 mmol, 2.2 eq.) was added. The reaction mixture was stirred at rt for 1 h. After this time TLC analysis indicated complete consumption of starting material (3:7 EtOH/CH_2Cl_2). MeOH (6.00 mL) was added and the reaction mixture stirred at rt for 2 h. After this time TLC analysis indicated complete consumption of the intermediate (3:7 EtOH/CH_2Cl_2). The reaction mixture was cooled on ice and the precipitate collected *via* filtration. The precipitate was washed with diethyl ether to yield the title compound as a light brown solid (1.23 g, 6.40 mmol, 97%): R_f 0.58 (3:7 EtOH/$CHCl_3$); m.p. >300 °C (from CH_2Cl_2) [lit.[222] 312–313 °C (from EtOH)]; $\tilde{\nu}_{max}$/cm^{-1} 3413 (br), 3157 (br), 2994 (br), 1758 (C=O, s), 1722 (C=O, s), 1632 (m), 1502 (w), 1473 (w), 1434 (m), 1366 (w), 1332 (w), 1299 (s), 1272 (m), 1226 (s), 1192 (w), 1113 (w), 1082 (m), 1029 (m), 964 (w), 929 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98 (2H, br s, N-H, N-H), 7.63 (1H, dd, *J* 8.2, 1.6, C^6-H), 7.46 (1H, d, *J* 1.6, C^4-H), 7.01 (1H, d, *J* 8.2, C^7-H), 3.81 (3H, s, C^9-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.7 (C^8), 155.7 (C^2), 134.1 (C^{7a}), 129.7 (C^{3a}), 123.2 (C^6), 122.0 (C^5), 109.3 (C^4), 108.5 (C^7), 52.1 (C^9); HRMS *m/z* (ESI$^+$) [Found: 193.06096, $C_9H_9N_2O_3$ requires $[M+H]^+$ 193.06077]; LRMS *m/z* (ESI$^-$) 191.0 ($[M–H]^-$, 100%). The spectroscopic data are in good agreement with literature values.[222,223]

5-(Hydroxymethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (114)[224]



Methyl 2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (**33**) (500 mg, 2.60 mmol, 1.0 eq.) was suspended in anhydrous toluene (18.0 mL). The reaction mixture was cooled to –78 °C and diisobutylaluminium hydride (1 M, hexanes) (18.2 mL, 18.2 mmol, 7.0 eq.) was added dropwise. The reaction mixture was stirred at –78 °C for 3.5 h. After this time TLC analysis indicated complete consumption of starting material (3:7 EtOH/CH_2Cl_2). The reaction was quenched at –78 °C with a saturated aqueous solution of potassium sodium tartrate (100 mL). The reaction mixture was warmed to rt and stirred for 1 h. The reaction mixture was extracted with EtOAc (6 × 100 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a colourless solid (145 mg, 0.883 mmol, 34%): R_f 0.16 (1:9 MeOH/CH_2Cl_2); m.p. 226–228 °C (from EtOAc); $\tilde{\nu}_{max}$/cm^{-1} 3123 (br), 2981 (br), 1678 (C=O, s), 1615 (w), 1507 (w), 1479 (w), 1388 (w), 1286 (w), 1263 (w), 1209 (w), 1148 (w), 1056 (w), 1023 (w), 1003 (w), 990 (m), 938 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.53 (1H, br s, N-H), 10.50 (1H, br s, N-H), 6.89 (1H, s, C^7-H), 6.89–6.81 (2H, m, C^4-H, C^6-H), 5.04 (1H, t, *J* 5.8, O-H), 4.43 (2H, d, *J* 5.8, C^8-H$_2$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.5 (C^2), 135.0 (C^5), 129.6 (C^{7a}), 128.5 (C^{3a}), 118.9 (C^6), 107.9 (C^4), 107.1 (C^7), 63.3 (C^8); HRMS *m/z* (ESI$^+$) [Found: 165.06597, $C_8H_9N_2O_2$ requires [M+H]$^+$ 165.06585]; LRMS *m/z* (ESI$^-$) 163.1 ([M–H]$^-$, 100%). The spectroscopic data are in good agreement with literature values.[224]

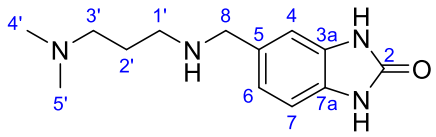
2-Oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (115)[225]



5-(Hydroxymethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (**114**) (0.10 g, 0.62 mmol, 1.0 eq.) was suspended in CH_2Cl_2 (15 mL) and MnO_2 (54 mg, 6.2 mmol, 10 eq.) was added. The reaction

mixture was stirred at rt for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was filtered through a glass microfibre filter and the filter washed with EtOH. The filtrate was concentrated *in vacuo* and the product purified using silica flash column chromatography (elution with 0–10% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless solid (28 mg, 0.17 mmol, 28%): R_f 0.22 (1:9 MeOH/CH_2Cl_2); d.p. >280 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3098 (br), 1759 (C=O, s), 1679 (C=O, s), 1633 (m), 1474 (w), 1384 (w), 1326 (w), 1280 (m), 1202 (m), 1139 (w), 1116 (w), 1031 (w), 960 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.07 (2H, br s, N-H, N-H), 9.86 (1H, s, C^8-H), 7.57 (1H, dd, *J* 7.9, 1.5, C^6-H), 7.39 (1H, d, *J* 1.5, C^4-H), 7.11 (1H, d, *J* 7.9, C^7-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.9 (C^8), 155.4 (C^2), 135.4 (C^{7a}), 130.2 (C^{3a}), 129.8 (C^5), 125.2 (C^6), 108.5 (C^7), 107.9 (C^4); HRMS *m/z* (ESI$^-$) [Found: 161.03531, $C_8H_5N_2O_2$ requires $[M–H]^-$ 161.03565]; LRMS *m/z* (ESI$^-$) 161.1 ($[M–H]^-$, 100%). The spectroscopic data are in good agreement with literature values.[225]

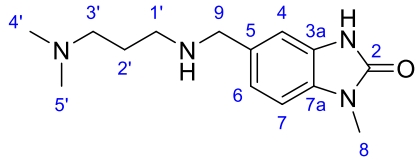
5-({[3'-(Dimethylamino)prop-1'-yl]amino}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (104)



2-Oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**115**) (42 mg, 0.26 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (6.0 mL) and 3 Å molecular sieves were added. *N*',*N*'-dimethylpropane-1,3-diamine (65 µL, 0.52 mmol, 2.0 eq.) and acetic acid (30 µL, 0.52 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 1.5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated partial imine formation. $NaBH(OAc)_3$ (0.27 g, 1.3 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 19 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL) and filtered through a pad of Celite®. The filtrate was washed with CH_2Cl_2 (2 × 15 mL). The aqueous fraction was concentrated *in vacuo*. The resulting residue was stirred in EtOH (20 mL) for 1 h and filtered

through filter paper. The filtrate was concentrated *in vacuo* and purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless oil (29 mg, 0.12 mmol, 45%): R_f 0.06 (EtOH + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 2944 (br), 2825 (br), 1689 (s), 1543 (w), 1504 (m), 1471 (w), 1372 (2), 1276 (m), 1208 (w), 1098 (w), 1012 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.08–6.96 (3H, m, C^4-H, C^6-H, C^7-H), 3.76 (2H, s, C^8-H$_2$), 2.61 (2H, d, *J* 7.5, C$^{1'}$-H$_2$), 2.35 (2H, d, *J* 7.5, C$^{3'}$-H$_2$), 2.23 (6H, s, C$^{4'}$-H$_3$, C$^{5'}$-H$_3$), 1.71 (2H, tt, *J* 7.5, 7.5, C$^{2'}$-H$_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 158.2 (C^2), 133.7 (C^5), 131.0 (C^{3a}), 130.1 (C^{7a}), 123.0 (C^6), 110.6 (C^4), 110.1 (C^7), 58.7 (C$^{3'}$), 54.5 (C^8), 48.0 (C$^{1'}$), 45.3 (C$^{4'}$, C$^{5'}$), 27.8 (C$^{2'}$); HRMS *m/z* (APCI) [Found: 249.17097, $C_{13}H_{21}N_4O$ requires $[M+H]^+$ 249.17099]; unable to detect by LRMS; unable to obtain a HPLC trace due to eluting in the injection peak.

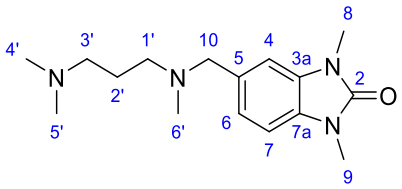
5-({[3'-(Dimethylamino)prop-1'-yl]amino}methyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (105)



1-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**65**) (200 mg, 1.14 mmol, 1.0 eq.) was suspended in anhydrous 1,2-dichloroethane (6.00 mL) and 3 Å molecular sieves were added. *N'*,*N'*-Dimethylpropane-1,3-diamine (286 μL, 2.28 mmol, 2.0 eq.) and acetic acid (130 μL, 2.28 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was warmed to rt and stirred for 6 h. After this time ^{1}H NMR analysis of the reaction mixture indicated partial imine formation. $NaBH(OAc)_3$ (1.21 g, 5.70 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 18 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL), filtered through a pad of Celite®, and the filter pad was washed with EtOH. The filtrate volume was reduced by concentration *in vacuo* and the reaction mixture extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1–10% Et_3N gradient) to yield the title compound as a colourless oil (147 mg, 0.560 mmol, 49%):

R_f 0.13 (EtOH + 1% Et_3N); $\tilde{\nu}_{max}$/cm^{-1} 2941 (br), 2816 (br), 1694 (C=O, s), 1633 (w), 1504 (w), 1463 (w), 1431 (w), 1385 (w), 1303 (w), 1262 (w), 1231 (w), 1182 (w), 1155 (w), 1136 (w), 1089 (w), 1039 (w), 927 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.03 (1H, d, *J* 1.6, C^4-H), 6.97 (1H, dd, *J* 8.0, 1.6, C^6-H), 6.83 (1H, d, *J* 8.0, C^7-H), 3.76 (2H, s, C^9-H_2), 3.34 (3H, s, C^8-H_3), 2.66 (2H, t, *J* 7.1, C$^{1'}$-H_2), 2.31 (2H, t, *J* 7.1, C$^{3'}$-H_2), 2.19 (6H, s, C$^{4'}$-H_3, C$^{5'}$-H_3), 1.68 (2H, tt, *J* 7.1, 7.1, C$^{2'}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.1 (C^2), 133.4 (C^5), 130.2 (C^{7a}), 128.6 (C^{3a}), 121.2 (C^6), 109.5 (C^4), 107.1 (C^7), 57.9 (C$^{3'}$), 54.0 (C^9), 47.6 (C$^{1'}$), 45.4 (C$^{4'}$, C$^{5'}$), 27.6 (C$^{2'}$), 26.8 (C^8); HRMS *m/z* (ESI$^-$) [Found: 261.17208, $C_{15}H_{24}N_4O$ requires [M–H]$^-$ 261.17208]; LRMS *m/z* (ESI$^-$) 261.1 ([M–H]$^-$, 100%); unable to obtain a HPLC trace due to eluting in the injection peak.

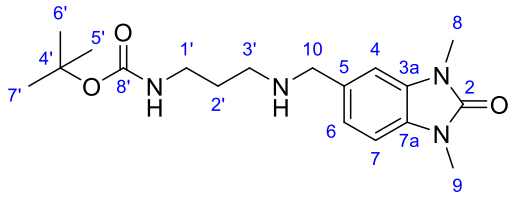
5-({[3'-(Dimethylamino)prop-1'-yl](methyl)amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (106)



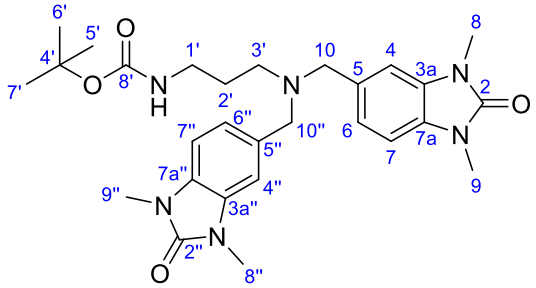
1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (0.12 g, 0.65 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (15 mL) and 3 Å molecular sieves were added. *N,N,N'*-Trimethyl-1,3-propane-diamine (0.29 mL, 2.0 mmol, 3.0 eq.) and Et_3N (0.27 mL, 2.0 mmol, 3.0 eq.) were added. The reaction mixture was stirred at rt for 2 h. After this time ^{1}H NMR analysis of the reaction mixture did not indicate imine formation. Additional *N,N,N'*-trimethyl-1,3-propane-diamine (0.29 mL, 2.0 mmol, 3.0 eq.) and Et_3N (0.27 mL, 2.0 mmol, 3.0 eq.) were added to help promote imine formation. The reaction mixture was stirred at rt for 1 h. $NaBH(OAc)_3$ (0.42 g, 2.0 mmol, 3.0 eq.) was added and the reaction mixture stirred at rt for 18 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (25 mL) and filtered through a pad of Celite®. The filter pad was washed with H_2O and the filtrate extracted with EtOAc (2 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N

isocratic) to yield the title compound as a colourless solid (75 mg, 0.26 mmol, 39%): R_f 0.057 (EtOH); m.p. 203–204 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 2949 (br), 2789 (br), 2642 (br), 2486 (br), 2207 (w), 1698 (C=O, s), 1608 (w), 1509 (m), 1460 (m), 1393 (w), 1350 (w), 1269 (w), 1186 (w), 1123 (w), 1081 (w), 1046 (w), 982 (w), 949 (w), 926 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01–6.95 (2H, m, C^4-H, C^6-H), 6.85 (1H, d, *J* 7.8, C^7-H), 3.52 (2H, s, C^{10}-H$_2$), 3.38 (3H, s, C$^{8/9}$-H$_3$), 3.36 (3H, s, C$^{8/9}$-H$_3$), 2.50 (2H, t, *J* 7.9, C$^{3'}$-H$_2$), 2.43 (2H, t, *J* 7.2, C$^{1'}$-H$_2$), 2.37 (6H, s, C$^{4'}$-H$_3$, C$^{5'}$-H$_3$), 2.19 (3H, s, C$^{6'}$-H$_3$), 1.85–1.75 (2H, m, C$^{2'}$-H$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.8 (C^2), 131.8 (C^5), 130.2 (C^{3a}), 129.3 (C^{7a}), 122.2 (C^6), 108.2 (C^4), 106.9 (C^7), 62.5 (C^{10}), 57.3 (C$^{3'}$), 54.9 (C$^{1'}$), 44.7 (C$^{4'}$, C$^{5'}$), 42.0 (C$^{6'}$), 27.3 (C$^{8/9}$), 27.2 (C$^{8/9}$), 24.3 (C$^{2'}$); HRMS *m/z* (ESI$^+$) [Found: 291.21805, $C_{16}H_{27}N_4O$ requires $[M+H]^+$ 291.21794]; LRMS *m/z* (ESI$^+$) 161.0 ($[M–C_7H_{17}N_2]^+$, 100%); HPLC retention time 4.6 min, >99.9%.

tert-Butyl (3'-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)carbamate (116)



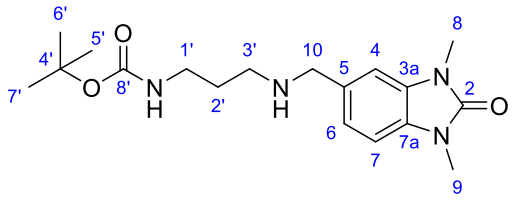
tert-Butyl (3'-{bis[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)carbamate (117)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (1.00 g, 2.63 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (25.0 mL) and 3 Å molecular sieves were added. *tert*-Butyl *N*-(3-aminopropyl)carbamate (1.37 g, 7.89 mmol, 1.5 eq.) and acetic acid

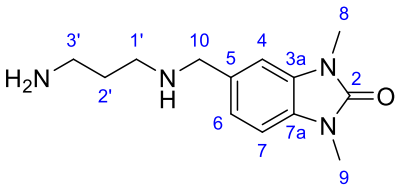
(455 μL, 7.89 mmol, 1.5 eq.) were added. The reaction mixture was stirred at rt for 6.5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated almost complete imine formation. $NaBH(OAc)_3$ (5.57 g, 26.3 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 16 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (100 mL), filtered through a pad of Celite® and the filter pad washed with CH_2Cl_2. The filtrate was diluted with a saturated aqueous solution of $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (3 × 125 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–6% EtOH/CH_2Cl_2 + 1% Et_3N gradient) to yield **116** as a pale yellow oil (406 mg, 1.17 mmol, 44%) and **117** as a colourless oil (770 mg, 1.47 mmol, 56%): **116**: R_f 0.57 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 3342 (br), 2934 (br), 2162 (br), 2029 (br), 1687 (C=O, s), 1508 (m), 1447 (w), 1395 (w), 1365 (w), 1268 (w), 1250 (w), 1167 (m), 1082 (w), 1028 (w), 976 (w), 953 (w), 921 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.03 (1H, dd, *J* 8.0, 1.5, C^{6}-H), 6.98 (1H, d, *J* 1.5, C^{4}-H), 6.88 (1H, d, *J* 8.0, C^{7}-H), 5.13 (1H, br s, N-H), 3.80 (2H, s, C^{10}-H_2), 3.40 (3H, s, C$^{8/9}$-H_3), 3.39 (3H, s, C$^{8/9}$-H_3), 3.23–3.16 (2H, m, C$^{1'}$-H_2), 2.69 (2H, t, *J* 6.6, C$^{3'}$-H_2), 1.68 (2H, tt, *J* 6.6, 6.6, C$^{2'}$-H_2), 1.41 (9H, s, C$^{5'}$-H_3, C$^{6'}$-H_3, C$^{7'}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.2 (C$^{8'}$), 154.9 (C^{2}), 133.5 (C^{5}), 130.5 (C^{3a}), 129.2 (C^{7a}), 121.3 (C^{6}), 107.5 (C^{4}), 107.1 (C^{7}), 79.1 (C$^{4'}$), 54.3 (C^{10}), 47.2 (C$^{3'}$), 39.2 (C$^{1'}$), 29.9 (C$^{2'}$), 28.5 (C$^{5'}$, C$^{6'}$, C$^{7'}$), 27.3 (C^{8}, C^{9}); HRMS *m/z* (ESI^{+}) [Found: 349.22341, $C_{18}H_{29}N_4O_3$ requires $[M+H]^+$ 349.22342]; LRMS *m/z* (ESI^{-}) 383.1 ($[M+Cl]^-$, 100%); HPLC retention time 6.3 min, >99.9%. **117**: R_f 0.50 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); m.p. 45–46 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 2936 (br), 2162 (br), 1979 (br), 1683 (C=O, s), 1630 (w), 1611 (w), 1506 (m), 1446 (w), 1394 (w), 1364 (w), 1269 (w), 1248 (w), 1169 (w), 1136 (w), 1081 (w), 1027 (w), 953 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14–7.03 (2H, m, C^{6}-H, C$^{6''}$-H), 7.01–6.89 (4H, m, C^{4}-H, C$^{4''}$-H, C^{7}-H, C$^{7''}$-H), 3.61 (4H, s, C^{10}-H_2, C$^{10''}$-H_2), 3.45 (6H, s, C$^{8/9}$-H_3, C$^{8''/9''}$-H_3), 3.43 (6H, s, C$^{8/9}$-H_3, C$^{8''/9''}$-H_3), 3.21–3.09 (2H, m, C$^{1'}$-H_2), 2.51 (2H, t, *J* 6.5, C$^{3'}$-H_2), 1.71 (2H, tt, *J* 6.5, 6.5 C$^{2'}$-H_2), 1.42 (9H, s, C$^{5'}$-H_3, C$^{6'}$-H_3, C$^{7'}$-H_3); HRMS *m/z* (ESI^{+}) [Found: 523.30243, $C_{28}H_{39}N_6O_4$ requires $[M+H]^+$ 523.30273]; LRMS *m/z* (ESI^{-}) 557.3 ($[M+Cl]^-$, 100%); HPLC retention time 6.7 min, 96.2%.

tert-Butyl (3'-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1'-yl)carbamate (116)



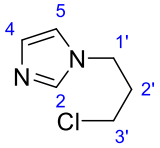
tert-Butyl (3-{bis[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino} propyl)carbamate (**117**) (300 mg, 0.574 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (15.0 mL). K_2CO_3 (157 mg, 1.15 mmol, 2.0 eq.) and 1-chloroethyl chloroformate (149 μL, 1.38 mmol, 2.4 eq.) were added. The reaction mixture was heated to 85 °C and stirred for 8 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + 1% Et_3N). The reaction mixture was cooled to rt and concentrated *in vacuo*. The resulting residue was dissolved in MeOH (15.0 mL). The reaction mixture was heated to 70 °C and stirred for 5.5 h. After this time TLC analysis indicated complete consumption of the intermediate (1:9 MeOH/CH_2Cl_2). The reaction mixture was cooled to rt and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–4% MeOH/CH_2Cl_2 + 1% Et_3N gradient) to yield the title compound as a colourless oil (117 mg, 0.471 mmol, 82%): R_f 0.59 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); ^{1}H NMR δ (400 MHz, $CDCl_3$) δ 7.03 (1H, dd, *J* 7.9, 1.5, C^6-H), 6.99 (1H, d, *J* 1.5, C^4-H), 6.86 (1H, d, *J* 7.9, C^7-H), 3.81 (2H, s, C^{10}-H_2), 3.37 (3H, s, $C^{8/9}$-H_3), 3.36 (3H, s, $C^{8/9}$-H_3), 3.23–3.13 (2H, m, $C^{1'}$-H_2), 2.70 (2H, t, *J* 6.7, $C^{3'}$-H_2), 1.68 (2H, tt, *J* 6.7, 6.7, $C^{2'}$-H_2), 1.39 (9H, s, $C^{5'}$-H_3, $C^{6'}$-H_3, $C^{7'}$-H_3); LRMS *m/z* (ESI^+) 175.0 ($[M–C_8H_{17}N_2O_2]^+$, 100%). The spectroscopic data are in good agreement with those previously obtained for **116**.

5-{[(3'-Aminoprop-1'-yl)amino]methyl}-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (107)



tert-Butyl (3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl) carbamate (**34**) (176 mg, 0.505 mmol, 1.0 eq.) was dissolved in HCl (4 M, dioxane) (3.79 mL, 15.2 mmol, 30 eq.). The reaction mixture was stirred at rt for 1 h. The reaction mixture was concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (7 M, MeOH)) to yield the title compound as a colourless solid (120 mg, 0.483 mmol, 96%): R_f 0.03 (EtOH + 1% Et_3N); d.p. >200 °C (from MeOH); $\tilde{\nu}_{max}$/cm^{-1} 3036 (br), 2925 (br), 2755 (br), 2161 (br), 1677 (C=O, s), 1631 (m), 1510 (w), 1483 (w), 1450 (m), 1392 (m), 1353 (w), 1269 (w), 1246 (w), 1204 (w), 1176 (w), 1129 (w), 1084 (w), 1028 (w), 1002 (w), 954 (w); ^{1}H NMR (400 MHz, CD_3OD) δ 7.33 (1H, d, *J* 1.6, C^4-H), 7.27 (1H, dd, *J* 8.0, 1.6, C^6-H), 7.18 (1H, d, *J* 8.0, C^7-H), 4.18 (2H, s, C^{10}-H_2), 3.44 (3H, s, C$^{8/9}$-H_3), 3.42 (3H, s, C$^{8/9}$-H_3), 3.12–3.01 (4H, m, C$^{1'}$-H_2, C$^{3'}$-H_2), 2.06 (2H, tt, *J* 8.6, 6.8, C$^{2'}$-H_2); ^{13}C NMR (126 MHz, CD_3OD) δ 156.4 (C^2), 131.8 (C^{7a}), 131.6 (C^{3a}), 127.6 (C^5), 124.4 (C^6), 110.3 (C^4), 109.1 (C^7), 53.2 (C^{10}), 45.9 (C$^{1'}$), 38.4 (C$^{3'}$), 27.6 (C$^{8/9}$), 27.5 (C$^{8/9}$), 26.0 (C$^{2'}$); HRMS *m/z* (APCI) [Found: 249.17109, $C_{13}H_{21}N_4O$ requires $[M+H]^+$ 249.17099]; unable to detect by LRMS; HPLC retention time 4.5 min, 98.4%.

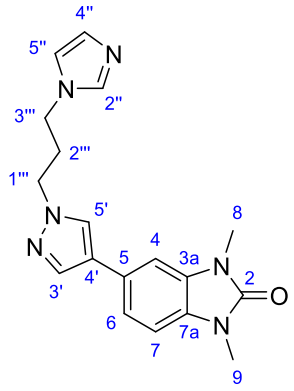
1-(3'-Chloropropyl)-1*H*-imidazole (120)[226,227]



Anhydrous tetrahydrofuran (11.0 mL) was cooled to 0 °C and sodium hydride (60% dispersion in mineral oil, 140 mg, 15.9 mmol, 1.2 eq.) was added. The reaction mixture was stirred at rt for

30 min. The reaction mixture was cooled to 0 °C and imidazole (900 mg, 13.2 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (3.00 mL) was added dropwise. The reaction mixture was warmed to rt and stirred for 1.5 h. 1-Bromo-3-chloropropane (1.30 mL, 13.2 mmol, 1.0 eq.) was added dropwise at 0 °C. The reaction mixture was stirred warmed to rt and stirred for 22.5 h. After this time TLC analysis indicated partial consumption of starting material (1:9 MeOH/CH_2Cl_2). MeOH (2.3 mL) was added to the reaction mixture, and the reaction mixture concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–5% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (547 mg, 3.78 mmol, 29%): R_f 0.31 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (1H, s, C^2-H), 7.05 (1H, br s, C^4-H), 6.91 (1H, br s, C^5-H), 4.14 (2H, t, *J* 6.3, $C^{1'}$-H_2), 3.45 (2H, t, *J* 6.3, $C^{3'}$-H_2), 2.18 (2H, tt, *J* 6.3, 6.3, $C^{2'}$-H_2); HRMS *m/z* (CI) [Found: 145.0529, $C_6H_{10}N_2Cl$ requires $[M+H]^+$ 145.0527]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[226,227]

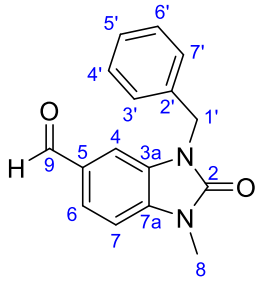
5-{1'-[3'''-(1''*H*-Imidazol-1''-yl)propyl]-1'*H*-pyrazol-4'-yl}-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (118)



1,3-Dimethyl-5-(1*H*-pyrazol-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (**92**) (28 mg, 0.12 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (0.50 mL) and cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 9.8 mg, 0.25 mmol, 2.0 eq.) was added. The reaction mixture was warmed to rt and stirred for 30 min. The reaction mixture was cooled to 0 °C and 1-(3-chloropropyl)-1*H*-imidazole (**120**) (40 mg, 0.27 mmol, 2.2 eq.) was added. The reaction mixture was warmed to rt and stirred for 67 h. After this time TLC analysis indicated complete

consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (2 × 10 mL). The organic fractions were combined and washed with an aqueous solution of LiCl (0.5 M, 3 × 10 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–10% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (6.7 mg, 0.020 mmol, 16%): R_f 0.13 (1:9 MeOH/CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3105 (w), 2937 (w), 2850 (w), 1696 (C=O, s), 1632 (w), 1608 (w), 1572 (w), 1507 (m), 1480 (m), 1448 (w), 1421 (w), 1394 (w), 1356 (w), 1284 (w), 1261 (w), 1230 (w), 1190 (w), 1131 (w), 1108 (w), 1078 (w), 1028 (w), 990 (w), 933 (w), 908 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (1H, s, C$^{3'}$-H), 7.56 (1H, s, C$^{5'}$-H), 7.50 (1H, s, C$^{2''}$-H), 7.21 (1H, dd, *J* 8.0, 1.5, C^6-H), 7.10 (1H, s, C$^{4''}$-H), 7.04 (1H, d, *J* 1.5, C^4-H), 6.98–6.92 (2H, m, C^7-H, C$^{5''}$-H), 4.11 (2H, t, *J* 6.5, C$^{1'''}$-H_2), 3.99 (2H, t, *J* 6.5, C$^{3'''}$-H_2), 3.45 (3H, s, C^8-H_3), 3.42 (3H, s, C^9-H_3), 2.41 (2H, tt, *J* 6.5, 6.5, C$^{2'''}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 137.5 (C$^{2''}$), 137.3 (C$^{3'}$), 130.8 (C^{3a}), 130.0 (C$^{4''}$), 129.1 (C^{7a}), 126.2 (C$^{5'}$), 126.1 (C^5), 123.7 (C$^{4'}$), 119.0 (C^6, C$^{5''}$), 107.8 (C^7), 104.8 (C^4), 48.6 (C$^{1'''}$), 43.8 (C$^{3'''}$), 31.5 (C$^{2'''}$), 27.38 (C$^{8/9}$), 27.36 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 337.17690, $C_{20}H_{22}N_4O$ requires [M+H]$^+$ 337.17714]; unable to detect by LRMS; HPLC retention time 5.8 min, 98.6%.

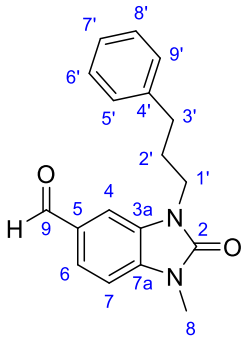
3-Benzyl-1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (127)



1-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**65**) (0.10 g, 0.57 mmol, 1.0 eq.) was dissolved in *N*,*N*-dimethylformamide (6.0 mL) and benzyl bromide (81 μL, 0.68 mmol, 1.2 eq.) and K_2CO_3 (0.12 g, 0.85 mmol, 1.5 eq.) were added. The reaction mixture was heated to 70 °C and stirred for 3.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (80 mL) and

extracted with EtOAc (2 × 80 mL). The organic fractions were combined and washed with an aqueous solution of LiCl (0.5 M, 3 × 100 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the title compound as a colourless solid (56 mg, 0.58 mmol, 99%): R_f 0.45 (3:2 EtOAc/petroleum ether); m.p. 158–160 °C (from EtOAc); $\tilde{\nu}_{max}$/cm^{-1} 1714 (C=O, s), 1681 (C=O, s), 1617 (w), 1508 (w), 1478 (w), 1443 (w), 1399 (w), 1349 (w), 1247 (w), 1195 (w), 1102 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.80 (1H, s, C^9-H), 7.55 (1H, dd, *J* 8.1, 1.5, C^6-H), 7.37 (1H, d, *J* 1.5, C^4-H), 7.31–7.16 (5H, m, C$^{3'}$-H, C$^{4'}$-H, C$^{5'}$-H, C$^{6'}$-H, C$^{7'}$-H), 7.01 (1H, d, *J* 8.1, C^7-H), 5.03 (2H, s, C$^{1'}$-H_2), 3.43 (3H, s, C^8-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.3 (C^9), 154.9 (C^2), 135.8 (C$^{2'}$), 135.5 (C^{7a}), 130.9 (C^5), 129.9 (C^{3a}), 129.0 (C$^{4'}$, C$^{6'}$), 128.1 (C$^{5'}$), 127.7 (C$^{3'}$, C$^{7'}$), 126.8 (C^6), 107.4, (C^4), 107.3 (C^7), 45.2 (C$^{1'}$), 27.7 (C^8); HRMS *m/z* (APCI) [Found: 267.11240, $C_{16}H_{15}N_2O_2$ requires $[M+H]^+$ 267.11280]; unable to detect by LRMS; HPLC retention time 8.6 min, 98.5%.

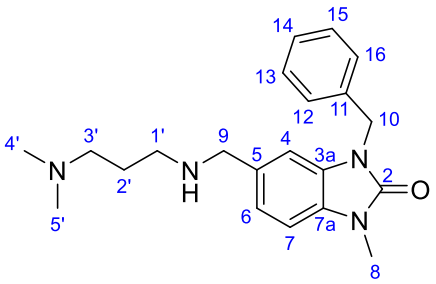
1-Methyl-2-oxo-3-(3'-phenylpropyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (128)



1-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**65**) (50 mg, 0.28 mmol, 1.0 eq.) was dissolved in *N*,*N*-dimethylformamide (3.5 mL) and 1-bromo-3-phenylpropane (52 µL, 0.34 mmol, 1.2 eq.) and K_2CO_3 (59 mg, 0.43 mmol, 1.5 eq.) were added. The reaction mixture was heated to 70 °C and stirred for 4.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was cooled to rt, quenched with H_2O (50 mL) and extracted with EtOAc (2 × 50 mL). The organic fractions were combined and washed with an aqueous solution of LiCl (0.5 M, 3 × 100 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column

chromatography (elution with 0–60% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (79 mg, 0.27 mmol, 95%): R_f 0.48 (3:2 EtOAc/petroleum ether); $\bar{\nu}_{max}$/cm^{-1} 2938 (br), 1711 (C=O, s), 1679 (C=O, s), 1615 (m), 1603 (m), 1508 (w), 1477 (w), 1453 (w), 1433 (w), 1398 (w), 1376 (w), 1349 (w), 1248 (w), 1187 (m), 1136 (w), 1104 (w), 1030 (w), 916 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.92 (1H, s, C^9-H), 7.63 (1H, dd, *J* 8.0, 1.5, C^6-H), 7.45 (1H, d, *J* 1.5, C^4-H), 7.30–7.22 (2H, m, C$^{5'/6'/8'/9'}$-H$_2$), 7.21–7.14 (3H, m, C$^{5'/6'/8'/9'}$-H$_2$, C$^{7'}$-H), 7.06 (1H, d, *J* 8.0, C^7-H), 3.97 (2H, t, *J* 7.5, C$^{1'}$-H$_2$), 3.45 (3H, s, C^8-H$_3$), 2.72 (2H, t, *J* 7.5, C$^{3'}$-H$_2$) 2.12 (2H, tt, *J* 7.5, 7.5, C$^{2'}$-H$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.4 (C^9), 154.7 (C^2), 140.8 (C$^{4'}$), 135.5 (C^{7a}), 130.8 (C^5), 130.1 (C^{3a}), 128.6 (C$^{6'}$, C$^{8'}$), 128.4 (C$^{5'}$, C$^{9'}$), 126.7 (C^6), 126.2 (C$^{7'}$), 107.2 (C^7), 106.8 (C^4), 41.2 (C$^{1'}$), 33.1 (C$^{3'}$), 29.7 (C$^{2'}$), 27.6 (C^8); HRMS *m/z* (APCI) [Found: 295.14381, $C_{18}H_{19}N_2O_2$ requires $[M+H]^+$ 295.14410]; unable to detect by LRMS; HPLC retention time 9.5 min, 99.0%.

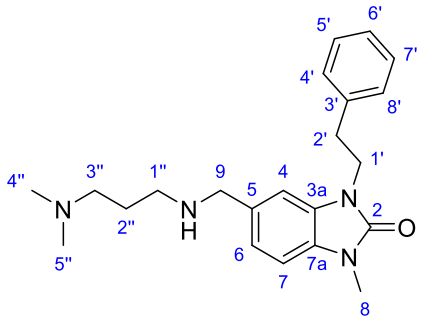
3-Benzyl-5-({[3'-(dimethylamino)prop-1'-yl]amino}methyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (121)



3-Benzyl-1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**127**) (50 mg, 0.19 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (4.0 mL) and 3 Å molecular sieves were added. *N'*,*N'*-Dimethylpropane-1,3-diamine (47 μL, 0.38 mmol, 2.0 eq.) and acetic acid (22 μL, 0.38 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was stirred at 0 °C for 3.5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated almost complete imine formation. $NaBH(OAc)_3$ (0.20 g, 0.94 mmol, 5.0 eq.) was added at 0 °C and the reaction mixture warmed to rt and stirred for 19 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (25 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2

(2 × 25 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless oil (31 mg, 0.087 mmol, 46%): R_f 0.03 (EtOH + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 2980 (w), 1700 (C=O, s), 1505 (w), 1443 (w), 1403 (w), 1254 (w), 1155 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.15 (5H, m, C^{12}-H, C^{13}-H, C^{14}-H, C^{15}-H, C^{16}-H), 6.97 (1H, dd, *J* 8.0, 1.5, C^6-H), 6.86 (1H, d, *J* 8.0, C^7-H), 6.82 (1H, d, *J* 1.5, C^4-H), 5.02 (2H, s, C^{10}-H$_2$), 3.69 (2H, s, C^9-H$_2$), 3.39 (3H, s, C^8-H$_3$), 2.56 (2H, t, *J* 7.1, C$^{1'}$-H$_2$), 2.23 (2H, t, *J* 7.1, C$^{3'}$-H$_2$), 2.14 (6H, s, C$^{4'}$-H$_3$, C$^{5'}$-H$_3$), 1.59 (2H, tt, *J* 7.1, 7.1, C$^{2'}$-H$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 136.5 (C^{11}), 134.0 (C^5), 129.5 (C^{7a}), 129.3 (C^{3a}), 128.8 (C^{13}, C^{15}), 127.7 (C^{14}), 127.5 (C^{12}, C^{16}), 121.4 (C^6), 108.1 (C^4), 107.3 (C^7), 58.1 (C$^{3'}$), 54.2 (C^9), 47.9 (C$^{1'}$), 45.6 (C$^{4'}$, C$^{5'}$), 44.9 (C^{10}), 28.0 (C$^{2'}$), 27.4 (C^8); HRMS *m/z* (ESI$^+$) [Found: 353.23375, $C_{21}H_{28}N_4O$ requires $[M+H]^+$ 353.23359]; LRMS *m/z* (ESI$^+$) 251.2 ($[M–C_5H_{12}N_2]^+$, 100%); HPLC retention time 5.7 min, 99.6%.

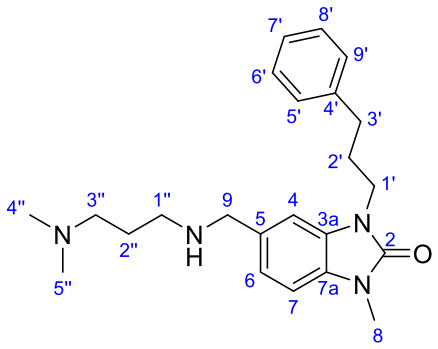
5-({[3''-(Dimethylamino)prop-1''-yl]amino}methyl)-1-methyl-3-(2'-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (122)



1-Methyl-2-oxo-3-(2-phenylethyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**66**) (50 mg, 0.18 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (4.0 mL) and 3 Å molecular sieves were added. *N'*,*N'*-Dimethylpropane-1,3-diamine (45 µL, 0.36 mmol, 2.0 eq.) and acetic acid (21 µL, 0.36 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was stirred at 0 °C for 6 h. After this time ^{1}H NMR analysis of the reaction mixture indicated almost complete imine formation. $NaBH(OAc)_3$ (0.19 g, 0.89 mmol, 5.0 eq.) was added at 0 °C and the reaction mixture warmed to rt and stirred for 17 h. The reaction was quenched with a saturated aqueous solution

of $NaHCO_3$ (25 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (2 × 25 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless oil (38 mg, 0.10 mmol, 59%): R_f 0.03 (EtOH + 1% Et_3N); $\tilde{\nu}_{max}$/cm^{-1} 2939 (br), 2817 (br), 1701 (C=O, s), 1505 (w), 1455 (w), 1404 (w), 1259 (w), 1195 (w), 1108 (w), 1039 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31–7.17 (5H, m, C$^{4'}$-H, C$^{5'}$-H, C$^{6'}$-H, C$^{7'}$-H, C$^{8'}$-H), 7.00 (1H, dd, *J* 7.9, 1.5, C^6-H), 6.89 (1H, d, *J* 7.9, C^7-H), 6.78 (1H, d, *J* 1.5, C^4-H), 4.09 (2H, t, *J* 7.6, C$^{1'}$-H$_2$), 3.76 (2H, s, C^9-H$_2$), 3.40 (3H, s, C^8-H$_3$), 3.03 (2H, t, *J* 7.6, C$^{2'}$-H$_2$), 2.66 (2H, t, *J* 7.1, C$^{1''}$-H$_2$), 2.32 (2H, t, *J* 7.1, C$^{3''}$-H$_2$), 2.22 (6H, s, C$^{4''}$-H$_3$, C$^{5''}$-H$_3$), 1.69 (2H, tt, *J* 7.1, 7.1, C$^{2''}$-H$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.6 (C^2), 138.6 (C$^{3'}$), 133.9 (C^5), 129.6 (C^{3a}), 129.1 (C^{7a}), 129.0 (C$^{5'}$, C$^{7'}$), 128.7 (C$^{4'}$, C$^{8'}$), 126.7 (C$^{6'}$), 121.1 (C^6), 107.6 (C^4), 107.2 (C^7), 58.2 (C$^{3''}$), 54.4 (C^9), 48.0 (C$^{1''}$), 45.7 (C$^{4''}$, C$^{5''}$), 42.9 (C$^{1'}$), 35.1 (C$^{2'}$), 28.2 (C$^{2''}$), 27.3 (C^8); HRMS *m/z* (ESI$^+$) [Found: 367.24964, $C_{22}H_{30}N_4O$ requires $[M+H]^+$ 367.24924]; LRMS *m/z* (ESI$^+$) 367.2 ($[M+H]^+$, 100%); HPLC retention time 5.9 min, 99.5%.

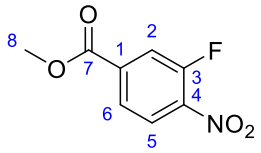
5-({[3''-(Dimethylamino)prop-1''-yl]amino}methyl)-1-methyl-3-(3'-phenylpropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (123)



1-Methyl-2-oxo-3-(3-phenylpropyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**128**) (50 mg, 0.17 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (4.0 mL) and 3 Å molecular sieves were added. *N',N'*-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 at 0 °C. The reaction mixture was stirred at 0 °C for 6 h. After this time ^{1}H NMR analysis of the reaction mixture indicated almost complete

imine formation. $NaBH(OAc)_3$ (0.19 g, 0.89 mmol, 5.0 eq.) was added at 0 °C and the reaction mixture warmed to rt and stirred for 17 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (25 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (2 × 25 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless oil (33 mg, 0.086 mmol, 51%): R_f 0.03 (EtOH + 1% Et_3N); $\tilde{\nu}_{max}$/cm^{-1} 2939 (br), 1700 (C=O, s), 1505 (w), 1453 (w), 1404 (w), 1259 (w), 1109 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.21 (5H, m, C$^{5'}$-H, C$^{6'}$-H, C$^{7'}$-H, C$^{8'}$-H, C$^{9'}$-H), 7.09 (1H, dd, *J* 8.0, 1.4, C^6-H), 6.98–6.93 (2H, m, C^4-H, C^7-H), 3.98 (2H, t, *J* 7.0, C$^{1'}$-H_2), 3.86 (2H, s, C^9-H_2), 3.46 (3H, s, C^8-H_3), 2.80–2.70 (4H, m, C$^{1''}$-H_2, C$^{3'}$-H_2), 2.38 (2H, t, *J* 7.2, C$^{3''}$-H_2), 2.27 (6H, s, C$^{4''}$-H_3, C$^{5''}$-H_3), 2.15 (2H, tt, *J* 9.4, 7.0, C$^{2'}$-H_2), 1.75 (2H, tt, *J* 7.2, 7.2, C$^{2''}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.7 (C^2), 141.3 (C$^{4'}$), 133.9 (C^5), 129.6 (C^{3a}), 129.3 (C^{7a}), 128.51 (C$^{5'/6'/8'/9'}$), 128.48 (C$^{5'/6'/8'/9'}$), 126.1 (C$^{7'}$), 121.1 (C^6), 107.6 (C^4), 107.2 (C^7), 58.2 (C$^{3''}$), 54.4 (C^9), 48.0 (C$^{1''}$), 45.7 (C$^{4''}$, C$^{5''}$), 40.9 (C$^{1'}$), 33.1 (C$^{3'}$), 30.0 (C$^{2'}$), 28.1 (C$^{2''}$), 27.3 (C^8); HRMS *m/z* (ESI$^+$) [Found: 381.26564, $C_{23}H_{32}N_4O$ requires $[M+H]^+$ 381.26489]; LRMS *m/z* (ESI$^+$) 279.2 ($[M–C_5H_{12}N_2]^+$, 100%); HPLC retention time 6.3 min, >99.9%.

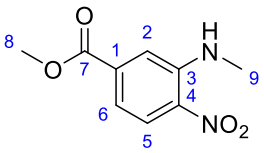
Methyl 3-fluoro-4-nitrobenzoate (146)[24,25]



3-Fluoro-4-nitrobenzoic acid (8.00 g, 43.2 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (80.0 mL) and K_2CO_3 (29.9 g, 216 mmol, 5.0 eq.) was added. The reaction mixture was cooled to 0 °C and methyl iodide (5.38 mL, 86.4 mmol, 2.0 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 2 h. After this time TLC analysis indicated complete consumption of starting material (4:1 EtOAc/petroleum ether). The reaction was cooled to 0 °C, quenched with H_2O (300 mL), warmed to rt, and extracted with EtOAc (300 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M,

3 × 200 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a pale green solid (7.23 g, 36.3 mmol, 84%): R_f 0.34 (3:7 EtOAc/petroleum ether); m.p. 44–46 °C (from CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (1H, dd, *J* 8.8, 7.1, C^6-H), 7.99–7.92 (2H, m, C^2-H, C^5-H), 3.98 (3H, s, C^8-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.2 (d, $J_{C\text{-}F}$ 2.0, C^7), 155.2 (d, $J_{C\text{-}F}$ 266.0, C^3), 140.2 (d, $J_{C\text{-}F}$ 7.9, C^4), 136.7 (d, $J_{C\text{-}F}$ 7.6, C^1), 126.3 (d, $J_{C\text{-}F}$ 2.7, C^6), 125.7 (d, $J_{C\text{-}F}$ 4.5, C^5), 119.9 (d, $J_{C\text{-}F}$ 22.5, C^2), 53.3 (C^8); ^{19}F NMR (376 MHz, $CDCl_3$) δ −116.5; HRMS *m/z* (APCI) [Found: 199.02833, $C_8H_6FNO_4$ requires $[M+H]^+$ 199.02863]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[24,25]

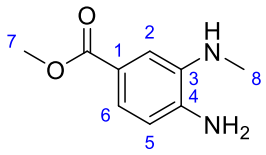
Methyl 3-(methylamino)-4-nitrobenzoate (147)[25,26]



Methyl 4-(methylamino)-3-nitrobenzoate (**146**) (7.23 g, 36.3 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (40.0 mL). Methylamine hydrochloride (4.90 g, 72.6 mmol, 2.0 eq.) and *N*,*N*-diisopropylethylamine (19.0 mL, 109 mmol, 3.0 eq.) were added. The reaction mixture was stirred at rt for 3.5 h. After this time TLC analysis indicated complete consumption of starting material (4:1 EtOAc/petroleum ether). The reaction was quenched with H_2O (200 mL) and extracted with EtOAc (200 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 3 × 300 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. ^{1}H NMR analysis of the reaction mixture indicated the presence of starting material, therefore the solid was redissolved in anhydrous *N*,*N*-dimethylformamide (40.0 mL). Methylamine hydrochloride (4.90 g, 72.6 mmol, 2.0 eq.) and *N*,*N*-diisopropylethylamine (19.0 mL, 109 mmol, 3.0 eq.) were added. The reaction mixture was stirred at rt for 6 h. The reaction was quenched with H_2O (200 mL) and extracted with EtOAc (200 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 3 × 300 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a bright

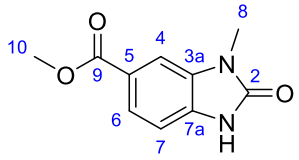
orange solid (7.24 g, 34.4 mmol, 95%): R_f 0.31 (3:7 EtOAc/petroleum ether); m.p. 122–124 °C (from CH_2Cl_2) [lit.[230] 129–130 °C (from EtOAc/petroleum ether)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (1H, d, *J* 8.9, C^5-H), 7.99 (1H, br s, N-H), 7.55 (1H, d, *J* 1.8, C^2-H), 7.24 (1H, dd, *J* 8.9, 1.8, C^6-H), 3.95 (3H, s, C^8-H_3), 3.09 (3H, d, *J* 5.1, C^9-H_3); HRMS *m/z* (APCI) [Found: 211.07106, $C_9H_{11}N_2O_4$ requires $[M+H]^+$ 211.07133]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[25,26]

Methyl 4-amino-3-(methylamino)benzoate (148)[231]



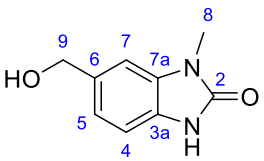
Methyl 3-(methylamino)-4-nitrobenzoate (**147**) (7.24 g, 34.5 mmol, 1.0 eq.) was suspended in EtOAc (114 mL) and EtOH (56.0 mL). The reaction mixture was purged with N_2. Pd on carbon (10% *w/w*, 1.83 g, 1.72 mmol, 0.05 eq.) was added and the reaction mixture purged with H_2. The reaction mixture was stirred at rt under an atmosphere of H_2 for 24 h. After this time TLC analysis indicated complete consumption of starting material (1:4 EtOAc/petroleum ether). The reaction mixture was purged with N_2, filtered through a pad of Celite®, and the filter pad washed with EtOH. The filtrate was concentrated *in vacuo* to yield the title compound as a pale yellow solid (6.22 g, 34.5 mmol, 100%): R_f 0.33 (4:1 EtOAc/petroleum ether); m.p. 76–78 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3446 (N-H, s), 3351 (N-H, s), 1671 (C=O, s), 1618 (m), 1579 (m), 1520 (m), 1478 (w), 1432 (m), 1369 (w), 1305 (s), 1268 (s), 1231 (s), 1191 (w), 1159 (w), 1139 (w), 1107 (w), 976 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (1H, dd, *J* 8.1, 1.9, C^6-H), 7.33 (1H, d, *J* 1.9, C^2-H), 6.67 (1H, d, *J* 8.1, C^5-H), 3.86 (3H, s, C^7-H_3), 2.89 (3H, s, C^8-H_3); HRMS *m/z* (APCI) [Found: 181.09680, $C_9H_{13}N_2O_2$ requires $[M+H]^+$ 181.09715]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[231]

Methyl 3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (149)



Methyl 4-amino-3-(methylamino)benzoate (**148**) (6.74 g, 37.4 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (50.0 mL) and 1,1'-carbonyldiimidazole (13.4 g, 82.3 mmol, 2.2 eq.) was added. The reaction mixture was stirred at rt for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:4 EtOAc/petroleum ether). The reaction was quenched with H_2O (100 mL). The precipitate was collected *via* filtration and washed with H_2O. The solid was crystallised from H_2O:EtOH (1:1) and lyophilised to yield the title compound as a colourless solid (4.80 g, 23.3 mmol, 62%): R_f 0.44 (1:9 MeOH/CH_2Cl_2); m.p. 222–224 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 1709 (C=O, s), 1626 (C=O, s), 1476 (m), 1439 (m), 1387 (w), 1309 (w), 1287 (w), 1257 (m), 1219 (w), 1180 (w), 1136 (w), 1112 (w), 1066 (w), 980 (m), 904 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.96 (1H, br s, N-H), 7.86 (1H, dd, *J* 8.2, 1.6, C^6-H), 7.69 (1H, d, *J* 1.6, C^4-H), 7.13 (1H, d, *J* 8.2, C^7-H), 3.93 (3H, s, C^{10}-H$_3$), 3.48 (3H, s, C^8-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2 (C^9), 156.0 (C^2), 131.9 (C^{7a}), 131.0 (C^{3a}), 124.4 (C^6), 123.7 (C^5), 109.2 (C^4), 109.1 (C^7), 52.3 (C^{10}), 27.2 (C^8); HRMS *m/z* (ESI$^+$) [Found: 207.07645, $C_{10}H_{11}N_2O_3$ requires $[M+H]^+$ 207.07642]; LRMS *m/z* (ESI$^-$) 205.1 ($[M-H]^-$, 100%); HPLC retention time 6.4 min, 98.8%.

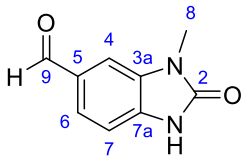
6-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (150)



Methyl 3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (**149**) (1.00 g, 4.85 mmol, 1.0 eq.) was suspended in anhydrous toluene (35.0 mL). The reaction mixture was cooled to 0 °C and diisobutylaluminium hydride (1 M, hexanes) (34.0 mL, 34.0 mmol, 7.0 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 18 h. After this time TLC analysis

indicated complete consumption of starting material (1:9 MeOH/petroleum ether). The reaction mixture was diluted with diethyl ether (60 mL) and cooled to 0 °C. H_2O (1.36 mL) was slowly added followed by an aqueous solution of NaOH (2 M, 2.55 mL) and H_2O (2.21 mL). The reaction mixture was warmed to rt and stirred for 15 min. $MgSO_4$ was added and the reaction mixture stirred for 15 min. The reaction mixture was filtered. The collected solid was stirred in EtOH (100 mL) for 40 min and filtered. The filtrates were combined, and concentrated *in vacuo* to yield the title compound as a colourless solid (860 mg, 4.83 mmol, 99%): R_f 0.29 (1:9 MeOH/CH_2Cl_2); m.p. 191–192 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3194 (O-H, br), 1677 (C=O, s), 1647 (s), 1502 (w), 1471 (w), 1448 (w), 1397 (w), 1310 (w), 1267 (w), 1237 (w), 1194 (w), 1085 (C-O, m), 1017 (m), 978 (w), 955 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.12 (1H, d, *J* 1.4, C^7-H), 7.09–7.00 (2H, m, C^4-H, C^5-H), 4.63 (2H, s, C^9-H$_2$), 3.39 (3H, s, C^8-H$_3$); ^{13}C NMR (126 MHz, CD_3OD) δ 157.2 (C^2), 136.4 (C^6), 132.4 (C^{7a}), 128.8 (C^{3a}), 122.0 (C^5), 110.1 (C^7), 108.1 (C^4), 65.5 (C^9), 27.0 (C^8); HRMS *m/z* (ESI$^+$) [Found: 179.08158, $C_9H_{11}N_2O_2$ requires $[M+H]^+$ 179.08150]; LRMS *m/z* (ESI$^-$) 177.1 ($[M–H]^-$, 100%); HPLC retention time 4.7 min, 94.8%.

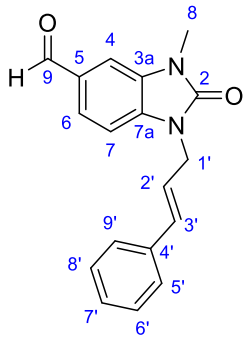
3-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (151)



6-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (**150**) (1.00 g, 5.61 mmol, 1.0 eq.) was suspended in CH_2Cl_2 (135 mL) and MnO_2 (4.88 g, 56.1 mmol, 10 eq.) was added. The reaction mixture was stirred at rt for 5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was filtered through a pad of Celite® and washed with CH_2Cl_2. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–7% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless solid (466 mg, 2.65 mmol, 47%): R_f 0.45 (1:9 MeOH/CH_2Cl_2); m.p. 213–214 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3008 (br), 1712 (C=O, s), 1627 (m), 1504 (w), 1477 (m), 1438 (m), 1387 (w), 1309 (w), 1288 (m), 1259 (m), 1220 (m), 1183 (m),

1135 (w), 1113 (w), 1067 (m), 981 (m), 905 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 9.91 (1H, s, C^{9}-H), 7.69 (1H, dd, *J* 8.0, 1.5, C^{6}-H), 7.64 (1H, d, *J* 1.5, C^{4}-H), 7.21 (1H, d, *J* 8.0, C^{7}-H), 3.44 (3H, s, C^{8}-H_3); ^{13}C NMR (126 MHz, CD_3OD) δ 193.3 (C^{9}), 157.2 (C^{2}), 135.3 (C^{7a}), 133.0 (C^{3a}), 132.3 (C^{5}), 127.3 (C^{6}), 110.2 (C^{7}), 108.8 (C^{4}), 27.2 (C^{8}); HRMS *m/z* (APCI) [Found: 177.06562, $C_9H_9N_2O_2$ requires $[M+H]^+$ 177.06585]; unable to detect by LRMS; HPLC retention time 5.6 min, 95.2%.

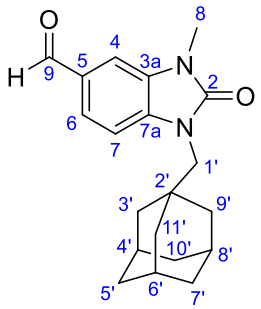
(*E*)-3-Methyl-2-oxo-1-[3'-phenylprop-2'-en-1'-yl]-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (152)



3-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**151**) (0.10 g, 0.57 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (6.0 mL). 3-Bromo-1-phenyl-1-propene (96 μL, 0.68 mmol, 1.2 eq.) and K_2CO_3 (0.12 g, 0.85 mmol, 1.5 eq.) were added. The reaction mixture was heated to 80 °C and stirred for 5 h. After this time TLC analysis indicated complete consumption of starting material (1:49 MeOH/CH_2Cl_2). The reaction was cooled to rt, quenched with H_2O (30 mL) and extracted with EtOAc (30 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 3 × 30 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 20–60% EtOAc/petroleum ether gradient). The product was dissolved in acetonitrile (20 mL) and washed with hexane (3 × 20 mL). The acetonitrile fraction was concentrated *in vacuo* to yield the title compound as a colourless solid (19 mg, 0.064 mmol, 11%): R_f 0.36 (4:1 EtOAc/petroleum ether); m.p. 114–116 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 2360 (s), 2341 (s), 1716 (C=O, s), 1686 (C=O, s), 1618 (w), 1506 (w), 1480

(w), 1188 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.96 (1H, s, C^{9}-H), 7.63 (1H, dd, *J* 8.0, 1.5, C^{6}-H), 7.58 (1H, d, *J* 1.5, C^{4}-H), 7.41–7.24 (5H, m, $C^{5'}$-H, $C^{6'}$-H, $C^{7'}$-H, $C^{8'}$-H, $C^{9'}$-H), 7.17 (1H, d, *J* 8.0, C^{7}-H), 6.66 (1H, dt, *J* 15.9, 1.6, $C^{3'}$-H), 6.27 (1H, dt, *J* 15.9, 6.1, $C^{2'}$-H), 4.75 (2H, dd, *J* 6.1, 1.6, $C^{1'}$-H_2), 3.53 (3H, s, C^{8}-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.4 (C^{9}), 154.5 (C^{2}), 136.0 ($C^{4'}$), 134.7 (C^{7a}), 133.7 ($C^{3'}$), 130.90 (C^{5}), 130.87 (C^{3a}), 128.8 ($C^{6'}$, $C^{8'}$), 128.3 ($C^{7'}$), 127.0 (C^{6}), 126.6 ($C^{5'}$, $C^{9'}$), 122.7 ($C^{2'}$), 107.9 (C^{7}), 106.6 (C^{4}), 43.6 ($C^{1'}$), 27.6 (C^{8}); HRMS *m/z* (ESI^+) [Found: 293.12845, $C_{18}H_{17}N_2O_2$ requires $[M+H]^+$ 293.12845]; LRMS *m/z* (ESI^+) 315.0 ($[M+Na]^+$, 100%); HPLC retention time 9.4 min, >99.9%.

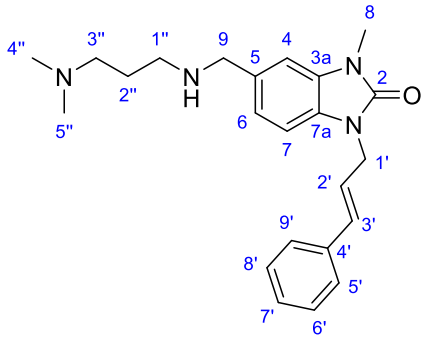
1-[(Adamantan-1'-yl)methyl]-3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (153)



3-Methyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**151**) (0.10 g, 0.57 mmol, 1.0 eq.) and 1-(bromomethyl)adamantane (0.16 g, 0.68 mmol, 1.0 eq.) were dissolved in *N*,*N*-dimethylformamide (3.0 mL). K_2CO_3 (0.16 g, 1.1 mmol, 2.0 eq.) was added and the reaction mixture heated to 70 °C and stirred for 90 h. After this time TLC analysis indicated complete consumption of starting material (EtOAc). The reaction was cooled to rt, quenched with H_2O (10 mL) and extracted with EtOAc (20 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 3 × 15 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 50–80% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (60 mg, 0.19 mmol, 33%): R_f 0.60 (3:2 EtOAc/petroleum ether); m.p. 208–210 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 1708 (C=O, s), 1683 (C=O, s), 1617 (m), 1508 (w), 1480 (w), 1467 (w), 1451 (w), 1432 (w), 1386 (m), 1352 (w), 1314 (w), 1297 (w), 1266 (w), 1189 (m), 1148 (w), 1087 (w),

1023 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.96 (1H, s, C^{9}-H), 7.63 (1H, dd, *J* 8.1, 1.5, C^{6}-H), 7.55 (1H, d, *J* 1.5, C^{4}-H), 7.16 (1H, d, *J* 8.1, C^{7}-H), 3.60 (2H, s, $C^{1'}$-H_2), 3.50 (3H, s, C^{8}-H_3), 2.04–1.98 (3H, m, $C^{4'}$-H, $C^{6'}$-H, $C^{8'}$-H), 1.75–1.59 (12H, m, $C^{3'}$-H_2, $C^{5'}$-H_2, $C^{7'}$-H_2, $C^{9'}$-H_2, $C^{10'}$-H_2, $C^{11'}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.3 (C^{9}), 155.7 (C^{2}), 136.6 (C^{7a}), 130.8 (C^{3a}), 130.5 (C^{5}), 126.6 (C^{6}), 108.5 (C^{7}), 106.4 (C^{4}), 54.0 ($C^{1'}$), 41.1 ($C^{3'}$, $C^{9'}$, $C^{11'}$), 36.7 ($C^{10'}$, $C^{5'}$, $C^{7'}$), 36.5 ($C^{2'}$), 28.3 ($C^{4'}$, $C^{6'}$, $C^{8'}$), 27.6 (C^{8}); HRMS *m/z* (ESI^+) [Found: 325.19095, $C_{20}H_{25}N_2O_2$ requires $[M+H]^+$ 325.19105]; LRMS *m/z* (ESI^+) 325.2 ($[M+H]^+$, 29%); HPLC retention time 11.6 min, >99.9%.

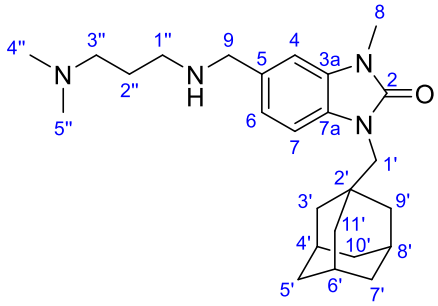
(*E*)-5-({[3''-(Dimethylamino)prop-1''-yl]amino}methyl)-3-methyl-1-[3'-phenylprop-2'-en-1'-yl]-1,3-dihydro-2*H*-benzimidazol-2-one (129)



(*E*)-3-Methyl-2-oxo-1-[3-phenylprop-2-en-1-yl]-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**152**) (26 mg, 0.090 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (4.0 mL) and 3 Å molecular sieves were added. *N*',*N*'-Dimethylpropane-1,3-diamine (27 μL, 0.18 mmol, 2.0 eq.) and acetic acid (10 μL, 0.18 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was warmed to rt and stirred for 5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated complete imine formation. $NaBH(OAc)_3$ (96 mg, 0.45 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 18 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL) and filtered through a pad of Celite®. The filter pad was washed with EtOH and the filtrate volume reduced by concentration *in vacuo*. The reaction mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 20–40% EtOH/$CHCl_3$ + 1% Et_3N gradient). The product was dissolved in

acetonitrile (10 mL) and washed with hexane (3 × 10 mL). The acetonitrile fraction was concentrated *in vacuo* to yield the title compound as a colourless oil (9.2 mg, 0.024 mmol, 36%): R_f 0.06 (EtOH + 2% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 2935 (br), 2815 (br), 1700 (C=O, s), 1628 (w), 1504 (m), 1439 (m), 1397 (m), 1358 (w), 1261 (w), 1175 (w), 1100 (w), 1026 (w), 966 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.22 (4H, m, C$^{5'}$-H, C$^{6'}$-H, C$^{8'}$-H, C$^{9'}$-H), 7.26–7.18 (1H, m, C$^{7'}$-H), 7.04 (1H, d, *J* 1.4, C^4-H), 7.02–6.93 (2H, m, C^6-H, C^7-H), 6.60 (1H, dt, *J* 15.9, 1.6, C$^{3'}$-H), 6.25 (1H, dt, *J* 15.9, 6.0, C$^{2'}$-H), 4.67 (2H, dd, *J* 6.0, 1.6, C$^{1'}$-H_2), 3.85 (2H, s, C^9-H_2), 3.45 (3H, s, C^8-H_3), 2.72 (2H, t, *J* 7.0, C$^{1''}$-H_2), 2.35 (2H, t, *J* 7.0, C$^{3''}$-H_2), 2.23 (6H, s, C$^{4''}$-H_3, C$^{5''}$-H_3), 1.72 (2H, tt, *J* 7.0, 7.0, C$^{2''}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.3 (C^2), 136.3 (C$^{4'}$), 133.2 (C^5), 133.0 (C$^{3'}$), 130.5 (C^{3a}), 128.7 (C$^{6'}$, C$^{8'}$), 128.6 (C^{7a}), 128.0 (C$^{7'}$, 126.6 (C$^{5'}$, C$^{9'}$), 123.7 (C$^{2'}$), 121.5 (C^6), 108.0 (C^7), 107.7 (C^4), 58.3 (C$^{3''}$), 54.1 (C$^{6'}$), 48.1 (C$^{1''}$), 45.6 (C$^{4''}$, C$^{5''}$), 43.4 (C$^{1'}$), 27.5 (C^9), 27.4 (C^8); HRMS *m/z* (APCI) [Found: 379.24851, $C_{23}H_{31}N_4O$ requires $[M+H]^+$ 379.24924]; unable to detect by LRMS; HPLC retention time 6.5 min, 98.8%.

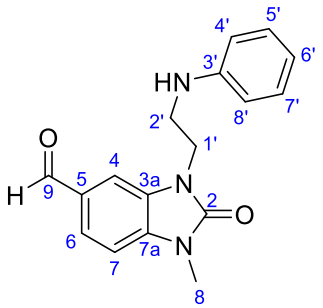
1-[(Adamantan-1'-yl)methyl]-5-({[3''-(dimethylamino)prop-1''-yl]amino}methyl)-3-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (130)



1-[(Adamantan-1-yl)methyl]-3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**153**) (58 mg, 0.18 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (7.0 mL) and 3 Å molecular sieves were added. *N*,*N*-Dimethyl-1-phenylpropane-1,3-dimaine (76 μL, 0.36 mmol, 2.0 eq.) and acetic acid (21 μL, 0.36 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 4.5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated complete imine formation. $NaBH(OAc)_3$ (0.19 g, 0.90 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 16 h. The reaction was quenched with H_2O (10 mL) and filtered through

a pad of Celite®. The filter pad was washed with a saturated aqueous solution of $NaHCO_3$ (10 mL) and CH_2Cl_2 (20 mL). The aqueous fraction of the filtrate was extracted with CH_2Cl_2 (2 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) to yield the title compound as a colourless oil (33 mg, 0.080 mmol, 45%): R_f 0.06 (EtOH + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 2902 (br), 2847 (br), 1704 (C=O, s), 1504 (w), 1438 (w), 1398 (w), 1370 (w), 1331 (w), 1265 (w), 1167 (w), 1107 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02–6.91 (3H, m, C^4-H, C^6-H, C^7-H), 3.82 (2H, s, C^9-H$_2$), 3.50 (2H, s, C$^{1'}$-H$_2$), 3.41 (3H, s, C^8-H$_3$), 2.70 (2H, t, *J* 7.1, C$^{3''}$-H$_2$), 2.37 (2H, t, *J* 7.1, C$^{1''}$-H$_2$), 2.21 (6H, s, C$^{4''}$-H$_3$, C$^{5''}$-H$_3$), 1.99–1.94 (3H, m, C$^{4'}$-H, C$^{6'}$-H, C$^{8'}$-H), 1.74–1.55 (14H, m, C$^{2''}$-H$_2$, C$^{3'}$-H$_2$, C$^{5'}$-H$_2$, C$^{7'}$-H$_2$, C$^{9'}$-H$_2$, C$^{10'}$-H$_2$, C$^{11'}$-H$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.7 (C^2), 133.7 (C^5), 130.4 (C$^{3a/7a}$), 130.3 (C$^{3a/7a}$), 120.9 (C^6), 108.5 (C^4), 107.2 (C^7), 58.2 (C$^{3''}$), 54.4 (C^9), 53.8 (C$^{1'}$), 48.1 (C$^{1''}$), 45.7 (C$^{4''}$, C$^{5''}$), 41.1 (C$^{3'}$, C$^{9'}$, C$^{11'}$), 36.8 (C$^{5'}$, C$^{7'}$, C$^{10'}$), 36.4 (C$^{2'}$), 28.4 (C$^{2''}$), 28.2 (C$^{4'}$, C$^{6'}$, C$^{8'}$), 27.4 (C^8); HRMS *m/z* (ESI$^+$) [Found: 411.31154, $C_{25}H_{39}N_4O$ requires $[M+H]^+$ 411.31184]; LRMS *m/z* (ESI$^+$) 411.8 ($[M+H]^+$, 92%); HPLC retention time 7.2 min, >99.9%.

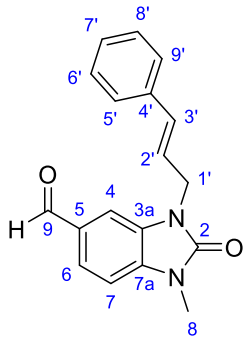
3-(2'-Anilinoethyl)-1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (154)



Sodium hydride (60% dispersion in mineral oil, 13 mg, 0.32 mmol, 1.2 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (0.50 mL). The reaction mixture was cooled to 0 °C and 1-methy-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**65**) (46 mg, 0.26 mmol, 1.0 eq.) was added. The reaction mixture was stirred at 0 °C for 1 h. *N*-(2-Chloroethyl)aniline (49 mg, 0.32 mmol, 1.2 eq.) in anhydrous *N*,*N*-dimethylformamide (0.30 mL) was added dropwise at 0 °C. The reaction mixture was warmed to rt and stirred for 5 h. After this time TLC analysis indicated

partial consumption of starting material (1:1 EtOAc/petroleum ether). The reaction mixture was heated to 70 °C and stirred for 15 h. After this time TLC analysis indicated partial consumption of starting material (1:1 EtOAc/petroleum ether). The reaction was cooled to rt and quenched with H_2O (30 mL). The reaction mixture was extracted with EtOAc (30 mL) and the organic fraction washed with an aqueous solution of LiCl (0.5 M, 3 × 30 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 50–80% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (31 mg, 0.11 mmol, 40%): R_f 0.29 (3:2 EtOAc/petroleum ether); m.p. 120–122 °C (from $CDCl_3$); $\tilde{\nu}_{max}/cm^{-1}$ 3334 (m), 2359 (w), 1718 (C=O, s), 1665 (C=O, s), 1601 (C=O, s), 1509 (m), 1470 (w), 1436 (w), 1398 (w), 1355 (w), 1316 (w), 1256 (w), 1241 (w), 1198 (m), 1168 (w), 1141 (w), 1123 (w), 1074 (w), 1038 (w), 995 (w), 955 (w), 900 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.88 (1H, s, C^9-H), 7.64 (1H, dd, *J* 8.0, 1.4, C^6-H), 7.46 (1H, d, *J* 1.4, C^4-H), 7.14 (2H, dd, *J* 7.4, 7.4, $C^{5'}$-H, $C^{7'}$-H), 7.07 (1H, d, *J* 8.0, C^7-H), 6.69 (1H, tt, *J* 7.4, 1.1, $C^{6'}$-H), 6.58 (2H, dd, *J* 7.4, 1.1, $C^{4'}$-H, $C^{8'}$-H), 4.19 (2H, t, *J* 5.9, $C^{1'}$-H_2), 4.13 (1H, br s, N-H), 3.54 (2H, t, *J* 5.9, $C^{2'}$-H_2), 3.45 (3H, s, C^8-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.3 (C^9), 155.2 (C^2), 147.4 ($C^{3'}$), 135.4 (C^{7a}), 130.9 (C^5), 130.0 (C^{3a}), 129.4 ($C^{5'}$, $C^{7'}$), 126.6 (C^6), 117.9 ($C^{6'}$), 112.8 ($C^{4'}$, $C^{8'}$), 107.5 (C^7), 106.9 (C^4), 42.8 ($C^{2'}$), 41.0 ($C^{1'}$), 27.6 (C^8); HRMS *m/z* (ESI^+) [Found: 296.13904, $C_{17}H_{18}N_3O_2$ requires $[M+H]^+$ 296.13935]; unable to detect by LRMS; HPLC retention time 7.2 min, 98.2%.

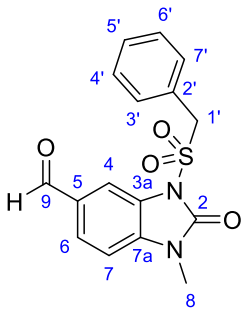
(*E*)-1-Methyl-2-oxo-3-[3'-phenylprop-2'-en-1'-yl]-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (155)



1-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**65**) (100 mg, 0.568 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (6.00 mL). 5-Bromo-1-phenyl-propene (95.8 μL, 0.682 mmol, 1.2 eq.) and K_2CO_3 (118 mg, 0.852 mmol, 1.5 eq.) were added. The reaction mixture was heated to 70 °C and stirred for 4 h. After this time TLC analysis indicated complete consumption of starting material (1:1 EtOAc/petroleum ether). The reaction was cooled to rt, quenched with H_2O (20 mL) and extracted with EtOAc (20 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 3 × 20 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–50% EtOAc/petroleum ether gradient) to yield the title compound as a light brown solid (92.2 mg, 0.315 mmol, 56%): R_f 0.26 (3:2 EtOAc/petroleum ether); m.p. 120–122 °C (from $CDCl_3$); $\bar{\nu}_{max}$/cm^{-1} 1693 (C=O, s), 1679 (C=O, s), 1619 (m), 1609 (m), 1507 (w), 1480 (w), 1442 (m), 1395 (w), 1349 (w), 1301 (w), 1271 (w), 1242 (w), 1197 (m), 1151 (w), 1138 (w), 1108 (w), 1081 (w), 1067 (w), 1017 (w), 1003 (w), 978 (m), 910 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.90 (1H, s, C^9-H), 7.64 (1H, dd, *J* 8.0, 1.5, C^6-H), 7.56 (1H, d, *J* 1.5, C^4-H), 7.36–7.29 (2H, m, C$^{5'}$-H, C$^{9'}$-H), 7.31–7.23 (2H, m, C$^{6'}$-H, C$^{8'}$-H), 7.25–7.17 (1H, m, C$^{7'}$-H), 7.09 (1H, d, *J* 8.0, C^7-H), 6.63 (1H, dd, *J* 15.9, 1.6, C$^{3'}$-H), 6.24 (1H, dt, *J* 15.9, 6.1, C$^{2'}$-H), 4.70 (2H, dd, *J* 6.1, 1.6, C$^{1'}$-H$_2$), 3.48 (3H, s, C^8-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.3 (C^9), 154.4 (C^2), 136.0 (C$^{4'}$), 135.4 (C^{7a}), 133.5 (C$^{3'}$), 130.8 (C^5), 129.8 (C^{3a}), 128.6 (C$^{6'}$, C$^{8'}$), 128.1 (C$^{7'}$), 126.7 (C^6), 126.6 (C$^{5'}$, C$^{9'}$), 122.7 (C$^{2'}$), 107.3 (C$^{4/7}$), 107.2 (C$^{4/7}$), 43.3 (C$^{1'}$), 27.6 (C^8);

HRMS *m/z* (ESI^+) [Found: 293.12782, $C_{18}H_{16}N_2O_2$ requires $[M+H]^+$ 293.12845]; unable to detect by LRMS; HPLC retention time 9.3 min, 99.1%.

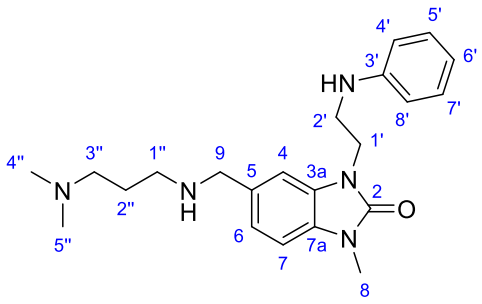
1-Methyl-2-oxo-3-(phenylmethanesulfonyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (156)



1-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**65**) (50 mg, 0.28 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (5.00 mL) and the reaction mixture cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 8.2 mg, 0.34 mmol, 1.2 eq.) was added and the reaction mixture stirred at rt for 1 h. Benzylsulfonyl chloride (65 mg, 0.34 mmol, 1.2 eq.) was added and the reaction mixture stirred at rt for 21 h. After this time TLC analysis indicated partial consumption of starting material (1:1 EtOAc/petroleum ether). The reaction was quenched with H_2O (15 mL) and the reaction volume reduced by concentration *in vacuo*. The reaction mixture was extracted with EtOAc (3 × 30 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 50–70% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (41 mg, 0.12 mmol, 44%): R_f 0.48 (3:2 EtOAc/petroleum ether); m.p. 168–170 °C (from $CDCl_3$); $\bar{\nu}_{max}$/cm^{-1} 2924 (br), 2360 (w), 2341 (w), 1713 (m), 1687 (s), 1603 (m), 1508 (w), 1459 (w), 1400 (w), 1367 (w), 1303 (w), 1260 (w), 1200 (w), 1158 (w), 1033 (w), 970 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.81 (1H, s, C^9-H), 7.87 (1H, d, *J* 1.6, C^4-H), 7.70 (1H, dd, *J* 8.2, 1.6, C^6-H), 7.35–7.28 (2H, m, $C^{3'}$-H, $C^{7'}$-H), 7.25–7.17 (3H, m, $C^{4'}$-H, $C^{5'}$-H, $C^{6'}$-H), 7.02 (1H, d, *J* 8.2, C^7-H), 4.87 (2H, s, $C^{1'}$-H_2), 3.46 (3H, s, C^8-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.7 (C^9), 151.7 (C^2), 134.4 (C^{7a}), 131.7 (C^5), 130.7 ($C^{3'}$, $C^{7'}$), 129.7 ($C^{5'}$), 129.0 ($C^{4'}$, $C^{6'}$), 127.1 (C^{3a}), 126.7 (C^6), 126.4 ($C^{2'}$), 114.3 (C^4), 108.2 (C^7), 59.8 ($C^{1'}$), 27.8 (C^8); HRMS *m/z*

(ESI$^+$) [Found: 331.07452, $C_{16}H_{14}N_2O_4S$ requires $[M+H]^+$ 331.07470]; unable to detect by LRMS; HPLC retention time 8.8 min, 98.4%.

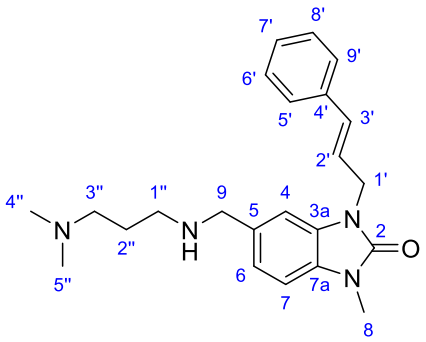
3-(2'-Anilinoethyl)-5-({[3''-(dimethylamino)prop-1''-yl]amino}methyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (141)



3-(2-Anilinoethyl)-1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**154**) (47 mg, 0.16 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (4.0 mL), and 3 Å molecular sieves were added. *N',N'*-Dimethylpropane-1,3-diamine (40 μL, 0.32 mmol, 2.0 eq.) and acetic acid (18 μL, 0.32 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was warmed to rt and stirred for 6.5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated complete imine formation. $NaBH(OAc)_3$ (0.17 mg, 0.79 mmol, 5.0 eq.) was added at 0 °C. The reaction was warmed to rt and stirred for 17.5 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL) and filtered through a pad of Celite®. The filter pad was washed with EtOH. The filtrate volume was reduced by concentration *in vacuo*, and then extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless oil (11 mg, 0.028 mmol, 17%): R_f 0.03 (EtOH + 1% Et_3N); $\tilde{\nu}_{max}$/cm^{-1} 2940 (w), 2821 (w), 2360 (w), 1689 (C=O, s), 1603 (m), 1505 (m), 1464 (w), 1405 (w), 1329 (w), 1260 (w), 1202 (w), 1179 (w), 1138 (w), 1102 (w), 1039 (w), 991 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19–7.11 (2H, m, C$^{5'}$-H, C$^{7'}$-H), 7.02 (1H, dd, *J* 7.9, 1.5, C^6-H), 6.93 (1H, d, *J* 1.5, C^4-H), 6.89 (1H, d, *J* 7.9, C^7-H), 6.69 (1H, tt, *J* 7.3, 1.1, C$^{6'}$-H), 6.63–6.56 (2H, m, C$^{4'}$-H, C$^{8'}$-H), 4.24 (1H, t, *J* 5.9, N-H), 4.14 (2H, t, *J* 5.9, C$^{1'}$-H$_2$), 3.78 (2H, s, C^9-H$_2$), 3.51 (2H, dt, *J* 5.9. 5.9, C$^{2'}$-H$_2$), 3.40 (3H, s, C^8-H$_3$), 2.67 (2H, t,

J 7.1, $C^{1''}$-H_2), 2.32 (2H, t, *J* 7.1, $C^{3''}$-H_2), 2.22 (6H, s, $C^{4''}$-H_3, $C^{5''}$-H_3), 1.69 (2H, tt, *J* 7.1, 7.1, $C^{2''}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.2 (C^2), 147.7 ($C^{3'}$), 133.7 (C^5), 130.0 (C^{3a}), 129.4 ($C^{5'}$, $C^{7'}$), 129.2 (C^{7a}), 121.4 (C^6), 117.6 ($C^{6'}$), 112.7 ($C^{4'}$, $C^{8'}$), 107.5 (C^4), 107.4 (C^7), 58.2 ($C^{3''}$), 54.2 (C^9), 48.0 ($C^{1''}$), 45.6 ($C^{4''}$, $C^{5''}$), 43.1 ($C^{2'}$), 40.6 ($C^{1'}$), 27.9 ($C^{2''}$), 27.3 (C^8); HRMS *m/z* (ESI^+) [Found: 382.25962, $C_{22}H_{32}N_5O$ requires $[M+H]^+$ 382.26349]; LRMS *m/z* (ESI^+) 382.2 ($[M+H]^+$, 100%); HPLC retention time 5.3 min, 98.5%.

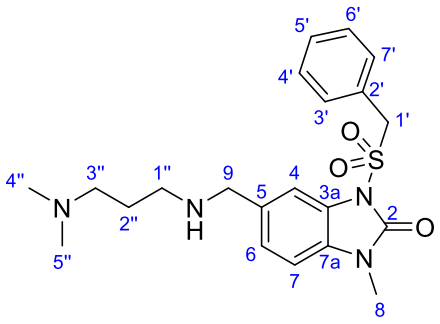
(*E*)-5-({[3''-(Dimethylamino)prop-1''-yl]amino}methyl)-1-methyl-3-[3'-phenylprop-2'-en-1'-yl]-1,3-dihydro-2*H*-benzimidazol-2-one (142)



(*E*)-1-Methyl-2-oxo-3-[3-phenylprop-2-en-1-yl]-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**155**) (69 mg, 0.24 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (2.0 mL) and 3 Å molecular sieves were added. *N',N'*-Dimethylpropane-1,3-diamine (59 μL, 0.47 mmol, 2.0 eq.) and acetic acid (27 μL, 0.47 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was warmed to rt and stirred for 7.5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated complete imine formation. $NaBH(OAc)_3$ (0.25 g, 1.2 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 19 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL) and filtered through a pad of Celite®. The filter pad was washed with EtOH. The filtrate volume was reduced by concentration *in vacuo*, and then extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless oil (31 mg, 0.083 mmol, 35%): R_f 0.03 (EtOH + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 2939 (w), 2815 (w), 1698 (C=O, s),

1627 (w), 1609 (w), 1504 (m), 1464 (w), 1440 (m), 1402 (w), 1359 (w), 1251 (w), 1197 (w), 1154 (w), 1137 (w), 1110 (w), 1028 (w), 965 (w), 924 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.34 (2H, m, $C^{5'}$-H', $C^{9'}$-H), 7.33–7.28 (2H, m, $C^{6'}$-H, $C^{8'}$-H), 7.28–7.19 (1H, m, $C^{7'}$-H), 7.08 (1H, dd, *J* 7.9, 1.5, C^{6}-H), 7.05 (1H, d, *J* 1.5, C^{4}-H), 6.96 (1H, d, *J* 7.9, C^{7}-H), 6.63 (1H, dt, *J* 15.9, 1.6 $C^{3'}$-H), 6.29 (1H, dt, *J* 15.9, 6.0, $C^{2'}$-H), 4.70 (2H, dd, *J* 6.0, 1.6, $C^{1'}$-H_2), 3.83 (2H, s, C^{9}-H_2), 3.47 (3H, s, C^{8}-H_3), 2.68 (2H, t, *J* 7.1, $C^{1''}$-H_2), 2.31 (2H, d, *J* 7.1, $C^{3''}$-H_2), 2.21 (6H, s, $C^{4''}$-H_3, $C^{5''}$-H_3), 1.68 (2H, tt, *J* 7.1, 7.1, $C^{2''}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.5 (C^{2}), 136.4 ($C^{4'}$), 134.0 (C^{5}), 132.8 ($C^{3'}$), 129.5 (C^{3a}), 129.3 (C^{7a}), 128.7 ($C^{6'}$, $C^{8'}$), 127.9 ($C^{7'}$), 126.6 ($C^{5'}$, $C^{9'}$), 123.7 ($C^{2'}$), 121.4 (C^{6}), 108.0 (C^{4}), 107.3 (C^{7}), 58.2 ($C^{3''}$), 54.3 (C^{9}), 48.0 ($C^{1''}$), 45.6 ($C^{4''}$, $C^{5''}$), 43.2 ($C^{1'}$), 28.1 ($C^{2''}$), 27.4 (C^{8}); HRMS *m/z* (APCI) [Found: 379.24924, $C_{23}H_{31}N_4O$ requires $[M+H]^+$ 379.24924]; unable to detect by LRMS; HPLC retention time 6.4 min, >99.9%.

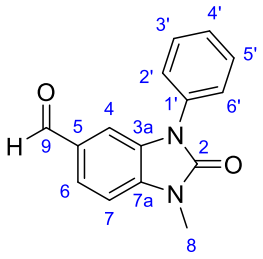
5-({[3''-(Dimethylamino)prop-1''-yl]amino}methyl)-1-methyl-3-(phenylmethanesulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (144)



1-Methyl-2-oxo-3-(phenylmethanesulfonyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**156**) (83 mg, 0.25 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (10 mL) and 3 Å molecular sieves were added. *N'*,*N'*-Dimethylpropane-1,3-diamine (63 µL, 0.50 mmol, 2.0 eq.) and acetic acid (29 µL, 0.50 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was warmed to rt and stirred for 8 h. After this time ^{1}H NMR analysis of the reaction mixture indicated complete imine formation. $NaBH(OAc)_3$ (0.27 g, 1.3 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 19 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL) and filtered through a pad of Celite®. The filter pad was washed with EtOH and the filtrate volume reduced by concentration *in vacuo*. The reaction mixture was extracted

with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless oil (70 mg, 0.17 mmol, 66%): R_f 0.03 (EtOH + 1% Et_3N); $\tilde{\nu}_{max}$/cm^{-1} 2980 (br), 2341 (br), 1732 (C=O, s), 1559 (w), 1499 (w), 1457 (w), 1368 (m), 1277 (w), 1162 (m), 972 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (1H, d, *J* 1.6, C^4-H), 7.34–7.26 (2H, m, C$^{4'}$-H, C$^{6'}$-H), 7.26–7.17 (3H, m, C$^{3'}$-H, C$^{5'}$-H, C$^{7'}$-H), 7.09 (1H, dd, *J* 8.0, 1.6, C^6-H), 6.83 (1H, d, *J* 8.0, C^7-H), 4.83 (2H, s, C$^{1'}$-H$_2$), 3.66 (2H, s, C^9-H$_2$), 3.37 (3H, s, C^8-H$_3$), 2.55 (2H, t, *J* 7.1, C$^{1''}$-H$_2$), 2.29 (2H, t, *J* 7.1, C$^{3''}$-H$_2$), 2.20 (6H, s, C$^{4''}$-H$_3$, C$^{5''}$-H$_3$), 1.63 (2H, tt, *J* 7.1, 7.1, C$^{2''}$-H$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.6 (C^2), 135.4 (C^5), 130.8 (C$^{4'}$, C$^{6'}$), 129.3 (C$^{5'}$), 128.8 (C$^{3'}$, C$^{7'}$), 128.6 (C^{7a}), 126.8 (C$^{3a/2'}$), 126.7 (C$^{3a/2'}$), 124.1 (C^6), 112.6 (C^4), 107.7 (C^7), 59.5 (C$^{1'}$), 58.1 (C$^{3''}$), 53.9 (C^9), 47.7 (C$^{1''}$), 45.6 (C$^{4''}$, C$^{5''}$), 28.0 (C$^{2''}$), 27.4 (C^8); HRMS *m/z* (ESI$^-$) [Found: 415.18084, $C_{21}H_{27}N_4O_3S$ requires [M–H]$^-$ 415.18093]; LRMS *m/z* (ESI$^-$) 330.0 ([M–$C_5H_{12}N$]$^-$, 100%); HPLC retention time 6.0 min, 96.2%.

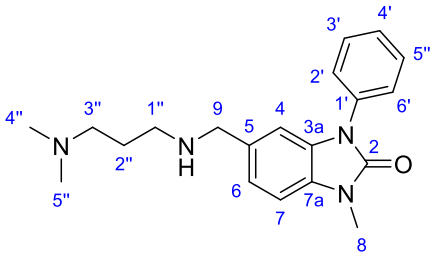
1-Methyl-2-oxo-3-phenyl-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (157)



1-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**65**) (0.10 g, 0.57 mmol, 1.0 eq.), phenylboronic acid (0.14 g, 1.1 mmol, 2.0 eq.) and copper(II) acetate monohydrate (0.13 g, 0.63 mmol, 1.1 eq.) were suspended in CH_2Cl_2 (0.15 mL). Et_3N (0.17 mL, 1.3 mmol, 2.2 eq.) was added and the reaction mixture stirred at rt for 18 h. After this time TLC analysis indicated partial consumption of starting material (EtOAc). The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL) and the resulting precipitate removed *via* filtration. The filtrate was extracted with EtOAc (15 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column

chromatography (elution with 30–50% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (80 mg, 0.32 mmol, 56%): R_f 0.49 (3:2 EtOAc/petroleum ether); m.p. 170–172 °C (from EtOAc); $\bar{\nu}_{max}$/cm^{-1} 3058 (br), 1725 (C=O, s), 1669 (C=O, s), 1613 (w), 1595 (m), 1510 (w), 1497 (w), 1471 (w), 1453 (m), 1418 (w), 1398 (w), 1353 (w), 1281 (w), 1261 (w), 1211 (m), 1188 (w), 1145 (w), 1088 (w), 1034 (w), 1001 (w), 953 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.94 (1H, s, C^9-H), 7.74 (1H, dd, *J* 8.0, 1.5, C^6-H), 7.63 (1H, d, *J* 1.5, C^4-H), 7.62–7.53 (4H, m, C$^{2'}$-H, C$^{3'}$-H, C$^{5'}$-H, C$^{6'}$-H), 7.50–7.45 (1H, m, C$^{4'}$-H), 7.19 (1H, d, *J* 8.0, C^7-H), 3.58 (3H, s, C^8-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.3 (C^9), 153.8 (C^2), 135.5 (C^{7a}), 134.1 (C$^{1'}$), 131.1 (C^5), 130.3 (C^{3a}), 129.9 (C$^{3'}$, C$^{5'}$), 128.4 (C$^{4'}$), 127.1 (C^6), 126.2 (C$^{2'}$, C$^{6'}$), 108.3 (C^4), 107.6 (C^7), 27.8 (C^8); HRMS *m/z* (ESI$^+$) [Found: 253.09721, $C_{15}H_{13}N_2O_2$ requires $[M+H]^+$ 253.09715]; LRMS *m/z* (ESI$^+$) 237.2 ($[M–O]^+$, 69%); HPLC retention time 8.4 min, 99.8%.

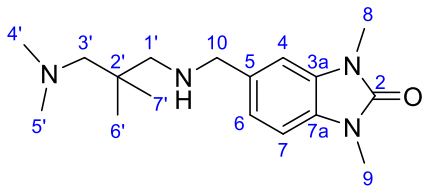
5-({[3''-(Dimethylamino)prop-1''-yl]amino}methyl)-1-methyl-3-phenyl-1,3-dihydro-2*H*-benzimidazol-2-one (143)



1-Methyl-2-oxo-3-phenyl-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**157**) (47 mg, 0.20 mmol, 1.0 eq.) was suspended in anhydrous 1,2-dichloroethane (7.5 mL) and 3 Å molecular sieves were added. *N',N'*-Dimethylpropane-1,3-diamine (50 μL, 0.40 mmol, 2.0 eq.) and acetic acid (23 μL, 0.40 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was warmed to rt and stirred for 6 h. After this time ^{1}H NMR analysis of the reaction mixture indicated complete imine formation. $NaBH(OAc)_3$ (0.21 g, 0.99 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 17 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL) and filtered through a pad of Celite®. The filter pad was washed with EtOH. The filtrate volume was reduced by concentration *in vacuo*, and then extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was

purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless oil (43 mg, 0.13 mmol, 64%): R_f 0.13 (EtOH + 1% Et_3N); $\tilde{\nu}_{max}$/cm^{-1} 2940 (br), 2814 (br), 2765 (br), 1704 (C=O, s), 1597 (w), 1507 (m), 1449 (w), 1429 (w), 1394 (m), 1268 (w), 1212 (w), 1146 (w), 1130 (w), 1086 (w), 1041 (w), 1028 (w), 1007 (w), 954 (w), 920 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56–7.48 (4H, m, C$^{2'}$-H, C$^{3'}$-H, C$^{5'}$-H, C$^{6'}$-H), 7.43–7.35 (1H, m, C$^{4'}$-H), 7.10 (1H, dd, *J* 8.0, 1.5, C^6-H), 7.05 (1H, d, *J* 1.5, C^4-H), 6.97 (1H, d, *J* 8.0, C^7-H), 3.77 (2H, s, C^9-H$_2$), 3.48 (3H, s, C^8-H$_3$), 2.64 (2H, t, *J* 7.0, C$^{1''}$-H$_2$), 2.29 (2H, t, *J* 7.0, C$^{3''}$-H$_2$), 2.18 (6H, s, C$^{4''}$-H$_3$, C$^{5''}$-H$_3$), 1.65 (2H, tt, *J* 7.0, 7.0, C$^{2''}$-H$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.8 (C^2), 134.9 (C$^{1'}$), 134.1 (C^5), 129.7 (C^{3a}), 129.6 (C$^{3'}$, C$^{5'}$), 129.3 (C^{7a}), 127.7 (C$^{4'}$), 126.2 (C$^{2'}$, C$^{6'}$), 122.1 (C^6), 108.6 (C^4), 107.5 (C^7), 58.2 (C$^{3''}$), 54.2 (C^9), 47.9 (C$^{1''}$), 45.5 (C$^{4''}$, C$^{5'}$), 27.8 (C$^{2''}$), 27.4 (C^8); HRMS *m/z* (ESI$^+$) [Found: 339.21731, $C_{20}H_{27}N_4O$ requires [M+H]$^+$ 339.21903]; LRMS *m/z* (ESI$^+$) 237.0 ([M–$C_5H_{13}N_2$]$^+$, 81%); HPLC retention time 5.5 min, 99.6%.

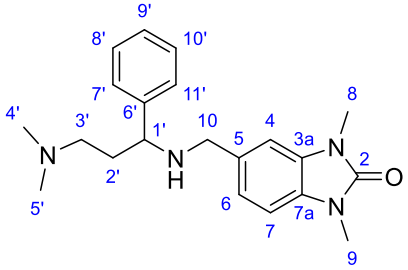
5-({[3'-(Dimethylamino)-2',2'-dimethylprop-1'-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (160)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (60 mg, 0.32 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (10 mL) and 3 Å molecular sieves were added. *N*,*N*,2,2-Tetramethyl-1,3-propanediamine (0.10 mL, 0.63 mmol, 2.0 eq.) and acetic acid (36 μL, 0.63 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 2.5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated complete imine formation. $NaBH(OAc)_3$ (0.33 g, 1.6 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 28 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL) and filtered through a pad of Celite®. The filter pad was washed with a saturated aqueous solution of $NaHCO_3$ (10 mL). The filtrate was extracted with CH_2Cl_2 (3 × 25 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using an ISOLUTE®

SCX-2 column (elution with NH_3 (2 M, MeOH)) to yield the title compound as a colourless oil (49 mg, 0.16 mmol, 51%): R_f 0.18 (EtOH + 1% Et_3N); $\tilde{\nu}_{max}$/cm^{-1} 2942 (br), 1704 (C=O, s), 1508 (m), 1456 (m), 1395 (w), 1267 (w), 1137 (w), 1043 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.03 (1H, dd, *J* 8.0, 1.4, C^6-H), 6.98 (1H, d, *J* 1.4, C^4-H), 6.88 (1H, d, *J* 8.0, C^7-H), 3.80 (2H, s, C^{10}-H_2), 3.39 (3H, s, C$^{8/9}$-H_3), 3.39 (3H, s, C$^{8/9}$-H_3), 2.43 (2H, s, C$^{1'}$-H_2), 2.25 (6H, s, C$^{4'}$-H_3, C$^{5'}$-H_3), 2.15 (2H, s, C$^{3'}$-H_2), 0.88 (6H, s, C$^{6'}$-H_3, C$^{7'}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 134.6 (C^5), 130.2 (C^{3a}), 129.0 (C^{7a}), 121.1 (C^6), 107.3 (C^4), 107.0 (C^7), 69.3 (C$^{3'}$), 58.9 (C$^{1'}$), 55.0 (C^{10}), 48.9 (C$^{4'}$, C$^{5'}$), 36.2 (C$^{2'}$) 27.3 (C$^{8/9}$), 27.2 (C$^{8/9}$), 25.0 (C$^{6'}$, C$^{7'}$); HRMS *m/z* (ESI$^+$) [Found: 305.23338, $C_{17}H_{29}N_4O$ requires $[M+H]^+$ 305.23359]; LRMS *m/z* (ESI$^+$) 175.0 ($[M-C_7H_{17}N_2]^+$, 100%); HPLC retention time 4.7 min, >99.9%.

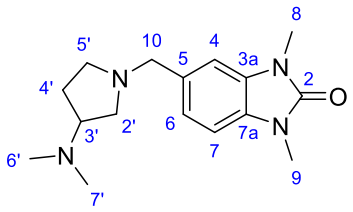
±-5-({[3'-(Dimethylamino)-1'-phenylprop-1'-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (161)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (60 mg, 0.32 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (12 mL) and 3 Å molecular sieves were added. *N*,*N*-Dimethyl-1-phenylpropane-1,3-diamine (0.12 mL, 0.63 mmol, 2.0 eq.) and acetic acid (36 μL, 0.63 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 45 min. After this time ^{1}H NMR analysis of the reaction mixture indicated complete imine formation. $NaBH(OAc)_3$ (0.33 g, 1.6 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 17 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL) and filtered through a pad of Celite®. The filter pad was washed with a saturated aqueous solution of $NaHCO_3$ and CH_2Cl_2. The aqueous fraction was extracted with CH_2Cl_2 (2 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified

using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) and further purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless oil (35 mg, 0.098 mmol, 31%): R_f 0.09 (EtOH + 1% Et_3N); $\tilde{\nu}_{max}$/cm^{-1} 1699 (C=O, s), 1629 (w), 1610 (w), 1507 (m), 1452 (m), 1443 (m), 1395 (w), 1266 (w), 1239 (w), 1191 (w), 1138 (w), 1080 (w), 1040 (w), 1028 (w), 950 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.29 (4H, m, C$^{7'}$-H, C$^{8'}$-H, C$^{10'}$-H, C$^{11'}$-H), 7.32–7.22 (1H, m, C$^{9'}$-H), 6.96 (1H, dd, *J* 7.9, 1.5, C^6-H), 6.90 (1H, d, *J* 1.5, C^4-H), 6.87 (1H, d, *J* 7.9, C^7-H), 3.72–3.64 (2H, m, C$^{1'}$-H, C^{10}-H$_a$), 3.56 (1H, d, *J* 13.0, C^{10}-H$_b$), 3.40 (6H, s, C^8-H$_3$, C^9-H$_3$), 2.35–2.20 (1H, m, C$^{3'}$-H$_a$), 2.17 (6H, s, C$^{4'}$-H$_3$, C$^{5'}$-H$_3$), 2.18–2.09 (1H, m, C$^{3'}$-H$_b$), 2.00–1.86 (1H, m, C$^{2'}$-H$_a$), 1.81–1.70 (1H, m, C$^{2'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 144.2 (C$^{6'}$), 134.3 (C^5), 130.2 (C^{3a}), 129.1 (C^{7a}), 128.5 (C$^{8'}$, C$^{10'}$), 127.4 (C$^{7'}$, C$^{11'}$), 127.2 (C$^{9'}$), 121.2 (C^6), 107.4 (C^4), 107.0 (C^7), 61.6 (C$^{1'}$), 57.3 (C$^{3'}$), 51.8 (C^{10}), 45.7 (C$^{4'}$, C$^{5'}$), 36.1 (C$^{2'}$), 27.28 (C$^{8/9}$), 27.26 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 353.23358, $C_{21}H_{29}N_4O$ requires $[M+H]^+$ 353.23359]; LRMS *m/z* (ESI$^+$) 353.2 ($[M+H]^+$, 100%); HPLC retention time 5.5 min, 98.7%.

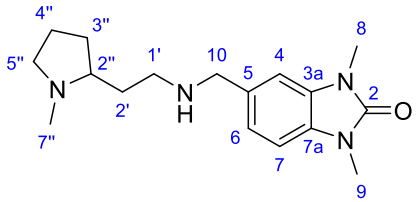
5-{[3'-(Dimethylamino)pyrrolidin-1'-yl]methyl}-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (163)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (60 mg, 0.32 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (10 mL) and 3 Å molecular sieves were added. 3-(Dimethylamino)pyrrolidine (80 µL, 0.63 mmol, 2.0 eq.) and acetic acid (36 µL, 0.63 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 30 min. $NaBH(OAc)_3$ (0.33 g, 1.6 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 17 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL) and filtered through a pad of Celite®. The filter pad was washed with a saturated aqueous solution of $NaHCO_3$. The filtrate

was extracted with CH_2Cl_2 (3 × 25 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) and further purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a pale orange oil (43 mg, 0.15 mmol, 47%): R_f 0.12 (EtOH + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 1698 (C=O, s), 1630 (w), 1610 (w), 1507 (m), 1443 (w), 1394 (w), 1268 (w), 1192 (w), 1136 (w), 1080 (w), 1041 (w), 911 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01 (1H, dd, *J* 7.9, 1.6, C^6-H), 6.97 (1H, d, *J* 1.6, C^4-H), 6.86 (1H, d, *J* 7.9, C^7-H), 3.64 (1H, d, *J* 12.8, C^{10}-H$_a$), 3.58 (1H, d, *J* 12.8, C^{10}-H$_b$), 3.39 (3H, s, C^8-H$_3$), 3.38 (3H, s, C^9-H$_3$), 2.84–2.77 (1H, m, C$^{2'}$-H$_a$), 2.78–2.66 (2H, m, C$^{5'}$-H$_a$, C$^{3'}$-H), 2.53–2.44 (1H, m, C$^{5'}$-H$_b$), 2.33–2.22 (1H, m, C$^{4'}$-H$_a$), 2.17 (6H, s, C$^{6'}$-H$_3$, C$^{7'}$-H$_3$), 2.06–1.92 (1H, m, C$^{2'}$-H$_b$), 1.76–1.66 (1H, m, C$^{4'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 132.6 (C^5), 130.2 (C^{3a}), 129.2 (C^{7a}), 121.8 (C^6), 107.9 (C^4), 106.9 (C^7), 65.6 (C$^{3'}$), 61.0 (C^{10}), 58.8 (C$^{2'}$), 53.5 (C$^{5'}$), 44.0 (C$^{6'}$, C$^{7'}$), 29.3 (C$^{4'}$), 27.30 (C$^{8/9}$), 27.26 (C$^{8/9}$); HRMS *m/z* (APCI) [Found: 289.20210, $C_{16}H_{25}N_4O$ requires $[M+H]^+$ 289.20229]; unable to detect by LRMS; HPLC retention time 4.6 min, >99.9%.

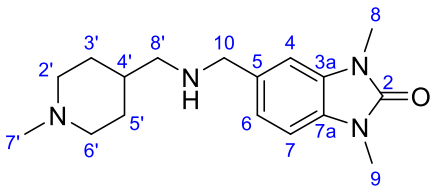
1,3-Dimethyl-5-({[2'-(1''-methylpyrrolidin-2''-yl)ethyl]amino}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (164)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (60 mg, 0.32 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (10 mL) and 3 Å molecular sieves were added. 2-(2-Aminoethyl)-*N*-methylpyrrolidine (90 μL, 0.63 mmol, 2.0 eq.) and acetic acid (36 μL, 0.63 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 30 min. After this time ^{1}H NMR analysis of the reaction mixture indicated complete imine formation. $NaBH(OAc)_3$ (0.33 g, 1.6 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 16 h. The reaction

mixture was quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL) and filtered through a pad of Celite®. The filter pad was washed with a saturated aqueous solution of $NaHCO_3$. The filtrate was extracted with CH_2Cl_2 (3 × 25 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) and further purified using silica flash column chromatography (elution with EtOH + 1% Et_3N isocratic) to yield the title compound as a colourless oil (3.1 mg, 0.010 mmol, 3%): R_f 0.06 (EtOH + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 1698 (s, C=O), 1509 (w), 1462 (m), 1396 (w), 1269 (w), 1242 (w), 1114 (w), 1037 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.03 (1H, dd, *J* 7.9, 1.5, C^6-H), 6.98 (1H, d, *J* 1.5, C^4-H), 6.89 (1H, d, *J* 7.9, C^7-H), 3.84 (1H, d, *J* 12.9, C^{10}-H$_a$), 3.81 (1H, d, *J* 12.9, C^{10}-H$_b$), 3.41 (3H, s, C$^{8/9}$-H$_3$), 3.40 (3H, s, C$^{8/9}$-H$_3$), 3.08–3.00 (1H, m, C$^{5''}$-H$_a$), 2.75–2.67 (1H, m, C$^{1'}$-H$_a$), 2.67–2.59 (1H, m, C$^{1'}$-H$_b$), 2.30 (3H, s, C$^{7''}$-H$_3$), 2.14–2.02 (2H, m, C$^{2''}$-H, C$^{5''}$-H$_b$), 2.01–1.84 (2H, m, C$^{3''}$-H$_a$, C$^{2'}$-H$_a$), 1.81–1.60 (2H, m, C$^{4''}$-H$_2$), 1.54–1.40 (2H, m, C$^{3''}$-H$_b$, C$^{2'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 133.9 (C^5), 130.3 (C^{3a}), 129.2 (C^{7a}), 121.3 (C^6), 107.4 (C^4), 107.1 (C^7), 64.7 (C$^{2''}$), 57.3 (C$^{5''}$), 54.6 (C^{10}), 47.0 (C$^{1'}$), 40.6 (C$^{7''}$), 34.1 (C$^{2'}$), 30.8 (C$^{3''}$), 27.3 (C^8, C^9), 22.1 (C$^{4''}$); HRMS *m/z* (APCI) [Found: 303.21790, $C_{17}H_{27}N_4O$ requires $[M+H]^+$ 303.21794]; unable to detect by LRMS; HPLC retention time 4.7 min, >99.9%.

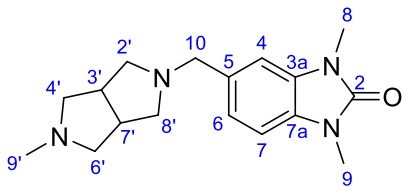
1,3-Dimethyl-5-({[(1'-methylpiperidin-4'-yl)methyl]amino}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (165)



1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**34**) (0.10 g, 0.53 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (13 mL) and 3 Å molecular sieves were added. (1-Methylpiperidin-4-yl)methanamine (0.14 g, 1.1 mmol, 2.0 eq.) and acetic acid (60 μL, 1.1 mmol, 2.0 eq.) were added at 0 °C. The reaction mixture was stirred at 0 °C for 2.5 h. After this time TLC analysis indicated complete imine formation (1:9 MeOH/CH_2Cl_2). $NaBH(OAc)_3$

(0.56 g, 2.6 mmol, 5.0 eq.) was added at 0 °C. The reaction mixture was warmed to rt and stirred for 18 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL), filtered through a pad of Celite®, and the filter pad washed with EtOH. The reaction volume was reduced by concentration *in vacuo*. The reaction mixture was extracted with CH_2Cl_2 (2 × 20 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with EtOH + 2% Et_3N isocratic) to yield the title compound as a colourless oil (29 mg, 0.097 mmol, 18%): R_f 0.07 (EtOH + 2% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 3469 (N-H, br), 2919 (br), 2788 (br), 1696 (C=O, s), 1508 (m), 1449 (m), 1396 (m), 1271 (w), 1188 (w), 1142 (w), 1079 (w), 980 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01 (1H, d, *J* 7.9, C^6-H), 6.97 (1H, s, C^4-H), 6.87 (1H, d, *J* 7.9, C^7-H), 3.79 (2H, s, C^{10}-H$_2$), 3.39 (3H, s, C$^{8/9}$-H$_3$), 3.38 (3H, s, C$^{8/9}$-H$_3$), 2.82 (2H, dd, *J* 12.3, 3.6, C$^{2'}$-H$_a$, C$^{6'}$-H$_a$), 2.51 (2H, d, *J* 6.7, C$^{8'}$-H$_2$), 2.23 (3H, s, C$^{7'}$-H$_3$), 1.89 (2H, ddd, *J* 12.3, 12.3, 3.0, C$^{2'}$-H$_b$, C$^{6'}$-H$_b$), 1.71 (2H, dd, *J* 12.3, 3.0, C$^{3'}$-H$_a$, C$^{5'}$-H$_a$), 1.50–1.38 (1H, m, C$^{4'}$-H), 1.27 (2H, ddd, *J* 12.3, 12.3, 3.6, C$^{3'}$-H$_b$, C$^{5'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 134.1 (C^5), 130.3 (C^{3a}), 129.1 (C^{7a}), 121.2 (C^6), 107.3 (C^4), 107.1 (C^7), 55.8 (C$^{2'}$, C$^{6'}$), 55.5 (C$^{8'}$), 54.5 (C^{10}), 46.6 (C$^{7'}$), 35.7 (C$^{4'}$), 30.8 (C$^{3'}$, C$^{5'}$), 27.3 (C^8, C^9); HRMS *m/z* (ESI$^+$) [Found: 303.21773, $C_{17}H_{26}N_4O$ requires $[M+H]^+$ 303.21794]; unable to detect by LRMS; HPLC retention time 4.8 min, 98.0%.

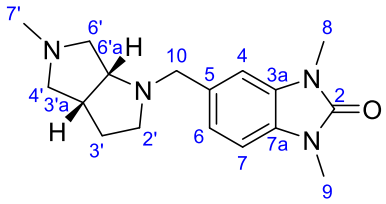
1,3-Dimethyl-5-[(5'-methylhexahydropyrrolo[3',4'-*c*]pyrrol-2'(1*H*)-yl)methyl]-1,3-dihydro-2*H*-benzimidazol-2-one (166)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (0.10 g, 0.53 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (5.0 mL) and 3 Å molecular sieves were added. 2-Methyloctahydropyrrolo[3,4-*c*]pyrrole (0.13 g, 1.1 mmol, 2.0 eq.) and acetic acid (60 μL, 1.1 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 3 h. $NaBH(OAc)_3$ (0.56 g, 2.6 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 18 h. After this

time LCMS analysis indicated complete consumption of starting material. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (3 × 10 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (7M, MeOH)) to yield the title compound as a colourless oil (97 mg, 0.32 mmol, 61%): R_f 0.16 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $\tilde{\nu}_{max}$/cm^{-1} 2945 (w), 2783 (w), 1696 (C=O, s), 1507 (w), 1444 (w), 1395 (w), 1337 (w), 1262 (w), 1137 (w), 922 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.00 (1H, dd, *J* 7.9, 1.3, C^6-H), 6.96 (1H, d, *J* 1.3, C^4-H), 6.85 (1H, d, *J* 7.9, C^7-H), 3.60 (2H, s, C^{10}-H$_2$), 3.375 (3H, s, C$^{8/9}$-H$_3$), 3.366 (3H, s, C$^{8/9}$-H$_3$), 2.77–2.66 (4H, m, C$^{2'}$-H$_a$, C$^{3'}$-H, C$^{7'}$-H, C$^{8'}$-H$_a$), 2.57–2.50 (2H, m, C$^{4'}$-H$_a$, C$^{6'}$-H$_a$), 2.45–2.38 (2H, m, C$^{4'}$-H$_b$, C$^{6'}$-H$_b$), 2.33 (3H, s, C$^{9'}$-H$_3$), 2.31–2.25 (2H, m, C$^{2'}$-H$_b$, C$^{8'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 132.7 (C^5), 130.2 (C^{3a}), 129.1 (C^{7a}), 121.8 (C^6), 107.9 (C^4), 106.8 (C^7), 61.8 (C^{10}), 59.9 (C$^{4'}$, C$^{6'}$), 59.8 (C$^{2'}$, C$^{8'}$), 42.5 (C$^{3'}$, C$^{7'}$), 41.8 (C$^{9'}$), 27.24 (C$^{8/9}$), 27.22 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 301.20217, $C_{17}H_{25}N_4O$ requires $[M+H]^+$ 301.20229]; LRMS *m/z* (ESI$^+$) 301.3 ($[M+H]^+$, 20%); LCMS (basic) retention time 0.66 min, >99%.

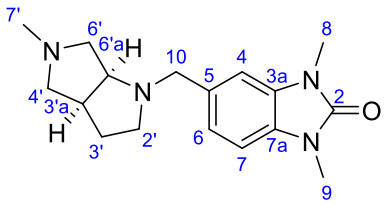
1,3-Dimethyl-5-{[(3'a*R*,6'a*R*)-5'-methylhexahydropyrrolo[3',4'-*b*]pyrrol-1'(2*H*)-yl]methyl}-1,3-dihydro-2*H*-benzimidazol-2-one ((*R*)-167)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (0.10 g, 0.53 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (5.0 mL) and 3 Å molecular sieves were added. (3a*R*,6a*R*)-5-Methyloctahydropyrrolo[3,4-*b*]pyrrole (66 mg, 0.53 mmol, 1.0 eq.) and acetic acid (60 μL, 1.1 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 30 min. $NaBH(OAc)_3$ (0.56 g, 2.6 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 18 h. After this time LCMS analysis indicated complete consumption of starting material. The

reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (3 × 10 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (7 M, MeOH)) to yield the title compound as a colourless oil (85 mg, 0.28 mmol, 54%): R_f 0.39 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = +7.7 (*c* 1.0, MeOH); $\bar{\nu}_{max}$/cm^{-1} 1694 (C=O, s), 1629 (w), 1611 (w), 1507 (w), 1446 (w), 1420 (w), 1395 (w), 1353 (w), 1254 (w), 1237 (w), 1216 (w), 1191 (w), 1137 (w), 1028 (w), 952 (w), 919 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02–6.96 (2H, m, C^4-H, C^6-H), 6.82 (1H, d, *J* 7.9, C^7-H), 3.72 (1H, d, *J* 12.5, C^{10}-H$_a$), 3.54 (1H, d, *J* 12.5, C^{10}-H$_b$), 3.38 (3H, s, C$^{8/9}$-H$_3$), 3.36 (3H, s, C$^{8/9}$-H$_3$), 3.03–2.98 (1H, m, C$^{6'a}$-H), 2.92–2.81 (1H, m, C$^{2'}$-H$_a$), 2.74–2.62 (1H, m, C$^{3'a}$-H), 2.58 (1H, dd, *J* 9.3, 2.0, C$^{4'}$-H$_a$), 2.53 (1H, d, *J* 9.7, C$^{6'}$-H$_a$), 2.25 (3H, s, C$^{7'}$-H$_3$), 2.24–2.14 (2H, m, C$^{2'}$-H$_b$, C$^{4'}$-H$_b$), 2.05–1.87 (2H, m, C$^{3'}$-H$_a$, C$^{6'}$-H$_b$), 1.61–1.49 (1H, m, C$^{3'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 132.5 (C^5), 130.2 (C^{3a}), 129.2 (C^{7a}), 122.3 (C^6), 108.6 (C^4), 106.7 (C^7), 68.9 (C$^{6'a}$), 62.5 (C$^{4'}$), 62.0 (C$^{6'}$), 60.4 (C^{10}), 55.3 (C$^{2'}$), 42.7 (C$^{3'a}$), 41.9 (C$^{7'}$), 31.7 (C$^{3'}$), 27.4 (C$^{8/9}$), 27.2 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 301.20224, $C_{17}H_{25}N_4O$ requires $[M+H]^+$ 301.20229]; LRMS *m/z* (ESI$^+$) 301.3 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.76 min, >99%; Chiral HPLC, Phenomonex C4 column (40 °C, 40% MeOH + 0.1% NH_3 in 60% $scCO_2$, 2.0 ml min^{-1}) retention time 2.71 min (**(*R*)-167**), 98% e.e. (other enantiomer (**(*S*)-167**), retention time 2.46 min).

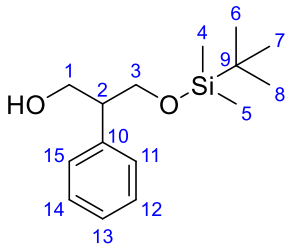
1,3-Dimethyl-5-{[(3'a*S*,6'a*S*)-5'-methylhexahydropyrrolo[3',4'-*b*]pyrrol-1'(2*H*)-yl]methyl}-1,3-dihydro-2*H*-benzimidazol-2-one ((*S*)-167)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (0.10 g, 0.53 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (5.0 mL) and 3 Å molecular sieves were

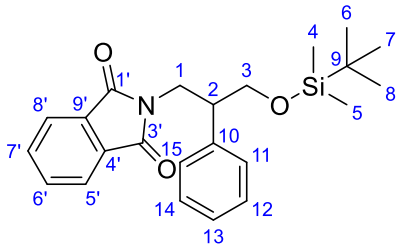
added. (3a*S*,6a*S*)-5-Methyloctahydropyrrolo[3,4-*b*]pyrrole (66 mg, 0.53 mmol, 1.0 eq.) and acetic acid (60 μL, 1.1 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 30 min. $NaBH(OAc)_3$ (0.56 g, 2.6 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 18 h. After this time LCMS analysis indicated complete consumption of starting material. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (3 × 10 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (7 M, MeOH)) to yield the title compound as a colourless oil (52 mg, 0.18 mmol, 34%): R_f 0.39 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = –5.1 (*c* 1.0, MeOH); $\bar{\nu}_{max}$/cm^{-1} 2938 (w), 2777 (w), 1693 (C=O, s), 1507 (m), 1446 (m), 1395 (w), 1353 (w), 1254 (w), 1192 (w), 1137 (w), 1020 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05–7.01 (2H, m, C^4-H, C^6-H), 6.86 (1H, d, *J* 8.3, C^7-H), 3.76 (1H, d, *J* 12.5, C^{10}-H$_a$), 3.59 (1H, d, *J* 12.5, C^{10}-H$_b$), 3.42 (3H, s, C$^{8/9}$-H$_3$), 3.41 (3H, s, C$^{8/9}$-H$_3$), 3.14–3.00 (1H, m, C$^{6'a}$-H), 2.97–2.90 (1H, m, C$^{2'}$-H$_a$), 2.75–2.64 (1H, m, C$^{3'a}$-H), 2.61 (1H, dd, *J* 9.2, 2.2, C$^{4'}$-H$_a$), 2.57 (1H, d, *J* 9.8, C$^{6'}$-H$_a$), 2.28 (3H, s, C$^{7'}$-H$_3$), 2.27–2.19 (2H, m, C$^{2'}$-H$_b$, C$^{4'}$-H$_b$), 2.03–1.91 (2H, m, C$^{3'}$-H$_a$, C$^{6'}$-H$_b$), 1.65–1.53 (1H, m, C$^{3'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.6 (C^2), 132.7 (C^5), 130.2 (C^{3a}), 129.3 (C^{7a}), 122.3 (C^6), 108.7 (C^4), 106.7 (C^7), 69.0 (C$^{6'a}$), 62.6 (C$^{4'}$), 62.1 (C$^{6'}$), 60.5 (C$^{8'}$), 55.3 (C$^{2'}$), 42.8 (C$^{3'a}$), 42.0 (C$^{7'}$), 31.7 (C$^{3'}$), 27.4 (C$^{8/9}$), 27.3 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 301.20224, $C_{17}H_{25}N_4O$ requires $[M+H]^+$ 301.20229]; LRMS *m/z* (ESI$^+$) 301.3 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.77 min, 95%; Chiral HPLC, Phenomonex C4 column (40 °C, 40% MeOH + 0.1% NH_3 in 60% $scCO_2$, 2.0 ml min^{-1}) retention time 2.46 min (**(*S*)-167**), 97% e.e. (other enantiomer (**(*R*)-167**), retention time 2.71 min).

3-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-phenylpropan-1-ol (172)[232]



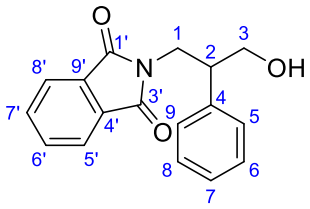
Sodium hydride (60% dispersion in mineral oil, 578 mg, 14.5 mmol, 1.1 eq.) was dissolved in anhydrous tetrahydrofuran (12.0 mL) and the reaction mixture cooled to 0 °C. 2-Phenylpropane-1,3-diol (2.00 g, 13.1 mmol, 1.0 eq.) in anhydrous tetrahydrofuran (10.0 mL) was added dropwise. The reaction mixture was warmed to rt and stirred for 1 h. The reaction mixture was cooled to 0 °C and *tert*-butyldimethylchlorosilane (2.18 g, 14.5 mmol, 1.1 eq.) in anhydrous tetrahydrofuran (12.0 mL) was added dropwise. The reaction mixture was warmed to rt and stirred for 2 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl (50 mL) and extracted with EtOAc (50 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a colourless oil (3.50 g, 13.1 mmol, 100%): R_f 0.78 (1:1 EtOAc/heptane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.22 (2H, m, C^{11}-H, C^{15}-H), 7.20–7.12 (3H, m, C^{12}-H, C^{13}-H, C^{14}-H), 4.00 (1H, dd, *J* 10.8, 7.6, C^1-H_a), 3.88–3.85 (2H, m, C^3-H_2), 3.85–3.77 (1H, m, C^1-H_b), 3.07–2.97 (1H, m, C^2-H), 2.68 (1H, br s, O-H), 0.85 (9H, s, C^6-H_3, C^7-H_3, C^8-H_3), 0.00 (6H, s, C^4-H_3, C^5-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.8 (C^{10}), 128.7 (C^{11}, C^{15}), 128.1 (C^{12}, C^{14}), 127.1 (C^{13}), 67.5 (C^3), 66.7 (C^1), 49.8 (C^2), 26.0 (C^6, C^7, C^8), 18.3 (C^9), –5.4 (C^4, C^5); LCMS *m/z* (ESI$^+$) 267.1 ($[M+H]^+$, 80%). The spectroscopic data are in good agreement with literature values.[232]

2'-(3-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-phenylpropyl)-1*H*-isoindole-1',3'(2*H*)-dione (173)[233]



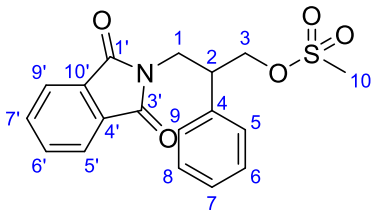
3-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-phenylpropan-1-ol **(172)** (1.00 g, 3.75 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (15.0 mL) and cooled to 0 °C. Triphenylphosphine (1.45 g, 5.63 mmol, 1.5 eq.) and phthalimide (0.663 g, 4.50 mmol, 1.2 eq.) were added. Diisopropyl azodicarboxylate (0.876 g, 4.50 mmol, 1.2 eq.) in anhydrous tetrahydrofuran (4.00 mL) was added dropwise. The reaction mixture was warmed to rt and stirred for 5 h. After this time TLC analysis (1:1 EtOAc/heptane) and LCMS analysis indicated complete consumption of starting material. The reaction mixture was diluted with EtOAc (30 mL) and washed sequentially with a saturated aqueous solution of Na_2CO_3 (30 mL), H_2O (30 mL), and brine (30 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–30% EtOAc/heptane gradient) to yield the title compound as a colourless oil (1.25 g, 3.16 mmol, 84%): R_f 0.22 (1:19 EtOAc/heptane); ^{1}H NMR (500 MHz, $CDCl_3$) 7.81–7.73 (2H, m, $C^{5'}$-H, $C^{8'}$-H), 7.69–7.61 (2H, m, $C^{6'}$-H, $C^{7'}$-H), 7.28–7.22 (4H, m, C^{11}-H, C^{12}-H, C^{14}-H, C^{15}-H), 7.22–7.14 (1H, m, C^{13}-H), 4.13–3.99 (2H, m, C^1-H_2), 3.88–3.79 (2H, m, C^3-H_2), 3.51–3.42 (1H, m, C^2-H), 0.82 (9H, s, C^6-H_3, C^7-H_3, C^8-H_3), –0.08 (6H, s, C^4-H_3, C^5-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.5 ($C^{1'}$, $C^{3'}$), 139.8 (C^{10}), 133.9 ($C^{6'}$, $C^{7'}$), 132.3 ($C^{4'}$, $C^{9'}$), 128.5 (C^{12}, C^{14}), 128.4 (C^{11}, C^{15}), 127.2 (C^{13}), 123.2 ($C^{5'}$, $C^{8'}$), 66.5 (C^3), 46.7 (C^2), 40.9 (C^1), 25.9 (C^6, C^7, C^8), 18.4 (C^9), –5.5 (C^4, C^5); LRMS *m/z* (ESI^+) 396.2 ($[M+H]^+$, 100%). The spectroscopic data are in good agreement with literature values.[233]

2'-(3-Hydroxy-2-phenylpropyl)-1*H*-isoindole-1',3'(2*H*)-dione (174)



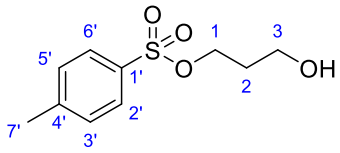
2-(3-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-phenylpropyl)-1*H*-isoindole-1,3(2*H*)-dione **(173)** (1.61 g, 4.07 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (2.00 mL) and cooled to 0 °C. An aqueous solution of HCl (2 M, 2.00 mL) was added. The reaction mixture was warmed to rt and stirred for 2 h. After this time TLC analysis indicated partial consumption of starting material (1:1 EtOAc/petroleum ether). The reaction mixture was heated to 66 °C and stirred for 5 h. After this time TLC analysis indicated complete consumption of starting material (1:1 EtOAc/petroleum ether). The reaction mixture was cooled to rt and concentrated *in vacuo*. The resulting solid was dissolved in a saturated aqueous solution of $NaHCO_3$ (100 mL) and extracted with EtOAc (3 × 100 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–70% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (918 mg, 3.26 mmol, 80%): R_f 0.23 (1:1 EtOAc/petroleum ether); $\tilde{\nu}_{max}$/cm^{-1} 3449 (w), 2980 (w), 2359 (w), 2163 (w), 1773 (w), 1697 (C=O, s), 1495 (w), 1465 (w), 1454 (w), 1433 (w), 1396 (m), 1352 (w), 1263 (w), 1190 (w), 1168 (w), 1111 (w), 1088 (w), 1058 (w), 1022 (w), 991 (w), 960 (w), 916 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89–7.82 (2H, m, C$^{5'}$-H, C$^{8'}$-H), 7.78–7.70 (2H, m, C$^{6'}$-H, C$^{7'}$-H), 7.40–7.32 (4H, m, C^5-H, C^6-H, C^8-H, C^9-H), 7.31–7.24 (1H, m, C^7-H), 4.08 (1H, dd, *J* 14.0, 8.8, C^1-H$_a$), 4.02 (1H, dd, *J* 14.0, 6.3, C^1-H$_b$), 3.89–3.84 (2H, m, C^3-H$_2$), 3.35–3.26 (1H, m, C^2-H), 2.38 (1H, br s, O-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.8 (C$^{1'}$, C$^{3'}$), 139.6 (C^4), 134.3 (C$^{6'}$, C$^{7'}$), 132.0 (C$^{4'}$, C$^{9'}$), 128.8 (C^6, C^8), 128.3 (C^5, C^9), 127.5 (C^7), 123.5 (C$^{5'}$, C$^{8'}$), 64.1 (C^3), 46.8 (C^2), 39.8 (C^1); HRMS *m/z* (ESI$^+$) [Found: 304.09451, $C_{17}H_{15}NO_3Na$ requires [M+Na]$^+$ 304.09441]; LRMS *m/z* (ESI$^+$) 304.0 ([M+Na]$^+$, 100%); HPLC retention time 8.4 min, 99.8%.

2'-(3-(Methylsulfonyl)-2-phenylpropyl)isoindoline-1,3-dione (175)



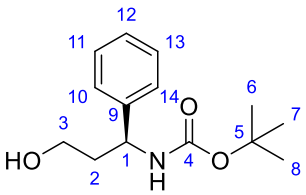
2-(3-Hydroxy-2-phenylpropyl)isoindoline-1,3-dione (**174**) (878 mg, 3.12 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (8.00 mL) and Et_3N (653 µL, 4.68 mmol, 1.5 eq.) was added. The reaction mixture was cooled to 0 °C. Methanesulfonyl chloride (362 µL, 4.68 mmol, 1.5 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 7 h. After this time TLC analysis indicated complete consumption of starting material (CH_2Cl_2). Ice/H_2O (20 mL) was added and the reaction mixture stirred for 5 min. The reaction mixture was extracted with CH_2Cl_2 (20 mL) and the organic fraction washed sequentially with aqueous HCl (1 M, 20 mL), and a saturated aqueous solution of $NaHCO_3$ (20 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a colourless solid (1.07 g, 3.12 mmol, 99%): R_f 0.50 (CH_2Cl_2); m.p. 120–121 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3015 (br), 2935 (br), 1770 (w), 1703 (C=O, s), 1607 (w), 1498 (w), 1469 (w), 1459 (w), 1437 (m), 1402 (w), 1349 (m), 1327 (w), 1172 (m), 1130 (w), 1084 (w), 1045 (w), 1027 (w), 1008 (m), 987 (m), 950 (w), 915 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87–7.79 (2H, m, $C^{5'}$-H, $C^{9'}$-H), 7.76–7.68 (2H, m, $C^{6'}$-H, $C^{7'}$-H), 7.38–7.25 (5H, m, C^5-H, C^6-H, C^7-H, C^8-H, C^9-H), 4.49 (1H, dd, *J* 10.1, 6.1, C^1-H_a), 4.43 (1H, dd, *J* 10.1, 6.7, C^1-H_b), 4.11–4.03 (2H, m, C^3-H_2), 3.79–3.67 (1H, m, C^2-H), 2.88 (3H, s, C^{10}-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.2 ($C^{1'}$, $C^{3'}$), 137.1 (C^4), 134.2 ($C^{6'}$, $C^{7'}$), 131.8 ($C^{4'}$, $C^{10'}$), 129.0 (C^6, C^8), 128.2 (C^5, C^9), 128.1 (C^7), 123.5 ($C^{5'}$, $C^{9'}$), 71.1 (C^3), 43.8 (C^2), 40.0 (C^1), 37.4 (C^{10}); HRMS *m/z* (ESI$^+$) [Found: 382.07188, $C_{18}H_{17}NO_5SNa$ requires $[M+Na]^+$ 382.07196]; LRMS *m/z* (ESI$^+$) 382.0 ($[M+Na]^+$, 100%); HPLC retention time 9.5 min, 99.4%.

3-Hydroxypropyl 4'-methylbenzenesulfonate (186)[234–236]



Propane-1,3-diol (5.65 mL, 78.7 mmol, 5.0 eq.) and Et_3N (2.41 mL, 17.3 mmol, 1.1 eq.) were added to CH_2Cl_2 (30.0 mL) and the reaction mixture cooled to 0 °C. Tosyl chloride (3.00 g, 15.7 mmol, 1.0 eq.) in CH_2Cl_2 (24.0 mL) was added over 20 min *via* a dropping funnel. The reaction mixture was warmed to rt and stirred for 24 h. After this time TLC analysis indicated complete consumption of starting material (3:2 EtOAc/heptane). The reaction mixture was concentrated *in vacuo*. The colourless oil was dissolved in a minimal amount of diethyl ether and filtered. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–100% EtOAc/heptane gradient) to yield the title compound as a colourless oil (1.40 g, 6.08 mmol, 39%): R_f 0.38 (3:2 EtOAc/heptane); $\tilde{\nu}_{max}$/cm^{-1} 1598 (w), 1350 (m), 1307 (w), 1292 (w), 1188 (w), 1172 (s), 1097 (w), 1042 (w), 1019 (w), 967 (w), 927 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81–7.74 (2H, m, C$^{2'}$-H, C$^{6'}$-H), 7.37–7.31 (2H, m, C$^{3'}$-H, C$^{5'}$-H), 4.16 (2H, t, *J* 6.0, C^1-H$_2$), 3.68 (2H, t, *J* 6.0, C^3-H$_2$), 2.43 (3H, s, C$^{7'}$-H$_3$), 1.88 (1H, br s, O-H), 1.87 (2H, tt, *J* 6.0, 6.0, C^2-H$_2$); LRMS *m/z* (ESI$^+$) 231.0 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[234–236]

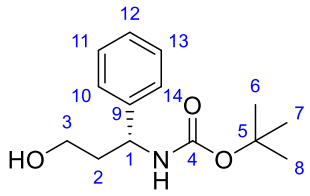
***tert*-Butyl [(1*S*)-3-hydroxy-1-phenylprop-1-yl]carbamate ((*S*)-194)**



(*S*)-3-Amino-3-phenylpropan-1-ol hydrochloride (1.00 g, 5.33 mmol, 1.0 eq.) and Et_3N (0.891 mL, 6.39 mmol, 1.2 eq.) were dissolved in CH_2Cl_2 (3.00 mL) and the reaction mixture cooled to 0 °C. Di-*tert*-butyl dicarbonate (1.73 mL, 7.46 mmol, 1.4 eq.) was added, and the reaction mixture warmed to rt and stirred for 1h. The reaction mixture was filtered, and the

precipitate washed with CH_2Cl_2 (10 mL). The filtrate was concentrated *in vacuo*. Diethyl ether (5 mL) was added and the resulting precipitate removed *via* filtration. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–100% EtOAc/heptane gradient) to yield the title compound as a colourless oil (1.16 g, 4.62 mmol, 87%): R_f 0.46 (1:1 EtOAc/heptane); $[\alpha]_D^{25}$ = –66.5 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 3328 (O-H, br), 1683 (C=O, s), 1510 (w), 1454 (w), 1391 (w), 1365 (m), 1286 (w), 1250 (w), 1165 (s), 1049 (m), 1029 (m), 985 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.33–7.25 (4H, m, C^{10}-H, C^{11}-H, C^{13}-H, C^{14}-H), 7.23–7.16 (1H, m, C^{12}-H), 4.60 (1H, dd, *J* 8.1, 8.1, C^1-H), 4.46 (1H, t, *J* 4.9, O-H), 3.42–3.25 (2H, m, C^3-H$_2$), 1.87–1.76 (1H, m, C^2-H$_a$), 1.75–1.65 (1H, m, C^2-H$_b$), 1.35 (9H, s, C^6-H$_3$, C^7-H$_3$, C^8-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.0 (C^4), 144.5 (C^9), 128.1 (C^{11}, C^{13}), 126.4 (C^{12}), 126.2 (C^{10}, C^{14}), 77.6 (C^5), 57.7 (C^3), 51.3 (C^1), 39.5 (C^2), 28.3 (C^6, C^7, C^8); HRMS *m/z* (ESI$^+$) [Found: 274.14134, $C_{14}H_{21}NO_3Na$ requires $[M+Na]^+$ 274.14136]; LRMS *m/z* (ESI$^+$) 252.2 ($[M+H]^+$, 60%); LCMS (basic) retention time 0.81 min, >99%; Chiral HPLC, Chiralpak AD-H column (90:10 hexane/isopropyl alcohol 1.0 mL min^{-1}) retention time 9.40 min (**(*S*)-194**), >99% e.e. (other enantiomer (**(*R*)-194**) not observed, retention time 15.7 min).

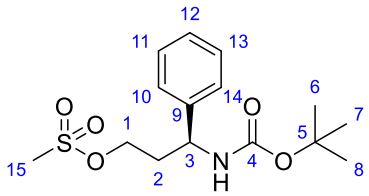
tert-Butyl [(1*R*)-3-hydroxy-1-phenylprop-1-yl]carbamate ((*R*)-194)



(*R*)-3-Amino-3-phenylpropan-1-ol (1.00 g, 6.61 mmol, 1.0 eq.) and Et_3N (1.11 mL, 7.94 mmol, 1.2 eq.) were dissolved in CH_2Cl_2 (3.00 mL) and the reaction mixture cooled to 0 °C. Di-*tert*-butyl dicarbonate (2.15 mL, 9.26 mmol, 1.4 eq.) was added, and the reaction mixture warmed to rt and stirred for 1h. After this time TLC analysis (1:1 EtOAc/heptane) and LCMS analysis indicated complete consumption of starting material. The reaction mixture was concentrated *in vacuo*. Diethyl ether (5 mL) was added and the resulting precipitate removed *via* filtration. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column

chromatography (elution with 0–100% EtOAc/heptane gradient) to yield the title compound as a colourless oil (1.20 g, 4.77 mmol, 72%): R_f 0.45 (1:1 EtOAc/heptane); $[\alpha]_D^{25}$ = +64.1 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 3368 (br), 2980 (w), 1682 (C=O, s), 1514 (s), 1457 (w), 1391 (w), 1363 (w), 1287 (w), 1247 (m), 1166 (s), 1049 (m), 1028 (w), 972 (m); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.35–7.24 (4H, m, C^{10}-H, C^{11}-H, C^{13}-H, C^{14}-H), 7.23–7.16 (1H, m, C^{12}-H), 4.60 (1H, dd, *J* 8.1, 8.1, C^1-H), 4.45 (1H, t, *J* 4.9, O-H), 3.41–3.25 (2H, m, C^3-H$_2$), 1.87–1.76 (1H, m, C^2-H$_a$), 1.75–1.65 (1H, m, C^2-H$_b$), 1.35 (9H, s, C^6-H$_3$, C^7-H$_3$, C^8-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.0 (C^4), 144.5 (C^9), 128.1 (C^{11}, C^{13}), 126.4 (C^{12}), 126.2 (C^{10}, C^{14}), 77.6 (C^5), 57.7 (C^3), 51.2 (C^1), 39.5 (C^2), 28.2 (C^6, C^7, C^8); HRMS *m/z* (ESI$^+$) [Found: 274.12493, $C_{14}H_{21}NO_3Na$ requires $[M+Na]^+$ 274.14136]; LCMS *m/z* (ESI$^+$) 252.2 ($[M+H]^+$, 60%); LCMS (basic) retention time 0.81 min, >99%; Chiral HPLC, Chiralpak AD-H column (90:10 hexane/isopropyl alcohol 1.0 mL min^{-1}) retention time 15.7 min (**(*R*)-194**), >99% e.e. (other enantiomer (**(*S*)-194**) not observed, retention time 9.40 min).

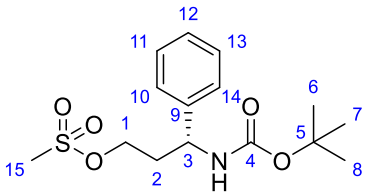
(3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-3-phenylprop-1-yl methanesulfonate ((*S*)-195)



tert-Butyl [(1*S*)-3-hydroxy-1-phenylpropyl]carbamate (**(*S*)-194**) (1.10 g, 4.38 mmol, 1.0 eq.) and Et_3N (0.671 mL, 4.81 mmol, 1.1 eq.) were dissolved in CH_2Cl_2 (10.0 mL) and the reaction mixture cooled to 0 °C. Methanesulfonyl chloride (0.375 mL, 4.81 mmol, 1.1 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 67 h. After this time TLC analysis (1:1 EtOAc/heptane) and LCMS analysis indicated partial consumption of starting material. The reaction mixture was cooled to 0 °C and additional methanesulfonyl chloride (0.375 mL, 4.81 mmol, 1.1 eq.) was added. The reaction mixture was warmed to rt and stirred for 18 h. Ice/H_2O (15 mL) was added and the reaction mixture stirred for 5 min. The reaction mixture was extracted with CH_2Cl_2 (2 × 20 mL). The organic fractions were combined and washed sequentially

with an aqueous solution of HCl (2 M, 30 mL), an aqueous solution of Na_2CO_3 (2 M, 30 mL), and brine (30 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–80% EtOAc/heptane gradient) to yield the title compound as a colourless solid (468 mg, 1.42 mmol, 33%): R_f 0.51 (1:1 EtOAc/heptane); $[\alpha]_D^{25}$ = –33.8 (*c* 1.0, MeOH); m.p. 90–91 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 2939 (w), 1692 (C=O, s), 1507 (m), 1453 (m), 1394 (w), 1266 (w), 1169 (w), 1028 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.51 (1H, d, *J* 8.7, N-H), 7.38–7.27 (4H, m, C^{10}-H, C^{11}-H, C^{13}-H, C^{14}-H), 7.28–7.20 (1H, m, C^{12}-H), 4.69–4.60 (1H, m, C^3-H), 4.27–4.18 (1H, m, C^1-H$_a$), 4.16–4.08 (1H, m, C^1-H$_b$), 3.16 (3H, s, C^{15}-H$_3$), 2.13–1.95 (2H, m, C^2-H$_2$), 1.37 (9H, s, C^6-H$_3$, C^7-H$_3$, C^8-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.0 (C^4), 143.3 (C^9), 128.3 (C^{11}, C^{13}), 126.9 (C^{12}), 126.2 (C^{10}, C^{14}), 77.9 (C^5), 67.5 (C^1), 50.7 (C^3), 36.5 (C^{15}), 35.4 (C^2), 28.2 (C^6, C^7, C^8); HRMS *m/z* (ESI$^+$) [Found: 352.11857, $C_{15}H_{23}NO_5Na$ requires $[M+Na]^+$ 352.11891]; LRMS *m/z* (ESI$^-$) 328.3 ($[M–H]^-$, 20%); LCMS (basic) retention time 0.92 min, 95%.

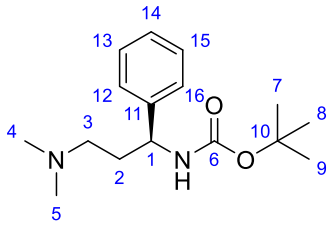
(*R*)-3-((*tert*-Butoxycarbonyl)amino)-3-phenylprop-1-yl methanesulfonate ((*R*)-195)



tert-Butyl [(1*R*)-3-hydroxy-1-phenylpropyl]carbamate (**(*R*)-194**) (932 mg, 3.71 mmol, 1.0 eq.) and Et_3N (0.569 mL, 4.08 mmol, 1.1 eq.) were dissolved in CH_2Cl_2 (10.0 mL) and the reaction mixture cooled to 0 °C. Methanesulfonyl chloride (0.318 mL, 4.08 mmol, 1.1 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 19 h. After this time LCMS analysis indicated complete consumption of starting material. Ice/H_2O (15 mL) was added and the reaction mixture stirred for 5 min. The reaction mixture was extracted with CH_2Cl_2 (2 × 20 mL). The organic fractions were combined and washed sequentially with an aqueous solution of HCl (2 M, 30 mL), an aqueous solution of Na_2CO_3 (2 M, 30 mL), and brine (30 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was

purified using silica flash column chromatography (elution with 0–80% EtOAc/heptane gradient) to yield the title compound as a colourless solid (876 mg, 2.66 mmol, 72%): R_f 0.51 (1:1 EtOAc/heptane); $[\alpha]_D^{25}$ = +33.9 (*c* 1.0, MeOH); m.p. 87–88 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 1681 (C=O, s), 1512 (w), 1457 (w), 1350 (m), 1290 (w), 1167 (s), 1080 (w), 1042 (w), 962 (w), 924 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.51 (1H, d, *J* 8.7, N-H), 7.38–7.28 (4H, m, C^{10}-H, C^{11}-H, C^{13}-H, C^{14}-H), 7.28–7.21 (1H, m, C^{12}-H), 4.67–4.58 (1H, m, C^3-H), 4.26–4.17 (1H, m, C^1-H$_a$), 4.15–4.07 (1H, m, C^1-H$_b$), 3.16 (3H, s, C^{15}-H$_3$), 2.12–1.94 (2H, m, C^2-H$_2$), 1.37 (9H, s, C^6-H$_3$, C^7-H$_3$, C^8-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.0 (C^4), 143.3 (C^9), 128.3 (C^{11}, C^{13}), 126.9 (C^{12}), 126.2 (C^{10}, C^{14}), 77.9 (C^5), 67.5 (C^1), 50.6 (C^3), 36.5 (C^{15}), 35.4 (C^2), 28.2 (C^6, C^7, C^8); HRMS *m/z* (ESI$^+$) [Found: 352.11858, $C_{15}H_{23}NO_5SNa$ requires $[M+Na]^+$ 352.11891]; LRMS *m/z* (ESI$^+$) 330.1 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.92 min, 95%.

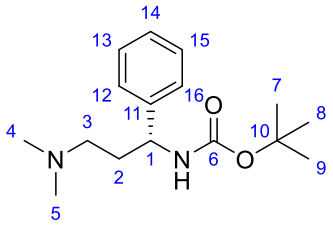
***tert*-Butyl [(1*S*)-3-(dimethylamino)-1-phenylprop-1-yl]carbamate ((*S*)-196)**



To a solution of dimethylamine (2 M, THF) (9.49 mL, 19.0 mmol, 25 eq.) was added (3*S*)-3-[(*tert*-butoxycarbonyl)amino]-3-phenylpropyl methanesulfonate (**(*S*)-195**) (250 mg, 0.760 mmol, 1.0 eq.). Tetrabutylammonium iodide (56.1 mg, 0.150 mmol, 0.2 eq.) was added and the reaction mixture heated at 150 °C for 30 min under microwave irradiation. After this time TLC analysis (1:1 EtOAc/heptane) and LCMS analysis indicated complete consumption of starting material. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (20 mL) and washed sequentially with H_2O (2 × 20 mL), and brine (15 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–30% (10% MeOH/CH_2Cl_2)/CH_2Cl_2 + 1% NH_3 (7 M, MeOH) gradient) to yield the title compound as a colourless oil (76.0 mg, 0.273 mmol, 36%): R_f 0.40 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = –36.1 (*c* 1.0, MeOH);

$\tilde{\nu}_{max}$/cm^{-1} 3376 (N-H, w), 1689 (C=O, s), 1521 (s), 1455 (w), 1389 (w), 1365 (w), 1345 (m), 1289 (w), 1251 (s), 1216 (w), 1168 (s), 1130 (w), 1098 (w), 1082 (w), 1056 (m), 1044 (m), 989 (w), 939 (w), 910 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.36 (1H, d, *J* 8.5, N-H), 7.33–7.24 (4H, m, C^{12}-H, C^{13}-H, C^{15}-H, C^{16}-H), 7.24–7.16 (1H, m, C^{14}-H), 4.56–4.48 (1H, m, C^{1}-H), 2.13 (2H, t, *J* 7.2, C^{3}-H$_2$), 2.09 (6H, s, C^{4}-H$_3$, C^{5}-H$_3$), 1.82–1.62 (2H, m, C^{2}-H$_2$), 1.35 (9H, s, C^{7}-H$_3$, C^{8}-H$_3$, C^{9}-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.0 (C^{6}), 144.4 (C^{11}), 128.1 (C^{13}, C^{15}), 126.5 (C^{14}), 126.2 (C^{12}, C^{16}), 77.6 (C^{10}), 56.1 (C^{3}), 52.5 (C^{1}), 45.2 (C^{4}, C^{5}), 34.4 (C^{2}), 28.2 (C^{7}, C^{8}, C^{9}); HRMS *m/z* (ESI$^+$) [Found: 279.20651, $C_{16}H_{27}N_2O_2$ requires [M+H]$^+$ 279.20670]; LRMS *m/z* (ESI$^+$) 279.3 ([M+H]$^+$, 100%); LCMS (basic) retention time 0.97 min, >99%.

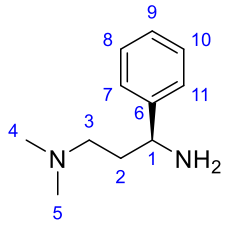
tert-Butyl [(1*R*)-3-(dimethylamino)-1-phenylprop-1-yl]carbamate ((*R*)-196)



(*S*)-3-((*tert*-Butoxycarbonyl)amino)-3-phenylpropyl methanesulfonate (**(*R*)-195**) (243 mg, 0.740 mmol, 1.0 eq.) was dissolved in dimethylamine (2 M, THF) (9.22 mL, 18.4 mmol, 25 eq.). Tetrabutylammonium iodide (54.5 mg, 0.150 mol, 0.2 eq.) was added and the reaction mixture heated at 150 °C for 30 min under microwave irradiation. After this time TLC analysis (1:1 EtOAc/heptane) and LCMS analysis indicated complete consumption of starting material. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (20 mL) and washed sequentially with H_2O (2 × 20 mL), and brine (15 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–40% (10% MeOH/CH_2Cl_2)/CH_2Cl_2 + 1% NH_3 (7 M, MeOH) gradient) to yield the title compound as a colourless oil (150 mg, 0.539 mmol, 73%): R_f 0.40 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = +41.6 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 2971 (w), 2813 (w), 1686 (C=O, s), 1521 (s), 1451 (w), 1364 (w), 1251 (m), 1167 (s), 1045 (w), 989 (w), 940 (w), 911 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.36 (1H, d, *J* 8.5, N-H), 7.33–7.24 (4H, m,

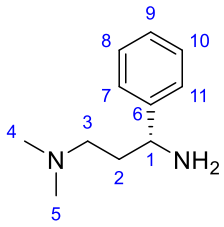
C^{12}-H, C^{13}-H, C^{15}-H, C^{16}-H), 7.24–7.16 (1H, m, C^{14}-H), 4.57–4.48 (1H, m, C^{1}-H), 2.13 (2H, t, *J* 7.2, C^{3}-H_2), 2.09 (6H, s, C^{4}-H_3, C^{5}-H_3), 1.82–1.62 (2H, m, C^{2}-H_2), 1.35 (9H, s, C^{7}-H_3, C^{8}-H_3, C^{9}-H_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.0 (C^{6}), 144.4 (C^{11}), 128.1 (C^{13}, C^{15}), 126.5 (C^{14}), 126.3 (C^{12}, C^{16}), 77.6 (C^{10}), 56.1 (C^{3}), 52.5 (C^{1}), 45.2 (C^{4}, C^{5}), 34.4 (C^{2}), 28.2 (C^{7}, C^{8}, C^{9}); HRMS *m/z* (ESI^+) [Found: 279.20654, $C_{16}H_{27}N_2O_2$ requires $[M+H]^+$ 279.20670]; LRMS *m/z* (ESI^+) 279.2 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.97 min, 97%.

(1*S*)-*N*^{3},*N*^{3}-Dimethyl-1-phenylpropane-1,3-diamine ((*S*)-186)



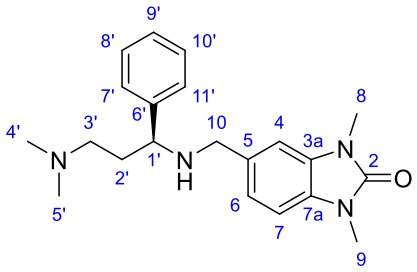
Hydrochloric acid (4 M, dioxane) (2.0 mL, 8.0 mmol, 36 eq.) was added to *tert*-butyl [(1*S*)-3-(dimethylamino)-1-phenylpropyl]carbamate (**(*S*)-196**) (54 mg, 0.19 mmol, 1.0 eq.). The reaction mixture was stirred at rt for 1 h. After this time LCMS analysis indicated complete consumption of starting material. The reaction mixture was concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (7 M, MeOH)) to yield the title compound as a colourless oil (31 mg, 0.17 mmol, 89%): R_f 0.08 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = +25.3 (*c* 0.2, MeOH); $\bar{\nu}_{max}$/cm^{-1} 2949 (w), 2773 (w), 2360 (w), 1602 (w), 1456 (w), 1383 (w), 1153 (w), 1038 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.40–7.30 (4H, m, C^{7}-H, C^{8}-H, C^{10}-H, C^{11}-H), 7.30–7.21 (1H, m, C^{9}-H), 3.89 (1H, t, *J* 7.0, C^{1}-H), 2.42–2.33 (1H, m, C^{3}-H_a), 2.24–2.13 (7H, m, C^{3}-H_b, C^{4}-H_3, C^{5}-H_3), 2.03–1.92 (1H, m, C^{2}-H_a), 1.93–1.82 (1H, m, C^{2}-H_b); ^{13}C NMR (126 MHz, CD_3OD) δ 145.8 (C^{6}), 129.7 (C^{8}, C^{10}), 128.4 (C^{9}), 127.6 (C^{7}, C^{11}), 57.8 (C^{3}), 55.9 (C^{1}), 45.4 (C^{4}, C^{5}), 36.9 (C^{2}); HRMS *m/z* (ESI^+) [Found: 179.15426, $C_{11}H_{19}N_2$ requires $[M+H]^+$ 179.15428]; LRMS *m/z* (ESI^+) 179.2 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.71 min, 98%.

(1*R*)-*N*^{3},*N*^{3}-Dimethyl-1-phenylpropane-1,3-diamine ((*R*)-186)



Hydrochloric acid (4 M, dioxane) (2.0 mL, 8.0 mL, 40 eq.) was added to *tert*-butyl [(1*R*)-3-(dimethylamino)-1-phenylpropyl]carbamate (**(*R*)-196**) (56 mg, 0.20 mmol, 1.0 eq.). The reaction mixture was stirred at rt for 1 h. After this time LCMS analysis indicated complete consumption of starting material. The reaction mixture was concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (7 M, MeOH)) to yield the title compound as a colourless oil (35 mg, 0.20 mmol, 98%): R_f 0.08 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = –18.3 (*c* 0.2, MeOH); $\bar{\nu}_{max}$/cm^{-1} 3407 (N-H, br), 1603 (br), 1494 (w), 1457 (m), 1388 (w), 1260 (w), 1155 (w), 1099 (w), 1072 (w), 1037 (w), 964 (w), 917 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.43–7.35 (4H, m, C^7-H, C^8-H, C^{10}-H, C^{11}-H), 7.35–7.27 (1H, m, C^9-H), 4.08 (1H, t, *J* 7.0, C^1-H), 2.56–2.46 (1H, m, C^3-H$_a$), 2.39–2.19 (7H, m, C^3-H$_b$, C^4-H$_3$, C^5-H$_3$), 2.13–2.02 (1H, m, C^2-H$_a$), 2.01–1.90 (1H, m, C^2-H$_b$); ^{13}C NMR (126 MHz, CD_3OD) δ 143.1 (C^6), 130.0 (C^8, C^{10}), 129.1 (C^9), 127.8 (C^7, C^{11}), 57.6 (C^3), 56.0 (C^1), 45.1 (C^4, C^5), 34.8 (C^2); HRMS *m/z* (ESI$^+$) [Found: 179.15406, $C_{11}H_{19}N_2$ requires $[M+H]^+$ 179.15428]; LRMS *m/z* (ESI$^+$) 179.2 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.73 min, 95%.

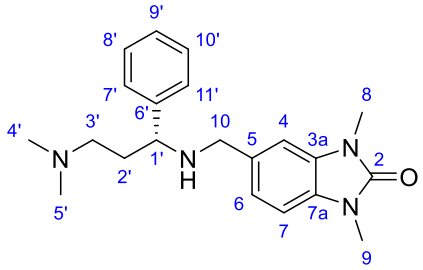
5-({[(1'*S*)-3'-(Dimethylamino)-1'-phenylprop-1'-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*S*)-161)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (17 mg, 0.090 mmol, 1.2 eq.) was dissolved in anhydrous 1,2-dichloroethane (2.0 mL) and 3 Å molecular sieves were added. (1*S*)-N^3,N^3-Dimethyl-1-phenylpropane-1,3-diamine (**(*S*)-186**) (13 mg, 0.070 mmol, 1.0 eq.) and acetic acid (5.0 μL, 0.090 mmol, 1.2 eq.) were added. The reaction mixture was stirred at rt for 30 min. $NaBH(OAc)_3$ (77 mg, 0.36 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 22 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (3 × 10 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (7 M, MeOH)) and further purified using silica flash column chromatography (elution with 0–100% (10% MeOH/CH_2Cl_2)/CH_2Cl_2 + 1% NH_3 (7 M, MeOH) gradient) to yield the title compound as a colourless oil (7.3 mg, 0.021 mmol, 29%): R_f 0.08 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = –35.7 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 2941 (w), 1695 (C=O, s), 1507 (m), 1452 (m), 1395 (w), 1266 (w), 1191 (w), 1138 (w), 1028 (w), 910 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.32 (4H, m, C$^{7'}$-H, C$^{8'}$-H, C$^{10'}$-H, C$^{11'}$-H), 7.32–7.26 (1H, m, C$^{9'}$-H), 6.97 (1H, dd, *J* 7.9, 1.6, C^6-H), 6.91 (1H, d, *J* 1.6, C^4-H), 6.88 (1H, d, *J* 7.9, C^7-H), 3.73–3.66 (2H, m, C$^{1'}$-H, C^{10}-H$_a$), 3.57 (1H, d, *J* 13.1, C^{10}-H$_b$), 3.41 (6H, s, C^8-H$_3$, C^9-H$_3$), 2.37–2.27 (1H, m, C$^{3'}$-H$_a$), 2.19 (6H, s, C$^{4'}$-H$_3$, C$^{5'}$-H$_3$), 2.18–2.11 (2H, m, C$^{3'}$-H$_b$, N-H), 1.97–1.90 (1H, m, C$^{2'}$-H$_a$), 1.83–1.72 (1H, m, C$^{2'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 144.2 (C$^{6'}$), 134.3 (C^5), 130.2 (C^{3a}), 129.1 (C^{7a}), 128.6 (C$^{7'}$, C$^{11'}$), 127.4 (C$^{8'}$, C$^{10'}$), 127.2 (C$^{9'}$), 121.2 (C^6), 107.4 (C^4), 107.1 (C^7), 61.7 (C$^{1'}$),

57.4 ($C^{3'}$), 51.8 (C^{10}), 45.7 ($C^{4'}$, $C^{5'}$), 36.1 ($C^{2'}$), 27.29 ($C^{8/9}$), 27.27 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 353.23355, $C_{21}H_{29}N_4O$ requires $[M+H]^+$ 353.23359]; LRMS *m/z* (ESI^+) 353.4 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.31 min, 99%; Chiral HPLC, Chiralpak IG column (40 °C, 40% MeOH + 0.1% NH_3 in 60% $scCO_2$, 2.0 ml min^{-1}) retention time 2.37 min (**(*S*)-161**), 98% e.e. (other enantiomer (**(*R*)-161**), retention time 2.05 min).

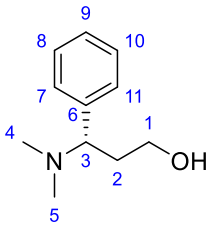
5-({[(1'*R*)-3'-(Dimethylamino)-1'-phenylprop-1'-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*R*)-161)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (24 mg, 0.13 mmol, 1.2 eq.) was dissolved in anhydrous 1,2-dichloroethane (2.0 mL) and 3 Å molecular sieves were added. (1*R*)-N^3,N^3-Dimethyl-1-phenylpropane-1,3-diamine (**(*R*)-186**) (19 mg, 0.10 mmol, 1.0 eq.) and acetic acid (6.0 µL, 0.10 mmol, 1.2 eq.) were added. The reaction mixture was stirred at rt for 30 min. $NaBH(OAc)_3$ (0.11 g, 0.52 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 22 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (3 × 10 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (7 M, MeOH)) and further purified using silica flash column chromatography (elution with 0–100% (10% MeOH/CH_2Cl_2)/CH_2Cl_2 + 1% NH_3 (7 M, MeOH) gradient) to yield the title compound as a colourless oil (15 mg, 0.042 mmol, 40%): R_f 0.08 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = +35.6 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 2939 (w), 1692 (C=O, s), 1507 (m), 1453 (m), 1394 (w), 1266 (w), 1169 (w), 1028 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.28 (4H, m, $C^{7'}$-H, $C^{8'}$-H, $C^{10'}$-H, $C^{11'}$-H), 7.31–7.22 (1H, m, $C^{9'}$-H), 6.97 (1H, dd, *J* 7.9, 1.5, C^6-H), 6.91 (1H, d, *J* 1.5,

C^4-H), 6.88 (1H, d, *J* 7.9, C^7-H), 3.73–3.65 (2H, m, $C^{1'}$-H, C^{10}-H_a), 3.57 (1H, d, *J* 13.1, C^{10}-H_b), 3.40 (6H, s, C^8-H_3, C^9-H_3), 2.37–2.26 (1H, m, $C^{3'}$-H_a), 2.18 (6H, s, $C^{4'}$-H_3, $C^{5'}$-H_3), 2.17–2.10 (1H, m, $C^{3'}$-H_b), 2.07 (1H, br s, N-H), 1.98–1.87 (1H, m, $C^{2'}$-H_a), 1.82–1.71 (1H, m, $C^{2'}$-H_b); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.0 (C^2), 144.2 ($C^{6'}$), 134.3 (C^5), 130.3 (C^{3a}), 129.1 (C^{7a}), 128.6 ($C^{7'}$, $C^{11'}$), 127.4 ($C^{8'}$, $C^{10'}$) 127.2 ($C^{9'}$), 121.2 (C^6), 107.4 (C^4), 107.1 (C^7), 61.7 ($C^{1'}$), 57.4 ($C^{3'}$), 51.8 (C^{10}), 45.7 ($C^{4'}$, $C^{5'}$), 36.2 ($C^{2'}$), 27.31 ($C^{8/9}$), 27.28 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 353.23297, $C_{21}H_{29}N_4O$ requires $[M+H]^+$ 353.23359]; LRMS *m/z* (ESI^+) 353.4 ($[M+H]^+$, 100%); LCMS (acidic) retention time 0.31 min, >99%; Chiral HPLC, Chiralpak IG column (40 °C, 40% MeOH + 0.1% NH_3 in 60% $scCO_2$, 2.0 ml min^{-1}) retention time 2.05 min (**(*R*)-161**), 98% e.e. (other enantiomer (**(*S*)-161**), retention time 2.37 min).

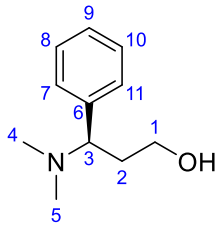
(3*S*)-3-(Dimethylamino)-3-phenylpropan-1-ol ((*S*)-197)



(*S*)-3-Amino-3-phenylpropan-1-ol (1.00 g, 6.66 mmol, 1.0 eq.) was dissolved in formic acid (0.780 mL, 20.3 mmol, 3.0 eq.) and aqueous formaldehyde was added (37%, 2.71 mL, 36.4 mmol, 5.5 eq.). The reaction mixture was heated to 100 °C and stirred for 18 h. After this time LCMS analysis indicated complete consumption of starting material. The reaction mixture was cooled to rt and an aqueous solution of NaOH (2 M) was added to adjust the pH to 12. The reaction mixture was extracted with EtOAc (3 × 30 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–100% (10% MeOH/CH_2Cl_2)/CH_2Cl_2 + 1% NH_3 (7 M, MeOH) gradient) to yield the title compound as a colourless oil (692 mg, 3.36 mmol, 51%): R_f 0.16 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = +22.1 (*c* 1.0, MeOH); $\bar{\nu}_{max}$/cm^{-1} 2956 (w), 1453 (w), 1160 (w), 1046 (w), 921 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.32 (2H, m, C^8-H, C^{10}-H), 7.31–7.23 (1H, m, C^9-H), 7.21–7.15 (2H, m, C^7-H, C^{11}-H), 5.23–5.18 (1H, m, O-H), 3.82–3.73

(2H, m, C^1-H_2), 3.69 (1H, dd, *J* 7.0, 3.4, C^3-H), 2.43–2.31 (1H, m, C^2-H_a), 2.16 (6H, s, C^4-H_3, C^5-H_3), 1.68 (1H, ddd, *J* 14.4, 3.9, 3.9, C^2-H_b); ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.5 (C^6), 128.8 (C^7, C^{11}), 127.9 (C^8, C^{10}), 127.4 (C^9), 69.7 (C^3), 62.9 (C^1), 41.2 (C^4, C^5), 32.6 (C^2); HRMS *m/z* (ESI$^+$) [Found: 180.13825, $C_{11}H_{18}NO$ requires $[M+H]^+$ 180.13829]; LRMS *m/z* (ESI$^+$) 180.2 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.66 min, >99%; Chiral HPLC, Chiralpak AS-H column (95:5 hexane/isopropyl alcohol + 0.1% ethanolamine 1.0 mL min^{-1}) retention time 7.34 min (**(*S*)-198**), >99% e.e. (other enantiomer (**(*R*)-198**) not observed, retention time 8.81 min).

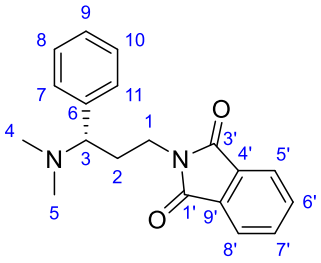
(3*R*)-3-(Dimethylamino)-3-phenylpropan-1-ol ((*R*)-197)



(*R*)-3-Amino-3-phenylpropan-1-ol (500 mg, 3.31 mmol, 1.0 eq.) was dissolved in formic acid (374 µL, 9.92 mmol, 3.0 eq.) and aqueous formaldehyde was added (37%, 1.48 mL, 18.2 mmol, 5.5 eq.). The reaction mixture was heated to 100 °C and stirred for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + 1% Et_3N). The reaction mixture was cooled to rt and an aqueous solution of NaOH (2 M) was added to adjust the pH to 12. H_2O (20 mL) was added and the reaction mixture was extracted with EtOAc (3 × 20 mL). The organic fractions were combined, washed with brine (50 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–6% MeOH/CH_2Cl_2 + 1% Et_3N gradient) to yield the title compound as a colourless oil (515 mg, 2.87 mmol, 87%): R_f 0.35 (1:19 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = –16.1 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 3374 (O-H, br), 2945 (w), 2867 (w), 2826 (w), 2780 (w), 1492 (w), 1453 (w), 1360 (w), 1161 (w), 1045 (m), 987 (w), 922 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41–7.35 (2H, m, C^8-H, C^{10}-H), 7.34–7.28 (1H, m, C^9-H), 7.24–7.18 (2H, m, C^7-H, C^{11}-H), 3.91–3.82 (2H, m, C^1-H_2), 3.79 (1H, dd, *J* 10.4, 3.9, C^3-H) 2.50–2.38 (1H, m,

C^2-H_a), 2.21 (6H, s, C^4-H_3, C^5-H_3), 1.75–1.66 (1H, m, C^2-H_b); ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.1 (C^6), 129.0 (C^7, C^{11}), 128.1 (C^8, C^{10}), 127.7 (C^9), 70.2 (C^3), 63.4 (C^1), 41.2 (C^4, C^5), 32.3 (C^2); HRMS *m/z* (ESI^+) [Found: 180.13814, $C_{11}H_{18}NO$ requires $[M+H]^+$ 180.13829]; LRMS *m/z* (ESI^+) 180.2 ($[M+H]^+$, 100%); HPLC retention time 4.8 min, >99.9%; Chiral HPLC, Chiralpak AS-H column (95:5 hexane/isopropyl alcohol, 1.5 mL min^{-1}) retention time 8.81 min (**(*R*)-198**), >99% e.e. (other enantiomer (**(*S*)-198**) not observed, retention time 7.34 min).

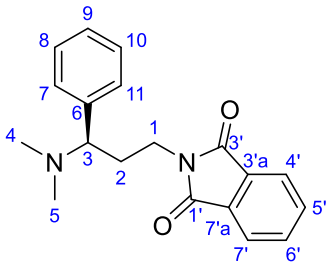
2'-[(3*S*)-3-(Dimethylamino)-3-phenylpropyl]-1*H*-isoindole-1',3'(2*H*)-dione ((*S*)-198)



(3*S*)-3-(Dimethylamino)-3-phenylpropan-1-ol (**(*S*)-198**) (300 mg, 1.67 mol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (3.40 mL) and cooled to 0 °C. Triphenylphosphine (527 mg, 2.01 mmol, 1.2 eq.) and phthalimide (295 mg, 2.01 mmol, 1.2 eq.) were added. Diisopropyl azodicarboxylate (0.390 mL, 2.01 mmol, 1.2 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 67 h. After this time LCMS analysis indicated complete consumption of starting material. The reaction mixture was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–50% (10% $MeOH/CH_2Cl_2$)/CH_2Cl_2 + 1% NH_3 (7 M, MeOH) gradient) to yield the title compound as a colourless oil (347 mg, 1.13 mmol, 67%): R_f 0.34 (1:19 $MeOH/CH_2Cl_2$); $[\alpha]_D^{25}$ = +14.1 (*c* 1.0, MeOH); $\tilde{\nu}_{max}/cm^{-1}$ 1771 (w), 1709 (C=O, s), 1439 (w), 1395 (m), 1370 (w), 1187 (w), 1074 (w), 1019 (w), 968 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83–7.74 (2H, m, $C^{5'}$-H, $C^{8'}$-H), 7.72–7.63 (2H, m, $C^{6'}$-H, $C^{7'}$-H), 7.32–7.25 (2H, m, C^8-H, C^{10}-H), 7.25–7.15 (3H, m, C^7-H, C^9-H, C^{11}-H), 3.75–3.56 (2H, m, C^1-H_2), 3.40 (1H, dd, *J* 8.4, 6.0, C^3-H), 2.32–2.22 (1H, m, C^2-H_a), 2.22–2.09 (7H, m, C^2-H_b, C^4-H_3, C^5-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.4 ($C^{1'}$, $C^{3'}$), 138.5 (C^6), 133.9 ($C^{6'}$, $C^{7'}$), 132.3 ($C^{4'}$, $C^{9'}$), 128.8 (C^7, C^{11}), 128.2 (C^8, C^{10}), 127.4 (C^9), 123.1 ($C^{5'}$, $C^{8'}$), 68.2 (C^3),

42.2 (C^4, C^5), 36.1 (C^1), 31.3 (C^2); HRMS *m/z* (ESI^+) [Found: 309.15935, $C_{19}H_{21}N_2O_2$ requires $[M+H]^+$ 309.15975]; LRMS *m/z* (ESI^+) 309.3 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.99 min, >99%; Chiral HPLC, Chiralpak AS-H column (90:10 hexane/isopropyl alcohol, 1.5 mL min^{-1}) retention time 7.70 min (**(*S*)-199**), >99% e.e. (other enantiomer (**(*R*)-199**) not observed, retention time 5.25 min).

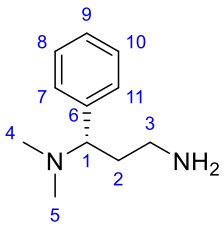
2'-[(3*R*)-3-(Dimethylamino)-3-phenylpropyl]-1*H*-isoindole-1',3'(2*H*)-dione ((*R*)-198)



(3*R*)-3-(Dimethylamino)-3-phenylpropan-1-ol (**(*R*)-198**) (313 mg, 1.74 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (3.40 mL). Triphenylphosphine (527 mg, 2.01 mmol, 1.2 eq.) and phthalimide (295 mg, 2.01 mmol, 1.2 eq.) were added. The reaction mixture was cooled to 0 °C and diisopropyl azodicarboxylate (0.390 mL, 2.01 mmol, 1.2 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 3 h. After this time TLC analysis indicated complete consumption of starting material (3:47 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–3% MeOH/CH_2Cl_2 + 1% Et_3N gradient). The product was further purified using silica flash column chromatography (elution with 0–100% EtOAc/petroleum ether + 1% Et_3N gradient) and further purified using silica flash column chromatography (elution with 0–100% diethyl ether/petroleum ether gradient) to yield the title compound as a pale yellow oil (397 mg, 1.29 mmol, 74%): R_f 0.09 (EtOAc); $[\alpha]_D^{25}$ = –16.1 (*c* 1.0, MeOH); $\bar{\nu}_{max}/cm^{-1}$ 3027 (w), 2939 (w), 2822 (w), 2779 (w), 2361 (w), 1772 (w), 1710 (C=O, s), 1439 (w), 1397 (w), 1371 (w), 1188 (w), 1019 (w); ^1H NMR (500 MHz, $CDCl_3$) δ 7.84–7.76 (2H, m, $C^{4'}$-H, $C^{7'}$-H), 7.73–7.65 (2H, m, $C^{5'}$-H, $C^{6'}$-H), 7.34–7.27 (2H, m, C^8-H, C^{10}-H), 7.27–7.17 (3H, m, C^7-H, C^9-H, C^{11}-H), 3.70 (1H, ddd, *J* 13.8, 8.8, 5.9, C^1-H_a), 3.62 (1H, ddd, *J* 13.8, 8.6, 6.3, C^1-H_b), 3.42 (1H, dd, *J* 8.4, 6.0,

C^3-H), 2.34–2.24 (1H, m, C^2-H_a), 2.24–2.13 (1H, m, C^2-H_b), 2.17 (6H, s, C^4-H_3, C^5-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.4 ($C^{1'}$, $C^{3'}$), 138.4 (C^6), 133.9 ($C^{5'}$, $C^{6'}$), 132.3 ($C^{3'a}$, $C^{7'a}$), 128.7 (C^7, C^{11}), 128.2 (C^8, C^{10}), 127.4 (C^9), 123.1 ($C^{4'}$, $C^{7'}$), 68.2 (C^3), 42.1 (C^4, C^5), 36.1 (C^1), 31.3 (C^2); HRMS [Found: 309.15983, $C_{19}H_{21}N_2O_2$ requires $[M+H]^+$ 309.15975]; LRMS *m/z* (ESI^+) 309.2 ($[M+H]^+$, 58%); HPLC retention time 6.8 min, >99.9%; Chiral HPLC, Chiralpak AS-H column (90:10 hexane/isopropyl alcohol, 1.5 mL min^{-1}) retention time 5.25 min (**(*R*)-199**), >99% e.e. (other enantiomer (**(*S*)-199**) not observed, retention time 7.70 min).

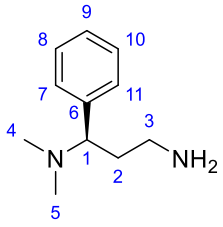
(1*S*)-*N*',*N*'-Dimethyl-1-phenylpropane-1,3-diamine ((*S*)-199)



2-[(3*S*)-3-(Dimethylamino)-3-phenylpropyl]-1*H*-isoindole-1,3(2*H*)-dione (**(*S*)-199**) (293 mg, 0.950 mmol, 1.0 eq.) was dissolved in EtOH (5.00 mL) and hydrazine monohydrate (0.709 mL, 9.50 mmol, 10 eq.) was added. The reaction mixture was heated to 70 °C and stirred for 1 h. After this time LCMS analysis indicated complete consumption of starting material. The reaction mixture was cooled to rt and an aqueous solution of HCl (2 M, 15 mL) was added. The reaction mixture was stirred at rt for 15 min. After this time the reaction mixture was filtered. The filtrate was extracted with a saturated aqueous solution of $NaHCO_3$ (15 mL). The aqueous fraction was concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (7 M, MeOH)) to yield the title compound as a colourless oil (125 mg, 0.701 mmol, 74%): R_f 0.13 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = +16.9 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 3347 (N-H, br), 1572 (br), 1453 (m), 1389 (w), 1313 (w), 1157 (w), 1097 (w), 1065 (w), 1032 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.39–7.31 (2H, m, C^8-H, C^{10}-H), 7.31–7.24 (3H, m, C^7-H, C^9-H, C^{11}-H), 3.33 (1H, dd, *J* 9.9, 5.0, C^1-H), 2.53–2.44 (1H, m, C^3-H_a), 2.44–2.35 (1H, m, C^3-H_b), 2.17 (6H, s, C^4-H_3, C^5-H_3), 2.15–2.07 (1H, m, C^2-H_a), 2.01–1.89 (1H, m, C^2-H_b); ^{13}C NMR (126 MHz, CD_3OD) δ 140.1 (C^6), 129.8 (C^7, C^{11}), 129.3 (C^8, C^{10}), 128.7 (C^9), 70.0 (C^1),

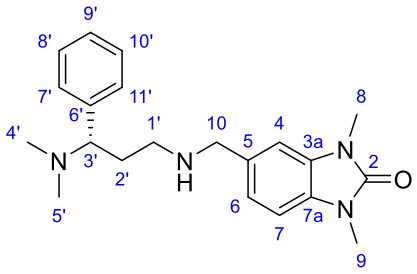
42.9 (C^4, C^5), 40.1 (C^3), 36.5 (C^2); HRMS *m/z* (ESI^+) [Found: 179.15417, $C_{11}H_{19}N_2$ requires $[M+H]^+$ 179.15428]; LRMS *m/z* (ESI^+) 179.1 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.73 min, 91%.

(1*R*)-*N*',*N*'-Dimethyl-1-phenylpropane-1,3-diamine ((*R*)-199)



2-[(3*R*)-3-(Dimethylamino)-3-phenylpropyl]-1*H*-isoindole-1,3(2*H*)-dione (**(*R*)-199**) (42 mg, 0.14 mmol, 1.0 eq.) was dissolved in EtOH (1.8 mL) and hydrazine monohydrate (75 µL, 1.5 mmol, 9.3 eq.) was added. The reaction mixture was heated to 70 °C and stirred for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + 1% Et_3N). The reaction mixture was cooled to 0 °C, filtered through a pad of Celite® and washed with cold EtOH (10 mL). The filtrate was concentrated *in vacuo*. The residue was suspended in CH_2Cl_2 (10 mL) and filtered through cotton wool. The filtrate was concentrated *in vacuo* to yield the title compound as a colourless oil (16 mg, 0.089 mmol, 64%): R_f 0.07 (EtOH + 1% Et_3N); $[\alpha]_D^{25}$ = –16.7 (*c* 1.0, MeOH); $\bar{\nu}_{max}/cm^{-1}$ 3027 (br), 2934 (br), 2860 (br), 2820 (br), 2775 (br), 2161 (br), 1977 (br), 1637 (w), 1566 (m), 1492 (m), 1452 (w), 1400 (w), 1256 (w), 1183 (w), 1155 (w), 1097 (w), 1042 (w), 917 (w); ^1H NMR (500 MHz, CD_3OD) δ 7.42–7.23 (5H, m, C^7-H, C^8-H, C^9-H, C^{10}-H, C^{11}-H), 3.37 (1H, dd, *J* 9.6, 5.3, C^1-H), 2.62–2.52 (1H, m, C^3-H_a), 2.51–2.42 (1H, m, C^3-H_b), 2.18 (6H, s, C^4-H_3, C^5-H_3), 2.22–2.11 (1H, m, C^2-H_a), 2.03–1.90 (1H, m, C^2-H_b); ^{13}C NMR (126 MHz, CD_3OD) δ 139.7 (C^6), 129.8 (C^7, C^{11}), 129.4 (C^8, C^{10}), 128.8 (C^9), 69.9 (C^1), 42.8 (C^4, C^5), 39.9 (C^3), 35.3 (C^2); HRMS *m/z* (ESI^+) [Found: 179.15417, $C_{11}H_{19}N_2$ requires $[M+H]^+$ 179.15428]; LRMS *m/z* (ESI^+) 179.2 ($[M+H]^+$, 100%); unable to obtain a HPLC trace due to multiple protonation states.

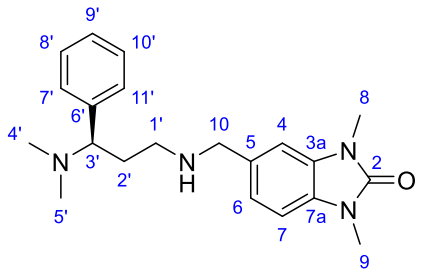
5-({[(3*S*)-3'-(Dimethylamino)-3'-phenylprop-1'-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*S*)-179)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (79 mg, 0.42 mmol, 1.2 eq.) was dissolved in anhydrous 1,2-dichloroethane (4.0 mL) and 3 Å molecular sieves were added. (1*S*)-*N*',*N*'-Dimethyl-1-phenylpropane-1,3-diamine (**(*S*)-200**) (62 mg, 0.35 mmol, 1.0 eq.) and acetic acid (24 μL, 0.42 mmol, 1.2 eq.) were added. The reaction mixture was stirred at rt for 30 min. $NaBH(OAc)_3$ (0.37 g, 1.7 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 22 h. After this time LCMS analysis indicated complete consumption of starting material. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (3 × 10 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (7 M, MeOH)) and further purified using silica flash column chromatography (elution with 0–100% (10% MeOH/CH_2Cl_2)/CH_2Cl_2 + 1% NH_3 (7 M, MeOH) gradient) to yield the title compound as a colourless oil (28 mg, 0.078 mmol, 23%): R_f 0.09 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = +11.4 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 2938 (w), 1695 (C=O, s), 1507 (w), 1450 (w), 1395 (w), 1267 (w), 1138 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.28 (2H, m, C$^{8'}$-H, C$^{10'}$-H), 7.28–7.23 (1H, m, C$^{9'}$-H), 7.23–7.17 (2H, m, C$^{7'}$-H, C$^{11'}$-H), 6.96 (1H, dd, *J* 7.9, 1.3, C^6-H), 6.91 (1H, d, *J* 1.3, C^4-H), 6.86 (1H, d, *J* 7.9, C^7-H), 3.75 (1H, d, *J* 12.9, C^{10}-H$_a$), 3.70 (1H, d, *J* 12.9, C^{10}-H$_b$), 3.398 (3H, s, C$^{8/9}$-H$_3$), 3.395 (3H, s, C$^{8/9}$-H$_3$), 3.28 (1H, dd, *J* 8.9, 5.6, C$^{3'}$-H), 2.59–2.44 (2H, m, C$^{1'}$-H$_2$), 2.17 (6H, s, C$^{4'}$-H$_3$, C$^{5'}$-H$_3$), 2.19–2.07 (1H, m, C$^{2'}$-H$_a$), 1.99–1.88 (1H, m, C$^{2'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^2), 139.9 (C$^{6'}$), 134.0 (C^5), 130.3 (C^{3a}), 129.1 (C^{7a}), 128.6 (C$^{7'}$, C$^{11'}$), 128.1 (C$^{8'}$, C$^{10'}$), 127.3 (C$^{9'}$),

121.2 (C^6), 107.3 (C^4), 107.0 (C^7), 69.2 ($C^{3'}$), 54.4 (C^{10}), 47.2 ($C^{1'}$), 42.8 ($C^{4'}$, $C^{5'}$), 33.5 ($C^{2'}$), 27.27 (C^8), 27.26 (C^9); HRMS *m/z* (ESI^+) [Found: 353.23306, $C_{21}H_{29}N_4O$ requires $[M+H]^+$ 353.23359]; LRMS *m/z* (ESI^+) 353.3 ($[M+H]^+$, 100%); LCMS (basic) retention time 0.86 min, >99%; Chiral HPLC, Phenomonex C4 column (40 °C, 45% MeOH in NH_3) retention time 2.08 min (**(*S*)-179**), >99% e.e. (other enantiomer (**(*R*)-179**) not observed, retention time 2.40 min).

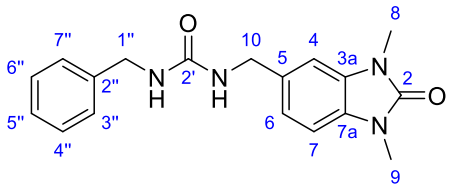
5-({[(3*R*)-3'-(Dimethylamino)-3'-phenylprop-1'-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*R*)-179)



1,3-Dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one-5-carbaldehyde (**34**) (32 mg, 0.17 mmol, 1.5 eq.) was dissolved in anhydrous 1,2-dichloroethane (4.0 mL) and 3 Å molecular sieves were added. (*R*)-*N*',*N*'-Dimethyl-1-phenylpropane-1,3-diamine (**(*R*)-200**) (20 mg, 0.11 mmol, 1.0 eq.) and acetic acid (9.8 μL, 0.17 mmol, 1.5 eq.) were added. The reaction mixture was stirred at rt for 4.5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated complete imine formation. $NaBH(OAc)_3$ (0.12 g, 0.57 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 17 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (3 × 30 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–10% MeOH/CH_2Cl_2 + 1% Et_3N gradient) to yield the title compound as a colourless oil (10 mg, 0.029 mmol, 26%): R_f 0.09 (1:9 MeOH/CH_2Cl_2 + 1% NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = –8.7 (*c* 1.0, MeOH); $\bar{\nu}_{max}$/cm^{-1} 2936 (br), 2822 (br), 2779 (br), 2360 (br), 2341 (br), 1698 (C=O, s), 1509 (w), 1453 (w), 1397 (w), 1267 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.24 (3H, m, $C^{8'}$-H, $C^{9'}$-H, $C^{10'}$-H), 7.22–7.16 (2H, m,

$C^{7'}$-H, $C^{11'}$-H), 6.98 (1H, dd, *J* 7.9, 1.4, C^{6}-H), 6.96 (1H, d, *J* 1.4, C^{4}-H), 6.88 (1H, d, *J* 7.9, C^{7}-H), 3.82 (1H, d, *J* 12.9, C^{10}-H_a), 3.74 (1H, d, *J* 12.9, C^{10}-H_b), 3.40 (6H, s, C^{8}-H_3, C^{9}-H_3), 3.35 (1H, dd, *J* 8.2, 6.3, $C^{3'}$-H), 2.60 (2H, t, *J* 7.3, $C^{1'}$-H_2), 2.31–2.20 (1H, m, $C^{2'}$-H_a), 2.17 (6H, s, $C^{4'}$-H_3, $C^{5'}$-H_3), 1.99–1.87 (1H, m, $C^{2'}$-H_b); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.9 (C^{2}), 138.9 ($C^{6'}$), 132.6 (C^{5}), 130.4 (C^{3a}), 129.4 (C^{7a}), 128.7 ($C^{7'}$, $C^{11'}$), 128.2 ($C^{8'}$, $C^{10'}$), 127.5 ($C^{9'}$), 121.4 (C^{6}), 107.6 (C^{4}), 107.2 (C^{7}), 69.3 ($C^{3'}$), 53.9 (C^{10}), 47.2 ($C^{1'}$), 42.4 ($C^{4'}$, $C^{5'}$), 32.4 ($C^{2'}$), 27.3 (C^{8}, C^{9}); HRMS *m/z* (ESI$^+$) [Found: 353.23373, $C_{21}H_{29}N_4O$ requires $[M+H]^+$ 353.23359]; LRMS *m/z* (ESI$^+$) 353.2 ($[M+H]^+$, 11%); HPLC retention time 5.2 min, 98.7%; Chiral HPLC, Phenomonex C4 column (40 °C, 45% MeOH in NH_3) retention time 2.40 min (**(*R*)-179**), >99% e.e. (other enantiomer (**(*S*)-179**) not observed, retention time 2.08 min).

***N*-Benzyl-*N*'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]urea (200)**

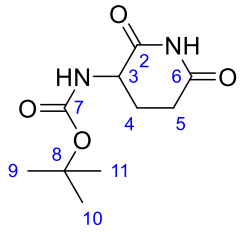


5-(Aminomethyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**26**) (23 mg, 0.12 mmol, 1.0 eq.) was suspended in tetrahydrofuran (1.0 mL) and benzyl isocyanate (15 μL, 0.12 mmol, 1.0 eq.) was added. The reaction mixture was stirred at rt for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:2:6 NH_4OH/EtOH/EtOAc). The reaction mixture was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−3% EtOH/EtOAc + 1% Et_3N gradient) and further purified using silica flash column chromatography (elution with 0−8% EtOH/CH_2Cl_2 gradient) to yield the title compound as a colourless solid (19 mg, 0.059 mmol, 49%): R_f 0.24 (1:24 EtOH/$CHCl_3$); m.p. 167–169 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 2954 (br), 2160 (br), 1722 (C=O, s), 1658 (m), 1432 (m), 1399 (m), 1234 (s), 1116 (s), 1035 (w), 979 (w), 942 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.33–7.23 (4H, m, $C^{3''}$-H, $C^{4''}$-H, $C^{6''}$-H, $C^{7''}$-H), 7.26–7.18 (1H, m, $C^{5''}$-H), 7.11–7.03 (3H, m, C^{4}-H, C^{6}-H, C^{7}-H), 4.38 (2H, s, C^{10}-H_2), 4.34 (2H, s, $C^{1''}$-H_2), 3.40 (3H, s, $C^{8/9}$-H_3), 3.38 (3H, s, $C^{8/9}$-H_3); ^{13}C NMR (126 MHz, CD_3OD) δ 161.0 ($C^{2'}$), 156.4 (C^{2}), 141.3 ($C^{2''}$), 135.3 (C^{5}), 131.3

($C^{3a/7a}$), 130.2 ($C^{3a/7a}$), 129.5 ($C^{4''}$, $C^{6''}$), 128.2 ($C^{3''}$, $C^{7''}$), 128.0 ($C^{5''}$), 121.9 (C^{6}), 108.8 (C^{7}), 108.0 (C^{4}), 45.0 (C^{10}), 44.8 ($C^{1''}$), 27.5 ($C^{8/9}$), 27.4 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 325.16613, $C_{18}H_{21}N_4O$ requires $[M+H]^+$ 325.16590]; LRMS *m/z* (ESI^+) 347.2 ($[M+Na]^+$, 100%); HPLC retention time 6.5 min, >99.9%.

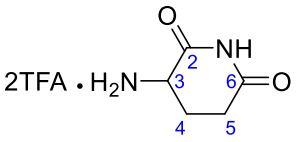
Chapter 4 Compounds

tert-Butyl (2,6-dioxopiperidin-3-yl)carbamate (224)[177,237,238]



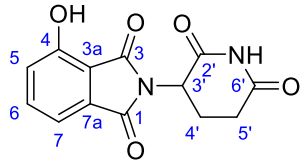
Boc-Gln-OH (9.00 g, 36.5 mmol, 1.0 eq.), 1,1'-carbonyldiimidazole (6.52 g, 40.2 mmol, 1.1 eq.) and 4-(dimethylamino)pyridine (18.0 mg, 0.146 mmol, 0.004 eq.) were dissolved in anhydrous tetrahydrofuran (90.0 mL). The reaction mixture was heated to 66 °C and stirred for 3 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + 1% Et_3N). The reaction mixture was concentrated *in vacuo*. The resulting solid was dissolved in CH_2Cl_2 (200 mL) and washed with H_2O (200 mL). The aqueous fraction was extracted with CH_2Cl_2 (2 × 200 mL). The organic fractions were combined and washed with brine (400 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a colourless solid (7.47 g, 32.7 mmol, 90%): R_f 0.53 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); $[\alpha]_D^{25}$ = –48.9 (*c* 1.0, MeOH); d.p. >160 °C (from CH_2Cl_2) [lit.[239] 143–144 °C (from EtOAc), lit.[237] 209–211 °C, lit.[240] 214–215 °C]; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.74 (1H, br s, N-H), 7.13 (1H, d, *J* 8.8, N-H), 4.28–4.18 (1H, m, C^3-H), 2.76–2.65 (1H, m, C^5-H_a), 2.51–2.42 (1H, m, C^5-H_b), 2.05–1.79 (2H, m, C^4-H_2), 1.39 (9H, s, C^9-H_3, C^{10}-H_3, C^{11}-H_3); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.0 (C^6), 172.5 (C^2), 155.4 (C^7), 78.2 (C^8), 50.4 (C^3), 31.0 (C^5), 28.2 (C^9, C^{10}, C^{11}), 24.4 (C^4); LRMS *m/z* (ESI$^-$) 227.1 ([M–H]$^-$, 100%). The spectroscopic data are in good agreement with literature values.[177,237,238]

3-Aminopiperidine-2,6-dione trifluoroacetate (225)[177,237,241]



tert-Butyl (2,6-dioxopiperidin-3-yl)carbamate (**224**) (7.45 g, 32.6 mmol, 1.0 eq.) was suspended in CH_2Cl_2 (45.0 mL). The reaction mixture was cooled to 0 °C. Trifluoroacetic acid (15.0 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 10 min then warmed to rt and stirred for 5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* to yield the title compound as a light pink solid (10.5 g, 29.4 mmol, 90%): R_f 0.13 (1:9 MeOH/CH_2Cl_2 + NH_3 (7 M, MeOH)); $[\alpha]_D^{25}$ = –28.7 (*c* 1.0, MeOH); m.p. 135–137 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3032 (br), 2840 (br), 2532 (br), 1724 (C=O, s), 1660 (C=O, s), 1528 (w), 1467 (w), 1434 (w), 1403 (w), 1370 (w), 1347 (w), 1262 (w), 1182 (C-F, s), 1121 (C-F, s), 1093 (w), 1040 (w), 1015 (w), 965 (w), 924 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27 (1H, br s, N-H), 8.46 (3H, br s, N-H, N-H$_2$), 4.25–4.17 (1H, m, C^3-H), 2.71 (1H, ddd, *J* 17.7, 13.3, 5.5, C^5-H$_a$), 2.66–2.54 (1H, m, C^5-H$_b$), 2.20–2.09 (1H, m, C^4-H$_a$), 2.08–1.92 (1H, m, C^4-H$_b$); ^{19}F NMR (376 MHz, DMSO-d_6) δ –74.8; LRMS *m/z* (ESI$^+$) 129.0 ([M+H]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[177,237,241]

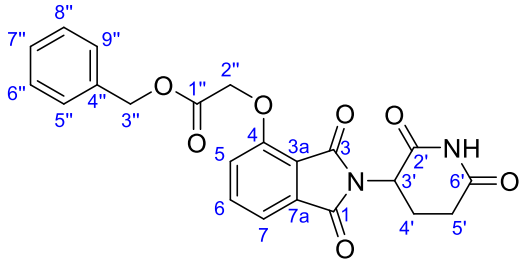
2-(2',6'-Dioxopiperidin-3'-yl)-4-hydroxy-1*H*-isoindole-1,3(2*H*)-dione (227)[177,183,242]



3-Hydroxyphthalic anhydride (1.50 g, 9.14 mmol, 1.0 eq.) and 3-aminopiperidine-2,6-dione trifluoroacetate (**225**) (3.25 g, 9.14 mmol, 1.0 eq.) were dissolved in anhydrous tetrahydrofuran (150 mL). Anhydrous Et_3N (3.82 mL, 27.4 mmol, 3.0 eq.) was added. The reaction mixture was heated to 70 °C and stirred for 4 h. The reaction mixture was cooled to rt and *N*,*N*′-dicyclohexylcarbodiimide (1.89 g, 9.14 mmol, 1.0 eq.) and 4-(dimethylamino)pyridine

(112 mg, 0.914 mmol, 0.1 eq.) were added. The reaction mixture was heated to 70 °C and stirred for 20 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was cooled to 0 °C and the precipitate removed *via* filtration. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–100% EtOAc/petroleum ether gradient) to yield the title compound as a light green solid (2.21 g, 8.04 mmol, 88%): R_f 0.15 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = –4.95 (*c* 1.0, DMSO); d.p. >250 °C [lit.[243] 275–276 °C, lit.[244] 281–282 °C]; $\tilde{\nu}_{max}$/cm^{-1} 3275 (br), 2161 (br), 2032 (br), 1978 (br), 1770 (w), 1694 (C=O, s), 1618 (w), 1461 (w), 1400 (w), 1358 (w), 1324 (w), 1302 (w), 1257 (w), 1199 (m), 1169 (w), 1114 (w), 1022 (w), 994 (w), 954 (w), 909 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 (1H, br s, O-H), 11.07 (1H, br s, N-H), 7.65 (1H, dd, *J* 8.4, 7.2, C^6-H), 7.32 (1H, d, *J* 7.2, C^7-H), 7.25 (1H, dd, *J* 8.4, 0.8, C^5-H), 5.07 (1H, dd, *J* 12.9, 5.4, C$^{3'}$-H), 2.95–2.81 (1H, m, C$^{4'}$-H$_a$), 2.64–2.44 (2H, m, C$^{5'}$-H$_2$), 2.08–1.96 (1H, m, C$^{4'}$-H$_b$); HRMS *m/z* (APCI) [Found: 275.06627, $C_{13}H_{11}N_2O_5$ requires $[M+H]^+$ 275.06625]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[177,183,242]

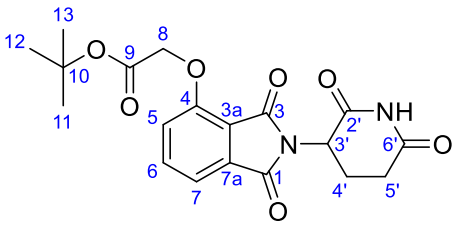
Benzyl {[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetate (228)[177]



2-(2,6-Dioxopiperidin-3-yl)-4-hydroxy-1*H*-isoindole-1,3(2*H*)-dione (**227**) (500 mg, 1.82 mmol, 1.0 eq.) was suspended in anhydrous tetrahydrofuran (20.0 mL). Triphenylphosphine (717 mg, 2.73 mmol, 1.5 eq.) and benzyl glycolate (389 µL, 2.00 mmol, 1.1 eq.) were added. The reaction mixture was cooled to 0 °C. Diisopropyl azodicarboxylate (415 µL, 2.00 mmol, 1.1 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 48 h. After this time TLC

analysis indicated complete consumption of starting material (1:1 EtOAc/petroleum ether). The reaction was quenched with H_2O (20 mL) and extracted with EtOAc (3 × 20 mL). The organic fractions were combined, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (512 mg, 1.21 mmol, 67%): R_f 0.35 (7:3 EtOAc/petroleum ether); $[\alpha]_D^{25}$ = +2.5 (*c* 1.0, MeOH); m.p. 110–112 °C (from EtOAc); $\tilde{\nu}_{max}$/cm^{-1} 2161 (br), 2032 (br), 1977 (br), 1703 (C=O, s), 1613 (w), 1484 (w), 1455 (w), 1394 (w), 1357 (w), 1323 (w), 1298 (w), 1259 (w), 1192 (m), 1122 (w), 1100 (w), 1083 (w), 1063 (w), 1022 (w), 988 (w), 910 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (1H, br s, N-H), 7.62 (1H, dd, *J* 8.4, 7.3, C^6-H), 7.52 (1H, d, *J* 7.3, C^5-H), 7.41–7.30 (5H, m, C$^{5''}$-H, C$^{6''}$-H, C$^{7''}$-H, C$^{8''}$-H, C$^{9''}$-H), 7.08 (1H, d, *J* 8.4, C^7-H), 5.24 (2H, s, C$^{3''}$-H$_2$), 5.08–4.93 (1H, m, C$^{3'}$-H), 4.94 (2H, s, C$^{2''}$-H$_2$), 2.94–2.68 (3H, m, C$^{4'}$-H$_a$, C$^{5'}$-H$_2$), 2.18–2.08 (1H, m, C$^{4'}$-H$_b$); HRMS *m/z* (APCI) [Found: 423.11859, $C_{22}H_{19}N_2O_7$ requires $[M+H]^+$ 423.11868]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[177]

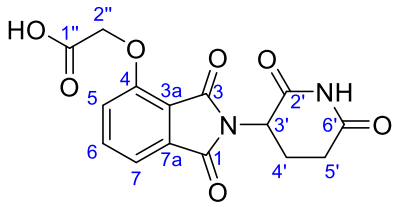
tert-Butyl {[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetate (230)[245,246]



2-(2,6-Dioxopiperidin-3-yl)-4-hydroxy-1*H*-isoindole-1,3(2*H*)-dione (**227**) (1.00 g, 3.64 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (30.0 mL). K_2CO_3 (503 mg, 5.46 mmol, 1.5 eq.) and *tert*-butyl bromoacetate (538 µL, 3.64 mmol, 1.0 eq.) were added. The reaction mixture was stirred at rt for 2 h. After this time TLC analysis indicated partial consumption of starting material (1:9 MeOH/CH_2Cl_2). Additional *tert*-butyl bromoacetate (269 µL, 1.82 mmol, 0.5 eq.) was added. The reaction mixture was stirred at rt for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The

reaction was quenched with an aqueous solution of LiCl (0.5 M, 50 mL) and extracted with EtOAc (50 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 50 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–4% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless solid (389 mg, 1.00 mmol, 28%): R_f 0.29 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = +3.16 (*c* 1.0, $CHCl_3$); m.p. 196–198 °C (from MeOH); $\tilde{\nu}_{max}$/cm^{-1} 3322 (m), 2928 (s), 2850 (s), 2161 (m), 2033 (m), 1978 (m), 1776 (w), 1709 (m), 1624 (C=O, s), 1571 (C=O, s), 1538 (m) 1488 (w), 1436 (w), 1394 (w), 1367 (w), 1357 (w), 1310 (w), 1270 (m), 1242 (w), 1229 (w), 1186 (m), 1159 (w), 1124 (w), 1089 (m), 1068 (w), 1046 (w), 1023 (w), 991 (w), 968 (w), 941 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (1H, br s, N-H), 7.96 (1H, br s, N-H), 7.61 (1H, dd, *J* 8.5, 7.3, C^6-H), 7.44 (1H, dd, *J* 7.3, 0.7, C^7-H), 7.05 (1H, dd, *J* 8.5, 0.7, C^5-H), 4.99–4.90 (1H, m, C$^{3'}$-H), 4.74 (2H, s, C^8-H_2), 2.86–2.64 (3H, m, C$^{4'}$-H_a, C$^{5'}$-H_2), 2.13–2.02 (1H, m, C$^{4'}$-H_b), 1.42 (9H, s, C^{11}-H_3, C^{12}-H_3, C^{13}-H_3); LRMS *m/z* (ESI$^-$) 387.0 ([M–H]$^-$, 95%). The spectroscopic data are in good agreement with literature values.[245,246]

{[2-(2',6'-Dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetic acid (229)[177,183,245]



Method 1

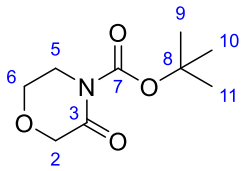
Benzyl {[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetate (**228**) (300 mg, 0.780 mmol, 1.0 eq.) was suspended in EtOAc (10.0 mL) and CH_2Cl_2 (4.00 mL). The reaction mixture was purged with N_2. Pd on carbon (10% *w*/*w*, 33.0 mg, 0.0313 mmol, 0.04 eq.) was added and the reaction mixture purged with H_2. The reaction mixture was stirred at rt under an atmosphere of H_2 for 2 h. After this time TLC analysis indicated complete consumption of starting material (EtOAc). The reaction mixture was purged with N_2. The reaction mixture was

diluted with MeOH (15 mL), heated to 60 °C and stirred for 1 h. The hot reaction mixture was filtered through a pad of Celite® and the filter pad washed with hot MeOH. The filtrate was concentrated *in vacuo*. The product was purified using an ISOLUTE® PE-AX column (elution with 1 M aqueous HCl) to yield the title compound as a colourless solid (194 mg, 0.584 mmol, 75%): R_f 0.10 (EtOH + 1% AcOH); $[\alpha]_D^{25}$ = –13.1 (*c* 1.0, DMSO); m.p. 221–223 °C (from H_2O); $\bar{\nu}_{max}$/cm^{-1} 3524 (br), 3242 (br), 3082 (br), 2899 (br), 2161 (br), 1768 (C=O, m), 1709 (C=O, s), 1658 (C=O, s), 1610 (m), 1517 (w), 1485 (w), 1444 (w), 1425 (w), 1399 (w), 1349 (w), 1322 (w), 1305 (w), 1284 (w), 1260 (w), 1205 (w), 1123 (s), 1101 (w), 1081 (w), 1059 (w), 1020 (w), 988 (w), 938 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.25 (1H, br s, O-H), 11.11 (1H, br s, N-H), 7.80 (1H, dd, *J* 8.6, 7.3, C^6-H), 7.48 (1H, d, *J* 7.3, C^5-H), 7.40 (1H, d, *J* 8.6, C^7-H), 5.11 (1H, dd, *J* 12.9, 5.4, C$^{3'}$-H), 4.99 (2H, s, C$^{2''}$-H$_2$), 2.97–2.83 (1H, m, C$^{4'}$-H$_a$), 2.66–2.52 (2H, m, C$^{5'}$-H$_2$), 2.11–1.99 (1H, m, C$^{4'}$-H$_b$); LRMS *m/z* (ESI$^-$) 331.0 ([M–H]$^-$, 100%). The spectroscopic data are in good agreement with literature values.[177,183,245]

Method 2

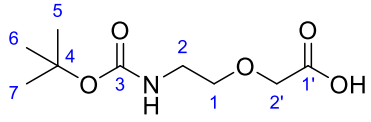
tert-Butyl {[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetate (**230**) (389 mg, 1.00 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (10.0 mL) and trifluoroacetic acid (5.00 mL) was added. The reaction mixture was stirred at rt for 45 min. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. The product was purified using an ISOLUTE® PE-AX column (elution with 1 M aqueous HCl) to yield the title compound as a colourless solid (180 mg, 0.542 mmol, 54%): R_f 0.10 (EtOH + 1% AcOH); $[\alpha]_D^{25}$ = –2.93 (c 1.0, DMSO); m.p. 220–221 °C (from CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.24 (1H, br s, O-H), 11.10 (1H, br s, N-H), 7.79 (1H, dd, *J* 8.5, 7.2, C^6-H), 7.48 (1H, d, *J* 7.2, C^5-H), 7.39 (1H, d, *J* 8.6, C^7-H), 5.10 (1H, dd, *J* 12.7, 5.2, C$^{3'}$-H), 4.99 (2H, s, C$^{2''}$-H$_2$), 2.96–2.82 (1H, m, C$^{4'}$-H$_a$), 2.64–2.51 (2H, m, C$^{5'}$-H$_2$), 2.11–1.99 (1H, m, C$^{4'}$-H$_b$); LRMS *m/z* (ESI$^-$) 331.0 ([M–H]$^-$, 100%). The spectroscopic data are in good agreement with those obtained for method 1.

tert-Butyl 3-oxomorpholine-4-carboxylate (235)[247]



Morpholin-3-one (1.00 g, 9.89 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (12.0 mL). Di-*tert*-butyl dicarbonate (3.24 g, 14.8 mmol, 1.5 eq.) and 4-dimethylaminopyridine (121 mg, 0.989 mmol, 0.1 eq.) were added. The reaction mixture was stirred at rt for 3.5 h. After this time TLC analysis indicated complete consumption of starting material (3:2 EtOAc/petroleum ether). The reaction mixture was cooled to 0 °C and imidazole (673 mg, 9.89 mmol, 1.0 eq.) was added. The reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was warmed to rt, diluted with EtOAc (30 mL) and washed sequentially with an aqueous solution of HCl (1 M, 30 mL), a saturated aqueous solution of $NaHCO_3$ (30 mL), and brine (30 mL). The organic fraction was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified through a plug of silica (elution with EtOAc) to yield the title compound as a colourless oil (1.50 g, 7.43 mmol, 75%): R_f 0.53 (3:2 EtOAc/petroleum ether); $\tilde{\nu}_{max}$/cm^{-1} 2980 (w), 1774 (C=O, m), 1715 (C=O, s), 1461 (w), 1396 (w), 1369 (w), 1344 (w), 1285 (s), 1239 (m), 1139 (s), 1105 (w), 1060 (w), 1004 (w), 952 (w), 915 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.08 (2H, s, C^2-H$_2$), 3.81–3.74 (2H, m, C^6-H$_2$), 3.66–3.59 (2H, m, C^5-H$_2$), 1.42 (9H, s, C^9-H$_3$, C^{10}-H$_3$, C^{11}-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.1 (C^3), 150.9 (C^7), 83.4 (C^8), 68.9 (C^2), 63.8 (C^6), 45.2 (C^5), 27.2 (C^9, C^{10}, C^{11}); LRMS *m/z* (ESI$^+$) 224.0 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[247]

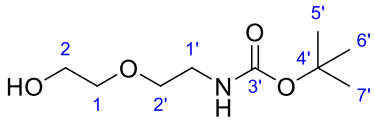
{2'-[(_tert_-Butoxycarbonyl)amino]ethoxy}acetic acid (231)[247,248]



tert-Butyl 3-oxomorpholine-4-carboxylate (**235**) (1.42 g, 7.05 mmol, 1.0 eq.) was dissolved in tetrahydrofuran (7.00 mL) and cooled to 0 °C. An aqueous solution of LiOH (1 M, 21.2 mL,

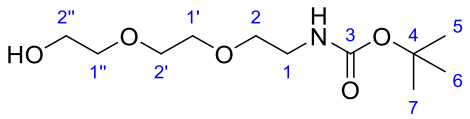
21.2 mmol, 3.0 eq.) was added. The reaction mixture was warmed to rt and stirred for 4 h. After this time TLC analysis indicated complete consumption of starting material (3:2 EtOAc/petroleum ether). The reaction mixture was diluted with an aqueous solution of $KHSO_4$ (0.5 M, 40 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a colourless oil (1.46 g, 6.66 mmol, 94%): R_f 0.30 (1:9 MeOH/CH_2Cl_2 + drop AcOH); $\bar{\nu}_{max}$/cm^{-1} 2979 (O-H, br), 1694 (C=O, s), 1521 (m), 1455 (w), 1429 (w), 1393 (w), 1367 (m), 1249 (m), 1133 (s), 1041 (w), 1024 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.19 (1H, br s, O-H), 5.29 (1H, br s, N-H), 4.09 (2H, s, C$^{2'}$-H$_2$), 3.59 (2H, t, *J* 5.1, C^1-H$_2$), 3.31 (2H, t, *J* 5.1, C^2-H$_2$), 1.41 (9H, s, C^5-H$_3$, C^6-H$_3$, C^7-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.2 (C$^{1'}$), 156.5 (C^3), 79.7 (C^4), 70.9 (C^1), 68.1 (C$^{2'}$), 40.4 (C^2), 28.4 (C^5, C^6, C^7); LRMS *m/z* (ESI$^-$) 218.1 ([M–H]$^-$, 100%). The spectroscopic data are in good agreement with literature values.[247,248]

***tert*-Butyl [2'-(2-hydroxyethoxy)ethyl]carbamate (238)**[249–251]



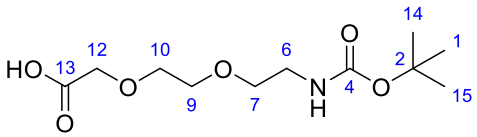
2-(2-Aminoethoxy)ethanol (1.00 mL, 9.97 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (20.0 mL). Di-*tert*-butyl dicarbonate (2.18 g, 9.97 mmol, 1.0 eq.) was added and the reaction mixture stirred at rt for 19 h. After this time TLC analysis indicated complete consumption of starting material (1:18 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* and purified using silica flash column chromatography (elution with 0–9% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (1.86 g, 9.08 mmol, 91%): R_f 0.34 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.10 (1H, br s, N-H), 3.76–3.67 (2H, m, C^2-H$_2$), 3.59–3.49 (4H, m, C^1-H$_2$, C$^{2'}$-H$_2$), 3.35–3.26 (2H, m, C$^{1'}$-H$_2$), 2.68 (1H, t, *J* 5.9, O-H), 1.42 (9H, s, C$^{5'}$-H$_3$, C$^{6'}$-H$_3$, C$^{7'}$-H$_3$); HRMS *m/z* (APCI$^+$) [Found: 206.13884, $C_9H_{20}NO_4$ requires [M+H]$^+$ 206.13868]; unable to detect by LRMS. The spectroscopic data are in good agreement with literature values.[249–251]

tert-Butyl {2-[2'-(2''-hydroxyethoxy)ethoxy]ethyl}carbamate (239)[252–255]



2-[2-(2-Aminoethoxy)ethoxy]ethanol (200 µL, 1.44 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (3.00 mL). Di-*tert*-butyl dicarbonate (315 mg, 1.44 mmol, 1.0 eq.) was added and the reaction mixture stirred at rt for 7 h. After this time TLC analysis indicated partial consumption of starting material (1:19 MeOH/CH_2Cl_2 + drop Et_3N). Additional di-*tert*-butyl dicarbonate (158 mg, 0.72 mmol, 0.5 eq.) was added and the reaction mixture stirred at rt for 17 h. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2 + drop Et_3N). The reaction mixture was concentrated *in vacuo* and purified using silica flash column chromatography (elution with 0–6% EtOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (343 mg, 1.38 mmol, 96%): R_f 0.56 (1:49 EtOH/$CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.29 (1H, br s, N-H), 3.70–3.61 (2H, m, $C^{2''}$-H_2), 3.61–3.52 (6H, m, C^2-H_2, $C^{2'}$-H_2, $C^{1'}$-H_2), 3.48 (2H, t, *J* 5.2, $C^{1''}$-H_2), 3.31–3.19 (2H, m, C^1-H_2), 3.16 (1H, br s, O-H), 1.37 (9H, s, C^5-H_3, C^6-H_3, C^7-H_3); LRMS *m/z* (ESI^+) 272.2 ($[M+H]^+$, 37%). The spectroscopic data are in good agreement with literature values.[252–255]

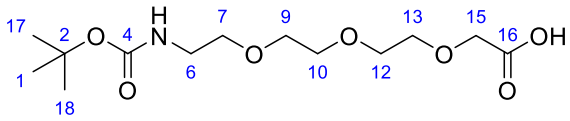
2,2-Dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-oic acid (232)[256–258]



tert-Butyl [2-(2-hydroxyethoxy)ethyl]carbamate (**238**) (500 mg, 2.44 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (5.00 mL). The reaction mixture was cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 390 mg, 9.76 mmol, 1.0 eq.) was added portionwise. Bromoacetic acid (509 mg, 3.66 mmol, 1.5 eq.) was added and the reaction mixture heated to 60 °C and stirred for 17 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was cooled to rt, quenched with H_2O

(40 mL) and washed with EtOAc (40 mL). The aqueous fraction was acidified with an aqueous solution of HCl (2 M) to pH 2–3 and washed with diethyl ether (3 × 40 mL). The aqueous fraction was neutralised with an aqueous solution of NaOH (1 M) and extracted with CH_2Cl_2 (3 × 50 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a colourless oil (223 mg, 0.847 mmol, 35%): R_f 0.10 (1:9 MeOH/CH_2Cl_2 + drop AcOH); $\bar{\nu}_{max}$/cm^{-1} 3352 (br), 2917 (br), 2160 (br), 1977 (br), 1690 (C=O, s), 1523 (m), 1456 (w), 1393 (w), 1366 (m), 1277 (m), 1249 (m), 1149 (C-O, s), 1116 (C-O, s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (2H, s, C^{12}-H_2), 3.73–3.68 (2H, m, $C^{6/7/9/10}$-H_2), 3.66–3.60 (2H, m, $C^{6/7/9/10}$-H_2), 3.58–3.48 (2H, m, $C^{6/7/9/10}$-H_2), 3.34–3.26 (2H, m, $C^{6/7/9/10}$-H_2), 1.41 (9H, s, C^1-H_3, C^{14}-H_3, C^{15}-H_3); LRMS *m/z* (ESI$^-$) 262.1 ([M–H]$^-$, 100%). The spectroscopic data are in good agreement with literature values.[256–258]

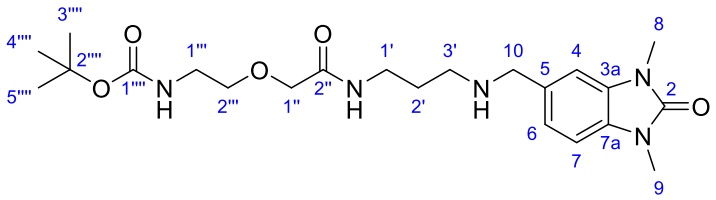
2,2-Dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azahexadecan-16-oic acid (233)



tert-Butyl (2-(2-(2-hydroxyethoxy)ethoxy)ethyl)carbamate (**239**) (343 mg, 1.38 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (4.00 mL). The reaction mixture was cooled to 0 °C and sodium hydride (60% dispersion in mineral oil, 220 mg, 5.50 mmol, 4.0 eq.) was added portionwise. Bromoacetic acid (288 mg, 2.07 mmol, 1.5 eq.) was added and the reaction mixture heated to 60 °C and stirred for 1.5 h. After this time TLC analysis indicated complete consumption of starting material (1:24 MeOH/CH_2Cl_2). The reaction was cooled to rt and quenched with H_2O (40 mL). The reaction mixture was washed with CH_2Cl_2 (40 mL). The aqueous fraction was acidified with an aqueous solution of HCl (1 M) to pH 2–3 and extracted with CH_2Cl_2 (40 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a colourless oil (233 mg, 0.758 mmol, 55%): R_f 0.50 (1:4 EtOH/$CHCl_3$); $\bar{\nu}_{max}$/cm^{-1} 3350 (br), 2873 (br), 2160 (br), 1977 (br), 1709 (C=O, s), 1522 (w), 1456 (w), 1393 (m), 1278 (w), 1249 (m), 1104 (C-O, s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.35 (1H, br s, O-H), 5.13 (1H, br s, N-H), 4.15 (2H, s, C^{15}-H_2), 3.77–3.70 (2H, m, C^{13}-H_2), 3.72–3.62 (4H, m, $C^{9/10/12}$-H_2,

$C^{9/10/12}$-H_2), 3.63–3.58 (2H, m, $C^{9/10/12}$-H_2), 3.52 (2H, t, *J* 5.2, C^{7}-H_2), 3.33–3.23 (2H, m, C^{6}-H_2), 1.42 (9H, s, C^{1}-H_3, C^{17}-H_3, C^{18}-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0 (C^{16}), 156.3 (C^{4}), 79.5 (C^{2}), 71.3 (C^{13}), 70.6 ($C^{7/9/10/12}$), 70.4 ($C^{7/9/10/12}$), 70.3 ($C^{7/9/10/12}$), 70.1 ($C^{7/9/10/12}$), 68.7 (C^{15}), 40.4 (C^{6}), 28.5 (C^{1}, C^{17}, C^{18}); HRMS *m/z* (ESI^+) [Found: 308.17023, $C_{13}H_{26}NO_7$ requires $[M+H]^+$ 308.17038]; LRMS *m/z* (ESI^-) 306.1 ($[M–H]^-$, 100%).

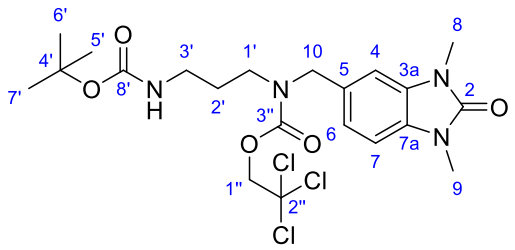
tert-Butyl (2'''-{2''-[(3'-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1'-yl)amino]-2''-oxoethoxy}ethyl)carbamate (240)



2-(2-((*tert*-Butoxycarbonyl)amino)ethoxy)acetic acid (**231**) (74 mg, 0.34 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (4.0 mL). 5-{[(3-Aminopropyl)amino]methyl}-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**107**) (0.10 g, 0.40 mmol, 1.2 eq.), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro phosphate (0.14 g, 0.37 mmol, 1.1 eq.) and *N*,*N*-diisopropylethylamine (0.12 mL, 0.67 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 18 h. After this time TLC analysis indicated complete consumption of starting material (1:2:4 NH_4OH/EtOH/EtOAc). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 25 mL) and extracted with EtOAc (3 × 25 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−8% MeOH/CH_2Cl_2 gradient) and further purified using silica flash column chromatography (elution with 0−8% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (13 mg, 0.029 mmol, 9%): R_f 0.24 (3:47 MeOH/CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3306 (br), 2932 (br), 1687 (C=O, s), 1509 (m), 1449 (m), 1395 (w), 1365 (w), 1250 (w), 1162 (m); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (1H, br s, N-H), 7.05 (1H, dd, *J* 7.9, 1.5, C^{6}-H), 7.01 (1H, s, C^{4}-H), 6.90 (1H, d, *J* 7.9, C^{7}-H), 4.96 (1H, br s, N-H), 3.94 (2H, s, $C^{1''}$-H_2), 3.86 (2H, s, C^{10}-H_2), 3.53 (2H, t, *J* 5.6, $C^{2'''}$-H_2), 3.42–3.35

(8H, m, C^{8}-H_3, C^{9}-H_3, $C^{1'}$-H_2), 3.27 (2H, t, *J* 5.6, $C^{1'''}$-H_2), 2.76 (2H, t, *J* 6.4, $C^{3'}$-H_2), 1.77 (2H, tt, *J* 6.4, 6.4, $C^{2'}$-H_2), 1.42 (9H, s, $C^{3''''}$-H_3, $C^{4''''}$-H_3, $C^{5''''}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.9 ($C^{2''}$), 156.0 ($C^{1''''}$), 154.9 (C^{2}), 132.3 (C^{5}), 130.4 (C^{3a}), 129.5 (C^{7a}), 121.6 (C^{6}), 107.7 (C^{4}), 107.2 (C^{7}), 79.7 ($C^{2''''}$), 71.1 ($C^{2'''}$), 70.5 ($C^{1''}$), 54.1 (C^{10}), 47.1 ($C^{3'}$), 40.4 ($C^{1'''}$), 37.7 ($C^{1'}$), 28.9 ($C^{2'}$), 28.5 ($C^{3''''}$, $C^{4''''}$, $C^{5''''}$), 27.31 ($C^{8/9}$), 27.28 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 450.27081, $C_{22}H_{36}N_5O_5$ requires $[M+H]^+$ 450.27110]; LRMS *m/z* (ESI^+) 472.2 ($[M+Na]^+$, 24%); HPLC retention time 5.8 min, >99.9%.

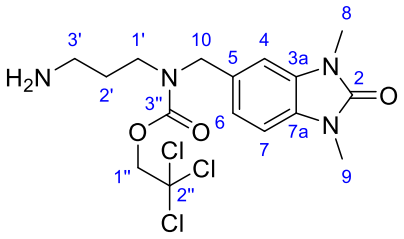
2'',2'',2''-Trichloroethyl {3'-[(*tert*-butoxycarbonyl)amino]prop-1'-yl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (242)



tert-Butyl (3-(((1,3-dimethyl-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one)5-methyl)amino)propyl) carbamate (**116**) (1.20 g, 3.44 mmol, 1.0 eq.) was dissolved in anhydrous CH_2Cl_2 (22.0 mL). 4-(Dimethylamino)pyridine (84.1 mg, 0.688 mmol, 0.2 eq.) and pyridine (388 µL, 4.821 mmol, 1.4 eq.) were added. The reaction mixture was cooled to 0 °C and 2,2,2-trichloroethoxycarbonyl chloride (664 µL, 4.82 mmol, 1.4 eq.) was added. The reaction mixture was warmed to rt and stirred for 15 h. After this time TLC analysis indicated complete consumption of starting material (2:25 MeOH/CH_2Cl_2). The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with H_2O (40 mL). The organic fraction was washed sequentially with an aqueous solution of HCl (1 M, 40 mL), a saturated aqueous solution of $NaHCO_3$ (40 mL), and brine (40 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless foam (292 mg, 0.557 mmol, 65%): R_f 0.38 (1:25 EtOH/$CHCl_3$); $\bar{\nu}_{max}$/cm^{-1} 2936 (br), 2161 (br), 1690 (C=O, s), 1509 (w), 1468 (w), 1443 (w), 1393 (w), 1365 (w), 1268 (w), 1246 (w), 1215 (w), 1167 (w), 1125 (w), 1060 (w), 961 (w); ^{1}H NMR

(500 MHz, DMSO-d_6, 363 K) δ 7.08–6.99 (3H, m, C^4-H, C^6-H, C^7-H), 6.32 (1H, br s, N-H), 4.89 (2H, s, $C^{1''}$-H_2), 4.55 (2H, s, C^{10}-H_2), 3.32 (3H, s, $C^{8/9}$-H_3), 3.31 (3H, s, $C^{8/9}$-H_3), 3.30–3.25 (2H, m, $C^{1'}$-H_2), 2.95 (2H, dt, *J* 7.0, 7.0, $C^{3'}$-H_2), 1.69 (2H, tt, *J* 7.0, 7.0, $C^{2'}$-H_2), 1.37 (9H, s, $C^{5'}$-H_3, $C^{6'}$-H_3, $C^{7'}$-H_3); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 155.1 ($C^{4'}$), 153.7 (C^2), 153.5 ($C^{3''}$), 129.9 (C^5), 129.7 (C^{3a}), 128.9 (C^{7a}), 120.0 (C^6), 106.8 (C^7), 106.4 (C^4), 95.8 ($C^{2''}$), 77.1 ($C^{4'}$), 74.1 ($C^{1''}$), 50.0 (C^{10}), 44.1 ($C^{1'}$), 37.5 ($C^{3'}$), 27.8 ($C^{2'}$, $C^{5'}$, $C^{6'}$, $C^{7'}$), 26.40 ($C^{8/9}$), 26.37 ($C^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 545.10932, $C_{21}H_{29}N_4O_5{}^{35}ClNa$ requires $[M+Na]^+$ 545.10957; Found: 547.10638, $C_{21}H_{29}N_4O_5{}^{37}ClNa$ requires $[M+Na]^+$ 547.10662]; LRMS *m/z* (ESI$^-$) 567.0 ($[M+CH_2O_2–H]^-$, 100%); HPLC retention time 10.4 min, 97.5%.

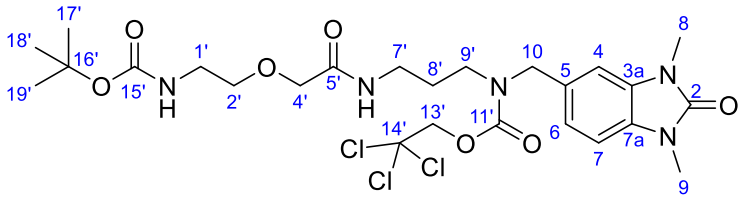
2'',2'',2''-Trichloroethyl (3'-aminoprop-1'-yl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (243)



2,2,2-Trichloroethyl (3-((*tert*-butoxycarbonyl)amino)propyl)((1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)methyl)carbamate (**242**) (0.10 g, 0.19 mmol, 1.0 eq.) was dissolved in HCl (4 M, dioxane) (1.5 mL). The reaction mixture was stirred at rt for 4.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (3 M, MeOH)) to yield the title compound as a colourless oil (55 mg, 0.13 mmol, 68%): R_f 0.10 (EtOH + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 2941 (br), 2872 (br), 2161 (br), 2034 (br), 1979 (br), 1687 (C=O, s), 1612 (w), 1510 (w), 1480 (w), 1466 (w), 1445 (w), 1427 (w), 1395 (w), 1353 (w), 1308 (w), 1274 (w), 1265 (m), 1226 (m), 1203 (m), 1122 (m), 1101 (w), 1084 (w), 1061 (w), 1040 (w), 1029 (w), 1018 (w), 990 (w), 974 (w), 948 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.14–6.96 (3H, m, C^4-H, C^6-H, C^7-H), 4.92 (2H, d, *J* 8.2, $C^{1''}$-H_2), 4.53 (2H, d, *J* 16.2, C^{10}-H_2), 3.43–3.24 (8H, m, C^8-H_3, C^9-H_3, $C^{1'}$-H_2), 2.60 (2H, t, *J* 7.1, $C^{3'}$-H_2),

1.75–1.63 (2H, m, $C^{2'}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.1 (C^{2}), 154.8 (C^{2}), 154.5 ($C^{3''}$), 130.64 ($C^{3a/7a/5}$), 130.58 ($C^{3a/7a/5}$), 130.5 ($C^{3a/7a/5}$), 130.4 ($C^{3a/7a/5}$), 129.8 ($C^{3a/7a/5}$) 121.2 (C^{6}), 121.0 (C^{6}), 107.2 ($C^{4/7}$), 107.1 ($C^{4/7}$), 107.0 ($C^{4/7}$), 95.9 ($C^{2''}$), 75.2 ($C^{1''}$), 51.2 (C^{10}), 50.3 ($C^{10'}$), 44.2 ($C^{1'}$), 39.6 ($C^{3'}$), 39.0 ($C^{3'}$), 32.0 ($C^{2'}$), 30.9 ($C^{2'}$), 27.4 ($C^{8/9}$), 27.3 ($C^{8/9}$), indistinguishable rotamers observed, however compound unstable at higher temperatures; HRMS *m/z* (ESI^+) [Found: 423.07472, $C_{16}H_{22}N_4O_3{}^{35}Cl_3$ requires $[M+H]^+$ 423.07520; Found: 425.07167, $C_{16}H_{22}N_4O_3{}^{37}Cl^{35}Cl_2$ requires $[M+H]^+$ 425.07225]; unable to detect by LRMS; HPLC retention time 6.9 min, 99.0%.

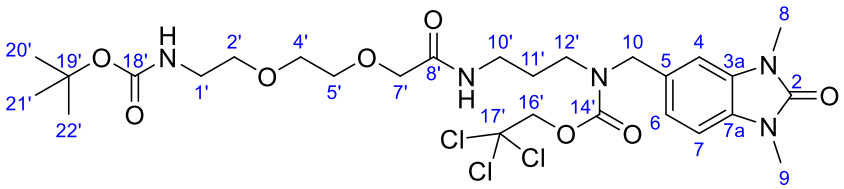
***tert*-Butyl {14',14',14'-trichloro-10'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-5',11'-dioxo-3',12'-dioxa-6',10'-diazatetradecan-1'-yl}carbamate (245)**



2-(2-((*tert*-Butoxycarbonyl)amino)ethoxy)acetic acid (**231**) (57 mg, 0.26 mmol, 1.1 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (2.0 mL). 2,2,2-Trichloroethyl (3-aminopropyl)((1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)methyl)carbamate (**243**) (0.10 g, 0.24 mmol, 1.0 eq.), *N*,*N*-diisopropylethylamine (82 µL, 0.42 mmol, 2.0 eq.) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro phosphate (99 mg, 0.26 mmol, 1.1 eq.) were added. The reaction mixture was stirred at rt for 17 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction volume was reduced by concentration *in vacuo*. The reaction mixture was diluted with an aqueous solution of LiCl (0.5 M, 30 mL) and extracted with EtOAc (3 × 30 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–4% EtOAc/petroleum ether gradient) and further purified using silica flash column chromatography (elution with 0–7% EtOH/CH_2Cl_2 gradient) to yield the title compound

as a colourless oil (93 mg, 0.15 mmol, 63%): R_f 0.19 (1:1 acetone/EtOAc); $\tilde{\nu}_{max}$/cm^{-1} 3323 (br), 2931 (br), 2542 (br), 2160 (br), 2022 (br), 1977 (br), 1696 (C=O, s), 1532 (w), 1509 (m), 1468 (w), 1443 (w), 1393 (w), 1365 (w), 1269 (w), 1246 (w), 1170 (w), 1125 (m), 1058 (w), 960 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.45 (1H, br s, N-H), 7.08–7.01 (3H, m, C^4-H, C^6-H, C^7-H), 6.44 (1H, br s, N-H), 4.89 (2H, s, C$^{13'}$-H$_2$), 4.56 (2H, s, C^{10}-H$_2$), 3.82 (2H, s, C$^{4'}$-H$_2$), 3.46 (2H, t, *J* 6.4, C$^{2'}$-H$_2$), 3.34–3.27 (8H, m, C^8-H$_3$, C^9-H$_3$, C$^{9'}$-H$_2$), 3.17–3.11 (4H, m, C$^{1'}$-H$_2$, C$^{7'}$-H$_2$), 1.75 (2H, tt, *J* 6.4, 6.4, C$^{8'}$-H$_2$), 1.39 (9H, s, C$^{17'}$-H$_3$, C$^{18'}$-H$_3$, C$^{19'}$-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.5 (C$^{5'}$), 155.1 (C$^{15'}$), 153.7 (C$^{2/11'}$), 153.6 (C$^{2/11'}$), 129.9 (C$^{3a/7a}$), 129.7 (C^5), 129.0 (C$^{3a/7a}$), 120.0 (C^6), 106.8 (C^7), 106.4 (C^4), 95.7 (C$^{14'}$), 77.3 (C$^{16'}$), 74.1 (C$^{13'}$), 69.68 (C$^{2'}$), 69.65 (C$^{4'}$), 49.9 (C^{10}), 44.1 (C$^{9'}$), 40.6 (C$^{1'}$), 35.5 (C$^{7'}$), 27.8 (C$^{17'}$, C$^{18'}$, C$^{19'}$), 27.4 (C$^{8'}$), 26.41 (C$^{8/9}$), 26.37 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 624.17511, $C_{25}H_{37}N_5O_7{}^{35}Cl_3$ requires [M+H]$^+$ 624.17531; Found: 626.17200, $C_{25}H_{37}N_5O_7{}^{35}Cl_2{}^{37}Cl$ requires [M+H]$^+$ 626.17236]; LRMS *m/z* (ESI$^+$) 646.2 ([M+Na]$^+$, 10%); HPLC retention time 9.2 min, >99.9%.

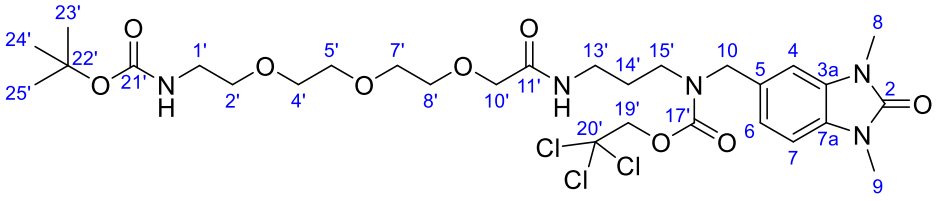
tert-Butyl {17',17',17'-trichloro-13'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-8',14'-dioxo-3',6',15'-trioxa-9',13'-diazaheptadecan-1'-yl}carbamate (246)



2,2,2-Trichloroethyl (3-aminopropyl)((1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)methyl)carbamate (**248**) (50 mg, 0.12 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (1.5 mL). 2,2-Dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-oic acid (**232**) (34 mg, 0.13 mmol, 1.1 eq.), *N*,*N*-diisopropylethylamine (41 μL, 0.24 mmol, 2.0 eq.) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro phosphate (49 mg, 0.13 mmol, 1.1 eq.) were added. The reaction mixture was stirred at rt for 16 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (3 × 20 mL). The organic fractions were combined, dried

(Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–10% EtOH/$CHCl_3$ gradient) to yield the title compound as a colourless oil (38 mg, 0.057 mmol, 48%): R_f 0.36 (3:47 MeOH/CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3340 (br), 2930 (br), 2160 (br), 1694 (C=O, s), 1509 (m), 1452 (w), 1391 (w), 1365 (w), 1271 (m), 1249 (m), 1170 (m), 1124 (s), 1064 (m); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 7.37 (1H, br s, N-H), 7.09–7.00 (3H, m, C^4-H, C^6-H, C^7-H), 6.28 (1H, br s, N-H), 4.90 (2H, s, $C^{16'}$-H_2), 4.56 (2H, br s, C^{10}-H_2), 3.85 (2H, s, $C^{7'}$-H_2), 3.61–3.52 (4H, m, $C^{4'}$-H_2, $C^{5'}$-H_2), 3.44 (2H, t, *J* 6.1, $C^{2'}$-H_2), 3.32 (3H, s, $C^{8/9}$-H_3), 3.31 (3H, s, $C^{8/9}$-H_3), 3.30–3.26 (2H, m, $C^{12'}$-H_2), 3.17–3.07 (4H, m, $C^{1'}$-H_2, $C^{10'}$-H_2), 1.74 (2H, tt, *J* 6.9, 6.9, $C^{11'}$-H_2), 1.40 (9H, s, $C^{20'}$-H_3, $C^{21'}$-H_3, $C^{22'}$-H_3); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 168.6 ($C^{8'}$), 155.1 ($C^{18'}$), 153.7 ($C^{14'}$), 153.6 (C^2), 129.9 ($C^{3a/7a}$), 129.7 (C^5), 128.9 ($C^{3a/7a}$), 120.0 (C^6), 106.8 ($C^{4/7}$), 106.4 ($C^{4/7}$), 95.7 ($C^{17'}$), 77.2 ($C^{19'}$), 74.1 ($C^{16'}$), 69.9 ($C^{7''}$) 69.8 ($C^{4'/5'}$), 69.0 ($C^{2'}$) 68.9 ($C^{4'/5'}$), 49.9 (C^{10}), 44.1 ($C^{12'}$), 40.5 ($C^{1'}$), 35.5 ($C^{10'}$), 27.8 ($C^{20'}$, $C^{21'}$, $C^{22'}$), 27.3 ($C^{11'}$), 26.3 ($C^{8/9}$), 26.4 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 668.20057, $C_{27}H_{41}N_5O_8{}^{35}Cl_3$ requires $[M+H]^+$ 668.20152; Found: 670.19762, $C_{27}H_{41}N_5O_8{}^{35}Cl_2{}^{37}Cl$ requires $[M+H]^+$ 670.19857]; LRMS *m/z* (ESI^-) 712.2 ($[M+CH_2O_2–H]^-$, 100%); HPLC retention time 9.1 min, 96.1%.

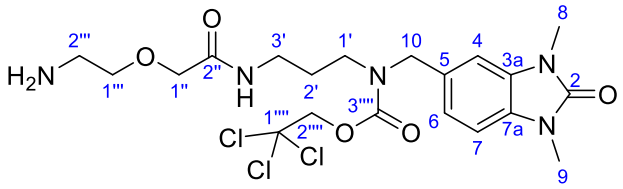
***tert*-Butyl {20',20',20'-trichloro-16'-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-11',17'-dioxo-3',6',9',18'-tetraoxa-12',16'-diazaicosan-1'-yl}carbamate (247)**



2,2-Dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azahexadecan-16-oic acid (**233**) (42 mg, 0.14 mmol, 1.1 eq.) was dissolved in *N*,*N*-dimethylformamide (1.5 mL) and 2,2,2-trichloroethyl (3-aminopropyl)((1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)methyl)carbamate (**248**) (53 mg, 0.12 mmol, 1.0 eq.), *N*,*N*-diisopropylethylamine (43 µL, 0.25 mmol, 2.0 eq.) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro

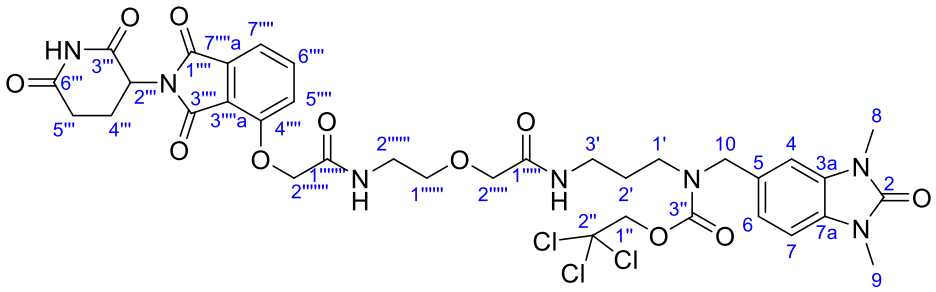
phosphate (50 mg, 0.13 mmol, 1.1 eq.) were added. The reaction mixture was stirred at rt for 21 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−6% EtOH/$CHCl_3$ gradient) and further purified using silica flash column chromatography (elution with 0−8% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (40 mg, 0.056 mmol, 45%): R_f 0.42 (3:47 MeOH/CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3340 (br), 2929 (br), 2160 (br), 1977 (br), 1698 (C=O, s), 1509 (m), 1443 (m), 1393 (w), 1365 (w), 1268 (m), 1247 (m), 1171 (m), 1124 (m), 921 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 7.37 (1H, br s, N-H), 7.09–7.00 (3H, m, C^4-H, C^6-H, C^7-H), 6.25 (1H, br s, N-H), 4.90 (2H, s, $C^{19'}$-H_2), 4.56 (2H, s, C^{10}-H_2), 3.85 (2H, s, $C^{10'}$-H_2), 3.61–3.56 (4H, m, $C^{4'/5'/7'/8'}$-H_2, $C^{4'/5'/7'/8'}$-H_2), 3.51–3.49 (4H, m, $C^{4'/5'/7'/8'}$-H_2, $C^{4'/5'/7'/8'}$-H_2), 3.43 (2H, t, *J* 6.1, $C^{2'}$-H_2), 3.32 (3H, s, $C^{8/9}$-H_3), 3.31 (3H, s, $C^{8/9}$-H_3), 3.30–3.26 (2H, m, $C^{15'}$-H_2), 3.19–3.12 (2H, m, $C^{13'}$-H_2), 3.12–3.06 (2H, m, $C^{1'}$-H_2), 1.74 (2H, tt, *J* 7.1, 7.1, $C^{14'}$-H_2), 1.39 (9H, s, $C^{23'}$-H_3, $C^{24'}$-H_3, $C^{25'}$-H_3); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 168.6 ($C^{11'}$), 155.1 ($C^{21'}$), 153.7 ($C^{17'}$), 153.6 (C^2), 129.9 ($C^{3a/7a}$), 129.7 (C^5), 128.9 ($C^{3a/7a}$), 120.0 (C^6), 106.8 ($C^{4/7}$), 106.4 ($C^{4/7}$), 95.7 ($C^{20'}$), 77.2 ($C^{22'}$), 74.1 ($C^{19'}$), 70.0 ($C^{2'/4'/5'/7'/8'}$), 69.8 ($C^{10'}$), 69.4 ($C^{2'/4'/5'/7'/8'}$), 69.2 ($C^{2'/4'/5'/7'/8'}$), 69.1 ($C^{2'/4'/5'/7'/8'}$), 68.9 ($C^{2'/4'/5'/7'/8'}$), 49.9 (C^{10}), 44.1 ($C^{15'}$), 40.7 ($C^{1'}$), 35.5 ($C^{13'}$), 27.8 ($C^{23'}$, $C^{24'}$, $C^{25'}$), 27.4 ($C^{14'}$), 26.40 ($C^{8/9}$), 26.36 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 712.22724, $C_{29}H_{45}5_5O_9{}^{35}Cl_3$ requires $[M+H]^+$ 712.22774; Found: 714.22432, $C_{29}H_{45}N_5O_9{}^{35}Cl_2{}^{37}Cl$ requires $[M+H]^+$ 714.22479]; LRMS *m/z* (ESI^+) 734.2 ($[M+Na]^+$, 62%); HPLC retention time 9.3 min, 95.4%.

2’’’’,2’’’’,2’’’’-Trichloroethyl {3’-[2’’-(2’’’-aminoethoxy)acetamido]prop-1’-yl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (248)



tert-Butyl {14,14,14-trichloro-10-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-5,11-dioxo-3,12-dioxa-6,10-diazatetradecan-1-yl}carbamate (**240**) (74 mg, 0.12 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (1.5 mL) and the reaction mixture cooled to 0 °C. Trifluoroacetic acid (1.5 mL) was added and the reaction mixture warmed to rt and stirred for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* and purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) to yield the title compound as a colourless oil (53 mg, 0.10 mmol, 86%): R_f 0.23 (1:9 MeOH/CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3412 (br), 2945 (br), 1689 (C=O, s), 1546 (w), 1509 (w), 1469 (w), 1398 (w), 1350 (w), 1270 (w), 1201 (m), 1129 (m), 1053 (w), 1026 (w), 1005 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 7.66 (1H, br s, N-H), 7.10–7.00 (3H, m, C^4-H, C^6-H, C^7-H), 4.90 (2H, s, C$^{2''''}$-H$_2$), 4.56 (2H, s, C^{10}-H$_2$), 3.87 (2H, s, C$^{1'''}$-H$_2$), 3.56 (2H, t, *J* 5.4, C$^{1'''}$-H$_2$), 3.34–3.26 (8H, m, C^8-H$_3$, C^9-H$_3$, C$^{1'}$-H$_2$), 3.18–3.10 (2H, m, C$^{3'}$-H$_2$), 2.90 (2H, t, *J* 5.4, C$^{2'''}$-H$_2$), 1.75 (2H, tt, *J* 7.1, 7.1, C$^{2'}$-H$_2$); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 168.6 (C$^{2''}$), 153.7 (C^2, C$^{3''''}$), 129.9 (C$^{3a/5/7a}$), 129.7 (C$^{3a/5/7a}$), 129.0 (C$^{3a/5/7a}$), 120.0 (C^6), 106.9 (C$^{4/7}$), 106.4 (C$^{4/7}$), 95.8 (C$^{2''''}$), 74.1 (C$^{1''''}$), 69.6 (C$^{1''}$, C$^{1'''}$), 49.9 (C^{10}), 44.2 (C$^{1'}$), 40.3 (C$^{2'''}$), 35.6 (C$^{3'}$), 27.4 (C$^{2'}$), 26.43 (C$^{8/9}$), 26.39 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 524.12270, $C_{20}H_{29}N_5O_5{}^{35}Cl$ requires [M+H]$^+$ 524.12288; Found: 526.11968, $C_{20}H_{29}N_5O_5{}^{35}Cl_2{}^{37}Cl$ requires [M+H]$^+$ 526.11993]; LRMS *m/z* (ESI$^+$) 548.0 ([M+Na]$^+$, 41%); HPLC retention time 7.8 min, >99.9%.

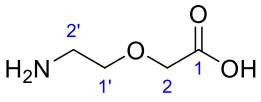
2'',2'',2''-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](3'-{2''''-[2''''''-(2'''''''-{[2''''-(2''',6'''-dioxopiperidin-3'''-yl)-1'''',3''''-dioxo-2'''',3''''-dihydro-1*H*-isoindol-4''''-yl]oxy}acetamido)ethoxy]acetamido}prop-1'-yl)carbamate (249)



2,2,2-Trichloroethyl {3-[2-(2-aminoethoxy)acetamido]propyl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (**248**) (52 mg, 0.099 mmol, 1.1 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (4.0 mL). 2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (**229**) (30 mg, 0.090 mmol, 1.0 eq.), *N*,*N*-diisopropylethylamine (31 µL, 0.18 mmol, 1.0 eq.) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (38 mg, 0.099 mmol, 1.1 eq.) were added. The reaction mixture was stirred at rt for 29 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–7% EtOH/$CHCl_3$ gradient) to yield the title compound as a yellow oil (65 mg, 0.078 mmol, 78%): R_f 0.52 (1:9 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = +3.4 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 2945 (br), 2362 (br), 2160 (br), 1771 (w), 1701 (C=O, s), 1613 (w), 1537 (w), 1509 (w), 1483 (w), 1442 (w), 1394 (w), 1356 (w), 1300 (w), 1265 (w), 1200 (w), 1123 (w), 1053 (w), 1025 (m), 1007 (w), 938 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 10.78 (1H, br s, N-H), 7.84 (1H, br s, N-H), 7.78 (1H, dd, *J* 8.5, 7.3, C$^{6''''}$-H), 7.48 (1H, d, *J* 7.3, C$^{7''''}$-H), 7.43 (2H, d, *J* 8.5, C$^{5''''}$-H$_2$), 7.07–6.98 (3H, m, C^4-H, C^6-H, C^7-H), 5.06 (1H, dd, *J* 12.6, 5.5, C$^{2'''}$-H), 4.88 (2H, s, C$^{1''}$-H$_2$), 4.76 (2H, s, C$^{2'''''''}$-H$_2$), 4.54

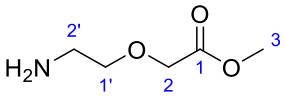
(2H, s, C^{10}-H_2), 3.85 (2H, s, $C^{2''''}$-H_2), 3.55 (2H, t, *J* 5.7, $C^{1''''''}$-H_2), 3.44–3.36 (2H, m, $C^{2''''''}$-H_2), 3.31 (3H, s, $C^{8/9}$-H_3), 3.30 (3H, s, $C^{8/9}$-H_3), 3.31–3.25 (2H, m, $C^{1'}$-H_2), 3.16–3.08 (2H, m, $C^{3'}$-H_2), 2.88 (1H, ddd, *J* 17.0, 13.5, 5.3, $C^{5'''}$-H_a), 2.68–2.52 (2H, m, $C^{4'''}$-H_a, $C^{5'''}$-H_b), 2.12–2.03 (1H, m, $C^{4'''}$-H_b), 1.73 (2H, tt, *J* 7.0, 7.0, $C^{2'}$-H_2); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 171.7 ($C^{6'''}$), 168.9 ($C^{3''''}$), 168.4 ($C^{1''''}$), 166.5 ($C^{1''''}$), 166.1 ($C^{1''''''}$), 165.9 ($C^{2'''}$), 154.7 ($C^{4''''}$), 153.7 (C^{2}), 153.5 ($C^{3''}$), 136.3 ($C^{6''''}$), 132.7 ($C^{7''''a}$), 129.9 ($C^{3a/7a}$), 129.7 (C^{5}), 128.9 ($C^{3a/7a}$), 120.6 ($C^{5''''}$), 120.0 (C^{6}), 116.9 ($C^{3''''a}$), 115.8 ($C^{7''''}$), 106.8 ($C^{4/7}$), 106.4 ($C^{4/7}$), 95.7 ($C^{2''}$), 74.1 ($C^{1''}$), 69.6 ($C^{2''''}$), 69.1 ($C^{1''''''}$), 68.0 ($C^{2''''''}$), 59.9 (C^{10}), 48.7 ($C^{3'''}$), 44.1 ($C^{1'}$), 38.0 ($C^{2''''''}$), 35.5 ($C^{3'}$), 30.5 ($C^{5'''}$), 27.6 ($C^{2'}$), 26.39 ($C^{8/9}$), 26.35 ($C^{8/9}$), 21.6 ($C^{4'''}$); HRMS *m/z* (ESI^+) [Found: 838.17668, $C_{35}H_{39}N_5O_{11}{}^{35}Cl_3$ requires $[M+H]^+$ 838.17676; Found: 840.17366, $C_{35}H_{39}N_7O_{11}{}^{35}Cl_2{}^{37}Cl$ requires $[M+H]^+$ 840.17381]; LRMS *m/z* (ESI^-) 836.0 ($[M–H]^-$, 93%); HPLC retention time 7.8 min, >99.9%.

2-(2'-Aminoethoxy)acetic acid (253)[259]



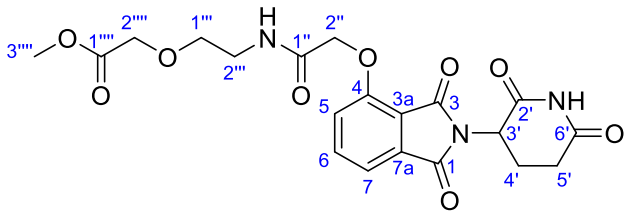
2-(2-((*tert*-Butoxycarbonyl)amino)ethoxy)acetic acid (**231**) (437 mg, 2.00 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (5.0 mL). The reaction mixture was cooled to 0 °C. Trifluoroacetic acid (5.00 mL) was added dropwise. The reaction mixture was warmed to rt and stirred for 1.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* and purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) to yield the title compound as colourless solid (188 mg, 1.58 mmol, 79%): R_f 0.36 (1:2:8 NH_4OH/EtOH/EtOAc); m.p. 160−162 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3452 (br), 2870 (br), 2550 (br), 23663 (m), 2032 (m), 1977 (m), 1722 (C=O, m), 1578 (s), 1455 (m), 1426 (m), 1400 (m), 1331 (m), 1281 (w), 1234 (w), 1112 (s), 1066 (m), 1016 (m), 935 (w); ^{1}H NMR (400 MHz, CD_3OD) δ 3.91 (2H, s, C^{2}-H_2), 3.72–3.65 (2H, m, $C^{1'}$-H_2), 3.13–3.05 (2H, m, $C^{2'}$-H_2); LRMS *m/z* (ESI^-) 118.1 ($[M–H]^-$, 100%). The spectroscopic data are in good agreement with literature values.[259]

Methyl 2-(2'-aminoethoxy)acetate (254)



2-(2-Aminoethoxy)acetic acid (**253**) (181 mg, 1.52 mmol, 1.0 eq.) was suspended in MeOH (3.00 mL). The reaction mixture was cooled to 0 °C and thionyl chloride (220 µL, 3.04 mmol, 2.0 eq.) was added dropwise. The reaction mixture was warmed to rt and stirred for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:2:6 NH_4OH/EtOH/EtOAc). The reaction mixture was concentrated *in vacuo* to yield the title compound as an orange oil (200 mg, 1.50 mmol, 99%): R_f 0.58 (1:2:4 NH_4OH/EtOH/EtOAc); $\bar{\nu}_{max}$/cm^{-1} 3437 (br), 3157 (br), 2955 (br), 2355 (br), 2161 (br), 2025 (br), 1979 (br), 1733 (C=O, s), 1722 (m), 1657 (m), 1495 (w), 1433 (m), 1399 (m), 1350 (w), 1300 (w), 1234 (s), 1148 (m), 1118 (s), 1035 (m), 979 (m), 942 (m); ^{1}H NMR (400 MHz, CD_3OD) δ 4.25 (2H, s, C^2-H_2), 3.82 (2H, t, *J* 4.3, C$^{1'}$-H_2), 3.77 (3H, s, C^3-H_3), 3.18 (2H, t, *J* 4.3, C$^{2'}$-H_2); ^{13}C NMR (126 MHz, CD_3OD) δ 173.0 (C^1), 68.8 (C^2), 68.3 (C$^{1'}$), 52.6 (C^3), 40.7 (C$^{2'}$); HRMS *m/z* (ESI$^+$) [Found: 134.08123, $C_5H_{12}NO_3$ requires $[M+H]^+$ 134.08117]; LRMS *m/z* (ESI$^+$) 134.0 ($[M+H]^+$, 100%).

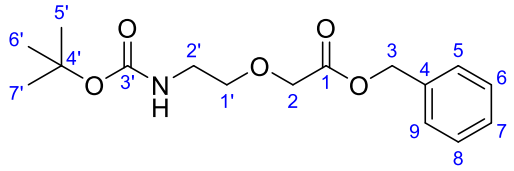
Methyl [2''''-(2'''-{[2''-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]acetate (255)



2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (**229**) (0.10 g, 0.30 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (3.0 mL). Methyl 2-(2-aminoethoxy)acetate (**254**) (48 mg, 0.36 mmol, 1.2 eq.), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (0.13 g, 0.33 mmol, 1.1 eq.) and *N*,*N*-diisopropylethylamine (0.11 mL, 0.60 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 17 h. After this time TLC analysis indicated complete consumption of starting

material (1:2:4 NH_4OH/EtOH/EtOAc). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−7% EtOH/CH_2Cl_2 gradient) and further purified using silica flash column chromatography (elution with 0−9% EtOH/EtOAc gradient) to yield the title compound as a colourless solid (66 mg, 0.15 mmol, 49%): R_f 0.17 (1:24 EtOH/$CHCl_3$); $[\alpha]_D^{25}$ = –5.18 (*c* 1.0, DMSO); m.p. 175–177 °C (from EtOH); $\tilde{\nu}_{max}$/cm^{-1} 2361 (s), 2342 (s), 1758 (C=O, s), 1717 (C=O, s), 1682 (C=O, s), 1540 (w), 1486 (w), 1449 (w), 1394 (w), 1360 (w), 1278 (w), 1205 (w), 1115 (w), 1057 (w), 1016 (w), 942 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (1H, br s, N-H), 8.07 (1H, t, *J* 5.7, N-H), 7.81 (1H, dd, *J* 8.5, 7.3, C^6-H), 7.50 (1H, d, *J* 7.3, C^7-H), 7.40 (1H, d, *J* 8.5, C^5-H), 5.12 (1H, dd, *J* 12.8, 5.4, C$^{3'}$-H), 4.79 (2H, s, C$^{2''}$-H_2), 4.13 (2H, s, C$^{2''''}$-H_2), 3.64 (3H, s, C$^{3''''}$-H_3), 3.53 (2H, t, *J* 5.6, C$^{1'''}$-H_2), 3.37–3.30 (2H, m, C$^{2'''}$-H_2), 2.95–2.84 (1H, m, C$^{5'}$-H_a), 2.64–2.52 (2H, m, C$^{4'}$-H_a, C$^{5'}$-H_b), 2.08–1.99 (1H, m, C$^{4'}$-H_b); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.8 (C$^{6'}$), 170.6 (C$^{1''''}$), 169.9 (C$^{2'}$), 167.0 (C$^{1''}$), 166.7 (C^1), 165.4 (C^3), 155.1 (C^4), 136.9 (C^6), 133.0 (C^{7a}), 120.4 (C^5), 116.8 (C^{3a}), 116.0 (C^7), 69.2 (C$^{1'''}$), 67.5 (C$^{2''''}$), 67.3 (C$^{2''}$), 51.4 (C$^{3''''}$), 48.8 (C$^{3'}$), 38.3 (C$^{2'''}$), 31.0 (C$^{5'}$), 22.0 (C$^{4'}$); HRMS *m/z* (ESI$^+$) [Found: 470.11673, $C_{20}H_{21}N_3O_9Na$ requires $[M+Na]^+$ 470.11700]; LRMS *m/z* (ESI$^-$) 446.0 ($[M-H]^-$, 10%); HPLC retention time 6.2 min, >99.9%.

Benzyl {2'-[(*tert*-butoxycarbonyl)amino]ethoxy}acetate (257)



Method 1

2-(2-((*tert*-Butoxycarbonyl)amino)ethoxy)acetic acid (**231**) (500 mg, 2.28 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (11.0 mL). Benzyl alcohol (237 µL, 2.28 mmol, 1.0 eq.), dicyclohexylcarbodiimide (139 mg, 1.14 mmol, 3.0 eq.) and 4-(dimethylamino)pyridine (139 mg,

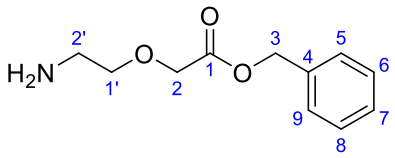
1.14 mmol, 0.5 eq.) were added. The reaction mixture was stirred at rt for 15 h. After this time TLC analysis indicated complete consumption of starting material (1:24 MeOH/CH_2Cl_2). The reaction mixture was filtered, and the filtrate diluted with a saturated aqueous solution of $NaHCO_3$ (50 mL). The reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic fractions were combined, washed with brine (100 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−4% MeOH/CH_2Cl_2 gradient) and further purified using silica flash column chromatography (elution with 0−40% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (591 mg, 1.91 mmol, 84%): R_f 0.71 (1:24 MeOH/CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3368 (br), 2976 (br), 2160 (br), 1753 (C=O, s), 1708 (C=O, s), 1508 (m), 1456 (w), 1391 (w), 1365 (m), 1249 (m), 1132 (s), 1001 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (5H, s, C^5-H, C^6-H, C^7-H, C^8-H, C^9-H), 5.17 (2H, s, C^3-H_2), 4.10 (2H, s, C^2-H_2), 3.58 (2H, t, *J* 5.1, $C^{1'}$-H_2), 3.35–3.28 (2H, m, $C^{2'}$-H_2), 1.43 (9H, s, $C^{5'}$-H_3, $C^{6'}$-H_3, $C^{7'}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.3 (C^1), 156.0 ($C^{3'}$), 135.3 (C^4), 128.6 ($C^{5/6/7/8/9}$) 128.5 ($C^{5/6/7/8/9}$), 128.4 ($C^{5/6/7/8/9}$), 79.2 ($C^{4'}$), 70.9 ($C^{1'}$), 68.2 (C^2), 66.6 (C^3), 40.4 ($C^{2'}$), 28.4 ($C^{5'}$, $C^{6'}$, $C^{7'}$); HRMS *m/z* (ESI^+) [Found: 332.14680, $C_{16}H_{23}NO_5Na$ requires $[M+Na]^+$ 332.14684]; LRMS *m/z* (ESI^+) 332.0 ($[M+Na]^+$, 54%); HPLC retention time 9.5 min, 98.8%.

Method 2

2-(2-((*tert*-Butoxycarbonyl)amino)ethoxy)acetic acid (**231**) (48 mg, 0.22 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (0.50 mL). 1,1′-Carbonyldiimidazole (39 mg, 0.24 mmol, 1.1 eq.) was added and the reaction mixture stirred at rt for 1 h. Benzyl alcohol (23 μL, 0.22 mmol, 1.0 eq.) was added and the reaction mixture stirred at rt for 2 h. After this time TLC analysis indicated complete consumption of the intermediate (3:2 EtOAc/petroleum ether). The reaction mixture was concentrated *in vacuo* and purified using silica flash column chromatography (elution with 0−4% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (54 mg, 0.17 mmol, 80%): R_f 0.70 (1:24 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.30 (5H, m, C^5-H, C^6-H, C^7-H, C^8-H, C^9-H), 5.19 (2H, s, C^3-H_2), 5.12 (1H, br s, N-H), 4.13 (2H, s, C^2-H_2), 3.61 (2H, t, *J* 5.1, $C^{1'}$-H_2), 3.38–3.29 (2H, m, $C^{2'}$-H_2), 1.44 (9H, s, $C^{5'}$-H_3, $C^{6'}$-H_3, $C^{7'}$-H_3); LRMS *m/z* (ESI^+)

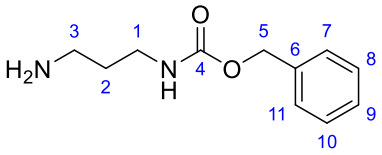
332.2 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with those obtained for method 1.

Benzyl 2-(2'-aminoethoxy)acetate (258)



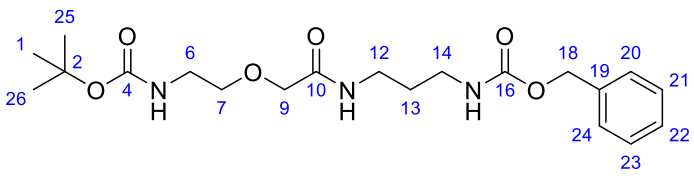
Benzyl 2-(2-((*tert*-butoxycarbonyl)amino)ethoxy)acetate (**257**) (375 mg, 1.21 mmol, 1.0 eq.) was dissolved in HCl (4 M, dioxane) (5.00 mL). The reaction mixture was stirred at rt for 1 h. After this time TLC analysis indicated complete consumption of starting material (2:3 EtOAc/petroleum ether). The reaction mixture was concentrated *in vacuo* and purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) and further purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) to yield the title compound as a colourless oil (227 mg, 1.08 mmol, 90%): R_f 0.67 (1:2:6 NH_4OH/EtOH/EtOAc); $\bar{\nu}_{max}$/cm^{-1} 3032 (br), 2977 (br), 2880 (br), 2321 (br), 1979 (br), 1662 (C=O, s), 1494 (m), 1454 (m), 1409 (s), 1346 (s), 1251 (m), 1208 (w), 1180 (w), 1121 (s), 1095 (m), 1023 (m), 983 (m), 942 (m), 909 (m); ^{1}H NMR (500 MHz, CD_3OD) δ 7.40–7.29 (4H, m, C^5-H, C^6-H, C^8-H, C^9-H), 7.28–7.21 (1H, m, C^7-H), 4.60 (2H, s, C^3-H$_2$), 4.09 (2H, s, C^2-H$_2$), 3.82–3.76 (2H, t, *J* 5.3, C$^{1'}$-H$_2$), 3.37–3.31 (2H, t, *J* 5.3, C$^{2'}$-H$_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 171.2 (C^1), 142.6 (C^4), 129.3 (C^6, C^8), 128.2 (C^7), 128.0 (C^5, C^9), 68.3 (C^2), 65.1 (C^3), 64.2 (C$^{1'}$), 42.1 (C$^{2'}$); HRMS *m/z* (ESI$^+$) [Found: 210.11262, $C_{11}H_{16}NO_3$ requires [M+H]$^+$ 210.11247]; LRMS *m/z* (ESI$^+$) 210.0 ([M+H]$^+$, 100%); HPLC retention time 6.0 min, 96.8%.

Benzyl (3-aminopropyl)carbamate (262)[260–262]



Propane-1,3-diamine (3.00 mL, 35.6 mmol, 3.0 eq.) was dissolved in $CHCl_3$ (20 mL). The reaction mixture was cooled to 0 °C. Benzyl chloroformate (1.68 mL, 11.9 mmol, 1.0 eq.) in $CHCl_3$ (75.0 mL) was added over 1 h *via* a dropping funnel. The reaction mixture was warmed to rt and stirred for 1.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc (50 mL) and washed with H_2O (3 × 20 mL). The organic fraction was extracted with an aqueous solution of HCl (2 M, 3 × 50 mL). The aqueous fraction was basified to pH 12 using solid NaOH, saturated with NaCl and extracted with EtOAc (3 × 50 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a colourless oil (840 mg, 4.03 mmol, 34%): R_f 0.25 (1:2:6 NH_4OH/EtOH/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.19 (5H, m, C^7-H, C^8-H, C^9-H, C^{10}-H, C^{11}-H), 5.70 (1H, br s, N-H), 5.07 (2H, s, C^5-H_2), 3.29–3.14 (2H, m, C^1-H_2), 2.77–2.64 (2H, m, C^3-H_2), 1.66–1.57 (2H, m, C^2-H_2); LRMS *m/z* (ESI^+) 209.2 ($[M+H]^+$, 30%). The spectroscopic data are in good agreement with literature values.[260–262]

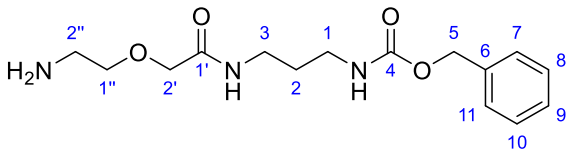
Benzyl (2,2-dimethyl-4,10-dioxo-3,8-dioxa-5,11-diazatetradecan-14-yl)carbamate (263)



2-(2-((*tert*-Butoxycarbonyl)amino)ethoxy)acetic acid (**231**) (632 mg, 2.88 mmol, 1.2 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (12.0 mL). Benzyl (3-aminopropyl)carbamate (**262**) (500 mg, 2.40 mmol, 1.0 eq.), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (1.00 g, 2.64 mmol, 1.1 eq.) and

N,*N*-diisopropylethylamine (836 µL, 4.80 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 22 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 70 mL) and extracted with CH_2Cl_2 (3 × 70 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 50−100% EtOAc/petroleum ether gradient then 1−7% EtOH/EtOAc gradient) and further purified using silica flash column chromatography (elution with 50−100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (708 mg, 1.73 mmol, 72%): R_f 0.52 (3:47 EtOH/CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3330 (br), 2934 (br), 2161 (br), 1978 (br), 1690 (C=O, s), 1529 (C=O, s), 1454 (w), 1391 (w), 1365 (w), 1337 (w), 1251 (s), 1135 (s), 1022 (w), 918 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (1H, br s, N-H), 7.37–7.25 (5H, m, C^{20}-H, C^{21}-H, C^{22}-H, C^{23}-H, C^{24}-H), 5.70 (1H, br s, N-H), 5.44 (1H, br s, N-H), 5.10 (2H, s, C^{18}-H_2), 3.92 (2H, s, C^9-H_2), 3.52 (2H, t, *J* 4.9, C^7-H_2), 3.38–3.28 (4H, m, C^6-H_2, C^{12}-H_2), 3.28–3.21 (2H, m, C^{14}-H_2), 1.63 (2H, tt, *J* 5.9, 5.9, C^{13}-H_2), 1.39 (9H, s, C^1-H_3, C^{25}-H_3, C^{26}-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.9 (C^{10}), 157.5 (C^{16}), 156.3 (C^4), 136.5 (C^{19}), 128.6 ($C^{20/21/22/23/24}$), 128.3 ($C^{20/21/22/23/24}$), 128.2 ($C^{20/21/22/23/24}$), 79.4 (C^2), 71.3 (C^7), 70.6 (C^9), 67.1 (C^{18}), 40.7 (C^6), 37.5 (C^{14}), 34.8 (C^{12}), 30.0 (C^{13}), 28.5 (C^1, C^{25}, C^{26}); HRMS *m/z* (ESI$^+$) [Found: 432.20974, $C_{20}H_{31}N_3O_6Na$ requires [M+Na]$^+$ 432.21051]; LRMS *m/z* (ESI$^+$) 432.2 ([M+Na]$^+$, 71%); HPLC retention time 6.6 min, 97.2%.

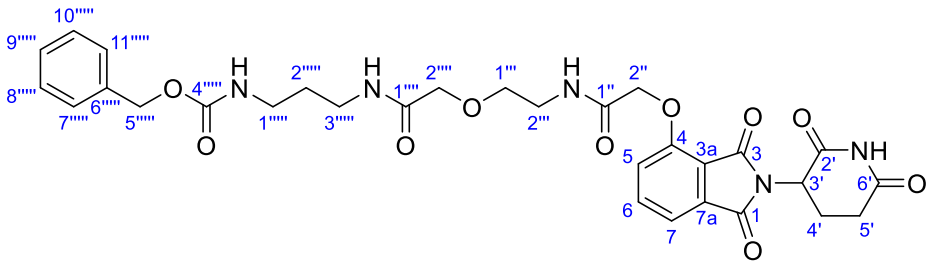
Benzyl {3-[2'-(2''-aminoethoxy)acetamido]propyl}carbamate (259)



Benzyl (2,2-dimethyl-4,10-dioxo-3,8-dioxa-5,11-diazatetradecan-14-yl)carbamate (**263**) (614 mg, 1.50 mmol, 1.0 eq.) was dissolved in HCl (4M, dioxane) (10.0 mL). The reaction mixture was stirred at rt for 1.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. The

product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) and further purified using silica flash column chromatography (elution with 1:2:6 NH_4OH/EtOH/EtOAc isocratic) to yield the title compound as a colourless oil (298 mg, 0.963 mmol, 64%): R_f 0.27 (1:2:6 NH_4OH/EtOH/EtOAc); $\tilde{\nu}_{max}$/cm^{-1} 3347 (br), 2940 (br), 2160 (br), 1977 (br), 1706 (C=O, s), 1656 (C=O, s), 1530 (s), 1454 (w), 1341 (w), 1256 (s), 1127 (m), 1025 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (1H, br s, N-H), 7.38–7.26 (5H, m, C^7-H, C^8-H, C^9-H, C^{10}-H, C^{11}-H), 5.39 (1H, br s, N-H), 5.08 (2H, s, C^5-H$_2$), 3.97 (2H, s, C$^{2'}$-H$_2$), 3.53 (2H, t, *J* 5.0, C$^{1''}$-H$_2$), 3.39–3.31 (2H, m, C^3-H$_2$), 3.27–3.18 (2H, m, C^1-H$_2$), 2.90 (2H, t, *J* 5.0, C$^{2''}$-H$_2$), 1.68 (2H, tt, *J* 7.3, 5.7, C^2-H$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6 (C$^{1'}$), 157.0 (C^4), 136.7 (C^6), 128.6 (C$^{7/8/9/10/11}$), 128.2 (C$^{7/8/9/10/11}$), 128.1 (C$^{7/8/9/10/11}$), 74.0 (C$^{1''}$), 70.5 (C$^{2'}$), 66.8 (C^5), 41.6 (C$^{2''}$), 37.8 (C^1), 35.4 (C^3), 30.0 (C^2); HRMS *m/z* (ESI$^+$) [Found: 310.17578, $C_{15}H_{24}N_3O_4$ requires $[M+H]^+$ 310.17613]; LRMS *m/z* (ESI$^+$) 310.2 ($[M+H]^+$, 100%); HPLC retention time 4.4 min, 93.6%.

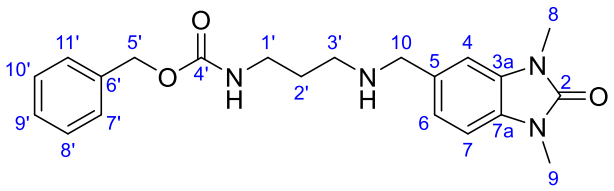
Benzyl (3’’’’’-{2’’’’-[2’’’-(2’’-{[2-(2’,6’-dioxopiperidin-3’-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]acetamido}propyl)carbamate (264)



Benzyl 2,2-dimethyl-4-oxo-3,8,11-trioxa-5-azatridecan-13-oate (**259**) (143 mg, 0.405 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (1.50 mL). 2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (**229**) (100 mg, 0.301 mmol, 1.2 eq.), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (105 mg, 0.276 mmol, 1.1 eq.) and *N*,*N*-diisopropylethylamine (87.4 µL, 0.502 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 18 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The

reaction was quenched with a saturated aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (2 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−8% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless solid (103 mg, 0.165 mmol, 66%): R_f 0.52 (1:9 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = –3.33 (*c* 1.0, DMSO); m.p. 153−155 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3288 (br), 2981 (br), 2552 (br), 2161 (s), 1978 (s), 1706 (C=O, s), 1658 (C=O, s), 1614 (w), 1544 (w), 1484 (w), 1434 (w), 1398 (w), 1349 (w), 1330 (w), 1263 (w), 1202 (w), 1145 (w), 1105 (s), 1057 (s), 1017 (s), 943 (s); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (1H, br s, N-H), 8.12 (1H, t, *J* 5.7, N-H), 7.79 (1H, dd, *J* 8.5, 7.3, C^6-H), 7.73 (1H, t, *J* 6.0, N-H), 7.48 (1H, d, *J* 7.3, C^7-H), 7.40–7.26 (6H, m, C^5-H, C$^{7'''''}$-H, C$^{8'''''}$-H, C$^{9'''''}$-H, C$^{10'''''}$-H, C$^{11'''''}$-H), 7.22 (1H, t, *J* 5.8, N-H), 5.11 (1H, dd, *J* 12.8, 5.4, C$^{3'}$-H), 4.99 (2H, s, C$^{5'''''}$-H$_2$), 4.80 (2H, s, C$^{2''}$-H$_2$), 3.85 (2H, s, C$^{2''''}$-H$_2$), 3.50 (2H, t, *J* 5.6, C$^{1'''}$-H$_2$), 3.40–3.33 (2H, m, C$^{2'''}$-H$_2$), 3.12–3.03 (2H, m, C$^{1'''''/3'''''}$-H$_2$), 3.02–2.94 (2H, m, C$^{1'''''/3'''''}$-H$_2$), 2.95–2.83 (1H, m, C$^{5'}$-H$_a$), 2.67–2.52 (2H, m, C$^{4'}$-H$_a$, C$^{5'}$-H$_b$), 2.07–1.98 (1H, m, C$^{4'}$-H$_b$), 1.53 (2H, tt, *J* 6.9, 6.9, C$^{2'''''}$-H$_2$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.8 (C$^{6'}$), 169.9 (C$^{2'}$), 169.0 (C$^{1''''}$), 167.0 (C$^{1''}$), 166.7 (C^1), 165.4 (C^3), 156.1 (C$^{4'''''}$), 155.1 (C^4), 137.2 (C$^{6'''''}$), 136.9 (C^6), 133.0 (C^{7a}), 128.4 (C$^{7'''''/8'''''/10'''''/11'''''}$), 127.8 (C$^{9'''''}$), 127.7 (C$^{7'''''/8'''''/10'''''/11'''''}$), 120.3 (C^5), 116.8 (C^{3a}), 116.0 (C^7), 69.8 (C$^{2''''}$), 69.4 (C$^{1'''}$), 67.8 (C$^{2''}$), 65.2 (C$^{5'''''}$), 48.8 (C$^{3'}$), 38.2 (C$^{2'''}$), 38.0 (C$^{1'''''/3'''''}$), 35.7 (C$^{1'''''/3'''''}$), 30.9 (C$^{5'}$), 29.6 (C$^{2'''''}$), 22.0 (C$^{4'}$); HRMS *m/z* (ESI$^+$) [Found: 624.23031, $C_{30}H_{34}N_5O_{10}$ requires [M+H]$^+$ 624.23002]; LRMS *m/z* (ESI$^+$) 646.2 ([M+Na]$^+$, 100%); HPLC retention time 6.2 min, >99.9%.

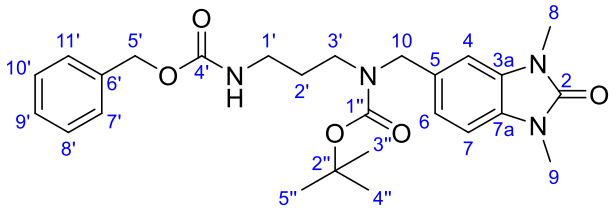
Benzyl (3'-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1'-yl)carbamate (270)



Benzyl (3-aminopropyl)carbamate (**262**) (131 mg, 0.631 mmol, 1.2 eq.) was dissolved in anhydrous 1,2-dichloroethane (4.00 mL) and 3 Å molecular sieves were added. 1,3-Dimethyl-1,3-dihydro-2*H*-benzo[*d*]imidazol-2-one-5-carbaldehyde (**34**) (100 mg, 0.526 mmol, 1.0 eq.) and acetic acid (45.0 µL, 0.789 mmol, 1.5 eq.) were added. The reaction mixture was stirred at rt for 3 h. After this time ^{1}H NMR analysis of the reaction mixture indicated almost complete imine formation. $NaBH(OAc)_3$ (557 mg, 2.63 mmol, 5.0 eq.) was added and the reaction mixture stirred at rt for 20 h. After this time TLC analysis indicated complete consumption of the intermediate (1:9 MeOH/CH_2Cl_2). The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL) and filtered through a pad of Celite®. The filter pad was washed with CH_2Cl_2. The filtrate was extracted with CH_2Cl_2 (2 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–25% EtOH/$CHCl_3$ gradient) to yield the title compound as a colourless oil (121 mg, 0.317 mmol, 60%): R_f 0.33 (1:9 MeOH/CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 1686 (C=O, s), 1630 (w), 1611 (w), 1508 (w), 1454 (m), 1396 (m), 1341 (w), 1250 (m), 1189 (w), 1138 (w), 953 (w), 909 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.27 (5H, m, C$^{7'}$-H, C$^{8'}$-H, C$^{9'}$-H, C$^{10'}$-H, C$^{11'}$-H), 7.00 (1H, dd, *J* 8.0, 1.6, C^{6}-H), 6.94 (1H, d, *J* 1.6, C^{4}-H), 6.81 (1H, d, *J* 8.0, C^{7}-H), 5.85 (1H, br s, N-H), 5.06 (2H, s, C$^{5'}$-H_2), 3.77 (2H, s, C^{10}-H_2), 3.35 (3H, s, C$^{8/9}$-H_3), 3.32–3.27 (5H, m, C$^{8/9}$-H_3, C$^{1'}$-H_2), 2.71 (2H, t, *J* 6.7, C$^{3'}$-H_2), 1.69 (2H, tt, *J* 6.7, 6.7, C$^{2'}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.6 (C$^{4'}$), 154.8 (C^{2}), 136.8 (C$^{6'}$), 133.5 (C^{5}), 130.2 (C^{3a}), 129.1 (C^{7a}), 128.5 (C$^{7'/8'/9'/10'/11'}$), 128.1 (C$^{7'/8'/9'/10'/11'}$), 121.2 (C^{6}), 107.3 (C^{4}), 107.0 (C^{7}), 66.5 (C$^{5'}$), 54.2 (C^{10}), 47.4 (C$^{3'}$), 40.1 (C$^{1'}$), 29.4 (C$^{2'}$), 27.2 (C$^{8/9}$), 27.1 (C$^{8/9}$); HRMS *m/z* (ESI^{+}) [Found: 383.20709,

$C_{27}H_{29}N_4O_3$ requires $[M+H]^+$ 383.20777]; LRMS *m/z* (ESI$^+$) 405.2 ($[M+Na]^+$, 12%); HPLC retention time 4.7 min, 97.5%.

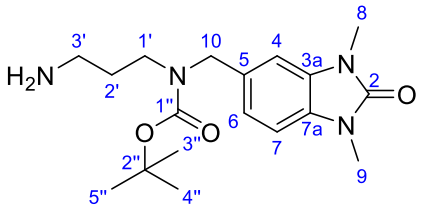
Benzyl (3'-{(*tert*-butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1'-yl)carbamate (271)



Benzyl (3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl) carbamate (**270**) (122 mg, 0.319 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (3.00 mL) and Et_3N (484 µL, 0.319 mmol, 1.0 eq.) was added. The reaction mixture was cooled to 0 °C and di-*tert*-butyl dicarbonate (97.5 mg, 0.447 mmol, 1.4 eq.) was added. The reaction mixture was warmed to rt and stirred for 3 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–6% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (123 mg, 0.255 mmol, 80%): R_f 0.27 (1:19 MeOH/CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3326 (br), 2933 (br), 1685 (C=O, s), 1612 (w), 1508 (m), 1454 (m), 1416 (w), 1394 (w), 1365 (w), 1302 (w), 1241 (m), 1165 (m), 1138 (m), 1080 (w), 996 (w), 905 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 7.39–7.24 (5H, m, $C^{12'}$-H, $C^{13'}$-H, $C^{14'}$-H, $C^{15'}$-H, $C^{16'}$-H), 7.06–7.01 (1H, m, $C^{4/7}$-H), 6.98–6.93 (2H, m, $C^{4/7}$-H, C^6-H), 6.78 (1H, br s, N-H), 5.02 (2H, s, $C^{10'}$-H_2), 4.41 (2H, s, $C^{9'}$-H_2), 3.31 (3H, s, $C^{8/9}$-H_3), 3.30 (3H, s, $C^{8/9}$-H_3), 3.17 (2H, t, *J* 7.3, $C^{3'}$-H_2), 3.00 (2H, t, *J* 7.3, $C^{1'}$-H_2), 1.65 (2H, tt, *J* 7.3, 7.3, $C^{2'}$-H_2), 1.44 (9H, s, $C^{3''}$-H_3, $C^{4''}$-H_3, $C^{5''}$-H_3); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 155.6 ($C^{8'}$), 154.6 ($C^{1''}$), 153.8 (C^2), 136.9 ($C^{11'}$), 131.2 (C^5), 129.6 ($C^{3a/7a}$), 128.7 ($C^{3a/7a}$), 127.8 ($C^{12'/13'/15'/16'}$), 127.1 ($C^{14'}$), 127.0 ($C^{12'/13'/15'/16'}$), 119.7 (C^6), 106.8 ($C^{4/7}$), 106.2 ($C^{4/7}$), 78.5 ($C^{2''}$), 64.8 ($C^{10'}$), 49.6 ($C^{9'}$), 43.7 ($C^{3'}$), 38.0 ($C^{1'}$), 27.9 ($C^{2'}$), 27.7 ($C^{3''}$, $C^{4''}$,

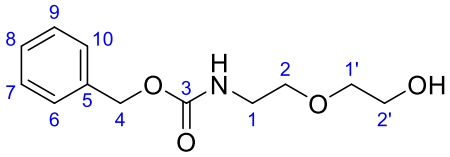
$C^{5''}$), 26.4 ($C^{8/9}$), 26.3 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 505.24148, $C_{26}H_{34}N_4O_5Na$ requires $[M+Na]^+$ 505.24214]; LRMS *m/z* (ESI^+) 505.2 ($[M+Na]^+$, 68%); HPLC retention time 9.5 min, 98.6%.

tert-Butyl (3'-aminoprop-1'-yl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (268)



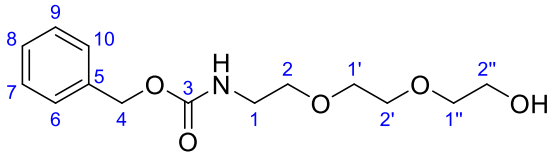
Benzyl (3-{(*tert*-butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl) methyl]amino}propyl)carbamate (**271**) (0.14 g, 0.30 mmol, 1.0 eq.) was dissolved in MeOH (1.5 mL) and the reaction mixture purged with N_2. Pd on carbon (10% *w/w*, 16 mg, 0.015 mmol, 0.05 eq.) was added. The reaction mixture was purged with H_2 and the reaction mixture stirred at rt under an atmosphere of H_2 for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:9 $MeOH/CH_2Cl_2$). The reaction mixture was purged with N_2, filtered through a pad of Celite® and the filter pad washed with MeOH. The filtrate was concentrated *in vacuo* to yield the title compound as a colourless oil (77 mg, 0.22 mmol, 74%): R_f 0.50 (1:9 $MeOH/CH_2Cl_2$ + 1% Et_3N); $\tilde{\nu}_{max}/cm^{-1}$ 2932 (br), 2161 (br), 1683 (C=O, s), 1509 (m), 1463 (m), 1416 (m), 1394 (m), 1365 (m), 1268 (m), 1239 (m), 1164 (m), 1081 (m), 1028 (w), 922 (w); ^1H NMR (500 MHz, DMSO-d_6, 373 K) δ 7.06 (1H, d, *J* 7.9, C^7-H), 7.03–6.95 (2H, m, C^4-H, C^6-H), 4.42 (2H, s, C^{10}-H_2), 3.32 (6H, s, C^8-H_3, C^9-H_3), 3.24 (2H, t, *J* 7.3, $C^{1'}$-H_2), 2.68 (2H, t, *J* 7.3, $C^{3'}$-H_2), 1.74 (2H, tt, *J* 7.3, 7.3, $C^{2'}$-H_2), 1.45 (9H, s, $C^{3''}$-H_3, $C^{4''}$-H_3, $C^{5''}$-H_3); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 154.7 ($C^{1''}$), 153.7 (C^2), 131.1 (C^5), 129.6 (C^{7a}), 128.7 (C^{3a}), 119.7 (C^6), 106.8 (C^4), 106.3 (C^7), 78.6 ($C^{2''}$), 49.7 (C^{10}), 43.5 ($C^{1'}$), 37.2 ($C^{3'}$), 27.7 ($C^{3''}$, $C^{4''}$, $C^{5''}$), 27.5 ($C^{2'}$), 26.4 ($C^{8/9}$), 26.3 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 349.22374, $C_{18}H_{29}N_4O_3$ requires $[M+H]^+$ 349.22342]; LRMS *m/z* (ESI^+) 175.0 ($[M–C_8H_{16}N_2O_2]^+$, 100%); HPLC retention time 3.3 min, 97.5%.

Benzyl [2-(2'-hydroxyethoxy)ethyl]carbamate (278)[263,264]



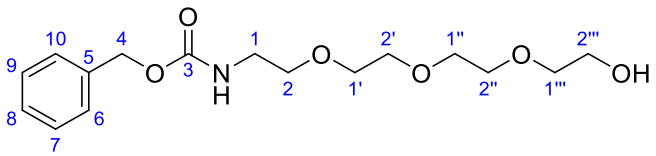
2-(2-Aminoethoxy)ethanol (3.00 mL, 27.2 mmol, 1.0 eq.) was dissolved in anhydrous CH_2Cl_2 (25.0 mL) and anhydrous Et_3N (3.79 mL, 27.2 mmol, 1.0 eq.) was added. The reaction mixture was cooled to 0 °C. Benzyl chloroformate (3.83 mL, 27.2 mmol, 1.0 eq.) in anhydrous CH_2Cl_2 (33.0 mL) was added slowly. The reaction mixture was warmed to rt and stirred for 5 h. After this time TLC analysis indicated partial consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). Additional benzyl chloroformate (1.15 mL, 8.16 mmol, 0.3 eq.) and Et_3N (1.14 mL, 8.16 mmol, 0.3 eq.) were added. The reaction mixture was stirred at rt for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with H_2O (75 mL) and the aqueous fraction extracted with CH_2Cl_2 (2 × 50 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 30–100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (6.33 g, 26.5 mmol, 97%): R_f 0.15 (3:2 EtOAc/petroleum ether); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41–7.22 (5H, m, C^6-H, C^7-H, C^8-H, C^9-H, C^{10}-H), 5.02 (2H, s, C^4-H_2), 4.58 (1H, t, *J* 5.5, O-H), 3.53–3.45 (2H, m, $C^{2'}$-H_2), 3.45–3.37 (4H, m, C^2-H_2, $C^{1'}$-H_2), 3.16 (2H, q, *J* 5.9, C^1-H_2); LRMS *m/z* (ESI$^+$) 262.2 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[263,264]

Benzyl {2-[2'-(2''-hydroxyethoxy)ethoxy]ethyl}carbamate (279)[265,266]



2-(2-(2-Amino(ethoxy)ethoxy) ethanol (390 µL, 2.82 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (2.00 mL) and Et_3N (815 µL, 3.67 mmol, 1.3 eq.) was added. The reaction mixture was cooled to 0 °C and benzyl chloroformate (517 µL, 3.67 mmol, 1.3 eq.) in CH_2Cl_2 (3.00 mL) was added slowly. The reaction mixture was warmed to rt and stirred for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 30–100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (459 mg, 1.62 mmol, 57%): R_f 0.29 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.26 (5H, m, C^6-H, C^7-H, C^8-H, C^9-H, C^{10}-H), 5.44 (1H, br s, O-H), 5.10 (2H, s, C^4-H_2), 3.78–3.68 (2H, m, $C^{1/2/1'/2'/1''/2''}$-H_2), 3.67–3.60 (4H, m, $C^{1/2/1'/2'/1''/2''}$-H_4), 3.60–3.54 (4H, m, $C^{1/2/1'/2'/1''/2''}$-H_4), 3.44–3.35 (2H, m, $C^{1/2/1'/2'/1''/2''}$-H_2), 2.50 (1H, t, *J* 6.1, N-H); LRMS *m/z* (ESI^+) 306.5 ($[M+Na]^+$, 100%). The spectroscopic data are in good agreement with literature values.[265,266]

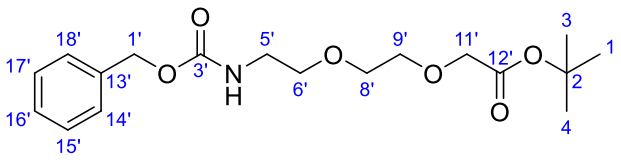
Benzyl (2-{2'-[2''-(2'''-hydroxyethoxy)ethoxy]ethoxy}ethyl)carbamate (280)[265,267]



2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethanol (500 µL, 2.59 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (2.00 mL) and Et_3N (746 µL, 3.36 mmol, 1.3 eq.) was added. The reaction mixture was cooled to 0 °C. Benzyl chloroformate (474 µL, 3.36 mmol, 1.3 eq.) in CH_2Cl_2 (3.00 mL) was added slowly. The reaction mixture was warmed to rt and stirred for 3 h. After this time TLC analysis indicated partial consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The

reaction mixture was cooled to 0 °C and additional Et_3N (287 µL, 1.30 mmol, 0.5 eq.) and benzyl chloroformate (182 µL, 1.30 mmol, 0.5 eq.) were added. The reaction mixture was warmed to rt and stirred for 17 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with H_2O (40 mL) and extracted with CH_2Cl_2 (3 × 40 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 30–100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (602 mg, 1.84 mmol, 71%): R_f 0.17 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.25 (5H, m, C^6-H, C^7-H, C^8-H, C^9-H, C^{10}-H), 6.04 (1H, br s, N-H), 5.10 (2H, s, C^4-H_2), 3.83–3.47 (14H, m, C^2-H_2, $C^{1'}$-H_2, $C^{2'}$-H_2, $C^{1''}$-H_2, $C^{2''}H_2$, $C^{1'''}$-H_2, $C^{2'''}$-H_2), 3.43–3.35 (2H, m, C^1-H_2), 3.07 (1H, t, *J* 6.1, O-H); LRMS *m/z* (ESI$^+$) 350.2 ([M+Na]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[265,267]

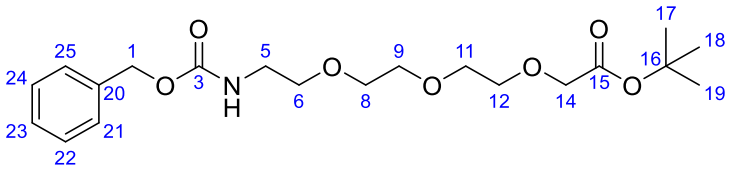
2,2-Dimethylpropyl 3'-oxo-1'-phenyl-2',7',10'-trioxa-4'-azadodecan-12'-oate (281)[263]



Benzyl [2-(2-hydroxyethoxy)ethyl]carbamate (**278**) (3.00 g, 12.5 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (60.0 mL). The reaction mixture was cooled to 0 °C and potassium *tert*-butoxide (1.41g, 12.5 mmol, 1.0 eq.) was added portionwise. The reaction mixture was stirred at 0 °C for 30 min. *tert*-Butyl bromoacetate (2.22 mL, 15.0 mmol, 1.2 eq.) was added at 0 °C and the reaction mixture stirred at 0 °C for 3 h. The reaction mixture was warmed to rt and stirred for 2 h. After this time TLC analysis indicated complete consumption of starting material (7:3 EtOAc/petroleum ether). The reaction was quenched with H_2O (100 mL) and the reaction volume reduced by concentration *in vacuo*. The reaction mixture was extracted with EtOAc (3 × 60 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–80% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (2.42 g,

6.59 mmol, 53%): R_f 0.55 (7:3 EtOAc/petroleum ether); $\bar{\nu}_{max}$/cm^{-1} 3353 (br), 2978 (br), 2933 (br), 1743 (C=O, s), 1719 (C=O, s), 1526 (m), 1455 (m), 1394 (m), 1368 (m), 1229 (s), 1142 (s), 1118 (s), 1042 (w), 1024 (w), 916 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41–7.28 (4H, m, C$^{14'}$-H, C$^{15'}$-H, C$^{17'}$-H, C$^{18'}$-H), 7.28–7.22 (1H, m, C$^{16'}$-H), 5.01 (2H, s, C$^{1'}$-H$_2$), 3.97 (2H, s, C$^{11'}$-H$_2$), 3.61–3.47 (4H, m, C$^{6'/8'/9'}$-H$_4$), 3.42 (2H, t, *J* 6.0, C$^{6'/8'/9'}$-H$_2$), 3.15 (2H, dt, *J* 5.9, 5.9 C$^{5'}$-H$_2$), 1.42 (9H, s, C^1-H$_3$, C^3-H$_3$, C^4-H$_3$); LRMS *m/z* (ESI$^+$) 254.2 ([M–Boc+H]$^+$, 100%). The spectroscopic data are in good agreement with literature values.[263]

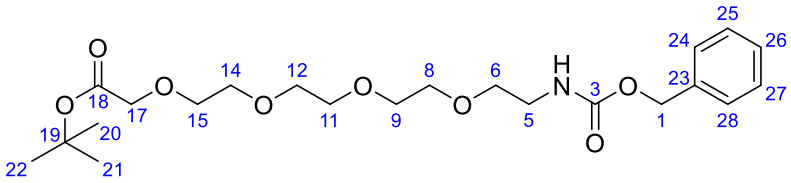
tert-Butyl 3-oxo-1-phenyl-2,7,10,13-tetraoxa-4-azapentadecan-15-oate (282)[248]



Benzyl {2-[2-(2-hydroxyethoxy)ethoxy]ethyl}carbamate (**279**) (362 mg, 1.27 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (6.00 mL) and the reaction mixture cooled to 0 °C. Potassium *tert*-butoxide (143 mg, 1.27 mmol, 1.0 eq.) was added portionwise. The reaction mixture was stirred at 0 °C for 30 min. *tert*-Butyl bromoacetate (225 µL, 1.52 mmol, 1.2 eq.) was added at 0 °C. The reaction mixture was warmed to rt and stirred for 6 h. After this time TLC analysis indicated partial consumption of starting material (1:9 MeOH/CH_2Cl_2). Additional *tert*-butyl bromoacetate (89.6 µL, 0.635 mmol, 0.5 eq.) was added and the reaction mixture stirred at rt for 15 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (40 mL) and the reaction volume reduced by concentration *in vacuo*. The reaction mixture was extracted with EtOAc (3 × 40 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (185 mg, 0.465 mmol, 37%): R_f 0.61 (7:3 EtOAc/petroleum ether); $\bar{\nu}_{max}$/cm^{-1} 3346 (br), 2872 (br), 2161 (br), 1978 (br), 1745 (C=O, s), 1718 (C=O, s), 1599 (w), 1527 (w), 1455 (w), 1393 (w), 1368 (w), 1231 (m), 1117 (C-O, s), 1025 (w), 942 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.24 (5H, m,

C^{21}-H, C^{22}-H, C^{23}-H, C^{24}-H, C^{25}-H), 5.43 (1H, br s, N-H), 5.09 (2H, s, C^{1}-H_2), 3.98 (2H, s, C^{14}-H_2), 3.75–3.62 (4H, m, $C^{6/8/9/11/12}$-H_4), 3.66–3.58 (4H, m, $C^{6/8/9/11/12}$-H_4), 3.55 (2H, t, *J* 5.1, $C^{6/8/9/11/12}$-H_2), 3.42–3.34 (2H, m, C^{5}-H_2), 1.46 (9H, s, C^{17}-H_3, C^{18}-H_3, C^{19}-H_3); LRMS *m/z* (ESI^+) 298.2 ($[M–Boc+H]^+$, 100%), 420.2 ($[M+Na]^+$, 29%). The spectroscopic data are in are in good agreement with literature values.[248]

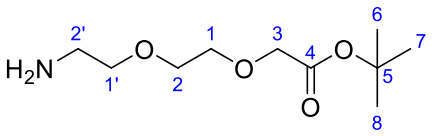
tert-Butyl 3-oxo-1-phenyl-2,7,10,13,16-pentaoxa-4-azaoctadecan-18-oate (283)



Benzyl (2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}ethyl)carbamate (**280**) (602 mg, 1.84 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (6.00 mL). The reaction mixture was cooled to 0 °C and potassium *tert*-butoxide was added. The reaction mixture was stirred at 0 °C for 30 min. *tert*-Butyl bromoacetate (327 µL, 2.22 mmol, 1.2 eq.) was added. The reaction mixture was warmed to rt and stirred for 16 h. After this time TLC analysis indicated partial consumption of starting material (1:19 MeOH/CH_2Cl_2). Additional potassium *tert*-butoxide (103 mg, 0.920 mmol, 0.5 eq.) and *tert*-butyl bromoacetate (136 µL, 0.920 mmol, 0.5 eq.) were added. The reaction mixture was stirred at rt for 3 h. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (20 mL) and the reaction volume reduced by concentration *in vacuo*. The reaction mixture was extracted with EtOAc (3 × 40 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated in vacuo. The product was purified using silica flash column chromatography (elution with 10–100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (250 mg, 0.566 mmol, 31%): R_f 0.36 (7:3 EtOAc/petroleum ether); $\tilde{\nu}_{max}$/cm^{-1} 3341 (br), 2872 (br), 2160 (br), 1977 (br), 1745 (C=O, s), 1718 (C=O, s), 1529 (m), 1455 (m), 1394 (m), 1246 (m), 1119 (C-O, s), 1026 (w), 943 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.27 (5H, m, C^{24}-H, C^{25}-H, C^{26}-H, C^{27}-H, C^{28}-H), 5.38 (1H, br s, N-H), 5.10 (2H, s, C^{1}-H_2), 3.99 (2H, s, C^{17}-H_2), 3.70–3.52 (12H, m, C^{8}-H_2, C^{9}-H_2, C^{11}-H_2, C^{12}-H_2, C^{14}-H_2, C^{15}-H_2), 3.49 (2H, t, *J* 5.2,

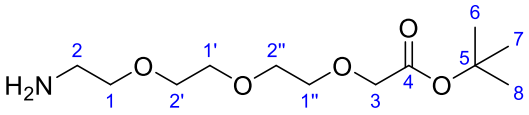
C^{6}-H_2), 3.42–3.36 (2H, m, C^{5}-H_2), 1.47 (9H, s, C^{20}-H_3, C^{21}-H_3, C^{22}-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.8 (C^{18}), 156.6 (C^{3}), 136.8 (C^{23}), 128.6 ($C^{24/25/26/26/28}$), 128.23 ($C^{24/25/26/27/28}$), 128.16 ($C^{24/25/26/27/28}$), 81.7 (C^{19}), 70.8 ($C^{6/8/9/11/12/14/15}$), 70.68 ($C^{6/8/9/11/12/14/15}$), 70.66 ($C^{6/8/9/11/12/14/15}$), 70.6 ($C^{6/8/9/11/12/14/15}$), 70.4 ($C^{6/8/9/11/12/14/15}$), 70.2 ($C^{6/8/9/11/12/14/15}$), 69.1 (C^{17}), 66.7 (C^{1}), 41.0 (C^{5}), 28.2 (C^{20}, C^{21}, C^{22}); HRMS *m/z* (ESI^+) [Found: 464.2252, $C_{22}H_{35}NO_8Na$ requires $[M+Na]^+$ 464.2255]; LRMS *m/z* (ESI^+) 464.2 ($[M+Na]^+$, 22%); HPLC retention time (Atlantis) 14.0 min, >99.9%.

***tert*-Butyl [2-(2'-aminoethoxy)ethoxy]acetate (274)**[263]



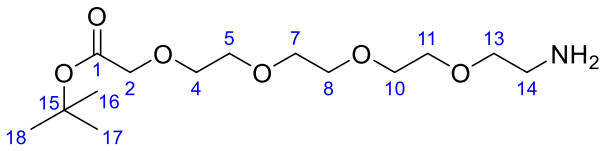
2,2-Dimethylpropyl 3-oxo-1-phenyl-2,7,10-trioxa-4-azadodecan-12-oate (**281**) (1.00 g, 2.83 mmol, 1.0 eq.) was dissolved in MeOH (14.0 mL) and the reaction mixture purged with N_2. Pd on carbon (10% *w/w*, 151 mg, 0.142 mmol, 0.05 eq.) was added. The reaction mixture was purged with H_2 and the reaction mixture stirred at rt under an atmosphere of H_2 for 2 h. After this time TLC analysis indicated complete consumption of starting material (4:1 EtOAc/petroleum ether). The reaction mixture was purged with N_2, filtered through a pad of Celite® and the filter pad washed with MeOH. The filtrate was concentrated *in vacuo* to yield the title compound as a colourless oil (586 mg, 2.67 mmol, 94%): R_f 0.43 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 2870 (br), 2160 (br), 1745 (C=O, s), 1671 (br), 1457 (w), 1393 (w), 1368 (m), 1301 (w), 1228 (m), 1141 (s), 1116 (s), 918 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.98 (2H, s, C^{3}-H_2), 3.61–3.54 (2H, m, $C^{1/2}$-H_2), 3.54–3.47 (2H, m, $C^{1/2}$-H_2), 3.36 (2H, t, *J* 5.8, $C^{1'}$-H_2), 2.65 (2H, t, *J* 5.8, $C^{2'}$-H_2), 1.42 (9H, s, C^{6}-H_3, C^{7}-H_3, C^{8}-H_3); LRMS *m/z* (ESI^+) 220.2 ($[M+H]^+$, 35%). The spectroscopic data are in good agreement with literature values.[263]

tert-Butyl {2''-[2'-(2-aminoethoxy)ethoxy]ethoxy}acetate (275)[181,268,269]



tert-Butyl 3-oxo-1-phenyl-2,7,10,13-tetraoxa-4-azapentadecan-15-oate (**282**) (0.13 g, 0.32 mmol, 1.0 eq.) was dissolved in MeOH (3.0 mL) and the reaction mixture purged with N_2. Pd on carbon (10% *w/w*, 17 mg, 0.016 mmol, 0.05 eq.) was added. The reaction mixture was purged with H_2 and the reaction mixture stirred at rt under an atmosphere of H_2 for 2 h. After this time TLC analysis indicated complete consumption of starting material (7:3 EtOAc/petroleum ether). The reaction mixture was purged with N_2, filtered through a pad of Celite® and the filter pad washed with MeOH. The filtrate was concentrated *in vacuo* to yield the title compound as a colourless oil (67 mg, 2.5 mmol, 80%): R_f 0.45 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 (2H, s, C^3-H_2), 3.76–3.60 (8H, m, $C^{1'}$-H_2, $C^{2'}$-H_2, $C^{1''}$-H_2, $C^{2''}$-H_2), 3.54 (2H, t, *J* 5.3, C^1-H_2), 2.88 (2H, t, *J* 5.3, C^2-H_2), 2.66 (2H, br s, N-H_2), 1.44 (9H, s, C^6-H_3, C^7-H_3, C^8-H_3); LRMS *m/z* (ESI^+) 286.5 ($[M+Na]^+$, 100%). The spectroscopic data are in good agreement with literature values.[181,268,269]

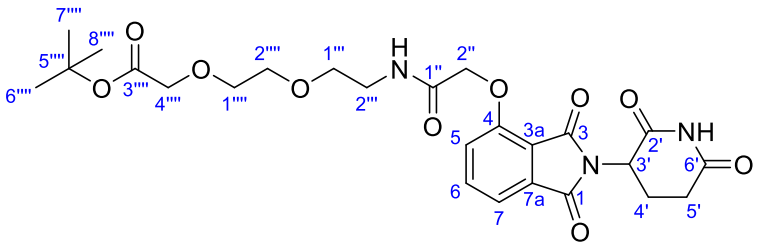
tert-Butyl 14-amino-3,6,9,12-tetraoxatetradecan-1-oate (276)[270]



tert-Butyl 3-oxo-1-phenyl-2,7,10,13,16-pentaoxa-4-azaoctadecan-18-oate (**283**) (243 mg, 0.550 mmol, 1.0 eq.) was dissolved in MeOH (3.00 mL). The reaction mixture was purged with N_2. Pd on carbon (10% *w/w*, 29.0 mg, 0.0275 mmol, 0.05 eq.) was added and the reaction mixture purged with H_2. The reaction mixture was stirred at rt under an atmosphere of H_2 for 4 h. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction mixture was purged with N_2. The reaction mixture was filtered through a pad of Celite® and the filter pad washed with MeOH. The filtrate was concentrated *in*

vacuo to yield the title compound as a colourless oil (143 mg, 0.465 mmol, 85%): R_f 0.50 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); $\bar{\nu}_{max}$/cm^{-1} 2870 (br), 1744 (C=O, s), 1673 (w), 1533 (w), 1457 (w), 1393 (w), 1368 (w), 1228 (w), 1113 (s), 940 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.01 (2H, s, C^2-H_2), 3.82–3.58 (16H, m, C^4-H_2, C^5-H_2, C^7-H_2, C^8-H_2, C^{10}-H_2, C^{11}-H_2, C^{13}-H_2, N-H_2), 3.01 (2H, d, *J* 5.3, C^{14}-H_2), 1.46 (9H, s, C^{16}-H_3, C^{17}-H_3, C^{18}-H_3); LRMS *m/z* (ESI$^+$) 308.2 ([M+H]$^+$, 21%). The spectroscopic data are in good agreement with literature values.[270]

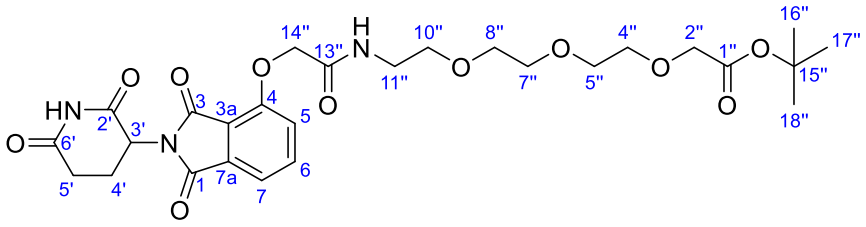
tert-Butyl {2''''-[2'''-(2''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]ethoxy}acetate (284)



{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetic acid (**229**) (163 mg, 0.491 mmol, 1.2 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (2.50 mL). 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro-phosphate (187 mg, 0.491 mmol, 1.2 eq.) and *N*,*N*-diisopropylethylamine (85.5 µL, 0.491 mmol, 1.2 eq.) were added. The reaction mixture was stirred at rt for 30 min. *tert*-Butyl [2-(2-aminoethoxy)ethoxy]acetate (**274**) (89.6 mg, 0.409 mmol, 1.0 eq.) was added and the reaction mixture stirred at rt for 23 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 25 mL) and extracted with EtOAc (75 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 75 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 50–100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless oil (131 mg, 0.246 mmol, 60%): R_f 0.52 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = –2.90 (*c* 1.0, $CHCl_3$); $\bar{\nu}_{max}$/cm^{-1} 2925 (br), 2361 (br), 2161 (br), 1979 (br), 1705 (C=O, s), 1615 (C=O, s), 1540 (m), 1484 (m), 1442 (w), 1394 (m), 1367 (w), 1324 (w), 1299 (m), 1259

(m), 1197 (m), 1118(m), 1055 (m), 1016 (m), 939 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (1H, br s, N-H), 7.72 (1H, dd, *J* 8.4, 7.4, C^{6}-H), 7.64 (1H, t, *J* 5.2, N-H), 7.52 (1H, dd, *J* 7.4, 0.6, C^{5}-H), 7.18 (1H, dd, *J* 8.4, 0.6, C^{7}-H), 5.02–4.91 (1H, m, $C^{3'}$-H), 4.64 (2H, s, $C^{2''}$-H_2), 4.00 (2H, s, $C^{4''''}$-H_2), 3.78–3.49 (8H, m, $C^{1'''}$-H_2, $C^{2'''}$-H_2, $C^{1''''}$-H_2, $C^{2''''}$-H_2), 2.96–2.68 (3H, m, $C^{4'}$-H_a, $C^{5'}$-H_2), 2.21–2.07 (1H, m, $C^{4'}$-H_b), 1.44 (9H, s, $C^{6''''}$-H_3, $C^{7''''}$-H_3, $C^{8''''}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2 ($C^{6'}$), 170.1 ($C^{3''''}$), 168.3 ($C^{2'}$), 167.0 ($C^{1''}$), 166.8 (C^{3}), 165.9 (C^{1}), 154.6 (C^{4}), 137.0 (C^{6}), 133.8 (C^{7a}), 119.5, (C^{7}), 118.2 (C^{3a}), 117.4 (C^{5}), 81.9 ($C^{5''''}$), 70.7 ($C^{1''''}$), 70.3 ($C^{1'''/2''''}$), 69.6 ($C^{1'''/2''''}$), 69.1 ($C^{4''''}$), 68.0 ($C^{2''}$), 49.4 ($C^{3'}$), 39.1 ($C^{2'''}$), 31.5 ($C^{5'}$), 28.2 ($C^{6''''}$, $C^{7''''}$, $C^{8''''}$), 22.8 ($C^{4'}$); HRMS *m/z* (ESI^+) [Found: 534.20745, $C_{25}H_{32}N_3O_{10}$ requires $[M+H]^+$ 534.20822]; LRMS *m/z* (ESI^-) 532.2 ($[M–H]^-$, 100%); HPLC retention time 6.3 min, >99.9%.

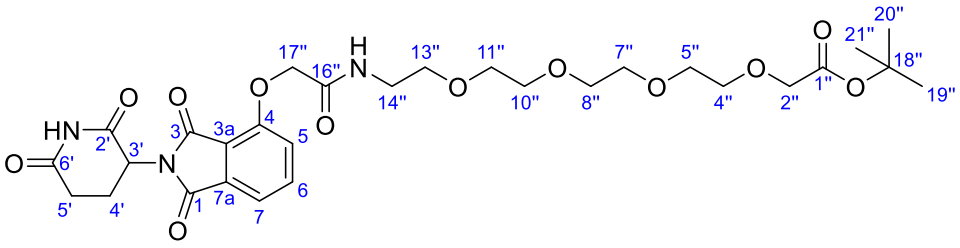
***tert*-Butyl 14''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-13''-oxo-3'',6'',9''-trioxa-12''-azatetradecan-1''-oate (285)**



{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetic acid (**229**) (91 mg, 0.27 mmol, 1.2 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (1.5 mL). 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro phosphate (0.10 g, 0.27 mmol, 1.2 eq.) and *N*,*N*-diisopropylethylamine (48 µL, 0.27 mmol, 1.2 eq.) were added. The reaction mixture was stirred at rt for 30 min. *tert*-Butyl {2-[2-(2-aminoethoxy)ethoxy]ethoxy}acetate (**275**) (60 mg, 0.23 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (1.0 mL) was added. The reaction mixture was stirred at rt for 17 h. After this time TLC analysis indicated complete consumption of starting material (1:19 $MeOH/CH_2Cl_2$). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (20 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 20 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in*

vacuo. The product was purified using silica flash column chromatography (elution with 0–5% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (31 mg, 0.054 mmol, 20%): R_f 0.52 (1:24 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = +1.75 (*c* 1.0, $CHCl_3$); $\tilde{\nu}_{max}$/cm^{-1} 2927 (br), 2572 (br), 2360 (m), 2034 (m), 1978 (m), 1706 (C=O, s), 1614 (m), 1541 (w), 1484 (w), 1451 (w), 1393 (m), 1368 (w), 1324 (w), 1300 (w), 1257 (w), 1198 (w), 1117 (m), 1055 (w), 1013 (w), 986 (w), 939 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.87 (1H, br s, N-H), 7.72 (1H, dd, *J* 8.4, 7.4, C^6-H), 7.62 (1H, t, *J* 5.5, N-H), 7.53 (1H, d, *J* 7.4, C^5-H), 7.18 (1H, d, *J* 8.4, C^7-H), 4.99–4.91 (1H, m, C$^{3'}$-H), 4.64 (2H, s, C$^{14''}$-H_2), 4.07–3.95 (2H, m, C$^{2''}$-H_2), 3.75–3.49 (12H, m, C$^{4''}$-H_2, C$^{5''}$-H_2, C$^{7''}$-H_2, C$^{8''}$-H_2, C$^{10''}$-H_2, C$^{11''}$-H_2), 2.97–2.69 (3H, m, C$^{4'}$-H_a, C$^{5'}$-H_2), 2.18–2.10 (1H, m, C$^{4'}$-H_b), 1.44 (9H, s, C$^{16''}$-H_3, C$^{17''}$-H_3, C$^{18''}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2 (C$^{6'}$), 169.8 (C$^{1''}$), 168.2 (C$^{2'}$), 166.9 (C$^{13''}$), 166.8 (C^3), 165.9 (C^1), 154.5 (C^4), 137.0 (C^6), 133.8 (C^{7a}), 119.4 (C^7), 118.2 (C^{3a}), 117.4 (C^5), 81.8 (C$^{15''}$), 71.0 (C$^{4''/5''/7''/8''}$), 70.54 (C$^{4''/5''/7''/8''}$), 70.50 (C$^{4''/5''/7''/8''}$), 70.4 (C$^{4''/5''/7''/8''}$), 69.5 (C$^{10''}$), 69.0 (C$^{2''}$), 68.0 (C$^{14''}$), 49.4 (C$^{3'}$), 39.2 (C$^{11''}$), 31.5 (C$^{5'}$), 28.2 (C$^{16''}$, C$^{17''}$, C$^{18''}$), 22.8 (C$^{4'}$); HRMS *m/z* (ESI$^+$) [Found: 578.2342, $C_{27}H_{36}N_3O_{11}$ requires $[M+H]^+$ 578.2344]; LRMS *m/z* (ESI$^-$) 576.2 ($[M–H]^-$, 100%); HPLC retention time 6.7 min, >99.9%.

tert-Butyl 17''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-16''-oxo-3'',6'',9'',12''-tetraoxa-15''-azaheptadecan-1''-oate (286)


HN
O
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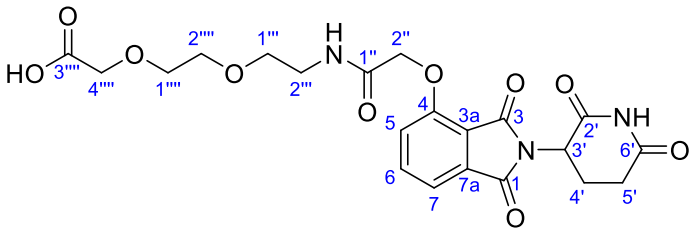
O

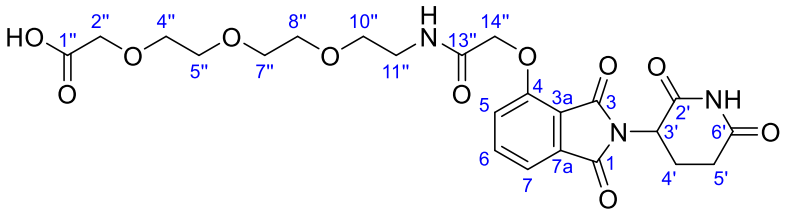
N,*N*-dimethylformamide (1.0 mL) was added. The reaction mixture was stirred at rt for 16 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (20 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 20 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–6% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (28 mg, 0.045 mmol, 20%): R_f 0.34 (1:19 MeOH/CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 22918 (br), 2161 (br), 1708 (C=O, s), 1684 (C=O, s), 1614 (w), 1540 (w), 1484 (m), 1445 (w), 1393 (m), 1367 (w), 1352 (w), 1324 (w), 1299 (m), 1260 (m), 1118 (m), 1099 (m), 1056 (m), 1016 (w), 988 (w), 939 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.02 (1H, br s, N-H), 7.71 (1H, dd, *J* 8.4, 7.3, C^6-H), 7.63 (1H, t, *J* 5.5, N-H), 7.52 (1H, d, *J* 7.3, C^5-H), 7.18 (1H, d, *J* 8.4, C^7-H), 4.99–4.91 (1H, m, $C^{3'}$-H), 4.63 (2H, s, $C^{17''}$-H_2), 3.98 (2H, s, $C^{2''}$-H_2), 3.73–3.49 (16H, m, $C^{4''}$-H_2, $C^{5''}$-H_2, $C^{7''}$-H_2, $C^{8''}$-H_2, $C^{10''}$-H_2, $C^{11''}$-H_2, $C^{13''}$-H_2, $C^{14''}$-H_2), 2.89–2.62 (3H, m, $C^{4'}$-H_a, $C^{5'}$-H_2), 2.18–2.09 (1H, m, $C^{4'}$-H_b), 1.44 (9H, s, $C^{19''}$-H_3, $C^{20''}$-H_3, $C^{21''}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3 ($C^{6'}$), 169.7 ($C^{1''}$), 168.3 ($C^{2'}$), 167.0 ($C^{16''}$), 166.8 (C^3), 165.9 (C^1), 154.5 (C^4), 137.0 (C^6), 133.8 (C^{7a}), 119.4 (C^7), 118.1 (C^{3a}), 117.3 (C^5), 81.7 ($C^{18''}$), 70.70 ($C^{4''/5''/7''/8''/10''/11''/13''}$), 70.68 ($C^{4''/5''/7''/8''/10''/11''/13''}$), 70.5 ($C^{4''/5''/7''/8''/10''/11''/13''}$), 70.40 ($C^{4''/5''/7''/8''/10''/11''/13''}$), 70.35 ($C^{4''/5''/7''/8''/10''/11''/13''}$), 69.6 ($C^{4''/5''/7''/8''/10''/11''/13''}$), 69.0 ($C^{2''}$), 68.0 ($C^{17''}$), 49.4 ($C^{3'}$), 39.2 ($C^{14''}$), 31.5 ($C^{5'}$), 28.2 ($C^{19''}$, $C^{20''}$, $C^{21''}$), 22.8 ($C^{4'}$); HRMS *m/z* (ESI^+) [Found: 644.2414, $C_{29}H_{39}N_3O_{12}Na$ requires $[M+Na]^+$ 644.2426]; LRMS *m/z* (ESI^+) 644.2 ($[M+Na]^+$, 18%); HPLC retention time 6.6 min, 98.9%.

{2’’’’-[2’’’-(2’’-{[2-(2’,6’-Dioxopiperidin-3’-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]ethoxy}acetic acid (287)



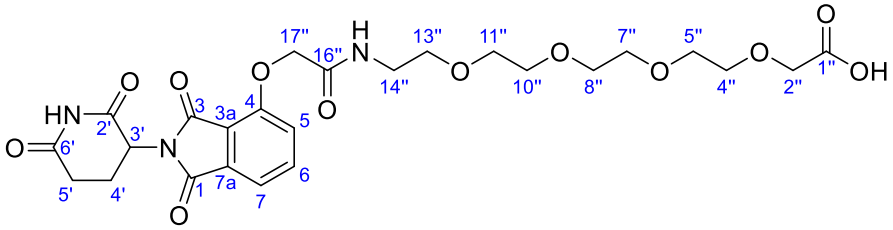
tert-Butyl {2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]ethoxy}acetate (**284**) (131 mg, 0.246 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (2.50 mL) and cooled to 0 °C. Trifluoroacetic acid (2.50 mL) was added. The reaction mixture was warmed to rt and stirred for 45 min. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* to yield the title compound as a colourless solid (113 mg, 0.237 mmol, 96%): R_f (reversed phase) 0.38 (4:1 H_2O/MeCN); $[\alpha]_D^{25}$ = –2.69 (*c* 1.0, DMSO); m.p. 163–165 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3378 (br), 2914 (br), 2546 (br), 2162 (br), 1978 (br), 1702 (C=O, s), 1646 (C=O, s), 1616 (m), 1539 (w), 1486 (w), 1437 (w), 1391 (m), 1351 (m), 1326 (w), 1299 (w), 1270 (m), 1211 (m), 1111 (m), 1081 (m), 1057 (w), 1038 (w), 1011 (w), 937 (w); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (1H, s, O-H), 11.11 (1H, br s, N-H), 8.01 (1H, t, *J* 5.6, N-H), 7.81 (1H, dd, *J* 8.5, 7.3, C^6-H), 7.49 (1H, d, *J* 7.3, C^5-H), 7.40 (1H, d, *J* 8.5, C^7-H), 5.11 (1H, dd, *J* 12.9, 5.3, C$^{3'}$-H), 4.79 (2H, s, C$^{2''}$-H$_2$), 4.01 (2H, s, C$^{4''''}$-H$_2$), 3.65–3.56 (2H, m, C$^{1''''}$-H$_2$), 3.56–3.50 (2H, m, C$^{2''''}$-H$_2$), 3.47 (2H, t, *J* 5.7, C$^{1'''}$-H$_2$), 3.35–3.29 (2H, m, C$^{2'''}$-H$_2$), 2.89 (1H, ddd, *J* 17.1, 13.9, 5.3, C$^{5'}$-H$_a$), 2.65–2.51 (2H, m, C$^{4'}$-H$_a$, C$^{5'}$-H$_b$), 2.11–1.99 (1H, m, C$^{4'}$-H$_b$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.8 (C$^{6'}$), 171.7 (C$^{3''''}$), 169.9 (C$^{2'}$), 166.9 (C$^{1''}$), 166.8 (C^3), 165.5 (C^1), 155.0 (C^4), 136.9 (C^6), 133.1 (C^{7a}), 120.3 (C^7), 116.8 (C^{3a}), 116.0 (C^5), 69.8 (C$^{1''''}$), 69.6 (C$^{2''''}$), 68.8 (C$^{1'''}$), 67.6 (C$^{4''''}$), 67.5 (C$^{2''}$), 48.8 (C$^{3'}$), 38.4 (C$^{2'''}$), 31.0 (C$^{5'}$), 22.0 (C$^{4'}$); HRMS *m/z* (ESI$^+$) [Found: 478.14555, $C_{21}H_{24}N_3O_{10}$ requires $[M+H]^+$ 478.14562]; LRMS *m/z* (ESI$^+$) 500.72 ($[M+Na]^+$, 100%); HPLC retention time 4.5 min, 99.1%.

14''-{[2-(2',6'-Dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-13''-oxo-3'',6'',9''-trioxa-12''-azatetradecan-1''-oic acid (288)



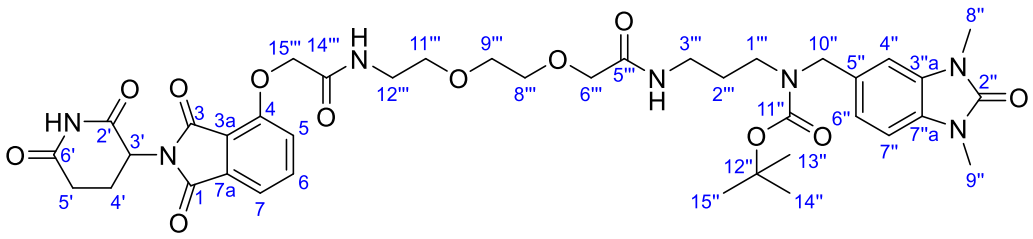
tert-Butyl 14-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-13-oxo-3,6,9-trioxa-12-azatetradecan-1-oate (**285**) (27 mg, 0.046 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (0.50 mL) and trifluoroacetic acid (0.50 mL) was added. The reaction mixture was stirred at rt for 15 min. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. The product was purified using an ISOLUTE® PE-AX column (elution with 1 M aqueous HCl) to yield the title compound as a colourless oil (24 mg, 0.046 mmol, 100%): R_f 0.06 (1:9 MeOH/CH_2Cl_2 + drop AcOH); $[\alpha]_D^{25}$ = +1.52 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 2917 (br), 2251 (br), 1707 (C=O, s), 1614 (C=O, s), 1484 (m), 1437 (w), 1394 (m), 1350 (m), 1325 (m), 1263 (m), 1199 (m), 1116 (s), 1056 (m), 1015 (m), 987 (w), 938 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (1H, dd, *J* 8.1, 8.1, C^6-H), 7.52 (1H, d, *J* 8.1, C^7-H), 7.42 (1H, dd, *J* 8.1, 1.8, C^5-H), 5.13 (1H, dd, *J* 12.6, 5.4, $C^{3'}$-H), 4.77 (2H, s, $C^{14''}$-H_2), 4.11 (2H, s, $C^{2''}$-H_2), 3.72–3.59 (10H, m, $C^{4''}$-H_2, $C^{5''}$-H_2, $C^{7''}$-H_2, $C^{8''}$-H_2, $C^{10''}$-H_2), 3.50 (2H, t, *J* 5.3, $C^{11''}$-H_2), 2.94–2.83 (1H, m, $C^{5'}$-H_a), 2.81–2.67 (2H, m, $C^{4'}$-H_a, $C^{5'}$-H_b), 2.20–2.11 (1H, m, $C^{4'}$-H_b); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.6 ($C^{6'}$), 174.0 ($C^{1''}$), 171.3 ($C^{2'}$), 170.0 (C^1), 168.3 ($C^{13''}$), 167.6 (C^3), 156.2 (C^4), 138.2 (C^6), 134.9 (C^{7a}), 121.5 (C^5), 119.2 (C^{3a}), 117.8 (C^7), 71.8 ($C^{4''/5''/7''/8''}$), 71.6 ($C^{4''/5''/7''/8''}$), 71.5 ($C^{4''/5''/7''/8''}$), 71.4 ($C^{4''/5''/7''/8''}$), 70.3 ($C^{10''}$), 69.2 ($C^{2''/14''}$), 69.1 ($C^{2''/14''}$), 50.5 ($C^{3'}$), 40.2 ($C^{11''}$), 32.2 ($C^{5'}$), 23.6 ($C^{4'}$); HRMS *m/z* (ESI$^+$) [Found: 522.1719, $C_{23}H_{28}N_3O_{11}$ requires $[M+H]^+$ 522.1718]; LRMS *m/z* (ESI$^+$) 544.2 ($[M+Na]^+$, 100%); HPLC retention time 4.6 min, 92.1%.

17’’-{[2-(2’,6’-Dioxopiperidin-3’-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-16’’-oxo-3’’,6’’,9’’,12’’-tetraoxa-15’’-azaheptadecan-1’’-oic acid (289)



tert-Butyl 17-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-16-oxo-3,6,9,12-tetraoxa-15-azaheptadecan-1-oate (**286**) (34 mg, 0.055 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (0.50 mL) and trifluoroacetic acid (0.50 mL) was added. The reaction mixture was stirred at rt for 15 min. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* to yield the title compound as colourless oil (34 mg, 0.050 mmol, 91%): R_f 0.10 (1:9 MeOH/CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 2874 (br), 2161 (br), 1706 (C=O, s), 1615 (m), 1547 (w), 1484 (m), 1441 (w), 1395 (m), 1352 (w), 1325 (w), 1262 (w), 1198 (m), 1096 (m), 1056 (m), 1017 (m), 939 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.19 (1H, br s, N-H), 8.99 (1H, br s, O-H), 7.89 (1H, t, *J* 5.6, N-H), 7.73 (1H, dd, *J* 8.4, 7.3, C^6-H), 7.53 (1H, d, *J* 7.3, C^7-H), 7.22 (1H, d, *J* 8.4, C^5-H), 4.98 (1H, dd, *J* 12.5, 5.6, C$^{3'}$-H), 4.73 (2H, s, C$^{17''}$-H$_2$), 4.13 (2H, s, C$^{2''}$-H$_2$), 3.75–3.70 (2H, m, C$^{4''}$H$_2$), 3.69–3.63 (12H, m, C$^{5''}$-H$_2$, C$^{7''}$-H$_2$, C$^{8''}$-H$_2$, C$^{10''}$-H$_2$, C$^{11''}$-H$_2$, C$^{13''}$-H$_2$), 3.62–3.52 (2H, m, C$^{14''}$-H$_2$), 2.92–2.84 (1H, m, C$^{5'}$-H$_a$), 2.82–2.71 (2H, m, C$^{4'}$-H$_a$, C$^{5'}$-H$_b$), 2.20–2.09 (1H, m, C$^{4'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.9 (C$^{1''}$), 172.1 (C$^{6'}$), 168.8 (C$^{2'}$), 168.5 (C$^{16''}$), 166.8 (C^1), 166.0 (C^3), 154.4 (C^4), 137.2 (C^6), 133.6 (C^{7a}), 119.8 (C^5), 118.2 (C^{3a}), 117.6 (C^7), 71.1 (C$^{4''}$), 70.6 (C$^{5''}$), 70.34 (C$^{7''/8''/10''/11''}$), 70.33 (C$^{7''/8''/10''/11''}$), 70.31 (C$^{7''/8''/10''/11''}$), 70.27 (C$^{7''/8''/10''/11''}$), 69.3 (C$^{13''}$), 68.8 (C$^{2''}$), 67.8 (C$^{17''}$), 49.3 (C$^{3'}$), 39.4 (C$^{14''}$), 31.4 (C$^{5'}$), 22.7 (C$^{4'}$); HRMS *m/z* (ESI$^+$) [Found: 588.1794, $C_{25}H_{31}N_3O_{12}Na$ requires [M+Na]$^+$ 588.1800]; LRMS *m/z* (ESI$^+$) 588.2 ([M+Na]$^+$, 100%); HPLC retention time (Atlantis) 10.0 min, 97.0%.

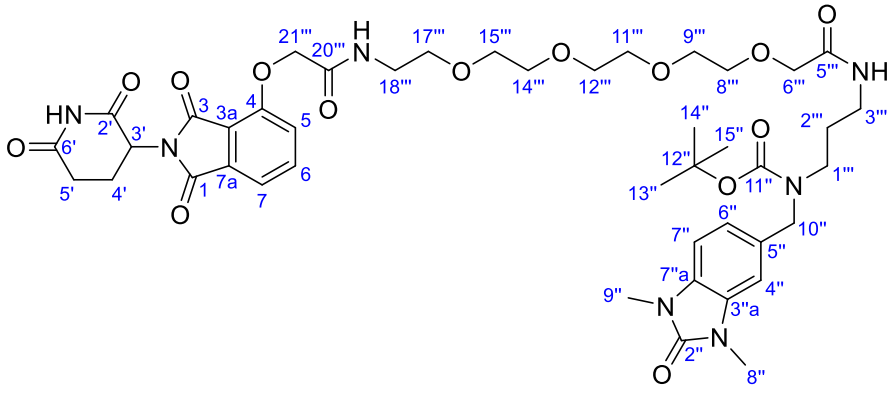
tert-Butyl [(1'',3''-dimethyl-2''-oxo-2'',3''-dihydro-1*H*-benzimidazol-5''-yl)methyl](15'''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-5''',14'''-dioxo-7''',10'''-dioxa-4''',13'''-diazapentadecan-1'''-yl)carbamate (290)



{2-[2-(2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido) ethoxy]ethoxy}acetic acid (**287**) (72 mg, 0.15 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (1.5 mL). 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (69 mg, 0.18 mmol, 1.2 eq.) and *N*,*N*-diisopropylethylamine (63 µL, 0.36 mmol, 3.0 eq.) were added. The reaction mixture was stirred at rt for 30 min. *tert*-Butyl (3-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (**268**) (63 mg, 0.18 mmol, 1.2 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (1.0 mL) was added. The reaction mixture was stirred at rt for 20 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 30 mL) and extracted with EtOAc (30 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 30 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–9% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (62 mg, 0.077 mmol, 51%): R_f 0.28 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = +1.30 (*c* 1.0, MeOH); $\tilde{\nu}_{max}$/cm^{-1} 2930 (br), 1706 (C=O, s), 1677 (C=O, s), 1614 (w), 1537 (w), 1509 (w), 1483 (w), 1394 (w), 1365 (w), 1299 (w), 1264 (w), 1196 (w), 1167 (w), 1121 (w), 1055 (w), 1016 (w), 911 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 373 K) δ 10.77 (1H, br s, N-H), 7.80 (1H, dd, *J* 8.5, 7.3, C^6-H), 7.74 (1H, br s, N-H), 7.49 (1H, d, *J* 7.3, C^7-H), 7.44 (1H, d, *J* 8.5, C^5-H), 7.35 (1H, br s, N-H), 7.06–7.01 (1H, m, C$^{4''}$-H), 6.97–6.94 (2H, m, C$^{6''}$-H, C$^{7''}$-H), 5.07 (1H, dd, *J* 12.6, 5.5, C$^{3'}$-H), 4.75 (2H, s, C$^{15'''}$-H$_2$),

4.41 (2H, s, $C^{10''}$-H_2), 3.85 (2H, s, $C^{6'''}$-H_2), 3.61–3.57 (4H, m, $C^{8'''}$-H_2, $C^{9'''}$-H_2), 3.53 (2H, t, *J* 5.8, $C^{11'''}$-H_2), 3.40–3.33 (2H, m, $C^{12'''}$-H_2), 3.31 (3H, s, $C^{8''/9''}$-H_3), 3.30 (3H, s, $C^{8''/9''}$-H_3), 3.16 (2H, t, *J* 7.1, $C^{1'''}$-H_2), 3.13–3.06 (2H, m, $C^{3'''}$-H_2), 3.00 (1H, br s, N-H), 2.94–2.83 (1H, m, $C^{5'}$-H_a), 2.68–2.52 (2H, m, $C^{4'}$-H_a, $C^{5'}$-H_b), 2.13–2.04 (1H, m, $C^{4'}$-H_b), 1.65 (2H, tt, *J* 7.1, 7.1, $C^{2'''}$-H_2), 1.44 (9H, s, $C^{13''}$-H_3, $C^{14''}$-H_3, $C^{15''}$-H_3); ^{13}C NMR (126 MHz, DMSO-d_6, 373 K) δ 171.7 ($C^{6'}$), 168.9 ($C^{2'}$), 168.5 ($C^{5'''}$), 166.4 (C^{1}), 166.1 ($C^{14'''}$), 165.0 (C^{3}), 154.6 (C^{4}, $C^{11''}$), 153.7 ($C^{2''}$), 136.3 (C^{6}), 132.8 (C^{7a}), 131.1 ($C^{5''}$), 129.6 ($C^{3''a}$), 128.7 ($C^{7''a}$), 120.5 (C^{5}), 119.6 ($C^{6''}$), 116.9 (C^{3a}), 115.8 (C^{7}), 106.7 ($C^{4''}$), 106.1 ($C^{7''}$), 78.4 ($C^{12''}$), 69.9 ($C^{8'''/9'''}$), 69.8 ($C^{6'''}$), 69.0 ($C^{8'''/9'''}$), 68.5 ($C1^{1'''}$), 67.9 ($C^{15'''}$), 49.5 ($C^{10''}$), 48.7 ($C^{3'}$), 43.7 ($C^{1'''}$), 38.1 ($C^{12'''}$), 35.6 ($C^{3'''}$), 30.5 ($C^{5'}$), 27.7 ($C^{13''}$, $C^{14''}$, $C^{15''}$), 27.6 ($C^{2'''}$), 26.4 ($C^{8''/9''}$), 26.3 ($C^{8''/9''}$), 21.6 ($C^{4'}$); HRMS *m/z* (ESI^+) [Found: 808.3507, $C_{39}H_{50}N_7O_{12}$ requires $[M+H]^+$ 808.3512]; LRMS *m/z* (ESI^+) 830.3 ($[M+Na]^+$, 100%); HPLC retention time 6.4 min, >99.9%.

***tert*-Butyl [(1'',3''-dimethyl-2''-oxo-2'',3''-dihydro-1*H*-benzimidazol-5''-yl)methyl](21'''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-5''',20'''-dioxo-7''',10''',13''',16'''-tetraoxa-4''',19'''-diazahenicosan-1'''-yl)carbamate (291)**


HN
O
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N
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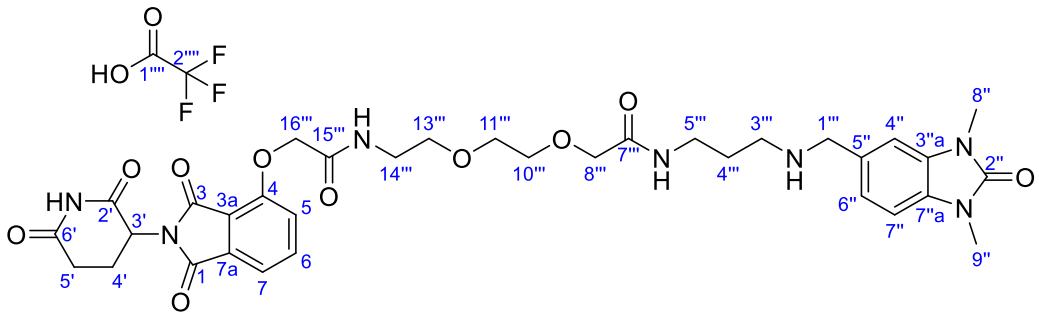
(3-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (**268**) (17 mg, 0.050 mmol, 1.0 e.q.) was added. The reaction mixture was stirred at rt for 22 h. After this time TLC analysis indicated partial consumption of starting material (1:9 EtOH/$CHCl_3$ + drop Et3N). Additional 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (10 mg, 0.025 mmol, 0.5 eq.) and hydroxybenzotriazole (3.5 mg, 0.025 mmol, 0.5 eq.) were added. The reaction mixture was stirred at rt for 21 h. After this time TLC analysis indicated complete consumption of starting material (1:9 EtOH/$CHCl_3$ + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (40 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 20 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–9% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (17 mg, 0.019 mmol, 38%): R_f 0.58 (1:9 MeOH/CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 2923 (br), 2562 (br), 2360 (m), 2342 (m), 2162 (s), 2029 (s), 1977 (s), 1706 (C=O, s), 1677 (C=O, s), 1615 (w), 1540 (w), 1509 (w), 1483 (w), 1394 (m), 1365 (m), 1299 (w), 1263 (w), 1196 (w), 1167 (w), 1121 (w), 1056 (w), 939 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.09 (1H, br s, N-H), 7.72 (1H, dd, *J* 8.4, 7.4, C^6-H), 7.64 (1H, t, *J* 5.5, N-H), 7.53 (1H, d, *J* 7.4, C^5-H), 7.33 (1H, br s, N-H), 7.18 (1H, d, *J* 8.4, C^7-H), 6.94 (1H, d, *J* 7.9, $C^{6''}$-H), 6.91–6.83 (2H, m, $C^{4''}$-H, $C^{7''}$-H), 4.98–4.91 (1H, m, $C^{3'}$-H), 4.63 (2H, s, $C^{21'''}$-H_2), 4.43 (2H, s, $C^{10''}$-H_2), 3.97 (2H, s, $C^{6'''}$-H_2), 3.68–3.49 (16H, m, $C^{8'''}$-H_2,$C^{9'''}$-H_2, $C^{11'''}$-H_2, $C^{12'''}$-H_2, $C^{14'''}$-H_2, $C^{15'''}$-H_2, $C^{17'''}$-H_2, $C^{18'''}$-H_2), 3.40 (3H, s, $C^{8''/9''}$-H_3), 3.39 (3H, s, $C^{8''/9''}$-H_3), 3.33–3.11 (4H, m, $C^{1'''}$-H_2, $C^{3'''}$-H_2), 2.90–2.57 (3H, m, $C^{4'}$-H_a, $C^{5'}$-H_2), 2.18–2.09 (1H, m, $C^{4'}$-H_b), 1.70 (2H, tt, *J* 6.9, 6.9, $C^{2'''}$-H_2), 1.47 (9H, s, $C^{13''}$-H_3, $C^{14''}$-H_3, $C^{15''}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3 ($C^{6'}$), 170.1 ($C^{5'''}$), 168.4 ($C^{2'}$), 167.0 ($C^{20'''}$), 166.7 ($C^{1/3}$), 166.0 ($C^{1/3}$), 156.1 ($C^{11''}$), 154.9 ($C^{2''}$), 154.6 (C^4), 137.1 (C^6), 133.8 (C^{7a}), 131.8 ($C^{5''}$), 130.5 ($C^{3''a}$), 129.5 ($C^{7''a}$), 120.5 ($C^{6''}$), 119.5 (C^7), 118.2 (C^{3a}), 117.4 (C^5), 107.2 ($C^{4''/7''}$) 106.5 ($C^{4''/7''}$), 80.1 ($C^{12''}$), 70.9 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 70.64 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 70.63 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$) 70.5 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 70.40 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 70.38 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 70.3 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 69.7 ($C^{17'''}$), 68.0 ($C^{21'''}$), 50.6 ($C^{10''}$),

49.4 ($C^{3'}$), 43.5 ($C^{1'''}$), 39.2 ($C^{18'''}$), 36.0 ($C^{3'''}$), 31.6 ($C^{5'}$), 28.6 ($C^{13''}$, $C^{14''}$, $C^{15''}$), 27.8 ($C^{2'''}$), 27.4 ($C^{8''/9''}$), 27.3 ($C^{8''/9''}$), 22.8 ($C^{4'}$), minor rotamer also observed; HRMS *m/z* (ESI^+) [Found: 896.4032, $C_{43}H_{58}N_7O_{14}$ requires $[M+H]^+$ 896.4036]; LRMS *m/z* (ESI^+) 918.4 ($[M+Na]^+$, 24%); HPLC retention time (Atlantis) 11.4 min, >99.9%.

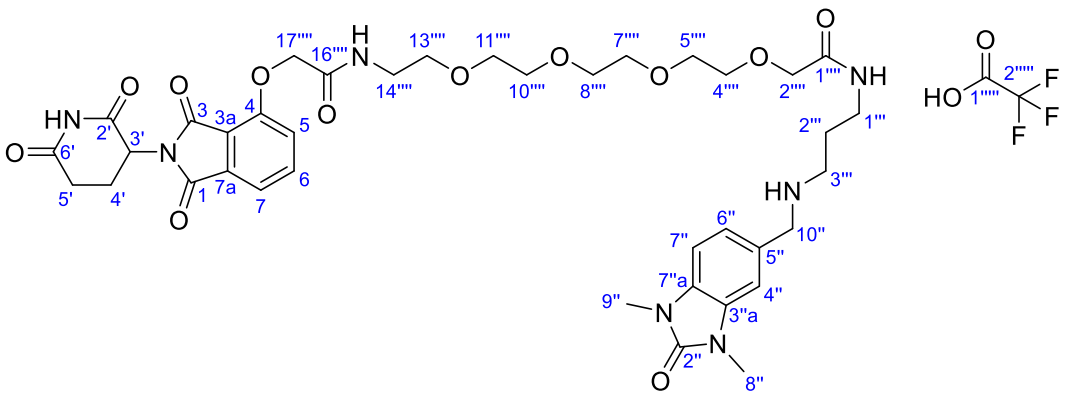
_N_-[1'''-(1'',3''-Dimethyl-2''-oxo-2'',3''-dihydro-1_H_-benzimidazol-5''-yl)-7'''-oxo-9''',12'''-dioxa-2''',6'''-diazatetradecan-14'''-yl]-2-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}acetamide trifluoroacetate (212)



tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](15-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-5,14-dioxo-7,10-dioxa-4,13-diazapentadecan-1-yl)carbamate (**290**) (58 mg, 0.072 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (1.0 mL) and trifluoroacetic acid (1.0 mL) was added. The reaction mixture was stirred at rt for 15 min. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* to yield the title compound as a colourless oil (59 mg, 0.22 mmol, 100%): R_f 0.38 (1:19 MeOH/CH_2Cl_2 + 1% Et_3N); $[\alpha]_D^{25}$ = –2.40 (*c* 1.0, $CHCl_3$); $\bar{\nu}_{max}$/cm^{-1} 3075 (br), 2924 (br), 2854 (br), 2361 (m), 2342 (m), 1773 (w), 1709 (C=O, s), 1616 (C=O, m), 1542 (w), 1513 (w), 1485 (w), 1448 (w), 1396 (w), 1357 (w), 1325 (w), 1300 (w), 1266 (w), 1199 (C-F, m), 1178 (C-F, m), 1141 (C-F, m), 1055 (w), 1025 (w), 939 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.21 (1H, br s, N-H), 7.74 (1H, t, *J* 5.7, N-H), 7.72 (1H, dd, *J* 8.4, 7.3, C^6-H), 7.63 (1H, t, *J* 6.4, N-H), 7.52 (1H, d, *J* 7.3, C^7-H), 7.18 (1H, d, *J* 8.4, C^5-H), 7.14–7.07 (2H, m, $C^{4''}$-H, $C^{6''}$-H), 6.92 (1H, d, *J* 7.9, $C^{7''}$-H), 4.95 (1H, dd, *J* 13.8, 6.1, $C^{3'}$-H), 4.60 (2H, s, $C^{16'''}$-H_2), 4.13 (2H, s, $C^{1'''}$-H_2), 3.99 (2H, s, $C^{8'''}$-H_2), 3.70–3.53 (8H, m, $C^{10'''}$-H_2,

$C^{11'''}$-H_2, $C^{13'''}$-H_2, $C^{14'''}H_2$), 3.43–3.28 (8H, m, $C^{8''}$-H_3, $C^{9''}$-H_3, $C^{5'''}$-H_2), 2.98 (2H, t, *J* 6.4, $C^{3'''}$-H_2), 2.90–2.63 (3H, m, $C^{4'}$-H_a, $C^{5'}$-H_2), 2.18–2.08 (1H, m, $C^{4'}$-H_b), 1.94 (2H, tt, *J* 6.4, 6.4, $C^{4''}$-H_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1 ($C^{7'''}$), 171.7 ($C^{6'}$), 168.7 ($C^{2'}$), 167.7 ($C^{15'''}$), 166.6 (C^{1}), 166.3 (C^{3}), 160.7 (q, $J_{C\text{-}F}$ 38.9, $C^{1''''}$), 154.8 ($C^{2''}$), 154.4 (C^{4}), 137.3 (C^{6}), 133.6 (C^{7a}), 131.0 ($C^{7''a}$), 130.6 ($C^{3''a}$), 123.7 ($C^{5''}$), 123.3 ($C^{6''}$), 119.7 (C^{5}), 118.0 (C^{3a}), 117.6 (C^{7}), 115.8 (q, $J_{C\text{-}F}$ 290.9, $C^{2''''}$), 109.0 ($C^{4''}$), 107.6 ($C^{7''}$), 71.2 ($C^{10'''}$), 70.1 ($C^{8'''}$), 70.0 ($C^{11'''/13'''}$), 69.8 ($C^{11'''/13'''}$), 67.9 ($C^{16'''}$), 52.2 ($C^{1'''}$), 49.4 ($C^{3'}$), 44.2 ($C^{3'''}$), 39.2 ($C^{14'''}$), 35.5 ($C^{5'''}$), 31.5 ($C^{5'}$), 27.4 ($C^{8''/9''}$), 27.3 ($C^{8''/9''}$), 26.6 ($C^{4'''}$), 22.7 ($C^{4'}$); ^{19}F NMR (376 MHz, $CDCl_3$) δ –76.0; HRMS *m/z* (ESI^+) [Found: 708.2980, $C_{34}H_{42}N_7O_{10}$ requires $[M+H]^+$ 708.2988]; LRMS *m/z* (ESI^+) 730.2 ($[M+Na]^+$, 100%); HPLC retention time (Atlantis) 8.8 min, 94.1%.

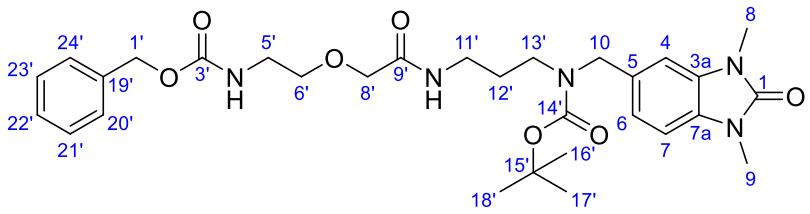
_N_-(3’’’-{[(1’’,3’’-Dimethyl-2’’-oxo-2’’,3’’-dihydro-1_H_-benzimidazol-5’’-yl)methyl]amino}prop-1’’’-yl)-17’’’’-{[2-(2’,6’-dioxopiperidin-3’-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}-16’’’’-oxo-3’’’’,6’’’’,9’’’’,12’’’’-tetraoxa-15’’’’-azaheptadecan-1’’’’-amide trifluoroacetate (214)



tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](21-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-5,20-dioxo-7,10,13,16-tetraoxa-4,19-diazahenicosan-1-yl)carbamate (**291**) (17 mg, 0.019 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (0.50 mL) and trifluoroacetic acid (0.50 mL) was added. The reaction mixture was stirred at rt for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* to yield the title compound as a colourless oil (17 mg, 0.019 mmol, 100%): R_f 0.24 (1:9 MeOH/CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1}

2922 (br), 2855 (br), 2160 (br), 2024 (br), 1977 (br), 1773 (w), 1706 (C=O, s), 1671 (C=O, s), 1615 (m), 1545 (w), 1512 (w), 1484 (w), 1448 (w), 1396 (m), 1350 (w), 1325 (w), 1299 (w), 1265 (w), 1198 (s), 1178 (s), 1123 (s), 989 (w), 939 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.77 (1H, dd, *J* 8.5, 7.3, C^{6}-H), 7.49 (1H, d, *J* 7.3, C^{7}-H), 7.37 (1H, d, *J* 8.5, C^{5}-H), 7.27–7.21 (2H, m, $C^{4''}$-H, $C^{6''}$-H), 7.17 (1H, d, *J* 7.9, $C^{7''}$-H), 5.12 (1H, dd, *J* 12.8, 5.5, $C^{3'}$-H), 4.70 (2H, s, $C^{17''''}$-H_2), 4.23 (2H, s, $C^{10''}$-H_2), 4.01 (2H, s, $C^{2''''}$-H_2), 3.68–3.59 (14H, m, $C^{4''''}$-H_2, $C^{5''''}$-H_2, $C^{7''''}$-H_2, $C^{8''''}$-H_2, $C^{10''''}$-H_2, $C^{11''''}$-H_2, $C^{13''''}$-H_2), 3.49 (2H, t, *J* 5.4, $C^{14''''}$-H_2), 3.41 (3H, s, $C^{8''/9''}$-H_3), 3.40 (3H, s, $C^{8''/9''}$-H_3), 3.37 (2H, t, *J* 7.3, $C^{1'''}$-H_2), 3.07 (2H, t, *J* 7.3, $C^{3'''}$-H_2), 2.93–2.82 (1H, m, $C^{5'}$-H_a), 2.81–2.64 (2H, m, $C^{4'}$-H_a, $C^{5'}$-H_b), 2.19–2.10 (1H, m, $C^{4'}$-H_b), 1.94 (2H, tt, *J* 7.3, 7.3, $C^{2'''}$-H_2); ^{13}C NMR (126 MHz, CD_3OD) δ 174.5 ($C^{6'}$), 174.0 ($C^{1''''}$), 171.4 ($C^{2'}$), 169.9 ($C^{16''''}$), 168.2 (C^{1}), 167.7 (C^{3}), 156.3 ($C^{2''}$), 156.1 (C^{4}), 138.2 (C^{6}), 134.9 (C^{7a}), 132.1 ($C^{7''a}$), 131.6 ($C^{3''a}$), 125.7 ($C^{5''}$), 124.7 ($C^{6''}$), 121.4 (C^{5}), 119.0 (C^{3a}), 117.8 (C^{7}), 110.4 ($C^{4''}$), 109.3 ($C^{7''}$), 72.0 ($C^{4''''}$), 71.5 ($C^{5''''/7''''/8''''/10''''/11''''/13''''}$), 71.44 ($C^{5''''/7''''/8''''/10''''/11''''/13''''}$), 71.42 ($C^{5''''/7''''/8''''/10''''/11''''/13''''}$), 71.38 ($C^{5''''/7''''/8''''/10''''/11''''/13''''}$), 71.3 ($C^{5''''/7''''/8''''/10''''/11''''/13''''}$), 71.1 ($C^{2''''}$), 70.3 ($C^{5''''/7''''/8''''/10''''/11''''/13''''}$), 69.1 ($C^{17''''}$), 52.8 ($C^{10''}$), 50.5 ($C^{3'}$), 45.8 ($C^{3'''}$), 40.2 ($C^{14''''}$), 36.5 ($C^{1'''}$), 32.2 ($C^{5'}$), 27.58 ($C^{8''/9''/2'''}$), 27.57 ($C^{8''/9''/2'''}$), 23.7 ($C^{4'}$); ^{19}F NMR (377 MHz, CD_3OD) δ –76.9; HRMS *m/z* (ESI^+) [Found: 796.3500, $C_{38}H_{50}N_7O_{12}$ requires $[M+H]^+$ 796.3512]; LRMS *m/z* (ESI^+) 818.3 ($[M+Na]^+$, 100%); HPLC retention time 5.0 min, 99.2%.

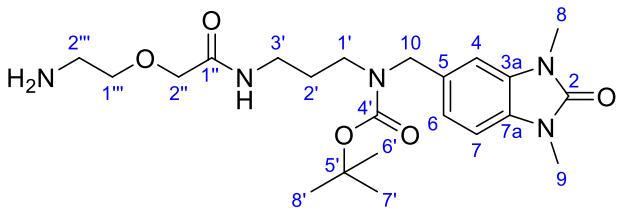
tert-Butyl ((1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)methyl)(3',9'-dioxo-1'-phenyl-2',7'-dioxa-4',10'-diazatridecan-13'-yl)carbamate (293)



2-(2-(((Benzyloxy)carbonyl)amino)ethoxy)acetic acid (87.0 mg, 0.344 mmol, 1.2 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (3.50 mL). 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (**268**) (131 mg, 0.344 mmol,

1.2 eq.) and *N*,*N*-diisopropylethylamine (60.0 µL, 0.344 mmol, 1.2 eq.) were added. The reaction mixture was stirred at rt for 30 min. *tert*-Butyl (3'aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (100 mg, 0.287 mmol, 1.0 eq.) was added. The reaction mixture was stirred at rt for 66 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (20 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 20 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–7% MeOH/CH_2Cl_2 gradient) to yield the title compound as a colourless oil (103 mg, 0.166 mmol, 58%): R_f 0.54 (1:9 MeOH/CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3338 (br), 2936 (br), 2161 (br), 1977 (br), 1663 (C=O, s), 1543 (m), 1509 (m), 1454 (m), 1422 (m), 1398 (w), 1367 (w), 1247 (m), 1163 (m), 1138 (m), 1027 (w), 909 (m); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 7.46 (1H, br s, N-H), 7.38–7.20 (5H, m, $C^{20'}$-H, $C^{21'}$-H, $C^{22'}$-H, $C^{23'}$-H, $C^{24'}$-H), 7.04 (1H, d, *J* 8.0, C^4-H), 7.00–6.93 (2H, m, C^6-H, C^7-H), 5.03 (2H, s, $C^{1'}$-H_2), 4.41 (2H, s, C^{10}-H_2), 3.83 (2H, s, $C^{8'}$-H_2), 3.50 (2H, t, *J* 5.7, $C^{6'}$-H_2), 3.31 (3H, s, $C^{8/9}$-H_3), 3.30 (3H, s, $C^{8/9}$-H_3), 3.24 (2H, dt, *J* 7.2, 7.2, $C^{11'}$-H_2), 3.17 (2H, t, *J* 7.2, $C^{13'}$-H_2), 3.10 (2H, dt, *J* 5.7, 5.7, $C^{5'}$-H_2), 2.90 (1H, br s, N-H), 2.75 (1H, s, N-H), 1.66 (2H, tt, *J* 7.2, 7.2, $C^{12'}$-H_2), 1.44 (9H, s, $C^{16'}$-H_3, $C^{17'}$-H_3, $C^{18'}$-H_3); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 168.4 ($C^{9'}$), 155.7 ($C^{3'}$), 154.6 ($C^{14'}$), 153.7 (C^1), 136.8 ($C^{19'}$), 131.1 (C^5), 129.6 (C^{7a}), 128.7 (C^{3a}), 127.8 ($C^{20'/21'/23'/24'}$), 127.2 ($C^{22'}$), 127.1 ($C^{20'/21'/23'/24'}$), 119.7 (C^6), 106.8 (C^4), 106.2 (C^7), 78.5 ($C^{15'}$), 69.7 ($C^{8'}$), 69.5 ($C^{6'}$), 64.9 ($C^{1'}$), 49.5 (C^{10}), 43.7 ($C^{13'}$), 40.6 ($C^{11'}$), 35.6 ($C^{5'}$), 27.7 ($C^{16'}$, $C^{17'}$, $C^{18'}$), 27.6 ($C^{12'}$), 26.4 ($C^{8/9}$), 26.3 ($C^{8/9}$); HRMS *m/z* (ESI^+) [Found: 584.3077, $C_{30}H_{42}N_5O_7$ requires $[M+H]^+$ 584.3079]; LRMS *m/z* (ESI^+) 606.2 ($[M+Na]^+$, 100%); HPLC retention time 7.4 min, 99.8%.

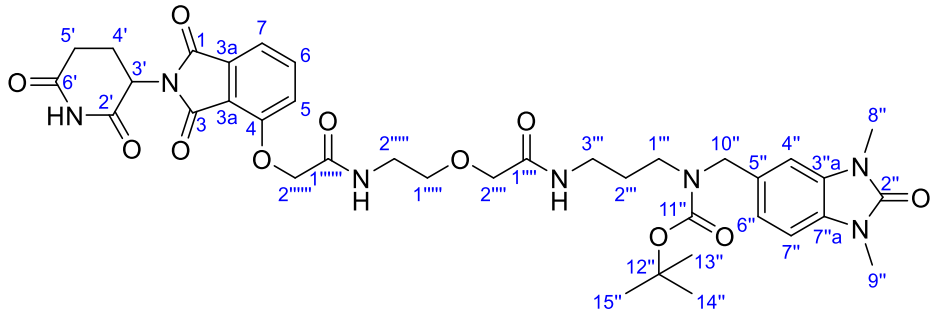
tert-Butyl {3'-[2''-(2'''-aminoethoxy)acetamido]prop-1'-yl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (294)



tert-Butyl ((1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)methyl)(3,9-dioxo-1phenyl-2,7-dioxa-4,10-diazatridecan-13-yl)carbamate (**293**) (94 mg, 0.16 mmol, 1.0 eq.) was dissolved in MeOH (1.5 mL). The reaction mixture was purged with N_2. Pd on carbon (10% *w/w*, 8.6 mg, 0.0081 mmol, 0.05 eq.) was added and the reaction mixture purged with H_2. The reaction mixture was stirred at rt under an atmosphere of H_2 for 3 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was purged with N_2. The reaction mixture was filtered through a pad of Celite® and the filter pad washed with MeOH. The filtrate was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–10% MeOH/CH_2Cl_2 + 1% Et_3N gradient) to yield the title compound as a colourless oil (55 mg, 0.12 mmol, 75%): R_f 0.25 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); $\tilde{\nu}_{max}$/cm^{-1} 3369 (br), 2972 (br), 2931 (br), 2161 (br), 2032 (br), 1675 (C=O, s), 1542 (m), 1508 (m), 1462 (m), 1418 (m), 1395 (m), 1366 (m), 1304 (w), 1269 (w), 1243 (w), 1164 (m), 1128 (m), 1082 (w), 1060 (w), 1027 (w), 966 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 7.75 (1H, br s, N-H), 7.06 (1H, d, *J* 7.8, C^7-H), 6.99–6.93 (2H, m, C^4-H, C^6-H), 4.42 (2H, s, C^{10}-H$_2$), 3.86 (2H, s, C$^{2''}$-H$_2$), 3.55 (2H, t, *J* 5.4, C$^{1'''}$-H$_2$), 3.32 (3H, s, C$^{8/9}$-H$_3$), 3.31 (3H, s, C$^{8/9}$-H$_3$), 3.17 (2H, t, *J* 7.1, C$^{1'}$-H$_2$), 3.11 (2H, td, *J* 7.1, 7.1, C$^{3'}$-H$_2$), 2.87 (2H, t, *J* 5.4, C$^{2'''}$-H$_2$), 1.68 (2H, tt, *J* 7.1, 7.1, C$^{2'}$-H$_2$), 1.45 (9H, s, C$^{6'}$-H$_3$, C$^{7'}$-H$_3$, C$^{8'}$-H$_3$); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 168.5 (C$^{1''}$), 154.6 (C$^{4'}$), 153.7 (C^2), 131.2 (C^5), 129.6 (C$^{3a/7a}$), 128.7 (C$^{3a/7a}$), 119.7 (C^6), 106.8 (C^7), 106.2 (C^4), 78.4 (C$^{5'}$), 70.0 (C$^{1'''}$), 69.7 (C$^{2''}$), 49.6 (C^{10}), 43.8 (C$^{1'}$), 40.4 (C$^{2'''}$), 35.6 (C$^{3'}$), 27.7 (C$^{6'}$, C$^{7'}$, C$^{8'}$), 27.6 (C$^{2'}$), 26.4 (C$^{8/9}$), 26.3 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 450.2699,

$C_{22}H_{36}N_5O_5$ requires $[M+H]^+$ 450.2711]; LRMS *m/z* (ESI^-) 484.1 ($[M+Cl]^-$, 100%); HPLC retention time 4.9 min, 95.1%.

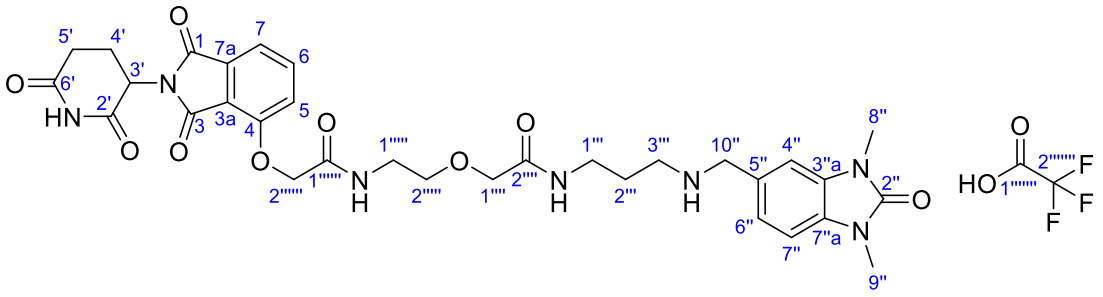
tert-Butyl [(1'',3''-dimethyl-2''-oxo-2'',3''-dihydro-1*H*-benzimidazol-5''-yl)methyl](3'''-{2''''-[2'''''-(2''''''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy}acetamido)ethoxy]acetamido}prop-1'''-yl)carbamate (295)



{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetic acid (**229**) (39 mg, 0.13 mmol, 1.2 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (1.5 mL). 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro phosphate (49 mg, 0.13 mmol, 1.2 eq.) and *N*,*N*-diisopropylethylamine (22 µL, 0.13 mmol, 1.2 eq.) were added. The reaction mixture was stirred at rt for 30 min. *tert*-Butyl {3'-[2''-(2'''-aminoethoxy)acetamido]propyl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl) methyl]carbamate (**294**) (48 mg, 0.11 mmol, 1.0 eq.) dissolved in anhydrous *N*,*N*-dimethylformamide (0.50 mL) was added. The reaction mixture was stirred at rt for 19 h. After this time TLC analysis indicated complete consumption of starting material (1:9 $MeOH/CH_2Cl_2$ + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (20 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 20 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–8% $MeOH/CH_2Cl_2$ gradient) to yield the title compound as a colourless oil (16 mg, 0.021 mmol, 20%): R_f 0.24 (1:19 $MeOH/CH_2Cl_2$); $[\alpha]_D^{25}$ = +7.76 (*c* 1.0, $CHCl_3$); $\tilde{\nu}_{max}/cm^{-1}$ 3015 (br), 2936 (br), 2855 (br), 2361 (br), 1772 (w), 1709 (C=O, s), 1684 (C=O, s), 1615 (w), 1541 (w), 1509 (w), 1483 (w), 1445 (w), 1420 (w), 1396 (w), 1365 (w), 1324 (w), 1301

(w), 1265 (w), 1198 (w), 1171 (w), 1138 (w), 1123 (w), 1079 (w), 1056 (w), 1016 (w), 989 (w), 913 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.20 (1H, br s, N-H), 7.79 (1H, t, *J* 5.3, N-H), 7.74 (1H, dd, *J* 7.9, 7.9, C^{6}-H), 7.55 (1H, d, *J* 7.9, C^{5}-H), 7.33 (1H, br s, N-H), 7.19 (1H, d, *J* 7.9, C^{7}-H), 6.95 (1H, d, *J* 8.0, $C^{6''}$-H), 6.92–6.85 (2H, m, $C^{4''}$-H, $C^{7''}$-H), 5.00–4.93 (1H, m, $C^{3'}$-H), 4.67 (2H, s, $C^{2''''''}$-H_2), 4.44 (2H, s, $C^{10''}$-H_2), 4.09–3.97 (2H, m, $C^{2''''}$-H_2), 3.76–3.52 (4H, m, $C^{1''''''}$-H_2, $C^{2''''''}$-H_2), 3.42 (3H, s, $C^{8''/9''}$-H_3), 3.40 (3H, s, $C^{8''/9''}$-H_3), 3.31–3.21 (4H, m, $C^{1'''}$-H_2, $C^{3'''}$-H_2), 2.94–2.64 (3H, m, $C^{4'}$-H_a, $C^{5'}$-H_2), 2.20–2.11 (1H, m, $C^{4'}$-H_b), 1.71 (2H, tt, *J* 6.9, 6.9, $C^{2'''}$-H_2), 1.49 (9H, s, $C^{13''}$-H_3, $C^{14''}$-H_3, $C^{15''}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2 ($C^{2'}$), 169.9 ($C^{1''''}$), 168.4 ($C^{6'}$), 167.1 ($C^{1''''''}$), 166.7 (C^{3}), 166.3 (C^{1}), 156.2 ($C^{11''}$), 154.9 ($C^{2''}$), 154.6 (C^{4}), 137.1 (C^{6}), 133.7 (C^{3a}), 131.6 ($C^{5''}$), 130.4 ($C^{3''a}$), 129.5 ($C^{7''a}$), 120.5 ($C^{6''}$), 119.8 (C^{7}), 118.3 (C^{3a}), 117.6 (C^{5}), 107.2 ($C^{7''}$), 106.5 ($C^{4''}$), 80.5 ($C^{12''}$), 70.7 ($C^{2''''}$), 70.1 ($C^{1''''''}$), 68.3 ($C^{2''''''}$), 50.5 ($C^{10''}$), 49.5 ($C^{3'}$), 43.3 ($C^{1'''}$), 39.2 ($C^{3'''}$), 35.9 ($C^{2''''''}$), 31.6 ($C^{5'}$), 28.6 ($C^{13''}$, $C^{14''}$, $C^{15''}$), 27.4 ($C^{2'''}$), 27.4 ($C^{8''/9''}$), 27.3 ($C^{8''/9''}$), 22.9 ($C^{4'}$), minor rotamer also observed; HRMS *m/z* (ESI^+) [Found: 764.3243, $C_{37}H_{46}N_7O_{11}$ requires $[M+H]^+$ 764.3250]; LRMS *m/z* (ESI^+) 802.2 ($[M+K]^+$, 100%); HPLC retention time 5.1 min, >99.9%.

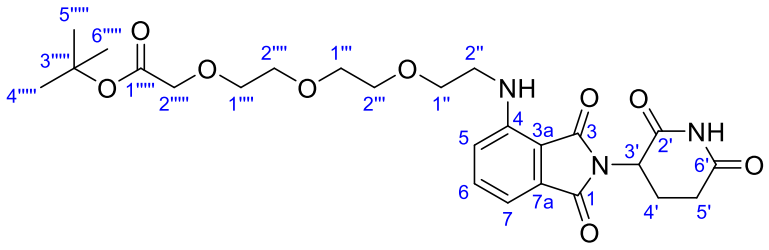
_N_-(2’’’’’-{2’’’’-[(3’’’-{[(1’’,3’’-Dimethyl-2’’-oxo-2’’,3’’-dihydro-1_H_-benzimidazol-5’’-yl)methyl]amino}prop-1’’’-yl)amino]-2’’’’-oxoethoxy}ethyl)-2’’’’’’-{[2-(2’,6’-dioxopiperidin-3’-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}acetamide trifluoroacetate (211)



tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](3-{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy] acetamido}propyl)carbamate (**295**) (16 mg, 0.021 mmol, 1.0 eq.) was dissolved in CH_2Cl_2

(0.50 mL) and trifluoroacetic acid (0.50 mL) was added. The reaction mixture was stirred at rt for 15 min. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* to yield the title compound as a colourless oil (15 mg, 0.019 mmol, 92%): R_f 0.42 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); $[\alpha]_D^{25}$ = +6.14 (*c* 1.0, $CHCl_3$); $\bar{\nu}_{max}$/cm^{-1} 3392 (br), 2362 (w), 2160 (w), 1977 (w), 1772 (w), 1672 (C=O, s), 1615 (m), 1545 (m), 1512 (w), 1484 (w), 1448 (m), 1397 (w), 1361 (w), 1265 (m), 1199 (m), 1127 (m), 1055 (w), 1017 (w), 940 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.79 (1H, dd, *J* 8.4, 7.3, C^6-H), 7.51 (1H, d, *J* 7.3, C^5-H), 7.41 (1H, d, *J* 8.4, C^7-H), 7.28–7.20 (2H, m, $C^{6''}$-H, $C^{7''}$-H), 7.18 (1H, d, *J* 8.0, $C^{4''}$-H), 5.11 (1H, dd, *J* 12.8, 5.5, $C^{3'}$-H), 4.78 (2H, s, $C^{2''''''}$-H_2), 4.22 (2H, s, $C^{10''}$-H_2), 4.03 (2H, s, $C^{1''''}$-H_2), 3.67 (2H, t, *J* 5.2, $C^{2''''}$-H_2), 3.55 (2H, t, *J* 5.2, $C^{1''''}$-H_2), 3.42 (3H, s, $C^{8''/9''}$-H_3), 3.42 (3H, s, $C^{8''/9''}$-H_3), 3.33 (2H, t, *J* 6.5, $C^{1'''}$-H_2), 3.06 (2H, t, *J* 6.5, $C^{3'''}$-H_2), 2.92–2.81 (1H, m, $C^{5'}$-H_a), 2.79–2.64 (2H, m, $C^{4'}$-H_a, $C^{5'}$-H_b), 2.21–2.09 (1H, m, $C^{4'}$-H_b), 1.90 (2H, tt, *J* 6.5, 6.5, $C^{2'''}$-H_2); ^{13}C NMR (126 MHz, CD_3OD) δ 174.5 ($C^{6'}$), 173.7 ($C^{2''''}$), 171.5 ($C^{2'}$), 170.3 ($C^{1''''''}$), 168.2 (C^3), 167.9 (C^1), 161.8 ($C^{1''''''}$), 156.4 ($C^{2''}$), 156.3 (C^4), 138.2 (C^6), 134.9 (C^{7a}), 132.1 ($C^{3''a}$), 131.6 ($C^{7''a}$), 129.8 (q, $J_{C\text{-}F}$ 104.1, $C^{2''''''}$), 125.7 ($C^{5''}$), 124.7 ($C^{6''}$), 121.7 (C^7), 119.2 (C^{3a}), 118.0 (C^5), 110.4 ($C^{7''}$), 109.3 ($C^{4''}$), 71.0 ($C^{2''''}$), 70.9 ($C^{1''''}$), 69.4 ($C^{2''''''}$), 52.8 ($C^{10''}$), 50.6 ($C^{3'}$), 45.8 ($C^{3'''}$), 40.0 ($C^{1''''}$), 36.4 ($C^{1'''}$), 32.2 ($C^{5'}$), 27.57 ($C^{8''}$, $C^{9''}$), 27.55 ($C^{2'''}$), 23.6 ($C^{4'}$); ^{19}F NMR (377 MHz, $CDCl_3$) δ –75.9; HRMS *m/z* (ESI^+) [Found: 664.2714, $C_{32}H_{38}N_7O_9$ requires $[M+H]^+$ 664.2726]; LRMS *m/z* (ESI^+) 686.2 ($[M+Na]^+$, 100%); HPLC retention time 4.6 min, 95.8%.

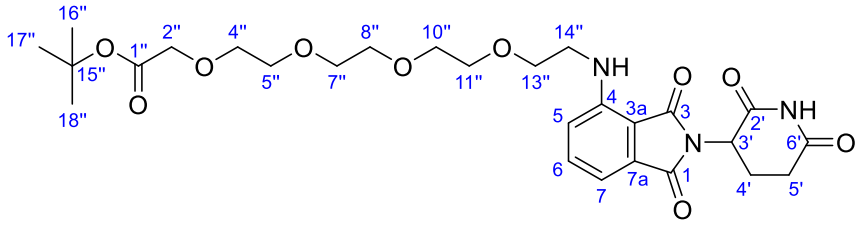
tert-Butyl {2''''-[2'''-(2''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetate (298)



tert-Butyl {2-[2-(2-aminoethoxy)ethoxy]ethoxy}acetate (**275**) (16 mg, 0.061 mmol, 1.1 eq.) was dissolved in *N*-methyl-2-pyrrolidinone (1.0 mL). *N*,*N*-Diisopropylethylamine (19 µL, 0.11 mmol, 2.0 eq.) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoro-1*H*-isoindole-1,3(2H)-dione (**297**) (15 mg, 0.055 mmol, 1.0 eq.) were added. The reaction mixture was heated to 90 °C and stirred for 23 h. After this time TLC analysis indicated complete consumption of starting material (1:19 $MeOH/CH_2Cl_2$). The reaction was cooled to rt, quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (40 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 20 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–90% EtOAc/petroleum ether gradient) to yield the title compound as a yellow oil (11 mg, 0.021 mmol, 38%): R_f 0.21 (1:19 $MeOH/CH_2Cl_2$); $[\alpha]_D^{25}$ = +3.59 (*c* 1.0, $CHCl_3$); $\tilde{\nu}_{max}/cm^{-1}$ 2871 (br), 2160 (br), 2026 (br), 1978 (br), 1694 (C=O, s), 1634 (m), 1602 (w), 1510 (w), 1482 (w), 1459 (w), 1407 (m), 1358 (m), 1323 (m), 1302 (m), 1258 (m), 1196 (m), 1172 (m), 1112 (m), 1054 (m), 1038 (w), 1023 (w), 990 (w), 940 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (1H, br s, N-H), 7.49 (1H, dd, *J* 8.5, 7.1, C^6-H), 7.10 (1H, d, *J* 7.1, C^7-H), 6.92 (1H, d, *J* 8.5, C^5-H), 6.49 (1H, t, *J* 5.7, N-H), 4.95–4.88 (1H, m, $C^{3'}$-H), 4.01 (2H, s, $C^{2''''}$-H_2), 3.75–3.62 (10H, m, $C^{1''}$-H_2, $C^{1'''}$-H_2, $C^{2'''}$-H_2, $C^{1''''}$-H_2, $C^{2''''}$-H_2), 3.47 (2H, dt, *J* 5.5, 5.5, $C^{2''}$-H_2), 2.92–2.67 (3H, m, $C^{4'}$-H_a, $C^{5'}$-H_2), 2.17–2.08 (1H, m, $C^{4'}$-H_b), 1.47 (9H, s, $C^{4''''}$-H_3, $C^{5''''}$-H_3, $C^{6''''}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.1 ($C^{6'}$), 169.9 ($C^{1''''}$), 169.4 (C^3), 168.4 ($C^{2'}$), 167.8 (C^1), 147.0 (C^4), 136.2 (C^6), 132.7 (C^{7a}), 116.9 (C^5), 111.8 (C^7), 110.4 (C^{3a}), 81.8 ($C^{3''''}$), 70.88 ($C^{1'''/2'''/1''''/2''''}$), 70.87 ($C^{1'''/2'''/1''''/2''''}$), 70.78 ($C^{1'''/2'''/1''''/2''''}$), 70.76 ($C^{1'''/2'''/1''''/2''''}$), 69.6 ($C^{1''}$), 69.2 ($C^{2''''}$), 49.0

($C^{3'}$), 42.5 ($C^{2''}$), 31.5 ($C^{5'}$), 28.3 ($C^{4''''}$, $C^{5''''}$, $C^{6''''}$), 23.0 ($C^{4'}$); HRMS *m/z* (ESI^+) [Found: 542.2104, $C_{25}H_{33}N_3O_9Na$ requires $[M+Na]^+$ 542.2109]; LRMS *m/z* (ESI^+) 486.2 ($[M–C_4H_9+Na]^+$, 100%); HPLC retention time 7.4 min, >99.9%.

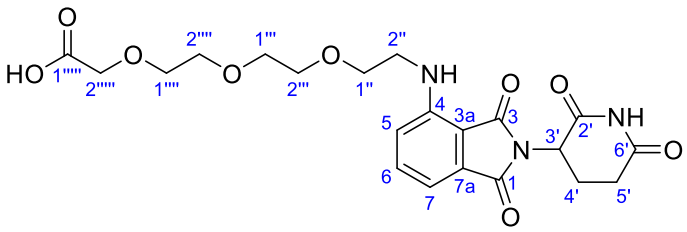
tert-Butyl 14''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-3'',6'',9'',12''-tetraoxatetradecan-1''-oate (299)



2-(2,6-Dioxopiperidin-3-yl)-4-fluoro-1*H*-isoindole-1,3(2*H*)-dione (**297**) (50 mg, 0.18 mmol, 1.0 eq.) was dissolved in *N*-methyl-2-pyrrolidinone (0.50 mL). *N*,*N*-Diisopropylethylamine (63 µL, 0.36 mmol, 2.0 eq.) and *tert*-butyl 14-amino-3,6,9,12-tetraoxatetradecan-1-oate (**276**) (72 mg, 0.23 mmol, 1.3 eq.) dissolved in *N*-methyl-2-pyrrolidinone (1.0 mL) were added. The reaction mixture was heated to 90 °C and stirred for 20 h. After this time TLC analysis indicated complete consumption of starting material (1:19 $MeOH/CH_2Cl_2$). The reaction was cooled to rt, quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (40 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 20 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–5% EtOH/EtOAc gradient) to yield the title compound as a bright yellow oil (26 mg, 0.046 mmol, 26%): R_f 0.23 (1:19 $MeOH/CH_2Cl_2$); $[\alpha]_D^{25}$ = +5.08 (*c* 1.0, $CHCl_3$); $\tilde{\nu}_{max}/cm^{-1}$ 2870 (br), 2161 (br), 1978 (br), 1694 (C=O, s), 1624 (m), 1510 (w), 1459 (w), 1407 (w), 1358 (m), 1323 (w), 1258 (w), 1109 (m), 942 (w); ^1H NMR (500 MHz, $CDCl_3$) δ 8.30 (1H, br s, N-H), 7.48 (1H, dd, *J* 8.5, 7.1, C^6-H), 7.09 (1H, d, *J* 7.1, C^7-H), 6.92 (1H, d, *J* 8.5, C^5-H), 6.49 (1H, t, *J* 5.6, N-H), 4.94–4.87 (1H, m, $C^{3'}$-H), 4.01 (2H, s, $C^{2''}$-H_2), 3.74–3.63 (14H, m, $C^{4''}$-H_2, $C^{5''}$-H_2, $C^{7''}$-H_2, $C^{8''}$-H_2, $C^{10''}$-H_2, $C^{11''}$-H_2, $C^{13''}$-H_2), 3.50–3.42 (2H, m, $C^{14''}$-H_2), 2.93–2.67 (3H, m, $C^{4'}$-H_a, $C^{5'}$-H_2), 2.17–2.08 (1H, m, $C^{4'}$-H_b), 1.46 (9H, s, $C^{16''}$-H_3, $C^{17''}$-H_3, $C^{18''}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2 ($C^{6'}$), 169.8 ($C^{1''}$), 169.4

(C^{3}), 168.5 ($C^{2'}$), 167.8 (C^{1}), 147.0 (C^{4}), 136.2 (C^{6}), 132.6 (C^{7a}), 116.9 (C^{5}), 111.8 (C^{7}), 110.4 (C^{3a}), 81.7 ($C^{15''}$), 70.9 ($C^{4''/5''/7''/8''/10''/11''}$), 70.76 ($C^{4''/5''/7''/8''/10''/11''}$), 70.75 ($C^{4''/5''/7''/8''10''/11''}$), 70.74 ($C^{4''/5''/7''/8''/10''/11''}$), 70.72 ($C^{4''/5''/7''/8''/10''/11''}$), 70.6 ($C^{4''/5''/7''/8''/10''/11''}$), 69.6 ($C^{13''}$), 69.1 ($C^{2''}$), 49.0 ($C^{3'}$), 42.5 ($C^{14''}$), 31.6 ($C^{5'}$), 28.2 ($C^{16''}$, $C^{17''}$, $C^{18''}$), 22.9 ($C^{4'}$); HRMS *m/z* (ESI^{+}) [Found: 564.2548, $C_{27}H_{38}N_3O_{10}$ requires $[M+H]^{+}$ 564.2552]; LRMS *m/z* (ESI^{+}) 508.2 ($[M–C_4H_9]^{+}$, 100%); HPLC retention time 7.5 min, >99.9%.

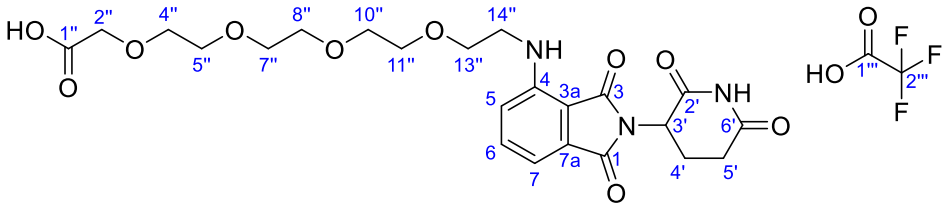
{2''''-[2'''-(2''-{[2-(2',6'-Dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetic acid (300)



tert-Butyl {2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetate (**298**) (12 mg, 0.023 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (0.50 mL) and trifluoroacetic acid (0.50 mL) was added. The reaction mixture was stirred at rt for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* to yield the title compound as a bright yellow oil (11 mg, 0.023 mmol, 100%): R_f 0.31 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); $[\alpha]_D^{25}$ = +6.99 (*c* 1.0, $CHCl_3$); $\tilde{\nu}_{max}/cm^{-1}$ 2918 (br), 2361 (w), 2160 (w), 1977 (w), 1692 (C=O, s), 1624 (C=O, m), 1510 (w), 1460 (w), 1408 (m), 1358 (m), 1324 (m), 1260 (m), 1197 (m), 1178 (m), 1112 (m), 1024 (w), 932 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (1H, br s, N-H), 7.52 (1H, dd, *J* 8.5, 7.0, C^{6}-H), 7.12 (1H, d, *J* 7.0, C^{7}-H), 6.95 (1H, d, *J* 8.5, C^{5}-H), 6.52 (1H, br s, N-H), 4.95 (1H, dd, *J* 12.0, 5.4 $C^{3'}$-H), 4.15 (2H, s, $C^{2''''}$-H_2), 3.79–3.68 (10H, m, $C^{1''}$-H_2, $C^{1'''}$-H_2, $C^{2'''}$-H_2, $C^{1''''}$-H_2, $C^{2''''}$-H_2), 3.50 (2H, t, *J* 5.2 $C^{2''}$-H_2), 2.94–2.68 (3H, m, $C^{4'}$-H_a, $C^{5'}$-H_2), 2.19–2.11 (1H, m, $C^{4'}$-H_b); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5 ($C^{6'}$), 169.5 (C^{3}), 168.9 ($C^{2'}$), 167.7 (C^{1}), 147.0 (C^{4}), 136.3 (C^{6}), 132.6 (C^{7a}), 117.0 (C^{5}), 111.9 (C^{7}), 110.4 (C^{3a}), 71.5 ($C^{1''/1'''/2'''/1''''/2''''}$), 70.7 ($C^{1''/1'''/2'''/1''''/2''''}$), 70.6 ($C^{1''/1'''/2'''/1''''/2''''}$), 70.3 ($C^{1''/1'''/2'''/1''''/2''''}$), 69.6

($C^{2'''''}$), 49.0 ($C^{3'}$), 42.4 ($C^{2''}$), 31.5 ($C^{5'}$), 22.9 ($C^{4'}$); HRMS *m/z* (ESI^+) [Found: 464.1664, $C_{21}H_{26}N_3O_9$ requires $[M+H]^+$ 464.1664]; LRMS *m/z* (ESI^+) 486.2 ($[M+Na]^+$, 100%); HPLC retention time 6.0 min, 98.1%.

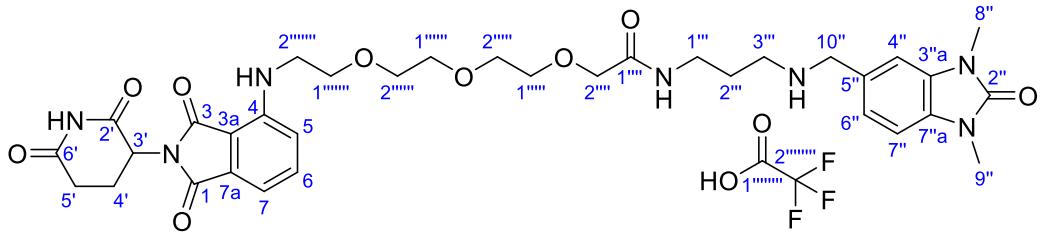
14''-{[2-(2',6'-Dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-3'',6'',9'',12''-tetraoxatetradecan-1''-oic acid trifluoro acetate (301)



tert-Butyl 14-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-3,6,9,12-tetraoxatetradecan-1-oate (**299**) (24 mg, 0.043 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (0.50 mL) and trifluoroacetic acid (0.50 mL) was added. The reaction mixture was stirred at rt for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in acetonitrile (20 mL) and washed with hexane (3 × 20 mL). The acetonitrile fraction was concentrated *in vacuo* to yield the title compound as a bright yellow oil (19 mg, 0.031 mmol, 71%): R_f 0.11 (1:9 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = +2.89 (*c* 1.0, $CHCl_3$); $\bar{\nu}_{max}$/cm^{-1} 3393 (br), 2918 (br), 2872 (br), 1753 (w), 1692 (C=O, s), 1624 (C=O, m), 1510 (w), 1482 (w), 1460 (w), 1408 (m), 1357 (m), 1324 (m), 1259 (m), 1197 (m), 1178 (m), 1108 (m), 1038 (m), 1024 (m), 991 (m), 944 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (1H, br s, N-H), 7.49 (1H, dd, *J* 8.5, 7.1, C^6-H), 7.09 (1H, d, *J* 7.1, C^7-H), 6.91 (1H, d, *J* 8.5, C^5-H), 6.37 (1H, br s, O-H), 4.92 (1H, dd, *J* 12.0, 5.4 $C^{3'}$-H), 4.13 (2H, s, $C^{2''}$-H_2), 3.79–3.63 (14H, m, $C^{4''}$-H_2, $C^{5''}$-H_2, $C^{7''}$-H_2, $C^{8''}$-H_2, $C^{10''}$-H_2, $C^{11''}$-H_2, $C^{13''}$-H_2), 3.47 (2H, t, *J* 5.1, $C^{14''}$-H_2), 2.90–2.83 (1H, m, $C^{5'}$-H_a), 2.81–2.68 (2H, m, $C^{4'}$-H_a, $C^{5'}$-H_b), 2.16–2.08 (1H, m, $C^{4'}$-H_b); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.7 ($C^{6'}$), 169.4 (C^3), 168.9 ($C^{2'}$), 167.7 (C^1), 146.9 (C^4), 136.2 (C^6), 132.6 (C^{7a}), 117.0 (C^5), 111.8 (C^7), 110.4 (C^{3a}), 71.4 ($C^{4''}$), 70.9 ($C^{5''/7''/8''/10''/11''/13''}$), 70.7 ($C^{5''/7''/8''/10''/11''/13''}$), 70.52 ($C^{5''/7''/8''/10''/11''/13''}$), 70.46 ($C^{5''/7''/8''/10''/11''/13''}$), 70.3 ($C^{5''/7''/8''/10''/11''/13''}$), 69.5 ($C^{2''}$), 48.9 ($C^{3'}$), 42.4 ($C^{14''}$), 31.5 ($C^{5'}$), 22.9

($C^{4'}$); ^{19}F NMR (377 MHz, $CDCl_3$) δ –75.8; HRMS *m/z* (ESI^+) [Found: 508.1923, $C_{23}H_{30}N_3O_{10}$ requires $[M+H]^+$ 508.1926]; LRMS *m/z* (ESI^+) 530.2 ($[M+Na]^+$, 100%); HPLC retention time (Atlantis) 10.5 min, >99.9%.

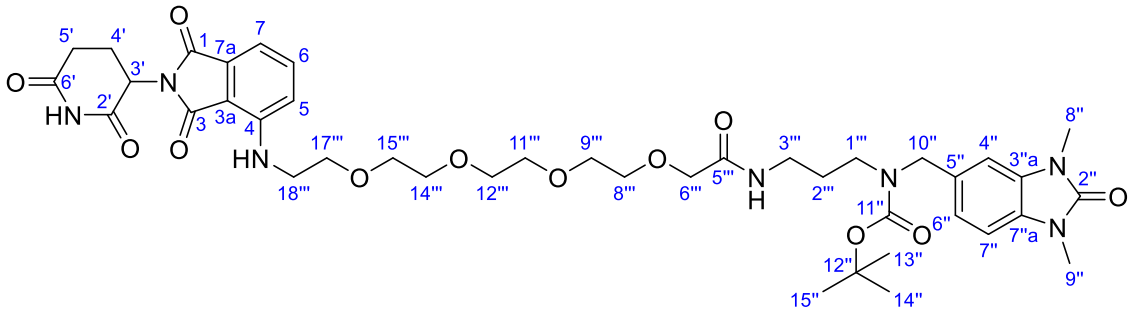
_N_-(3’’’-{[(1’’,3’’-Dimethyl-2’’-oxo-2’’,3’’-dihydro-1_H_-benzimidazol-5’’-yl)methyl]amino}prop-1’’’-yl)-2’’’’-{2’’’’’-[2’’’’’’-(2’’’’’’’-{[2-(2’,6’-dioxopiperidin-3’-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetamide trifluoroacetate (217)



{2-[2-(2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy) ethoxy]ethoxy}acetic acid trifluoroacetate (**300**) (11 mg, 0.023 mmol, 1.1 eq.) was dissolved in *N*,*N*-dimethylformamide (1.0 mL). 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (8.7 mg, 0.023 mmol, 1.1 eq.), *N*,*N*-diisopropylethylamine (4.0 µL, 0.010 mmol, 1.0 eq.) and hydroxybenzotriazole (3.1 mg, 0.010 mmol, 1.0 eq.) were added. The reaction mixture was stirred at rt for 30 min. *tert*-Butyl (3-aminoprop-1-yl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (**268**) (6.7 mg, 0.019 mmol, 1.0 eq.) was added. The reaction mixture was stirred at rt for 43 h. After this time TLC analysis indicated complete consumption of starting material (1:9 EtOH/$CHCl_3$ + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (20 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 20 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–6% MeOH/CH_2Cl_2 gradient) and further purified using silica flash column chromatography (elution with 0–5% MeOH/CH_2Cl_2 + 1% Et_3N gradient) to yield *tert*-butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](15-{[2-(2,6-dioxopiperidin-3-yl)-

1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-5-oxo-7,10,13-trioxa-4-azapentadecan-1-yl)carbamate (**302**) as a bright yellow oil (3.0 mg, 0.0038 mmol, 20%). **302** was dissolved in CH_2Cl_2 (0.50 mL) and trifluoroacetic acid (0.50 mL) was added. The reaction mixture was stirred at rt for 30 min. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* to yield the title compound as a bright yellow oil (3.0 mg, 0.0038 mmol, 100%): R_f 0.53 (1:19 MeOH/CH_2Cl_2 + 1% Et_3N); $[\alpha]_D^{25}$ = +12.00 (*c* 0.5, MeOH); $\bar{\nu}_{max}$/cm^{-1} 2924 (br), 2854 (br), 1684 (C=O, s), 1559 (w), 1511 (w), 1458 (w), 1408 (w), 1326 (w), 1265(w), 1202 (m), 1134 (m), 1029 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.53 (1H, dd, *J* 8.6, 7.1, C^6-H), 7.31–7.16 (3H, m, C$^{4''}$-H, C$^{6''}$-H, C$^{7''}$-H), 7.06 (1H, d, *J* 8.6, C^5-H), 7.03 (1H, d, *J* 7.1, C^7-H), 5.04 (1H, dd, *J* 12.8, 5.5, C$^{3'}$-H), 4.20 (2H, s, C$^{10''}$-H$_2$), 4.01 (2H, s, C$^{2''''}$-H$_2$), 3.73 (2H, t, *J* 5.2, C$^{1'''''''}$-H$_2$), 3.70–3.64 (8H, m, C$^{1'''''}$-H$_2$, C$^{2'''''}$-H$_2$, C$^{1''''''}$-H$_2$, C$^{2''''''}$-H$_2$), 3.50 (2H, t, *J* 5.2, C$^{1'''''''}$-H$_2$), 3.43 (3H, s, C$^{8''/9''}$-H$_3$), 3.42 (3H, s, C$^{8''/9''}$-H$_3$), 3.37–3.31 (2H, m, C$^{1'''}$-H$_2$), 3.02 (2H, t, *J* 7.3, C$^{3'''}$-H$_2$), 2.91–2.80 (1H, m, C$^{5'}$-H$_a$), 2.78–2.61 (2H, m, C$^{4'}$-H$_a$, C$^{5'}$-H$_b$), 2.15–2.06 (1H, m, C$^{4'}$-H$_b$), 1.88 (2H, tt, *J* 7.3, 7.3, C$^{2'''}$-H$_2$); ^{13}C NMR (126 MHz, CD_3OD) δ 174.6 (C$^{6'}$), 174.3 (C$^{1''''}$), 171.8 (C$^{2'}$), 170.7 (C^3), 169.2 (C^1), 156.4 (C$^{2''}$), 148.1 (C^4), 137.2 (C^6), 133.8 (C^{7a}), 132.1 (C$^{3''a/7''a}$), 131.6 (C$^{3''a/7''a}$), 125.7 (C$^{5''}$), 124.6 (C$^{6''}$), 118.2 (C^5), 112.0 (C^7), 111.3 (C^{3a}), 110.3 (C$^{4''}$), 109.3 (C$^{7''}$), 72.0 (C$^{1'''''/2'''''/1''''''/2''''''}$), 71.5 (C$^{1'''''/2'''''/1''''''/2''''''}$), 71.4 (C$^{1'''''/2'''''/1''''''/2''''''}$), 71.1 (C$^{2''''}$), 70.5 (C$^{1'''''''}$), 52.8 (C$^{10''}$), 50.2 (C$^{3'}$), 45.6 (C$^{3'''}$), 43.2 (C$^{2'''''''}$), 36.3 (C$^{1'''}$), 32.2 (C$^{5'}$), 27.7 (C$^{2'''}$), 27.6 (C$^{8''}$, C$^{9''}$), 22.4 (C$^{4'}$); HRMS *m/z* (ESI$^+$) [Found: 694.3187, $C_{34}H_{44}N_7O_9$ requires $[M+H]^+$ 694.3195]; LRMS *m/z* (ESI$^+$) 716.3 ($[M+Na]^+$, 42%); HPLC retention time 3.3 min, 96.4%.

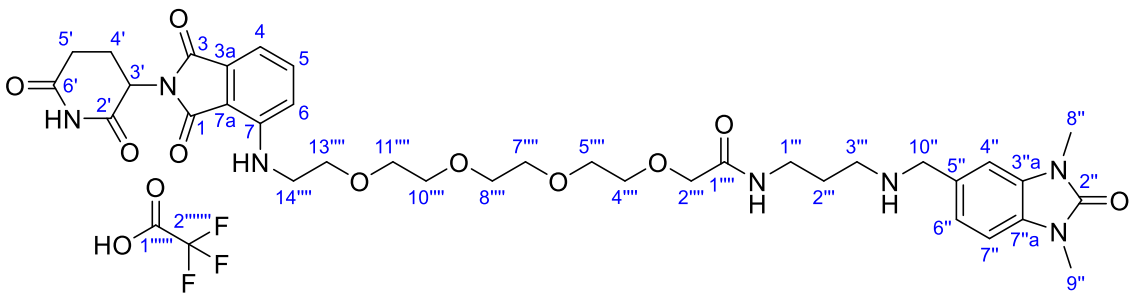
tert-Butyl [(1'',3''-dimethyl-2''-oxo-2'',3''-dihydro-1_H_-benzimidazol-5''-yl)methyl](18'''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}-5'''-oxo-7''',10''',13''',16'''-tetraoxa-4'''-azaoctadecan-1'''-yl)carbamate (303)



14-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-3,6,9,12-tetraoxatetradecan-1-oic acid (**301**) (18 mg, 0.036 mmol, 1.2 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (1.0 mL). 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (13 mg, 0.036 mmol, 1.2 eq.), *N*,*N*-diisopropylethylamine (17 µL, 0.036 mmol, 1.2 eq.) and hydroxybenzotriazole (5.0 mg, 0.036 mmol, 1.2 eq.) were added. The reaction mixture was stirred at rt for 30 min. *tert*-Butyl (3-aminopropyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (**268**) (10 mg, 0.30 mmol, 1.0 eq.) was added and the reaction mixture stirred at rt for 3.5 h. After this time additional 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (5.0 mg, 0.015 mmol, 0.5 eq.) and hydroxybenzotriazole (2.0 mg, 0.015 mmol, 0.5 eq.) were added. The reaction mixture was stirred at rt for 19 h. After this time TLC analysis indicated complete consumption of starting material (1:9 EtOH/$CHCl_3$ + drop Et_3N). The reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (30 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 20 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–10% EtOH/EtOAc gradient then 10% EtOH/CH_2Cl_2 isocratic) to yield the title compound as a yellow oil (9.0 mg, 0.011 mmol, 36%): R_f 0.20 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = +4.35 (*c* 1.0, $CHCl_3$); $\tilde{\nu}_{max}$/cm^{-1} 2921 (br), 2160 (m), 2031 (m), 1977 (m), 1698 (C=O, s), 1626 (w), 1558 (w), 1541 (w),

1508 (w), 1458 (w), 1410 (w), 1362 (w), 1262 (w), 1171 (w), 1138 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (1H, br s, N-H), 7.48 (1H, dd, *J* 8.5, 7.1, C^{6}-H), 7.09 (1H, d, *J* 7.1, C^{7}-H), 6.97–6.83 (4H, m, C^{5}-H, $C^{4''}$-H, $C^{6''}$-H, $C^{7''}$-H), 6.48 (1H, t, *J* 5.6, N-H), 4.90 (1H, dd, *J* 12.2, 5.4, $C^{3'}$-H), 4.44 (2H, s, $C^{10''}$-H_2), 3.97 (2H, s, $C^{6'''}$-H_2), 3.71 (2H, t, *J* 5.5, $C^{17'''}$-H_2), 3.68–3.61 (12H, m, $C^{8'''}$-H_2, $C^{9'''}$-H_2, $C^{11'''}$-H_2, $C^{12'''}$-H_2, $C^{14'''}$-H_2, $C^{15'''}$-H_2,), 3.45 (2H, dt, *J* 5.5, 5.5, $C^{18'''}$-H_2), 3.40 (3H, s, $C^{8''/9''}$-H_3), 3.39 (3H, s, $C^{8''/9''}$-H_3), 3.29–3.22 (4H, m, $C^{1'''}$-H_2, $C^{3'''}$-H_2), 2.90–2.66 (3H, m, $C^{4'}$-H_a, $C^{5'}$-H_2), 2.16–2.07 (1H, m, $C^{4'}$-H_b), 1.75–1.66 (2H, m, $C^{2'''}$-H_2), 1.48 (9H, s, $C^{13''}$-H_3, $C^{14''}$-H_3, $C^{15''}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3 ($C^{6'}$), 170.1 ($C^{5'''}$), 169.4 ($C^{1/3}$), 168.6 ($C^{2'}$), 167.7 ($C^{1/3}$), 156.1 ($C^{11''}$), 154.9 ($C^{2''}$), 146.9 (C^{4}), 136.2 (C^{6}), 132.7 (C^{7a}), 131.8 ($C^{5''}$), 130.5 ($C^{3''a/7''a}$), 129.5 ($C^{3''a/7''a}$), 120.5 ($C^{6''}$), 116.9 (C^{5}), 111.8 (C^{7}), 110.4 (C^{3a}), 107.2 ($C^{4''/7''}$), 106.5 ($C^{4''/7''}$), 80.1 ($C^{12''}$), 71.1 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 70.9 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 70.74 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 70.68 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 70.4 ($C^{6'''/8'''/9'''/11'''/12'''/14'''/15'''}$), 69.6 ($C^{17'''}$), 50.5 ($C^{10''}$), 49.0 ($C^{3'}$), 43.5 ($C^{1'''}$), 42.5 ($C^{18'''}$), 36.0 ($C^{3'''}$), 31.6 ($C^{5'}$), 28.6 ($C^{13''}$, $C^{14''}$, $C^{15''}$), 27.8 ($^{2'''}$), 27.4 ($C^{8''/9''}$), 27.3 ($C^{8''/9''}$), 23.0 ($C^{4'}$), minor rotamer also observed; HRMS *m/z* (ESI^{+}) [Found: 838.3975, $C_{41}H_{56}N_7O_{12}$ requires $[M+H]^{+}$ 838.3981]; LRMS *m/z* (ESI^{+}) 860.3 ($[M+Na]^{+}$, 100%); HPLC retention time 6.4 min, 97.0%.

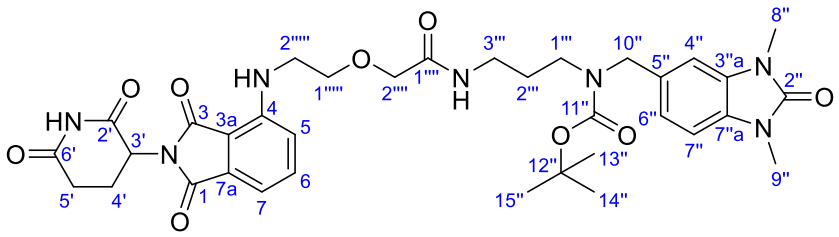
_N_-(3'''-{[(1'',3''-Dimethyl-2''-oxo-2'',3''-dihydro-1_H_-benzimidazol-5''-yl)methyl]amino}propyl)-14''''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}-3'''',6'''',9'''',12''''-tetraoxatetradecan-1''''-amide (218)



tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](18-{[2-(,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-5-oxo-7,10,13,16-tetraoxa-4-azaoctadecan-1-yl)carbamate (**303**) (10 mg, 0.012 mmol, 1.0 eq.) was dissolved in

CH_2Cl_2 (0.50 mL) and trifluoroacetic acid (0.50 mL) was added. The reaction mixture was stirred at rt for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* to yield the title compound as a yellow oil (10 mg, 0.012 mmol, 100%): R_f 0.67 (1:9 MeOH/CH_2Cl_2 + 1% Et_3N); $[\alpha]_D^{25}$ = +2.89 (*c* 1.0, $CHCl_3$); $\tilde{\nu}_{max}$/cm^{-1} 3392 (br), 2360 (w), 2341 (w), 2160 (w), 2027 (w), 1977 (w), 1756 (w), 1692 (C=O, s), 1625 (m), 1547 (w), 1511 (w), 1462 (w), 1408 (w), 1359 (w), 1324 (w), 1262 (w), 1199 (m), 1175 (m), 1114 (m), 948 (m); ^{1}H NMR (500 MHz, CD_3OD) δ 7.52 (1H, dd, *J* 8.6, 7.1, C^5-H), 7.26–7.21 (2H, m, $C^{6''}$-H, $C^{4''}$-H), 7.20–7.15 (1H, m, $C^{7''}$-H), 7.05 (1H, d, *J* 8.6, C^6-H), 7.02 (1H, d, *J* 7.1, C^4-H), 5.04 (1H, dd, *J* 12.7, 5.5, $C^{3'}$-H), 4.21 (2H, s, $C^{10''}$-H_2), 4.00 (2H, s, $C^{2''''}$-H_2), 3.71 (2H, t, *J* 5.2, $C^{13''''}$-H_2), 3.69–3.61 (12H, m, $C^{4''''}$-H_2, $C^{5''''}$-H_2, $C^{7''''}$-H_2, $C^{8''''}$-H_2, $C^{10''''}$-H_2, $C^{11''''}$-H_2), 3.47 (2H, t, *J* 5.2, $C^{14''''}$-H_2), 3.42 (3H, s, $C^{8''/9''}$-H_3), 3.41 (3H, s, $C^{8''/9''}$-H_3), 3.36 (2H, t, *J* 6.5, $C^{1'''}$-H_2), 3.05 (2H, t, *J* 7.3, $C^{3'''}$-H_2), 2.91–2.80 (1H, m, $C^{5'}$-H_a), 2.78–2.63 (2H, m, $C^{4'}$-H_a, $C^{5'}$-H_b), 2.15–2.06 (1H, m, $C^{4'}$-H_b), 1.91 (2H, tt, *J* 7.3, 6.5, $C^{2'''}$-H_2); ^{13}C NMR (126 MHz, CD_3OD) δ 174.6 ($C^{6'}$), 174.2 ($C^{1''''}$), 171.7 ($C^{2'}$), 170.7 (C^1), 169.2 (C^3), 156.4 ($C^{2''}$), 148.1 (C^7), 137.2 (C^5), 133.8 (C^{3a}), 132.1 ($C^{7''a}$), 131.6 ($C^{3''a}$), 125.7 ($C^{5''}$), 124.7 ($C^{6''}$), 118.2 (C^6), 112.0 (C^4), 111.2 (C^{7a}), 110.4 ($C^{4''}$), 109.3 ($C^{7''}$), 72.0 ($C^{4''''/5''''/7''''/8''''/10''''/11''''}$), 71.6 ($C^{4''''/5''''/7''''/8''''/10''''/11''''}$), 71.51 ($C^{4''''/5''''/7''''/8''''/10''''/11''''}$), 71.47 ($C^{4''''/5''''/7''''/8''''/10''''/11''''}$), 71.45 ($C^{4''''/5''''/7''''/8''''/10''''/11''''}$), 71.3 ($C^{4''''/5''''/7''''/8''''/10''''/11''''}$), 71.0 ($C^{2''''}$), 70.5 ($C^{13''''}$), 52.8 ($C^{10''}$), 50.2 ($C^{3'}$), 45.7 ($C^{3'''}$), 43.2 ($C^{14''''}$), 36.4 ($C^{1'''}$), 32.2 ($C^{5'}$), 27.58 ($C^{8''}$, $C^{9''}$), 27.56 ($C^{2'''}$), 23.8 ($C^{4'}$); ^{19}F NMR (377 MHz, CD_3OD) δ –77.5; HRMS *m/z* (ESI$^+$) [Found: 738.3446, $C_{36}H_{48}N_7O_{10}$ requires $[M+H]^+$ 738.3457]; LRMS *m/z* (ESI$^+$) 760.4 ($[M+Na]^+$, 100%); HPLC retention time 4.6 min, 99.7%.

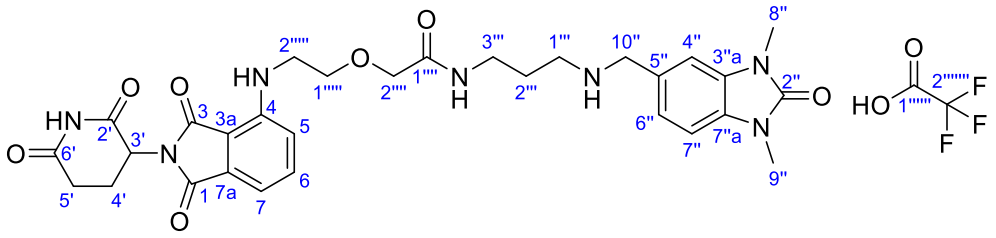
tert-Butyl [(1'',3''-dimethyl-2''-oxo-2'',3''-dihydro-1_H_-benzimidazol-5''-yl)methyl]{3'''-[2''''-(2'''''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}ethoxy)acetamido]prop-1'''-yl}carbamate (306)



tert-Butyl {3-[2-(2-aminoethoxy)acetamido]propyl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]carbamate (**294**) (0.11 g, 0.24 mmol, 1.2 eq.) was dissolved in _N_-methyl-2-pyrrolidinone (1.0 mL). 2-(2,6-Dioxopiperidin-3-yl)-4-fluoro-1_H_-isoindole-1,3(2H)-dione (**297**) (55 mg, 0.20 mmol, 1.0 eq.) and _N,N_-diisopropylethylamine (70 µL, 0.40 mmol, 2.0 eq.) were added. The reaction mixture was heated to 90 °C and stirred for 17 h. After this time TLC analysis indicated complete consumption of starting material (1:19 MeOH/CH_2Cl_2). The reaction was cooled to rt, quenched with an aqueous solution of LiCl (0.5 M, 30 mL) and extracted with EtOAc (50 mL). The organic fraction was washed with an aqueous solution of LiCl (0.5 M, 2 × 30 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated _in vacuo_. The product was purified using silica flash column chromatography (elution with 0–6% MeOH/CH_2Cl_2 gradient) to yield the title compound as a bright green oil (33 mg, 0.047 mmol, 23%): R_f 0.29 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = +2.41 (_c_ 1.0, $CHCl_3$); $\bar{\nu}_{max}$/cm^{-1} 2929 (br), 2541 (br), 2161 (m), 2029 (m), 1977 (m), 1696 (C=O, s), 1624 (w), 1508 (w), 1458 (w), 1408 (w), 1362 (w), 1324 (w), 1260 (w), 1169 (w), 1117 (w), 1025 (w), 914 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (1H, dd, _J_ 7.8, 7.1, C^6-H), 7.12 (1H, d, _J_ 7.1, C^7-H), 6.97–6.91 (2H, m, C^5-H, $C^{6''}$-H), 6.90–6.85 (2H, m, $C^{4''}$-H, $C^{7''}$-H), 6.57 (1H, br s, N-H), 4.92–4.84 (1H, m, $C^{3'}$-H), 4.43 (2H, s, $C^{10''}$-H_2), 3.99 (2H, s, $C^{2''''}$-H_2), 3.74 (2H, t, _J_ 5.2, $C^{1'''''}$-H_2), 3.57–3.49 (2H, m, $C^{2'''''}$-H_2), 3.40 (3H, s, $C^{8''/9''}$-H_3), 3.38 (3H, s, $C^{8''/9''}$-H_3), 3.33–3.07 (4H, m, $C^{1'''}$-H_2, $C^{3'''}$-H_2), 2.88–2.79 (1H, m, $C^{5'}$-H_a), 2.78–2.65 (2H, m, $C^{4'}$-H_a, $C^{5'}$-H_b), 2.16–2.05 (1H, m, $C^{4'}$-H_b), 1.70 (2H, tt, _J_ 7.3, 7.3, $C^{2'''}$-H_2), 1.46 (9H, s, $C^{13''}$-H_3, $C^{14''}$-H_3, $C^{15''}$-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4 ($C^{6'}$),

169.7 (C^{3}), 169.3 ($C^{1''''}$), 168.6 ($C^{2'}$), 167.6 (C^{1}), 156.2 ($C^{11''}$), 154.9 ($C^{2''}$), 146.8 (C^{4}), 136.3 (C^{6}), 132.7 (C^{7a}), 131.6 ($C^{5''}$), 130.5 ($C^{3''a/7''a}$) 129.5 ($C^{3''a/7''a}$), 120.5 ($C^{6''}$), 116.9 (C^{5}), 112.2 (C^{7}), 110.8 (C^{3a}), 107.2 ($C^{4''/7''}$), 106.6 ($C^{4''/7''}$), 80.5 ($C^{12''}$), 70.7 ($C^{2''''}$), 69.8 ($C^{1''''}$), 50.5 ($C^{10''}$), 49.1 ($C^{3'}$), 43.4 ($C^{1'''}$), 42.2 ($C^{2''''}$), 36.0 ($C^{3'''}$), 31.5 ($C^{5'}$), 28.6 ($C^{13''}$, $C^{14''}$, $C^{15''}$), 27.7 ($C^{2'''}$), 27.4 ($C^{8''/9''}$), 27.3 ($C^{8''/9''}$), 23.0 ($C^{4'}$); HRMS *m/z* (ESI^{+}) [Found: 706.3190, $C_{35}H_{44}N_7O_9$ requires $[M+H]^{+}$ 706.3195]; LRMS *m/z* (ESI^{+}) 728.3 ($[M+Na]^{+}$, 100%); HPLC retention time 6.3 min, 98.5%.

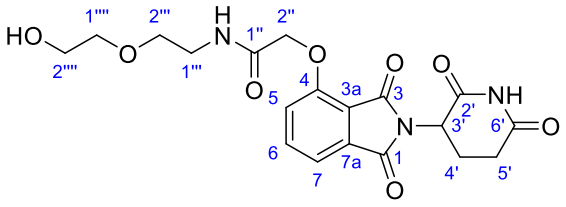
_N_-(3'''-{[(1'',3''-Dimethyl-2''-oxo-2'',3''-dihydro-1_H_-benzimidazol-5''-yl)methyl]amino}propyl)-2''''-(2'''''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}ethoxy)acetamide trifluoroacetate (215)



tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{3-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)acetamido]prop-1-yl}carbamate (**306**) (33 mg, 0.047 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (0.50 mL) and trifluoroacetic acid (0.50 mL) was added. The reaction mixture was stirred at rt for 1 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo* to yield the title compound as a bright green oil (34 mg, 0.047 mmol, 100%): R_f 0.22 (1:9 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$= +4.18 (*c* 1.0, $CHCl_3$); $\bar{\nu}_{max}$/cm^{-1} 3385 (br), 2560 (br), 2160 (br), 2031 (m), 1978 (m), 1755 (w), 1687 (C=O, s), 1625 (m), 1542 (w), 1511 (w), 1461 (w), 1408 (w), 1359 (w), 1324 (w), 1262 (w), 1198 (m), 1175 (m), 1124 (m); ^{1}H NMR (500 MHz, CD_3OD) δ 7.55 (1H, dd, *J* 8.6, 7.1 C^{6}-H), 7.29–7.21 (2H, m, $C^{4''}$-H, $C^{6''}$-H), 7.15 (1H, d, *J* 8.0, $C^{7''}$-H), 7.10 (1H, d, *J* 8.6, C^{5}-H), 7.05 (1H, d, *J* 7.1, C^{7}-H), 5.02 (1H, dd, *J* 12.7, 5.5, $C^{3'}$-H), 4.22 (2H, s, $C^{10''}$-H_2), 4.03 (2H, s, $C^{2''''}$-H_2), 3.76 (2H, t, *J* 5.2, $C^{2'''''}$-H_2), 3.56 (2H, t, *J* 5.2, $C^{2'''''}$-H_2), 3.40 (3H, s, $C^{8''/9''}$-H_3), 3.39 (3H, s, $C^{8''/9''}$-H_3), 3.37–3.32 (2H, m, $C^{3'''}$-H_2), 3.04 (2H, t, *J* 7.3, $C^{1'''}$-H_2), 2.86–2.76 (1H, m, $C^{5'}$-H_a), 2.75–2.61 (2H, m, $C^{4'}$-H_a, $C^{5'}$-H_b),

2.13–2.04 (1H, m, $C^{4'}$-H_b), 1.90 (2H, tt, *J* 7.3, 7.3, $C^{2'''}$-H_2); ^{13}C NMR (126 MHz, CD_3OD) δ 174.5 ($C^{6'}$), 173.5 ($C^{1''''}$), 171.7 ($C^{2'}$), 170.8 (C^{1}), 169.1 (C^{3}), 156.3 ($C^{2''}$), 148.0 (C^{4}), 137.3 (C^{6}), 133.8 (C^{7a}), 132.1 ($C^{3''a}$), 131.6 ($C^{7''a}$), 125.6 ($C^{5''}$), 124.7 ($C^{6''}$), 118.3 (C^{5}), 112.3 (C^{7}), 111.4 (C^{3a}), 110.4 ($C^{4''}$), 109.2 ($C^{7''}$), 71.2 ($C^{1''''}$), 70.9 ($C^{2''''}$), 52.8 ($C^{10''}$), 50.2 ($C^{3'}$), 45.6 ($C^{1'''}$), 43.0 ($C^{2''''}$), 36.4 ($C^{3'''}$), 32.1 ($C^{5'}$), 27.6 ($C^{8''}$, $C^{9''}$), 27.5 ($C^{2'''}$), 23.8 ($C^{4'}$); ^{19}F NMR (377 MHz, CD_3OD) δ –77.4; HRMS *m/z* (ESI^+) [Found: 606.2661, $C_{30}H_{36}N_7O_7$ requires $[M+H]^+$ 606.2671]; LRMS *m/z* (ESI^+) 628.2 ($[M+Na]^+$, 100%); HPLC retention time 4.5 min, 99.6%.

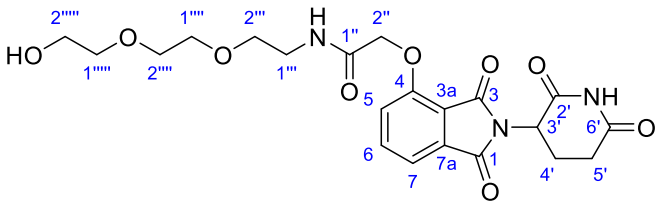
2'''-{[2-(2',6'-Dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-*N*-[2'''-(2''''-hydroxyethoxy)ethyl]acetamide (311)



{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetic acid (**229**) (0.10 g, 0.31 mmol, 1.0 eq.) was dissolved in *N*,*N*-dimethylformamide (4.0 mL). 2-(2-Aminoethoxy)ethanol (34 μL, 0.34 mmol, 1.1 eq.), *N*,*N*-diisopropylethylamine (0.11 mL, 0.62 mmol, 2.0 eq.) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (0.13 g, 0.34 mmol, 1.1 eq.) were added. The reaction mixture was stirred at rt for 17 h. After this time TLC analysis indicated complete consumption of starting material (EtOH + drop AcOH). The reaction volume was reduced by concentration *in vacuo*. The residue was diluted with an aqueous solution of LiCl (0.5 M, 30 mL) and extracted with EtOAc (3 × 40 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–8% MeOH/CH_2Cl_2 gradient) and further purified using silica flash column chromatography (elution with 0–9% EtOH/$CHCl_3$ gradient) to yield the title compound as a colourless solid (38 mg, 0.091 mmol, 29%): R_f 0.47 (2:3 EtOH/$CHCl_3$); $[\alpha]_D^{25}$ = –2.43 (*c* 1.0, DMSO); m.p. 177–178 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 3538 (br), 3393 (br), 3104 (br), 2894 (br), 2563 (br), 2160 (br), 2030

(br), 1977 (br), 1776 (w), 1703 (C=O, s), 1655 (C=O, m), 1614 (w), 1550 (w), 1481 (w), 1437 (w), 1393 (w), 1352 (w), 1324 (w), 1270 (m), 1201 (w), 1116 (m), 1074 (w), 1057 (w), 1036 (w), 1013 (w), 988 (w), 939 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (1H, br s, N-H), 8.02 (1H, t, *J* 5.6, N-H), 7.81 (1H, dd, *J* 8.5, 7.3, C^{6}-H), 7.50 (1H, d, *J* 7.3, C^{7}-H), 7.40 (1H, d, *J* 8.5, C^{5}-H), 5.11 (1H, dd, *J* 12.8, 5.4, C$^{3'}$-H), 4.79 (2H, s, C$^{2''}$-H$_2$), 4.56 (1H, t, *J* 5.5, O-H), 3.53–3.44 (4H, m, C$^{2'''}$-H$_2$, C$^{2''''}$-H$_2$), 3.44–3.39 (2H, m, C$^{1''''}$-H$_2$), 3.35–3.28 (2H, m, C$^{1'''}$-H$_2$), 2.89 (1H, ddd, *J* 16.9, 13.8, 5.5, C$^{5'}$-H$_a$), 2.64–2.52 (2H, m, C$^{4'}$-H$_a$, C$^{5'}$-H$_b$), 2.08–1.99 (1H, m, C$^{4'}$-H$_b$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.8 (C$^{6'}$), 169.9 (C$^{2'}$), 166.9 (C$^{1''}$), 166.7 (C^{1}), 165.4 (C^{3}), 155.0 (C^{4}), 136.9 (C^{6}), 133.0 (C^{7a}), 120.4 (C^{5}), 116.8 (C^{3a}), 116.0 (C^{7}), 72.1 (C$^{1''''}$), 68.8 (C$^{2'''}$), 67.5 (C$^{2''}$), 60.2 (C$^{2''''}$), 48.8 (C$^{3'}$), 38.5 (C$^{1'''}$), 30.9 (C$^{5'}$), 22.0 (C$^{4'}$); HRMS *m/z* (ESI$^-$) [Found: 418.12545, $C_{19}H_{20}N_3O_8$ requires [M–H]$^-$ 418.12559]; LRMS *m/z* (ESI$^-$) 418.0 ([M–H]$^-$, 100%); HPLC retention time 5.6 min, 97.5%.

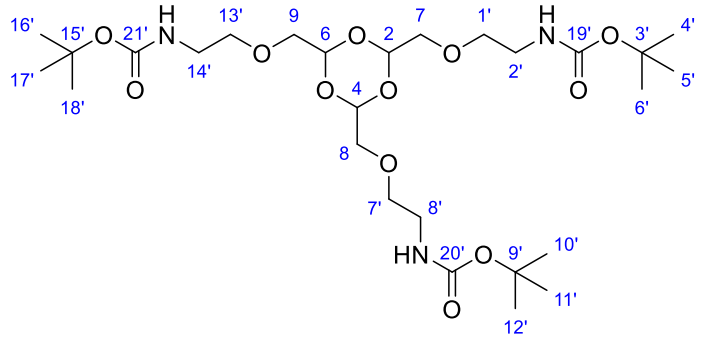
2''-{[2-(2',6'-Dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-*N*-{2'''-[2''''-(2'''''-hydroxyethoxy)ethoxy]ethyl}acetamide (312)



{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetic acid (**229**) (0.10 g, 0.31 mmol, 1.0 eq.) was dissolved in *N*,*N*-dimethylformamide (4.0 mL). 1,11-Diamino-3,6,9-trioxaundecane (47 μL, 0.34 mmol, 1.1 eq.), *N*,*N*-diisopropylethylamine (0.11 mL, 0.62 mmol, 2.0 eq.) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (0.13 g, 0.34 mmol, 1.1 eq.) were added. The reaction mixture was stirred at rt for 17 h. After this time TLC analysis indicated complete consumption of starting material (EtOH + drop AcOH). The reaction volume was reduced by concentration *in vacuo*. The residue was diluted with an aqueous solution of LiCl (0.5 M, 30 mL) and extracted with EtOAc (3 × 40 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in*

vacuo. The product was purified using silica flash column chromatography (elution with 0–8% MeOH/CH_2Cl_2 gradient) and further purified using silica flash column chromatography (elution with 0–9% EtOH/$CHCl_3$ gradient) to yield the title compound as a colourless solid (43 mg, 0.10 mmol, 33%): R_f 0.53 (2:3 EtOH/$CHCl_3$); $[\alpha]_D^{25}$ –10.3 (*c* 1.0, DMSO); m.p. 143–144 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 3387 (br), 3096 (br), 2921 (br), 2568 (br), 2162 (br), 2033 (br), 1979 (br), 1773 (w), 1707 (C=O, s), 1657 (C=O, s), 1618 (w), 1546 (w), 1485 (w), 1448 (w), 1396 (w), 1347 (w), 1327 (w), 1300 (w), 1273 (w), 1199 (w), 1118 (w), 1100 (w), 1054 (w), 1014 (w), 988 (w), 940 (w); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (1H, br s, N-H), 8.00 (1H, t, *J* 5.7, N-H), 7.81 (1H, dd, *J* 8.5, 7.3, C^6-H), 7.50 (1H, d, *J* 7.3, C^5-H), 7.40 (1H, d, *J* 8.5, C^7-H), 5.11 (1H, dd, *J* 12.8, 5.4, C$^{3'}$-H), 4.79 (2H, s, C$^{2''}$-H$_2$), 4.57 (1H, t, *J* 5.5, O-H), 3.57–3.36 (10H, m, C$^{2'''}$-H$_2$, C$^{1''''}$-H$_2$, C$^{2''''}$-H$_2$, C$^{1'''''}$-H$_2$, C$^{2'''''}$-H$_2$), 3.35–3.28 (2H, m, C$^{1'''}$-H$_2$), 2.90 (1H, ddd, *J* 16.9, 13.8, 5.4, C$^{5'}$-H$_a$), 2.65–2.50 (2H, m, C$^{4'}$-H$_a$, C$^{5'}$-H$_b$), 2.09–2.00 (1H, m, C$^{4'}$-H$_b$); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.8 (C$^{6'}$), 169.9 (C$^{2'}$), 166.9 (C$^{1''}$), 166.7 (C^3), 165.4 (C^1), 155.0 (C^4), 136.9 (C^6), 133.0 (C^{7a}), 120.3 (C^7), 116.8 (C^{3a}), 116.0 (C^5), 72.3 (C$^{1'''''}$), 69.7 (C$^{1''''/2''''}$), 69.6 (C$^{1''''/2''''}$), 68.8 (C$^{2'''}$), 67.5 (C$^{2''}$), 60.2 (C$^{2'''''}$), 48.8 (C$^{3'}$), 38.4 (C$^{1'''}$), 30.9 (C$^{5'}$), 22.0 (C$^{4'}$); HRMS *m/z* (ESI$^+$) [Found: 464.16659, $C_{21}H_{26}N_3O_9$ requires $[M+H]^+$ 464.16636]; LRMS *m/z* (ESI$^-$) 462.1 ([M–H]$^-$, 100%); HPLC retention time 5.5 min, >99.9%.

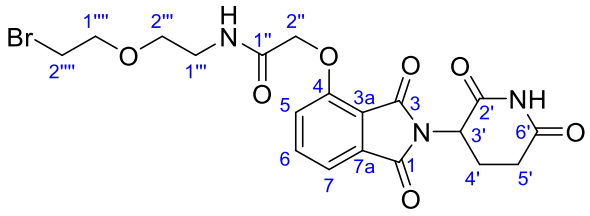
Tri-*tert*-butyl [1,3,5-trioxane-2,4,6-triyltris(methyleneoxyethane-2,1-diyl)]triscarbamate (318)



Oxalyl chloride (288 μL, 3.36 mmol, 2.3 eq.) was dissolved in anhydrous CH_2Cl_2 (3.00 mL) and the reaction mixture cooled to –78 °C. Anhydrous DMSO (570 μL) in anhydrous CH_2Cl_2

(3.00 mL) was added dropwise. The reaction mixture was stirred at –78 °C for 30 min. *tert*-Butyl (2-(2-hydroxyethoxy)ethyl)carbamate (**238**) (300 mg, 1.46 mmol, 1.0 eq.) dissolved in anhydrous CH_2Cl_2 (3.00 mL) was added dropwise. Et_3N (1.80 mL) was added dropwise and the reaction mixture stirred at –78 °C for 30 min. The reaction mixture was warmed to rt and stirred for 16.5 h. After this time TLC analysis indicated complete consumption of starting material (3:2 EtOAc/petroleum ether). The reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–100% EtOAc/petroleum ether gradient) to yield the title compound as a colourless solid (198 mg, 0.325 mmol, 67%): R_f 0.32 (3:2 EtOAc/petroleum ether); m.p. 55–57 °C (from EtOAc); $\bar{\nu}_{max}$/cm^{-1} 2902 (m), 2846 (w), 2815 (br), 2759 (br), 2361 (br), 2161 (br), 2030 (br), 1977 (br), 1703 (C=O, s), 1628 (w), 1608 (w), 1504 (m), 1478 (m), 1439 (w), 1397 (m), 1370 (w), 1331 (w), 1316 (w), 1265 (w), 1190 (w), 1166 (m), 1107 (w), 1042 (w), 1029 (w), 978 (w), 948 (w), 924 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.33 (3H, s, C^2-H, C^4-H, C^6-H), 3.95–3.76 (6H, m, C^7-H$_a$, C^8-H$_a$, C^9-H$_a$, C$^{1'}$-H$_a$, C$^{7'}$-H$_a$, C$^{13'}$-H$_a$), 3.64–3.49 (3H, m, C$^{2'}$-H$_a$, C$^{8'}$-H$_a$, C$^{14'}$-H$_a$), 3.50 (3H, dd, *J* 12.0, 2.2, C^7-H$_b$, C^8-H$_b$, C^9-H$_b$), 3.45 (3H, td, *J* 11.6, 2.9, C$^{1'}$-H$_b$ C$^{7'}$-H$_b$, C$^{13'}$-H$_b$), 3.36–3.17 (3H, m, C$^{2'}$-H$_b$, C$^{8'}$H$_b$, C$^{14'}$-H$_b$), 1.41 (27H, s, C$^{4'}$-H$_3$, C$^{5'}$-H$_3$, C$^{6'}$-H$_3$, C$^{10'}$-H$_3$, C$^{11'}$-H$_3$, C$^{12'}$-H$_3$, C$^{16'}$-H$_3$, C$^{17'}$-H$_3$, C$^{18'}$-H$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.1 (C$^{19'}$, C$^{20'}$, C$^{21'}$), 80.9 (C$^{3'}$, C$^{9'}$, C$^{15'}$), 72.9 (C^2, C^4, C^6), 70.5 (C^7, C^8, C^9), 66.4 (C$^{1'}$, C$^{7'}$, C$^{13'}$), 39.4 (C$^{2'}$, C$^{8'}$, C$^{14'}$), 28.3 (C$^{4'}$, C$^{5'}$, C$^{6'}$, C$^{10'}$, C$^{11'}$, C$^{12'}$, C$^{16'}$, C$^{17'}$, C$^{18'}$); unable to detect by mass spectrometry; HPLC retention time 7.0 min, 98.5%.

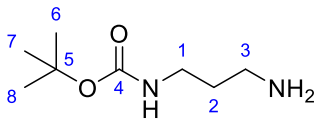
_N_-[2’’’-(2’’’’-Bromoethoxy)ethyl]-2’’-{[2-(2’,6’-dioxopiperidin-3’-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}acetamide (323)



2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-*N*-[2-(2-hydroxy ethoxy)ethyl]acetamide (**311**) (28 mg, 0.067 mmol, 1.0 eq.) was suspended in CH_2Cl_2 (0.60 mL) and PPh_3 (44 mg, 0.17 mmol, 2.5 eq.) was added. The reaction mixture was cooled to 0 °C and *N*-bromosuccinimide (30 mg, 0.17 mmol, 2.5 eq.) was added portionwise. The reaction mixture was stirred at 0 °C for 1 h, then warmed to rt and stirred for 4 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–100% EtOAc/petroleum ether gradient) and further purified using silica flash column chromatography (elution with 0–2% EtOH/$CHCl_3$ gradient) to yield the title compound as a colourless solid (27 mg, 0.056 mmol, 84%): R_f 0.31 (3:50 EtOH/$CHCl_3$); $[\alpha]_D^{25}$ = –7.98 (*c* 1.0, DMSO); m.p. 140–142 °C (from $CHCl_3$); $\tilde{\nu}_{max}$/cm^{-1} 3397 (br), 3104 (br), 2160 (br), 2031 (br), 1779 (w), 1704 (C=O, s), 1658 (C=O, s), 1614 (w), 1543 (w), 1484 (w), 1438 (m), 1392 (w), 1354 (w), 1325 (m), 1262 (m), 1198 (w), 1179 (w), 1114 (m), 1057 (w), 1014 (w), 983 (w), 939 (w); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (1H, br s, N-H), 7.73 (1H, dd, *J* 8.4, 7.4, C^6-H), 7.62 (1H, t, *J* 5.5, N-H), 7.54 (1H, dd, *J* 7.4, 0.6, C^5-H), 7.19 (1H, d, *J* 8.4, C^7-H), 5.02–4.94 (1H, m, C$^{3'}$-H), 4.65 (2H, s, C$^{2''}$-H$_2$), 3.80 (2H, t, *J* 6.4, C$^{1''''}$-H$_2$), 3.71–3.63 (2H, m, C$^{2'''}$-H$_2$), 3.66–3.55 (2H, m, C$^{1'''}$-H$_2$), 3.59–3.44 (2H, m, C$^{2''''}$-H$_2$), 2.95–2.70 (3H, m, C$^{4'}$-H$_a$, C$^{5'}$-H$_2$), 2.21–2.11 (1H, m, C$^{4'}$-H$_b$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0 (C$^{6'}$), 168.2 (C$^{2'}$), 167.0 (C^3), 166.8 (C$^{1''}$), 166.0 (C^1), 154.5 (C^4), 137.1 (C^6), 133.8 (C^{7a}), 119.4 (C^7), 118.1 (C^{3a}), 117.5 (C^5), 71.0 (C$^{1''''}$), 69.4 (C$^{2'''}$), 67.9 (C$^{2''}$), 49.4 (C$^{3'}$), 39.0 (C$^{1'''}$), 31.6 (C$^{5'}$), 30.3 (C$^{2''''}$), 22.8 (C$^{4'}$); HRMS *m/z* (ESI$^+$) [Found: 482.05573, $C_{19}H_{21}N_3O_7{}^{79}Br$ requires $[M+H]^+$ 482.05574; Found: 484.05369,

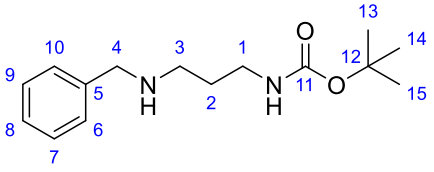
$C_{19}H_{21}N_3O_7{}^{81}Br$ requires $[M+H]^+$ 484.05369]; LRMS *m/z* (ESI^+) 504.0 ($[M+Na]^+$, 93%), 505.0 ($[M+Na]^+$, 100%); HPLC retention time 6.7 min, 96.0%.

***tert*-Butyl (3-aminopropyl)carbamate (324)**[271–273]



1,3-Diaminopropane (1.00 mL, 15.2 mmol, 5.0 eq.) was dissolved in $CHCl_3$ (65.0 mL). The reaction mixture was cooled to 0 °C and di-*tert*-butyl dicarbonate (663 mg, 3.04 mmol, 1.0 eq.) in $CHCl_3$ (53.0 mL) was added over 1 h *via* a dropping funnel. The reaction mixture was warmed to rt and stirred for 22 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with a saturated aqueous solution of K_2CO_3 (200 mL) and the organic fraction washed sequentially with a saturated aqueous solution of K_2CO_3 (200 mL), and H_2O (2 × 200 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–10% MeOH/CH_2Cl_2 + 1% Et_3N gradient) to yield the title compound as a colourless oil (437 mg, 2.51 mmol, 83%): R_f 0.29 (1:9 MeOH/CH_2Cl_2 + drop Et_3N); ^1H NMR (400 MHz, $CDCl_3$) δ 5.01 (1H, br s, N-H), 3.23–3.09 (2H, m, C^1-H_2), 2.73 (2H, t, *J* 6.7, C^3-H_2), 1.79 (2H, br s, N-H_2), 1.58 (2H, tt, *J* 6.6, 6.6, C^2-H_2), 1.40 (9H, s, C^6-H_3, C^7-H_3, C^8-H_3); LRMS *m/z* (ESI^+) 175.2 ($[M+Na]^+$, 17%). The spectroscopic data are in good agreement with literature values.[271–273]

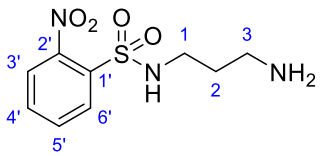
***tert*-Butyl [3-(benzylamino)propyl]carbamate (325)**[274]



tert-Butyl (3-aminopropyl)carbamate (**324**) (200 mg, 1.15 mmol, 1.0 eq.) was dissolved in anhydrous MeOH (1.50 mL). Et_3N (64.0 µL, 0.460 mmol, 0.4 eq.), $MgSO_4$ (208 mg, 1.73 mmol, 1.5 eq.) and benzaldehyde (129 µL, 1.27 mmol, 1.04 eq.) were added. The reaction mixture was

stirred at rt for 1.5 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction mixture was cooled to –5 °C and $NaBH_4$ (218 mg, 5.75 mmol, 5.0 eq.) was added portionwise. The reaction mixture was stirred at –5 °C for 1 h. Anhydrous MeOH (1.50 mL) was added and the reaction mixture warmed to 0 °C and stirred for 1 h. After this time TLC analysis indicated complete consumption of the intermediate (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The reaction was quenched with H_2O (15 mL) and extracted with EtOAc (3 × 15 mL). The organic fractions were combined and washed with H_2O (4 × 20 mL). The organic fraction was extracted with an aqueous solution of HCl (1 M, 4 × 20 mL). The aqueous fractions were combined, cooled to 0 °C and neutralised with 30% NH_4OH. The aqueous fraction was extracted with $CHCl_3$ (3 × 20 mL). The organic fractions were combined and washed with H_2O (3 × 15 mL). The organic fraction was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the title compound as a colourless oil (172 mg, 0.651 mmol, 57%): R_f 0.24 (1:9 MeOH/CH_2Cl_2 + drop Et_3N); $\bar{\nu}_{max}$/cm^{-1} 3334 (br), 2975 (br), 2930 (br), 2361 (br), 2342 (br), 2161 (br), 2027 (br), 1977 (br), 1693 (C=O, s), 1512 (m), 1454 (m), 1391 (w), 1365 (m), 1273 (m), 1270 (s), 1251 (m), 1170 (s), 1043 (w), 1028 (w); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.13 (5H, m, C^6-H, C^7-H, C^8-H, C^9-H, C^{10}-H), 5.45 (1H, br s, N-H), 3.70 (2H, s, C^4-H_2), 3.19–3.09 (2H, m, C^1-H_2), 2.63 (2H, t, *J* 6.5, C^3-H_2), 1.66 (1H, br s, N-H), 1.60 (2H, tt, *J* 6.5, 6.5, C^2-H_2), 1.39 (9H, s, C^{13}-H_3, C^{14}-H_3, C^{15}-H_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.2 (C^{11}), 140.2 (C^5), 128.5 ($C^{6/7/9/10}$), 128.3 ($C^{6/7/9/10}$), 127.1 (C^8), 79.0 (C^{12}), 54.1 (C^4), 47.5 (C^3), 39.5 (C^1), 29.7 (C^2), 28.5 (C^{13}, C^{14}, C^{15}); LRMS *m/z* (ESI$^+$) 646.2 ([M+Na]$^+$, 10%). The spectroscopic data are in good agreement with literature values.[274]

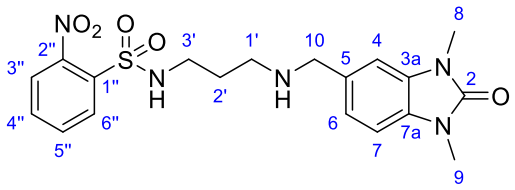
***N*-(3-Aminopropyl)-2'-nitrobenzenesulfonamide (330)**[275,276]



Propane-1,3-diamine (3.00 mL, 35.6 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (20.0 mL). The reaction mixture was cooled to −5 °C and 2-nitrobenzenesulfonyl chloride (2.63 g, 11.9 mmol,

1.0 eq.) in CH_2Cl_2 (75.0 mL) was added over 1 h *via* a dropping funnel. The reaction mixture was warmed to rt and stirred for 1 h. After this time TLC analysis indicated complete consumption of starting material (3:2 EtOAc/petroleum ether). The reaction mixture was filtered through a pad of Celite® and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−10% EtOH/EtOAc gradient then 1:2:8 NH_4OH/EtOH/EtOAc isocratic) and further purified using silica flash column chromatography (elution with 1:2:12 NH_4OH/EtOH/EtOAc isocratic). The product was further purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) to yield the title compound as yellow oil (1.56 g, 6.02 mmol, 51%): R_f 0.18 (1:2:8 NH_4OH/EtOH/EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02–7.93 (2H, m, $C^{3'/4'/5'/6'}$-H_2), 7.89–7.80 (2H, m, $C^{3'/4'/5'/6'}$-H_2), 2.95 (2H, t, *J* 6.7, C^1-H_2), 2.54 (2H, t, *J* 6.7, C^3-H_2), 1.47 (2H, tt, *J* 6.7, 6.7, C^2-H_2); LRMS *m/z* (ESI^+) 260.0 ($[M+H]^+$, 100%). The spectroscopic data are in good agreement with literature values.[275,276]

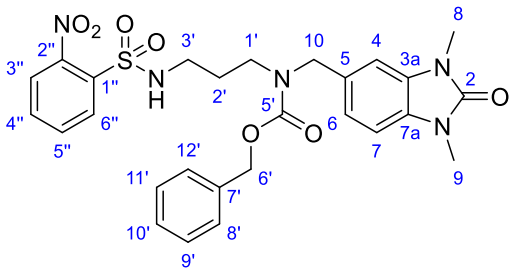
_N_-(3'-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}prop-1'-yl)-2''-nitrobenzene-1-sulfonamide (331)



1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (**34**) (200 mg, 1.05 mmol, 1.0 eq.) was dissolved in anhydrous 1,2-dichloroethane (5.00 mL) and 3 Å molecular sieves were added. *N*-(3-Aminopropyl)-2-nitrobenzenesulfonamide (**330**) (409 mg, 1.58 mmol, 1.5 eq.) and acetic acid (90.4 μL, 1.58 mmol, 1.5 eq.) were added. The reaction mixture was stirred at rt for 5.5 h. After this time ^{1}H NMR analysis of the reaction mixture indicated partial imine formation. $NaBH(OAc)_3$ (1.11 g, 5.25 mmol, 5.0 eq.) was added and the reaction mixture was stirred at rt for 17 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL) and filtered through a pad of Celite®. The filtrate was extracted with CH_2Cl_2 (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in*

vacuo. The product was purified using silica flash column chromatography (elution with EtOAc isocratic then 1:2:12 NH_4OH/EtOH/EtOAc isocratic) and further purified using silica flash column chromatography (elution with 0−15% EtOH/CH_2Cl_2 gradient) to yield the title compound as a yellow solid (219 mg, 0.505 mmol, 48%): R_f 0.52 (1:2:8 NH_4OH/EtOH/EtOAc); m.p. 173−175 °C (from CH_2Cl_2); $\tilde{\nu}_{max}$/cm^{-1} 2554 (br), 2161 (s), 2027 (s), 1978 (s), 1687 (m), 1537 (m), 1508 (m), 1463 (m), 1399 (m), 1323 (S=O, s), 1266 (s), 1160 (m), 1092 (s), 1009 (w), 967 (m); ^{1}H NMR (500 MHz, CD_3OD) δ 8.09–8.01 (1H, m, C$^{6''}$-H), 7.87–7.71 (3H, m, C$^{3''}$-H, C$^{4''}$-H, C$^{5''}$-H), 7.19–7.15 (1H, m, C^4-H), 7.13–7.06 (2H, m, C^6-H, C^7-H), 3.84 (2H, s, C^{10}-H_2), 3.42 (3H, s, C$^{8/9}$-H_3), 3.41 (3H, s, C$^{8/9}$-H_3), 3.11 (2H, t, *J* 6.8, C$^{3'}$-H_2), 2.72 (2H, t, *J* 6.8, C$^{1'}$-H_2), 1.76 (2H, tt, *J* 6.8, 6.8, C$^{2'}$-H_2); ^{13}C NMR (126 MHz, CD_3OD) δ 156.4 (C^2), 149.6 (C$^{2''}$), 134.9 (C$^{4''}$), 134.7 (C$^{1''}$), 133.5 (C$^{5''}$), 133.1 (C^5), 131.5 (C$^{6''}$), 131.4 (C^{3a}), 130.6 (C^{7a}), 125.9 (C$^{3''}$), 123.5 (C^6), 109.4 (C^4), 108.8 (C^7), 54.3 (C^{10}), 46.9 (C$^{1'}$), 42.5 (C$^{3'}$), 29.7 (C$^{2'}$), 27.49 (C$^{8/9}$), 27.47 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 434.14927, $C_{19}H_{24}N_5O_5S$ requires $[M+H]^+$ 434.14927]; LRMS *m/z* (ESI$^-$) 432.1 ($[M–H]^-$, 100%); HPLC retention time 5.5 min, 94.2%.

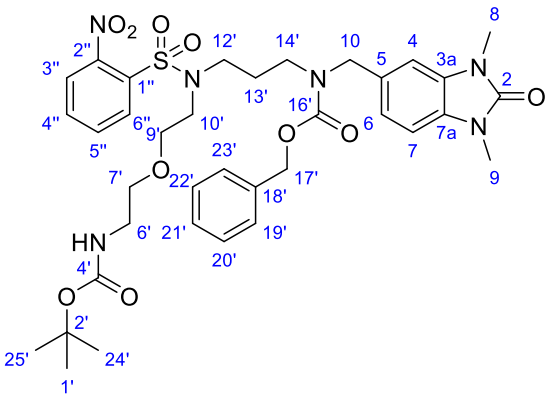
Benzyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{3'-[(2''-nitrobenzene-1''-sulfonyl)amino]prop-1'-yl}carbamate (327)



N-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)-2-nitro benzene-1-sulfonamide (**331**) (239 mg, 0.551 mmol, 1.0 eq.) was suspended in CH_2Cl_2 (3.00 mL). The reaction mixture was cooled to 0 °C and benzyl chloroformate (101 μL, 0.717 mmol, 1.3 eq.) and Et_3N (99.9 μL, 0.717 mmol, 1.3 eq.) were added dropwise. The reaction mixture was warmed to rt and stirred for 4 h. After this time TLC analysis indicated partial consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction mixture was cooled to 0 °C and additional benzyl

chloroformate (70.7 μL, 0.502 mmol, 0.7 eq.) and Et_3N (69.9 μL, 0.502 mmol, 0.7 eq.) were added dropwise. The reaction mixture was warmed to rt and stirred for 23 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2). The reaction was quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (20 mL). The organic fraction was washed with a saturated aqueous solution of $NaHCO_3$ (20 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−5% MeOH/CH_2Cl_2 gradient) to yield the title compound as a yellow solid (242 mg, 0.426 mmol, 77%): R_f 0.57 (3:47 MeOH/CH_2Cl_2); m.p. 56−58 °C (from CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 2941 (br), 2160 (br), 1688 (C=O, s), 1540 (m), 1509 (m), 1442 (m), 1423 (w), 1396 (m), 1365 (m), 1339 (w), 1299 (m), 1267 (m), 1217 (m), 1163 (m), 1124 (w), 1058 (w), 1027 (m), 1006 (w), 957 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 8.03–7.96 (1H, m, C$^{6''}$-H), 7.90–7.85 (1H, m, C$^{3''}$-H), 7.85–7.76 (2H, m, C$^{4''}$-H, C$^{5''}$-H), 7.66 (1H, br s, N-H), 7.43–7.27 (5H, m, C$^{8'}$-H, C$^{9'}$-H, C$^{10'}$-H, C$^{11'}$-H, C$^{12'}$-H), 7.00 (1H, d, *J* 7.8, C^7-H), 6.98–6.89 (2H, m, C^4-H, C^6-H), 5.14 (2H, s, C$^{6'}$-H_2), 4.47 (2H, s, C^{10}-H_2), 3.31 (3H, s, C$^{8/9}$-H_3), 3.29–3.24 (5H, m, C$^{8/9}$-H_3, C$^{1'}$-H_2), 2.95 (2H, t, *J* 7.1, C$^{3'}$-H_2), 1.72 (2H, tt, *J* 7.1, 7.1, C$^{2'}$-H_2); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 155.2 (C$^{5'}$), 153.7 (C^2), 147.4 (C$^{2''}$), 136.6 (C$^{7'}$), 133.3 (C$^{4''}$), 132.7 (C$^{1''}$), 131.8 (C$^{5''}$), 130.5 (C$^{6''}$), 129.6 (C^5), 129.1 (C^{3a}), 128.8 (C^{7a}), 127.8 (C$^{9'}$, C$^{11'}$), 127.2 (C$^{10'}$), 127.0 (C$^{8'}$, C$^{12'}$), 123.8 (C$^{3''}$), 119.8 (C^6), 106.7 (C^7), 106.2 (C^4), 66.0 (C$^{6'}$), 49.9 (C^{10}), 43.8 (C$^{1'}$), 40.3 (C$^{3'}$), 27.7 (C$^{2'}$), 26.4 (C$^{8/9}$), 26.3 (C$^{8/9}$); HRMS *m/z* (ESI$^+$) [Found: 568.18614, $C_{27}H_{30}N_5O_7S$ requires $[M+H]^+$ 568.18605]; LRMS *m/z* (ESI$^+$) 590.2 ($[M+Na]^+$, 100%); HPLC retention time 6.7 min, 95.4%.

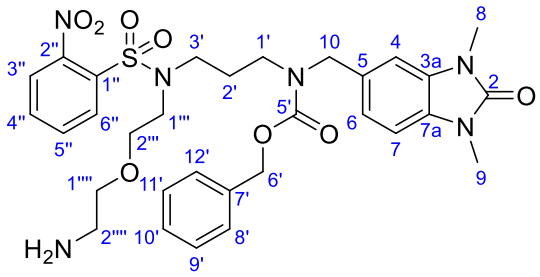
Benzyl [2',2'-dimethyl-11'-(2''-nitrobenzene-1''-sulfonyl)-4'-oxo-3',8'-dioxa-5',11'-diazatetradecan-14'-yl][(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (332)



Benzyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{3-[(2-nitrobenzene-1-sulfonyl)amino]propyl}carbamate (**327**) (254 mg, 0.448 mmol, 1.0 eq.) was dissolved in anhydrous tetrahydrofuran (4.00 mL). The reaction mixture was cooled to 0 °C. *tert*-Butyl (2-(2-hydroxyethoxy)ethyl)carbamate (**238**) (230 mg, 1.12 mmol, 2.5 eq.), diisopropyl azodicarboxylate (221 μL, 1.12 mmol, 2.5 eq.) and triphenylphosphine (294 mg, 1.12 mmol, 2.5 eq.) were added. The reaction mixture was warmed to rt and stirred for 23 h. After this time TLC analysis indicated complete consumption of starting material (1:24 MeOH/CH_2Cl_2). The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL) and extracted with EtOAc (20 mL). The organic fraction was washed with brine (20 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0−5% EtOH/$CHCl_3$ gradient) to yield the title compound as a colourless oil (234 mg, 0.310 mmol, 69%): R_f 0.48 (1:24 MeOH/CH_2Cl_2); $\bar{\nu}_{max}$/cm^{-1} 2161 (br), 1693 (C=O, s), 1543 (m), 1509 (m), 1443 (w), 1366 (w), 1267 (m), 1227 (m), 1162 (m), 1123 (m), 992 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 7.97–7.91 (1H, m, C$^{6''}$-H), 7.89–7.74 (3H, m, C$^{3''}$-H, C$^{4''}$-H, C$^{5''}$-H), 7.41–7.27 (5H, m, C$^{19'}$-H, C$^{20'}$-H, C$^{21'}$-H, C$^{22'}$-H, C$^{23'}$-H), 7.02 (1H, d, *J* 7.9, C^{7}-H), 6.97–6.91 (2H, m, C^{4}-H, C^{6}-H), 6.24 (1H, br s, N-H), 5.15 (2H, s, C$^{17'}$-H_2), 4.47 (2H, s, C^{10}-H_2), 3.45 (2H, t, *J* 5.7, C$^{9'}$-H_2), 3.40–3.30 (7H, m, C$^{8/9}$-H_3, C$^{7'}$-H_2, C$^{12'}$-H_2), 3.30–3.23 (5H, m, C$^{8/9}$-H_3, C$^{10'}$-H_2), 3.21 (2H, t, *J* 7.2, C$^{14'}$-H_2), 3.08–2.99 (2H, m, C$^{6'}$-H_2), 1.76 (2H, tt, *J* 7.4, 7.2, C$^{13'}$-H_2),

1.38 (9H, s, $C^{1'}$-H_3, $C^{24'}$-H_3, $C^{25'}$-H_3); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 155.2 ($C^{16'}$), 155.1 ($C^{4'}$), 153.7 (C^{2}), 147.4 ($C^{2''}$), 136.5 ($C^{18'}$), 133.7 ($C^{4''/5''}$), 131.8 ($C^{1''}$), 131.7 ($C^{4''/5''}$), 130.5 (C^{5}), 129.6 ($C^{3a/7a}$), 129.3 ($C^{6''}$), 128.8 ($C^{3a/7a}$), 127.8 ($C^{19'/20'/22'/23'}$), 127.3 ($C^{21'}$), 127.1 ($C^{19'/20'/22'/23'}$), 123.7 ($C^{3''}$), 119.9 (C^{6}), 106.8 (C^{7}), 106.3 (C^{4}), 77.3 ($C^{2'}$), 68.9 ($C^{7'}$), 67.9 ($C^{9'}$), 66.0 ($C^{17'}$), 49.9 (C^{10}), 46.4 ($C^{12'}$), 45.6 ($C^{10'}$), 43.7 ($C^{14'}$), 40.4 ($C^{6'}$), 27.8 ($C^{1'}$, $C^{24'}$, $C^{25'}$), 26.4 ($C^{8/9}$), 26.29 ($C^{8/9}$), 26.26 ($C^{13'}$); HRMS *m/z* (ESI$^+$) [Found: 755.30700, $C_{36}H_{47}N_6O_{10}S$ requires $[M+H]^+$ 755.30689]; LRMS *m/z* (ESI$^+$) 777.2 ($[M+Na]^+$, 25%); HPLC retention time 9.3 min, 98.1%.

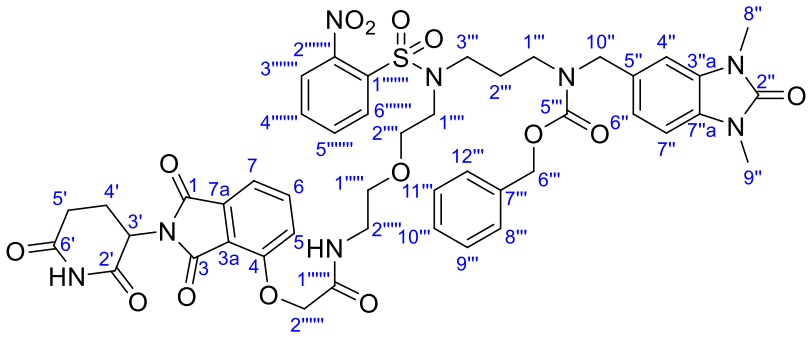
Benzyl (3'-{[2'''-(2''''-aminoethoxy)ethyl](2''-nitrobenzene-1''-sulfonyl)amino}prop-1'-yl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (333)



Benzyl [2,2-dimethyl-11-(2-nitrobenzene-1-sulfonyl)-4-oxo-3,8-dioxa-5,11-diazatetradecan-14-yl][(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (**332**) (171 mg, 0.227 mmol, 1.0 eq.) was dissolved in hydrochloric acid (4 M, dioxane) (1.50 mL). The reaction mixture was stirred at rt for 2 h. After this time TLC analysis indicated complete consumption of starting material (1:24 MeOH/CH_2Cl_2). The reaction mixture was concentrated *in vacuo*. The product was purified using an ISOLUTE® SCX-2 column (elution with NH_3 (2 M, MeOH)) to yield the title compound as a colourless oil (128 mg, 0.195 mmol, 86%): R_f 0.43 (1:2:6 NH_4OH/EtOH/EtOAc); $\bar{\nu}_{max}$/cm^{-1} 2942 (br), 2160 (br), 2027 (br), 1687 (C=O, s), 1542 (m), 1509 (w), 1466 (w), 1342 (w), 1268 (w), 1227 (w), 1160 (w), 1141 (w), 1122 (w), 1059 (w), 1024 (w), 912 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 7.98–7.93 (1H, m, $C^{6''}$-H), 7.90–7.77 (3H, m, $C^{3''}$-H, $C^{4''}$-H, $C^{5''}$-H), 7.38–7.27 (5H, m, $C^{8'}$-H, $C^{9'}$-H, $C^{10'}$-H, $C^{11'}$-H, $C^{12'}$-H), 7.03 (1H, d, *J* 8.2, C^{7}-H), 6.97–6.91 (2H, m, C^{4}-H, C^{6}-H), 5.14 (2H, s, $C^{6'}$-H_2), 4.47 (2H, s, C^{10}-H_2), 3.57 (2H, t,

J 5.6, $C^{1''''}$-H_2), 3.52 (2H, t, *J* 5.9, $C^{2'''}$-H_2), 3.42 (2H, t, *J* 5.9, $C^{1'''}$-H_2), 3.34–3.24 (8H, m, C^{8}-H_3, C^{9}-H_3, $C^{3'}$-H_2), 3.21 (2H, t, *J* 7.5, $C^{1'}$-H_2), 2.88 (2H, t, *J* 5.6, $C^{2''''}$-H_2), 1.76 (2H, tt, *J* 7.5, 7.5, $C^{2'}$-H_2); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 155.1 ($C^{5'}$), 153.7 (C^{2}), 147.3 ($C^{2''}$), 136.5 ($C^{7'}$), 133.8 ($C^{4''/5''}$), 131.8 ($C^{4''/5''}$), 131.6 ($C^{1''}$), 130.5 (C^{5}), 129.6 ($C^{3a/7a}$), 129.3 ($C^{6''}$), 128.8 ($C^{3a/7a}$), 127.8 ($C^{9'}$, $C^{11'}$), 127.2 ($C^{10'}$), 127.0 ($C^{8'}$, $C^{12'}$), 123.7 ($C^{3''}$), 119.8 (C^{6}), 106.8 (C^{7}), 106.3 (C^{4}), 68.0 ($C^{2'''}$), 67.0 ($C^{1''''}$), 66.0 ($C^{6'}$), 49.9 (C^{10}), 46.3 ($C^{1'''}$), 45.7 ($C^{3'}$), 43.7 ($C^{1'}$), 38.2 ($C^{2''''}$), 26.4 ($C^{8/9/2'}$), 26.32 ($C^{8/9/2'}$), 26.31 ($C^{8/9/2'}$); HRMS *m/z* (ESI^+) [Found: 655.25390, $C_{31}H_{39}N_6O_8S$ requires $[M+H]^+$ 655.25446]; LRMS *m/z* (ESI^+) 677.2 ($[M+Na]^+$, 100%); HPLC retention time 7.6 min, 98.0%.

Benzyl [(1'',3''-dimethyl-2''-oxo-2'',3''-dihydro-1*H*-benzimidazol-5''-yl)methyl]{3'''-[{2''''-[2'''''-(2''''''-{[2-(2',6'-dioxopiperidin-3'-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]ethyl}(2'''''''-nitrobenzene-1'''''''-sulfonyl)amino]prop-1'''-yl}carbamate (334)



Benzyl (3-{[2-(2-aminoethoxy)ethyl](2-nitrobenzene-1-sulfonyl)amino}propyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (**333**) (63 mg, 0.096 mmol, 1.0 eq.) was dissolved in anhydrous *N*,*N*-dimethylformamide (0.60 mL). Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (**229**) (51 mg, 0.15 mmol, 1.2 eq.), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro phosphate (40 mg, 0.11 mmol, 1.1 eq.) and *N*,*N*-diisopropylethylamine (24 µL, 0.19 mmol, 2.0 eq.) were added. The reaction mixture was stirred at rt for 96 h. After this time TLC analysis indicated complete consumption of starting material (1:9 MeOH/CH_2Cl_2 + drop Et_3N). The

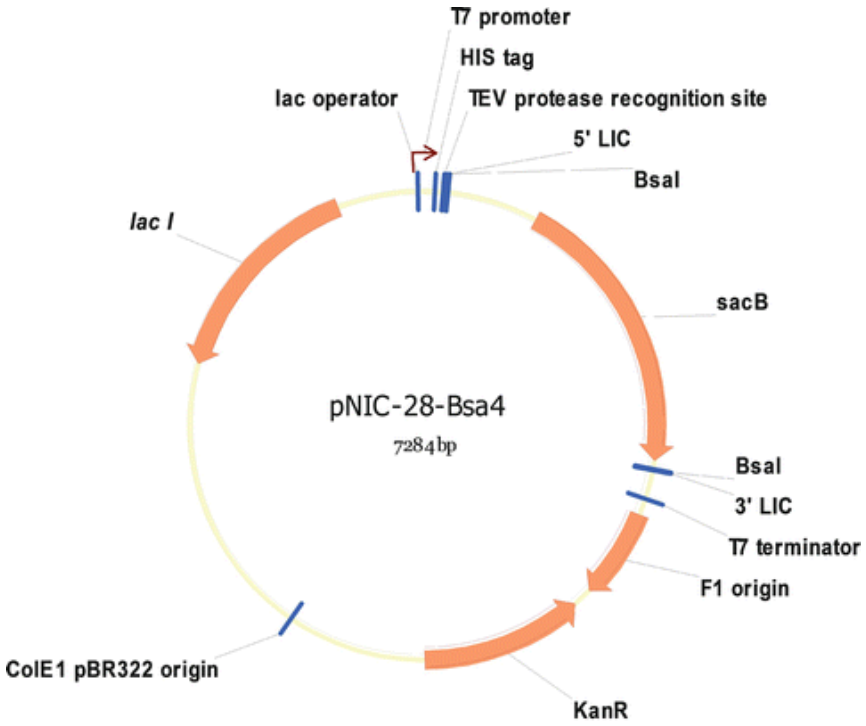
reaction was quenched with an aqueous solution of LiCl (0.5 M, 20 mL) and extracted with EtOAc (3 × 20 mL). The organic fractions were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The product was purified using silica flash column chromatography (elution with 0–6% MeOH/CH_2Cl_2 gradient) to yield the title compound as a yellow oil (47 mg, 0.049 mmol, 50%): R_f 0.48 (1:9 MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ = +5.40 (*c* 1.0, DMSO); $\tilde{\nu}_{max}$/cm^{-1} 2923 (br), 2161 (br), 2032 (br), 1978 (br), 1772 (w), 1703 (C=O, s), 1614 (w), 1542 (m), 1509 (w), 1483 (w), 1441 (w), 1394 (w), 1351 (w), 1299 (w), 1262 (w), 1229 (w), 1197 (w), 1163 (w), 1122 (w), 1079 (w), 1057 (w), 1016 (w), 940 (w), 913 (w); ^{1}H NMR (500 MHz, DMSO-d_6, 363 K) δ 10.78 (1H, br s, N-H), 7.96–7.90 (1H, m, C^6-H), 7.88–7.73 (4H, m, C$^{3'''''''}$-H, C$^{4'''''''}$-H, C$^{5'''''''}$-H, C$^{6'''''''}$-H), 7.68 (1H, br s, N-H), 7.47 (1H, d, *J* 7.3, C^5-H), 7.42 (1H, d, *J* 8.5, C^7-H), 7.37–7.26 (5H, m, C$^{8'''}$-H, C$^{9'''}$-H, C$^{10'''}$-H, C$^{11'''}$-H, C$^{12'''}$-H), 7.00 (1H, d, *J* 7.8, C$^{7''}$-H), 6.94–6.89 (2H, m, C$^{4''}$-H, C$^{6''}$-H), 5.13 (2H, s, C$^{6'''}$-H$_2$), 5.10–5.00 (1H, m, C$^{3'}$-H), 4.74 (2H, s, C$^{2'''''}$-H$_2$), 4.45 (2H, s, C$^{10''}$-H$_2$), 3.48 (2H, t, *J* 5.7, C$^{1'''''}$-H$_2$), 3.48–3.36 (4H, m, C$^{1''''}$-H$_2$, C$^{2''''}$-H$_2$), 3.36–3.23 (10H, m, C$^{8''}$-H$_3$, C$^{9''}$-H$_3$, C$^{3'''}$-H$_2$, C$^{2'''''}$-H$_2$), 3.19 (2H, t, *J* 7.3, C$^{1'''}$-H$_2$), 2.92–2.81 (1H, m, C$^{5'}$-H$_a$), 2.67–2.51 (2H, m, C$^{4'}$-H$_a$, C$^{5'}$-H$_b$), 2.11–2.02 (1H, m, C$^{4'}$-H$_b$), 1.75 (2H, tt, *J* 7.3, 7.3, C$^{2'''}$-H$_2$); ^{13}C NMR (126 MHz, DMSO-d_6, 363 K) δ 171.7 (C$^{2'}$), 168.9 (C$^{6'}$), 166.4 (C$^{1''''''}$), 166.1 (C^1), 165.0 (C^3), 155.1 (C$^{5'''}$), 154.6 (C^4), 153.7 (C$^{2''}$), 147.3 (C$^{2'''''''}$), 136.5 (C$^{4'''''''}$), 136.3 (C$^{7'''}$), 133.7 (C$^{5'''''''/6'''''''}$), 132.8 (C$^{1'''''''}$), 131.7 (C^{7a}), 131.7 (C$^{5'''''''/6'''''''}$), 130.4 (C$^{5''}$), 129.6 (C$^{3''a/7''a}$), 129.3 (C^6), 128.8 (C$^{3''a/7''a}$), 127.8 (C$^{9'''}$, C$^{11'''}$), 127.2 (C$^{10'''}$), 127.0 (C$^{8'''}$, C$^{12'''}$), 123.7 (C$^{3'''''''}$), 120.5 (C^7), 119.8 (C$^{6''}$), 116.9 (C^{3a}), 115.8 (C^5), 106.8 (C$^{7''}$), 106.2 (C$^{4''}$), 68.4 (C$^{2''''}$), 68.0 (C$^{1'''''}$), 67.9 (C$^{2''''''}$), 66.0 (C$^{6'''}$), 49.9 (C$^{10''}$), 48.7 (C$^{3'}$), 46.4 (C$^{1''''}$), 45.6 (C$^{2'''''}$), 43.7 (C$^{1'''}$), 38.0 (C$^{3'''}$), 30.5 (C$^{5'}$), 26.4 (C$^{8''/9''}$), 26.29 (C$^{8''/9''}$), 26.25 (C$^{2'''}$), 21.6 (C$^{4'}$); HRMS *m/z* (ESI$^+$) [Found: 969.3091, $C_{48}H_{49}N_8O_{14}S$ requires $[M+H]^+$ 969.30835]; LRMS *m/z* (ESI$^+$) 992.4 ($[M+Na]^+$, 30%); HPLC retention time 8.4 min, 97.1%.

6.3 General Biology Methods

6.3.1 Protein Production

6.3.1.1 Plasmids

The pNIC28-Bsa4 TRIM24, pNIC28-Bsa4 TRIM33α and pNIC28-Bsa4 TRIM33β plasmid constructs were provided by Dr Angelina Sekirnik.



6.3.1.2 pH Measurement

Buffer pH was determined using a VWR pH 100 pH meter. Calibration was carried out between pH 7.0 (phosphate buffer solution) and pH 10.0 (borate buffer solution). Electrodes were stored at pH 4.0 (phthalate buffer solution).

6.3.1.3 Protein Concentration Measurement

Protein concentration was calculated using the Beer-Lambert law (TRIM24, ε = 25160 $M^{-1}cm^{-1}$; TRIM33α, ε = 26650 $M^{-1}cm^{-1}$; TRIM33β, ε = 25160 $M^{-1}cm^{-1}$). The absorbance was measured at 280 nm using a Thermo Scientific Nanodrop Lite. Protein molecular weight and molar extinction coefficient were calculated using ProtParam on the ExPASy Bioinformatics Resource Portal.

6.3.1.4 Bacterial Transformation

Plasmid DNA was added to *Escherichia coli* BL21 (DE3) competent cells thawed on ice. The mixture was flicked 10 times then incubated on ice for 20 min. The cells were heat shocked by heating them to 42 °C for 30 s, then returned to ice for 5 min. SOC outgrowth media (500 μL) was added and the cells incubated at 37 °C for 1 h. Under aseptic conditions, the bacteria (10 μL and 40 μL) were streaked on solid 2× TY medium (Table 6.1) supplemented with kanamycin (50 μg/mL). The plate cultures were inverted and grown overnight at 37 °C, then stored at 4 °C.

Table 6.1: Growth media composition (1 L).

Media	Reagent	Amount required per litre (g)
2× TY	Tryptone	16.0
	Yeast extract	10.0
	NaCl	5.0
Agar bacteriological		15.0

6.3.1.5 Protein Expression

All growth media (Table 6.1) were autoclaved (121 °C, 45 min) (*Thermo Life Sciences* MAT 490 LEI) before use.

For the starter culture (500 mL baffled flask), 2× TY media (100 mL) containing kanamycin (50 μg/mL) was inoculated with a single colony and incubated at 37 °C overnight.

Large-scale recombinant protein expression was performed in 2 L PYREX® narrow-mouth baffled Tunair™ flasks. To 2× TY media (1000 mL) containing kanamycin (50 μg/mL) was added the bacterial starter culture (10 mL). Cultures were grown at 37 °C (180 rpm) until an OD_{600} of 1.0 was reached. The absorbance was measured at 600 nm using a NovaSpec™ II spectrophotometer with 2× dilution in 2× TY (2× TY growth media reference). Cultures were then equilibrated to 16 °C, induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) (0.1 mM) and supplemented with $ZnCl_2$ (0.2 mM). The cultures were then incubated for 21 h. Following incubation, the cultures were centrifuged at 11325 ×g, 4 °C for 5 min (Rotor: Beckman F10BCI-6×500y, Avanti™ J-25 centrifuge). The resulting bacterial pellet was then frozen at –80 °C.

6.3.1.6 Protein Purification

Buffers (Table 6.2) were stored at 4 °C and filtered through a 0.2 µm filter paper (Sartorius UK) under vacuum before use.

Table 6.2: Protein purification buffer composition.

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	–

Cell pellets were resuspended in extraction buffer (1 Sigma*FAST*™ protease inhibitor cocktail tablet EDTA free (Sigma-Aldrich) dissolved in 100 mL binding buffer), 100 mL per 20 g cell mass. Benzonase® nuclease (Novagen®), 10 µL per 7 g cell mass was added. The cells were lysed by sonication (50% amplitude) (Fisherbrand™ Q500 Sonicator). 30 cycles of 5 s bursts, interrupted by 5 s pauses, were applied. Polyethyleneimine (0.15% *v/v*) was added from a 5% (*v/v*) stock and the DNA precipitated by incubation on ice (15 min). The lysate was centrifuged and the supernatant decanted and filtered through a 0.45 µm syringe filter (Merck Millipore). 10 mM β-Mercaptoethanol (β-ME) (Bio-Rad Laboratories) and 20 mM imidazole were added.

The protein was purified at 4 °C by immobilised metal affinity chromatography (IMAC) and size-exclusion chromatography (SEC), both operated through a computerised ÄKTA fast protein liquid chromatography (ÄKTAFPLC) system (GE Healthcare). The IMAC was performed with a HisTrap™ HP 5 mL column (GE Healthcare). The column was rinsed with 50 mL (10 CV) of Milli-Q water, charged with 10 mL (2 CV) of 100 mM $NiSO_4$ and equilibrated with 50 mL (10 CV) of binding buffer (supplemented with 10 mM β-ME). The cell lysate was then loaded (1 mL min^{-1}). The column was then washed with a step gradient of 50 mL (10 CV) 9% elution buffer in binding buffer (45 mM imidazole) and 50 mL (10 CV) 12% elution buffer in binding buffer (60 mM imidazole). The protein was then batch-eluted with 20 mL (4 CV) 60% elution

buffer in binding buffer (300 mM imidazole) followed by 10 mL (2 CV) 100% elution buffer (500 mM imidazole). Eluted protein was collected in 2 mL fractions. The column was then washed with 50 mL Milli-Q water and stripped with 50 mL (10 CV) of strip buffer to remove the bound Ni^{2+} ions for storage at 4 °C.

Protein containing fractions 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) to a volume of 4–6 mL.

The protein was further purified using SEC. The gel filtration column XK 16/70 (GE Healthcare) packed with 120 mL Superdex 75 resin (GE Healthcare) was washed with 150 mL Milli-Q water and equilibrated with 150 mL degassed gel filtration buffer. The concentrated fractions were then loaded *via* a 2 mL injection loop onto the column. Protein was eluted with 150 mL degassed gel filtration buffer (1 mL min^{-1}) and collected in 5 mL fractions. Fraction collection began at 20 mL and finished at 120 mL. Pure fractions (identified on the SEC trace) were then spin concentrated at 3270 ×g, 4 °C until an appropriate concentration was reached (Spin-X UF, 6 mL Concentrators, 10K Cutoff, NS, Ind. Wrap). The protein was aliquoted, frozen, and stored at –80 °C.

Protein purity was evaluated using SDS-PAGE 16% with Coomassie Blue staining. Protein mass spectrometry was carried out on a Waters LCT Premier mass spectrometer (0.02 mg/mL and 0.1 mg/mL).

6.3.1.7 Sodium Dodecyl Sulfate-Polyacrylamide (SDS) Gel Electrophoresis

The gel composition is given in Table 6.3. 70 mm × 100 mm glass plates, with 0.75 mm spacers, were used to make the gels. TEMED and 10% ammonium persulfate were added just before casting gels. The resolving gel was cast with the addition of a layer of methanol which was removed following polymerisation and before casting the stacking gel. The protein samples were prepared by mixing with Laemmli sample loading buffer (4×, with addition of 10% β-ME), and incubating at 100 °C (10 min). Gels were loaded with colour protein standard, broad range (NEB) molecular weight ladder and run on a mini-PROTEAN Tetra Electrophoresis System (Bio-Rad)

at a constant potential of 100 V. Gels were then stained with InstantBlue™ Coomasie® stain (15 min) and subsequently rinsed with Milli-Q water. The gels were then destained multiple times with destaining solution (40% methanol and 10% acetic acid in Milli-Q water).

Table 6.3: Sodium Dodecyl Sulfate-Polyacrylamide (SDS) gel composition (2 gels).

Reagents	Resolving Gel (16%)	Stacking Gel (4%)
Milli-Q water	2.10 mL	2.90 mL
1.5 M Tris-HCl, pH 8.8	2.50 mL	-
0.5 M Tris-HCl, pH 6.8	-	1.30 mL
30% Acrylamide/bis	5.30 mL	0.70 mL
10% Sodium dodecyl sulfate (SDS)	100 µL	50.0 µL
TEMED	8.00 µL	5.00 µL
10% APS	100 µL	50.0 µL

6.3.2 AlphaScreen™

6.3.2.1 Peptides

Peptides (Table 6.4) were supplied at >95% purity (Cambridge Biochemicals or GenScript) and stored as either 1 mM or 0.1 mM stocks (Milli-Q water) at –80 °C.

Table 6.4: Peptide amino acid sequences.

Peptide	Amino acid sequence
$H3_{1–27}K9Me_3$	H_2N-ARTKQTARK(Me_3)STGGKAPRKQLATKAARKY-CO_2H
$H3_{1–27}K18Ac$	H_2N-ARTKQTARKSTGGKAPRK(Ac)QLATKAARKY-CO_2H
$H3_{1–27}K9Me_3K18Ac$	H_2N-ARTKQTARK(Me_3)STGGKAPRK(Ac)QLATKAARKY-CO_2H
$H4KAc_4$	H_2N-YSGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RHRK-$CONH_2$

6.3.2.2 Typical Procedure for AlphaScreen™

The assay buffer (Table 6.5) was stored as a 5× solution (without BSA) at 4 °C. BSA was added and the buffer filtered through a 0.22 µM filter prior to use. Incubation refers to sealing the plates, shaking at 600 rpm (10 s) on a plate oscillator and storing at rt in the dark for the specified amount of time. The compounds, proteins (synthesised in collaboration with Dr Angelina Sekirnik, Dr Larissa See and Dr Matthias Schiedel), and peptides were dispensed into a ProxiPlate-384 Plus

(Pekin Elmer) using a Thermo-Fisher electronic multi-channel pipette. Plates were read using an Envision 2104 Multilabel Plate Reader (Perkin Elmer) using the inbuilt AlphaScreen™ 384 ProxiPlate function (excitation 680 nm, 0.18 s; emission 570 nm, 0.37 s). Ni^{2+} chelate acceptor beads and streptavidin-coated donor beads were prepared as a mixture in a 1:300 dilution (0.007 mg/mL final assay concentration (FAC)).

Table 6.5: HEPES buffer composition for AlphaScreen™ (50 mL volume).

Assay Buffer (pH 7.6)		
	Composition	**Mass (mg)**
HEPES	25 mM	298
NaCl	100 mM	292
CHAPS	0.05% (*w/v*)	25
BSA	0.1% (*w/v*)	50

Table 6.6: FAC of protein-peptide combinations.

Protein	**Peptide**	**Peptide FAC (nM)**	**Protein FAC (nM)**
TRIM24	$H3K9Me_3$ (H3PHD)	4	100
	H3K18Ac (H3BRD)	8	25
	$H3K9Me_3K18Ac$ (H3DUAL)	4	25
TRIM33α	$H3K9Me_3$ (H3PHD)	15	500
	H3K18Ac (H3BRD)	15	500
	$H3K9Me_3K18Ac$ (H3DUAL)	8	250
TRIM33β	$H3K9Me_3$ (H3PHD)	8	500
	H3K18Ac (H3BRD)	15	125
	$H3K9Me_3K18Ac$ (H3DUAL)	8	125
BRD4	$H4KAc_4$	10	4

6.3.2.3 Signal Response Experiment

Proteins (7 µL) were serially diluted 1:2 from 100 nM FAC for TRIM24 and 500 nM FAC for TRIM33α and TRIM33β and dispensed into wells. Peptides (5 µL) were serially diluted 1:2 from 250 nM FAC, dispensed into wells and plates incubated for 30 min. Assay beads (8 µL) were added and plates incubated for 1 h.

6.3.2.4 Single Concentration Screening Experiments

Compounds (stored as 50 mM DMSO stock solutions) (5 µL) were screened in triplicate at 30 µM and 150 µM FAC. Protein-peptide mixes (7 µL) for each combination were prepared (Table 6.6), dispensed into wells and the plates incubated for 30 min. Assay beads (8 µL) were added and the plates incubated for 1 h. The emission intensity as a percentage of the average DMSO control (100%) was calculated for each protein-peptide combination.

6.3.2.5 Dose Response Experiments

Compounds (stored as 50 mM DMSO stock solutions) (5 µL) were serially diluted 1:2 from 500 µM and dispensed into wells. The protein-peptide mixes (7 µL) for each combination were prepared (Table 6.6), dispensed into wells and the plates incubated for 30 min. Assay beads (8 µL) were added and the plates incubated for 1 h. Each compound was tested in triplicate. Data was processed by fitting a four-parameter equation (Equation 5) to calculate IC_{50} values using Prism software.

$$\text{Response} = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{((\log IC_{50} - X) * \text{HillSlope})}} \qquad (5)$$

$$X = \textit{Log of concentration}$$

6.3.2.6 TruHits™ Experiments

Compounds (stored as 50 mM DMSO stock solutions) (5 µL) were serially diluted 1:2 from 500 µM and dispensed into wells. For the first incubation method, assay beads (15 µL) were preincubated for 30 min, added to the plate, and then incubated for a further 10 min. For the second incubation method, streptavidin-coated donor beads (7.5 µL) were added to the compounds, and the plate incubated for 30 min. Biotinylated acceptor beads (7.5 µL) were then added, and the plate incubated for a further 30 min. Data were processed by calculating the percentage decrease in emission intensity with respect to the average DMSO control (100%).

6.3.3 WaterLOGSY NMR Experiments

Buffer (Table 6.7) was filtered through a 0.2 µM filter paper (Sartorius UK) under vacuum. Proteins were buffer exchanged from HEPES storage buffer into PBS buffer using GE Healthcare PD SpinTrap G-25 columns. For each compound, a water-supressed ^{1}H NMR spectrum was recorded, followed by a WaterLOGSY spectrum of the compound in the absence of protein, and then in the presence of the protein of interest. Samples were prepared (160 µL) in PBS buffer (pH 7.6), with 500 µM compound (stored as 50 mM DMSO stock solutions), 10% D_2O for signal locking, and 20 µM protein for samples with protein. Where compounds formed soluble aggregates, the compound and protein concentrations were decreased as necessary. 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.[115]

Table 6.7: 10 mM Na/K phosphate buffer (PBS) composition (pH 7.6).

Reagent	Amount required per litre (g)
KH_2PO_4	0.26
Na_2HPO_4	1.15
NaCl	8.71

6.3.4 Isothermal Titration Calorimetry (ITC) Experiments

ITC experiments were performed on a MicroCal PEAQ-ITC Automated (Malvern) and analysed with the MicroCal PEAQ-ITC Analysis software (Malvern 1.1.0.1262) using a single binding site model. The first data point was excluded from the analysis. Buffer (Table 6.8) was filtered through a 0.2 µM filter paper (Sartorius UK) under vacuum before use. Proteins and peptides were dialysed at 4 °C overnight in a Slide-A-Lyzer™ Mini Dialysis Device (2K MWCO, Thermo Fisher Scientific) into ITC HEPES buffer (Table 6.8) which was supplemented with 0.2% DMSO. Proteins were centrifuged to remove aggregates (3 min, 3000 rpm, 25 °C). Concentrations after dialysis were calculated by measuring the absorbance at 280 nM using a Thermo Scientific Nanodrop Lite. Compounds (stored as 50 mM DMSO stock solutions) were diluted to the desired

concentration using the dialysis buffer. The cell was stirred at 750 rpm, reference power set to 5 µcal/s and temperature held at 25 °C. After an initial delay of 60 s, 20 × 2 µL injections (first injection 0.4 µL) were performed with a spacing of 150 s. Heats of dilutions were measured under the same conditions and subtracted for analysis. Compound solutions in the calorimetric cell (250 µL, 40 µM, 0.2% DMSO) were titrated with the protein solutions in the syringe (60 µL, 363 µM).

Table 6.8: ITC HEPES buffer composition (pH 7.6).

Reagent	Composition	Amount required per litre (g)
HEPES	50 mM	11.9
NaCl	150 mM	8.77

6.3.5 Docking

CLC Drug Discovery Workbench 3.0 was used to dock ligands. The protonation state of the protein residues and the ligands were calculated within CLC. For TRIM33, the region for docking was specified as a 13 Å sphere centred on E981. The structural water molecules in the binding site were maintained. Different combinations of the ZA channel water molecules were also maintained. An ensemble of low-energy confirmations for each ligand were generated (usually one to ten) and imported to a molecule table. The ligands in the molecule table were then docked in the protein. For each ligand in the molecule table, the docking simulation searched for the optimal binding mode to the biding site. 100 iterations were carried out. During docking, the conformation of the ligand is changed *via* rotation around flexible bonds and the protein remains rigid. 10 docking results were presented for each ligand. The docking score presented is the $PLANTS_{PLP}$ score.[125] Only binding poses with the benzimidazolone core orientated as predicted were considered.[79,80] Both the CLC score and manual inspection were then used to filter the results.

References

1 C. H. Waddington, *Endeavour*, 1942, **1**, 18–20.

2 C. H. Waddington, *Int. J. Epidemiol.*, 2012, **41**, 10–13.

3 S. L. Berger, T. Kouzarides, R. Shiekhattar and A. Shilatifard, *Genes Dev.*, 2009, **23**, 781–783.

4 V. Alva, M. Ammelburg, J. Söding and A. N. Lupas, *BMC Struct. Biol.*, 2007, **7**, 1–10.

5 C. Zheng and J. J. Hayes, *J. Biol. Chem.*, 2003, **278**, 24217–24224.

6 D. V. Fyodorov, B-R. Zhou, A. I. Skoultchi and Y. Bai, *Nat. Rev. Mol. Cell Biol.*, 2018, **19**, 192–206.

7 D. Holoch and D. Moazed, *Nat. Rev. Genet.*, 2015, **16**, 71–84.

8 I. Donkin and R. Barrès, *Mol. Metab.*, 2018, **14**, 1–11.

9 V. Kumar, T. Khare, V. Shriram and S. H. Wani, *Plant Cell Rep.*, 2018, **37**, 61–75.

10 L. D. Moore, T. Le and G. Fan, *Neuropsychopharmacology*, 2013, **38**, 23–38.

11 P. Tessarz and T. Kouzarides, *Nat. Rev. Mol. Cell Biol.*, 2014, **15**, 703–708.

12 S. I. S. Grewal and D. Moazed, *Science*, 2003, **301**, 798–802.

13 S. Hatakeyama, *Trends Biochem. Sci.*, 2017, **42**, 297–311.

14 J. S. Schneekloth, F. N. Fonseca, M. Koldobskiy, A. Mandal, R. Deshaies, K. Sakamoto and C. M. Crews, *J. Am. Chem. Soc.*, 2004, **126**, 3748–3754.

15 X. Huang and V. M. Dixit, *Cell Res.*, 2016, **26**, 484–498.

16 T. Jenuwein and C. D. Allie, *Science*, 2001, **293**, 1074–1080.

17 S. B. Rothbart and B. D. Strahl, *Biochem. Biophys. Acta*, 2014, **1839**, 627–643.

18 C. P. Bagowski, W. Bruins and A. J. W. te Velthuis, *Curr. Genomics*, 2010, **11**, 368–376.

19 X. J. Yang and E. Seto, *Oncogene*, 2007, **26**, 5310–5318.

20 H. Li, S. Ilin, W. Wang, E. M. Duncan, J. Wysocka, C. D. Allis and D. J. Patel, *Nature*, 2006, **442**, 91–95.

21 K. J. Falkenberg and R. W. Johnstone, *Nat. Rev. Drug Discov.*, 2014, **13**, 673–691.

22 L. R. Vidler, N. Brown, S. Knapp and S. Hoelder, *J. Med. Chem.*, 2012, **55**, 7346–7359.

23 S. Muller, P. Filippakopoulos and S. Knapp, *Expert Rev. Mol. Med.*, 2011, **13**, 1–21.

24 M. Moustakim, P. G. K. Clark, D. A. Hay, D. J. Dixon and P. E. Brennan, *Medchemcomm*, 2016, **7**, 2246–2264.

25 T. Fujisawa and P. Filippakopoulos, *Nat. Rev. Mol. Cell Biol.*, 2017, **18**, 246–262.

26 R. Sanchez and M-M. Zhou, *Trends Biochem. Sci.*, 2011, **36**, 364–372.

27 J. Meslamani, S. G. Smith, R. Sanchez and M-M. Zhou, *Drug Discov. Today Technol.*, 2016, **19**, 3–15.

28 C. A. Musselman and T. G. Kutateladze, *Nucleic Acids Res.*, 2011, **39**, 9061–9071.

29 C. A. Musselman and T. G. Kutateladze, *Mol. Interv.*, 2009, **9**, 314–323.

30 S. Sharma, T. K. Kelly and P. A. Jones, *Carcinogenesis*, 2009, **31**, 27–36.

31 B. Molnár, O. Galamb, B. Péterfia, B. Wichmann, I. Csabai, A. Bodor, A. Kalmár, K. A. Szigeti, B. K. Barták, Z. B. Nagy, G. Valcz, Á V. Patai, P. Igaz and Z. Tulassay, *BMC Cancer*, 2018, **18**, 1–14.

32 B. P. de Almeida, J. D. Apolónio, A. Binnie and P. Castelo-Branco, *BMC Cancer*, 2019, **19**, 1–12.

33 G. Fan, Y. Tu, C. Chen, H. Sun, C. Wan and X. Cai, *Cancer Cell Int.*, 2018, **18**, 1–13.

34 R. Kandimalla, A. A. van Tilborg and E. C. Zwarthoff, *Nat. Rev. Urol.*, 2013, **10**, 327–335.

35 J. Hentze, C. Hogdall and E. Hogdall, *Mol. Clin. Oncol.*, 2019, **10**, 323–330.

36 P. A. Jones and S. B. Baylin, *Nat. Rev. Genet.*, 2002, **3**, 415–428.

37 R. Delgado-Morales, *Neuroepigenomics in Aging and Disease*; Springer International Publishing, 2017.

38 A. S. Yokoyama, J. C. Rutledge and V. Medici, *Environ. Epigenetics*, 2018, **3**, 1–11.

39 M. W. Tremblay and Y. Jiang, *Annu. Rev. Med.*, 2019, **70**, 151–166.

40 J. Li, G. Zhao and X. Gao, *J. Neurodev. Disord.*, 2013, **5**, 1–11.

41 A. Maksylewicz, A. Bysiek, K. B. Lagosz, J. M. Macina, M. Kantorowicz, G. Bereta, M. Sochalska, K. Gawron, M. Chomyszyn-Gajewska, J. Potempa and A. M. Grabiec, *Front. Immunol.*, 2019, **10**, 1–12.

42 K. M. Keck, S. A. Moquin, A. He, S. G. Fernandez, J. J. Somberg, S. M. Liu, D. M. Martinez and J. L. Miranda, *J. Biol. Chem.*, 2017, **292**, 13284–13295.

43 S. Yang, K. Bansal, J. Lopes, C. Benoist and D. Mathis, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 1833–1838.

44 S. Adhikary, D. Chakravarti, C. Terranova, I. Sengupta, M. Maitituoheti, A. Dasgupta, D. K. Srivastava, J. Ma, A. T. Raman, E. Tarco, A. A. Sahin, R. Bassett, F. Yang, C. Tapia, S. Roy, K. Rai and C. Das, *Nat. Commun.*, 2019, **10**, 1–15.

45 J. Douglas, S. Hanks, I. K. Temple, S. Davies, A. Murray, M. Upadhyaya, S. Tomkins, H. E. Hughes, T. R. P. Cole and N. Rahman, *Am. J. Hum. Genet.*, 2003, **72**, 132–143.

46 P. Filippakopoulos, J. Qi, S. Picaud, Y. Shen, W. B. Smith, O. Fedorov, E. M. Morse, T. Keates, T. T. Hickman, I. Felletar, M. Philpott, S. Munro, M. R. McKeown, Y. Wang, A. L. Christie, N. West, M. J. Cameron, B. Schwartz, T. D. Heightman, N. L. Thangue, C. A. French, O. Wiest, A. L. Kung and S. Knapp, *Nature*, 2010, **468**, 1067–1073.

47 E. Nicodeme, K. L. Jeffrey, U. Schaefer, S. Beinke, S. Dewell, C. Chung, R. Chandwani, I. Marazzi, P. Wilson, H. Coste, J. White, J. Kirilovsky, C. M. Rice, J. M. Lora, R. K. Prinjha, K. Lee and A. Tarakhovsky, *Nature*, 2010, **468**, 1119–1123.

48 S. C. Hsu and G. A. Blobel, *Cold Spring Harb. Symp. Quant. Biol.*, 2017, **82**, 37–43.

49 H. Dombret, C. Preudhomme, C. Berthon, E. Raffoux, X. Thomas, N. Vey, M. Ethell, C. Gomez-Roca, K. Yee, F. Bourdel, P. Herait, M. Michallet, R. Christian, R. Christophe and B. Quesnel, *Blood*, 2014, **124**, 117.

50 Y. Zhao, C. Y. Yang and S. Wang, *J. Med. Chem.*, 2013, **56**, 7498–7500.

51 S. J. Nicholls, A. Gordon, J. Johansson, K. Wolski, C. M. Ballantyne, J. J. P. Kastelein, A. Taylor, M. Borgman and S. E. Nissen, *J. Am. Coll. Cardiol.*, 2011, **57**, 1111–1119.

52 S. J. Nicholls, A. Gordon, J. Johannson, C. M. Ballantyne, P. J. Barter, H. B. Brewer, J. J. P. Kastelein, N. C. Wong, M. R. N. Borgman and S. E. Nissen, *Cardiovasc. Drugs Ther.*, 2012, **26**, 181–187.

53 M. A. Dawson, R. K. Prinjha, A. Dittmann, G. Giotopoulos, M. Bantscheff, W. I. Chan, S. C. Robson, C. W. Chung, C. Hopf, M. M. Savitski, C. Huthmacher, E. Gudgin, D. Lugo, S. Beinke, T. D. Chapman, E. J. Roberts, P. E. Soden, K. R. Auger, O. Mirguit, K. Doehner, R. Delwel, A. K. Burnett, P. Jeffrey, G. Drewes, K. Lee, B. J. P. Huntly and T. Kouzarides, *Nature*, 2011, **478**, 529–533.

54 O. Mirguet, R. Gosmini, J. Toum, C. A. Clément, M. Barnathan, J-M. Brusq, J. E. Mordaunt, R. M. Grimes, M. Crowe, O. Pineau, M. Ajakane, A. Daugan, P. Jeffrey, L. Cutler, A. C. Haynes, N. N. Smithersm C. Chung, P. Bamborough, I. J. Uings, A. Lewis, J. Witherington, N. Parr, R. K. Prinjha and E. Nicodème, *J. Med. Chem.*, 2013, **56**, 7501–7515.

55 M. Pervaiz, P. Mishra and S. Günther, *Chem. Rec.*, 2018, **18**, 1808–1817.

56 A. Hopkins and C. R. Groom, *Nat. Rev. Drug Discov.*, 2002, **1**, 727–730.

57 T. A. Halgren, *J. Chem. Inf. Model.*, 2009, **49**, 377–389.

58 E. K. Wagner, N. Nath, R. Flemming, J. B. Feltenberger and J. M. Denu, *Biochemistry*, 2012, **51**, 8293–8306.

59 A. Amato, X. Lucas, A. Bortoluzzi, D. Wright and A. Ciulli, *ACS Chem. Biol.*, 2018, **13**, 915–921.

60 S. Hatakeyama, *Nat. Rev. Cancer*, 2011, **11**, 792–804.

61 K. M. Short and T. C. Cox, *J. Biol. Chem.*, 2006, **281**, 8970–8980.

62 Y. Li and H. Li, *Acta Biochim. Biophys. Sin.*, 2012, **4**, 28–39.

63 L. Carthagena, A. Bergamaschi, J. M. Luna, A. David, P. D. Uchil, F. Margottin-Goguet, W. Mothes, U. Hazan, C. Transy, G. Pancino and S. Nisole, *PLoS One*, 2009, **4**, e4894.

64 M. Watanabe and S. Hatakeyama, *J. Biochem.*, 2017, **161**, 135–144.

65 P. D. Uchil, A. Hinz, S. Siegel, A. Coenen-Stass, T. Pertel, J. Luban and W. Mothes, *J. Virol.*, 2013, **87**, 257–272.

66 F. E. Morreale, H. Walden, R. Es and A. Ring, *Cell*, 2016, **165**, 248.

67 E. Agricola, R. A. Randall, T. Gaarenstroom, S. Dupont and C. S. Hill, *Mol. Cell*, 2011, **43**, 85–96.

68 R. V Stevens, D. Esposito and K. Rittinger, *Life Sci. Alliance*, 2019, **2**, 1–14.

69 A. Kulkarni, J. Oza, M. Yao, H. Sohail, V. Ginjala, A. Tomas-loba, Z. Horejsi, A. R. Tan, S. J. Boulton and S. Ganesan, *J. Biol. Chem.*, 2013, **288**, 32357–32369.

70 H-J. Lee, *J. Cancer Prev.*, 2018, **23**, 162–169.

71 B. Herquel, K. Ouararhni, K. Khetchoumian, M. Ignat, M. Teletin, M. Mark, G. Béchade, A. V. Dorsselaer, S. Sanglier-Cianférani, A. Hamiche, F. Cammas, I. Davidson and R. Losson, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 8212–8217.

72 D. F. Vincent, K. P. Yan, I. Treilleux, F. Gay, V. Arfi, B. Kaniewsky, J. C. Marie, F. Lepinasse, S. Martel, S. Goddard-Leon, J. L. Iovanna, P. Dubus, S. Garcia, A. Puisieux, R. Rimokh, N. Bardeesy, J-Y. Scoazec, R. Losson and L. Bartholin, *PLoS Genet.*, 2009, **5**, 1–9.

73 R. Aucagne, N. Droin, J. Paggetti, B. Lagrange, A. Largeot, A. Hammann, A. Bataille, L. Martin, K. P. Yan, P. Fenaux, R. Losson, E. Solary, J-N. Bastie and L. Delva, *J. Clin. Invest.*, 2011, **121**, 2361–2370.

74 G. G. Sedgwick, K. Townsend, A. Martin, N. J. Shimwell, R. J. A. Grand, G. S. Stewart, J. Nilsson and A. S. Turnell, *Oncogene*, 2013, **32**, 4622–4633.

75 X. Bai, J. Kim, Z. Yang, M. J. Jurynec, T. E. Akie, J. Lee, J. LeBlanc, A. Sessa, H. Jiang, A. DiBiase, Y. Zhou, D. J. Grunwald, S. Lun, A. B. Cantor, S. H. Orkin and L. I. Zon, *Cell*, 2010, **142**, 133–143.

76 D. G. Ransom, N. Bahary, K. Niss, D. Traver, C. Burns, N. S. Trede, N. Paffett-Lugassy, W. J. Saganic, C. A. Lim, C. Hersey, Y. Zhou, B. A. Barut, S. Lin, P. D. Kingsley, J. Palis, S. H. Orkin and L. I. Zon, *PLoS Biol.*, 2004, **2**, 1188–1196.

77 C. H. Arrowsmith, J. E. Audia, C. Austin, J. Baell, J. Bennett, J. Blagg, C. Bountra, P. E. Brennan, P. J. Brown, M. E. Bunnage, C. Buser-Deopner, R. M. Campbell, A. J. Carter, P. Cohen, R. A. Copeland, B. Cravatt, J. L. Dahlin, D. Dhanak, A. M. Edwards, M. Frederiksen, S. V. Frye, N. Gray, C. E. Grimshaw, D. Hepworth, T. Howe, K. V. M. Huber, J. Jim, S. Knapp, J. D. Kotz, R. G. Kruger, D. Lowe, M. M. Mader, B. Marsden, A. Mueller-Fahrnow, S. Müller, R. C. O'Hagam, J. P. Overington, D. R. Owen, S. H. Rosenberg, R. Ross, B. Roth, M. Schapira, S. L. Schreiber, B. Shoichet, M. Sundström, G. Superti-Furga, J. Taunton, L. Toledo-Sherman, C. Walpole, M. A. Walters, T. M. Wilson, P. Workman, R. N. Young and W. J. Zuercher, *Nat. Chem. Biol.*, 2015, **11**, 536–541.

78 J. Qi and C-K. Pei, Inhibitors of TRIM33 and Methods of Use, WO2018/200988A1, 2018.

79 W. S. Palmer, G. Poncet-Montange, G. Liu, A. Petrocchi, N. Reyna, G. Subramanian, J. Theroff, A. Yau, M. Kost-Alimova, J. P. Bardenhagen, E. Leo, H. E. Shepard, T. N. Tieu, X. Shi, Y. Zhan, S. Zhao, M. C. Barton, G. Draetta, C. Toniatti, P. Jones, M. G. Do and J. N. Andersen, *J. Med. Chem.*, 2016, **59**, 1440–1454.

80 J. Bennett, O. Fedorov, C. Tallant, O. Monteiro, J. Meier, V. Gamble, P. Savitsky, G. A. Nunez-Alonso, B. Haendler, C. Rogers, P. E. Brennan, S. Müller and S. Knapp, *J. Med. Chem.*, 2016, **59**, 1642–1647.

81 K. M. Sakamoto, K. B. Kim, A. Kumagai, F. Mercurio, C. M. Crews and R. J. Deshaies, *Proc. Natl. Acad. Sci.*, 2001, **98**, 8554–8559.

82 M. Pettersson and C. M. Crews, *Drug Discov. Today Technol.*, 2019, **31**, 15–27.

83 S. An and L. Fu, *EBioMedicine*, 2018, **36**, 553–562.

84 A. R. Schneekloth, M. Pucheault, H. S. Tae and C. M. Crews, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 5904–5908.

85 J. Lu, Y. Qian, M. Altieri, H. Dong, J. Wang, K. Raina, J. Hines, J. D. Winkler, A. P. Crew, K. Coleman and C. M. Crews, *Chem. Biol.*, 2015, **22**, 755–763.

86 D. L. Buckley, I. V. Molle, P. C. Gareiss, H. S. Tae, J. Michel, D. J. Noblin, W. L. Jorgensen, A. Ciulli and C. M. Crews, *J. Am. Chem. Soc.*, 2012, **134**, 4465–4468.

87 D. L. Buckley, J. L. Gustafson, I. V. Molle, A. G. Roth, H. S. Tae, P. C. Gareiss, W. L. Jorgensen, A. Ciulli and C. M. Crews, *Angew. Chem. Int. Ed.*, 2012, **51**, 11463–11467.

88 C. Galdeano, M. S. Gadd, P. Soares, S. Scaffidi, I. V. Molle, I. Birced, S. Hewitt, D. M. Dias and A. Ciulli, *J. Med. Chem.*, 2014, **57**, 8657–8663.

89 T. Ito, H. Ando, T. Suzuki, T. Ogura, K. Hotta, Y. Imamura, Y. Yamaguchi and H. Handa, *Science*, 2010, **327**, 1345–1350.

90 A. Lopez-Girona, D. Mendy, T. Ito, K. Miller, A. K. Gandhi, J. Kang, S. Karasawa, G. Carmel, P. Jackson, M. Abbasian, A. Mahmoudi, B. Cathers, E. Rychak, S. Gaidarova, R. Chen, P. H. Schafer, H. Handa, T. O. Daniel, J. F. Evans and R. Chopra, *Leukemia*, 2012, **26**, 2326–2335.

91 M. Toure and C. M. Crews, *Angew. Chem. Int. Ed.*, 2016, **55**, 1966–1973.

92 K. Okuhira, N. Ohoka, K. Sai, T. Nishimaki-Mogami, Y. Itoh, M. Ishikawa, Y.

Hashimoto and M. Naito, *FEBS Lett.*, 2011, **585**, 1147–1152.

93 N. Ohoka, K. Okuhira, M. Ito, K. Nagai, N. Shibata, T. Hattori, O. Ujikawa, K. Shimokawa, O. Sano, R. Koyama, H. Fujia, M. Teratani, H. Matsumoto, Y. Imaeda, H. Nara, N. Cho and M. Naito, *J. Biol. Chem.*, 2017, **292**, 4556–4570.

94 A. D. Buhimschi, H. A. Armstrong, M. Toure, S. Jaime-Figueroa, T. L. Chen, A. M. Lehman, J. A. Woyach, A. J. Johnson, J. C. Byrd and C. M. Crews, *Biochemistry*, 2018, **57**, 3564–3575.

95 M. Zengerle, K-H. Chan and A. Ciulli, *ACS Chem. Biol.*, 2015, **10**, 1770–1777.

96 B. E. Smith, S. L. Wang, S. Jaime-Figueroa, A. Harbin, J. Wang, B. D. Hamman and C. M. Crews, *Nat. Commun.*, 2019, **10**, 1–13.

97 D. Houzelstein, S. L. Bullock, D. E. Lynch, E. F. Grigorieva, V. A. Wilson and R. S. P. Beddington, *Mol. Cell. Biol.*, 2002, **22**, 3794–3802.

98 E. Shang, X. Wang, D. Wen, A. Greenberg, David and J. Wolgemuth, Debra, *Dev. Dyn.*, 2009, **238**, 908–917.

99 M. Reynders, B. Matsuura, M. Bérouti, D. Simoneschi, A. Marzio, M. Pagano and D. Trauner, *ChemRxiv*, 2019, doi: 10.26434/chemrxiv.8206688.

100 D. Lv, Y. Li, W. Zhang, A. A. Alvarez, L. Song, J. Tang, W. Q. Gao, B. Hu, S. Y. Cheng and H. Feng, *Nat. Commun.*, 2017, **8**, 1–13.

101 L. N. Gechijian, D. L. Buckley, M. A. Lawlor, J. M. Reyes, J. Paulk, C. J. Ott, G. E. Winter, M. A. Erb, T. G. Scott, M. Xu, H-S. Seo, S. Dhe-Paganon, N. P. Kwiatkowski, J. A. Perry, J. Qi, N. S. Gray and J. E. Bradner, *Nat. Chem. Biol.*, 2018, **14**, 405–412.

102 BLAST: Basic Local Alignment Search Tool, https://blast.ncbi.nlm.nih.gov/Blast.cgi, (accessed 14 July 2019).

103 T. K. Neklesa, J. D. Winkler and C. M. Crews, *Pharmacol. Ther.*, 2017, **174**, 138–144.

104 M. J. Roy, S. Winkler, S. J. Hughes, C. Whitworth, M. Galant, W. Farnaby, K. Rumpel and A. Ciulli, *ACS Chem. Biol.*, 2019, **14**, 361–368.

105 A. C. Lai and C. M. Crews, *Nat. Rev. Drug Discov.*, 2017, **16**, 101–114.

106 Y. Zou, D. Ma and Y. Wang, *Cell Biochem. Funct.*, 2019, **37**, 21–30.

107 H. Pei, Y. Peng, Q. Zhao and Y. Chen, *RSC Adv.*, 2019, **9**, 16967–16976.

108 A. Mullard, *Nat. Rev. Drug Discov.*, 2019, **18**, 237–239.

109 R. M. Eglen, T. Reisine, P. Roby, N. Rouleau, C. Illy, R. Bossé and M. Bielefeld, *Curr. Chem. Genomics*, 2008, **1**, 2–10.

110 M. Philpott, J. Yang, T. Tumber, O. Fedorov, S. Uttarkar, P. Filippakopoulos, S. Picaud, T. Keates, I. Felletar, A. Ciulli, S. Knapp and T. D. Heightman, *Mol. Biosyst.*, 2011, **7**, 2899–2908.

111 Using the AlphaScreen™ TruHits™ Kit, http://www.perkinelmer.com/CMSResources/Images/44-73574MAN_AlphaScreenTruHitsKit.pdf, (accessed 9 July 2019).

112 M. Schiedel, M. Moroglu, D. M. H. Ascough, A. E. R. Chamberlain, J. J. A. G. Kamps, A. R. Sekirnik and S. J. Conway, *Angew. Chem. Int. Ed.*, 10.1002/anie.201812164.

113 A. Sekirnik, DPhil Thesis, University of Oxford, 2016.

114 L. See, DPhil Thesis, University of Oxford, 2018.

115 C. Dalvit, G. P. Fogliatto, A. Stewart, M. Veronesi and B. Stockman, *J. Biomol. NMR*, 2001, **21**, 349–359.

116 L-H. Zhang, A-A. Yin, J-X. Cheng, H-Y. Huang, X-M. Li, Y-Q. Zhang, N. Han and X. Zhang, *Oncogene*, 2015, **34**, 600–610.

117 M. d. C. Fernández-Alonso, D. Díaz, M. Á. Berbis, F. Marcelo, J. Cañada and J. Jiménez-Barbero, *Curr. Protein Pept. Sci.*, 2012, **13**, 816–830.

118 X. Bian and S. W. Lockless, *Anal. Chem.*, 2016, **88**, 5549–5553.

119 T. Wiseman, S. Williston, J. F. Brandts and L. N. Lin, *Anal. Biochem.*, 1989, **179**, 131–137.

120 K. Bouchemal and S. Mazzaferro, *Drug Discov. Today*, 2012, **17**, 623–629.

121 PPI-Net, https://ppi-net.org/, (accessed 8 July 2019).

122 C. H. Reynolds, B. A. Tounge and S. D. Bembenek, *J. Med. Chem.*, 2008, **51**, 2432–2438.

123 S. Schultes, C. D. Graaf, E. E. J. Haaksma, I. J. P. D. Esch, R. Leurs and O. Krämer, *Drug Discov. Today Technol.*, 2010, **7**, 157–162.

124 M. L. Verdonk and D. C. Rees, *ChemMedChem*, 2008, **3**, 1179–1180.

125 O. Korb, T. Stützle and T. E. Exner, *J. Chem. Inf. Model*, 2009, **49**, 84–96.

126 J. Baell and M. A. Walters, *Nature*, 2014, **513**, 481–483.

127 A. P. Hill and R. J. Young, *Drug Discov. Today*, 2010, **15**, 648–655.

128 T. J. Ritchie and S. J. F. Macdonald, *Drug Discov. Today*, 2009, **14**, 1011–1020.

129 A. Yasgar, A. Jadhav, A. Simeonov and N. P. Coussens, *Methods Mol. Biol.*, 2016, **1439**, 77–98.

130 ACD I-Lab, http://ilab.cds.rsc.org, (accessed 9 August 2019).

131 R. J. Gritter, G. D. Dupre and T. J. Wallace, *Nature*, 1964, **202**, 179–181.

132 E. H. Demont, P. Bamborough, C-W. Chung, P. D. Craggs, D. Fallon, L. J. Gordon, P. Grandi, C. I. Hobbs, J. Hussain, E. J. Jones, A. L. Gall, A-M. Michon, D. J. Mitchell, R. K. Prinjha, A. D. Roberts, R. J. Sheppard, and R. J. Watson, *ACS Med. Chem. Lett.*, 2014, **5**, 1190–1195.

133 R. Huang and I. K. H. Leung, *Protein–Small Molecule Interactions by WaterLOGSY* in *Methods in Enzymology*, Elsevier, 2019, vol. 615, 477–500.

134 J. P. Hughes, S. S. Rees, S. B. Kalindjian and K. L. Philpott, *Br. J. Pharmacol.*, 2011, **162**, 1239–1249.

135 F. F. Hefti, *BMC Neurosci.*, 2008, **9**, 1–7.

136 J. Blagg and P. Workman, *Cancer Cell*, 2017, **32**, 9–25.

137 D. S. Hewings, M. Wang, M. Philpott, O. Fedorov, S. Uttarkar, P. Filippakopoulos, S. Picaud, C. Vuppusetty, B. Marsden, S. Knapp, S. J. Conway and T. D. Heightman, *J. Med. Chem.*, 2011, **54**, 6761–6770.

138 K. Fries and G. Finck, *Ber. Dtsch. Chem. Ges.*, 1908, **41**, 4271–4284.

139 J. Al-Nu'Airat, M. Altarawneh, X. Gao, P. R. Westmoreland and B. Z. Dlugogorski, *J. Phys. Chem. A*, 2017, **121**, 3199–3206.

140 K. Briviba, T. P. A. Devasagayam, H. Sies and S. Steenken, *Chem. Res. Toxicol.*, 1993, **6**, 548–553.

141 R. S. Shamsiev, O. L. Kaliya and V. R. Flid, *Russ. Chem. Bull.*, 2018, **67**, 1567–1572.

142 K. Groebke, L. Weber and F. Mehlin, *Synlett*, 1998, **6**, 661–663.

143 C. Blackburn, B. Guan, P. Fleming, K. Shiosaki and S. Tsai, *Tetrahedron Lett.*, 1998, **39**, 3635–3638.

144 H. Bienayme and K. Bouzid, *Angew. Chem. Int. Ed.*, 1998, **37**, 2234.

145 W. B. Turnbull and A. H. Daranas, *J. Am. Chem. Soc.*, 2003, **125**, 14859–14866.

146 M. M. Pierce, C. S. Raman and B. T. Nall, *Methods*, 1999, **19**, 213–221.

147 D. S. Hewings, T. P. C. Rooney, L. E. Jennings, D. A. Hay, C. J. Schofield, P. E. Brennan, S. Knapp and S. J. Conway, *J. Med. Chem.*, 2012, **55**, 9393–9413.

148 T. P. C. Rooney, P. Filippakopoulos, O. Fedorov, S. Picaud, W. A. Cortopassi, D. A. Hay, S. Martin, A. Tumber, C. M. Rogers, M. Philpott, M. Wang, A. L. Thompson, T. D. Heightman, D. C. Pryde, A. Cook, R. S. Paton, S. Müller, S. Knapp, P. E. Brennan and S. J. Conway, *Angew. Chem. Int. Ed.*, 2014, **53**, 6126–6130.

149 N. Miyaura and A. Suzuki, *Tetrahedron Lett.*, 1979, **36**, 3437–3440.

150 N. Miyaura and A. Suzuki, *J. Chem. Soc. Chem. Commun.*, 1979, 866–867.

151 A. L. Campbell, D. R. Pilipauaskas, I. K. Khanna and R. A. Rhodes, *Tetrahedron Lett.*, 1987, **28**, 2331–2334.

152 V. Yang, Bingwei, D. O'Rourke and J. Li, *Synlett*, 1993, **3**, 195–196.

153 W. B. Wright and H. J. Brabander, *J. Org. Chem.*, 1961, **26**, 4057–4060.

154 D. M. T. Chan, K. L. Monaco, R-P. Wang and M. P. Winters, *Tetrahedron Lett.*, 1998, **39**, 2933–2936.

155 D. A. Evans, J. L. Katz and T. R. West, *Tetrahedron Lett.*, 1998, **39**, 2937–2940.

156 P. Y. S. Lam, C. G. Clark, S. Saubern, J. Adams, M. P. Winters, D. M. T. Chan and A. Combs, *Tetrahedron Lett.*, 1998, **39**, 2941–2944.

157 O. Mitsunobu and M. Yamada, *Bull. Chem. Soc. Jpn.*, 1967, **40**, 2380–2382.

158 G. Liu, D. A. Cogan and J. A. Ellman, *J. Am. Chem. Soc.*, 1997, **119**, 9913–9914.

159 C. Mannich and W. Krösche, *Arch. Pharm.*, 1912, **250**, 647–667.

160 W. Eschweiler, *Ber. Dtsch. Chem. Ges.*, 1905, **38**, 880–882.

161 H. T. Clarke, B. Gillespie and S. Z. Weisshaus, *J. Am. Chem. Soc.*, 1933, **55**, 4571–4587.

162 E. Haggi, N. Blasich, L. Gutiérrez, G. Vázquez, S. Criado, S. Miskoski, G. Ferrari, M. P. Montaña and N. A. García, *J. Photochem. Photobiol. B*, 2012, **113**, 22–28.

163 I. Saito and T. Matsuura, *Acc. Chem. Res.*, 1977, **10**, 346–352.

164 D. Costa, A. Gomes, J. L. F. C. Lima and E. Fernandes, *Redox Rep.*, 2008, **13**, 153–160.

165 H. H. Wasserman, K. Stiller and M. B. Floyd, *Tetrahedron Lett.*, 1968, **29**, 3277–3280.

166 M. Aldeghi, G. A. Ross, M. J. Bodkin, J. W. Essex, S. Knapp and P. C. Biggin, *Commun. Chem.*, 2018, **1**, 1–12.

167 ProtoMS, http://www.essexgroup.soton.ac.uk/ProtoMS/, (accessed 19 July 2019).

168 A. Alqahtani, K. Choucair, M. Ashraf, D. M. Hammouda, A. Alloghbi, T. Khan, N. Senzer and J. Nemunaitis, *Future Sci. OA*, 2019, **5**, FSO372.

169 A. Stathis and F. Bertoni, *Cancer Discov.*, 2018, **8**, 24–36.

170 G. W. Rhyasen, M. M. Hattersley, Y. Yao, A. Dulak, W. Wang, P. Petteruti, I. L. Dale, S. Boiko, T. Cheung, J. Zhang, S. Wen, L. Castriotta, D. Lawson, M. Collins, L. Bao, M. J. Ahdesmaki, G. Walker, G. O'Connor, T. C. Yeh, A. A. Rabow, J. R. Dry, C. Reimer, P. Lyne, G. B. Mills, S. E. Fawell, M. J. Waring, M. Zinda, E. Clark and H. Chen, *Mol.*

Cancer Ther., 2016, **15**, 2563–2574.

171 S. Angers, T. Li, X. Yi, M. J. MacCoss, R. T. Moon and N. Zheng, *Nature*, 2006, **443**, 590–593.

172 D. P. Bondeson, B. E. Smith, G. M. Burslem, A. D. Buhimschi, J. Hines, S. Jaime-Figueroa, J. Wang, B. D. Hamman, A. Ishchenko and C. M. Crews, *Cell Chem. Biol.*, 2018, **25**, 78–87.

173 N. W. Pierce, G. Kleiger, S. O. Shan and R. J. Deshaies, *Nature*, 2009, **462**, 615–619.

174 E. S. Fischer, K. Böhm, J. R. Lydeard, H. Yang, M. B. Stadler, S. Cavadini, J. Nagel, F. Serluca, V. Acker, G. M. Lingaraju, R. B. Tichkule, M. Schebesta, W. C. Forrester, M. Schirle, U. Hassiepen, J. Ottl, M. Hild, R. E. J. Beckwith, J. W. Harper, J. L. Jenkins and N. H. Thomä, *Nature*, 2014, **512**, 49–53.

175 J. J. Flanagan and T. K. Neklesa, *Mol. Cell. Endocrinol.*, 2019, **493**, 110452.

176 M. Schiedel, D. Herp, S. Hammelmann, S. Swyter, A. Lehotzky, D. Robaa, J. Oláh, J. Ovádi, W. Sippl and M. Jung, *J. Med. Chem.*, 2018, **61**, 482–491.

177 J. Lohbeck and A. K. Miller, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 5260–5262.

178 X. H. Yuan, M. J. Zhang, C. Q. Kang, H. Q. Guo, X. P. Qiu and L. X. Gao, *Synth. Commun.*, 2006, **36**, 435–444.

179 G. A. Olah and S. C. Narang, *Tetrahedron*, 1982, **38**, 1339–1388.

180 M. E. Jung and M. A. Lyster, *J. Chem. Soc. Chem. Commun.*, 1978, 315–316.

181 J. E. Chojnacki, K. Liu, J. M. Saathoff and S. Zhang, *Bioorg. Med. Chem.*, 2015, **23**, 7324–7331.

182 M. Saitoh, J. Kunitomo, E. Kimura, Y. Hayase, H. Kobayashi, N. Uchiyama, T. Kawamoto, T. Tanaka, C. D. Mol, D. R. Dougan, G. S. Textor, G. P. Snell and F. Itoh, *Bioorg. Med. Chem.*, 2009, **17**, 2017–2029.

183 B. Zhou, J. Hu, F. Xu, Z. Chen, L. Bai, E. Fernandez-Salas, M. Lin, L. Liu, C. Y. Yang, Y. Zhao, D. McEachern, S. Przybranowski, B. Wen, D. Sun and S. Wang, *J. Med. Chem.*, 2018, **61**, 462–481.

184 A. J. Mancuso, D. S. Brownfain and D. Swern, *J. Org. Chem.*, 1979, **44**, 4148–4150.

185 K. Omura and D. Swern, *Tetrahedron*, 1978, **34**, 1651–1660.

186 T. D. Ros, M. Bergamin, E. Vazquez, G. Spalluto, B. Baiti, S. Moro, A. Boutorine and M. Prato, *Eur. J. Org. Chem.*, 2002, **2002**, 405–413.

187 R. Appel, *Angew. Chem. Int. Ed.*, 1975, **14**, 801–811.

188 D. S. Coffey, A. I. McDonald and L. E. Overman, *J. Org. Chem.*, 1999, **64**, 8741–8742.

189 E. T. Silva, A. S. Cunha and E. L. S. Lima, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 3207–3208.

190 S. Fletcher, *Org. Chem. Front.*, 2015, **2**, 739–752.

191 A. A. Akuffo, A. Y. Alontaga, R. Metcalf, M. S. Beatty, A. Becker, J. M. McDaniel, R. S. Hesterberg, W. E. Goodheart, S. Gunawan, M. Ayaz, Y. Yang, M. R. Karim, M. E. Orobello, K. Daniel, W. Guida, J. A. Yoder, A. M. Rajadhyaksha, E. Schönbrunn, H. R. Lawrence, N. J. Lawrence and P. K. Epling-Burnette, *J. Biol. Chem.*, 2018, **293**, 6187–6200.

192 G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176–2179.

193 K. Ryu, B. Kim, G. Sung, H. Yoon and Y-J. Yoon, *Synlett*, 2015, **26**, 1985–1990.

194 T. Kimura, K. Kamata and N. Mizuno, *Angew. Chem. Int. Ed.*, 2012, **51**, 6700–6703.

195 X. Diao, Y. Wang, Y. Jiang and D. Ma, *J. Org. Chem.*, 2009, **74**, 7974–7977.

196 A. N. Kost, Z. F. Solomko, V. A. Budylin and T. S. Semenova, *Chem. Heterocycl. Compd.*, 1972, **8**, 632–635.

197 R. S. Goudie and P. N. Preston, *J. Chem. Soc. C*, 1971, 1139–1142.

198 S. M. Abdelaal, S-B. Kong and L. Bauer, *J. Heterocycl. Chem.*, 1992, **29**, 1069–1076.

199 N. N. Smolyar, O. Y. Pankina, A. I. Bondarenko, Y. S. Borodkin and A. I. Khizhan, *Russ. J. Org. Chem.*, 2011, **47**, 1190–1193.

200 M. T. Conconi, G. Marzaro, L. Urbani, I. Zanusso, R. D. Liddo, I. Castagliuolo, P. Brun, F. Tonus, A. Ferrarese, A. Guiotto and A. Chilin, *Eur. J. Med. Chem.*, 2013, **67**, 373–383.

201 R. L. Clark and A. A. Pessolano, *J. Am. Chem. Soc.*, 1958, **80**, 1662–1664.

202 N. N. Smolyar, O. Y. Pankina, A. I. Bondarenko, Y. S. Borodkin and A. I. Khizhan, *Russ. J. Org. Chem.*, 2011, **47**, 1190–1193.

203 C. Ren, S. Xu, J. Xu, H. Chen and H. Zeng, *Org. Lett.*, 2011, **13**, 3840–3843.

204 M. Mochizuki, T. Kojima, K. Kobayashi, E. Kotani, Y. Ishichi, N. Kanzaki, H. Nakagawa, T. Okuda, Y. Kosugi, T. Yano, Y. Sako, M. Tanaka and K. Aso, *Bioorg. Med. Chem.*, 2017, **25**, 1556–1570.

205 K. Aso, M. Mochizuki and K. Kobayashi, Fused Heterocyclic Compounds having CRF Antagonistic Activity, WO2008/082003A1, 2008.

206 S. A. Shintre, D. Ramjugernath, P. Singh, C. Mocktar and N. A. Koorbanally, *Med. Chem. Res.*, 2017, **26**, 484–498.

207 D-D. Li, Z-H. Wang, W-L. Chen, Y-Y. Xie, Q-D. You and X-K. Guo, *Bioorg. Med. Chem.*, 2016, **24**, 6109–6118.

208 N. Soundararajan and M. S. Platz, *J. Org. Chem.*, 1990, **55**, 2034–2044.

209 S. Munack, V. Leroux, K. Roderer, L. Gundersen, U. Krengel and P. Kast, *Chem. Biodivers.*, 2012, **9**, 2507–2527.

210 H. Yin and K. Cheng, 2-(4-Aryl-1*H*-imidazol-1-yl)aniline Compounds, WO2015/171951A1, 2015.

211 K. N. More and J. Lee, *Bull. Korean Chem. Soc.*, 2017, **38**, 70–77.

212 R. S. Givens, K. Stensrud, P. G. Conrad, A. L. Yousef, C. Perera, S. N. Senadheera, D. Heger and J. Wirz, *Can. J. Chem.*, 2011, **89**, 364–384.

213 T. Nagano and K. Matsumura, *J. Am. Chem. Soc.*, 1953, **75**, 6237–6238.

214 J. N. Chakor, L. Merlini and S. Dallavalle, *Tetrahedron*, 2011, **67**, 6300–6307.

215 K. Hirota, Y. Kitade and S. Senda, *J. Org. Chem.*, 1981, **46**, 3949–3953.

216 A. Padwa, M. Dimitroff, A. G. Waterson and W. Tianhua, *J. Org. Chem.*, 1997, **62**, 4088–4096.

217 J. McCall, R. C. Kelly and D. L. Romero, Heterocyclic Compounds for the Inhibition of PASK, WO2014/066795A1, 2014.

218 P. S. Branco, S. Prabhakar, A. M. Lobo and D. J. Williams, *Tetrahedron*, 1992, **48**, 6335–6360.

219 B. Yu, H. Zhang, Y. Zhao, S. Chen, J. Xu, L. Hao and Z. Liu, *ACS Catal.*, 2013, **3**, 2076–2082.

220 F. Kilchmann, M. J. Marcaida, S. Kotak, T. Schick, S. D. Boss, M. Awale, P. Gönczy and J-L. Reymond, *J. Med. Chem.*, 2016, **59**, 7188–7211.

221 D. B. Nale and B. M. Bhanage, *Green Chem.*, 2015, **17**, 2480–2486.

222 J. P. English, R. C. Clapp, Q. P. Cole, I. F. Halverstadt, J. O. Lampen and R. O. Roblin, *J. Am. Chem. Soc.*, 1945, **67**, 295–302.

223 F. M. Hocutt, B. E. Leonard, H. M. Peltier, V. K. Phuong, M. H. Rabinowitz, M. D. Rosen, K. T. Tarantino, H. Venkatesan and L. X. Zhao, Benzoimidazoles as Prolyl Hydroxylase Inhibitors, WO2009/134750A1, 2009.

224 C. R. Hurt, A. M. K. Pennell, J. J. Wright, Q. Wang, M. R. Leleti, W. D. Thomas, Y. Li and D. R. Dragoli, Substituted Dihydropyridines and Methods of Use, WO2007/051062A2, 2007.

225 B. Herbert, T. M. Nguyen, A. Tehim, A. T. Hopper and W. Xie, Anabaseine Derivatives Useful in the Treatment of Neurodegenerative Diseases, WO2004/019943A1, 2004.

226 Y-N. Hsieh, C-H. Kuei, Y-K. Chou, C-C. Liu, K-L. Leu, T-H. Yang, M-Y. Wang and W-Y. Ho, *Tetrahedron Lett.*, 2010, **51**, 3666–3669.

227 P. D. Pham, J. Vitz, C. Chamignon, A. Martel and S. Legoupy, *Eur. J. Org. Chem.*, 2009, **2009**, 3249–3257.

228 E. Ayral, P. Gloanec, G. Bergé, G. D. Nanteuil, P. Mennecier, A. Rupin, T. J. Verbeuren, P. Fulcrand, J. Martinez and J-F. Hernandez, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1386–1391.

229 M. Saitoh, J. Kunitomo, E. Kimura, Y. Hayase, H. Kobayashi, N. Uchiyama, T. Kawamoto, T. Tanaka, C. D. Mol, D. R. Dougan, G. S. Textor, G. P. Snell and F. Itoh, *Bioorg. Med. Chem.*, 2009, **17**, 2017–2029.

230 M. P. Hay, B. M. Sykes, W. A. Denny and C. J. O'Connor, *J. Chem. Soc. Perkin Trans. 1*, 1999, 2759–2770.

231 Y. Li, I. Sorribes, T. Yan, K. Junge and M. Beller, *Angew. Chem. Int. Ed.*, 2013, **52**, 12156–12160.

232 Y. Yuasa, N. Fujimaki, T. Yokomatsu, J. Ando and S. Shibuya, *J. Chem. Soc. Perkin Trans. 1*, 1998, 3577–3584.

233 D. L. J. Clive and X. Huang, *J. Org. Chem.*, 2004, **69**, 1872–1879.

234 D. H. Burns, H-K. Chan, J. D. Miller, C. L. Jayne and D. M. Eichhorn, *J. Org. Chem.*, 2000, **65**, 5185–5196.

235 J. J. Kasper, J. E. Hitro, S. R. Fitzgerald, J. M. Schnitter, J. J. Rutowski, J. A. Heck and J. L. Steinbacher, *J. Org. Chem.*, 2016, **81**, 8095–8103.

236 I. A. Moussa, S. D. Banister, C. Beinat, N. Giboureau, A. J. Reynolds and M. Kassiou, *J. Med. Chem.*, 2010, **53**, 6228–6239.

237 S. M. Capitosti, T. P. Hansen and M. L. Brown, *Org. Lett.*, 2003, **5**, 2865–2867.

238 G. M. Burslem, P. Ottis, S. Jaime-Figueroa, A. Morgan, P. M. Cromm, M. Toure and C. M. Crews, *ChemMedChem*, 2018, **13**, 1508–1512.

239 V. E. Schnubel, J. Stoltefuß, H. A. Offe and E. Kluuke, *Liebigs Ann. Chem.*, 1971, **743**, 57–68.

240 N. H. Greig, H. Holloway, A. Brossi, X. Zhu, T. Giordano, Q-S. Yu and W. D. Figg, Thalidomide Analogs, WO2005/028436A2, 2005.

241 S. G. Stewart, C. J. Braun, S. L. Ng, M. E. Polomska, M. Karimi and L. J. Abraham, *Bioorg. Med. Chem.*, 2010, **18**, 650–662.

242 N. E. A. Chessum, S. Y. Sharp, J. J. Caldwell, A. E. Pasqua, B. Wilding, G. Colombano, I. Collins, B. Ozer, M. Richards, M. Rowlands, M. Stubbs, R. Burke, P. C. McAndrew, P. A. clarke, P. Workman, M. D. Cheeseman and K. Jones, *J. Med. Chem.*, 2018, **61**, 918–933.

243 W. Luo, Q. S. Yu, D. Tweedie, J. Deschamps, D. Parrish, H. W. Holloway, Y. Li, A. Brossi and N. H. Greig, *Synthesis*, 2008, **21**, 3415–3422.

244 T. Nakamura, T. Noguchi, H. Miyachi and Y. Hashimoto, *Chem. Pharm. Bull. (Tokyo)*, 2007, **55**, 651–654.

245 D. Remillard, D. L. Buckley, J. Paulk, G. L. Brien, M. Sonnett, H. S. Seo, S. Dastjerdi, M. Wühr, S. Dhe-Paganon, S. A. Armstrong and J. E. Bradner, *Angew. Chem. Int. Ed.*, 2017, **56**, 5738–5743.

246 J. Bradner, D. Buckley and G. Winter, Methods to Induce Targeted Protein Degradation Through Bifunctional Molecules, WO2017/024317A2, 2017.

247 N. S. Chandrakumar, A. Stapelfeld, P. M. Beardsley, O. T. Lopez, B. Drury, E. Anthony, M. A. Savage, M. Reichman, L. N. Williamson and M. Reichman, *J. Med. Chem.*, 1992, **35**, 2928–2938.

248 M. Adamczyk, J. R. Fishpaugh and M. Thiruvazhi, *Org. Prep. Proced. Int.*, 2002, **34**, 326–331.

249 B. Söveges, T. Imre, A. L. Póti, P. Sok, Z. Kele, A. Alexa, P. Kele and K. Németh, *Org. Biomol. Chem.*, 2018, **16**, 5756–5763.

250 V. Singh, S. Wang and E. T. Kool, *J. Am. Chem. Soc.*, 2013, **135**, 6184–6191.

251 B. Fabre, J. Pícha, V. Vaněk, I. Selicharová, M. Chrudinová, M. Collinsová, L. Žáková, M. Buděšínský and J. Jiráček, *ACS Comb. Sci.*, 2016, **18**, 710–722.

252 T. Anabuki, M. Tsukahara, H. Matsuura and K. Takahashi, *Biosci. Biotechnol. Biochem.*, 2016, **80**, 432–439.

253 N. M. Shukla, S. S. Malladi, C. A. Mutz, R. Balakrishna and S. A. David, *J. Med. Chem.*, 2010, **53**, 4450–4465.

254 M. Dakanali, T. H. Do, A. Horn, A. Chongchivivat, T. Jarusreni, D. Lichlyter, G. Guizzunti, M. A. Haidekker and E. A. Theodorakis, *Bioorg. Med. Chem.*, 2012, **20**, 4443–4450.

255 J. Simonin, S. K. V. Vernekar, A. J. Thompson, J. D. Hothersall, C. N. Connolly, S. C. R. Lummis and M. Lochner, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 1151–1155.

256 Z. B. Hill, B. Gayani, K. Perera and D. J. Maly, *J. Am. Chem., Soc.* 2009, **131**, 6686–6688.

257 G. Clave, H. Boutal, A. Hoang, F. Perraut, H. Volland, P-Y. Renard and A. Romieu, *Org. Biomol. Chem.*, 2008, **6**, 3065–3078.

258 K. Ogawa, T. Takeda, M. Yokokawa, J. Yu, A. Makino, Y. Kiyono, K. Shiba, S. Kinuya and A. Odani, *Chem. Pharm. Bull.*, 2018, **66**, 651–659.

259 A. Späth, J. Gonschor and B. König, *Monatsh. Chem.*, 2011, **142**, 1289–1308.

260 L. M. Pardo, I. Tellitu and E. Domínguez, *Tetrahedron*, 2010, **66**, 5811–5818.

261 R. J. Middleton, S. J. Briddon, Y. Cordeaux, A. S. Yates, C. L. Dale, M. W. George, J. G. Baker, S. J. Hill and B. Kellam, *J. Med. Chem.*, 2007, **50**, 782–793.

262 J. Wagner, J. Kallen, C. Ehrhardt, J. P. Evenou and D. Wagner, *J. Med. Chem.*, 1998, **41**, 3664–3674.

263 F. Kohl, J. Schmitz, N. Furtmann, A. C. Schulz-Fincke, M. D. Mertens, J. Küppers, M.

Benkhoff, E. Tobiasch, U. Bartz, J. Bajorath, M. Stirnberg and M. Gütschow, *Org. Biomol. Chem.*, 2015, **13**, 10310–10323.

264 J. Marchand-Brynaert, M. Bouchet, R. Touillaux, C. Beauve and J. Fastrez, *Tetrahedron*, 1996, **52**, 5591–5606.

265 A. Favre, J. Grugier, A. Brans, B. Joris and J. Marchand-Brynaert, *Tetrahedron*, 2012, **68**, 10818–10826.

266 H. Stockmann, V. Todorovic, P. L. Richardson, V. Marin, V. Scott, C. Gerstein, M. Lake, L. Wang, R. Sadhukhan and A. Vasudevan, *J. Am. Chem. Soc.*, 2017, **139**, 16822–16829.

267 S. S. Jalisatgi, V. S. Kulkarni, B. Tang, Z. H. Houston, M. W. Lee and M. F. Hawthorne, *J. Am. Chem. Soc.*, 2011, **133**, 12382–12385.

268 M-P. Chien and N. C. Gianneschi, Nanofibers and Morphology Shifting Micelles, US2012/149843A1, 2012.

269 D. V. Filippov, G. P. P. Gential, G. V. d. Marel and F. Ossendorp, Adjuvant Compounds, WO2018/197582A1, 2018.

270 X. Yue, Y. Feng and Y. B. Yu, *J. Fluor. Chem.*, 2013, **152**, 173–181.

271 A. Milelli, C. Marchetti, M. L. Greco, F. Moraca, G. Costa, E. Turrini, E. Catanzaro, N. Betari, C. Calcabrini, C. Sissi, S. Alcaro, C. Fimognari, V. Tumiatti and A. Minarini, *Eur. J. Med. Chem.*, 2017, **128**, 107–122.

272 T. Aubineau and J. Cossy, *Chem. Commun.*, 2013, **49**, 3303–3305.

273 A. P. Ingale, V. K. More, U. S. Gangarde and S. V. Shinde, *New J. Chem.*, 2018, **42**, 10142–10147.

274 K. Maruyoshi, K. Nonaka, T. Sagane, T. Demura, T. Yamaguchi, N. Matsumori, T. Oishi and M. Murata, *Chem.: Eur. J.*, 2009, **15**, 1618–1626.

275 T. Nishimaru, M. Sano, Y. Yamaguchi and T. Wakamiya, *Bioorg. Med. Chem.*, 2009, **17**, 57–63.

276 Y. Hidai, T. Kan and T. Fukuyama, *Chem. Pharm. Bull.*, 2000, **48**, 1570–1576.

APPENDICES

A. Protein Characterisation

TRIM33α

Protein	His_6-TRIM33α
Plasmid vector	pNIC28-Bsa4 (kanamycin)
DNA sequence	ATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGA GAACCTGTACTTCCAATCCATGGACTGGTGTGCTGTCTGCCAAAACG GAGGAGATCTCTTGTGCTGCGAAAAATGTCCAAAGGTCTTTCATCTA ACTTGTCATGTTCCAACACTACTTAGCTTTCCAAGTGGGGACTGGAT ATGCACATTTTGTAGAGATATTGGAAAGCCAGAAGTTGAATATGATT GTGATAATTTGCAACATAGTAAGAAGGGGAAAACTGCGCAGGGGTT AAGCCCCGTGGACCAAAGGAAATGTGAACGTCTTCTGCTTTACCTCT ATTGCCATGAATTAAGTATTGAATTCCAGGAGCCTGTTCCTGCTTCG ATACCAAACTACTATAAAATTATAAAGAAACCAATGGATTTATCCAC CGTGAAAAAGAAGCTTCAGAAAAAACATTCCCAACACTACCAAATC CCGGATGACTTTGTGGCCGATGTCCGTTTGATCTTCAAGAACTGTGA AAGGTTTAATGAAATGATGAAAGTTGTTCAAGTTTATGCAGACACAC AAGAGATTAATTTGAAGGCTGATTCAGAAGTAGCTCAGGCAGGGAA AGCAGTTGCATTGTACTTTGAAGATAAACTCACAGAGATCTACTCAG ACAGGACCTTCGCACCTTTGCCAGAGTTTGAGCAGGAAGAGGATGA TGGTGAGGTAACTGAGGACTCTGATGAAGACTTTATACAGCCCCGC AGAAAACGCCTAAAGTCAGATGAGAGACCAGTACATATAAAGTAA
Amino acid sequence	MHHHHHHSSGVDLGTENLYFQSMDWCAVCQNGGDLLCCEKCPKVFH LTCHVPTLLSFPSGDWICTFCRDIGKPEVEYDCDNLQHSKKGKTAQGLS PVDQRKCERLLLYLYCHELSIEFQEPVPASIPNYYKIIKKPMDLSTVKKK LQKKHSQHYQIPDDFVADVRLIFKNCERFNEMMKVVQVYADTQEINLK ADSEVAQAGKAVALYFEDKLTEIYSDRTFAPLPEFEQEEDDGEVTEDSD EDFIQPRRKRLKSDERPVHIK
MW (Da)	30502

TRIM33β

Protein	His_6-TRIM33β
Plasmid vector	pNIC28-Bsa4 (kanamycin)
DNA sequence	ATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGA GAACTGTACTTCCAATCCATGGACTGGTGTGCTGTCTGCCAAAACGG AGGAGATCTCTTGTGCTGCGAAAAATGTCCAAAGGTCTTTCATCTAA CTTGTCATGTTCCAACACTACTTAGCTTTCCAAGTGGGGACTGGATA TGCACATTTTGTAGAGATATTGGAAAGCCAGAAGTTGAATATGATTG TGATAATTTGCAACATAGTAAGAAGGGGAAAACTGCGCAGGGGTTA AGCCCCGTGGACCAAAGGAAATGTGAACGTCTTCTGCTTTACCTCTA TTGCCATGAATTAAGTATTGAATTCCAGGAGCCTGTTCCTGCTTCGA TACCAAACTACTATAAAATTATAAAGAAACCAATGGATTTATCCACC GTGAAAAAGAAGCTTCAGAAAAAACATTCCCAACACTACCAAATCC CGGATGACTTTGTGGCCGATGTCCGTTTGATCTTCAAGAACTGTGAA AGGTTTAATGAGGCTGATTCAGAAGTAGCTCAGGCAGGGAAAGCAG TTGCATTGTACTTTGAAGATAAACTCACAGAGATCTACTCAGACAGG ACCTTCGCACCTTTGCCAGAGTTTGAGCAGGAAGAGGATGATGGTG AGGTAACTGAGGACTCTGATGAAGACTTTATACAGCCCCGCAGAAA ACGCCTAAAGTCAGATGAGAGACCAGTACATATAAAGTAA
Amino acid sequence	MHHHHHHSSGVDLGTENLYFQSMDWCAVCQNGGDLLCCEKCPKVFH LTCHVPTLLSFPSGDWICTFCRDIGKPEVEYDCDNLQHSKKGKTAQGLS PVDQRKCERLLLYLYCHELSIEFQEPVPASIPNYYKIIKKPMDLSTVKKK LQKKHSQHYQIPDDFVADVRLIFKNCERFNEADSEVAQAGKAVALYFE DKLTEIYSDRTFAPLPEFEQEEDDGEVTEDSDEDFIQPRRKRLKSDERPV HIK
MW (Da)	28509

Molecular weights of proteins were predicted using the ProtParam tool (available at: http://web.expasy.org/protparam/).

B. AlphaScreen™ Data

Table B1: IC_{50} values (μM) determined using a dose-response AlphaScreen™ assay (Chapter 2, Table 2.3). Compounds were serially diluted 1:2 from 500 μM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
23	>250	40.3 ± 1.88 (0.24)	>250	10.8 ± 1.21 (0.27)	3.88 ± 1.03 (0.29)	9.40 ± 1.12 (0.27)	24.4 ± 1.24 (0.25)	11.9 ± 1.09 (0.27)	62.1 ± 1.11 (0.23)
25	>250	22.5 ± 1.45 (0.50)	>250	>250	41.8 ± 1.13 (0.47)	244 ± 119 (0.39)	>250	168 ± 1.96 (0.41)	>250
26	>250	>250	>250	9.63 ± 1.18 (0.50)	7.83 ± 1.10 (0.51)	16.3 ± 1.12 (0.48)	19.1 ± 3.13 (0.47)	30.6 ± 2.60 (0.45)	86.4 ± 24.1 (0.41)
27	>250	>250	>250	>250	65.1 ± 12.4 (0.39)	>250	>250	>250	>250

Table B2: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 2, Table 2.5). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
46	>250	>250	>250	>250	>250	>250	>250	>250	>250
47	>250	>250	>250	>250	12.1 ± 1.12 (0.33)	19.7 ± 1.86 (0.31)	>250	>250	>250
48	>250	>250	>250	>250	23.3 ± 2.34 (0.31)	39.2 ± 5.97 (0.29)	>250	>250	>250
49	>250	>250	>250	>250	>250	>250	>250	>250	>250

Table B3: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 2, Table 2.8). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
76	3.57 ± 0.521 (0.35)	4.21 ± 0.665 (0.34)	3.33 ± 0.226 (0.35)	2.71 ± 0.166 (0.35)	2.74 ± 0.529 (0.35)	1.68 ± 0.407 (0.37)	3.37 ± 0.619 (0.35)	3.16 ± 0.545 (0.35)	3.11 ± 0.342 (0.35)
77	1.86 ± 0.182 (0.32)	1.76 ± 0.100 (0.32)	1.46 ±.0840 (0.33)	1.89 ± 0.118 (0.32)	1.58 ± 0.165 (0.32)	1.15 ± 0.120 (0.33)	1.40 ± 0.194 (0.33)	1.49 ± 0.218 (0.33)	1.82 ± 0.206 (0.32)
78	105 ± 26.7 (0.24)	43.8 ± 10.9 (0.27)	58.0 ± 20.3 (0.26)	>250	>250	>250	>250	>250	>250
79	17.1 ± 6.15 (0.26)	20.0 ± 2.95 (0.25)	11.1 ± 1.81 (0.27)	>250	16.6 ± 6.10 (0.26)	75.1 ± 20.5 (0.24)	>250	7.91 ± 1.84 (0.27)	8.86 ± 2.41 (0.27)
80	52.0 ± 5.56 (0.25)	18.6 ± 0.727 (0.28)	23.1 ± 0.740 (0.27)	>250	>250	>250	>250	55.3 ± 4.68 (0.25)	58.7 ± 6.00 (0.25)

Table B4: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 3, Table 3.3). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses. *IC_{50} values are averaged over 14 repeats.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
23	>250	40.3 ± 1.88 (0.24)	>250	10.8 ± 1.21 (0.27)	3.88 ± 1.03 (0.29)	9.40 ± 1.12 (0.27)	24.4 ± 1.24 (0.25)	11.9 ± 1.09 (0.27)	62.1 ± 1.11 (0.23)
89	>250	>250	>250	75.4 ± 1.66 (0.26)	7.97 ± 1.06 (0.32)	3.37 ± 1.20 (0.35)	54.3 ± 1.28 (0.27)	11.9 ± 1.12 (0.31)	72.8 ± 1.19 (0.26)
***90**	>250	>250	>250	75.0 ± 0.810 (0.38)	3.61 ± 0.352 (0.38)	5.74 ± 0.597 (0.37)	6.74 ± 1.05 (0.36)	13.7 ± 1.53 (0.35)	66.3 ± 3.91 (0.32)
91	>250	>250	>250	>250	10.5 ± 1.09 (0.28)	33.3 ± 1.32 (0.25)	>250	65.5 ± 1.18 (0.23)	>250
92	>250	92.1 ± 1.23 (0.33)	>250	>250	5.34 ± 1.10 (0.43)	6.51 ± 1.07 (0.43)	>250	23.3 ± 1.27 (0.38)	>250
94	>250	52.4 ± 1.21 (0.35)	>250	>250	3.35 ± 1.11 (0.45)	10.4 ± 1.12 (0.41)	>250	26.5 ± 1.10 (0.38)	177 ± 1.34 (0.31)

Table B5: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 3, Table 3.6). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses. *IC_{50} values are averaged over 14 repeats.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
*90	>250	>250	>250	75.0 ± 0.810 (0.38)	3.61 ± 0.352 (0.38)	5.74 ± 0.597 (0.37)	6.74 ± 1.05 (0.36)	13.7 ± 1.53 (0.35)	66.3 ± 3.91 (0.32)
104	>250	>250	>250	>250	>250	>250	>250	>250	>250
105	>250	>250	>250	>250	>250	>250	>250	>250	>250
106	>250	>250	>250	9.42 ± 1.41 (0.34)	5.69 ± 0.334 (0.35)	12.8 ± 1.32 (0.33)	15.5 ± 1.79 (0.32)	23.5 ± 2.80 (0.31)	>250
107	>250	>250	>250	>250	3.73 ± 0.383 (0.42)	>250	7.71 ± 0.786 (0.40)	13.8 ± 1.00 (0.38)	37.5 ± 8.01 (0.34)
108	4.54 ± 1.37 (0.34)	24.3 ± 1.36 (0.29)	16.0 ± 1.61 (0.31)	5.06 ± 1.54 (0.34)	4.11 ± 1.31 (0.34)	6.65 ± 1.48 (0.33)	5.32 ± 1.47 (0.34)	9.30 ± 1.47 (0.32)	12.9 ± 1.48 (0.31)
109	22.7 ± 1.16 (0.28)	130 ± 10.3 (0.24)	102 ± 1.12 (0.24)	4.60 ± 1.14 (0.32)	2.71 ± 1.08 (0.34)	4.89 ± 1.12 (0.32)	3.81 ± 1.08 (0.33)	9.24 ± 1.06 (0.31)	24.6 ± 1.12 (0.28)
110	8.72 ± 1.19 (0.37)	71.9 ± 1.30 (0.31)	45.1 ± 1.08 (0.32)	17.1 ± 1.17 (0.35)	5.89 ± 1.09 (0.39)	9.68 ± 1.11 (0.37)	6.79 ± 1.11 (0.38)	15.2 ± 1.07 (0.35)	26.1 ± 1.05 (0.34)
111	>250	>250	>250	2.38 ± 1.12 (0.36)	4.74 ± 1.14 (0.34)	1.65 ± 1.08 (0.37)	1.58 ± 1.28 (0.37)	2.02 ± 1.06 (0.36)	6.24 ± 1.04 (0.33)
112	>250	>250	>250	4.22 ± 1.11 (0.36)	7.96 ± 1.11 (0.34)	2.39 ± 1.12 (0.37)	2.67 ± 1.22 (0.37)	2.73 ± 1.14 (0.37)	3.86 ± 1.07 (0.36)
113	>250	>250	>250	>250	>250	>250	>250	>250	>250

Table B6: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 3, Table 3.8). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
118	>250	34.5 ± 1.35 (0.25)	>250	9.49 ± 1.37 (0.28)	2.46 ± 1.07 (0.31)	5.56 ± 1.11 (0.29)	21.1 ± 1.40 (0.26)	15.3 ± 1.10 (0.27)	43.6 ± 1.22 (0.24)

Table B7: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 3, Table 3.9). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses. *IC_{50} values are averaged over 14 repeats.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
*90	>250	>250	>250	75.0 ± 0.810 (0.38)	3.61 ± 0.352 (0.38)	5.74 ± 0.597 (0.37)	6.74 ± 1.05 (0.36)	13.7 ± 1.53 (0.35)	66.3 ± 3.91 (0.32)
121	>250	>250	>250	>250	14.8 ± 1.12 (0.26)	41.9 ± 1.18 (0.24)	>250	>250	>250
122	>250	>250	>250	>250	5.37 ± 1.10 (0.27)	12.8 ± 1.08 (0.25)	>250	>250	>250
123	>250	>250	>250	>250	8.76 ± 1.15 (0.25)	22.8 ± 1.08 (0.23)	>250	>250	>250
124	>250	>250	>250	2.39 ± 1.20 (0.30)	3.64 ± 1.11 (0.29)	5.77 ± 1.10 (0.28)	35.7 ± 1.12 (0.24)	86.0 ± 1.15 (0.22)	>250
125	>250	>250	>250	2.81 ± 1.19 (0.29)	4.34 ± 1.08 (0.28)	6.07 ± 1.10 (0.27)	19.2 ± 1.30 (0.24)	59.7 ± 1.12 (0.22)	150 ± 1.26 (0.20)
126	>250	>250	>250	0.710 ± 2.56 (0.31)	2.47 ± 1.08 (0.28)	3.89 ± 1.05 (0.27)	27.8 ± 1.14 (0.23)	100 ± 1.10 (0.20)	233 ± 1.49 (0.18)

Table B8: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 3, Table 3.11). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses. *IC_{50} values are averaged over 14 repeats.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
*90	>250	>250	>250	75.0 ± 0.810 (0.38)	3.61 ± 0.352 (0.38)	5.74 ± 0.597 (0.37)	6.74 ± 1.05 (0.36)	13.7 ± 1.53 (0.35)	66.3 ± 3.91 (0.32)
129	>250	>250	>250	>250	2.01 ± 0.240 (0.28)	5.94 ± 0.605 (0.26)	>250	53.3 ± 7.89 (0.21)	>250
130	>250	>250	>250	>250	>250	23.8 ± 4.58 (0.22)	>250	>250	>250
131	>250	>250	>250	>250	>250	>250	>250	>250	>250
132	>250	>250	>250	7.83 ± 2.16 (0.30)	4.32 ± 0.351 (0.31)	15.6 ± 1.93 (0.28)	58.9 ± 20.3 (0.25)	60.2 ± 7.42 (0.20)	>250
133	>250	>250	>250	>250	9.87 ± 2.16 (0.24)	26.3 ± 8.79 (0.22)	>250	>250	>250
134	>250	>250	>250	>250	16.4 ± 1.08 (0.29)	28.0 ± 8.35 (0.28)	>250	>250	>250
135	>250	>250	>250	>250	8.74 ± 0.819 (0.32)	29.6 ± 3.23 (0.29)	14.4 ± 2.93 (0.31)	20.6 ± 1.94 (0.30)	>250
136	>250	>250	>250	>250	2.07 ± 0.200 (0.38)	5.99 ± 0.410 (0.35)	10.5 ± 1.31 (0.33)	14.7 ± 1.47 (0.32)	>250
137	>250	>250	>250	>250	3.22 ± 0.246 (0.35)	7.47 ± 0.589 (0.33)	15.6 ± 2.71 (0.31)	19.6 ± 1.64 (0.30)	>250

Table B9: IC_{50} values (μM) determined using a dose-response AlphaScreen™ assay (Chapter 3, Table 3.11 continued). Compounds were serially diluted 1:2 from 500 μM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
138	>250	124 ± 32.3 (0.25)	155 ± 36.7 (0.24)	9.51 ± 1.04 (0.33)	9.01 ± 1.66 (0.32)	22.1 ± 3.59 (0.30)	81.3 ± 16.6 (0.26)	96.0 ± 24.9 (0.26)	>250
139	>250	>250	>250	>250	1.42 ± 0.131 (0.30)	3.62 ± 0.236 (0.28)	>250	>250	>250
140	>250	>250	>250	>250	>250	>250	>250	>250	>250
141	>250	>250	>250	>250	>250	>250	>250	>250	>250
142	>250	>250	>250	>250	>250	38.6 ± 1.28 (0.22)	>250	>250	>250
143	>250	>250	>250	>250	38.8 ± 1.15 (0.25)	>250	>250	>250	>250
144	>250	>250	>250	>250	>250	>250	>250	>250	>250
158	>250	>250	>250	>250	41.4 ± 0.317 (0.32)	118 ± 71.0 (0.29)	>250	182 ± 50.9 (0.28)	>250

Table B10: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 3, Table 3.13). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses. *IC_{50} values are averaged over 14 repeats.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
*90	>250	>250	>250	75.0 ± 0.810 (0.38)	3.61 ± 0.352 (0.38)	5.74 ± 0.597 (0.37)	6.74 ± 1.05 (0.36)	13.7 ± 1.53 (0.35)	66.3 ± 3.91 (0.32)
160	>250	>250	>250	>250	19.8 ± 3.56 (0.30)	43.3 ± 11.7 (0.28)	51.6 ± 5.24 (0.27)	39.2 ± 8.41 (0.28)	>250
161	>250	>250	>250	6.56 ± 1.63 (0.28)	1.56 ± 0.100 (0.31)	3.61 ± 0.290 (0.29)	5.36 ± 0.497 (0.28)	4.83 ± 0.508 (0.29)	12.1 ± 1.39 (0.26)
163	>250	>250	>250	>250	6.61 ± 0.535 (0.35)	15.2 ± 3.61 (0.32)	12.6 ± 1.04 (0.33)	12.8 ± 2.09 (0.33)	38.5 ± 17.3 (0.29)
164	>250	>250	>250	4.97 ± 0.742 (0.34)	1.52 ± 0.0940 (0.37)	2.29 ± 0.255 (0.36)	2.83 ± 0.201 (0.35)	1.62 ± 0.140 (0.37)	7.50 ± 0.543 (0.33)
165	>250	>250	>250	5.82 ± 1.17 (0.33)	5.49 ± 1.12 (0.33)	17.1 ± 1.07 (0.30)	12.2 ± 1.15 (0.31)	12.0 ± 1.12 (0.31)	51.4 ± 1.27 (0.27)
166	>250	>250	>250	43.0 ± 7.00 (0.28)	25.6 ± 6.82 (0.29)	70.3 ± 12.2 (0.26)	>250	100 ± 20.7 (0.25)	>250
(*R*)-167	45.7 ± 5.04 (0.28)	44.3 ± 3.43 (0.28)	55.6 ± 7.54 (0.27)	4.52 ± 0.618 (0.34)	3.97 ± 0.858 (0.34)	16.2 ± 1.41 (0.30)	13.8 ± 3.74 (0.31)	12.3 ± 3.13 (0.31)	>250
(*S*)-167	>250	>250	>250	7.91 ± 0.807 (0.32)	5.79 ± 0.841 (0.33)	10.3 ± 2.21 (0.32)	12.8 ± 3.04 (0.31)	9.57 ± 1.74 (0.32)	>250

Table B11: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 3, Table 3.13 continued). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses.

Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
168	>250	>250	>250	20.9 ± 1.12 (0.26)	7.96 ± 1.11 (0.29)	31.1 ± 1.28 (0.25)	30.9 ± 1.17 (0.25)	40.7 ± 1.09 (0.25)	>250
169	>250	>250	>250	11.9 ± 1.78 (0.31)	5.81 ± 0.210 (0.33)	11.6 ± 0.974 (0.31)	18.3 ± 4.38 (0.30)	15.2 ± 0.787 (0.31)	72.8 ± 30.1 (0.26)
170	>250	>250	>250	2.84 ± 0.471 (0.37)	1.60 ± 0.137 (0.39)	4.58 ± 0.231 (0.36)	7.46 ± 0.539 (0.34)	8.53 ± 0.558 (0.34)	29.7 ± 3.37 (0.30)

Table B12: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 3, Table 3.15). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$. Ligand efficiencies are given in parentheses.

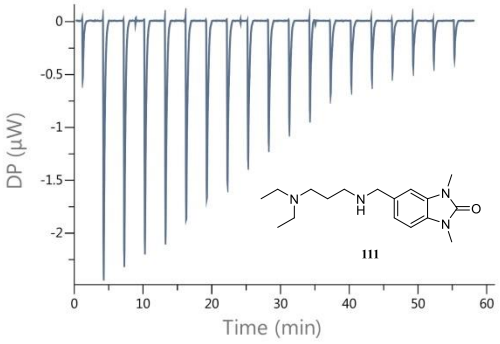
Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
161	>250	>250	>250	6.56 ± 1.63 (0.28)	1.56 ± 0.100 (0.31)	3.61 ± 0.290 (0.29)	5.36 ± 0.497 (0.28)	4.83 ± 0.508 (0.29)	12.1 ± 1.39 (0.26)
(*S*)-161	>250	>250	>250	1.97 ± 0.101 (0.31)	1.32 ± 0.0630 (0.32)	2.70 ± 0.237 (0.30)	4.32 ± 1.25 (0.29)	4.52 ± 0.596 (0.29)	29.0 ± 2.69 (0.24)
(*R*)-161	>250	>250	>250	4.44 ± 0.654 (0.28)	2.45 ± 0.241 (0.30)	5.89 ± 0.648 (0.28)	9.21 ± 2.60 (0.27)	7.75 ± 0.599 (0.28)	>250
(*S*)-179	>250	>250	>250	>250	5.94 ± 0.619 (0.28)	>250	41.9 ± 8.57 (0.24)	9.63 ± 2.00 (0.27)	96.8 ± 19.1 (0.22)
(*R*)-179	>250	>250	>250	>250	5.56 ± 0.559 (0.28)	>250	15.8 ± 1.46 (0.26)	12.2 ± 1.55 (0.26)	105 ± 25.8 (0.21)

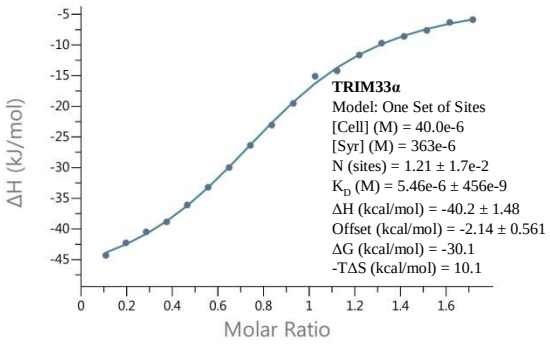
Table B13: IC_{50} values (µM) determined using a dose-response AlphaScreen™ assay (Chapter 4, Table 4.1). Compounds were serially diluted 1:2 from 500 µM highest compound concentration. Colour scale from low IC_{50} value (red) to high IC_{50} value (green). Quoted IC_{50} values are a mean of three technical repeats. H3(PHD) – $H3_{1–27}K9Me_3$, H3(BRD) – $H3_{1–27}K18Ac$, H3(DUAL) – $H3_{1–27}K9Me_3K18Ac$.

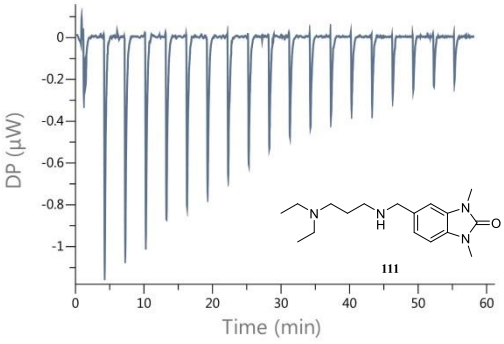
Compound	TRIM24			TRIM33α			TRIM33β		
	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)	H3(PHD)	H3(BRD)	H3(DUAL)
90	>250	>250	>250	>250	0.597 ± 0.161	1.82 ± 0.369	5.93 ± 0.867	1.82 ± 0.286	>250
227	>250	>250	>250	>250	>250	>250	>250	>250	>250
338	>250	>250	>250	>250	>250	>250	>250	>250	>250
202	>250	>250	>250	>250	2.81 ± 0.452	12.3 ± 3.00	>250	6.26 ± 1.75	>250
207	>250	>250	>250	>250	0.913 ± 0.179	3.58 ± 0.569	15. 5 ± 4.64	6.15 ± 1.28	>250

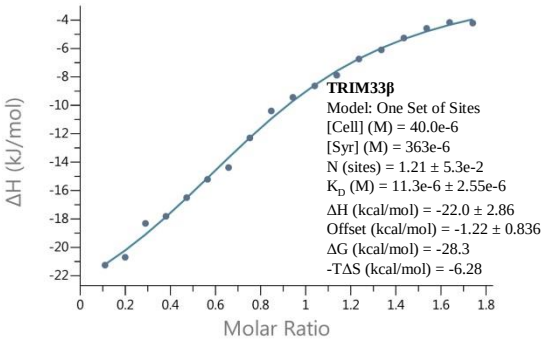
C. ITC Curves

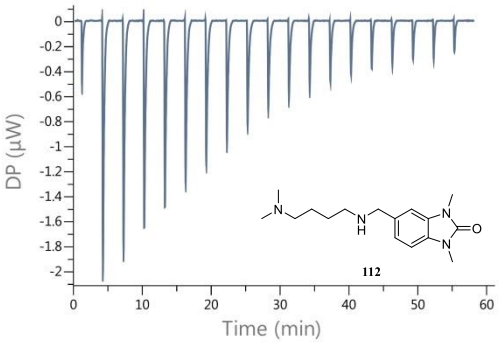
Shown are the heat effects for each injection (above) and the binding isotherms (below) including the fitted function (solid line).

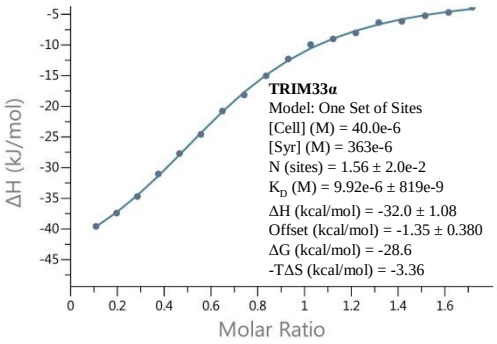

DP (µW)
Time (min)
111

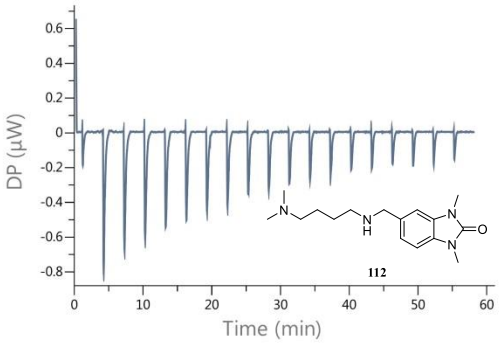

ΔH (kJ/mol)
Molar Ratio
TRIM33α
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.21 ± 1.7e-2
K_D (M) = 5.46e-6 ± 456e-9
ΔH (kcal/mol) = -40.2 ± 1.48
Offset (kcal/mol) = -2.14 ± 0.561
ΔG (kcal/mol) = -30.1
-TΔS (kcal/mol) = 10.1

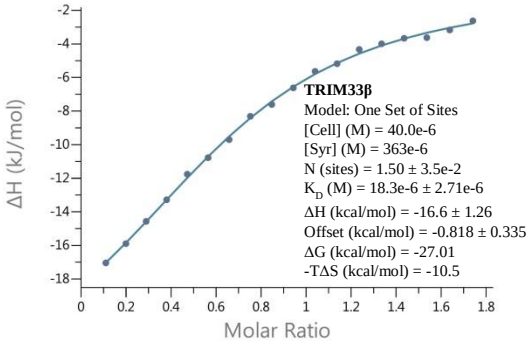

DP (µW)
Time (min)
111

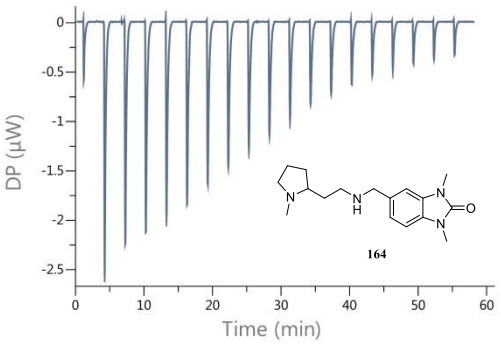

ΔH (kJ/mol)
Molar Ratio
TRIM33β
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.21 ± 5.3e-2
K_D (M) = 11.3e-6 ± 2.55e-6
ΔH (kcal/mol) = -22.0 ± 2.86
Offset (kcal/mol) = -1.22 ± 0.836
ΔG (kcal/mol) = -28.3
-TΔS (kcal/mol) = -6.28

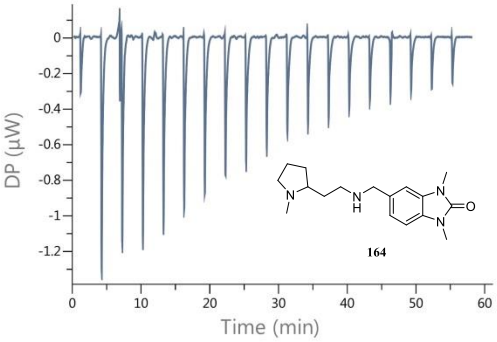

DP (µW)
Time (min)
112

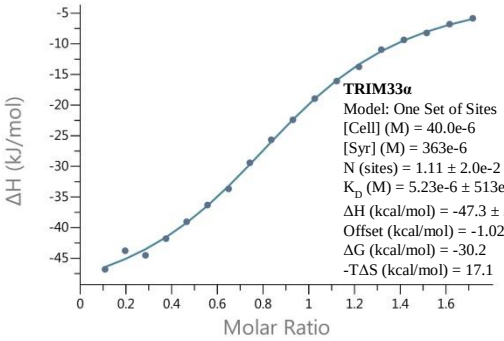

ΔH (kJ/mol)
Molar Ratio
TRIM33α
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.56 ± 2.0e-2
K_D (M) = 9.92e-6 ± 819e-9
ΔH (kcal/mol) = -32.0 ± 1.08
Offset (kcal/mol) = -1.35 ± 0.380
ΔG (kcal/mol) = -28.6
-TΔS (kcal/mol) = -3.36

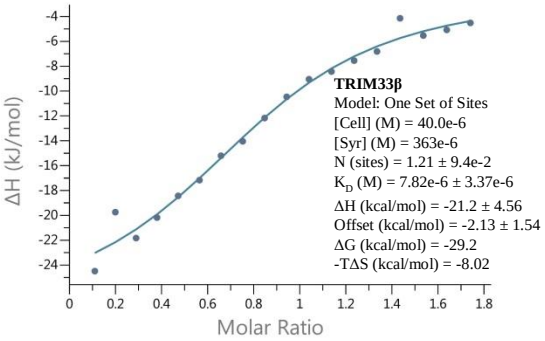

DP (µW)
Time (min)
112

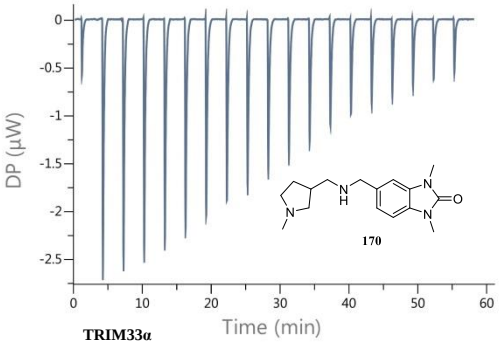

ΔH (kJ/mol)
Molar Ratio
TRIM33β
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.50 ± 3.5e-2
K_D (M) = 18.3e-6 ± 2.71e-6
ΔH (kcal/mol) = -16.6 ± 1.26
Offset (kcal/mol) = -0.818 ± 0.335
ΔG (kcal/mol) = -27.01
-TΔS (kcal/mol) = -10.5

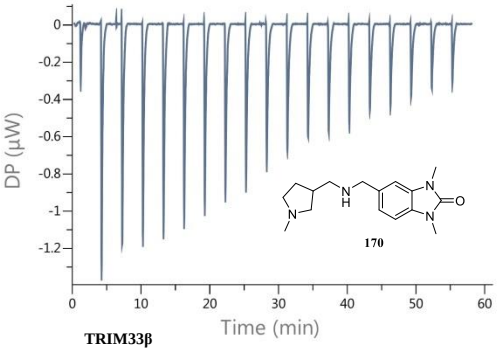

DP (µW)
Time (min)
164

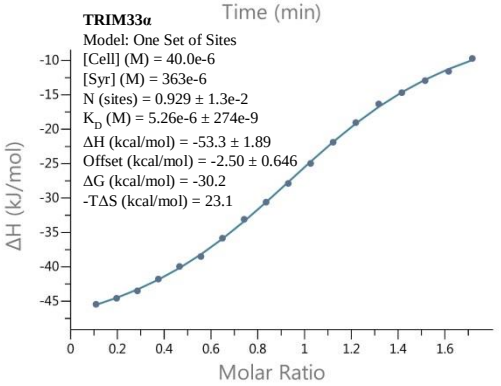

DP (µW)
Time (min)
164

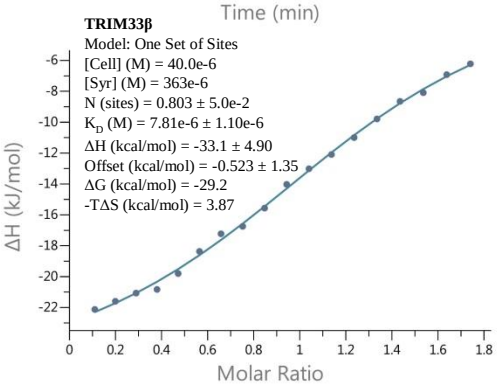

ΔH (kJ/mol)
Molar Ratio
TRIM33α
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 1.11 ± 2.0e-2
K_D (M) = 5.23e-6 ± 513e-9
ΔH (kcal/mol) = -47.3 ± 2.28
Offset (kcal/mol) = -1.02 ± 0.843
ΔG (kcal/mol) = -30.2
-TΔS (kcal/mol) = 17.1


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


DP (µW)
Time (min)
170

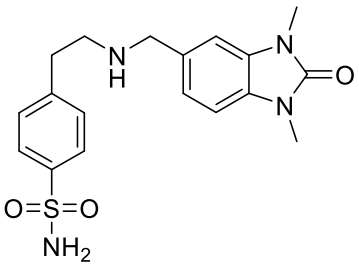

DP (µW)
Time (min)
170


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

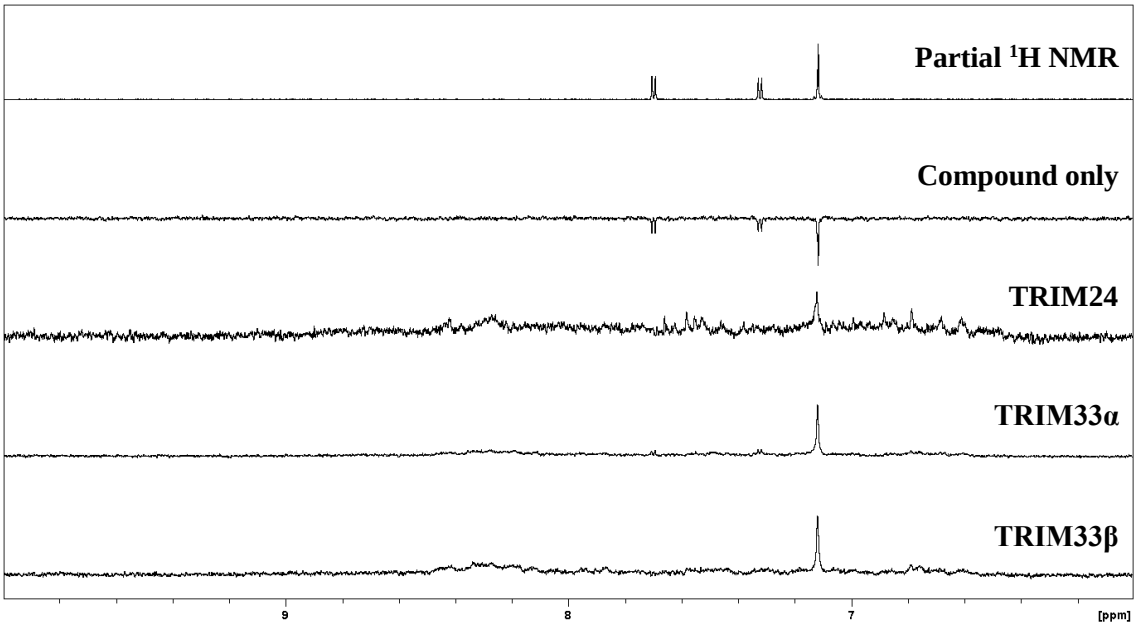

ΔH (kJ/mol)
Molar Ratio
TRIM33β
Model: One Set of Sites
[Cell] (M) = 40.0e-6
[Syr] (M) = 363e-6
N (sites) = 0.803 ± 5.0e-2
K_D (M) = 7.81e-6 ± 1.10e-6
ΔH (kcal/mol) = -33.1 ± 4.90
Offset (kcal/mol) = -0.523 ± 1.35
ΔG (kcal/mol) = -29.2
-TΔS (kcal/mol) = 3.87

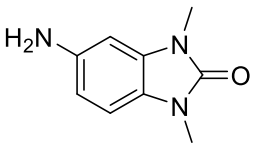
D. WaterLOGSY NMR Spectra

Water-supressed ^{1}H NMR spectra and waterLOGSY NMR spectra with and without protein are presented.

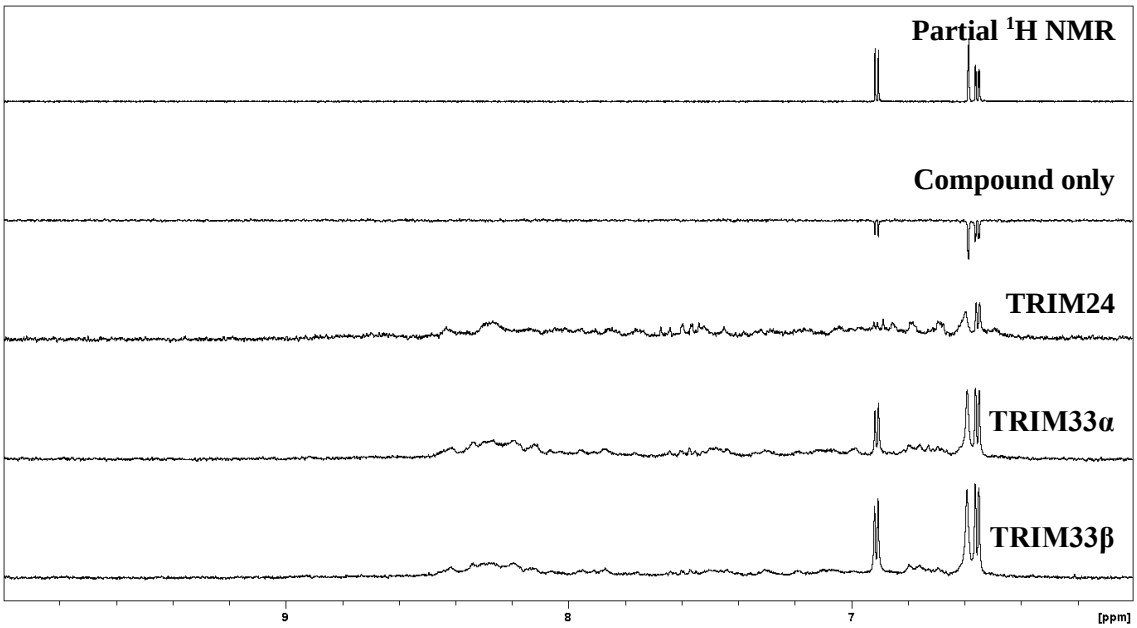


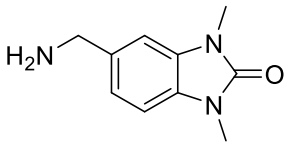
23


Partial ¹H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
9
8
7
[ppm]

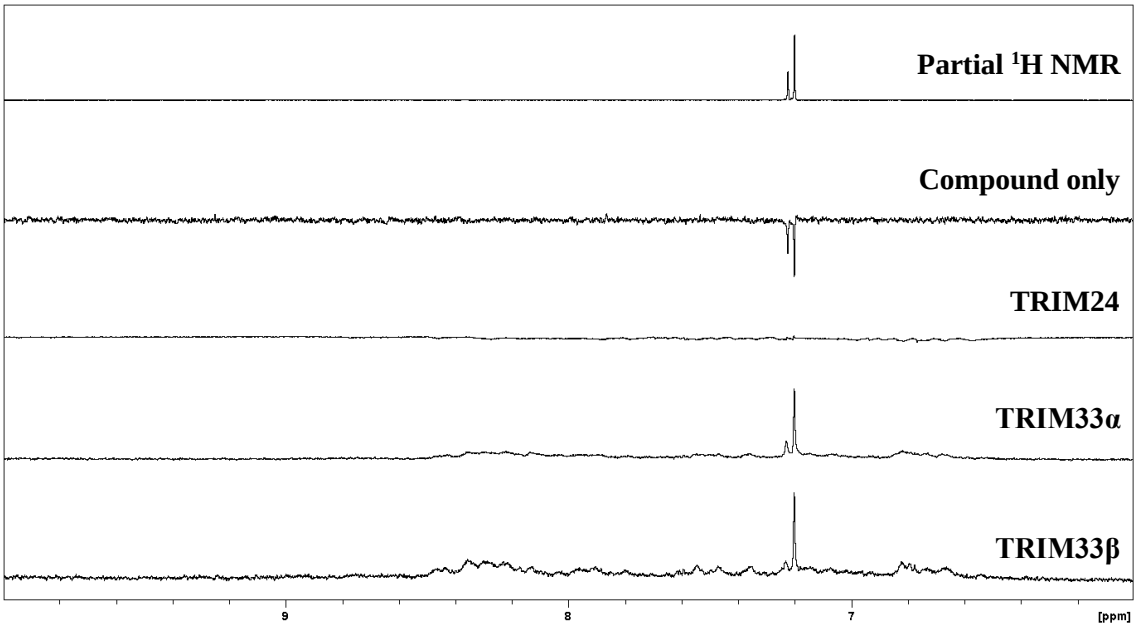


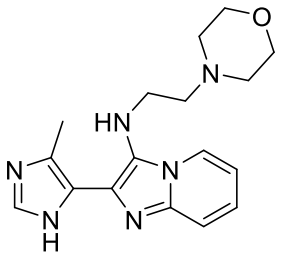
25


Partial ¹H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
9
8
7
[ppm]

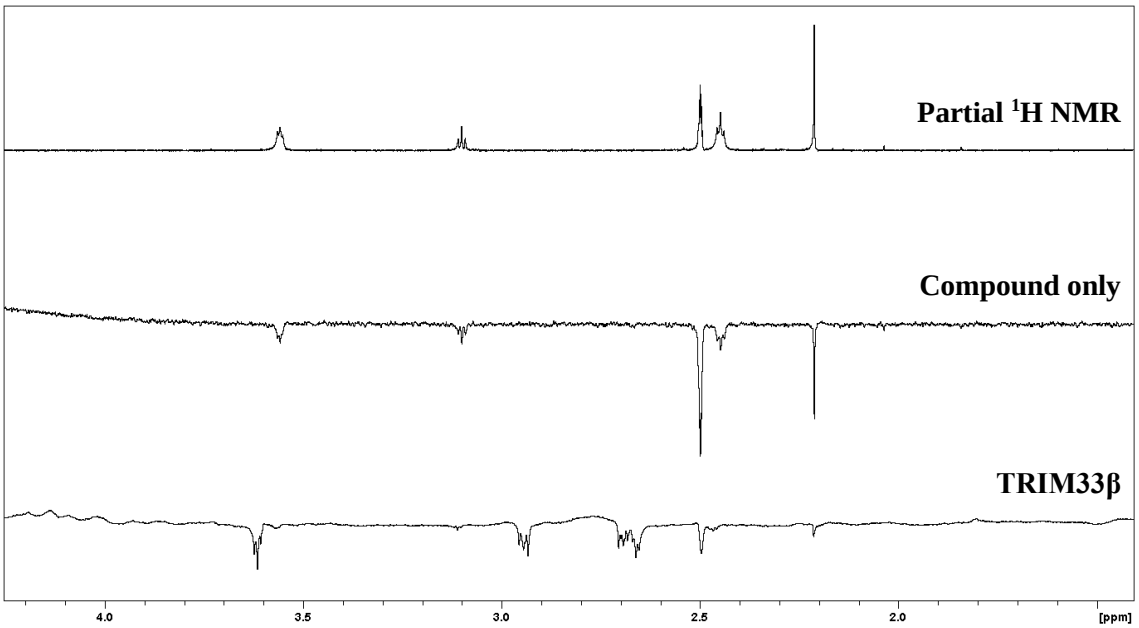

H2N
N
O
N

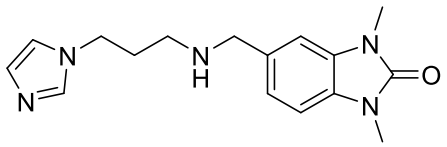
26


Partial 1H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
9
8
7
[ppm]

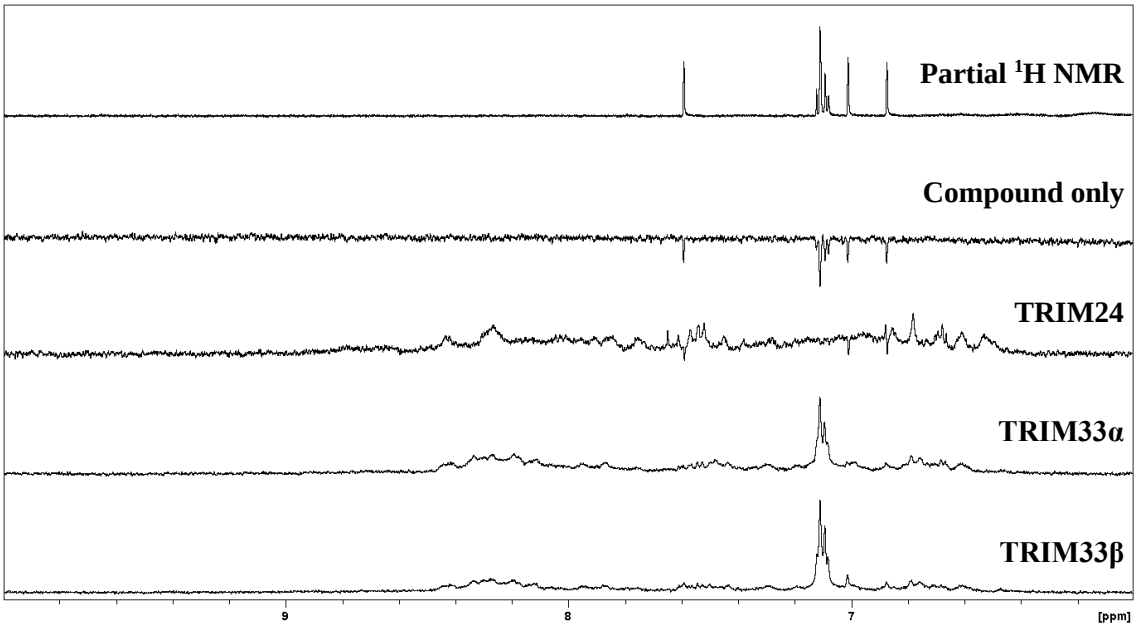

O
N
HN
N
N
N
H

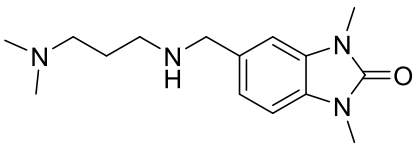
79


Partial 1H NMR
Compound only
TRIM33β
4.0
3.5
3.0
2.5
2.0
[ppm]

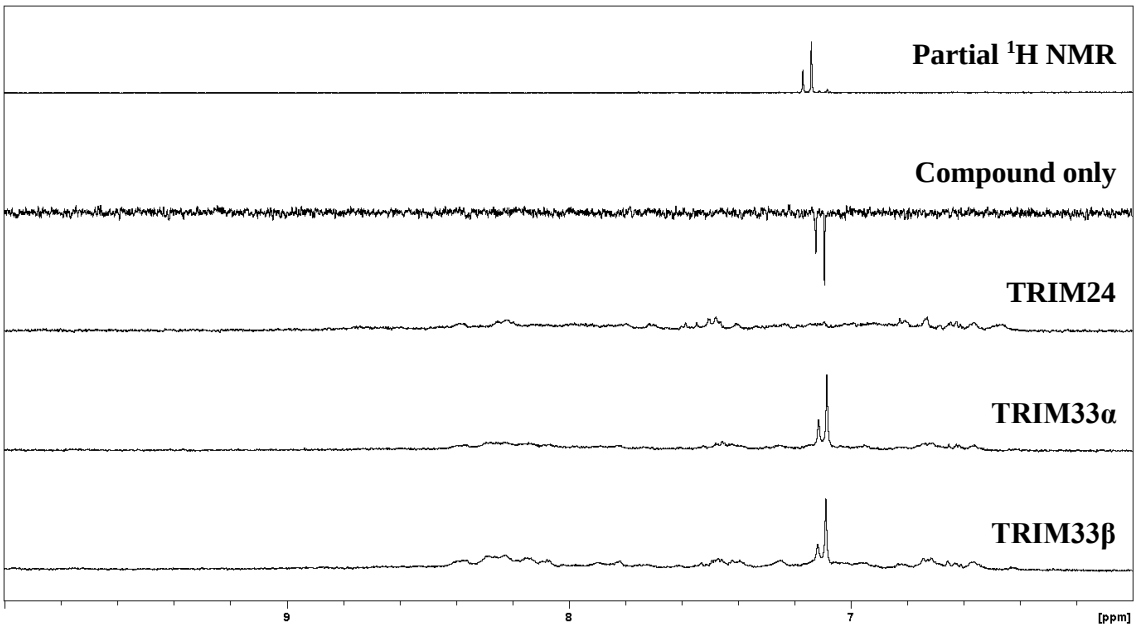


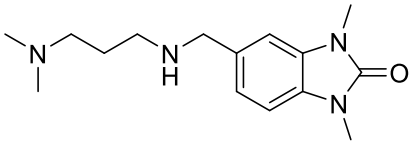
89


Partial ¹H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
9
8
7
[ppm]

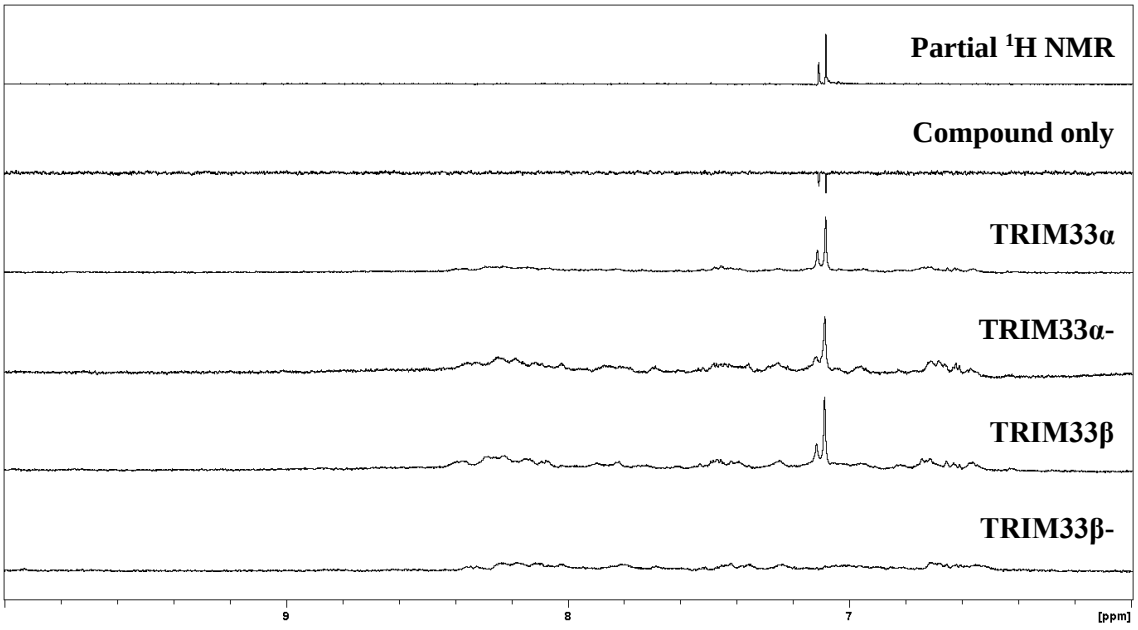


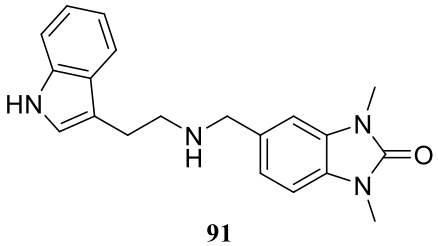
90


Partial ¹H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
9
8
7
[ppm]



90


Partial ¹H NMR
Compound only
TRIM33α
TRIM33α-
TRIM33β
TRIM33β-
9
8
7
[ppm]



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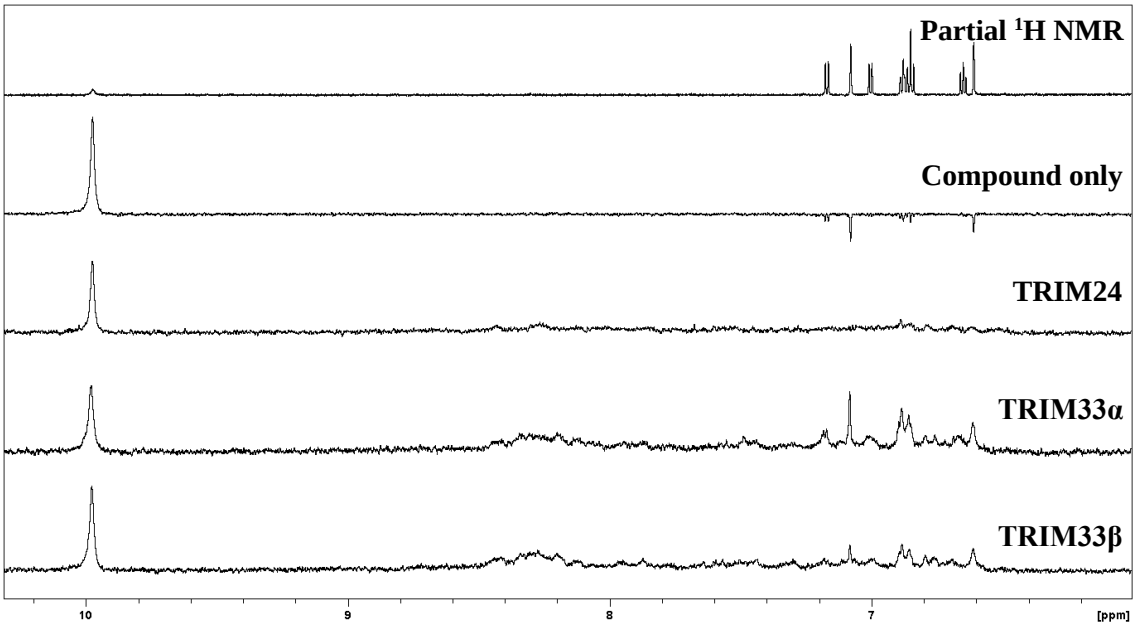
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Compound only
TRIM24
TRIM33α
TRIM33β
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9
8
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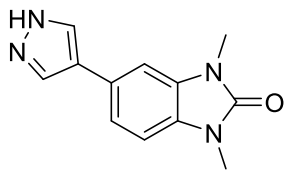

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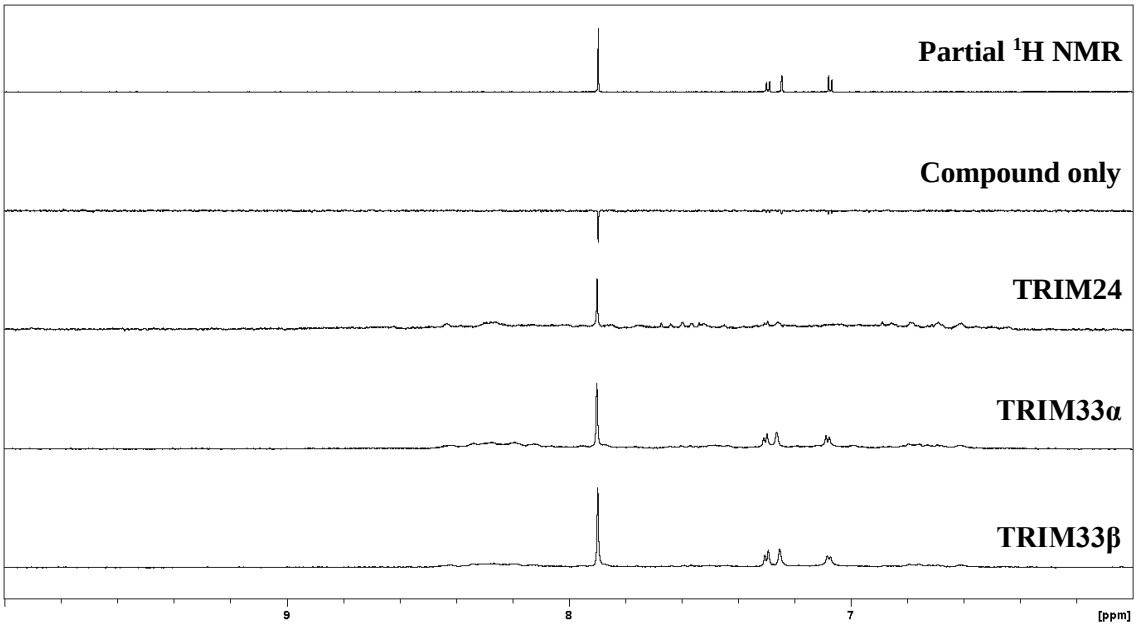
Partial ^{1}H NMR
Compound only
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TRIM33α
TRIM33β
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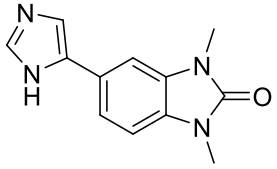

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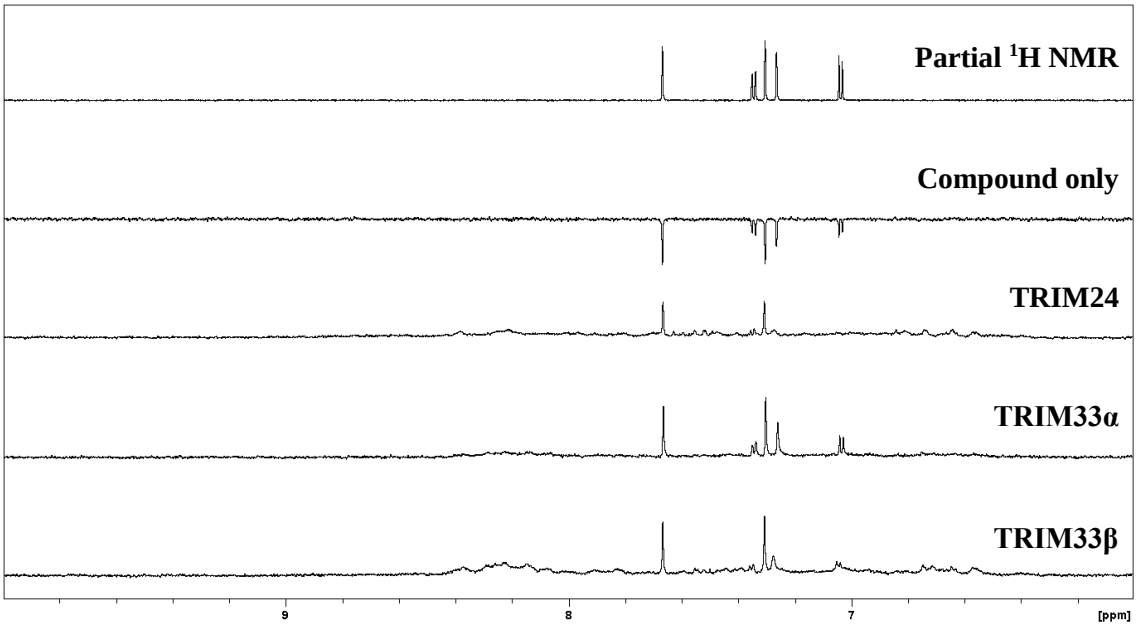
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Compound only
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TRIM33α
TRIM33β
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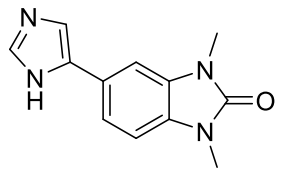

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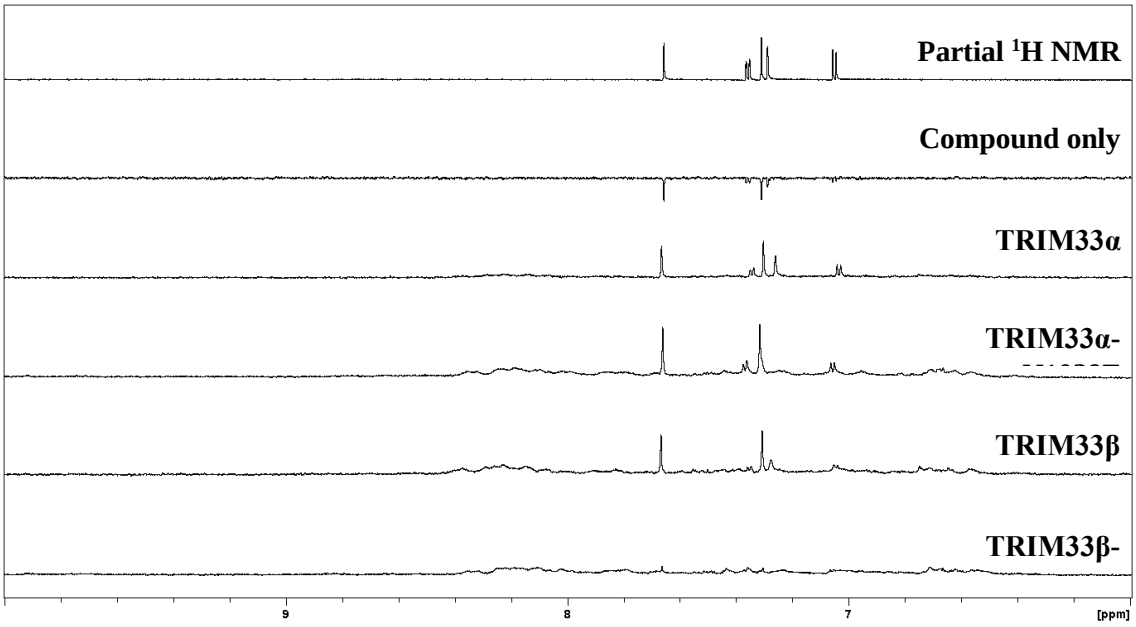
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Compound only
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TRIM33α-
TRIM33β
TRIM33β-
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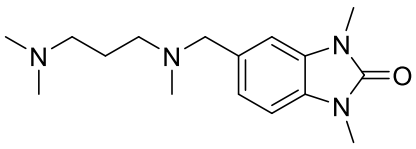

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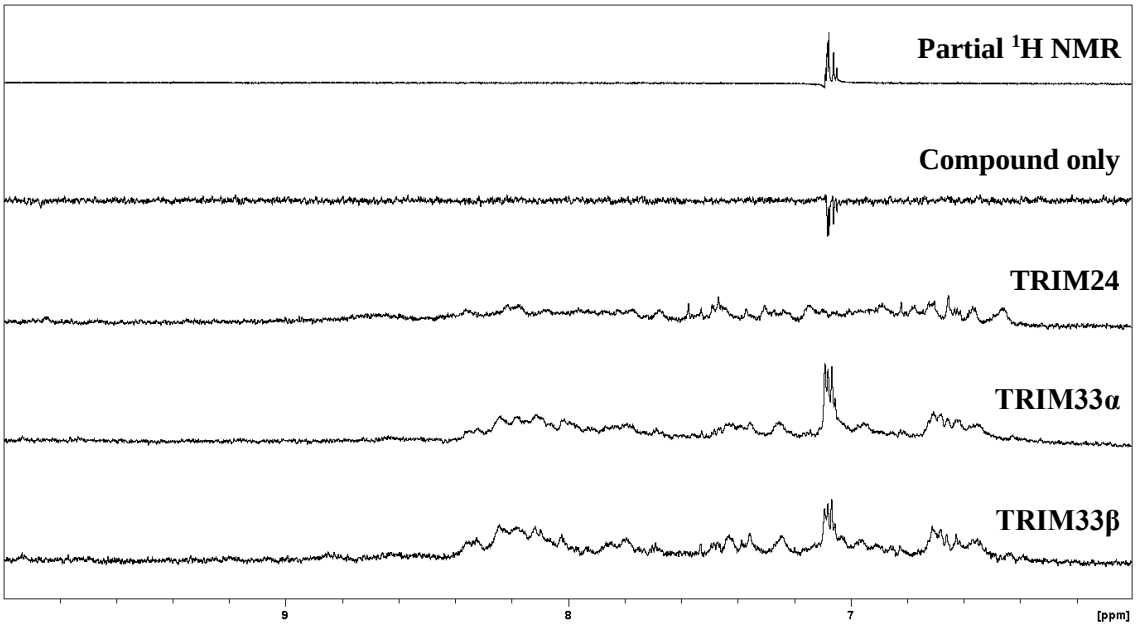
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Compound only
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TRIM33α
TRIM33β
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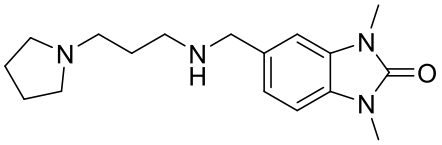

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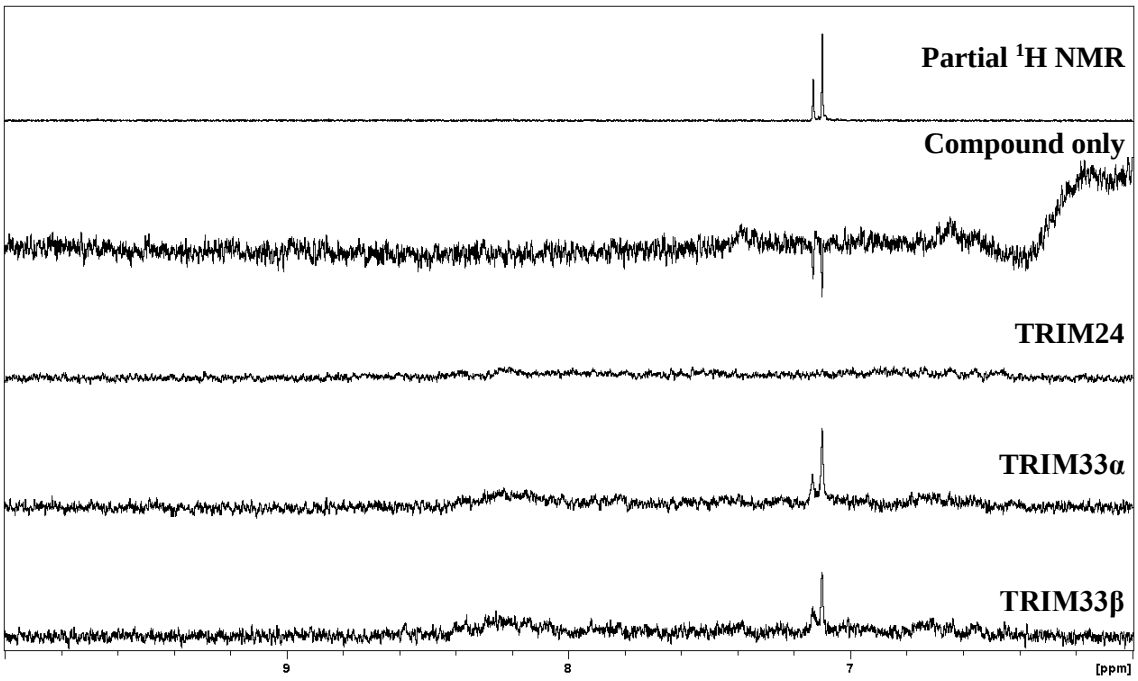
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Compound only
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TRIM33α
TRIM33β
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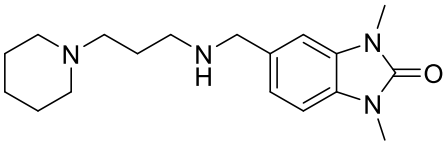

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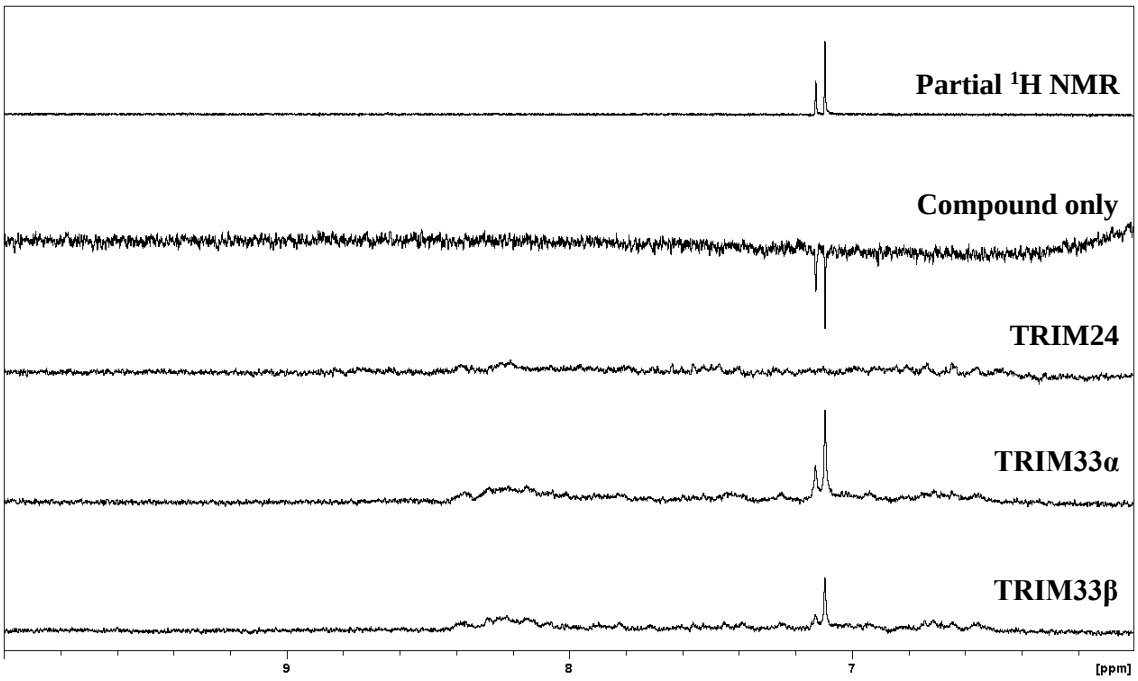
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Compound only
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TRIM33α
TRIM33β
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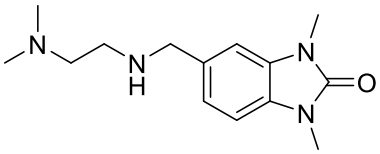

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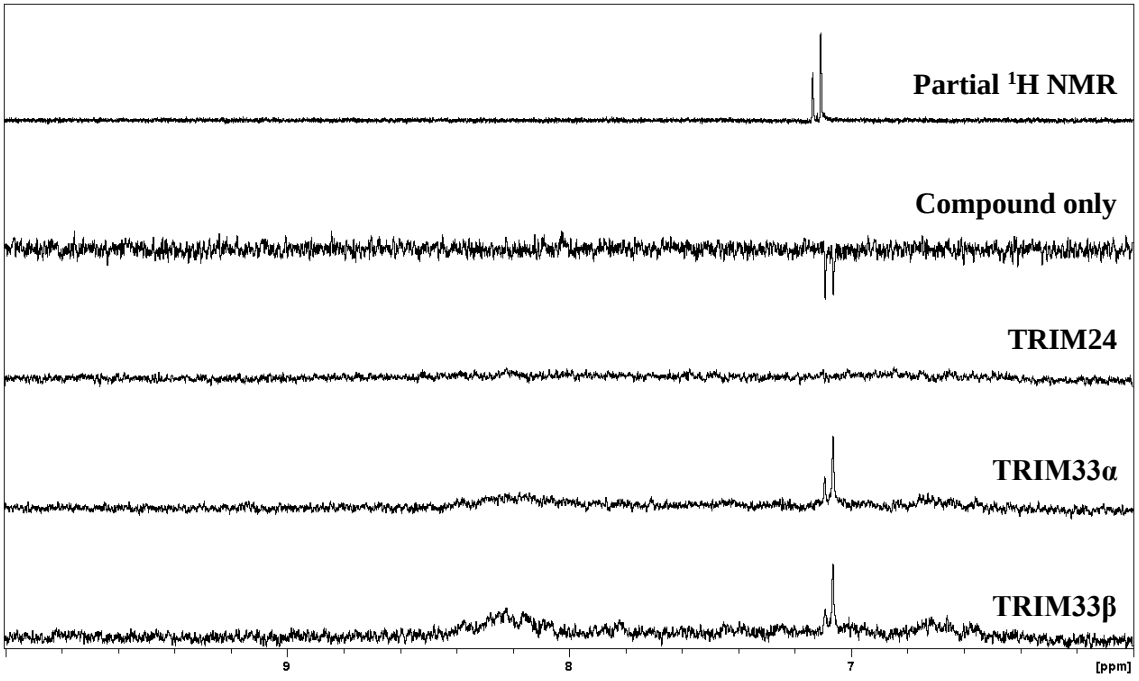
Partial ^{1}H NMR
Compound only
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TRIM33α
TRIM33β
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8
7
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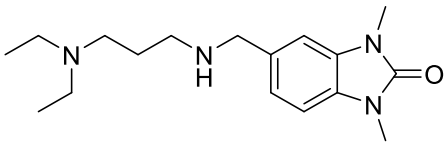

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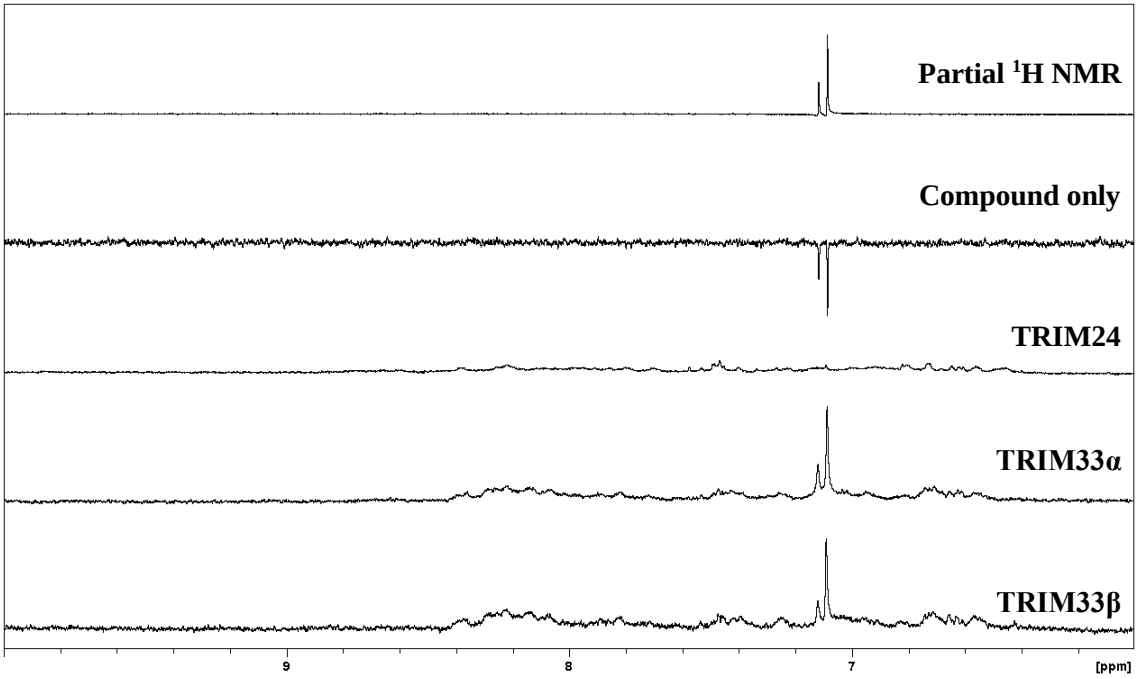
Partial ^{1}H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
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8
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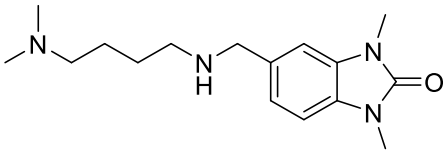
[ppm]



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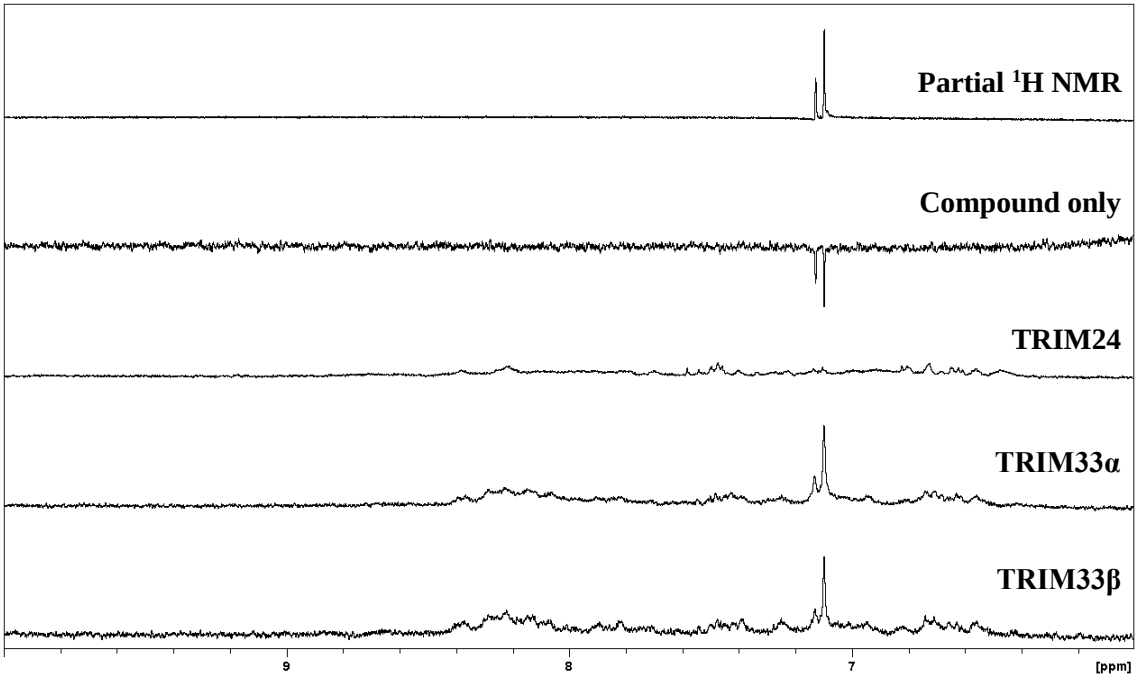
Partial ^{1}H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
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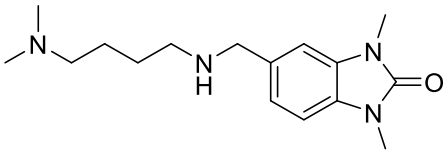
8
7
[ppm]



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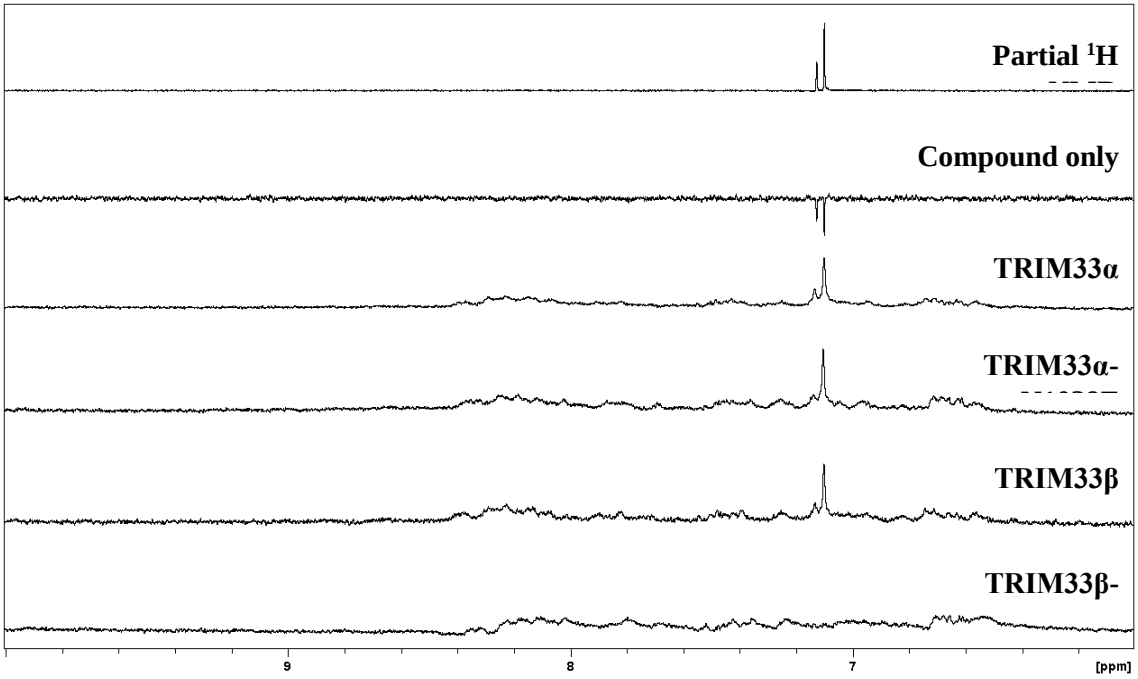
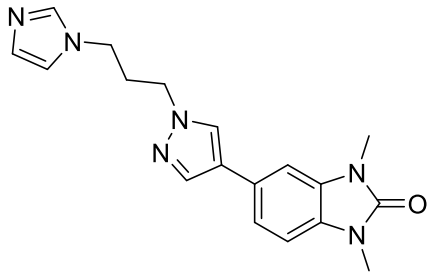

Partial ^{1}H
Compound only
TRIM33α
TRIM33α-
TRIM33β
TRIM33β-
9
8
7
[ppm]



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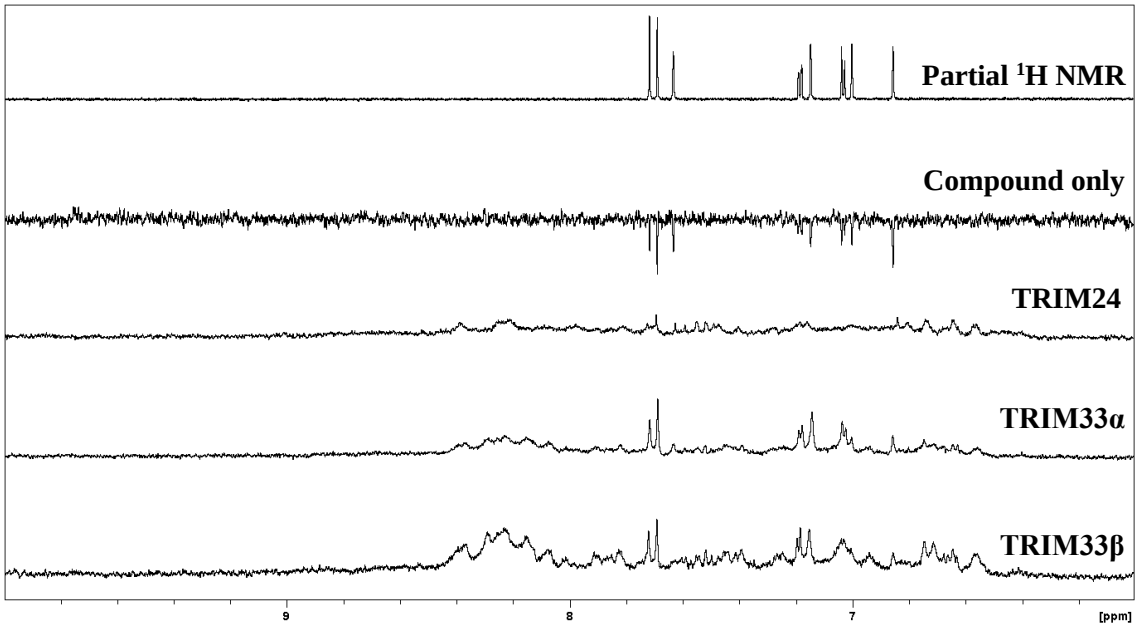
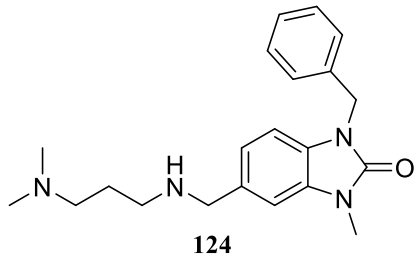

Partial ¹H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
9
8
7
[ppm]



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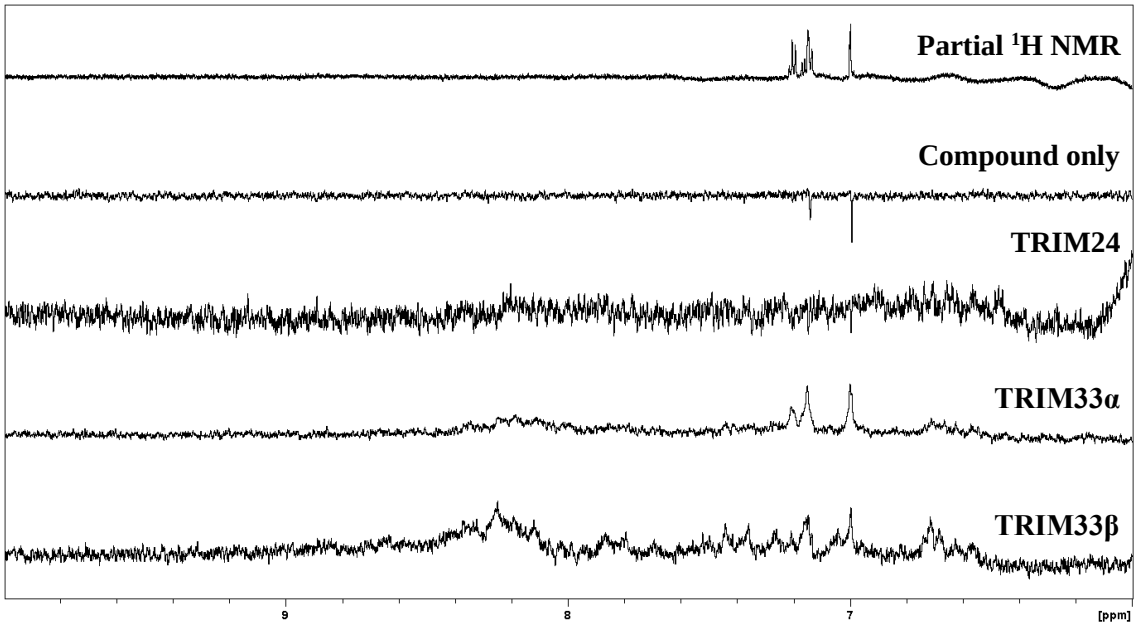
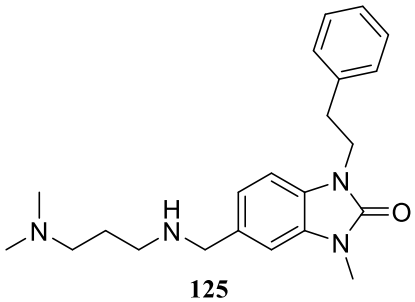

Partial ¹H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
9
8
7
[ppm]



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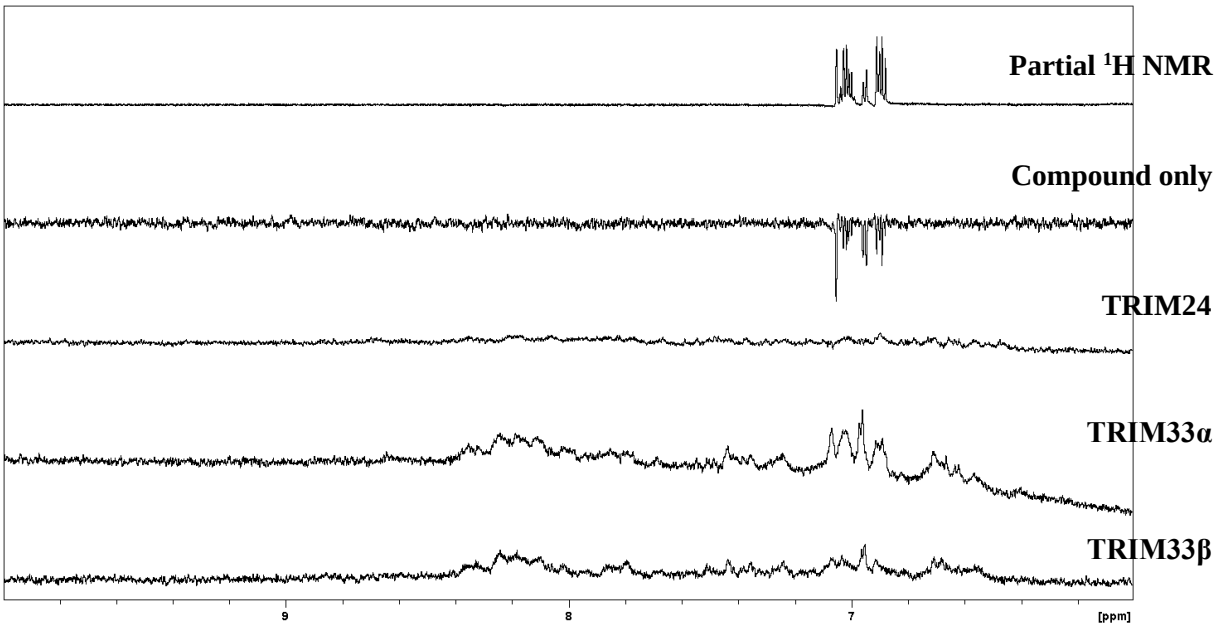
Partial ¹H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
9
8
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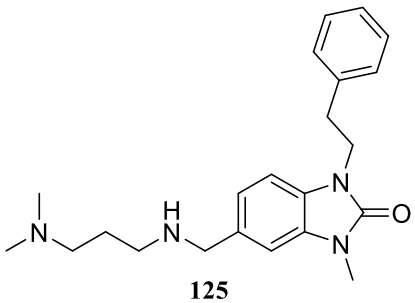
[ppm]



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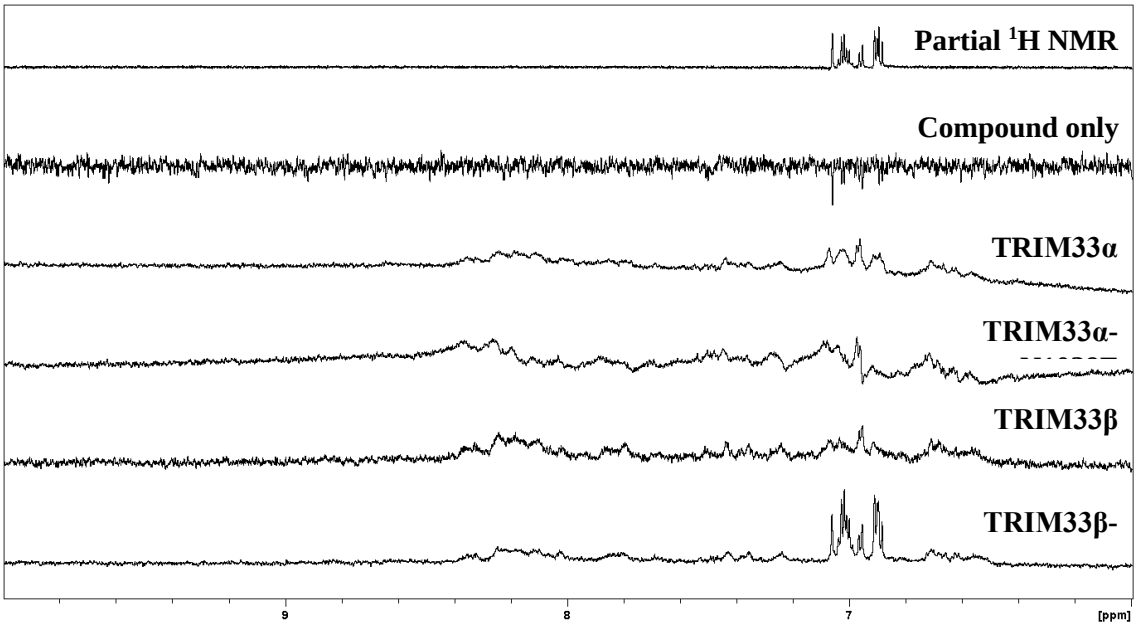
Partial ¹H NMR
Compound only
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TRIM33α-
TRIM33β
TRIM33β-
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7
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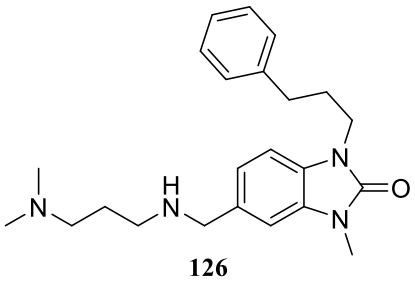

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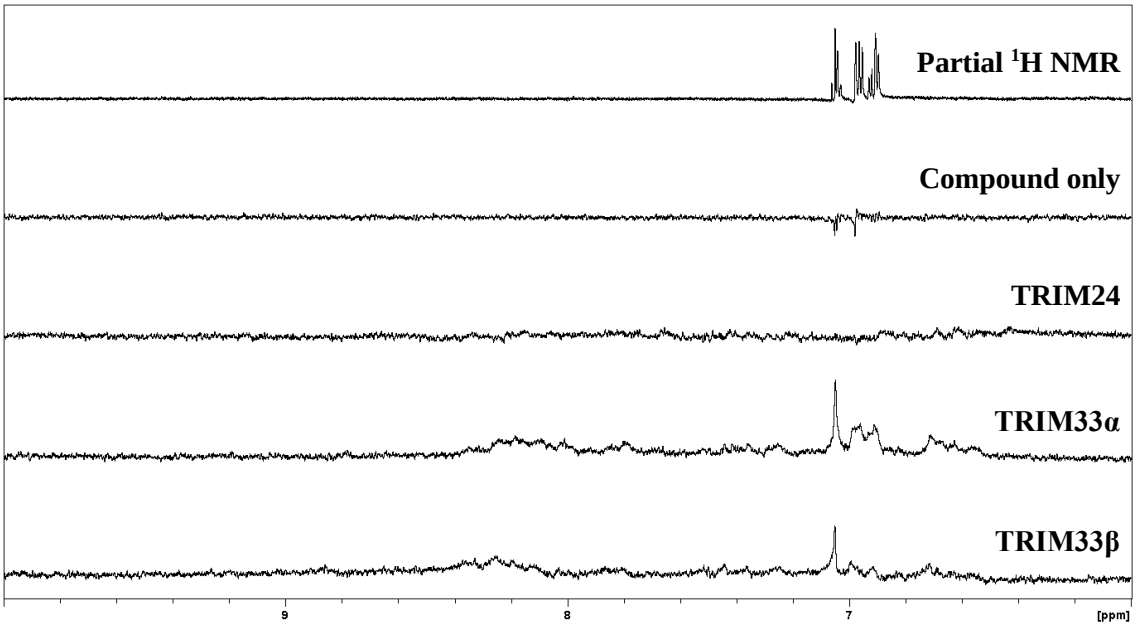
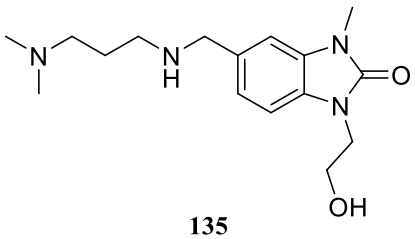

Partial ¹H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
9
8
7
[ppm]



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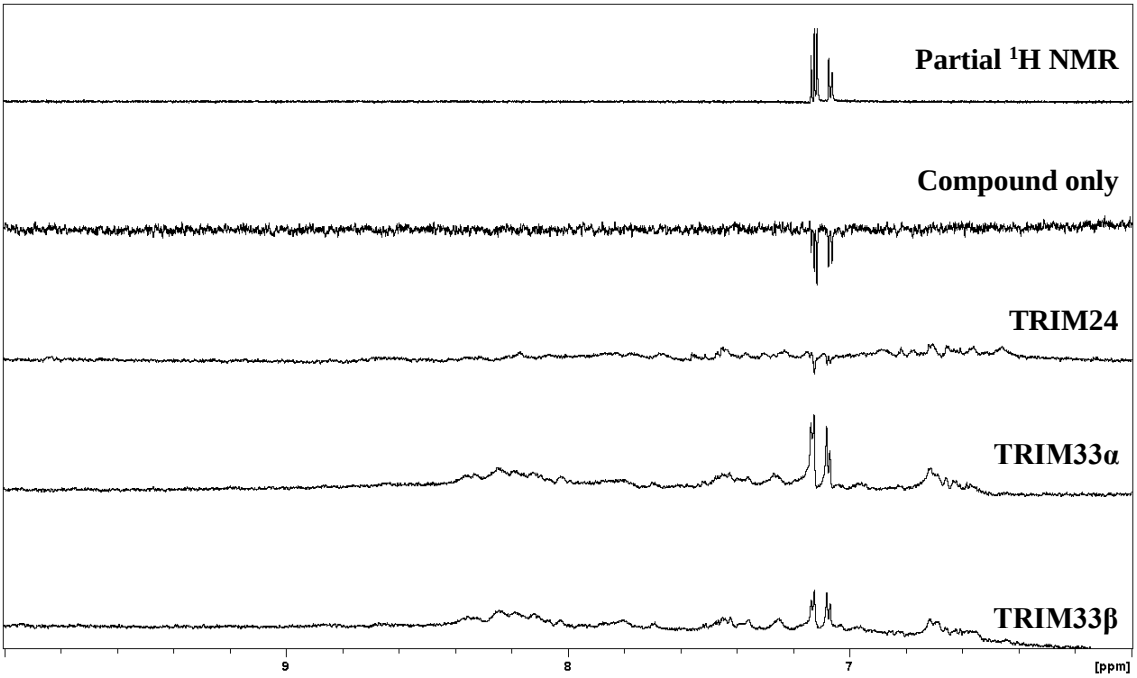
Partial ¹H NMR
Compound only
TRIM24
TRIM33α
TRIM33β
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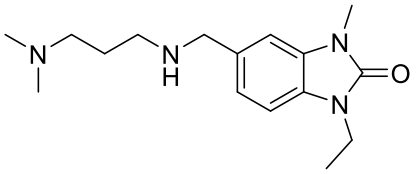

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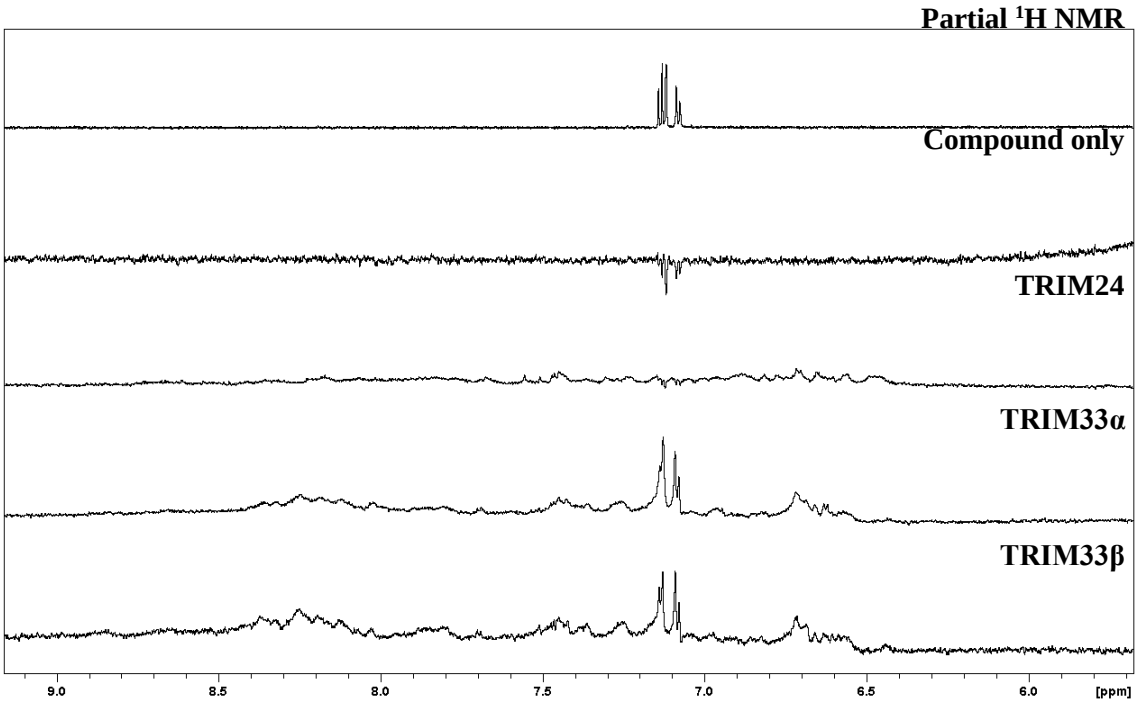
Partial ^{1}H NMR
Compound only
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TRIM33α
TRIM33β
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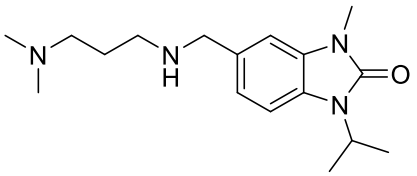
6.5
6.0
[ppm]



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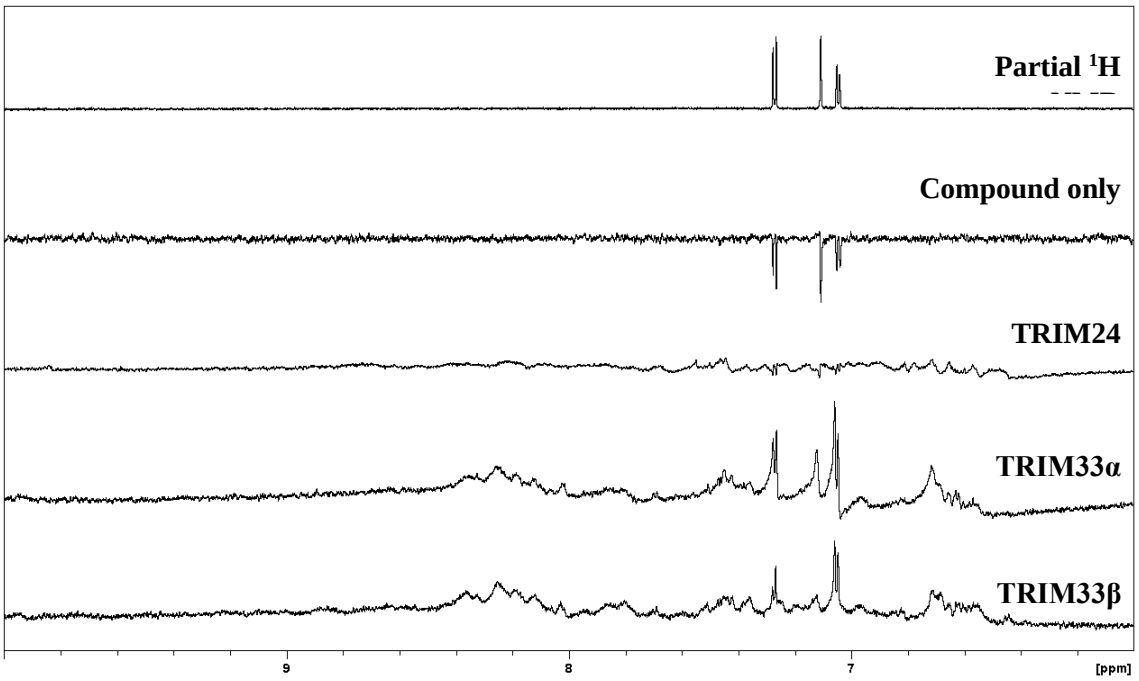
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Compound only
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TRIM33α
TRIM33β
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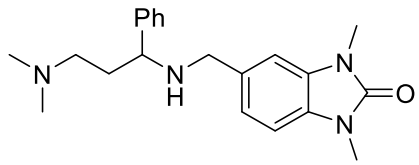
8
7
[ppm]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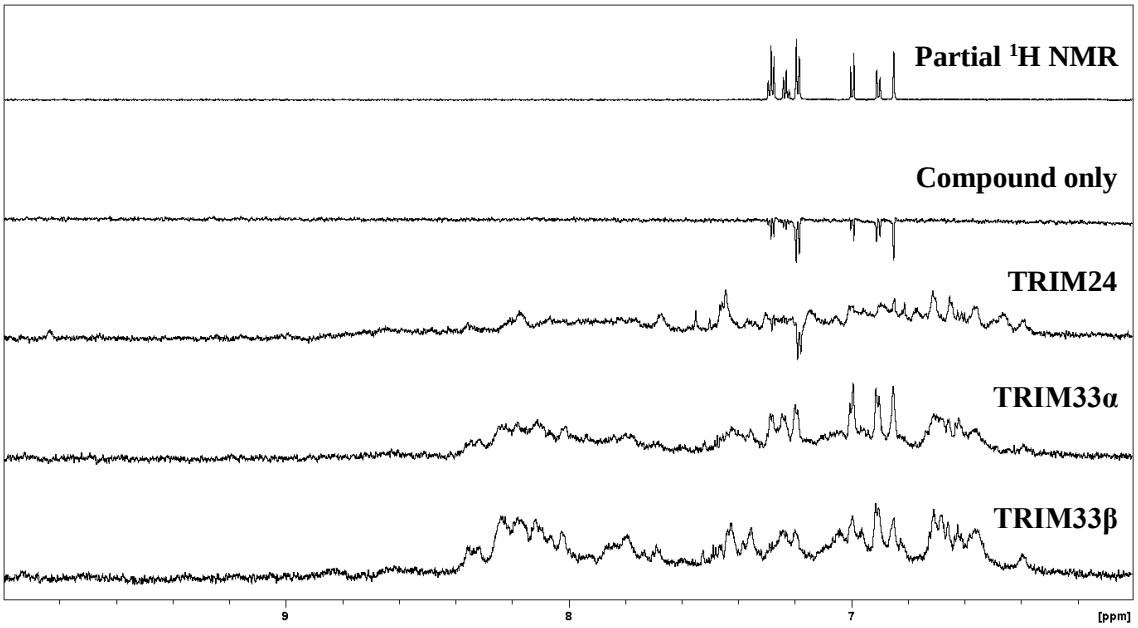
N
H
N
O
N

161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Compound only
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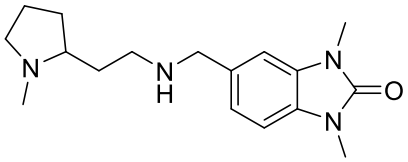
TRIM33α
TRIM33β
9
8
7
[ppm]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H
N
O
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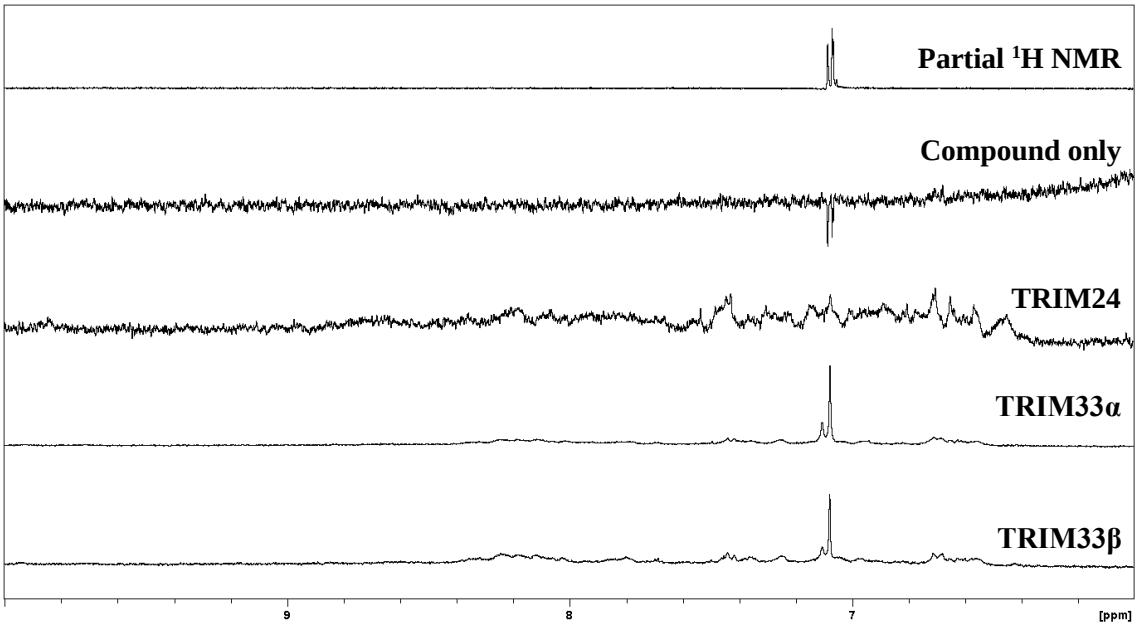
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Compound only
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TRIM33α
TRIM33β
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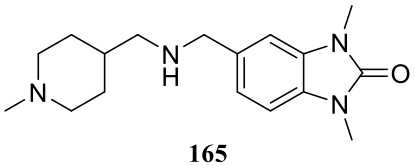

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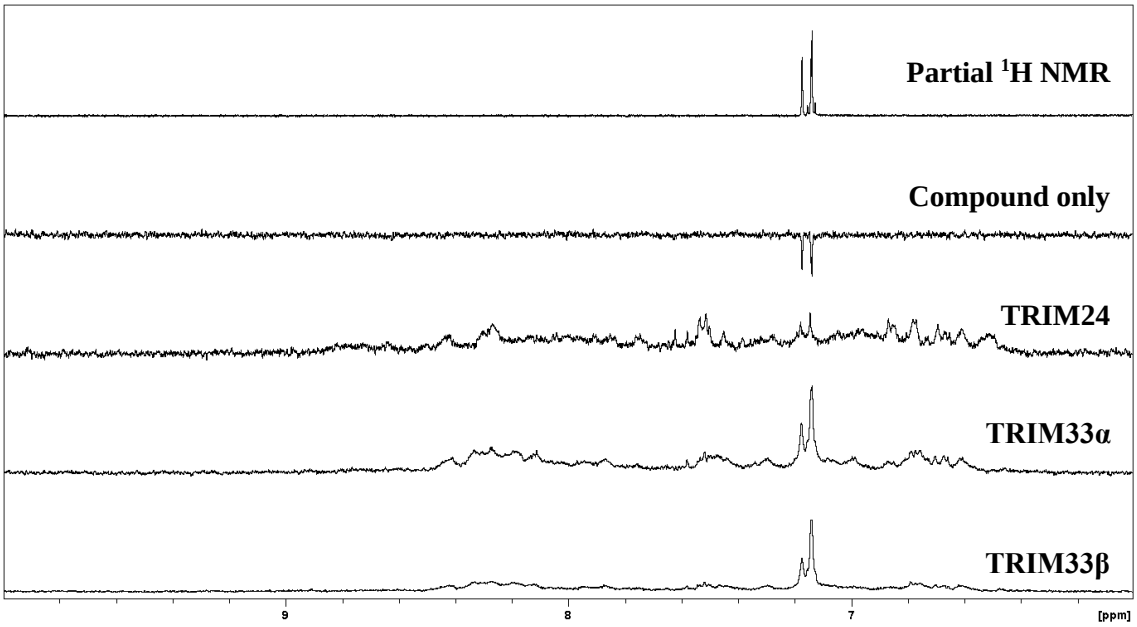
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Compound only
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TRIM33α
TRIM33β
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8
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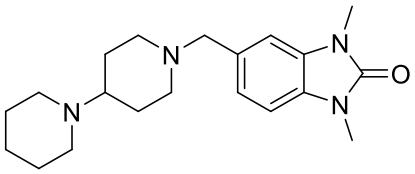

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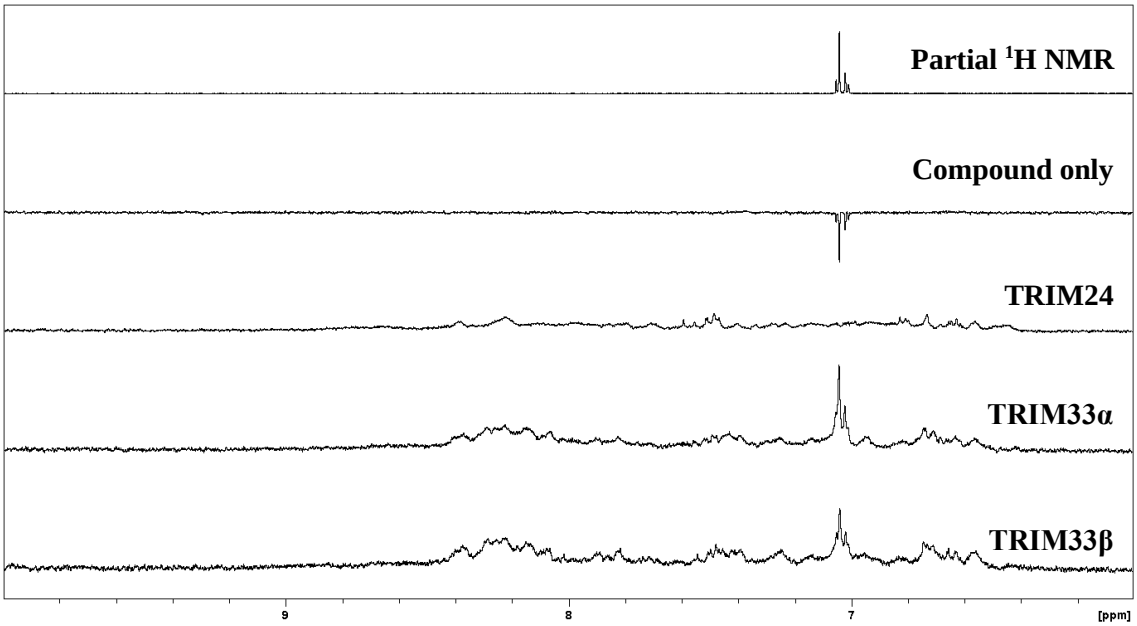
Partial ^{1}H NMR
Compound only
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TRIM33α
TRIM33β
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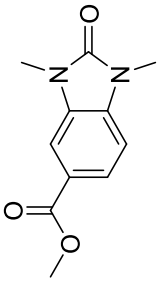
8
7
[ppm]

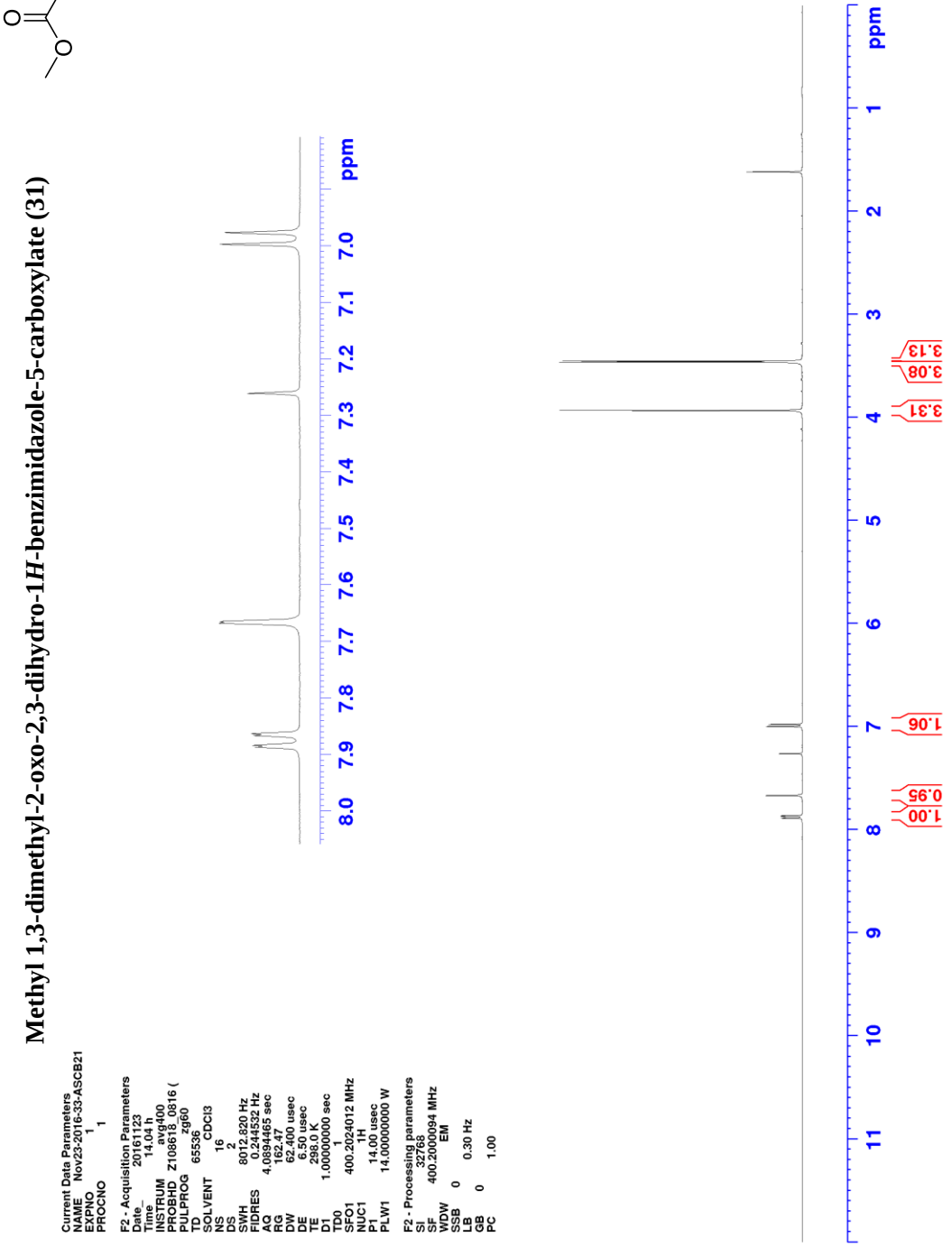
E. NMR Spectra of Novel Compounds

^{1}H, ^{13}C and ^{19}F (where relevant) NMR spectra are presented for all novel compounds.

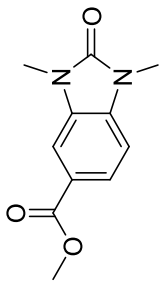
Chapter 2 Compounds

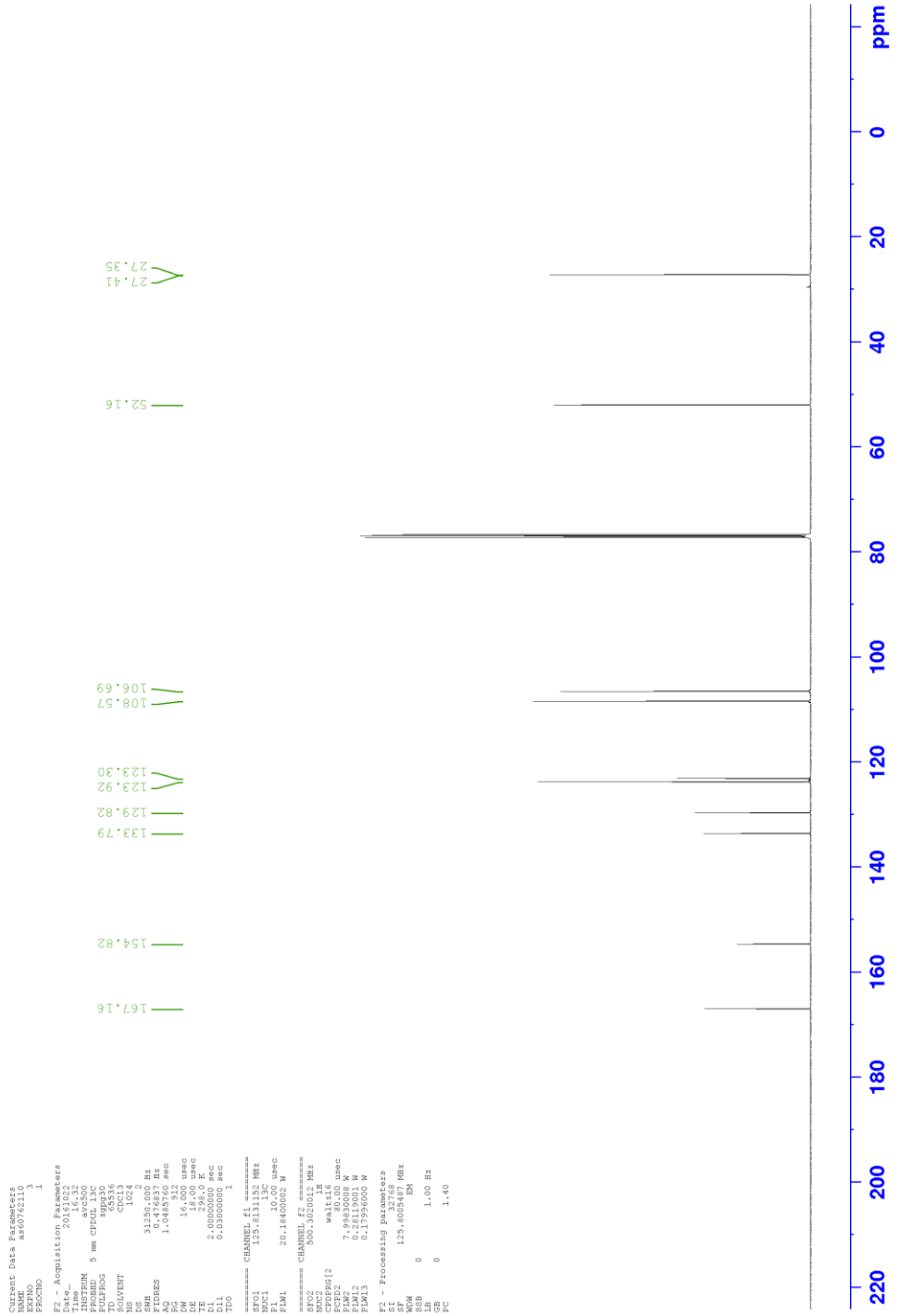


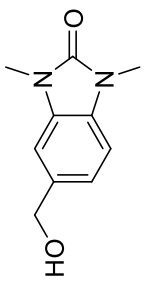
Methyl 1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (31)



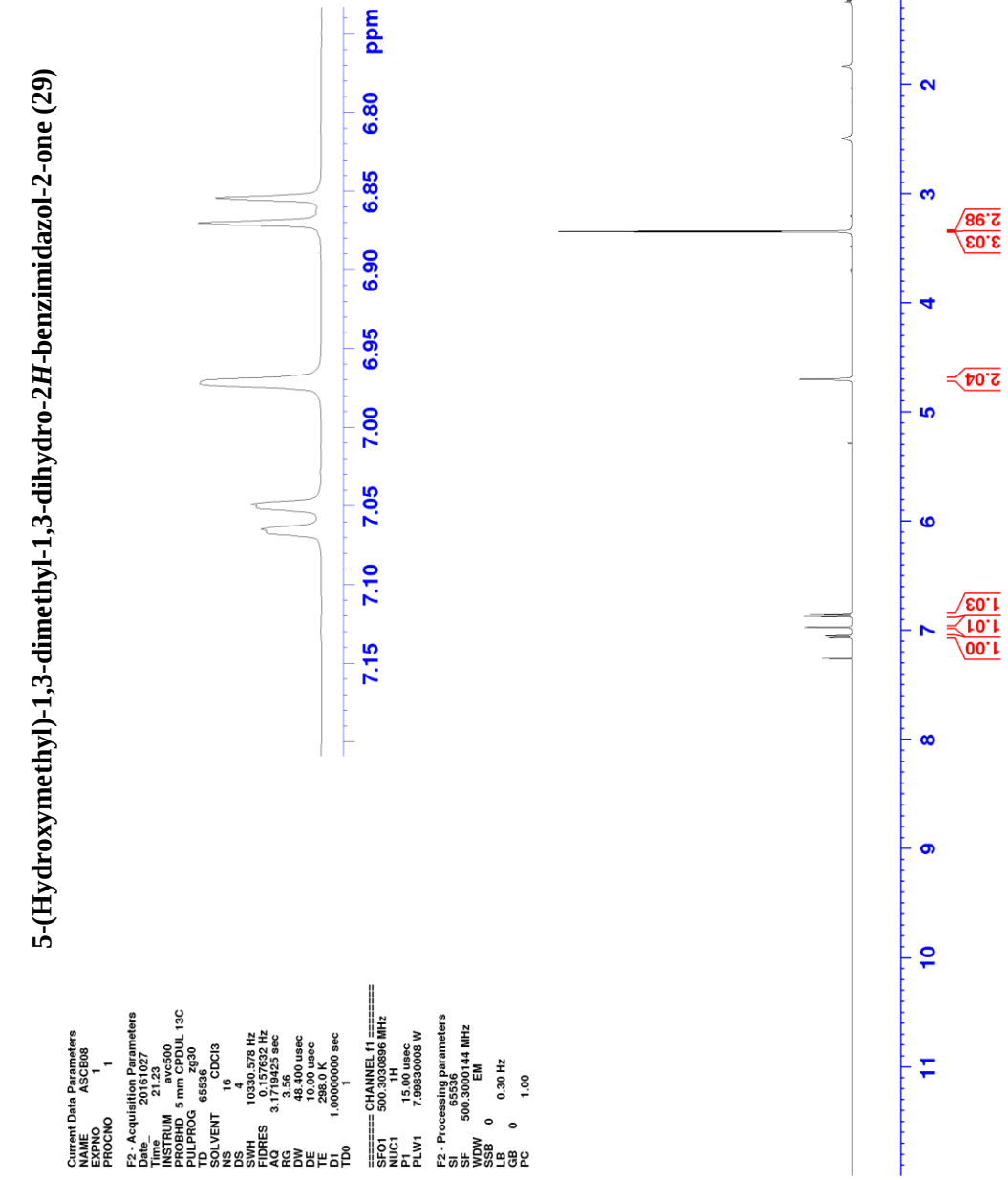
Methyl 1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (31)



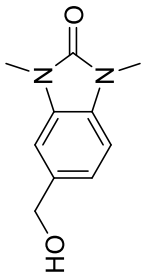


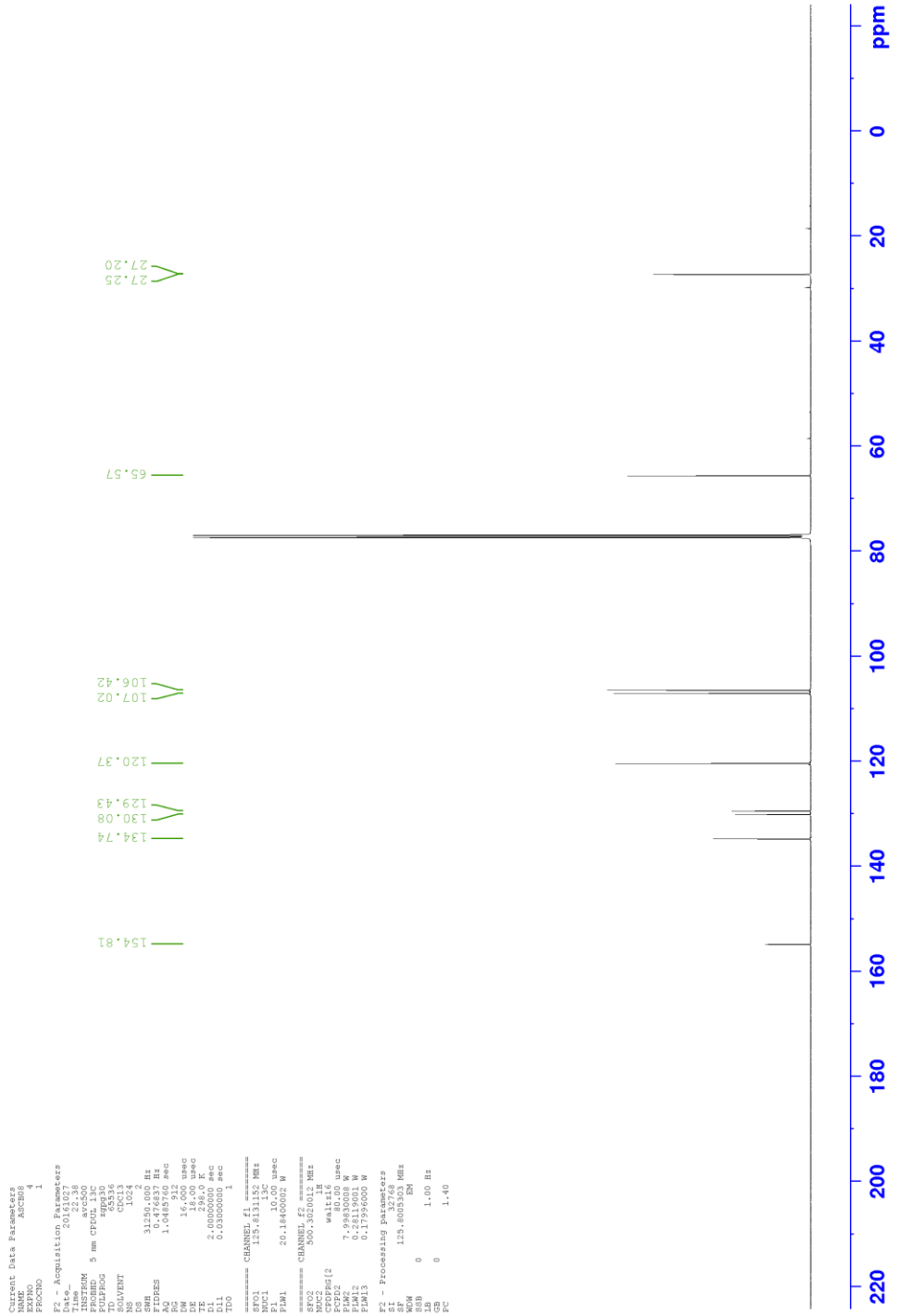


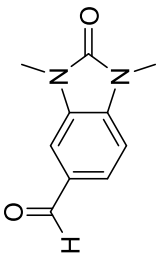
5-(Hydroxymethyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (29)



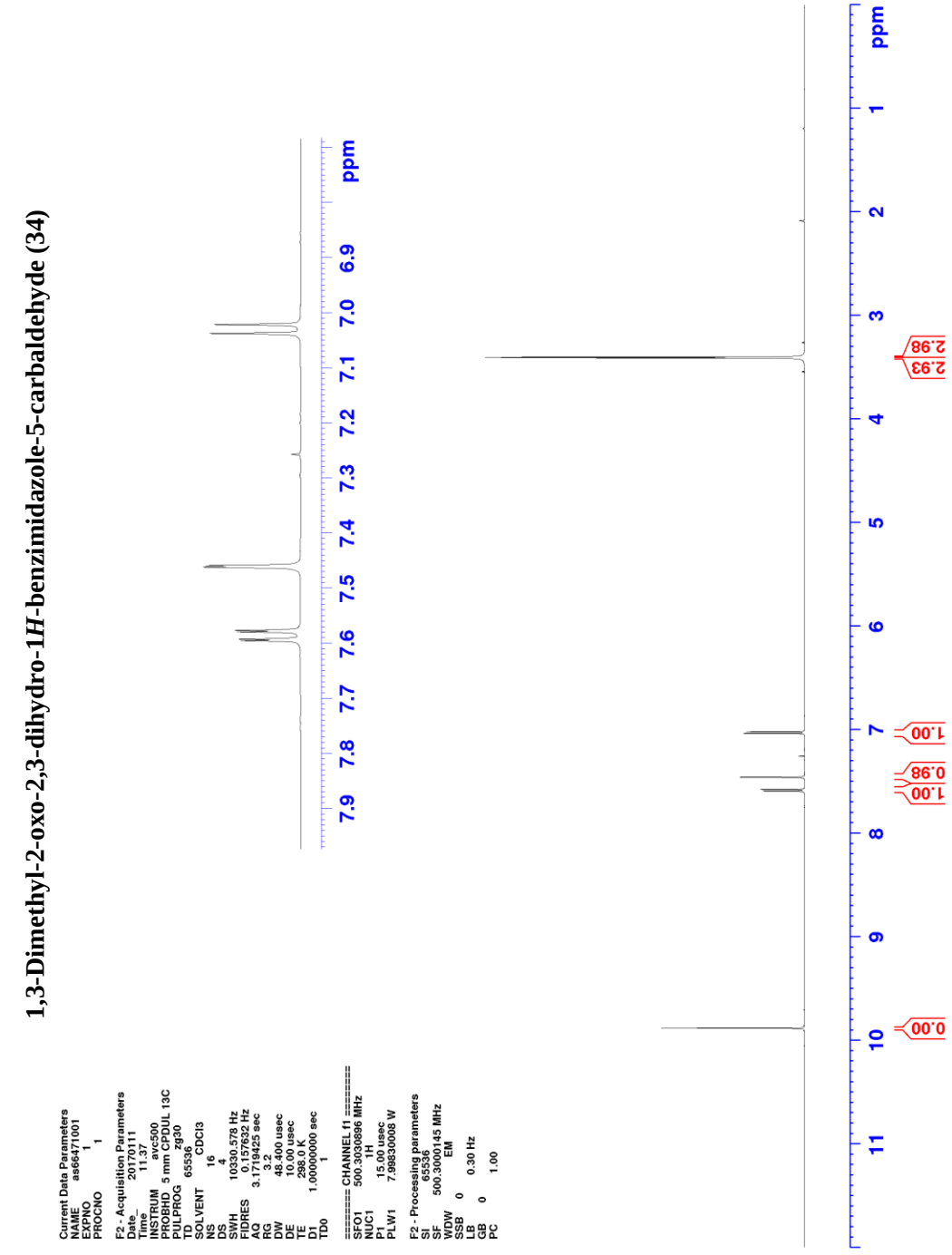
5-(Hydroxymethyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (29)



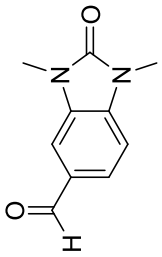


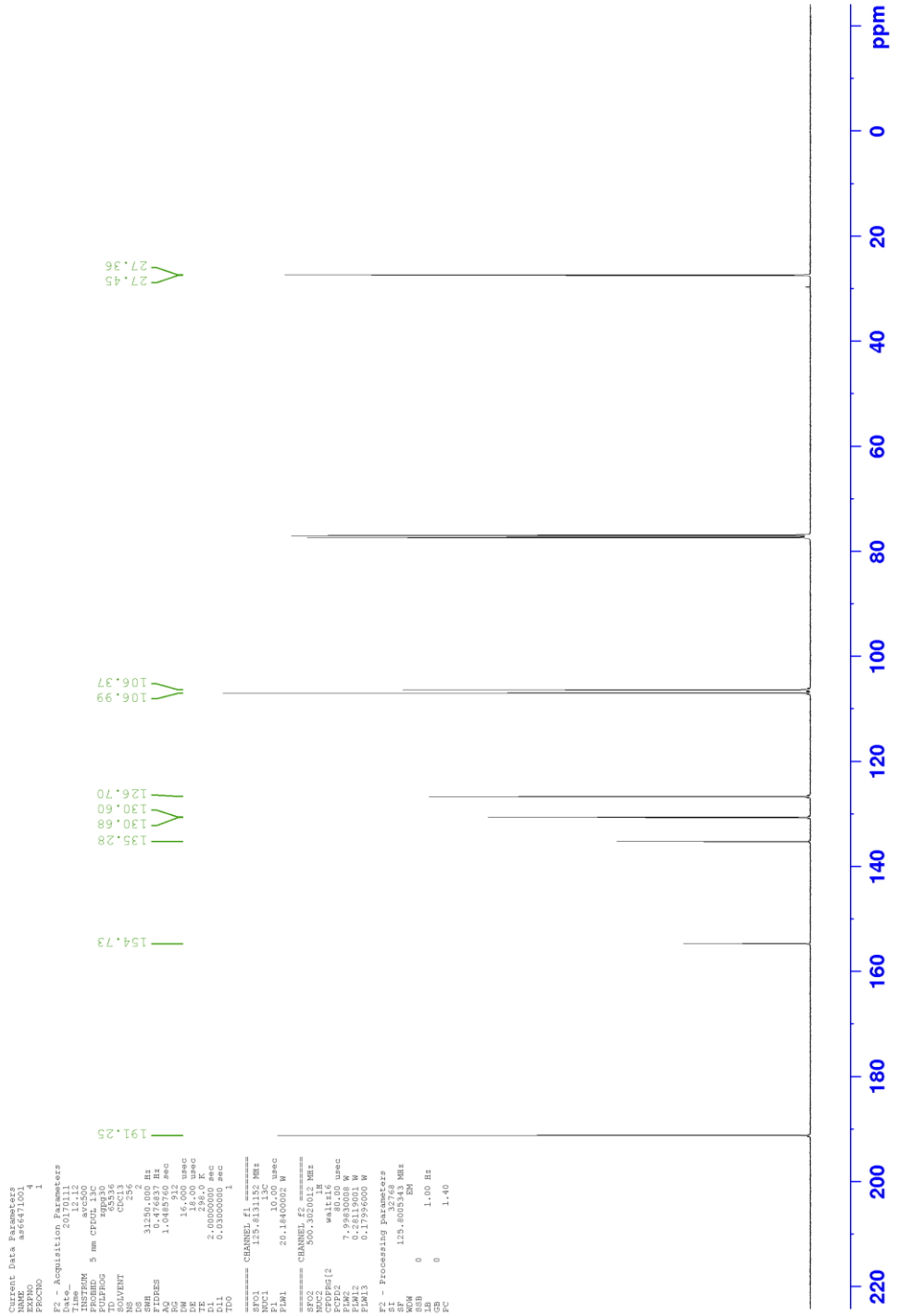


1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (34)

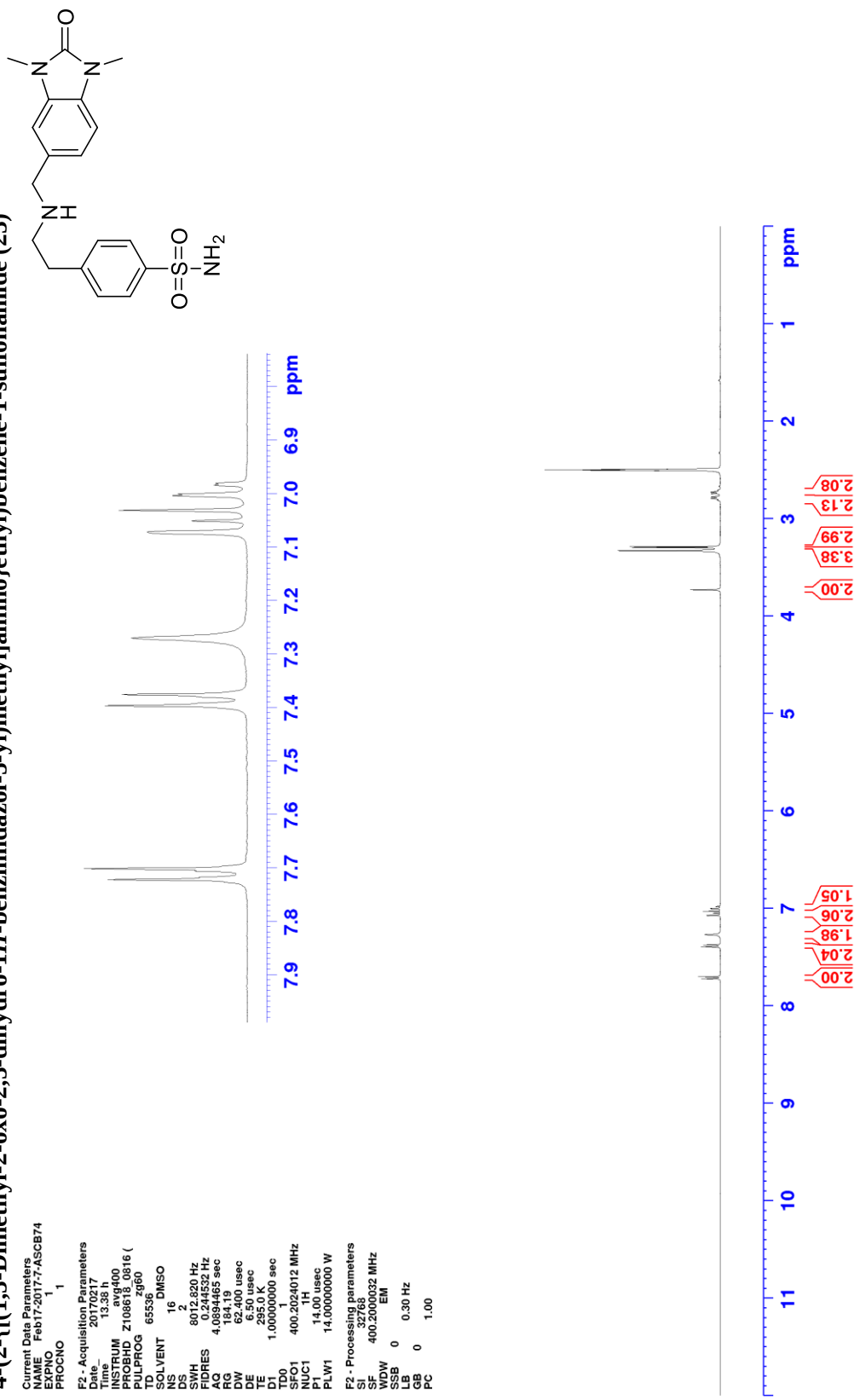


1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (34)

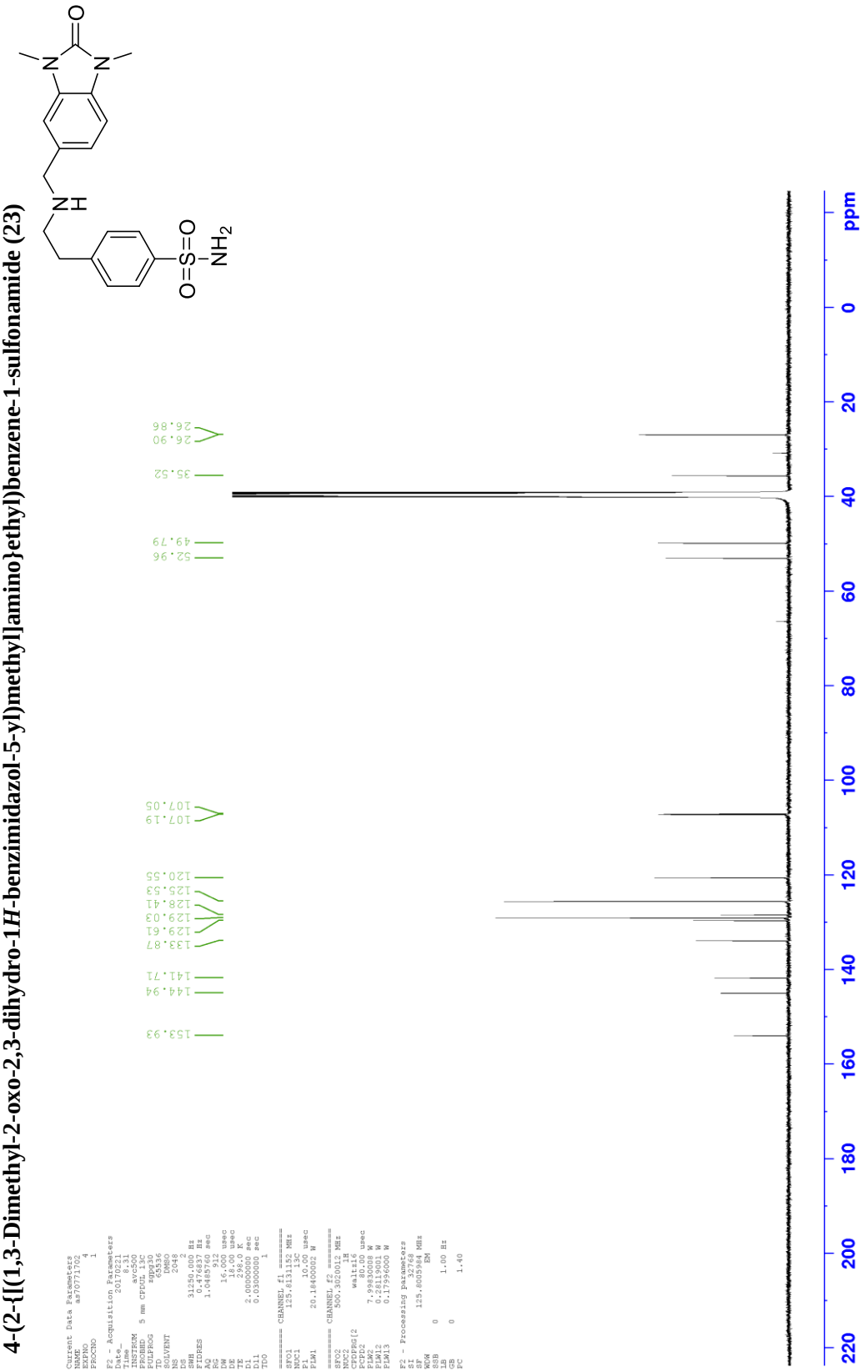




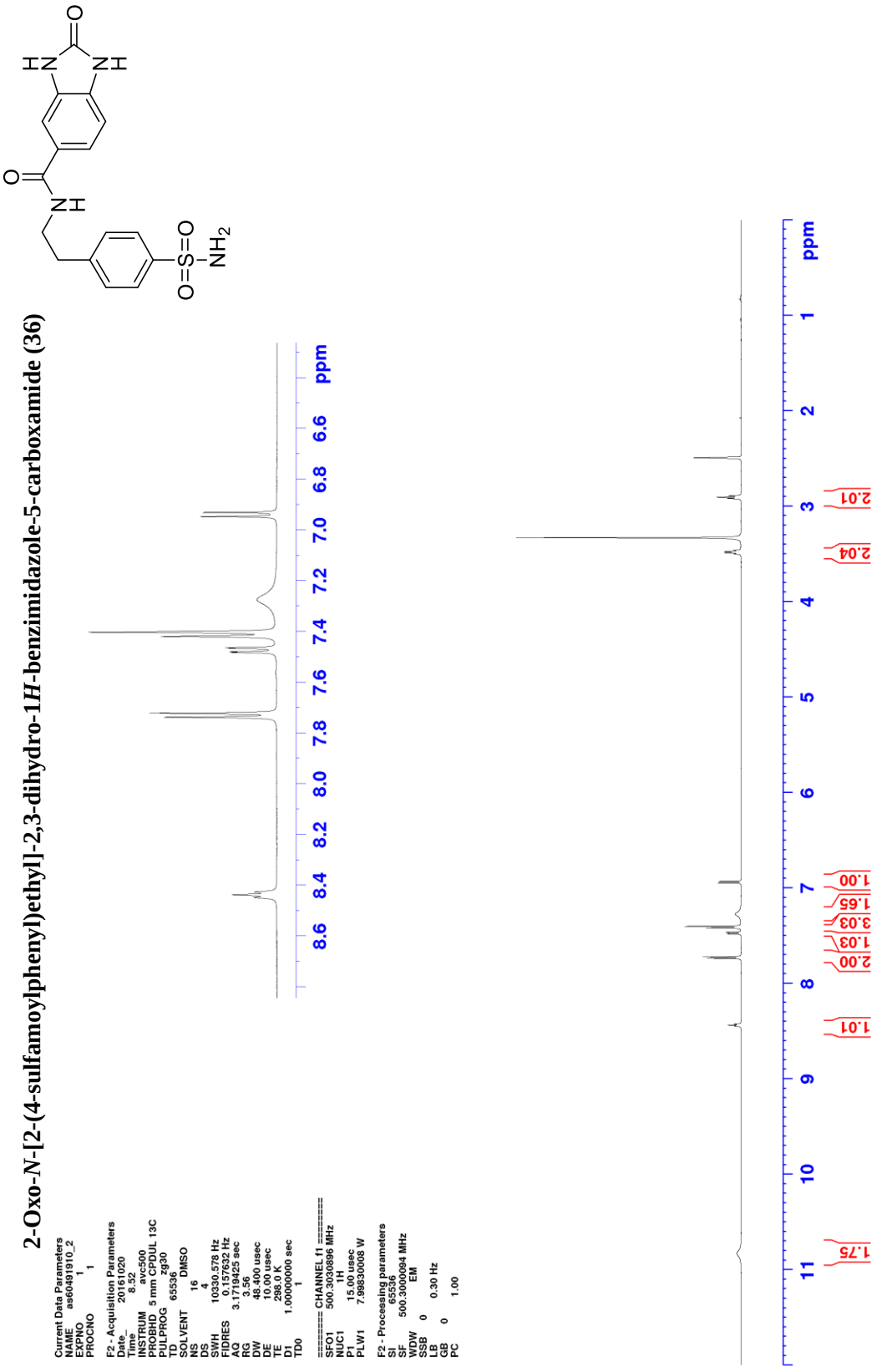
4-(2-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}ethyl)benzene-1-sulfonamide (23)



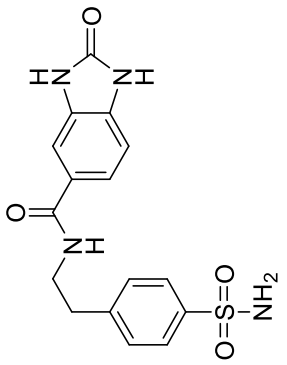
4-(2-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}ethyl)benzene-1-sulfonamide (23)

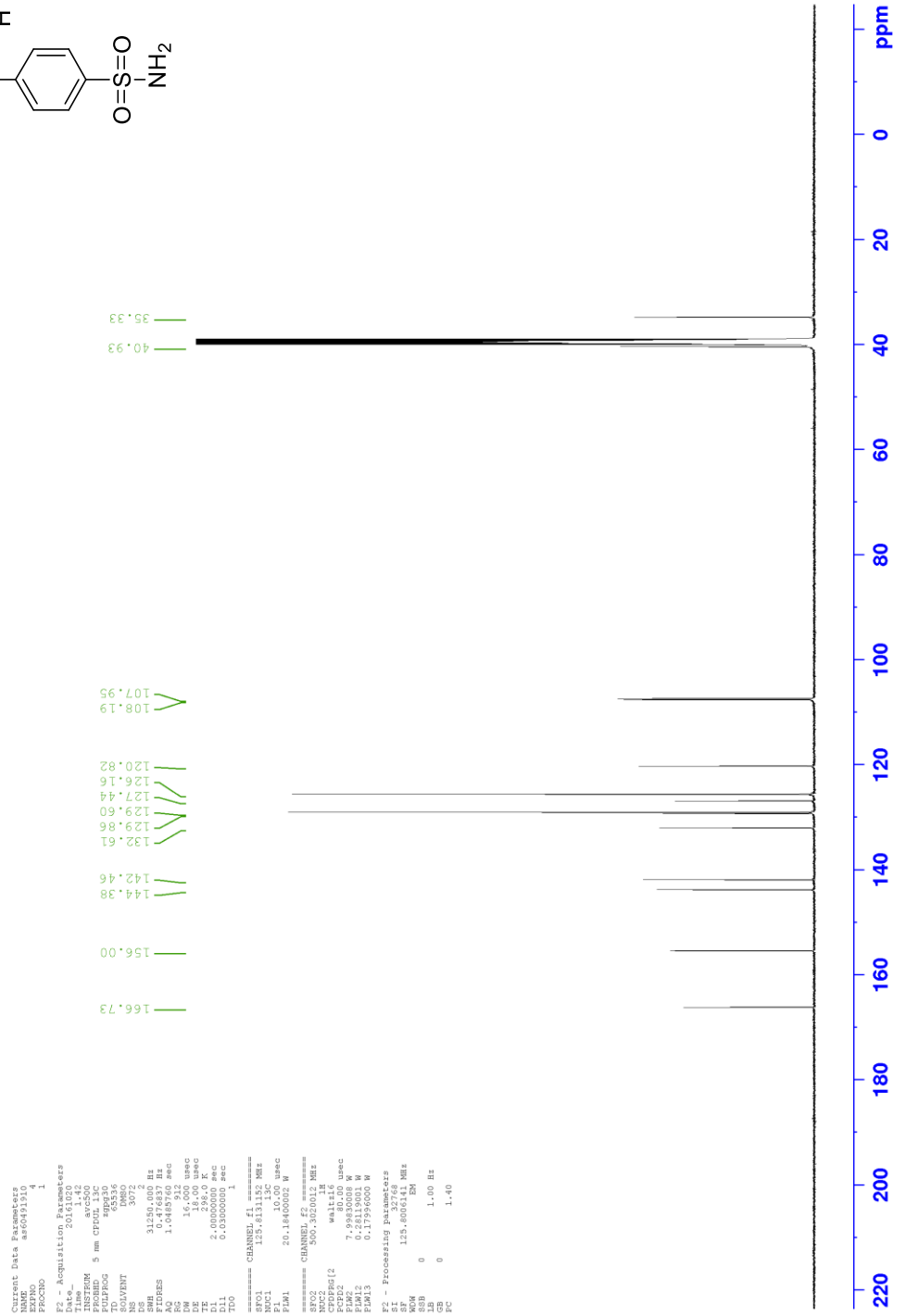


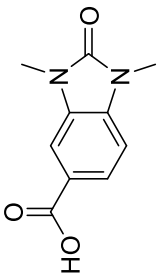
2-Oxo-*N*-[2-(4-sulfamoylphenyl)ethyl]-2,3-dihydro-1*H*-benzimidazole-5-carboxamide (36)



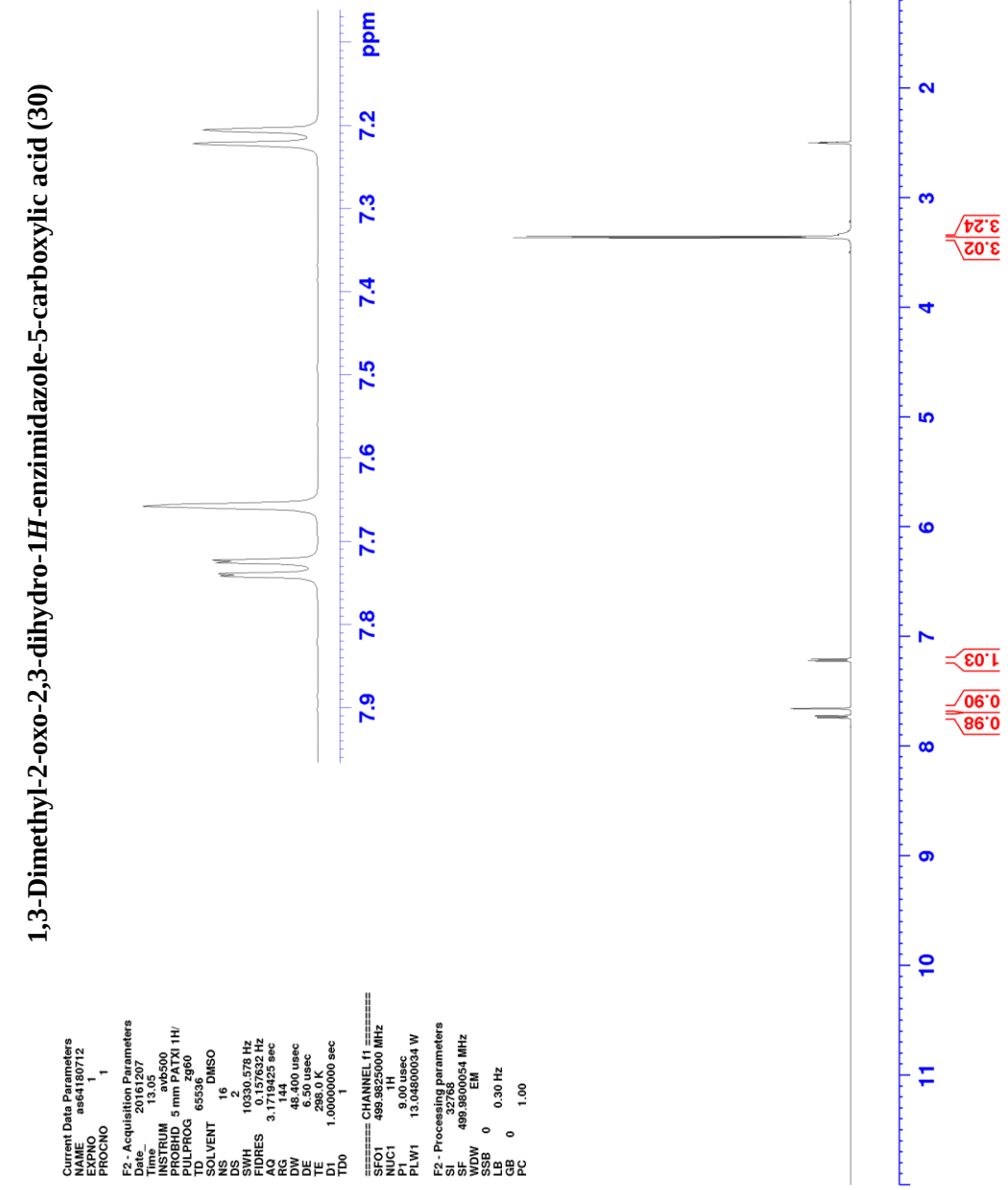
2-Oxo-*N*-[2-(4-sulfamoylphenyl)ethyl]-2,3-dihydro-1*H*-benzimidazole-5-carboxamide (36)



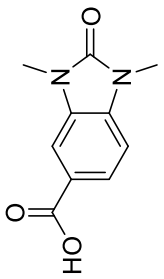


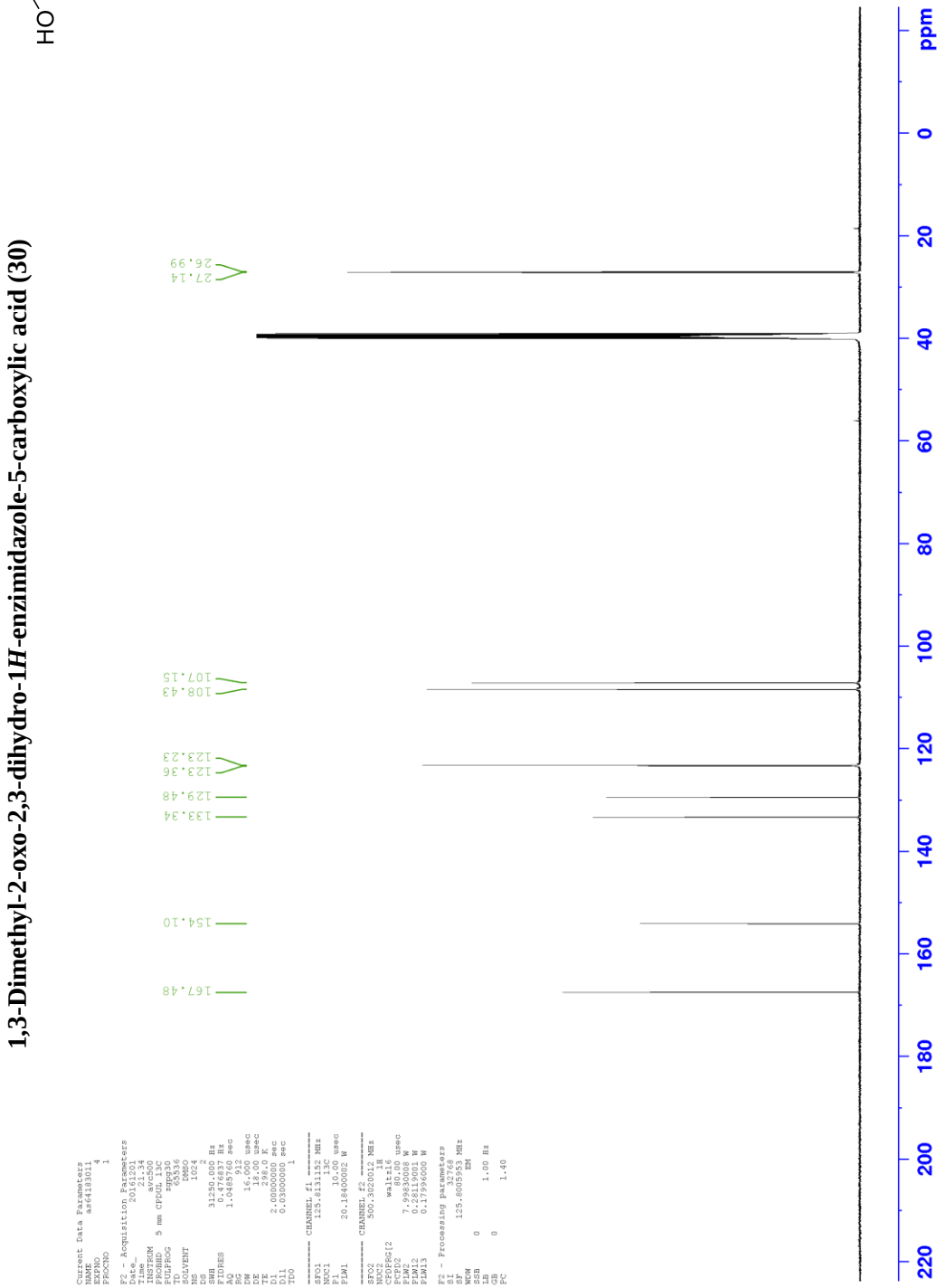


1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-enzimidazole-5-carboxylic acid (30)

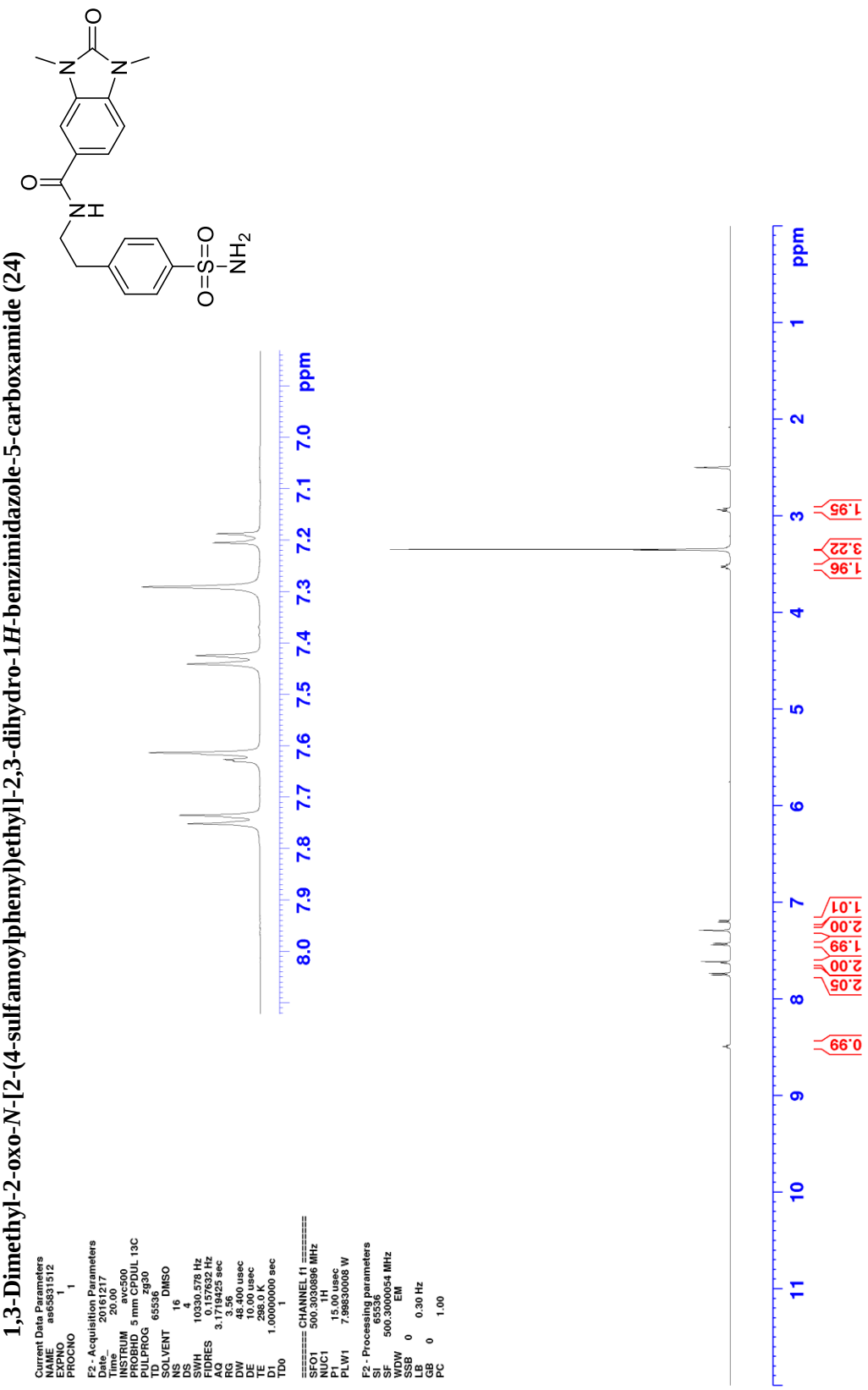


1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-enzimidazole-5-carboxylic acid (30)

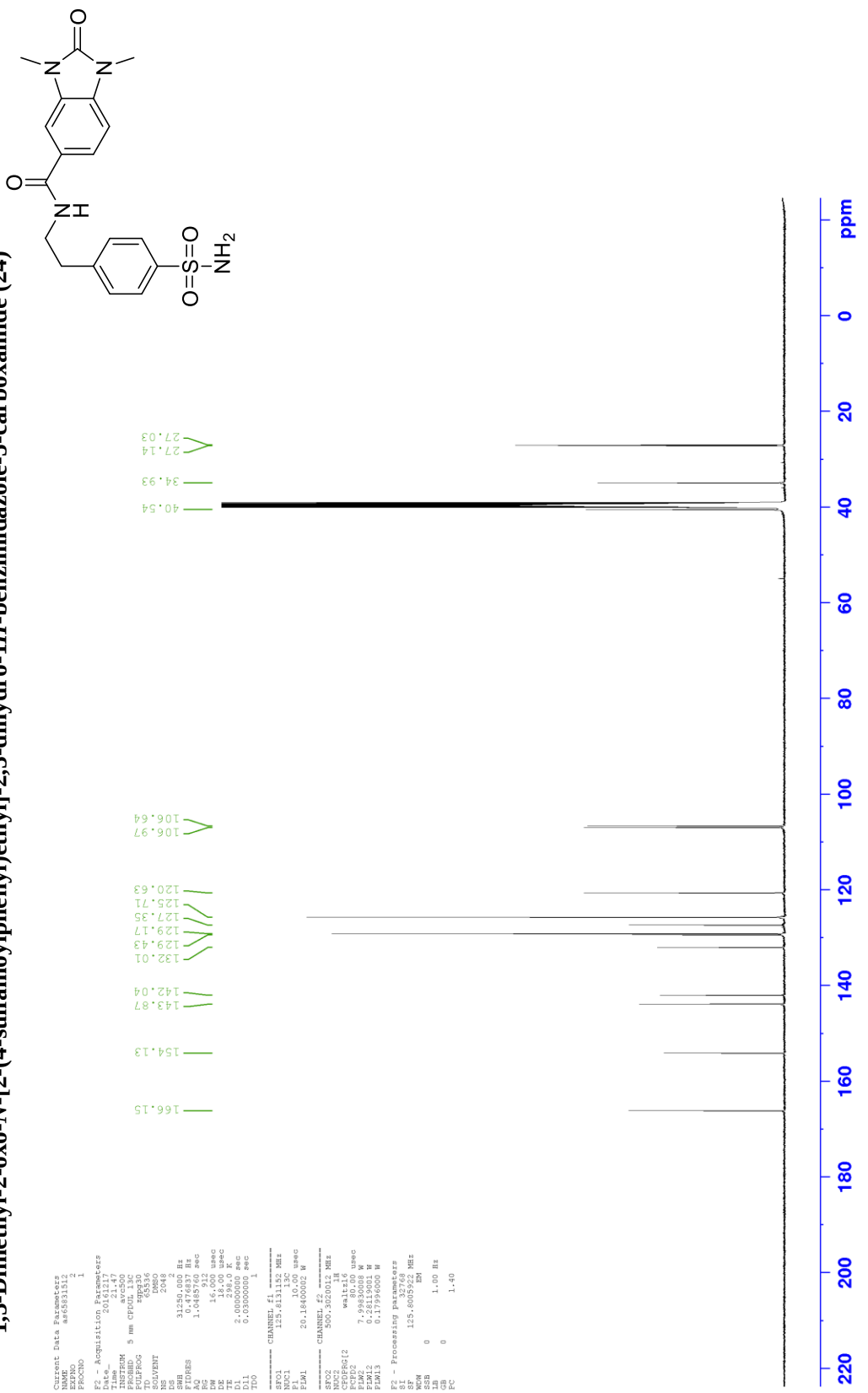


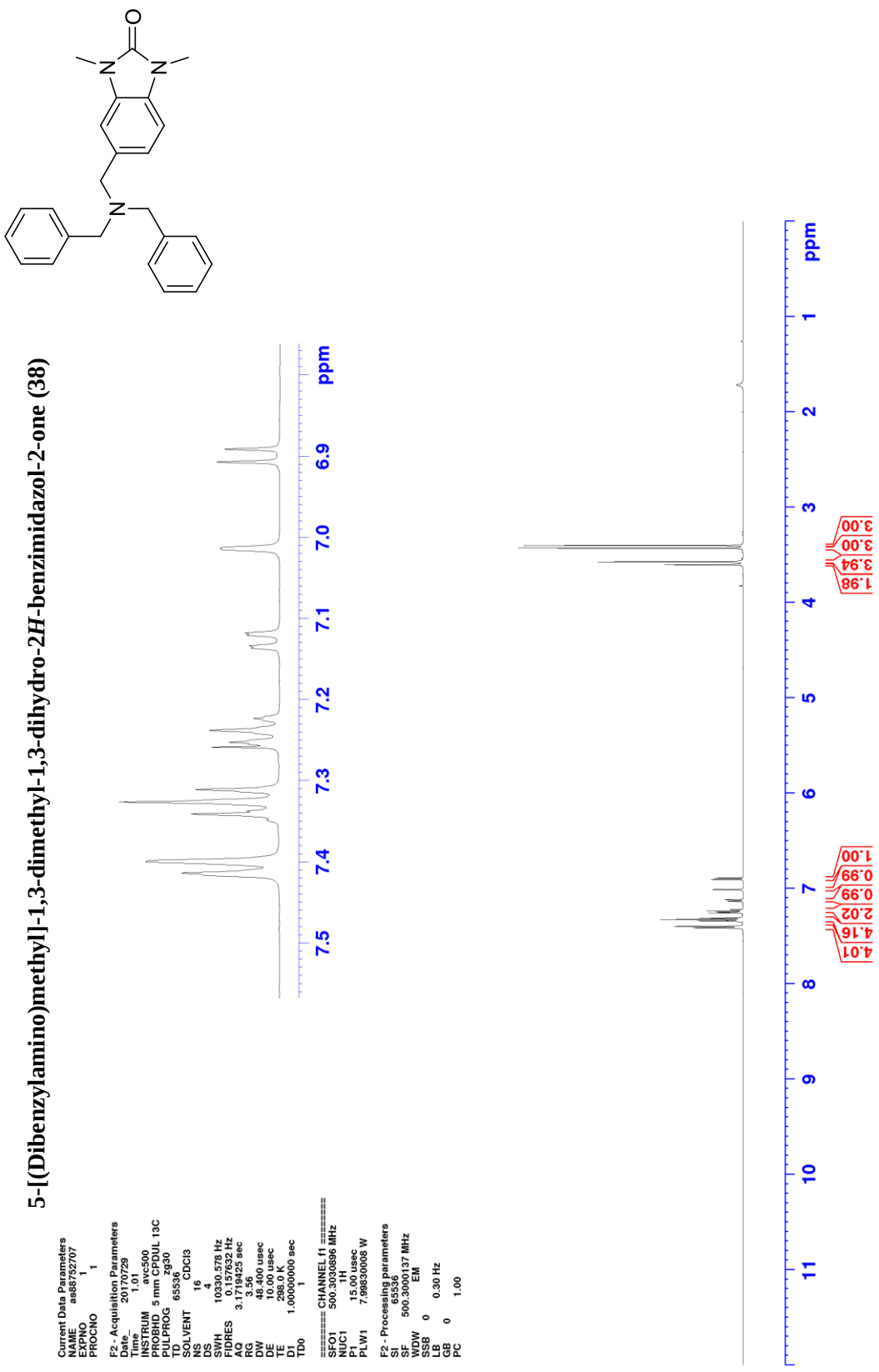


1,3-Dimethyl-2-oxo-*N*-[2-(4-sulfamoylphenyl)ethyl]-2,3-dihydro-1*H*-benzimidazole-5-carboxamide (24)



1,3-Dimethyl-2-oxo-*N*-[2-(4-sulfamoylphenyl)ethyl]-2,3-dihydro-1*H*-benzimidazole-5-carboxamide (24)




5-[(Dibenzylamino)methyl]-1,3-dimethyl-1,3-dihydro-2H-benzimidazol-2-one (38)
Current Data Parameters
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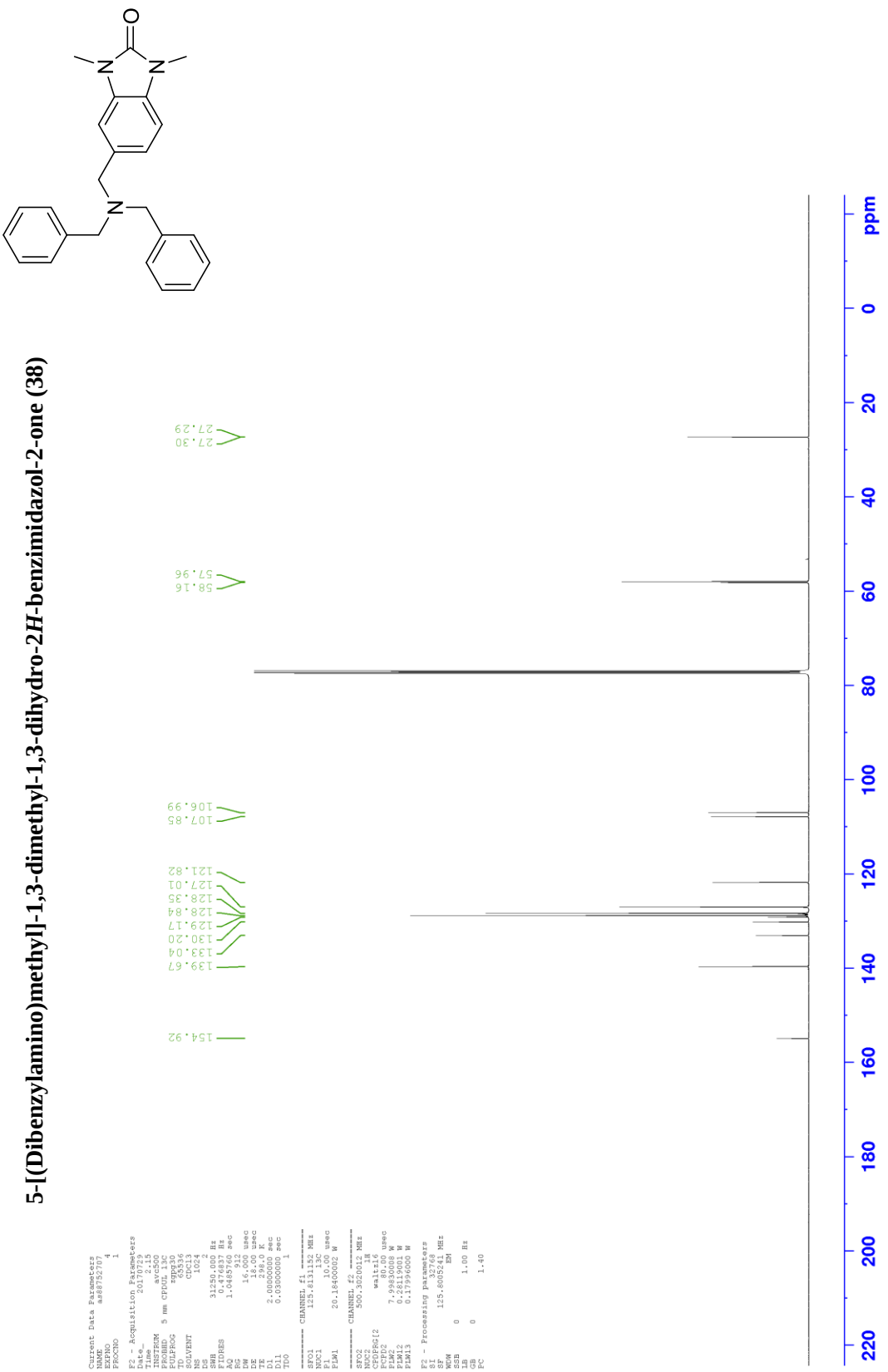
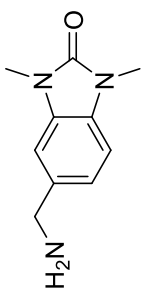
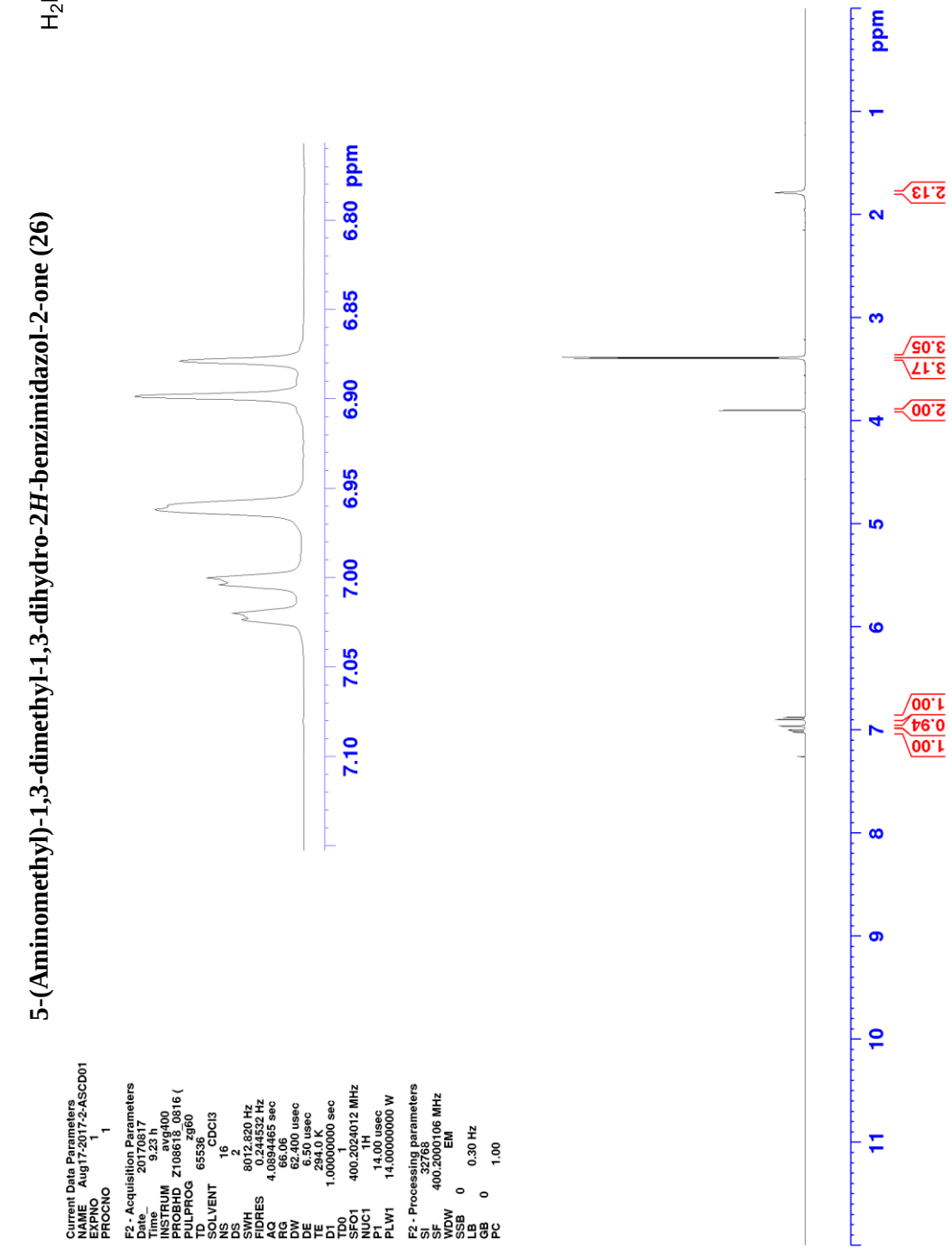
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170729
Time 1.01
INSTRUM av-500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1770425 sec
RG 3.55
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
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
7.5
7.4
7.3
7.2
7.1
7.0
6.9
ppm
1.98
3.94
3.00
3.00
4.01
4.16
2.02
0.99
0.99
1.00
11
10
9
8
7
6
5
4
3
2
1
ppm

5-[(Dibenzylamino)methyl]-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (38)

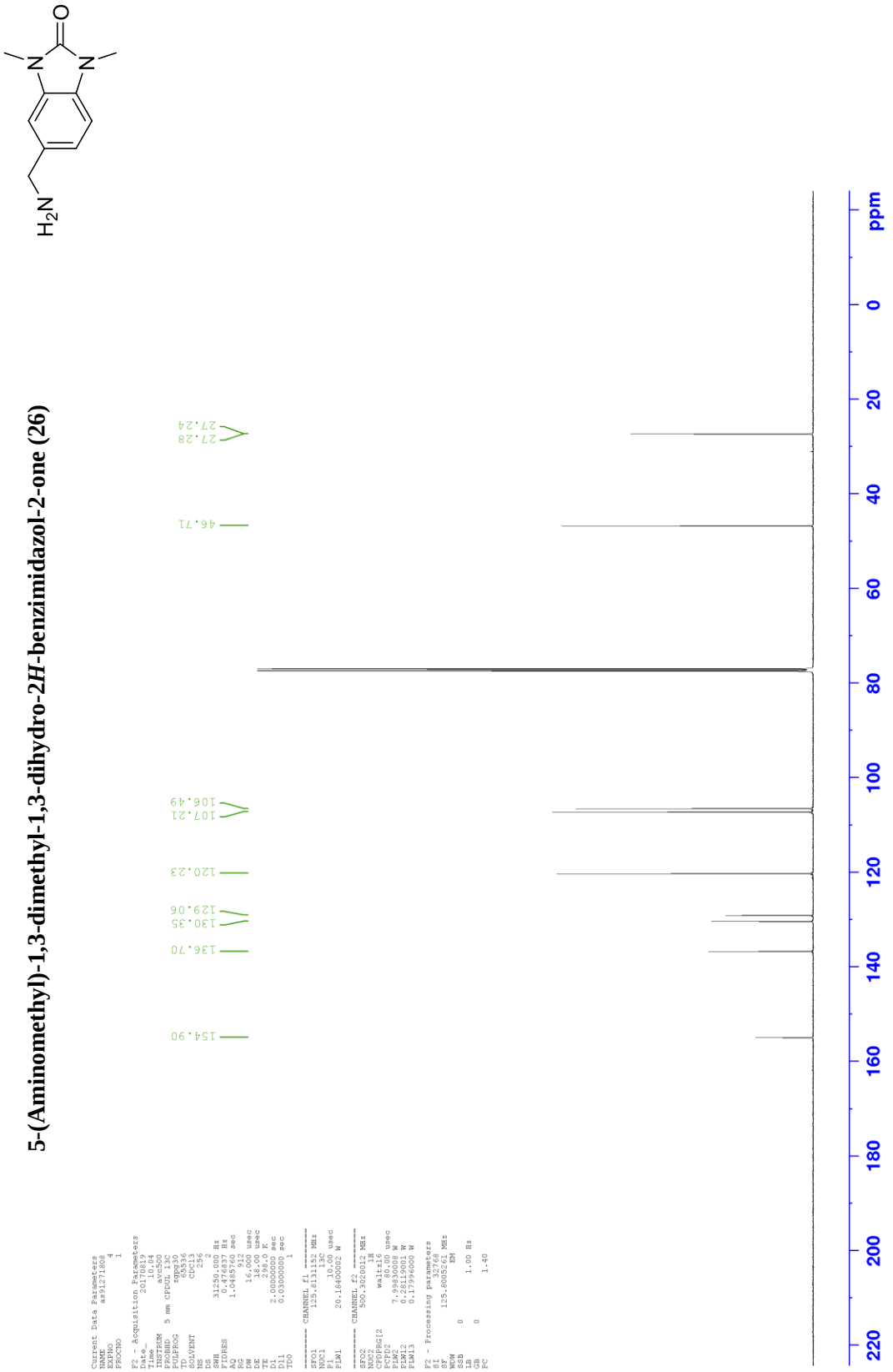


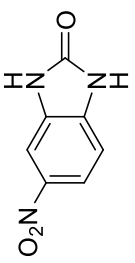


5-(Aminomethyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (26)

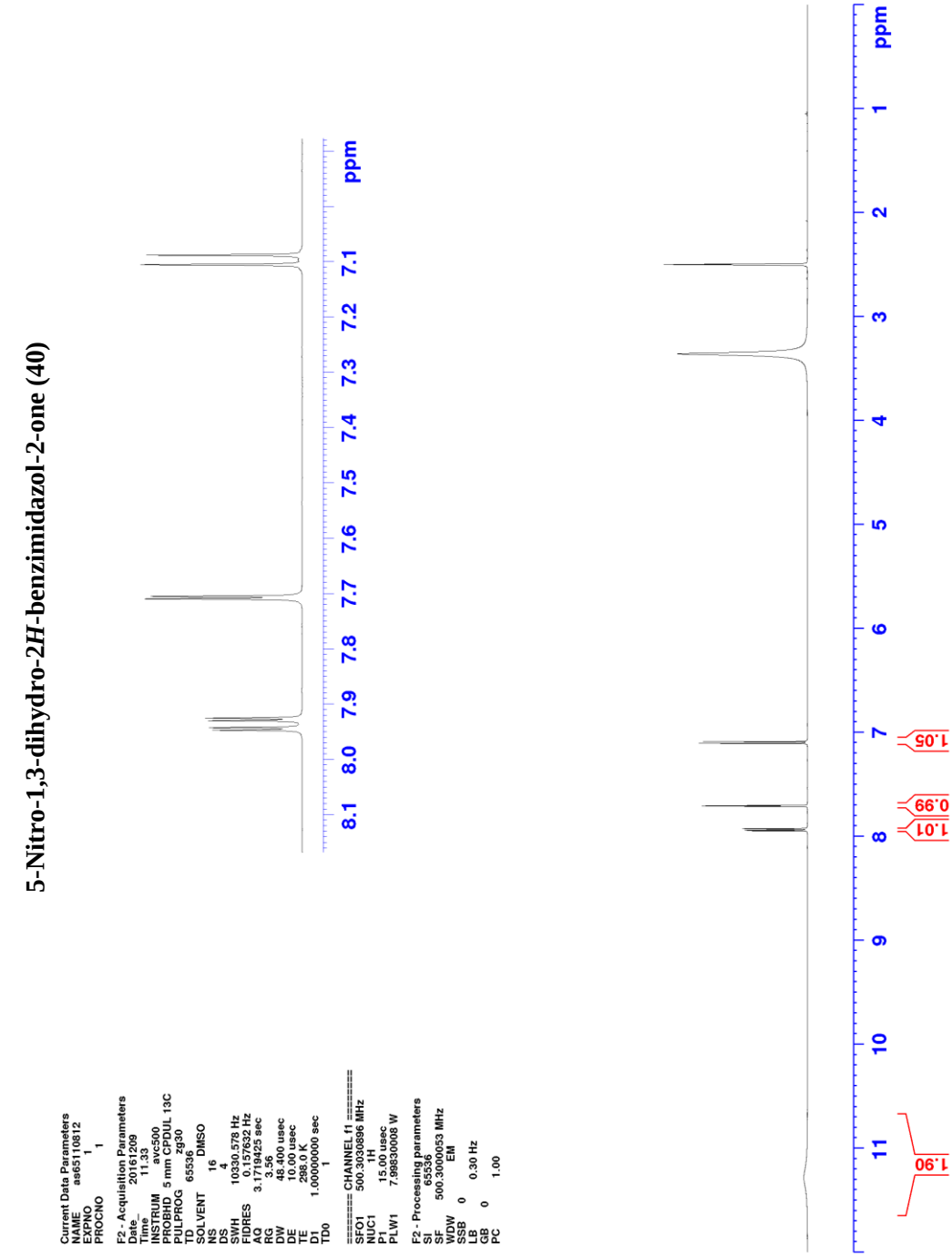


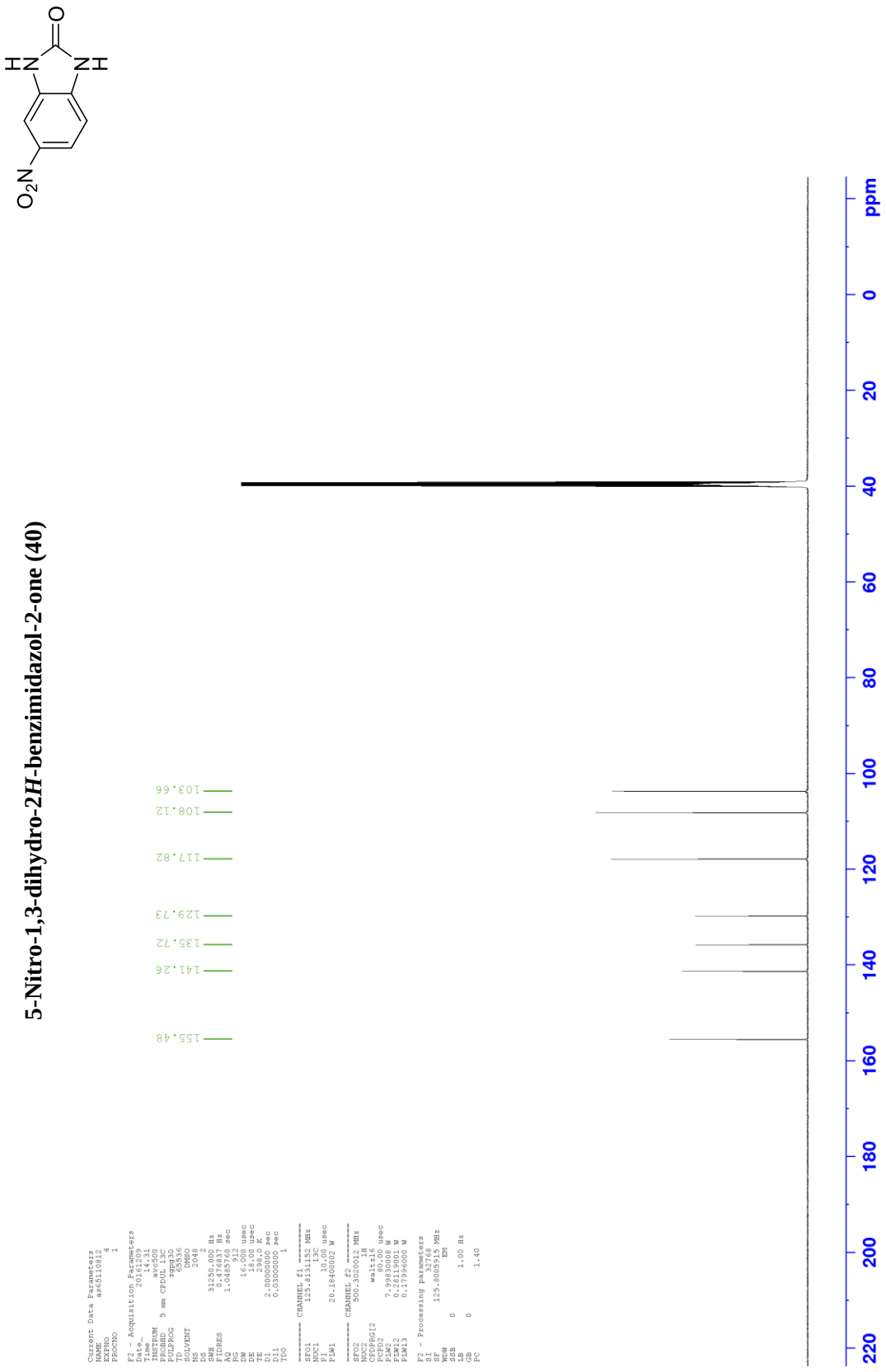
5-(Aminomethyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (26)

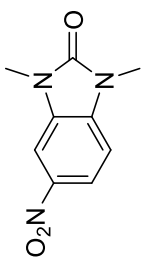




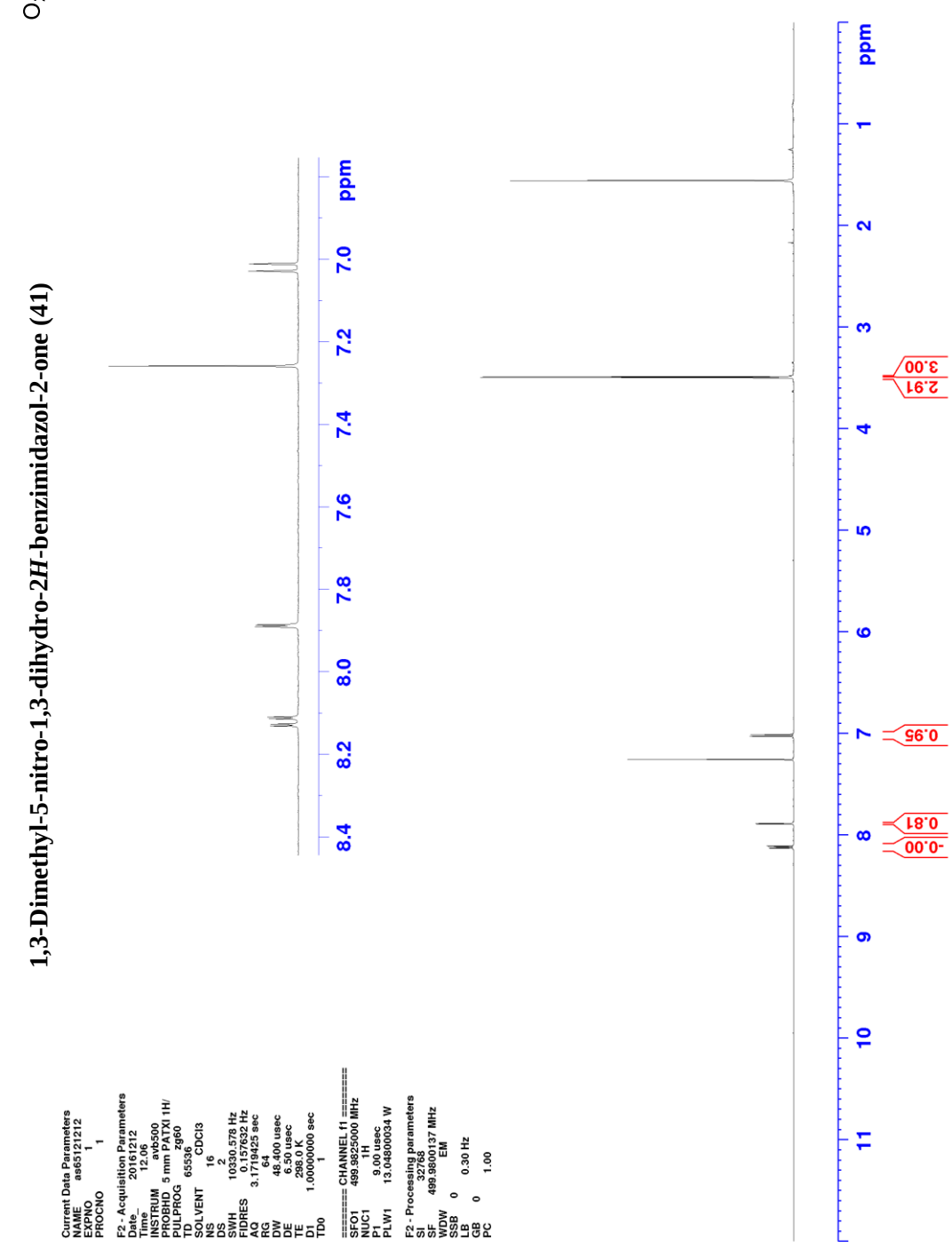
5-Nitro-1,3-dihydro-2*H*-benzimidazol-2-one (40)



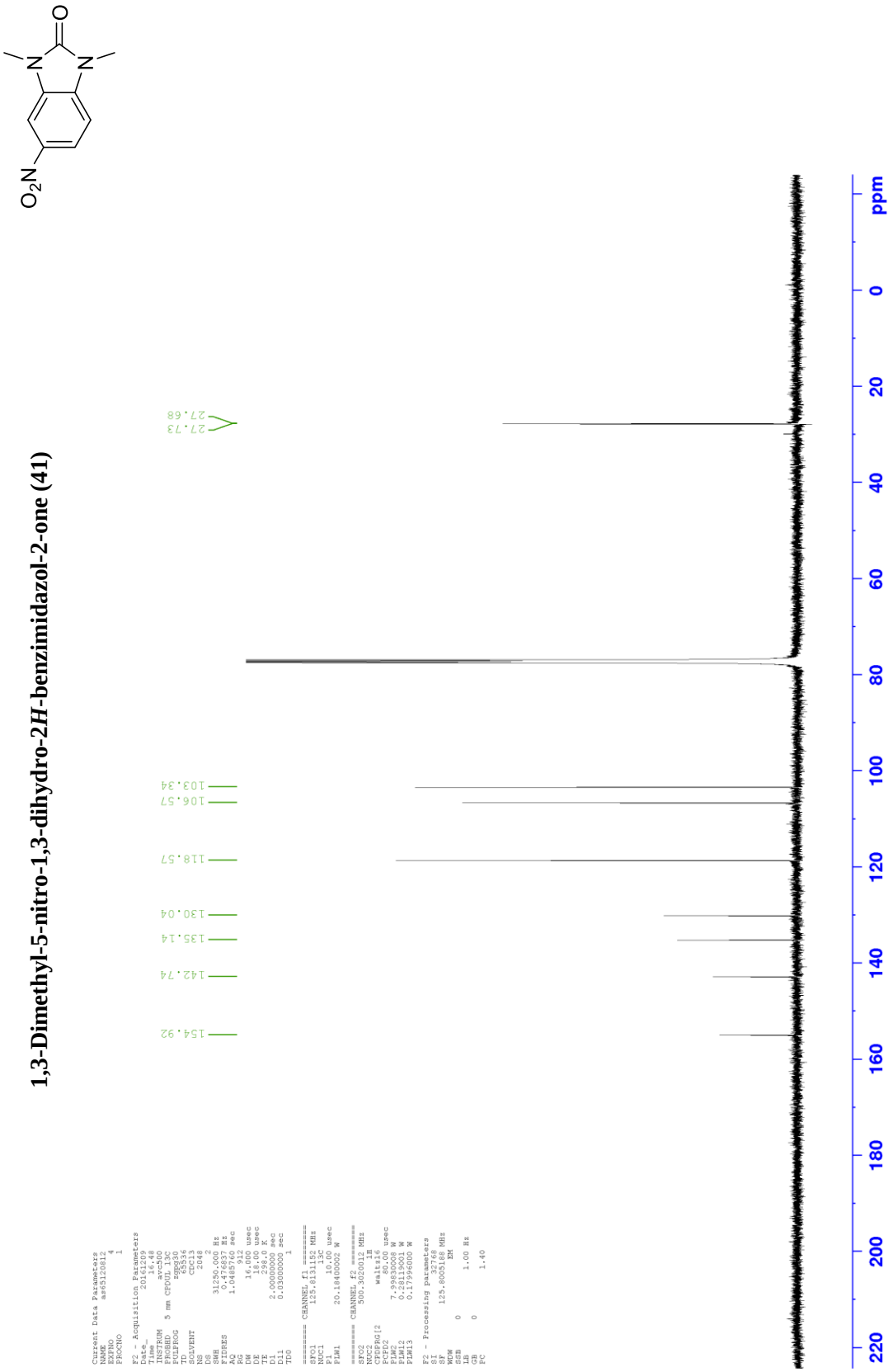

5-Nitro-1,3-dihydro-2H-benzimidazol-2-one (40)
O2N
H
N
O
N
H
103.66
108.12
117.82
129.73
135.72
141.26
155.48
ppm
0
20
40
60
80
100
120
140
160
180
200
220

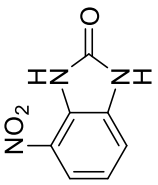


1,3-Dimethyl-5-nitro-1,3-dihydro-2*H*-benzimidazol-2-one (41)

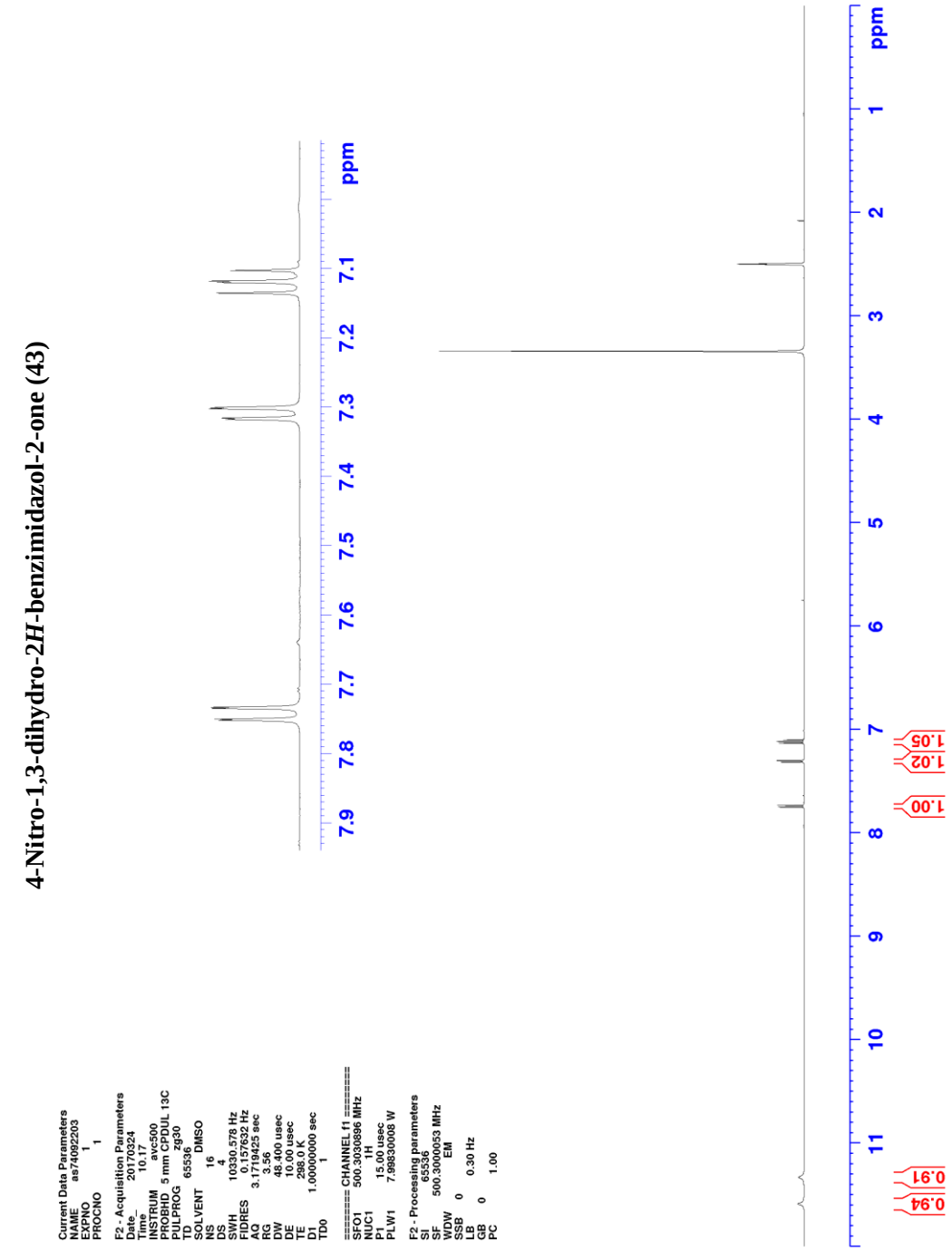


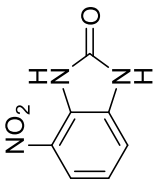
1,3-Dimethyl-5-nitro-1,3-dihydro-2*H*-benzimidazol-2-one (41)



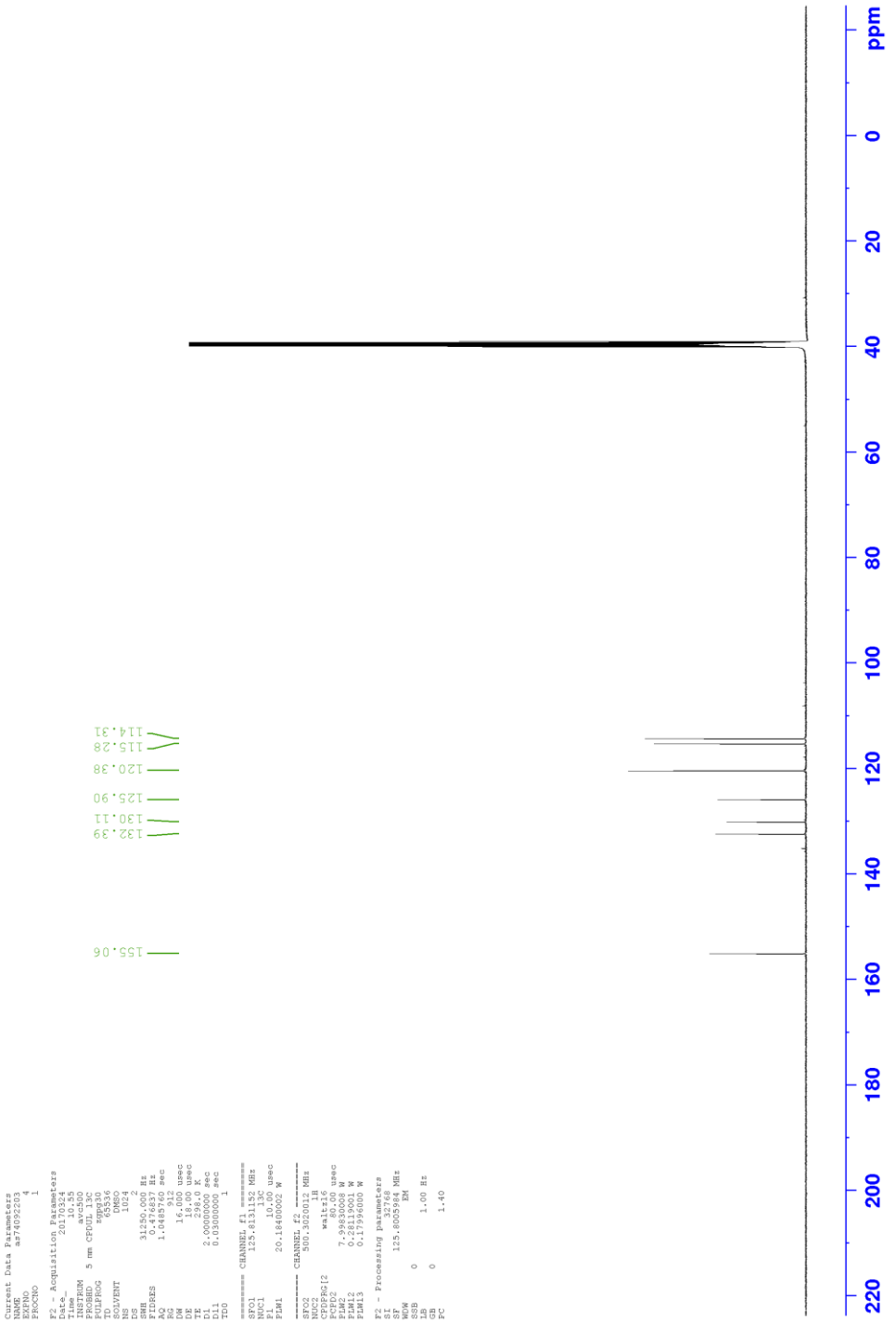


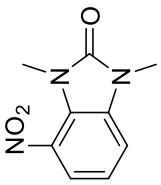
4-Nitro-1,3-dihydro-2*H*-benzimidazol-2-one (43)



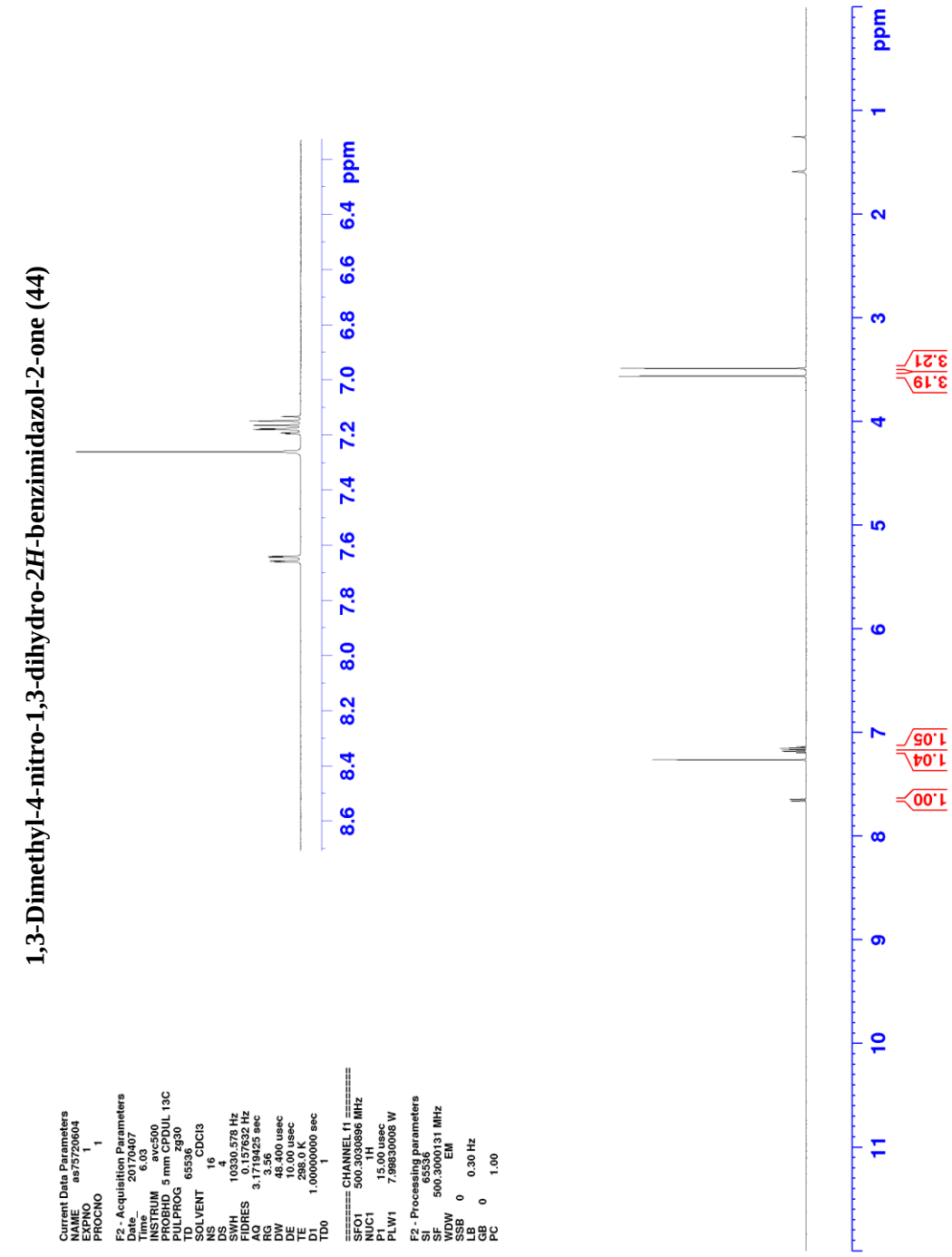


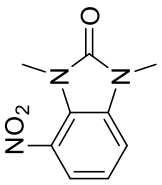
4-Nitro-1,3-dihydro-2*H*-benzimidazol-2-one (43)



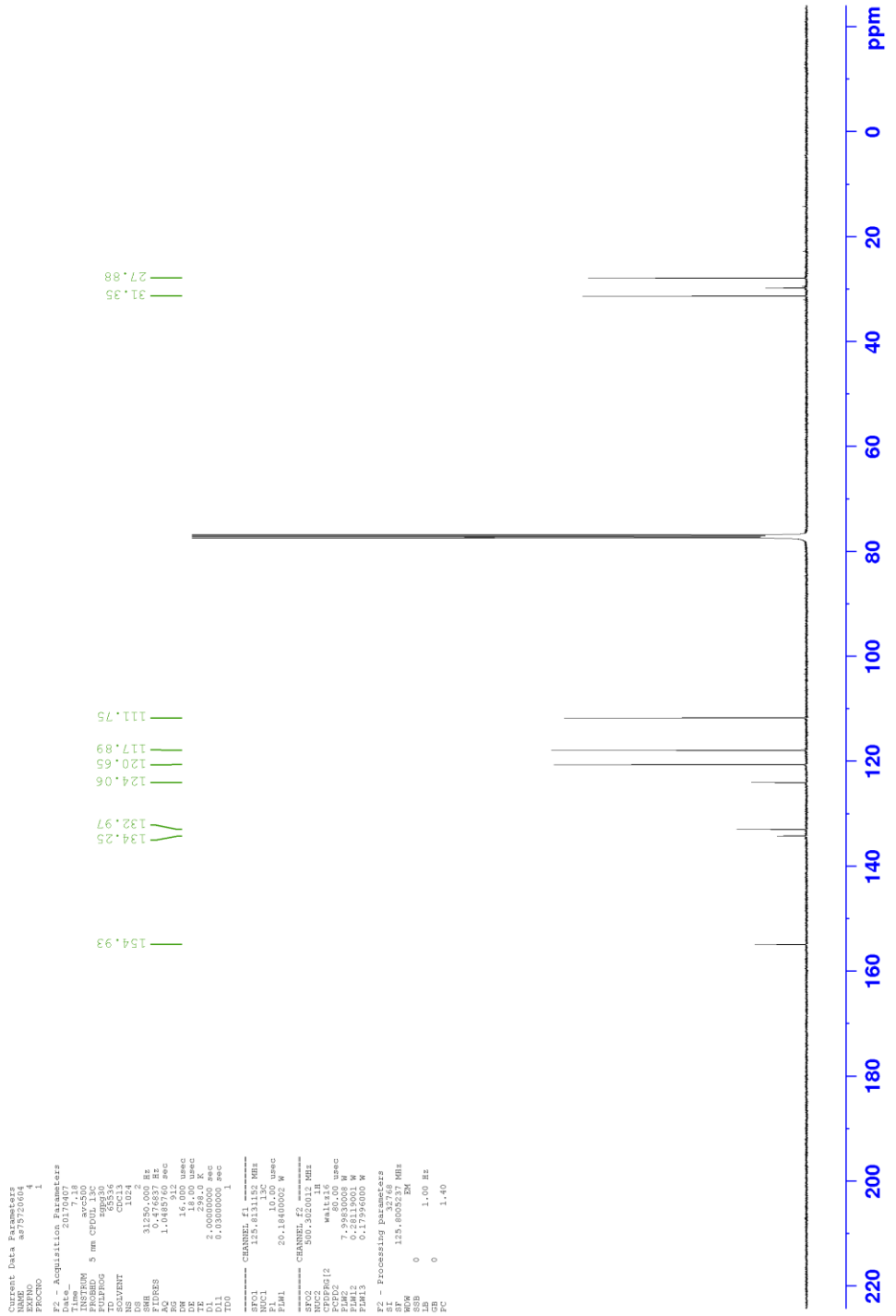


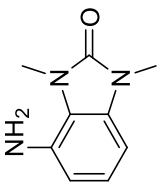
1,3-Dimethyl-4-nitro-1,3-dihydro-2*H*-benzimidazol-2-one (44)



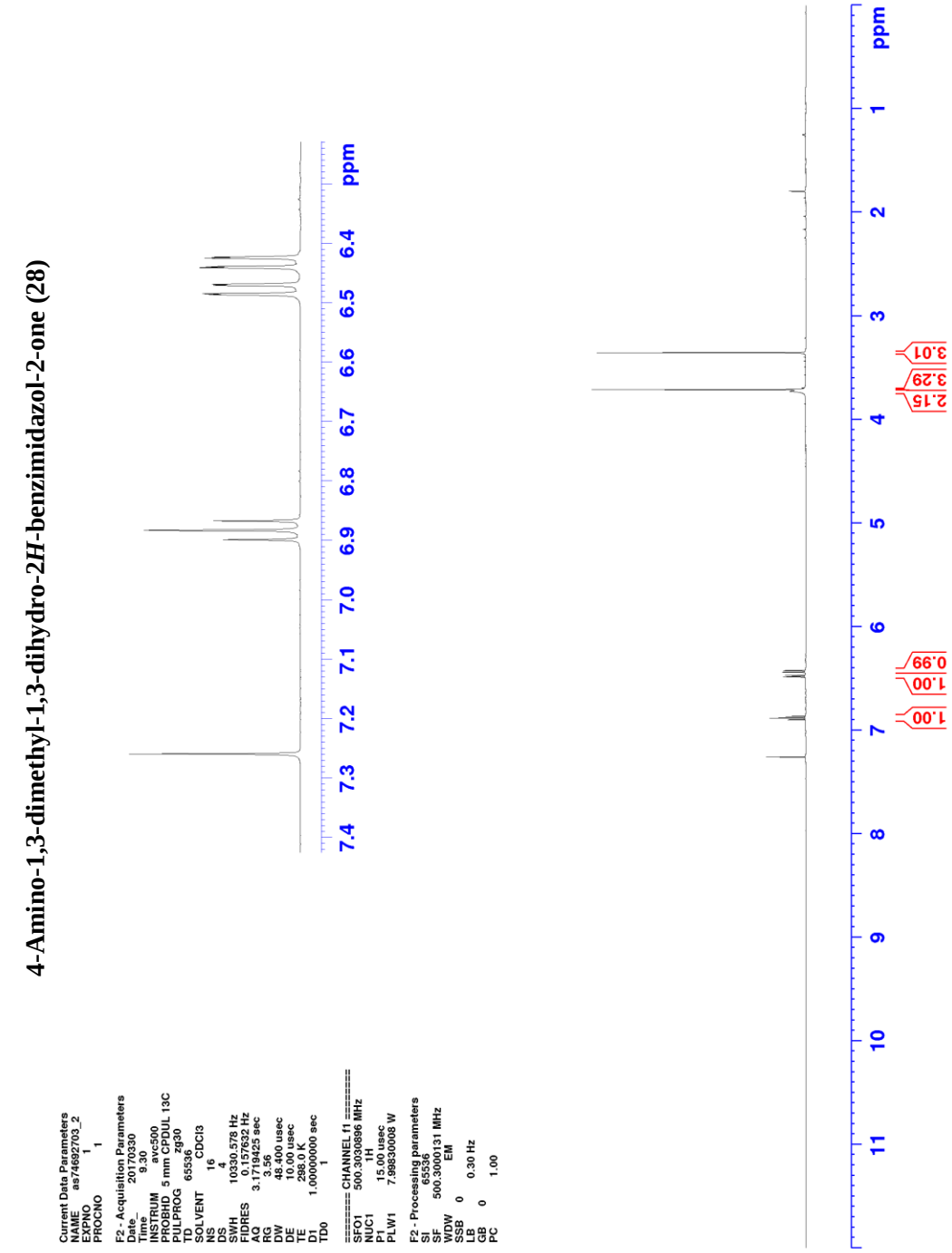


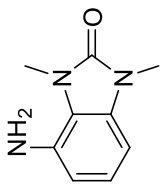
1,3-Dimethyl-4-nitro-1,3-dihydro-2*H*-benzimidazol-2-one (44)



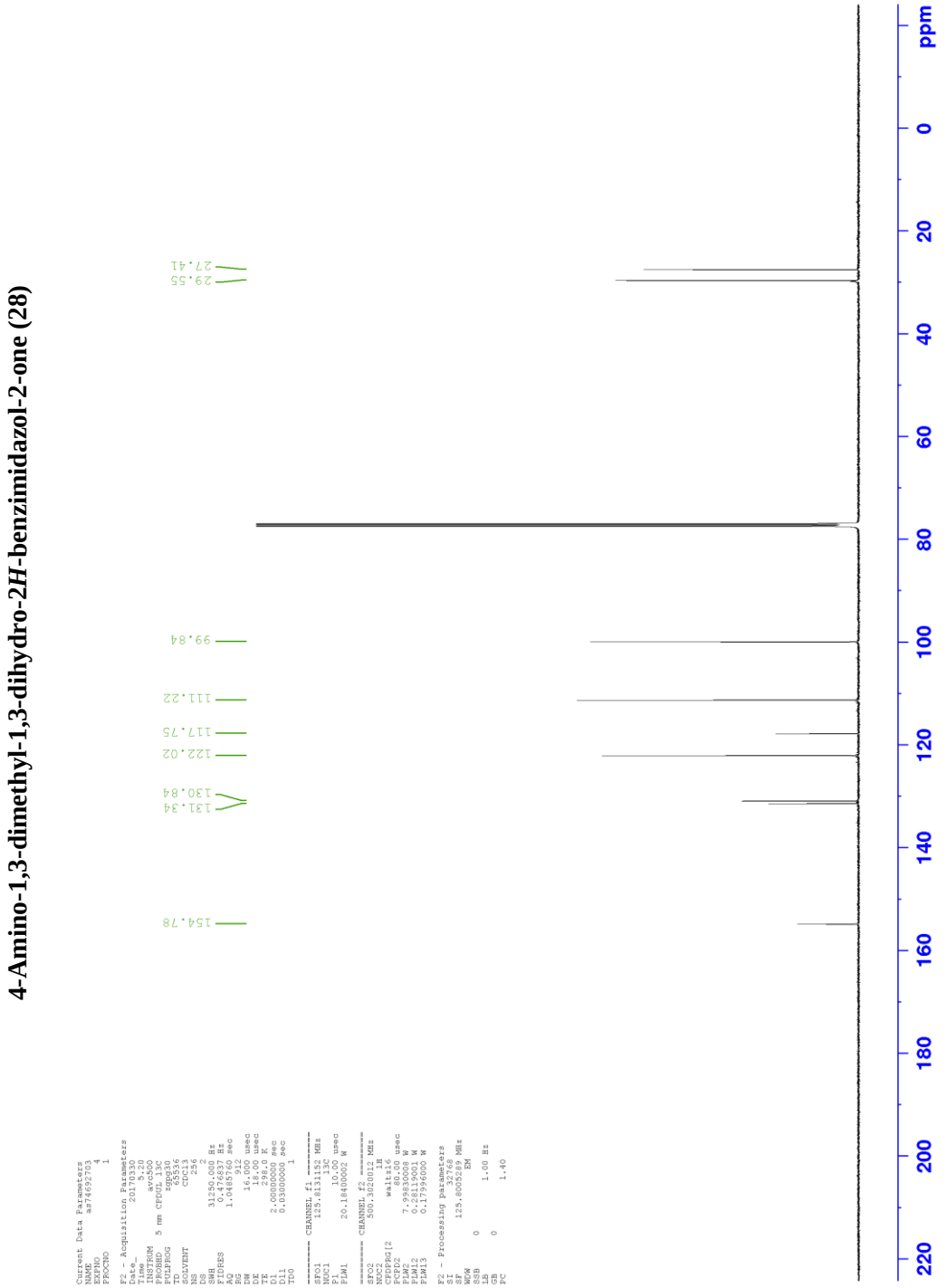


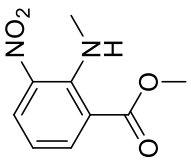
4-Amino-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (28)



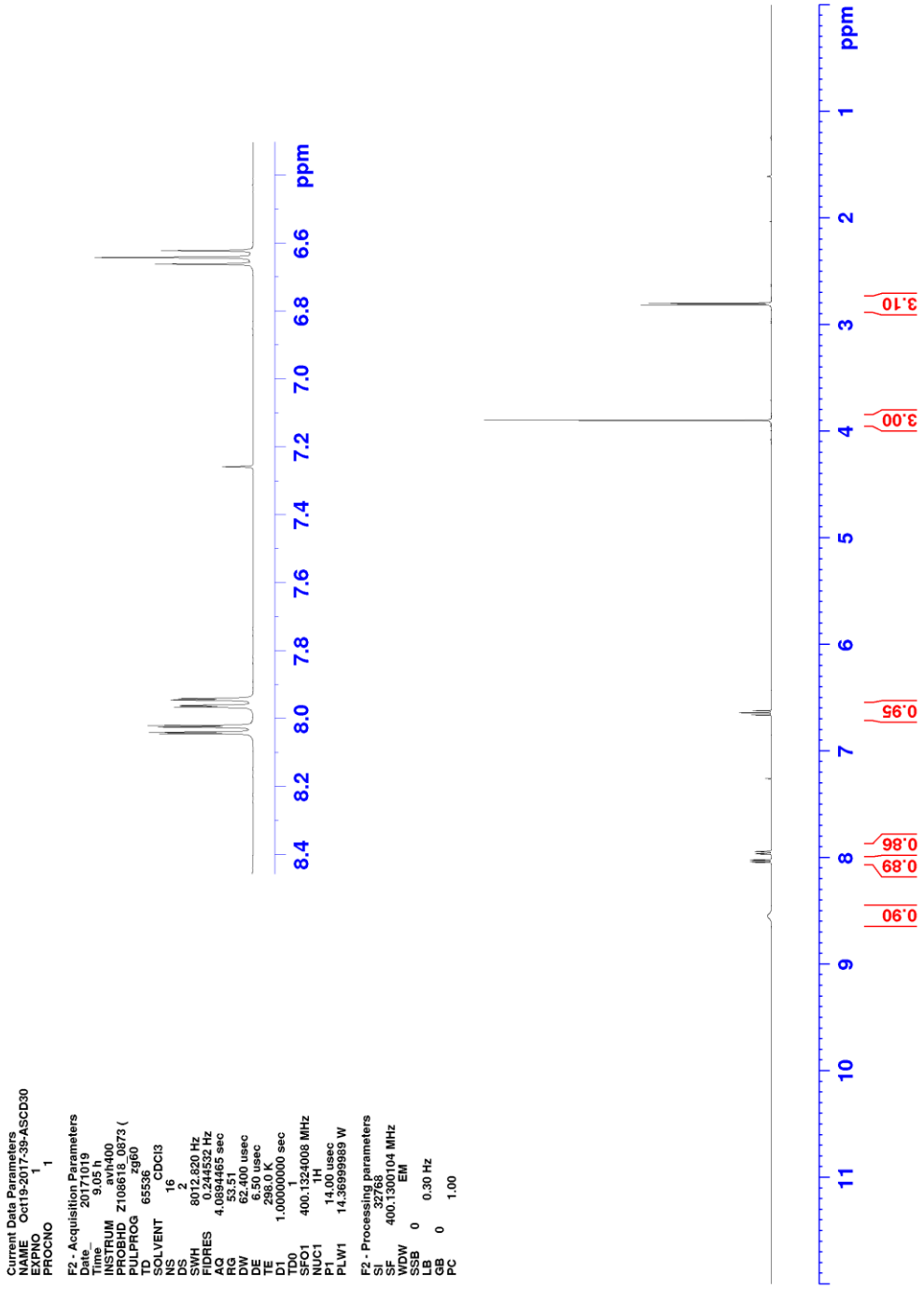


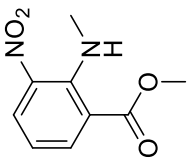
4-Amino-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (28)



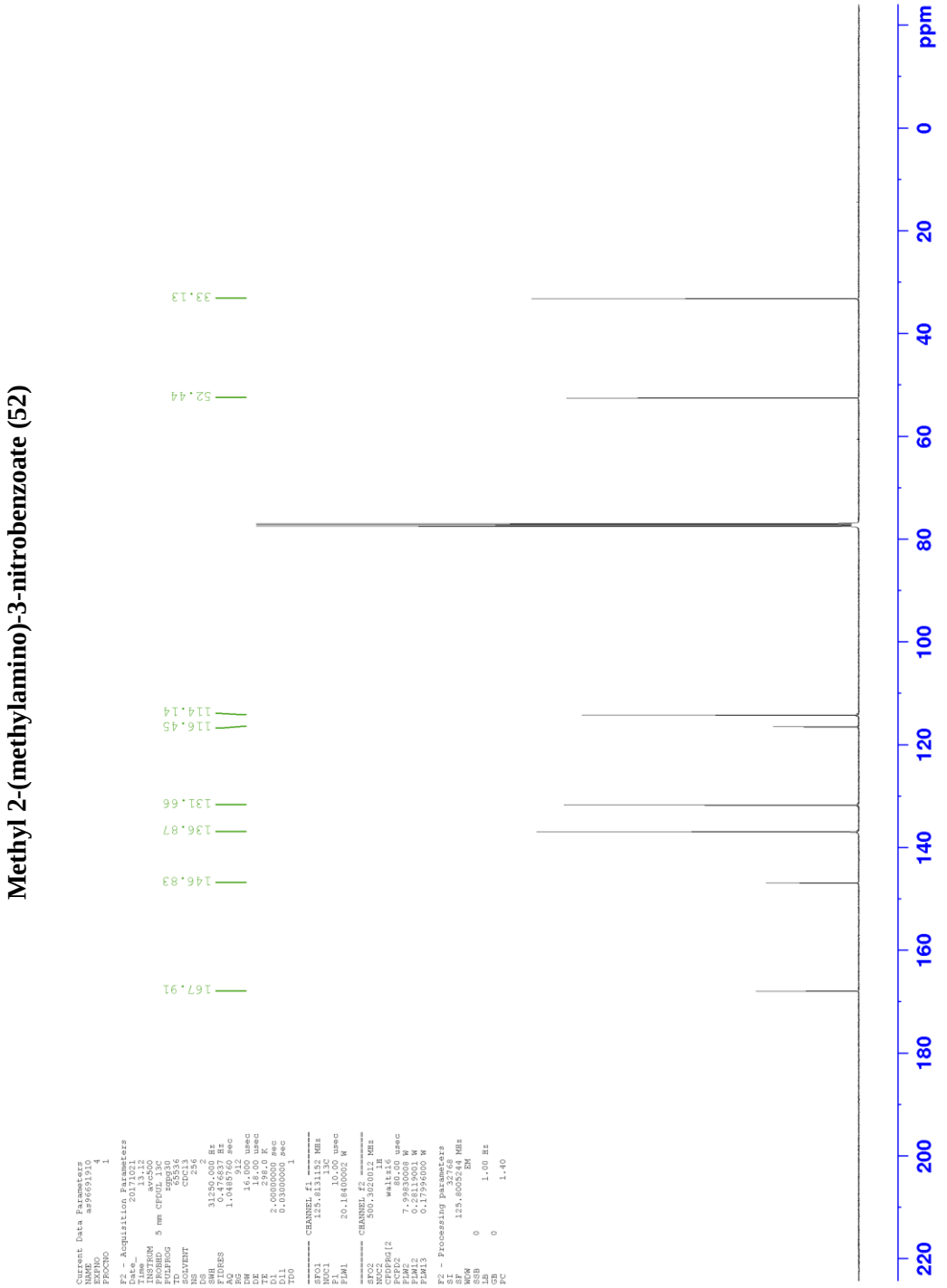


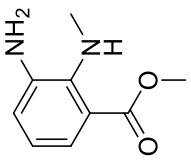
Methyl 2-(methylamino)-3-nitrobenzoate (52)



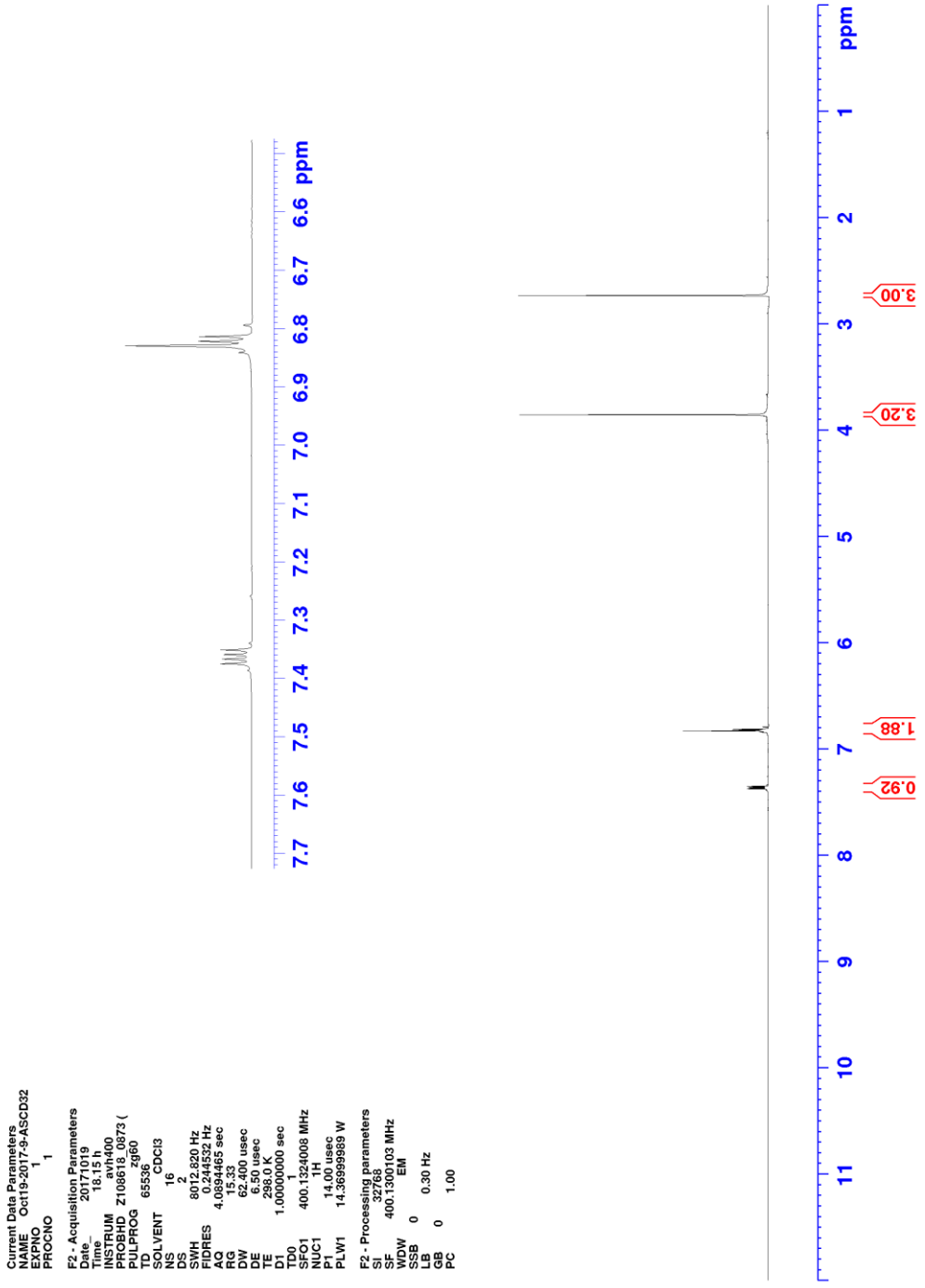


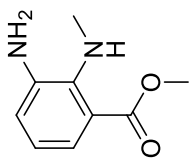
Methyl 2-(methylamino)-3-nitrobenzoate (52)



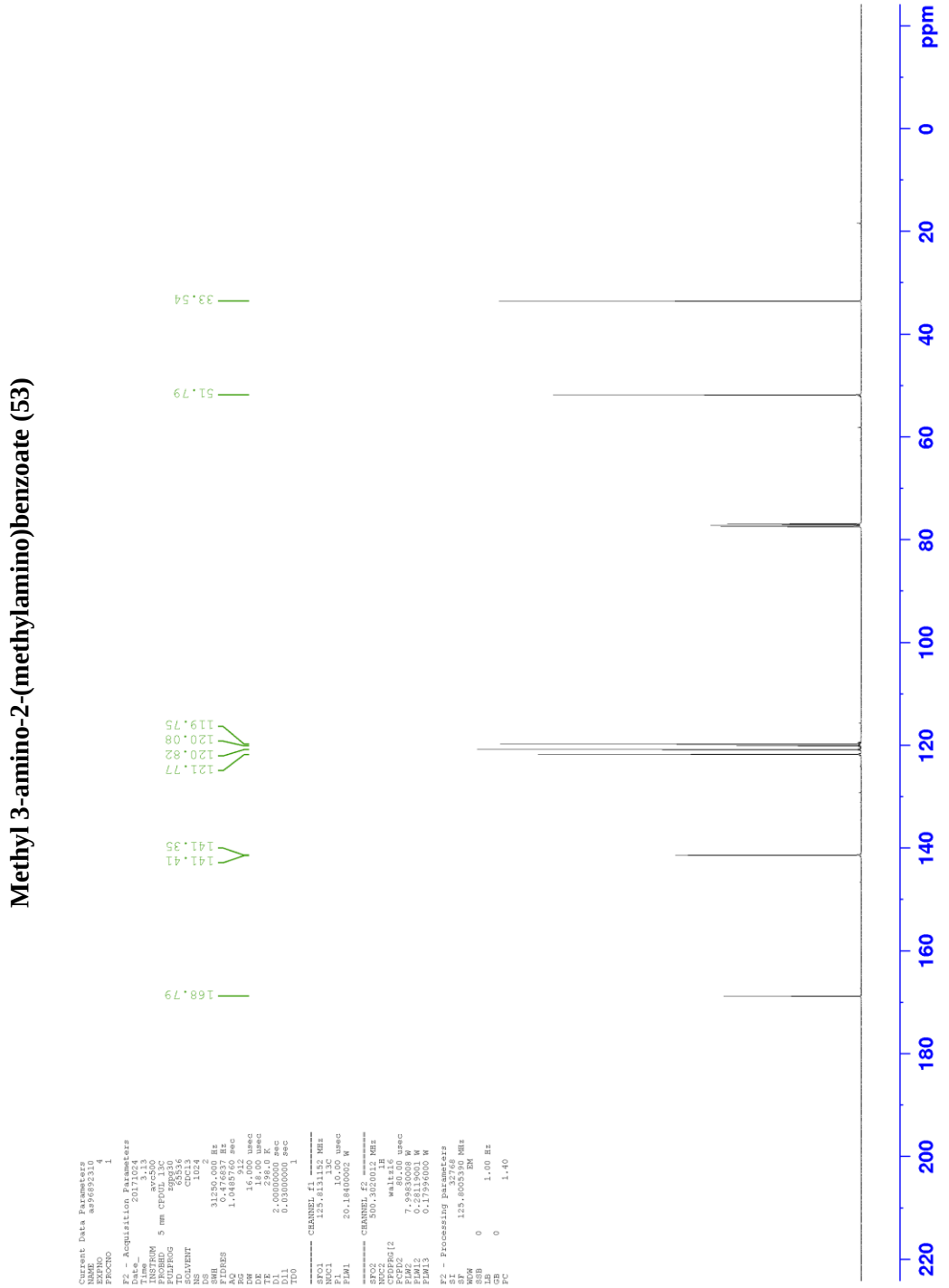


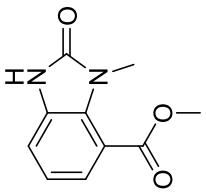
Methyl 3-amino-2-(methylamino)benzoate (53)



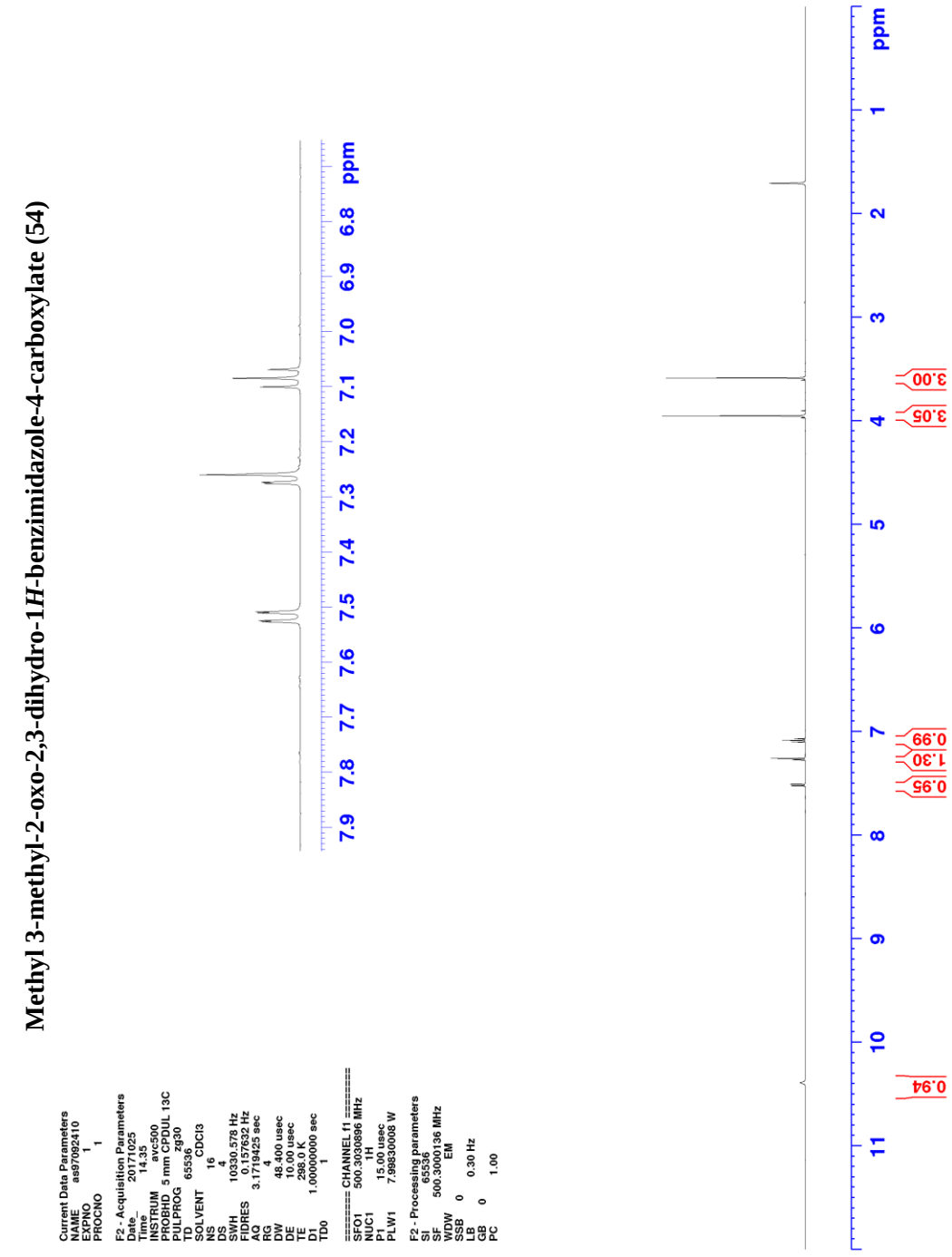


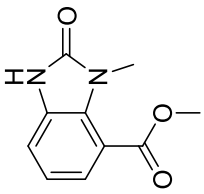
Methyl 3-amino-2-(methylamino)benzoate (53)



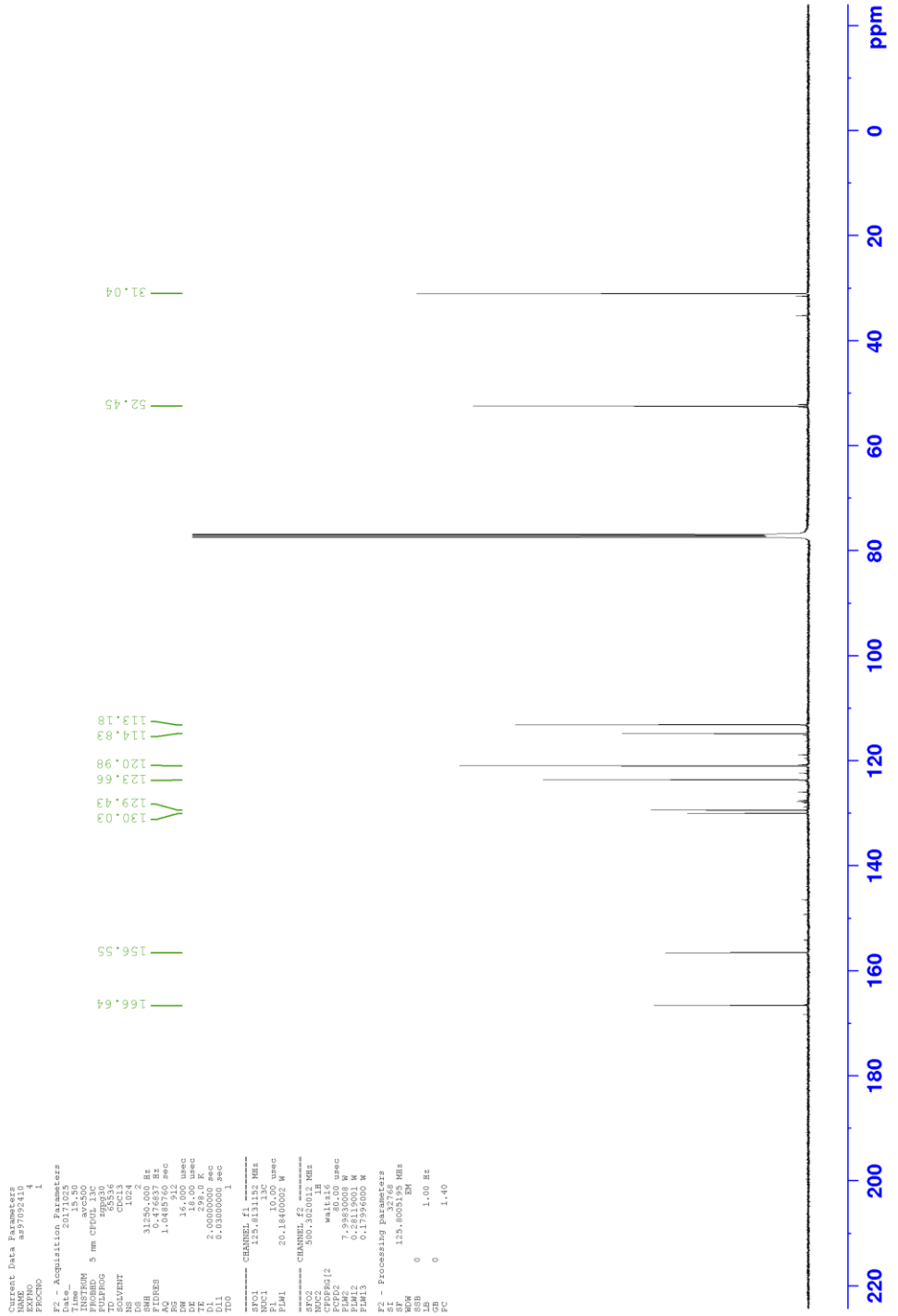


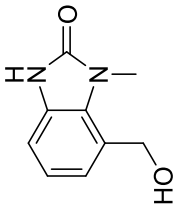
Methyl 3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate (54)



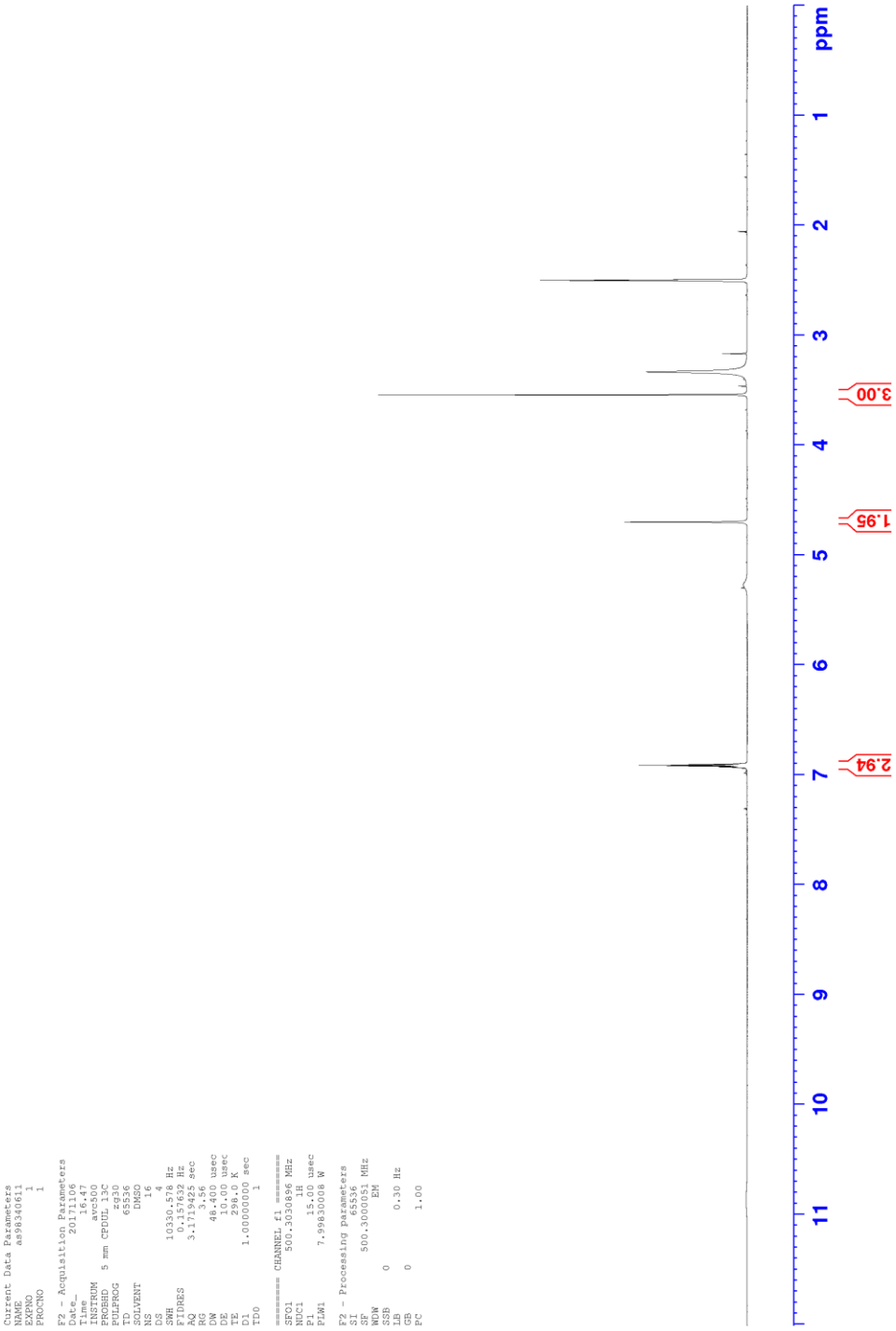


Methyl 3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate (54)

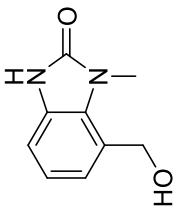


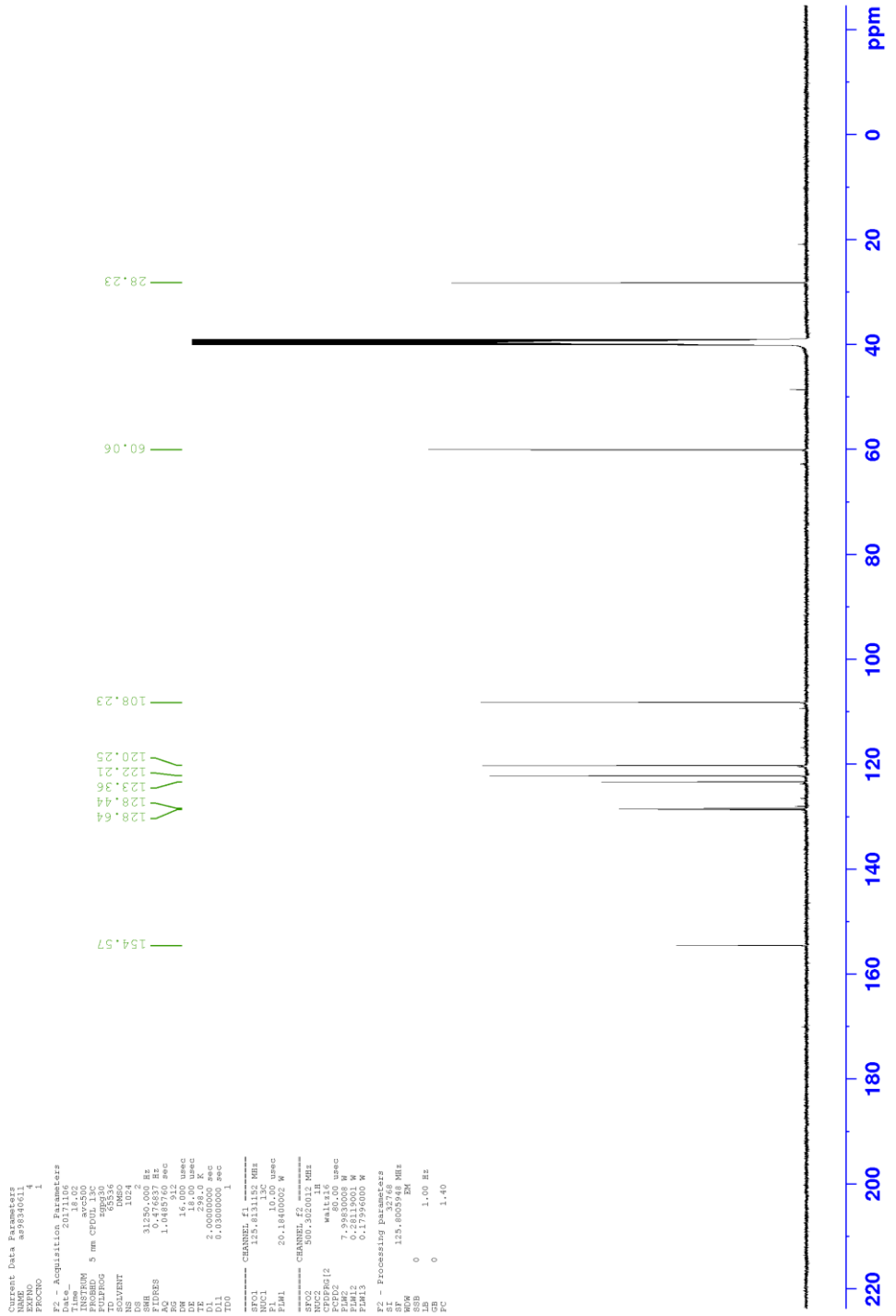


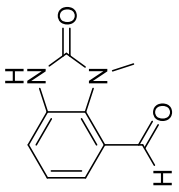
7-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (55)



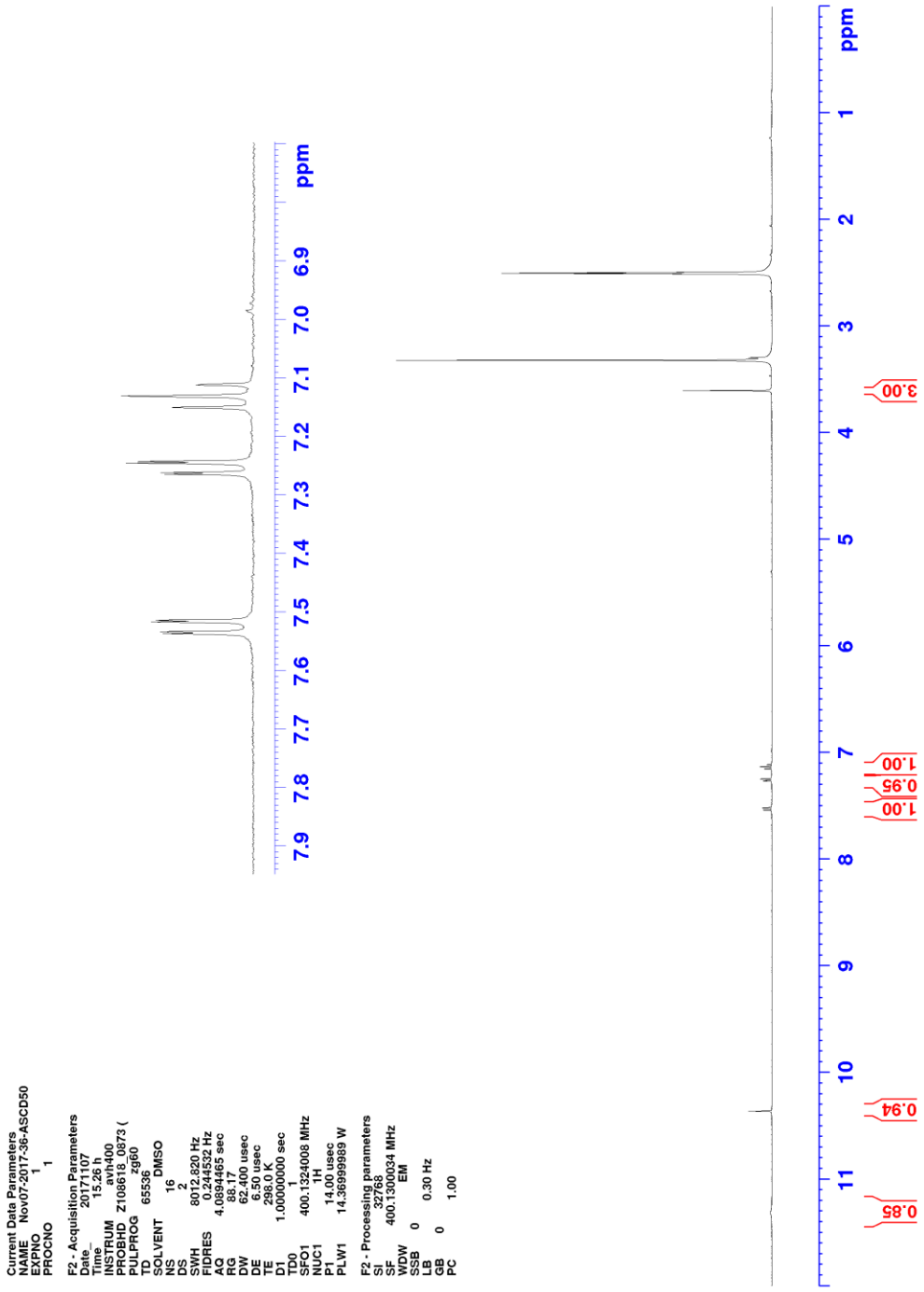
7-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (55)

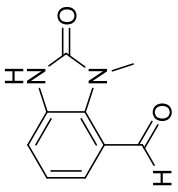




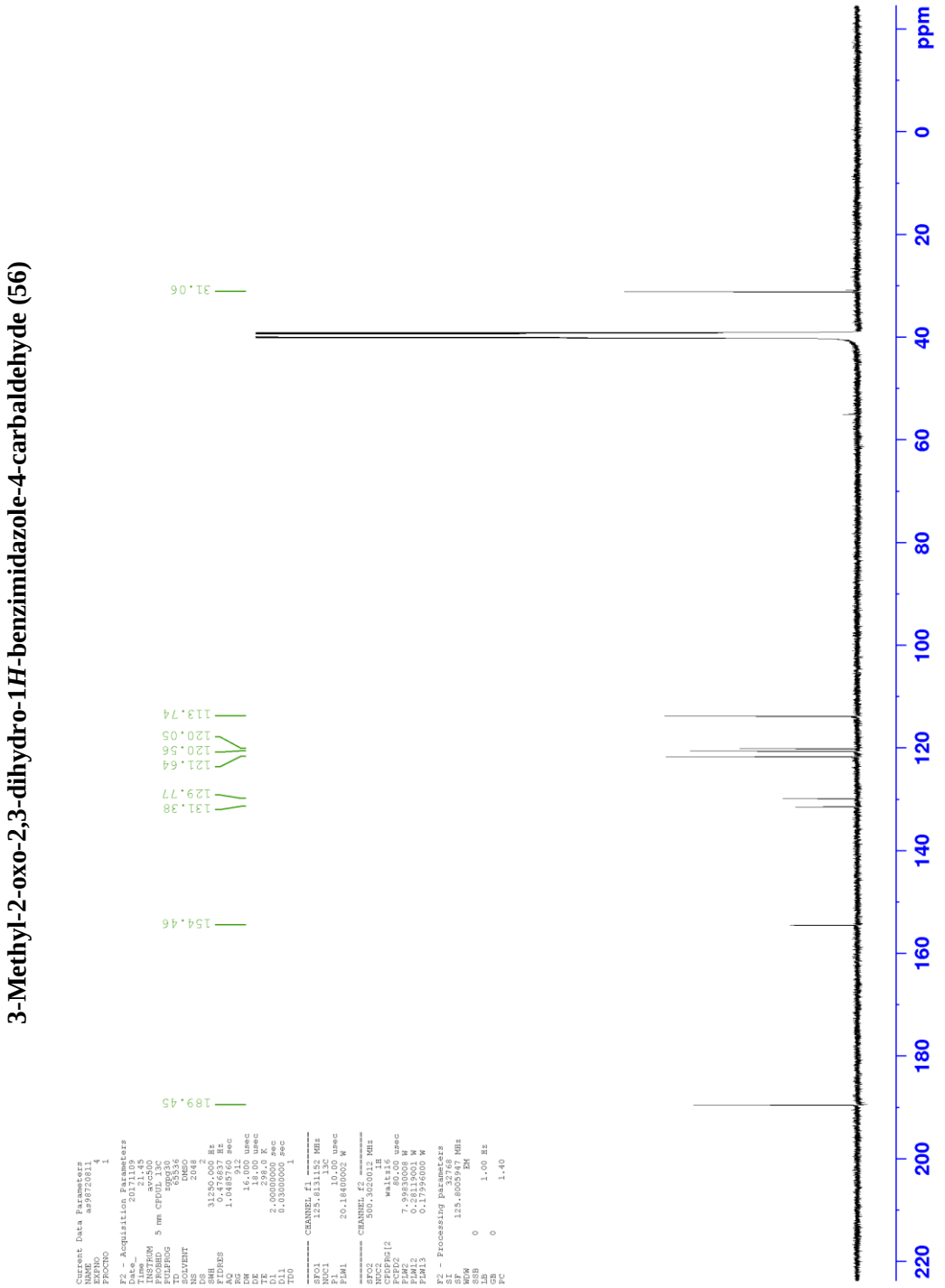


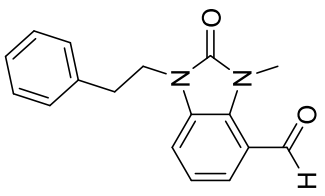
3-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carbaldehyde (56)



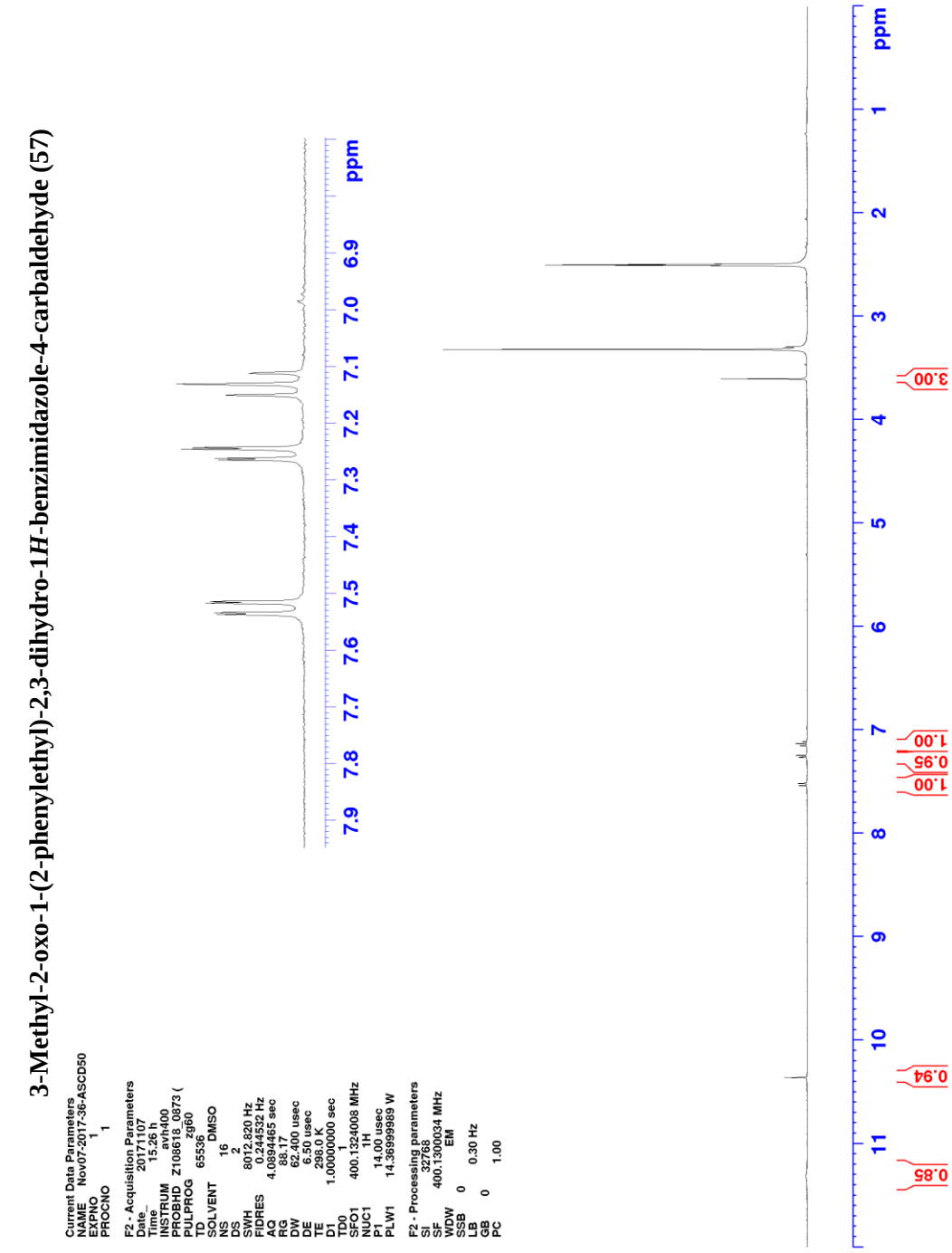


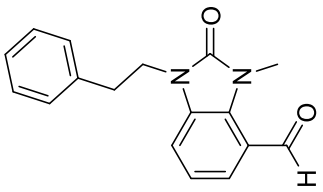
3-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carbaldehyde (56)



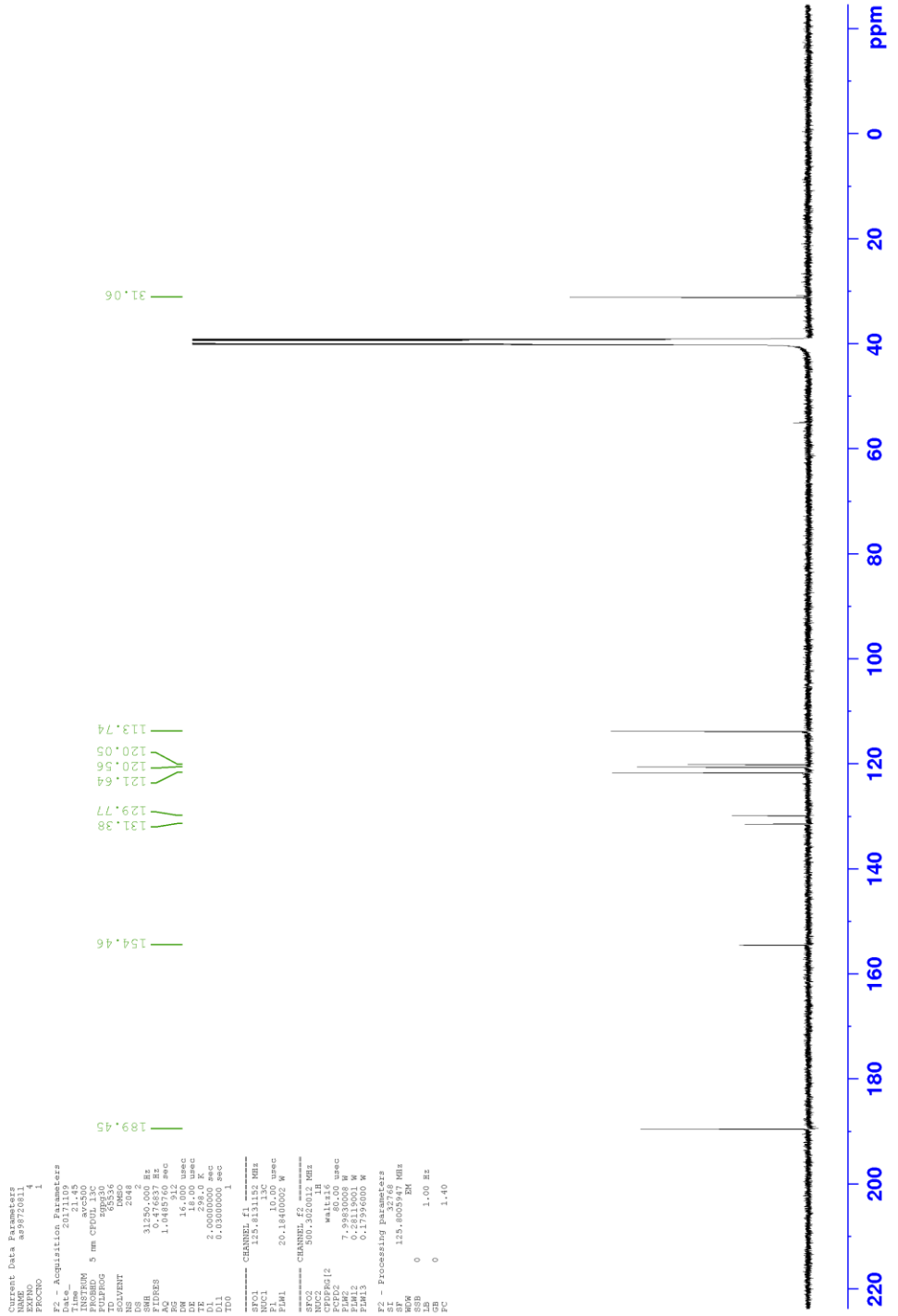


3-Methyl-2-oxo-1-(2-phenylethyl)-2,3-dihydro-1*H*-benzimidazole-4-carbaldehyde (57)

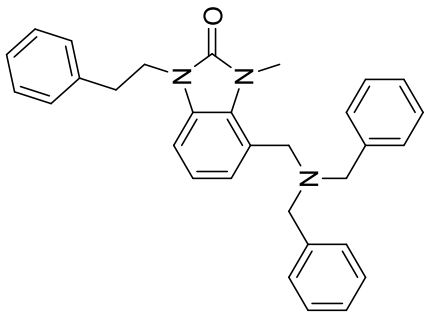


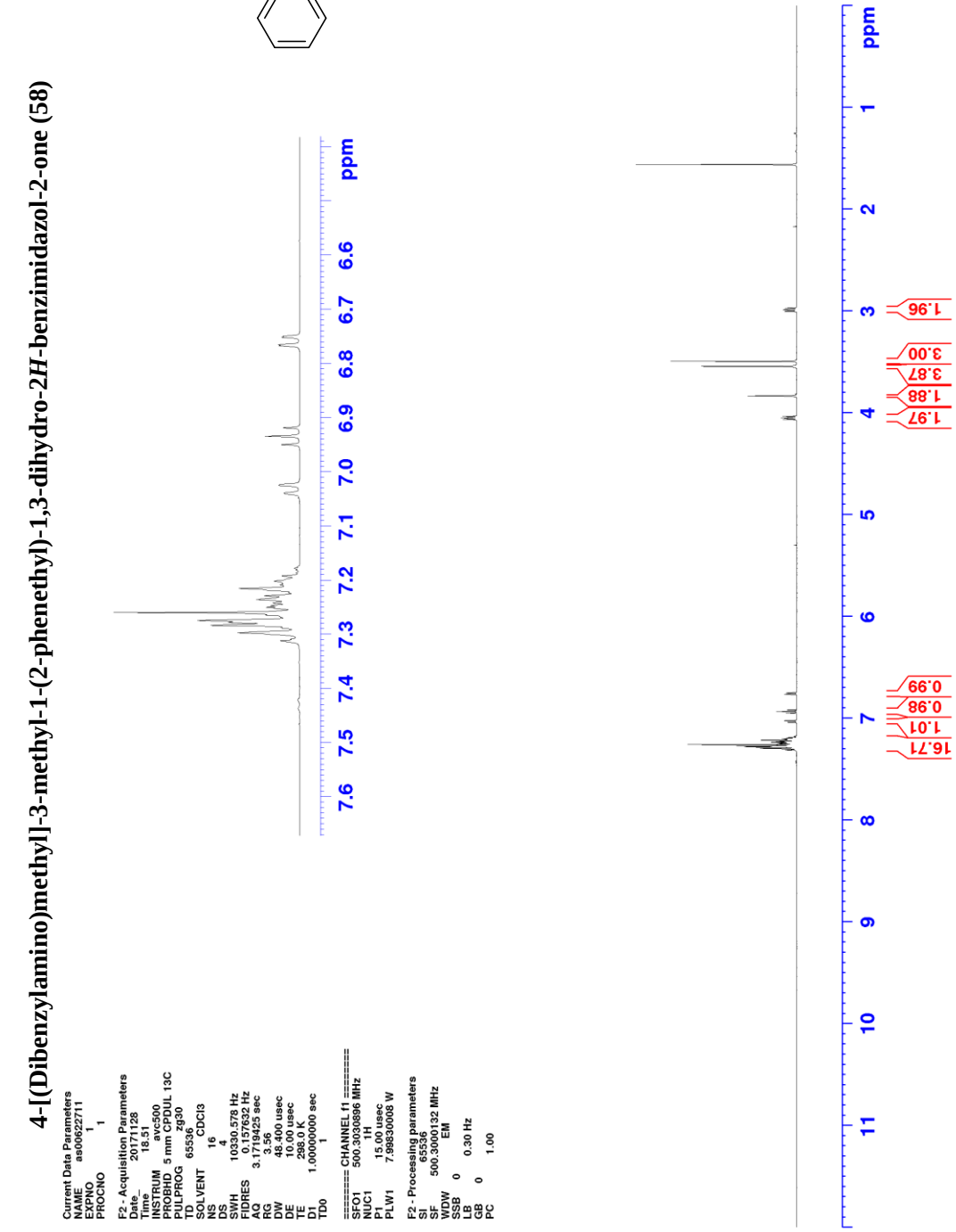


3-Methyl-2-oxo-1-(2-phenylethyl)-2,3-dihydro-1*H*-benzimidazole-4-carbaldehyde (57)

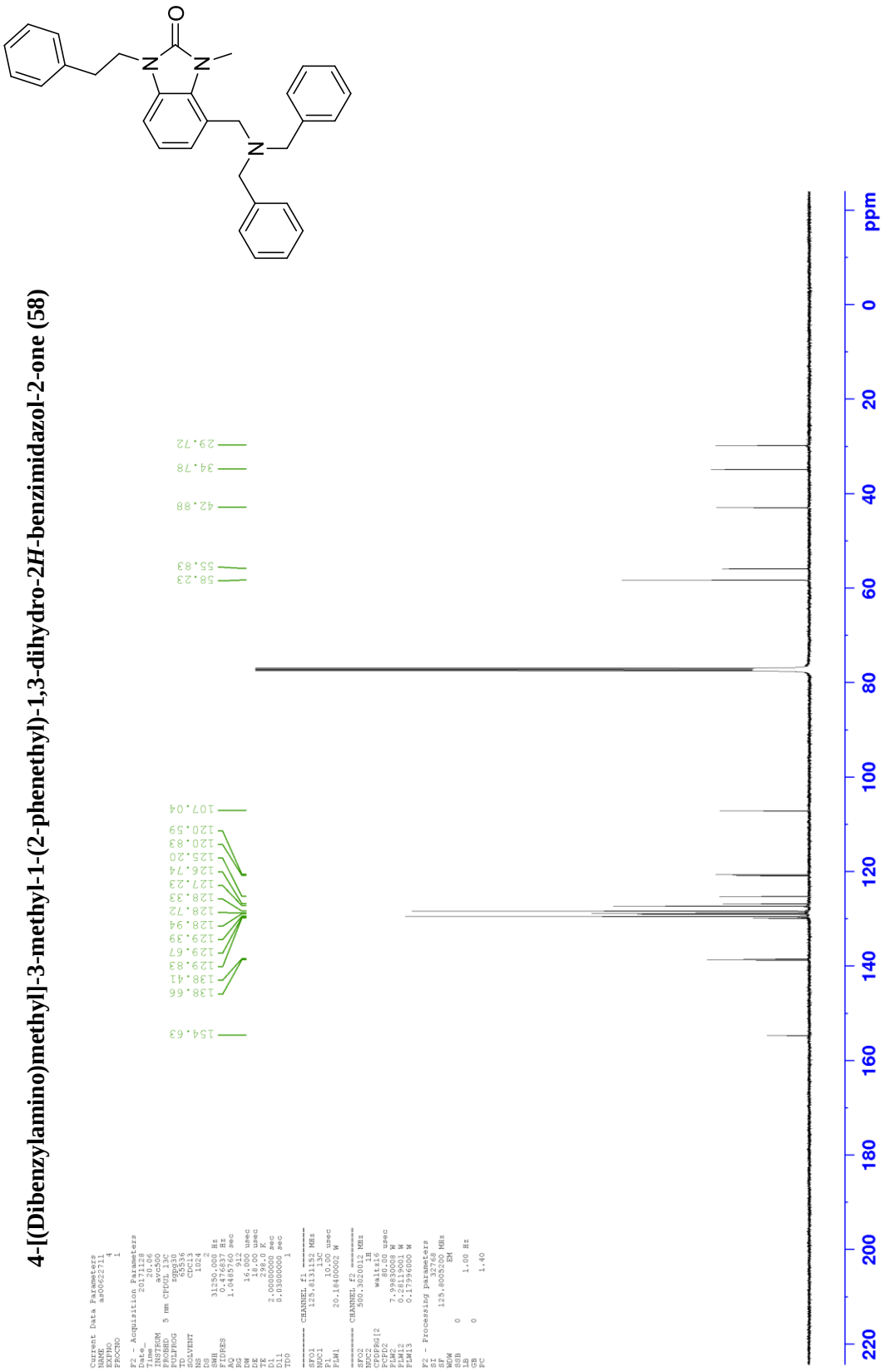


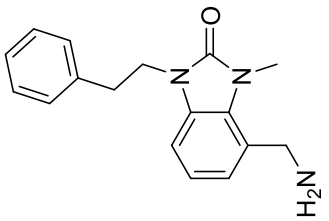
4-[(Dibenzylamino)methyl]-3-methyl-1-(2-phenethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (58)



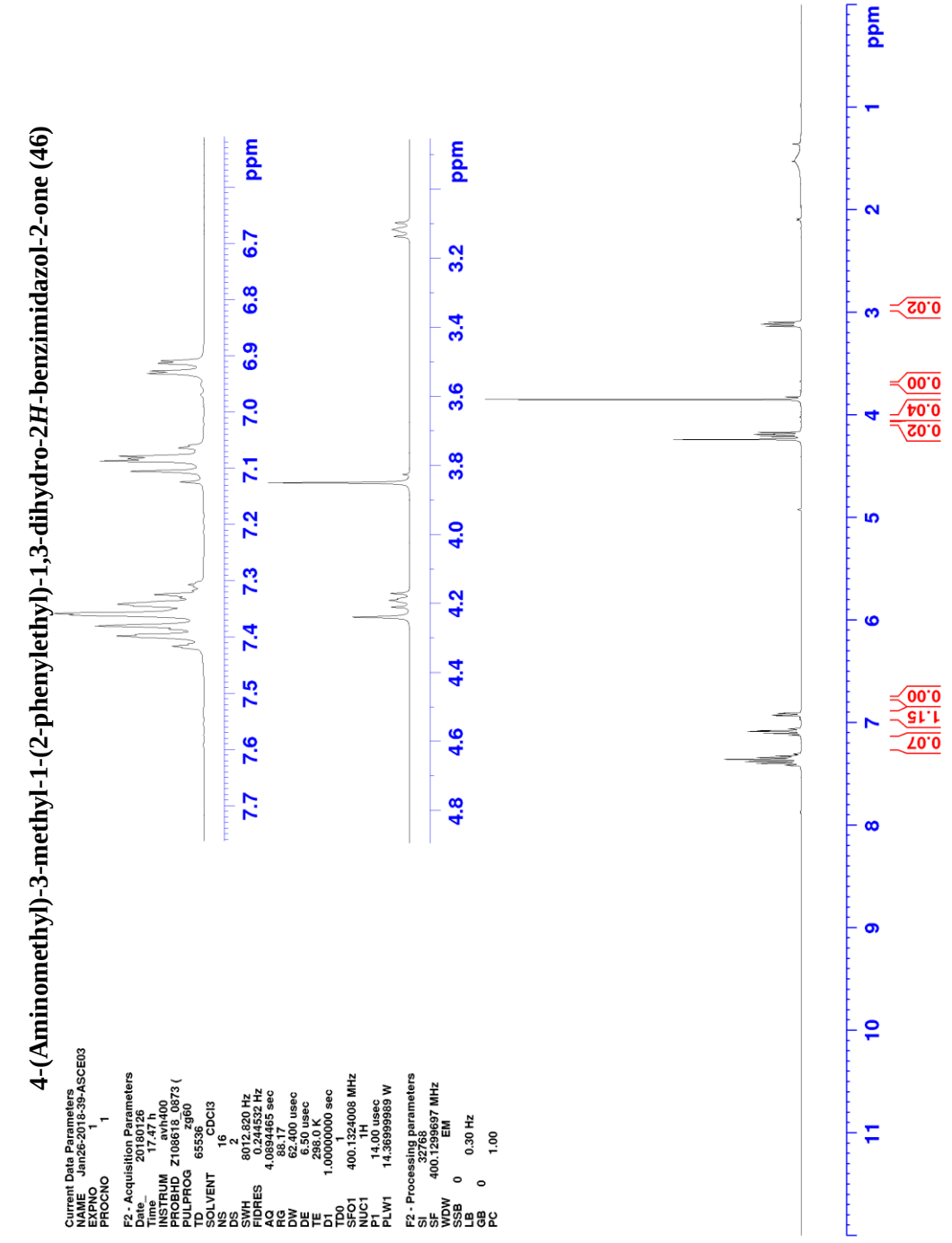


4-[(Dibenzylamino)methyl]-3-methyl-1-(2-phenethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (58)

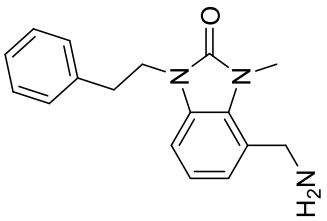


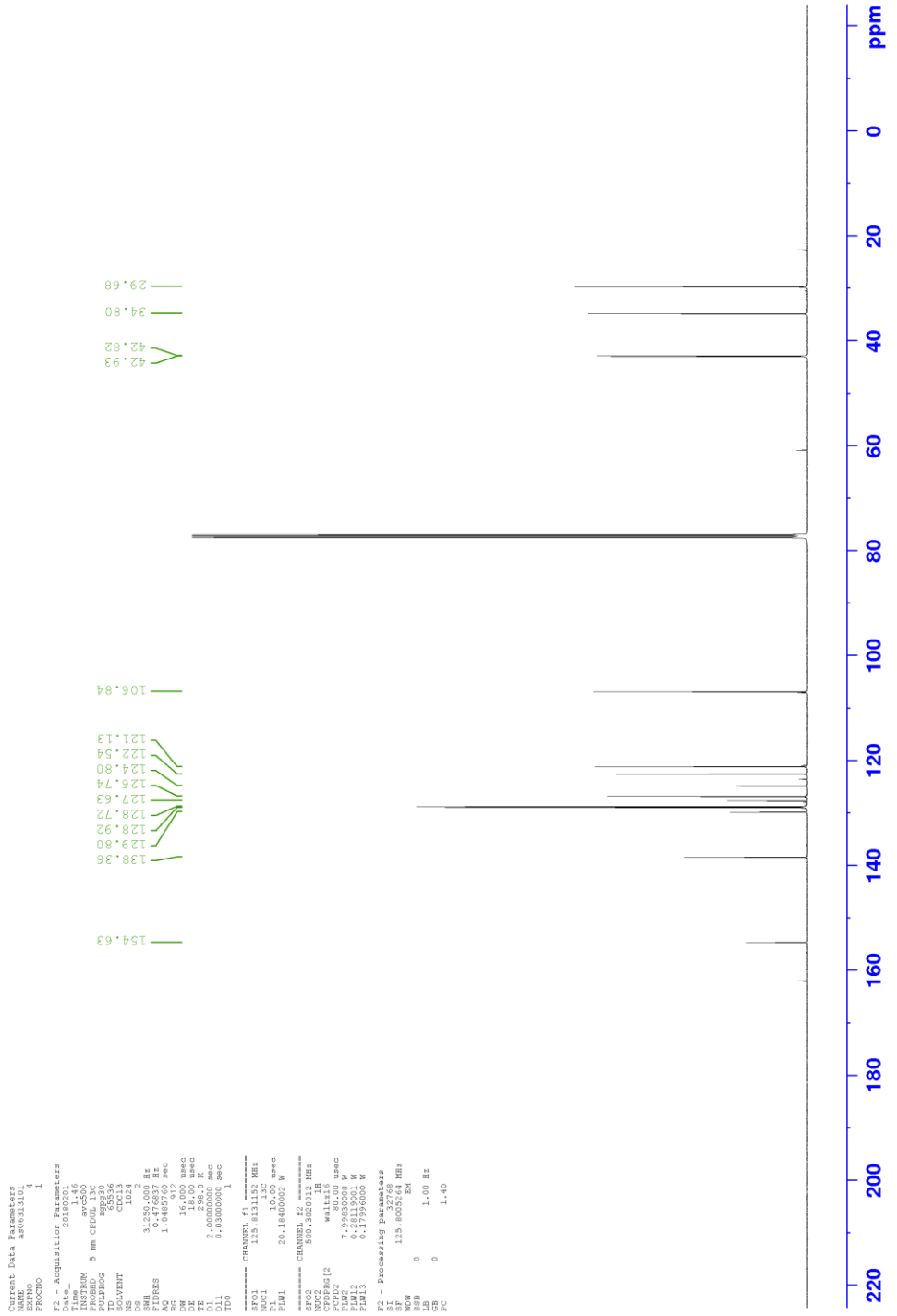


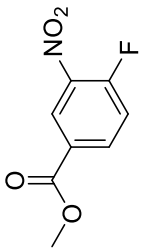
4-(Aminomethyl)-3-methyl-1-(2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (46)



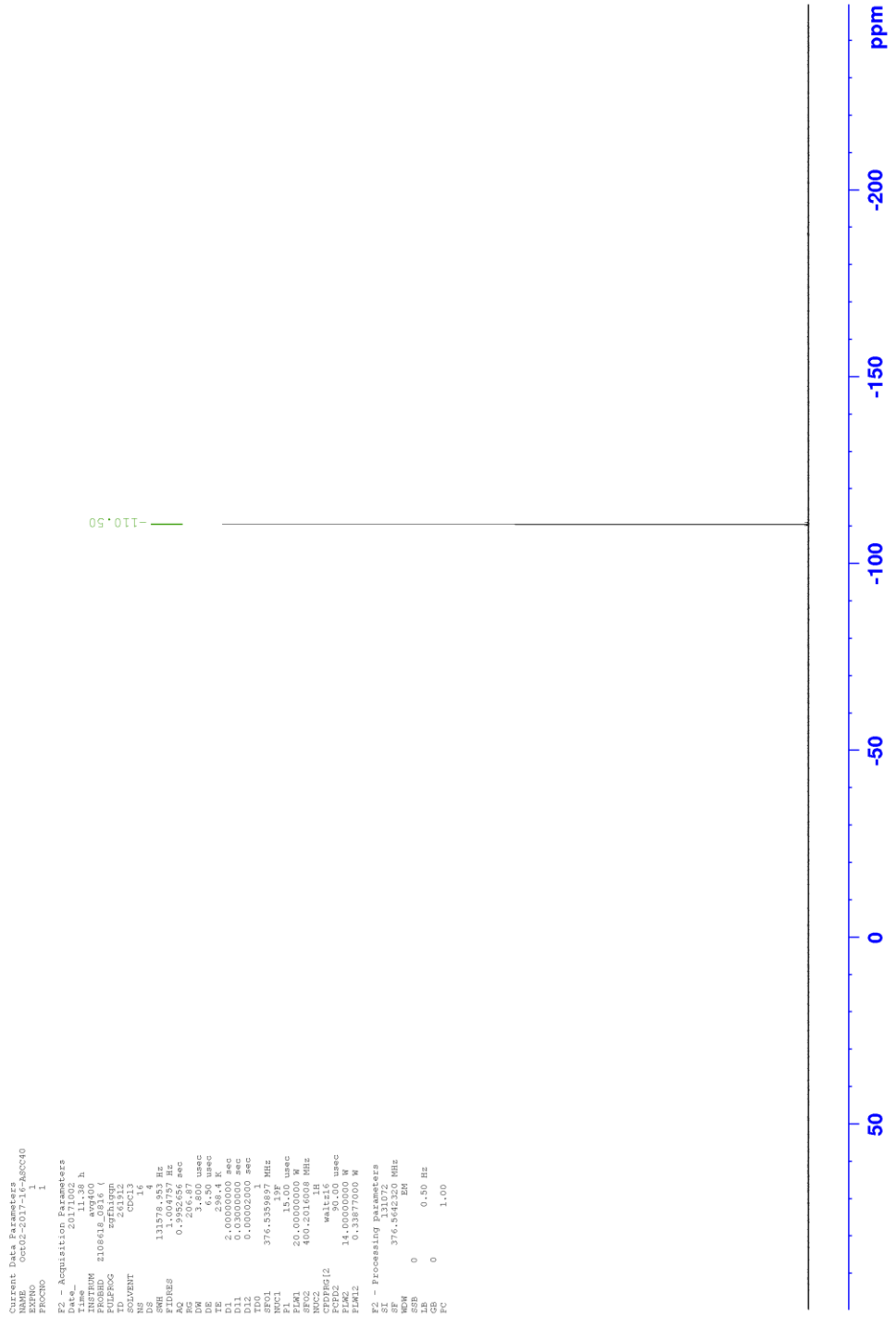
4-(Aminomethyl)-3-methyl-1-(2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (46)







Methyl 4-fluoro-3-nitrobenzoate (60)

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PROCNO         1

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PROBHD  2108618_0816 (
PULPROG        zgfhigqn
TD             21472
SOLVENT          CDCl3
NS               16
DS
SWH        131578.947 Hz
FIDRES       1.004757 Hz
AQ           0.9952656 sec
RG             206.07
DW            3.800 usec
DE               6.50 usec
TE             298.4 K
D1           2.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
TD0                1

SFO1       376.5359897 MHz
NUC1              19F
P1               15.50 usec
PLW1      20.00000000 W
SFO2     400.2016008 MHz
NUC2              1H
CPDPRG2         waltz16
PCPD2            90.00 usec
PLW2      14.00000000 W
PLW12      0.33877000 W

F2 - Processing parameters
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SF         376.5642320 MHz
WDW              EM
SSB        0
LB                 0.50 Hz
GB         0
PC                 1.00
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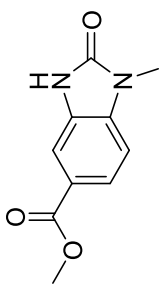
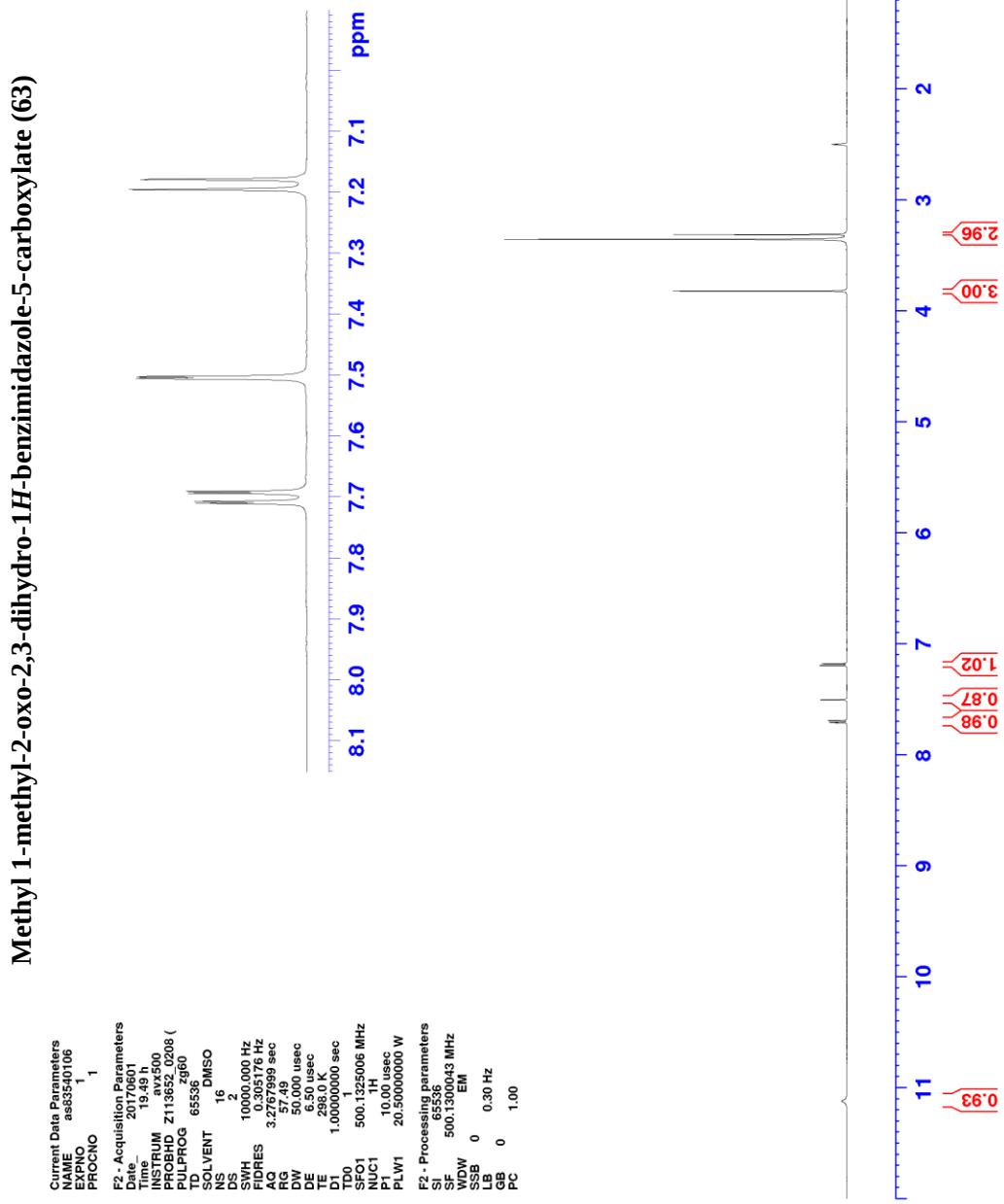
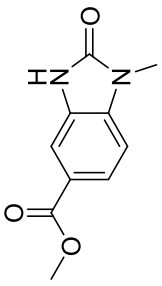
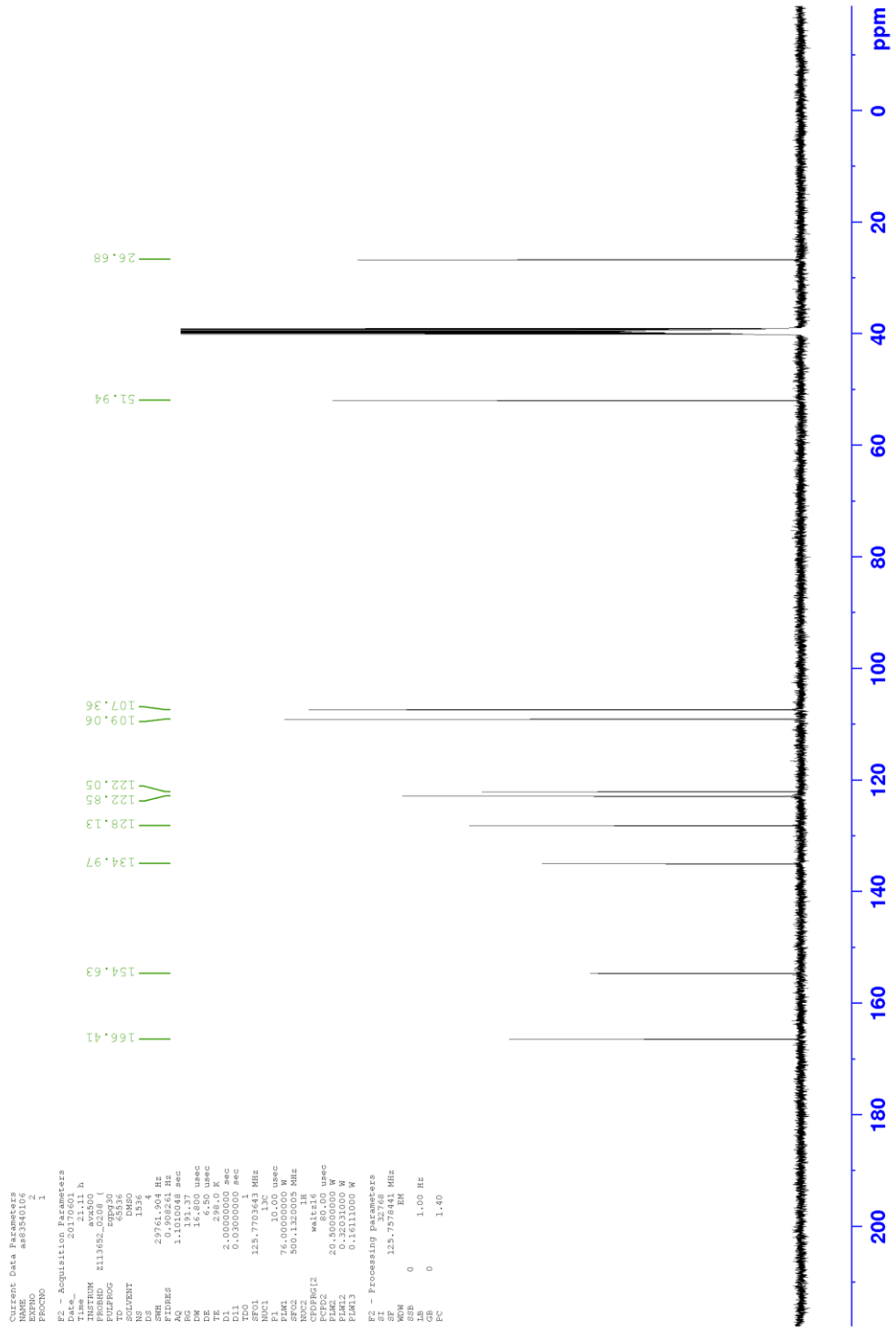
Methyl 1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (63)



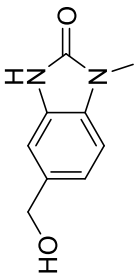


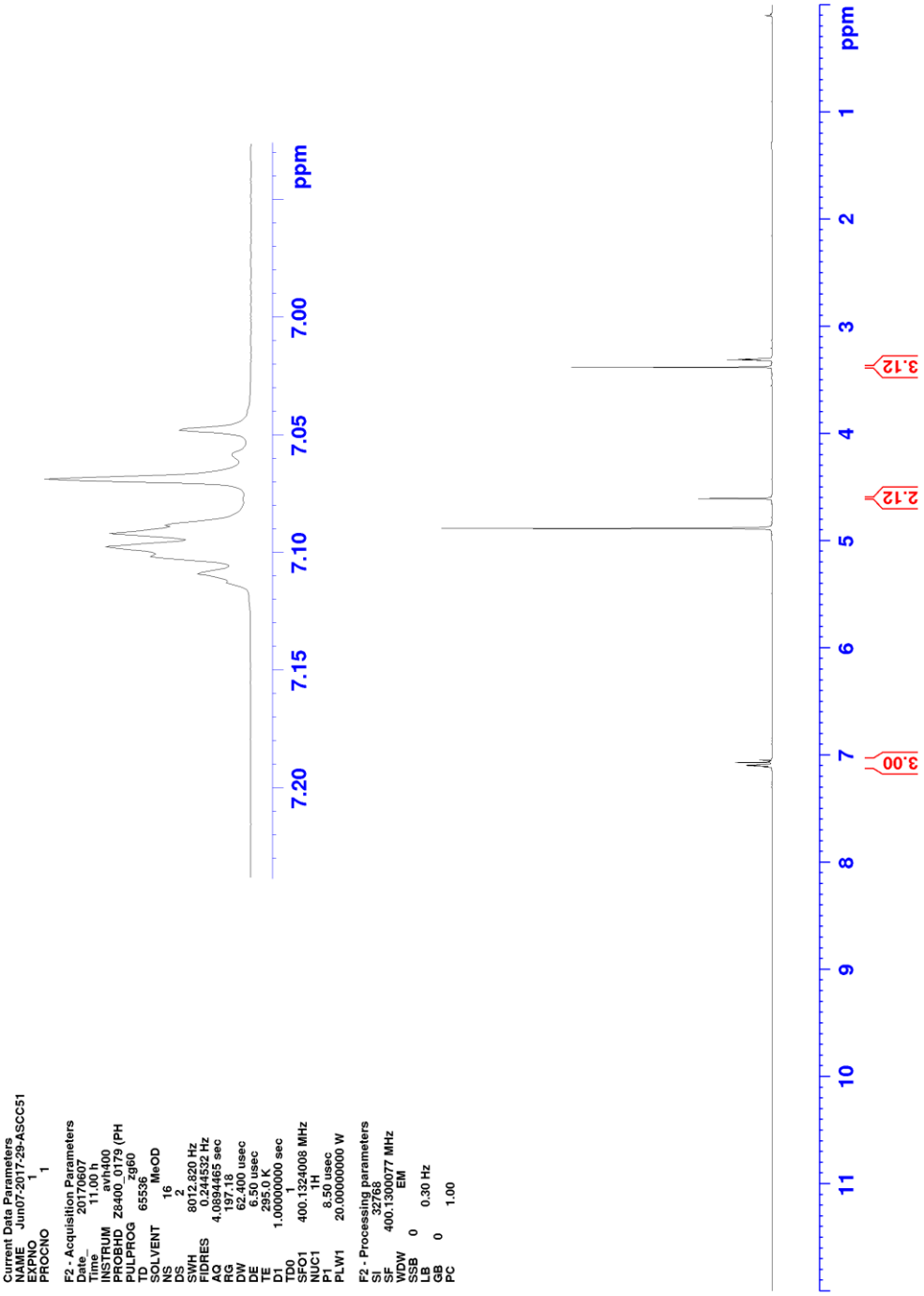


Methyl 1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (63)



5-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (64)





Current Data Parameters
NAME Jun07-2017-29-ASCC51
EXPNO 1
PROCNO 1

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Time 11.00 h
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TD 65536
SOLVENT MeOD
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DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 197.18
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
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F2 - Processing parameters
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GB 0
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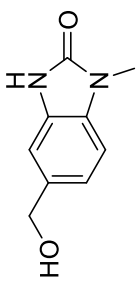
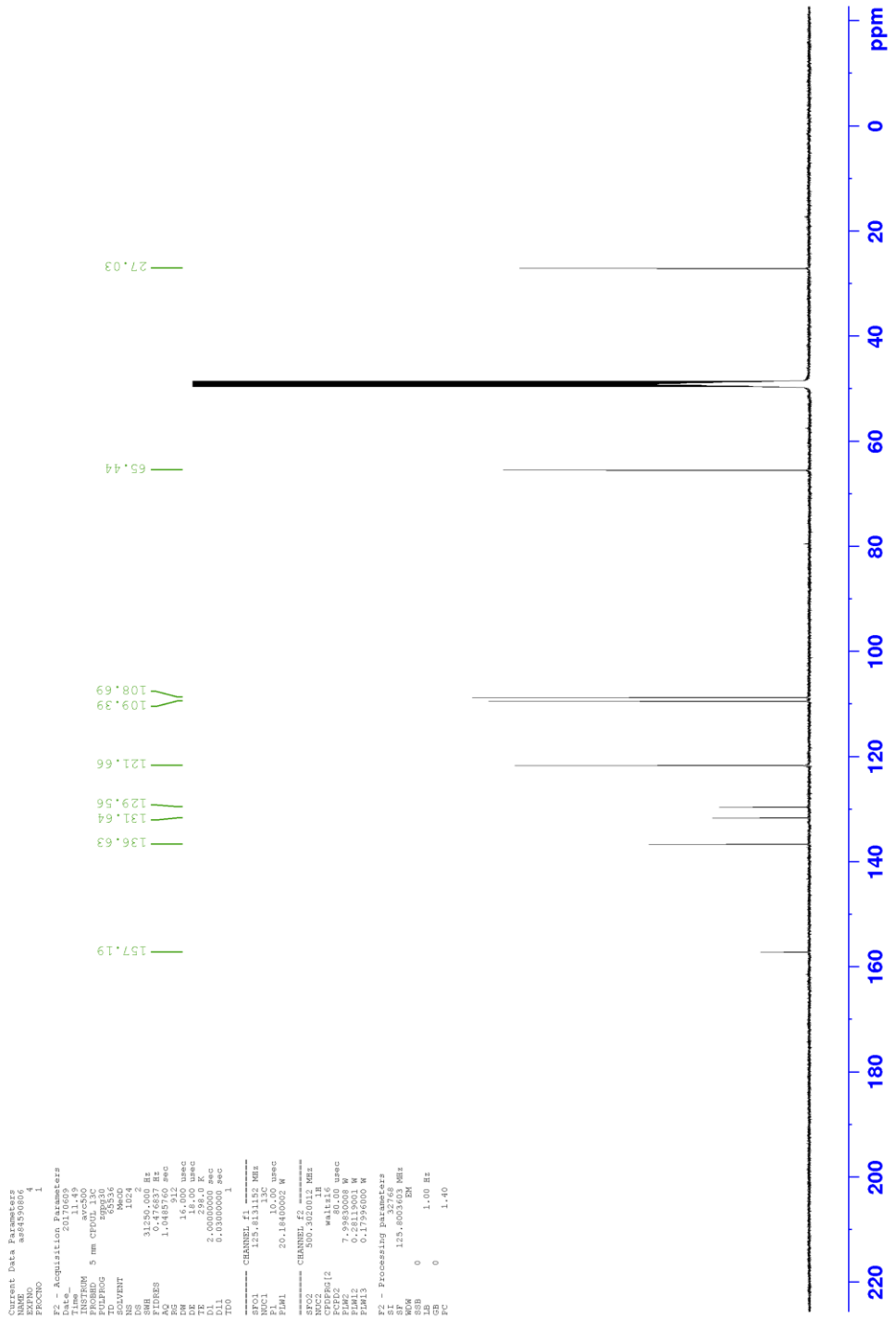
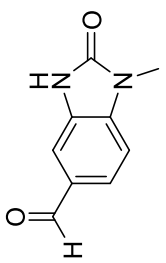
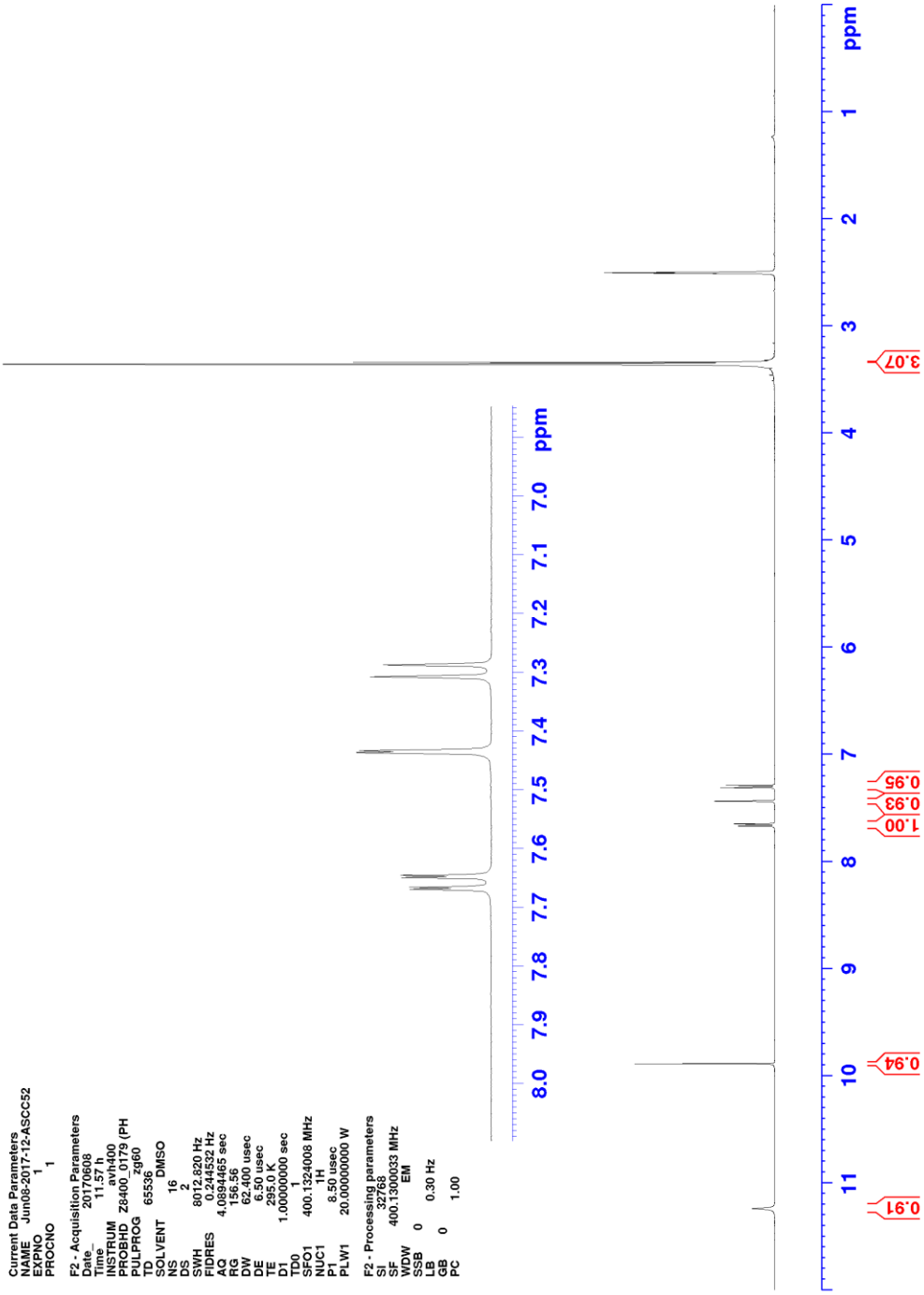


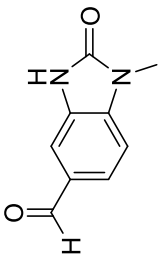
5-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (64)



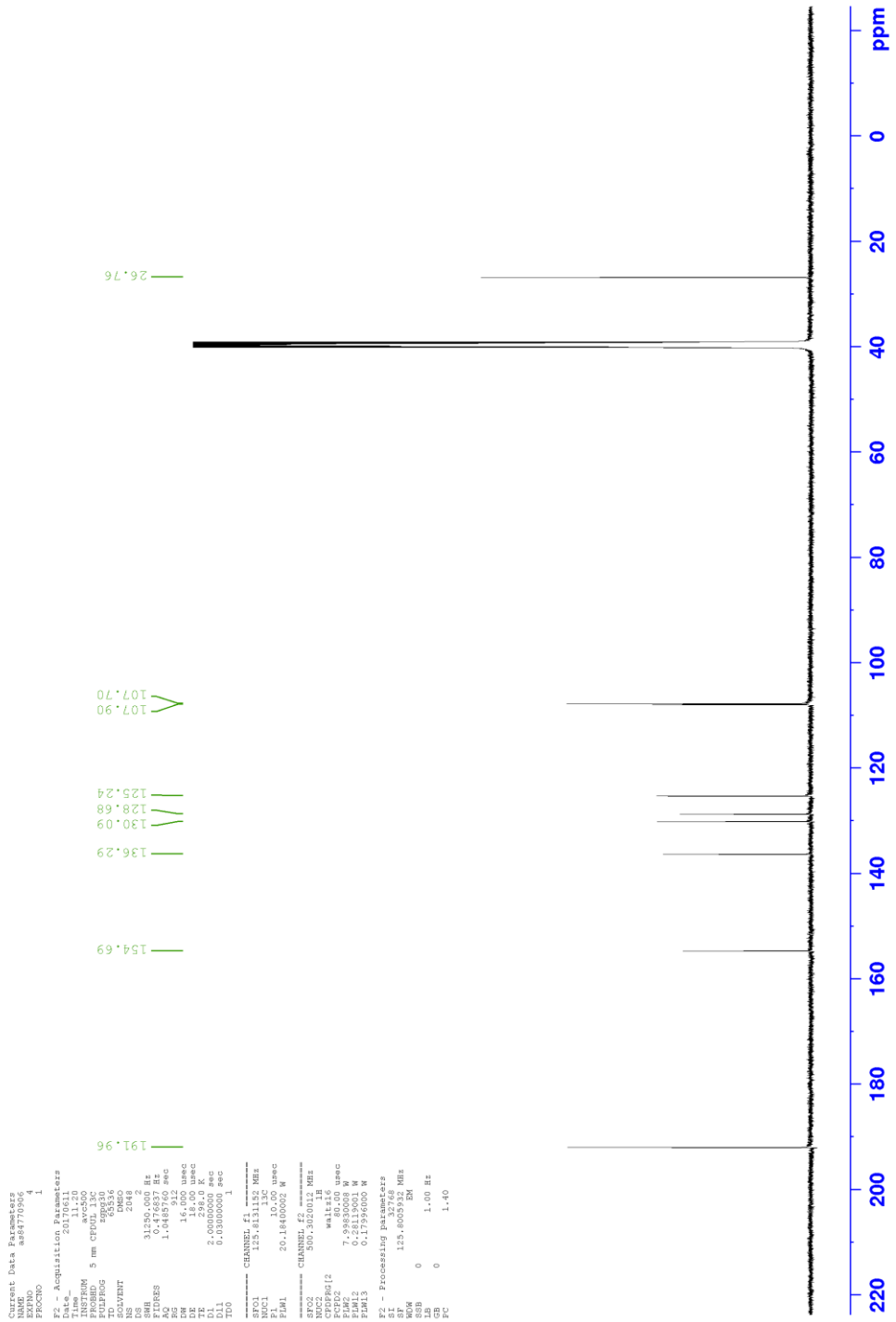


1-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (65)

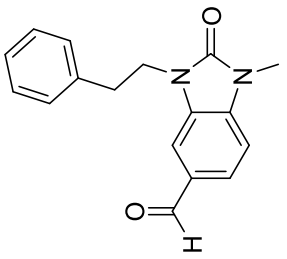


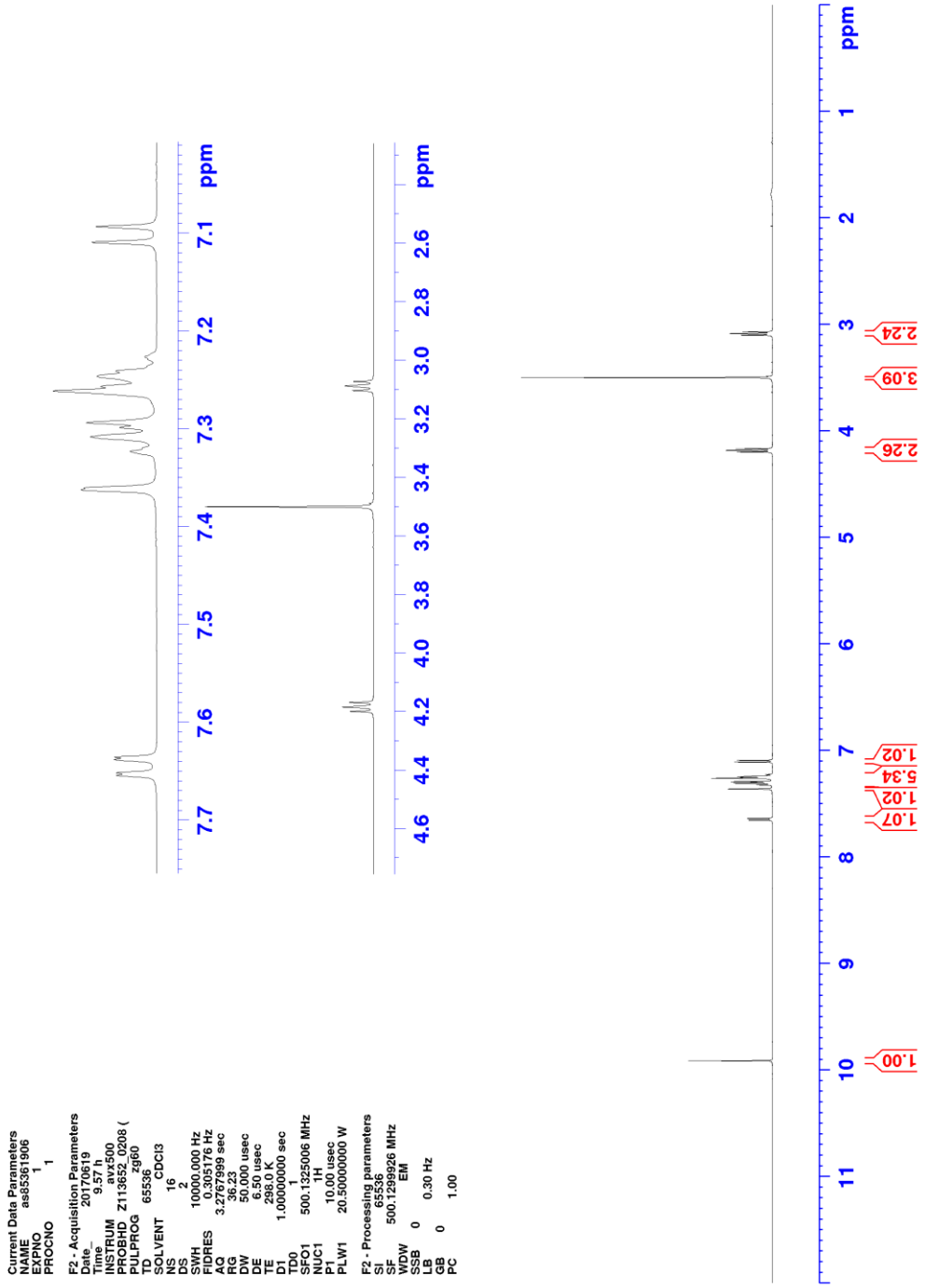


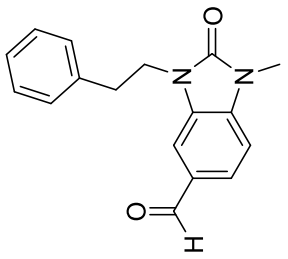
1-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (65)



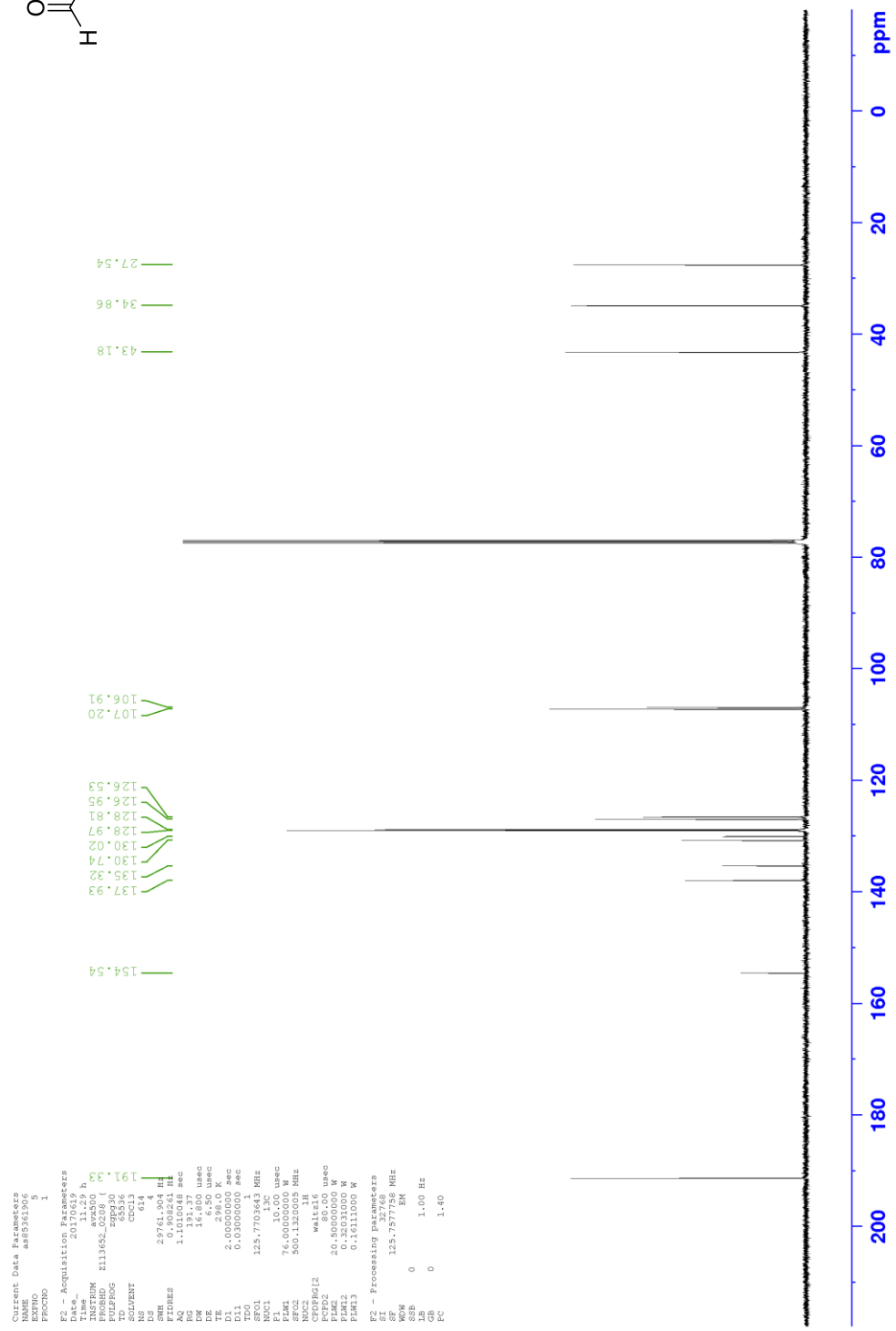
1-Methyl-2-oxo-3-(2-phenylethyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (66)



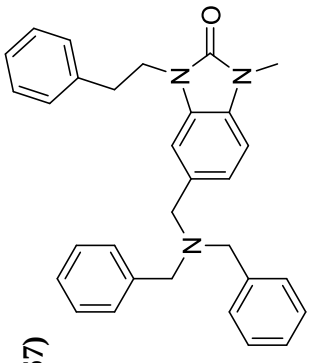


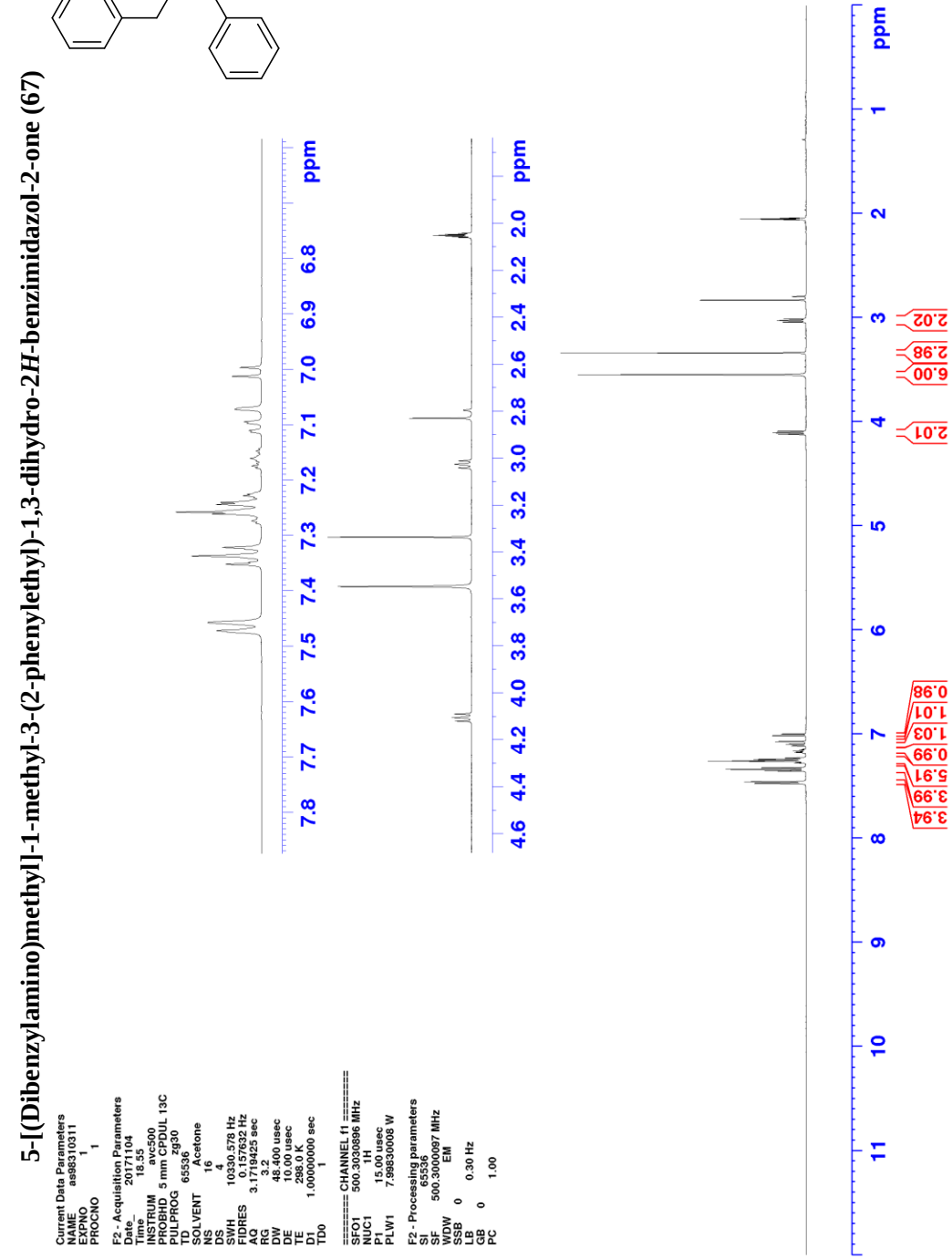


1-Methyl-2-oxo-3-(2-phenylethyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (66)

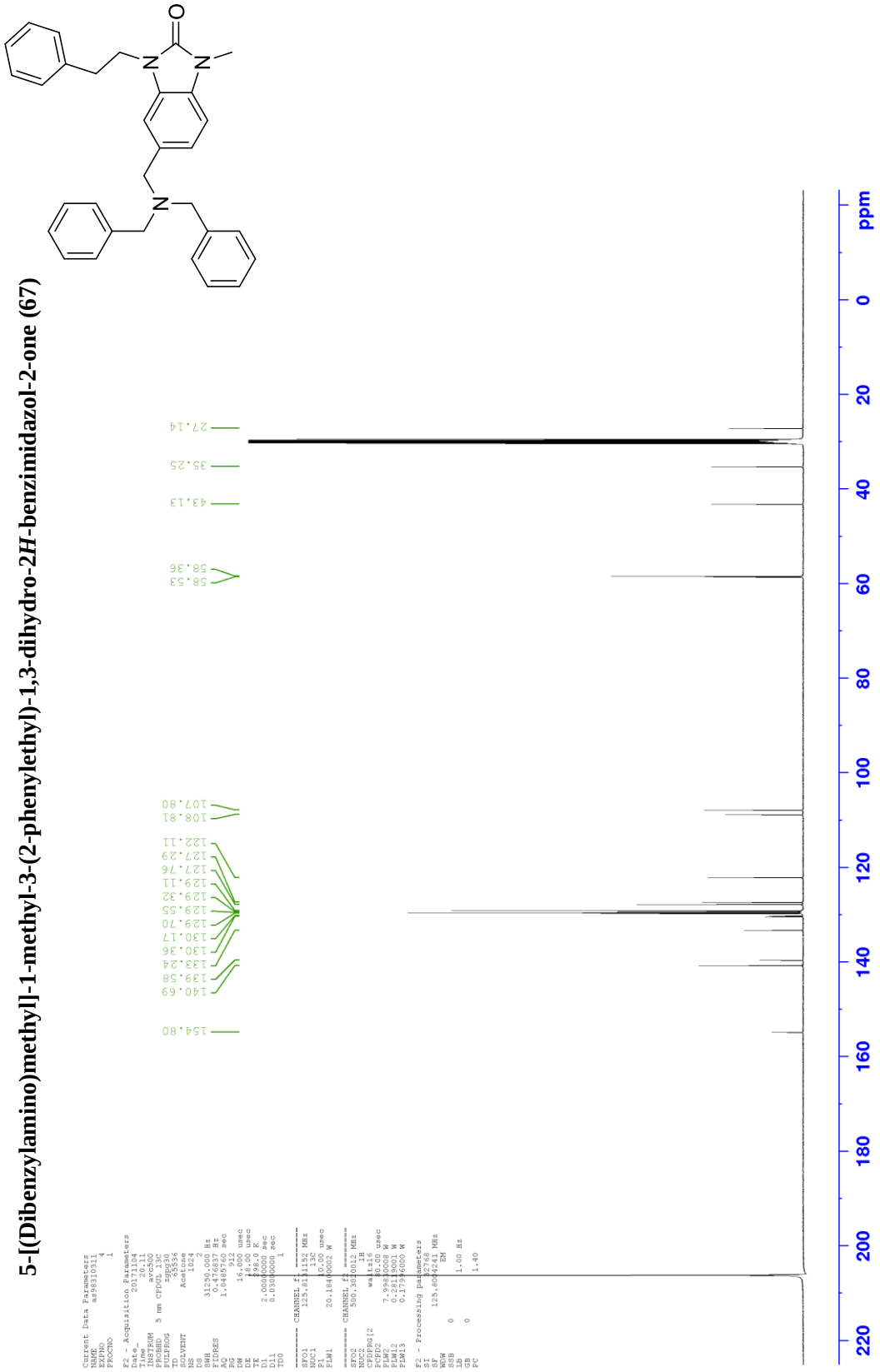


5-[(Dibenzylamino)methyl]-1-methyl-3-(2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (67)

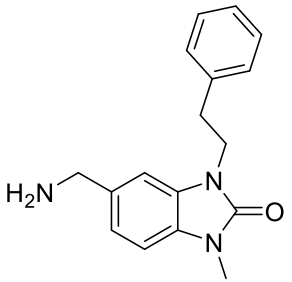




5-[(Dibenzylamino)methyl]-1-methyl-3-(2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (67)



5-(Aminomethyl)-1-methyl-3-(2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (64)

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PROCNO             1

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SOLVENT         MeOD
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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 ========
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NUC1               1H
P1              15.00 usec
PLW1        7.99830008 W

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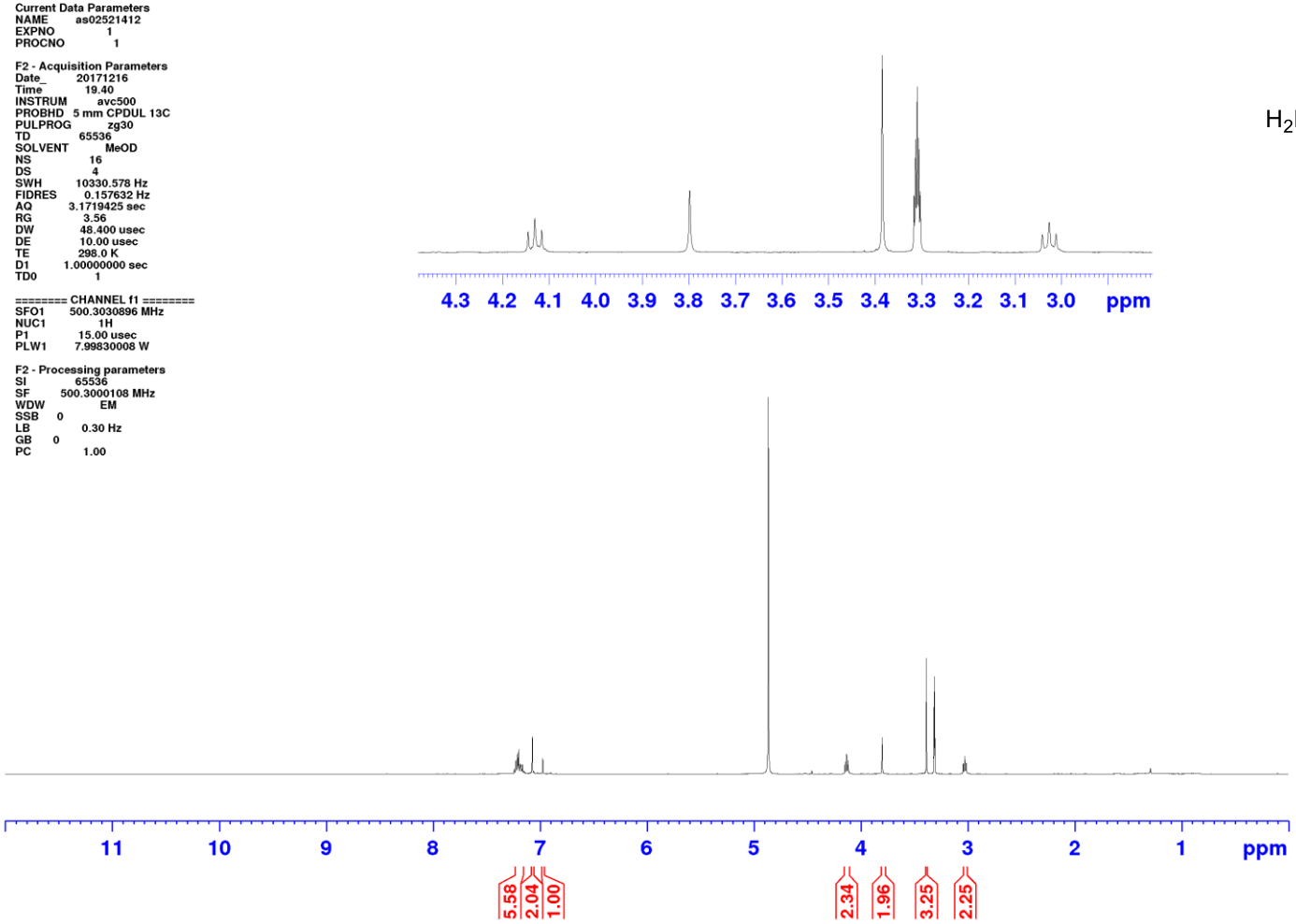
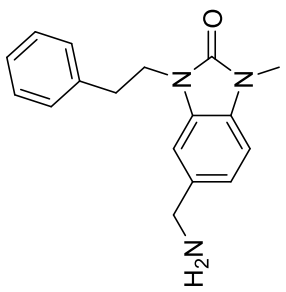
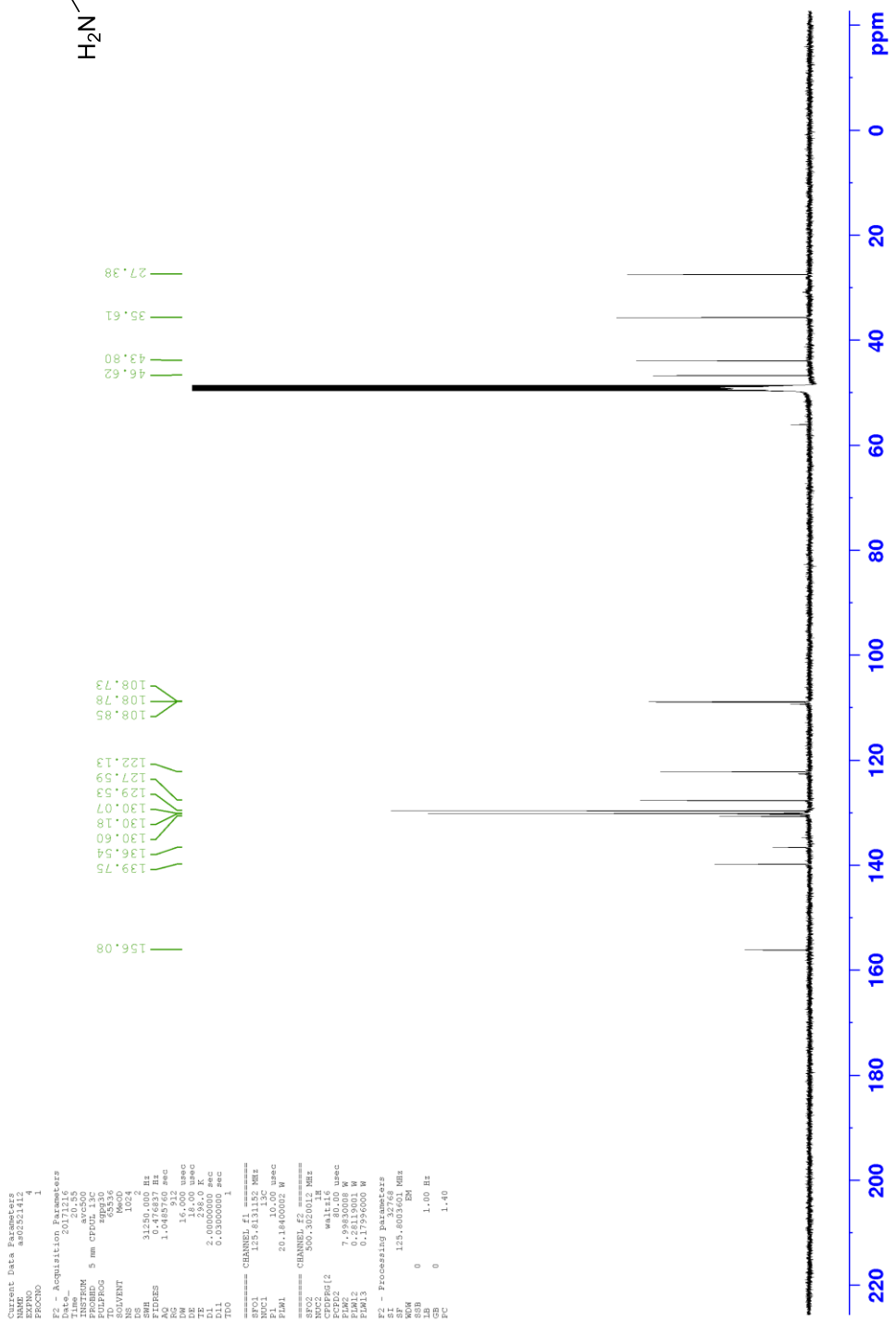
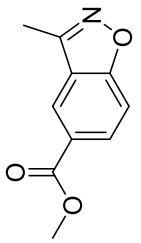


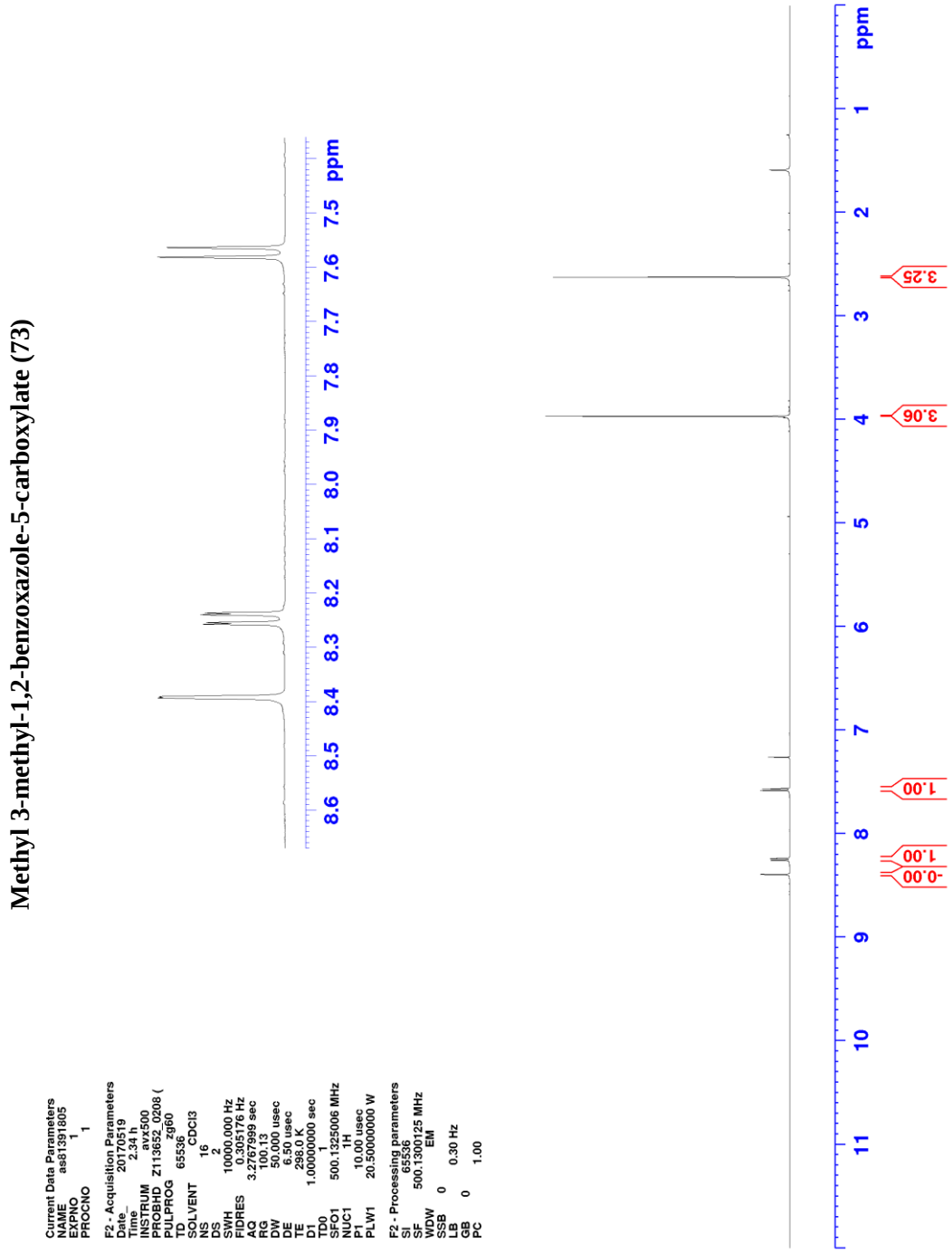
5-(Aminomethyl)-1-methyl-3-(2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (64)

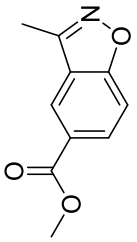




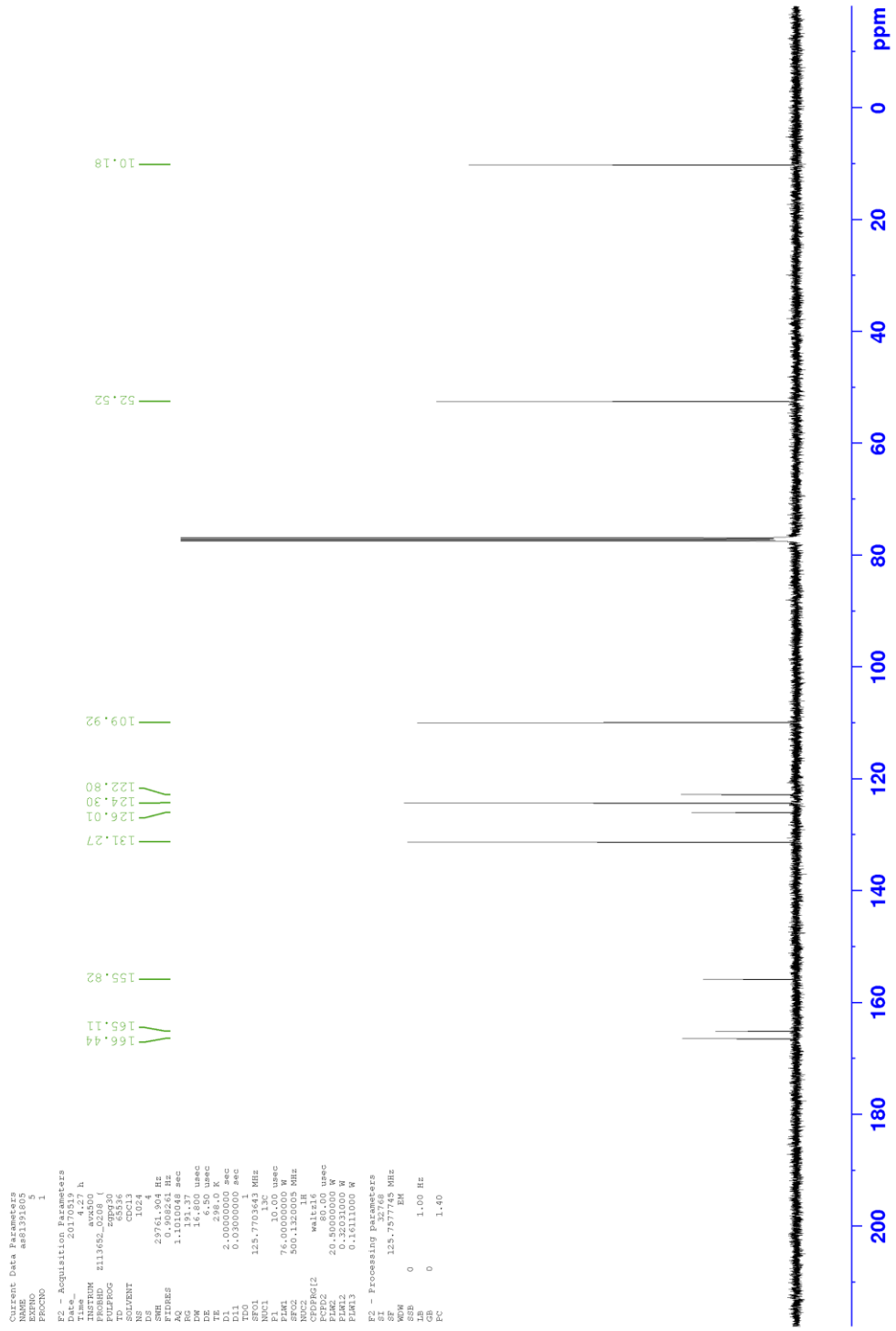


Methyl 3-methyl-1,2-benzoxazole-5-carboxylate (73)

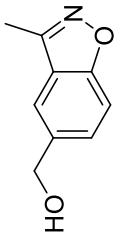


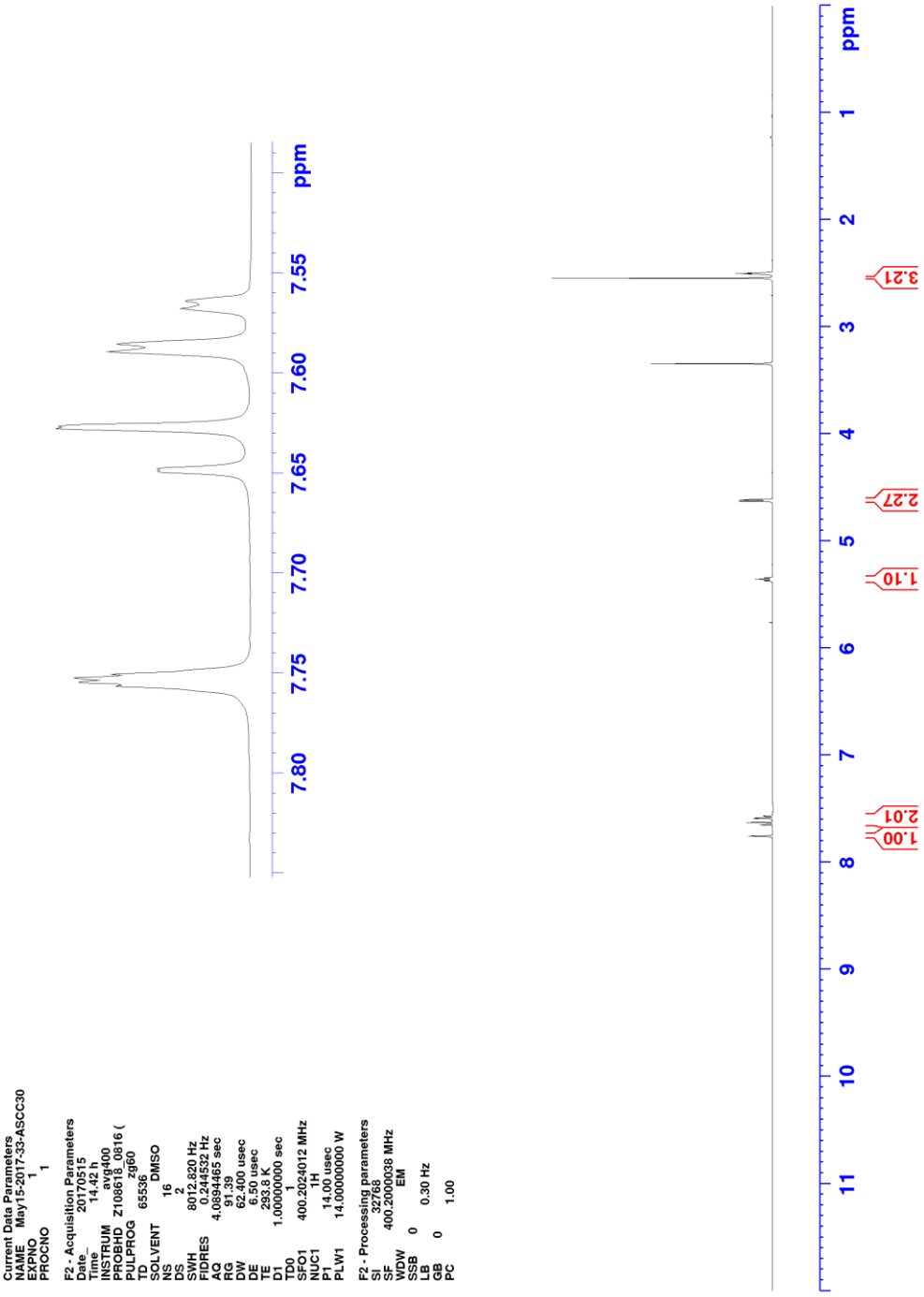


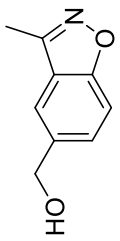
Methyl 3-methyl-1,2-benzoxazole-5-carboxylate (73)



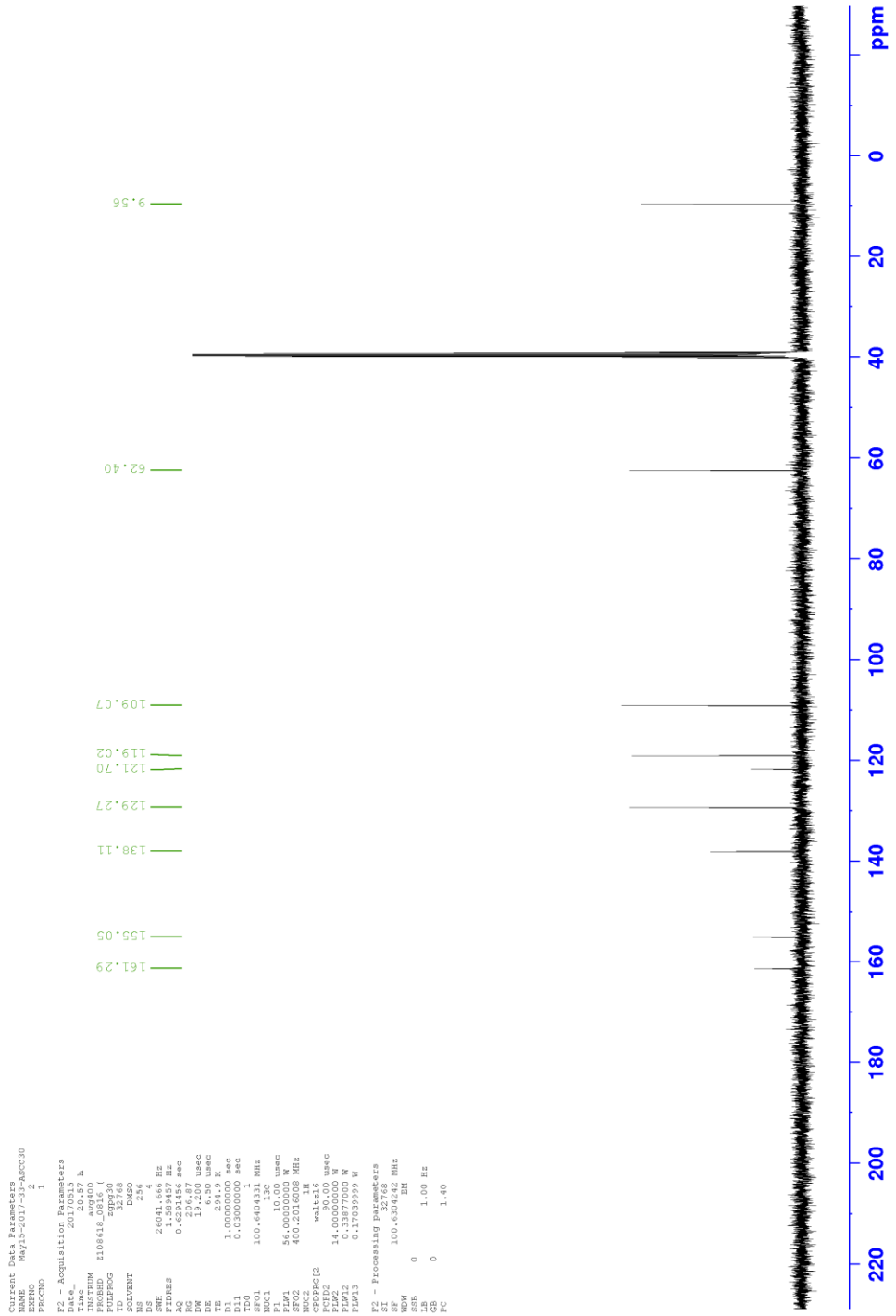
(3-Methyl-1,2-benzoxazol-5-yl)methanol (74)

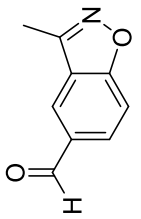




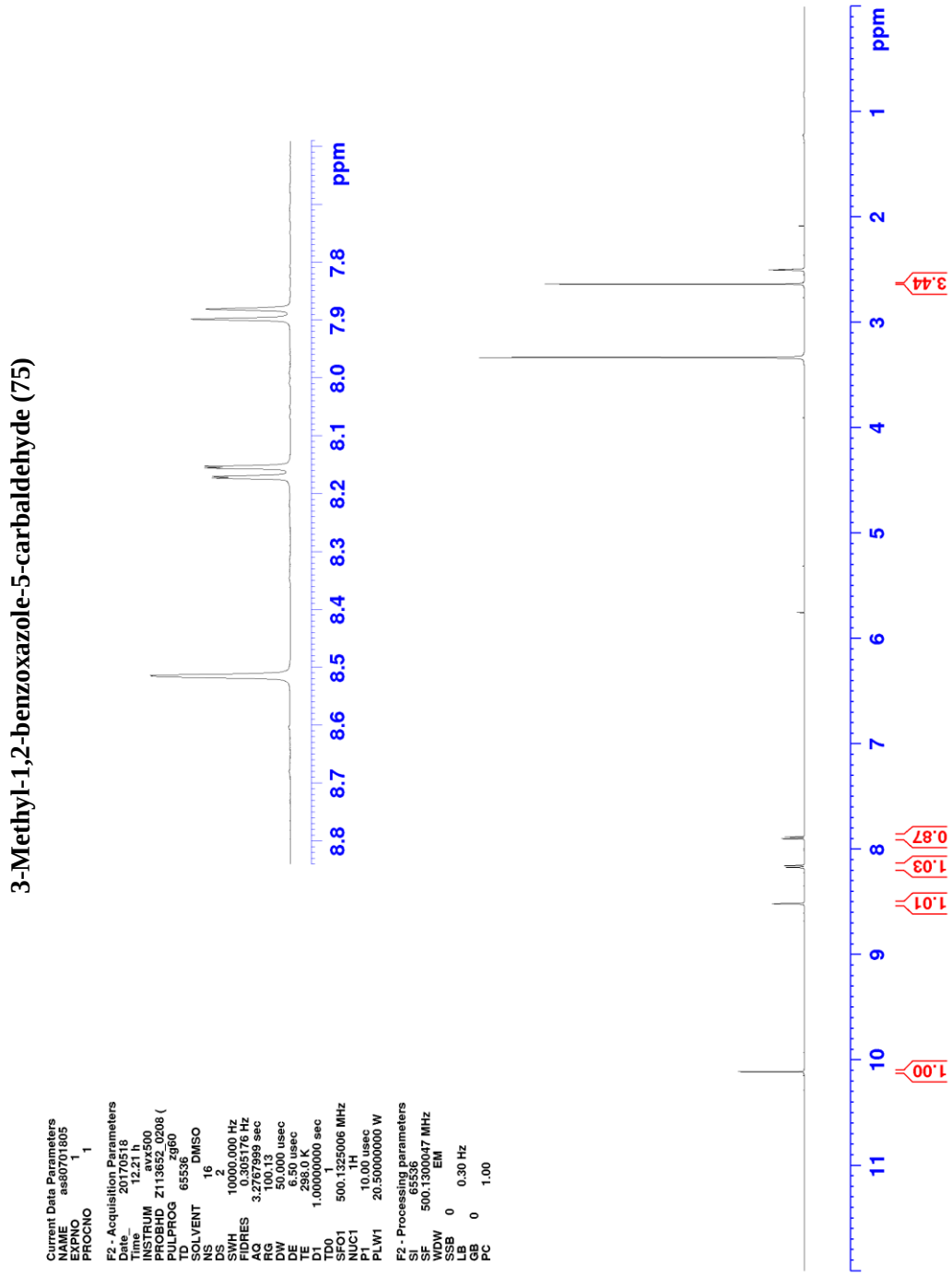


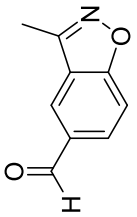
(3-Methyl-1,2-benzoxazol-5-yl)methanol (74)



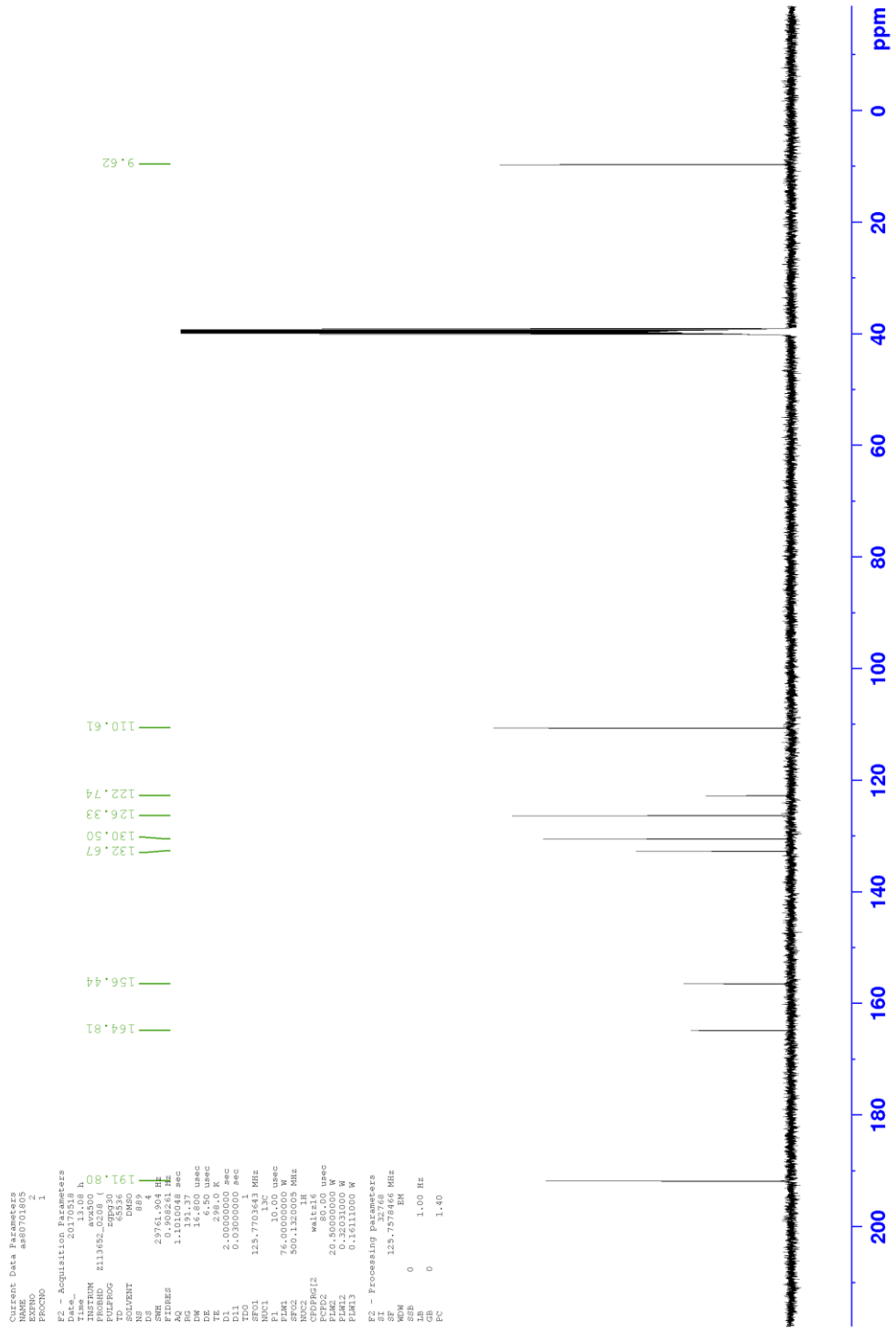


3-Methyl-1,2-benzoxazole-5-carbaldehyde (75)

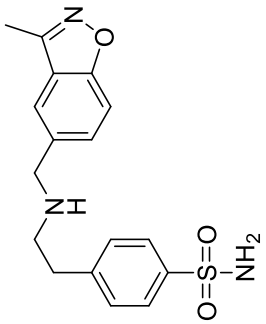


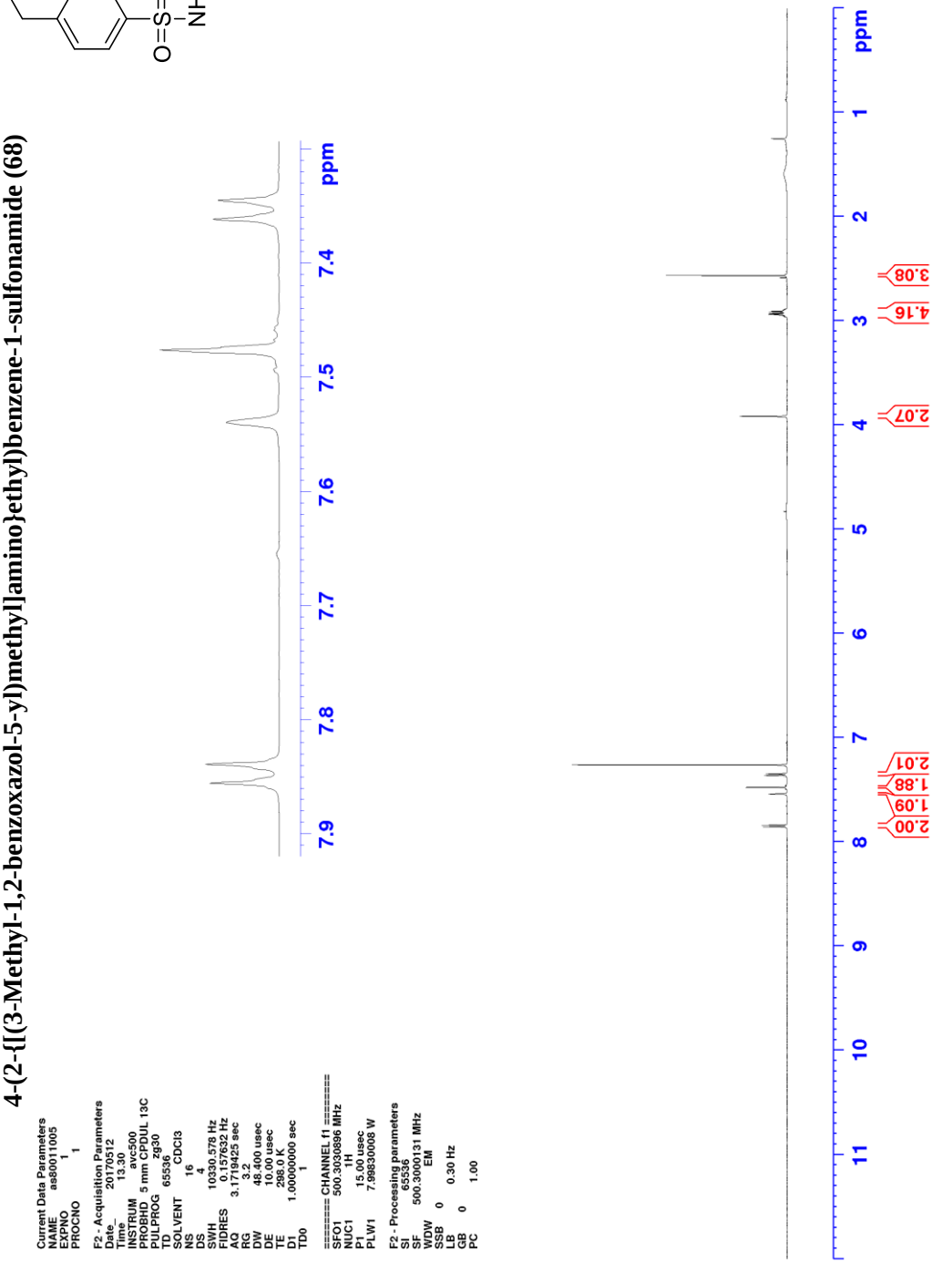


3-Methyl-1,2-benzoxazole-5-carbaldehyde (75)

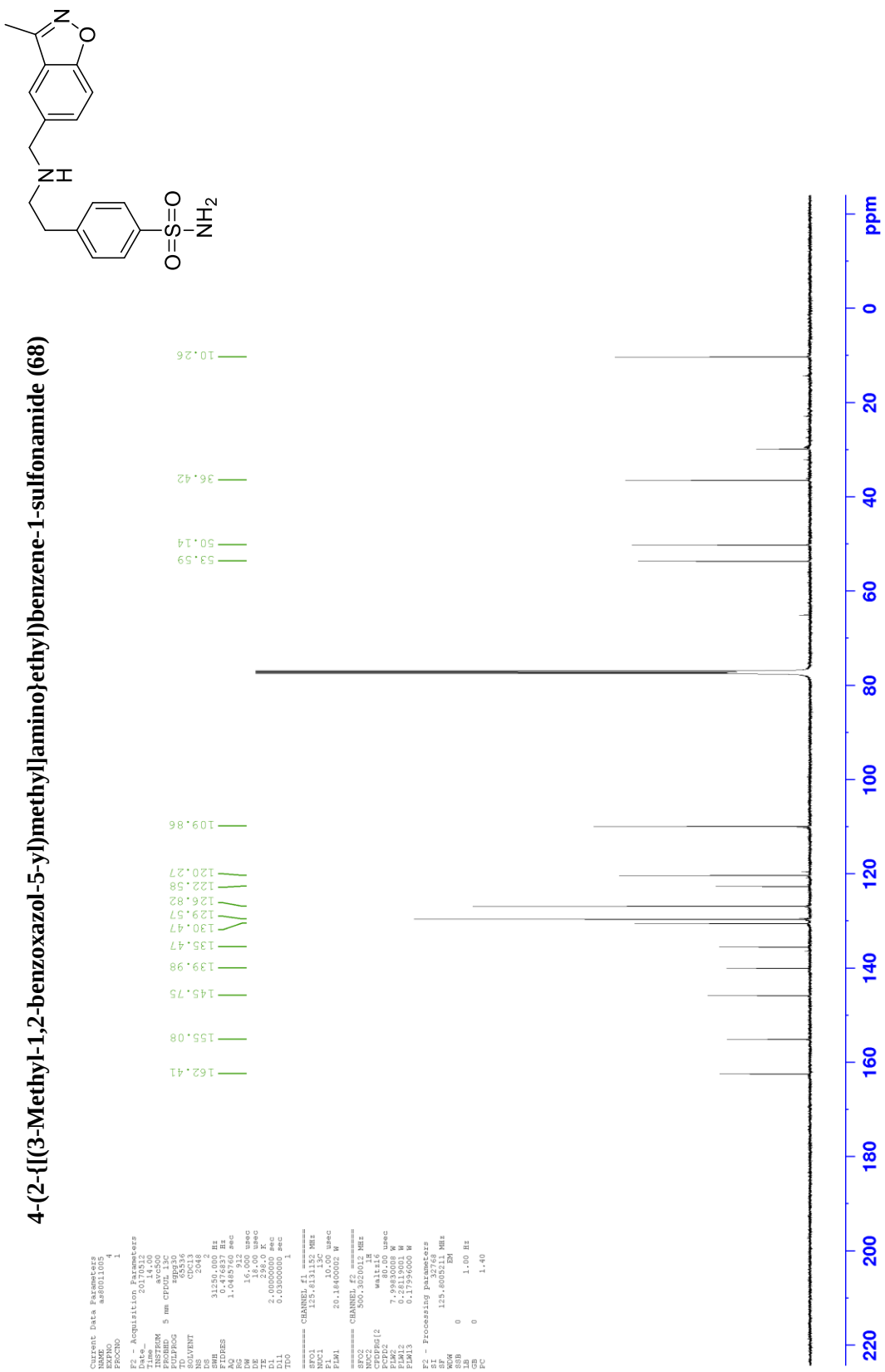


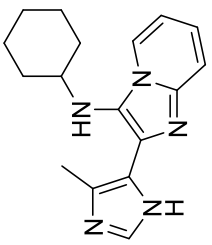
4-(2-{[(3-Methyl-1,2-benzoxazol-5-yl)methyl]amino}ethyl)benzene-1-sulfonamide (68)



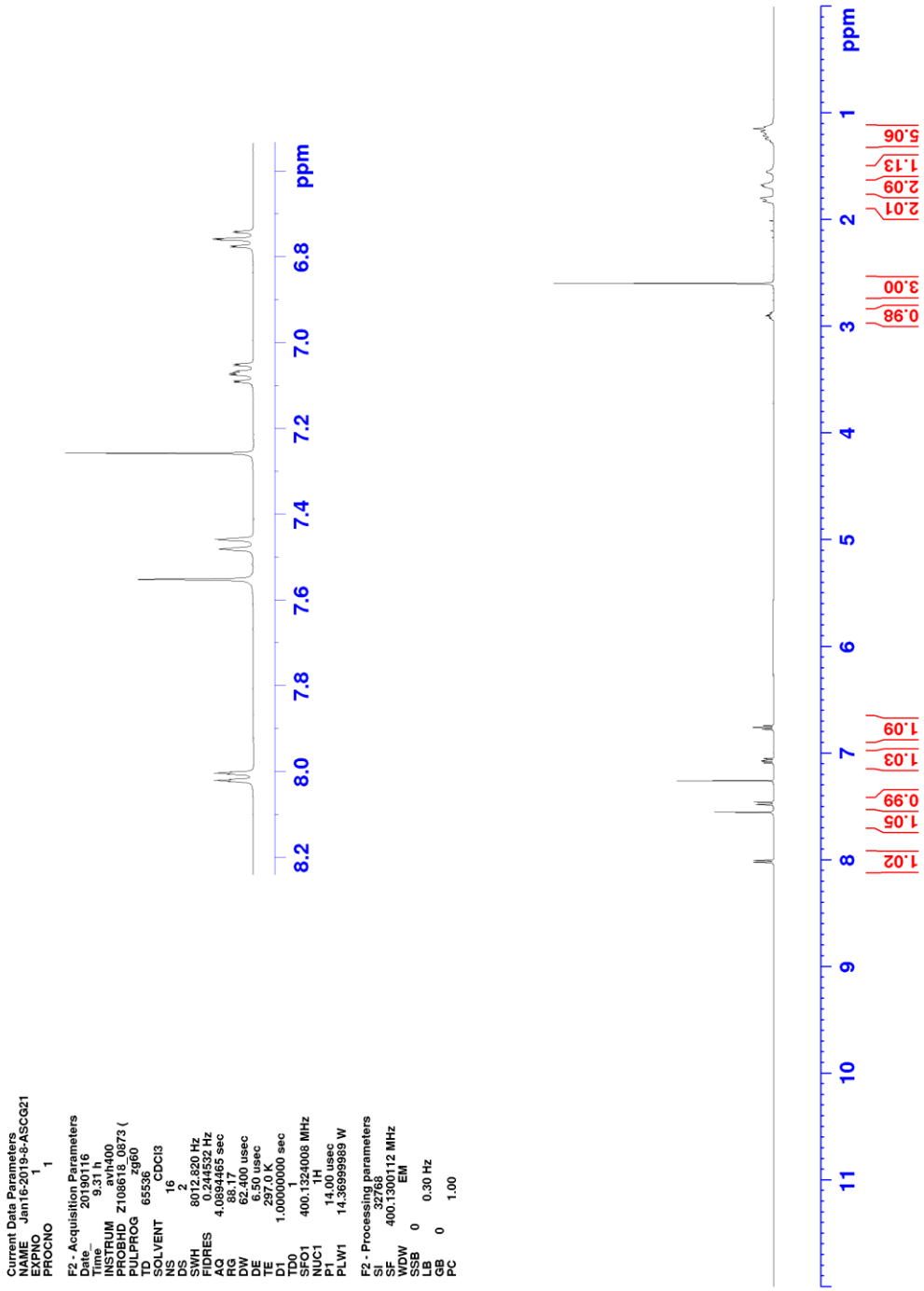


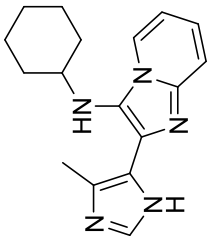
4-(2-{[(3-Methyl-1,2-benzoxazol-5-yl)methyl]amino}ethyl)benzene-1-sulfonamide (68)



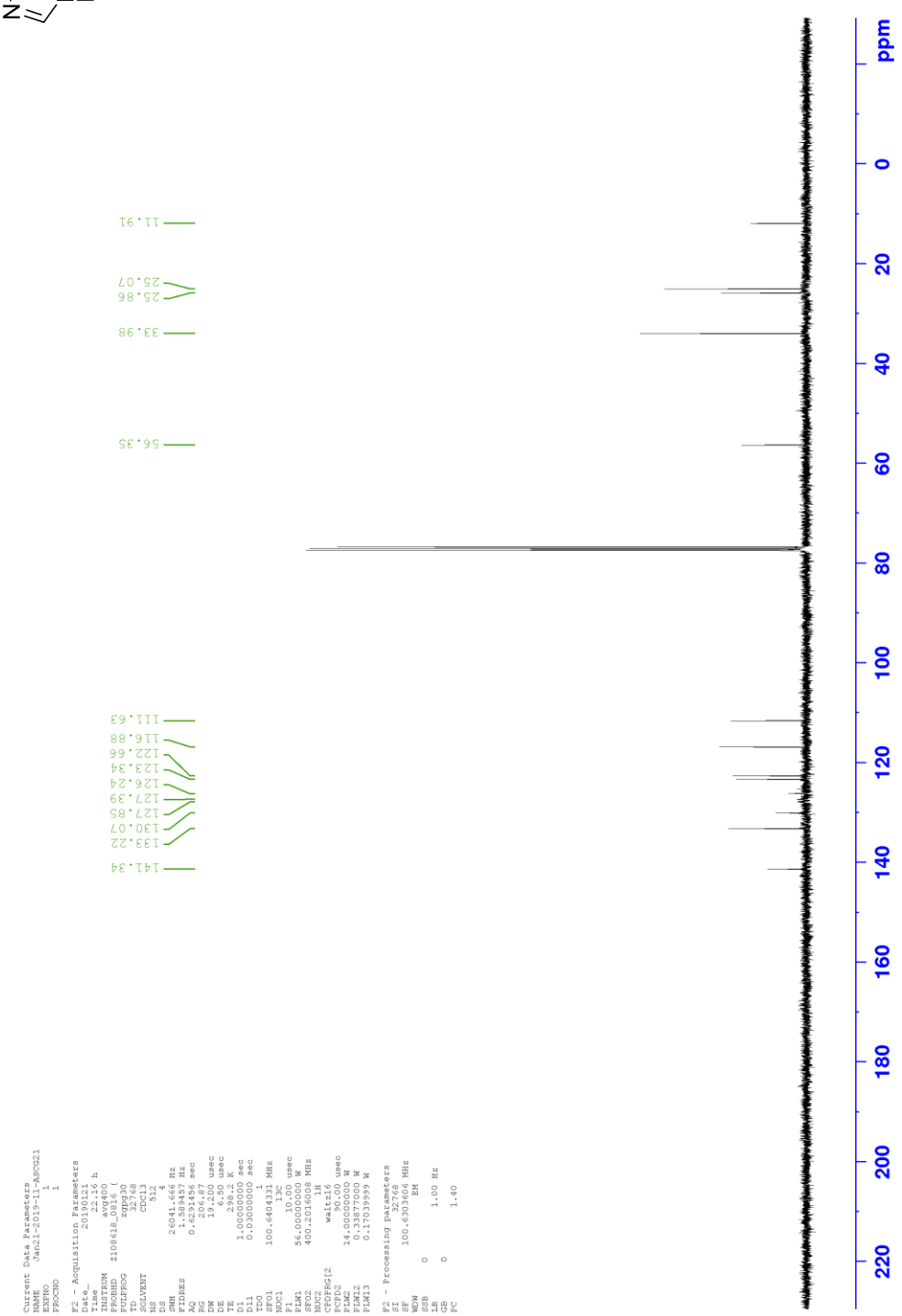


_N_-Cyclohexyl-2-(4-methyl-1_H_-imidazol-5-yl)imidazo[1,2-_a_]pyridin-3-amine (76)

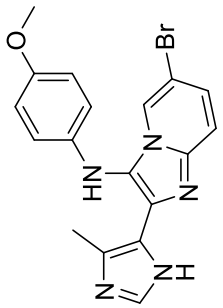


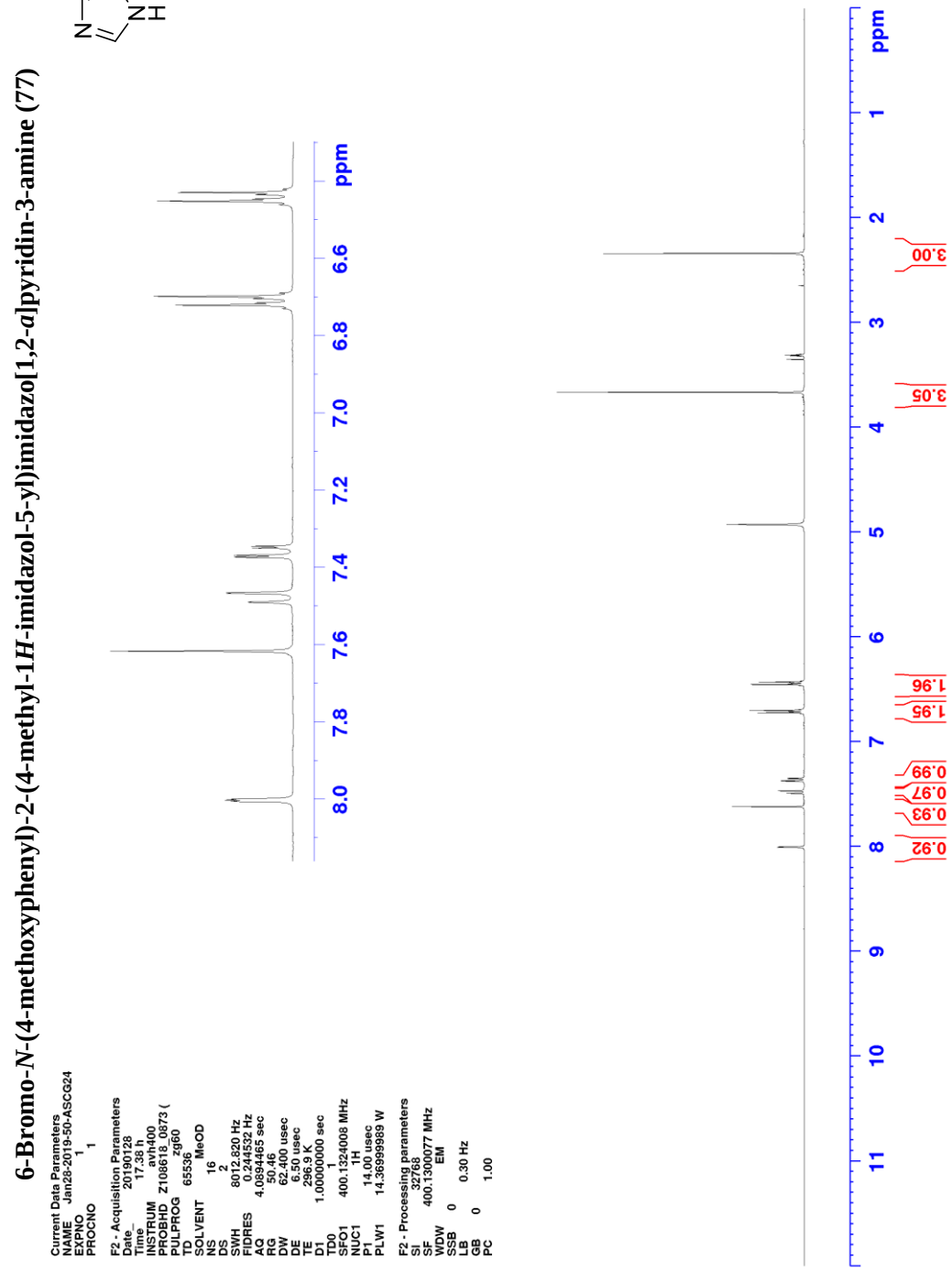


_N_-Cyclohexyl-2-(4-methyl-1*H*-imidazol-5-yl)imidazo[1,2-*a*]pyridin-3-amine (76)

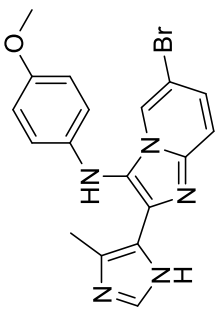


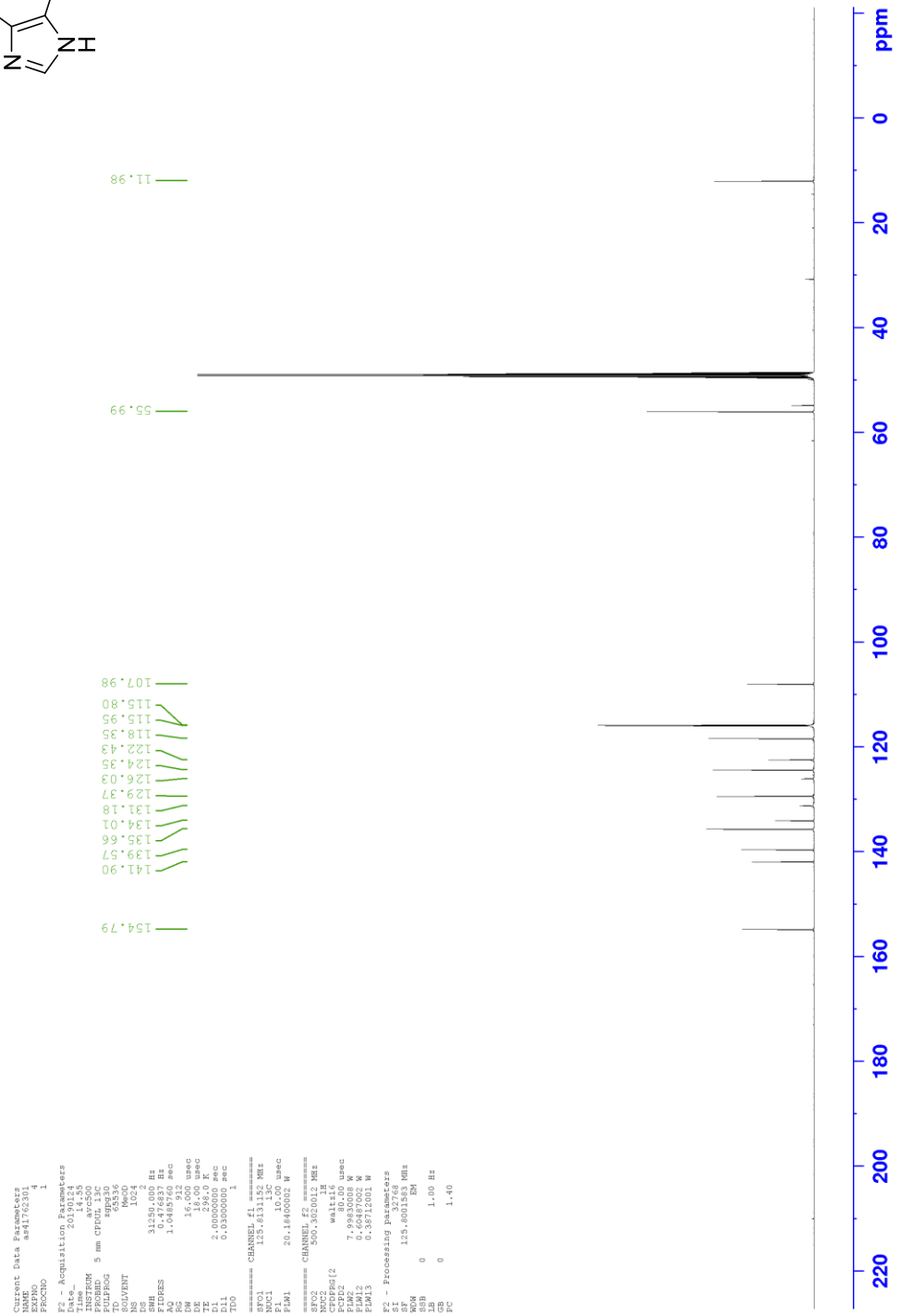
6-Bromo-*N*-(4-methoxyphenyl)-2-(4-methyl-1*H*-imidazol-5-yl)imidazo[1,2-*a*]pyridin-3-amine (77)

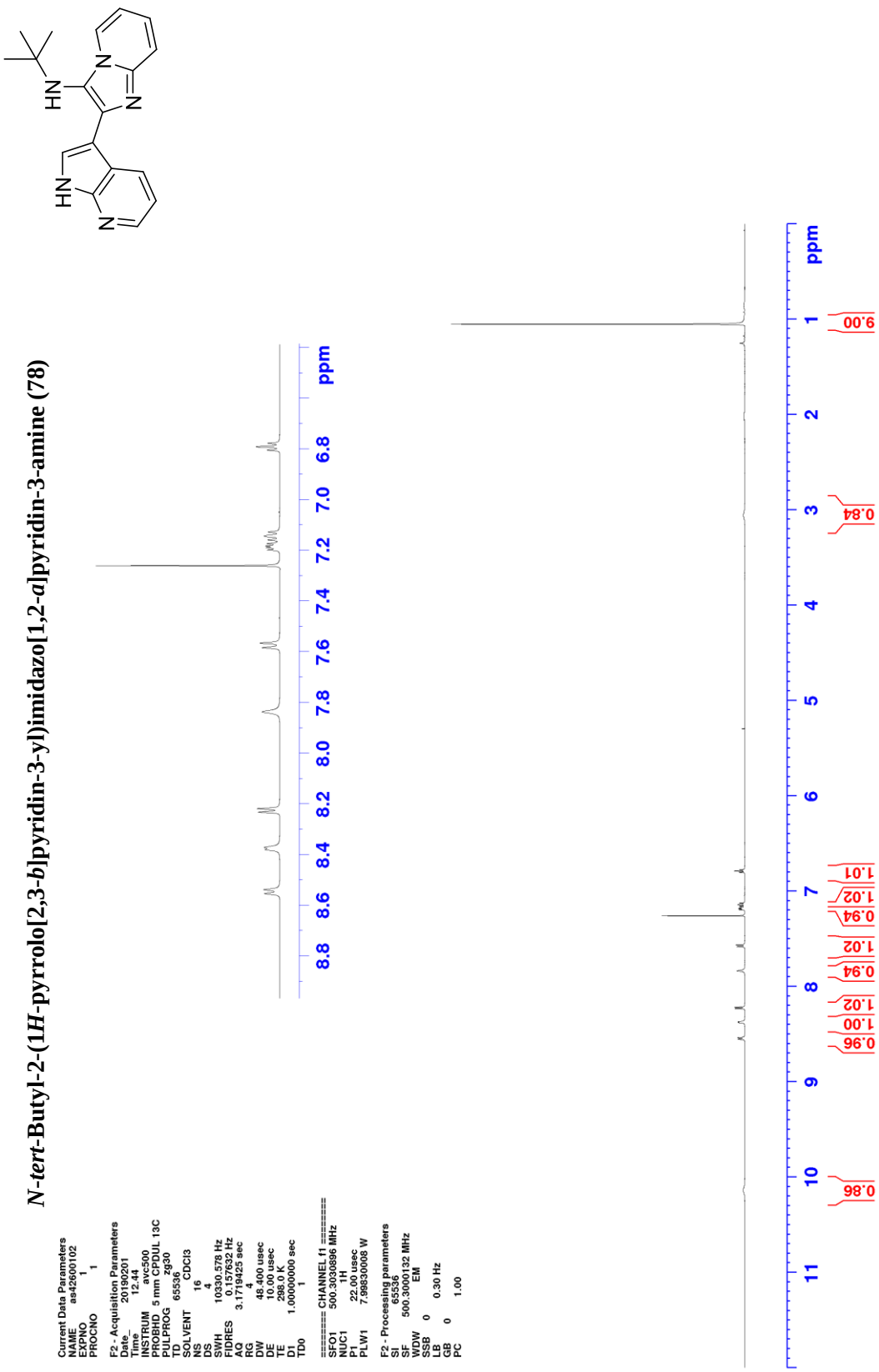




6-Bromo-*N*-(4-methoxyphenyl)-2-(4-methyl-1*H*-imidazol-5-yl)imidazo[1,2-*a*]pyridin-3-amine (77)






N-tert-Butyl-2-(1H-pyrrolo[2,3-b]pyridin-3-yl)imidazo[1,2-a]pyridin-3-amine (78)
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PROCNO 1
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TD 65536
SOLVENT CDCl3
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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 ========
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NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W
F2 - Processing parameters
SI 65536
SF 500.30000132 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
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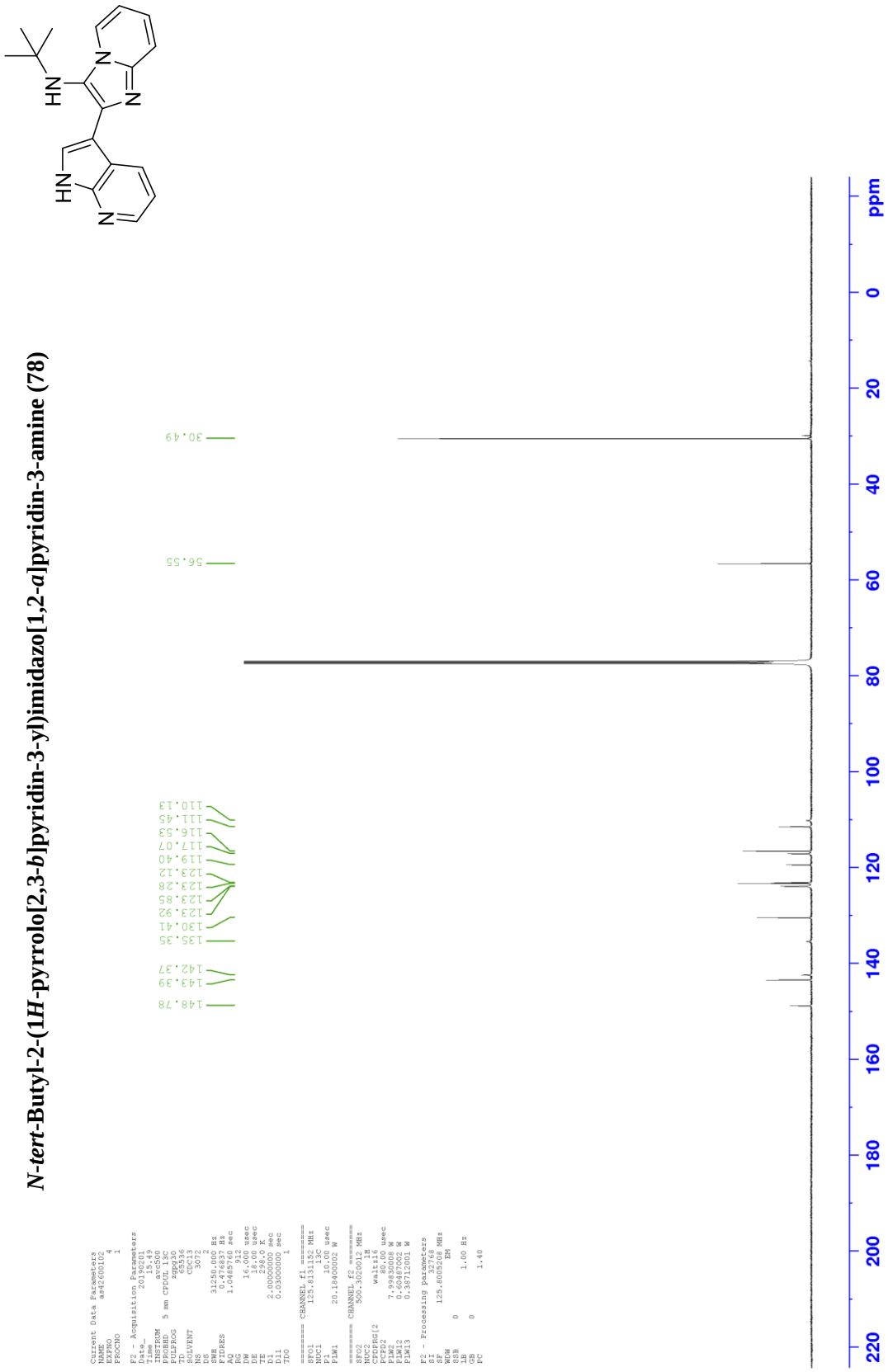
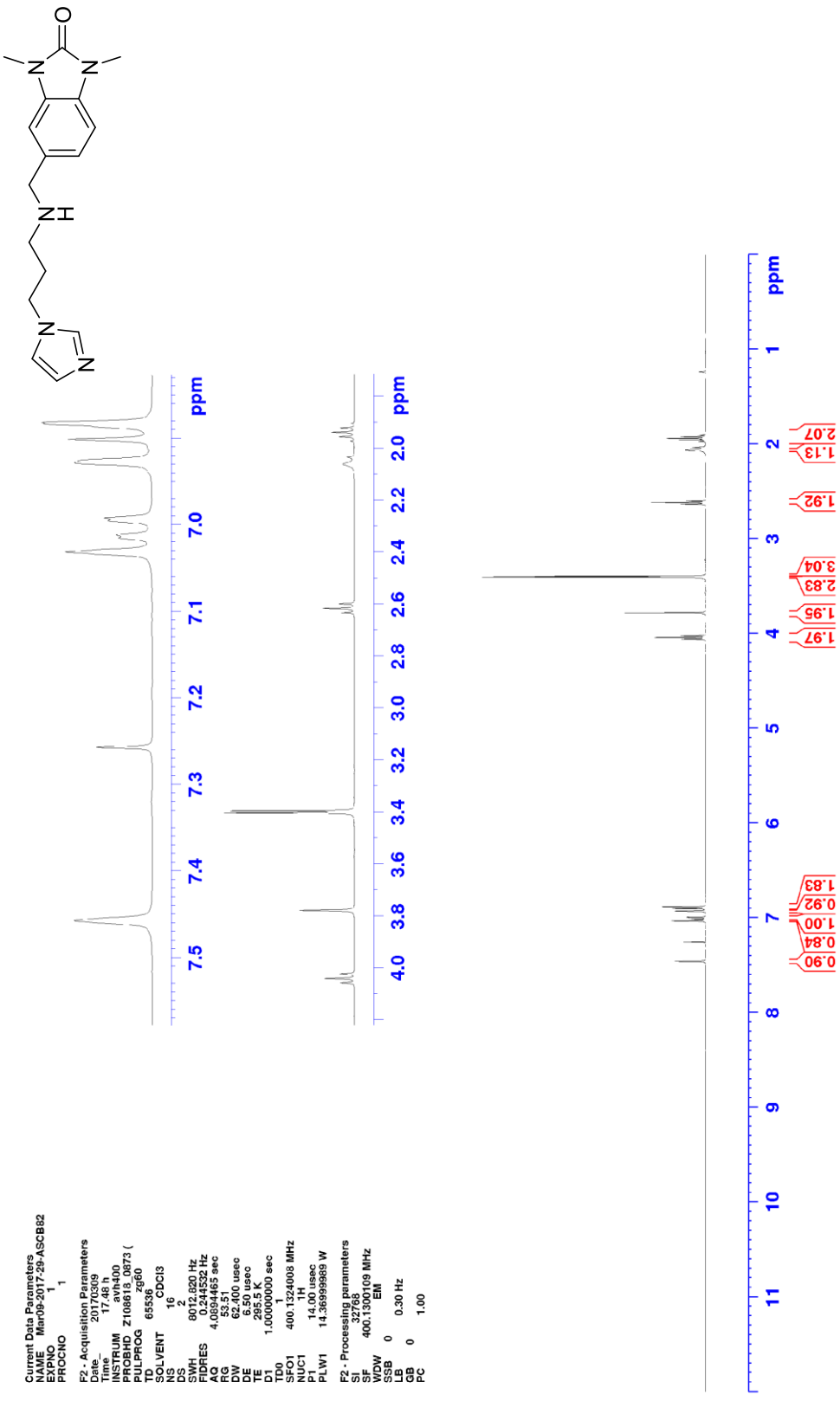
11 10 9 8 7 6 5 4 3 2 1 ppm
0.86
0.96
1.00
1.02
0.94
1.02
0.94
1.02
1.01
0.84
9.00

N-tert-Butyl-2-(1_H_-pyrrolo[2,3-_b_]pyridin-3-yl)imidazo[1,2-_a_]pyridin-3-amine (78)

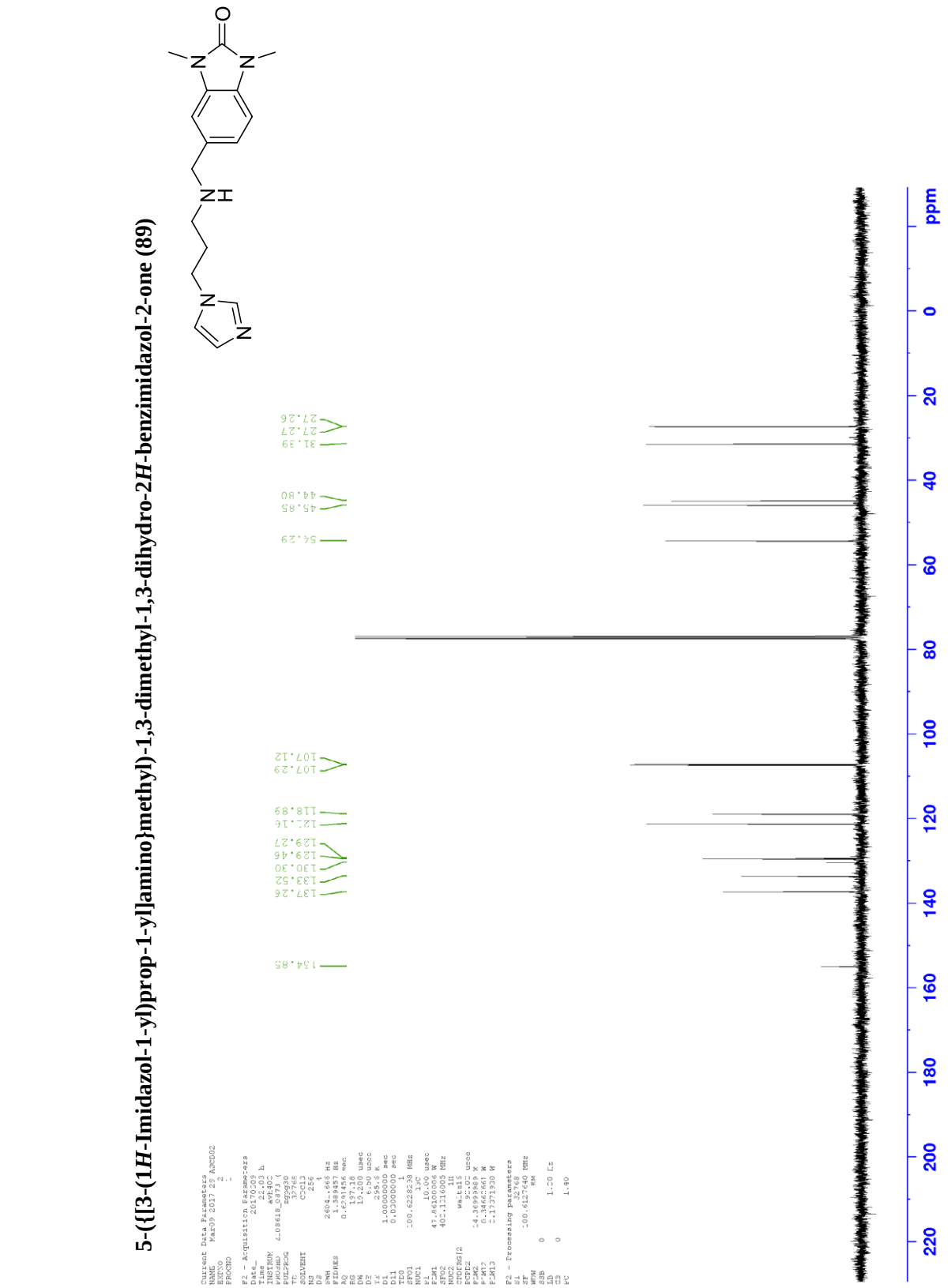


Chapter 3 Compounds

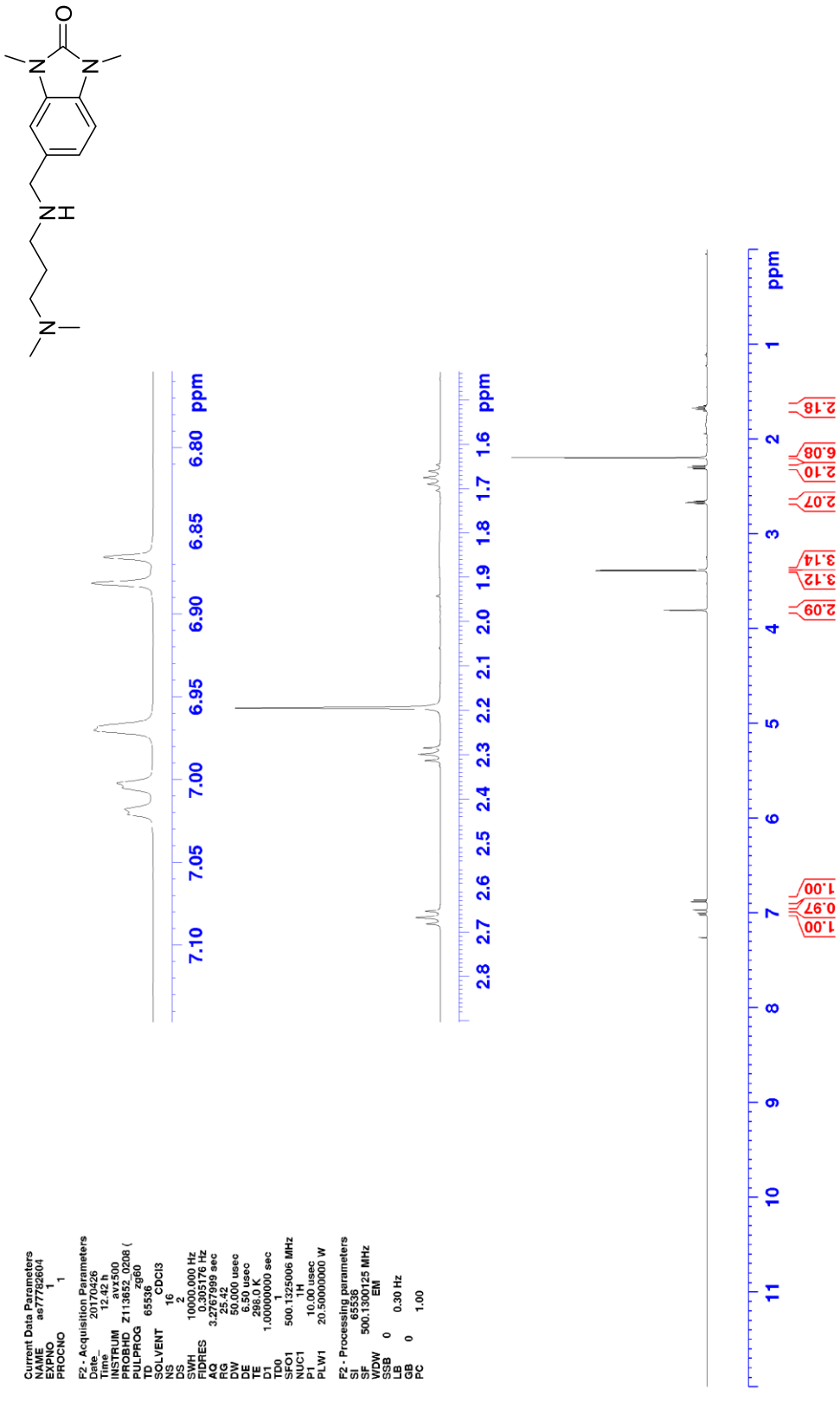
5-({[3-(1*H*-Imidazol-1-yl)prop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (89)



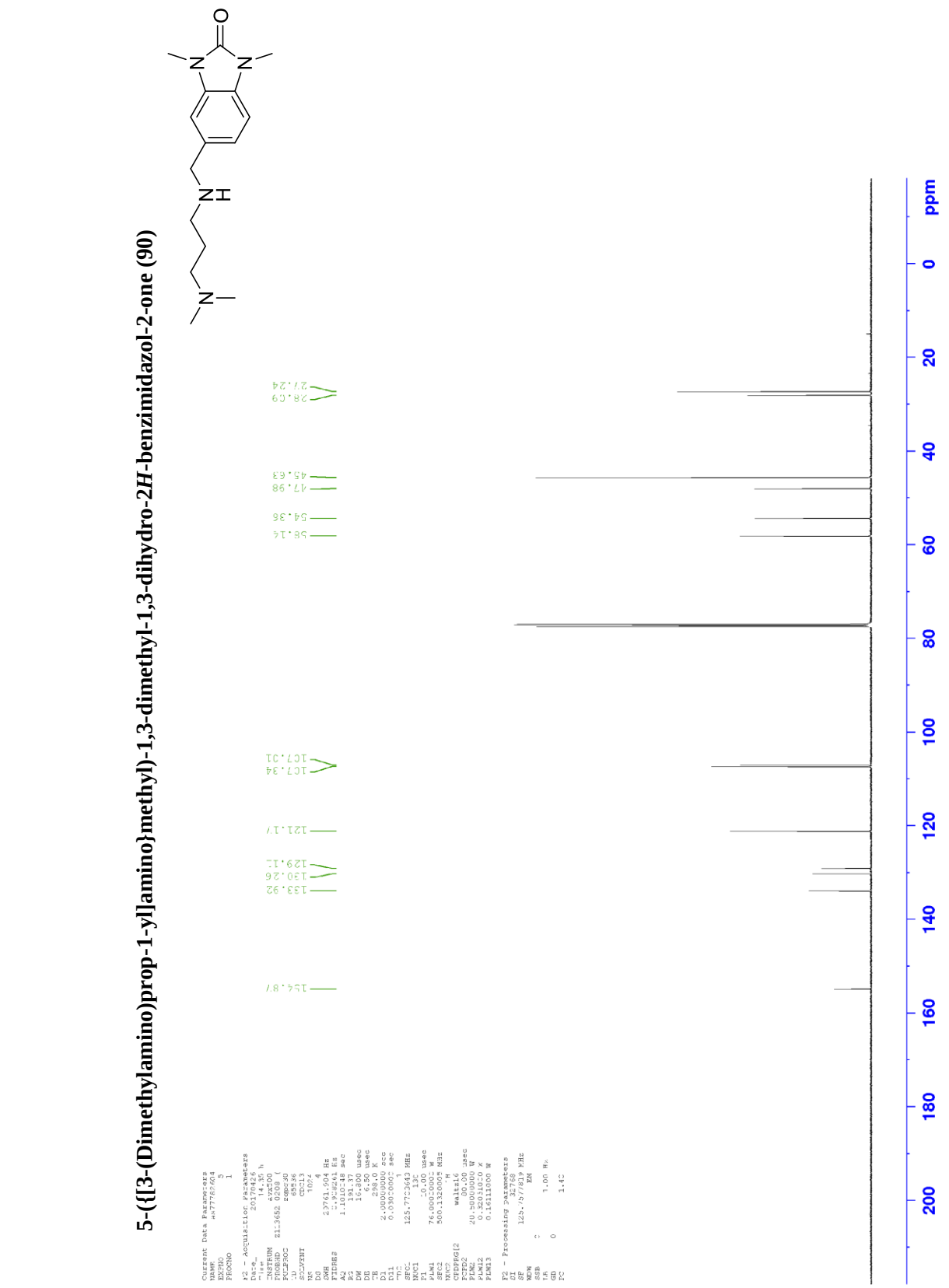
5-({[3-(1*H*-Imidazol-1-yl)prop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (89)



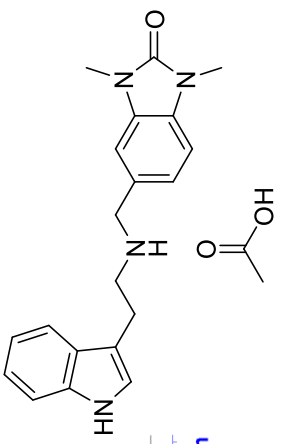
5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (90)

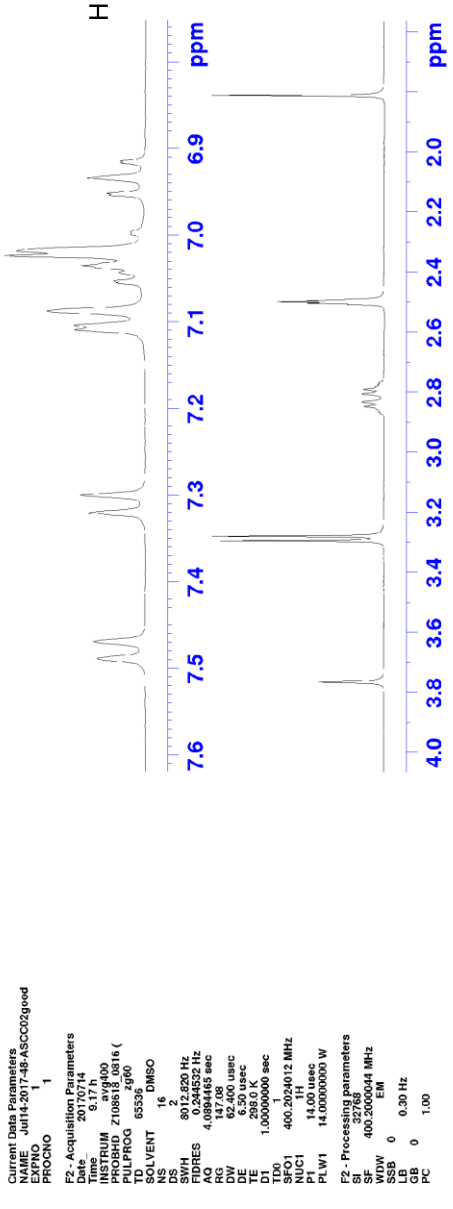


5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (90)



5-({[2-(1*H*-Indol-3-yl)ethyl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one acetate (91)





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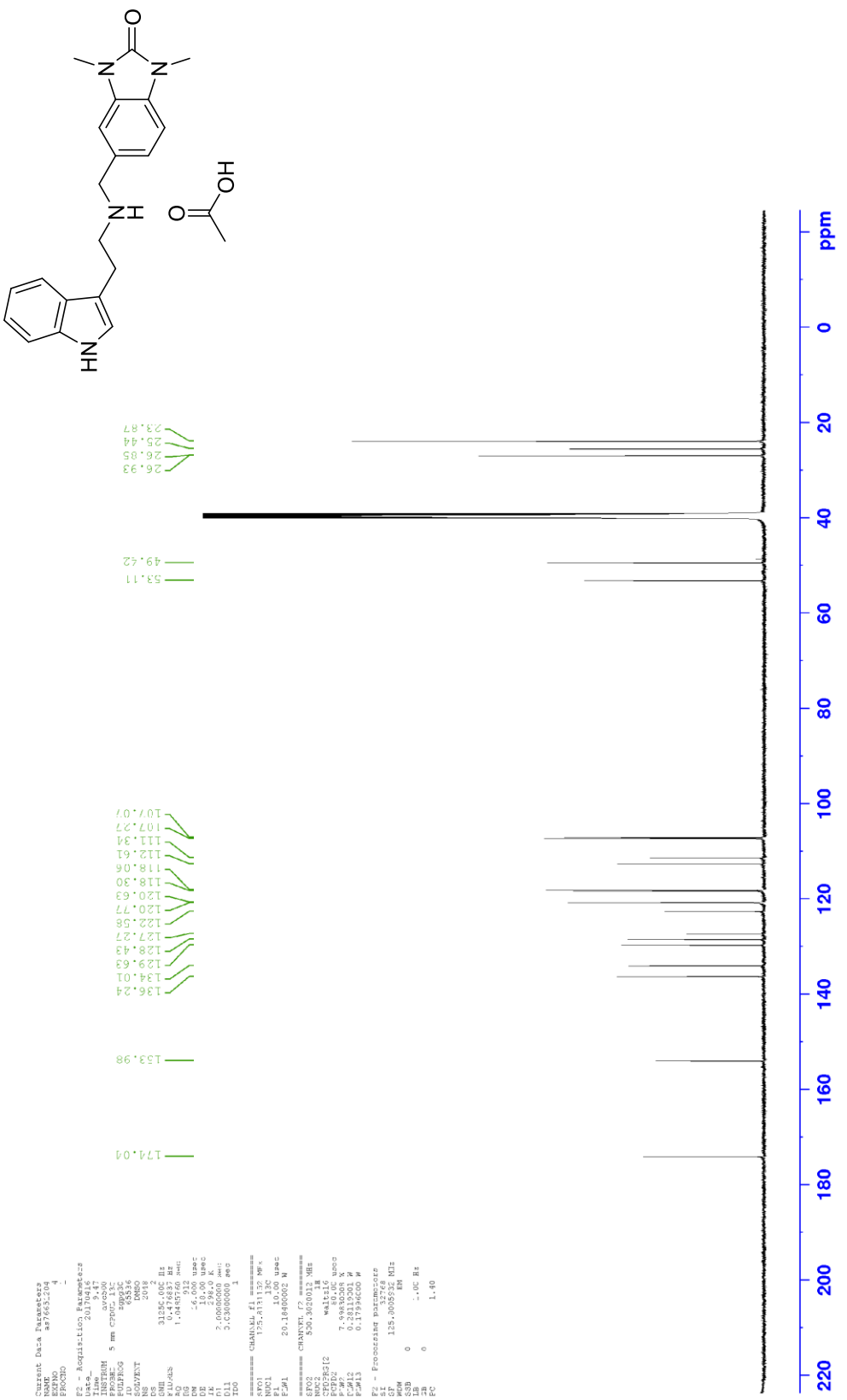
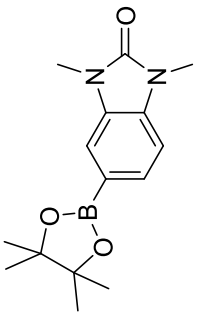
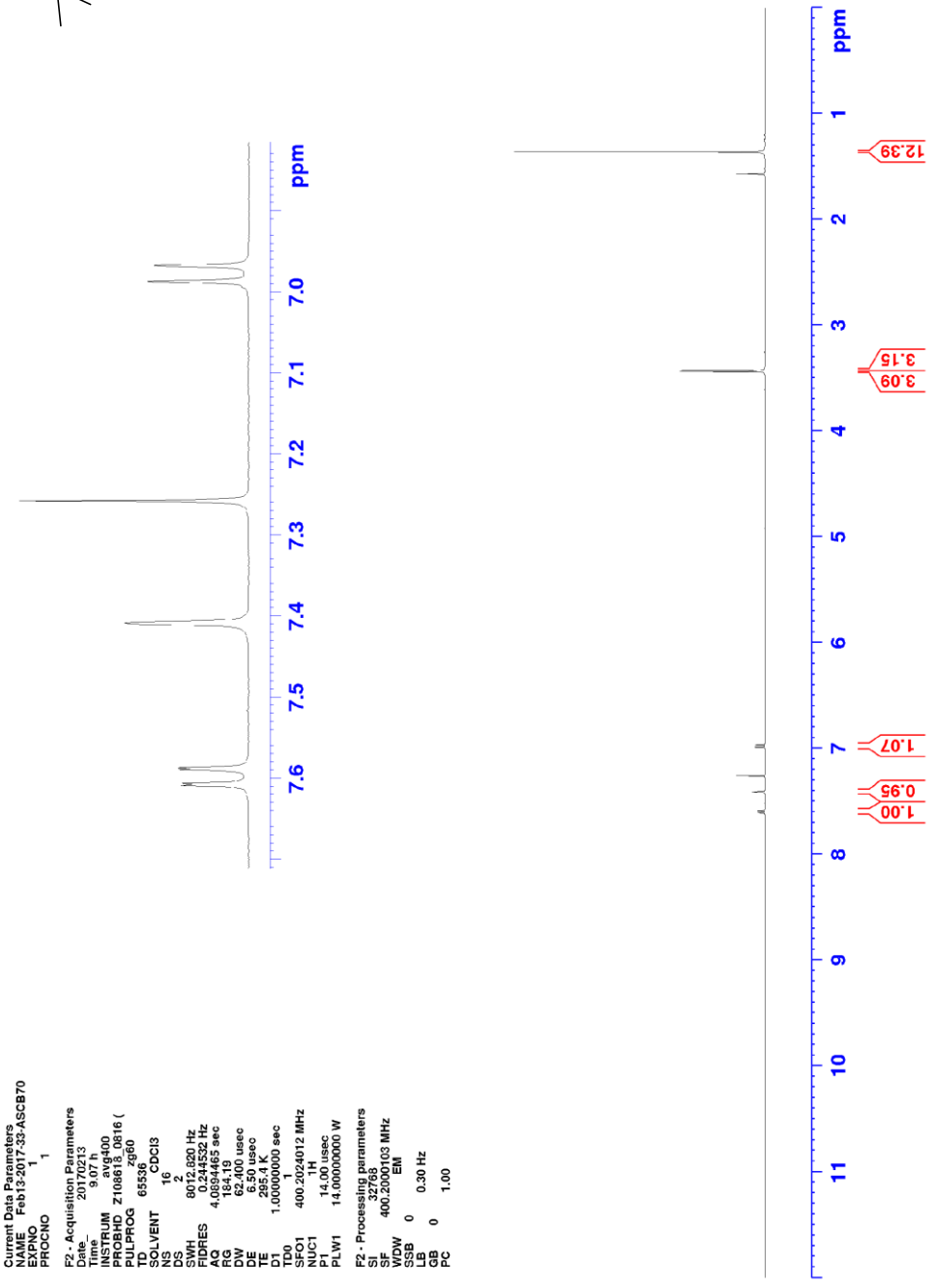
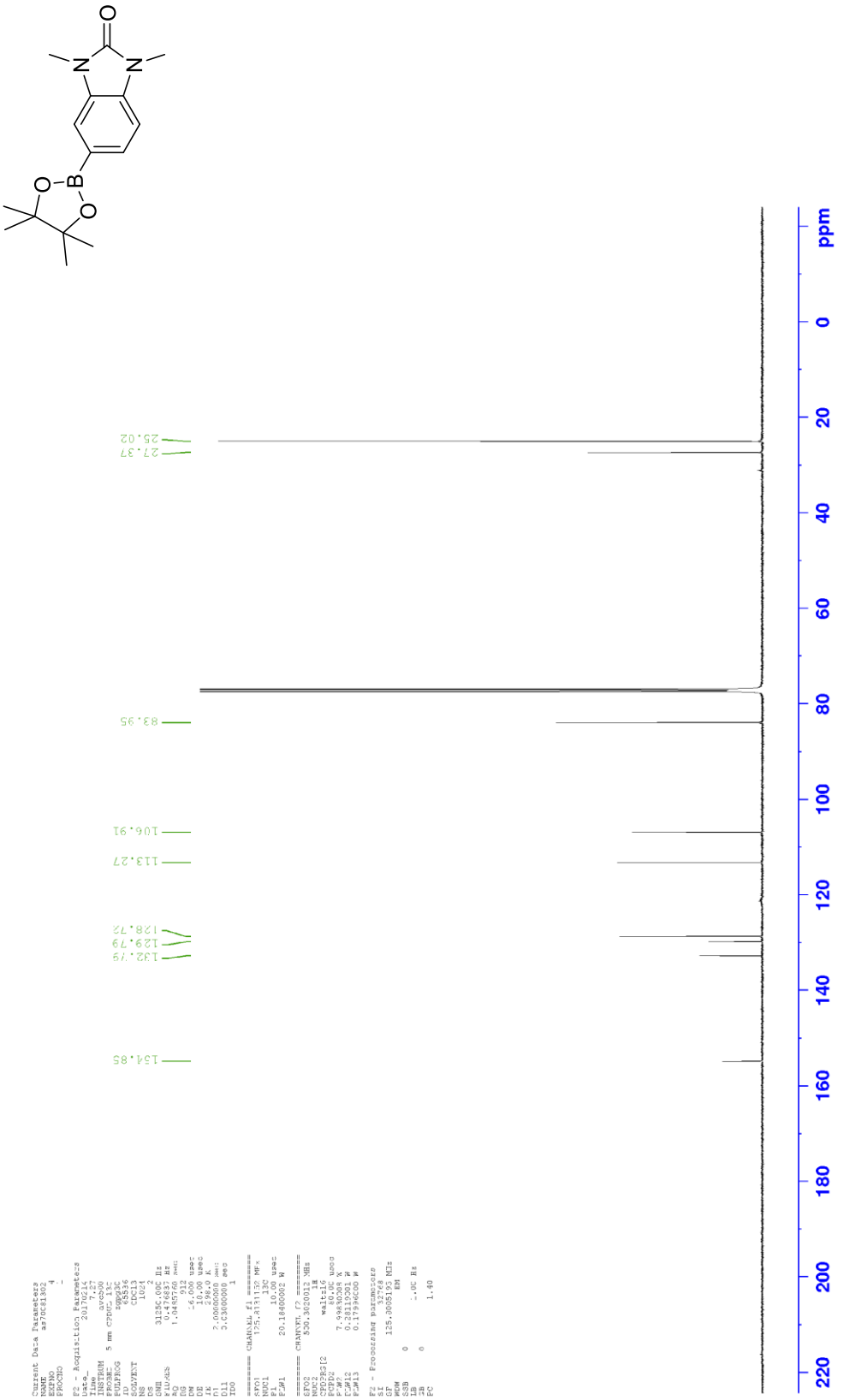


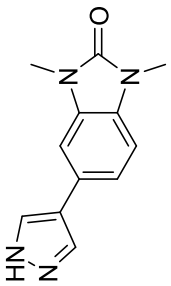
1,3-Dimethyl-5-(4,4,5,5-tetramethyl-1,3-dioxaborolan-2-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (99)



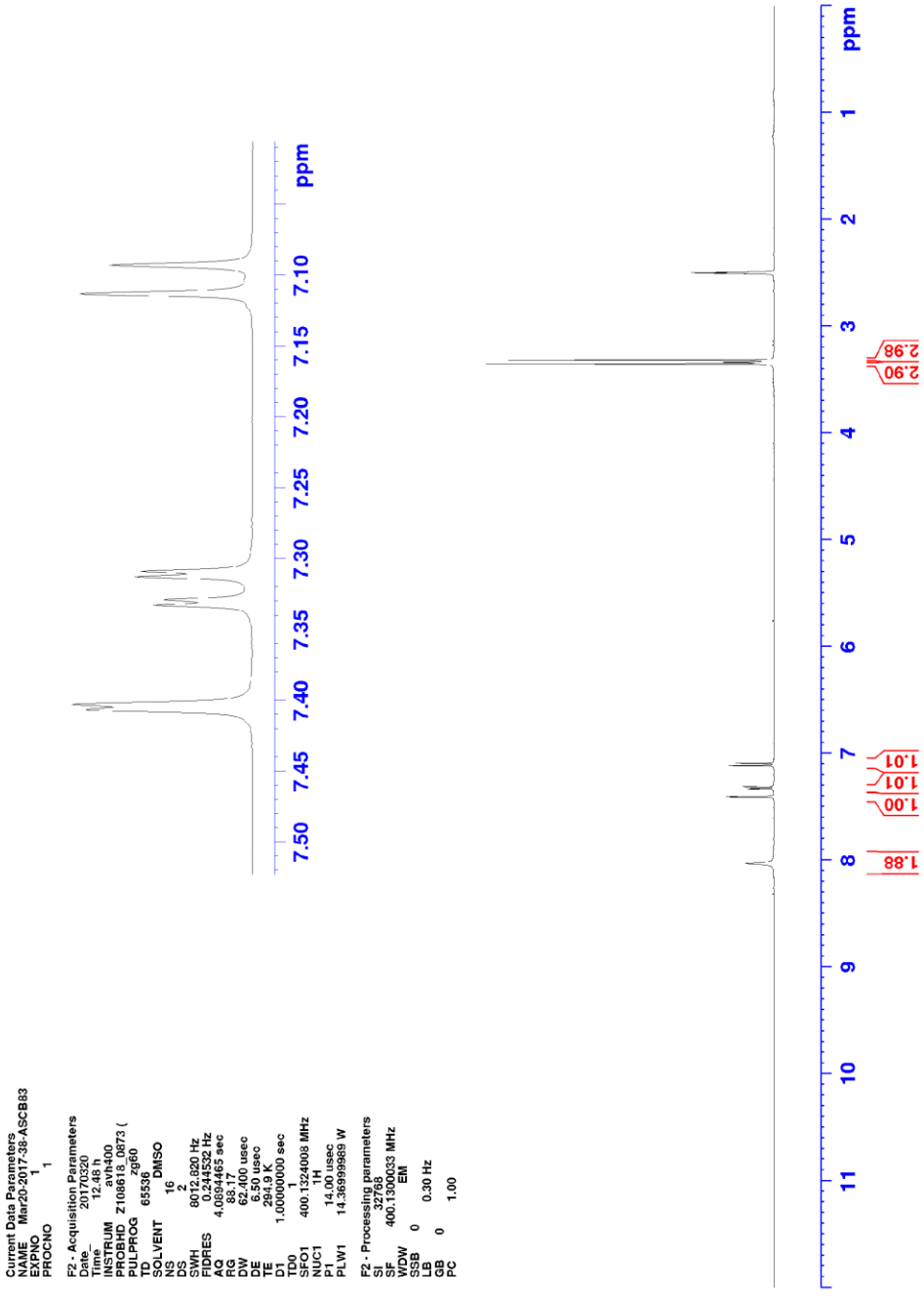


1,3-Dimethyl-5-(4,4,5,5-tetramethyl-1,3-dioxaborolan-2-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (99)

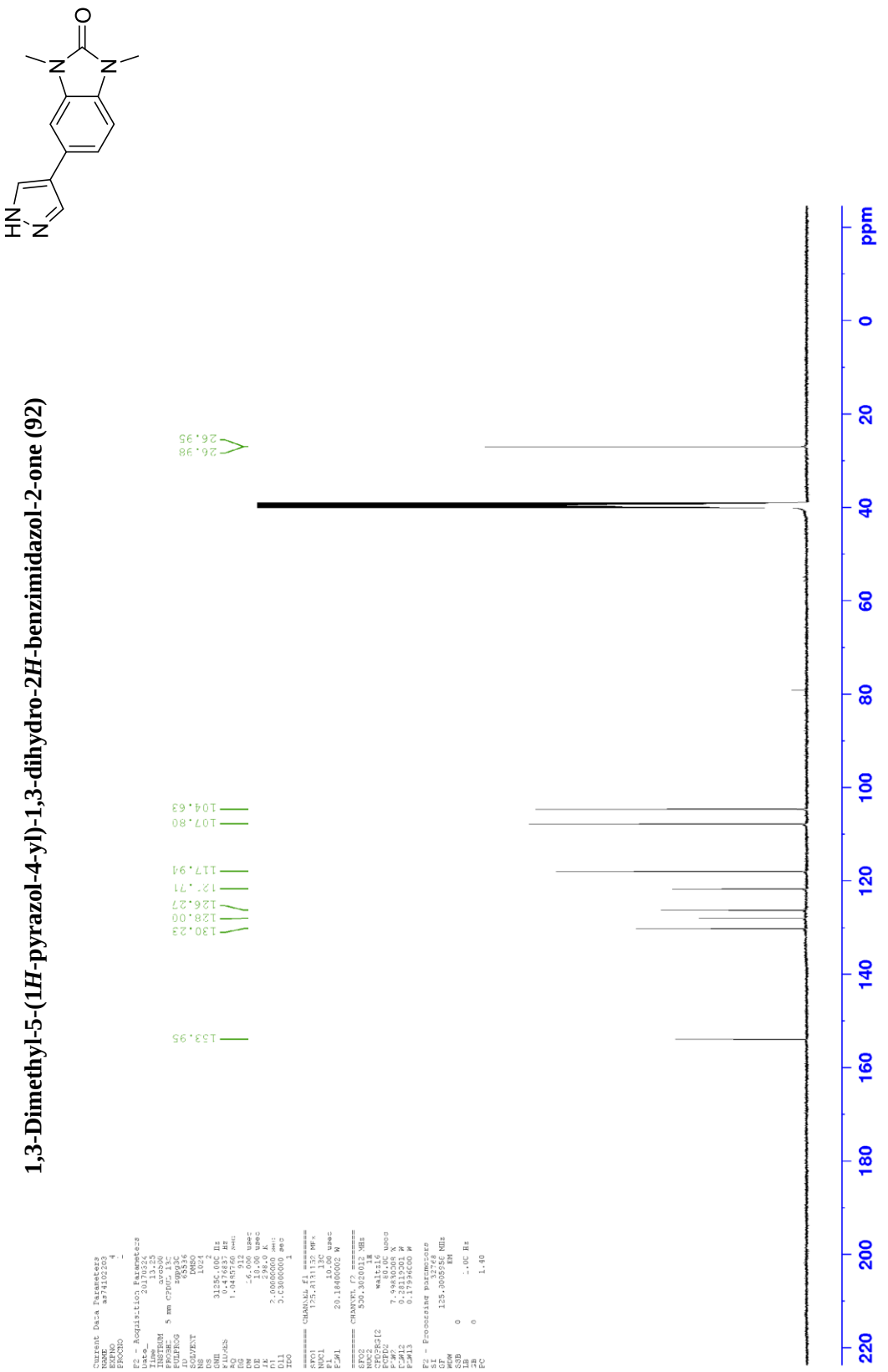


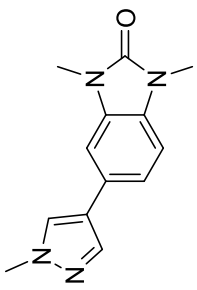


1,3-Dimethyl-5-(1*H*-pyrazol-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (92)

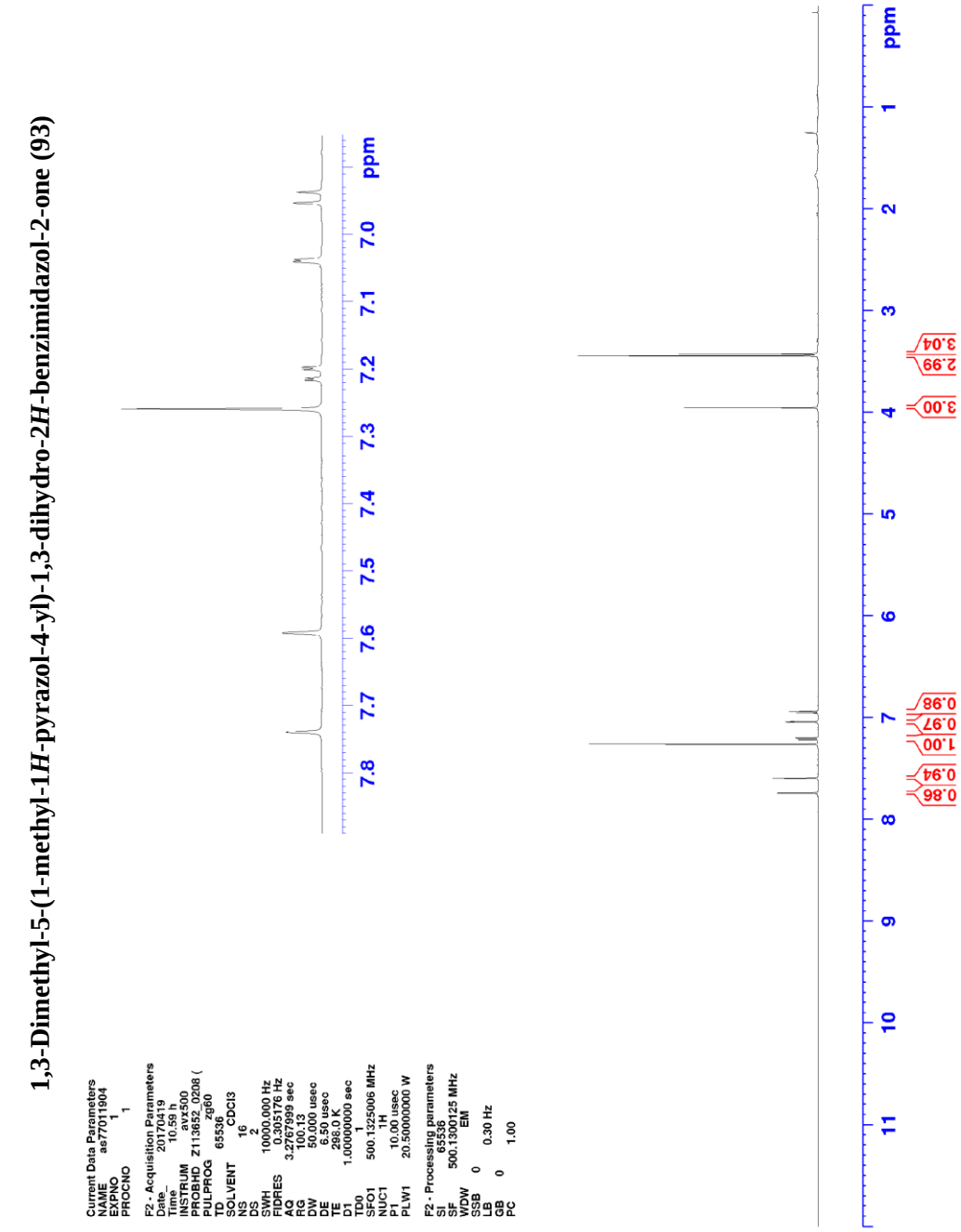


1,3-Dimethyl-5-(1*H*-pyrazol-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (92)

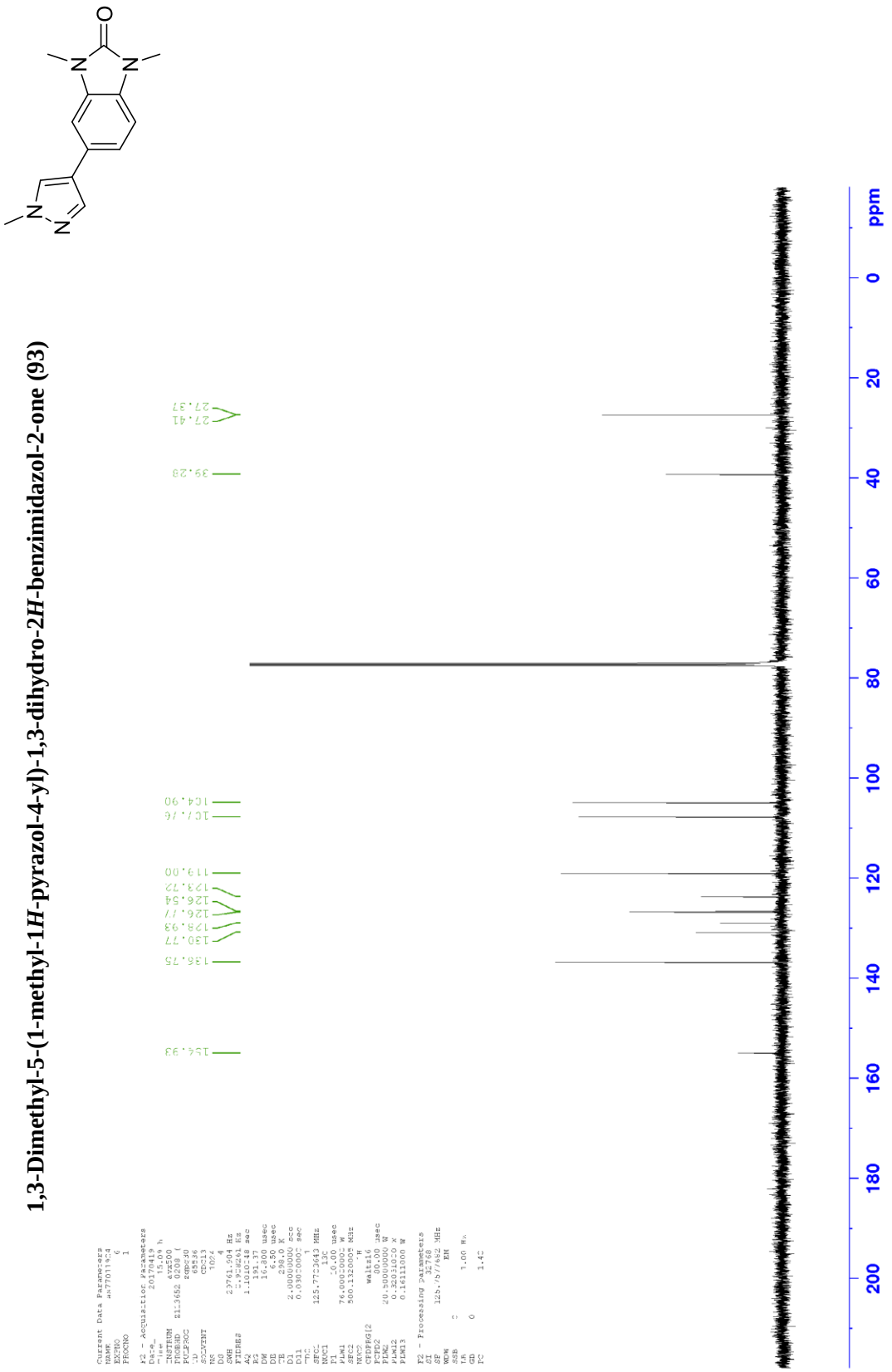


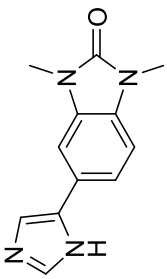


1,3-Dimethyl-5-(1-methyl-1*H*-pyrazol-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (93)

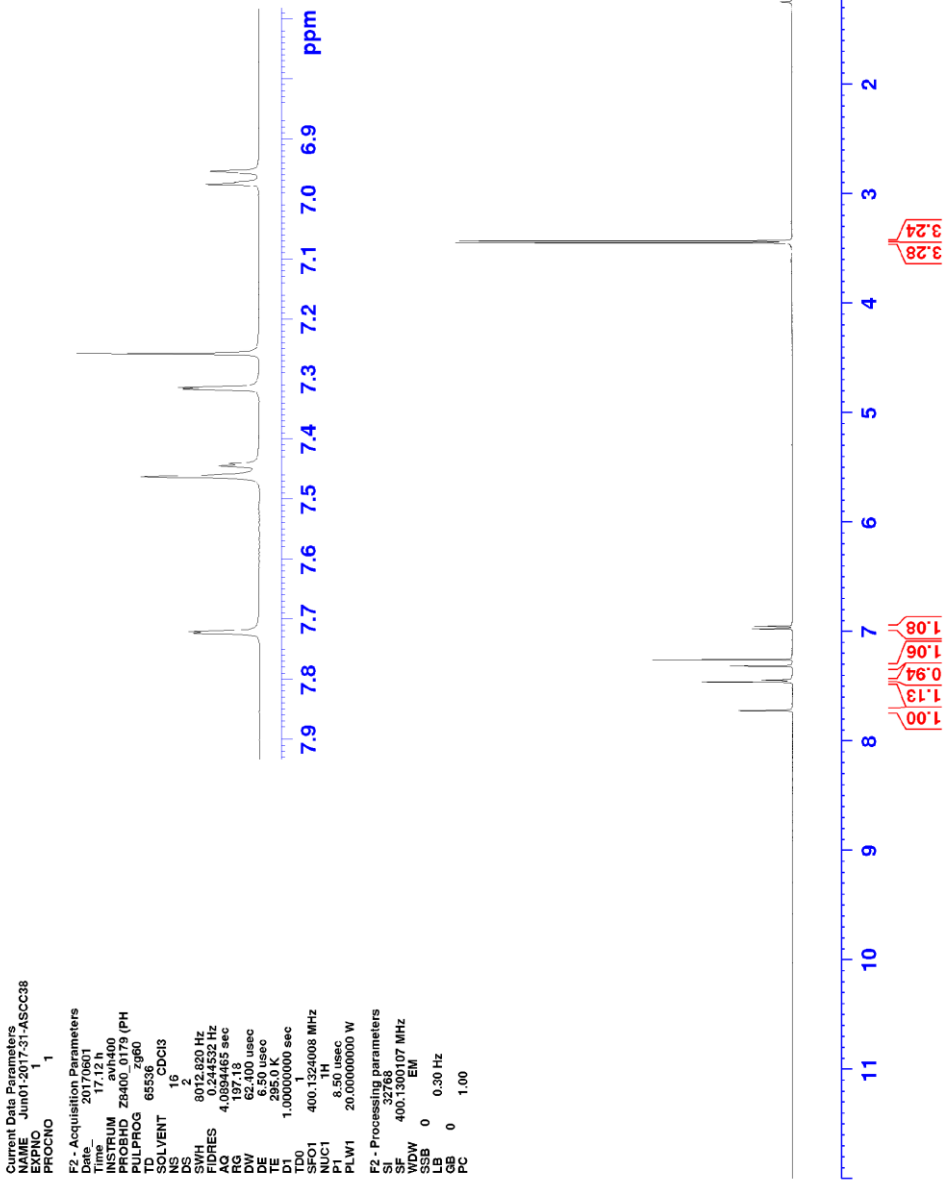


1,3-Dimethyl-5-(1-methyl-1*H*-pyrazol-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (93)

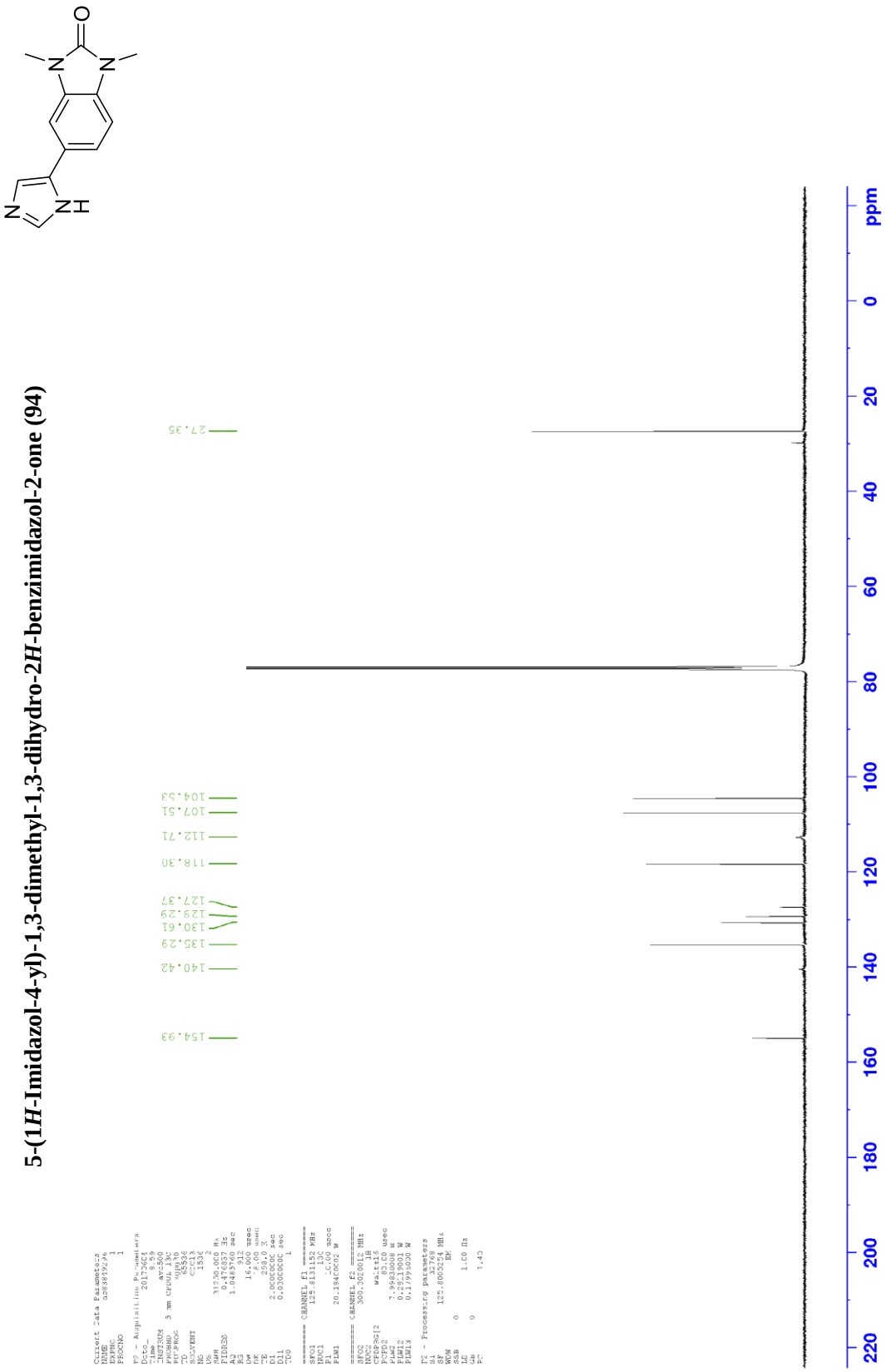


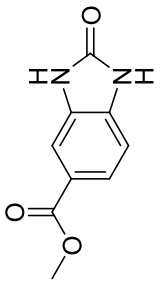


5-(1*H*-Imidazol-4-yl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (94)

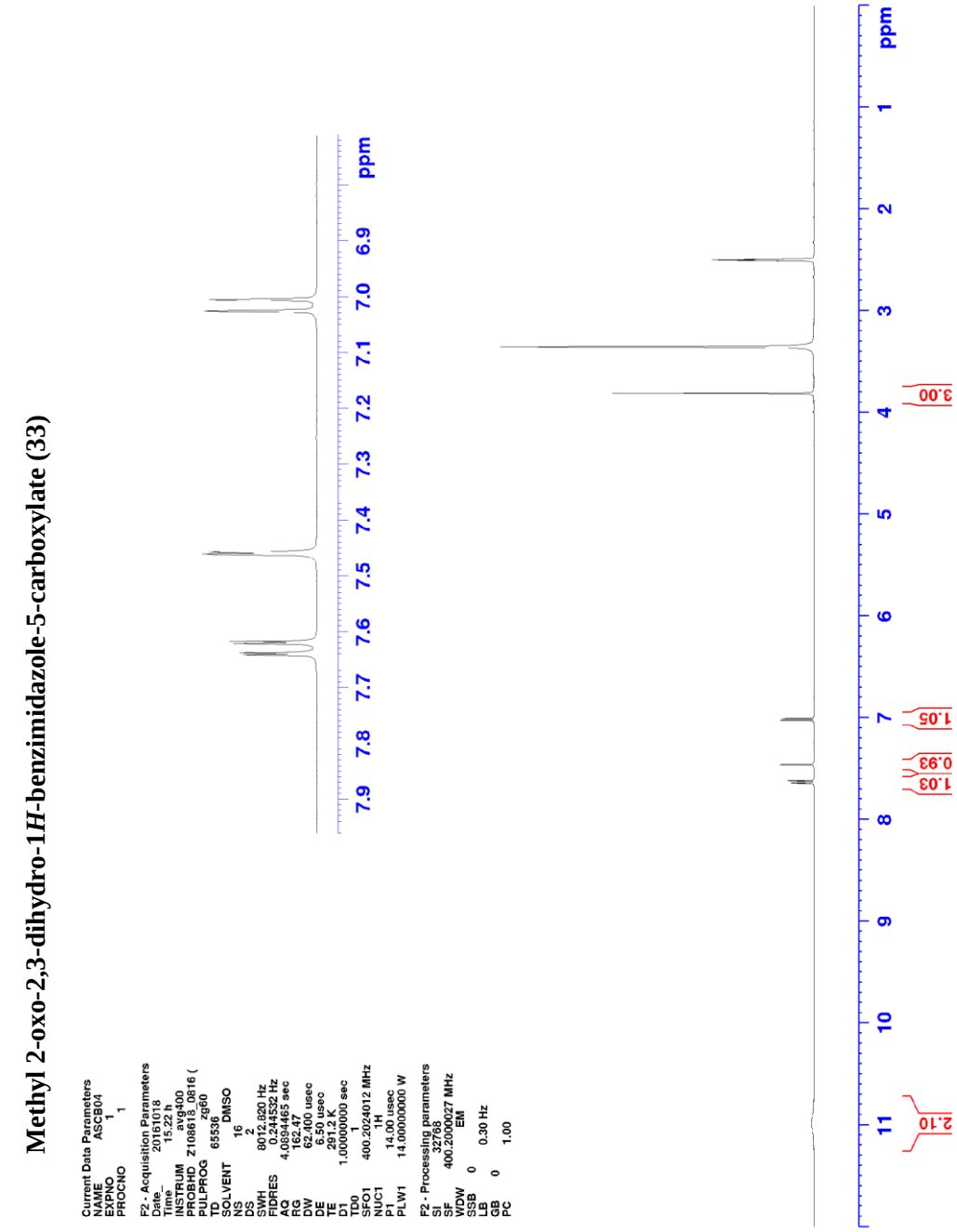


5-(1*H*-Imidazol-4-yl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (94)

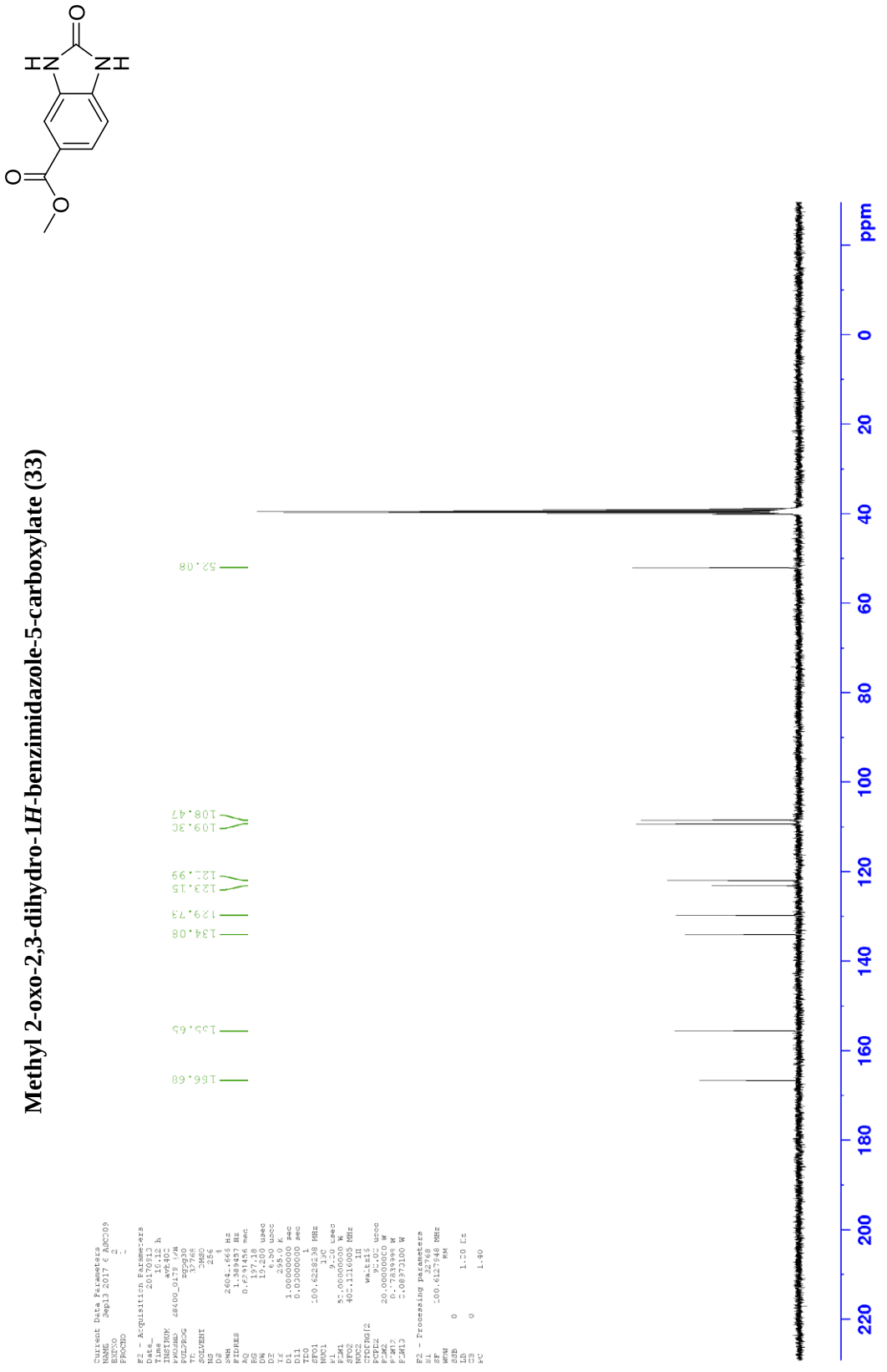


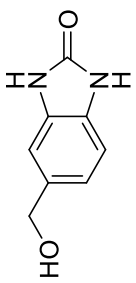


Methyl 2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (33)

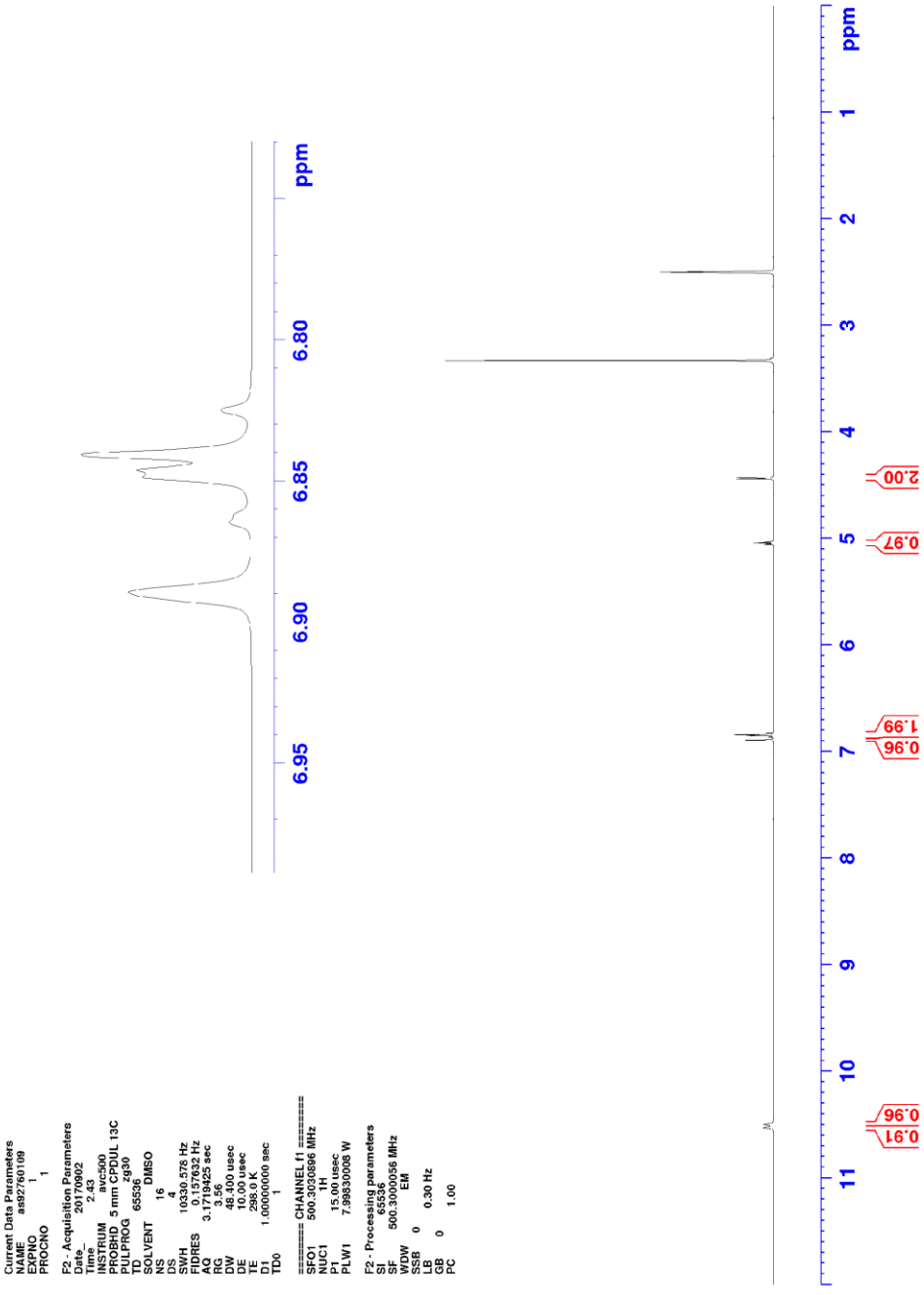


Methyl 2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (33)

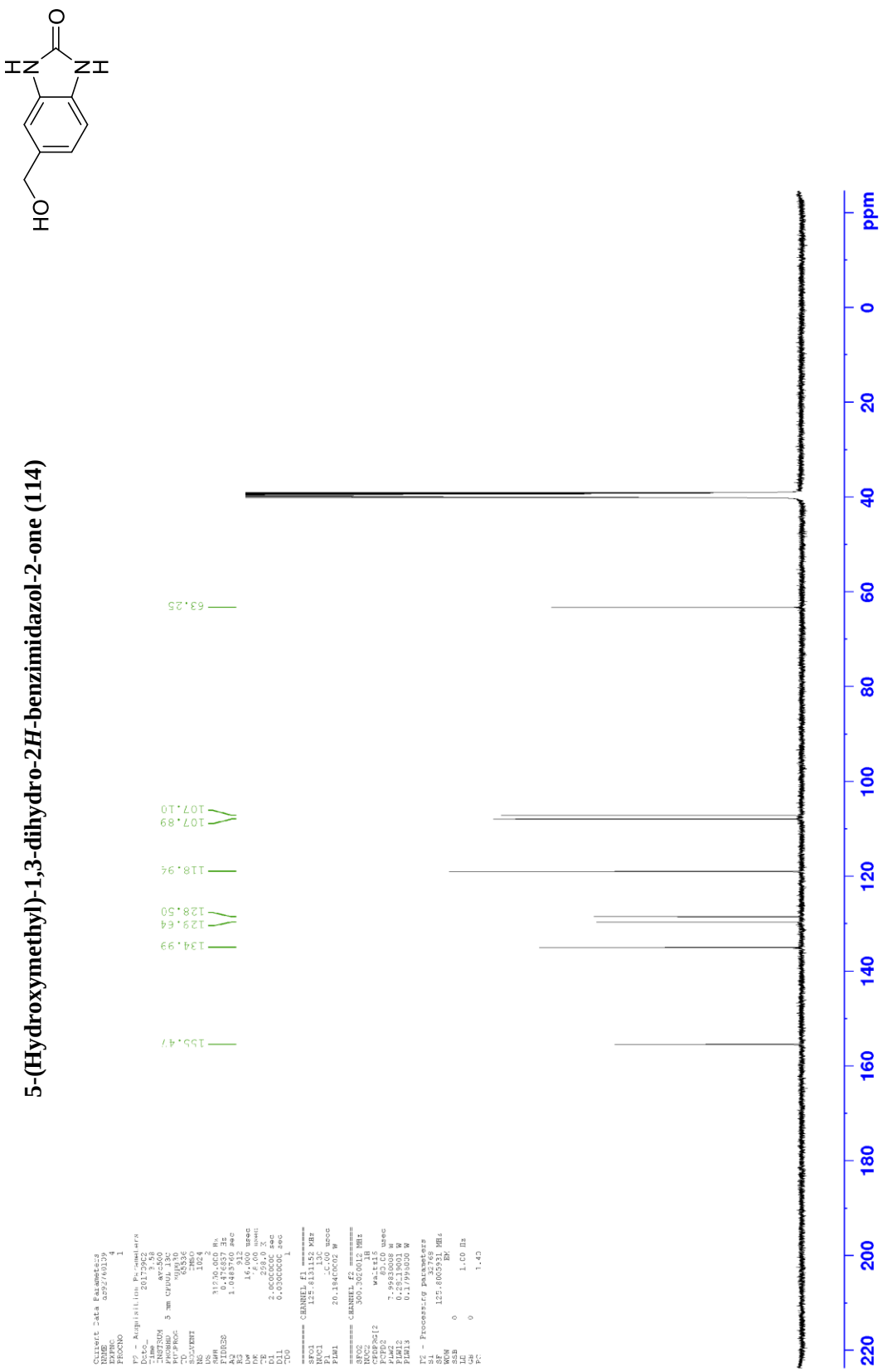


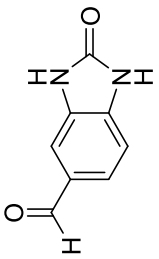


5-(Hydroxymethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (114)

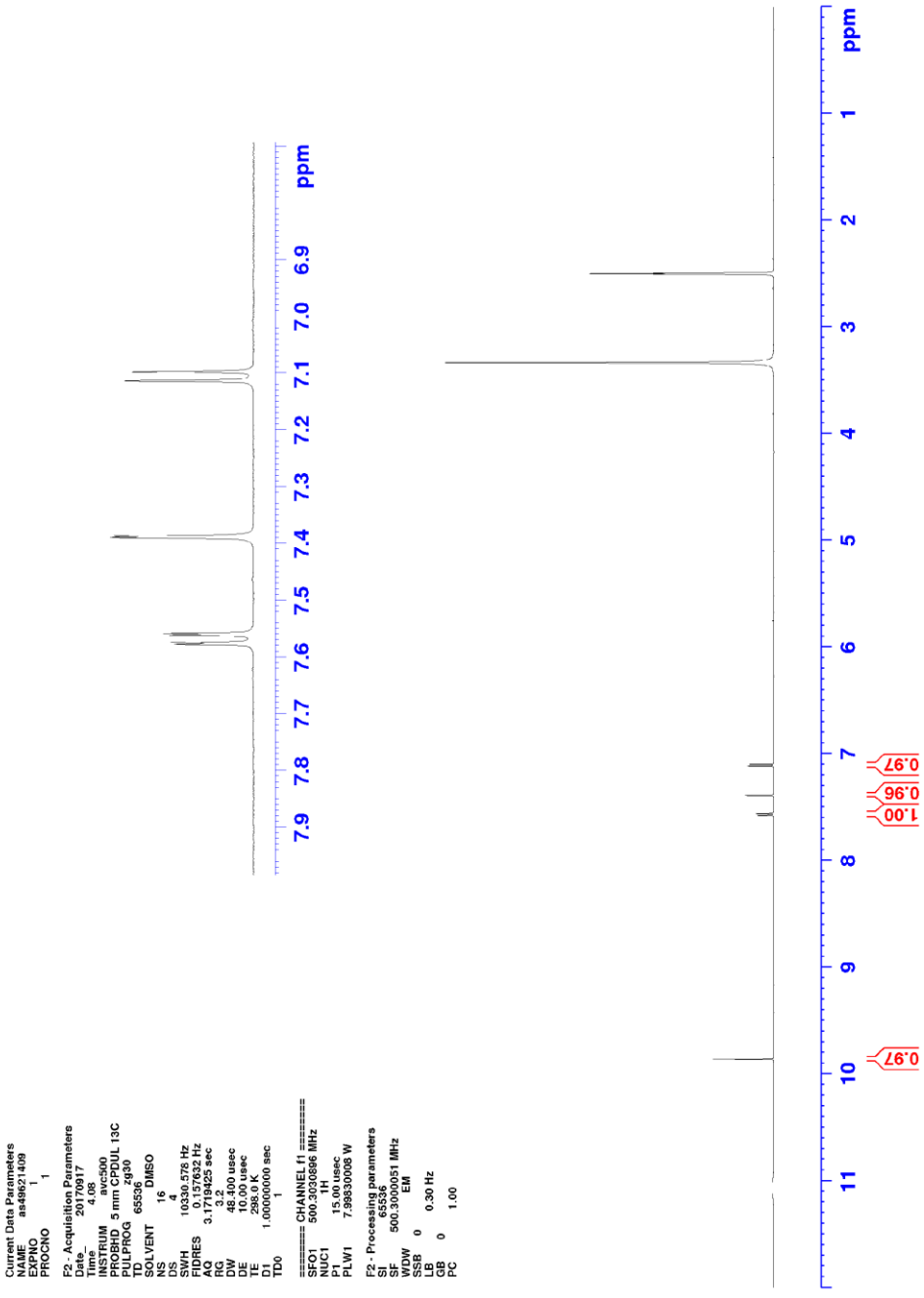


5-(Hydroxymethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (114)

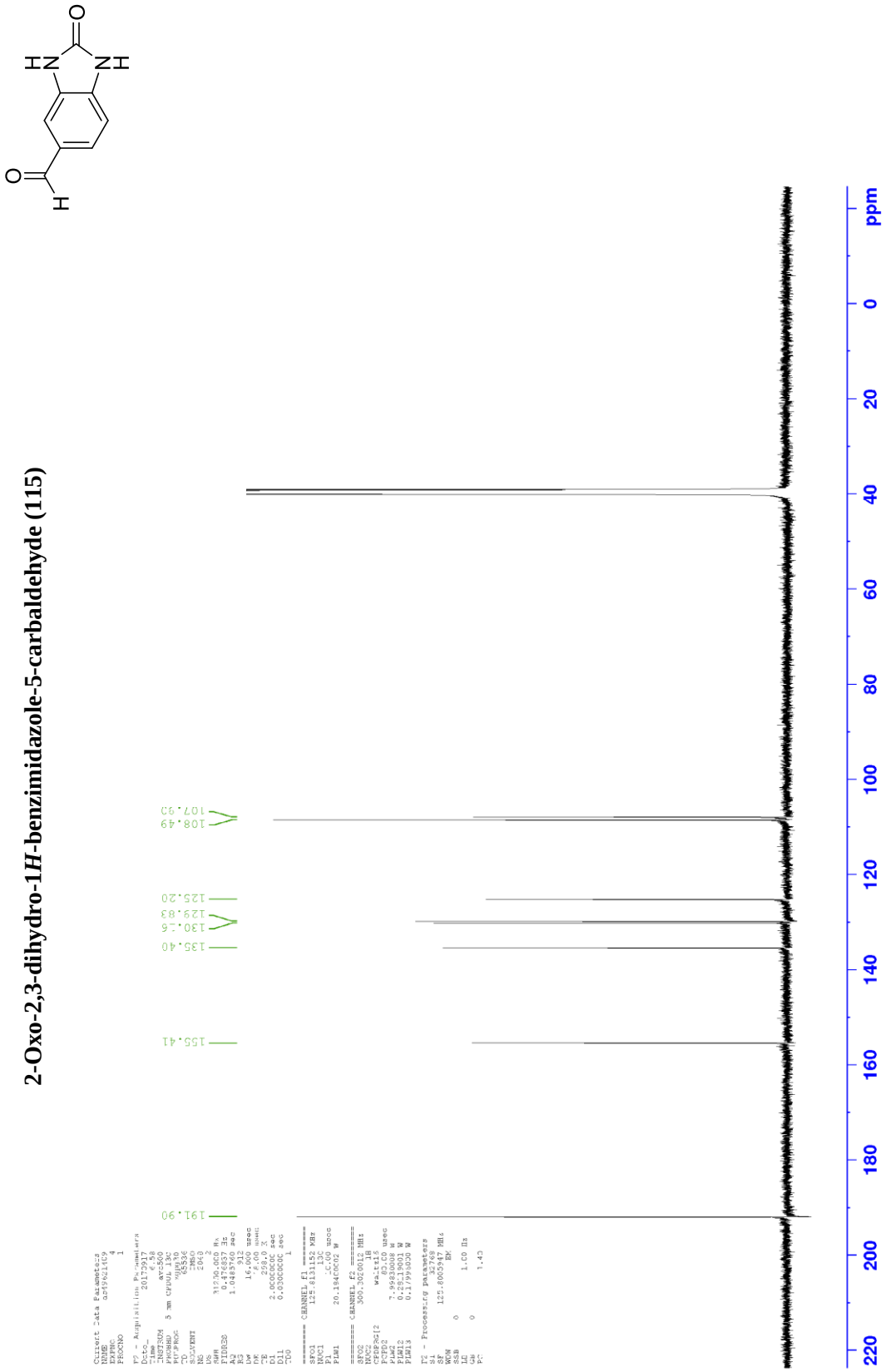




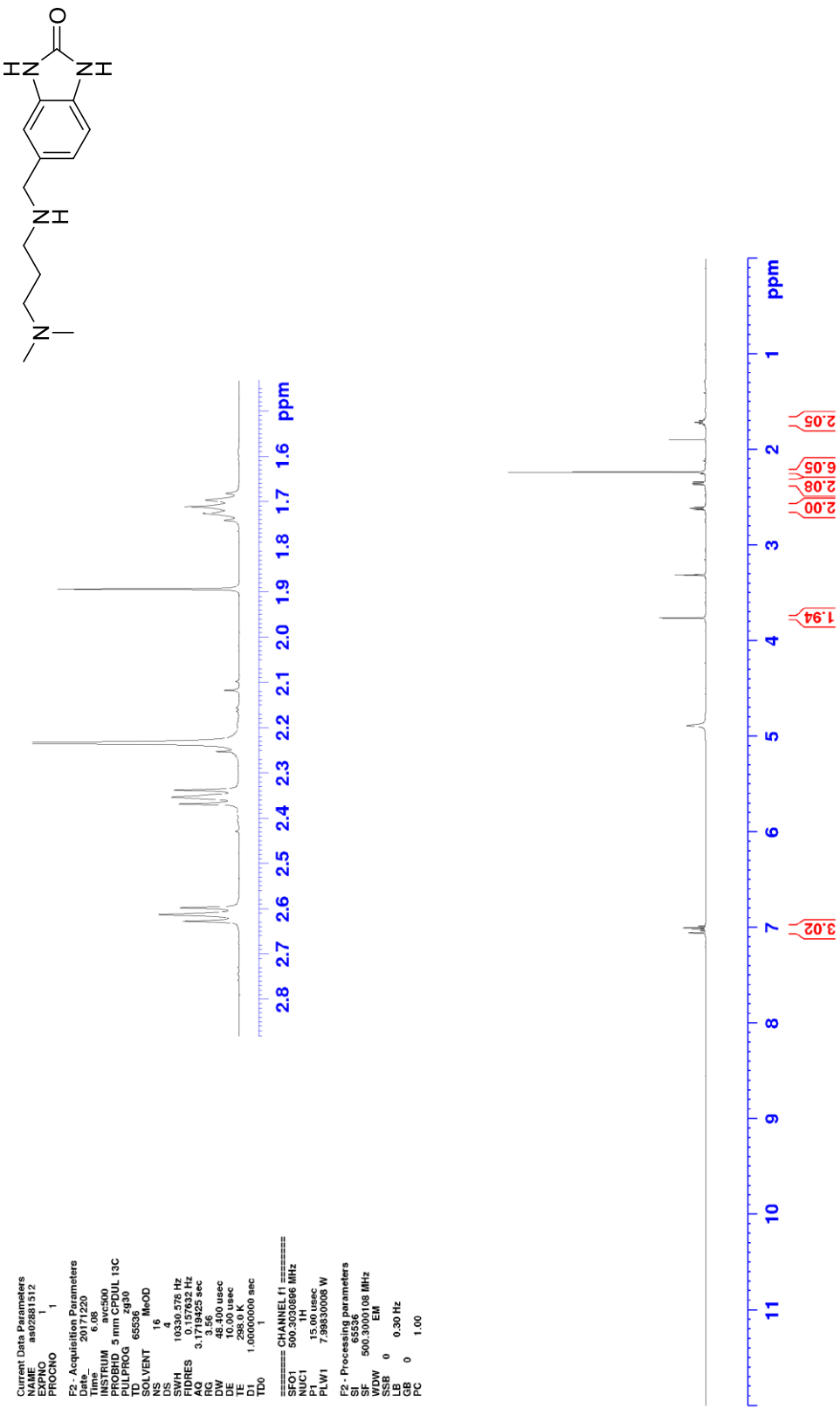
2-Oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (115)



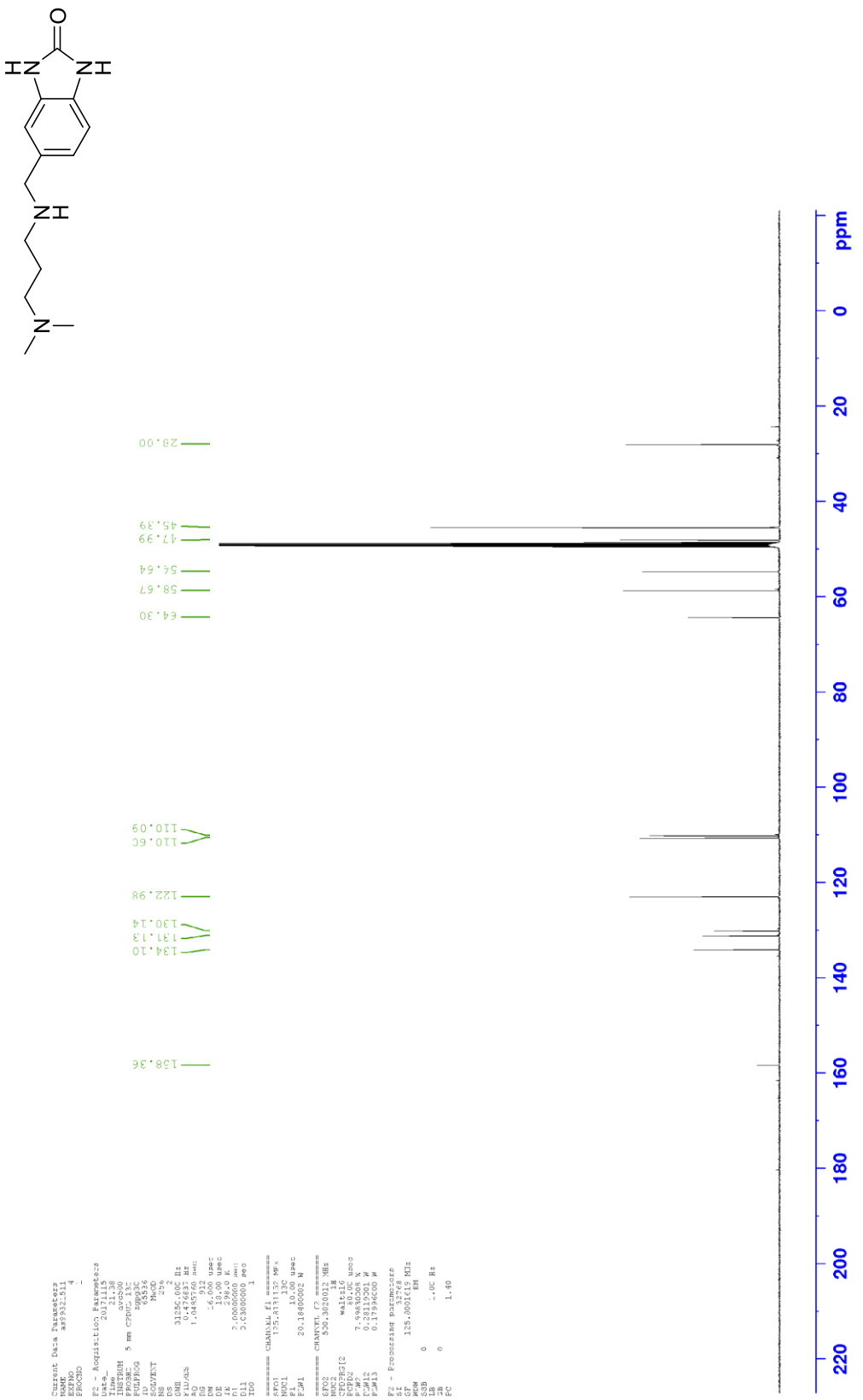
2-Oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (115)



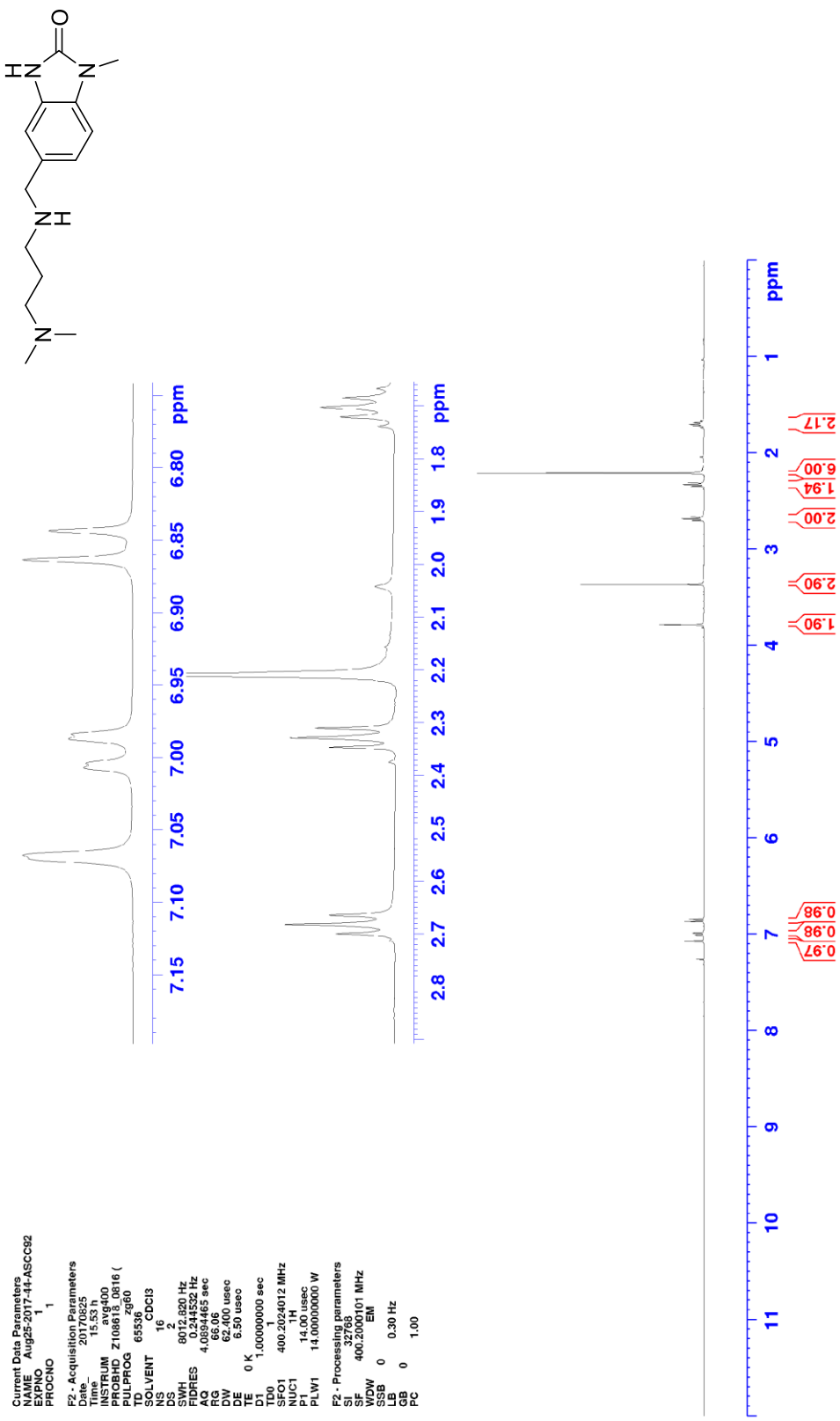
5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (104)



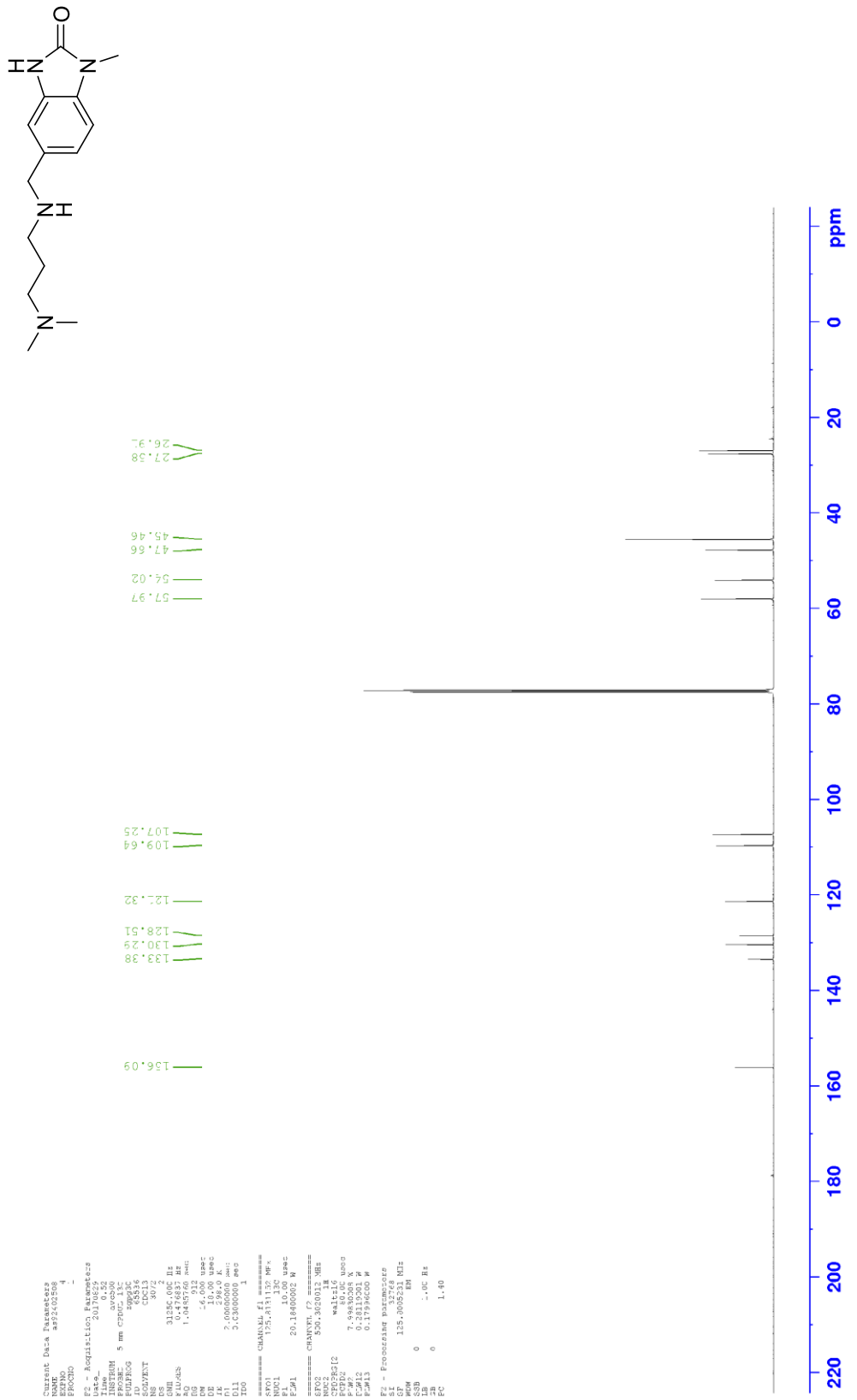
5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (104)



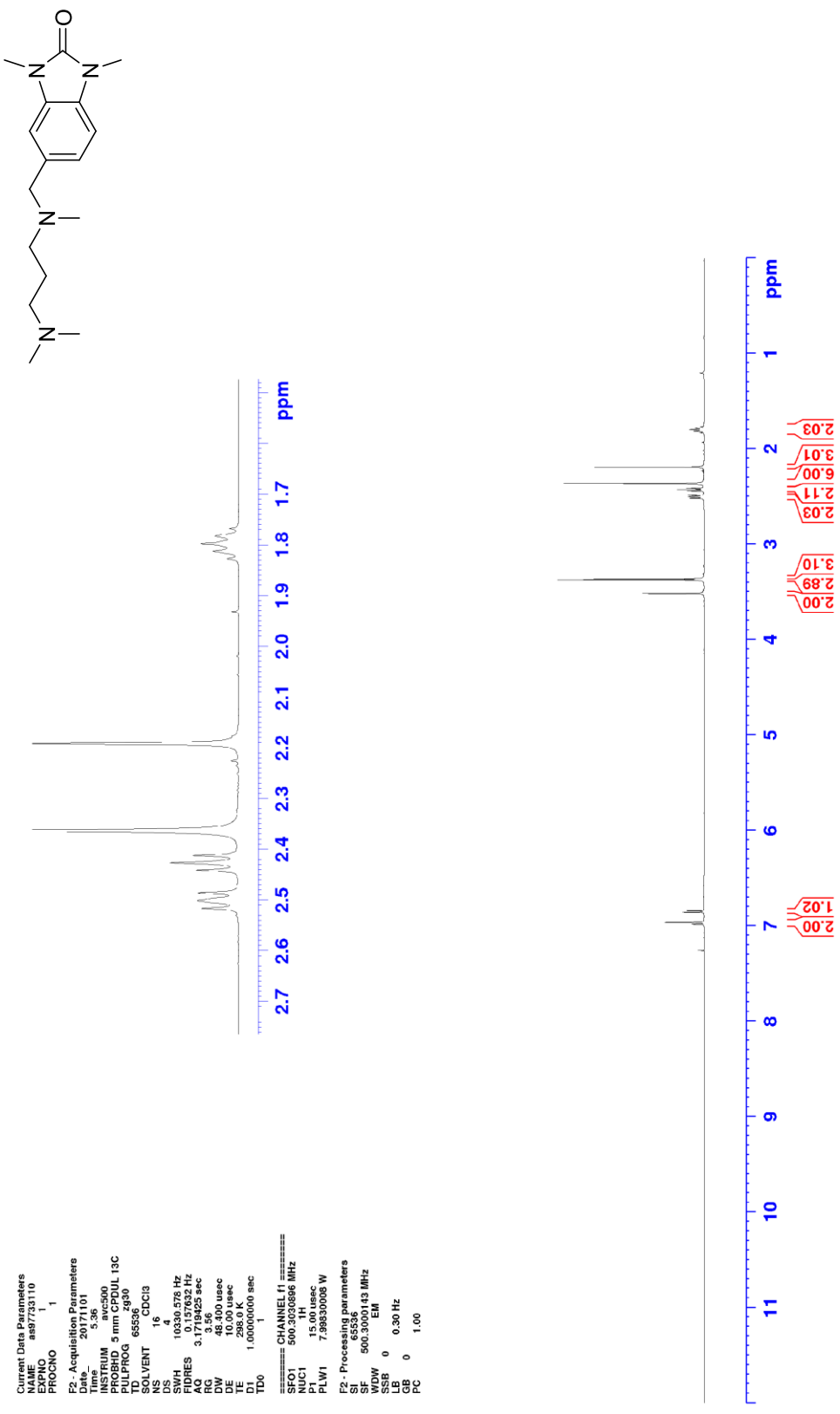
5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (105)



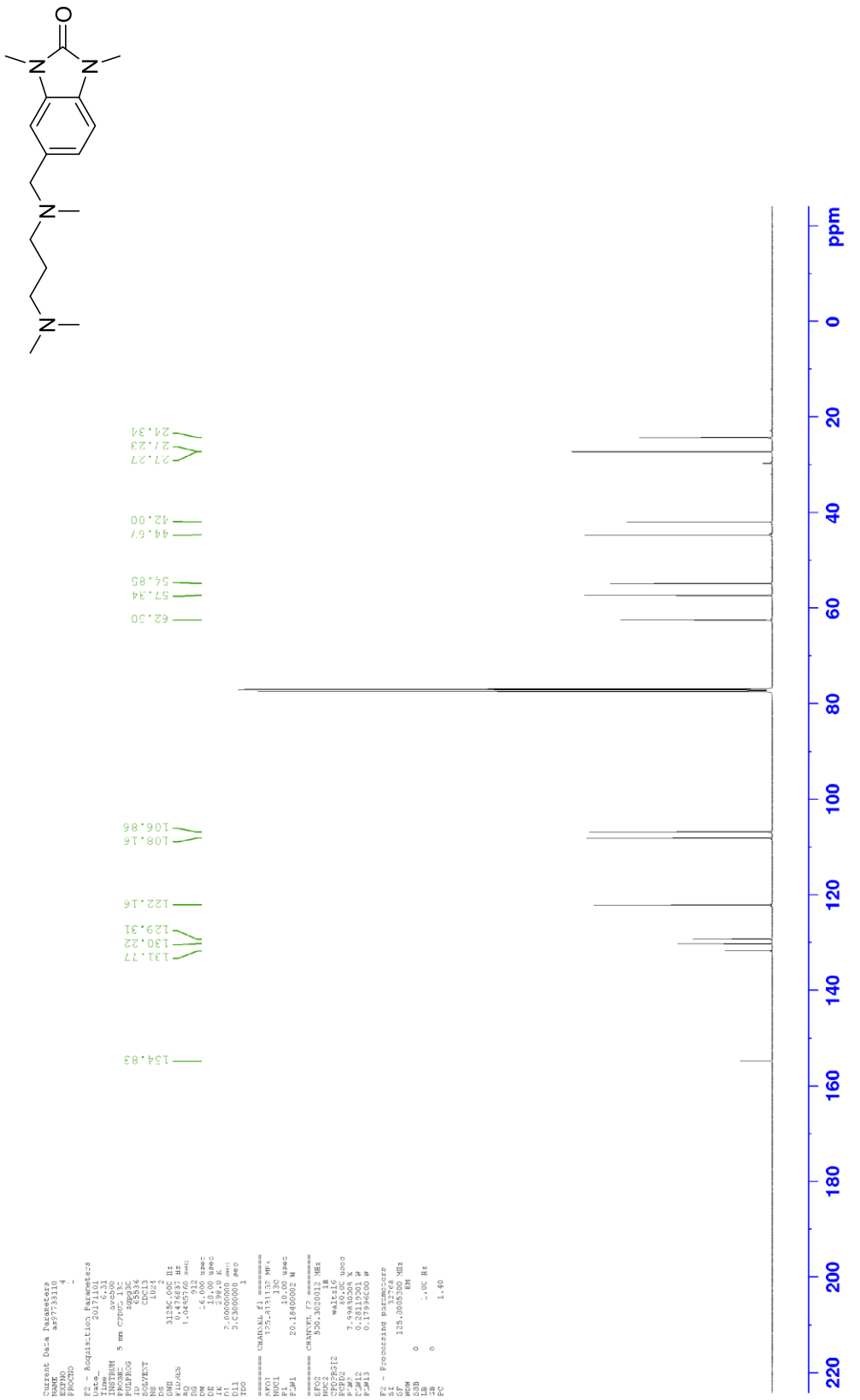
5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (105)



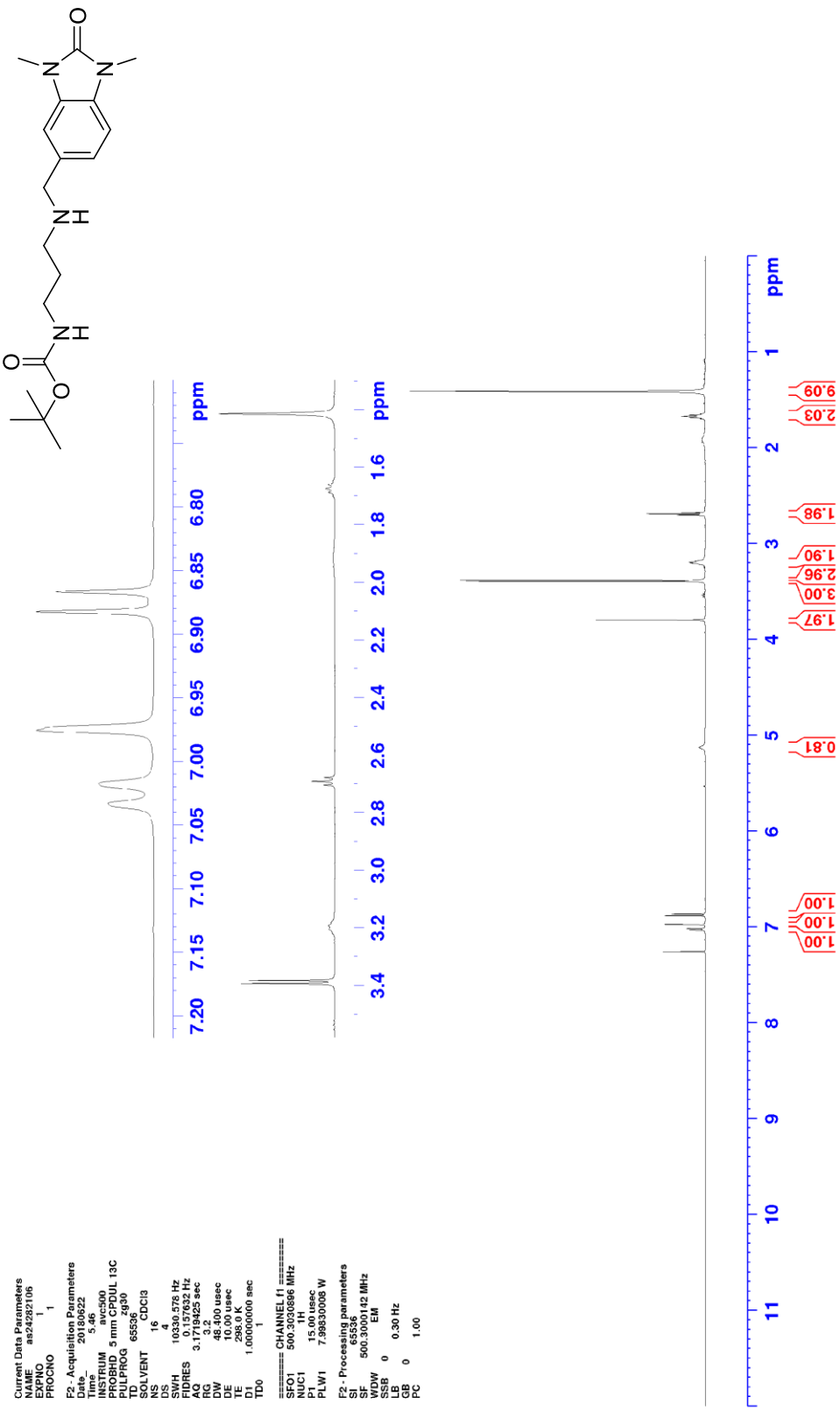
5-({[3-(Dimethylamino)prop-1-yl](methyl)amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (106)



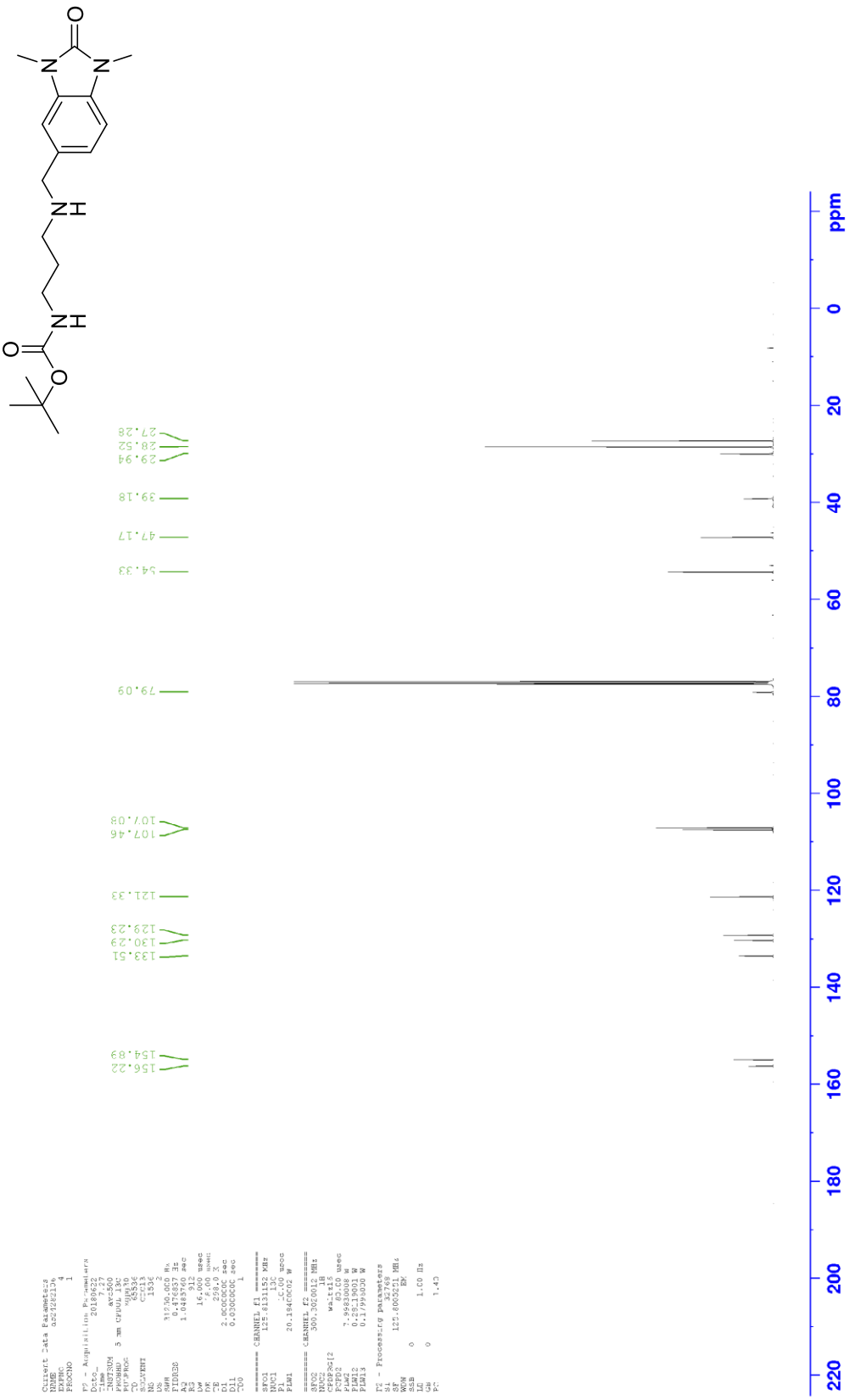
5-({[3-(Dimethylamino)prop-1-yl](methyl)amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (106)



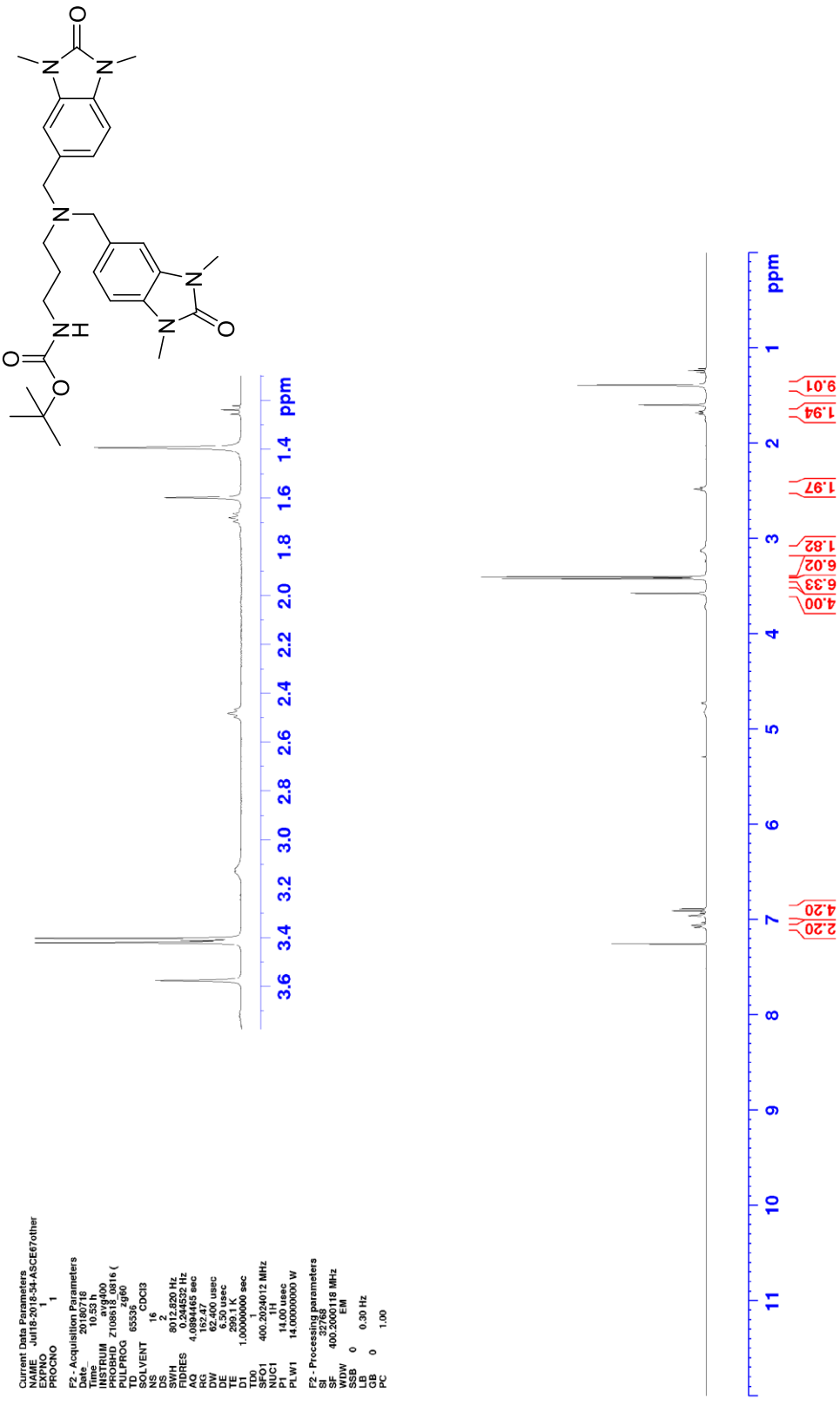
***tert*-Butyl (3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)carbamate (116)**



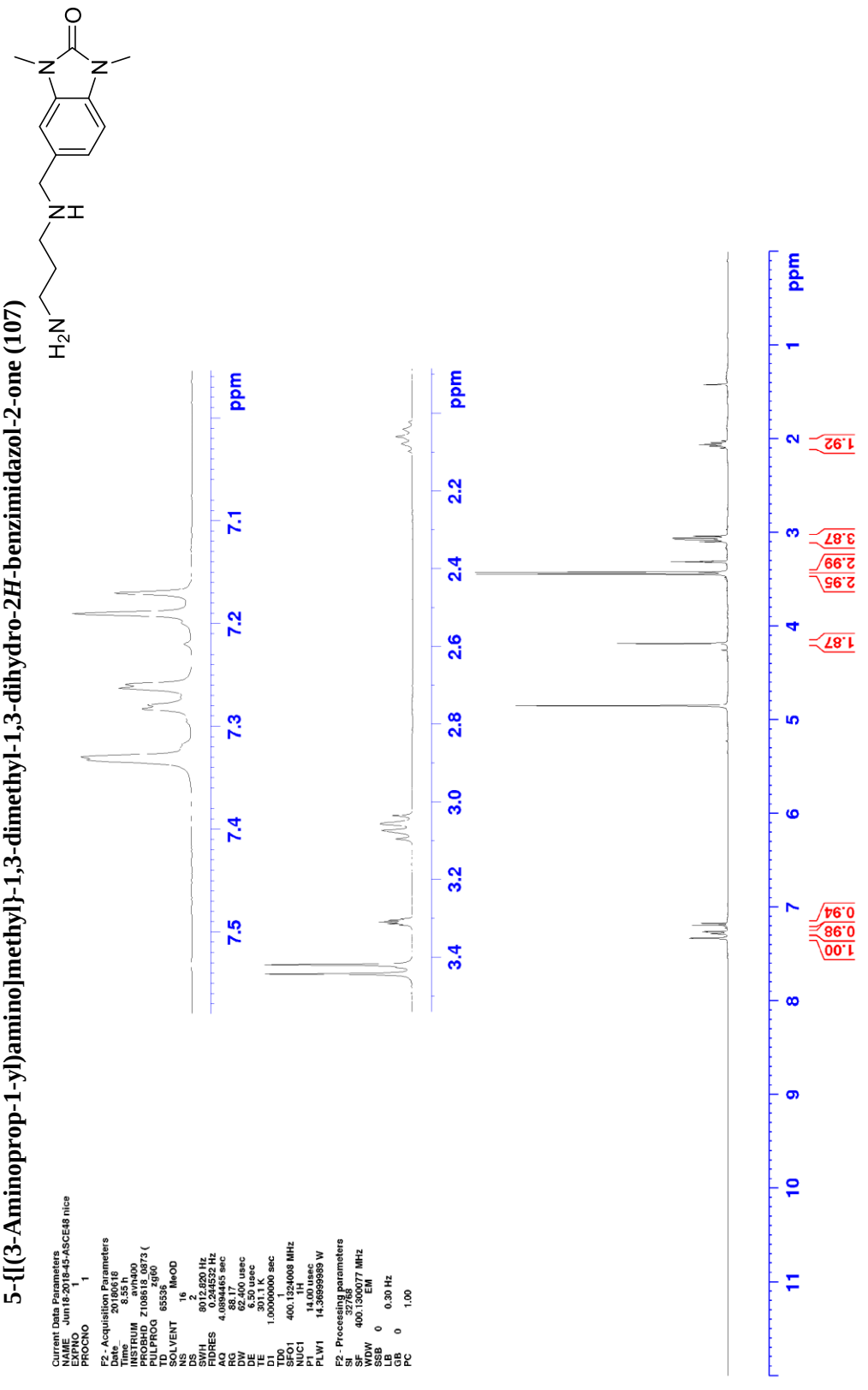
***tert*-Butyl (3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)carbamate (116)**



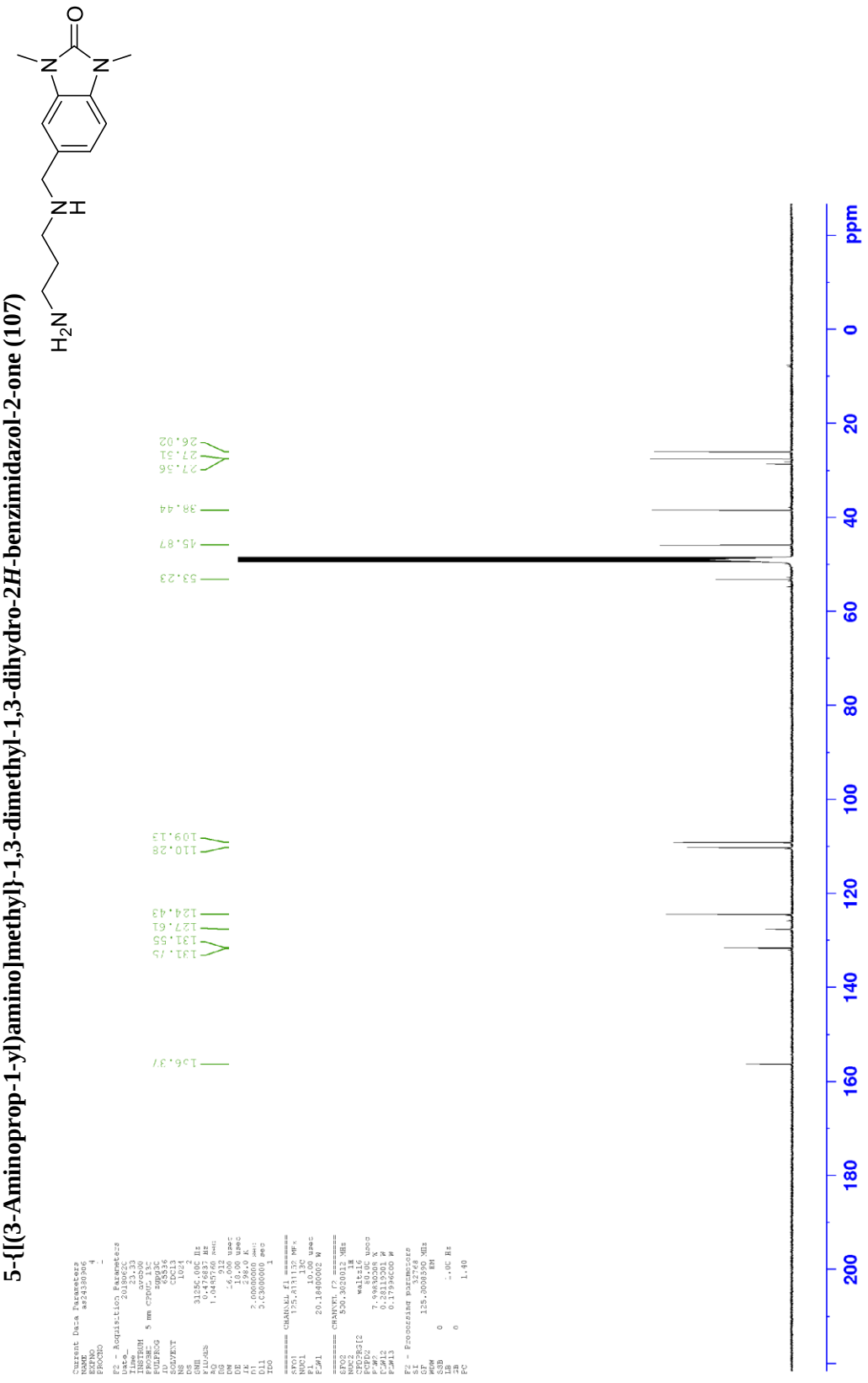
***tert*-Butyl (3-{bis[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)carbamate (117)**



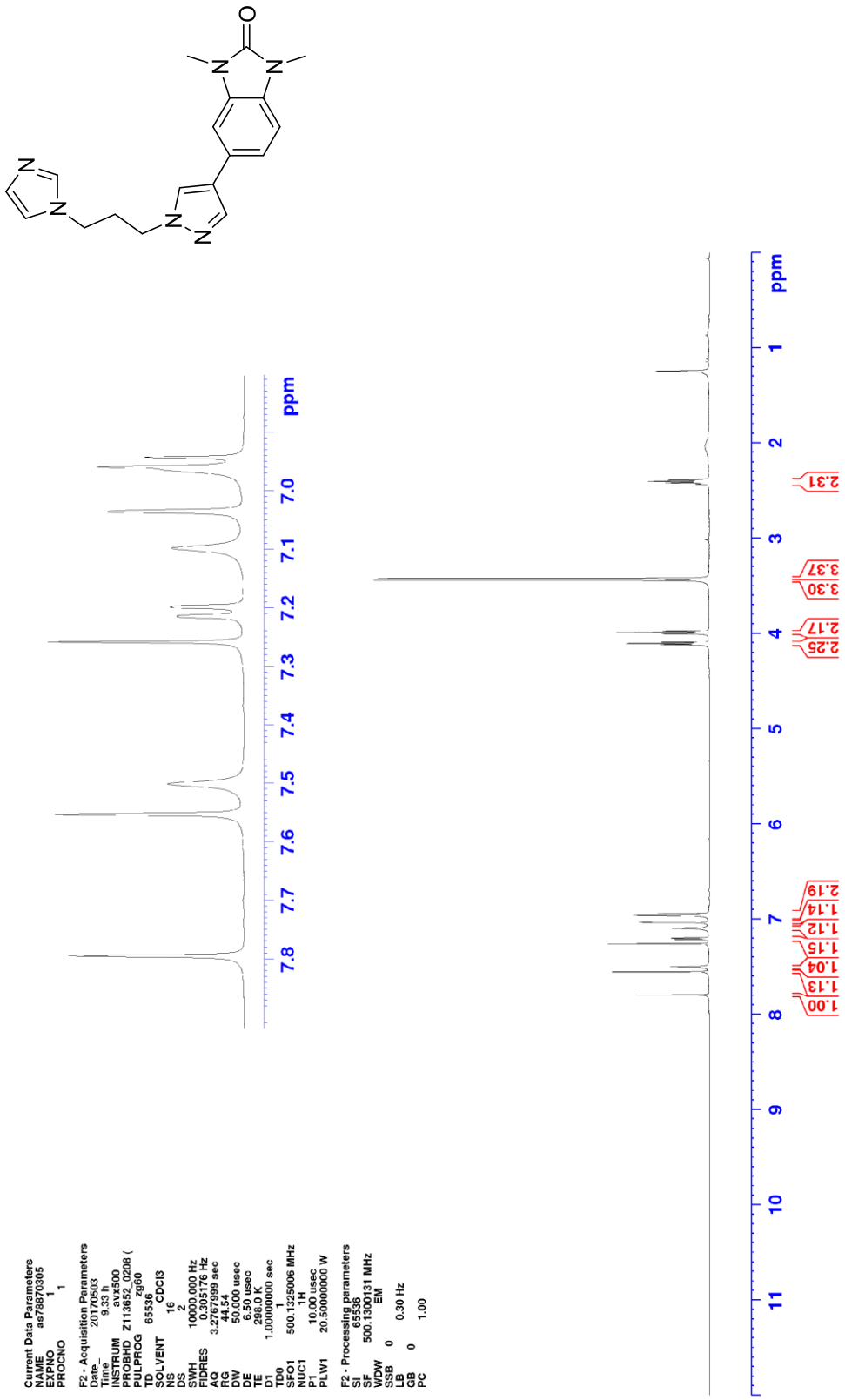
5-{[(3-Aminoprop-1-yl)amino]methyl}-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (107)



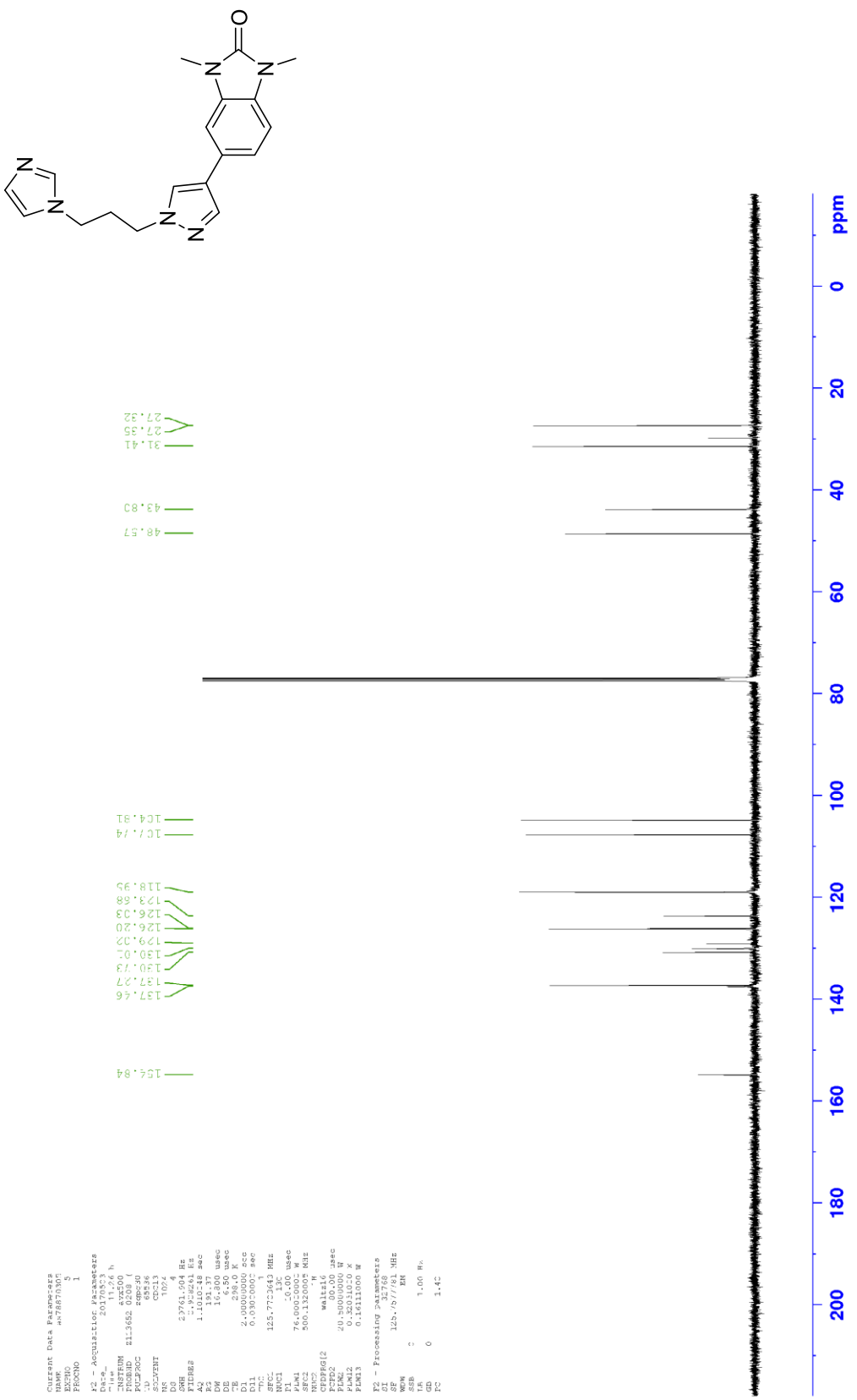
5-{[(3-Aminoprop-1-yl)amino]methyl}-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (107)



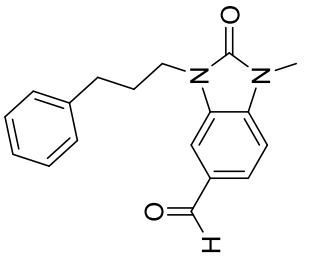
5-{1-[3-(1*H*-Imidazol-1-yl)propyl]-1*H*-pyrazol-4-yl}-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (118)

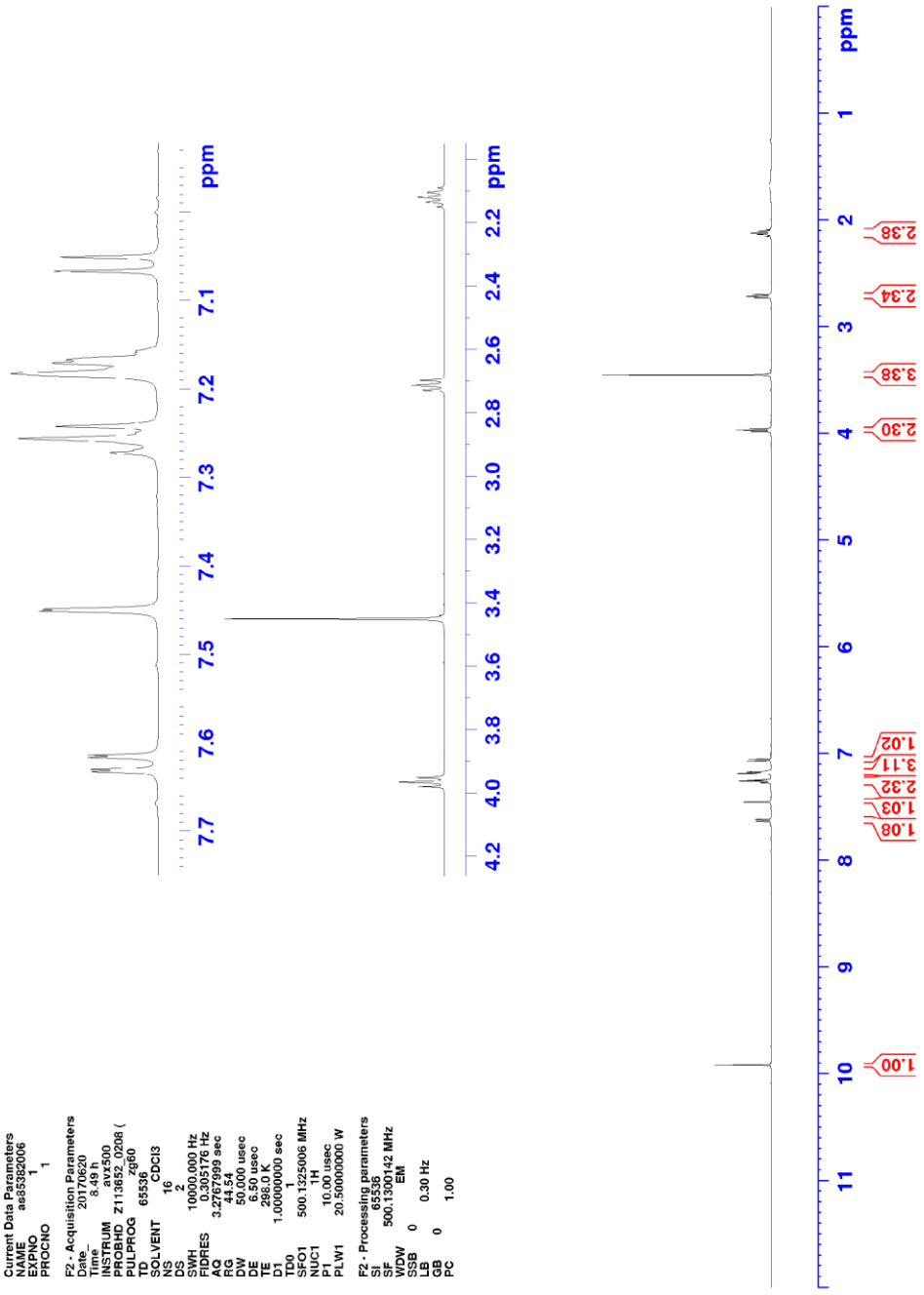


5-{1-[3-(1*H*-Imidazol-1-yl)propyl]-1*H*-pyrazol-4-yl}-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (118)

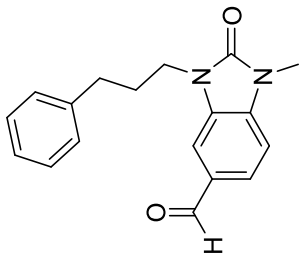


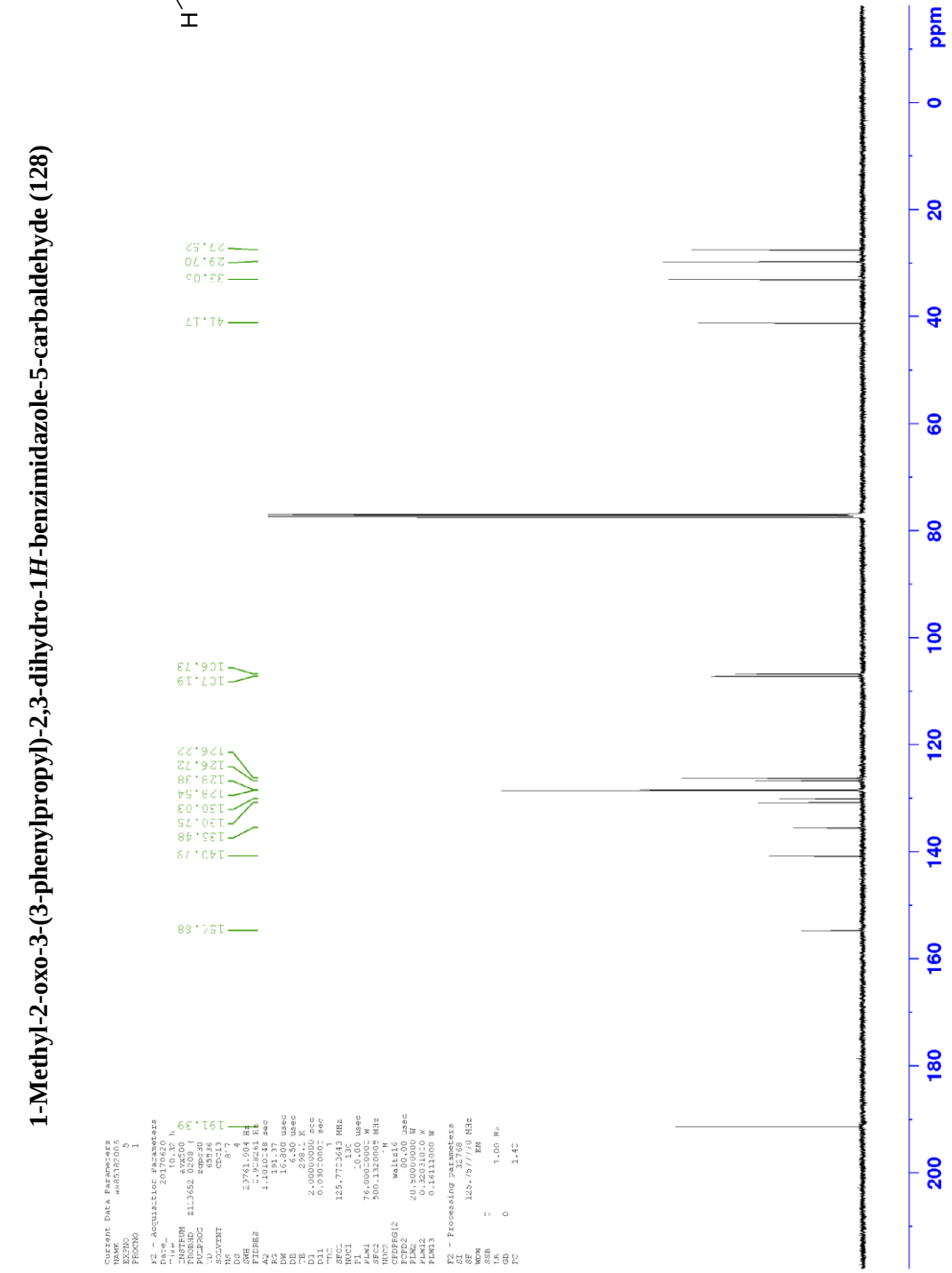
1-Methyl-2-oxo-3-(3-phenylpropyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (128)



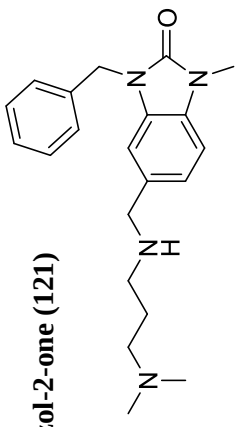


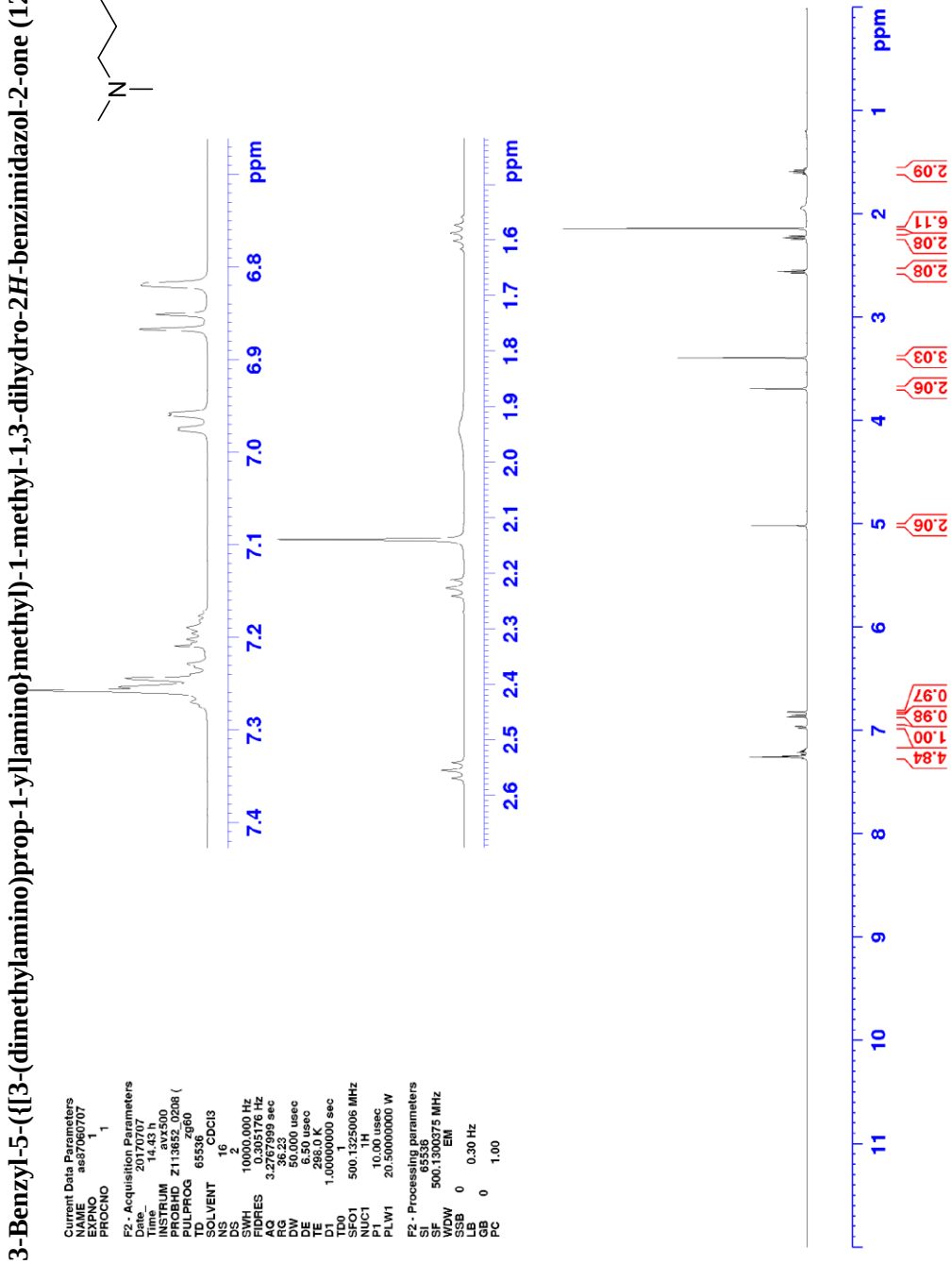
1-Methyl-2-oxo-3-(3-phenylpropyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (128)



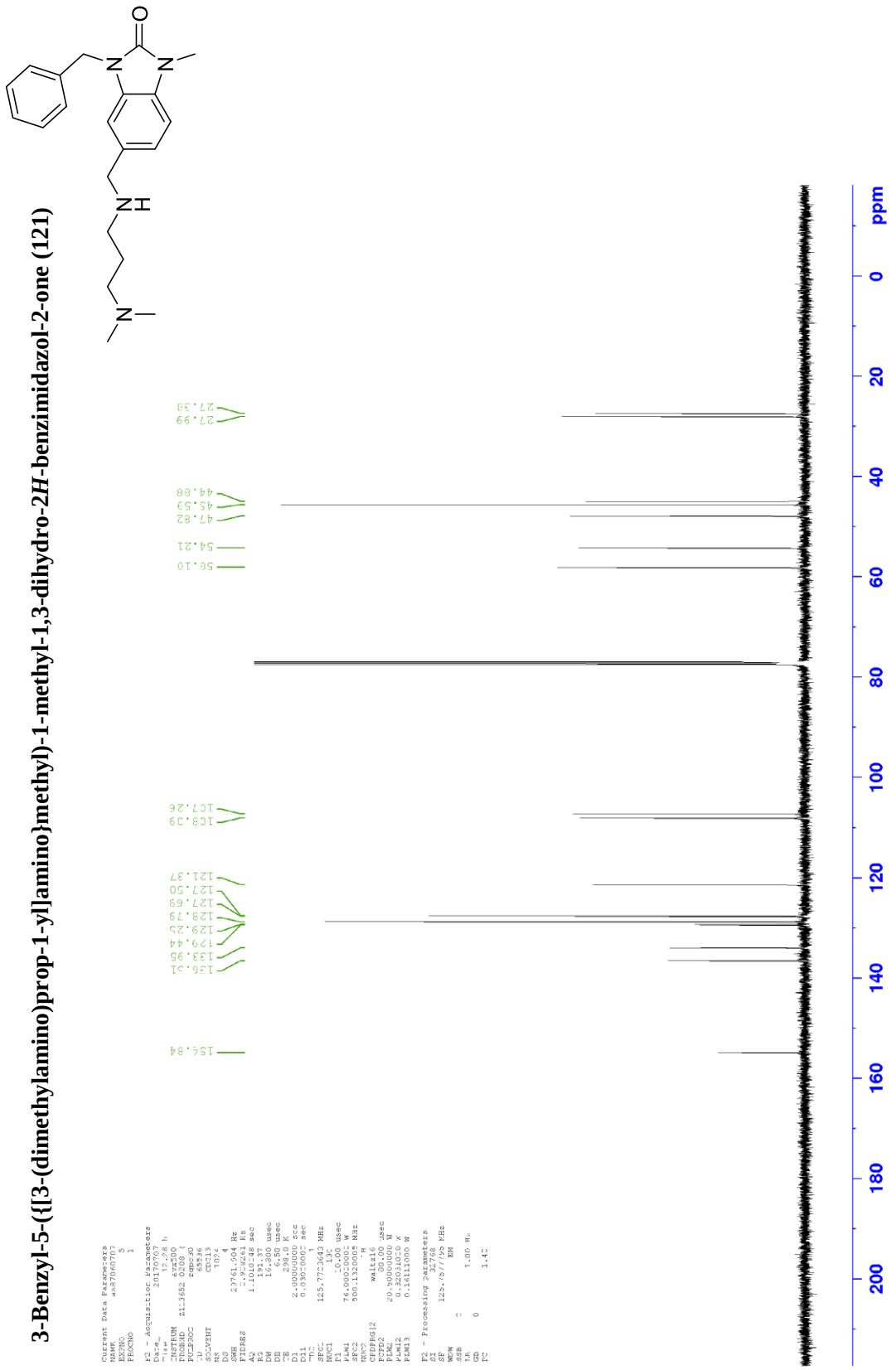


3-Benzyl-5-({[3-(dimethylamino)prop-1-yl]amino}methyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (121)

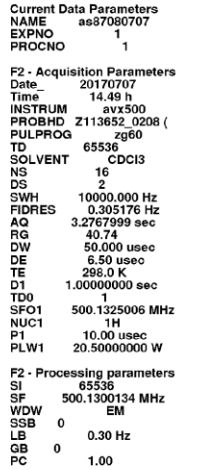




3-Benzyl-5-({[3-(dimethylamino)prop-1-yl]amino}methyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (121)



5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-3-(2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (122)



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DE 6.50 usec
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F2 - Processing parameters
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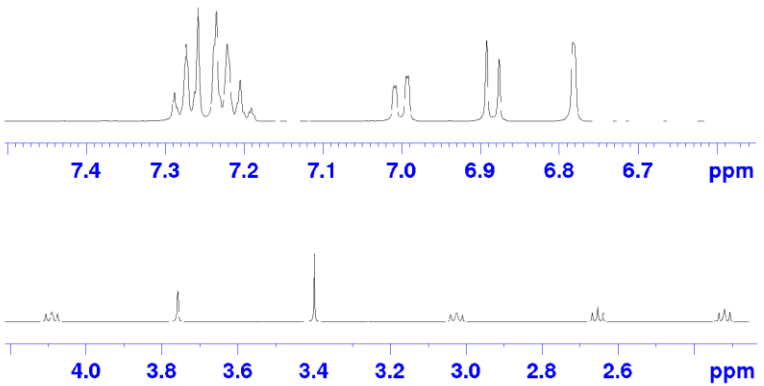
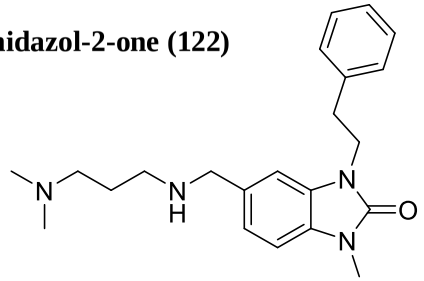
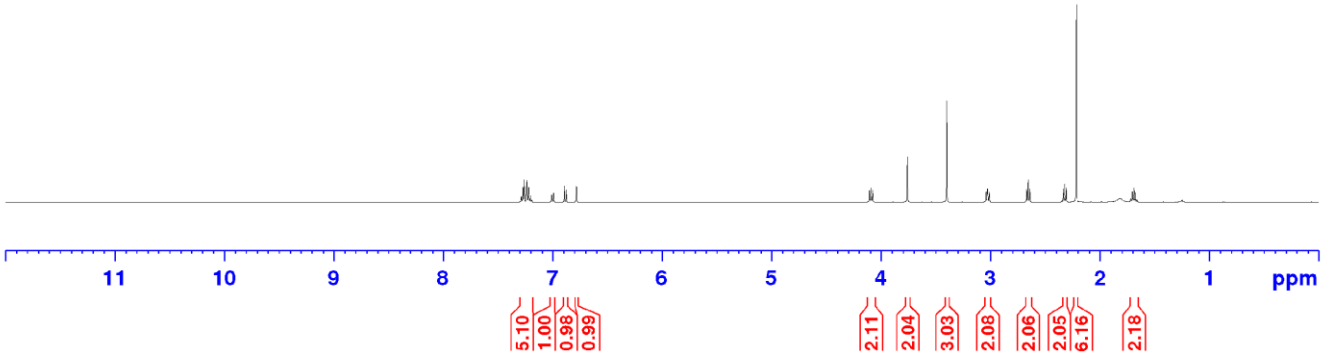
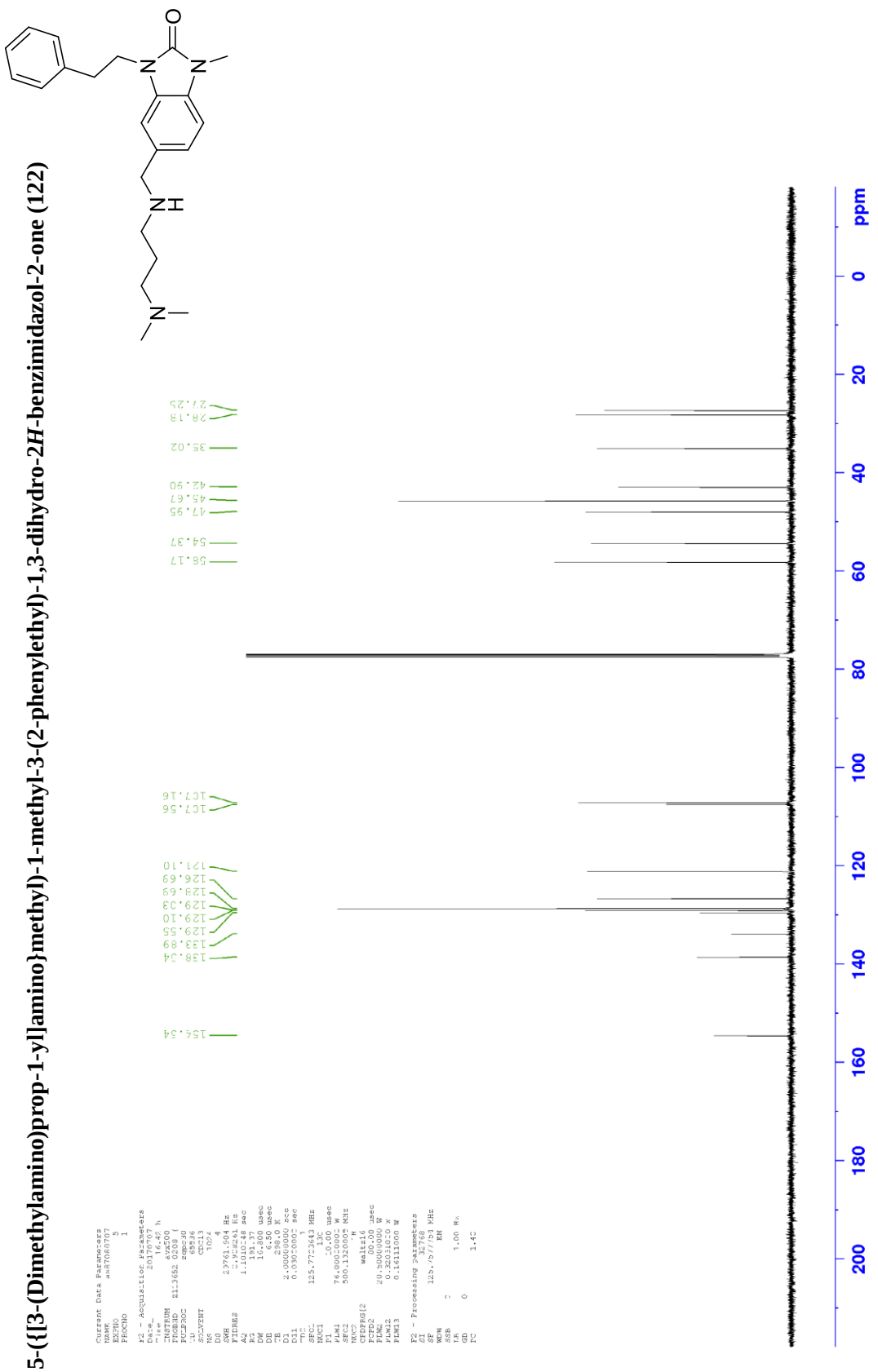


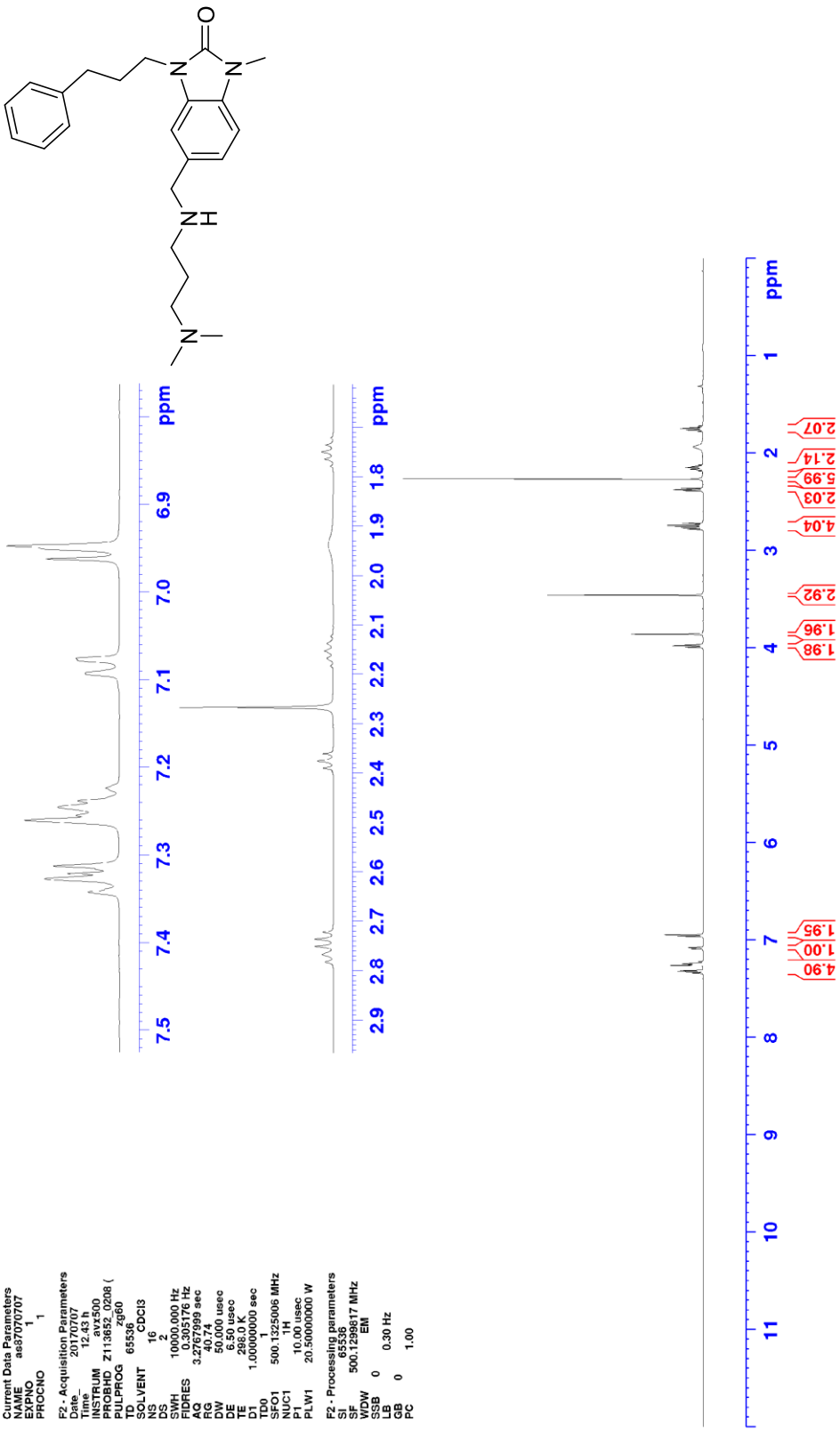




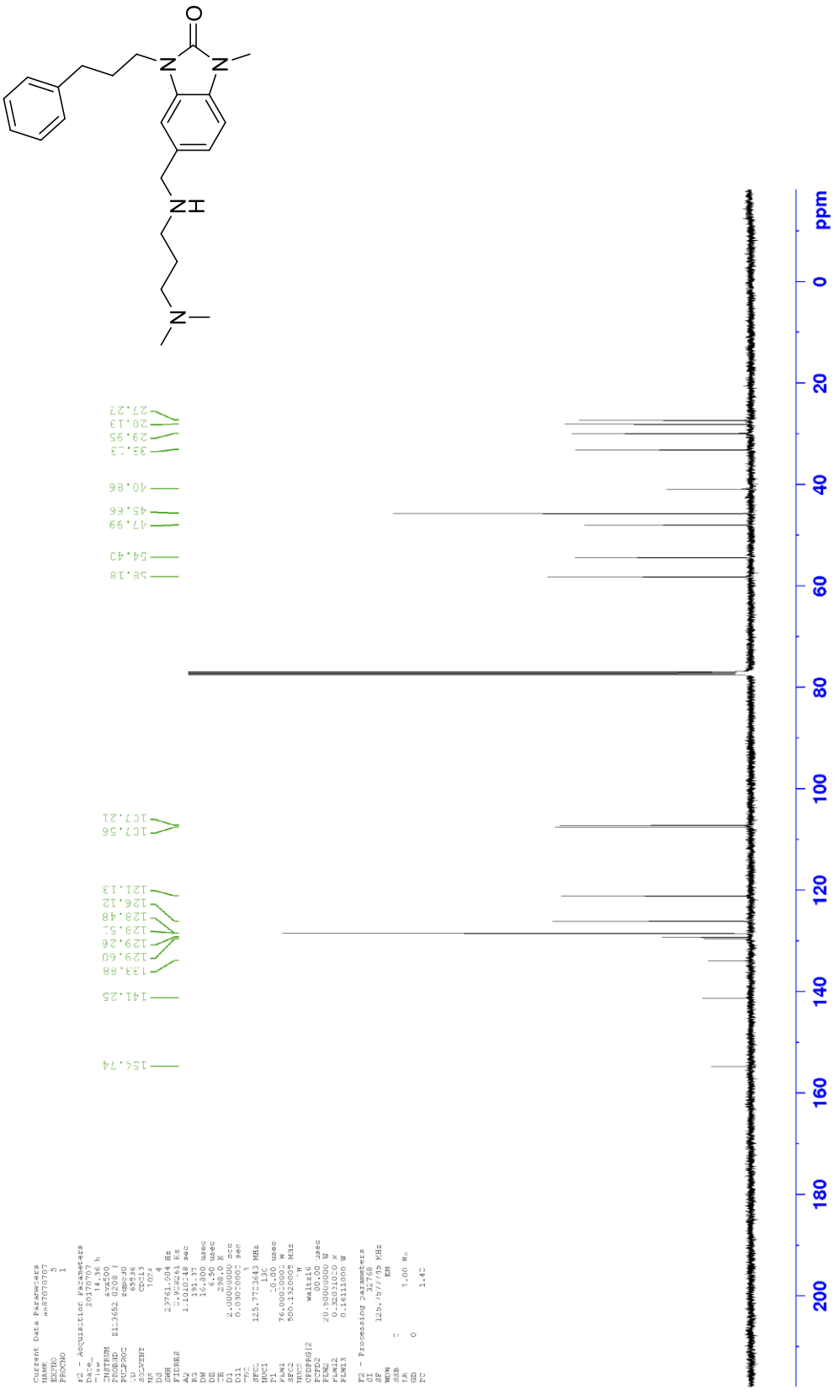
5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-3-(2-phenylethyl)-1,3-dihydro-2*H*-benzimidazol-2-one (122)



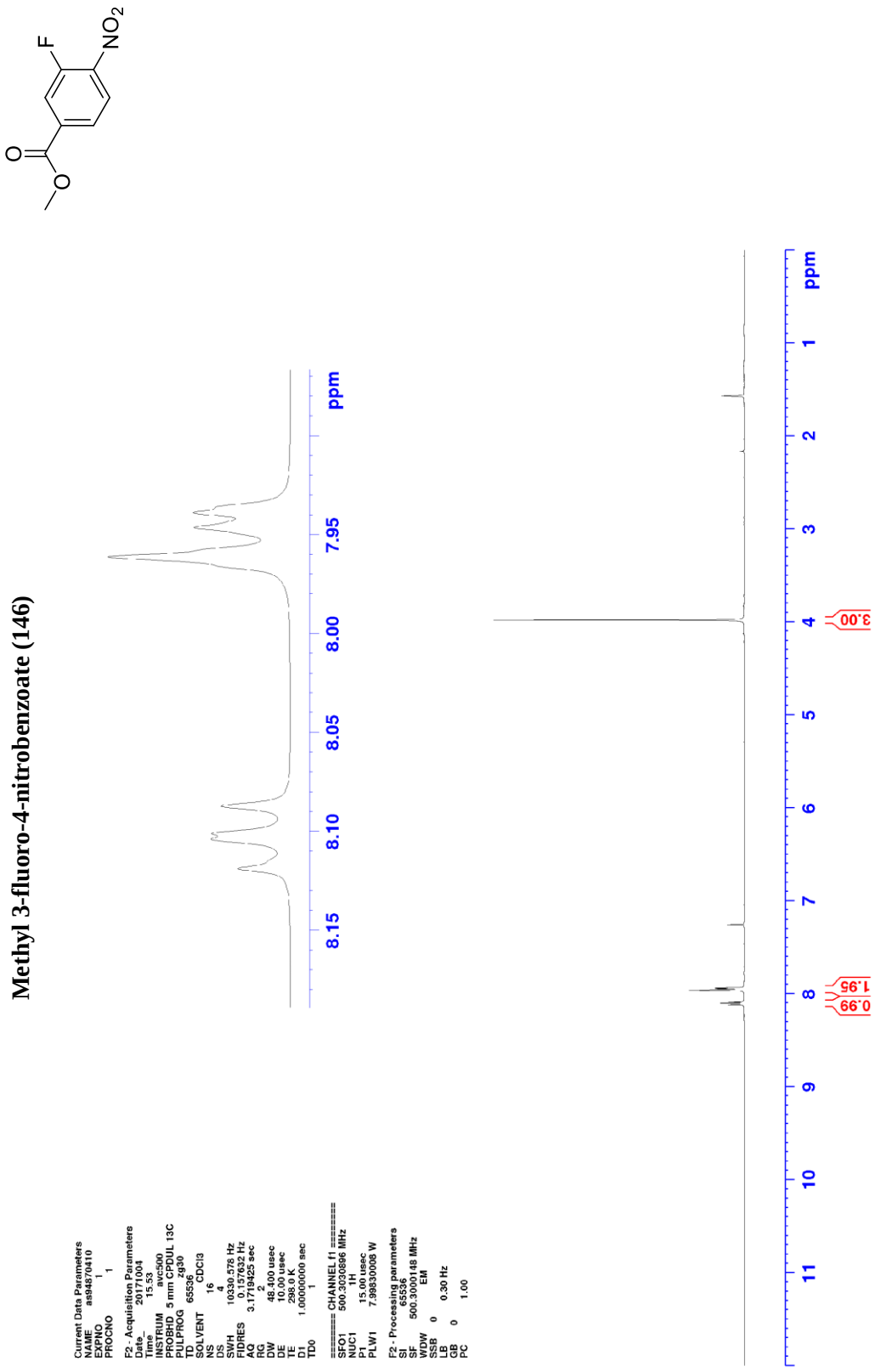
5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-3-(3-phenylpropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (123)



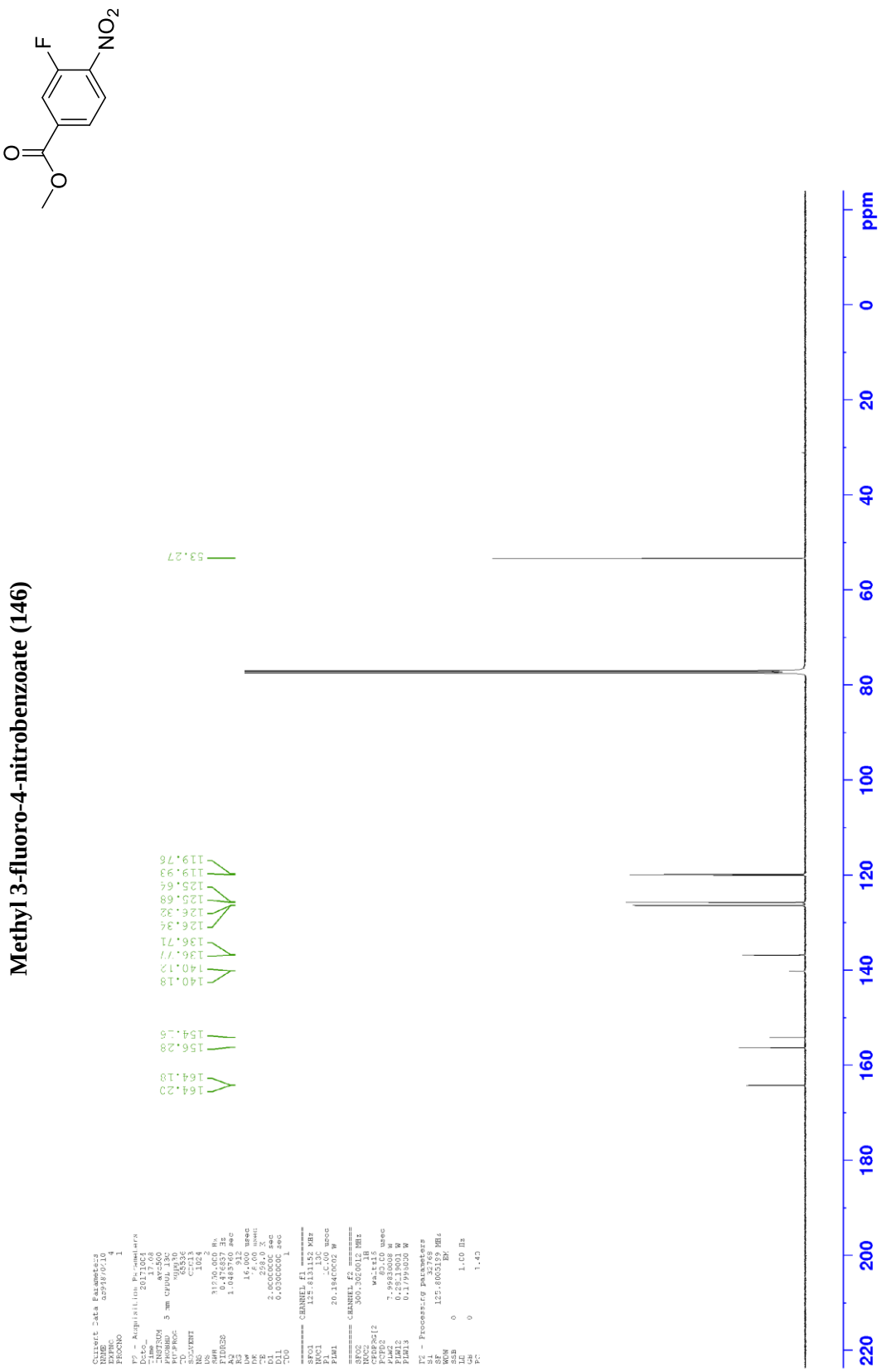
5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-3-(3-phenylpropyl)-1,3-dihydro-2*H*-benzimidazol-2-one (123)



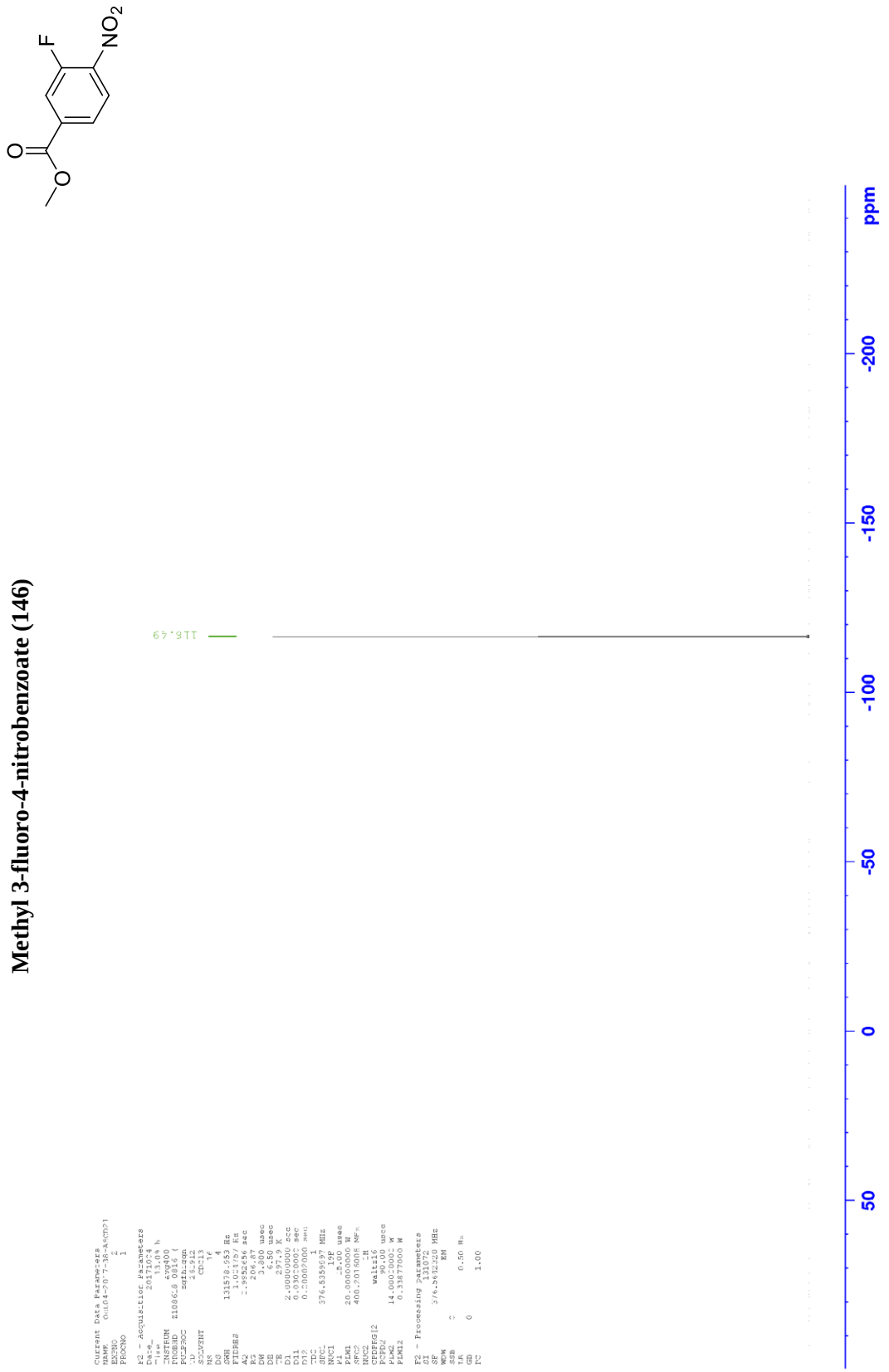
Methyl 3-fluoro-4-nitrobenzoate (146)

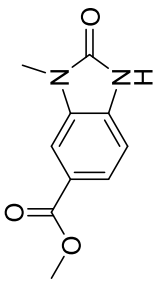


Methyl 3-fluoro-4-nitrobenzoate (146)

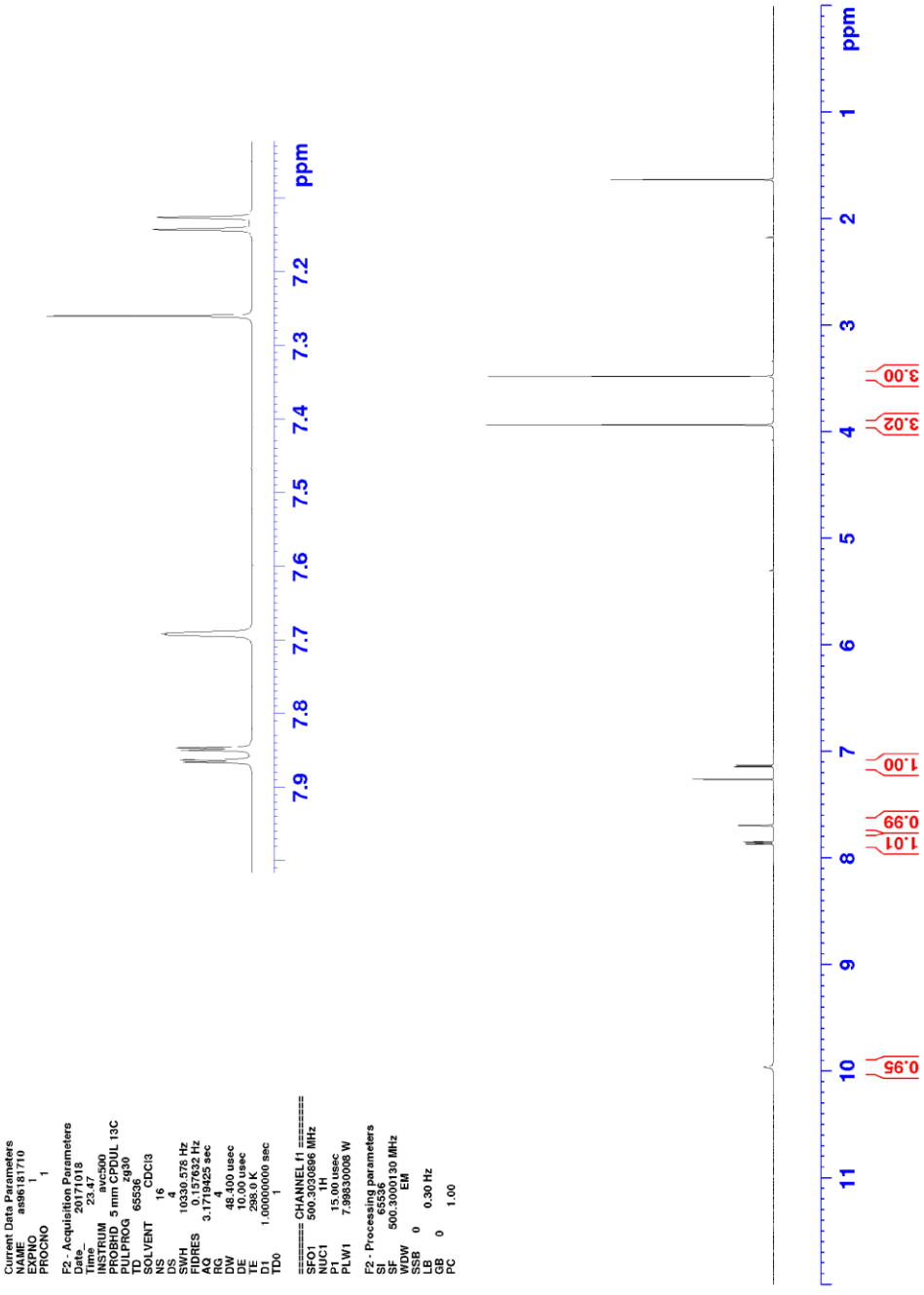


Methyl 3-fluoro-4-nitrobenzoate (146)

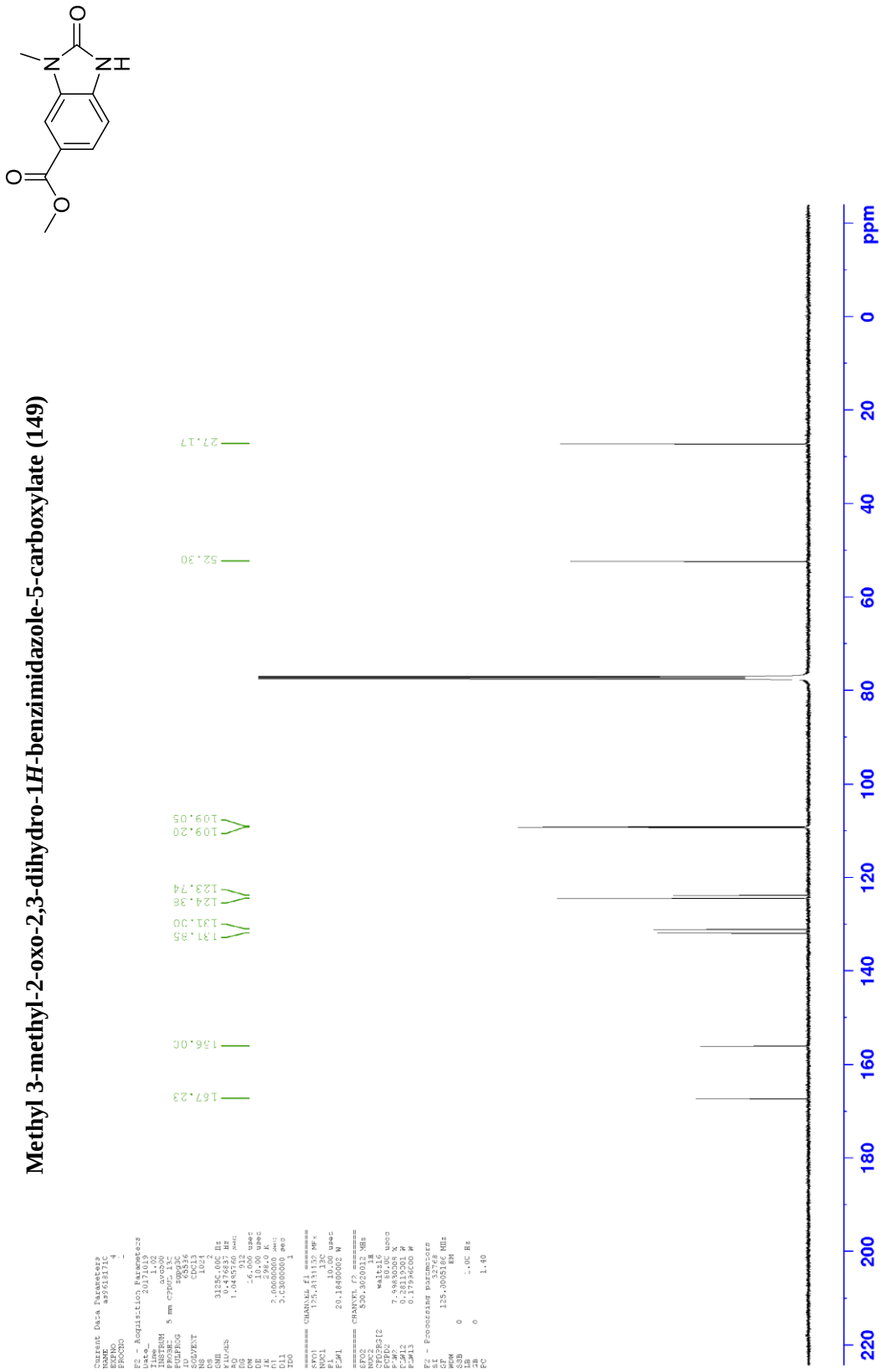


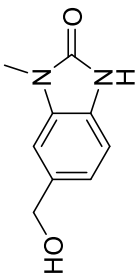


Methyl 3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (149)

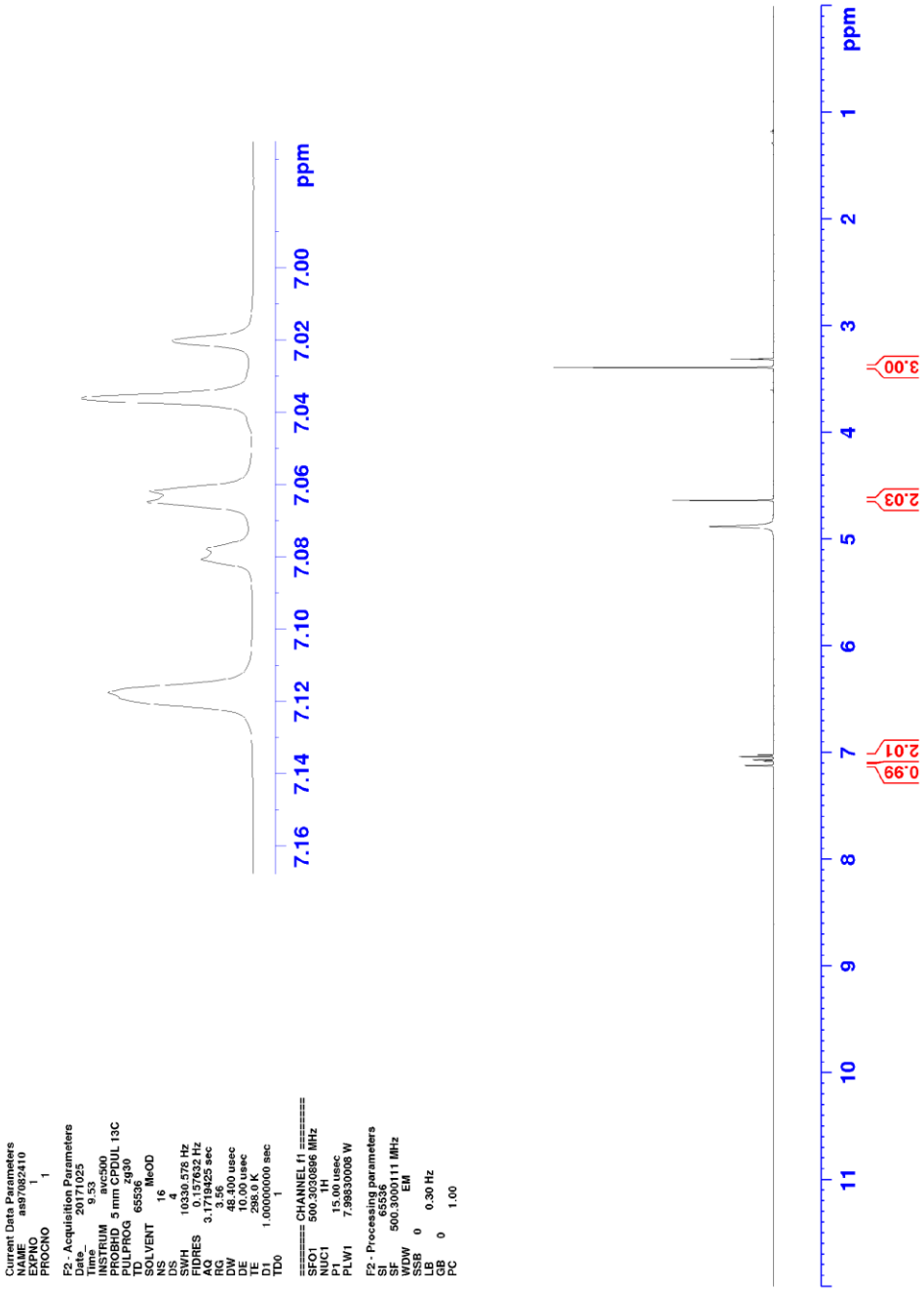


Methyl 3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carboxylate (149)

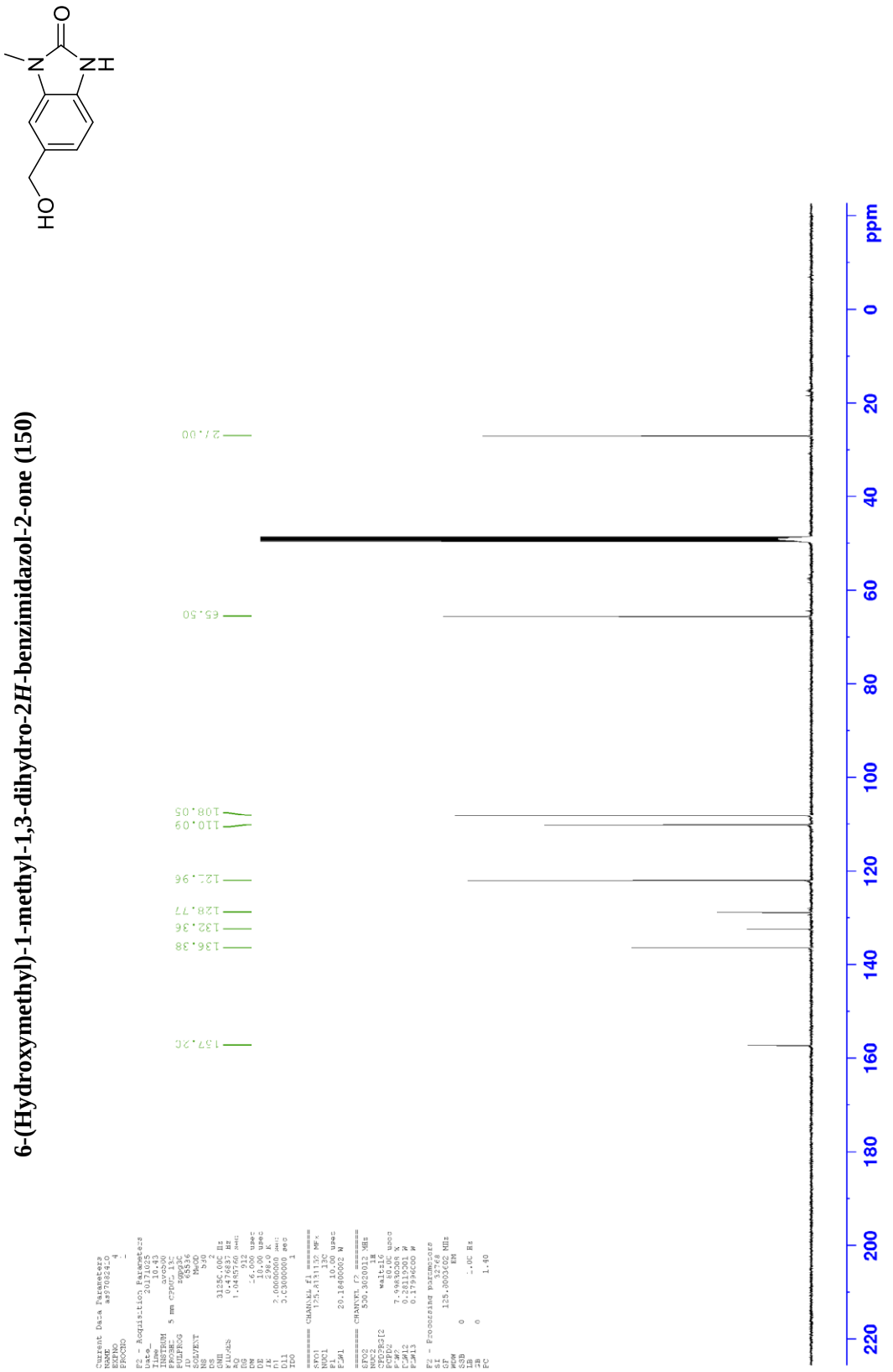


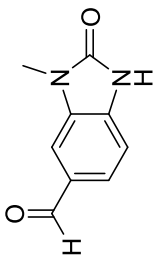


6-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (150)

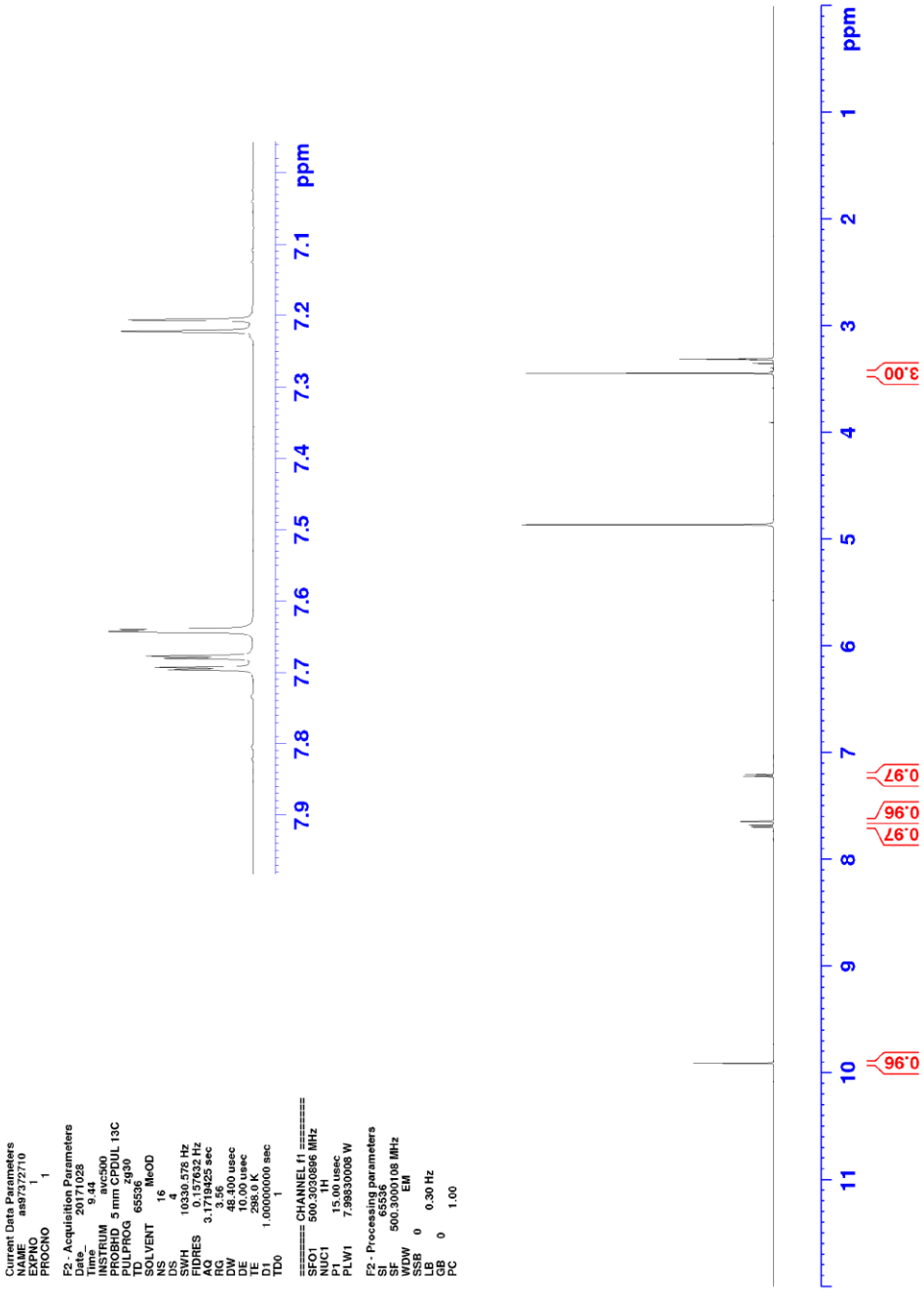


6-(Hydroxymethyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (150)

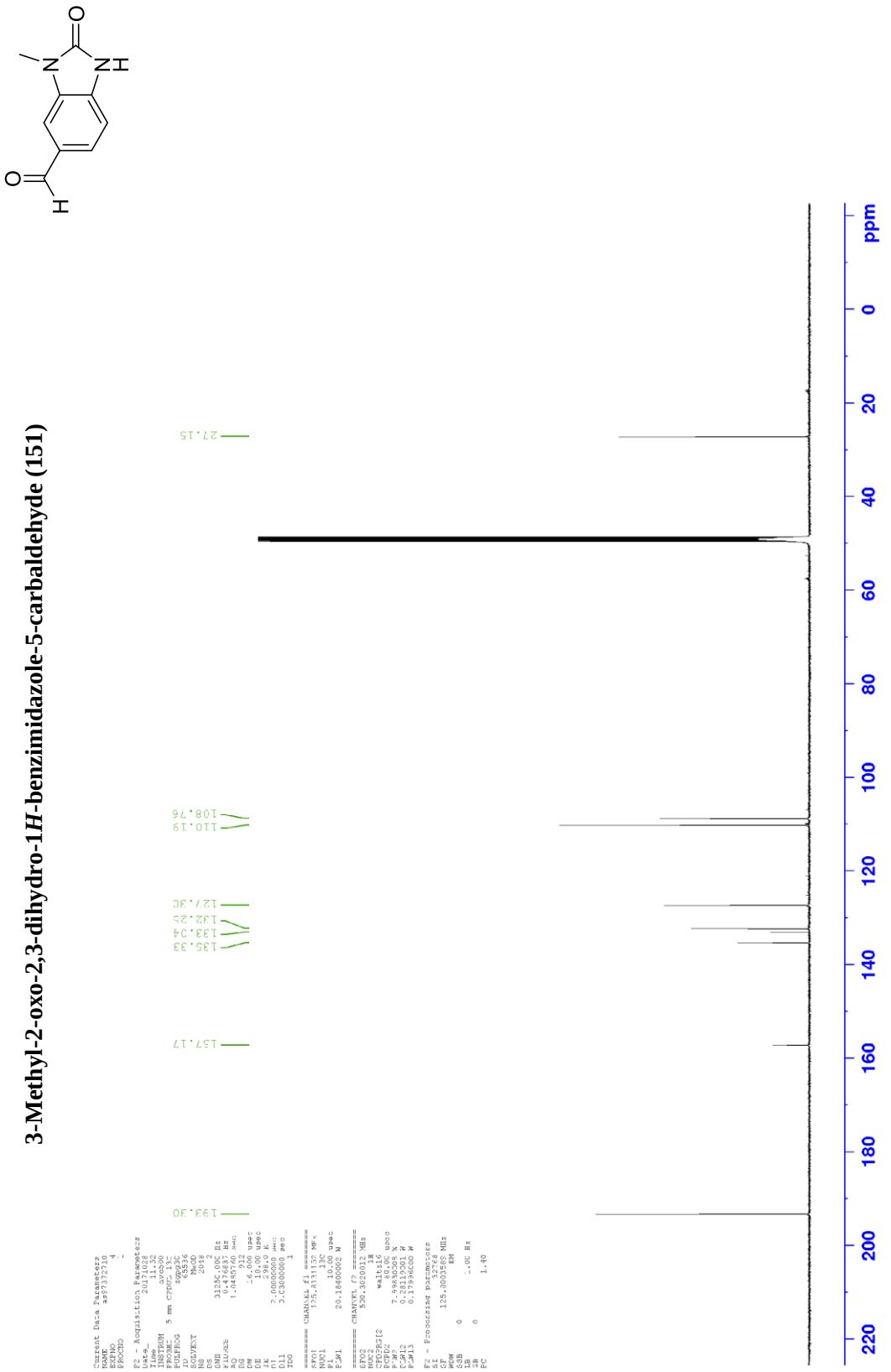


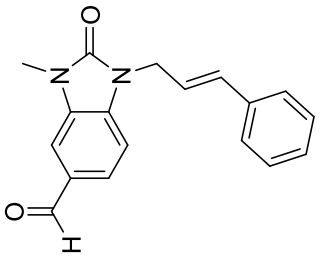


3-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (151)

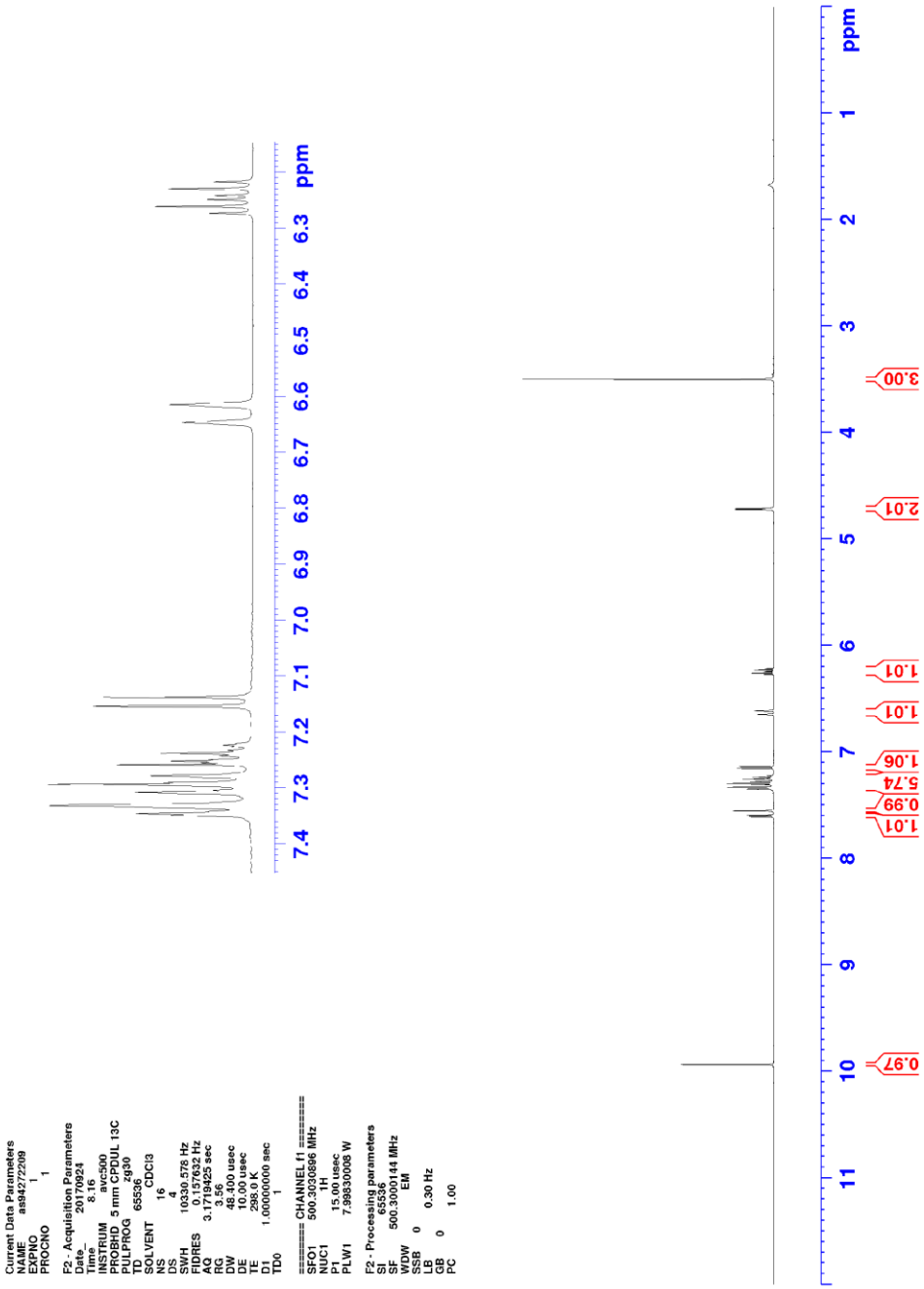


3-Methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (151)

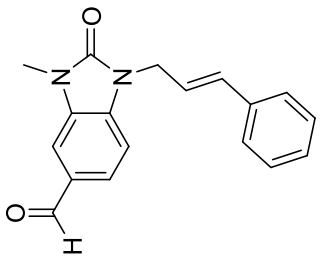


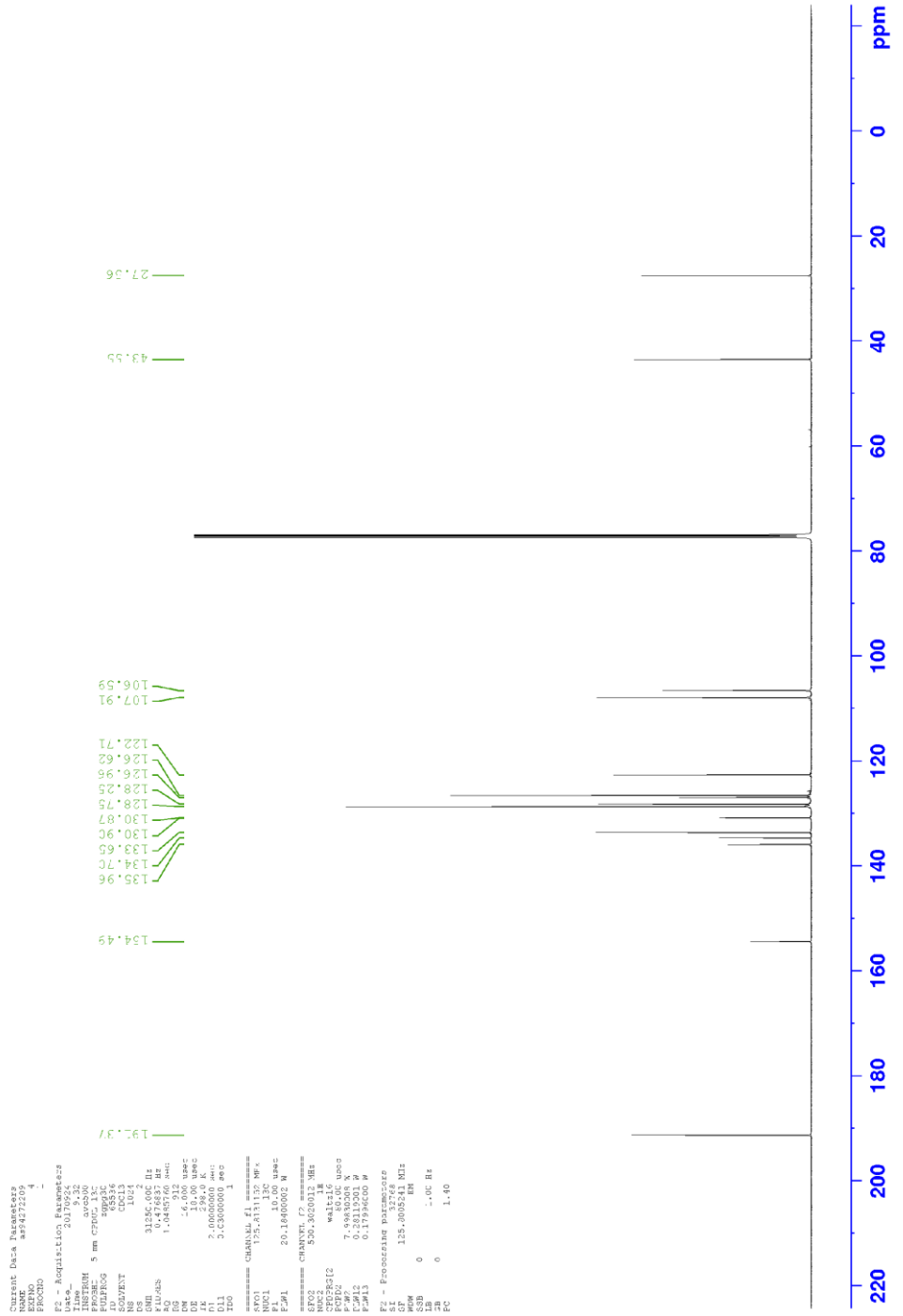


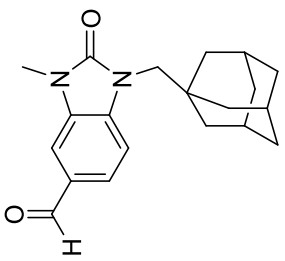
(*E*)-3-Methyl-2-oxo-1-[3-phenylprop-2-en-1-yl]-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (152)



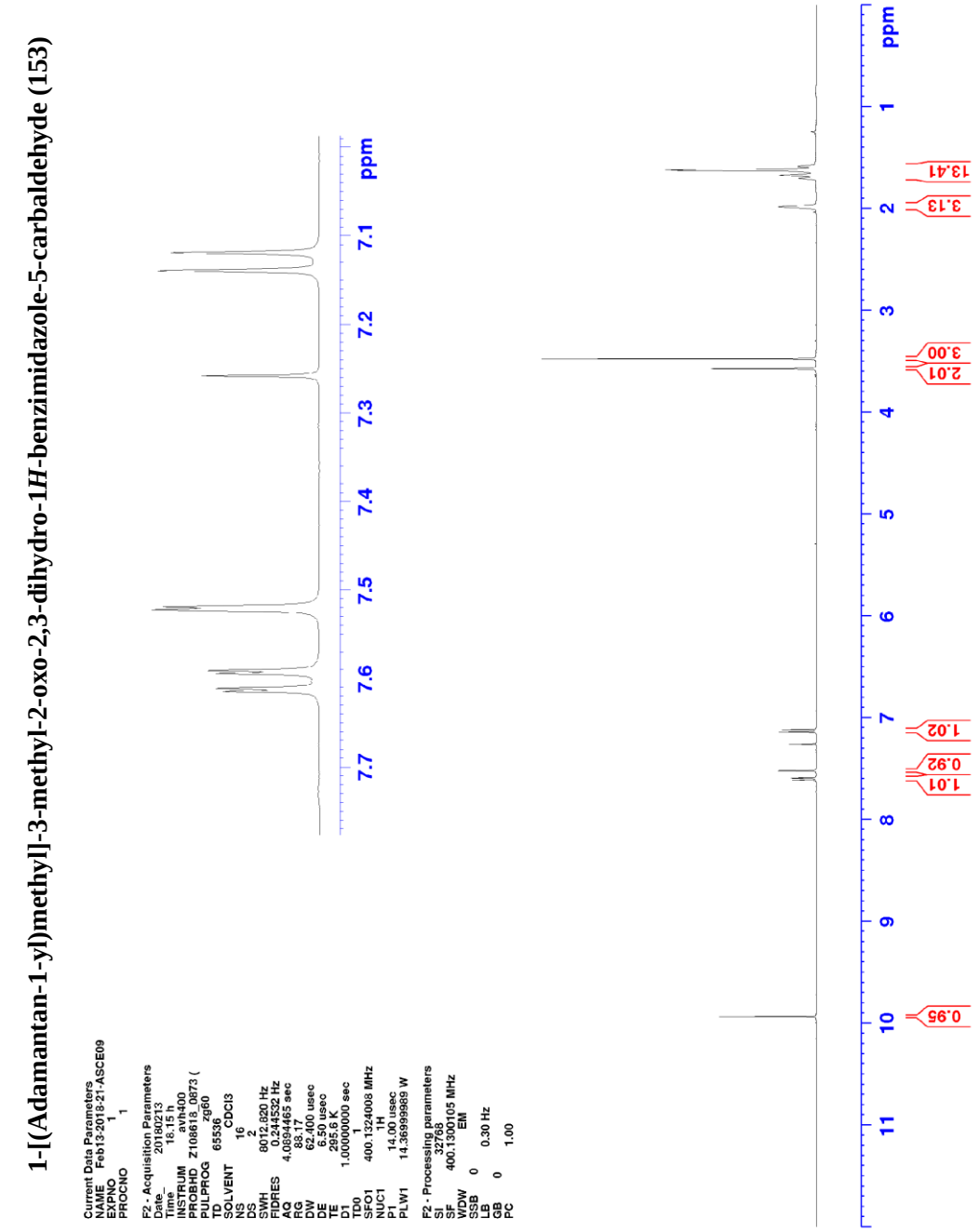
(*E*)-3-Methyl-2-oxo-1-[3-phenylprop-2-en-1-yl]-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (152)



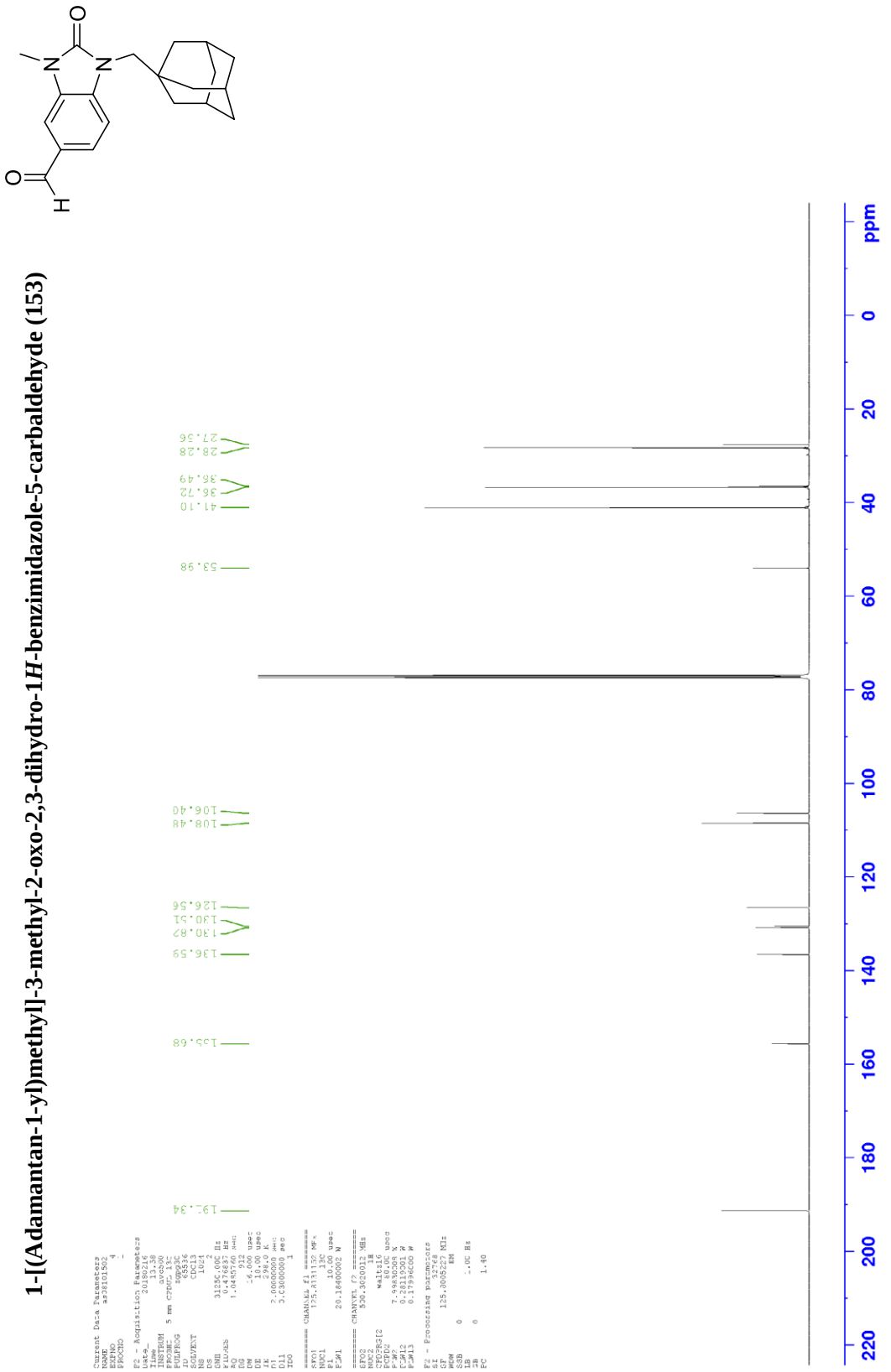




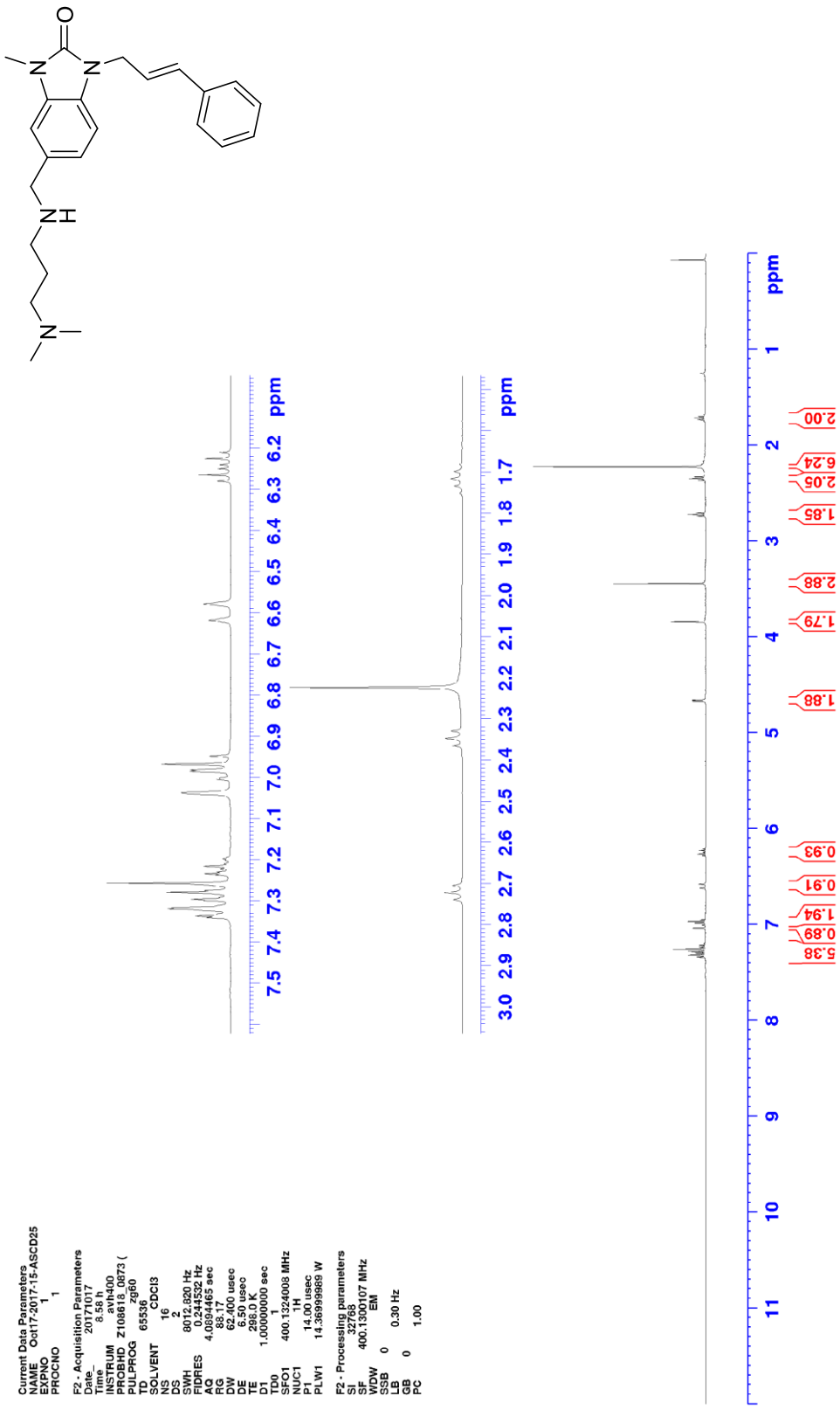
1-[(Adamantan-1-yl)methyl]-3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (153)



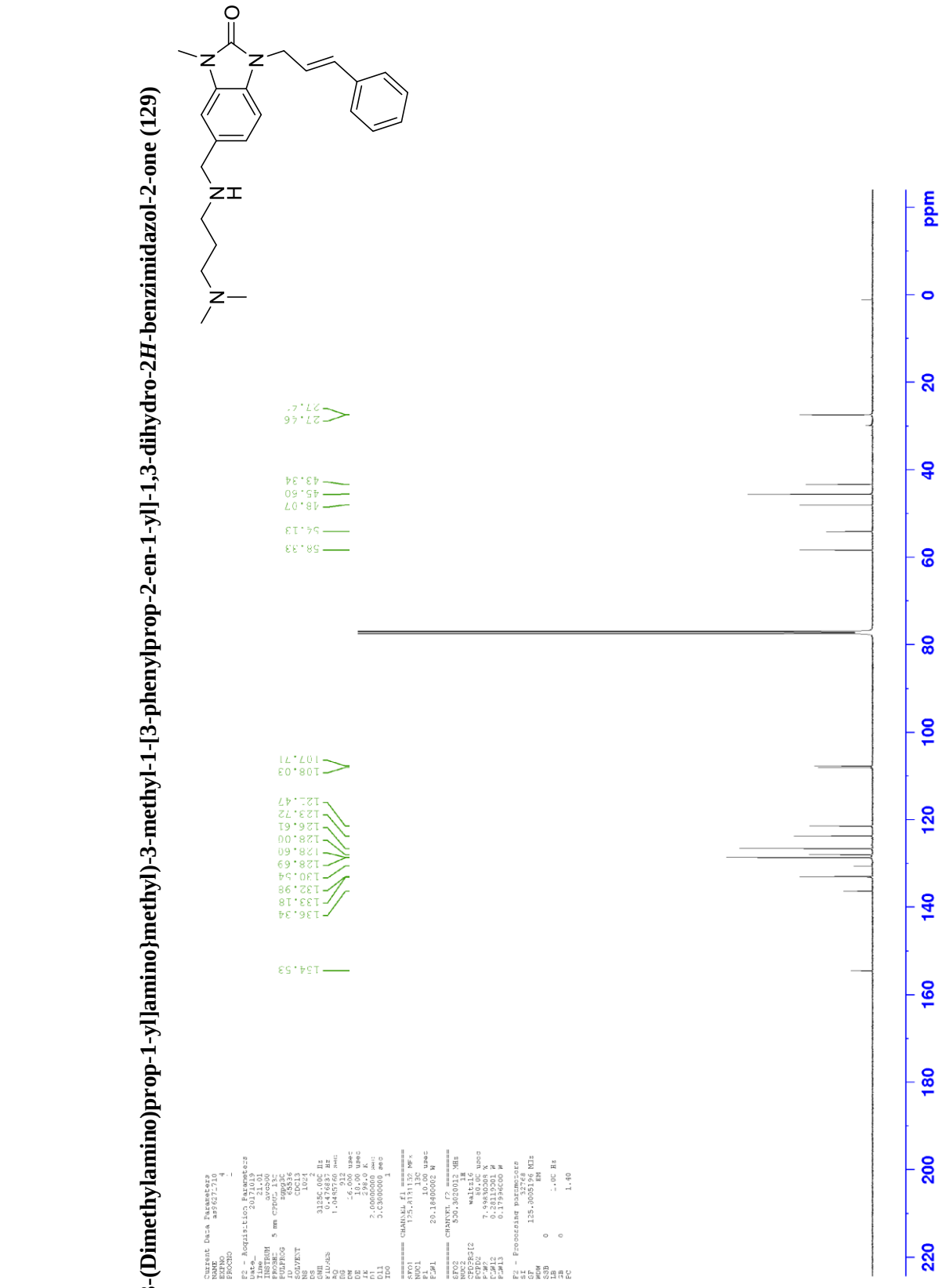
1-[(Adamantan-1-yl)methyl]-3-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (153)



(*E*)-5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-3-methyl-1-[3-phenylprop-2-en-1-yl]-1,3-dihydro-2*H*-benzimidazol-2-one (129)



(*E*)-5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-3-methyl-1-[3-phenylprop-2-en-1-yl]-1,3-dihydro-2*H*-benzimidazol-2-one (129)



1-[(Adamantan-1-yl)methyl]-5-({[3-(dimethylamino)prop-1-yl]amino}methyl)-3-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (130)

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PROCNO             1

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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 ========
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NUC1               1H
P1             15.00 usec
PLW1       7.99830008 W

F2 - Processing parameters
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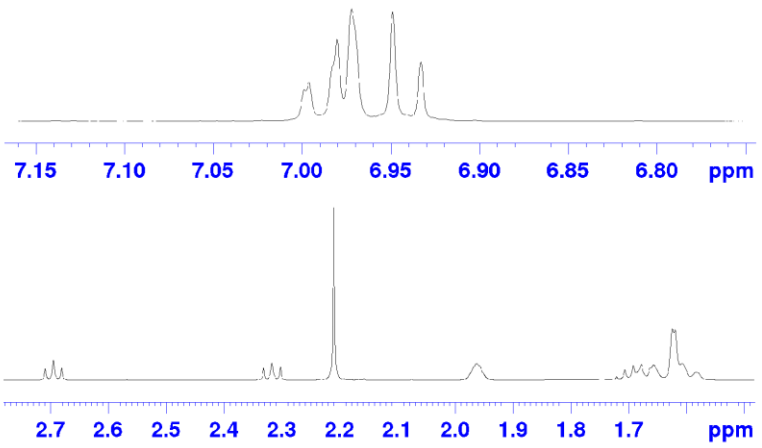
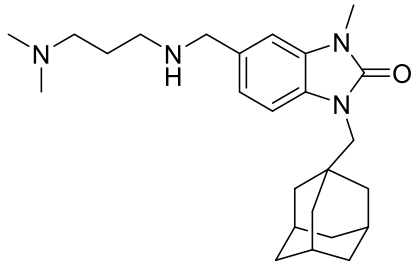
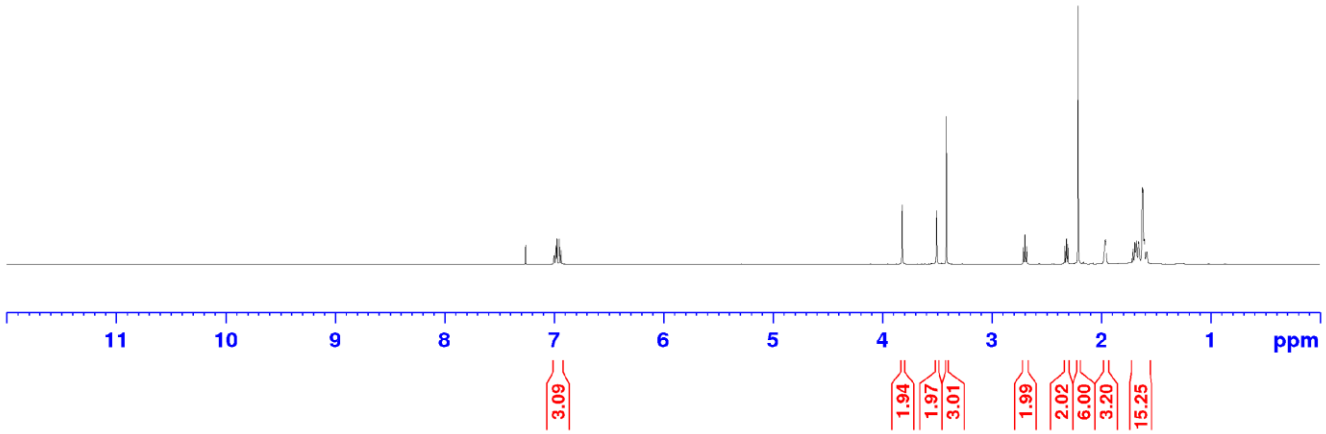
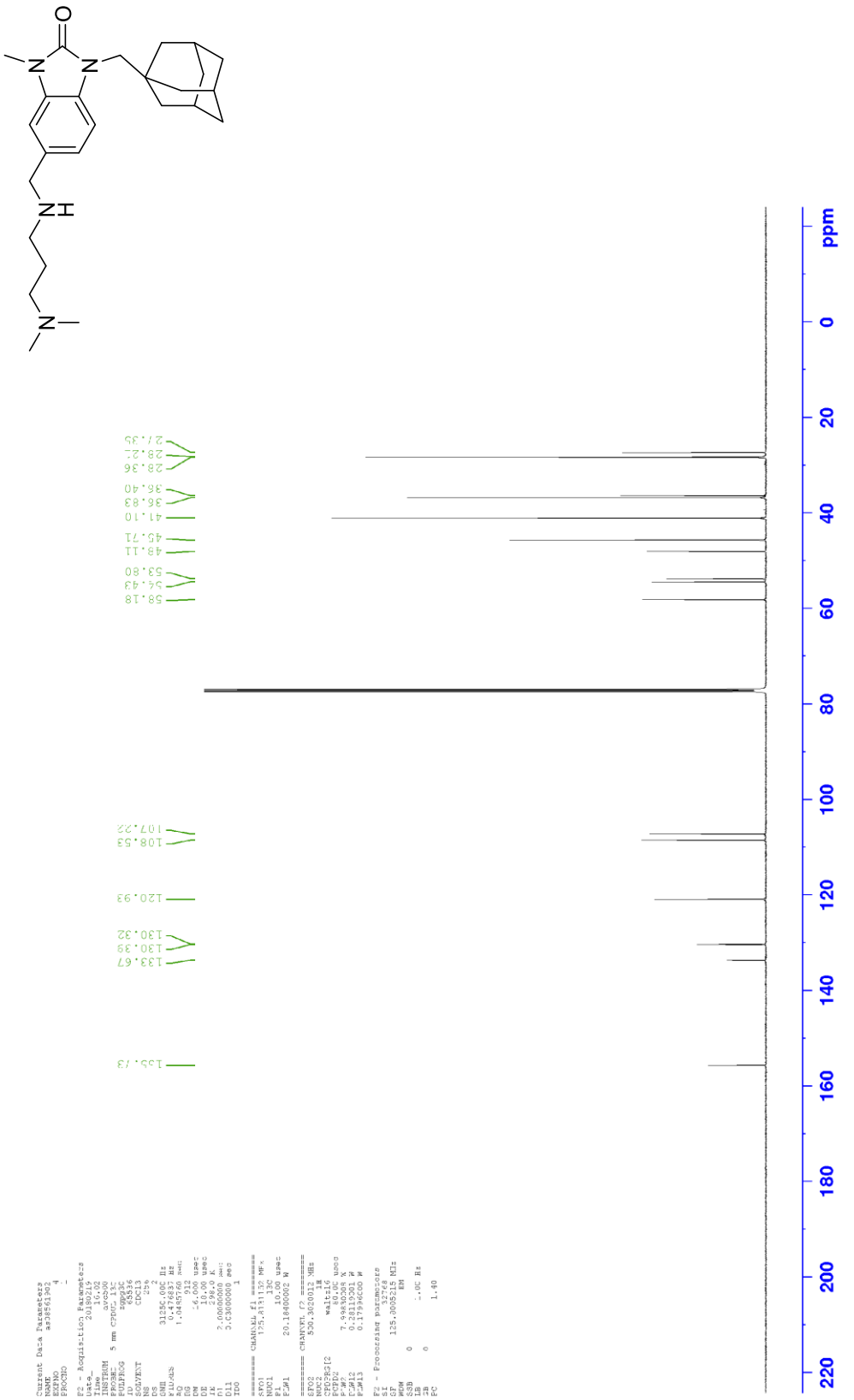


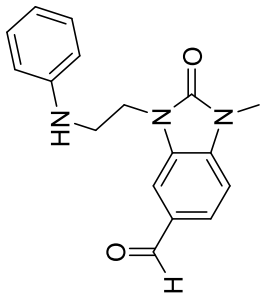


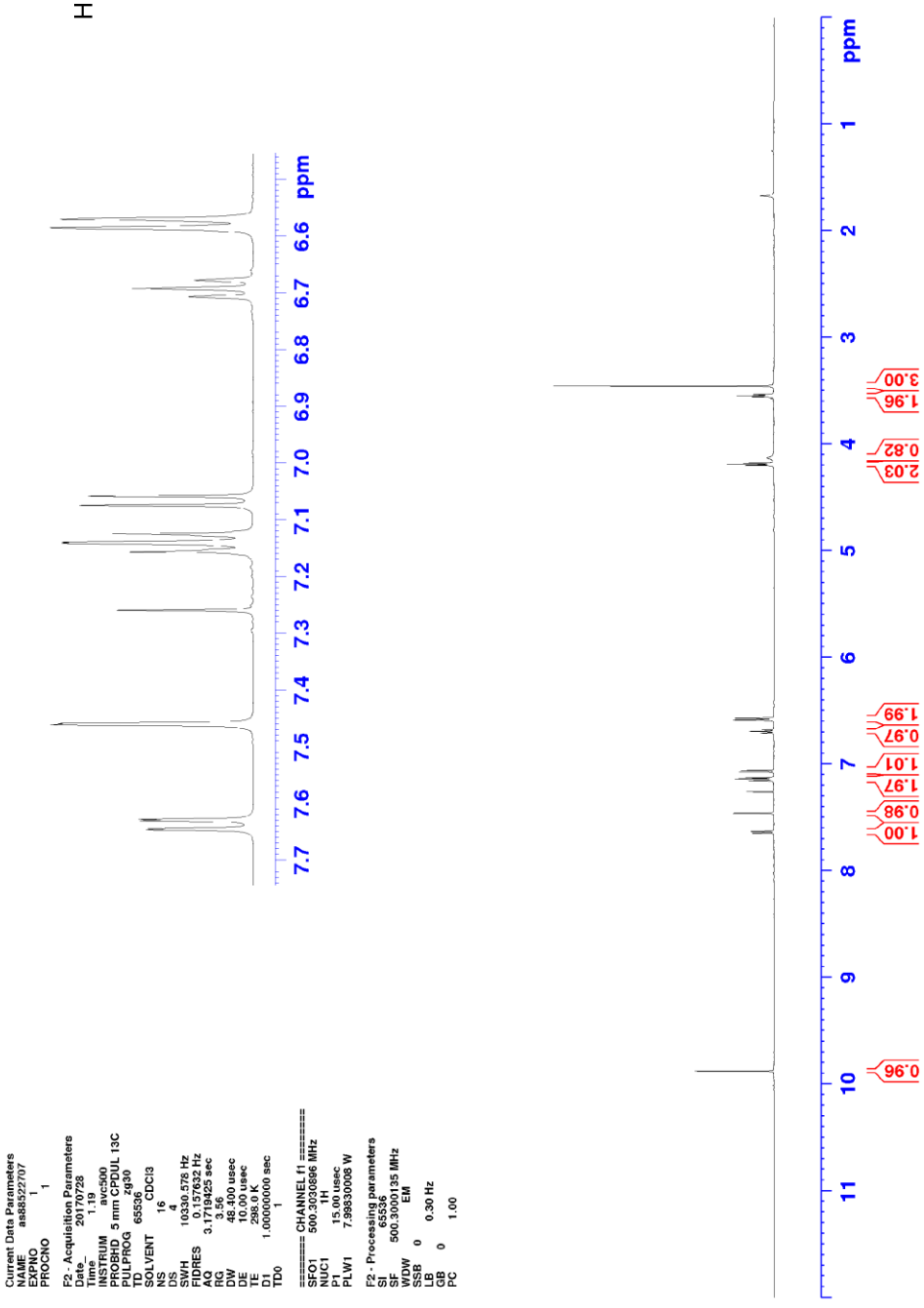


1-[(Adamantan-1-yl)methyl]-5-({[3-(dimethylamino)prop-1-yl]amino}methyl)-3-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (130)

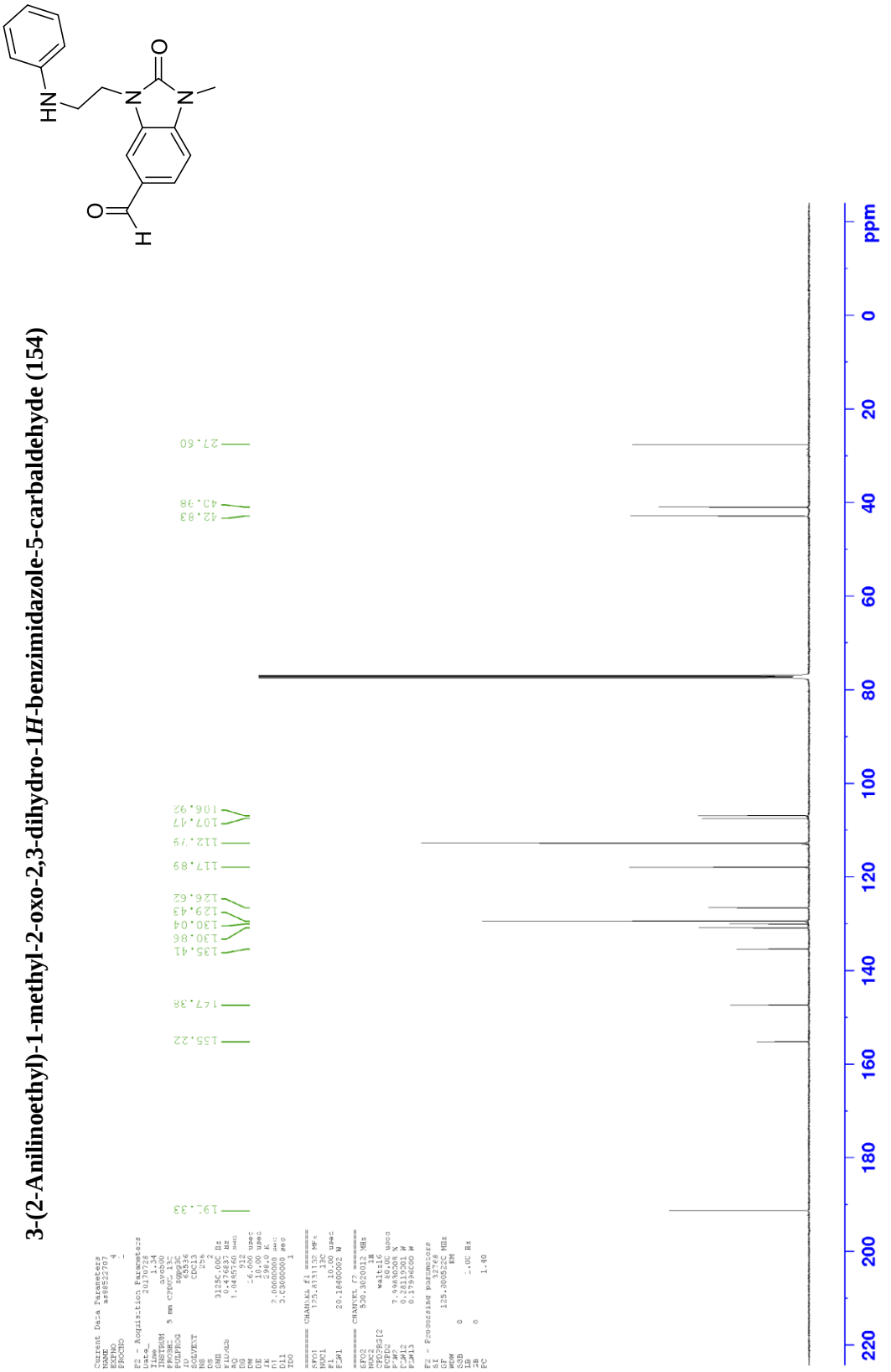


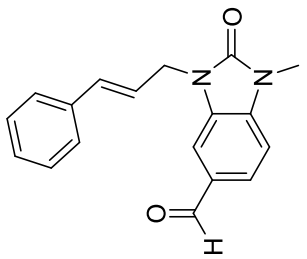
3-(2-Anilinoethyl)-1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (154)



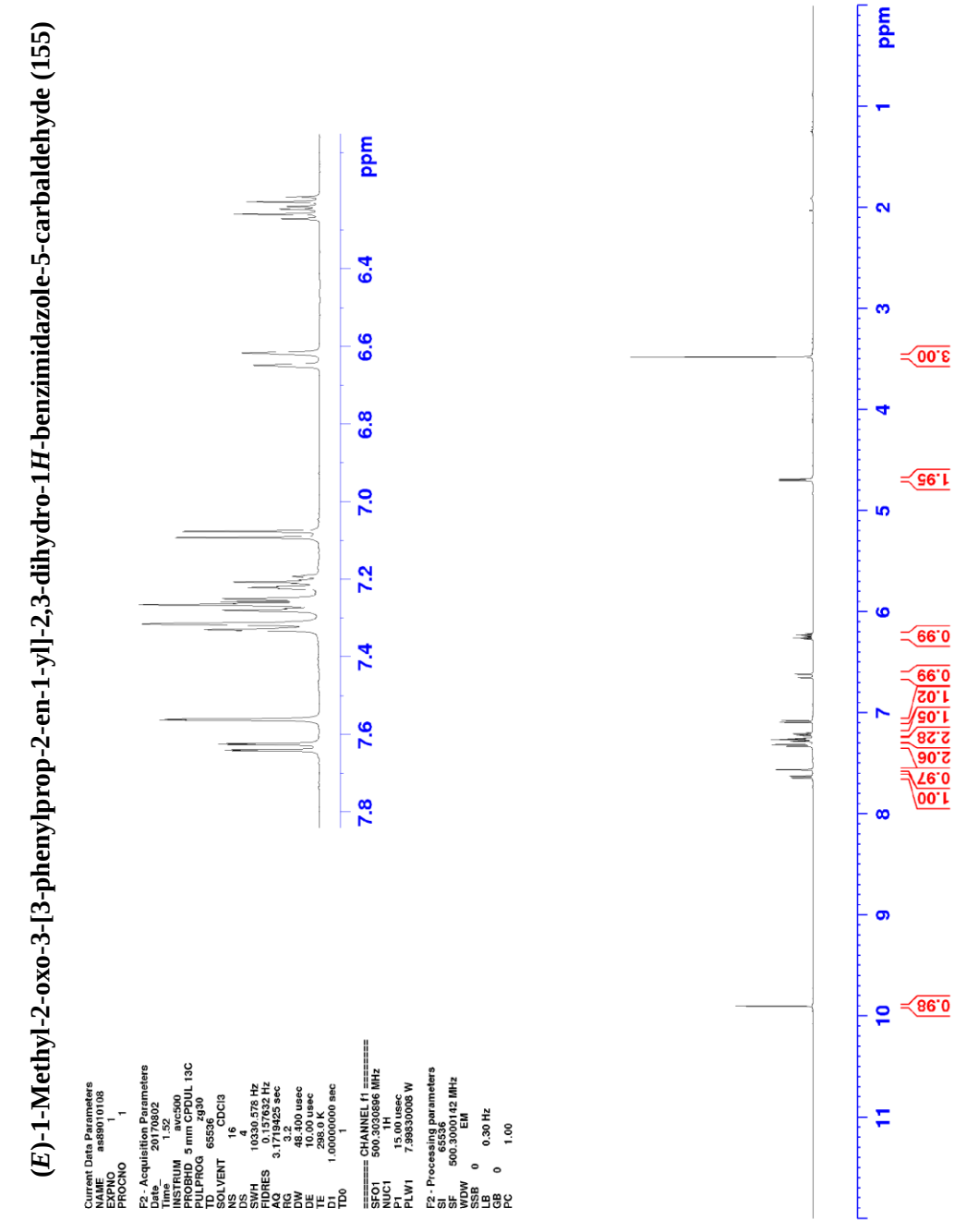


3-(2-Anilinoethyl)-1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (154)

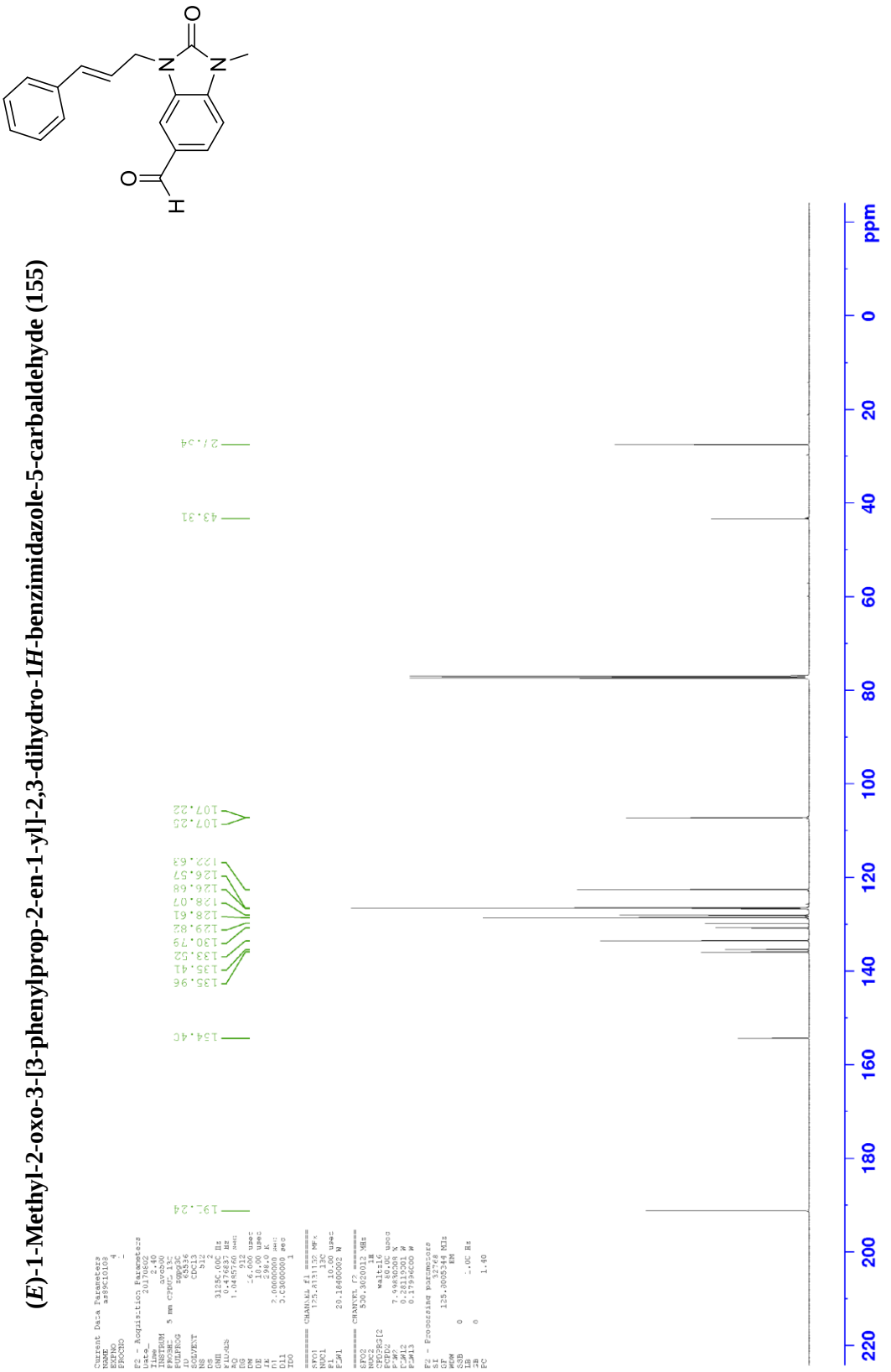


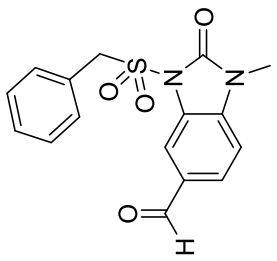


(*E*)-1-Methyl-2-oxo-3-[3-phenylprop-2-en-1-yl]-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (155)

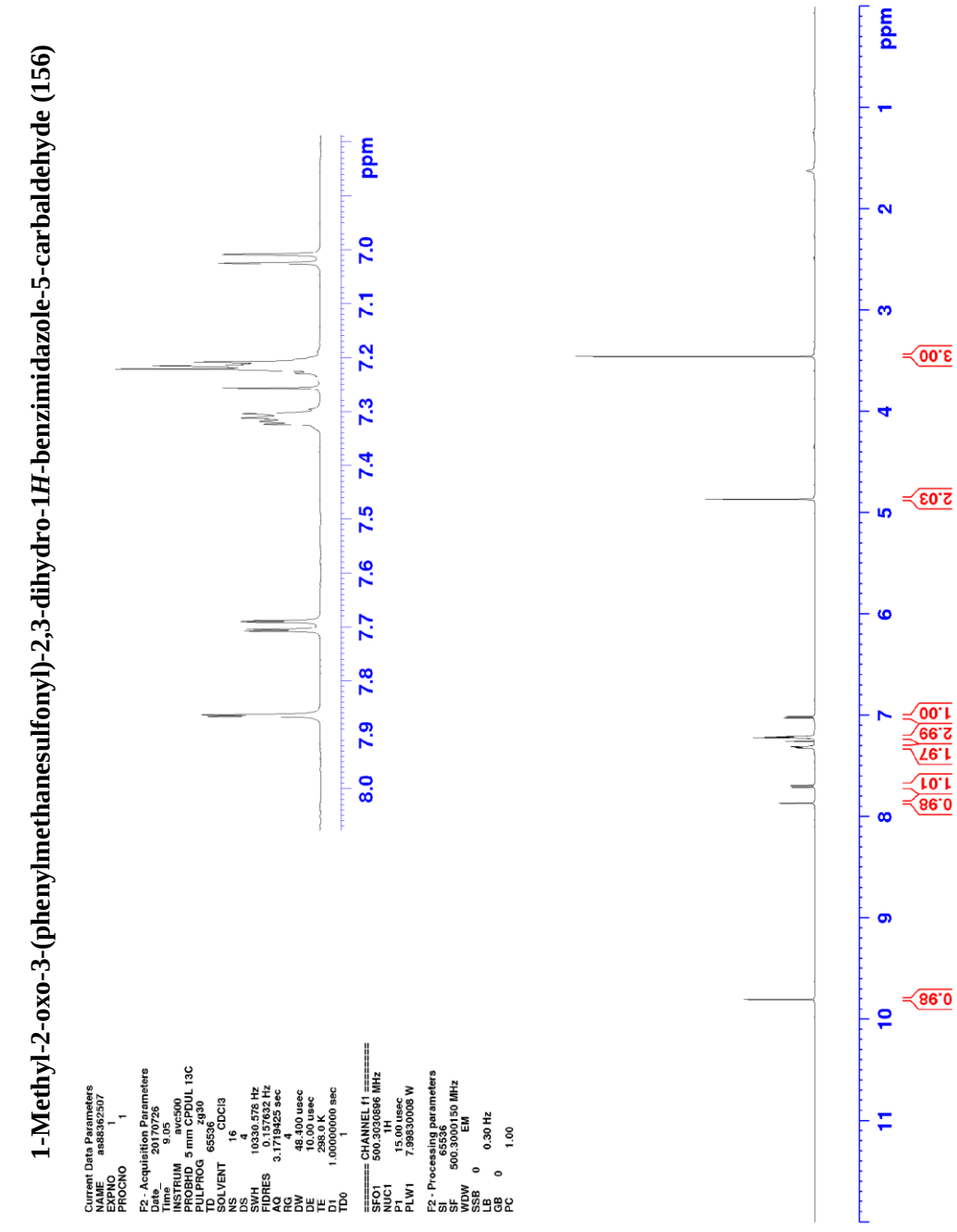


(*E*)-1-Methyl-2-oxo-3-[3-phenylprop-2-en-1-yl]-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (155)

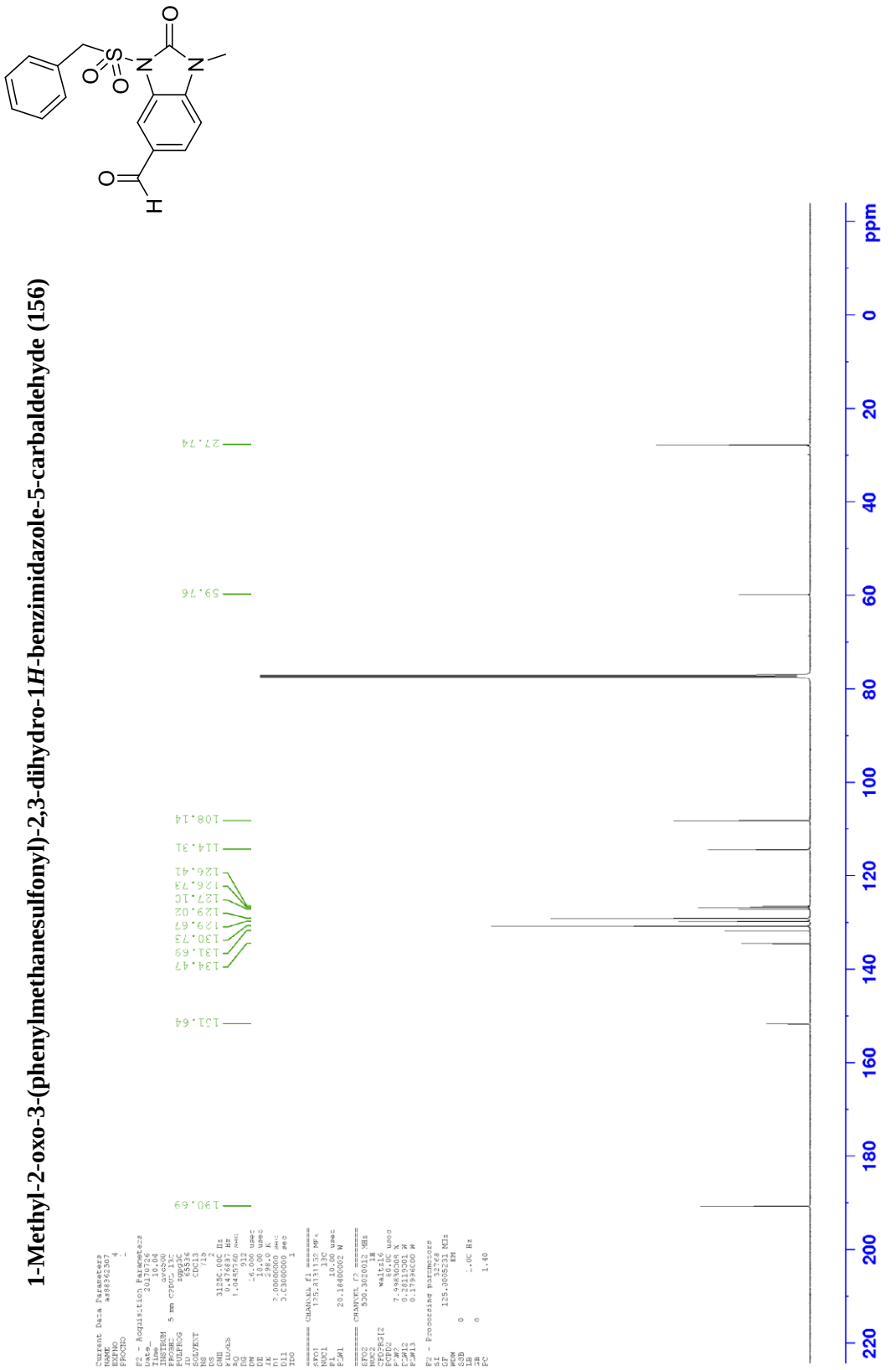




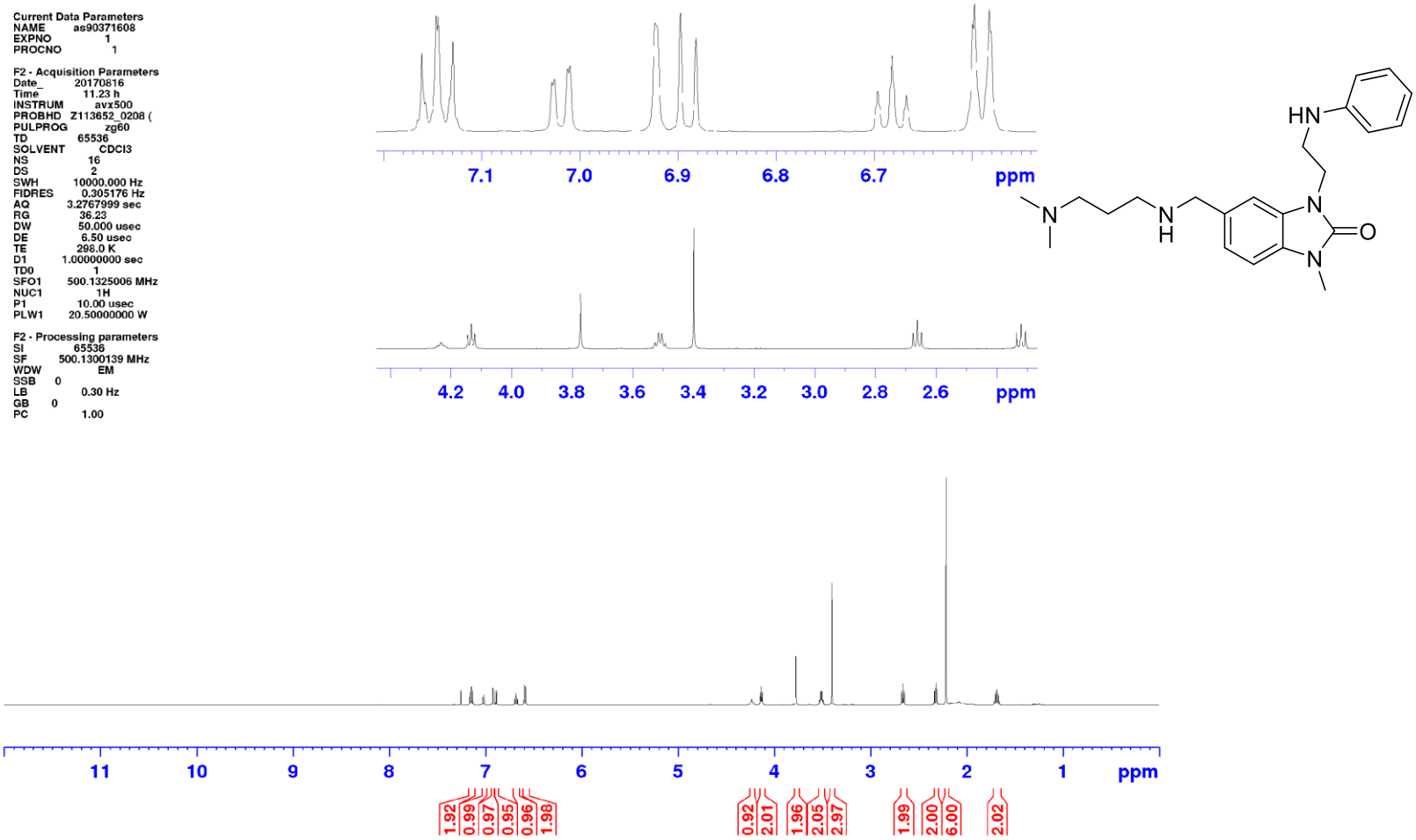
1-Methyl-2-oxo-3-(phenylmethanesulfonyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (156)



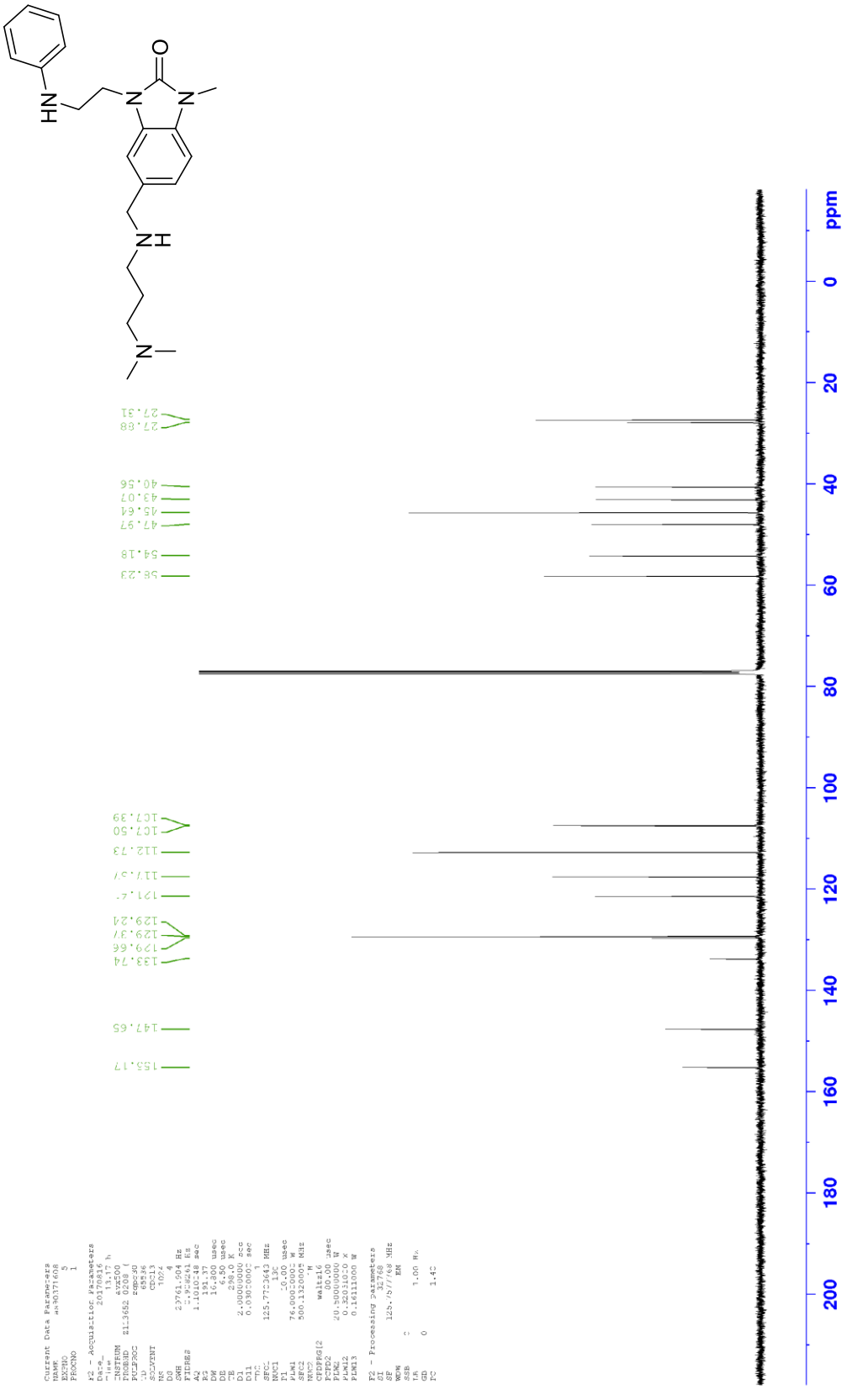
1-Methyl-2-oxo-3-(phenylmethanesulfonyl)-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (156)



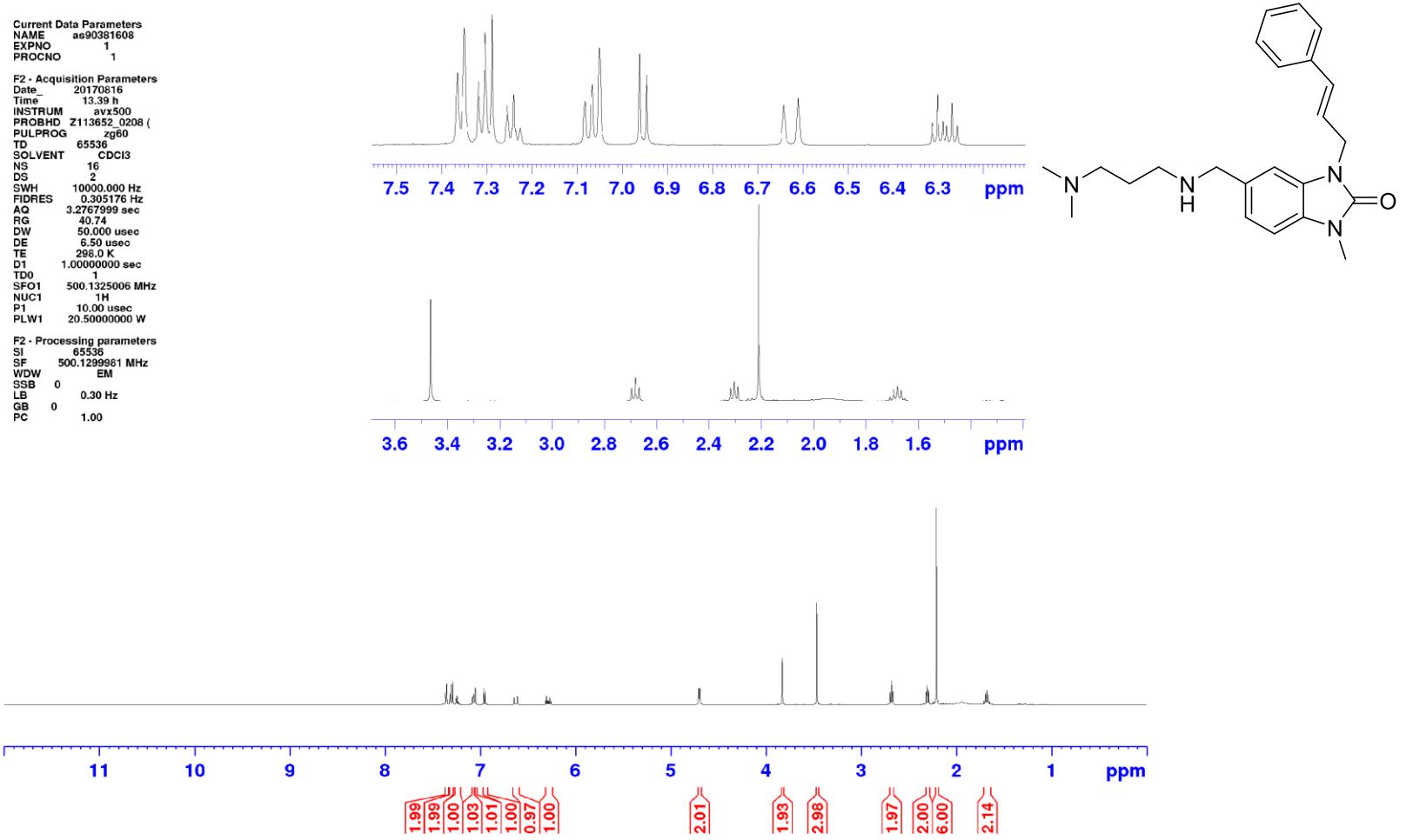
3-(2-Anilinoethyl)-5-({[3-(dimethylamino)prop-1-yl]amino}methyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (141)



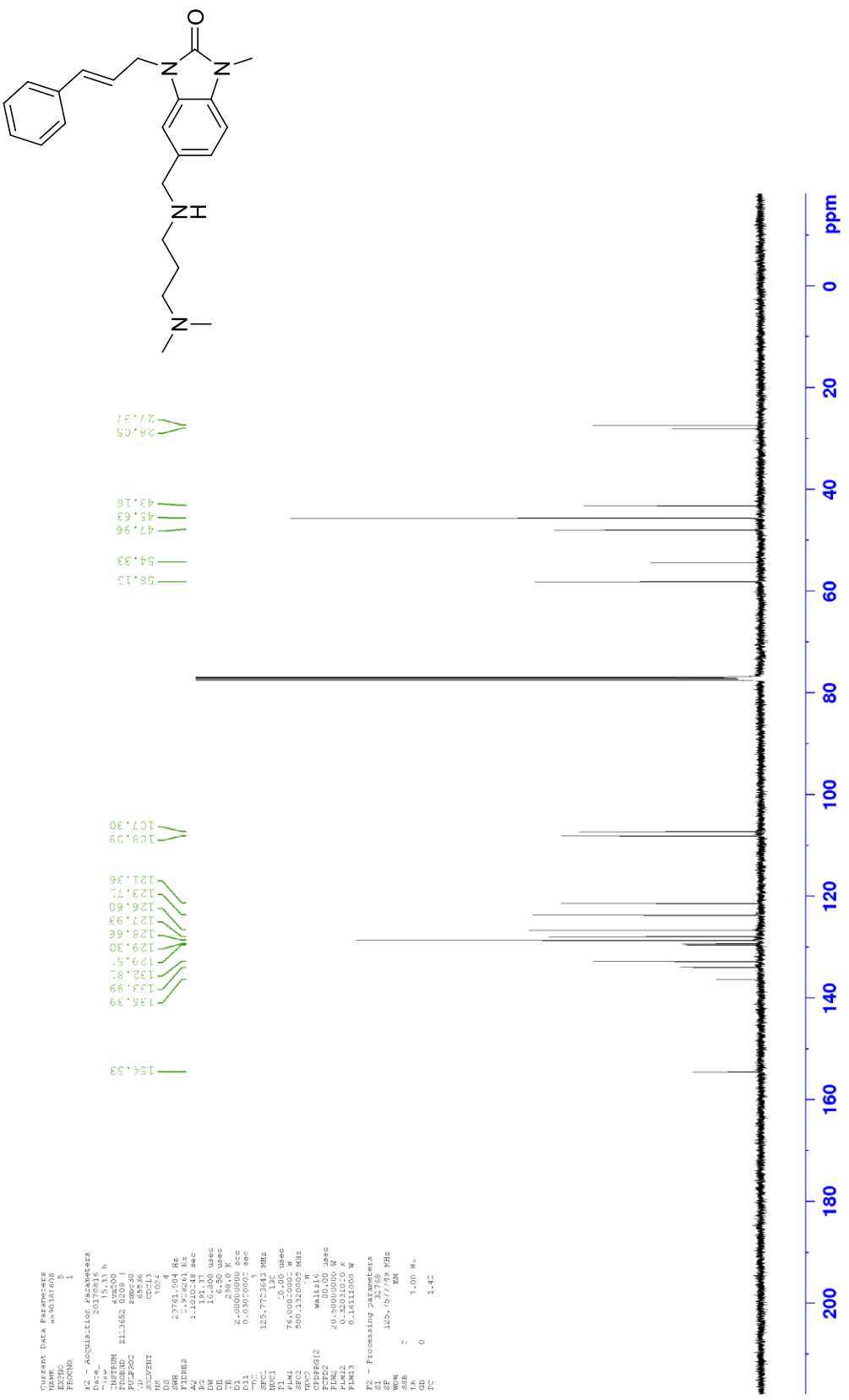
3-(2-Anilinoethyl)-5-({[3-(dimethylamino)prop-1-yl]amino}methyl)-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one (141)



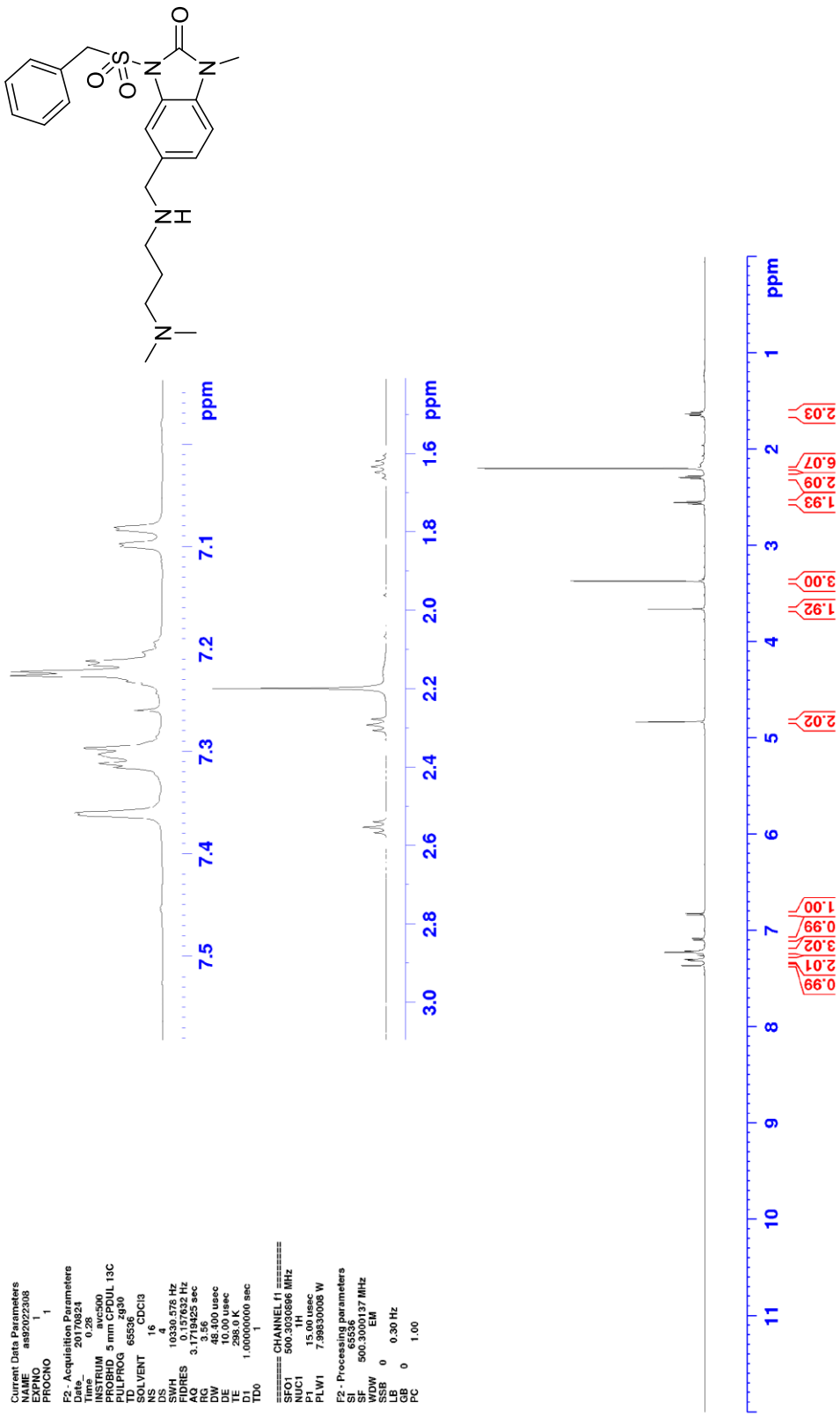
(*E*)-5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-3-[3-phenylprop-2-en-1-yl]-1,3-dihydro-2*H*-benzimidazol-2-one (142)



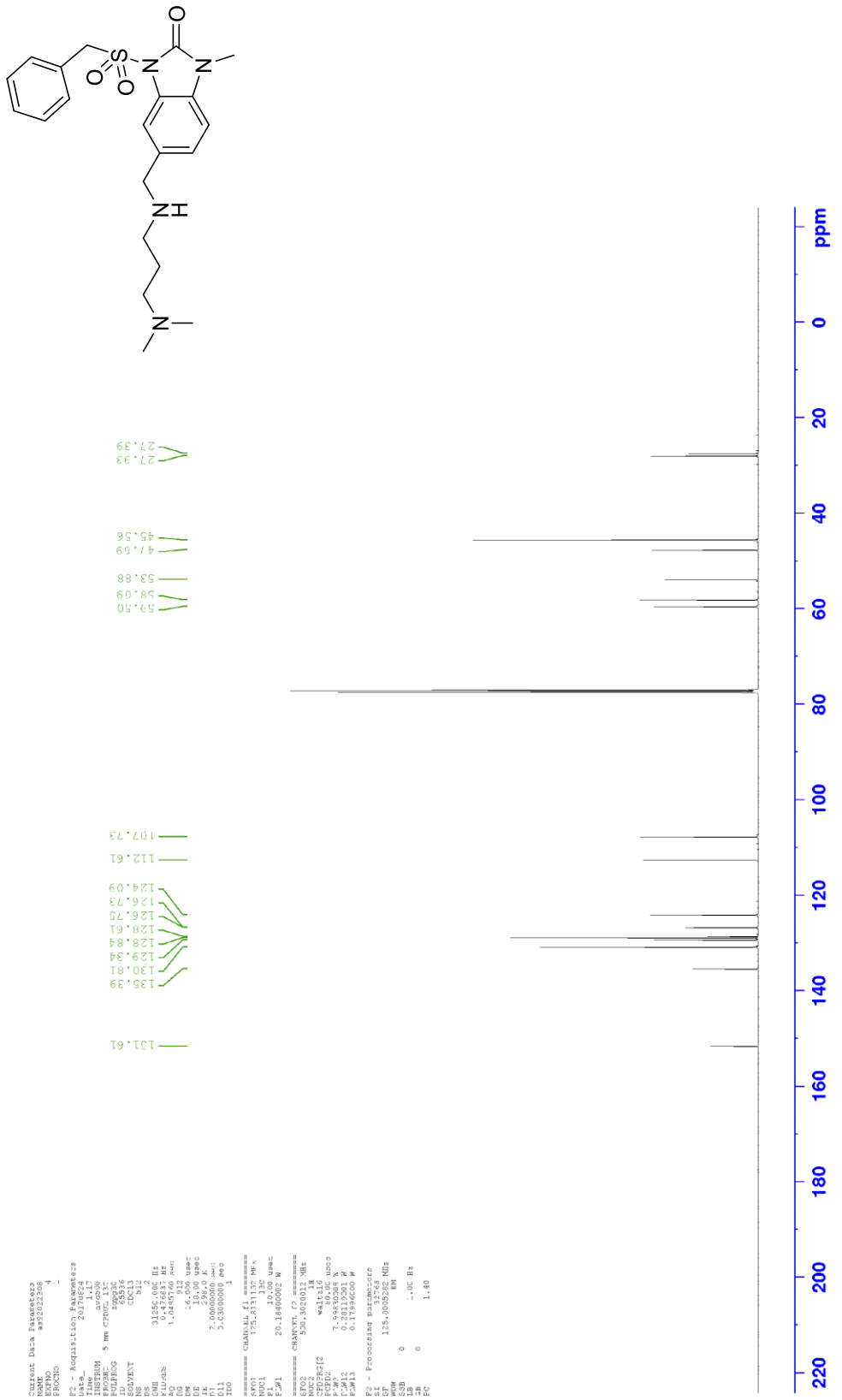
(*E*)-5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-3-[3-phenylprop-2-en-1-yl]-1,3-dihydro-2*H*-benzimidazol-2-one (142)

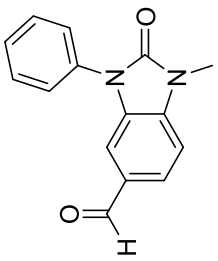


5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-3-(phenylmethanesulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (144)

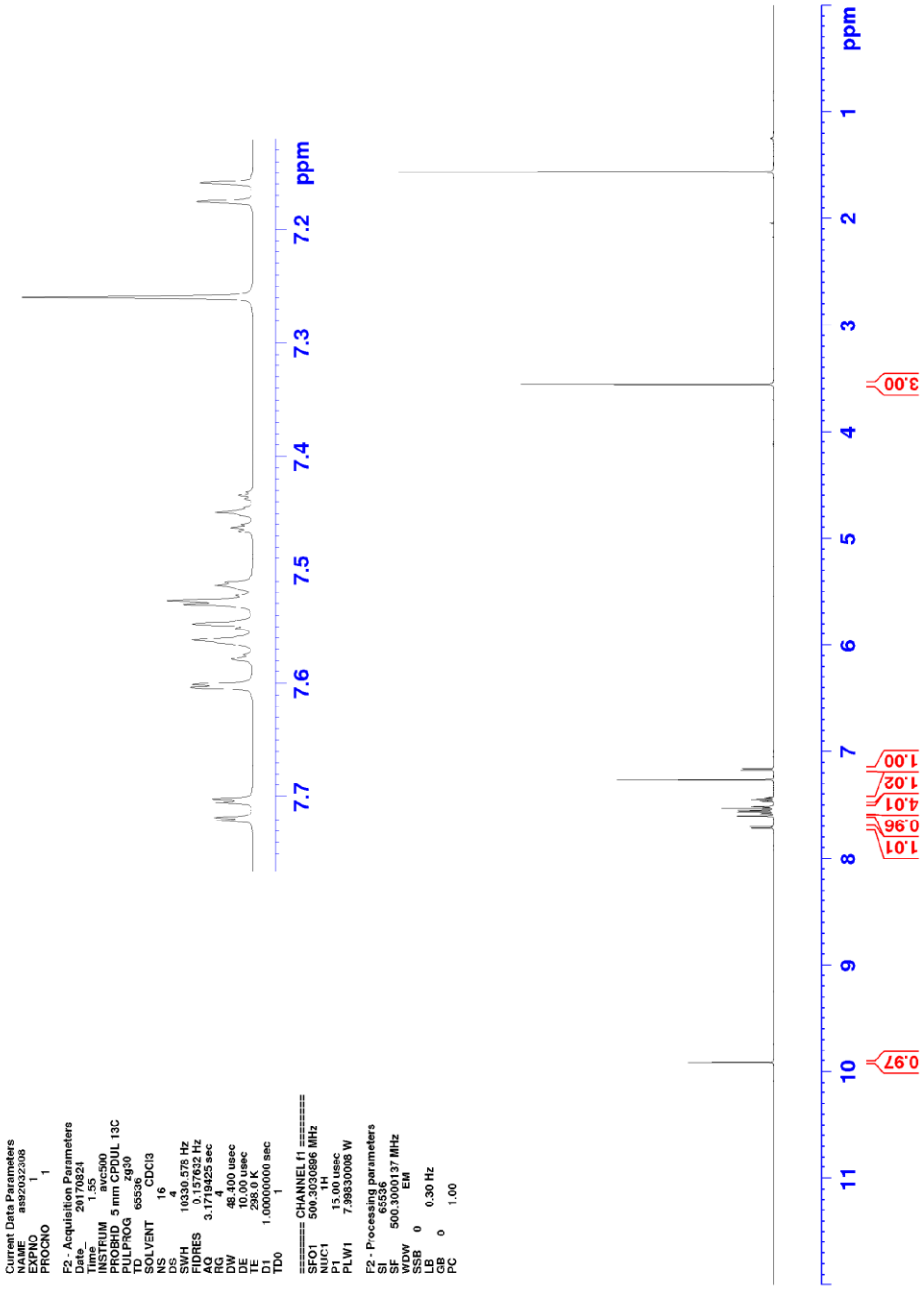


5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-3-(phenylmethanesulfonyl)-1,3-dihydro-2*H*-benzimidazol-2-one (144)

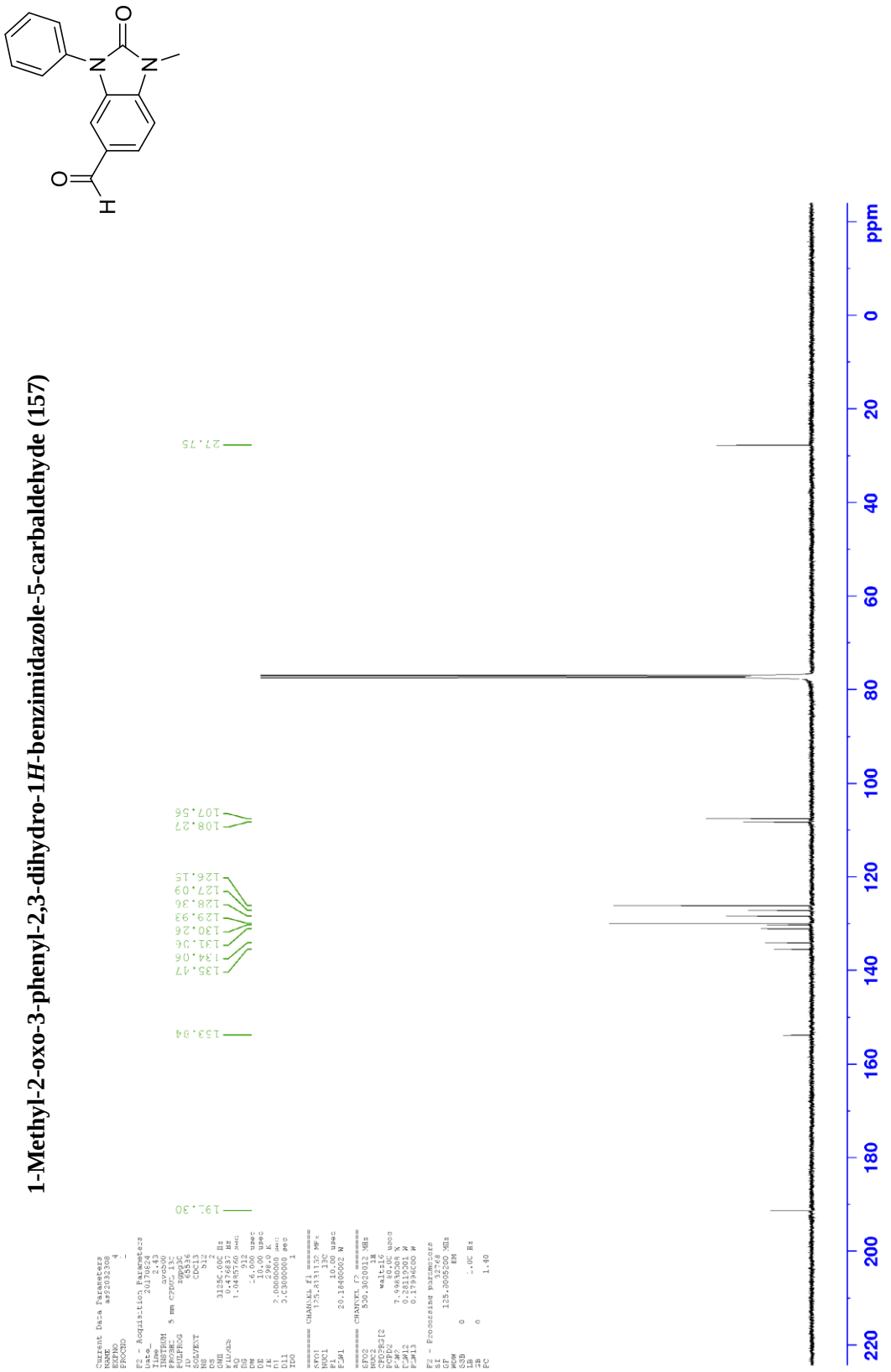




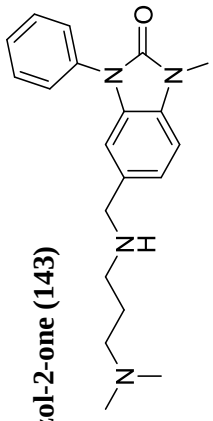
1-Methyl-2-oxo-3-phenyl-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (157)

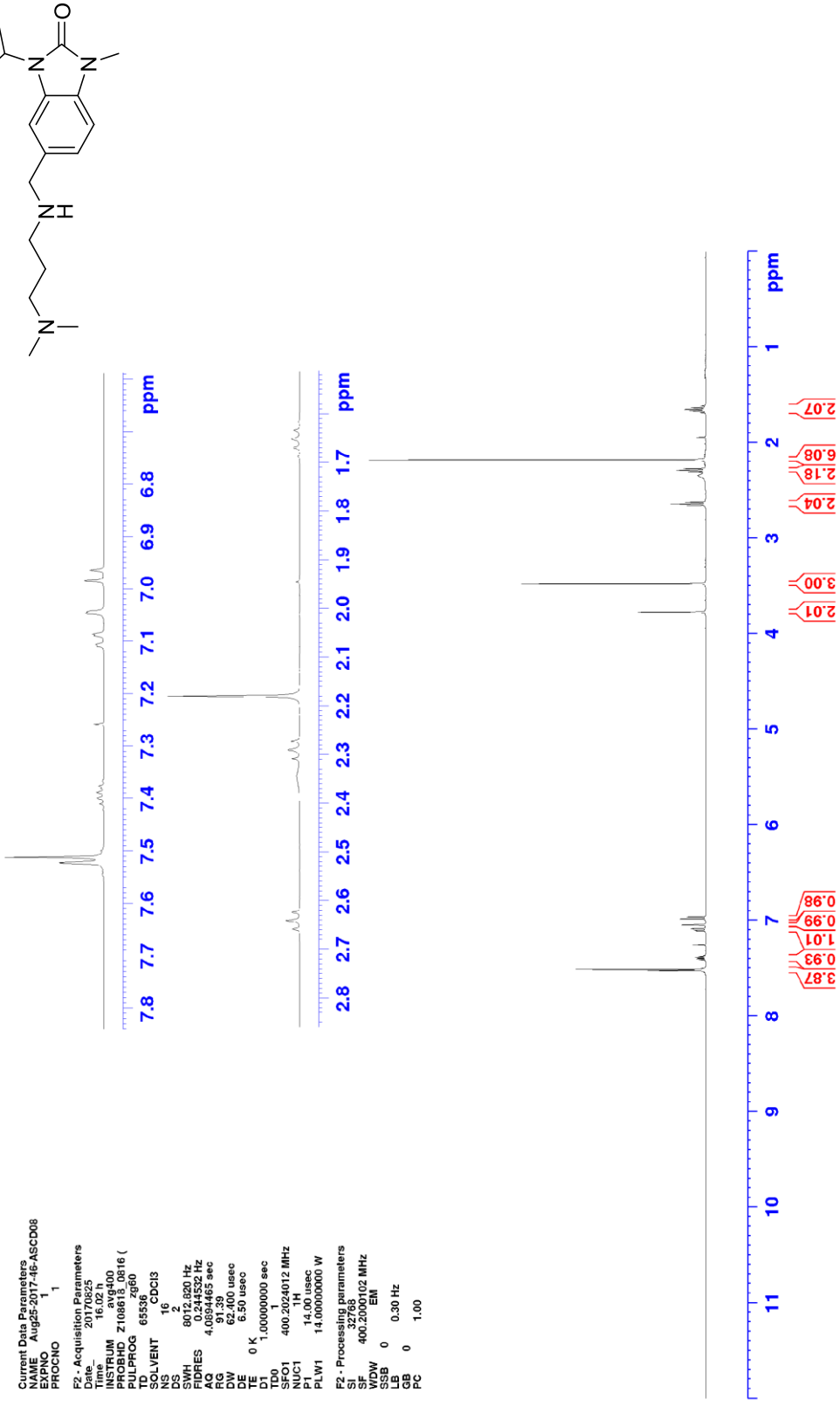


1-Methyl-2-oxo-3-phenyl-2,3-dihydro-1*H*-benzimidazole-5-carbaldehyde (157)

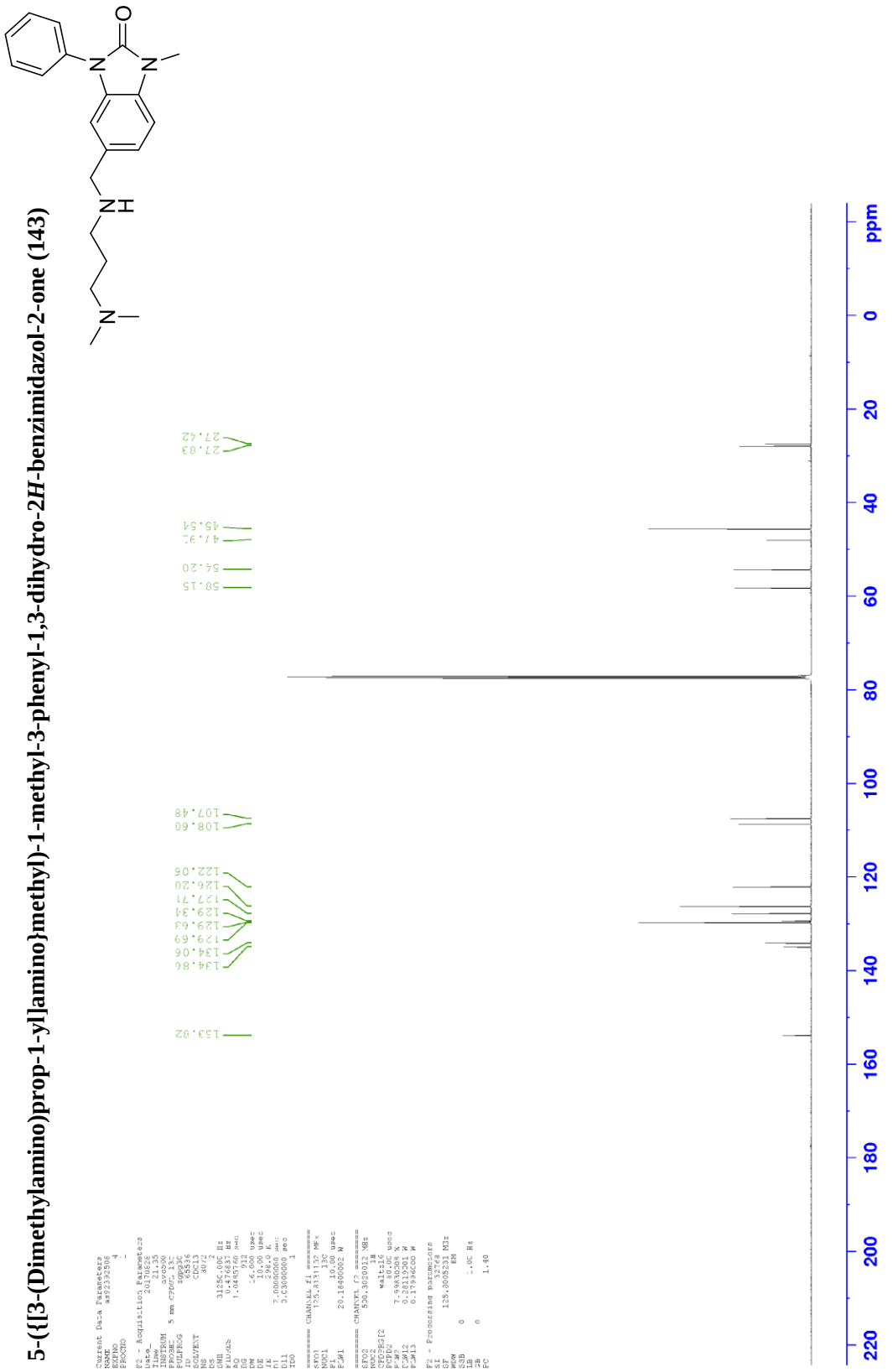


5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-3-phenyl-1,3-dihydro-2*H*-benzimidazol-2-one (143)

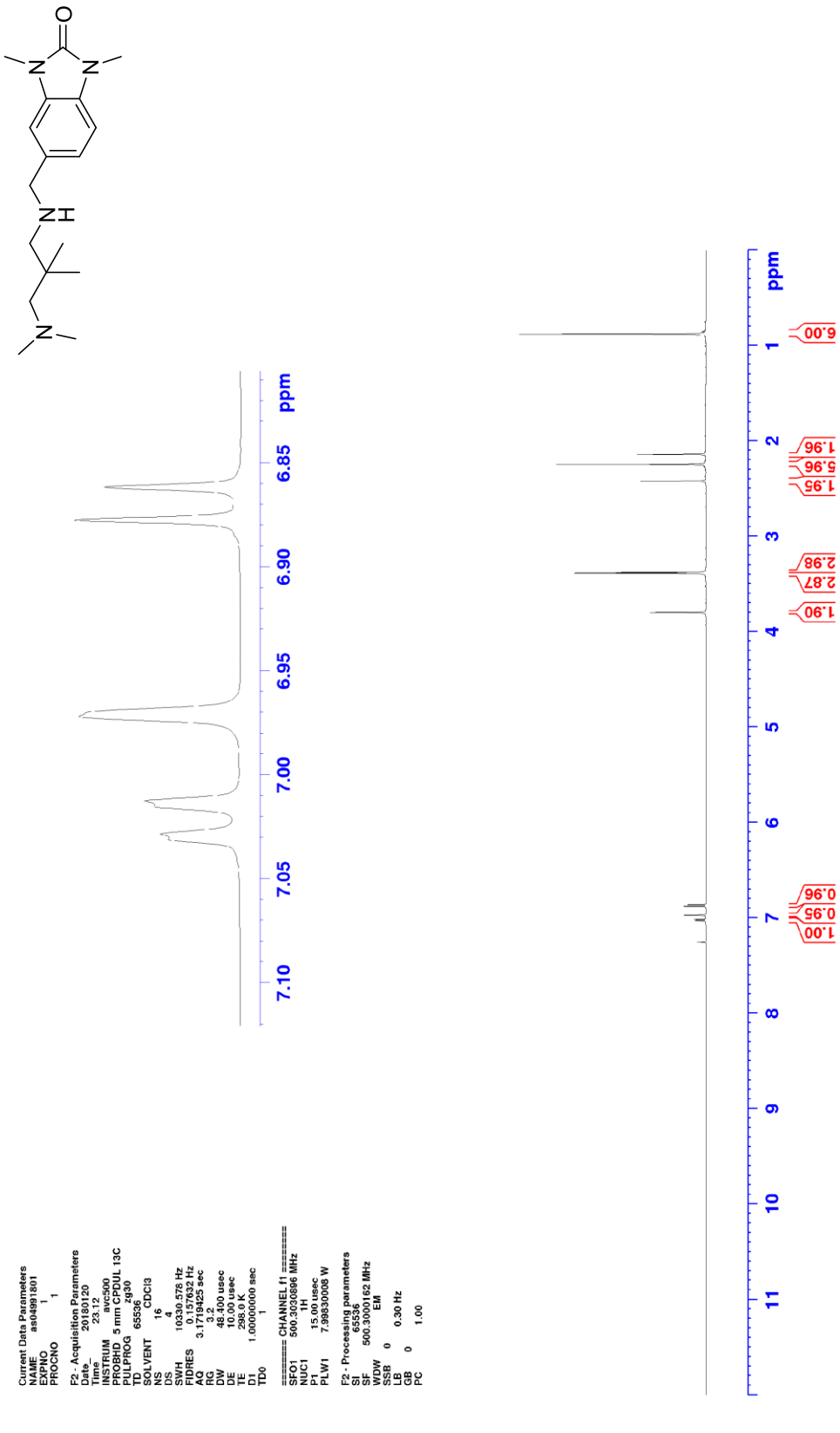




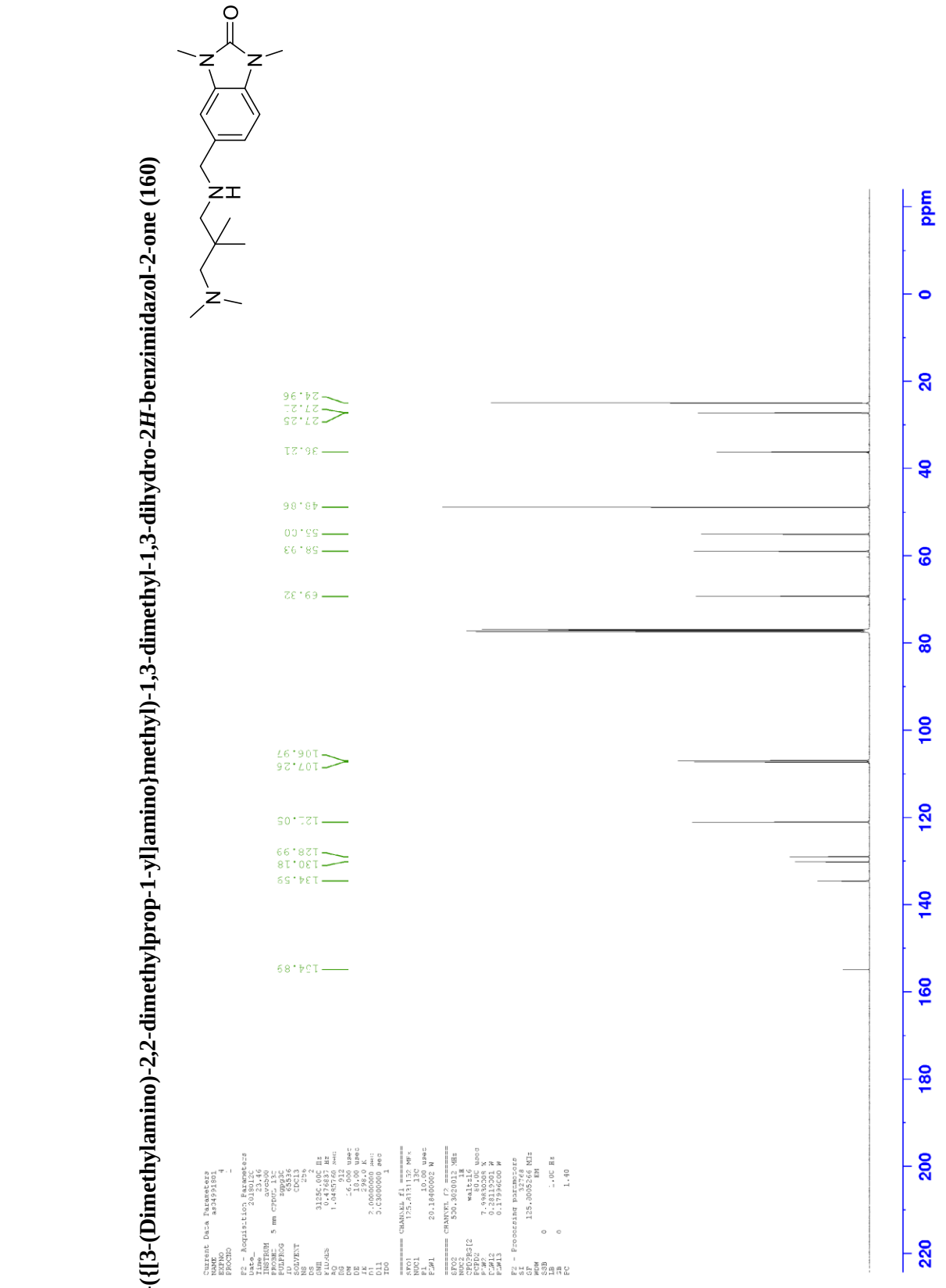
5-({[3-(Dimethylamino)prop-1-yl]amino}methyl)-1-methyl-3-phenyl-1,3-dihydro-2*H*-benzimidazol-2-one (143)



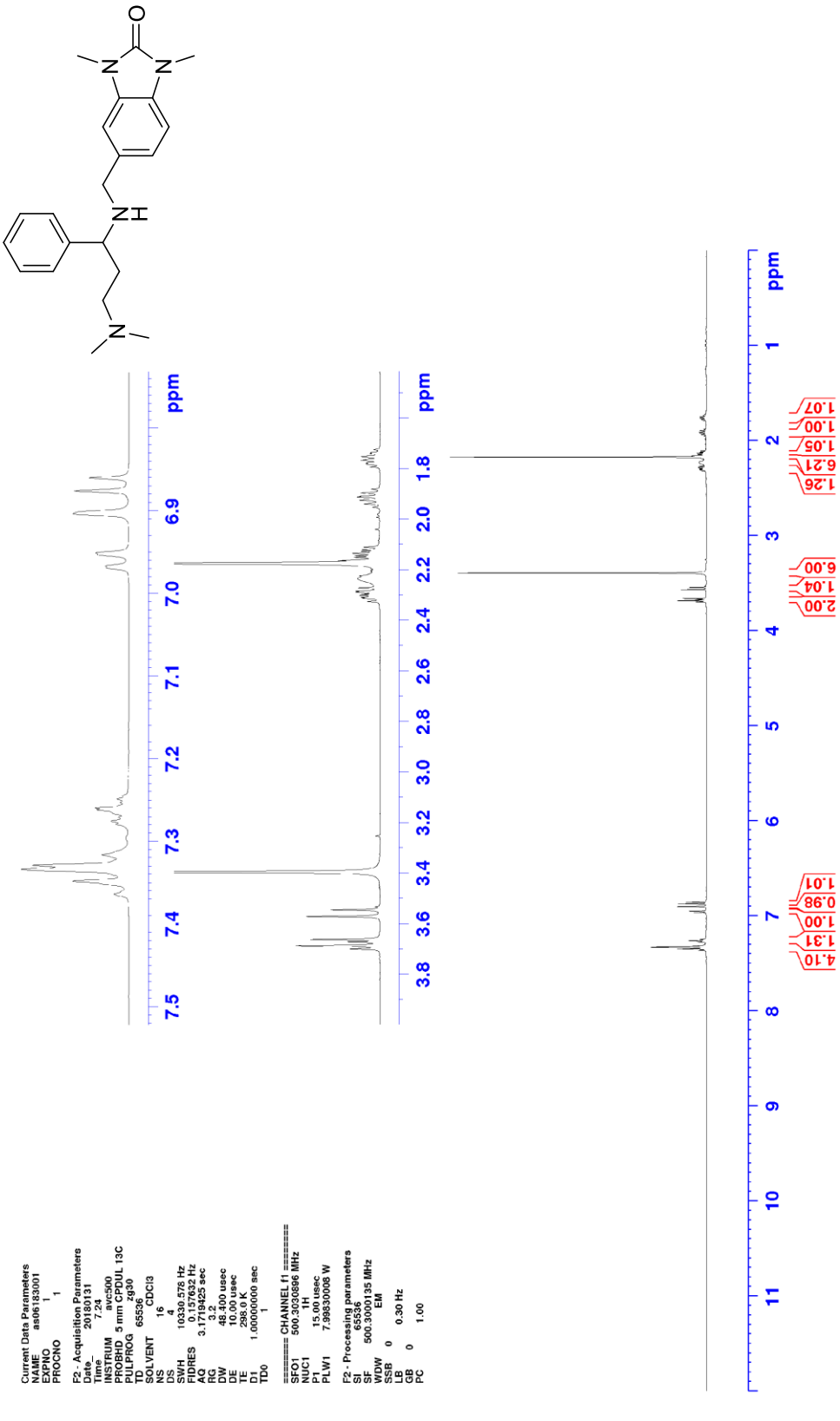
5-({[3-(Dimethylamino)-2,2-dimethylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (160)



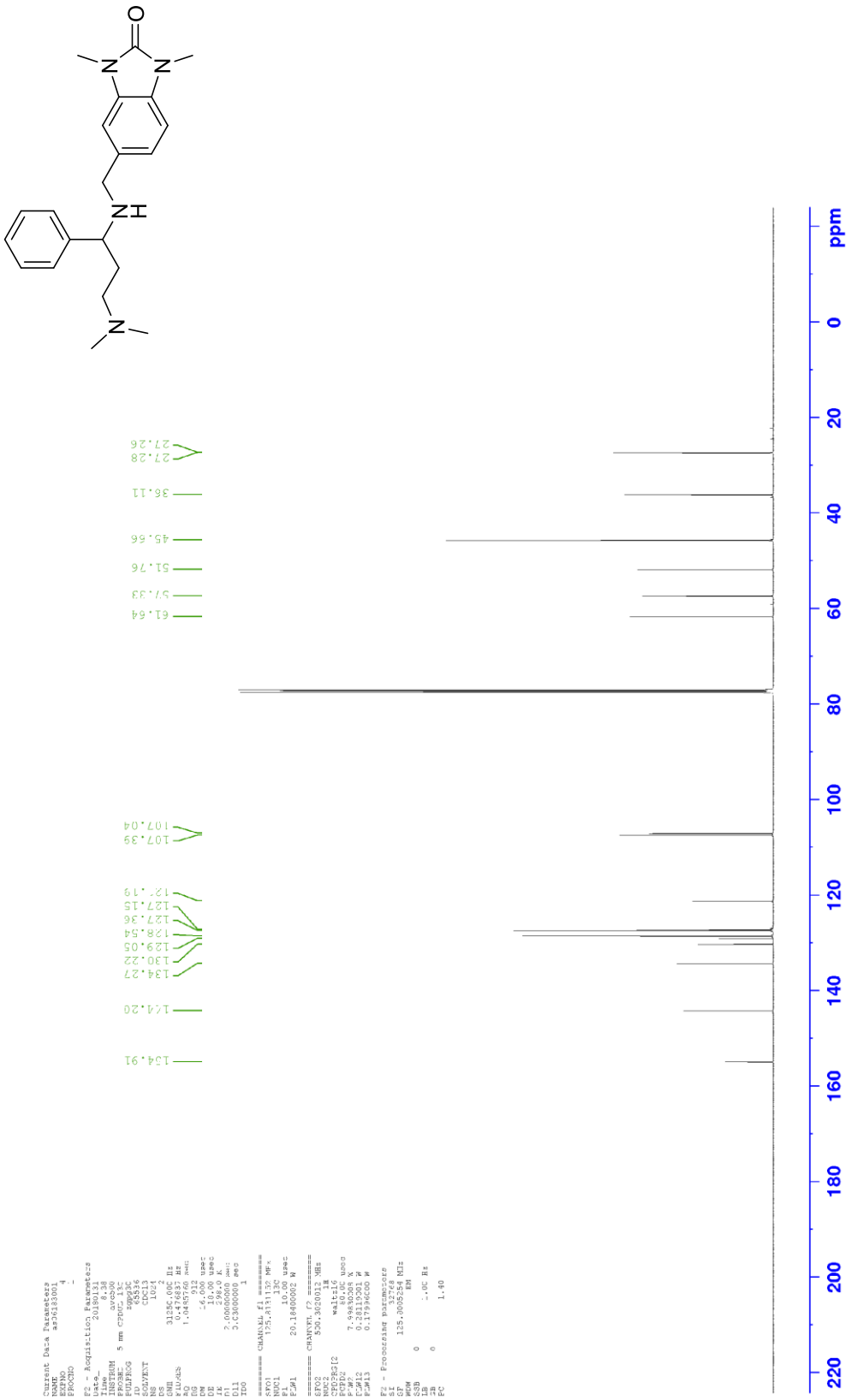
5-({[3-(Dimethylamino)-2,2-dimethylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (160)



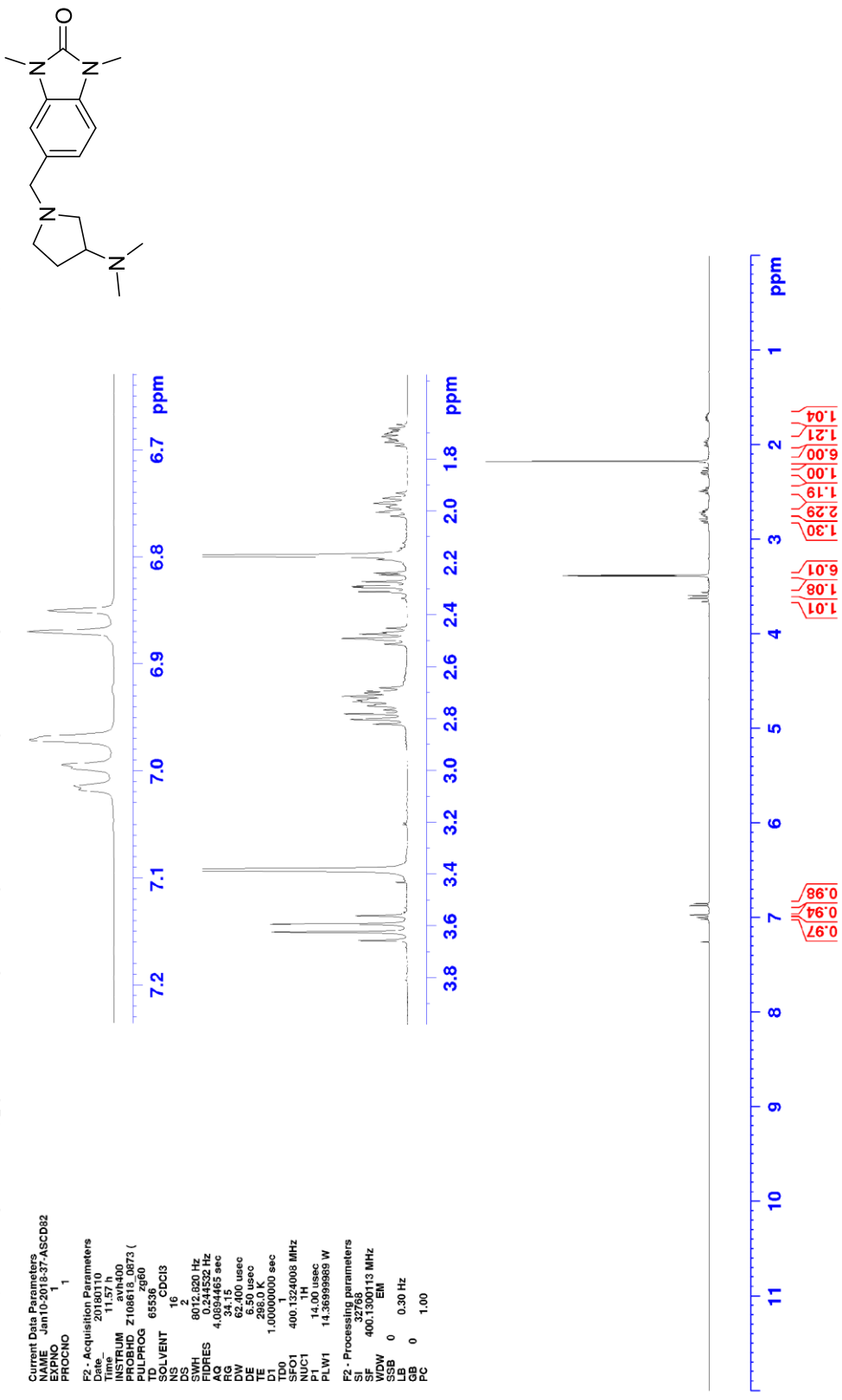
±-5-({[3-(Dimethylamino)-1-phenylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (161)



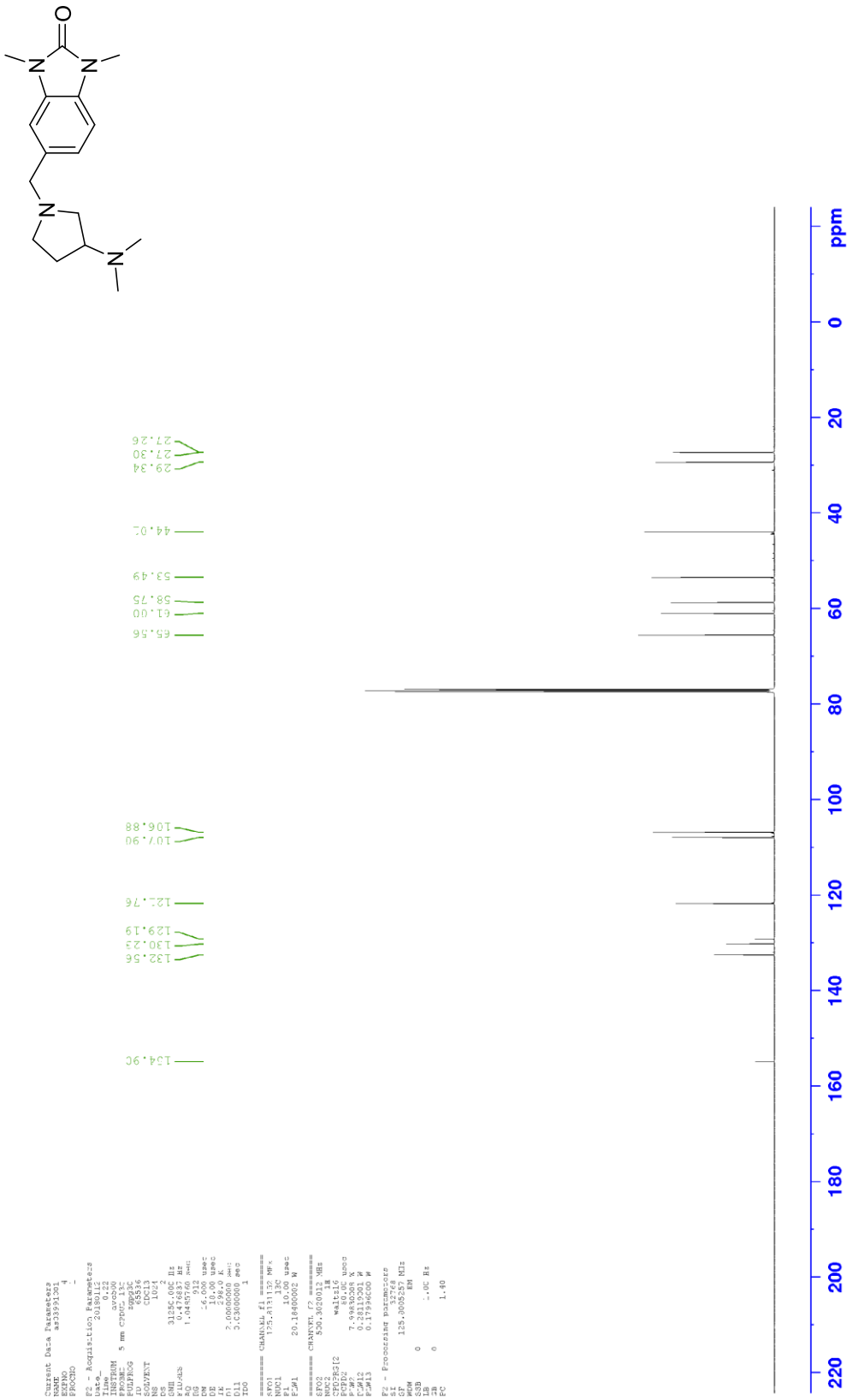
±-5-({[3-(Dimethylamino)-1-phenylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (161)



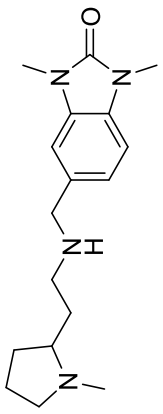
5-{[3-(Dimethylamino)pyrrolidin-1-yl]methyl}-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (163)

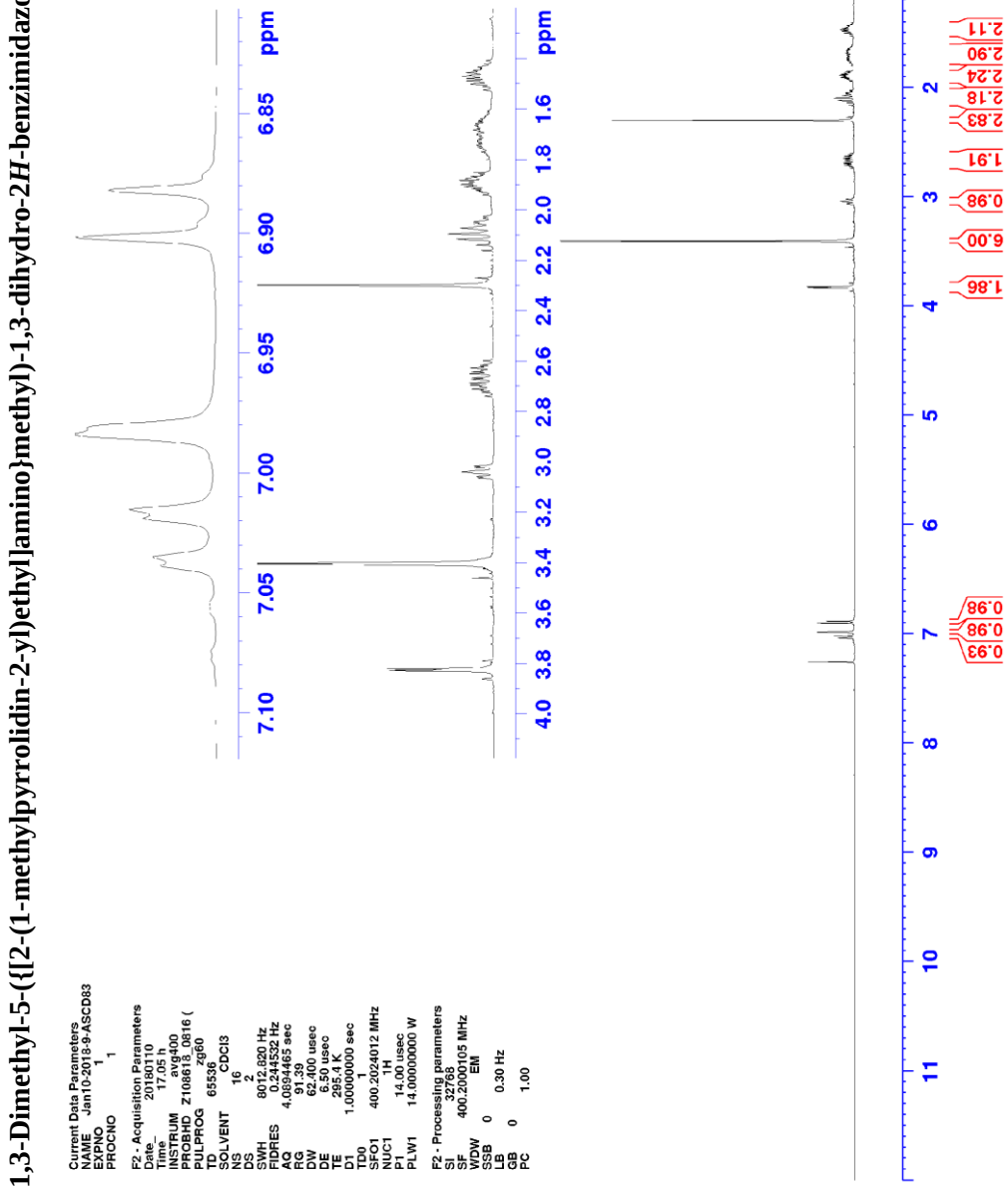


5-{[3-(Dimethylamino)pyrrolidin-1-yl]methyl}-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one (163)



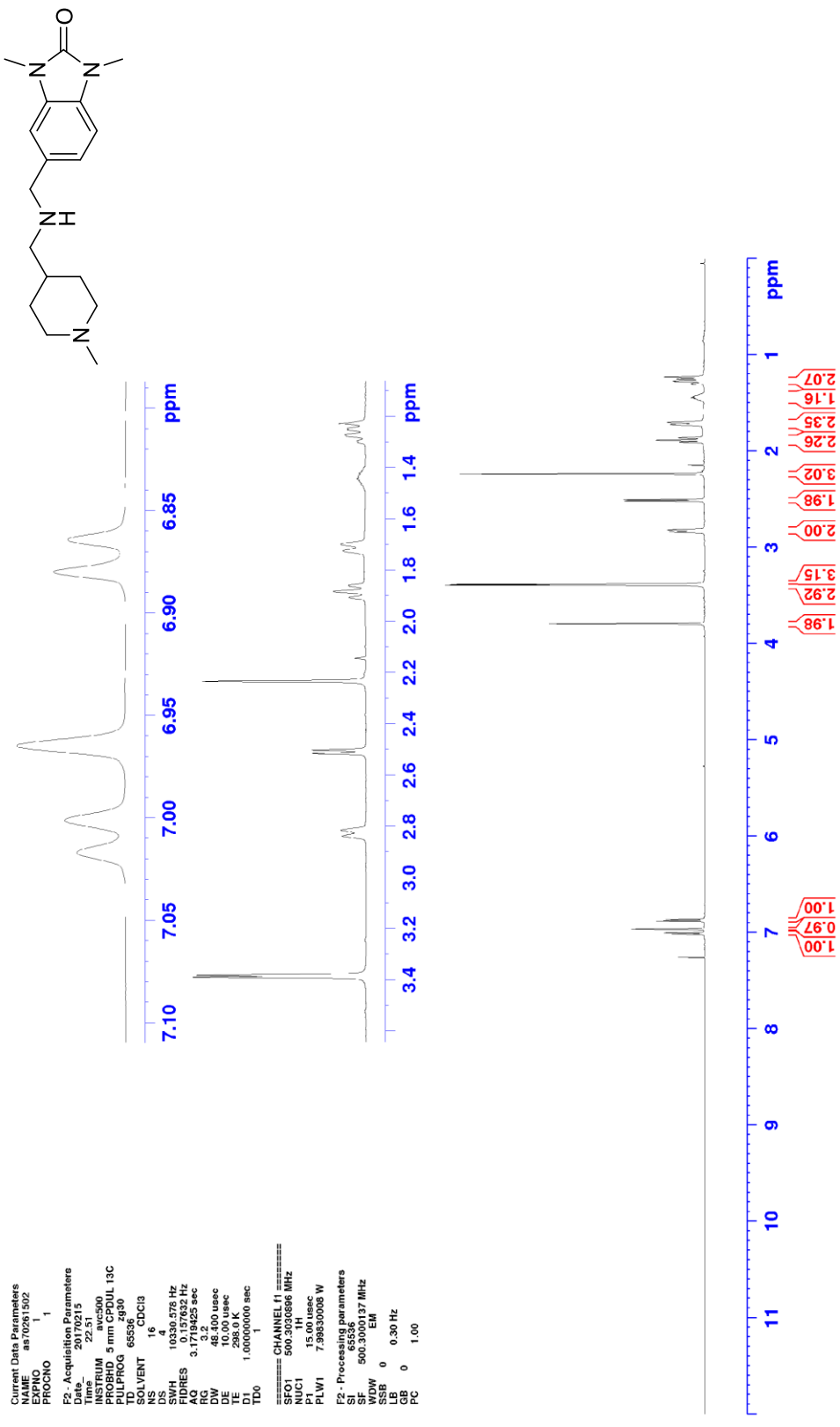
1,3-Dimethyl-5-({[2-(1-methylpyrrolidin-2-yl)ethyl]amino}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (164)



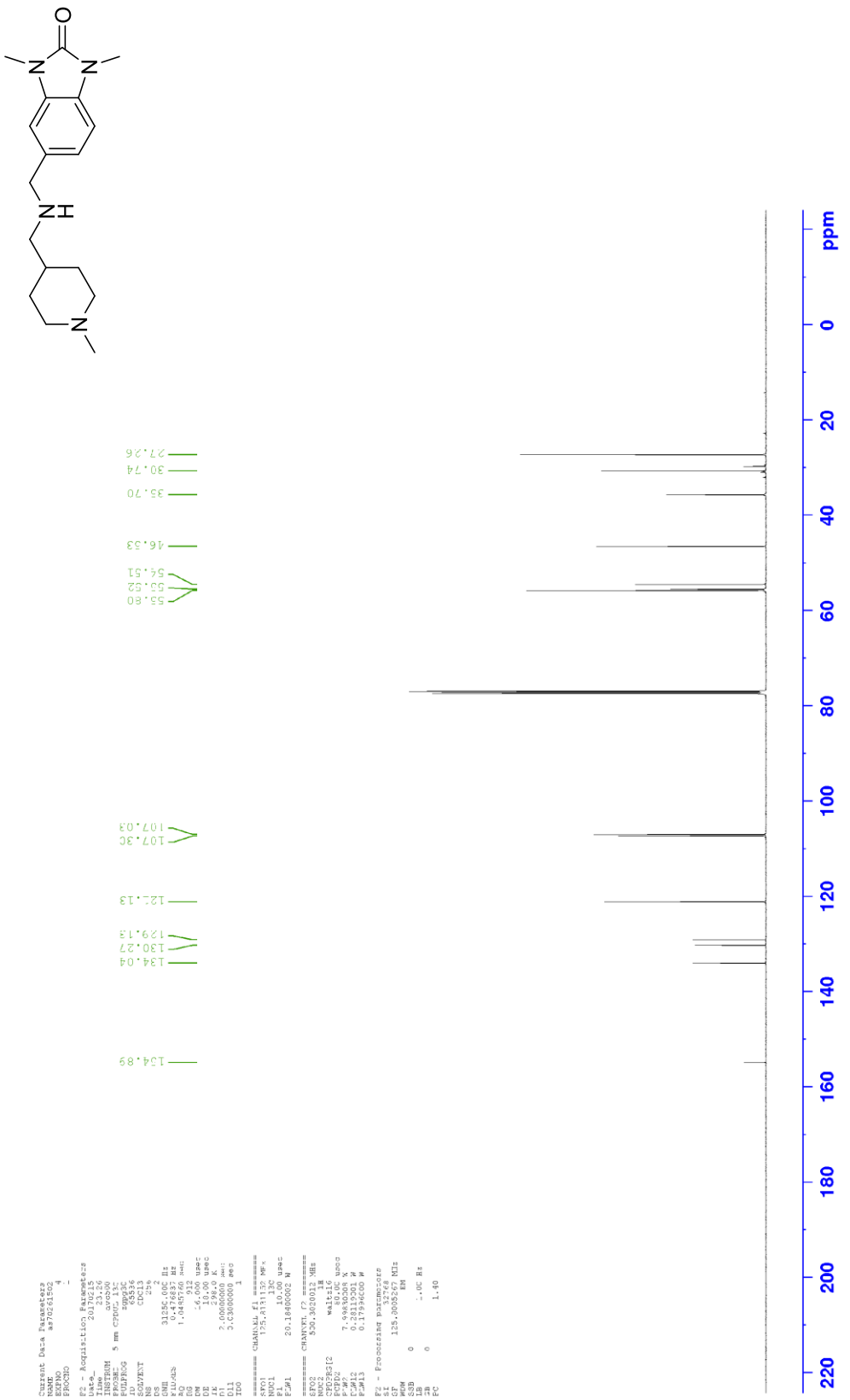


1,3-Dimethyl-5-({[2-(1-methylpyrrolidin-2-yl)ethyl]amino}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (164)

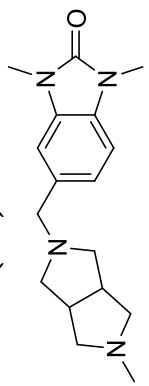
1,3-Dimethyl-5-({[(1-methylpiperidin-4-yl)methyl]amino}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (165)

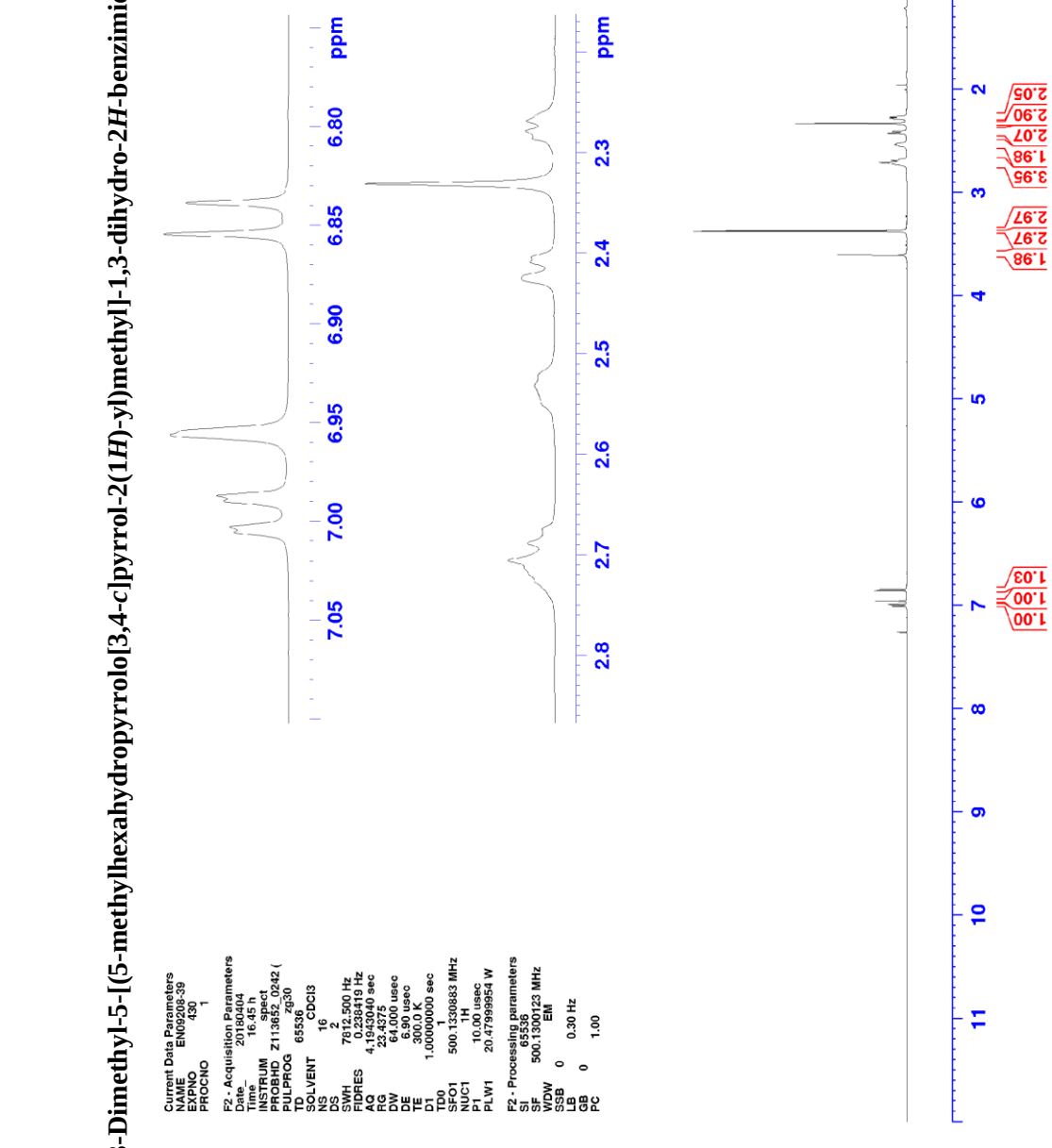


1,3-Dimethyl-5-({[(1-methylpiperidin-4-yl)methyl]amino}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (165)

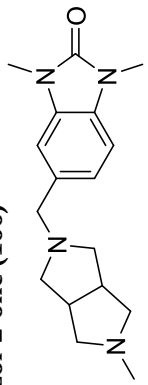


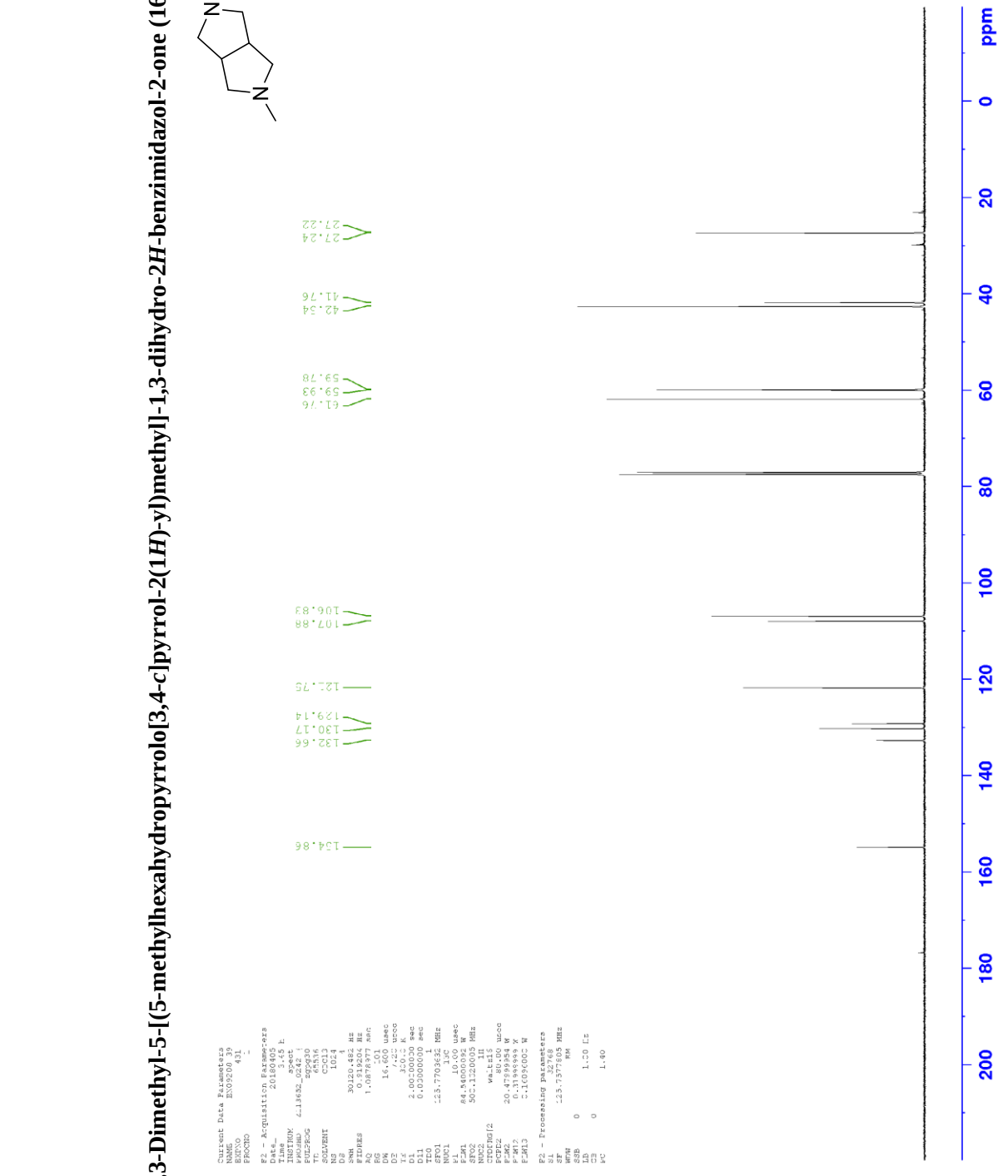
1,3-Dimethyl-5-[(5-methylhexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-yl)methyl]-1,3-dihydro-2*H*-benzimidazol-2-one (166)



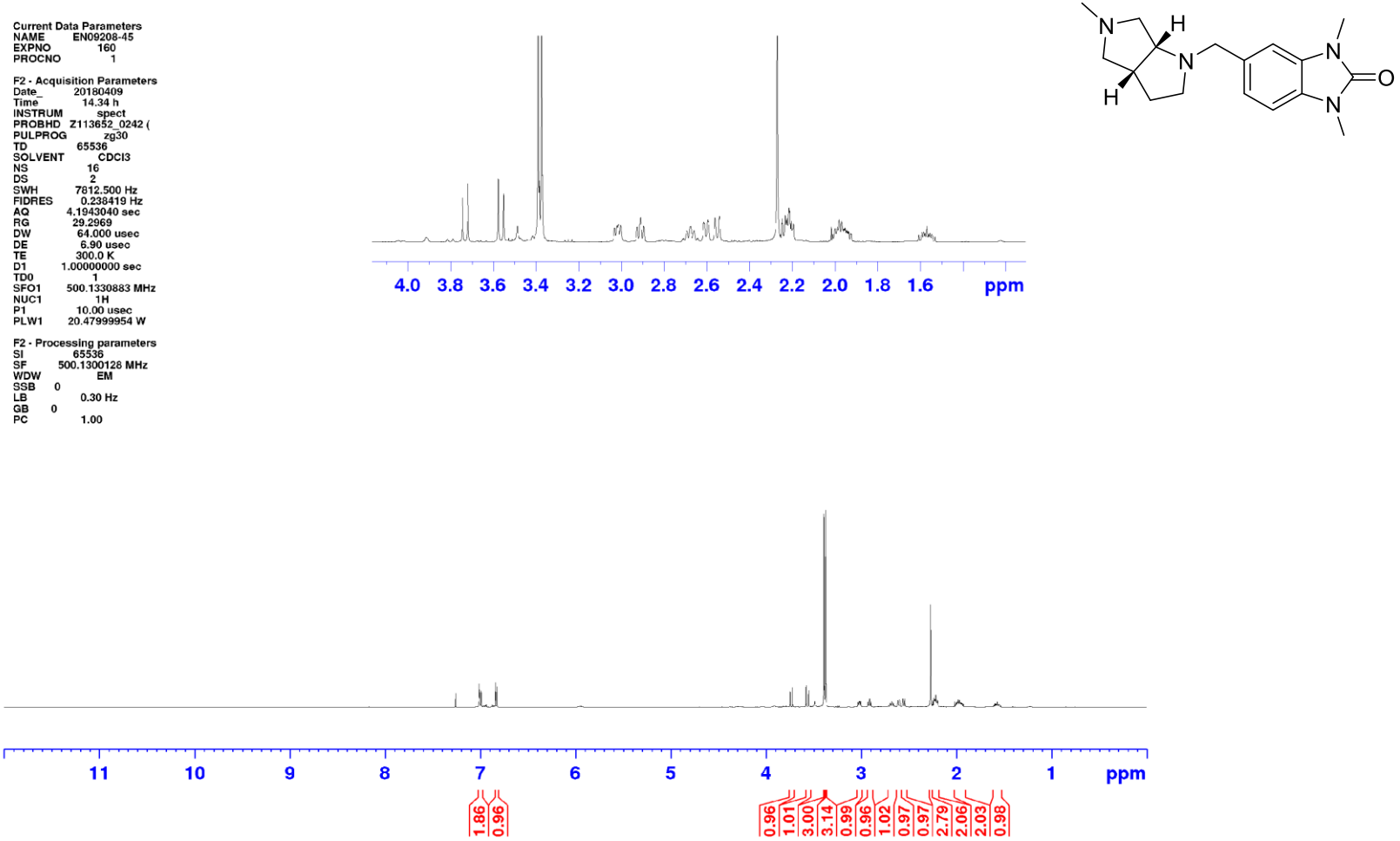


1,3-Dimethyl-5-[(5-methylhexahydropyrrolo[3,4-*c*]pyrrol-2(1*H*)-yl)methyl]-1,3-dihydro-2*H*-benzimidazol-2-one (166)

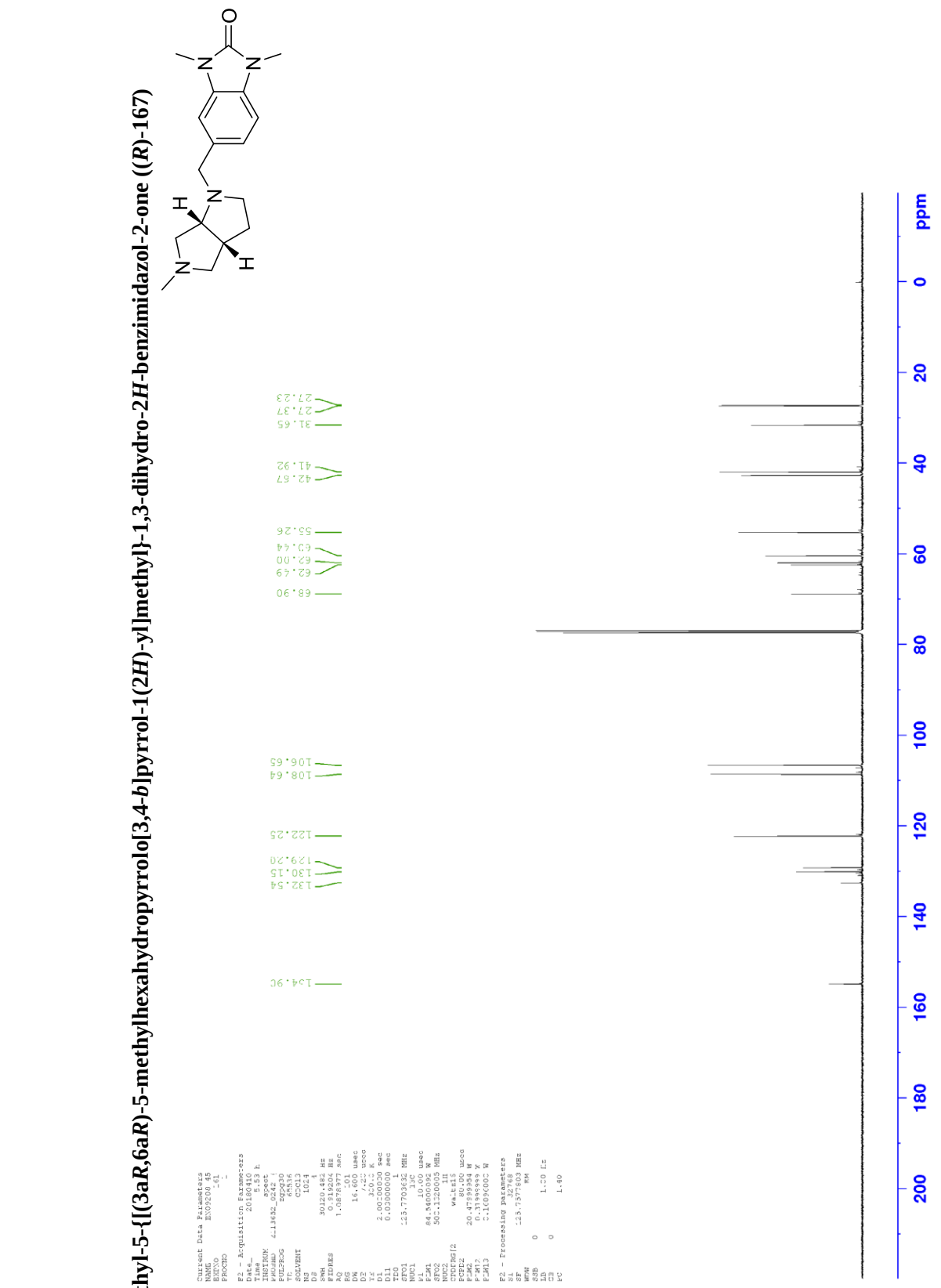




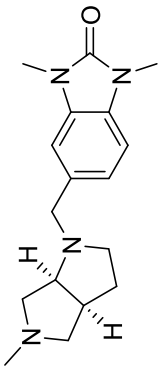
1,3-Dimethyl-5-{[(3a*R*,6a*R*)-5-methylhexahydropyrrolo[3,4-*b*]pyrrol-1(2*H*)-yl]methyl}-1,3-dihydro-2*H*-benzimidazol-2-one ((*R*)-167)

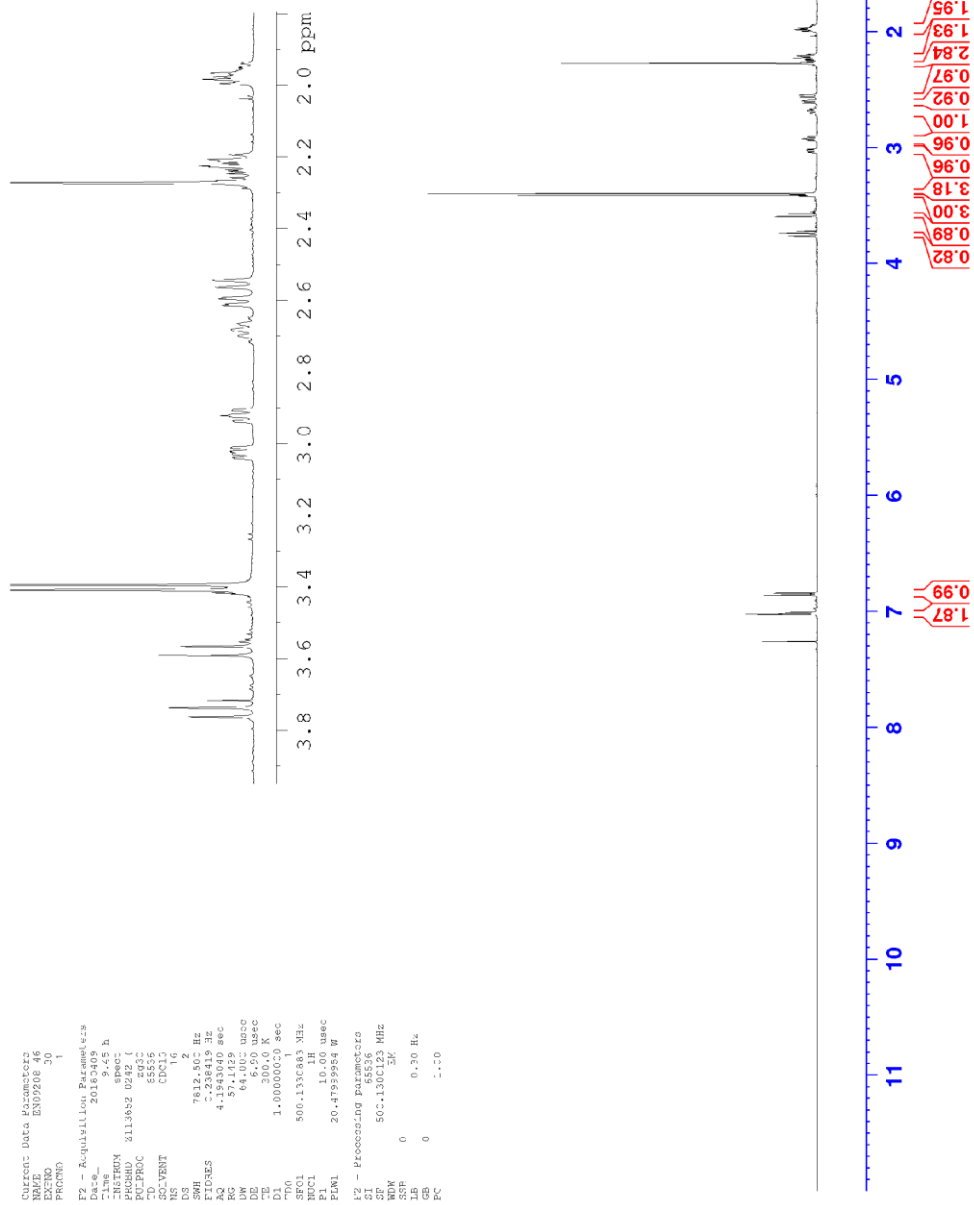


1,3-Dimethyl-5-{[(3a*R*,6a*R*)-5-methylhexahydropyrrolo[3,4-*b*]pyrrol-1(2*H*)-yl]methyl}-1,3-dihydro-2*H*-benzimidazol-2-one ((*R*)-167)

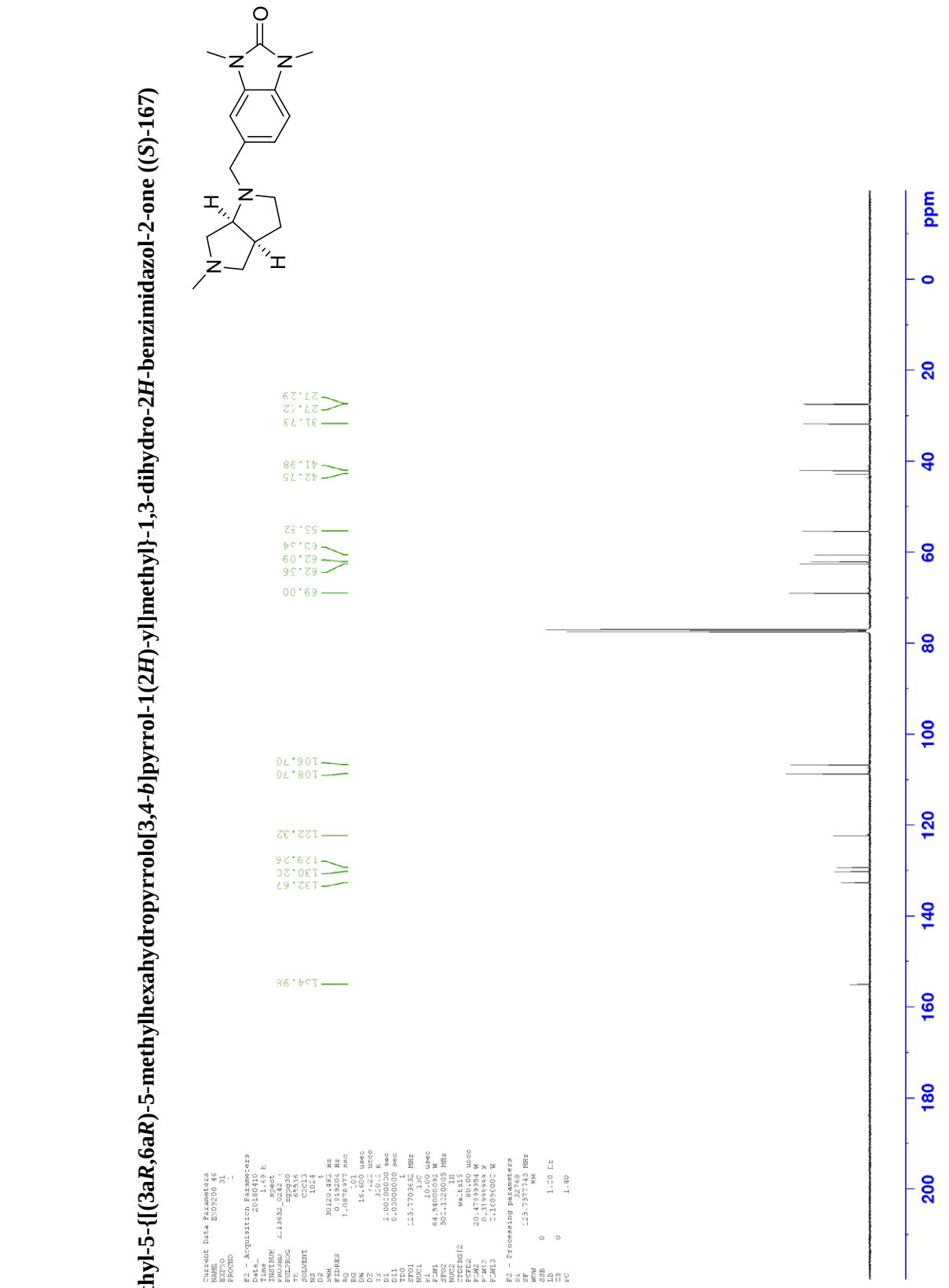


1,3-Dimethyl-5-{[(3a*R*,6a*R*)-5-methylhexahydropyrrolo[3,4-*b*]pyrrol-1(2*H*)-yl]methyl}-1,3-dihydro-2*H*-benzimidazol-2-one ((*S*)-167)

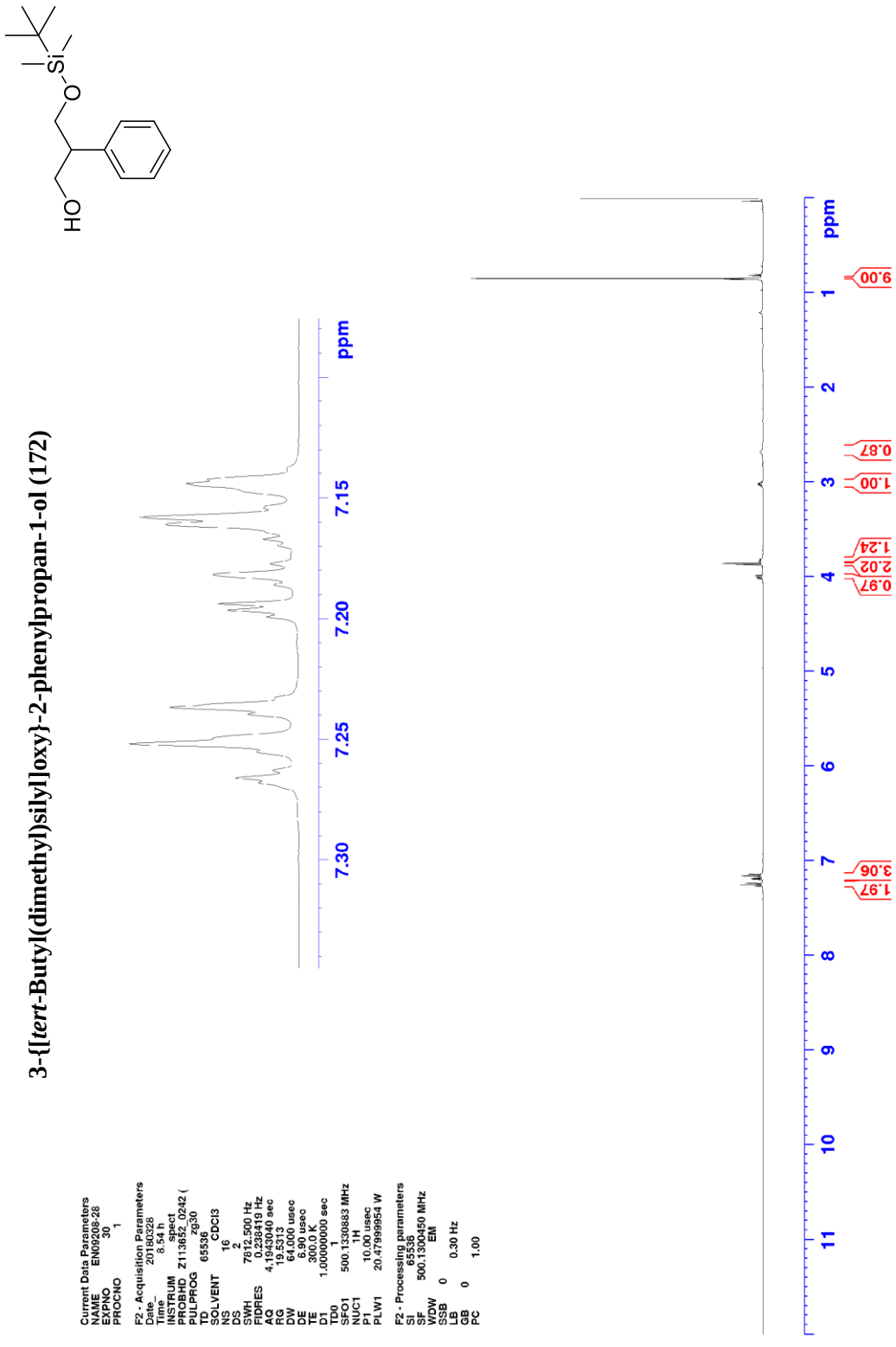




1,3-Dimethyl-5-{[(3a*R*,6a*R*)-5-methylhexahydropyrrolo[3,4-*b*]pyrrol-1(2*H*)-yl]methyl}-1,3-dihydro-2*H*-benzimidazol-2-one ((*S*)-167)



3-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-phenylpropan-1-ol (172)



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EXPNO 30
PROCNO 1

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PROBHD Z113652_0242 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 7812.500 Hz
FIDRES 0.238419 Hz
AQ 4.1943040 sec
RG 19.5313
DW 64.000 usec
DE 6.90 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330883 MHz
NUC1 1H
P1 10.00 usec
PLW1 20.47999954 W

F2 - Processing parameters
SI 65536
SF 500.1300450 MHz
WDW EM
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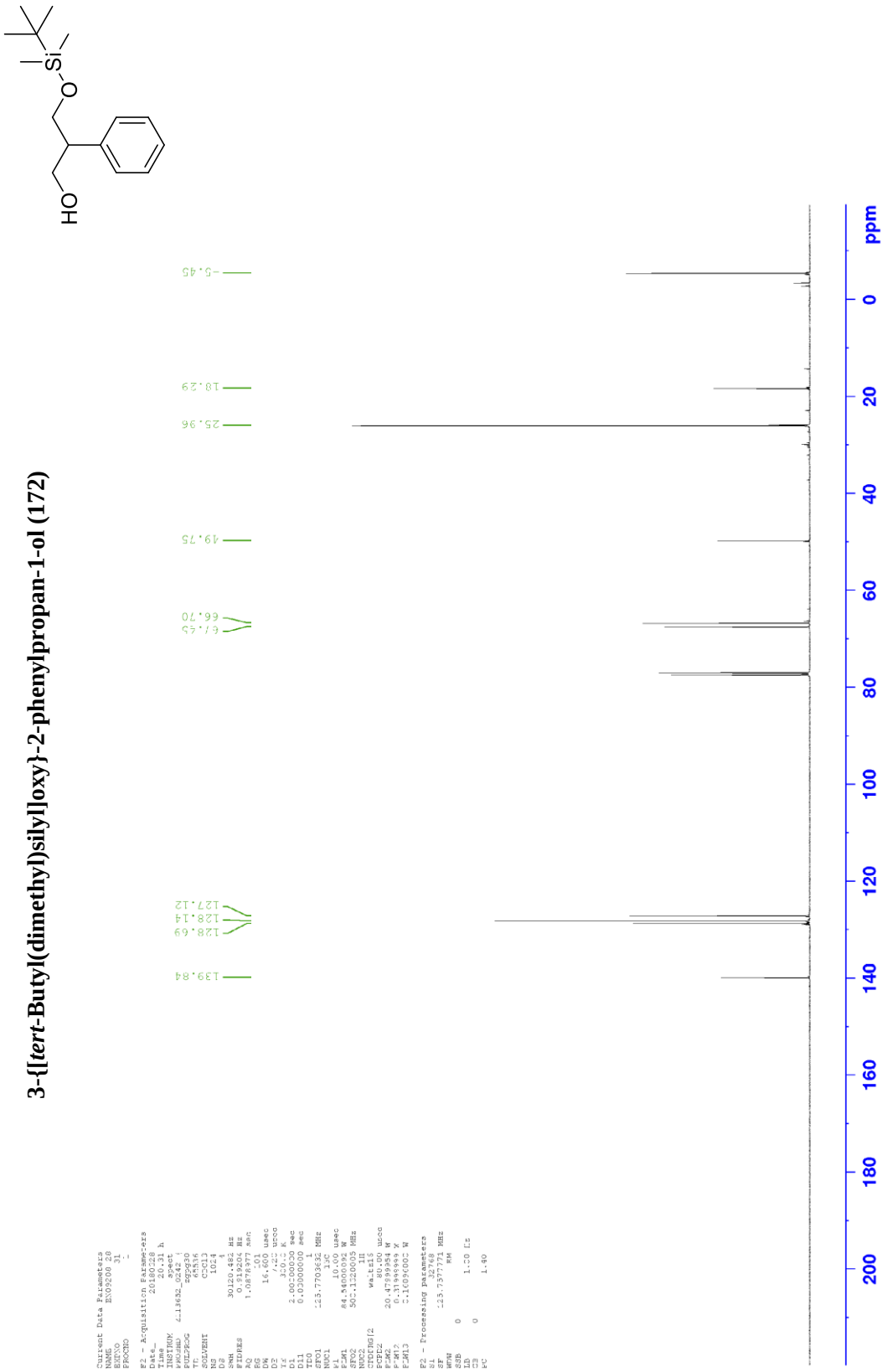
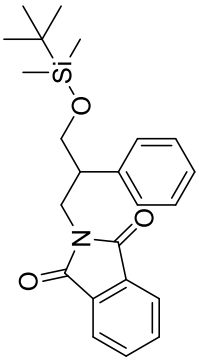
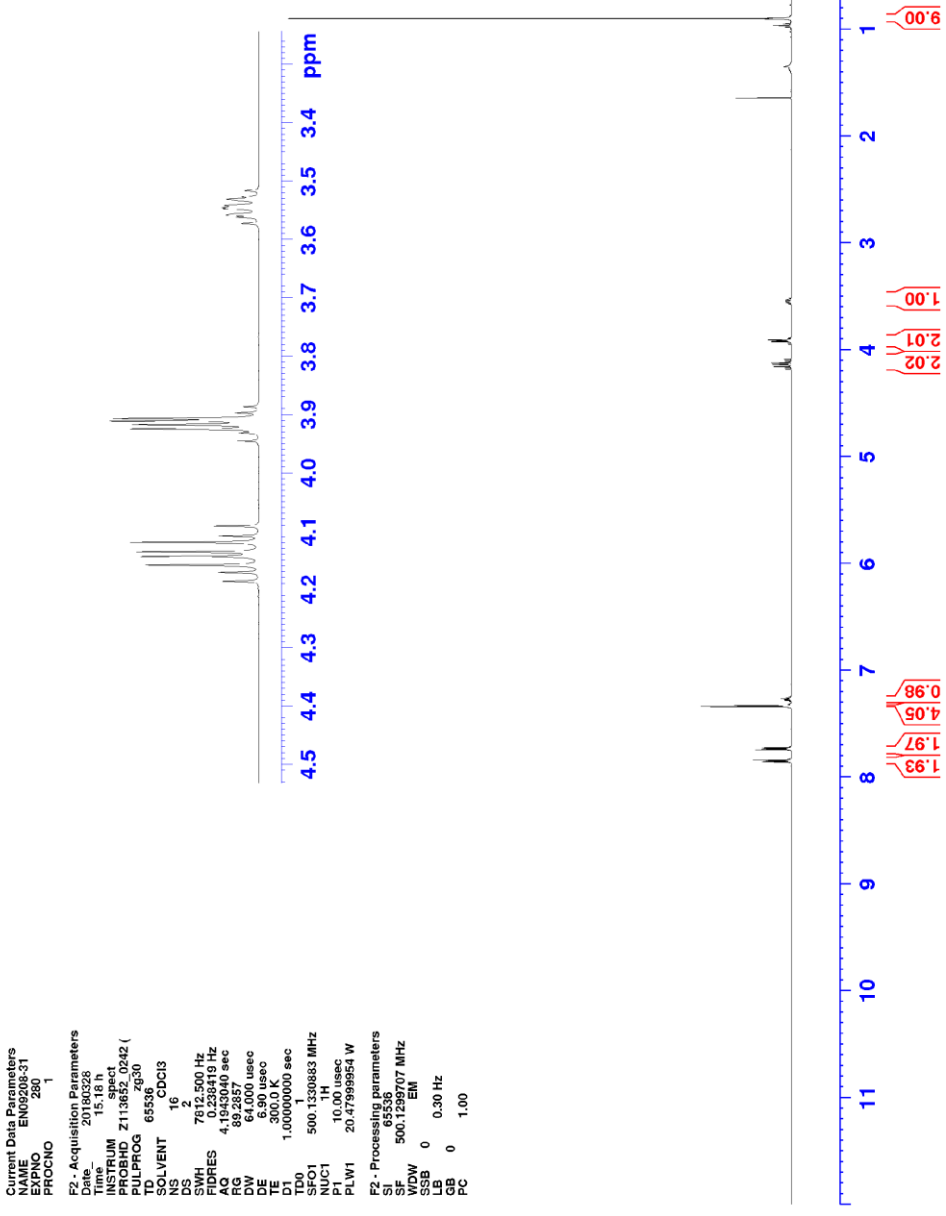
LB 0.30 Hz
GB 0
PC 1.00

3-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-phenylpropan-1-ol (172)

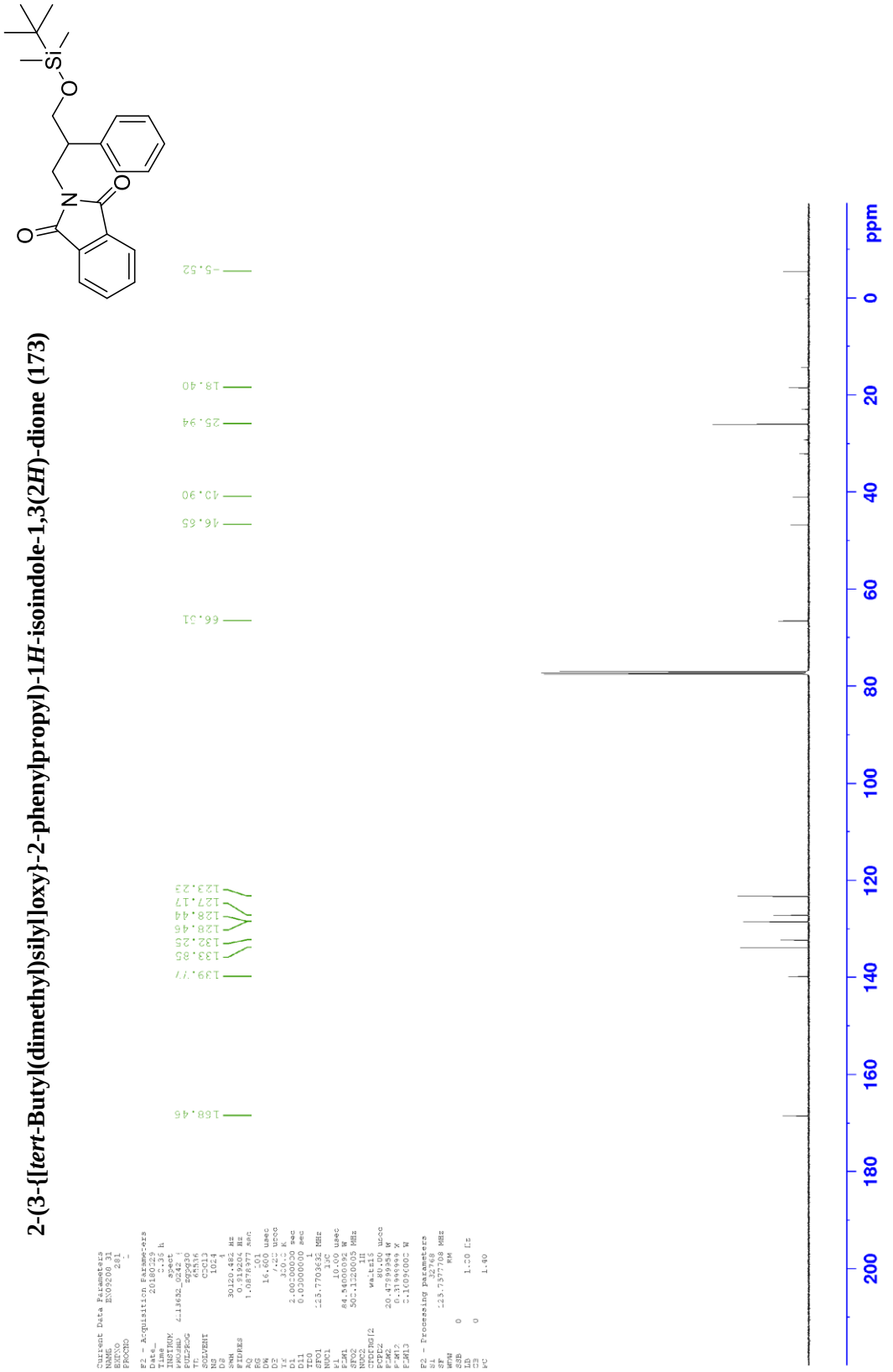


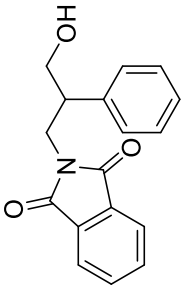


2-(3-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-phenylpropyl)-1*H*-isoindole-1,3(2*H*)-dione (173)

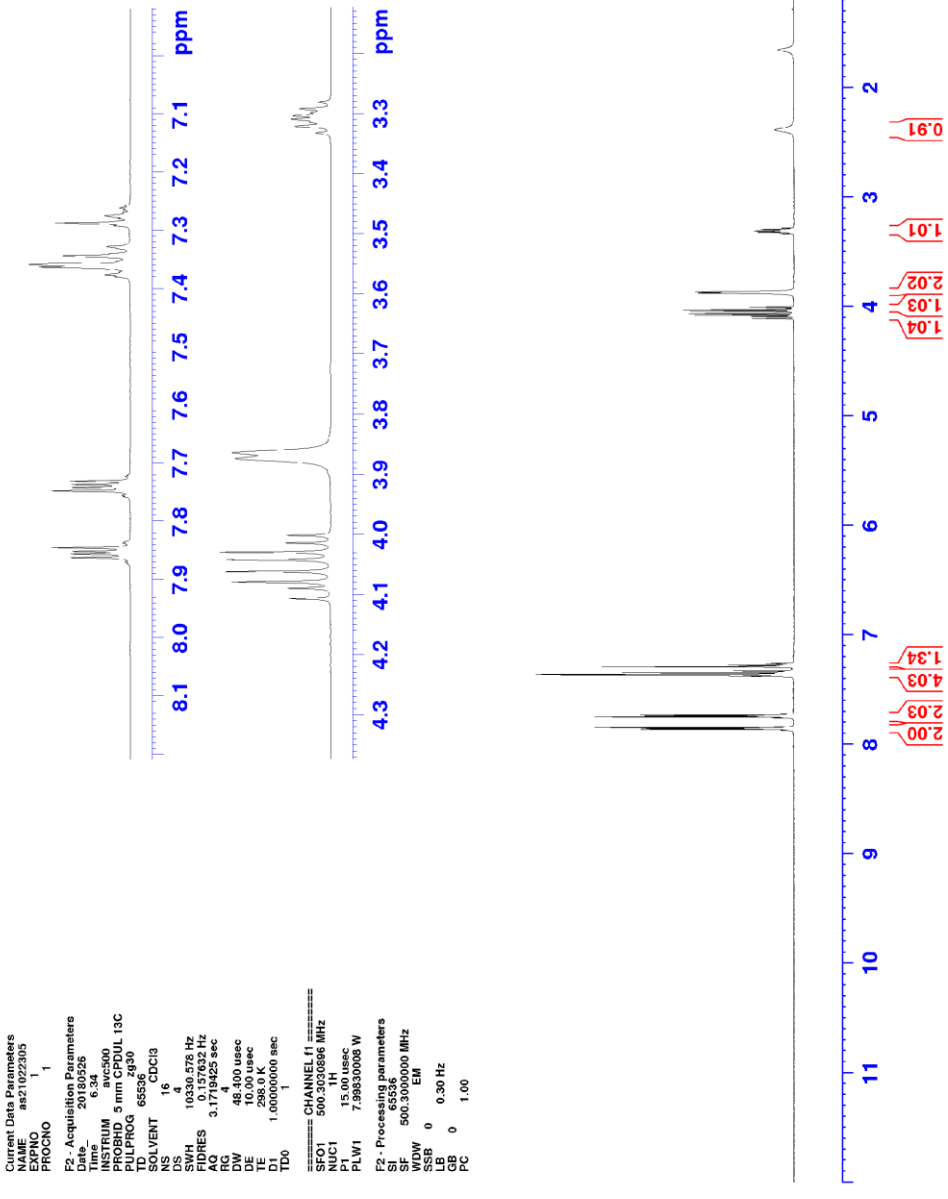


2-(3-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-phenylpropyl)-1*H*-isoindole-1,3(2*H*)-dione (173)

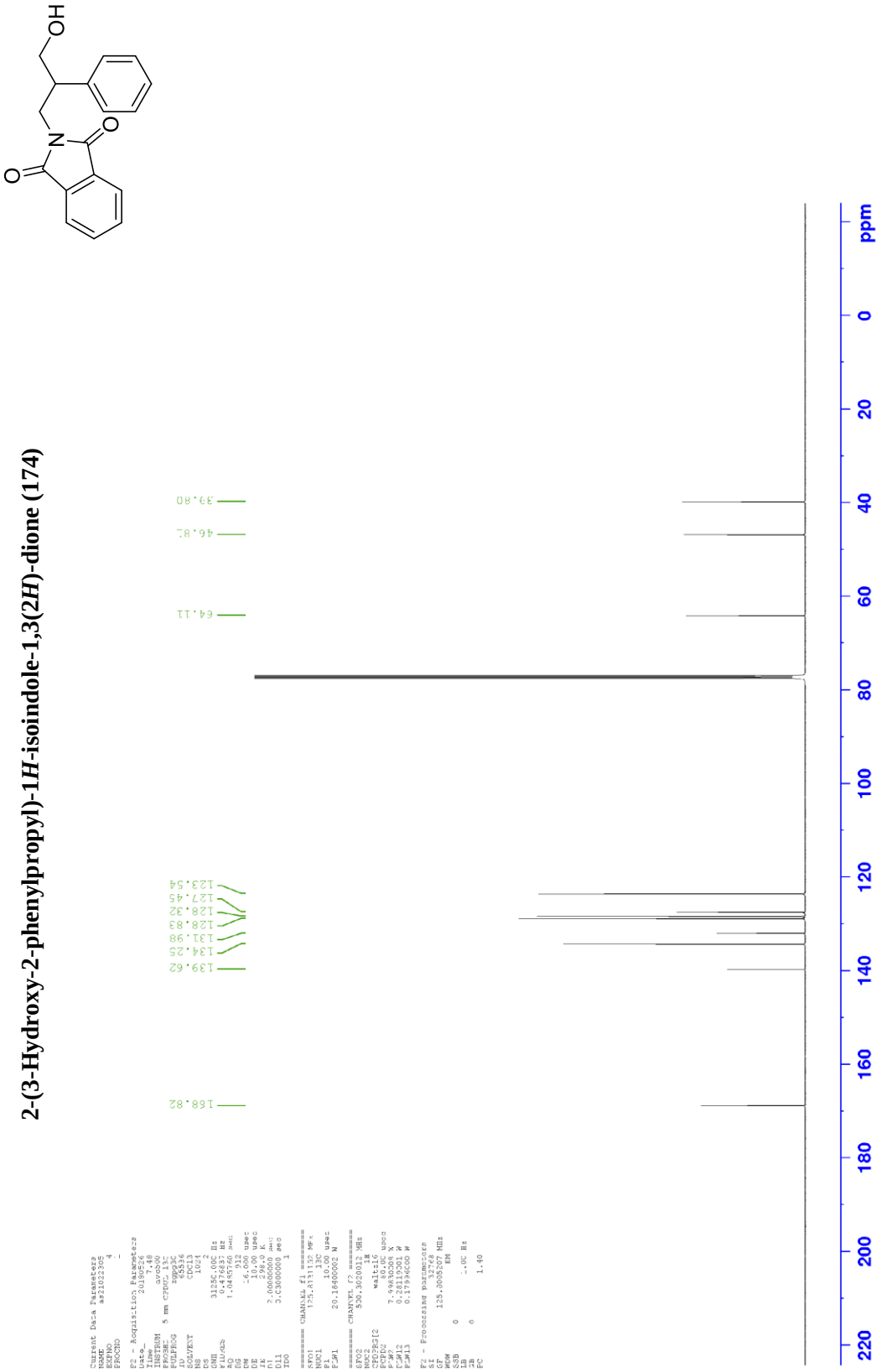


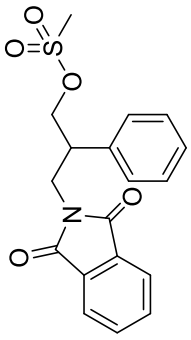


2-(3-Hydroxy-2-phenylpropyl)-1*H*-isoindole-1,3(2*H*)-dione (174)

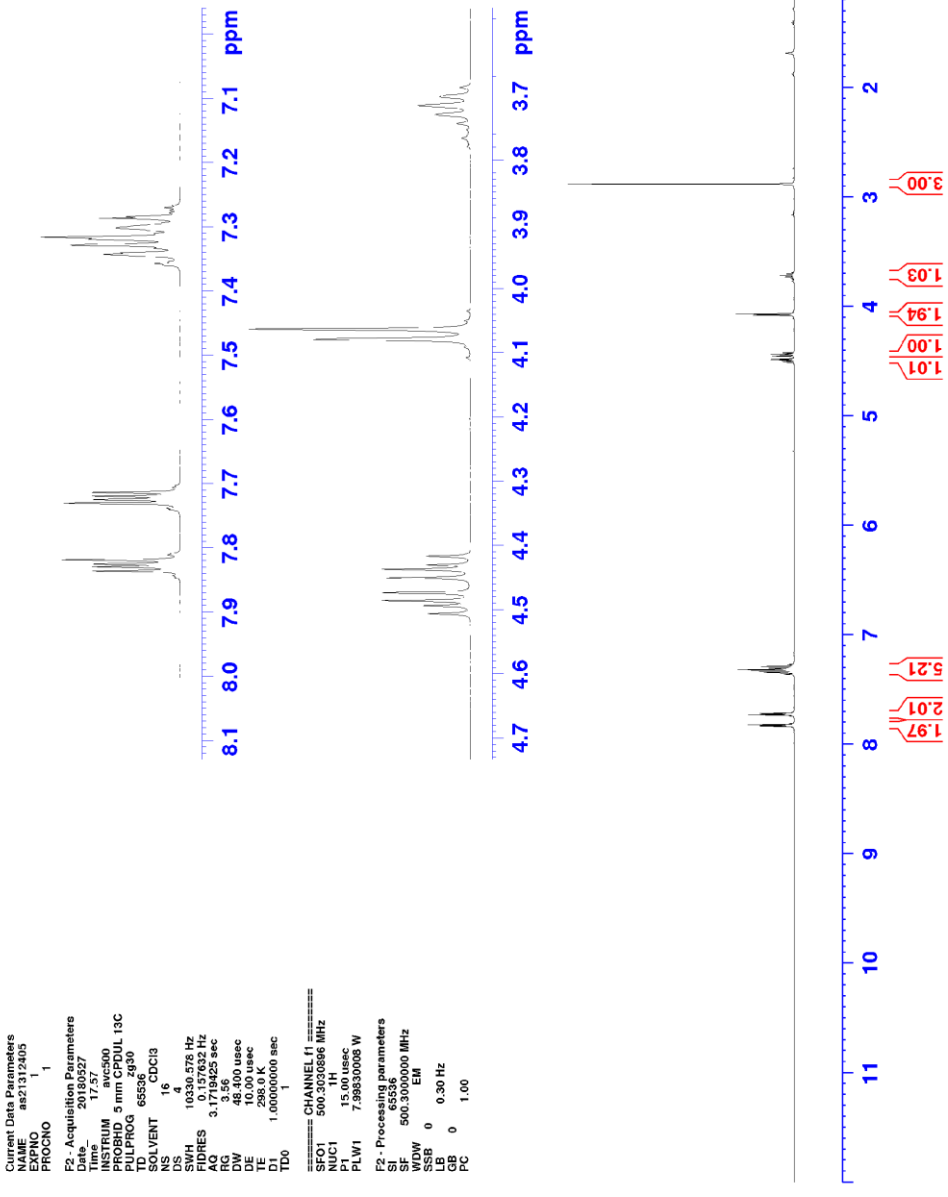


2-(3-Hydroxy-2-phenylpropyl)-1*H*-isoindole-1,3(2*H*)-dione (174)

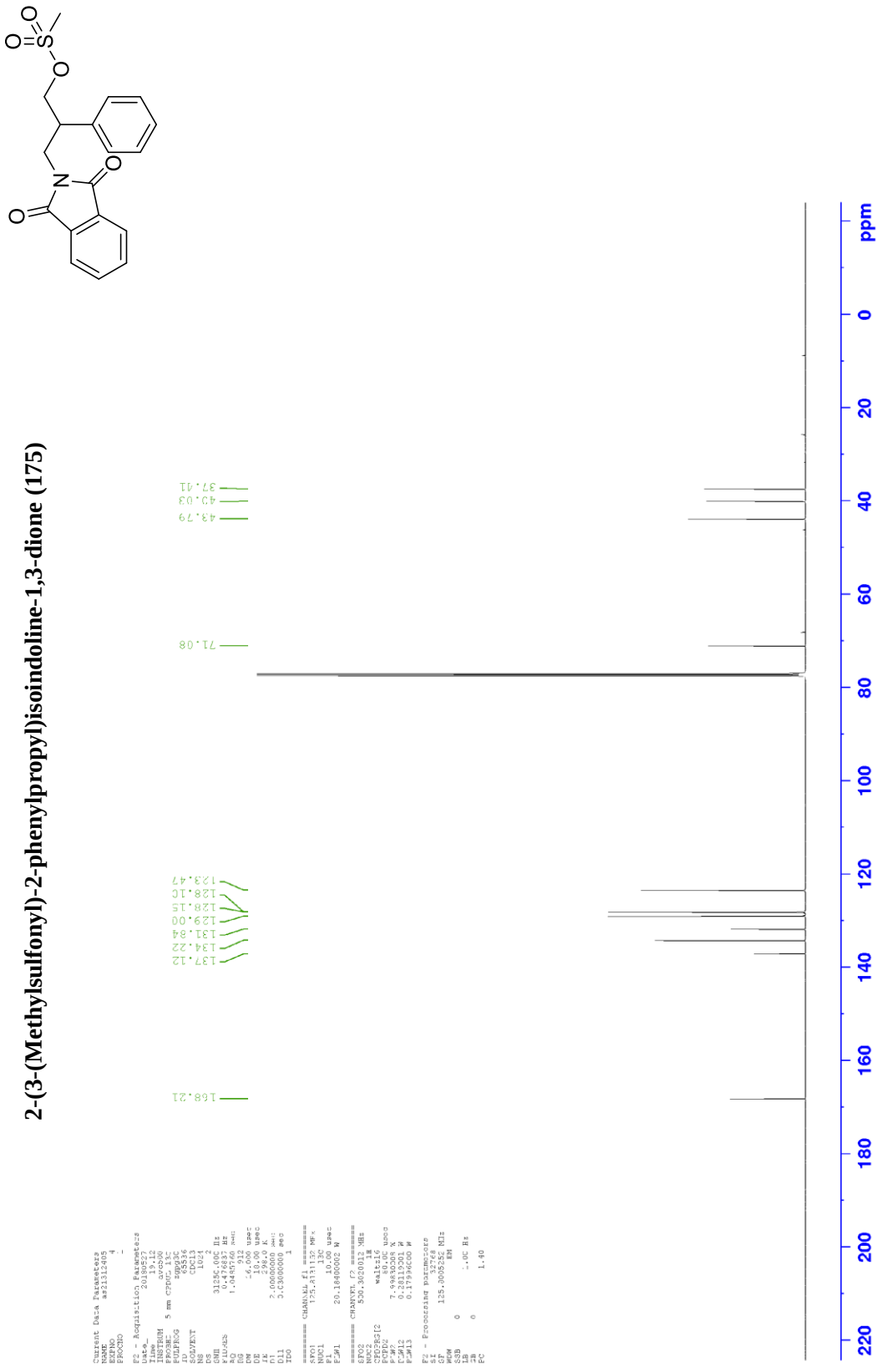


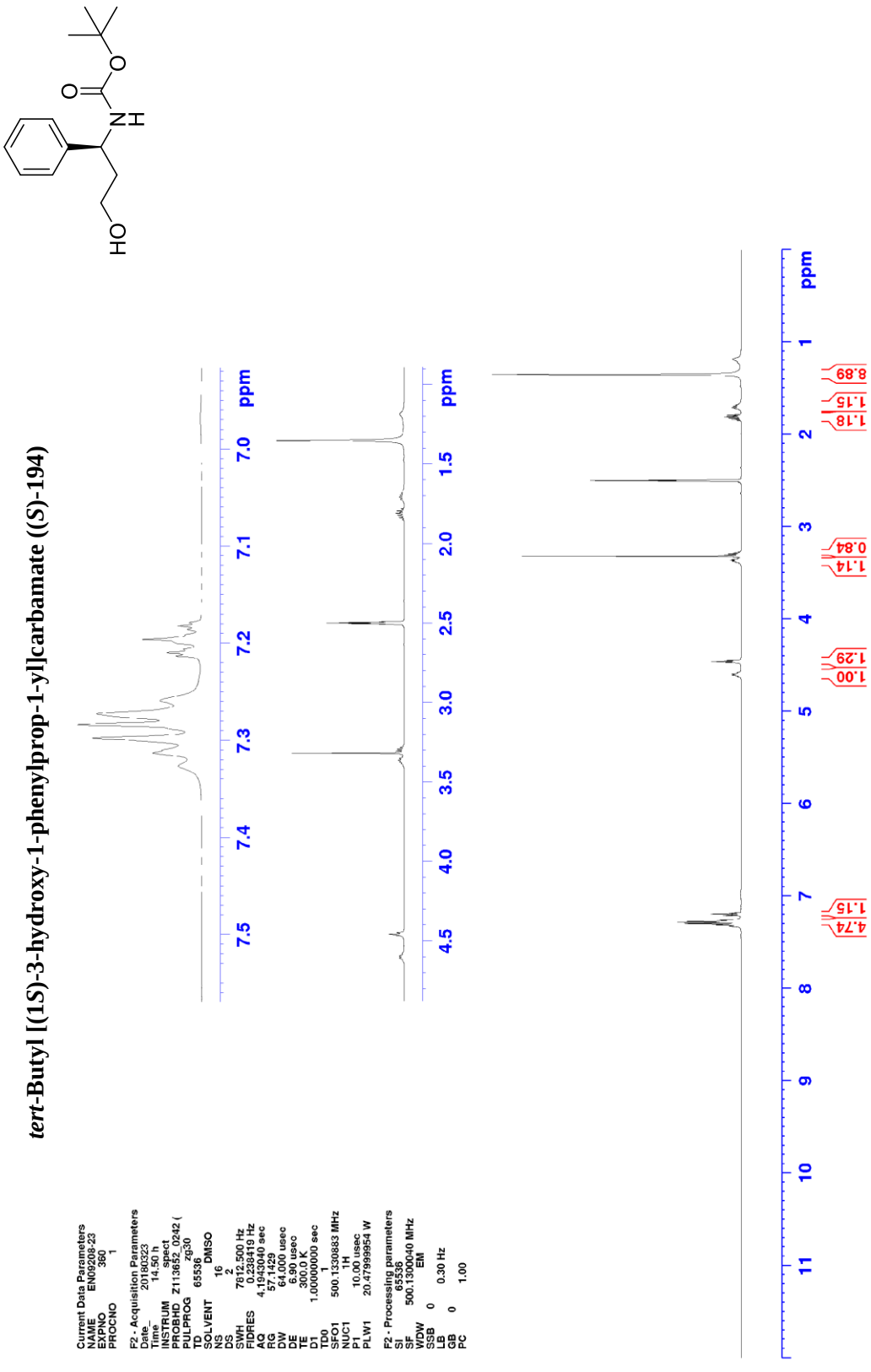


2-(3-(Methylsulfonyl)-2-phenylpropyl)isoindoline-1,3-dione (175)



2-(3-(Methylsulfonyl)-2-phenylpropyl)isoindoline-1,3-dione (175)




tert-Butyl [(1S)-3-hydroxy-1-phenylprop-1-yl]carbamate ((S)-194)
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PROCNO 1
F2 - Acquisition Parameters
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Time 14.50 h
INSTRUM spect
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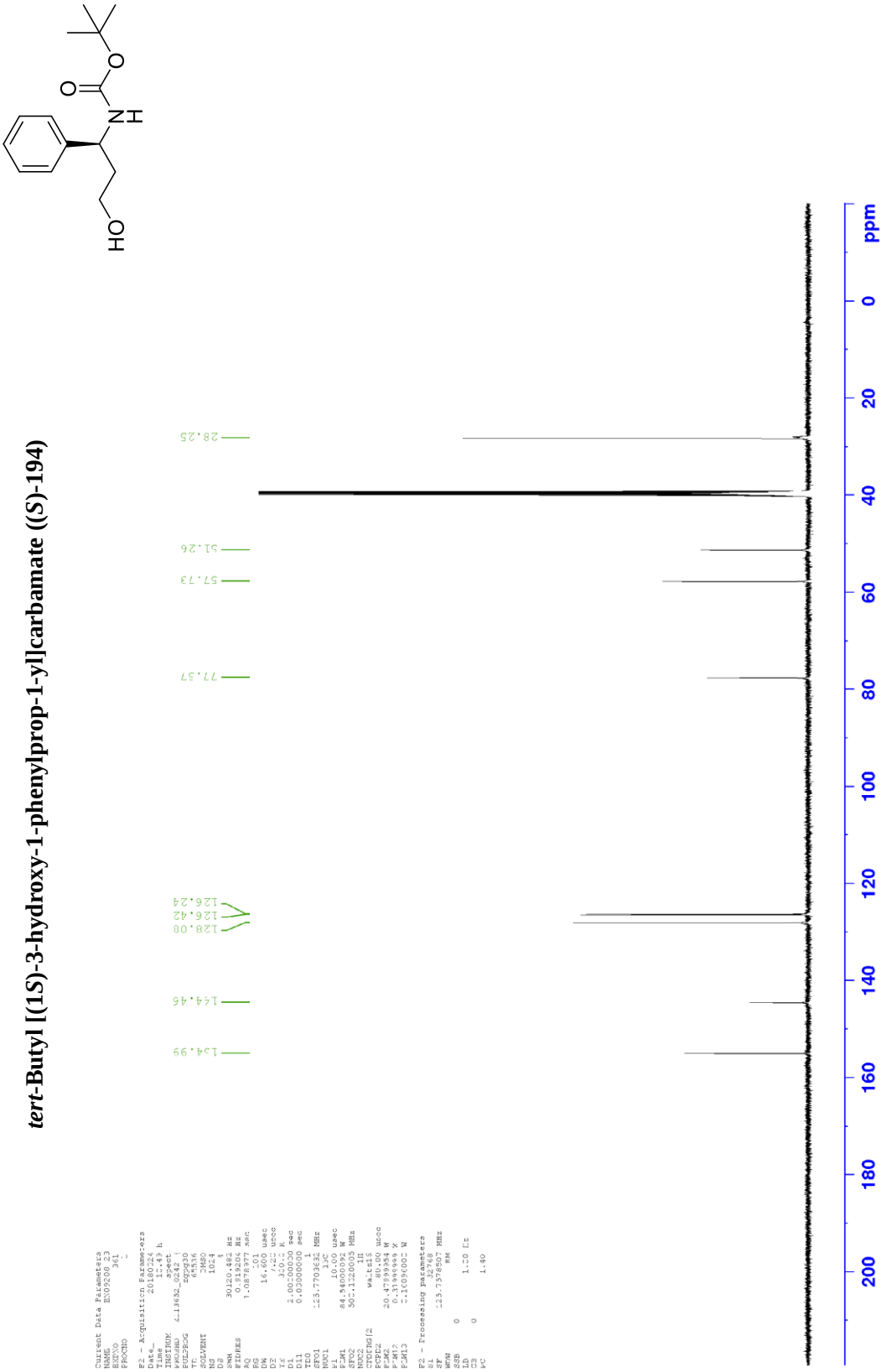
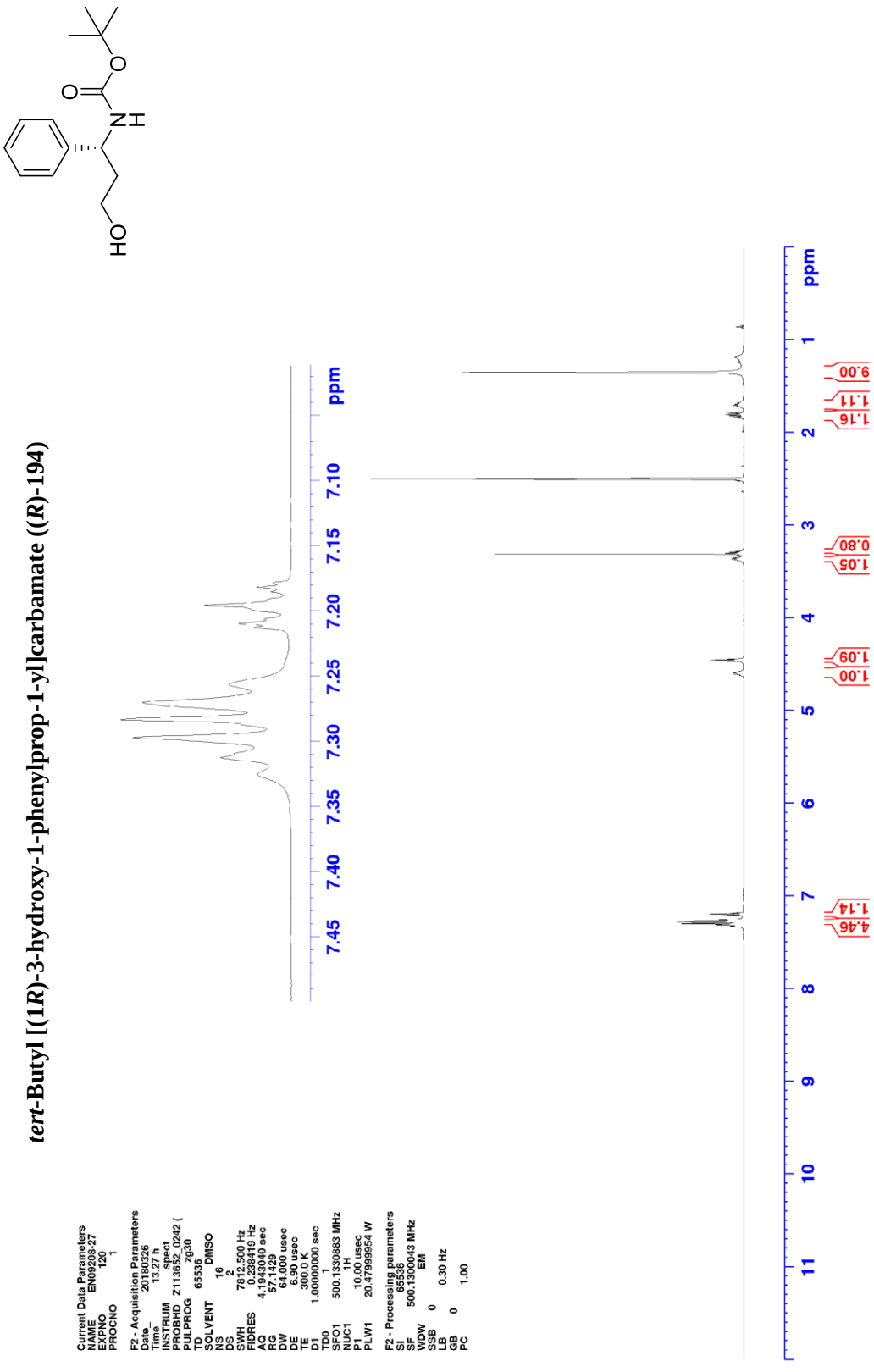
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 7812.500 Hz
FIDRES 0.238419 Hz
AQ 4.1943040 sec
RG 57.1429
DW 64.000 usec
DE 6.90 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330883 MHz
NUC1 1H
P1 10.00 usec
PLW1 20.47999954 W
F2 - Processing parameters
SI 65536
SF 500.1300040 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.5
7.4
7.3
7.2
7.1
7.0
ppm
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
11
10
9
8
7
6
5
4
3
2
1
ppm
4.74
1.15
1.00
1.29
1.14
0.84
1.18
1.15
8.89

tert-Butyl [(1S)-3-hydroxy-1-phenylprop-1-yl]carbamate ((S)-194)




tert-Butyl [(1R)-3-hydroxy-1-phenylprop-1-yl]carbamate ((R)-194)
Current Data Parameters
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EXPNO 120
PROCNO 1
F2 - Acquisition Parameters
Date_ 20190226
Time 13.27 h
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PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 7812.500 Hz
FIDRES 0.238419 Hz
AQ 4.1943040 sec
RG 57.1429
DW 64.000 usec
DE 6.90 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330883 MHz
NUC1 1H
P1 10.00 usec
PLW1 20.47999954 W
F2 - Processing parameters
SI 65536
SF 500.1300043 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.45
7.40
7.35
7.30
7.25
7.20
7.15
7.10
ppm
4.46
1.14
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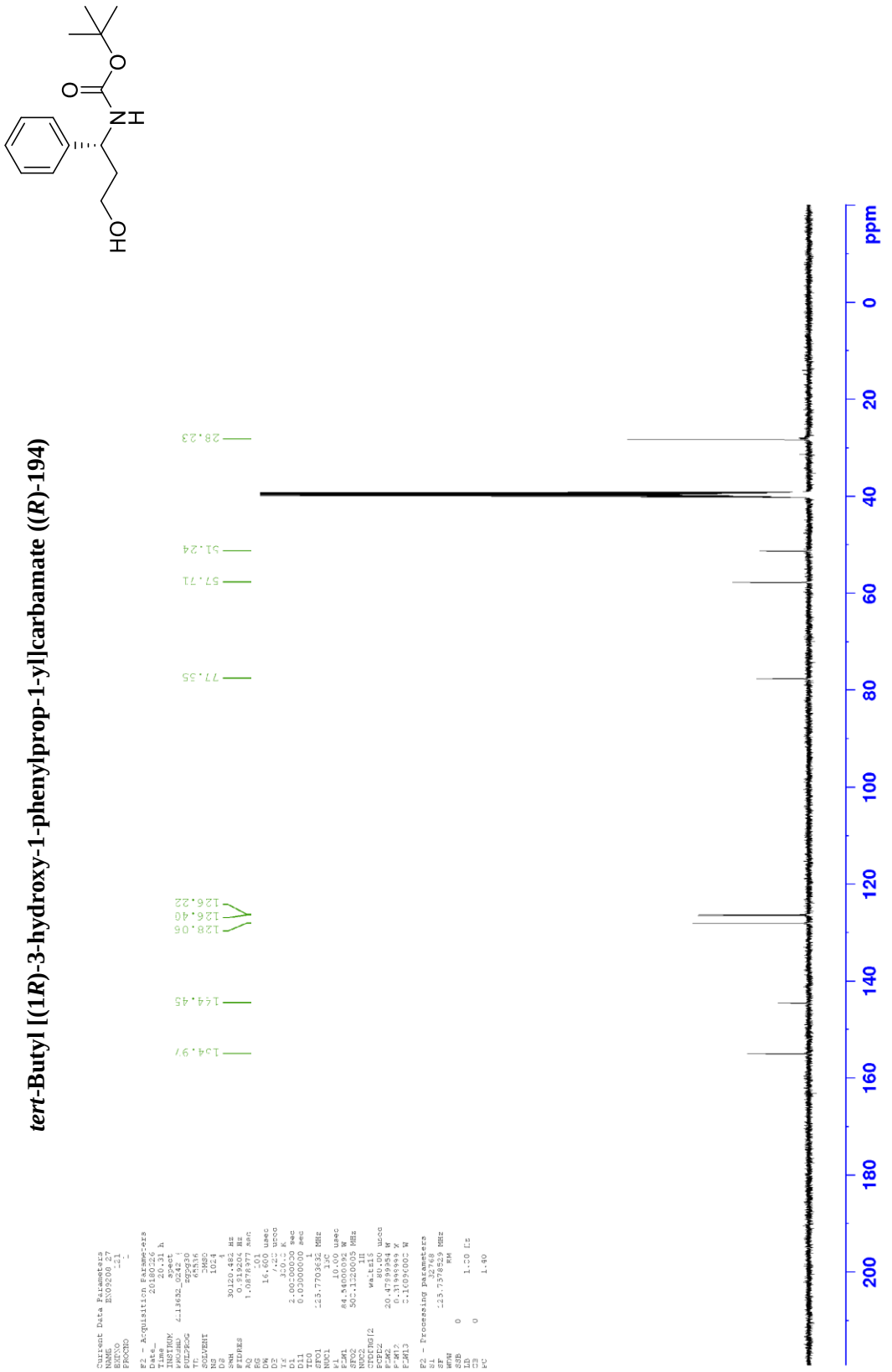
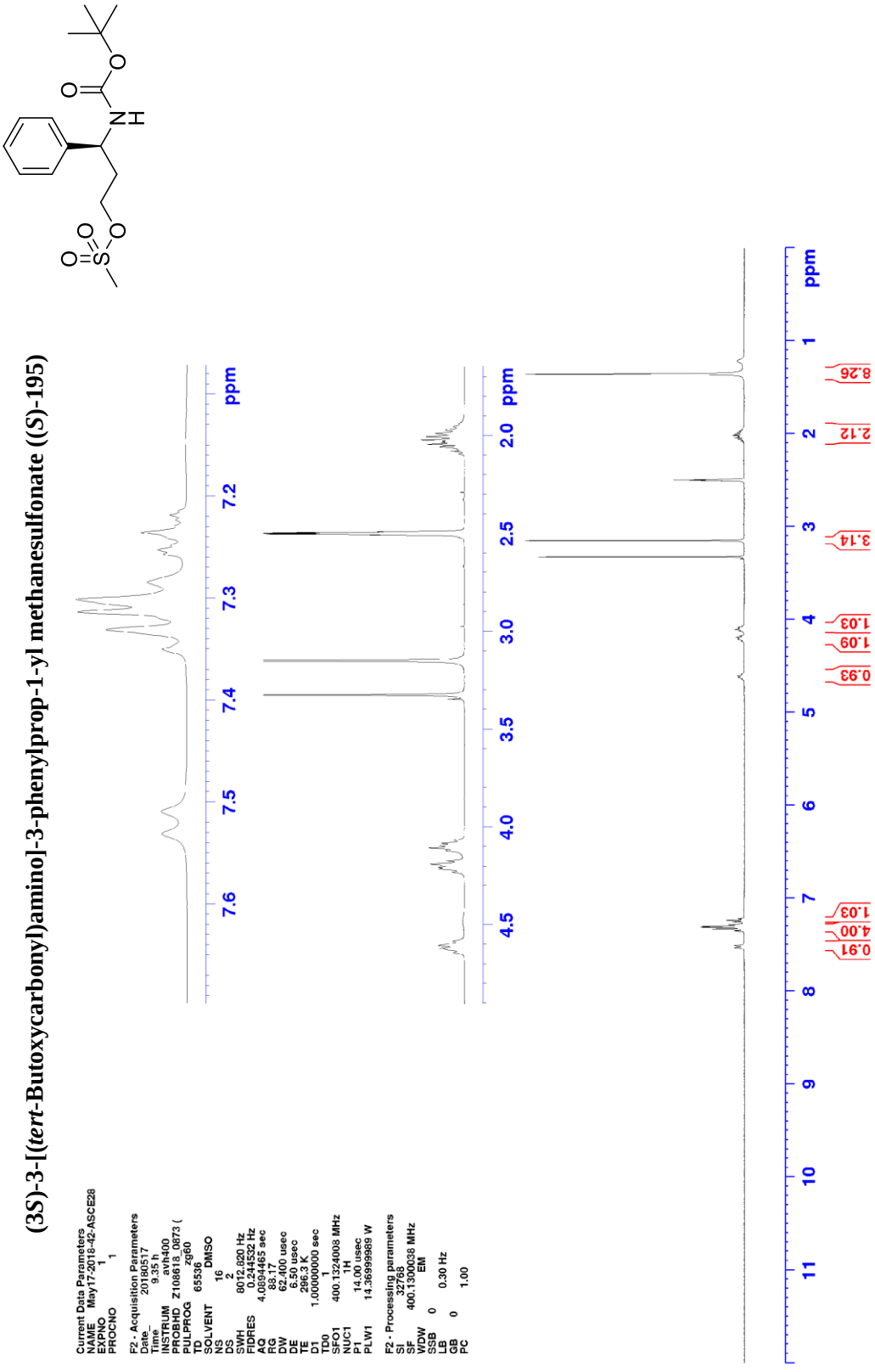
1.09
1.05
0.80
1.16
1.11
9.00
11
10
9
8
7
6
5
4
3
2
1
ppm

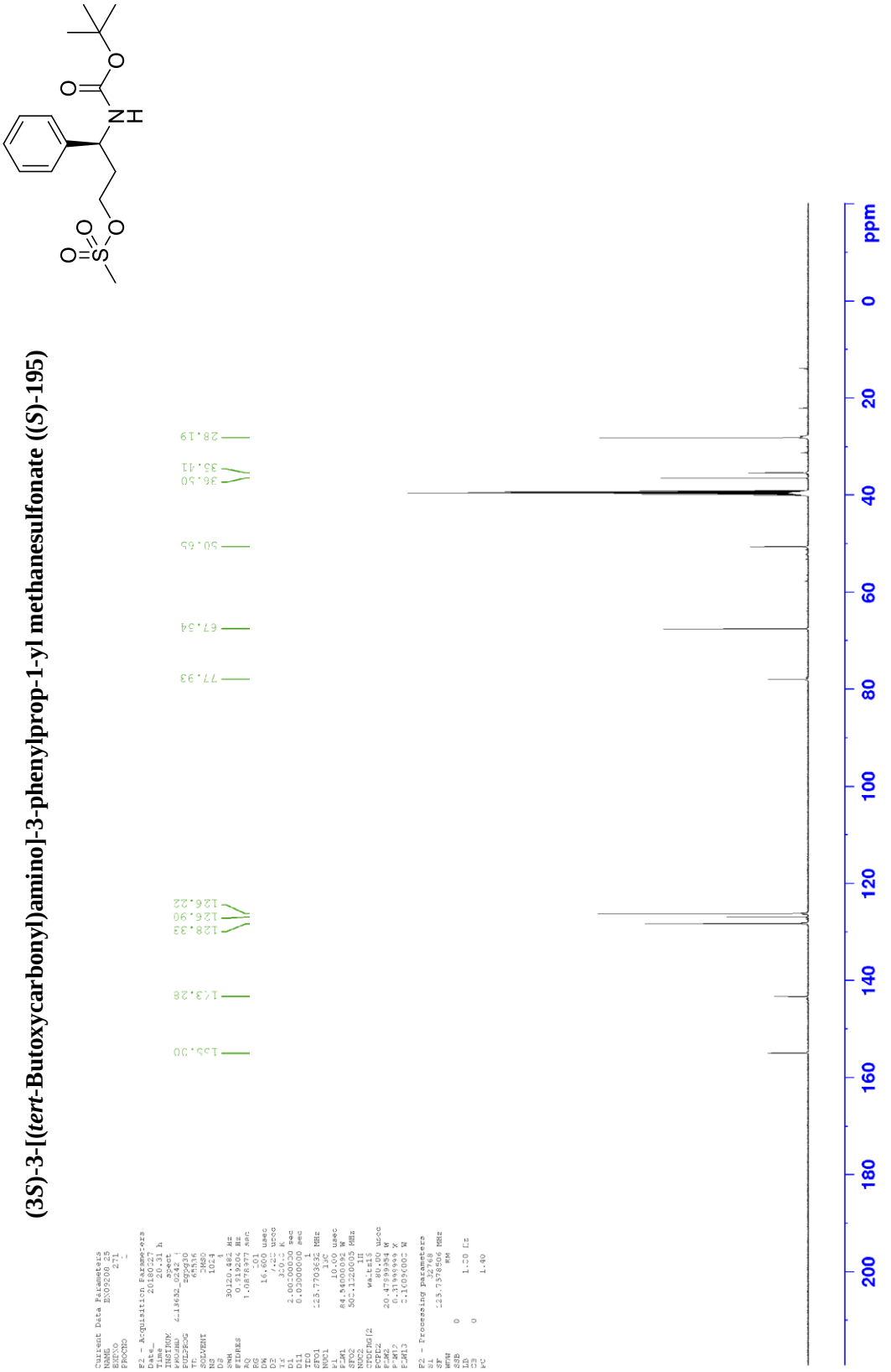
tert-Butyl [(1*R*)-3-hydroxy-1-phenylprop-1-yl]carbamate ((*R*)-194)



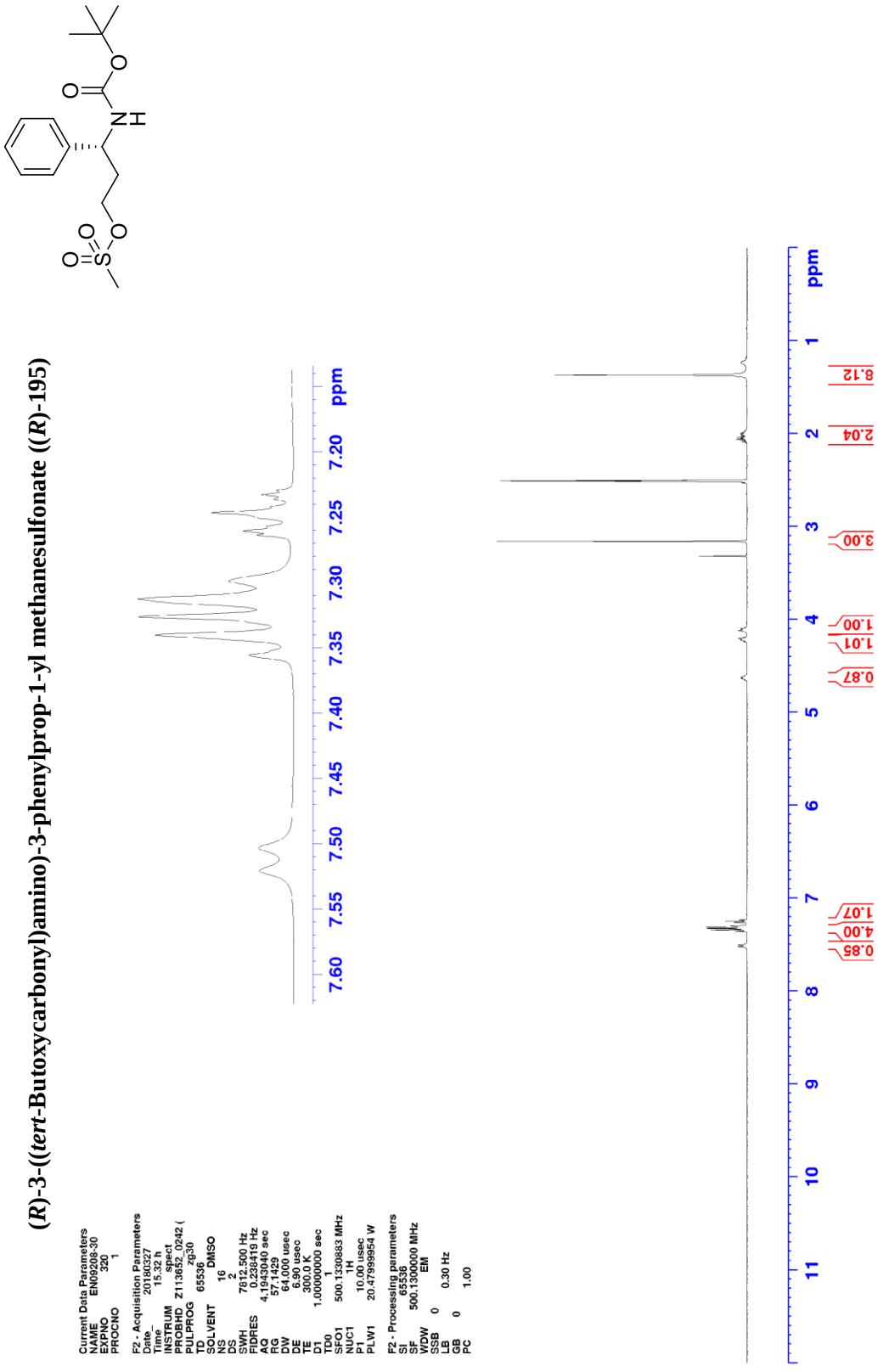
(3S)-3-[(*tert*-Butoxycarbonyl)amino]-3-phenylprop-1-yl methanesulfonate ((S)-195)



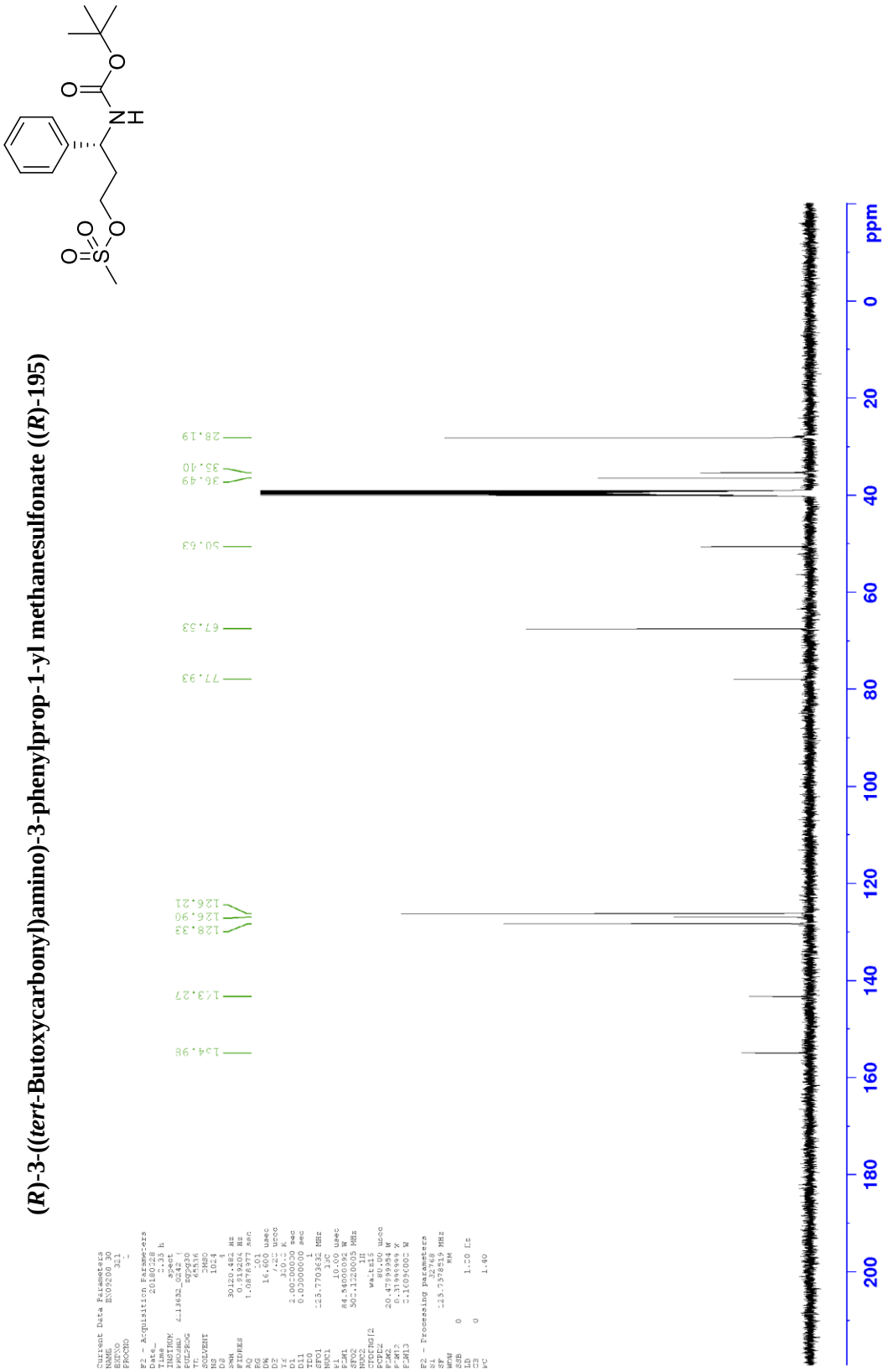
(3S)-3-[(*tert*-Butoxycarbonyl)amino]-3-phenylprop-1-yl methanesulfonate ((*S*)-195)



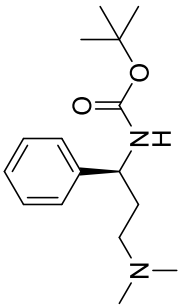
(*R*)-3-((*tert*-Butoxycarbonyl)amino)-3-phenylprop-1-yl methanesulfonate ((*R*)-195)

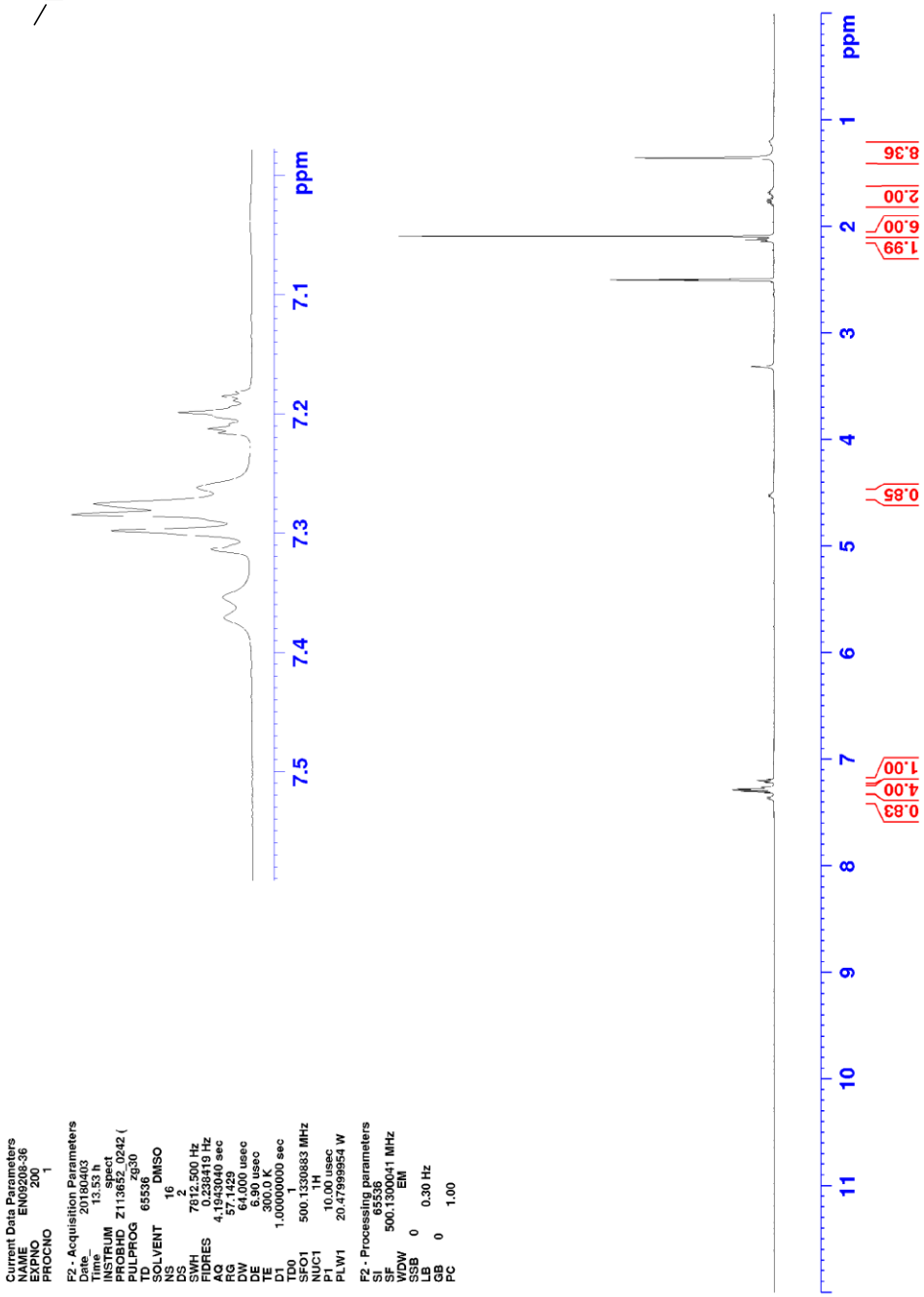


(*R*)-3-((*tert*-Butoxycarbonyl)amino)-3-phenylprop-1-yl methanesulfonate ((*R*)-195)

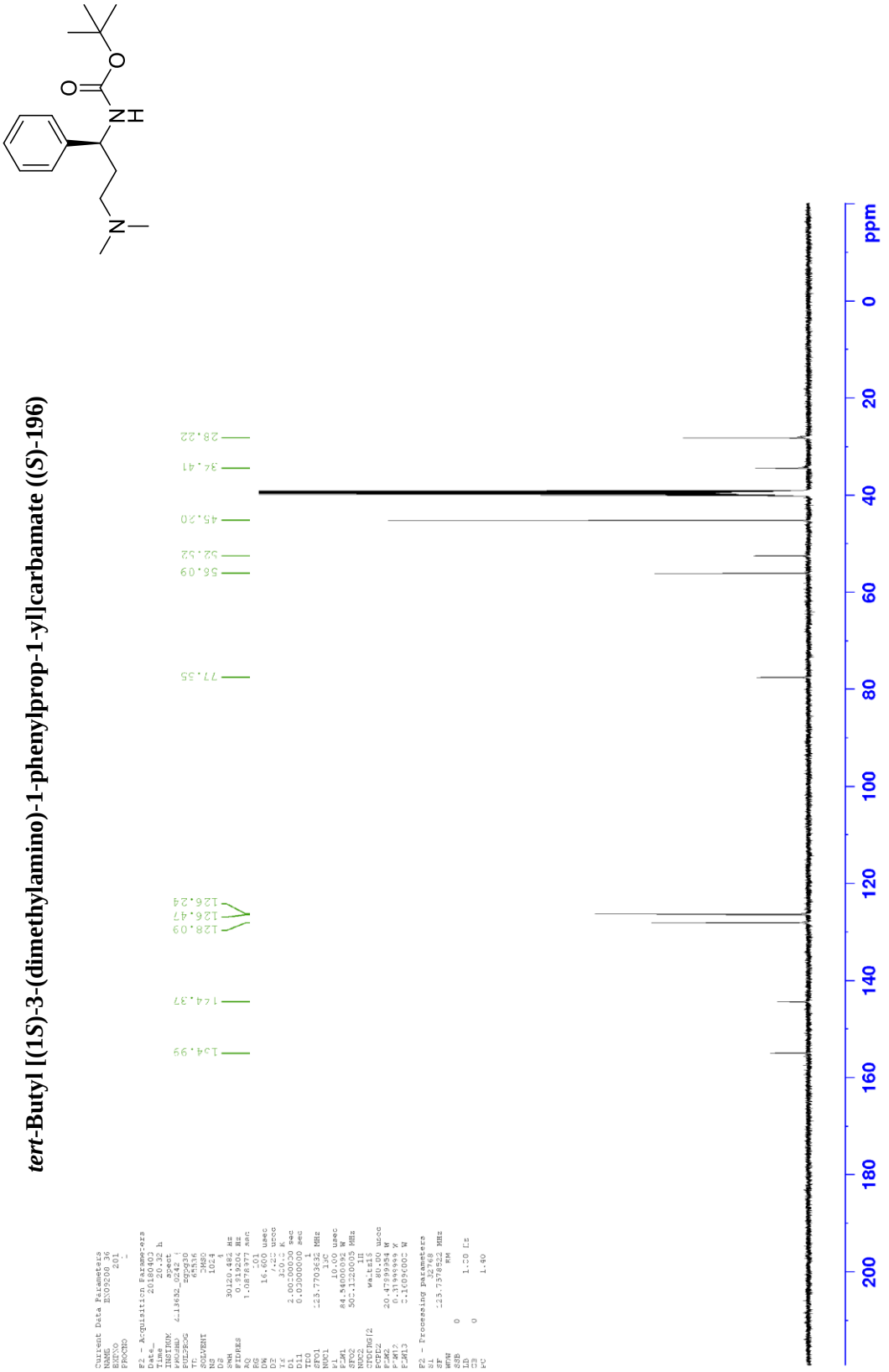


tert-Butyl [(1*S*)-3-(dimethylamino)-1-phenylprop-1-yl]carbamate ((*S*)-196)

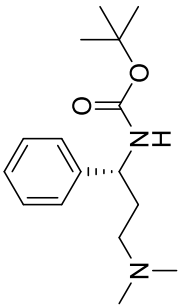


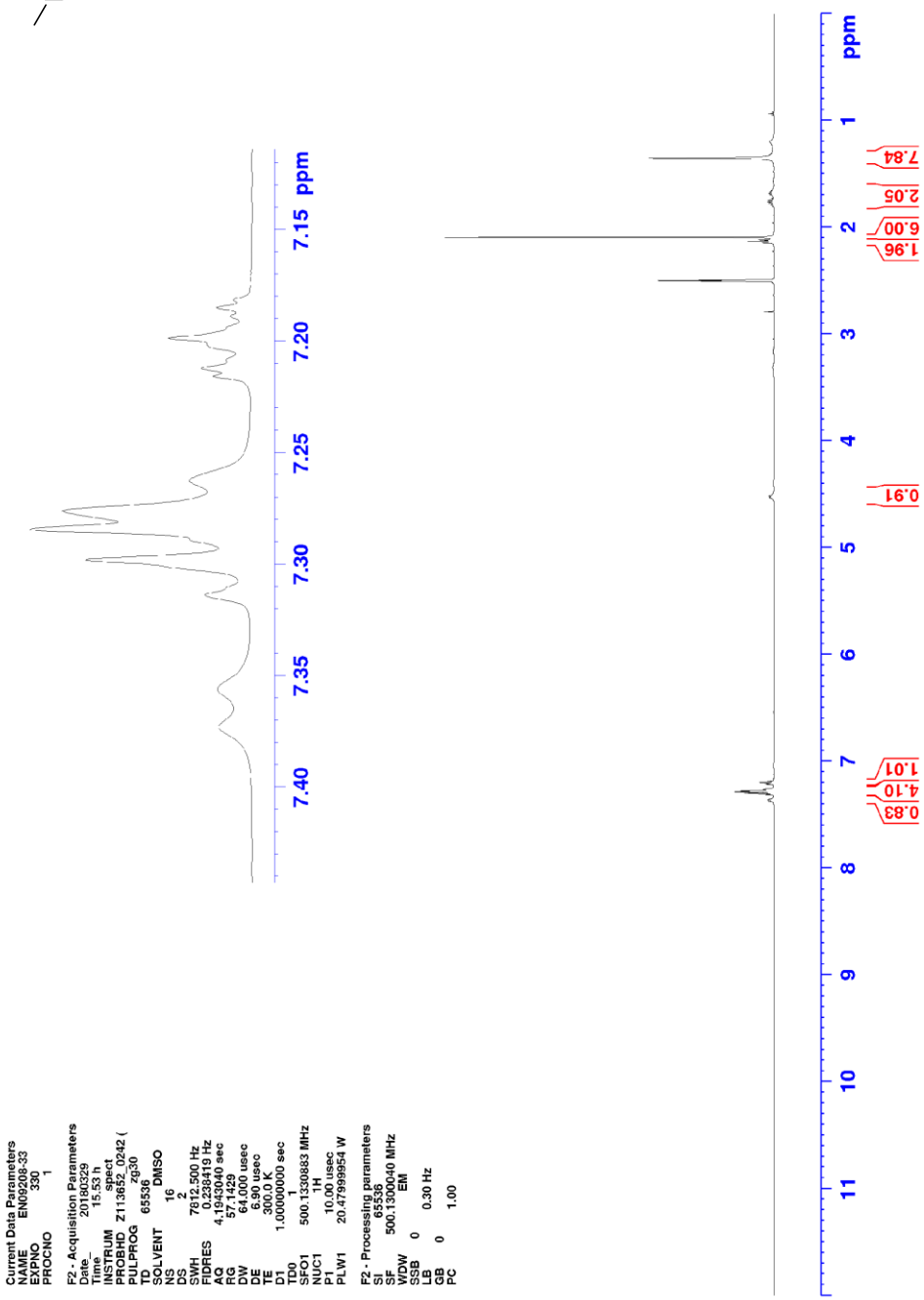


tert-Butyl [(1S)-3-(dimethylamino)-1-phenylprop-1-yl]carbamate ((S)-196)

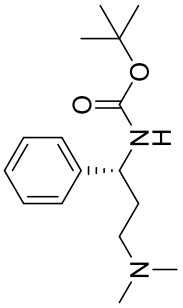


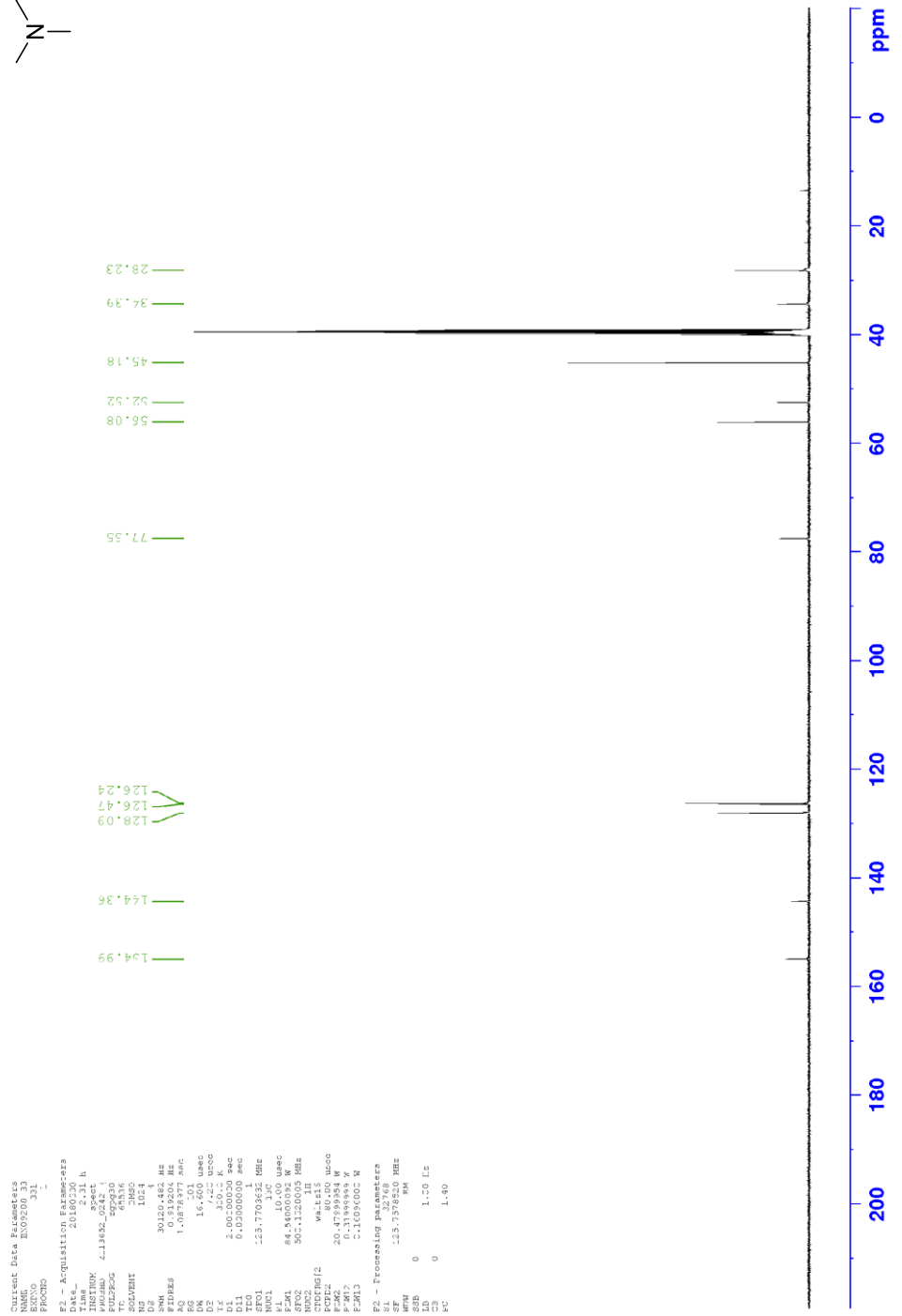
tert-Butyl [(1*R*)-3-(dimethylamino)-1-phenylprop-1-yl]carbamate ((*R*)-196)

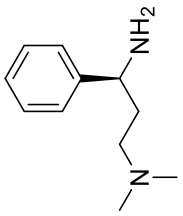




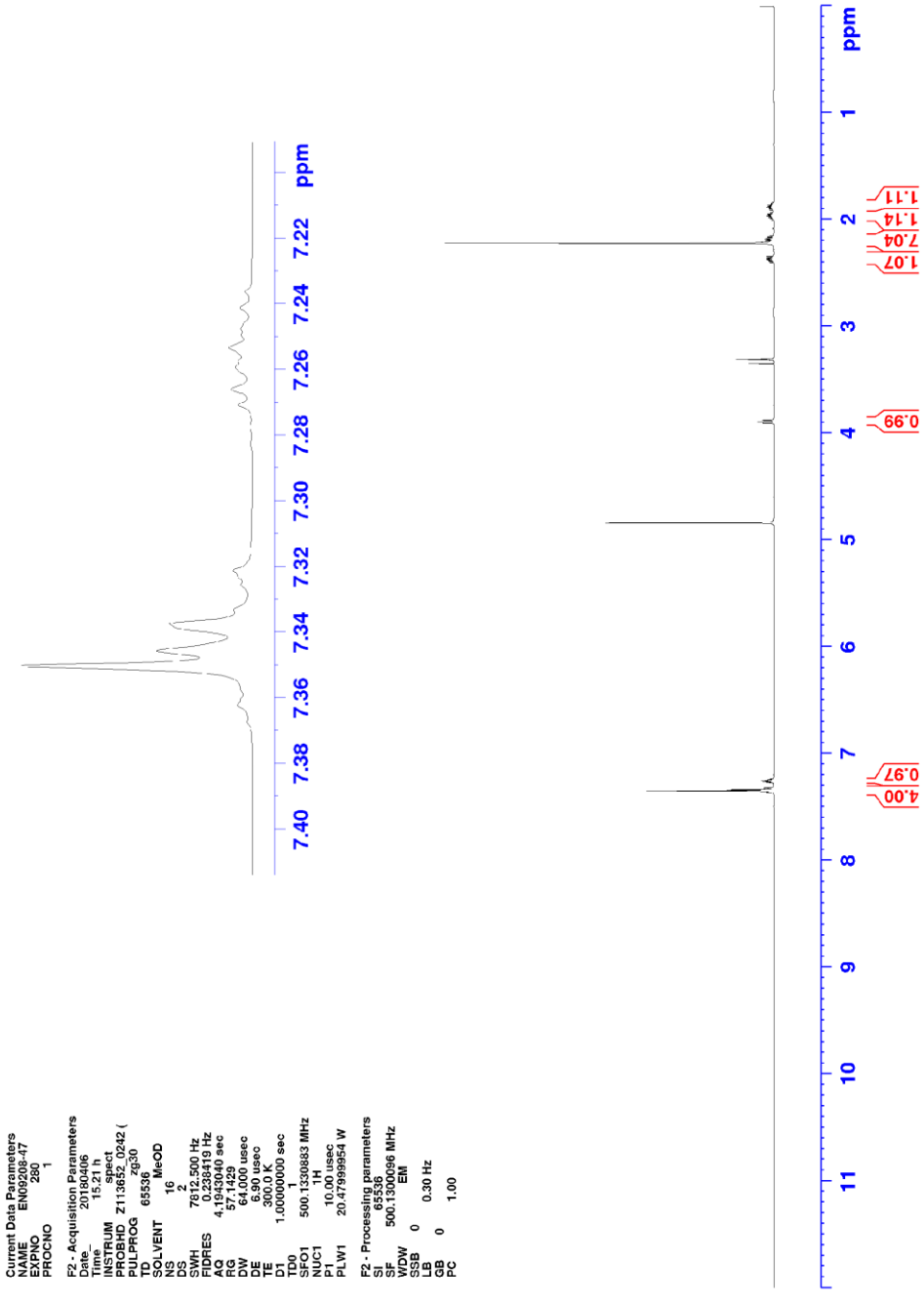
***tert*-Butyl [(1*R*)-3-(dimethylamino)-1-phenylprop-1-yl]carbamate ((*R*)-196)**

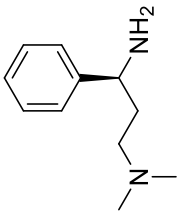




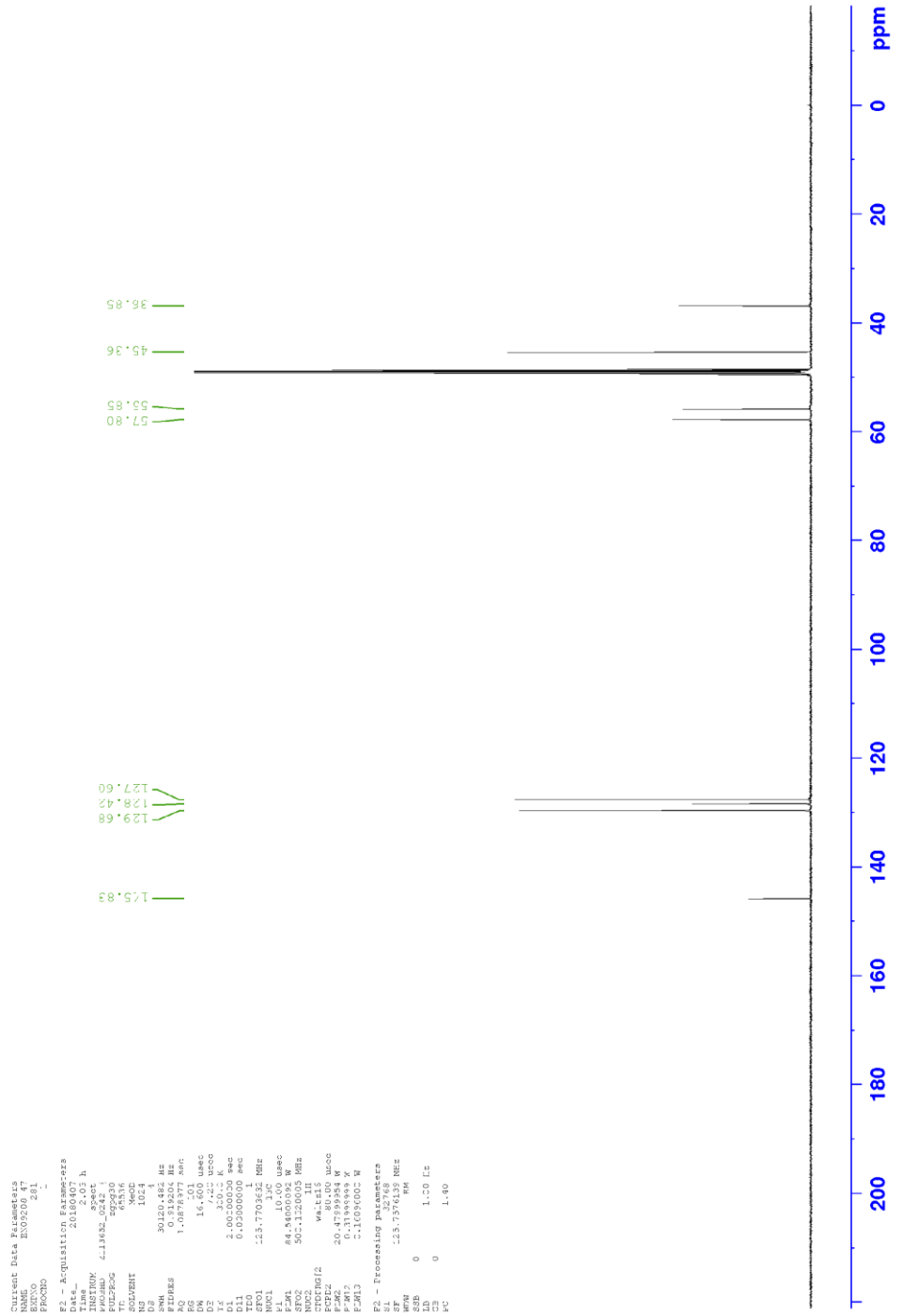


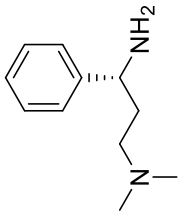
(1S)-N^3,N^3-Dimethyl-1-phenylpropane-1,3-diamine ((*S*)-186)



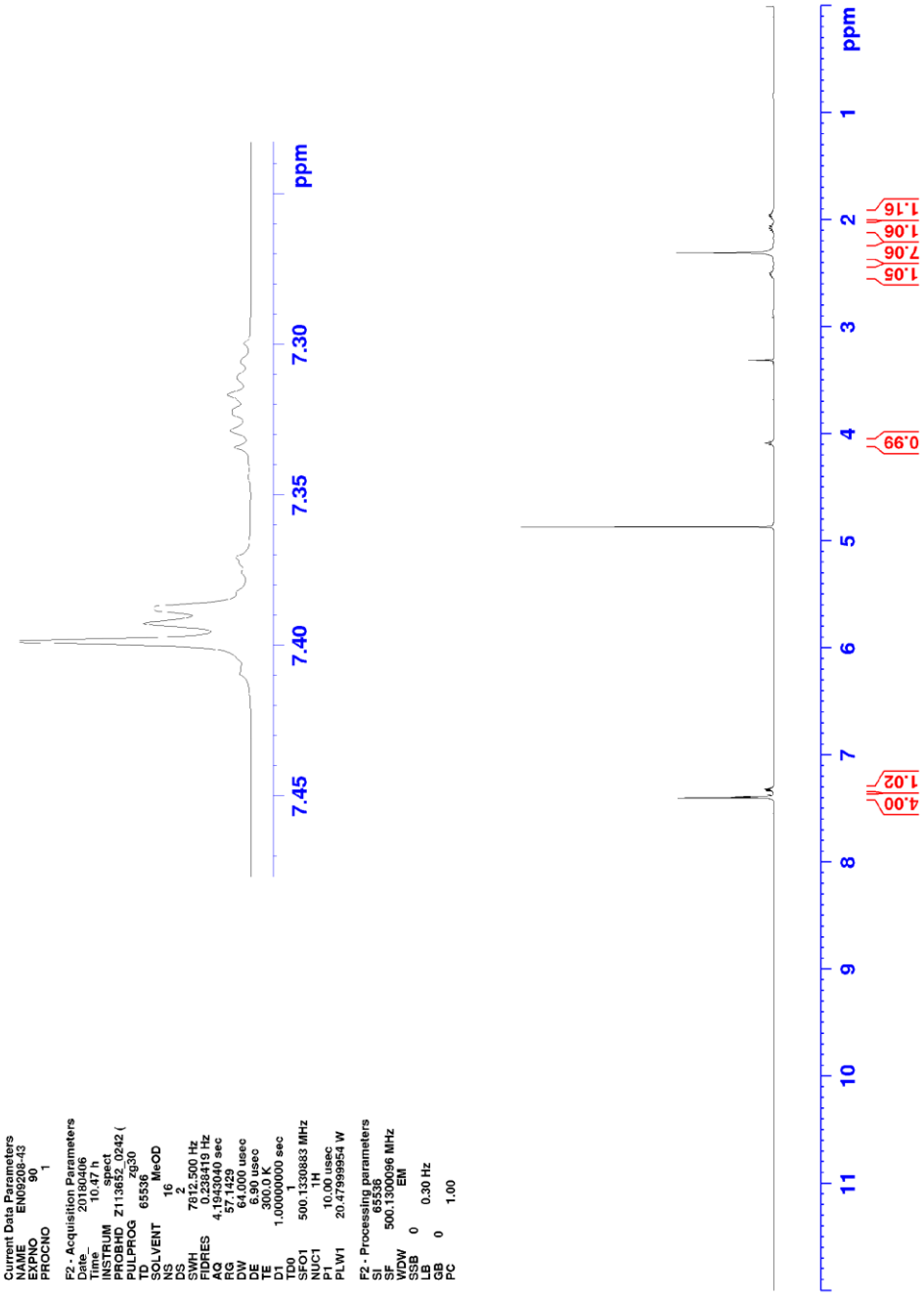


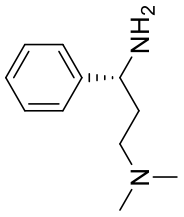
(1S)-N^3,N^3-Dimethyl-1-phenylpropane-1,3-diamine ((*S*)-186)



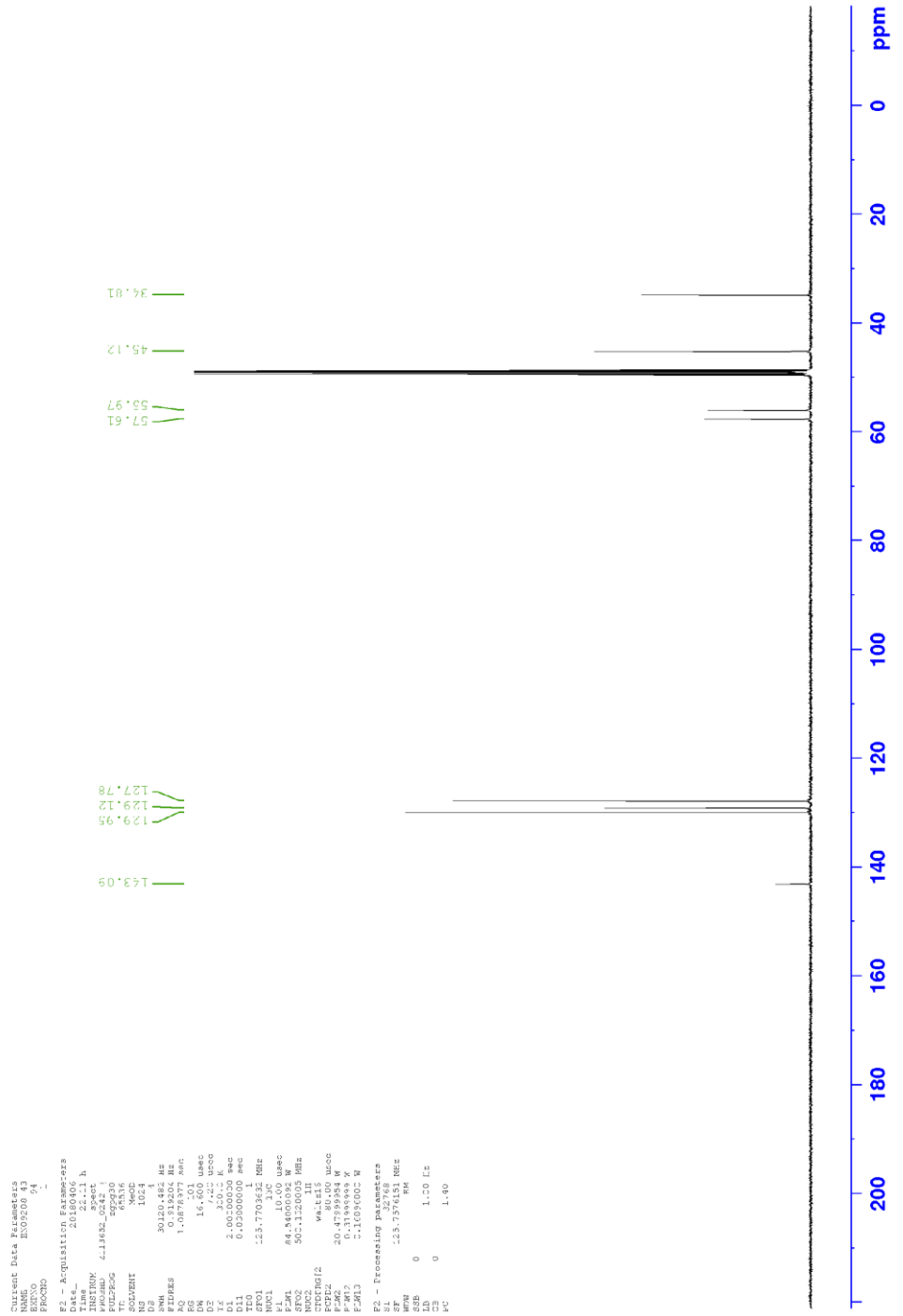


(1*R*)-N^3,N^3-Dimethyl-1-phenylpropane-1,3-diamine ((*R*)-186)

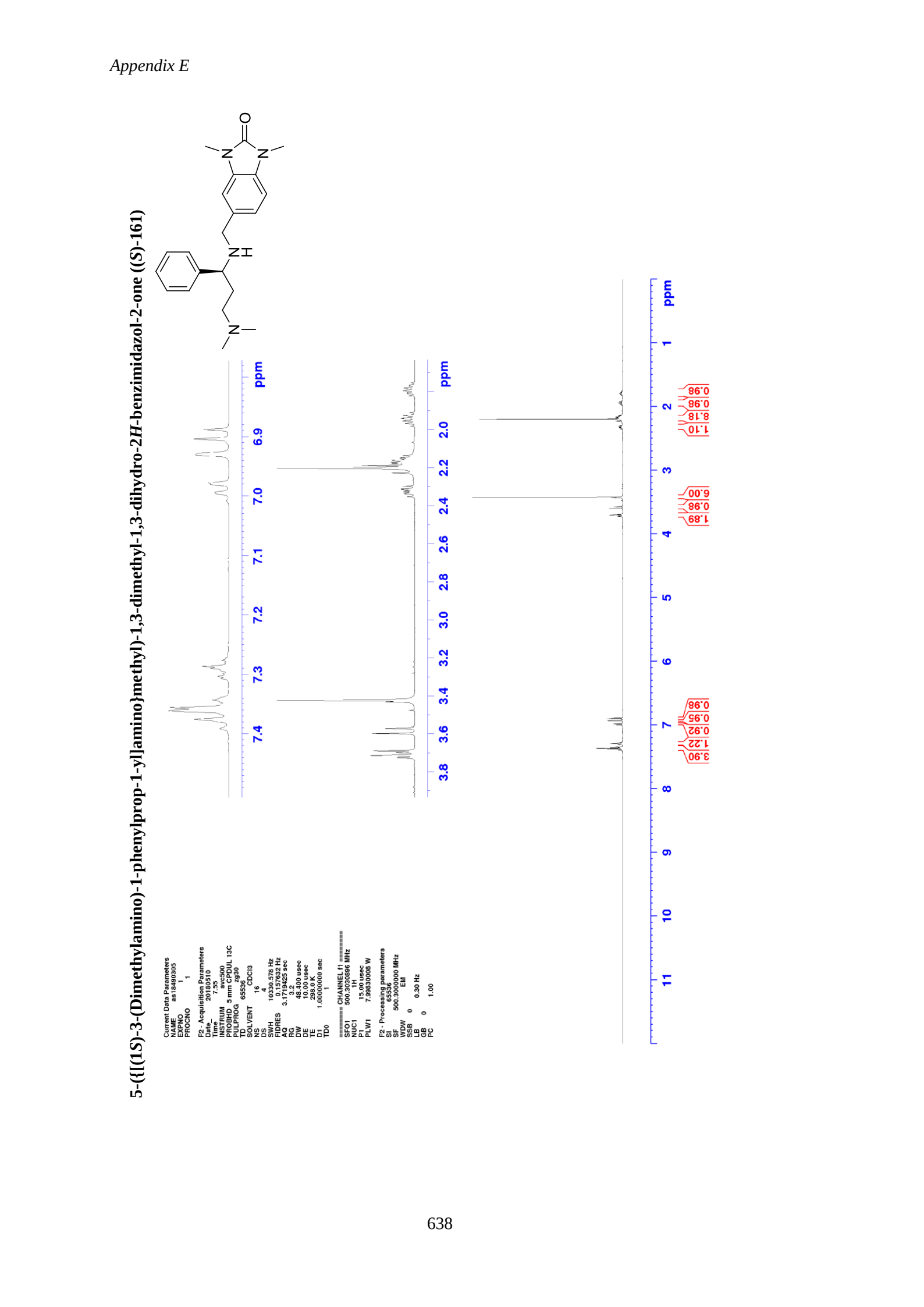




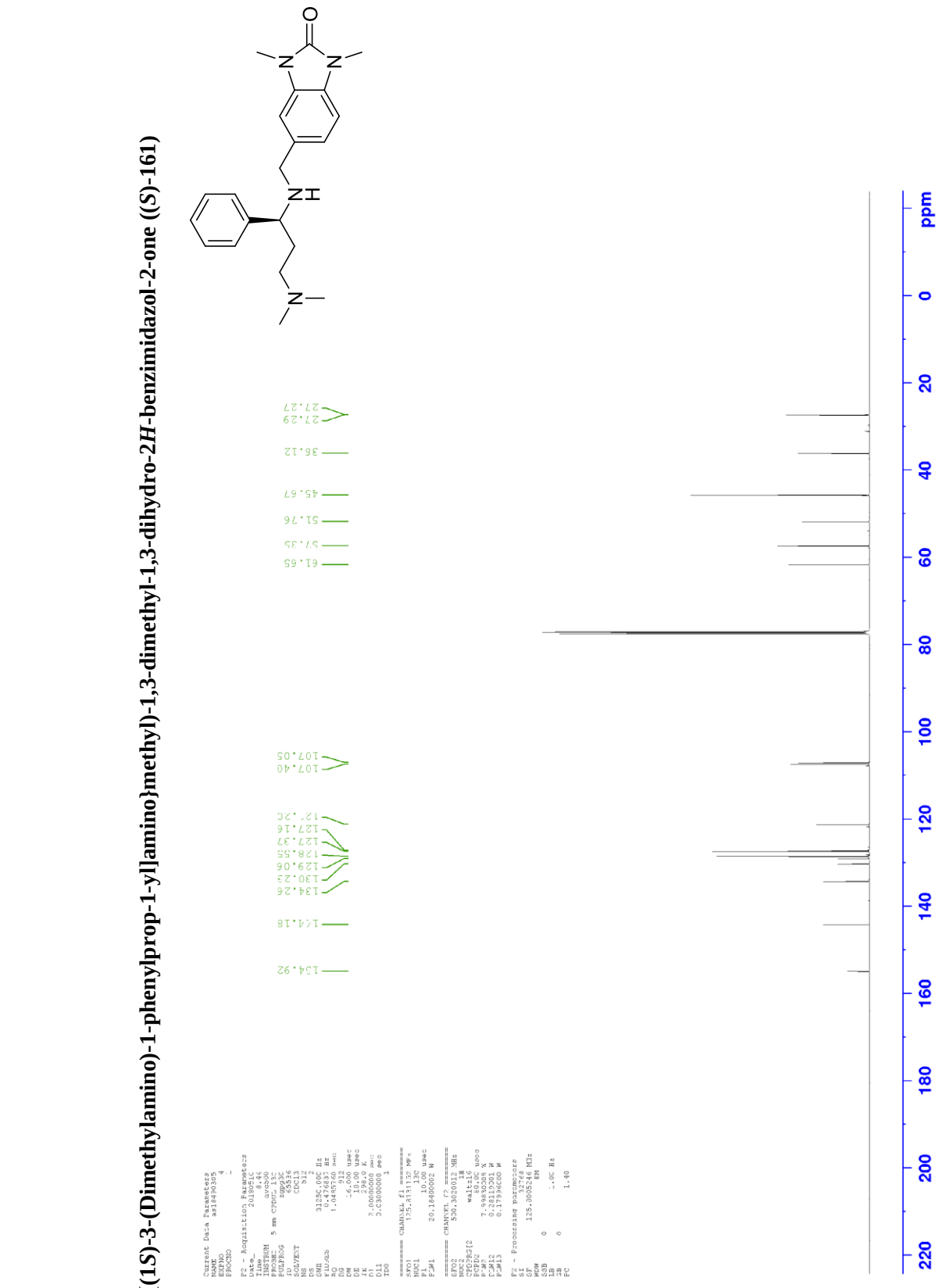
(1*R*)-N^3,N^3-Dimethyl-1-phenylpropane-1,3-diamine ((*R*)-186)



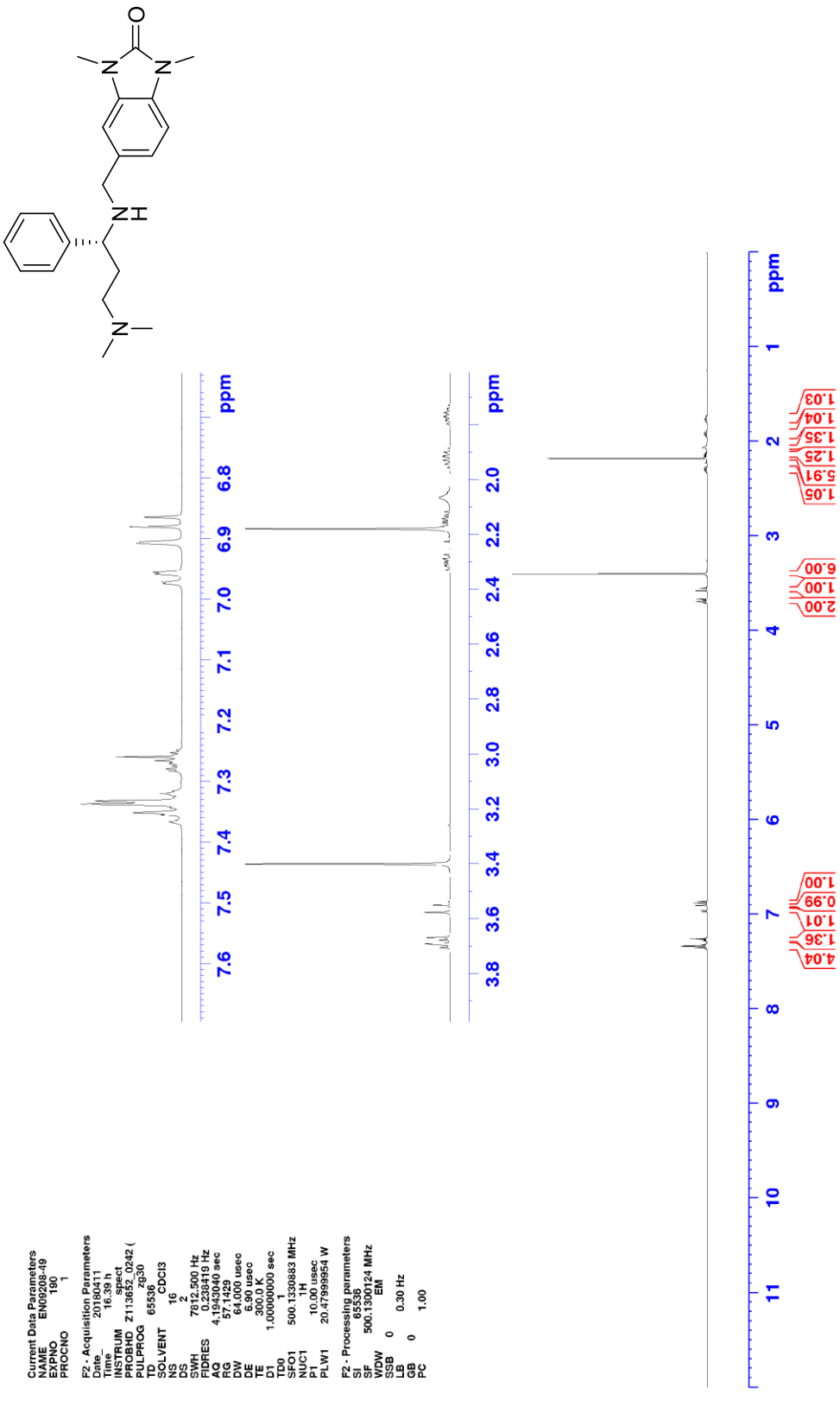
5-({[(1*S*)-3-(Dimethylamino)-1-phenylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*S*)-161)



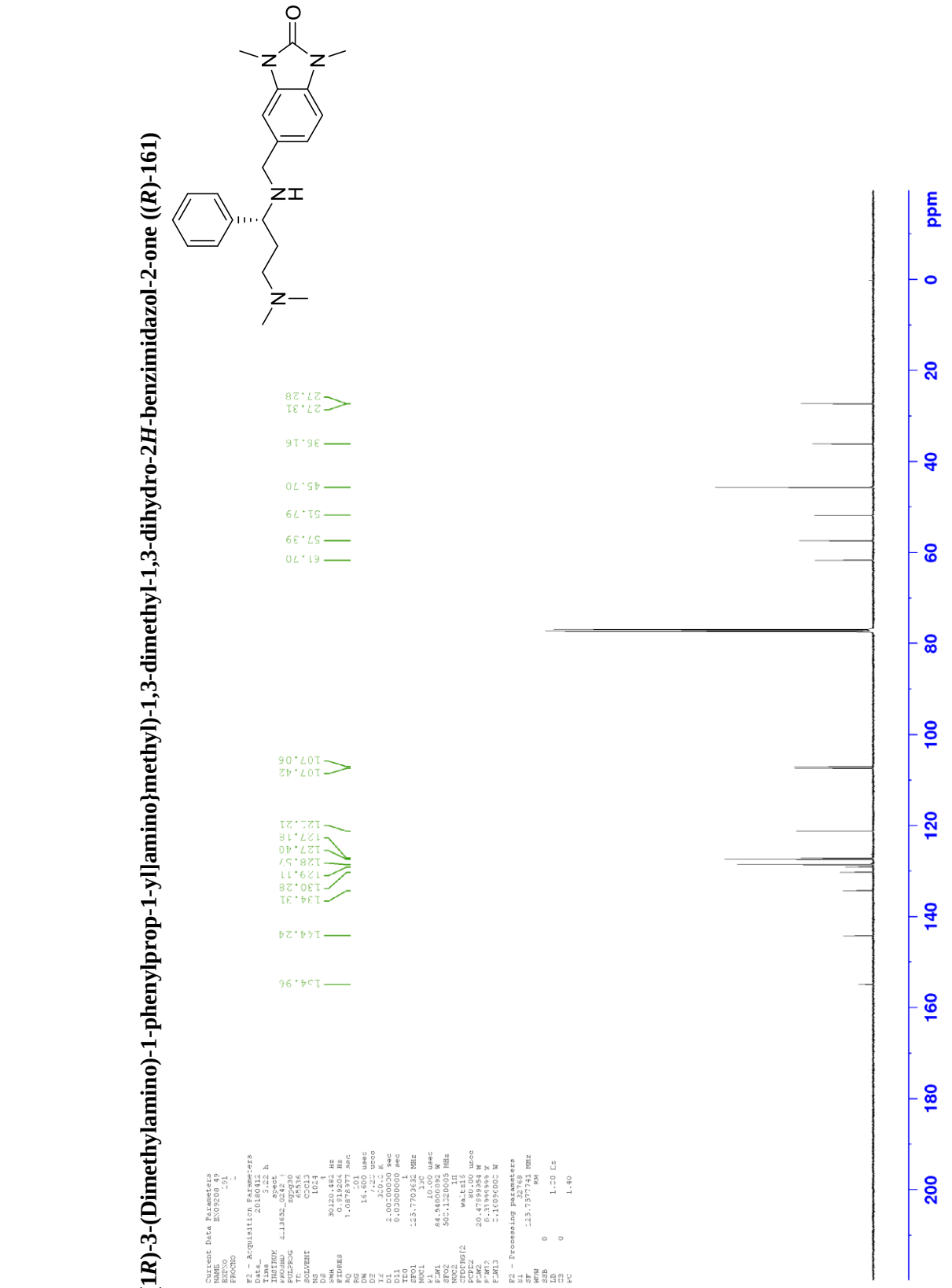
5-({[(1*S*)-3-(Dimethylamino)-1-phenylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*S*)-161)

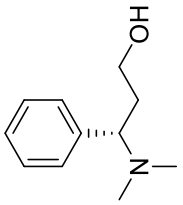


5-({[(1*R*)-3-(Dimethylamino)-1-phenylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*R*)-161)

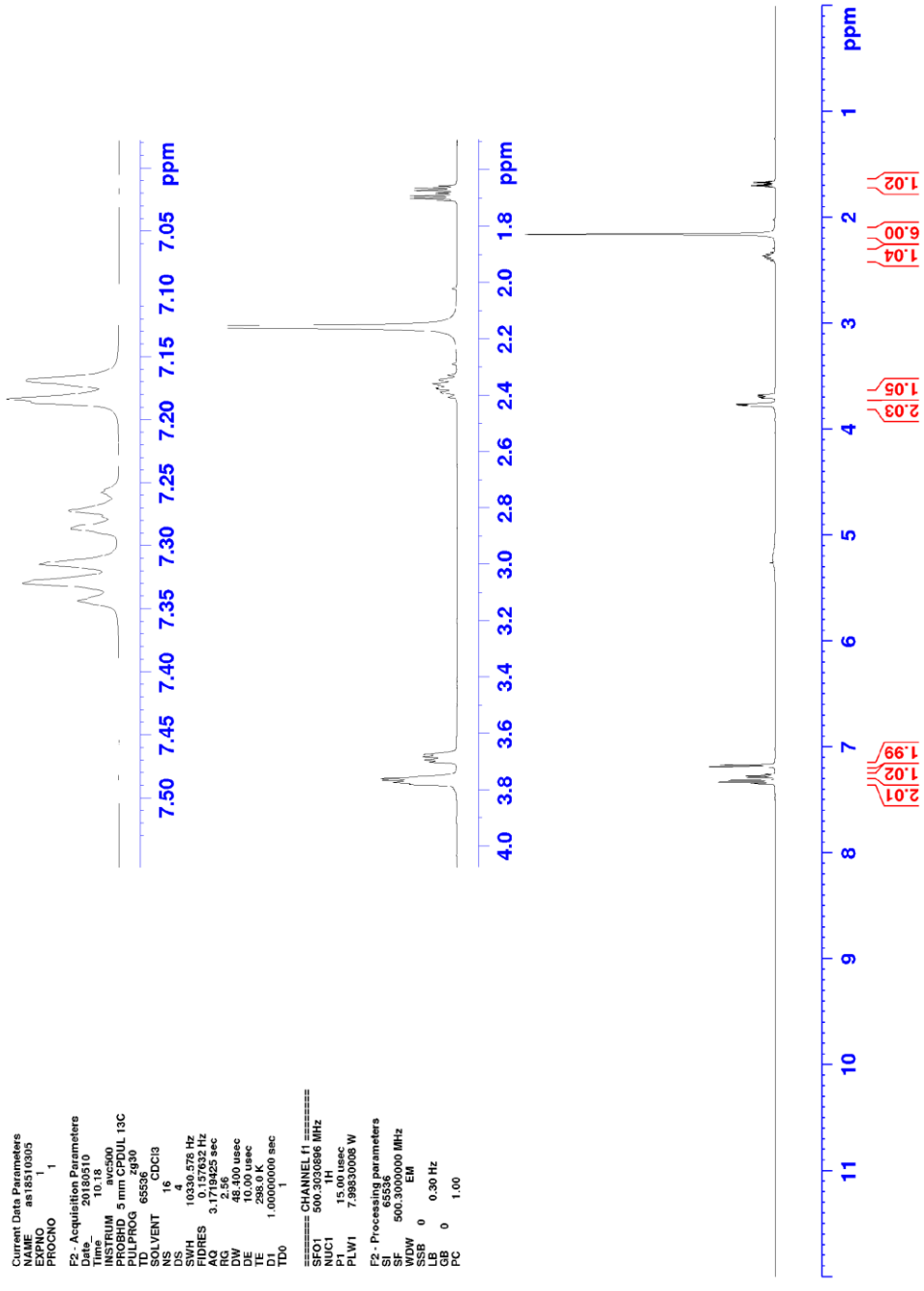


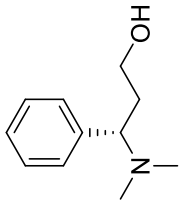
5-({[(1*R*)-3-(Dimethylamino)-1-phenylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*R*)-161)



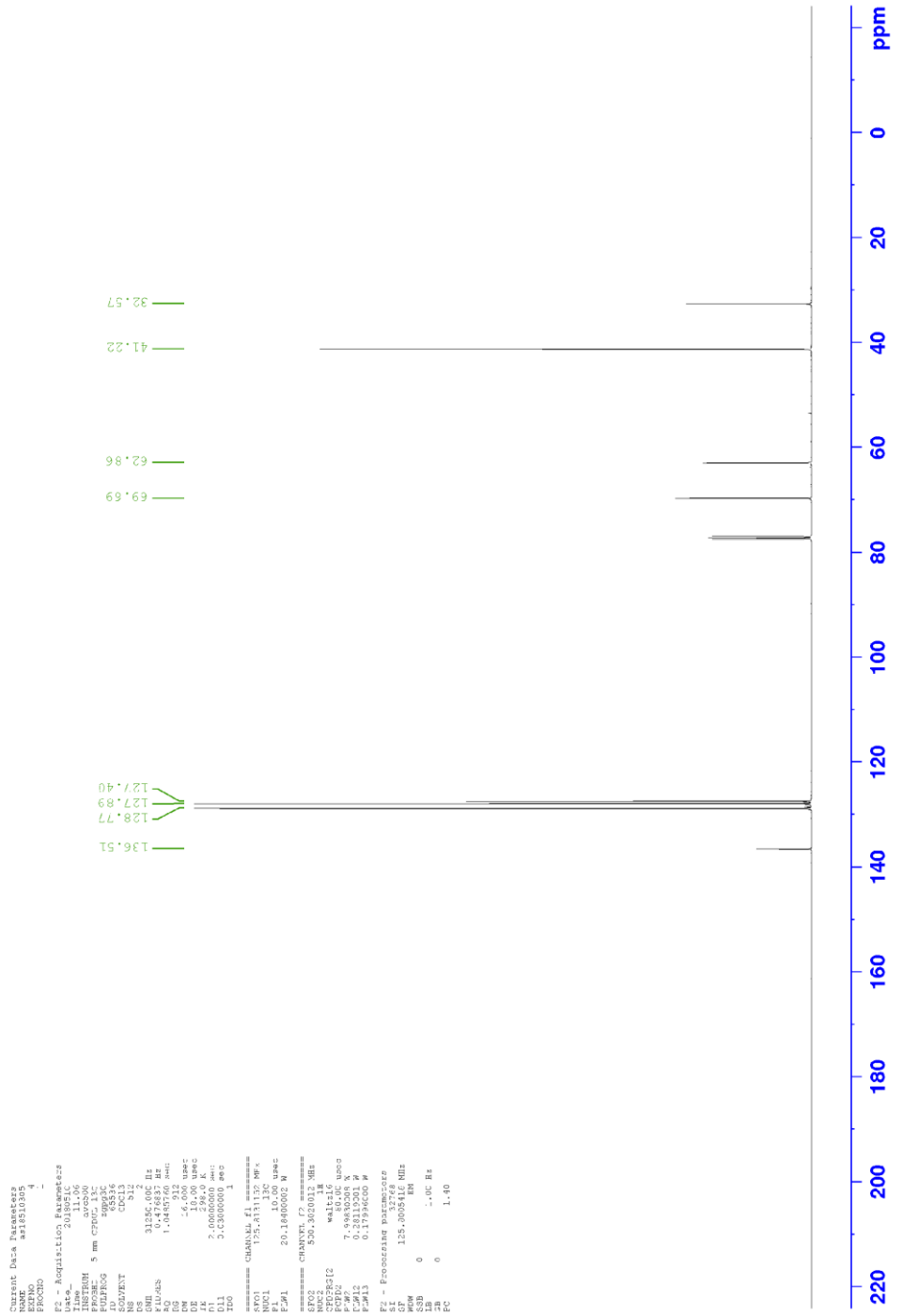


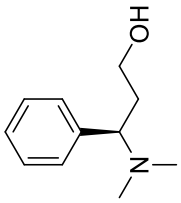
(3S)-3-(Dimethylamino)-3-phenylpropan-1-ol ((S)-197)



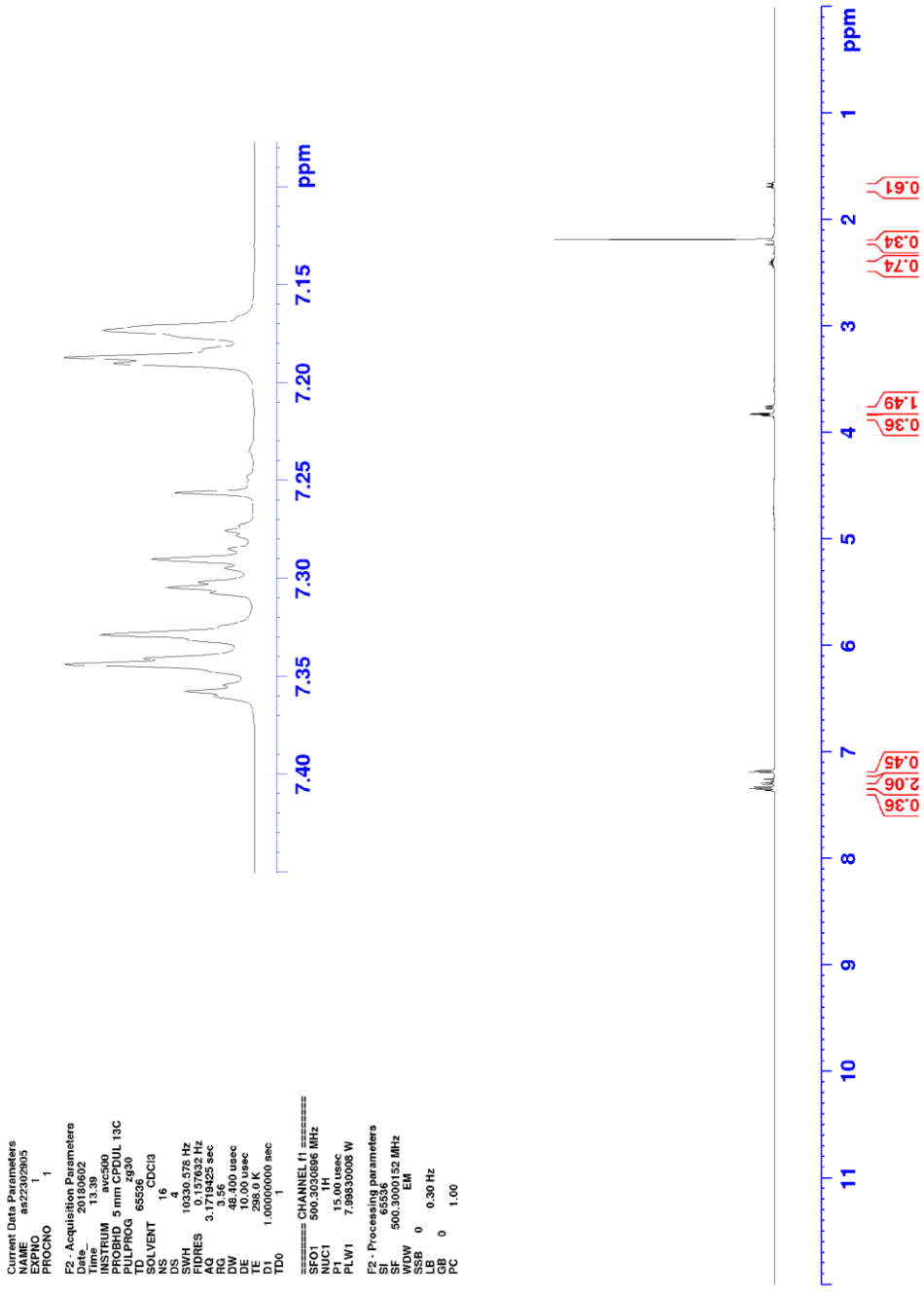


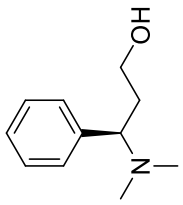
(3*S*)-3-(Dimethylamino)-3-phenylpropan-1-ol ((*S*)-197)



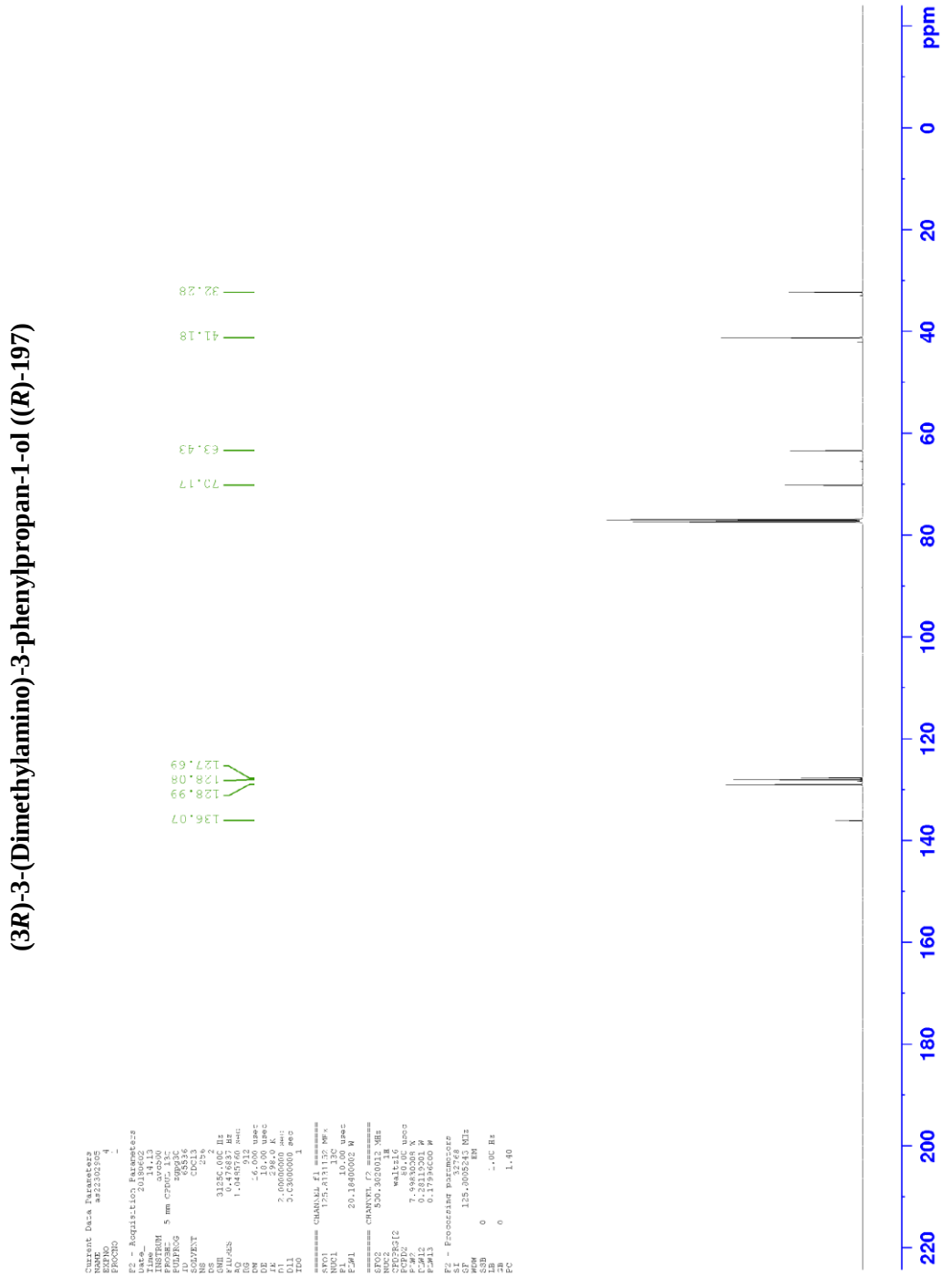


(3*R*)-3-(Dimethylamino)-3-phenylpropan-1-ol ((*R*)-197)

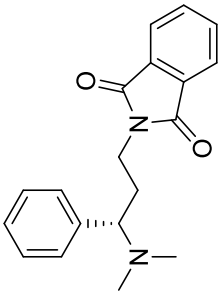


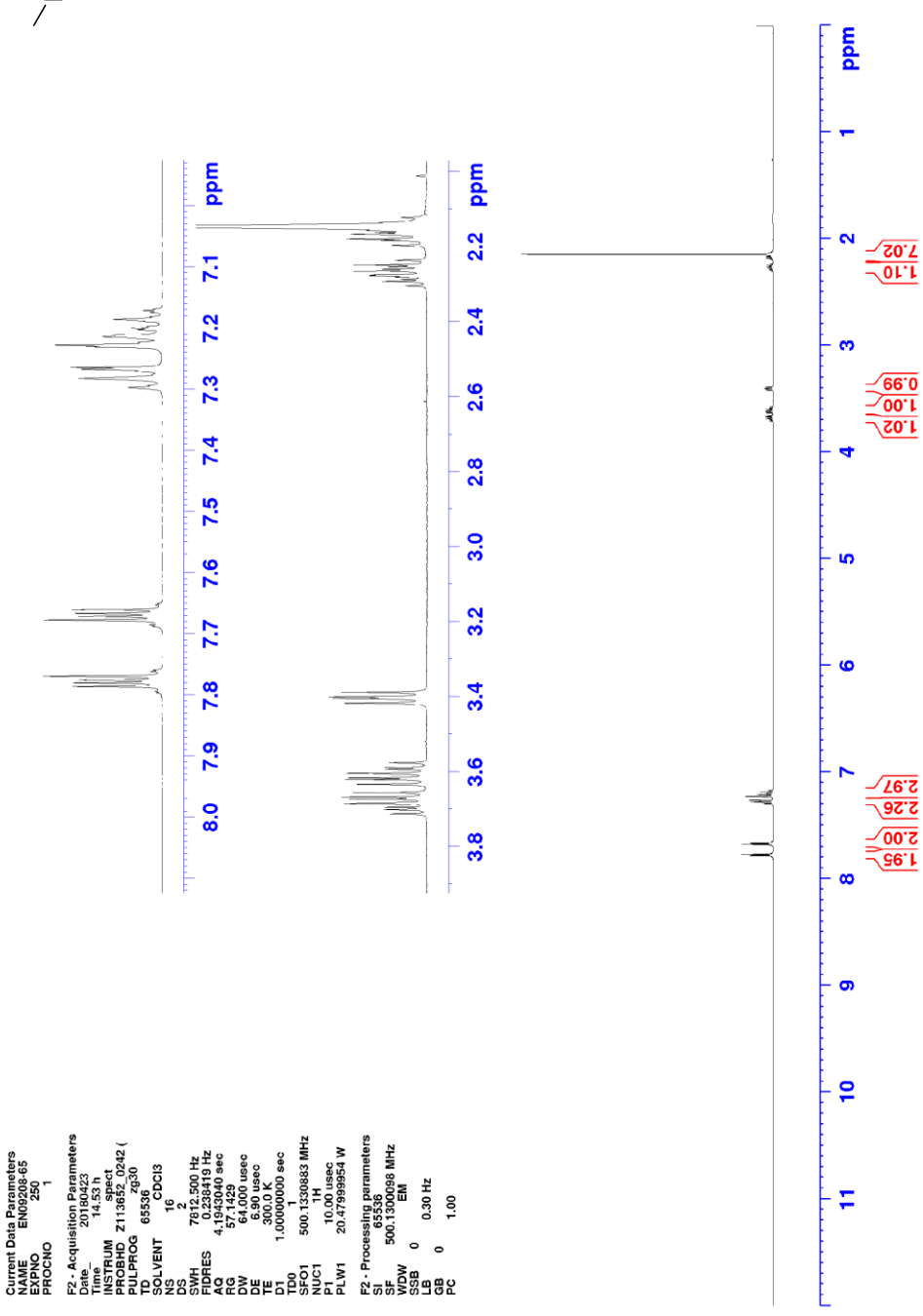


(3*R*)-3-(Dimethylamino)-3-phenylpropan-1-ol ((*R*)-197)

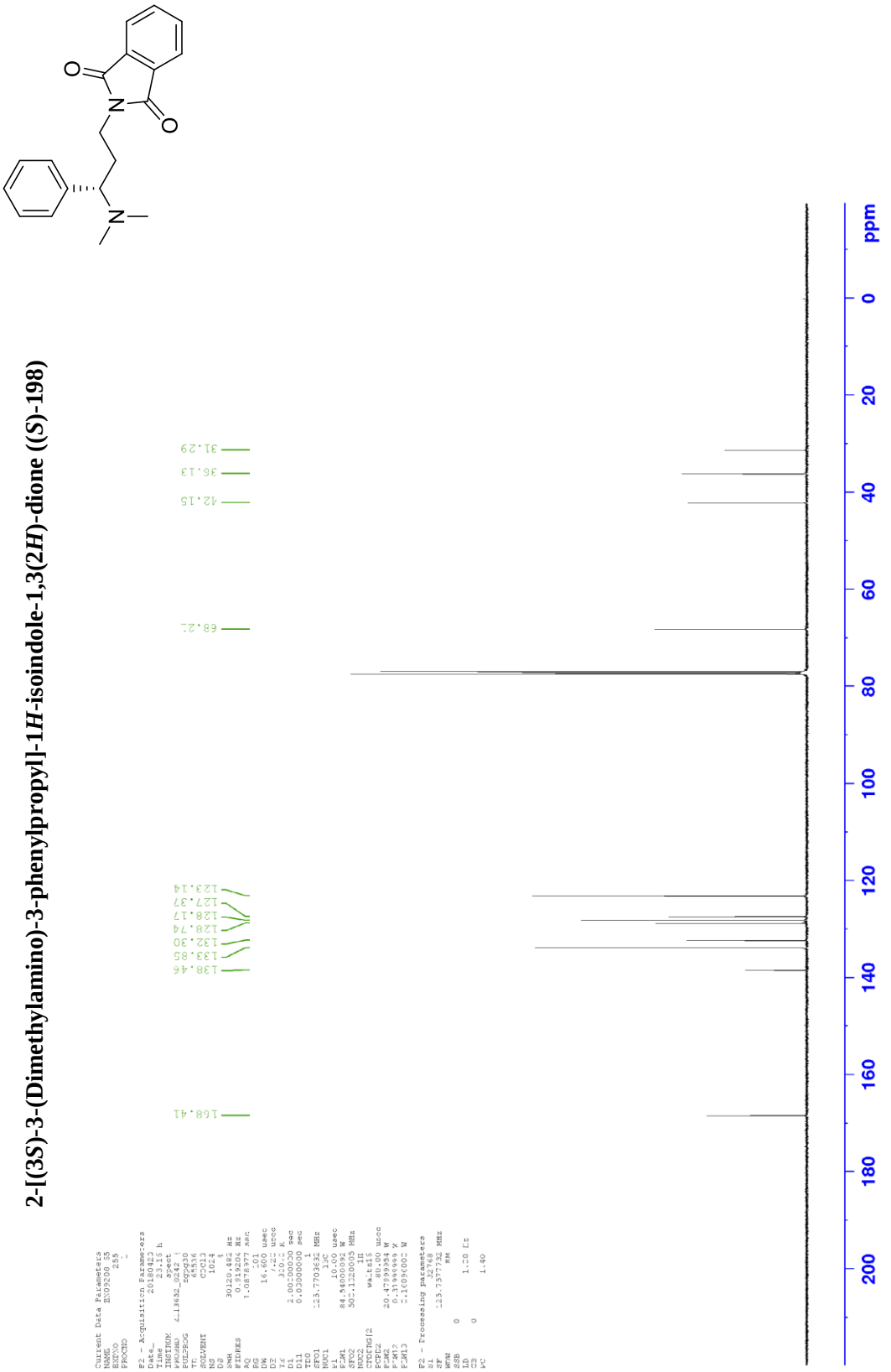


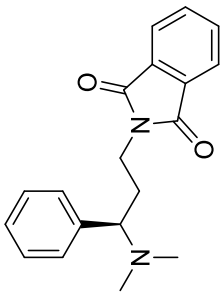
2-[(3S)-3-(Dimethylamino)-3-phenylpropyl]-1*H*-isoindole-1,3(2*H*)-dione ((*S*)-198)



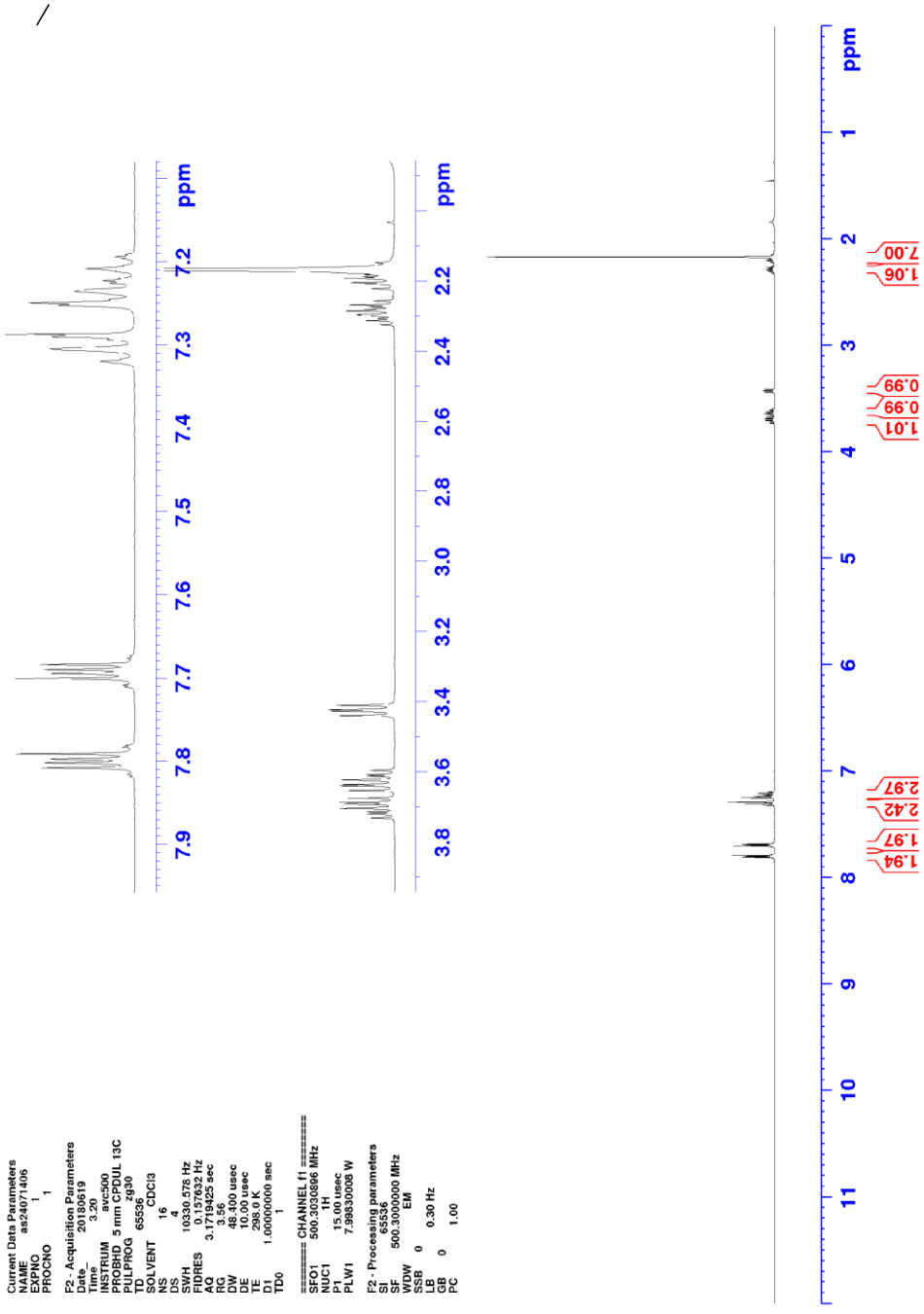


2-[(3*S*)-3-(Dimethylamino)-3-phenylpropyl]-1*H*-isoindole-1,3(2*H*)-dione ((*S*)-198)

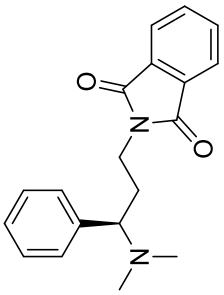


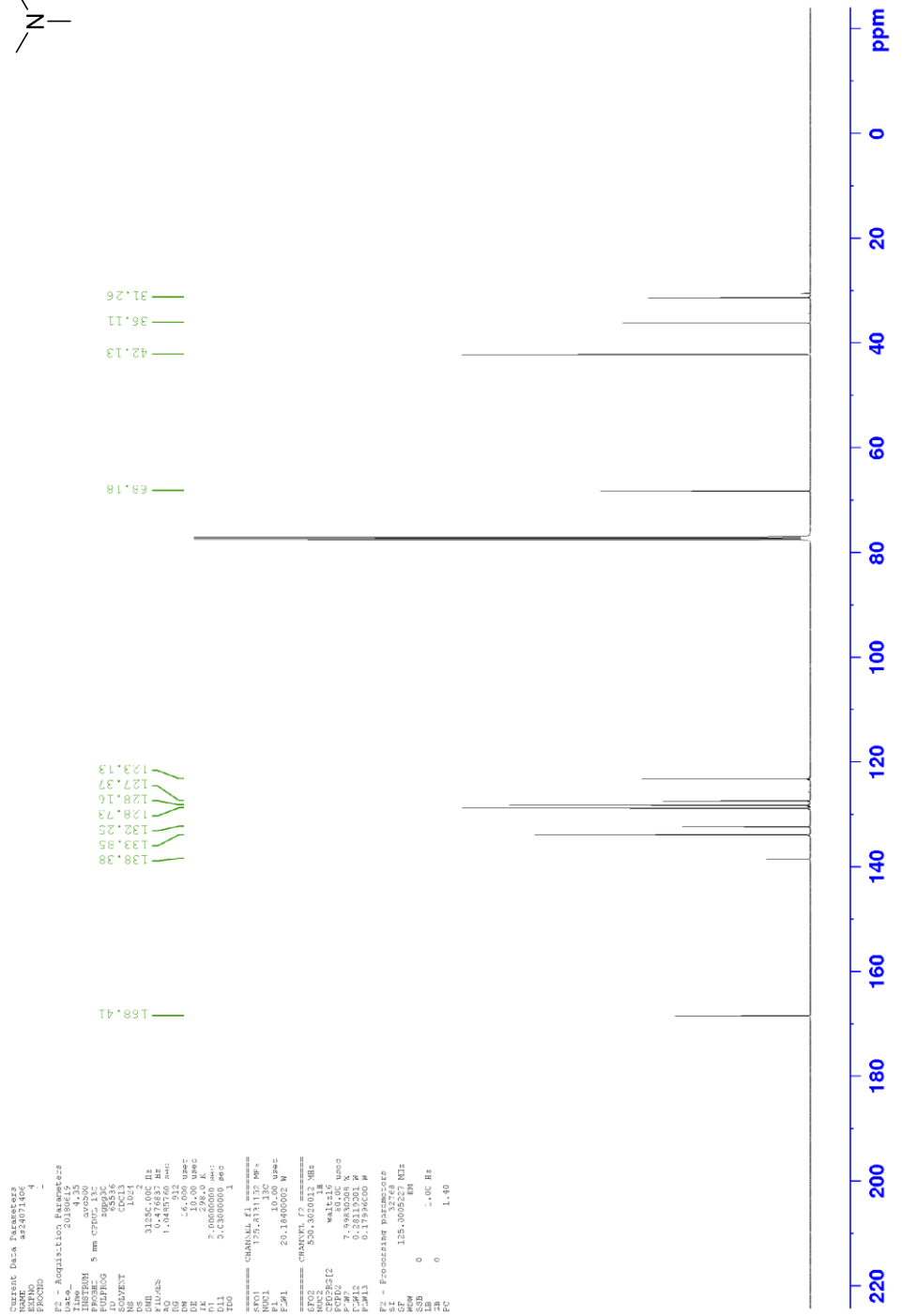


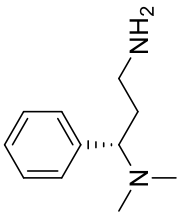
2-[(3*R*)-3-(Dimethylamino)-3-phenylpropyl]-1*H*-isoindole-1,3(2*H*)-dione ((*R*)-198)



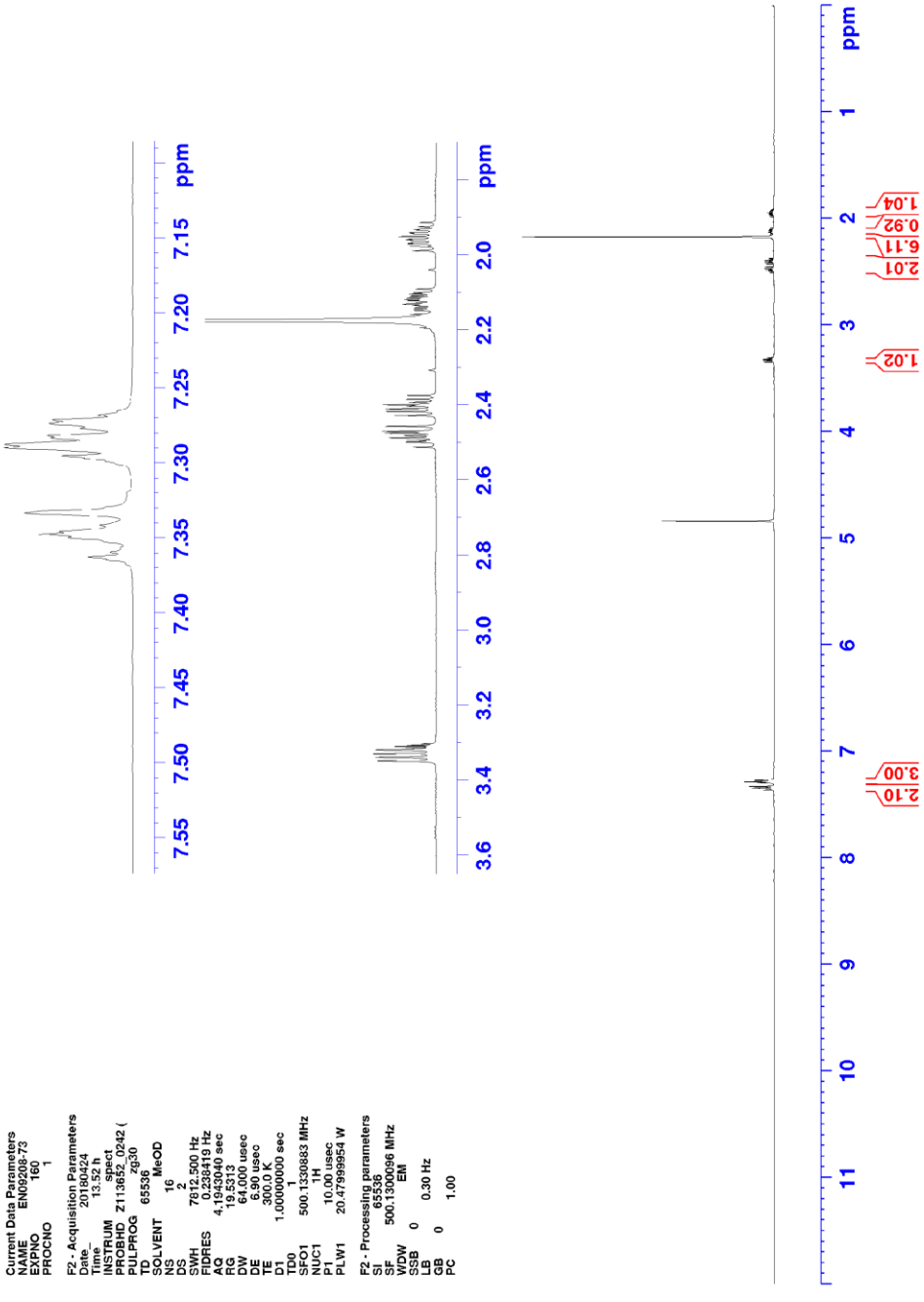
2-[(3*R*)-3-(Dimethylamino)-3-phenylpropyl]-1*H*-isoindole-1,3(2*H*)-dione ((*R*)-198)



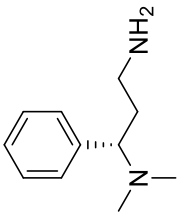


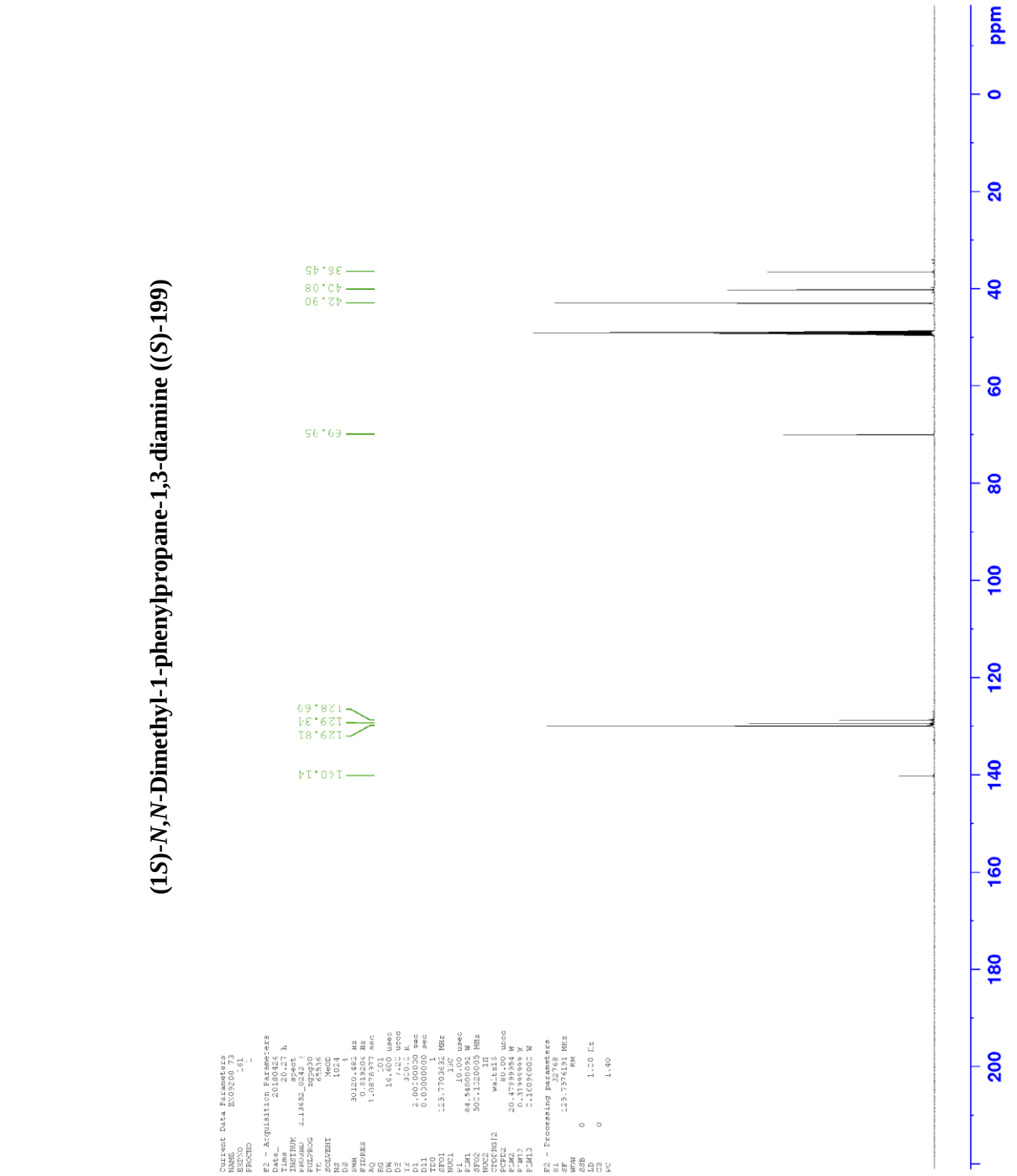


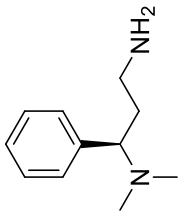
(1S)-*N*,*N*-Dimethyl-1-phenylpropane-1,3-diamine ((S)-199)



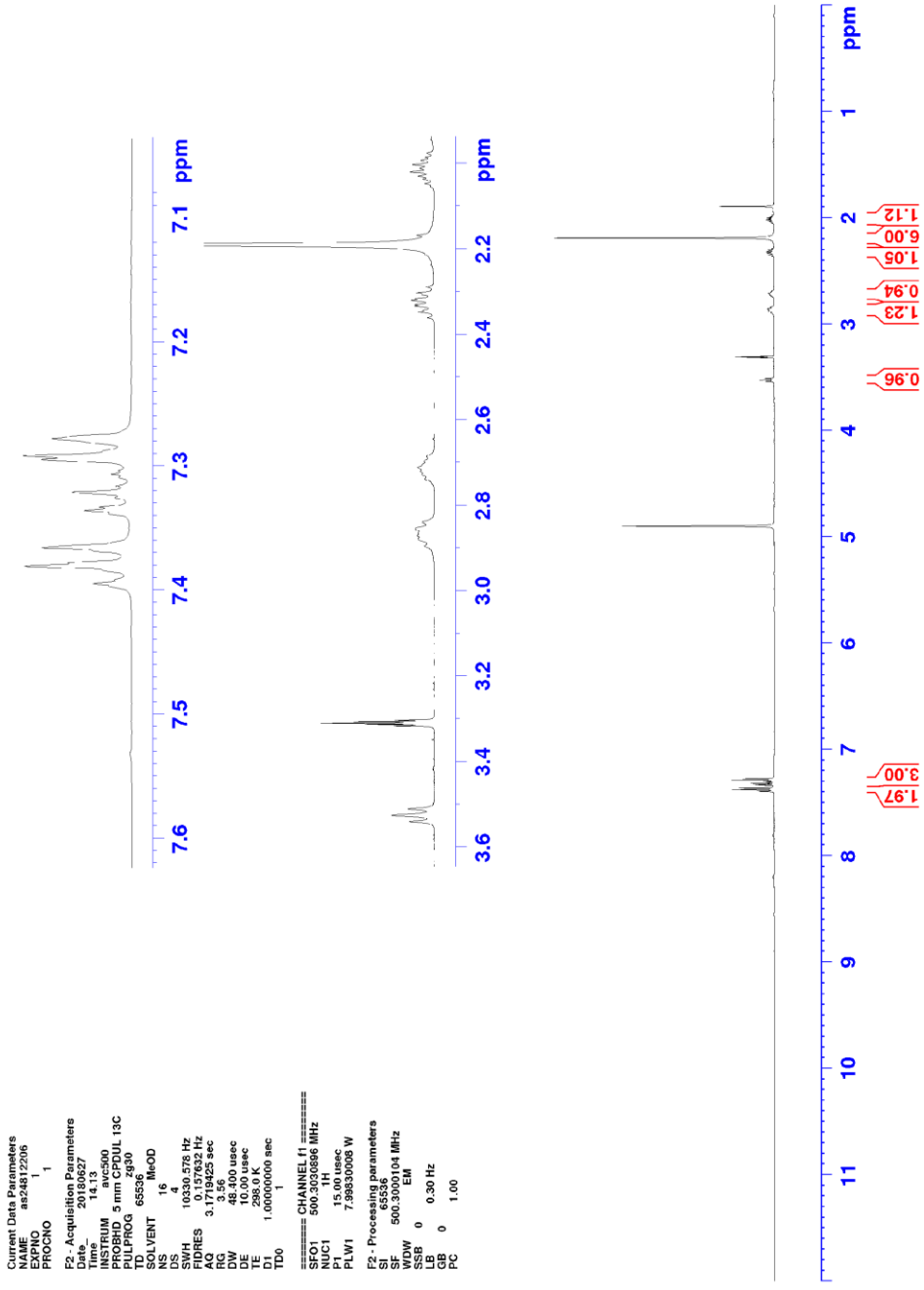
(1*S*)-*N*,*N*-Dimethyl-1-phenylpropane-1,3-diamine ((*S*)-199)



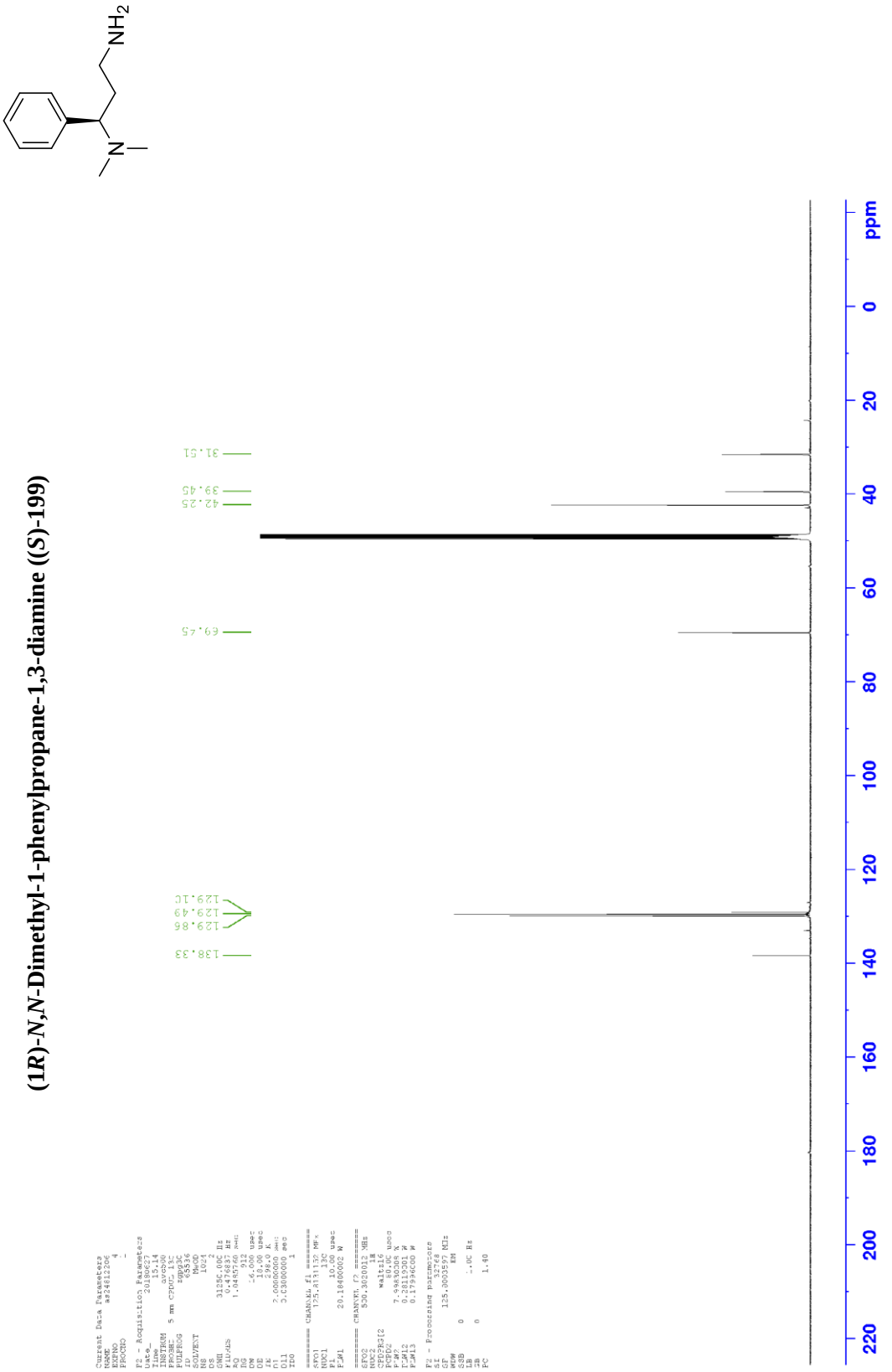




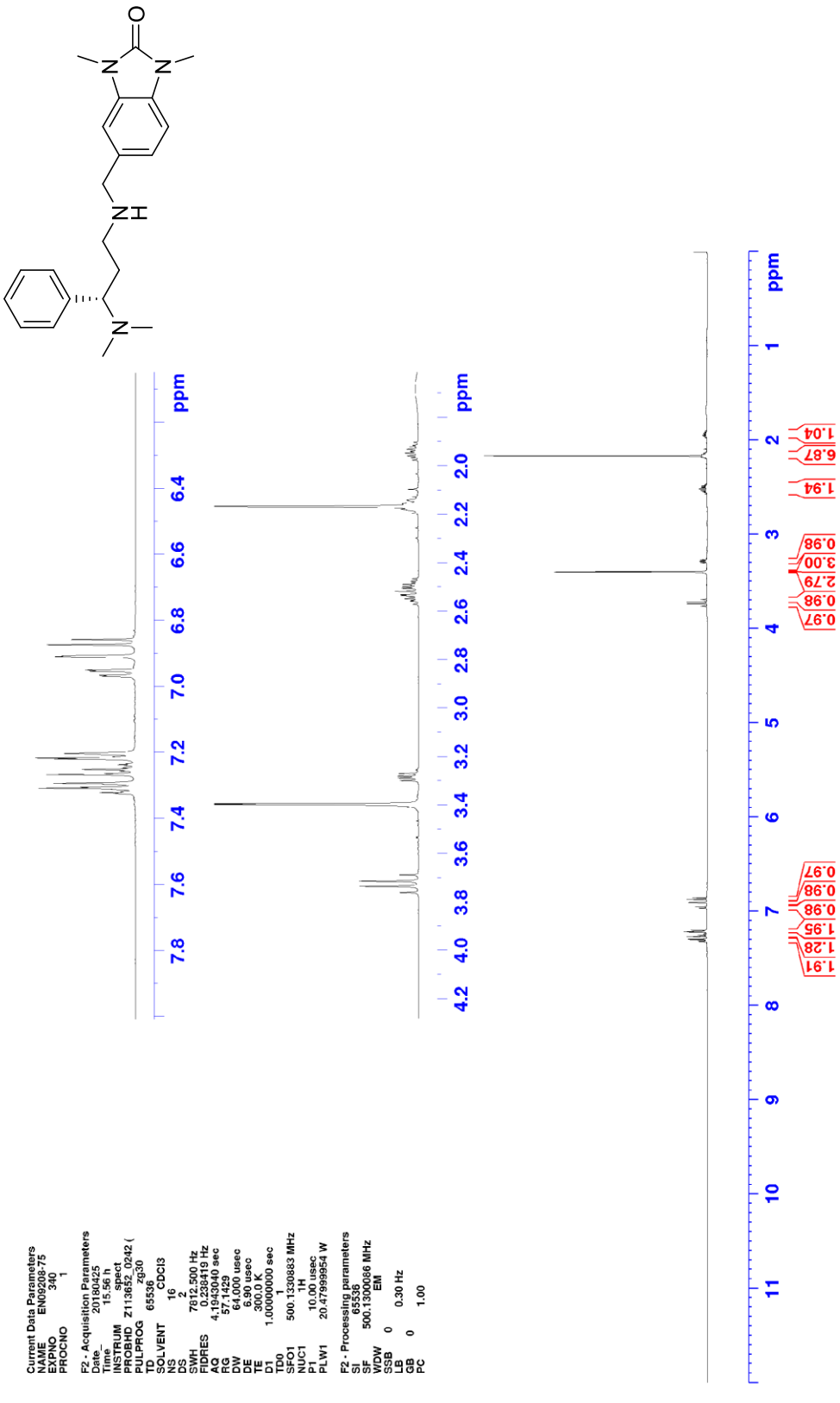
(1*R*)-*N*,*N*-Dimethyl-1-phenylpropane-1,3-diamine ((*R*)-199)



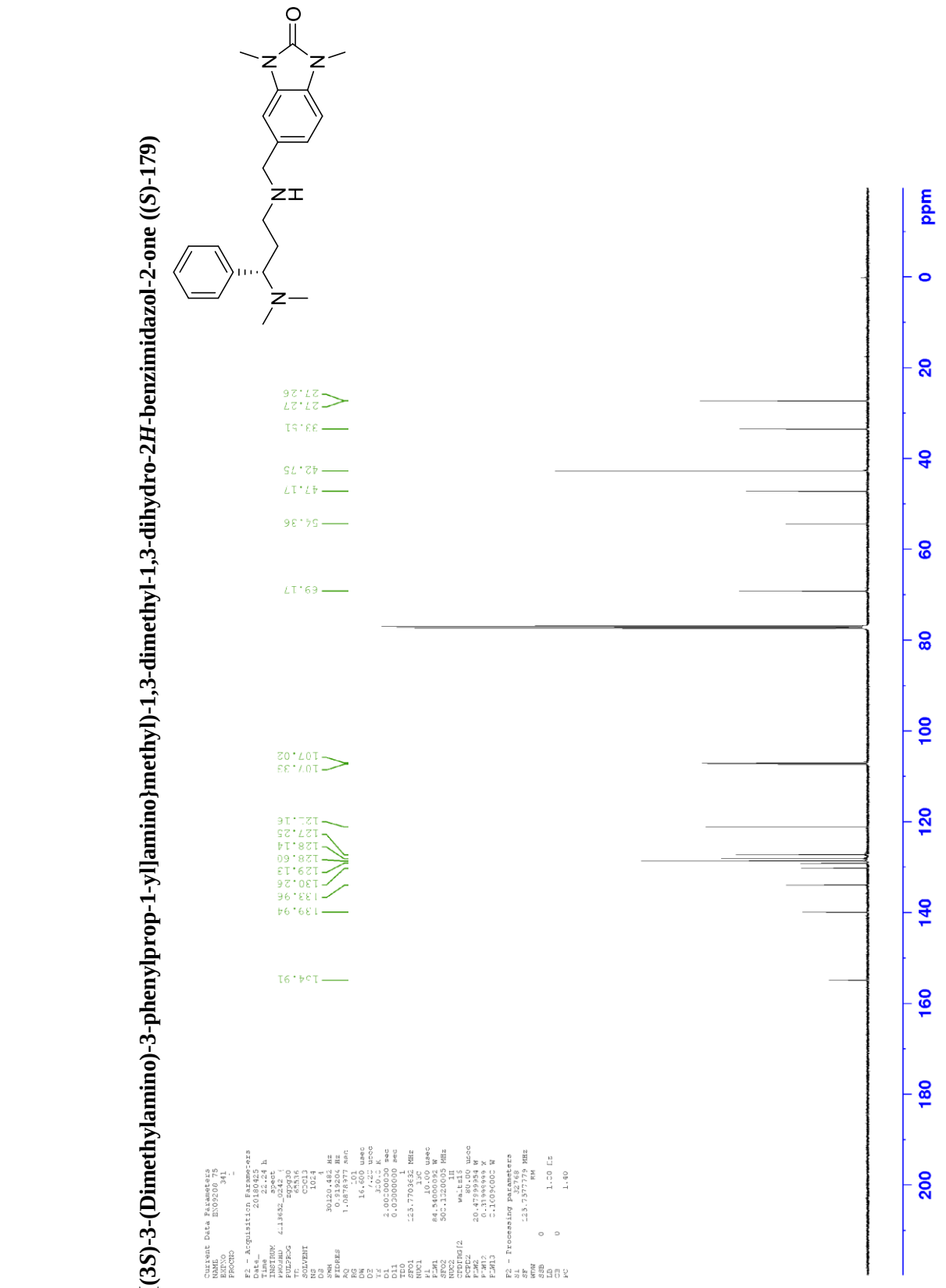
(1*R*)-*N*,*N*-Dimethyl-1-phenylpropane-1,3-diamine ((*S*)-199)



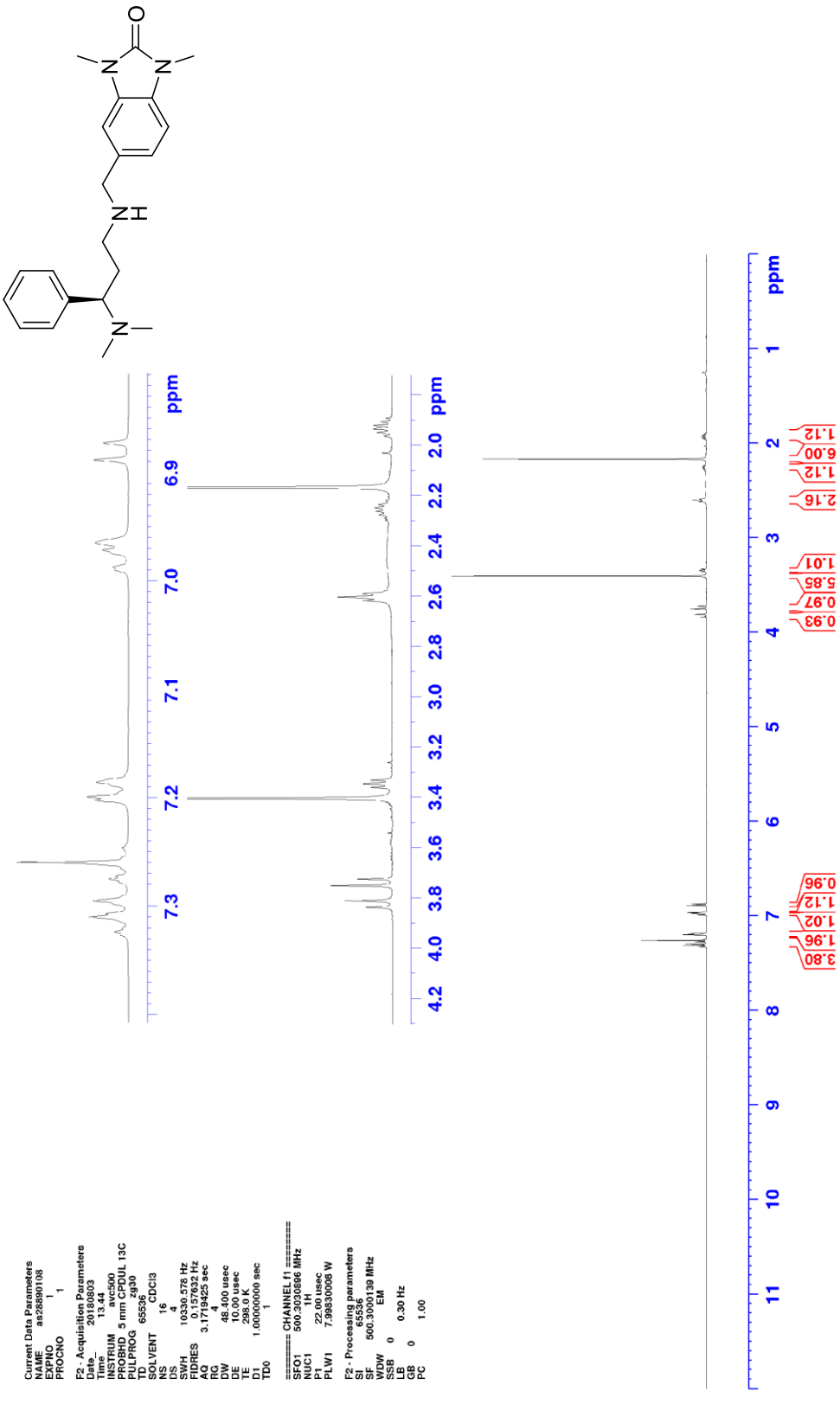
5-({[(3*S*)-3-(Dimethylamino)-3-phenylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*S*)-179)



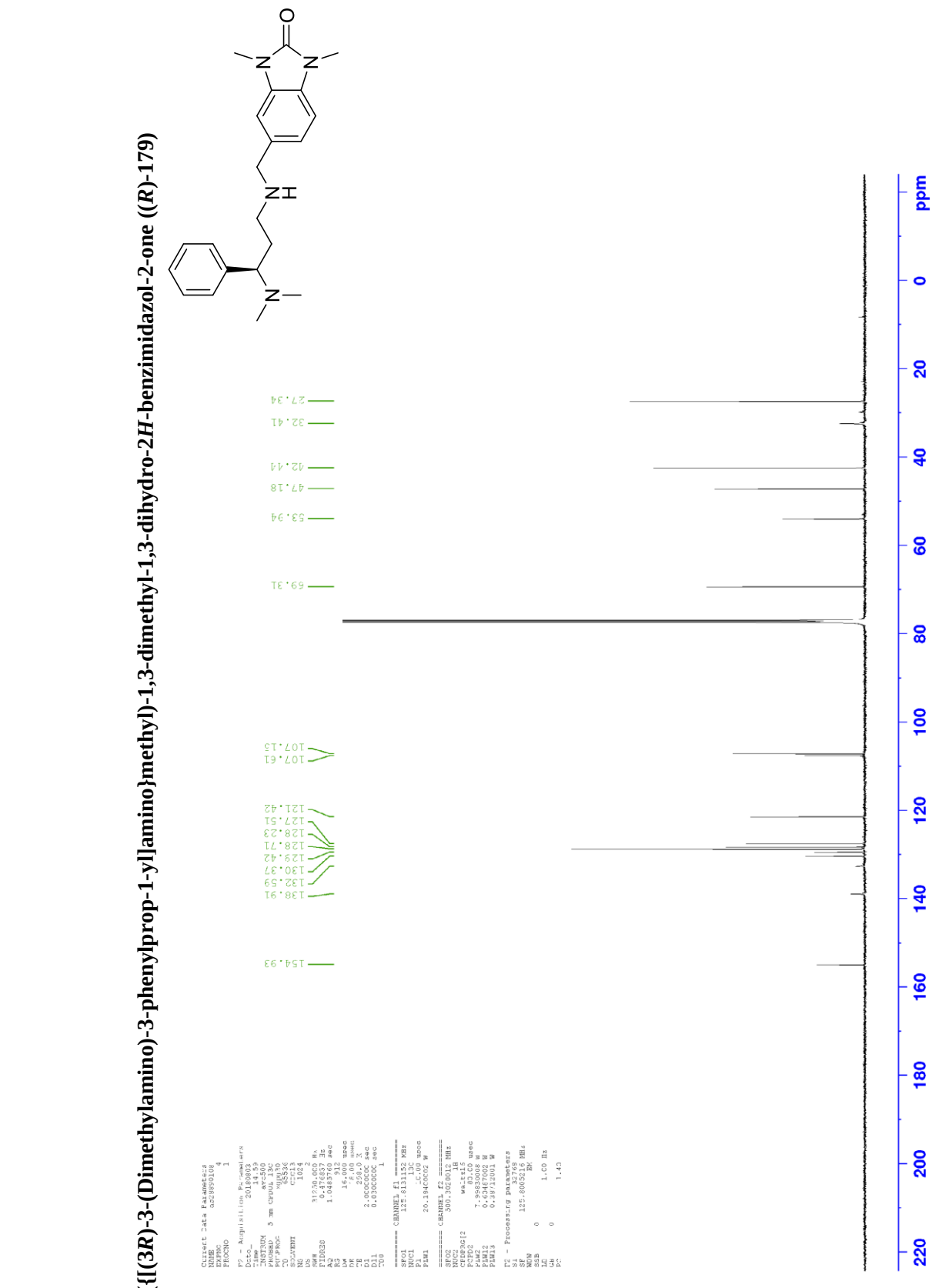
5-({[(3*S*)-3-(Dimethylamino)-3-phenylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*S*)-179)



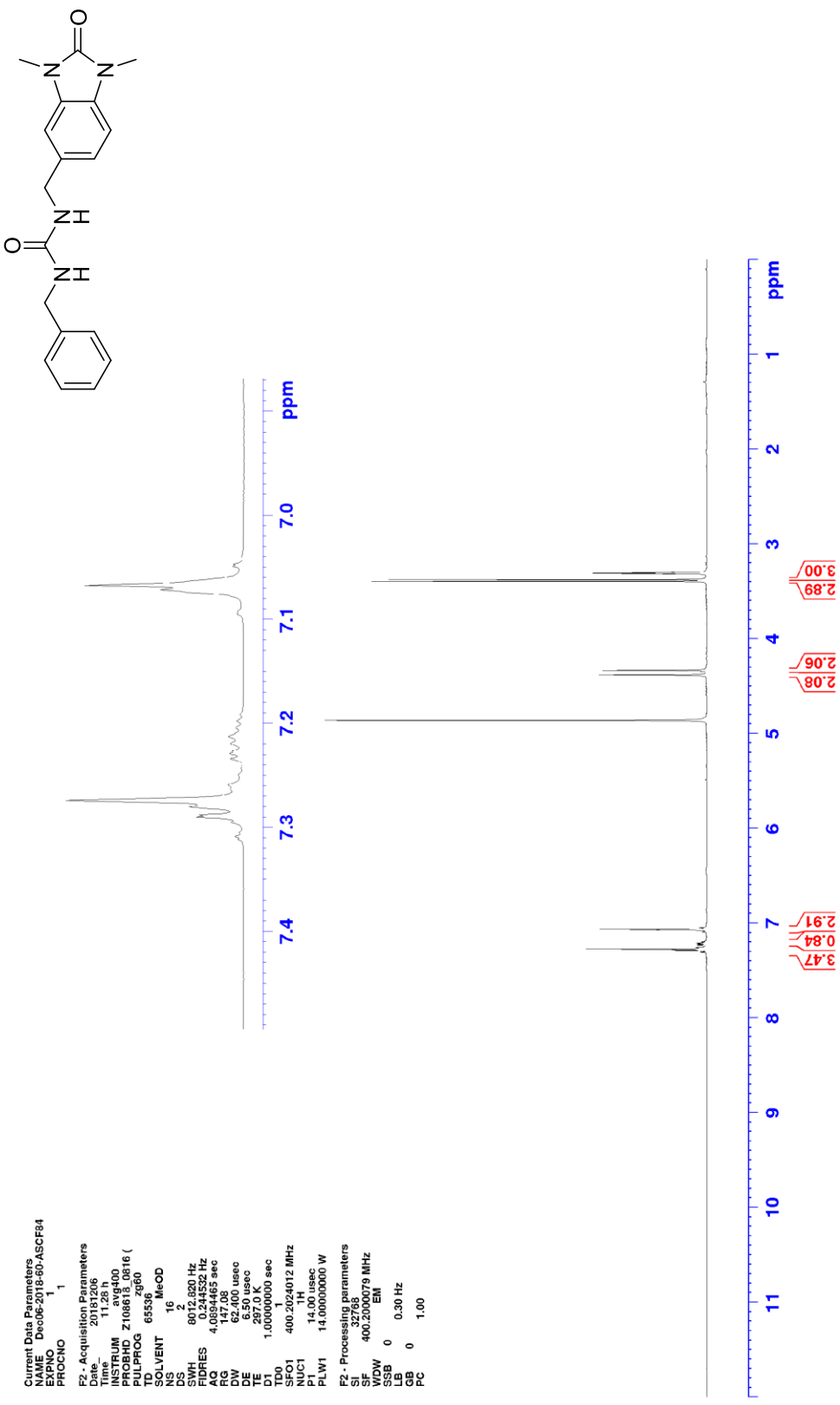
5-({[(3*R*)-3-(Dimethylamino)-3-phenylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*R*)-179)



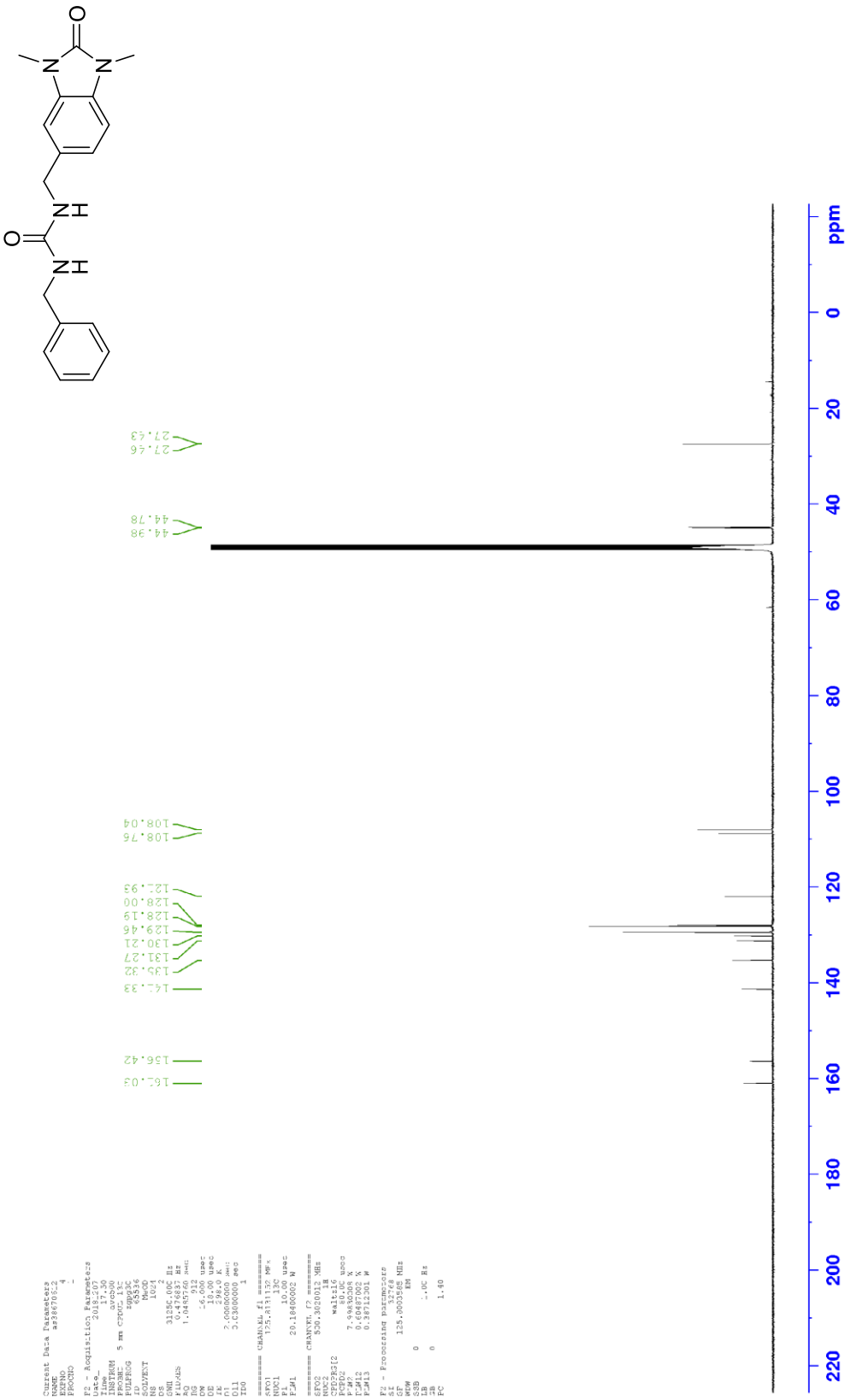
5-({[(3*R*)-3-(Dimethylamino)-3-phenylprop-1-yl]amino}methyl)-1,3-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one ((*R*)-179)



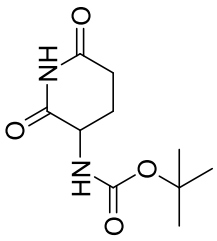
_N_-Benzyl-_N_-[(1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]urea (200)



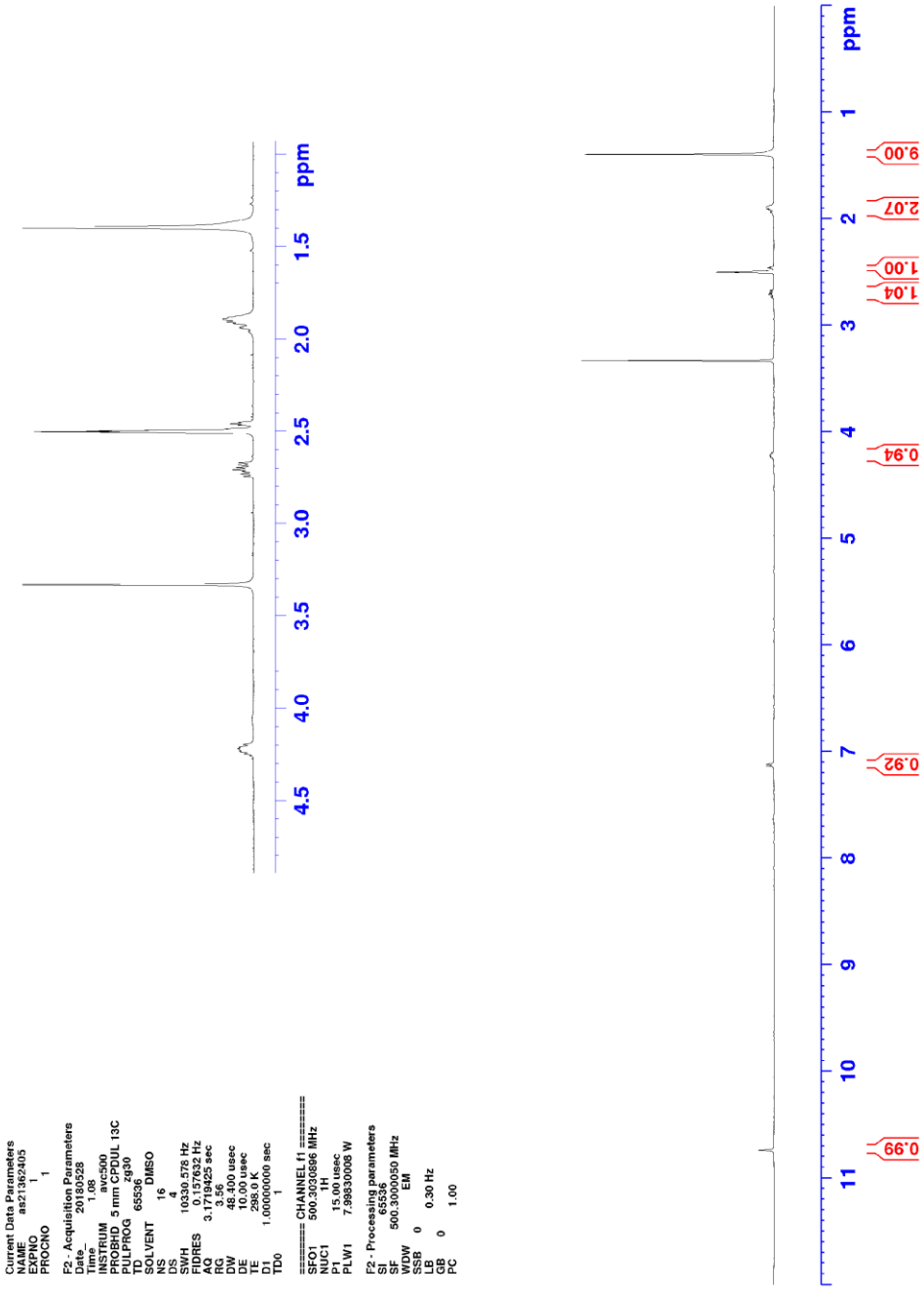
_N_-Benzyl-_N_-[(1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]urea (200)

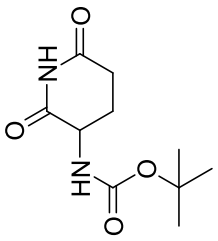


Chapter 4 Compounds

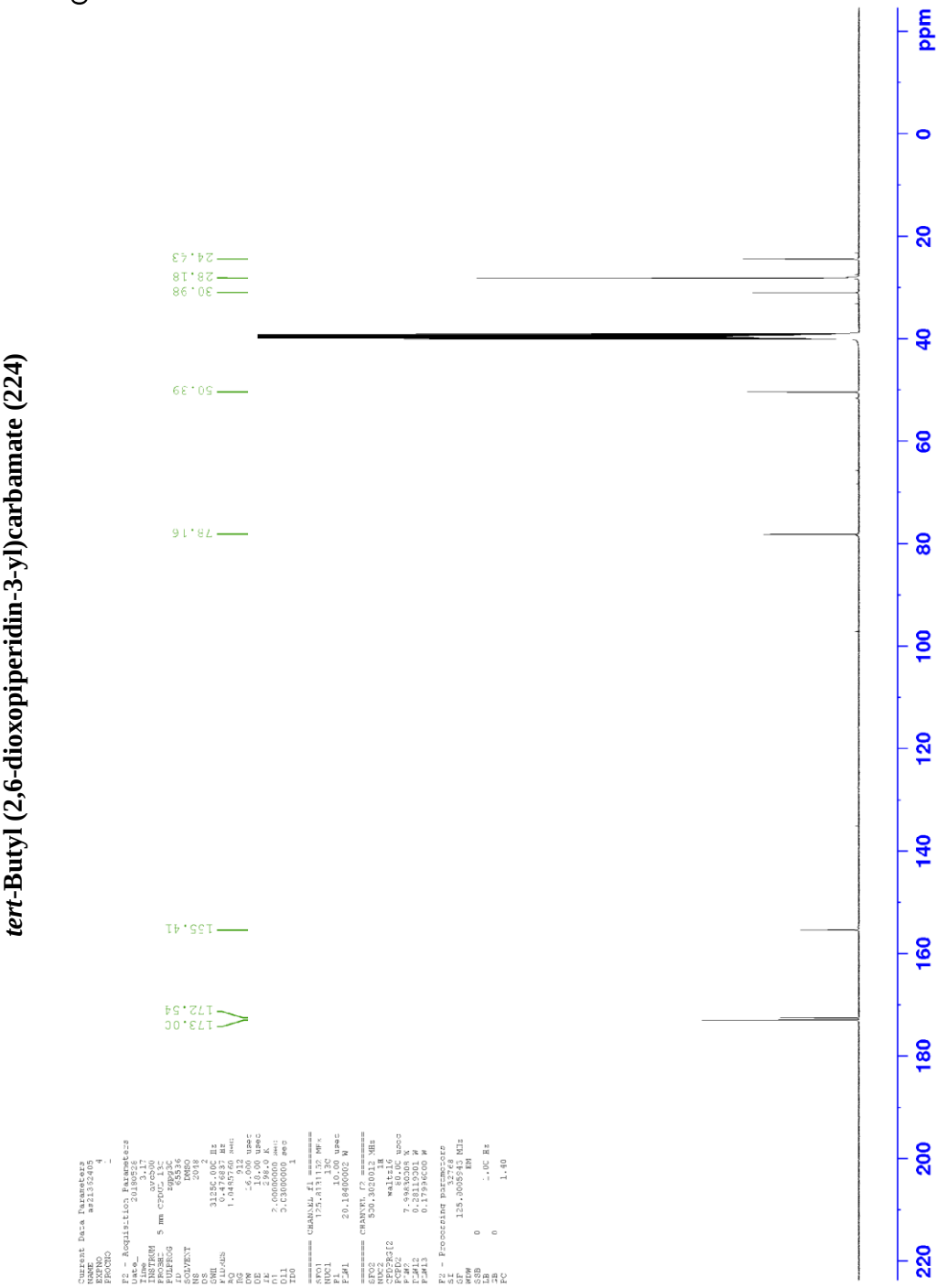


tert-Butyl (2,6-dioxopiperidin-3-yl)carbamate (224)

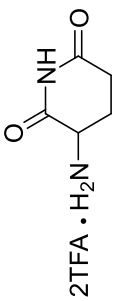


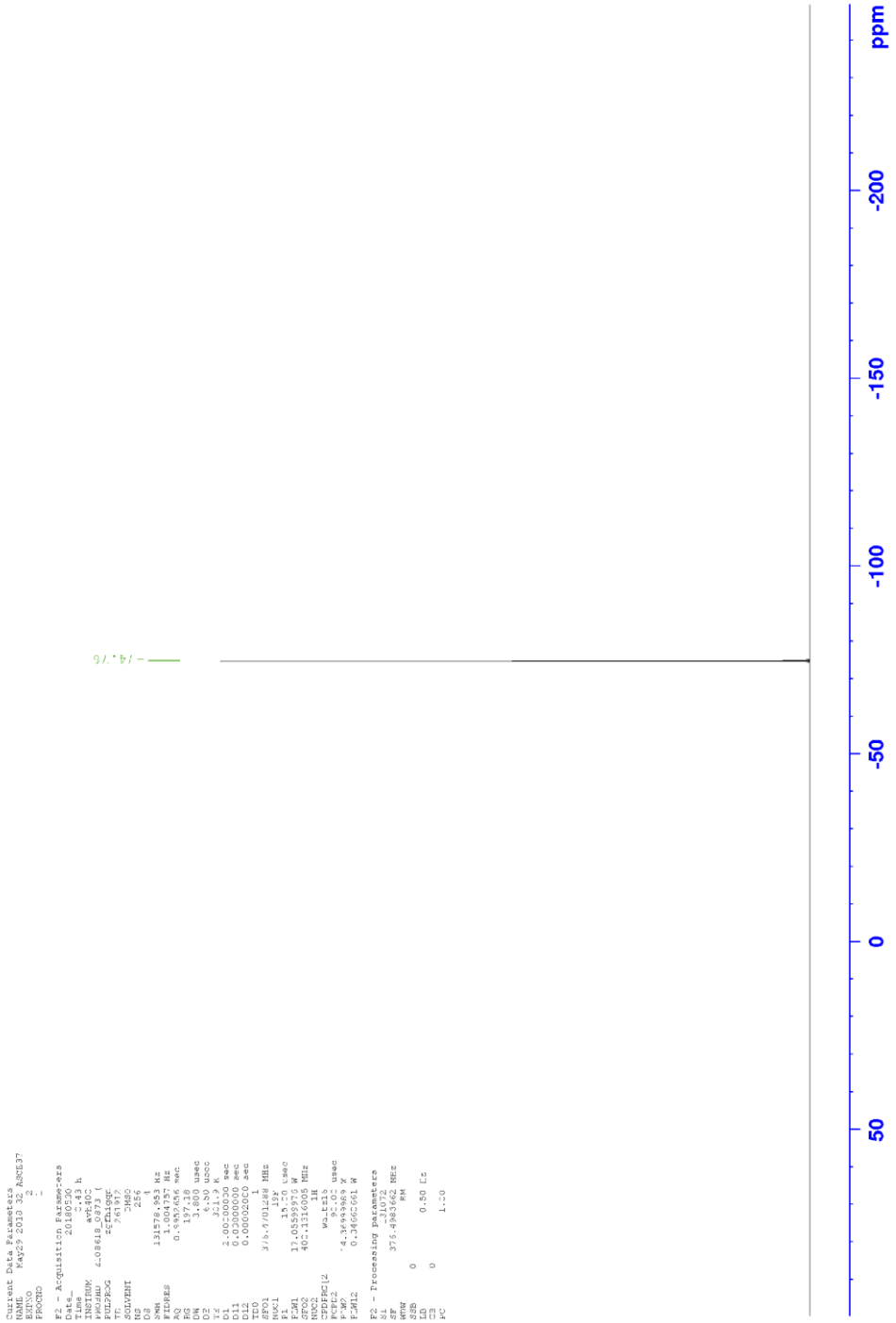


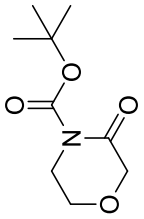
tert-Butyl (2,6-dioxopiperidin-3-yl)carbamate (224)



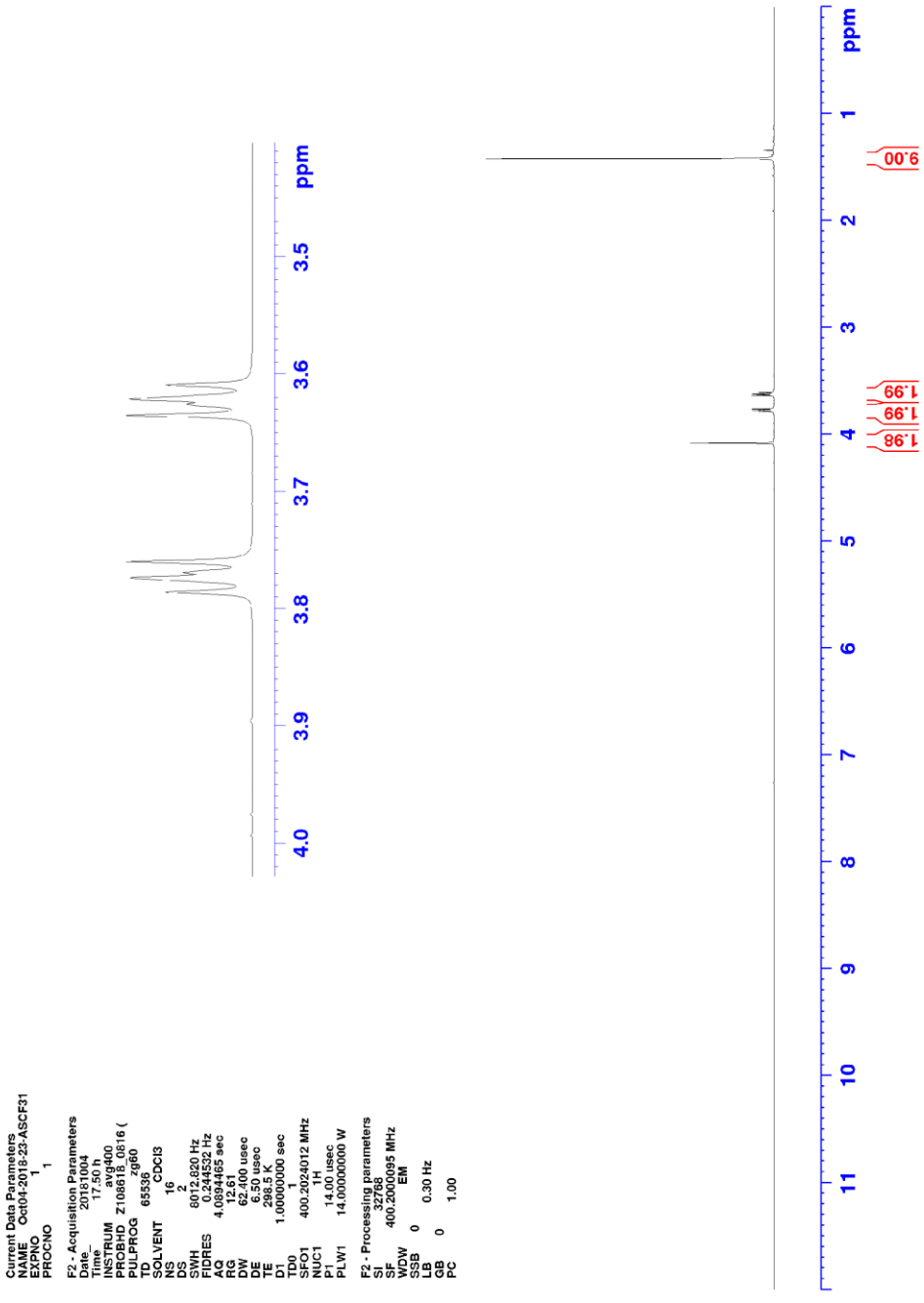
3-Aminopiperidine-2,6-dione trifluoroacetate (225)

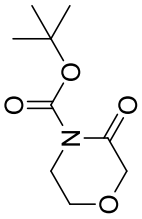




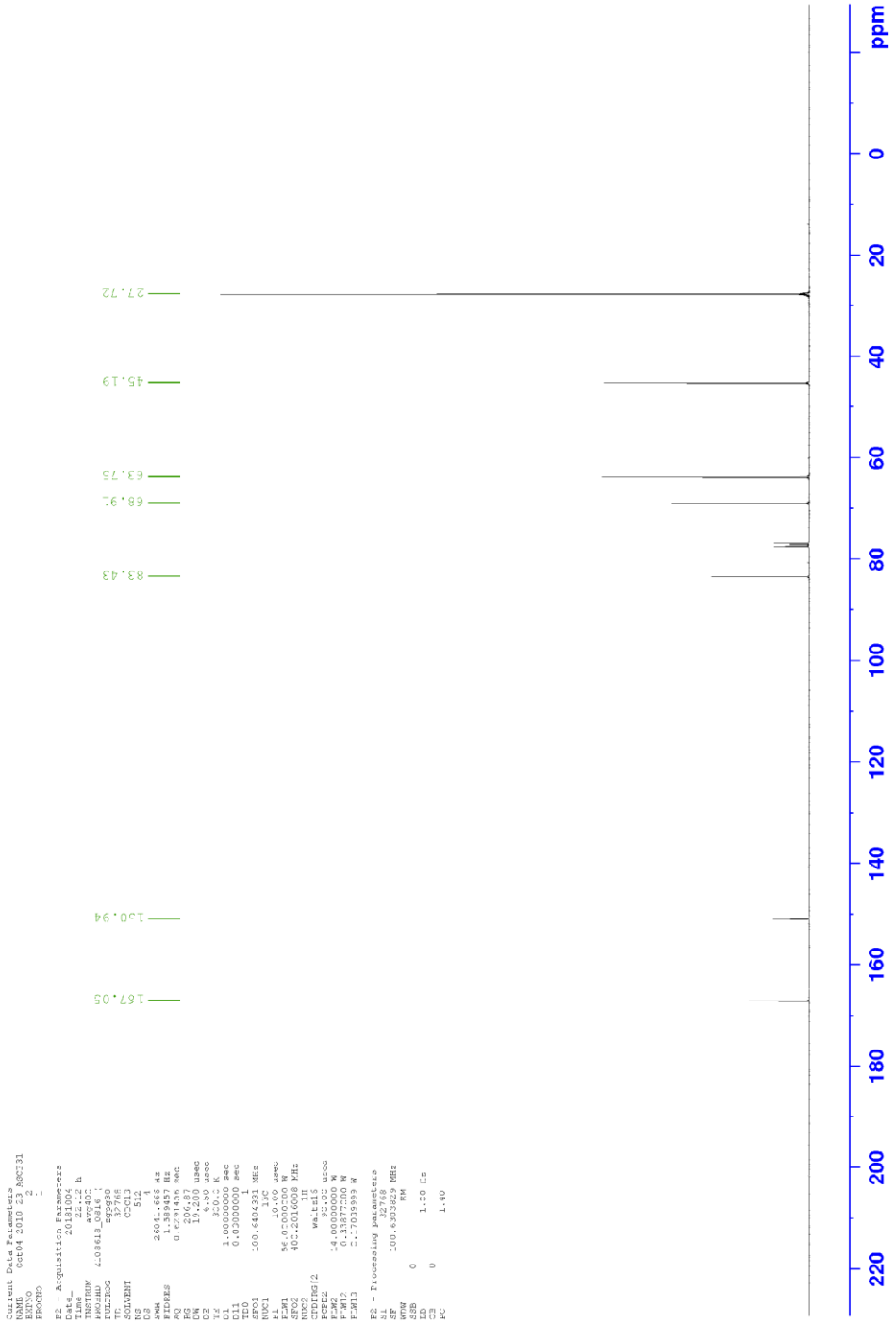


{2-[(*tert*-Butoxycarbonyl)amino]ethoxy}acetic acid (235)

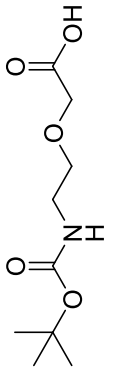


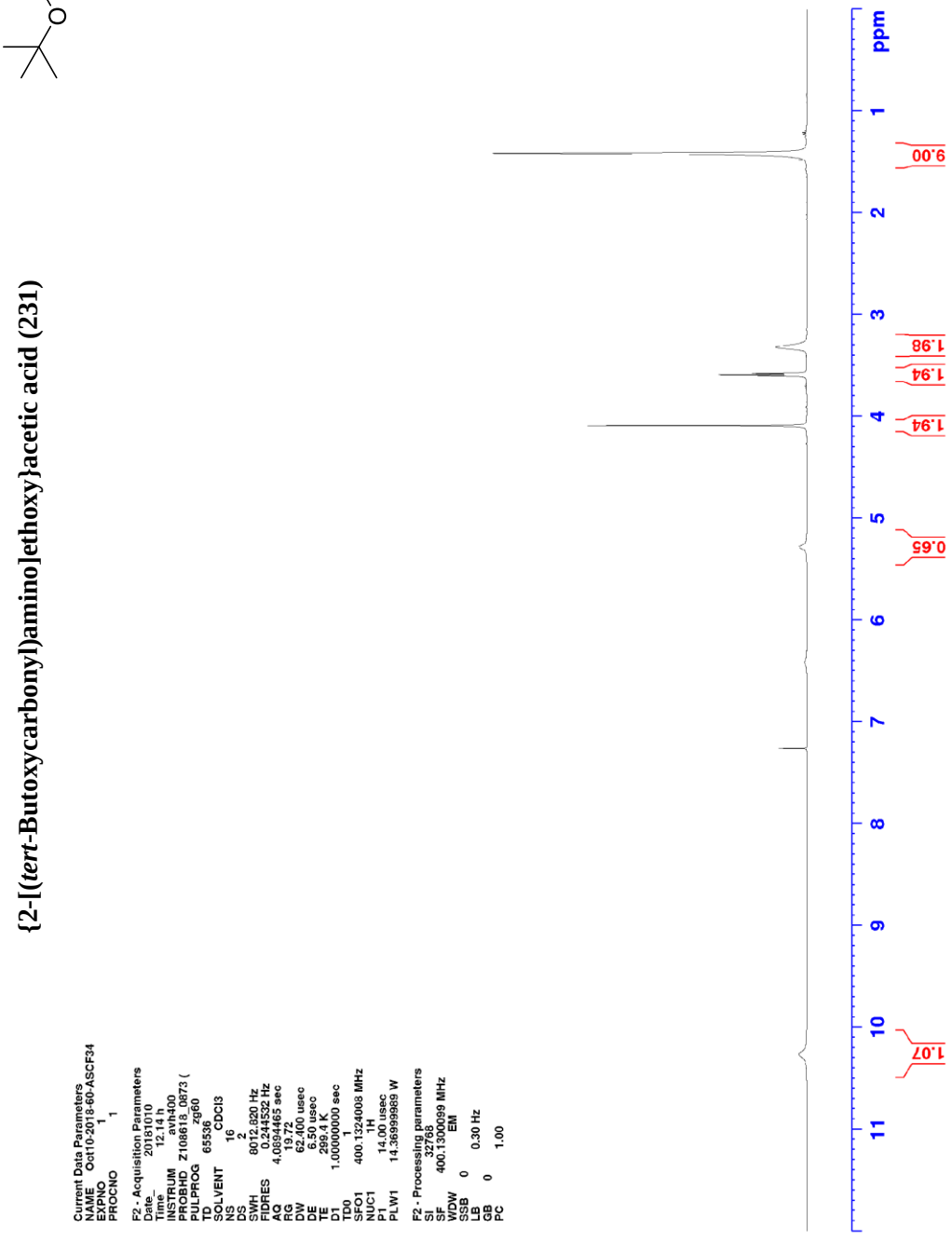


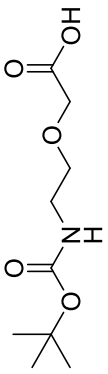
{2-[(*tert*-Butoxycarbonyl)amino]ethoxy}acetic acid (235)



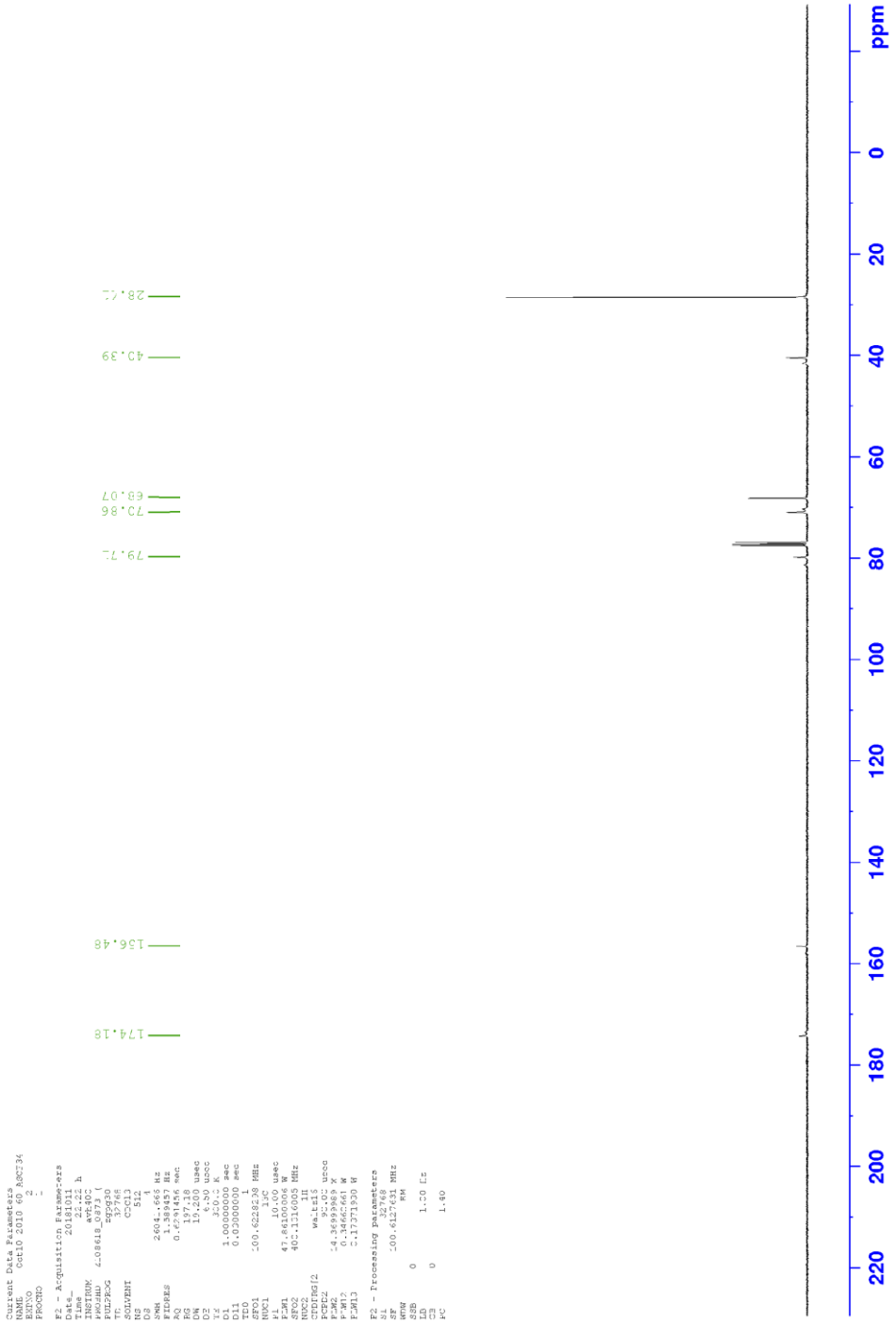
{2-[(*tert*-Butoxycarbonyl)amino]ethoxy}acetic acid (231)



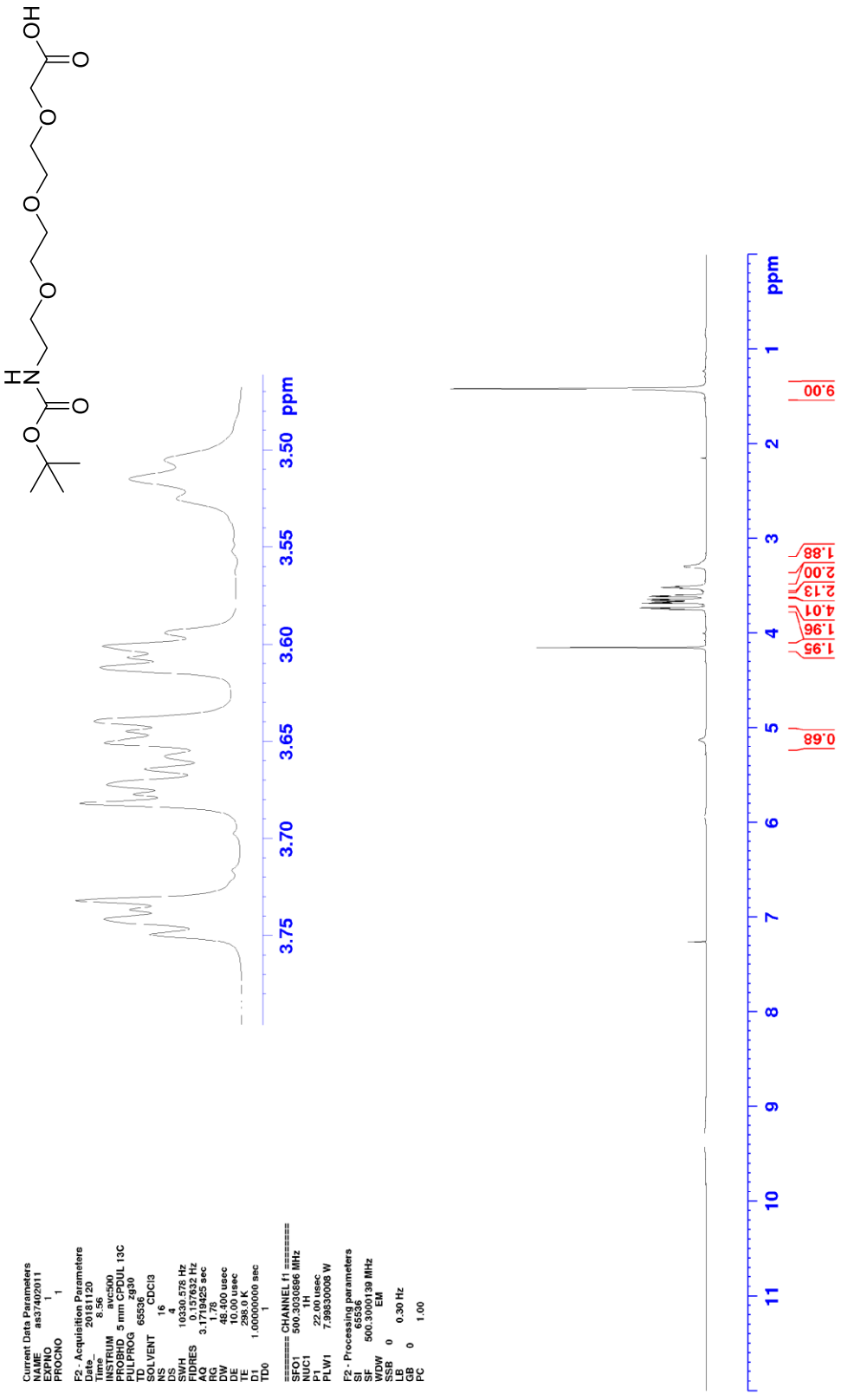




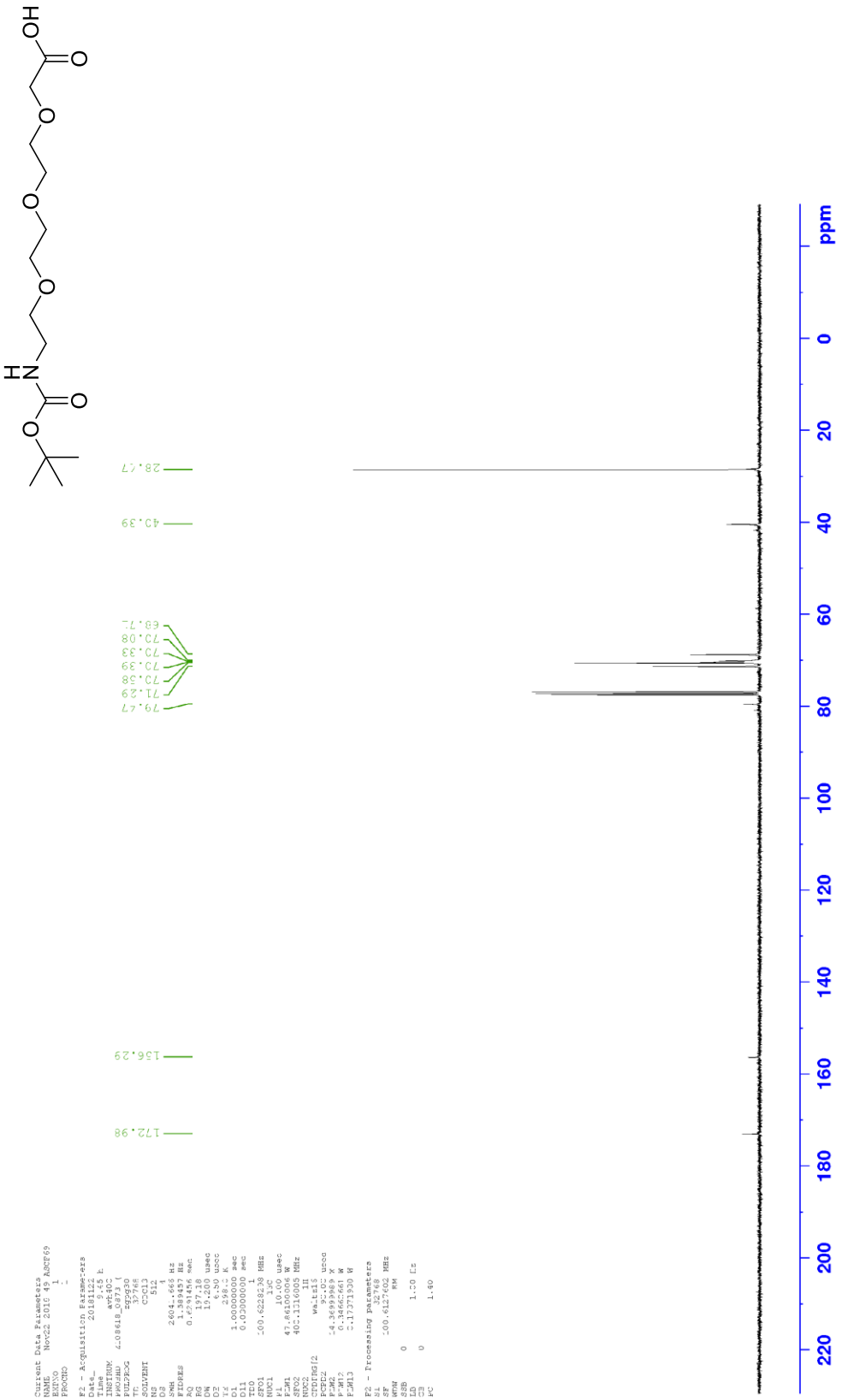
{2-[(*tert*-Butoxycarbonyl)amino]ethoxy}acetic acid (231)



2,2-Dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azahexadecan-16-oic acid (233)

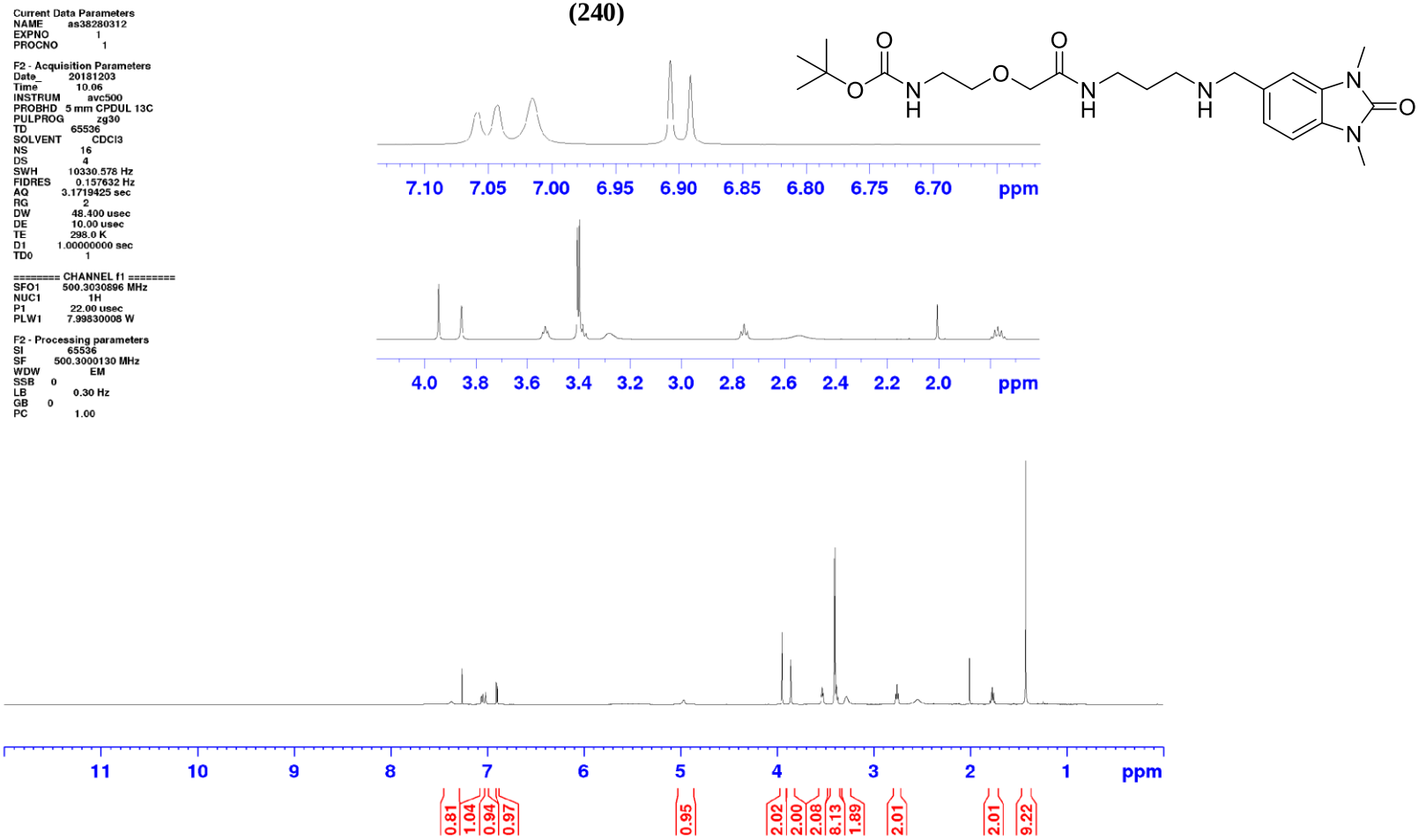


2,2-Dimethyl-4-oxo-3,8,11,14-tetraoxa-5-azahexadecan-16-oic acid (233)



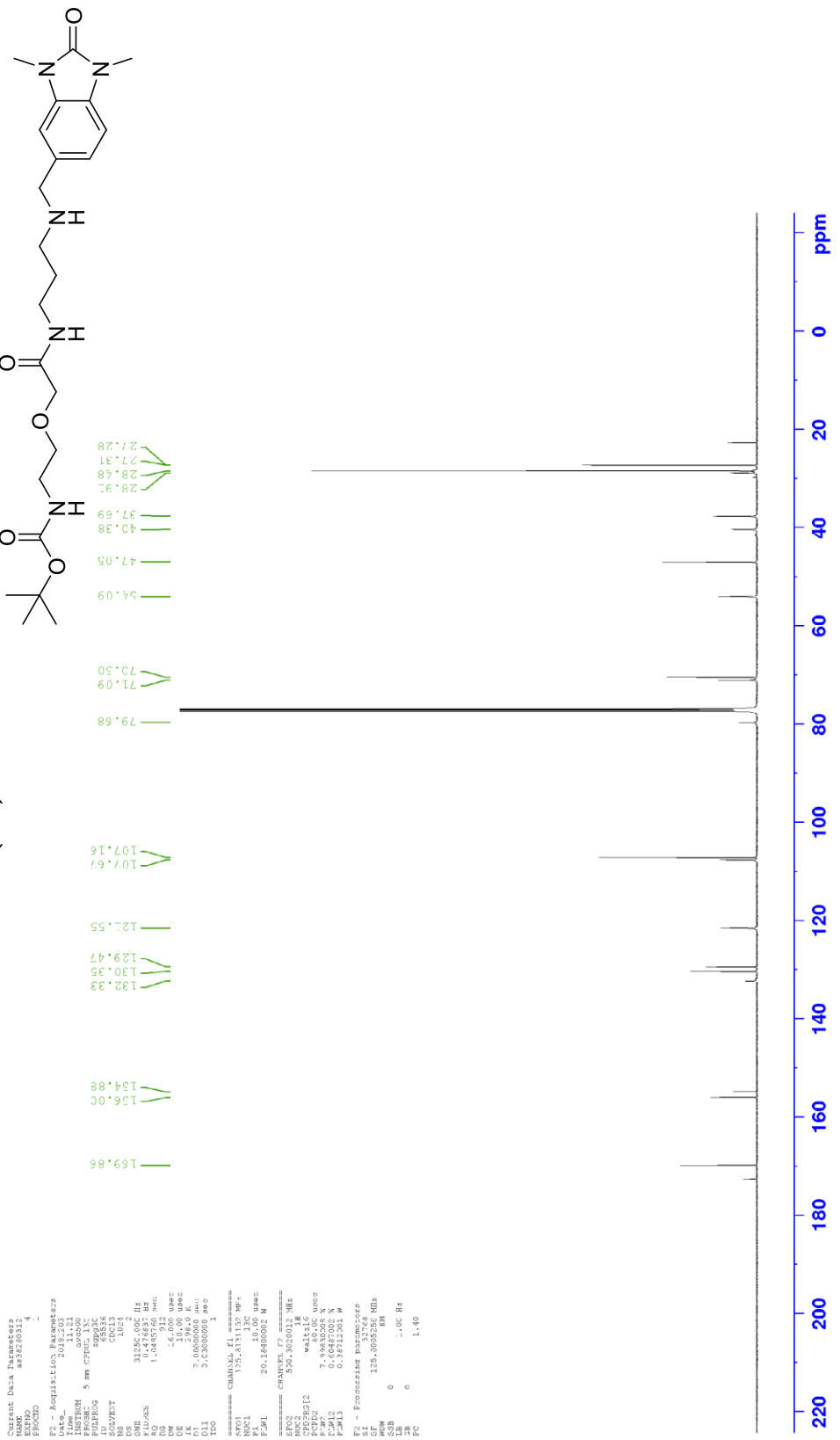
tert-Butyl (2-{2-[(3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1-yl)amino]-2-oxoethoxy}ethyl)carbamate

(240)

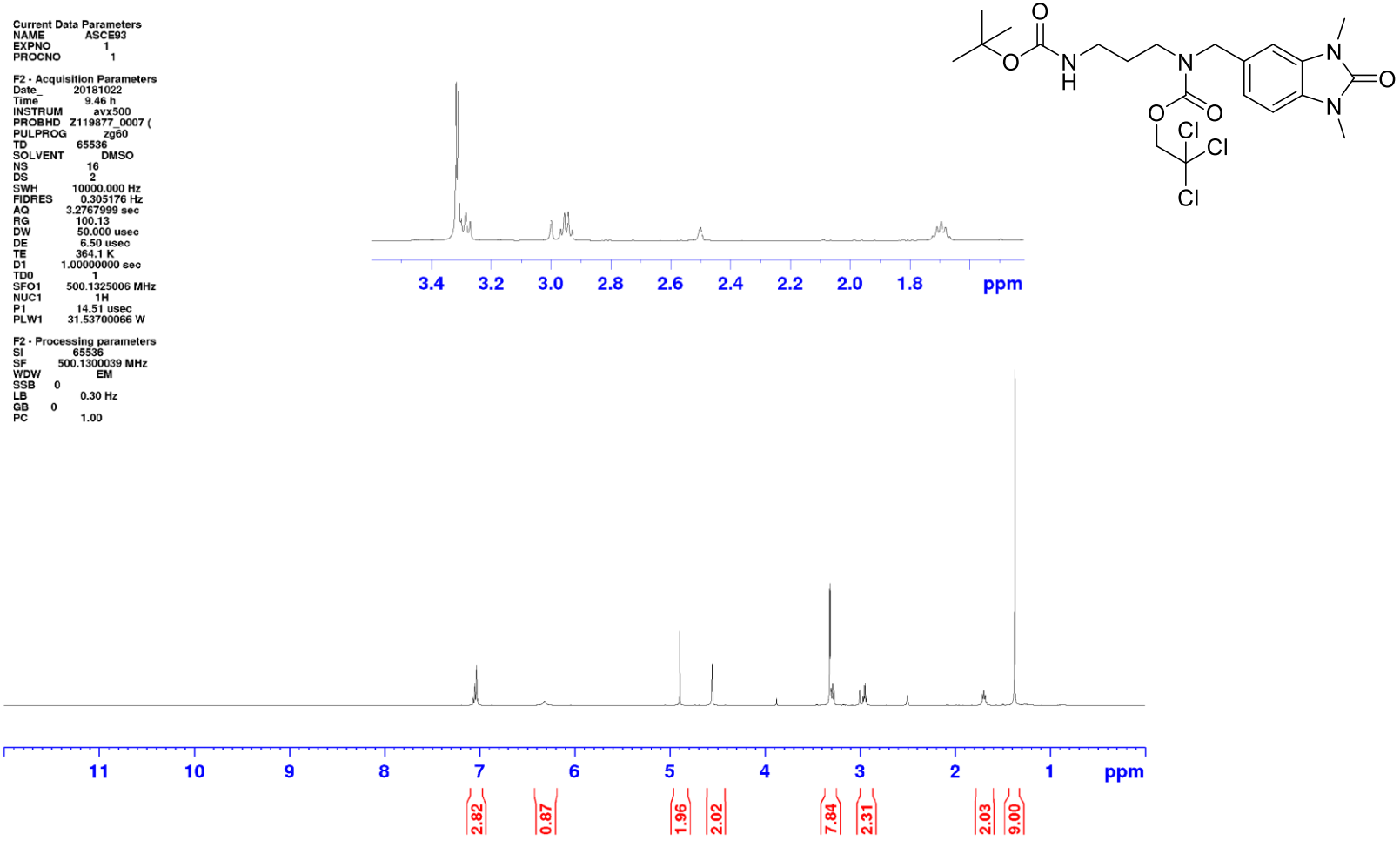


***tert*-Butyl (2-{2-[(3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1-yl)amino]-2-oxoethoxy}ethyl)carbamate**

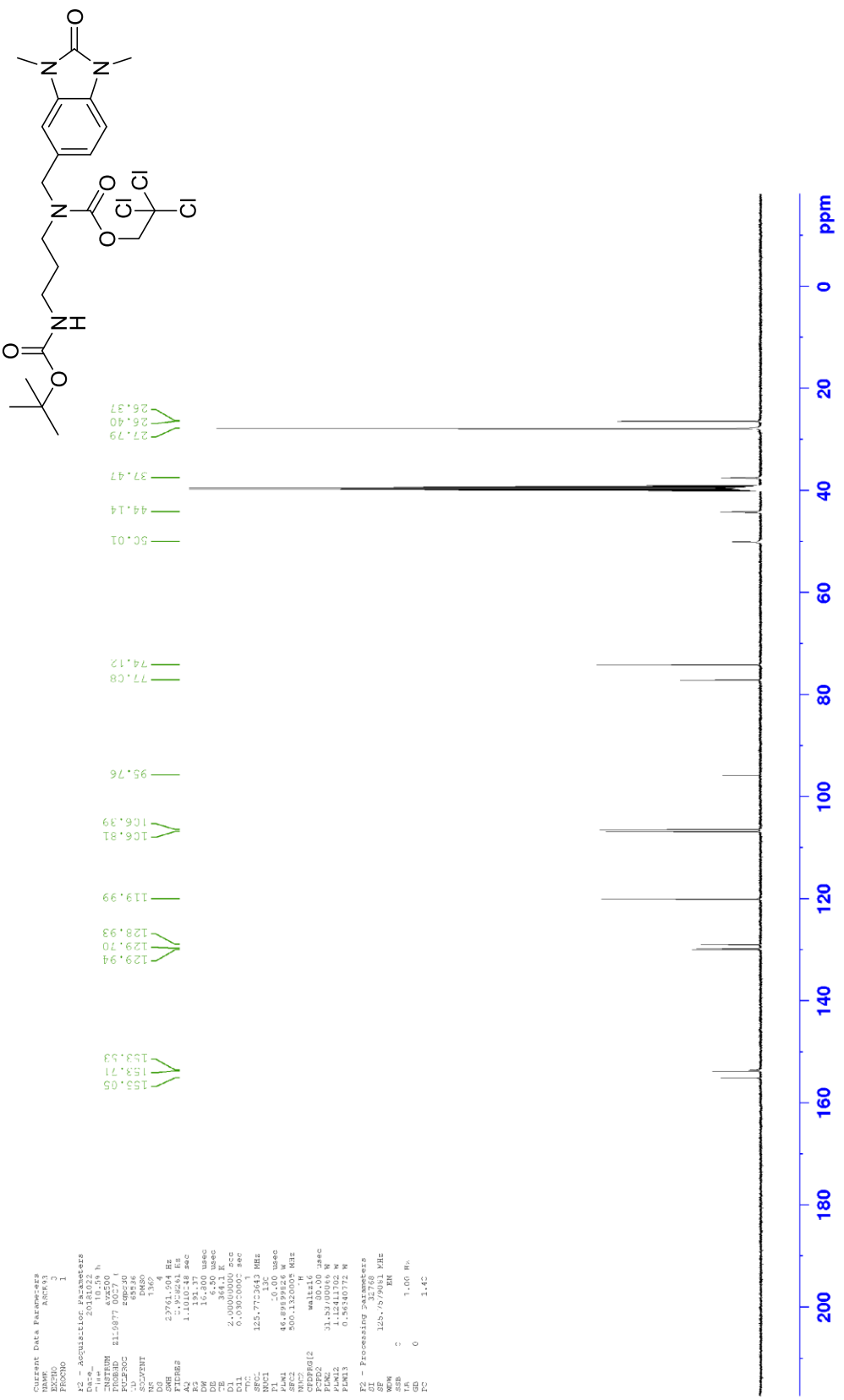
(240)



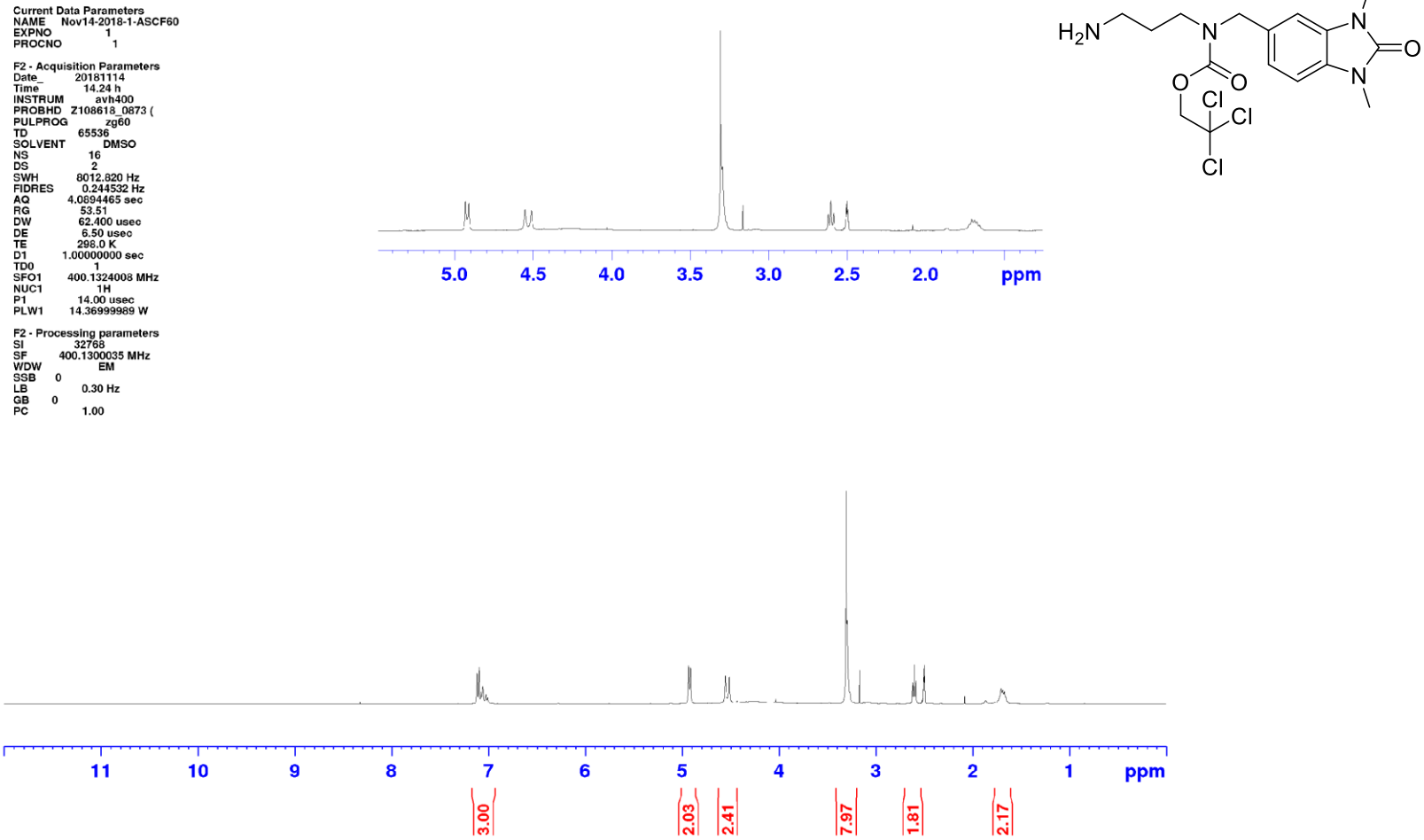
2,2,2-Trichloroethyl {3-[(*tert*-butoxycarbonyl)amino]prop-1-yl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (242)



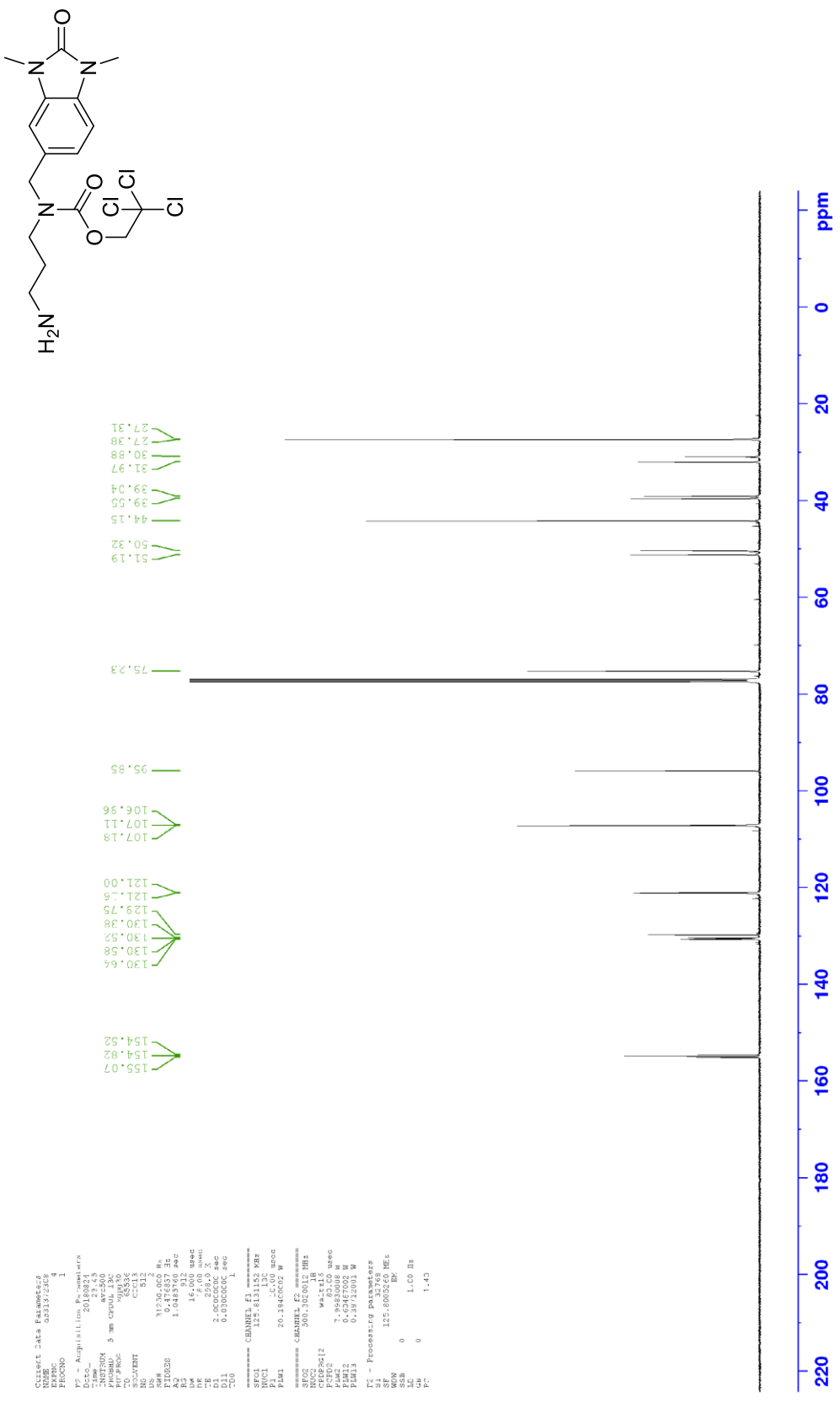
2,2,2-Trichloroethyl {3-[(*tert*-butoxycarbonyl)amino]prop-1-yl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (242)



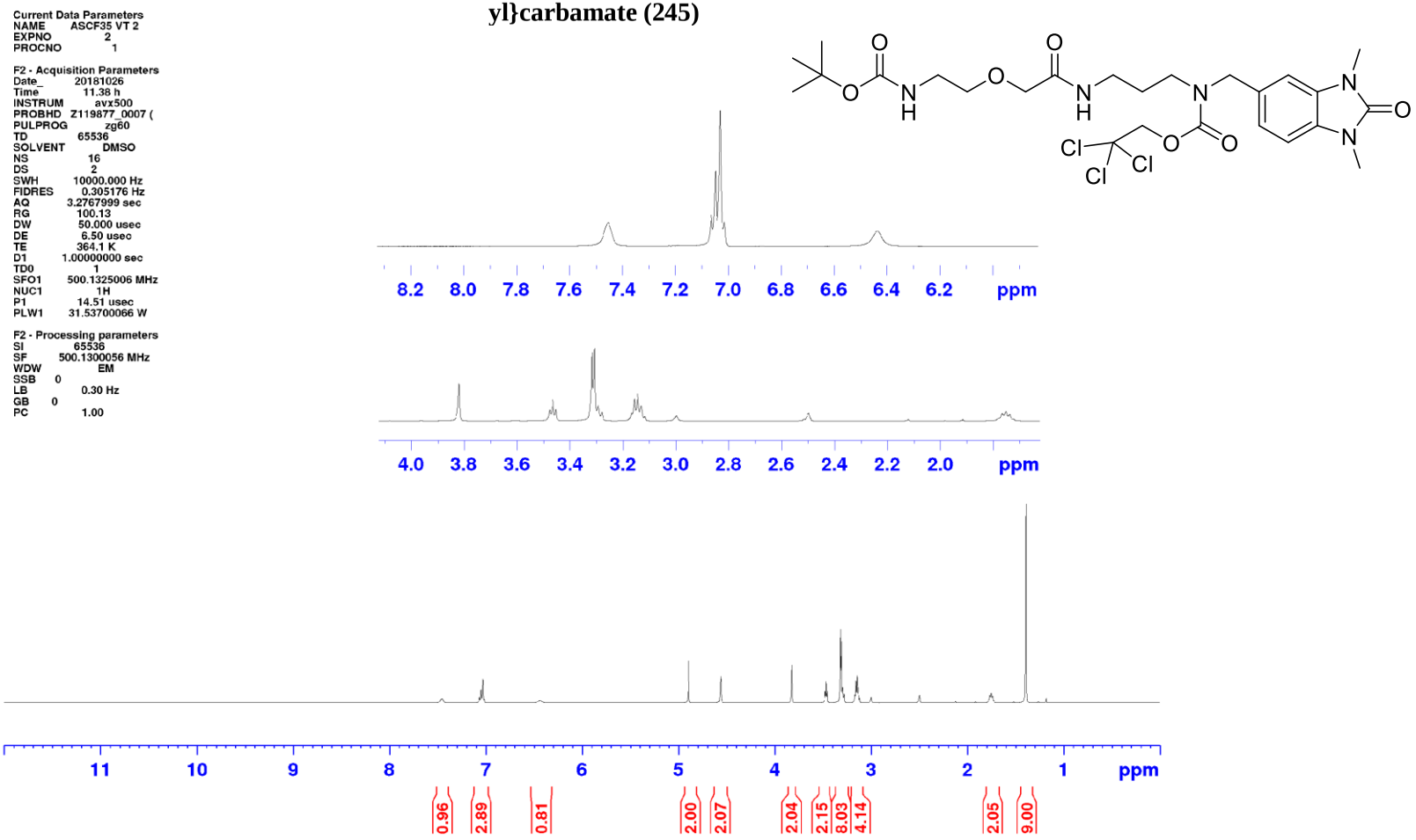
2,2,2-Trichloroethyl (3-aminoprop-1-yl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (243)



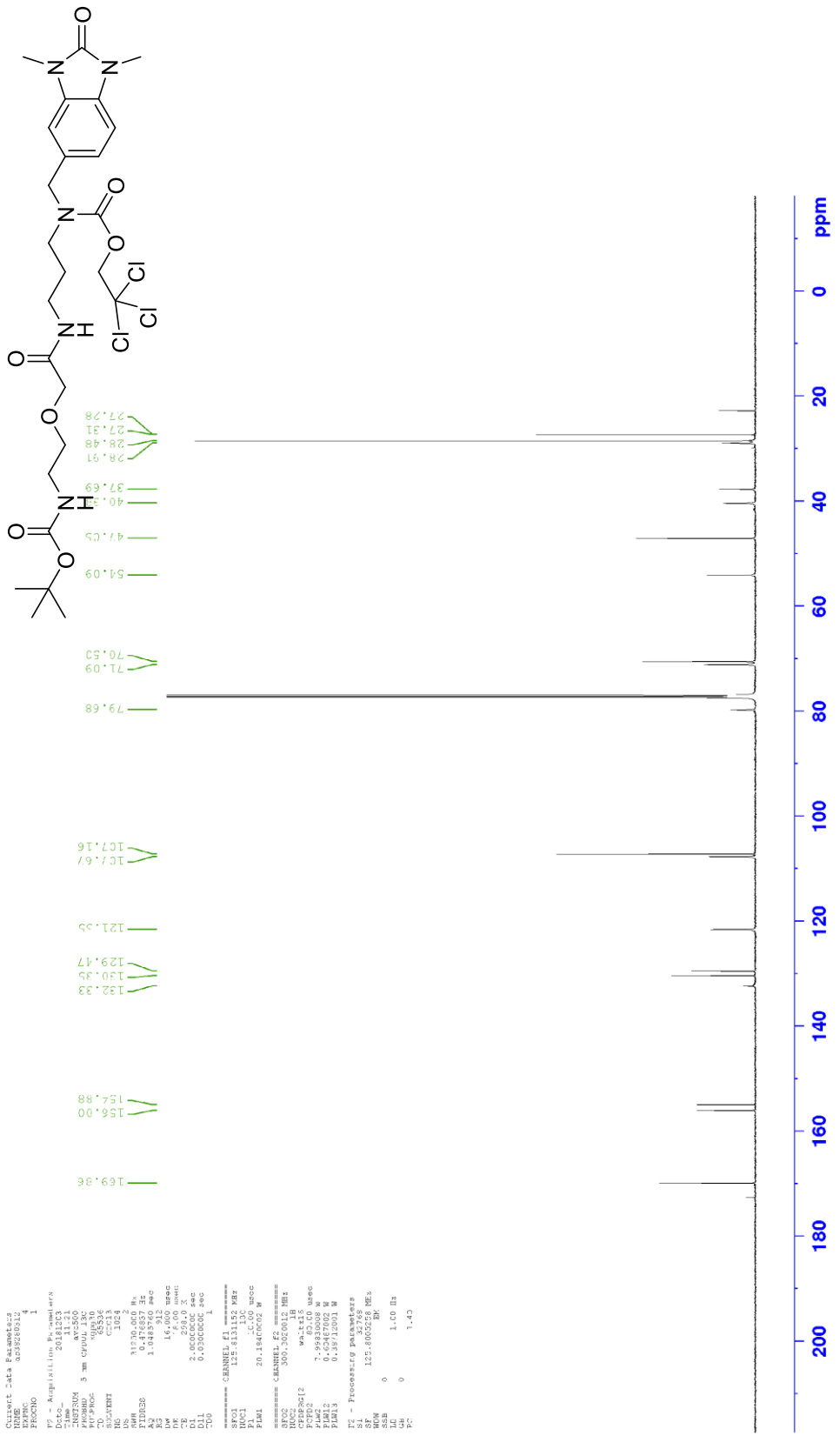
2,2,2-Trichloroethyl (3-aminoprop-1-yl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (243)



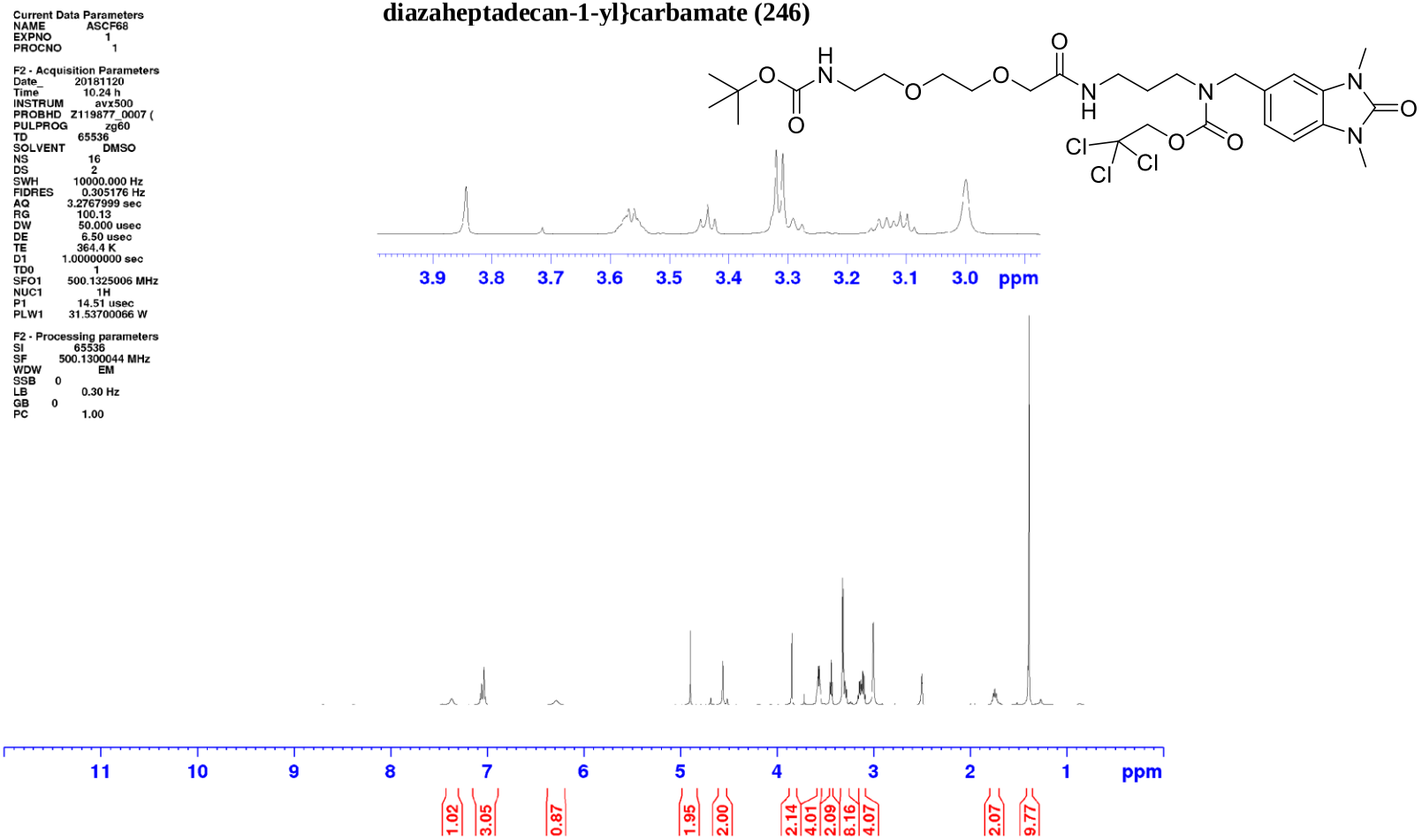
tert-Butyl {14,14,14-trichloro-10-[(1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]-5,11-dioxo-3,12-dioxa-6,10-diazatetradecan-1-yl}carbamate (245)



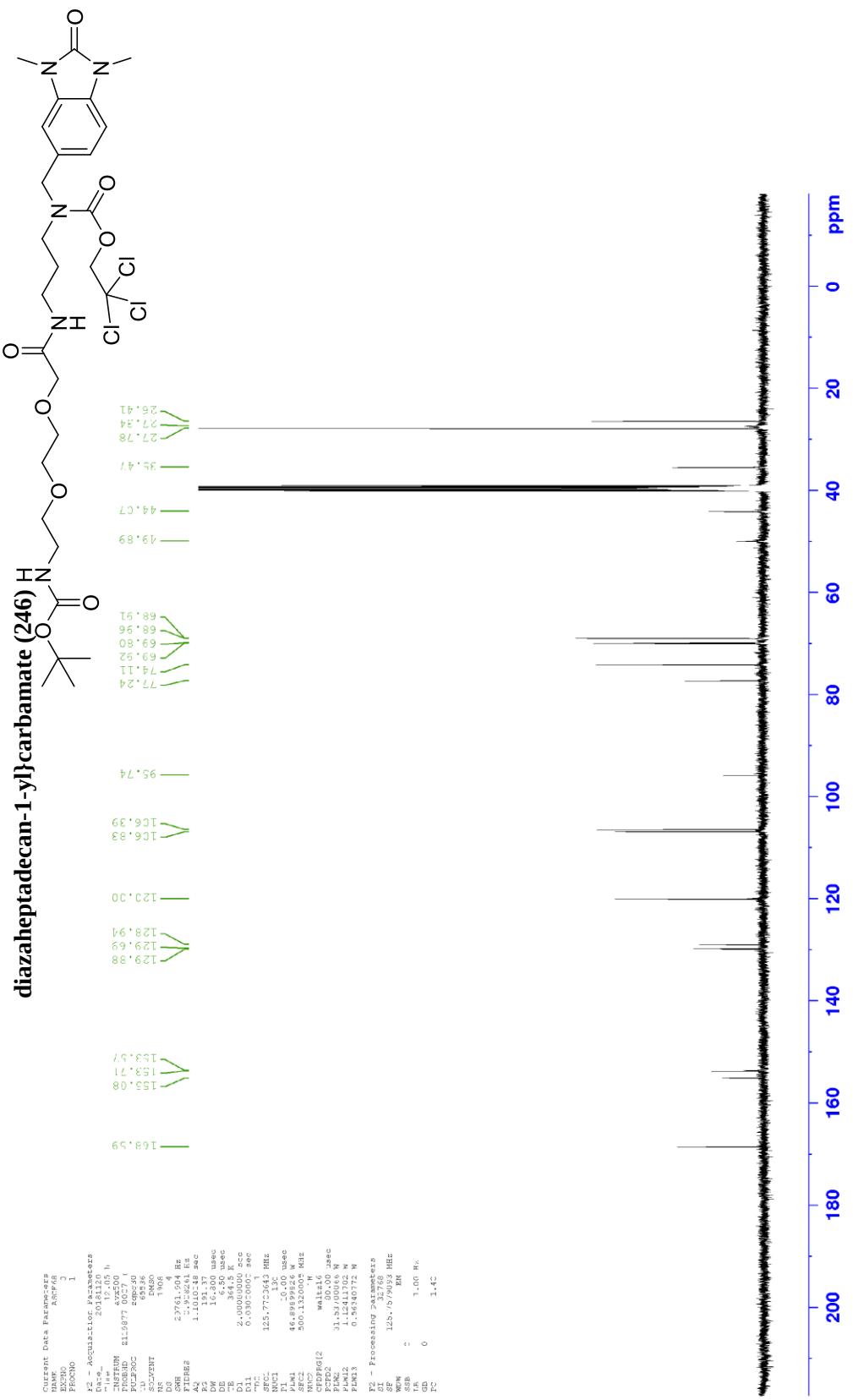
***tert*-Butyl {14,14,14-trichloro-10-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-5,11-dioxo-3,12-dioxa-6,10-diazatetradecan-1-yl}carbamate (245)**



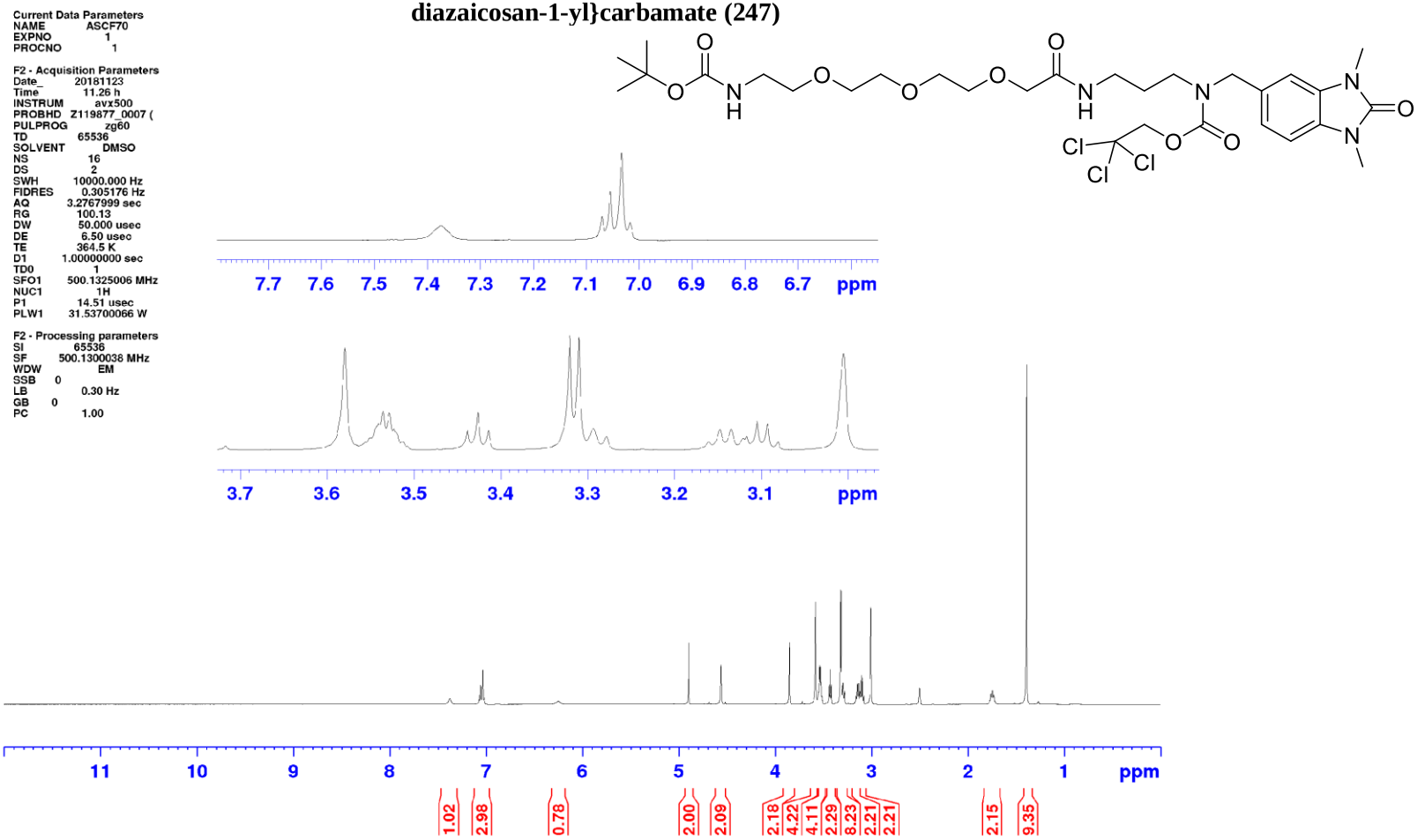
tert-Butyl {17,17,17-trichloro-13-[(1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]-8,14-dioxo-3,6,15-trioxa-9,13-diazaheptadecan-1-yl}carbamate (246)



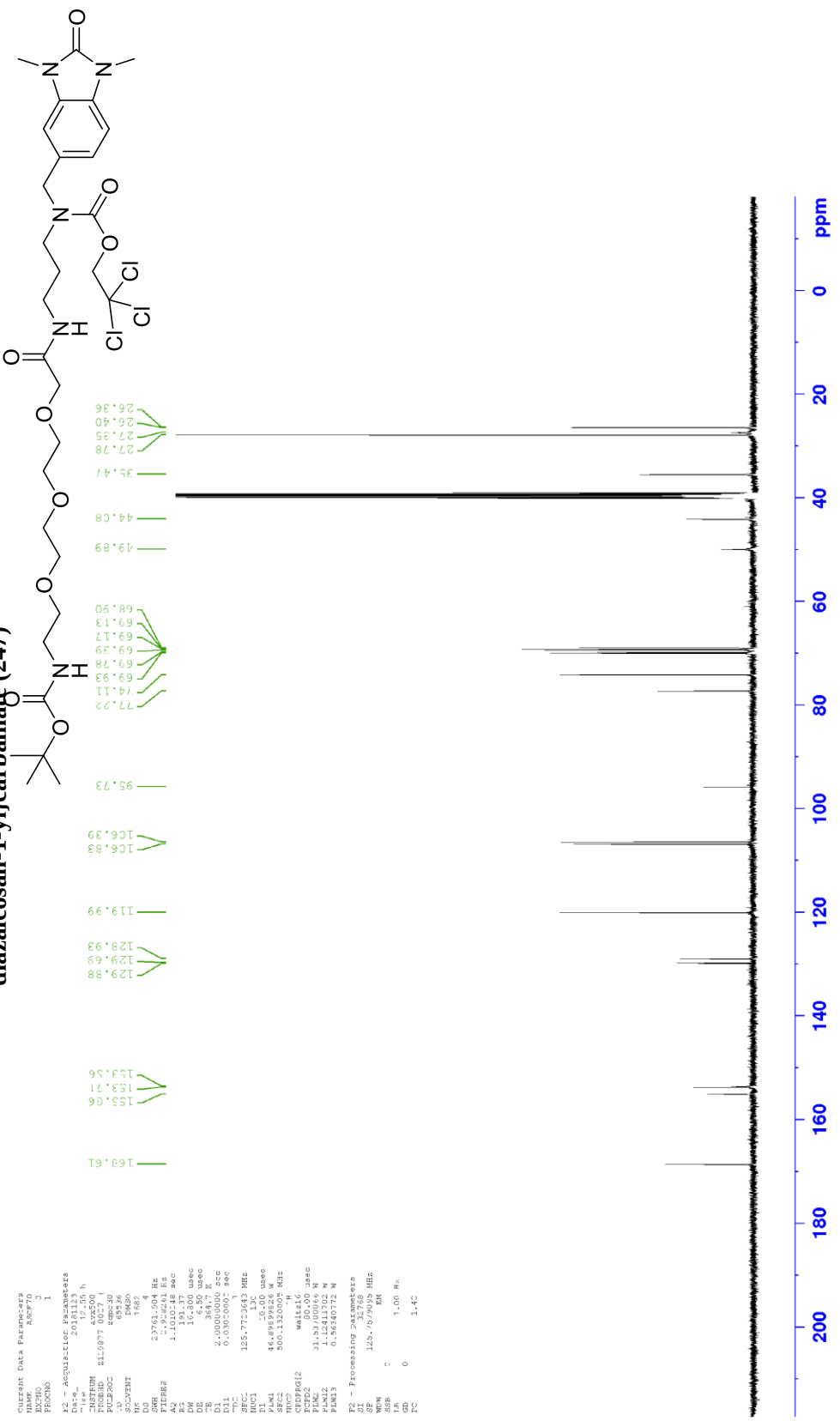
***tert*-Butyl {17,17,17-trichloro-13-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-8,14-dioxo-3,6,15-trioxa-9,13-diazaheptadecan-1-yl}carbamate (246)**



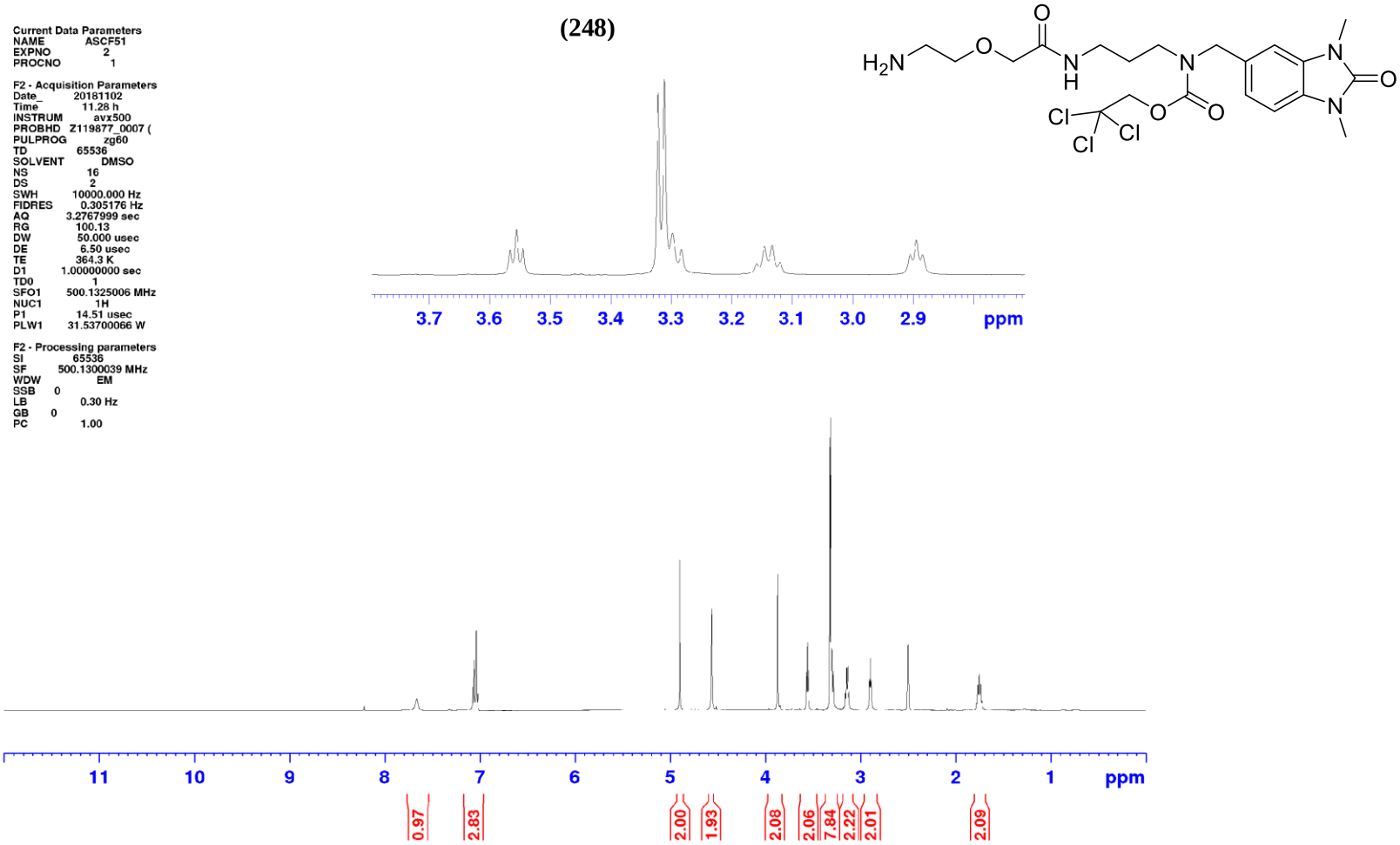
tert-Butyl {20,20,20-trichloro-16-[(1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]-11,17-dioxo-3,6,9,18-tetraoxa-12,16-diazaicosan-1-yl}carbamate (247)



***tert*-Butyl {20,20,20-trichloro-16-[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]-11,17-dioxo-3,6,9,18-tetraoxa-12,16-diazaicosan-1-yl}carbamate (247)**

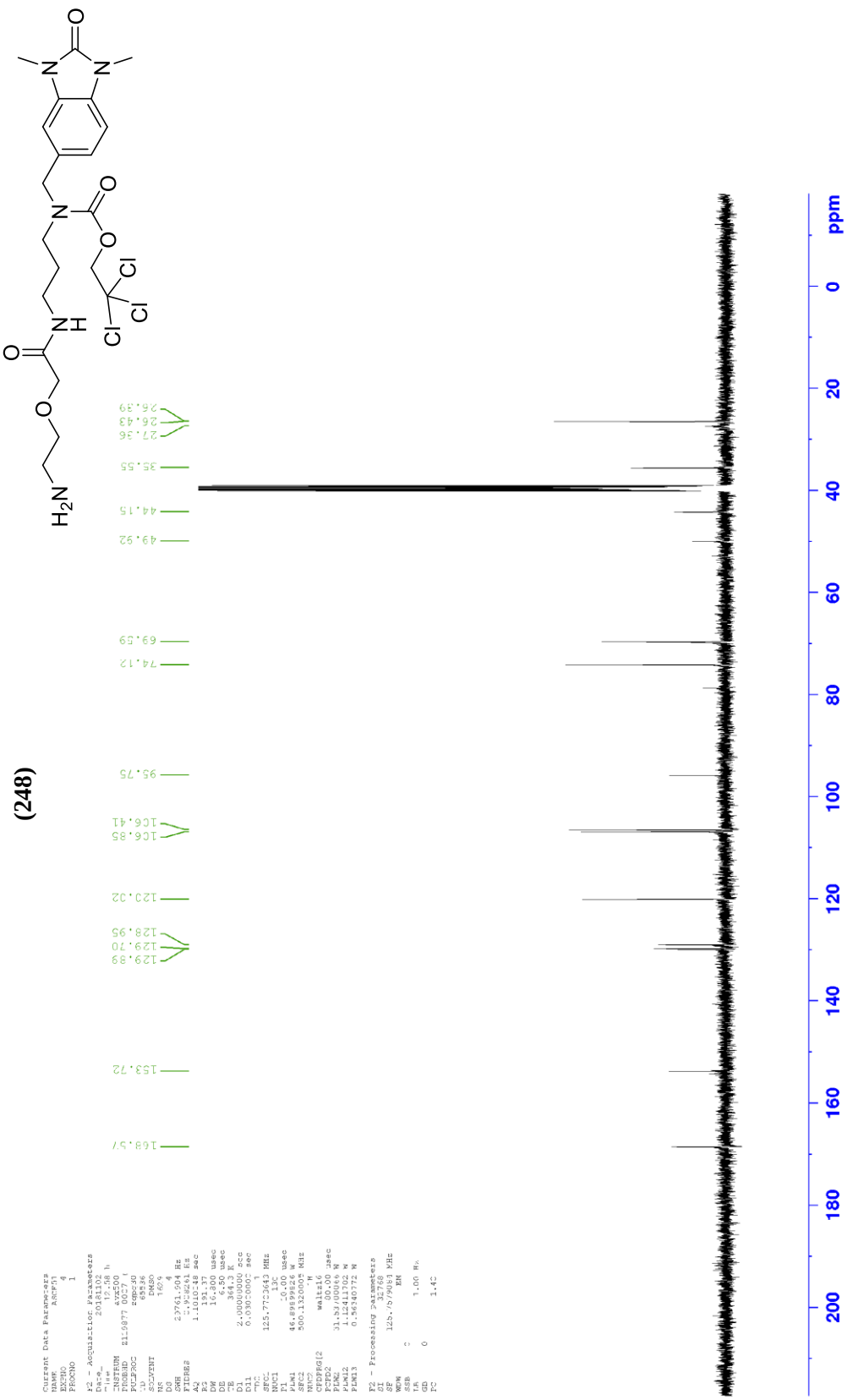


2,2,2-Trichloroethyl {3-[2-(2-aminoethoxy)acetamido]prop-1-yl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (248)

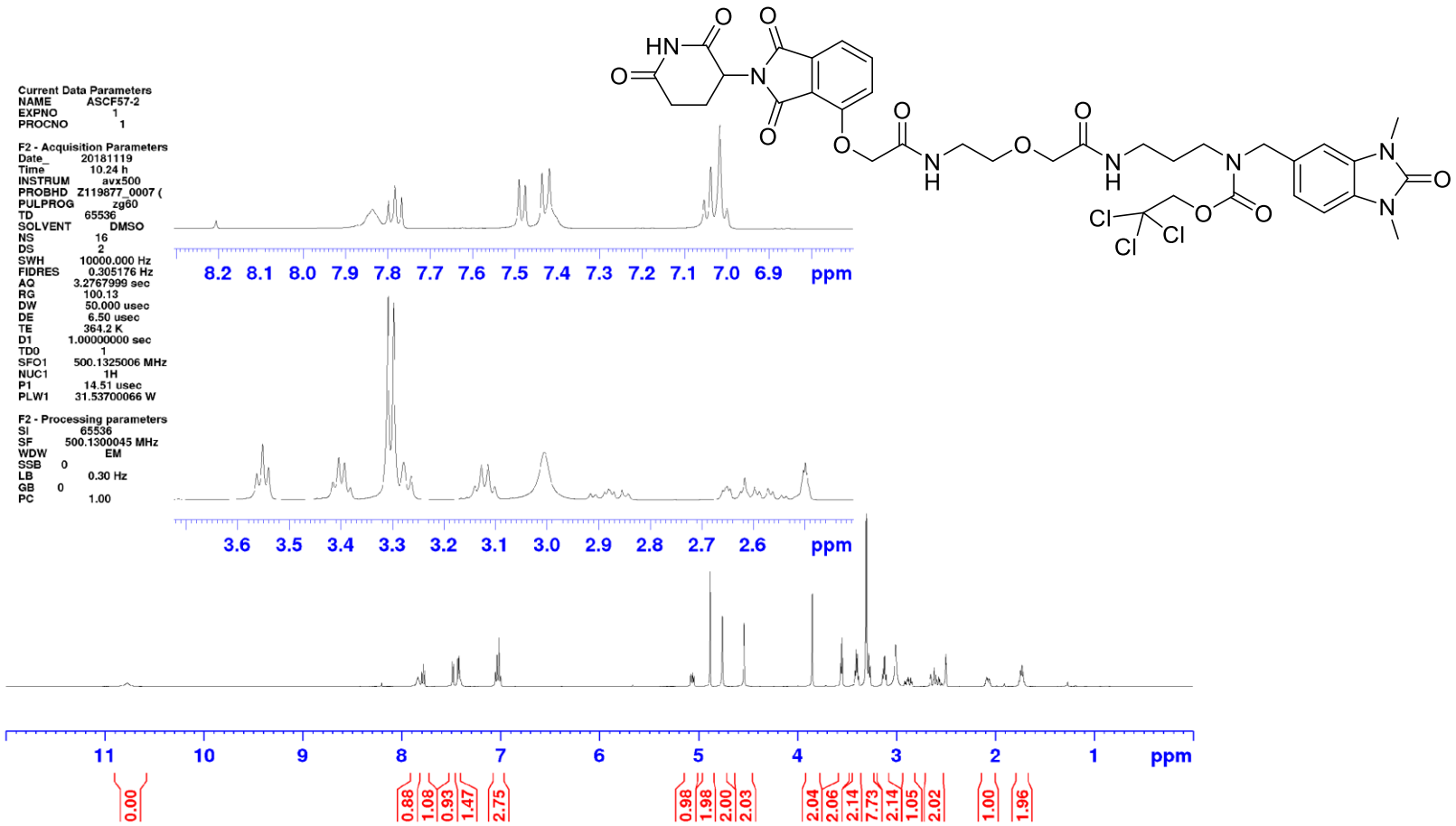


2,2,2-Trichloroethyl {3-[2-(2-aminoethoxy)acetamido]prop-1-yl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate

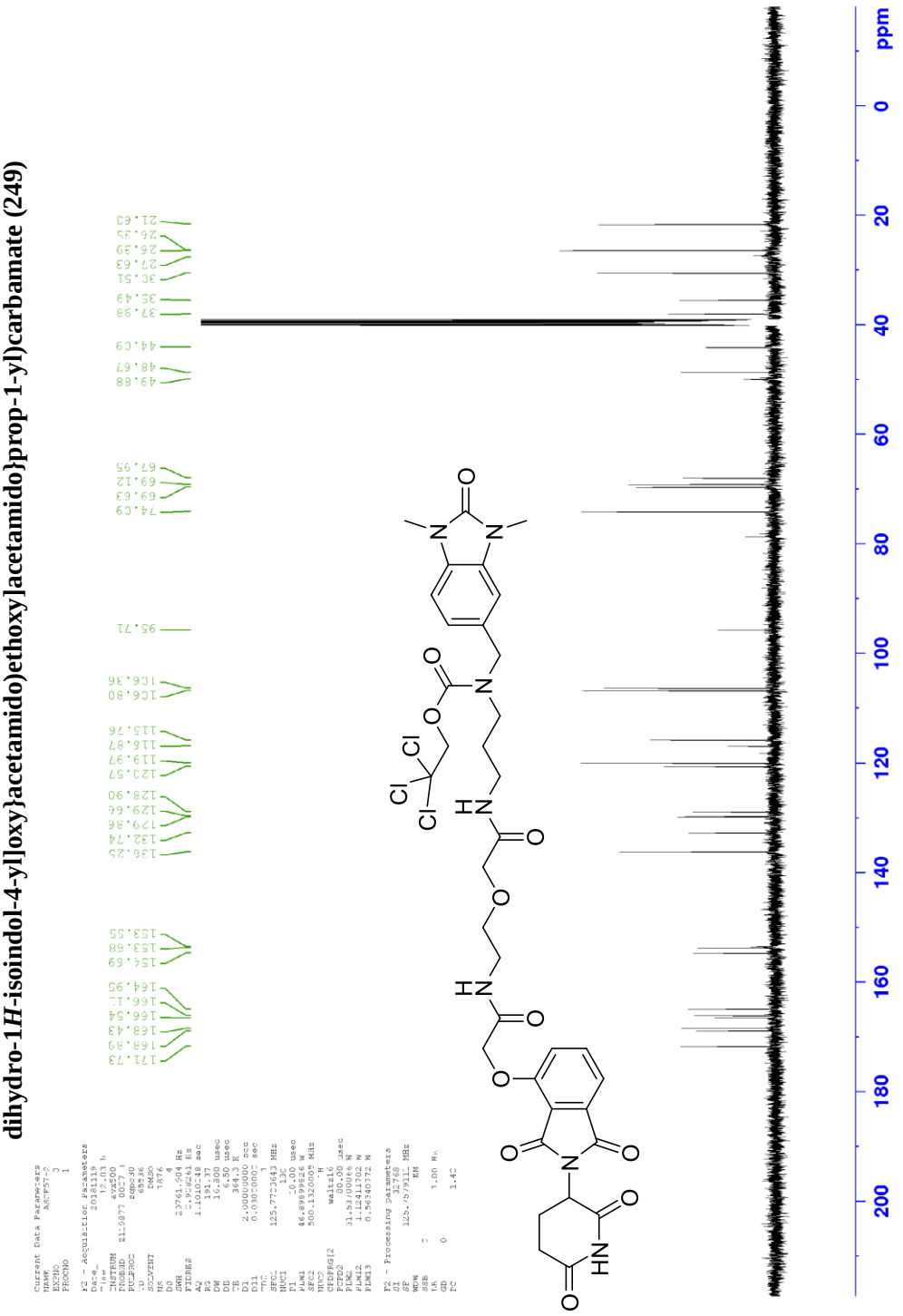
(248)



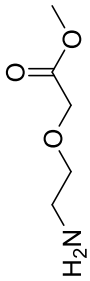
2,2,2-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](3-{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]acetamido}prop-1-yl)carbamate (249)

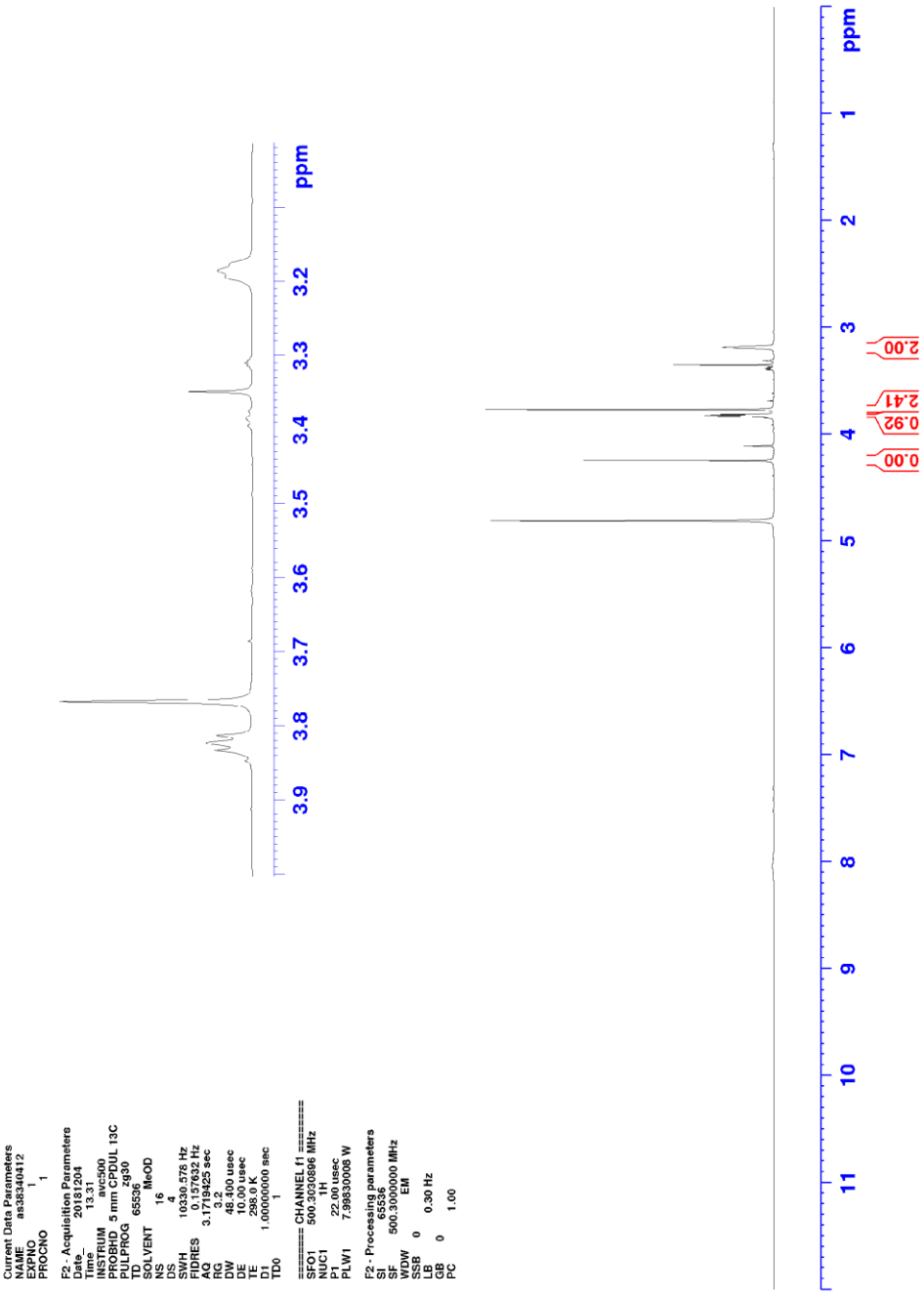


2,2,2-Trichloroethyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](3-{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]acetamido}prop-1-yl)carbamate (249)

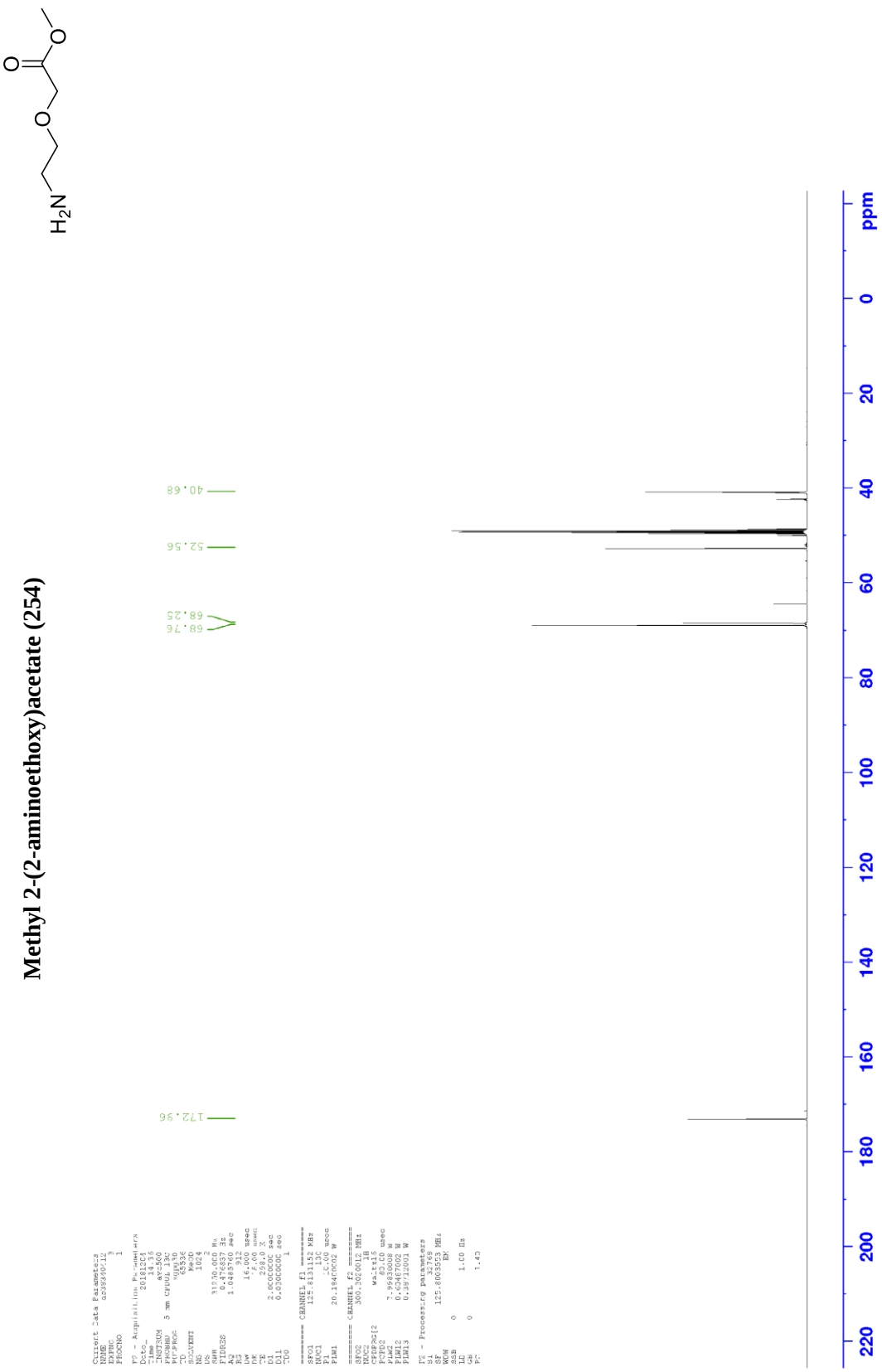


Methyl 2-(2-aminoethoxy)acetate (254)

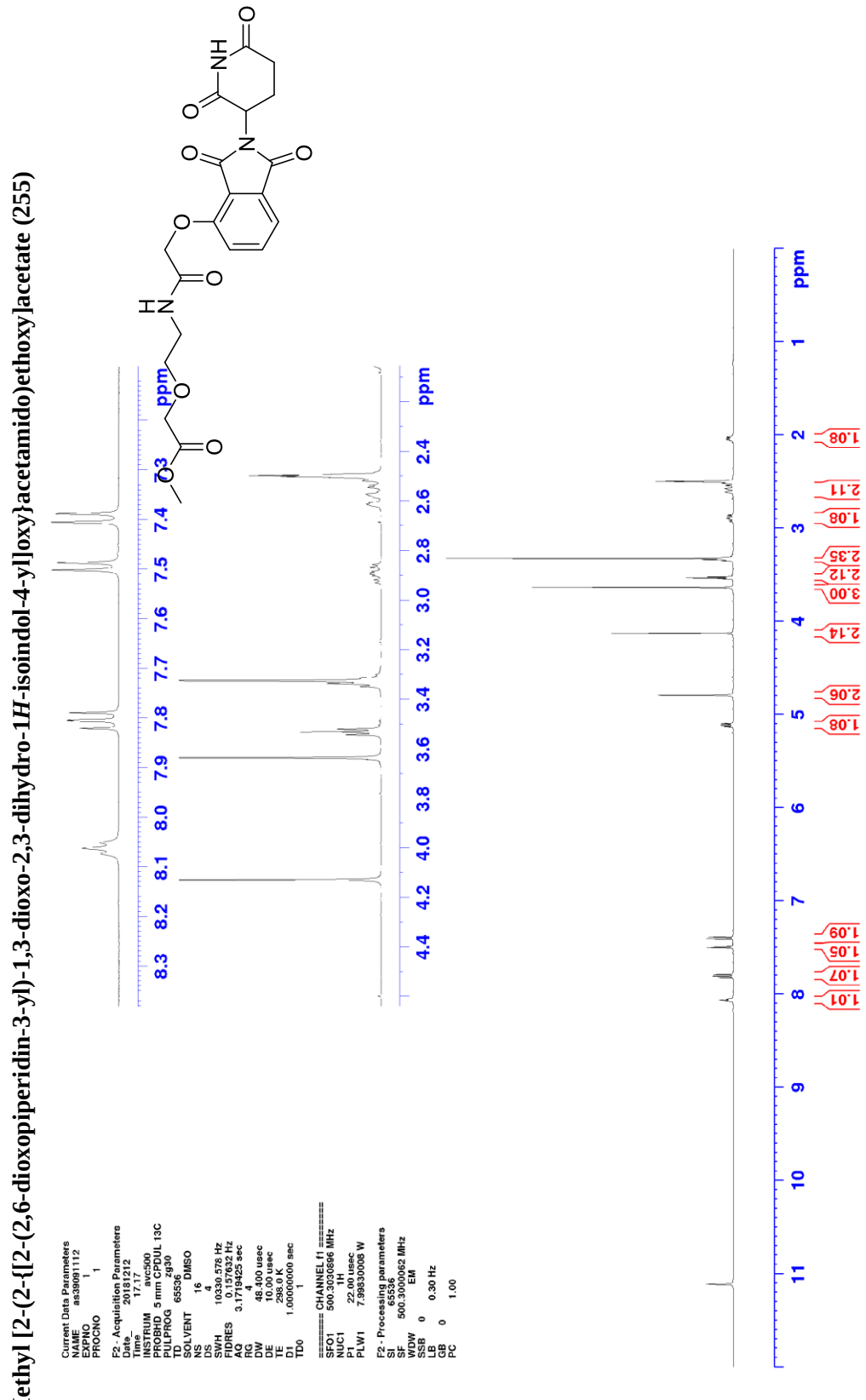




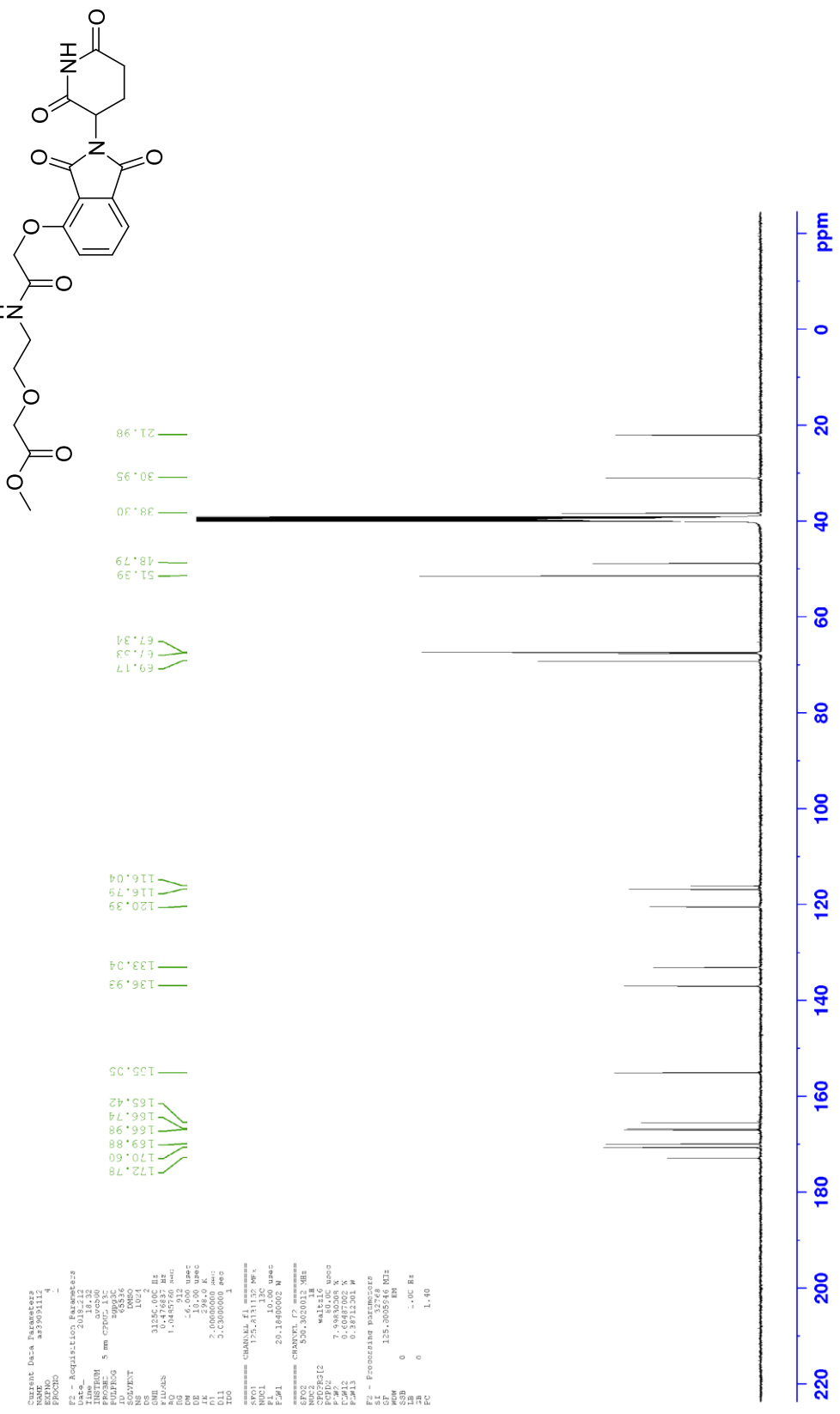
Methyl 2-(2-aminoethoxy)acetate (254)



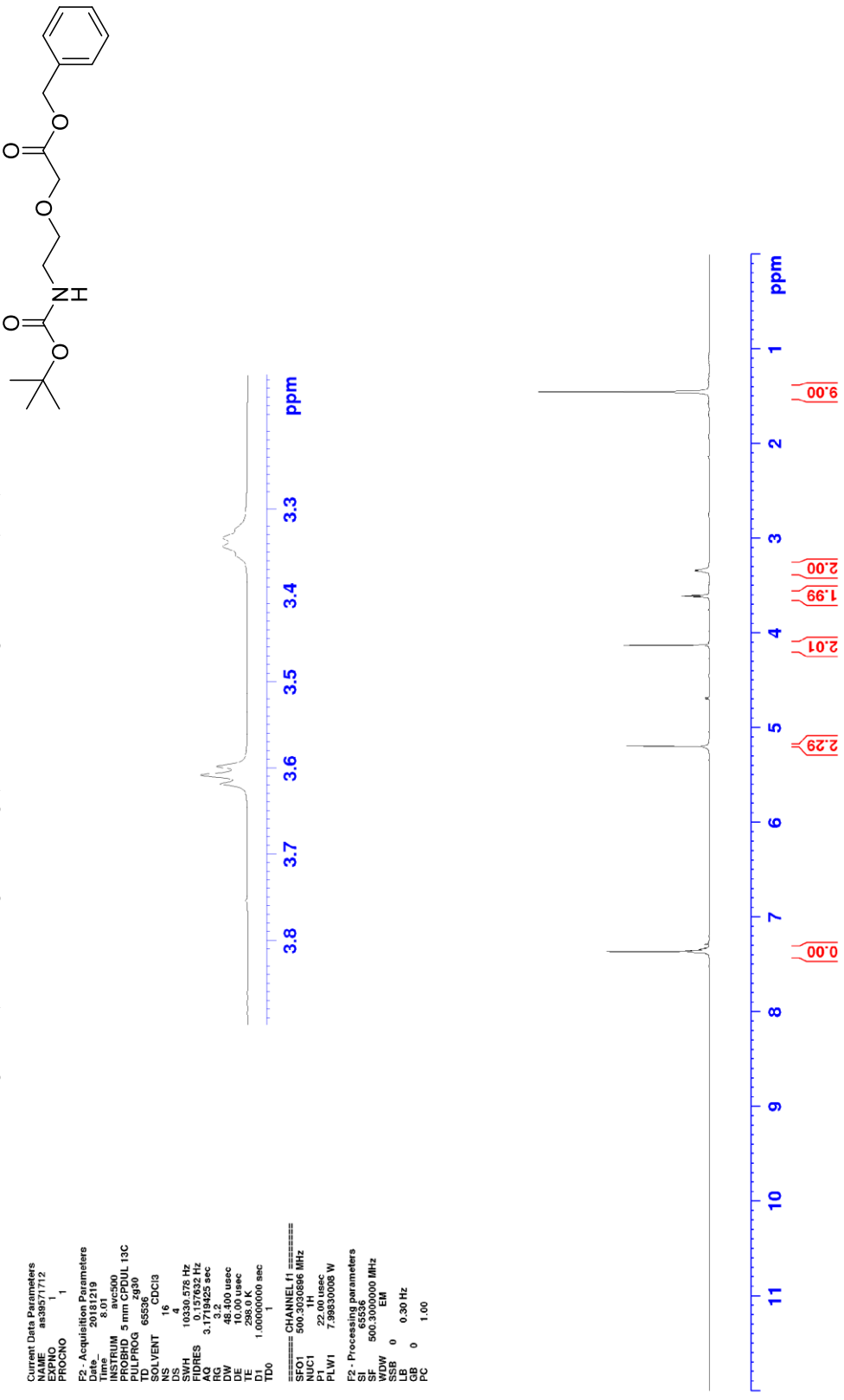
Methyl [2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]acetate (255)



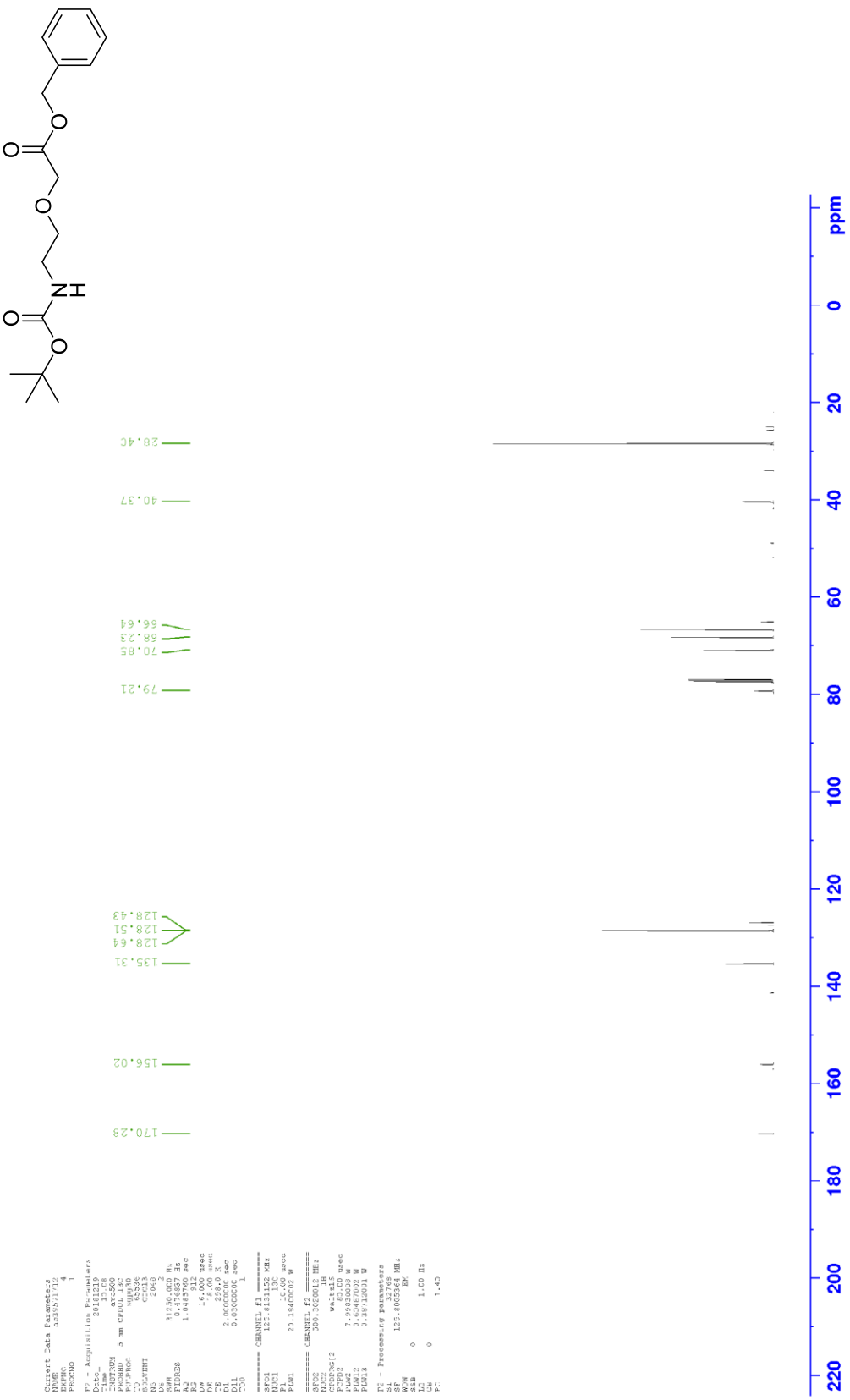
Methyl [2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]acetate (255)



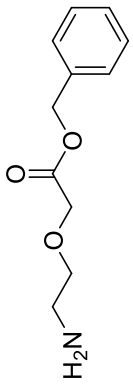
Benzyl {2-[(*tert*-butoxycarbonyl)amino]ethoxy}acetate (257)

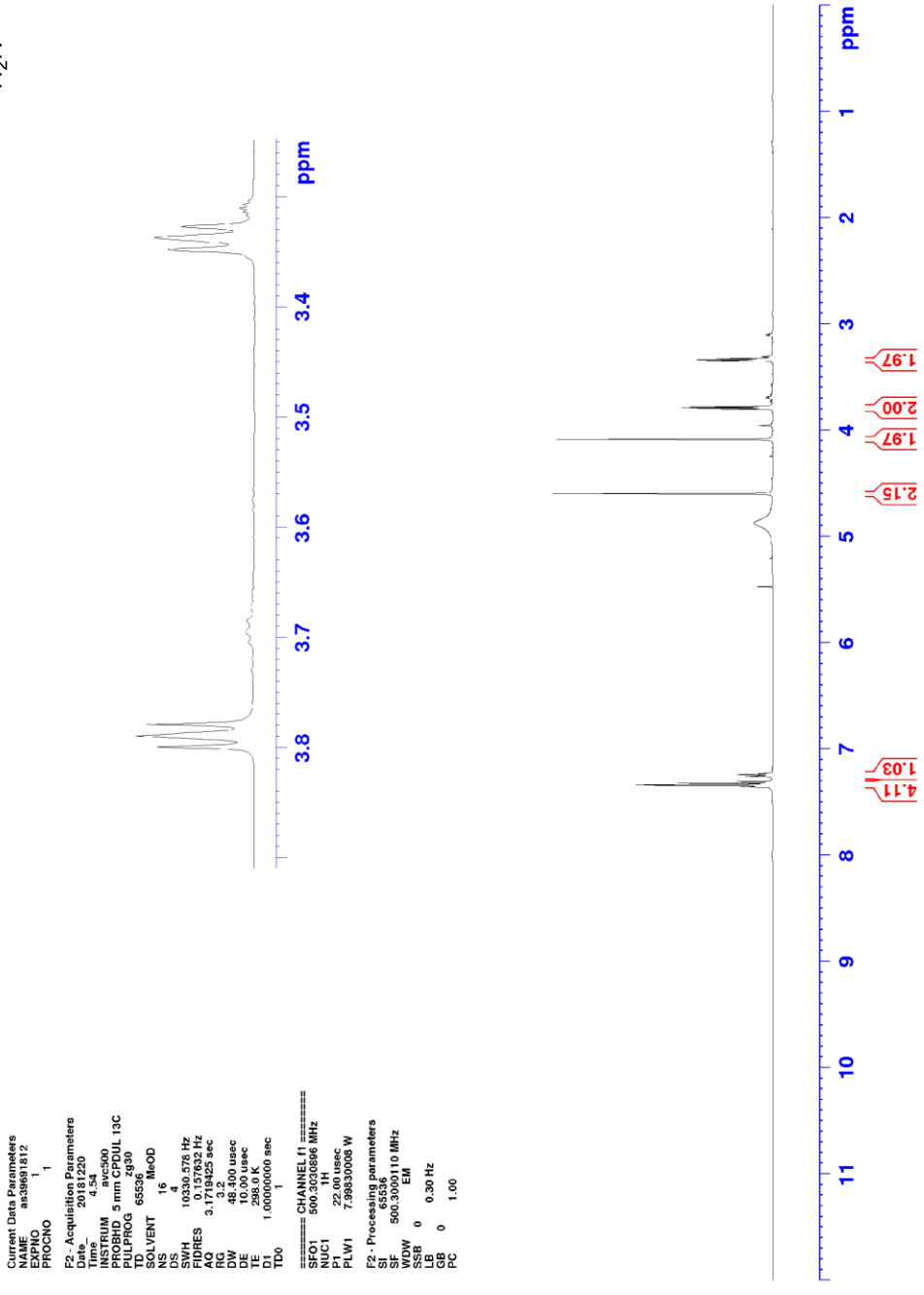


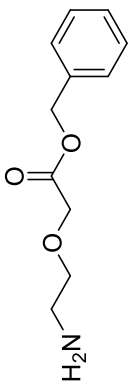
Benzyl {2-[(*tert*-butoxycarbonyl)amino]ethoxy}acetate (257)



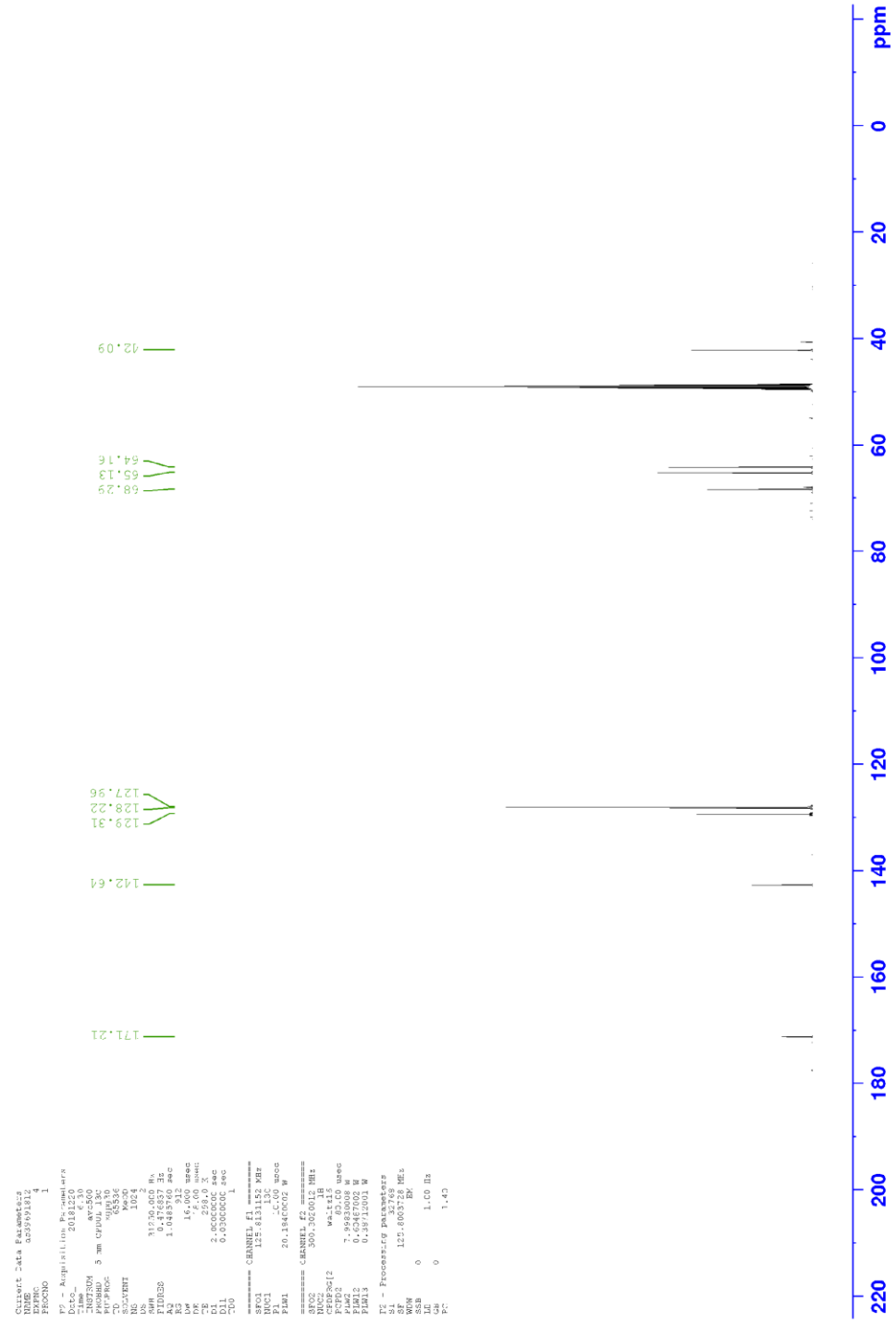
Benzyl 2-(2-aminoethoxy)acetate (258)



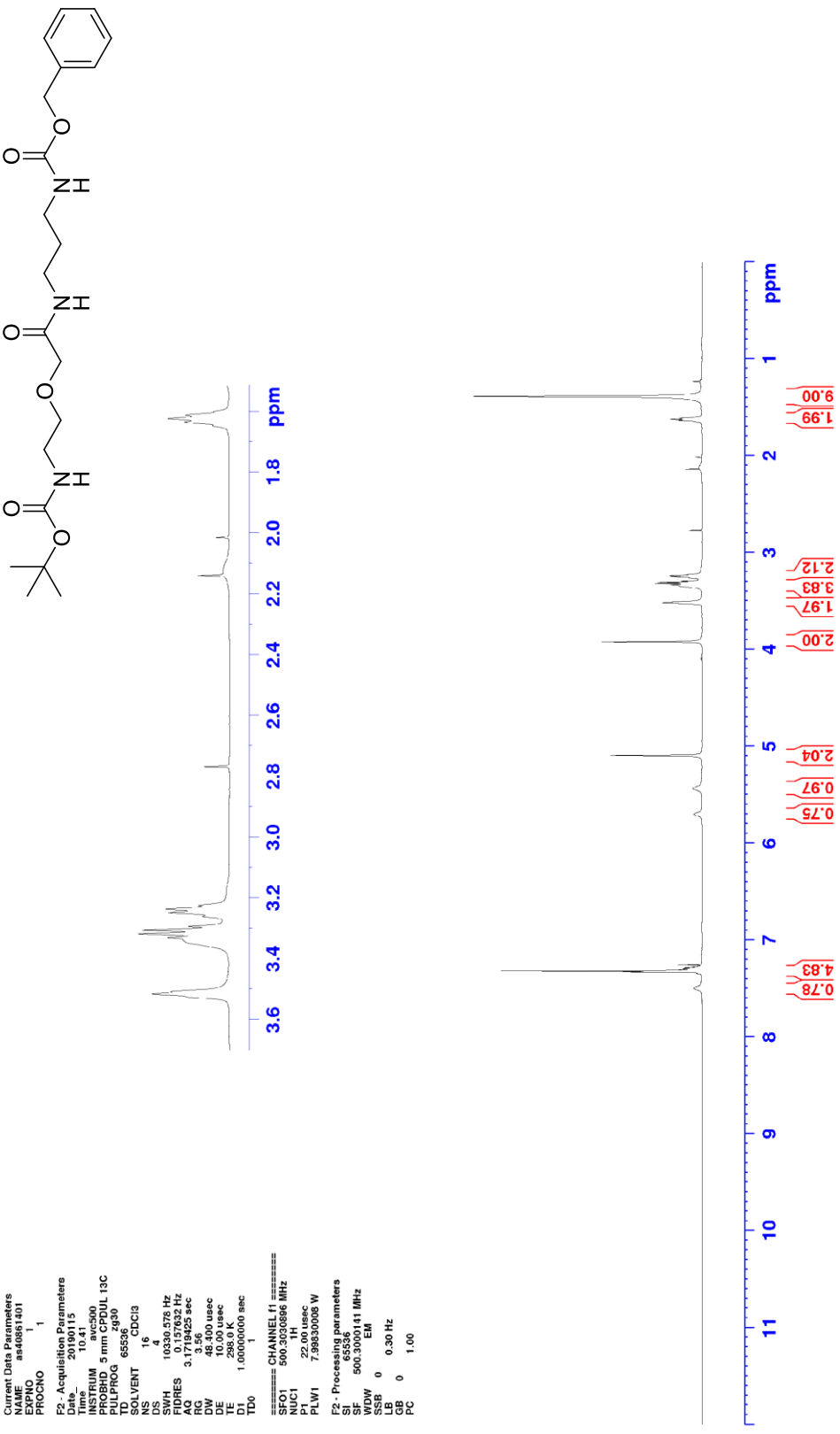




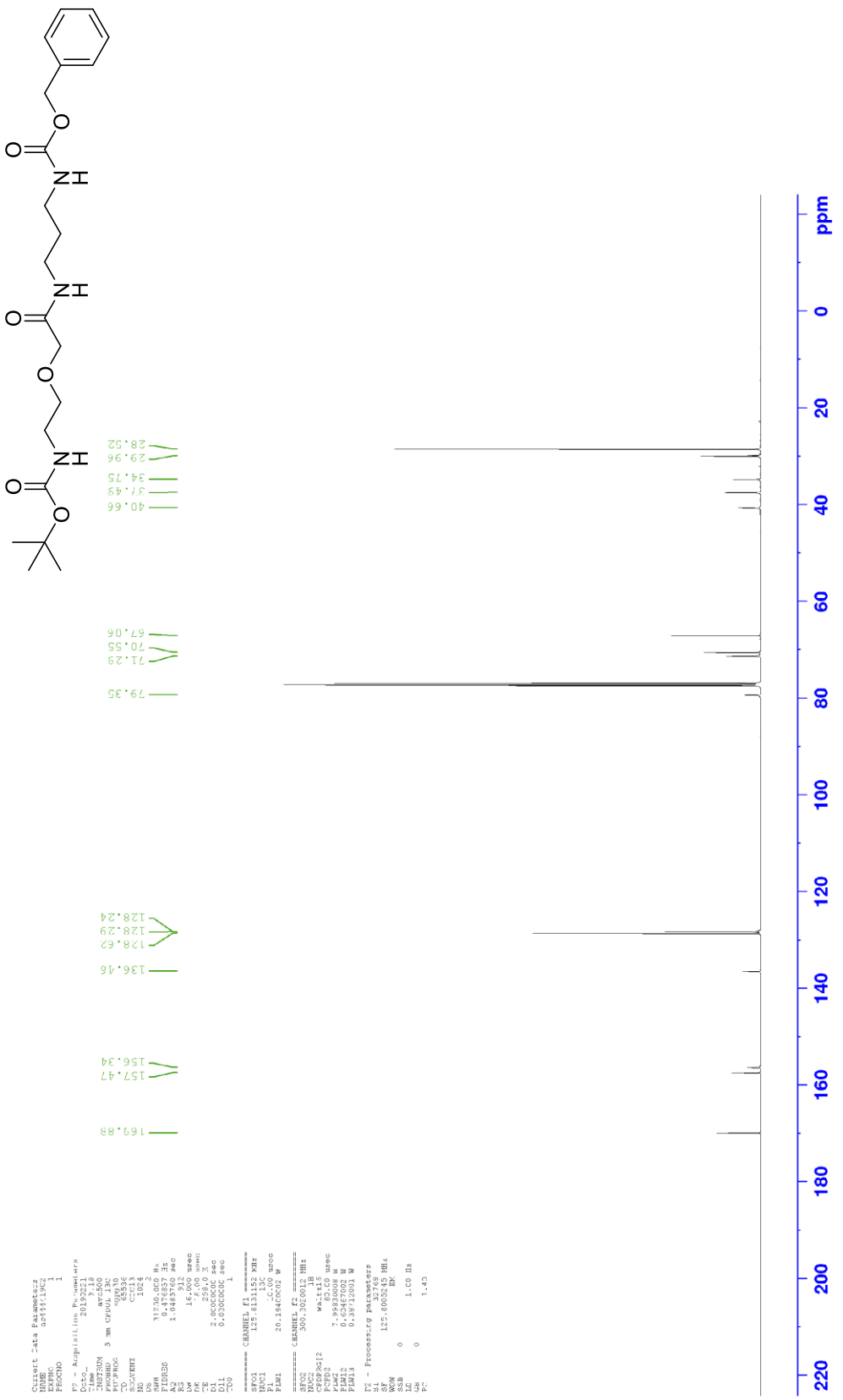
Benzyl 2-(2-aminoethoxy)acetate (258)



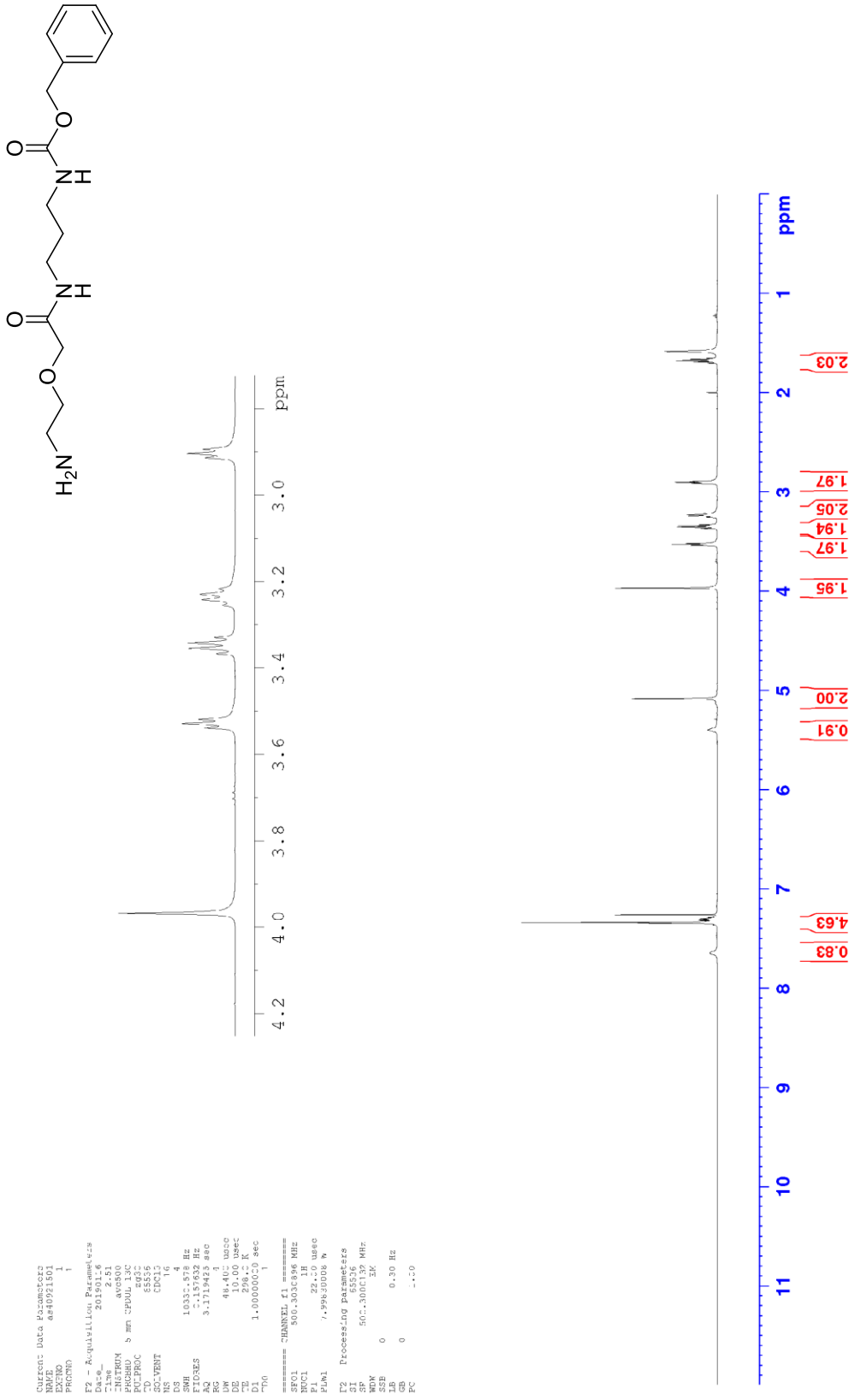
Benzyl (2,2-dimethyl-4,10-dioxo-3,8-dioxa-5,11-diazatetradecan-14-yl)carbamate (263)



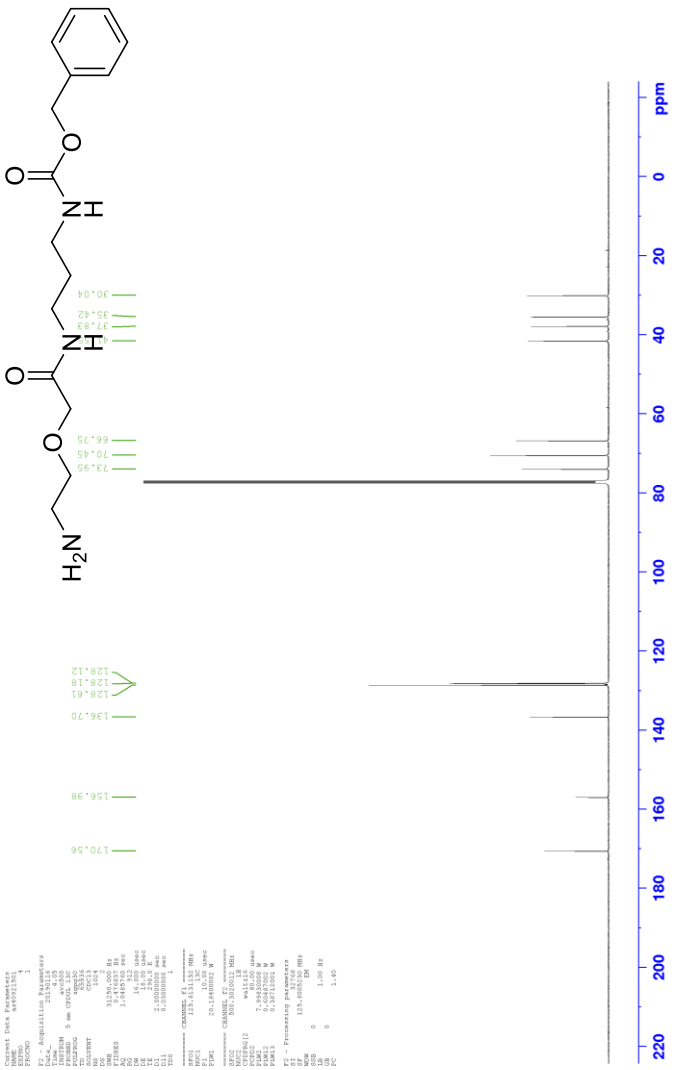
Benzyl (2,2-dimethyl-4,10-dioxo-3,8-dioxa-5,11-diazatetradecan-14-yl)carbamate (263)



Benzyl {3-[2-(2-aminoethoxy)acetamido]propyl}carbamate (259)

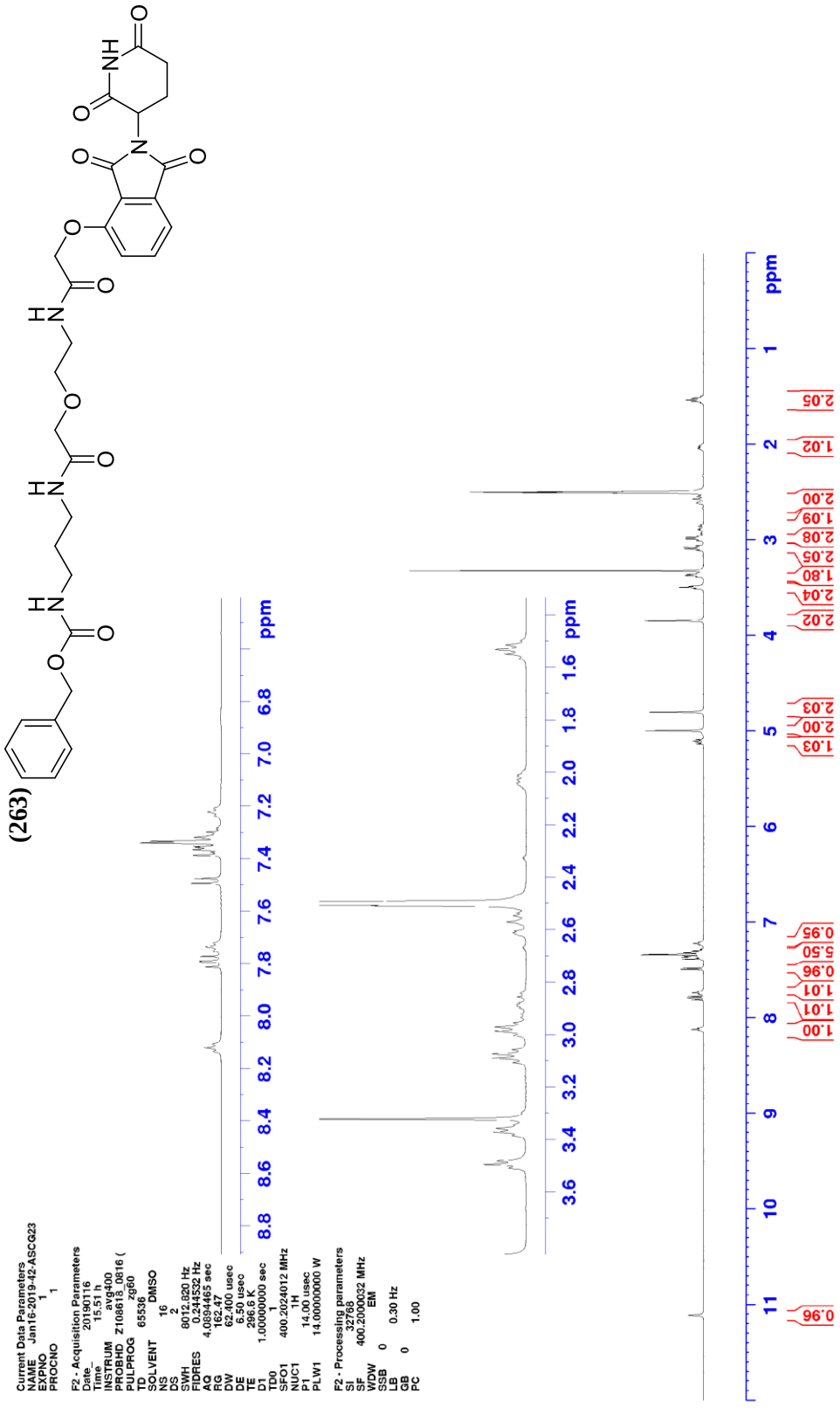


Benzyl {3-[2-(2-aminoethoxy)acetamido]propyl}carbamate (259)



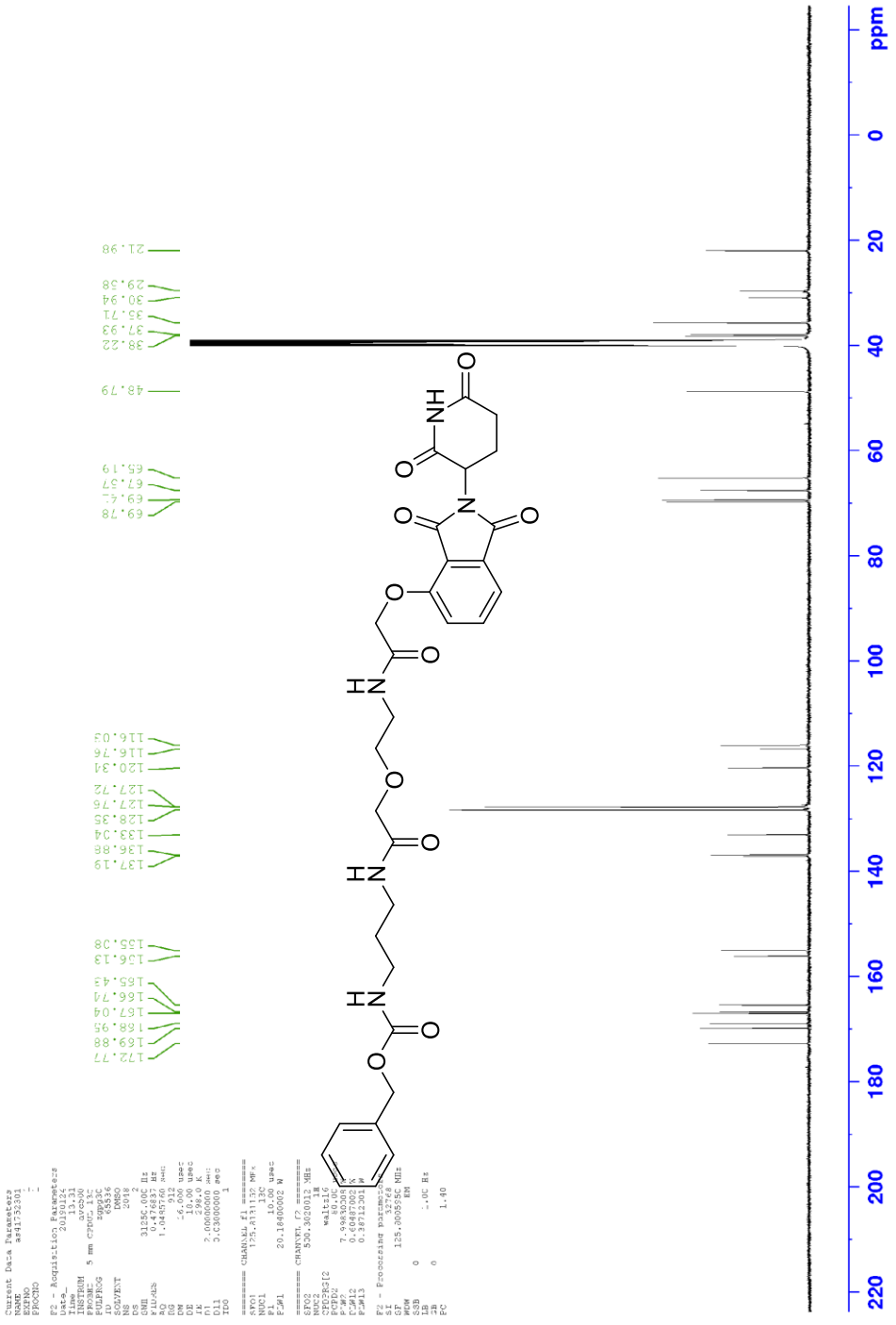
Benzyl (3-{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]acetamido}propyl)carbamate

(263)

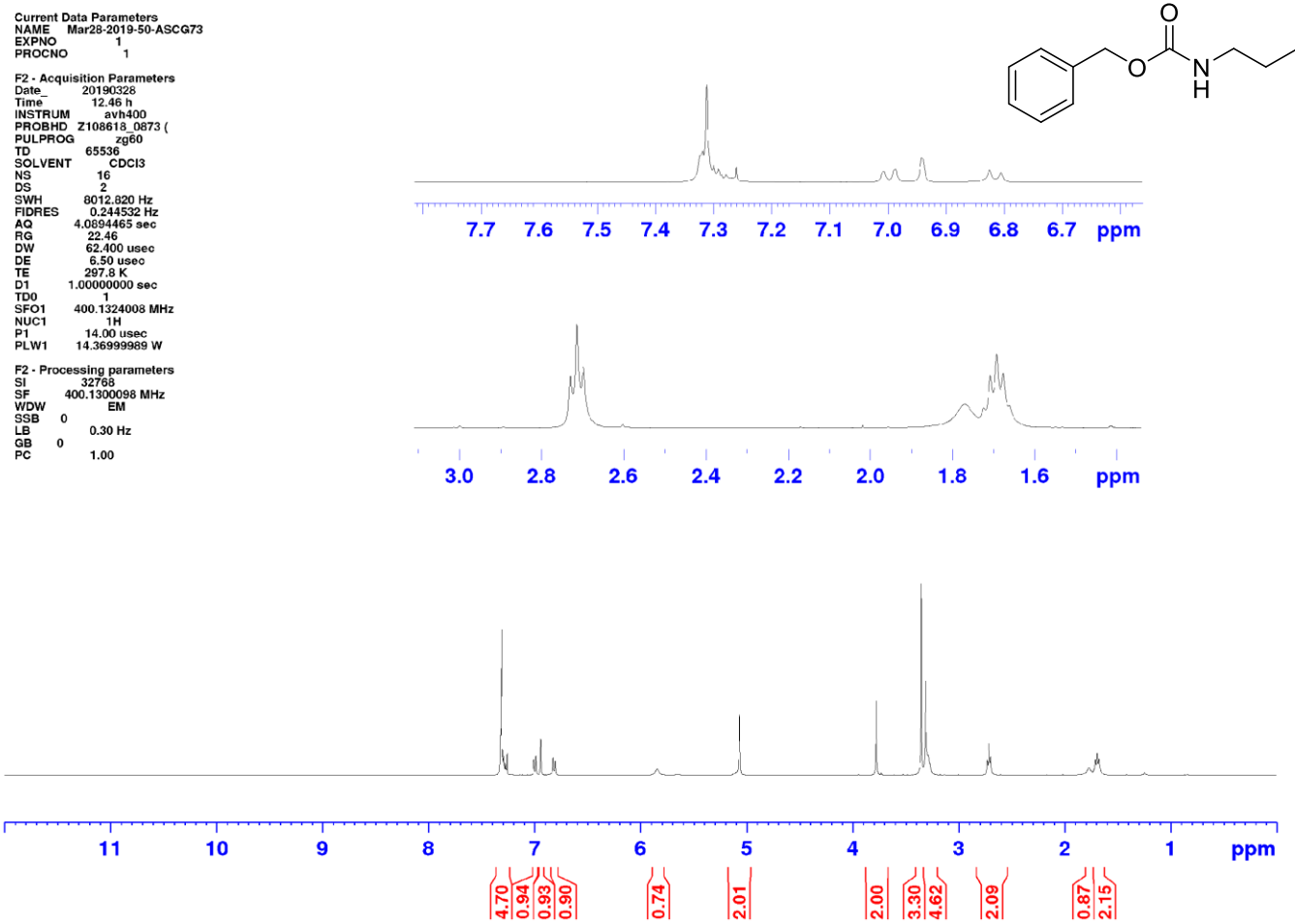


Benzyl (3-{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]acetamido}propyl)carbamate

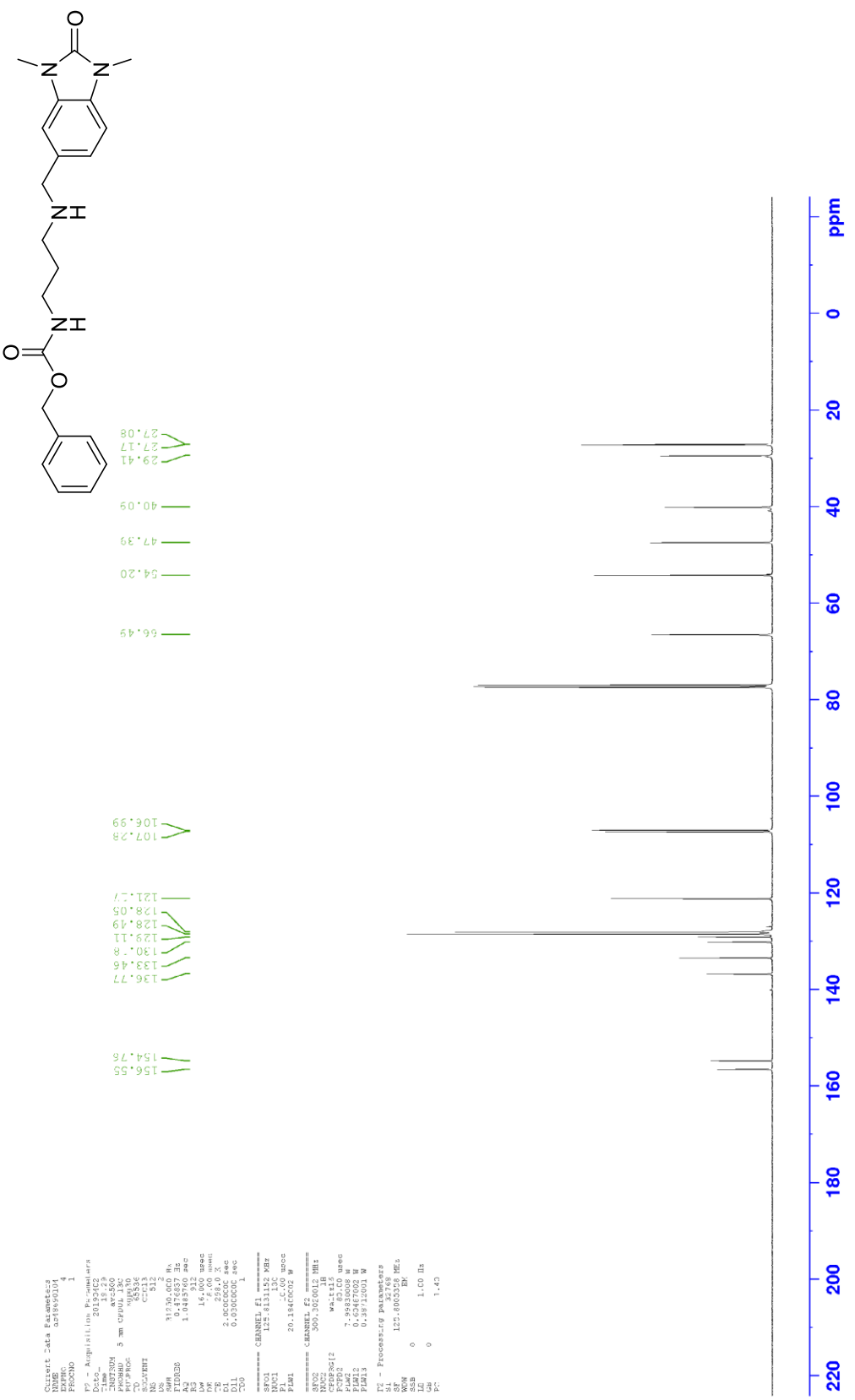
(264)



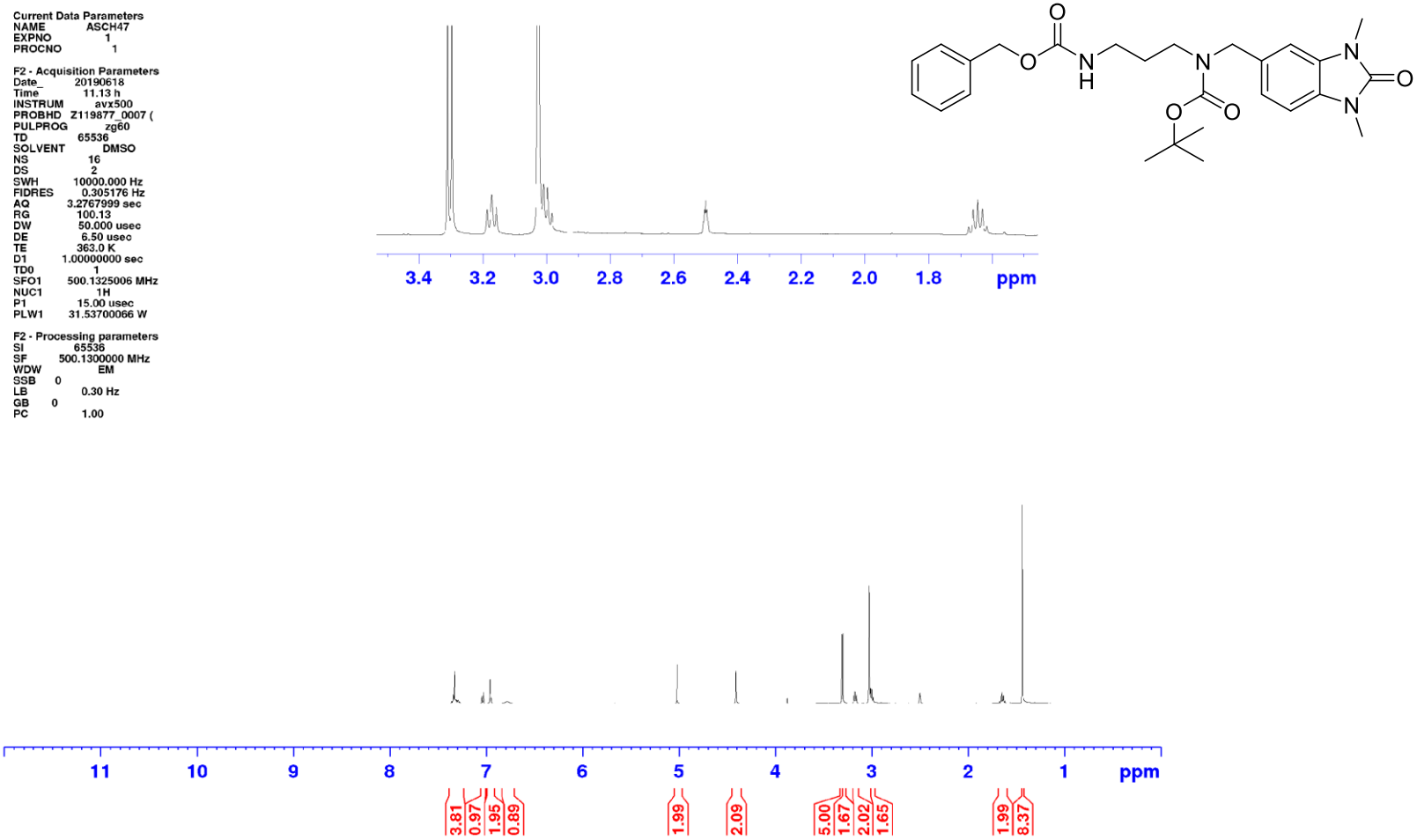
Benzyl (3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1-yl)carbamate (270)



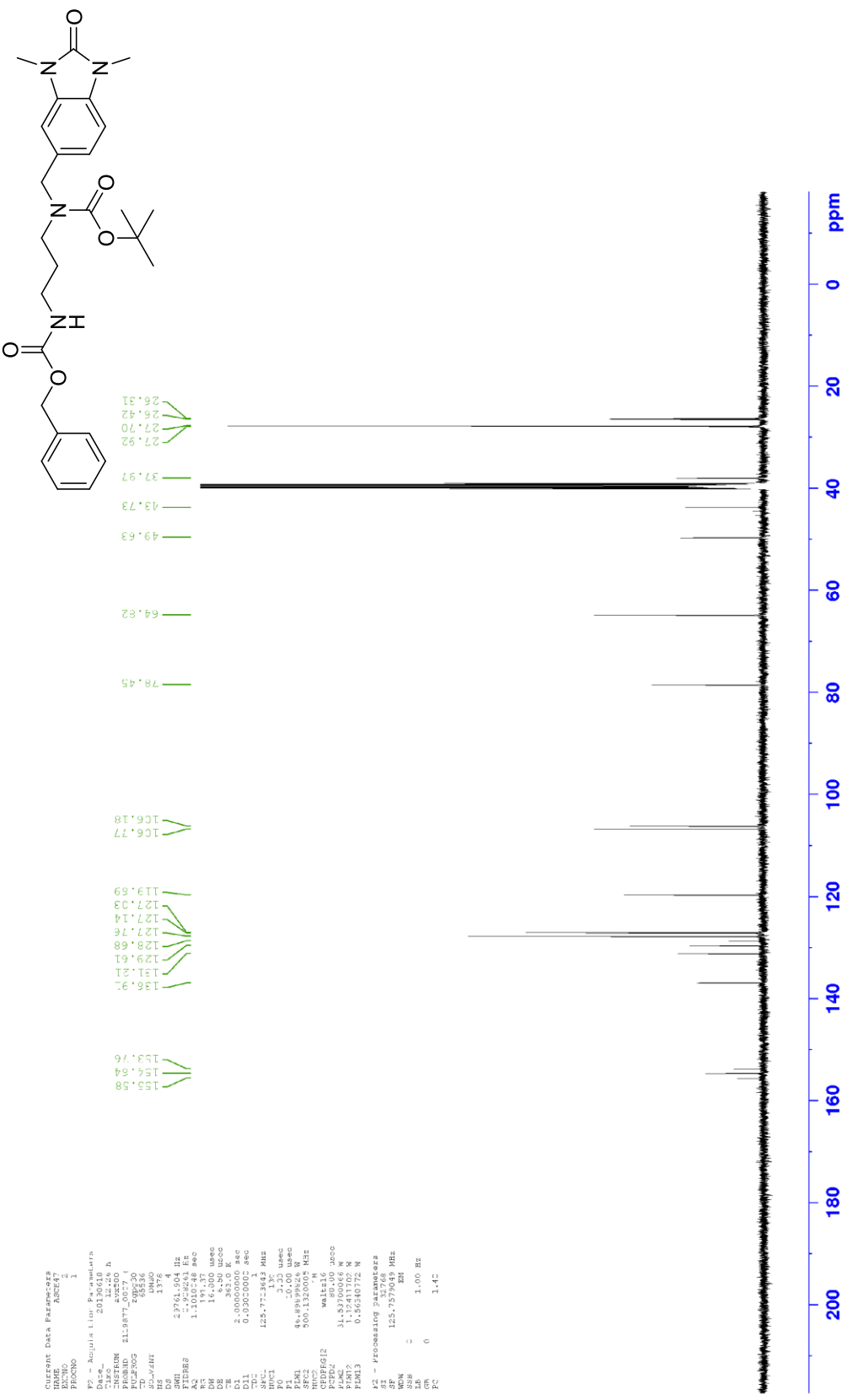
Benzyl (3-{[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1-yl)carbamate (270)



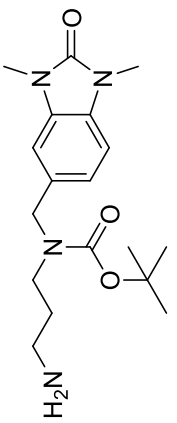
Benzyl (3-{(*tert*-butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1-yl)carbamate (271)

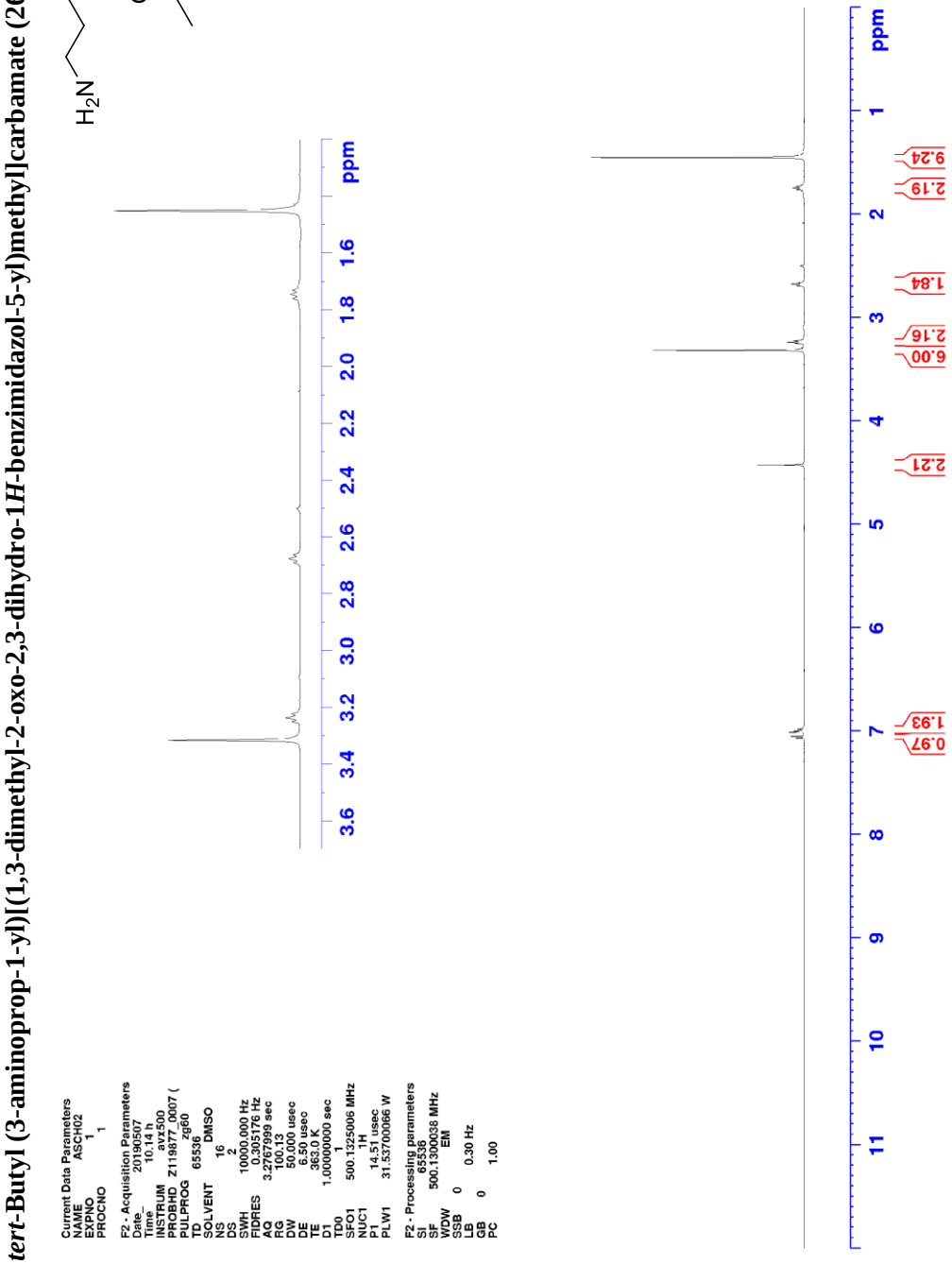


Benzyl (3-{(*tert*-butoxycarbonyl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1-yl)carbamate (271)

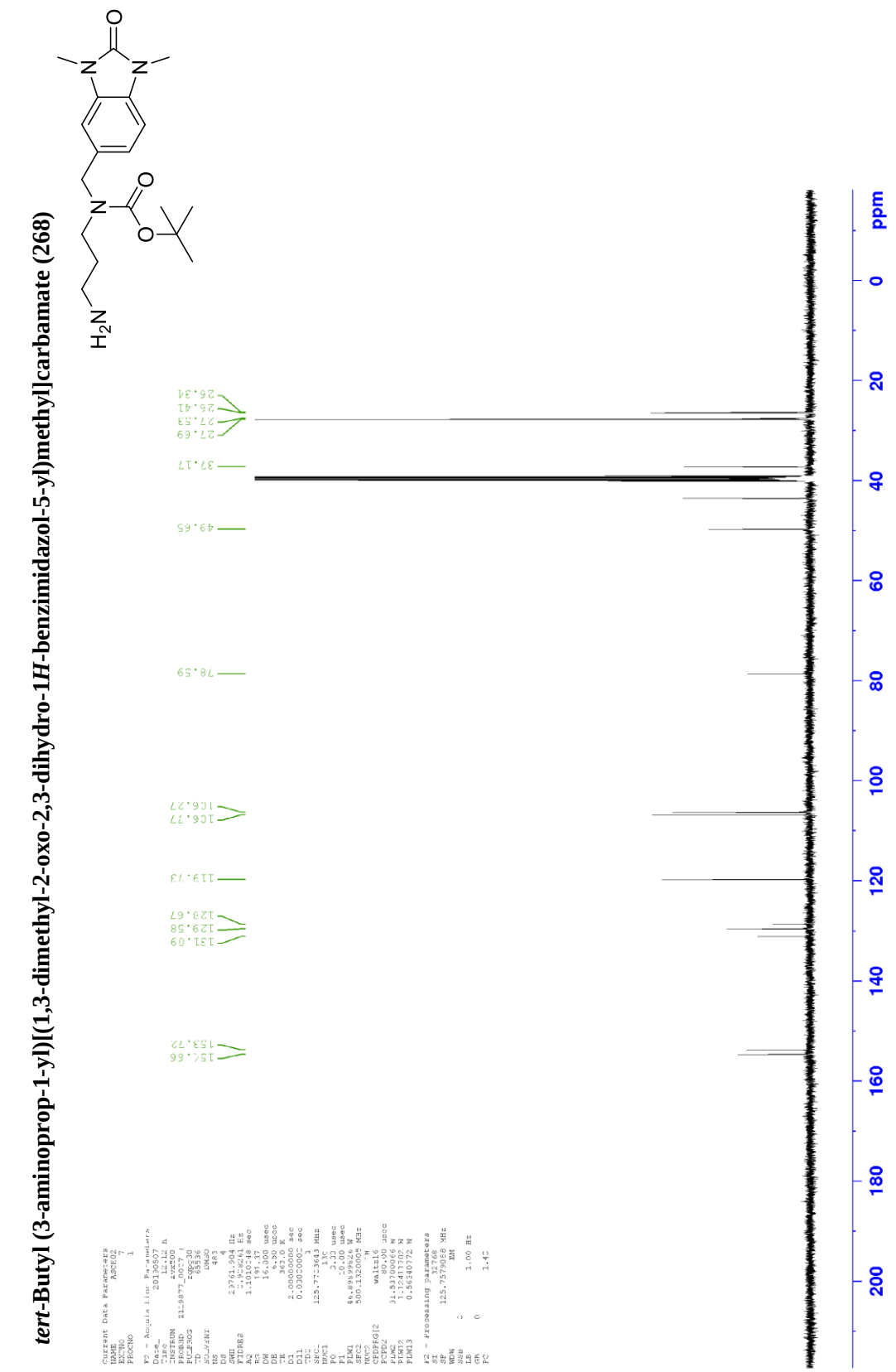


tert-Butyl (3-aminoprop-1-yl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (268)

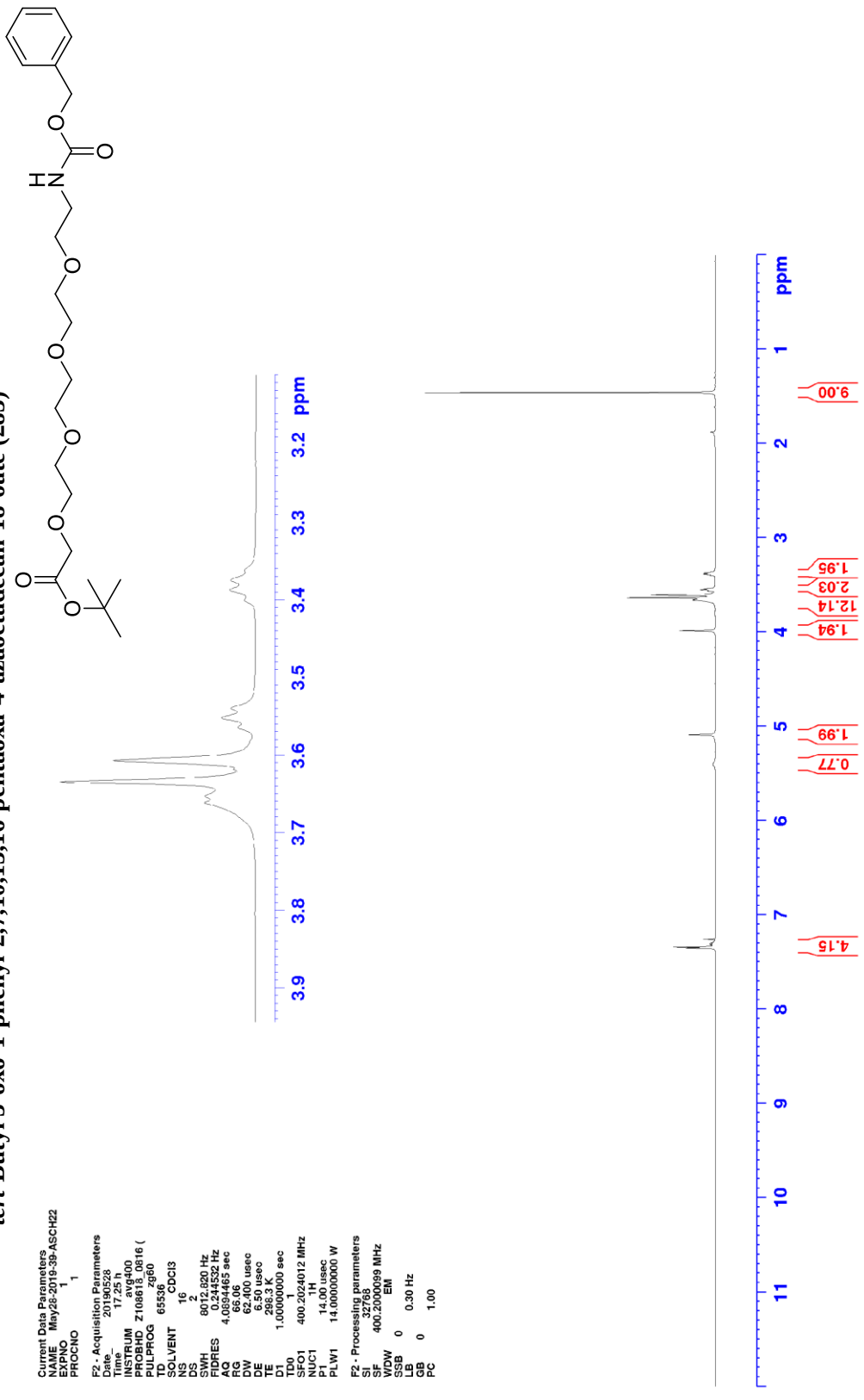




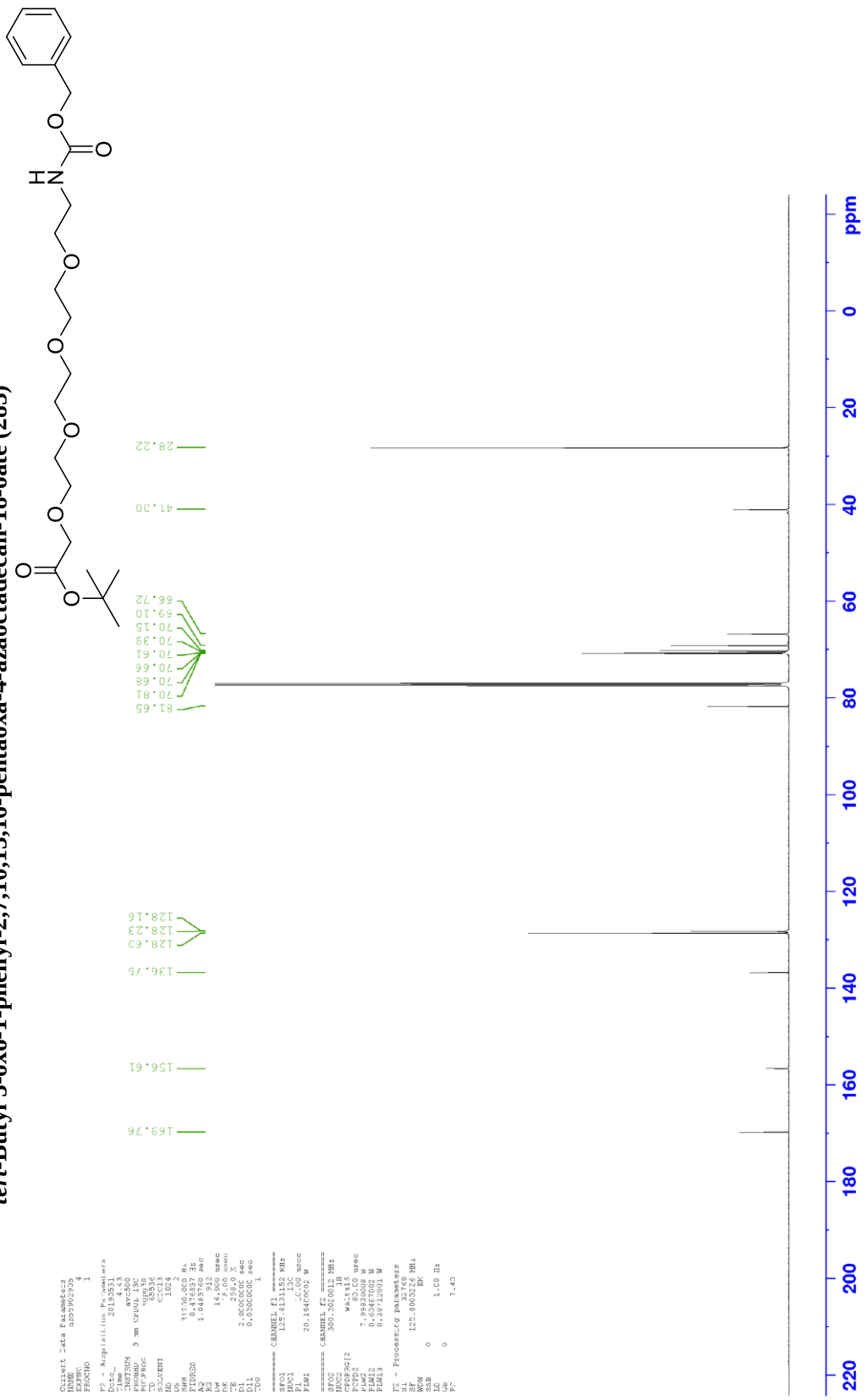
tert-Butyl (3-aminoprop-1-yl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (268)



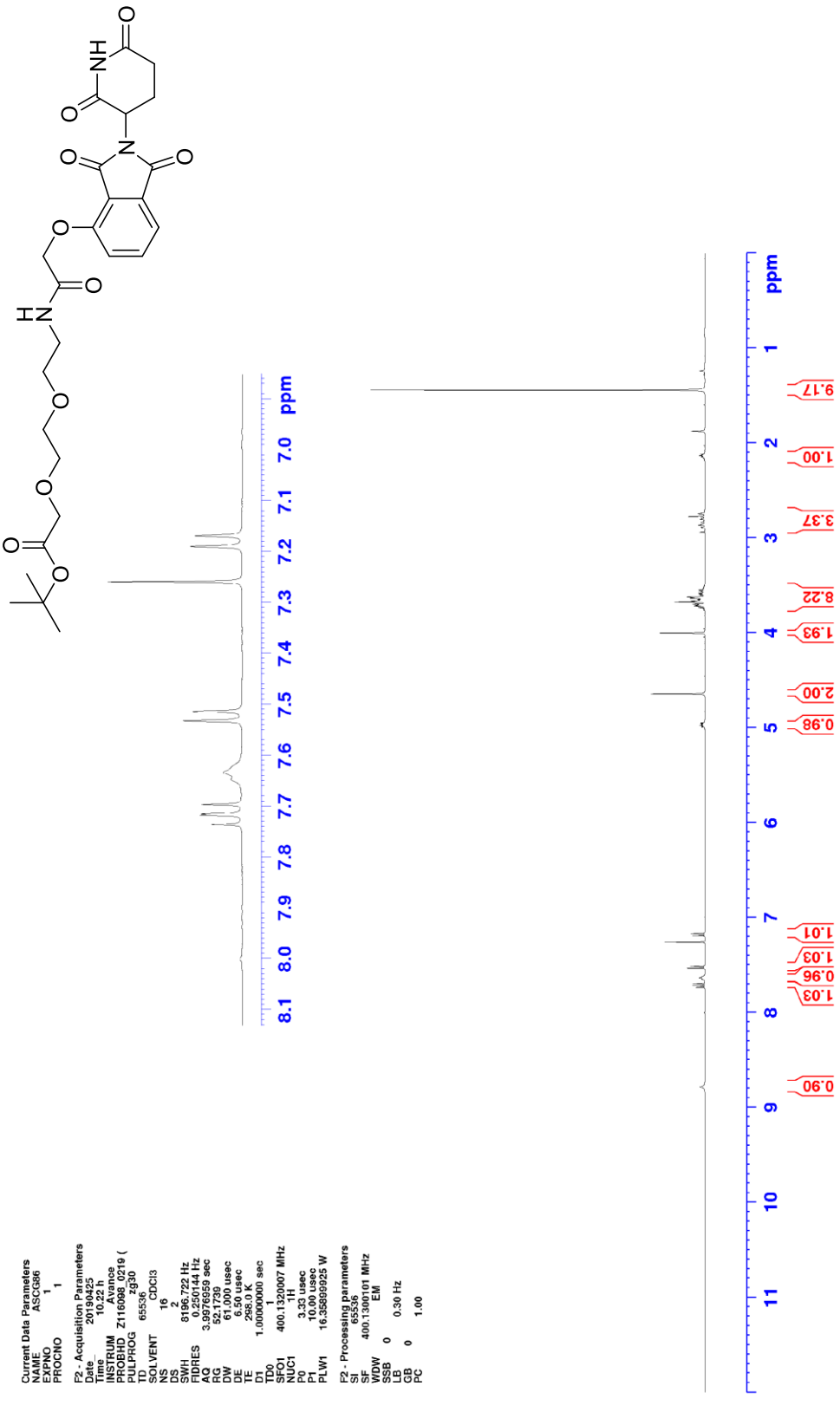
tert-Butyl 3-oxo-1-phenyl-2,7,10,13,16-pentaoxa-4-azaoctadecan-18-oate (283)



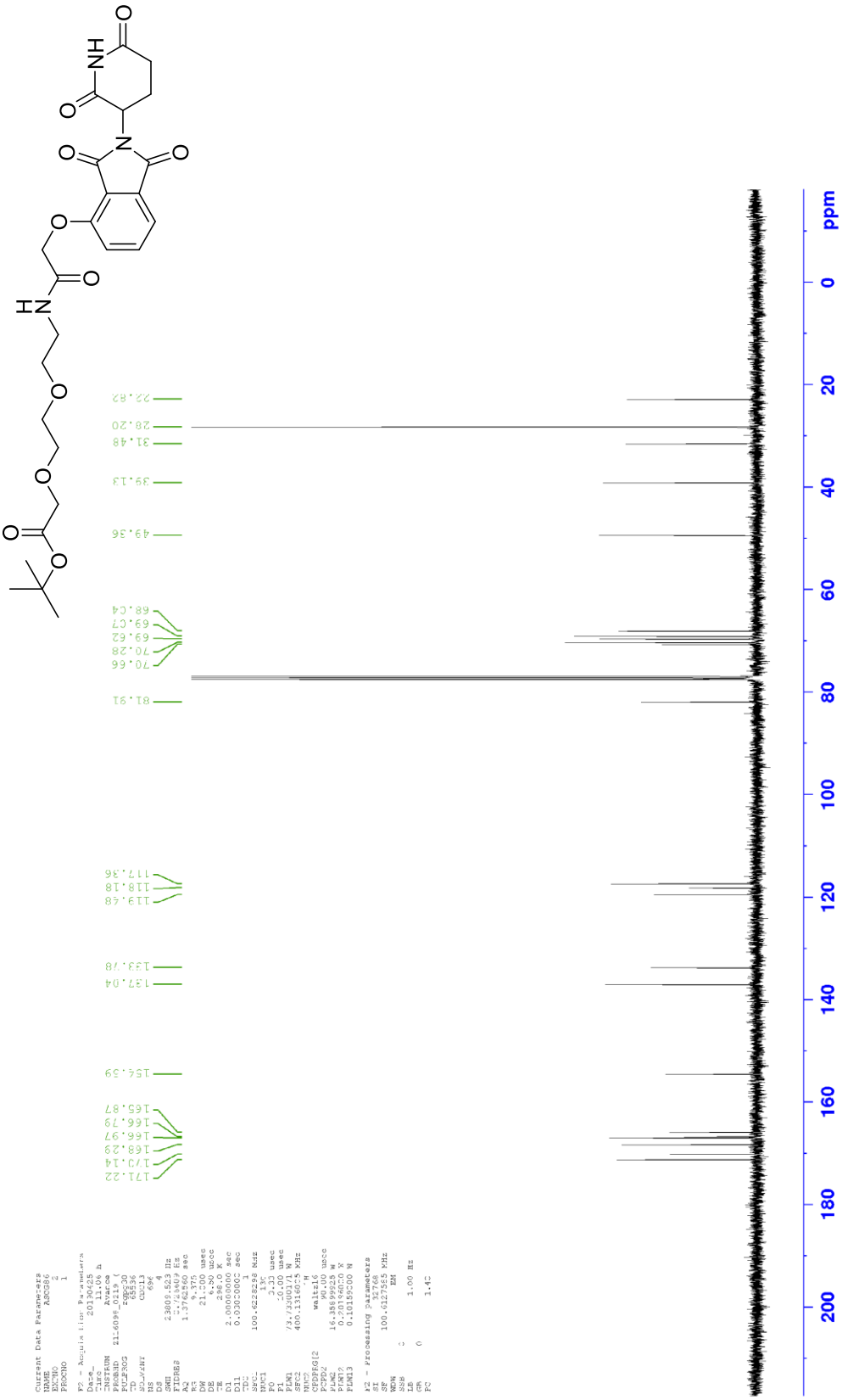
tert-Butyl 3-oxo-1-phenyl-2,7,10,13,16-pentaoxa-4-azaoctadecan-18-oate (283)



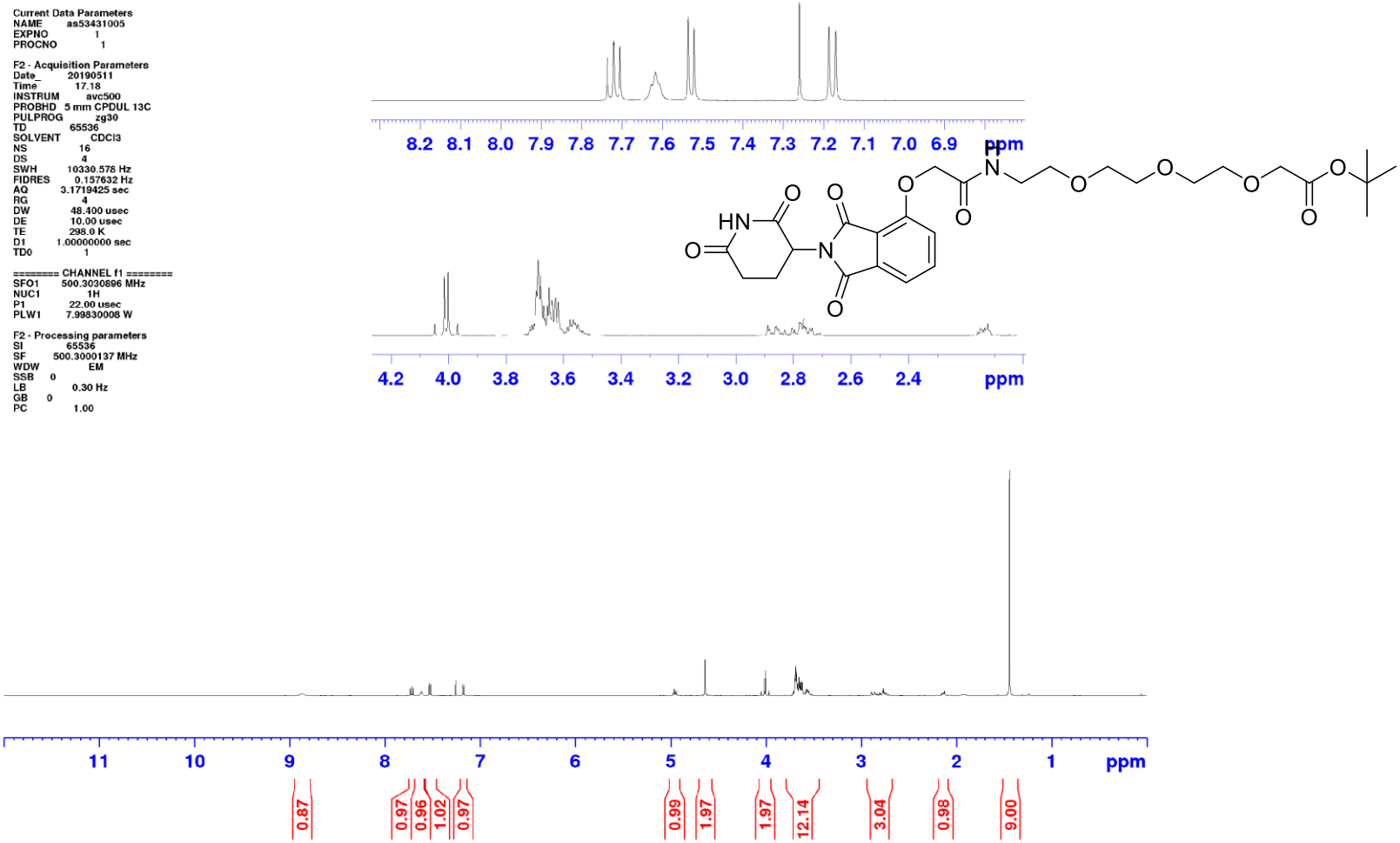
***tert*-Butyl {2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]ethoxy}acetate (284)**



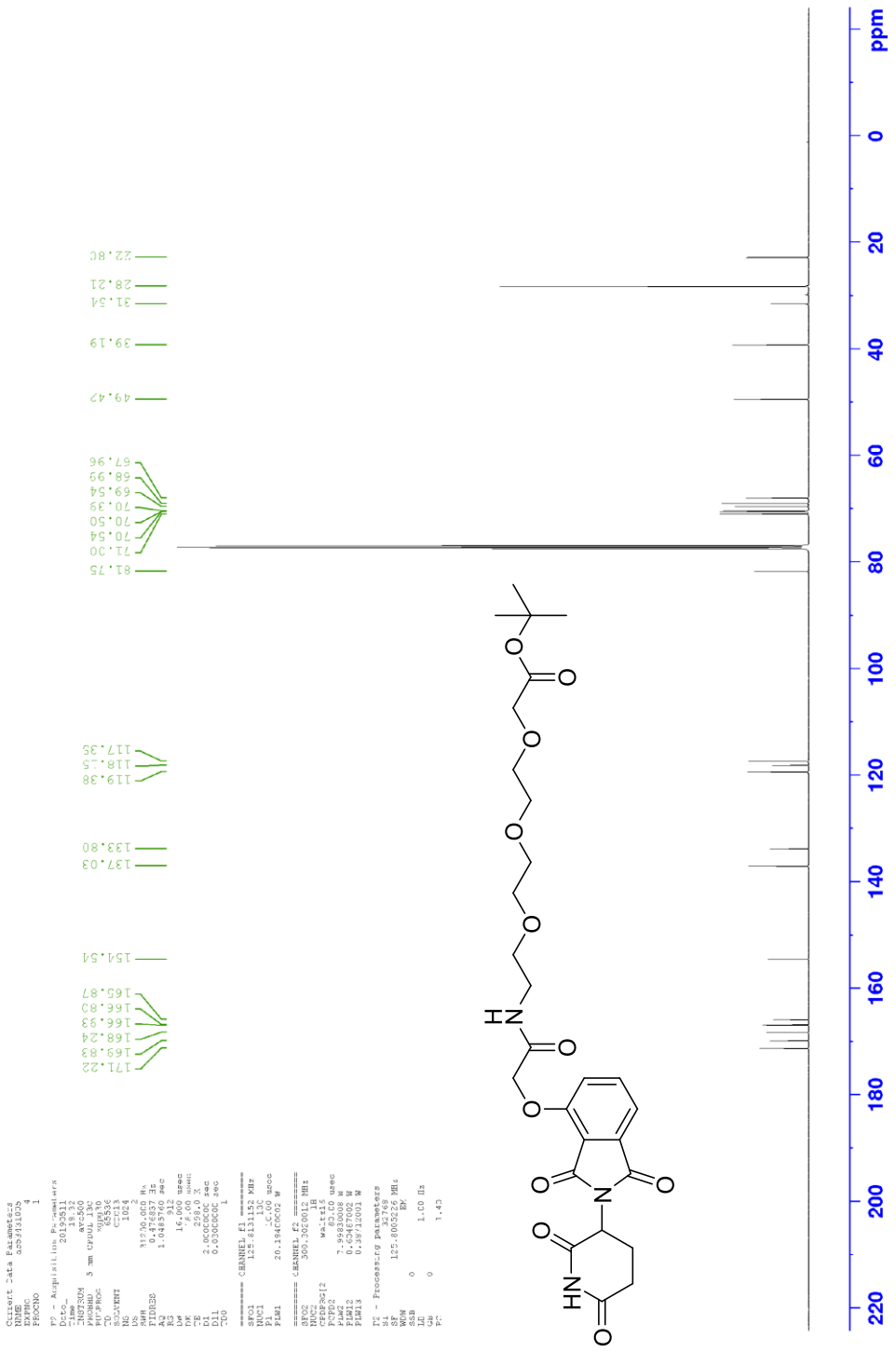
tert-Butyl {2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]ethoxy}acetate (284)



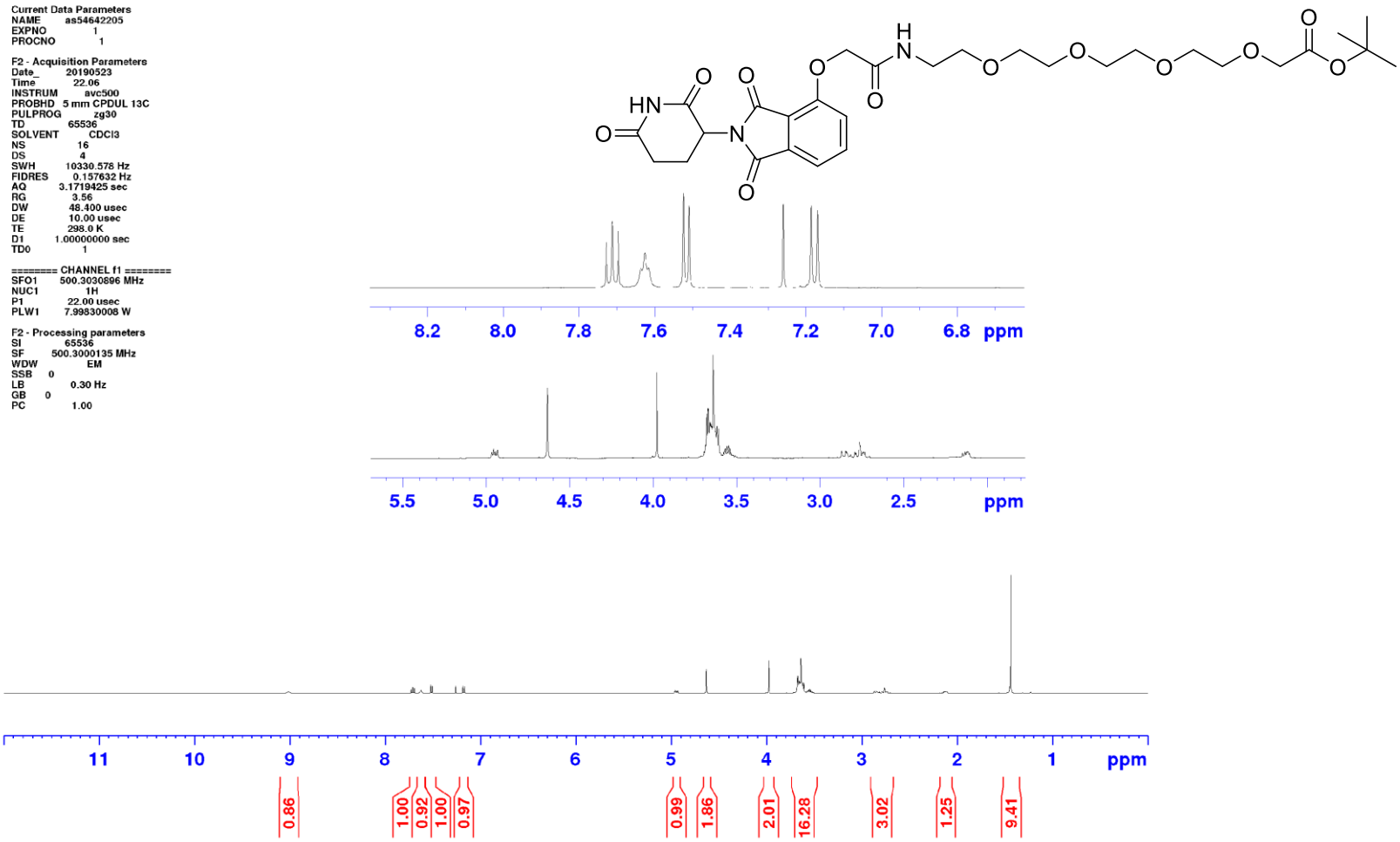
tert-Butyl 14-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}-13-oxo-3,6,9-trioxa-12-azatetradecan-1-oate (285)



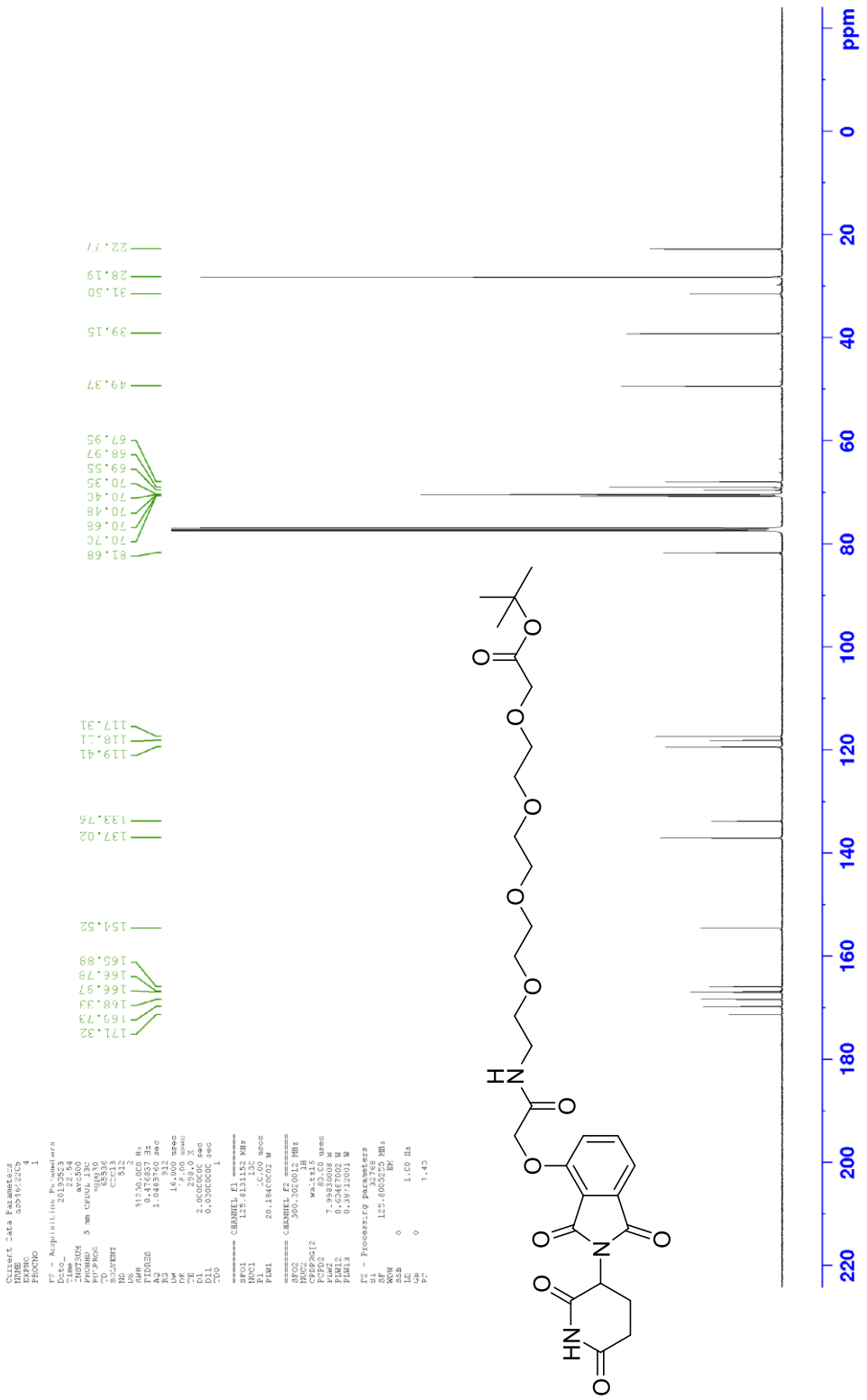
tert-Butyl 14-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-13-oxo-3,6,9-trioxa-12-azatetradecan-1-oate (285)



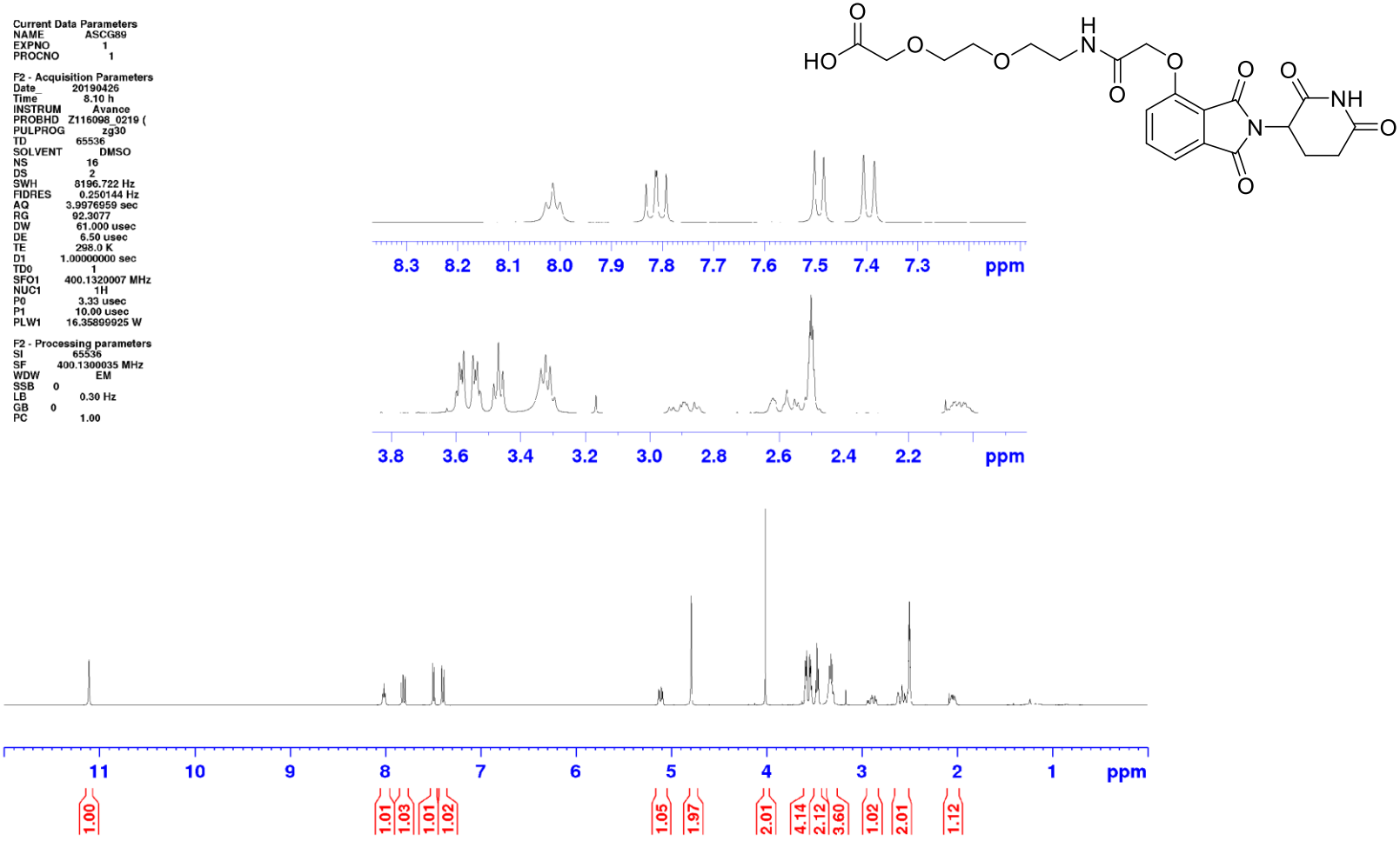
tert-Butyl 17-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}-16-oxo-3,6,9,12-tetraoxa-15-azaheptadecan-1-oate (286)



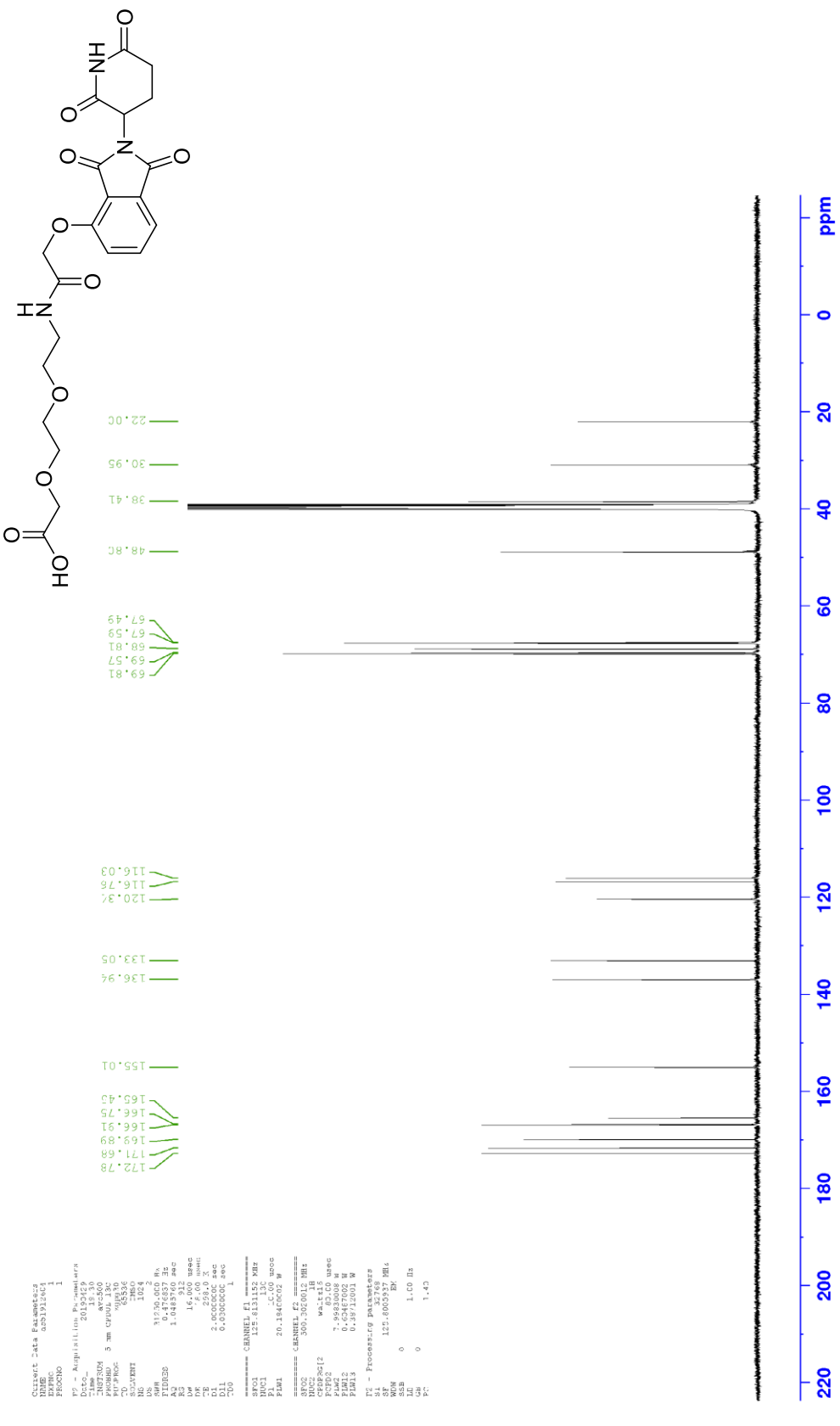
tert-Butyl 17-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-16-oxo-3,6,9,12-tetraoxa-15-azaheptadecan-1-oate (286)



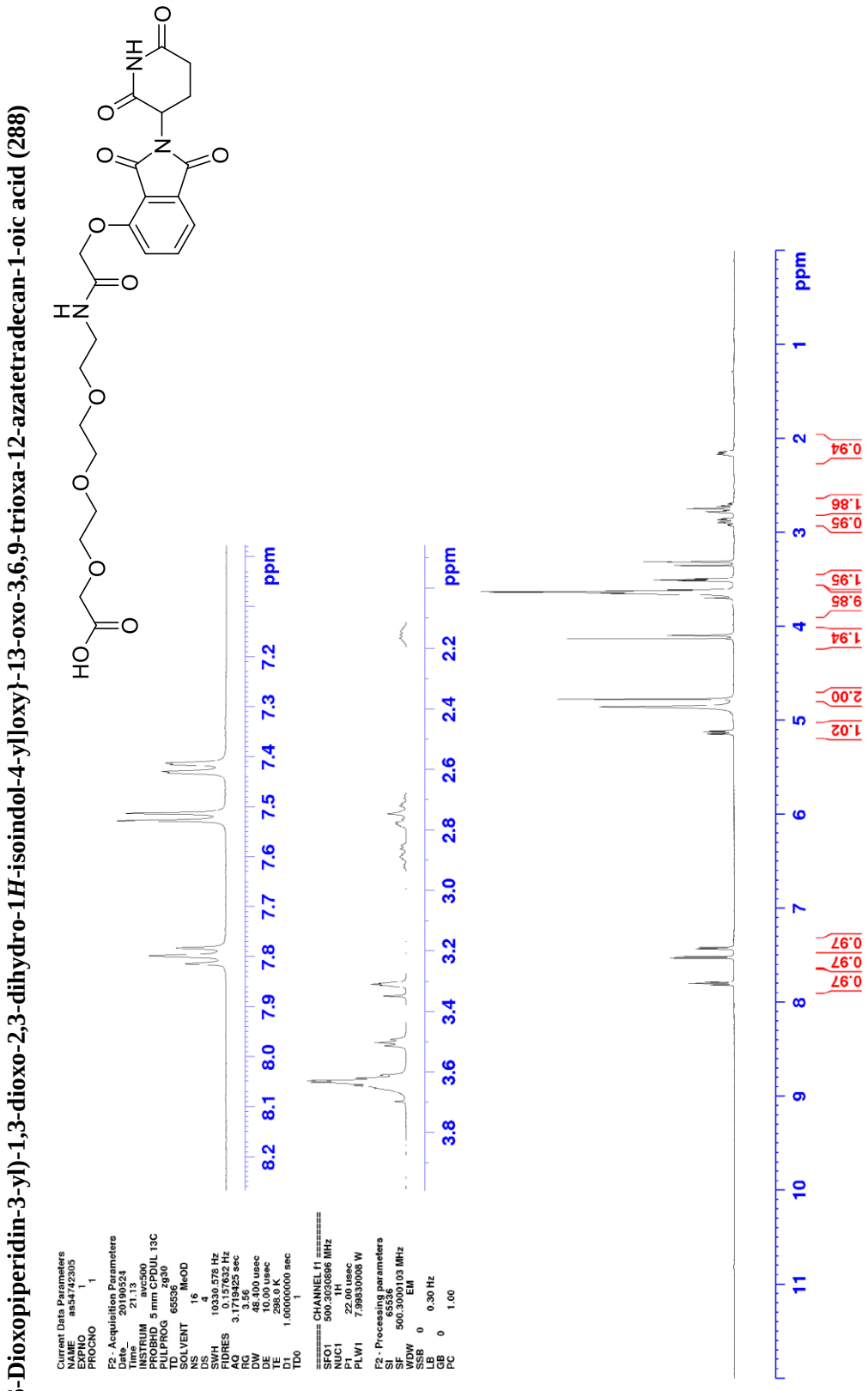
{2-[2-(2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]ethoxy}acetic acid (287)



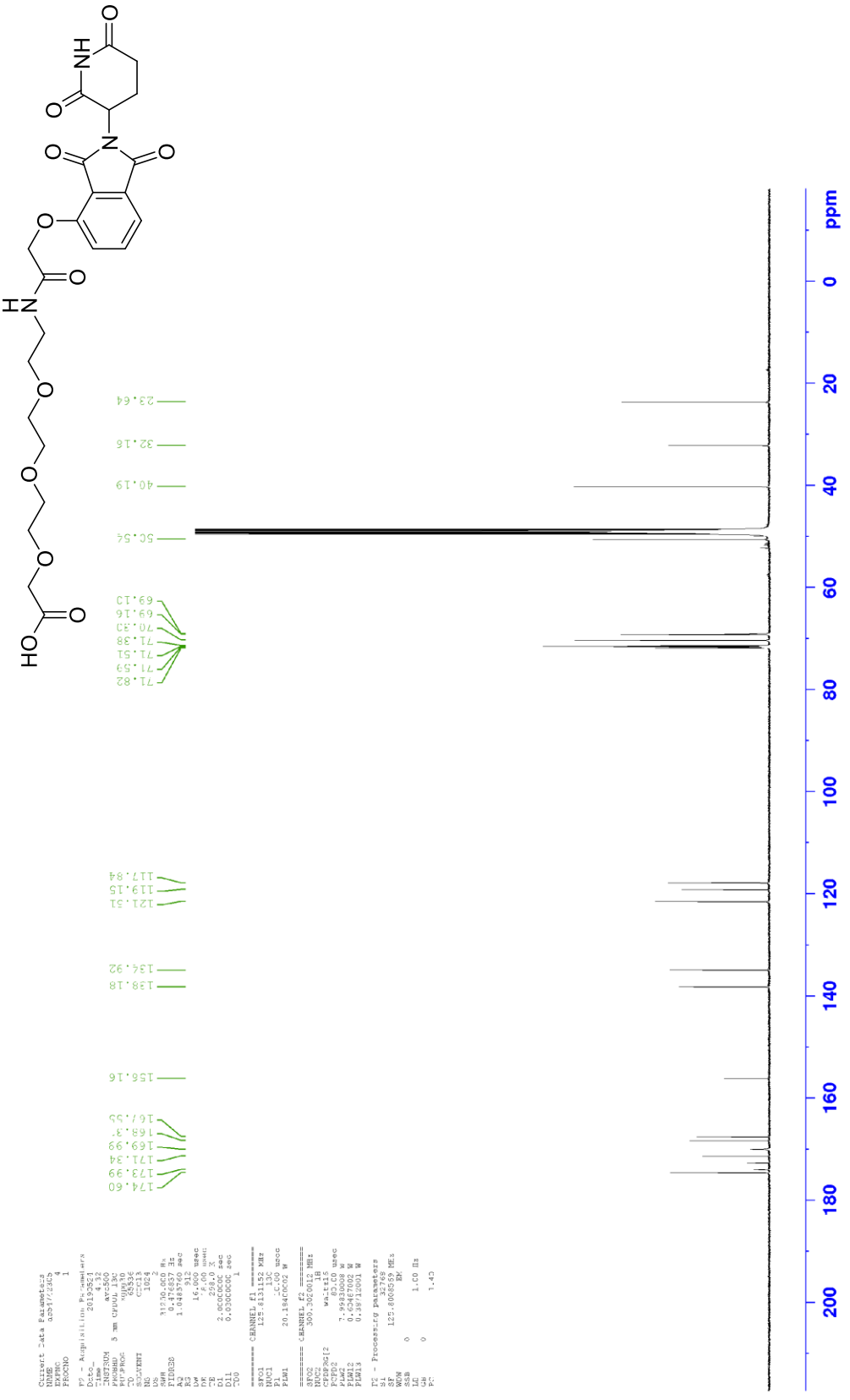
{2-[2-(2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]ethoxy}acetic acid (287)



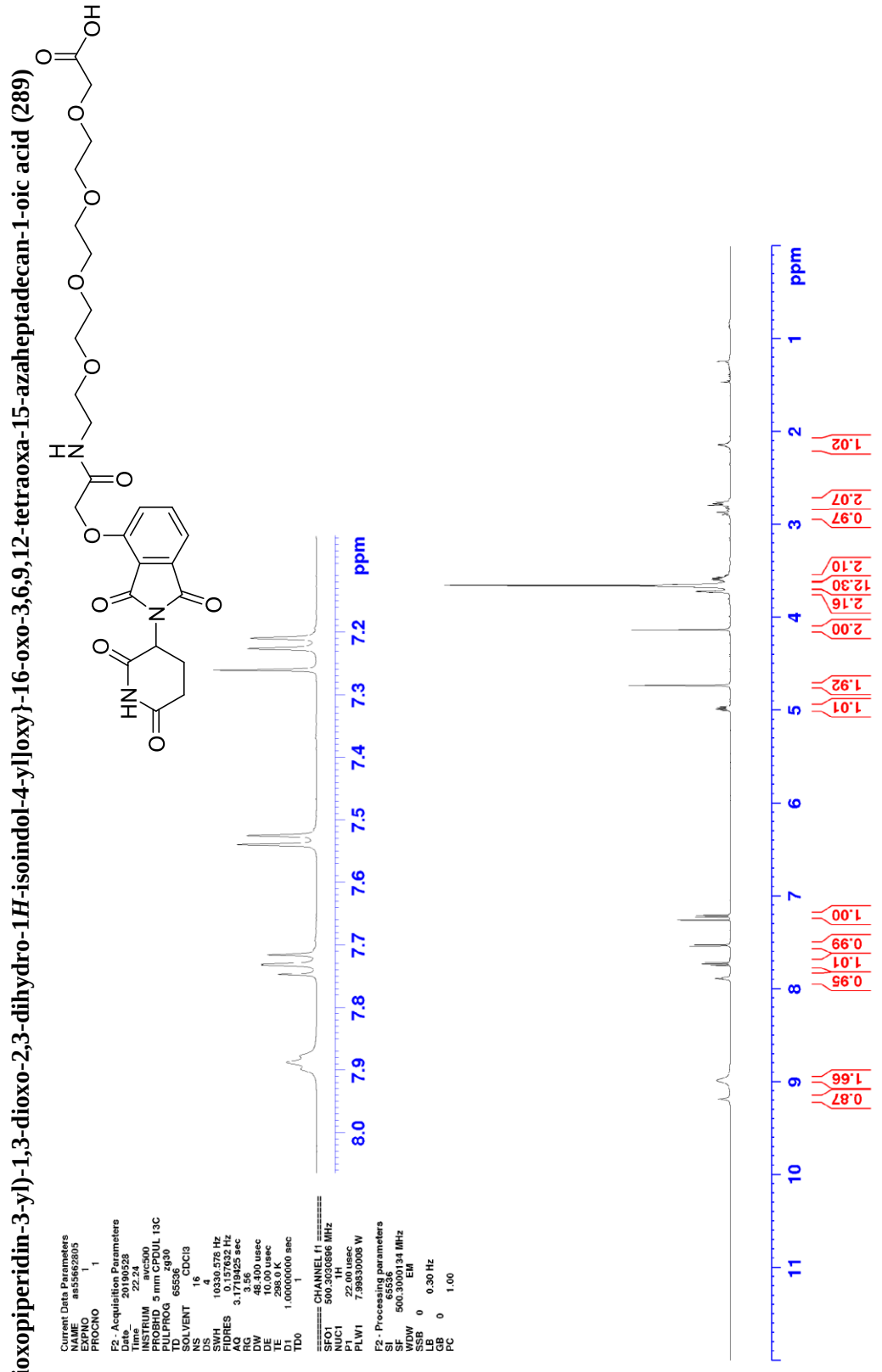
14-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-13-oxo-3,6,9-trioxa-12-azatetradecan-1-oic acid (288)



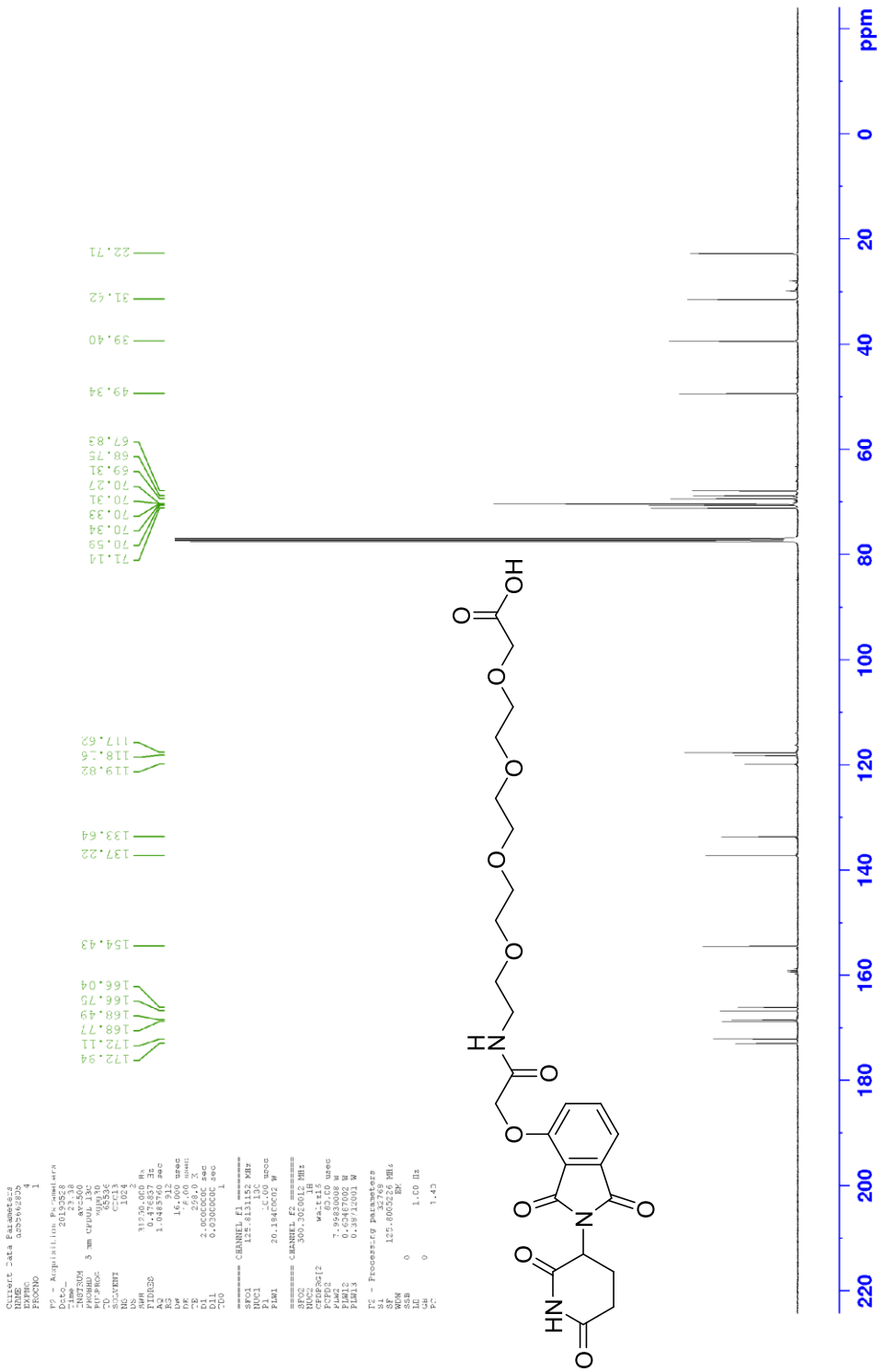
14-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-13-oxo-3,6,9-trioxa-12-azatetradecan-1-oic acid (288)



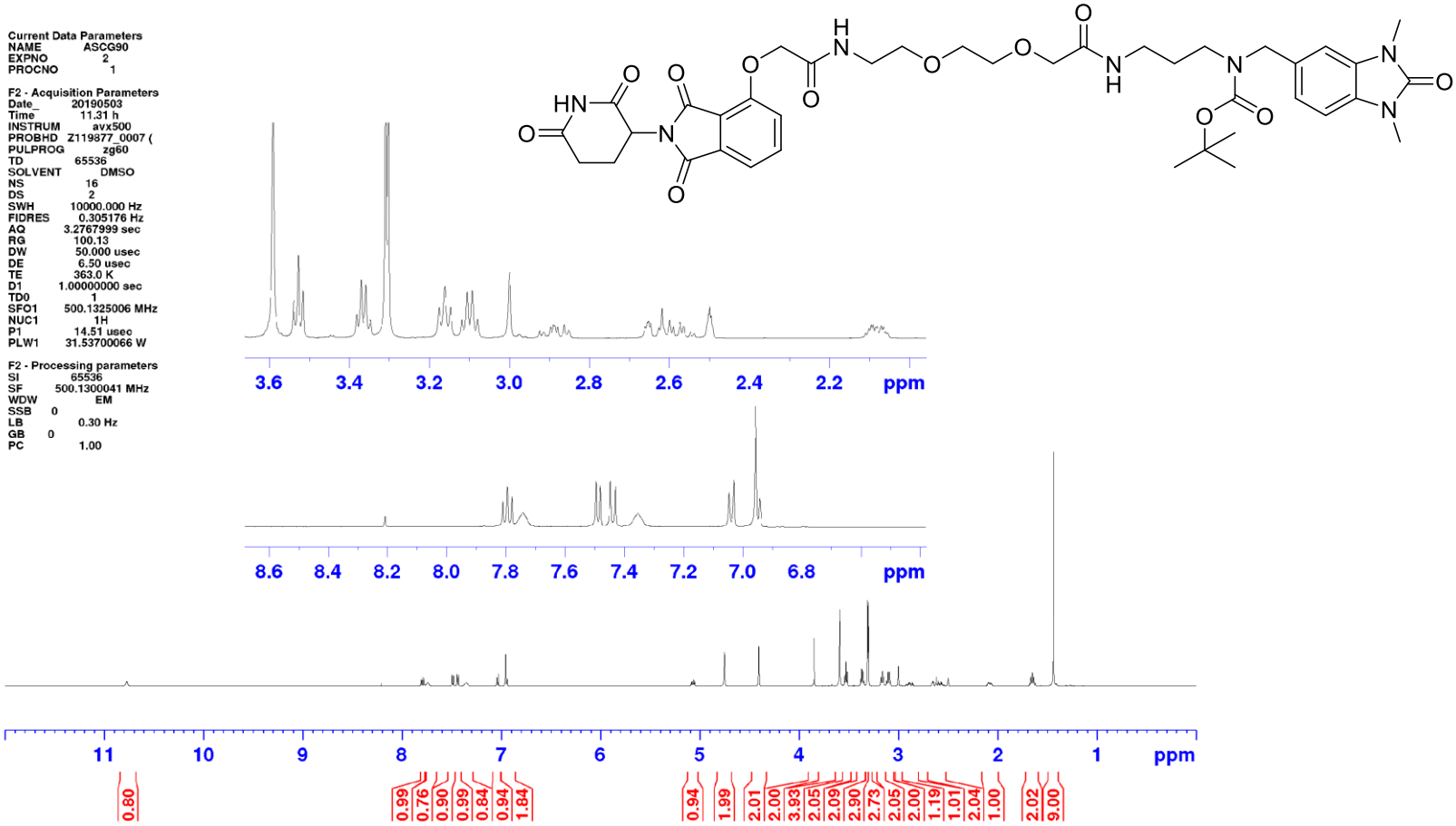
17-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-16-oxo-3,6,9,12-tetraoxa-15-azaheptadecan-1-oic acid (289)



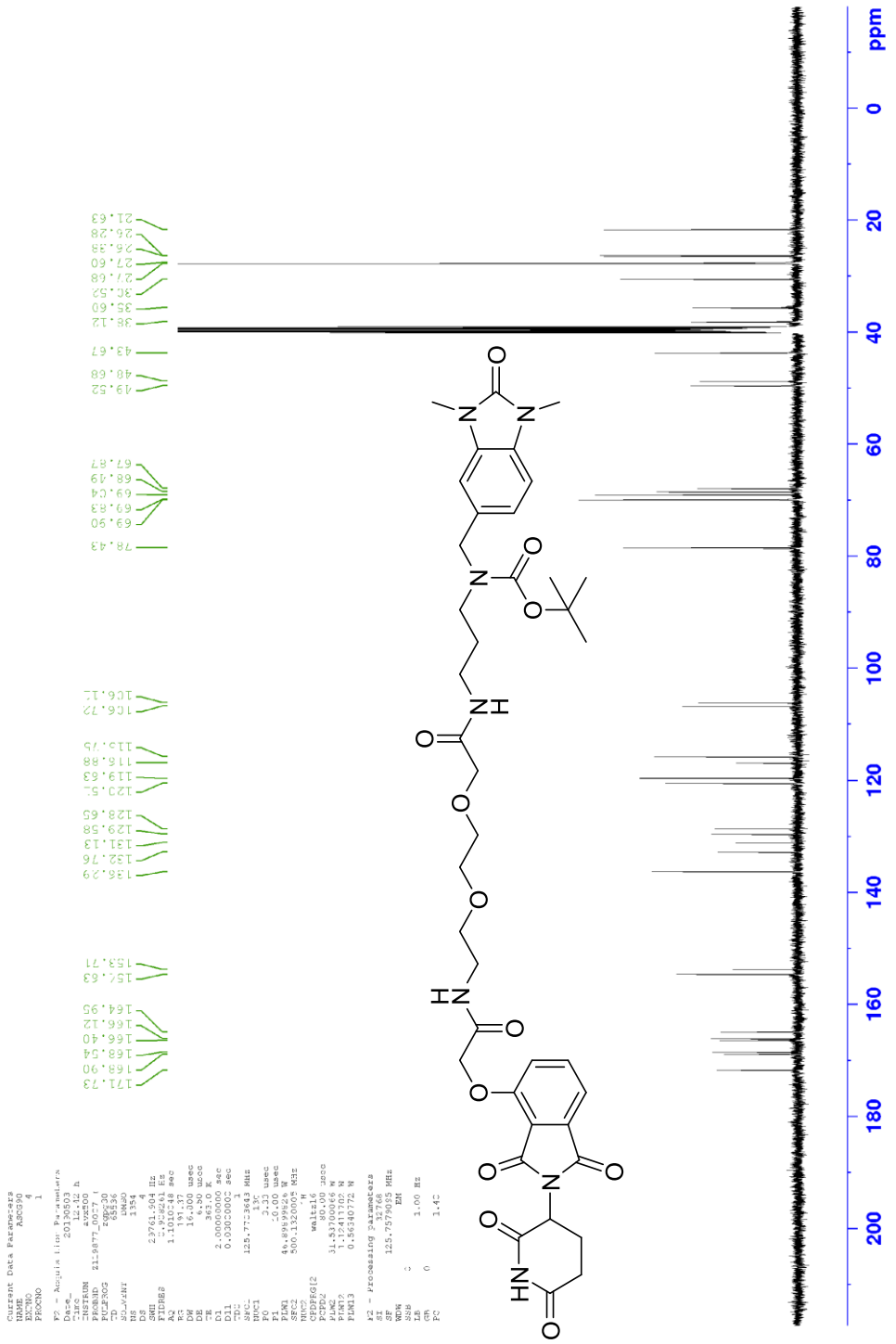
17-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-16-oxo-3,6,9,12-tetraoxa-15-azaheptadecan-1-oic acid (289)



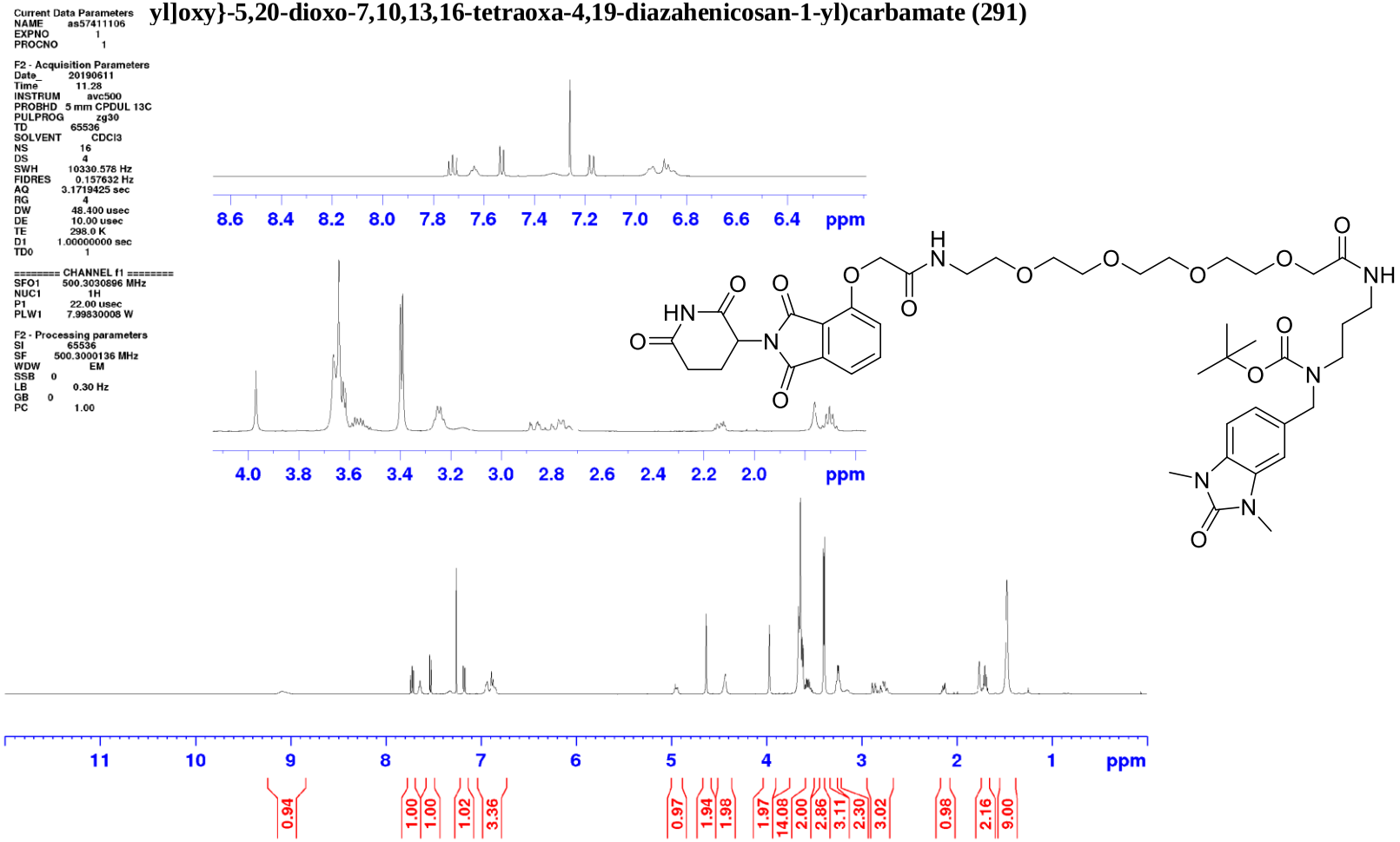
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](15-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-5,14-dioxo-7,10-dioxa-4,13-diazapentadecan-1-yl)carbamate (290)



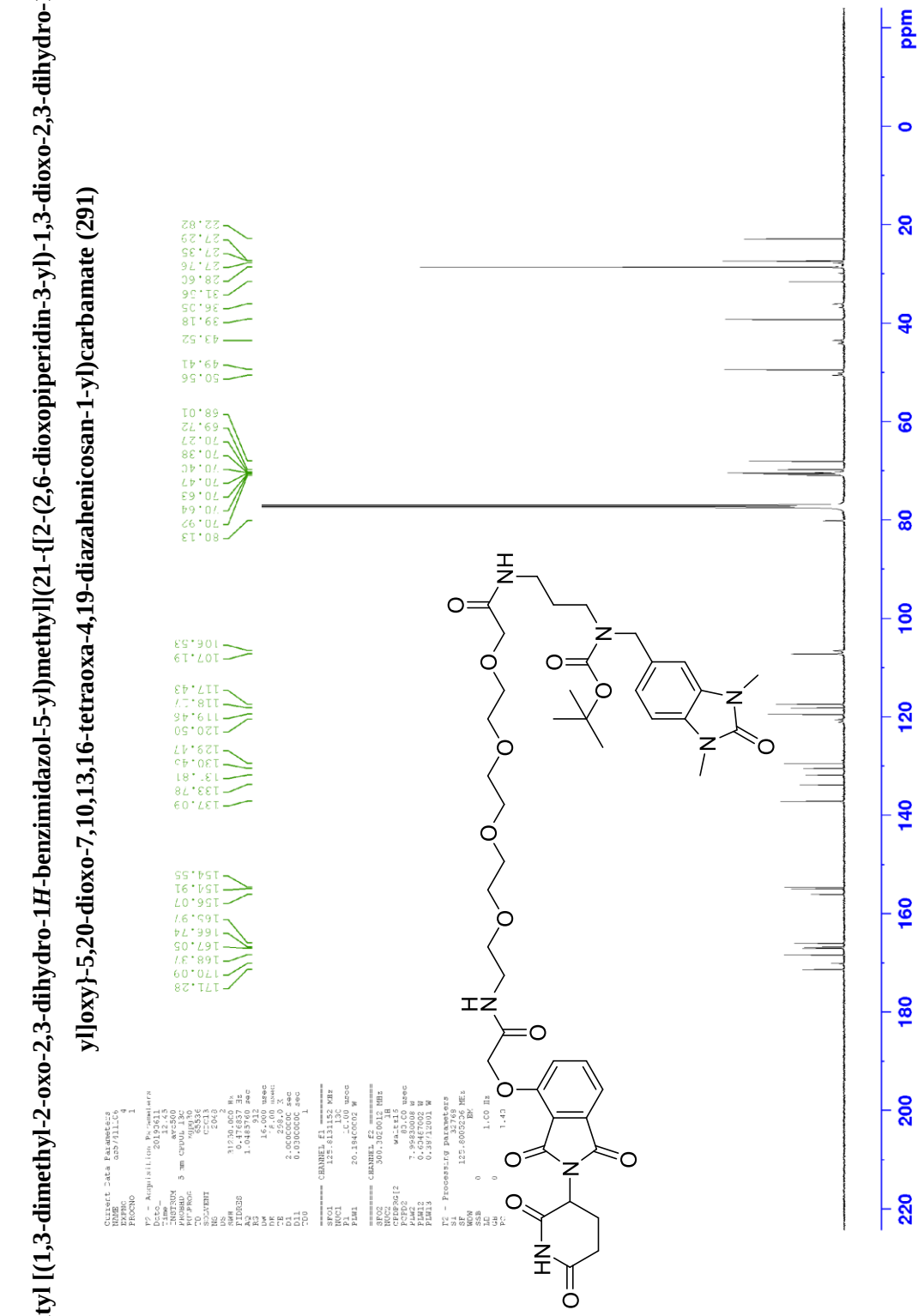
***tert*-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](15-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-5,14-dioxo-7,10-dioxa-4,13-diazapentadecan-1-yl)carbamate (290)**



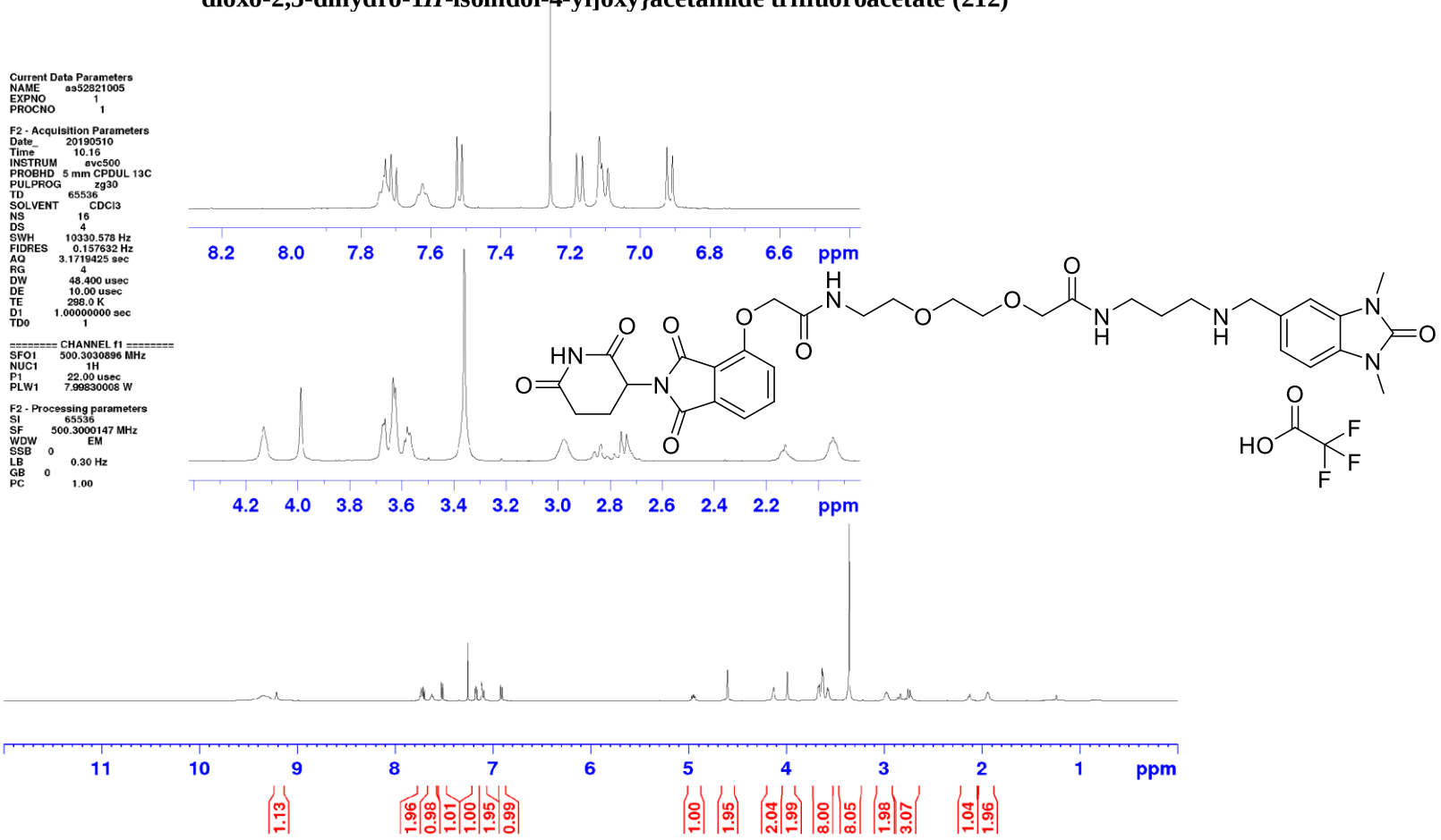
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](21-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-5,20-dioxo-7,10,13,16-tetraoxa-4,19-diazahenicosan-1-yl)carbamate (291)



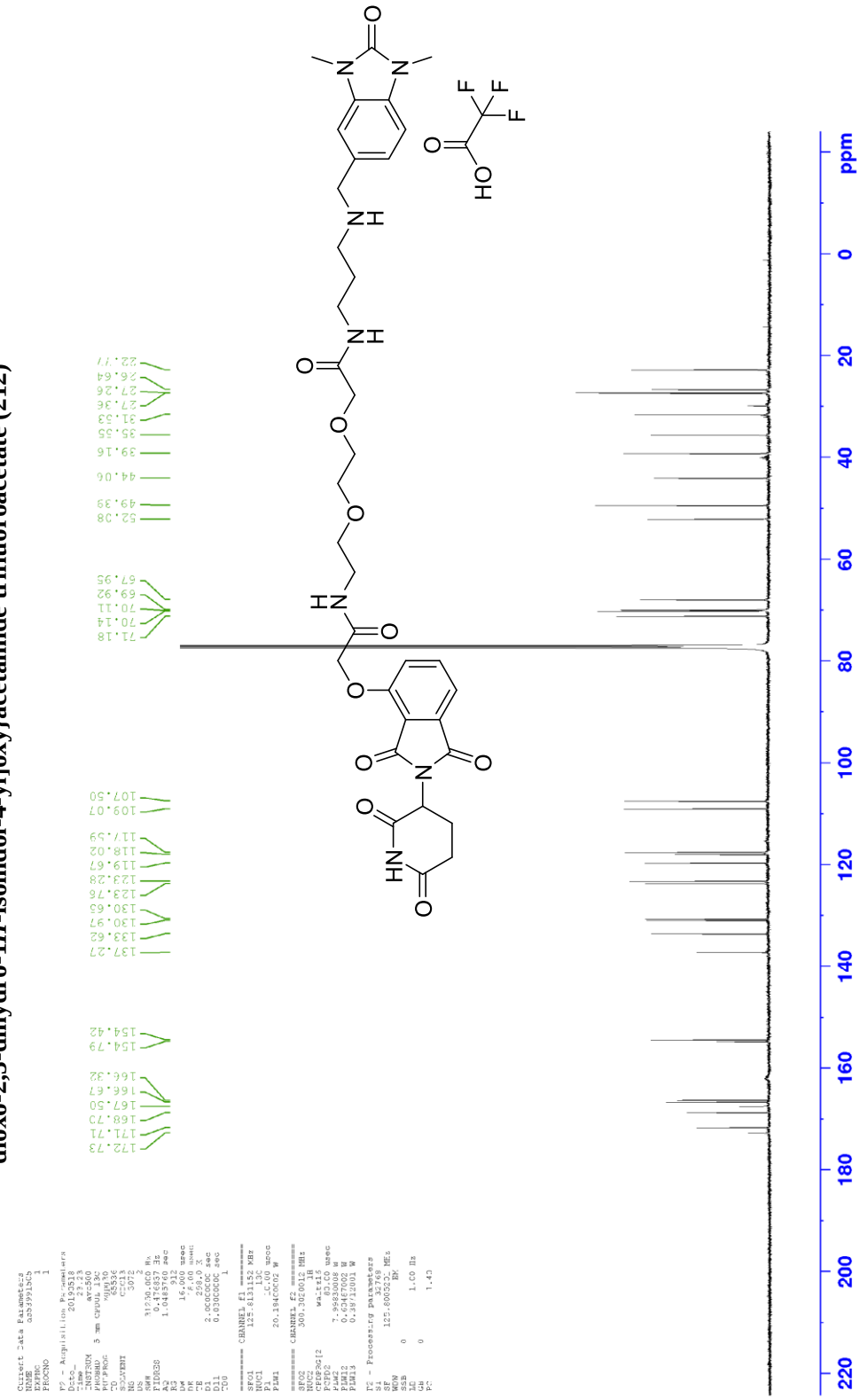
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](21-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-5,20-dioxo-7,10,13,16-tetraoxa-4,19-diazahenicosan-1-yl)carbamate (291)



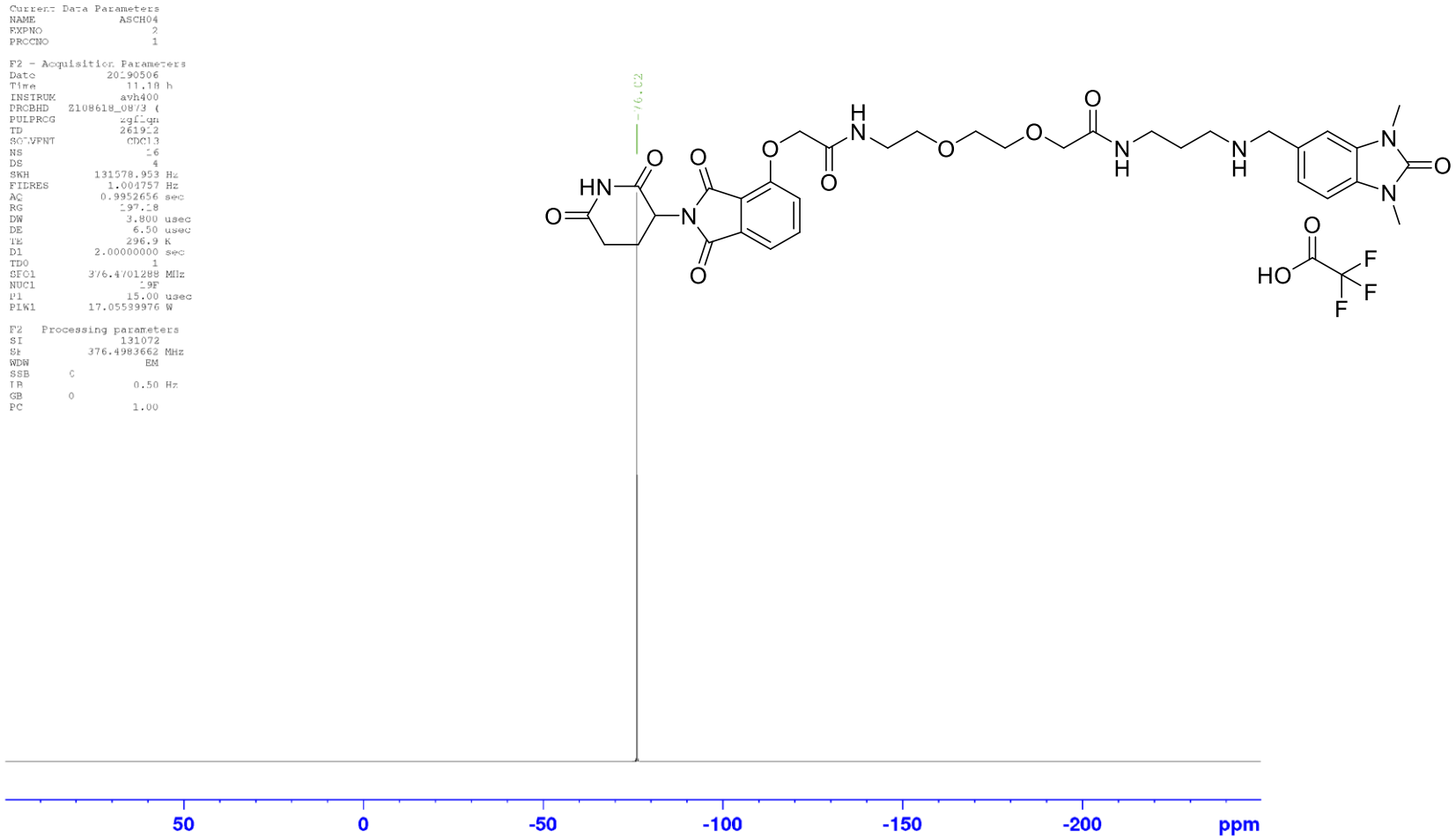
_N_-[1-(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)-7-oxo-9,12-dioxa-2,6-diazatetradecan-14-yl]-2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}acetamide trifluoroacetate (212)



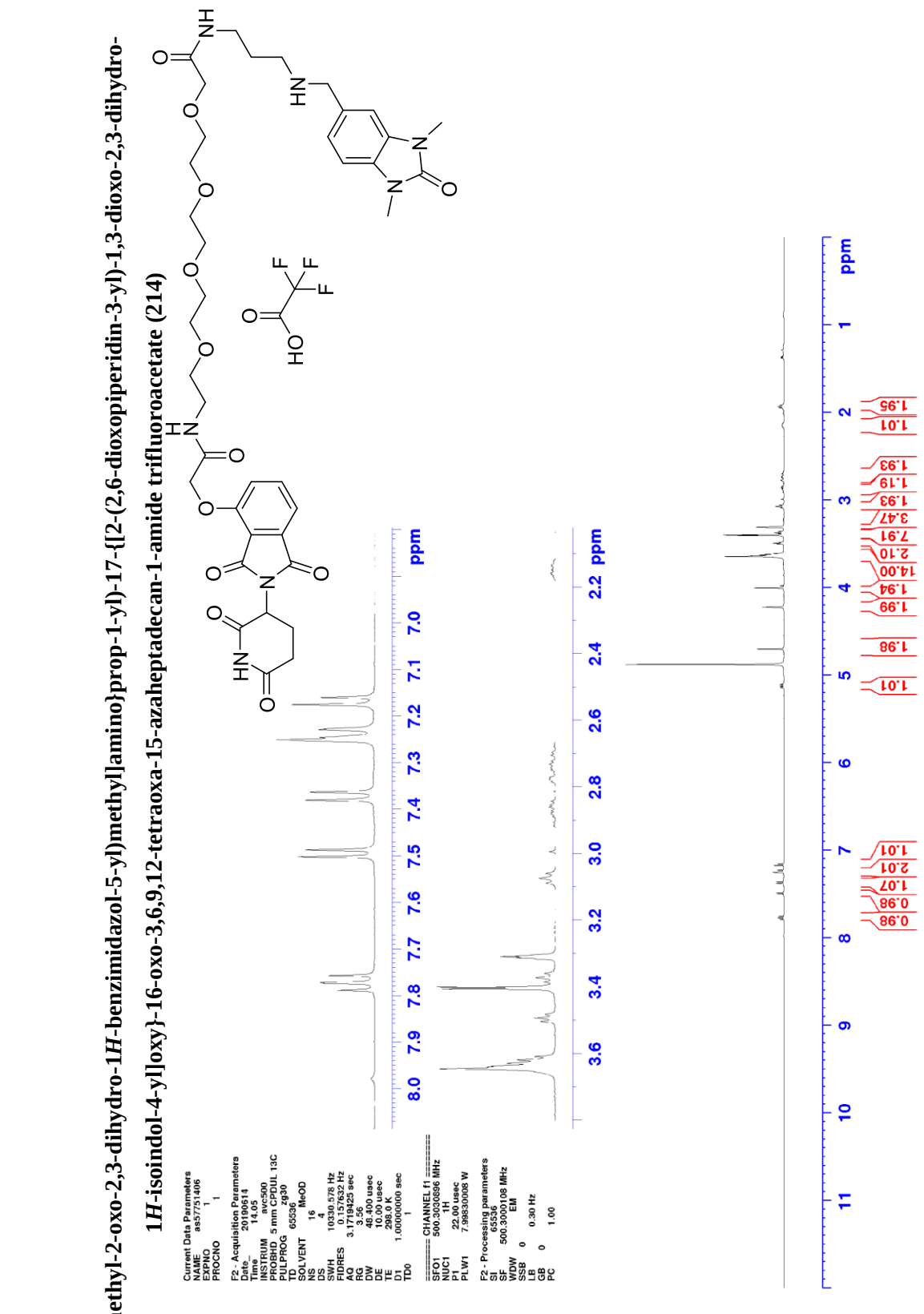
***N*-[1-(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)-7-oxo-9,12-dioxa-2,6-diazatetradecan-14-yl]-2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamide trifluoroacetate (212)**



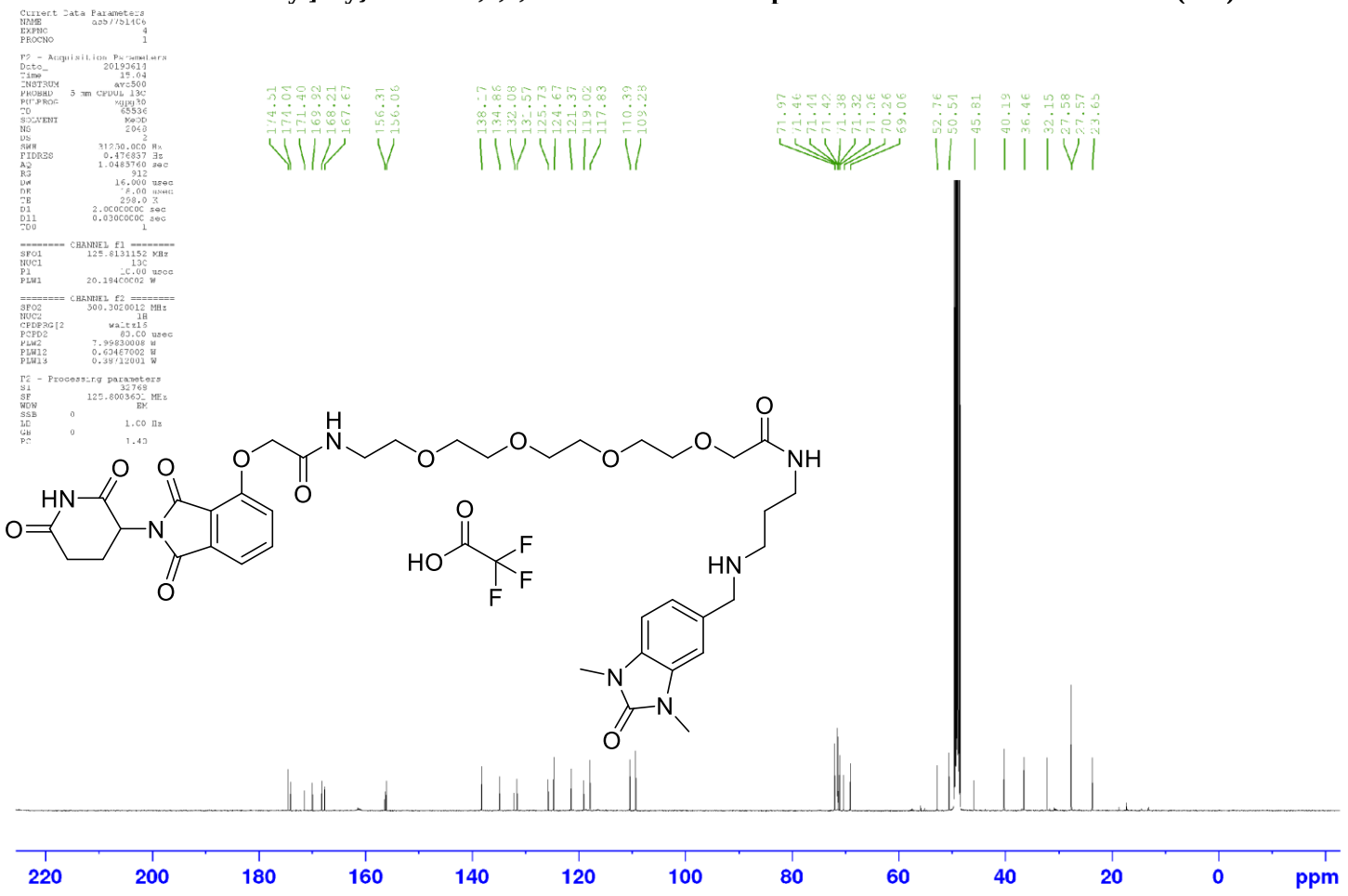
_N_-[1-(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)-7-oxo-9,12-dioxa-2,6-diazatetradecan-14-yl]-2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}acetamide trifluoroacetate (212)



_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1-yl)-17-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-16-oxo-3,6,9,12-tetraoxa-15-azaheptadecan-1-amide trifluoroacetate (214)

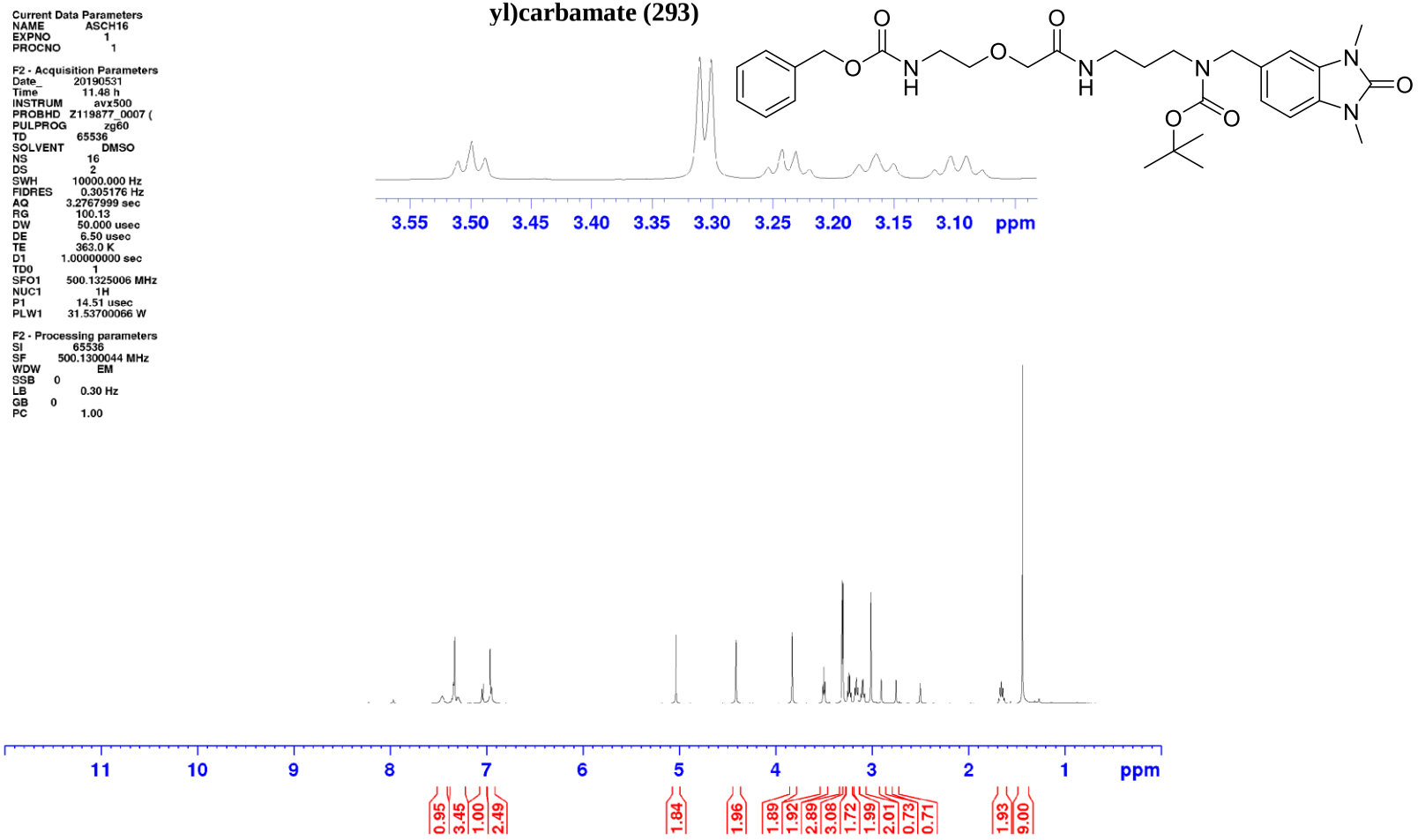


_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}prop-1-yl)-17-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}-16-oxo-3,6,9,12-tetraoxa-15-azaheptadecan-1-amide trifluoroacetate (214)

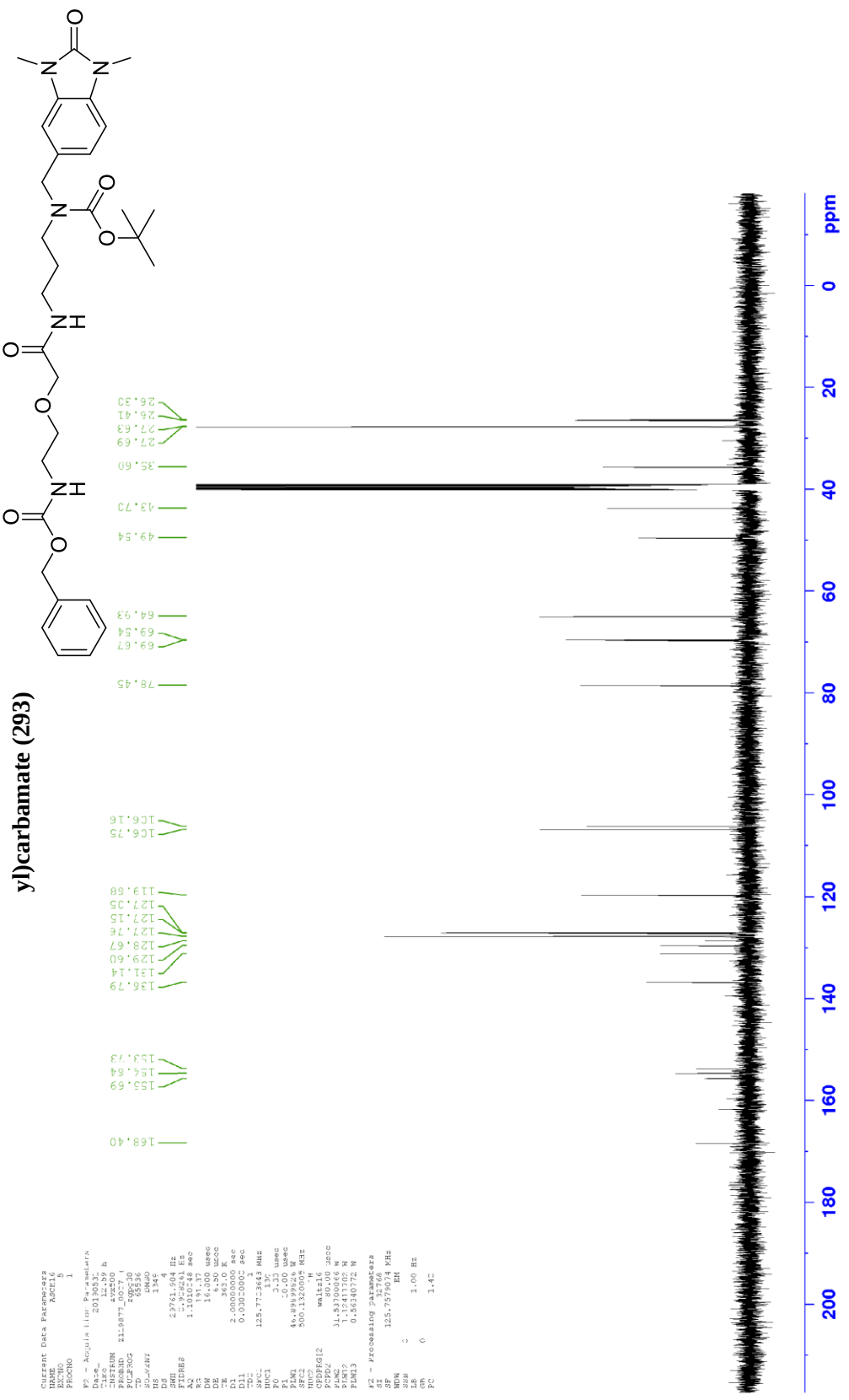


_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}prop-1-yl)-17-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}-16-oxo-3,6,9,12-tetraoxa-15-azaheptadecan-1-amide trifluoroacetate (214)

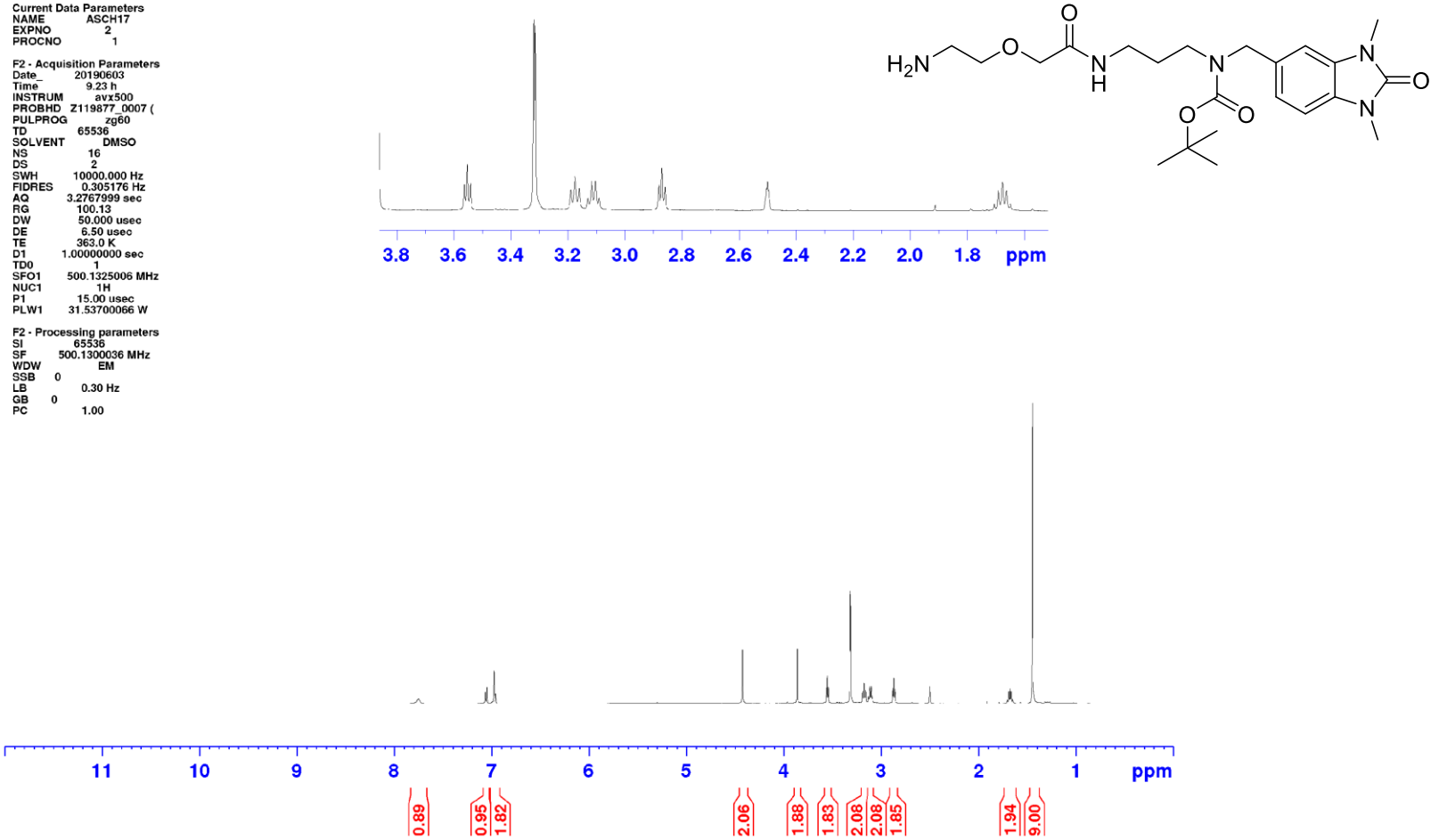
tert-Butyl ((1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzo[_d_]imidazol-5-yl)methyl)(3,9-dioxo-1-phenyl-2,7-dioxa-4,10-diazatridecan-13-yl)carbamate (293)



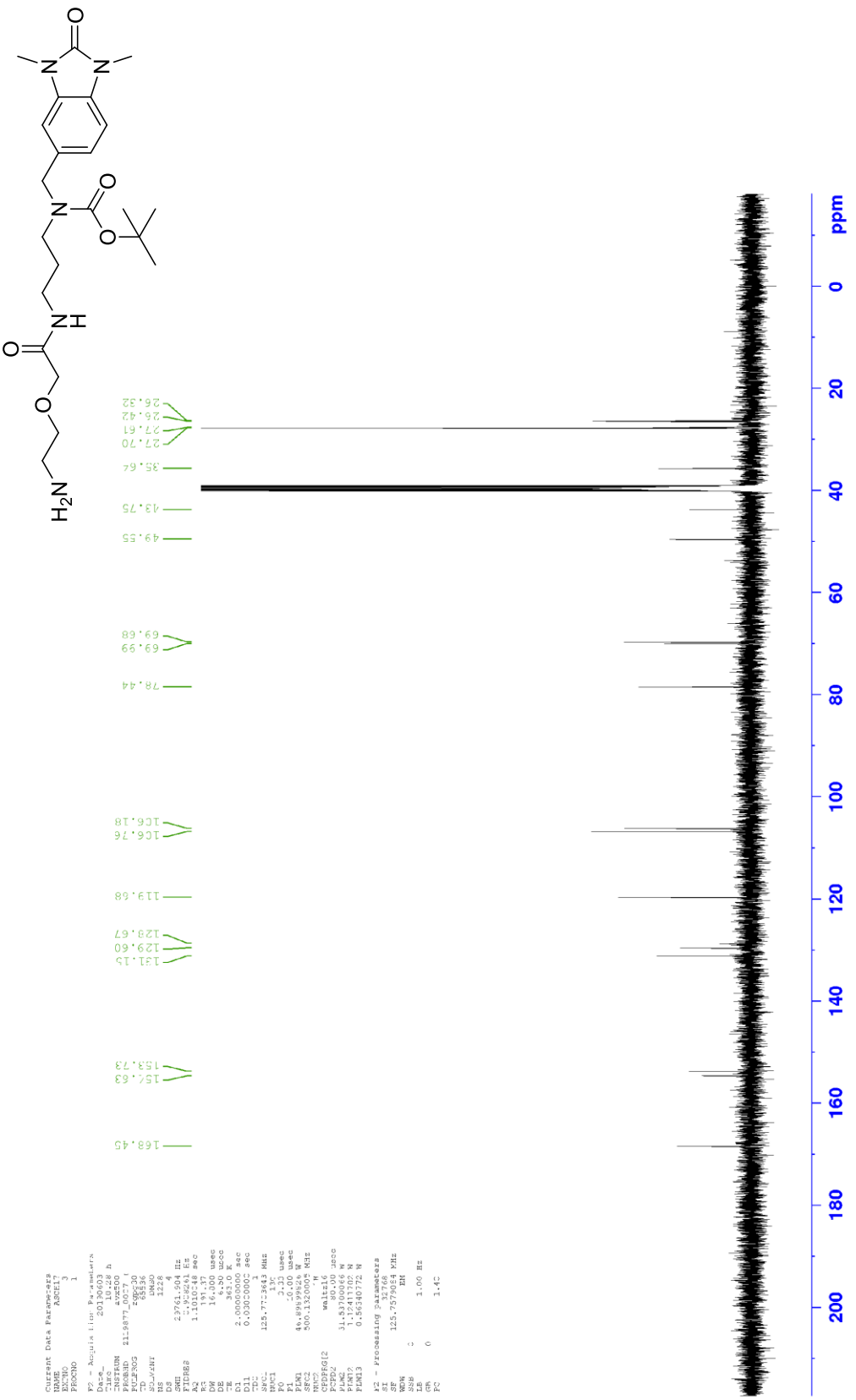
***tert*-Butyl ((1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)methyl)(3,9-dioxo-1-phenyl-2,7-dioxa-4,10-diazatridecan-13-yl)carbamate (293)**



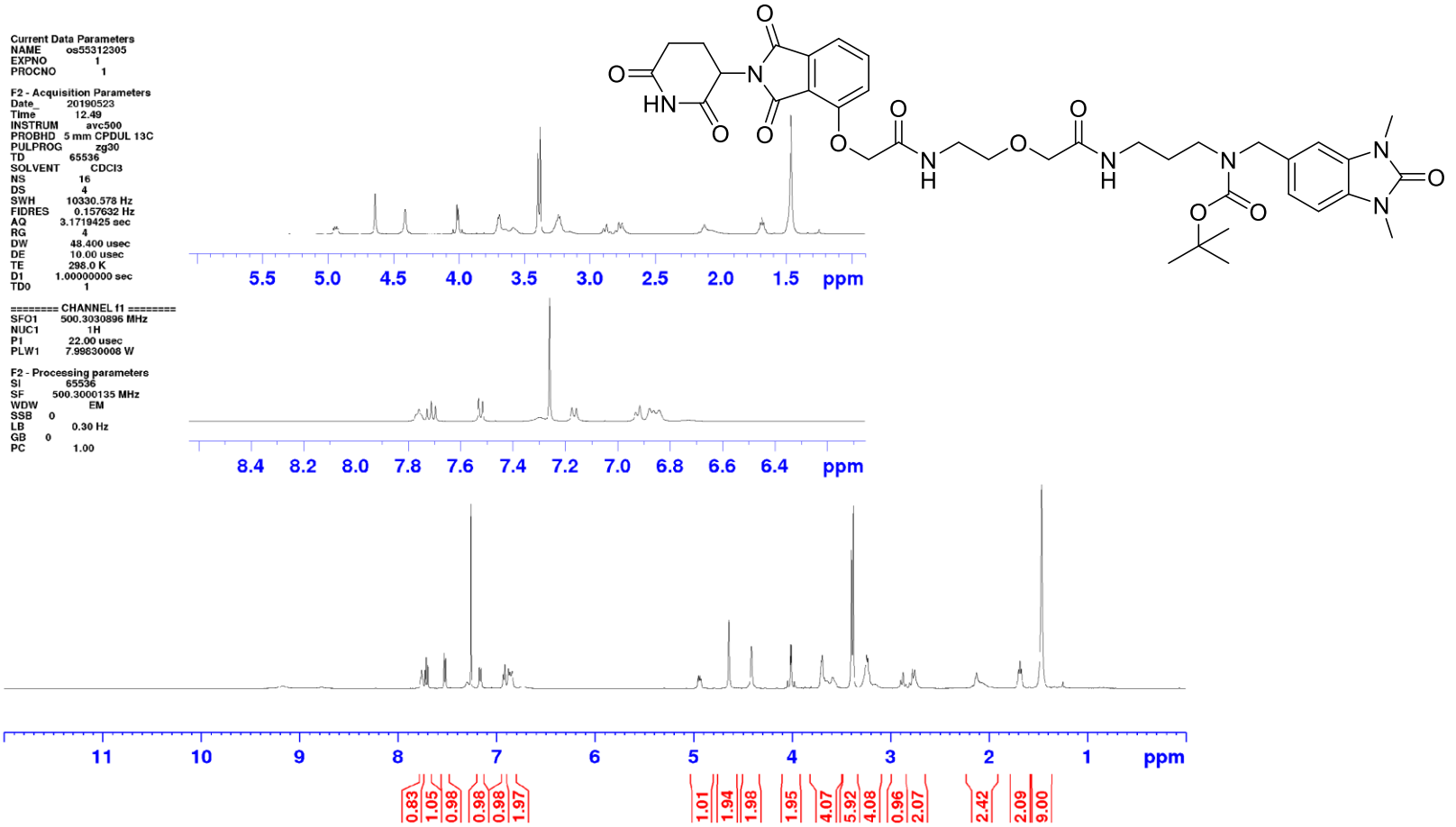
tert-Butyl {3-[2-(2-aminoethoxy)acetamido]prop-1-yl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]carbamate (294)



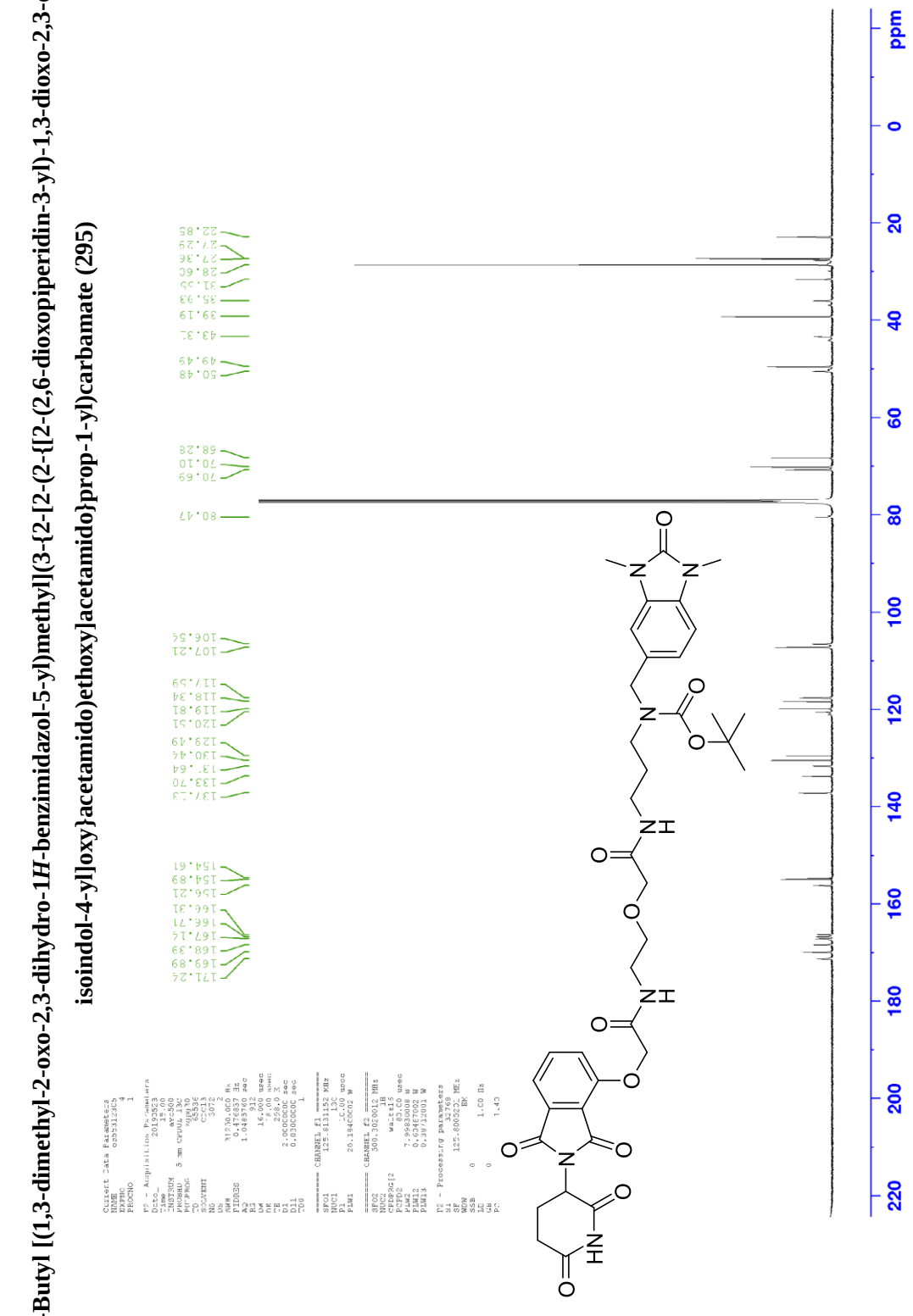
***tert*-Butyl {3-[2-(2-aminoethoxy)acetamido]prop-1-yl}[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (294)**



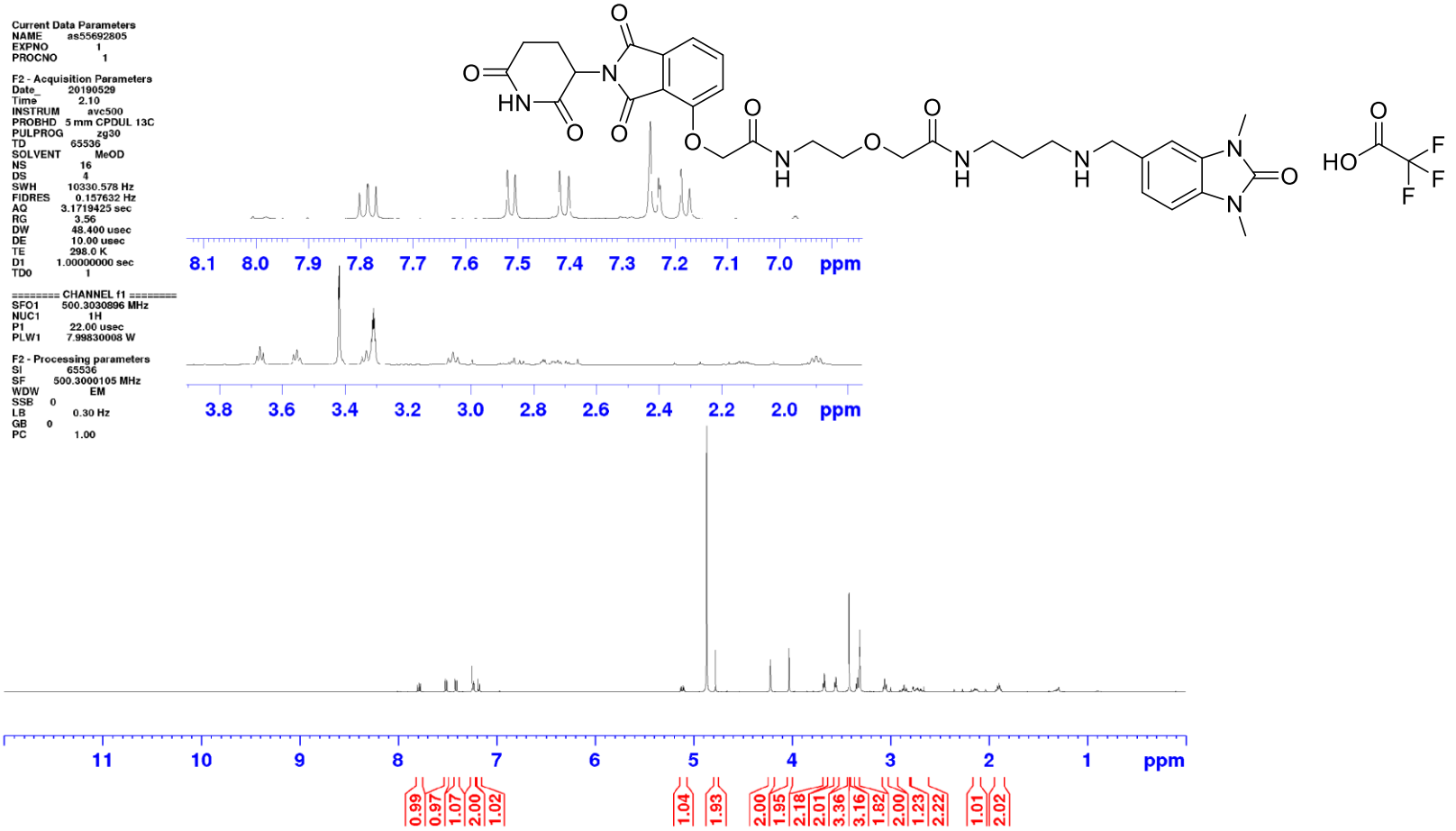
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl](3-{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy}acetamido)ethoxy]acetamido}prop-1-yl)carbamate (295)



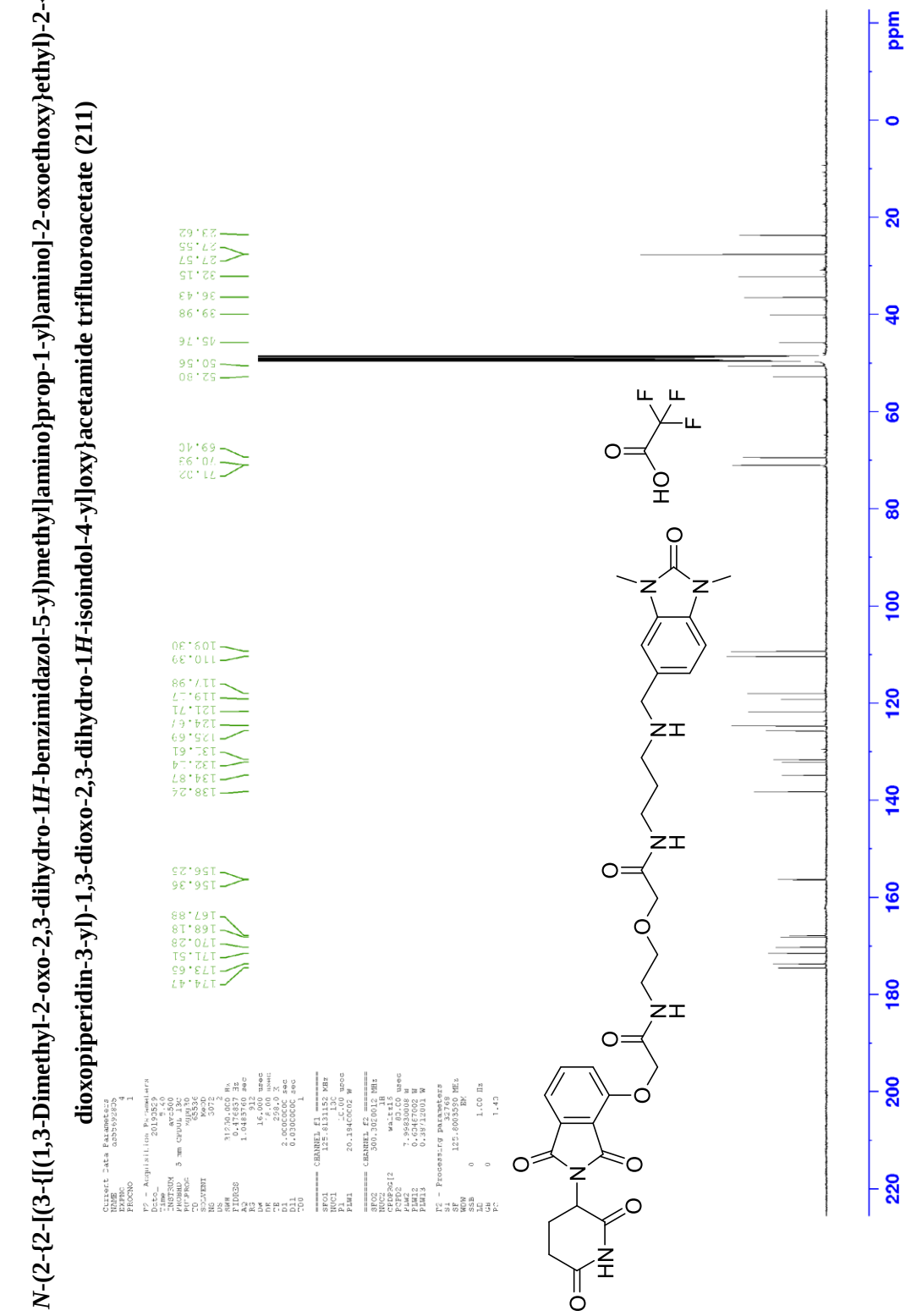
***tert*-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](3-{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy}acetamido)ethoxy]acetamido}prop-1-yl)carbamate (295)**



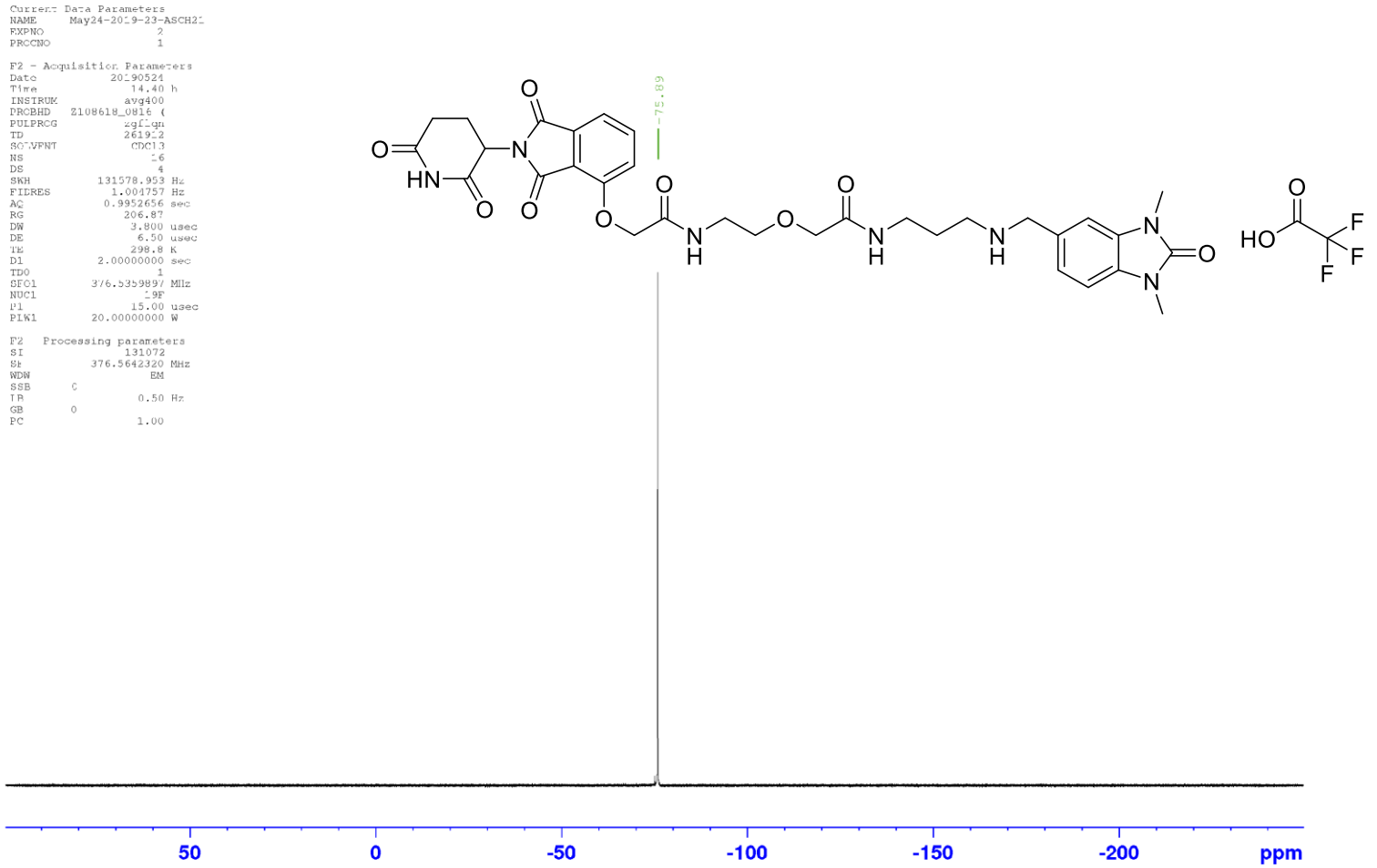
_N_-(2-{2-[(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}prop-1-yl)amino]-2-oxoethoxy}ethyl)-2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}acetamide trifluoroacetate (211)



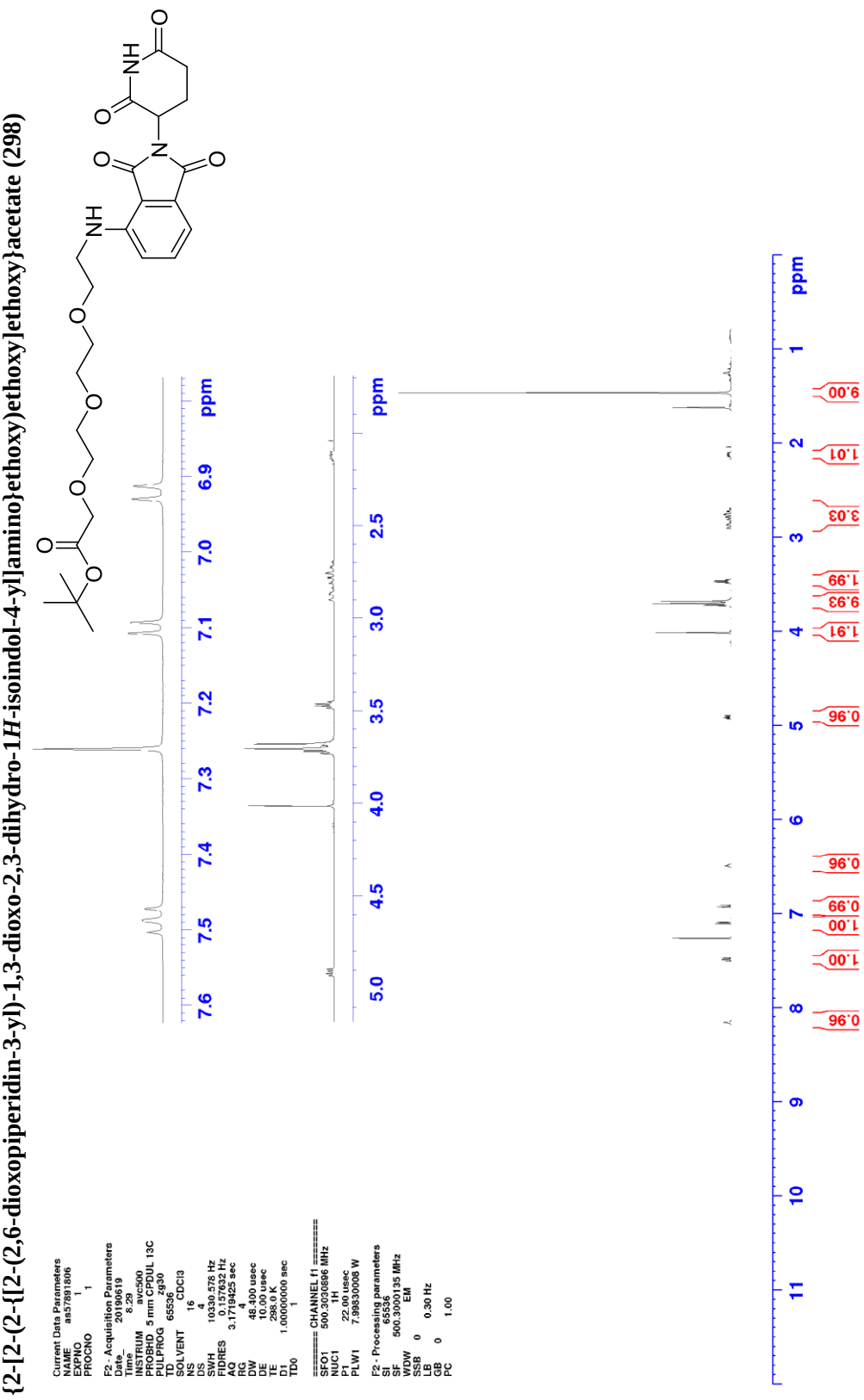
_N_-(2-{2-[(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1-yl)amino]-2-oxoethoxy}ethyl)-2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamide trifluoroacetate (211)



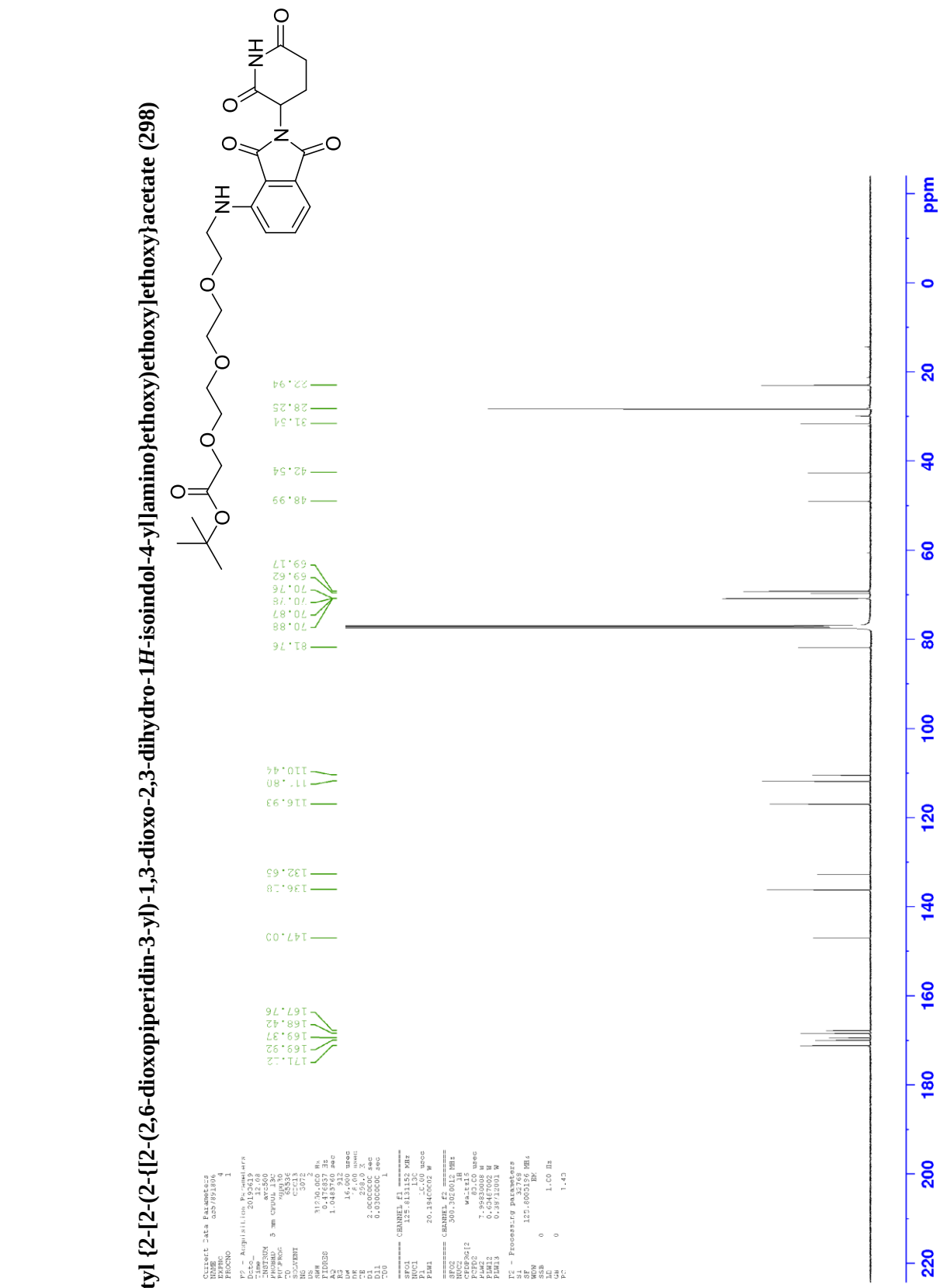
_N_-(2-{2-[(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}prop-1-yl)amino]-2-oxoethoxy}ethyl)-2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}acetamide trifluoroacetate (211)



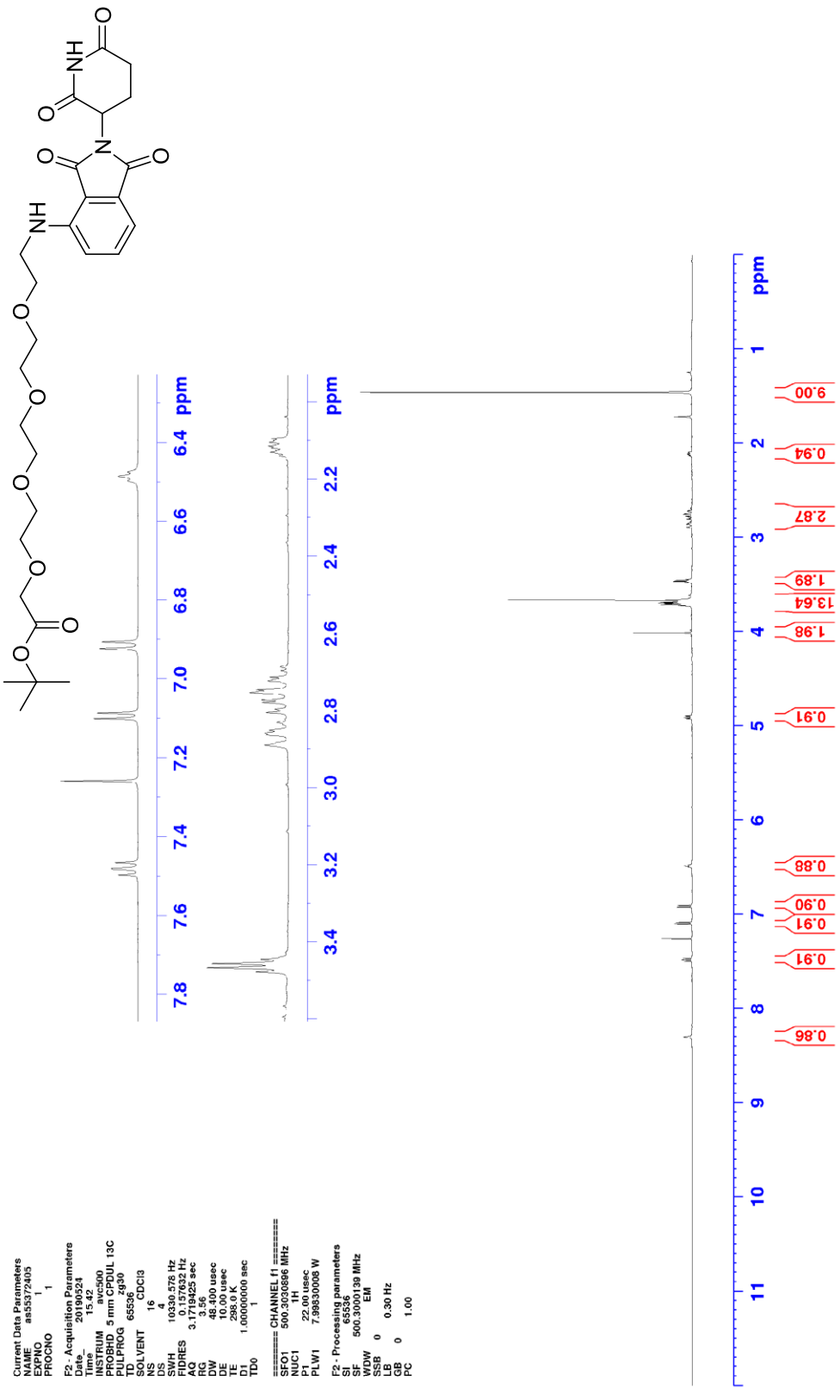
***tert*-Butyl {2-[2-(2-{2-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetate (298)**



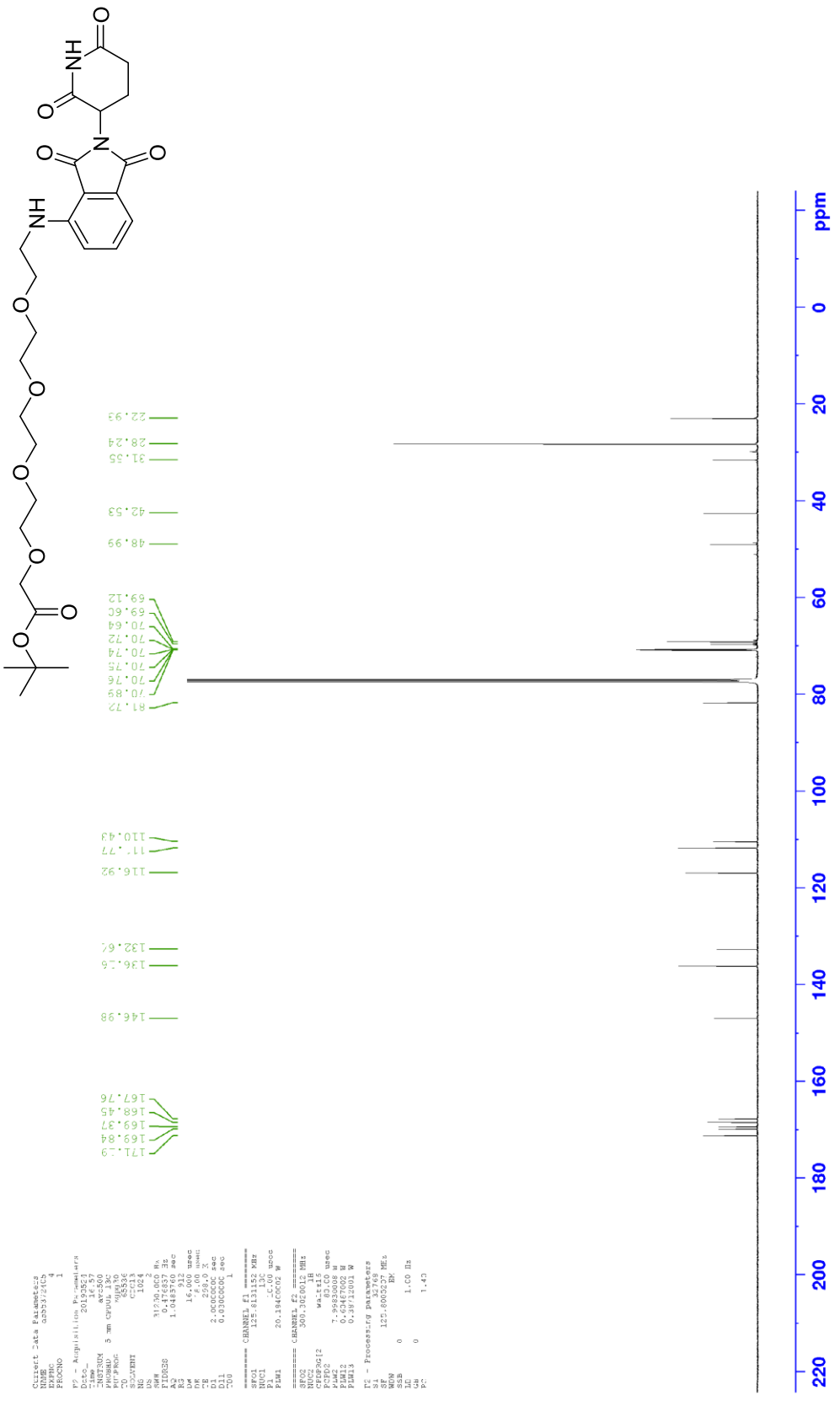
***tert*-Butyl {2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetate (298)**



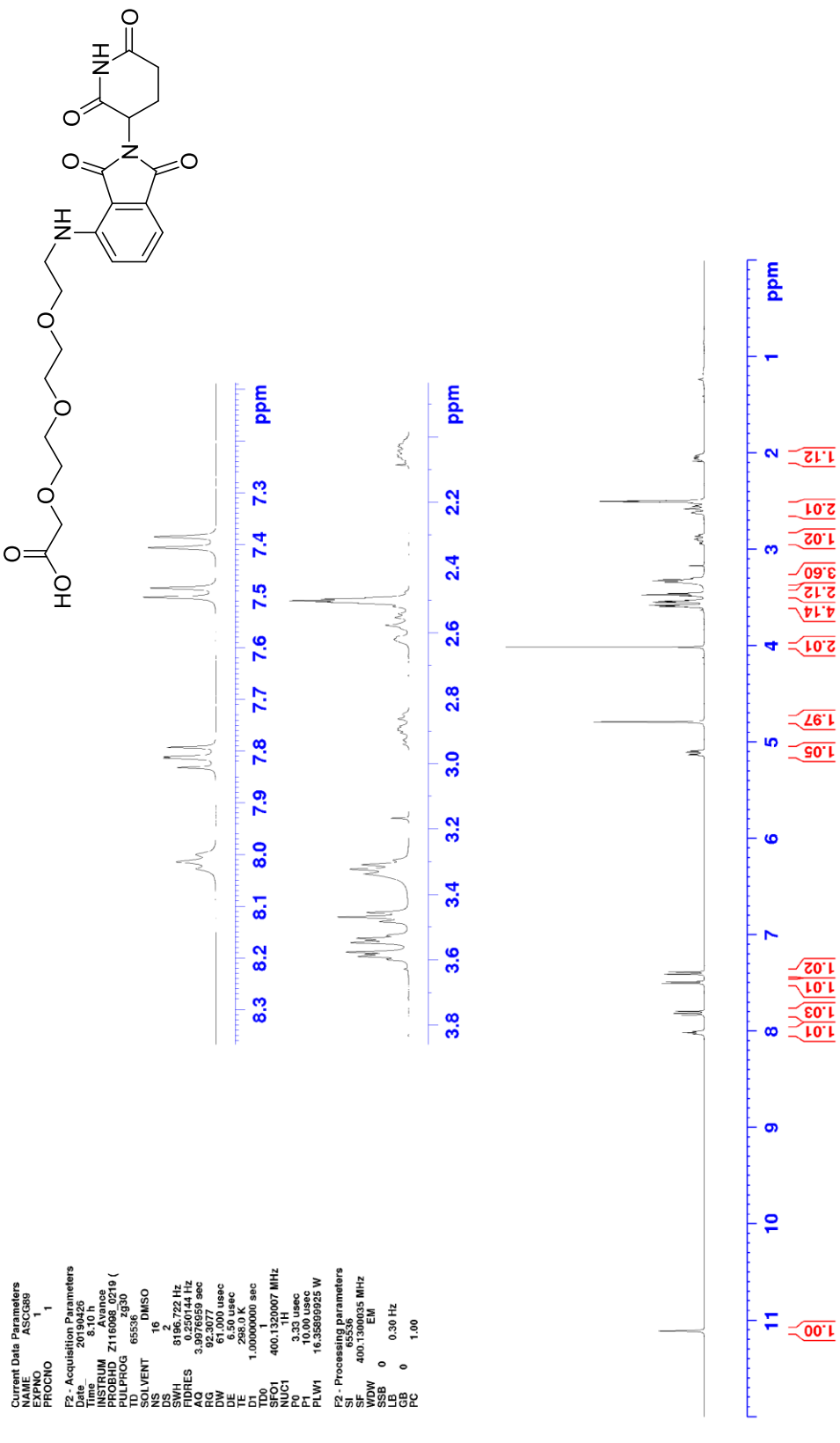
***tert*-Butyl 14-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-3,6,9,12-tetraoxatetradecan-1-oate (299)**



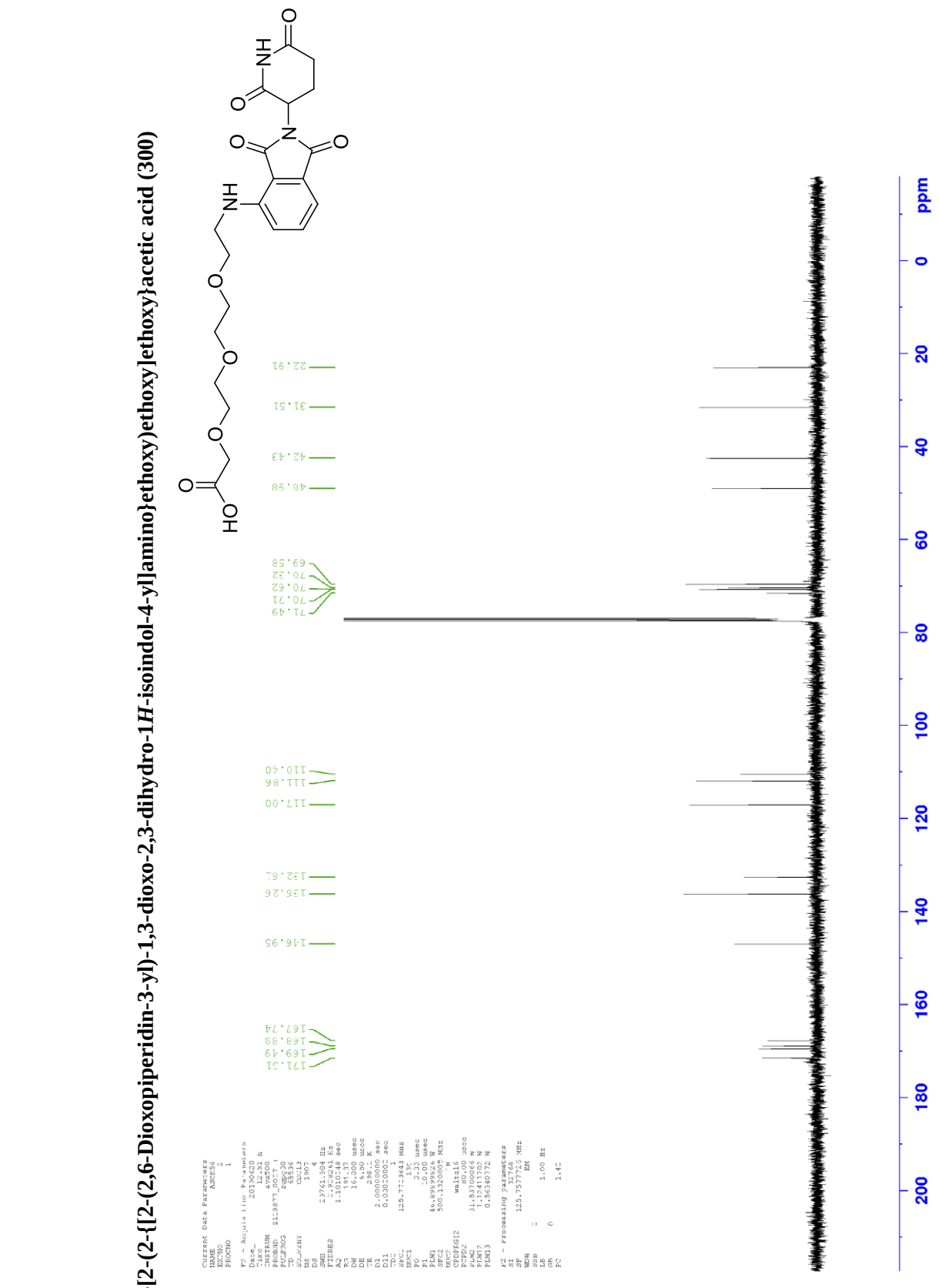
***tert*-Butyl 14-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-3,6,9,12-tetraoxatetradecan-1-oate (299)**



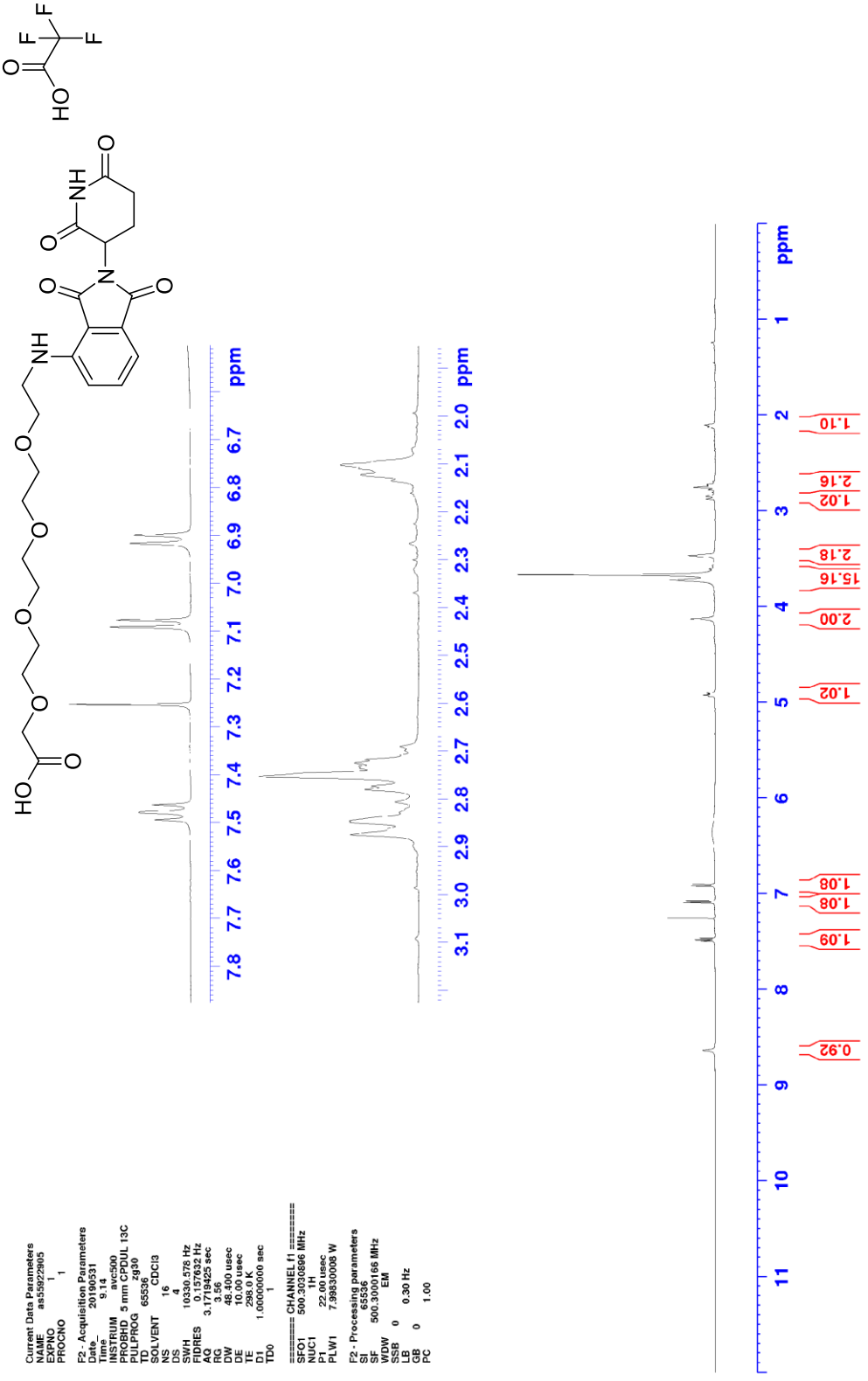
{2-[2-(2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetic acid (300)



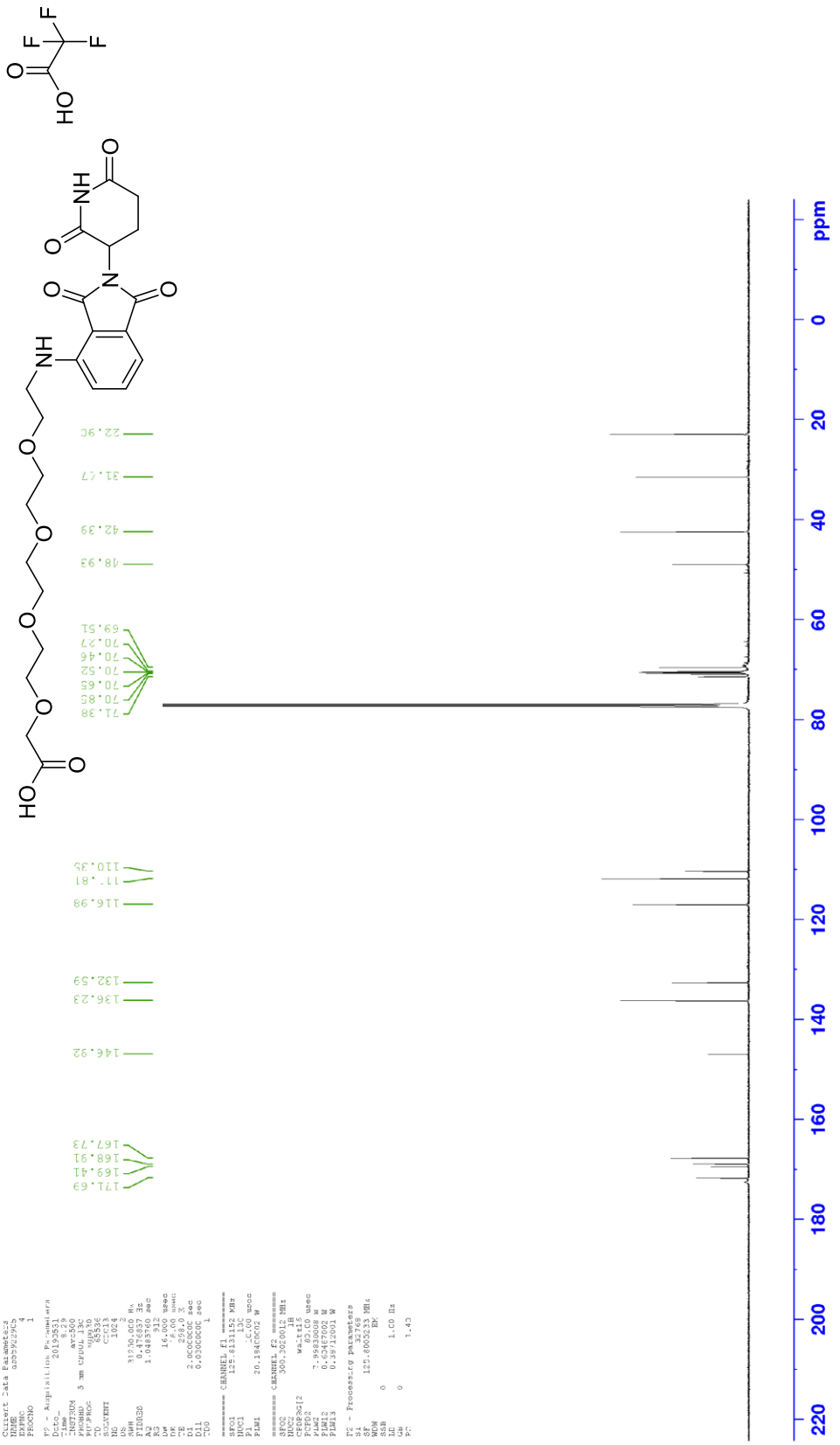
{2-[2-(2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetic acid (300)



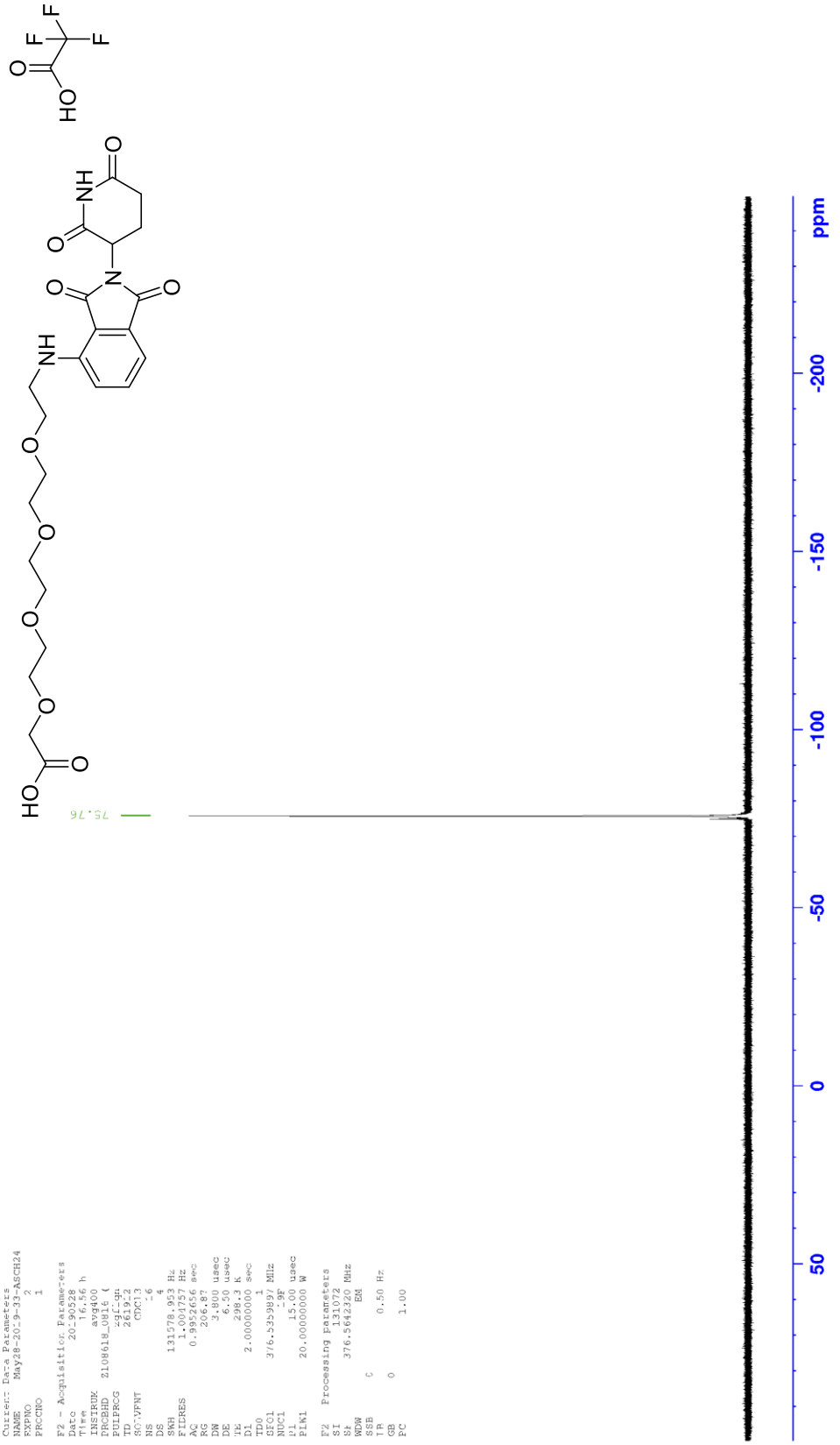
14-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-3,6,9,12-tetraoxatetradecan-1-oic acid trifluoro acetate (301)



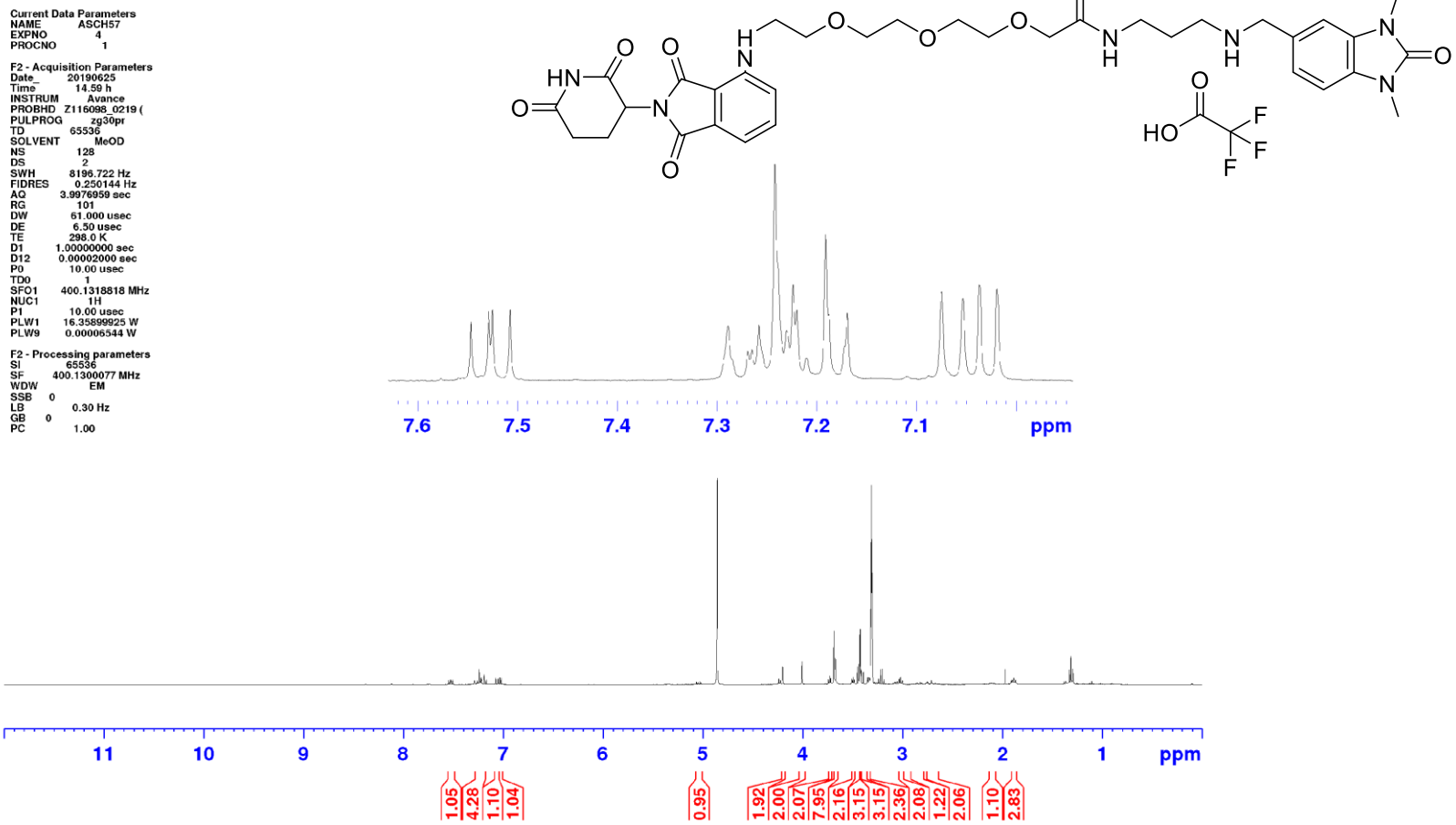
14-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-3,6,9,12-tetraoxatetradecan-1-oic acid trifluoro acetate (301)



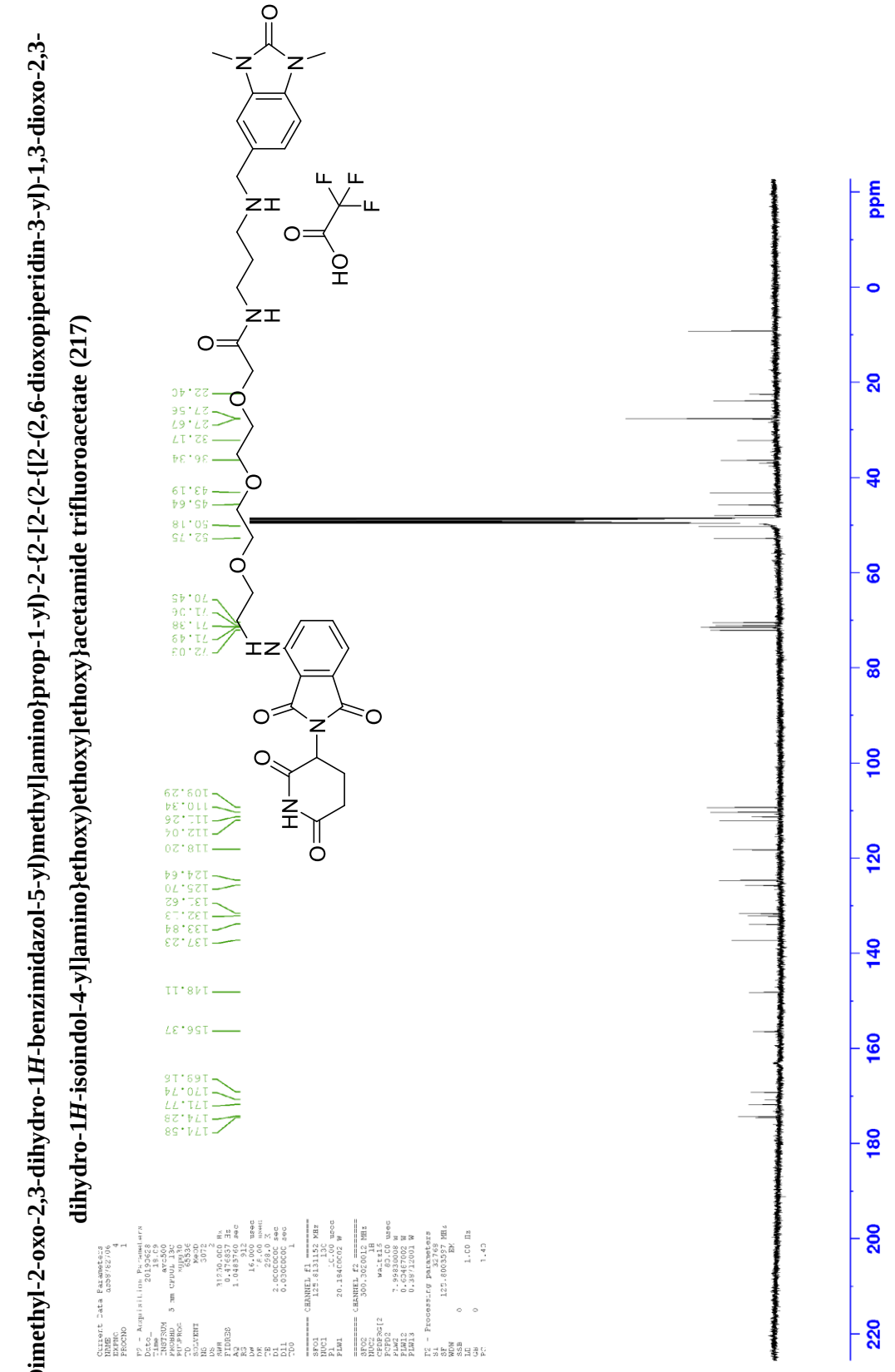
14-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-3,6,9,12-tetraoxatetradecan-1-oic acid trifluoro acetate (301)



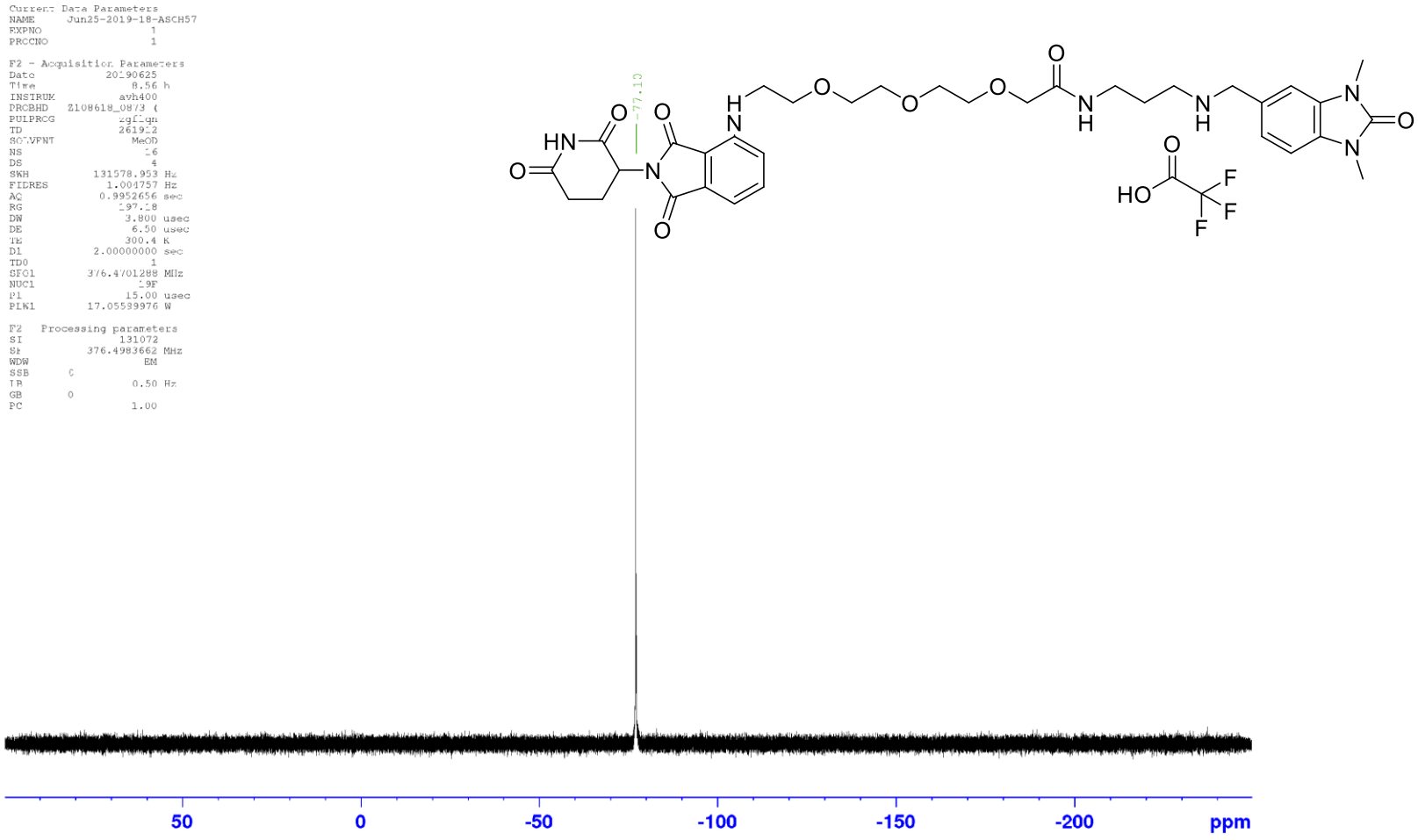
_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}prop-1-yl)-2-{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetamide trifluoroacetate (217)



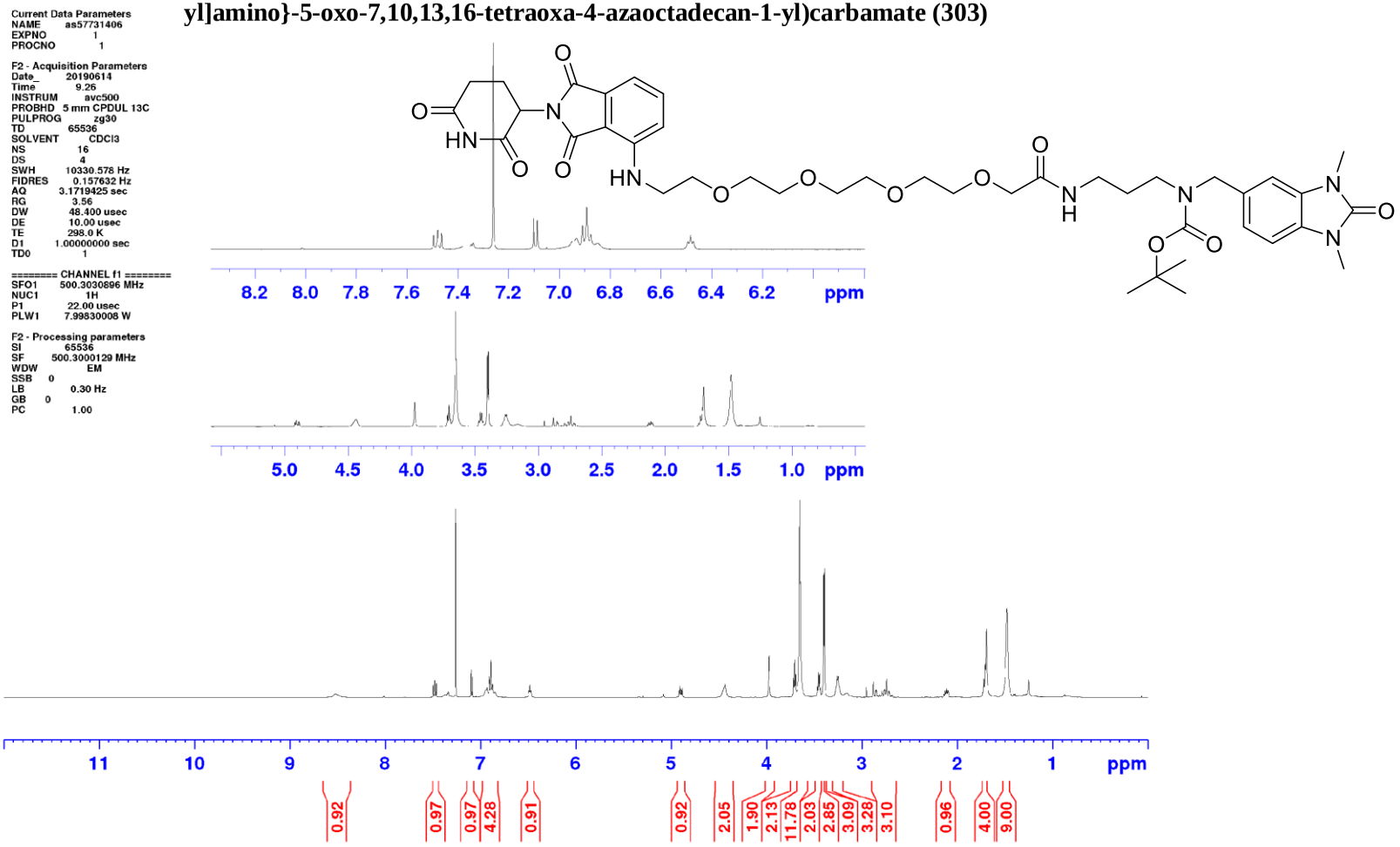
***N*-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}prop-1-yl)-2-{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetamide trifluoroacetate (217)**



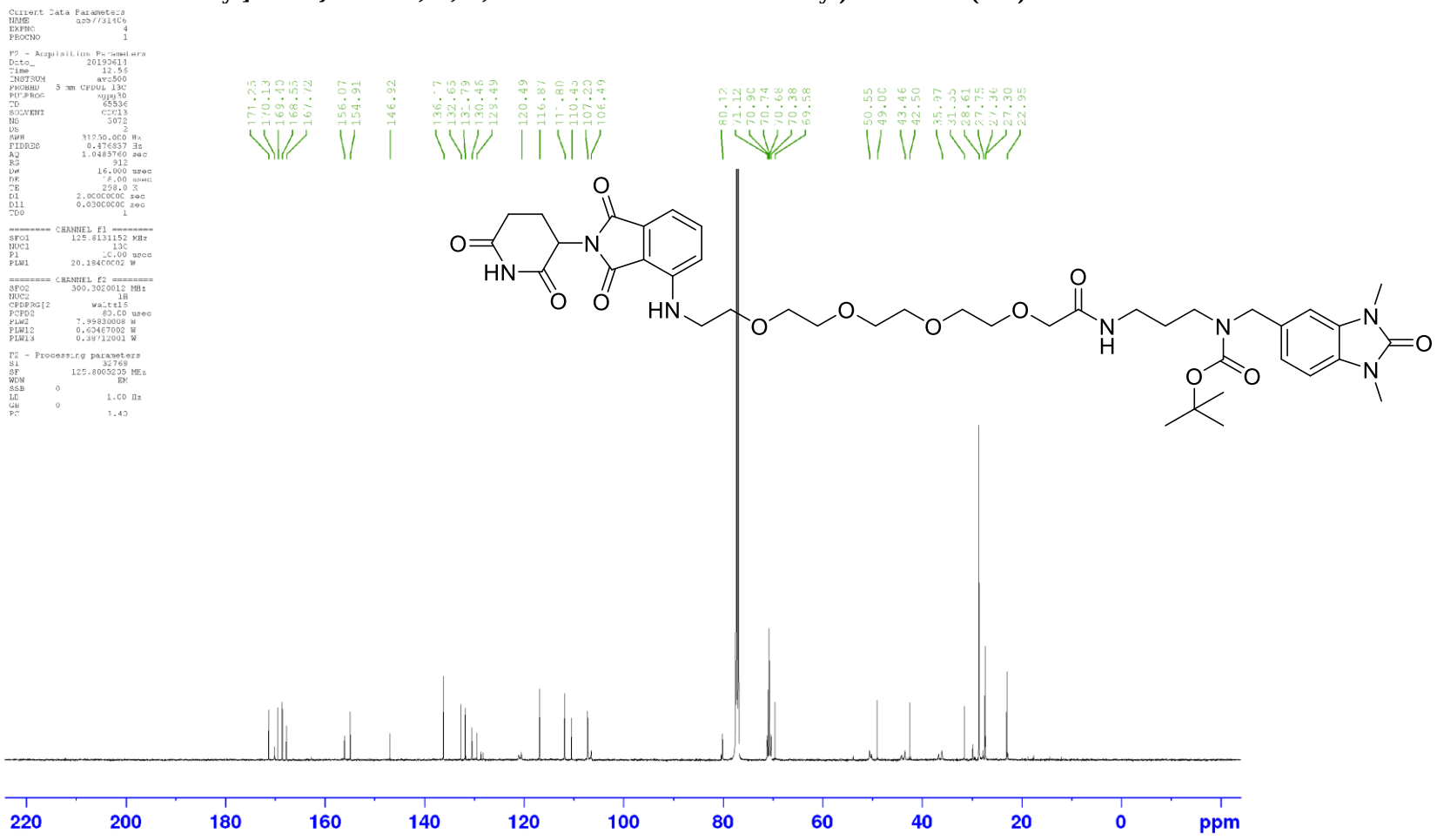
_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}prop-1-yl)-2-{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}ethoxy)ethoxy]ethoxy}acetamide trifluoroacetate (217)



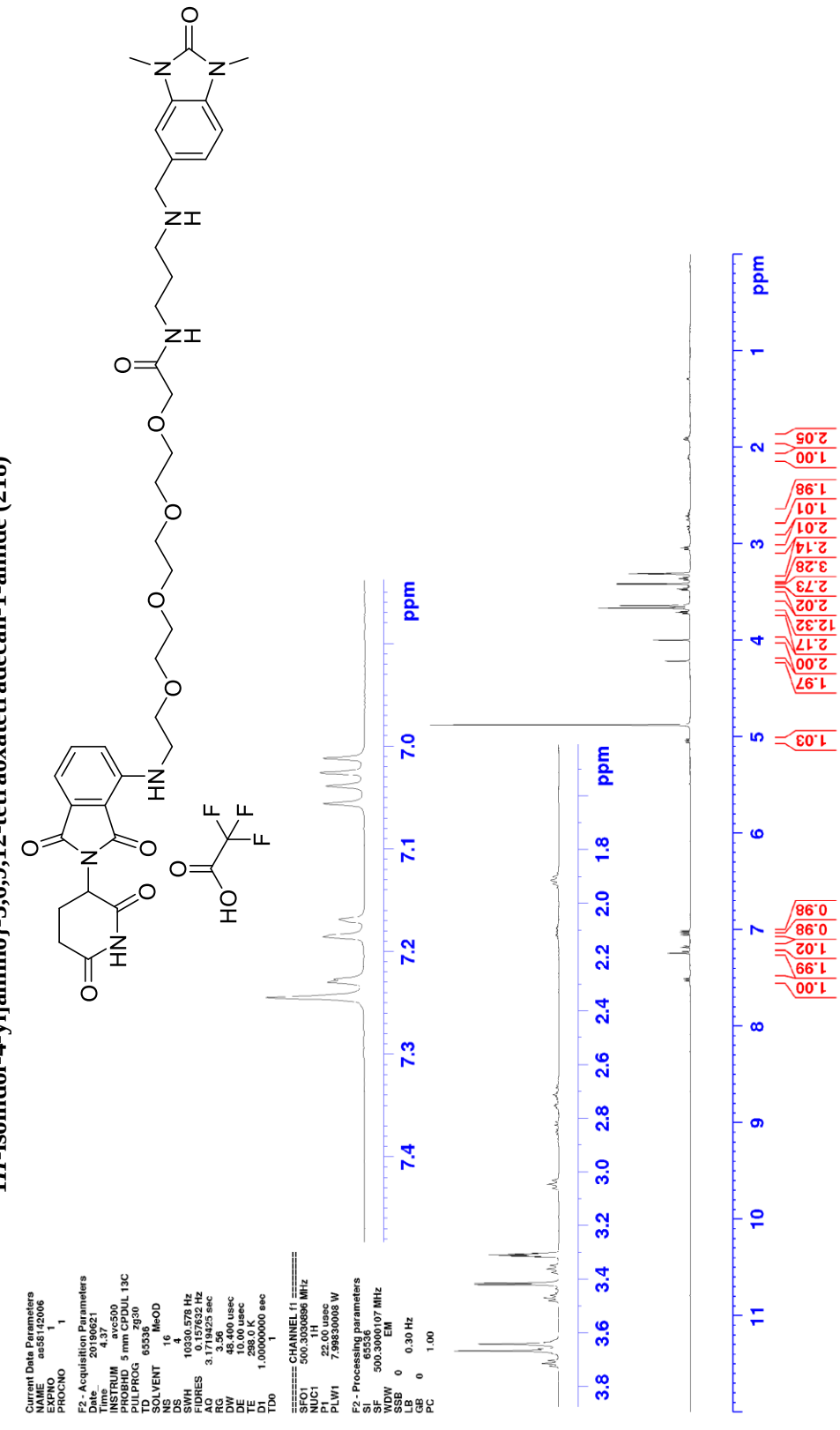
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl](18-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}-5-oxo-7,10,13,16-tetraoxa-4-azaoctadecan-1-yl)carbamate (303)



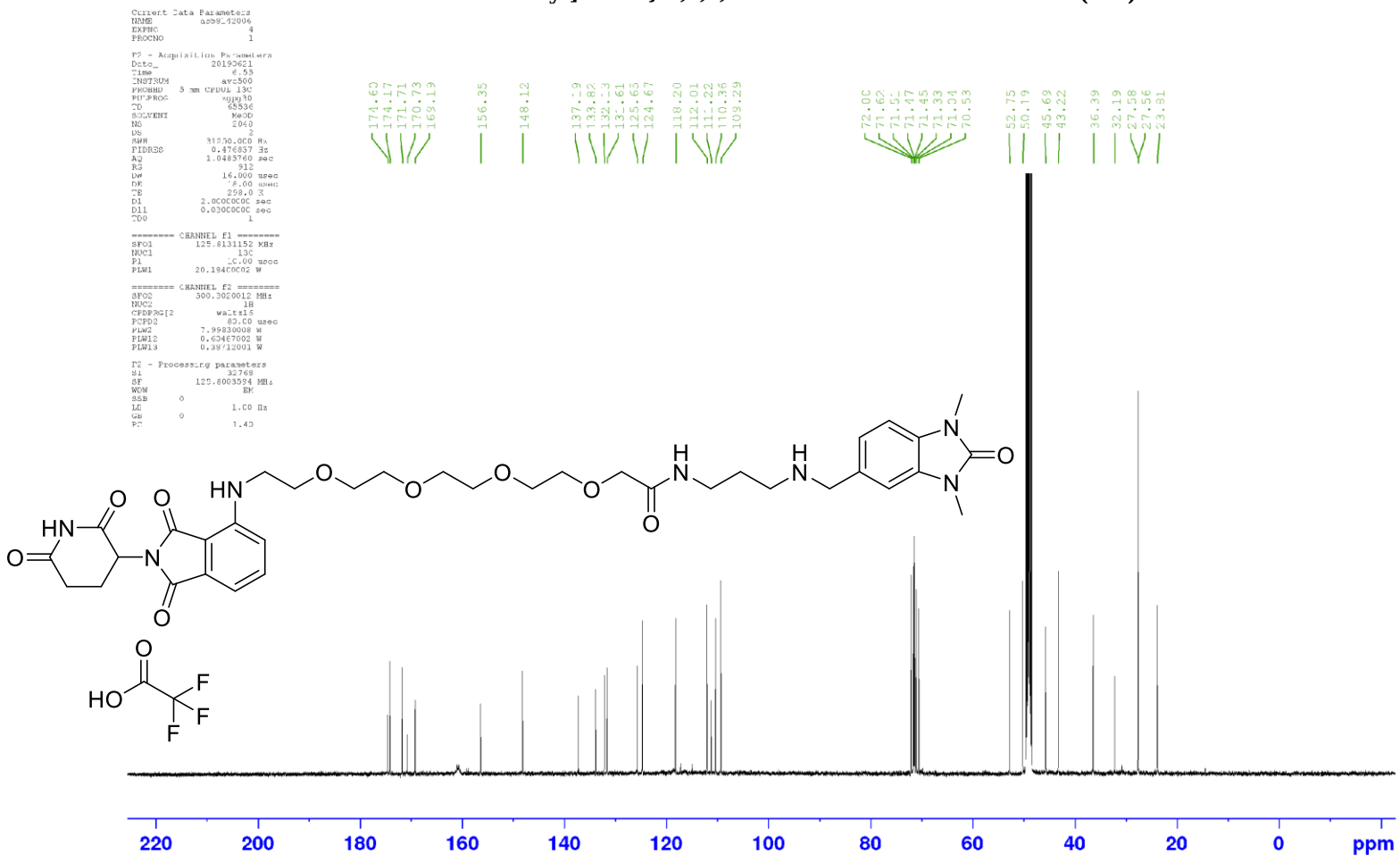
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl](18-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-5-oxo-7,10,13,16-tetraoxa-4-azaoctadecan-1-yl)carbamate (303)



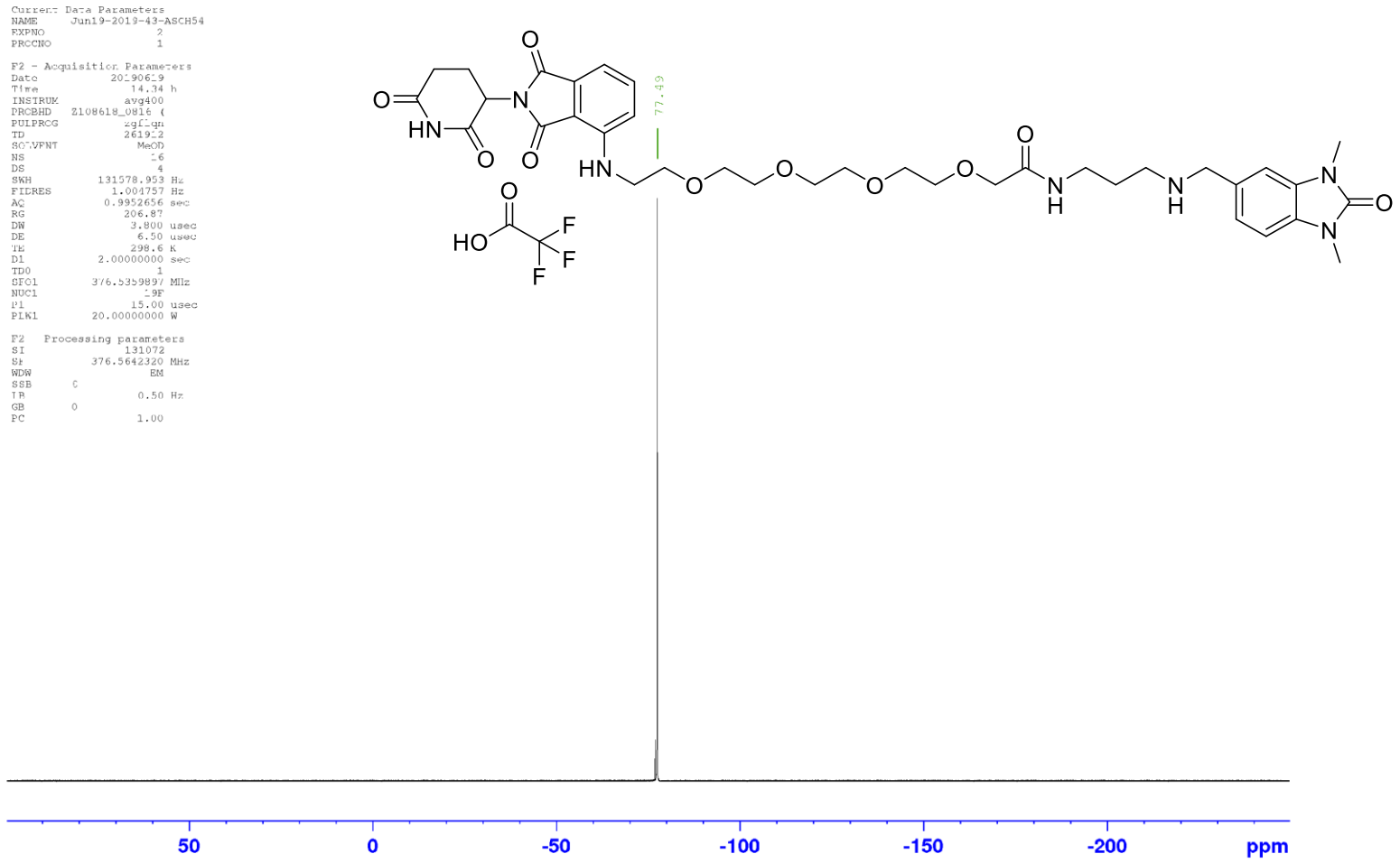
***N*-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)-14-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}-3,6,9,12-tetraoxatetradecan-1-amide (218)**



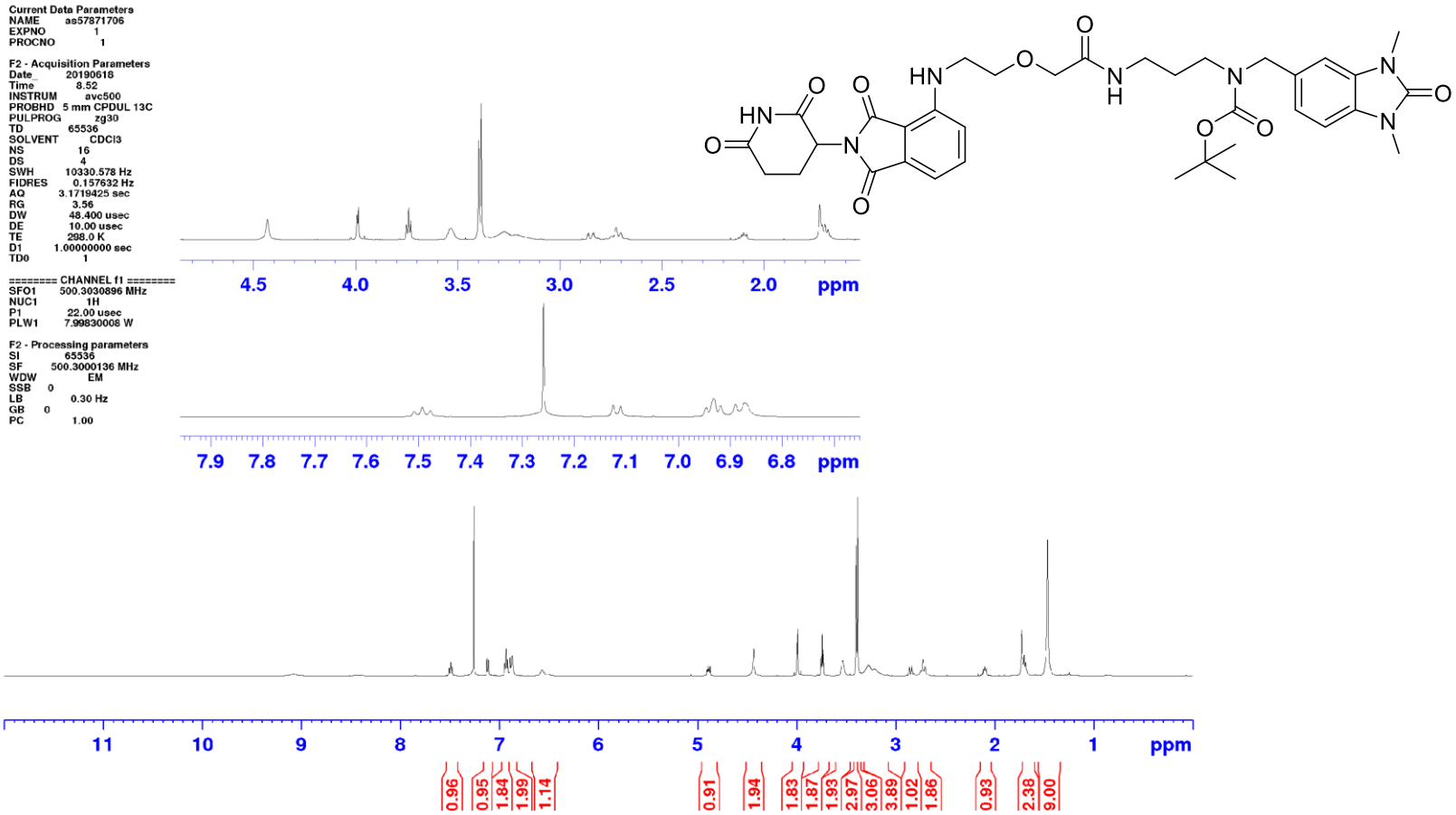
_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}propyl)-14-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}-3,6,9,12-tetraoxatetradecan-1-amide (218)



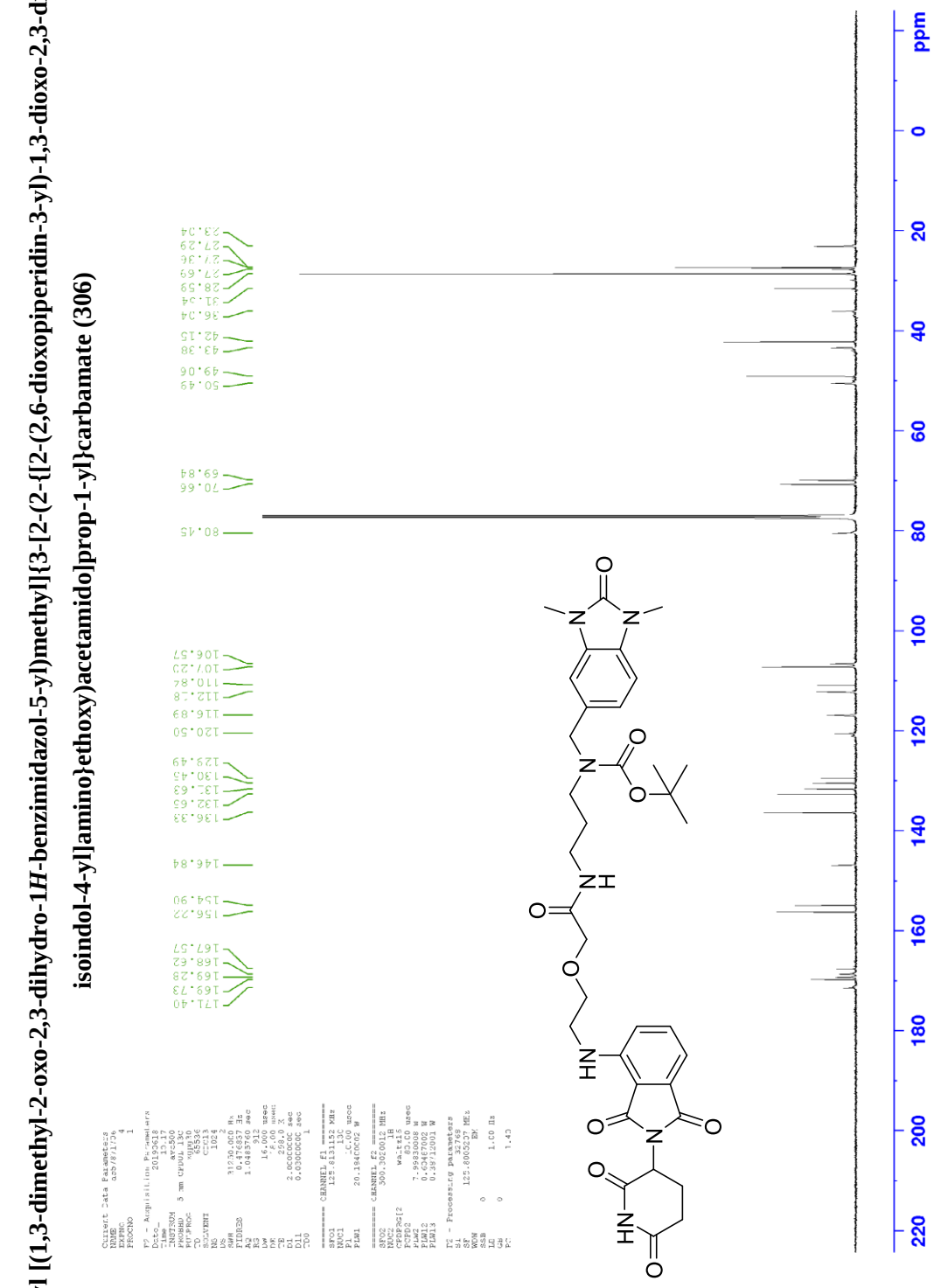
_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}propyl)-14-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}-3,6,9,12-tetraoxatetradecan-1-amide (218)



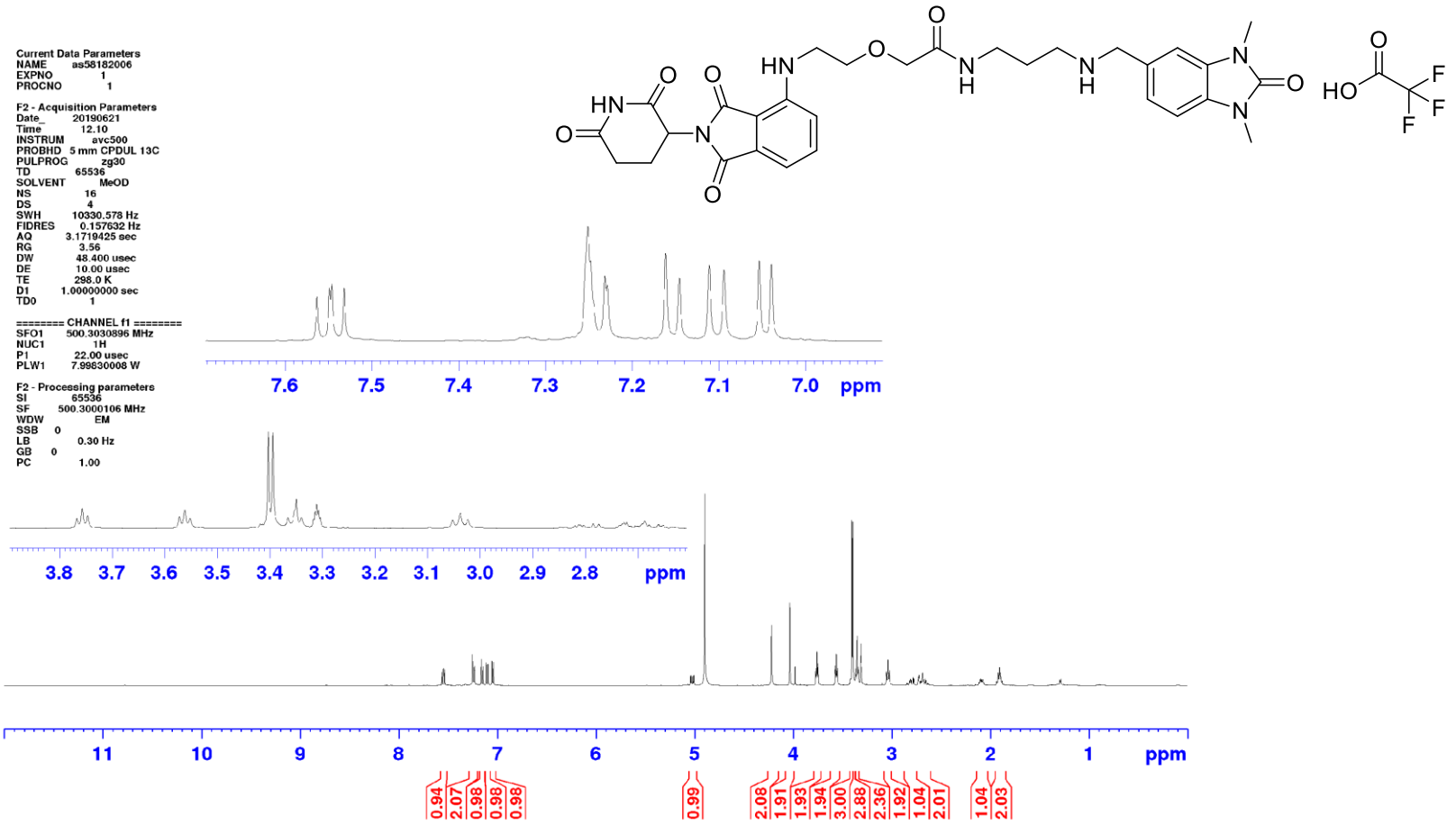
tert-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{3-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)acetamido]prop-1-yl}carbamate (306)



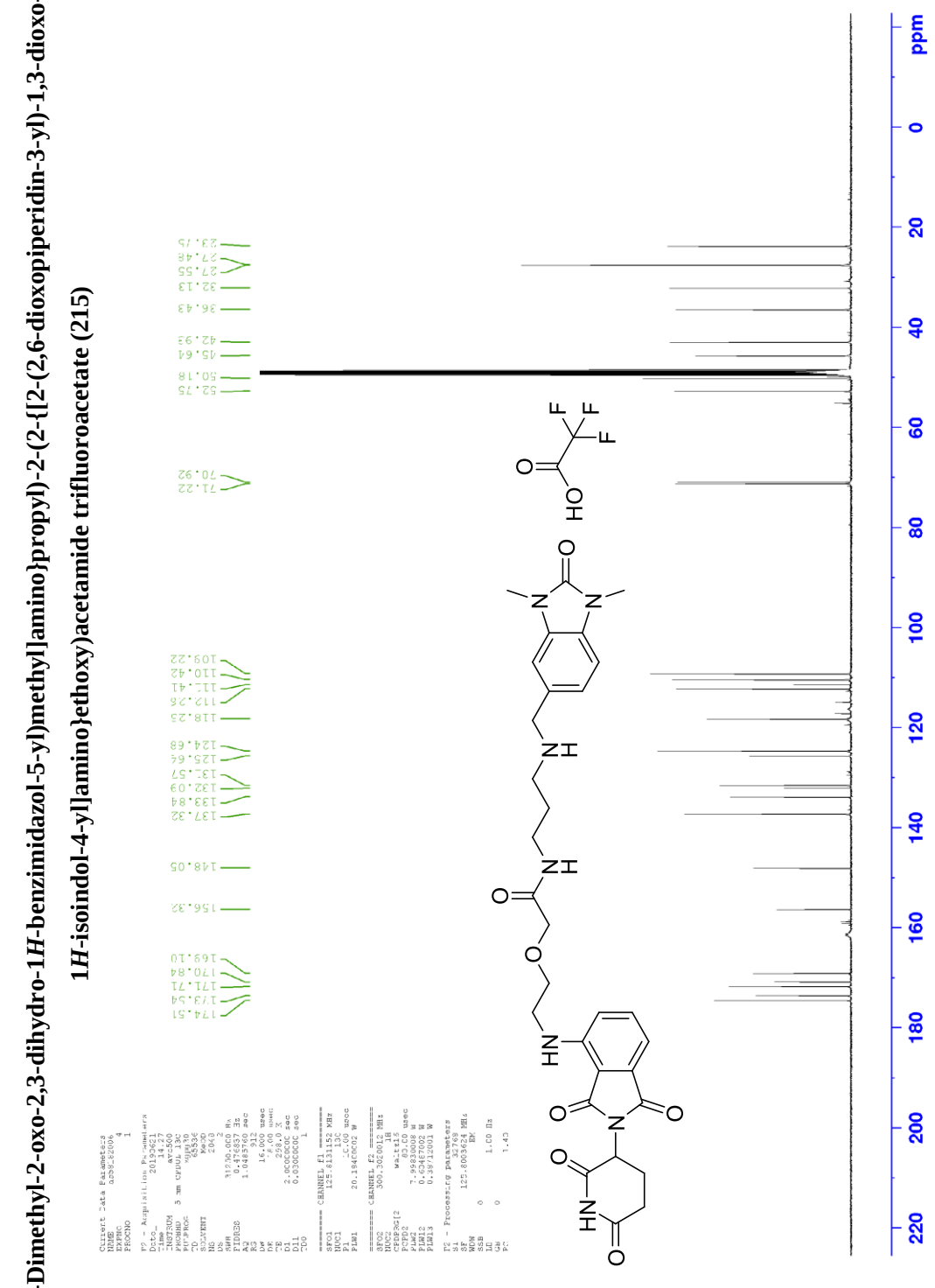
***tert*-Butyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{3-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)acetamido]prop-1-yl}carbamate (306)**



_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}propyl)-2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}ethoxy)acetamide trifluoroacetate (215)

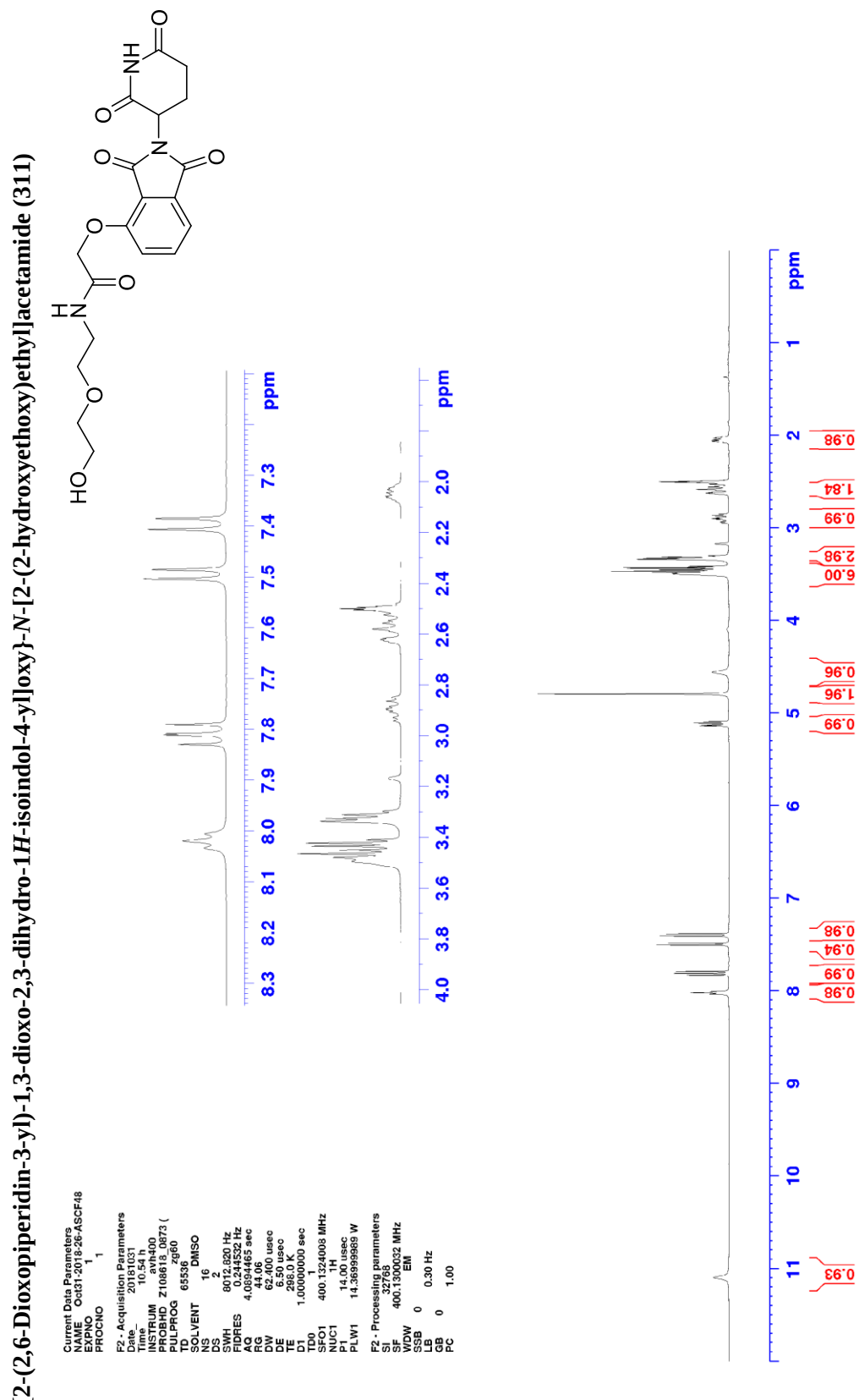


***N*-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]amino}propyl)-2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino}ethoxy)acetamide trifluoroacetate (215)**

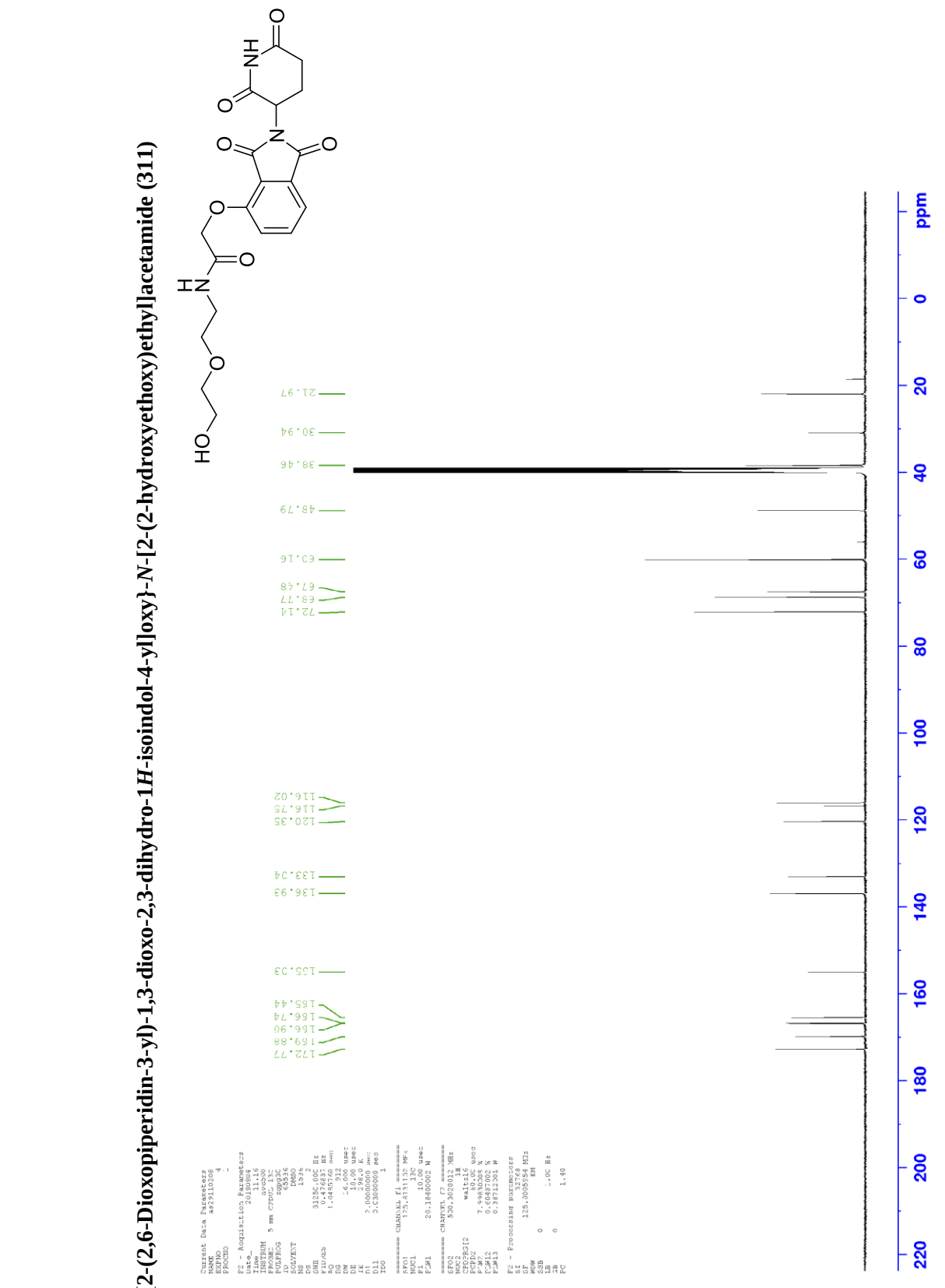


_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}propyl)-2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]amino}ethoxy)acetamide trifluoroacetate (215)

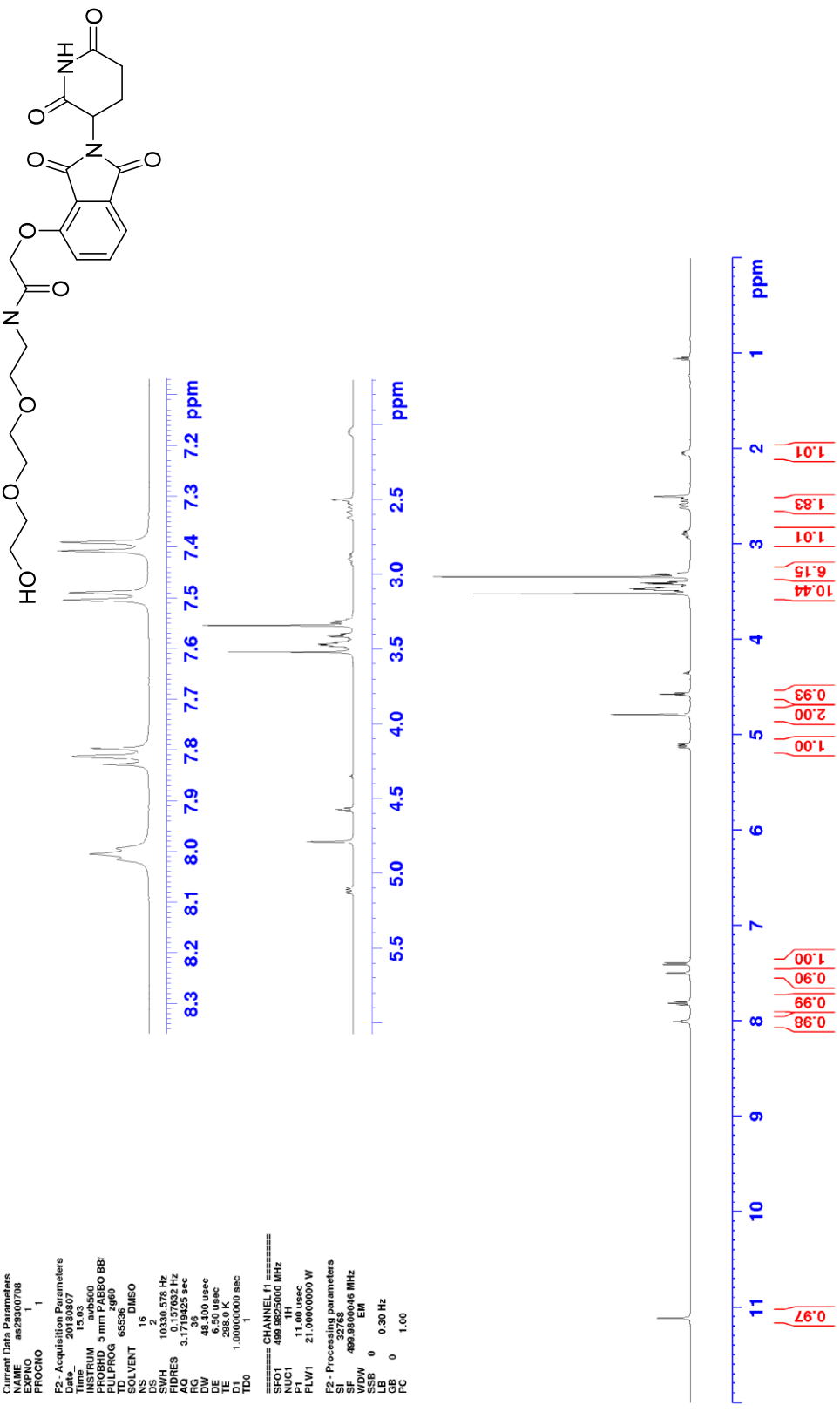
2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-*N*-[2-(2-hydroxyethoxy)ethyl]acetamide (311)



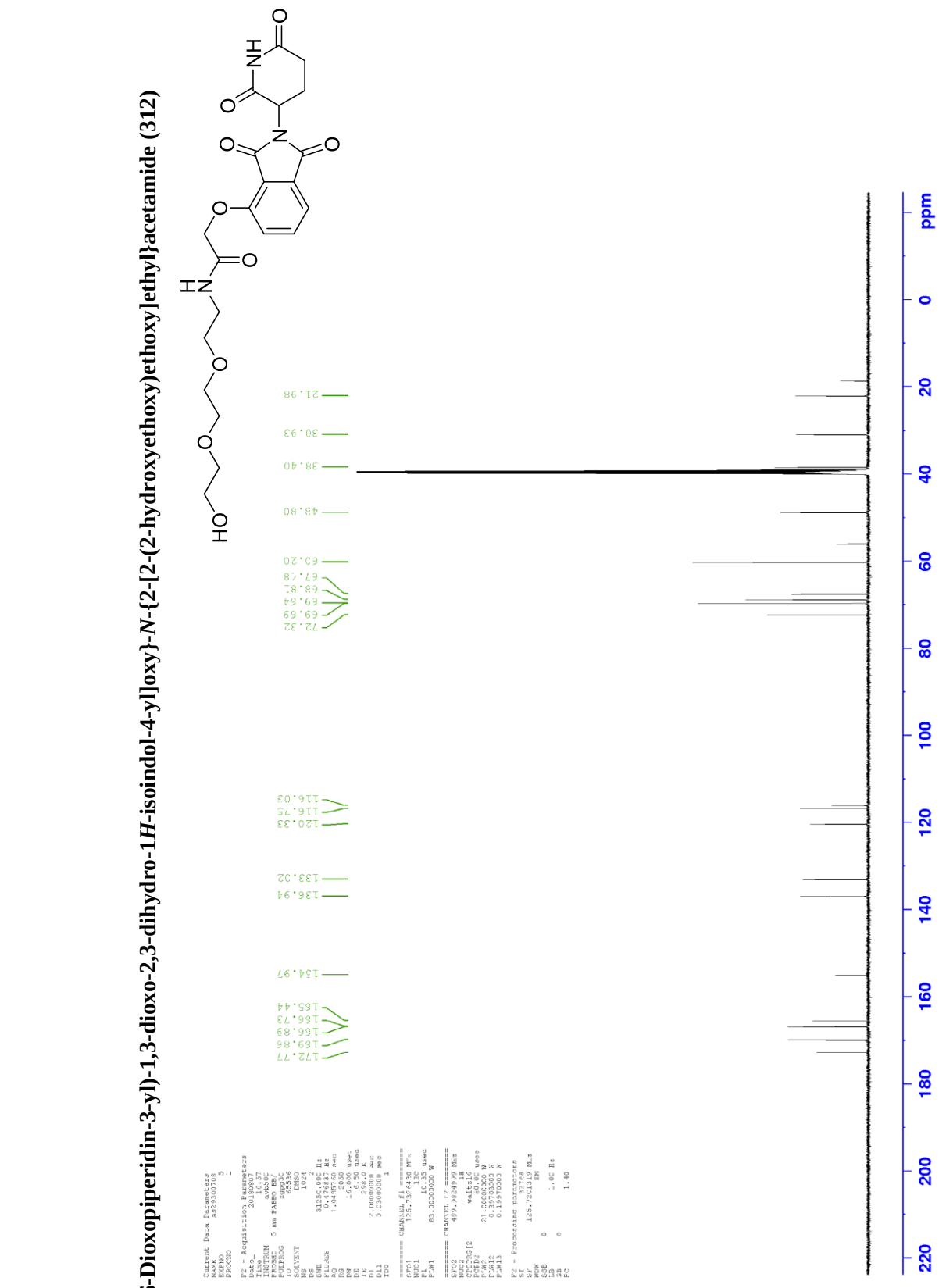
2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-*N*-[2-(2-hydroxyethoxy)ethyl]acetamide (311)



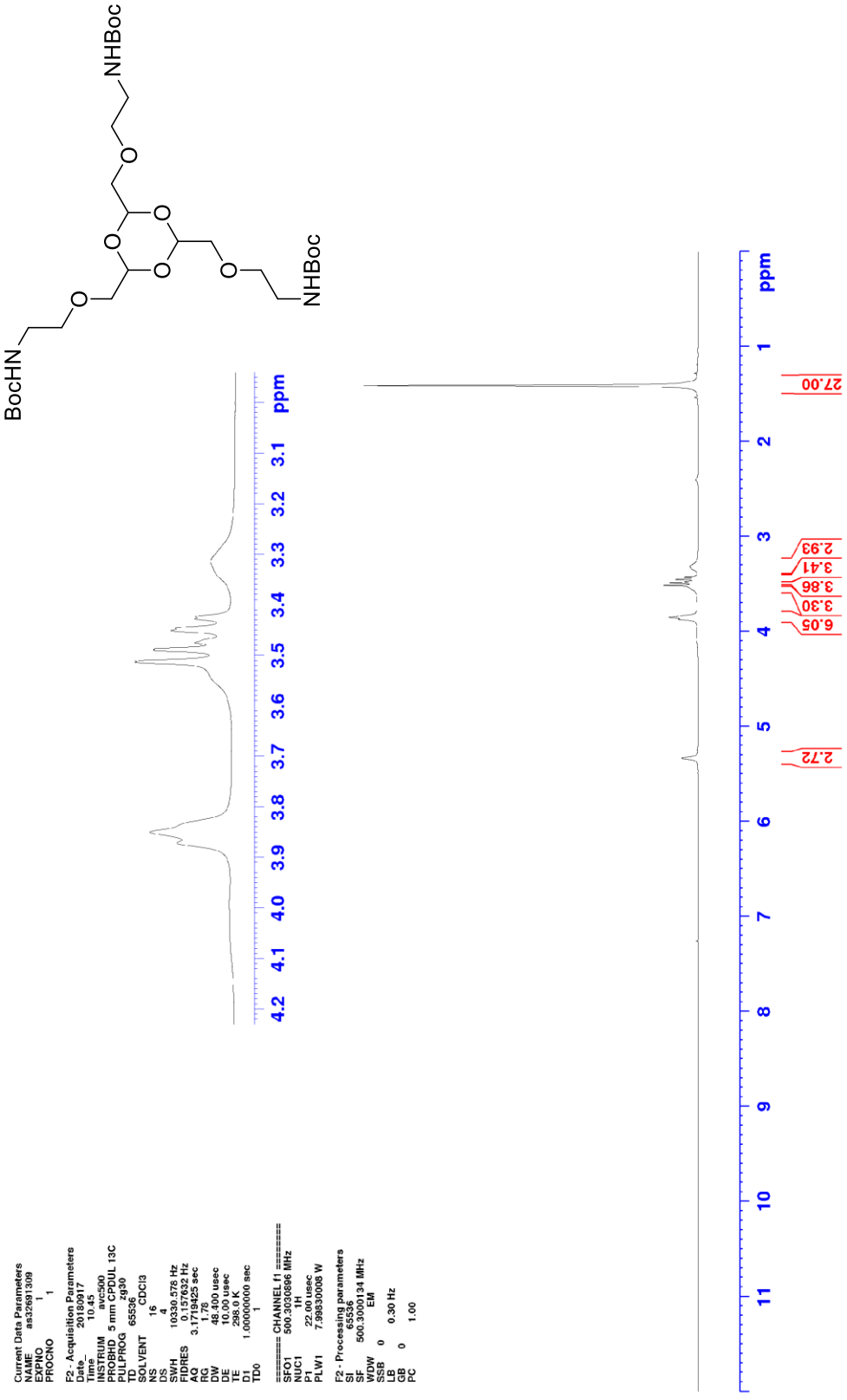
2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-*N*-{2-[2-(2-hydroxyethoxy)ethoxy]ethyl}acetamide (312)



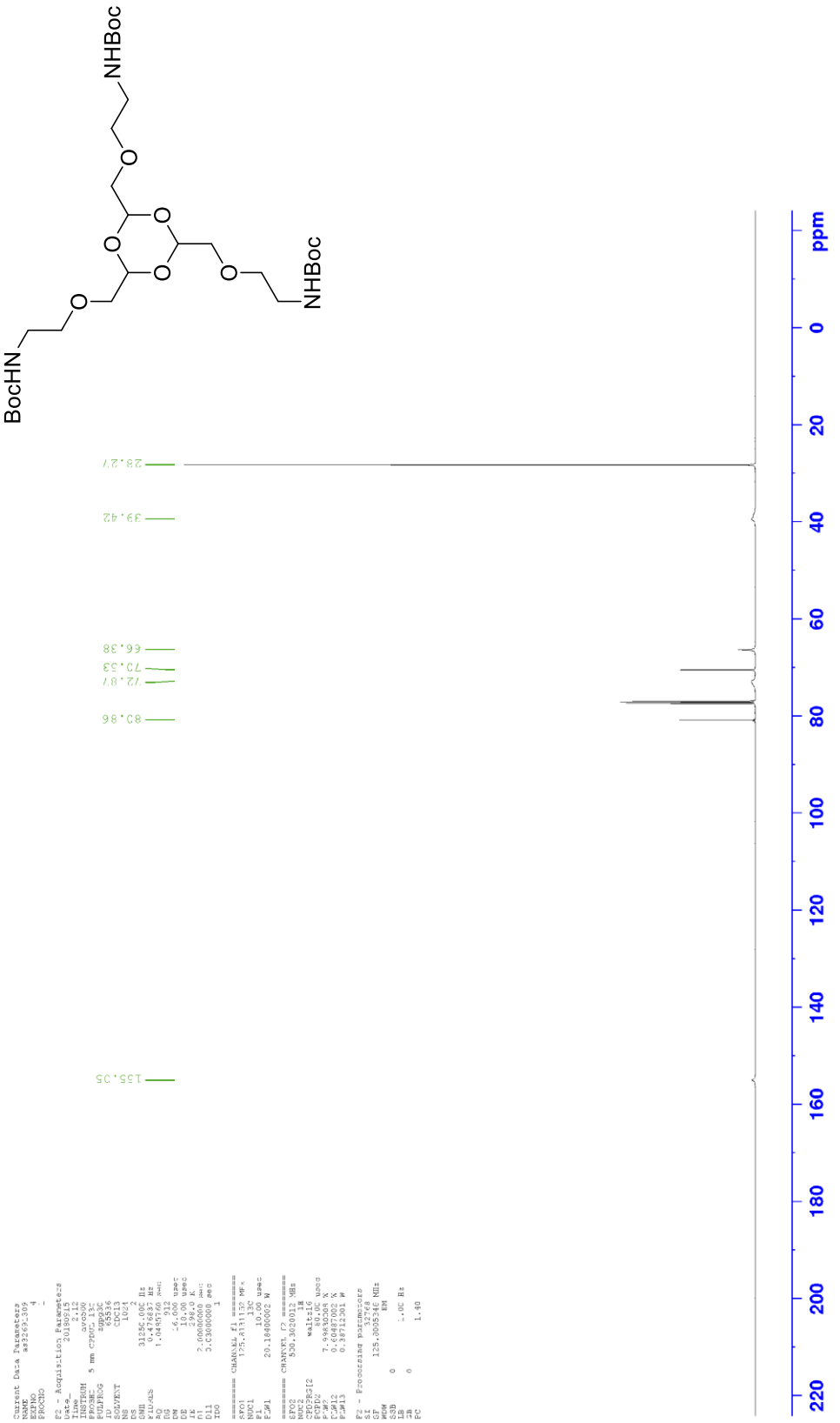
2-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-*N*-{2-[2-(2-hydroxyethoxy)ethoxy]ethyl}acetamide (312)



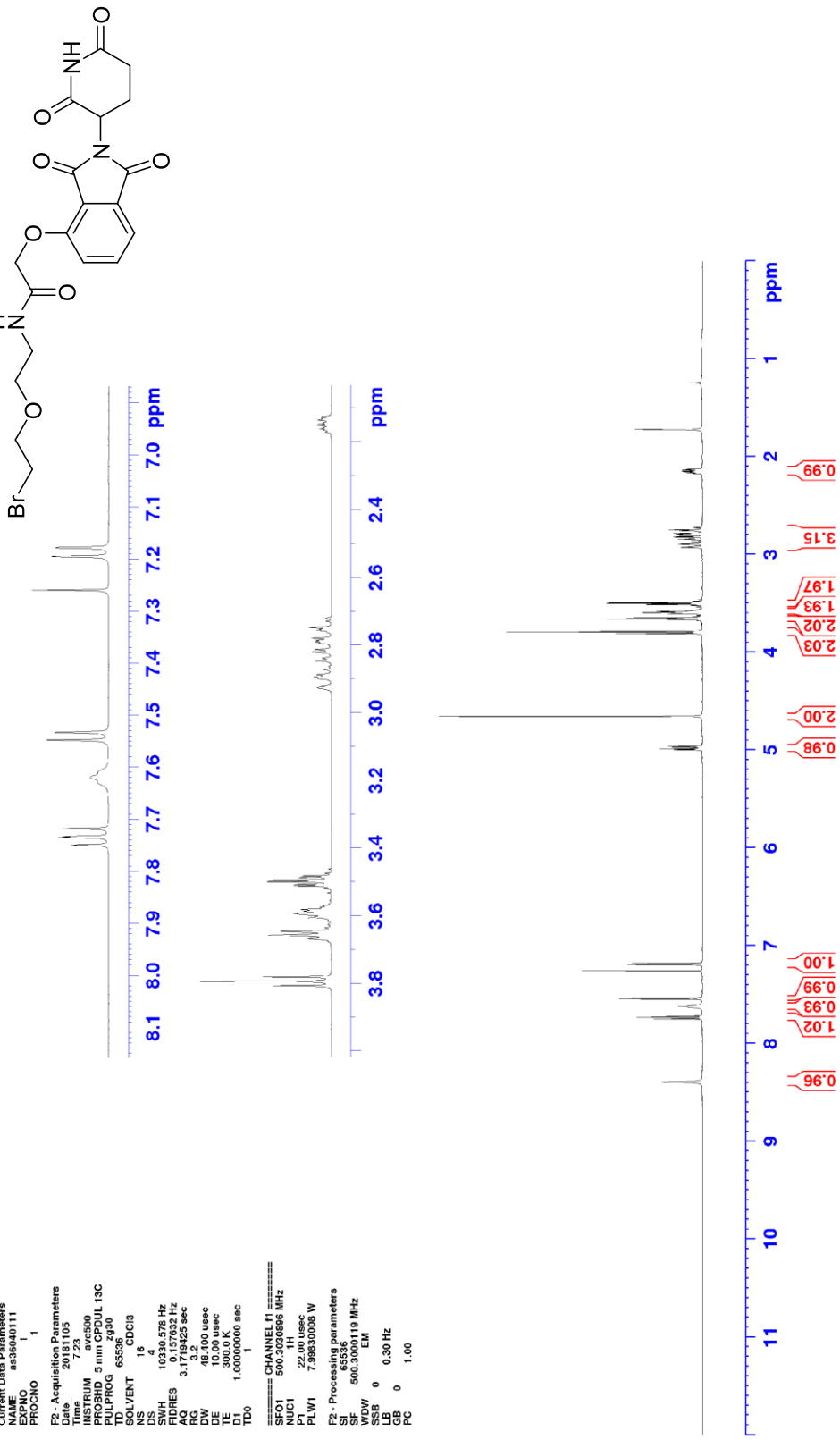
Tri-*tert*-butyl [1,3,5-trioxane-2,4,6-triyltris(methyleneoxyethane-2,1-diyl)]triscarbamate (318)



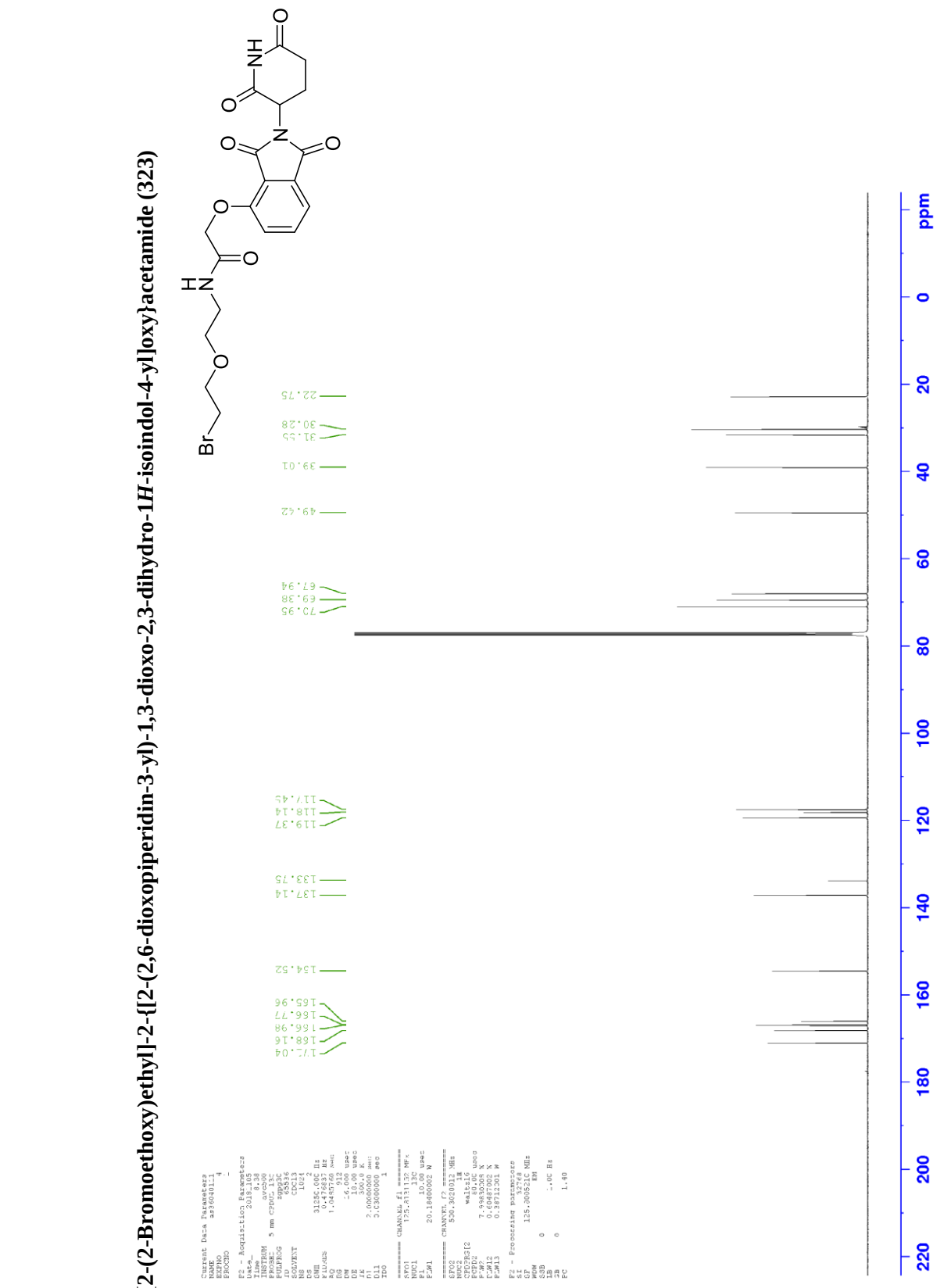
Tri-*tert*-butyl [1,3,5-trioxane-2,4,6-triyltris(methyleneoxyethane-2,1-diyl)]triscarbamate (318)



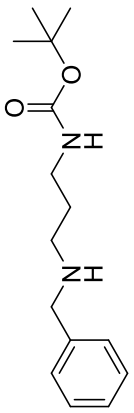
_N_-[2-(2-Bromoethoxy)ethyl]-2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}acetamide (323)

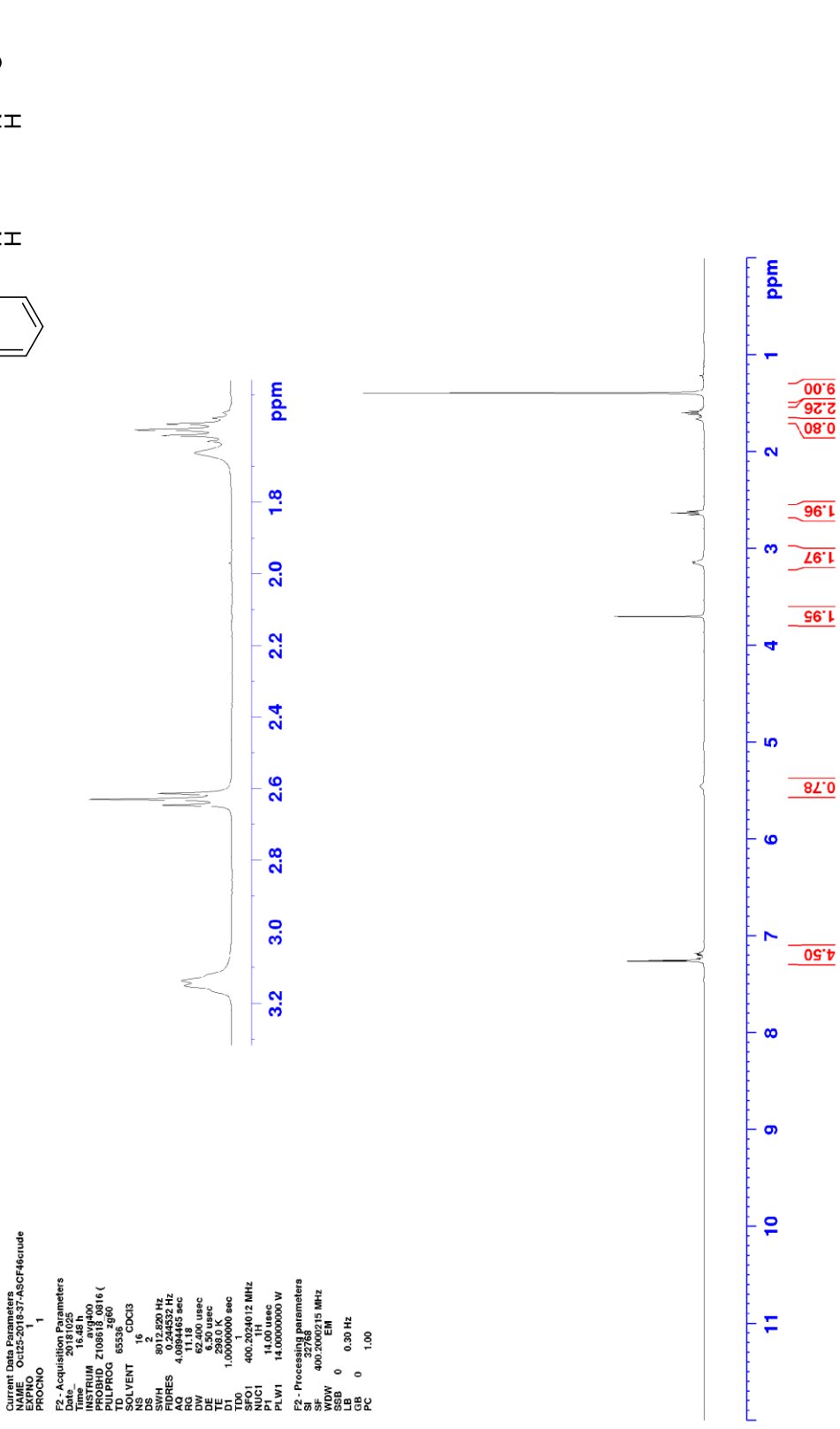


_N_-[2-(2-Bromoethoxy)ethyl]-2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1_H_-isoindol-4-yl]oxy}acetamide (323)

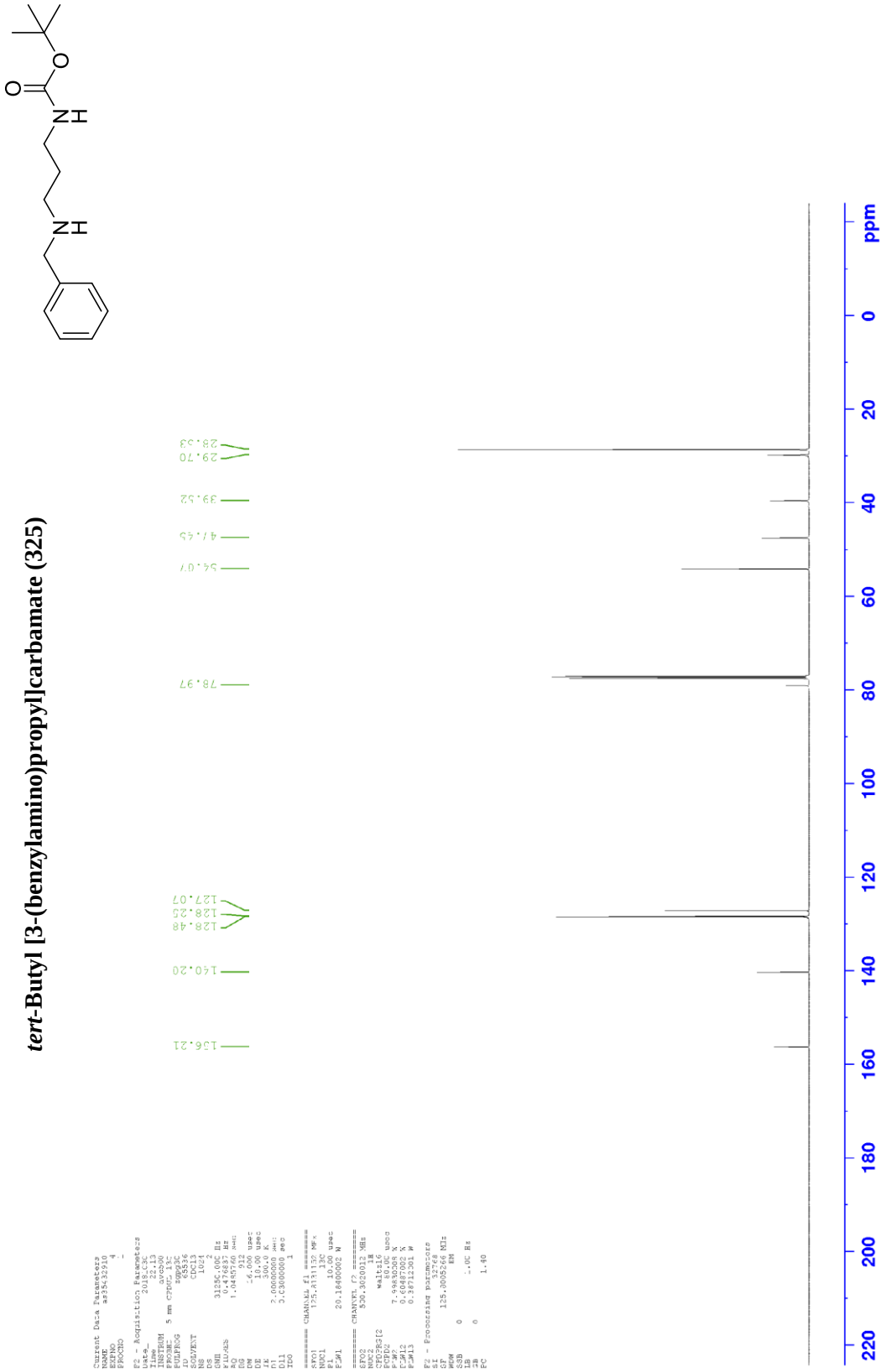


tert-Butyl [3-(benzylamino)propyl]carbamate (325)

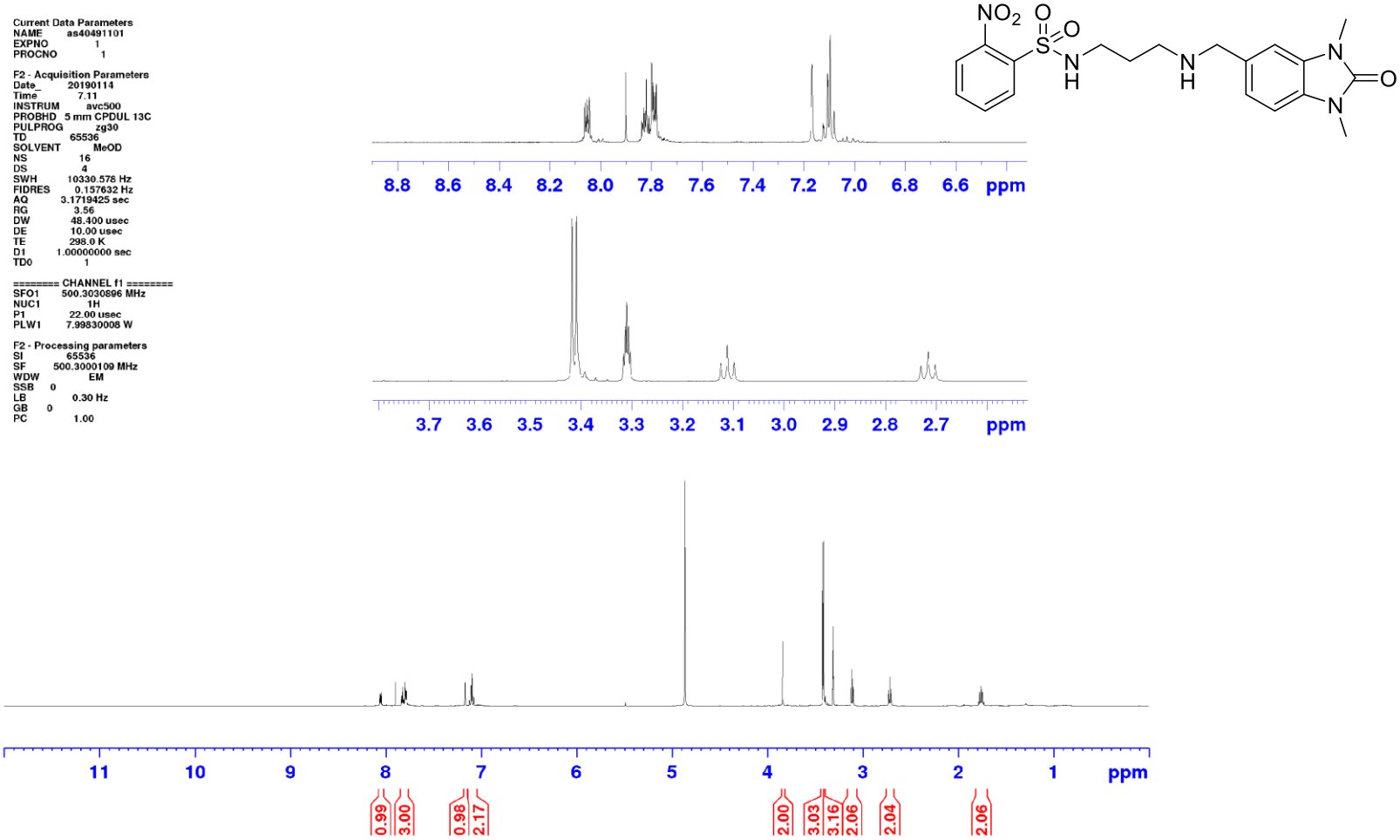




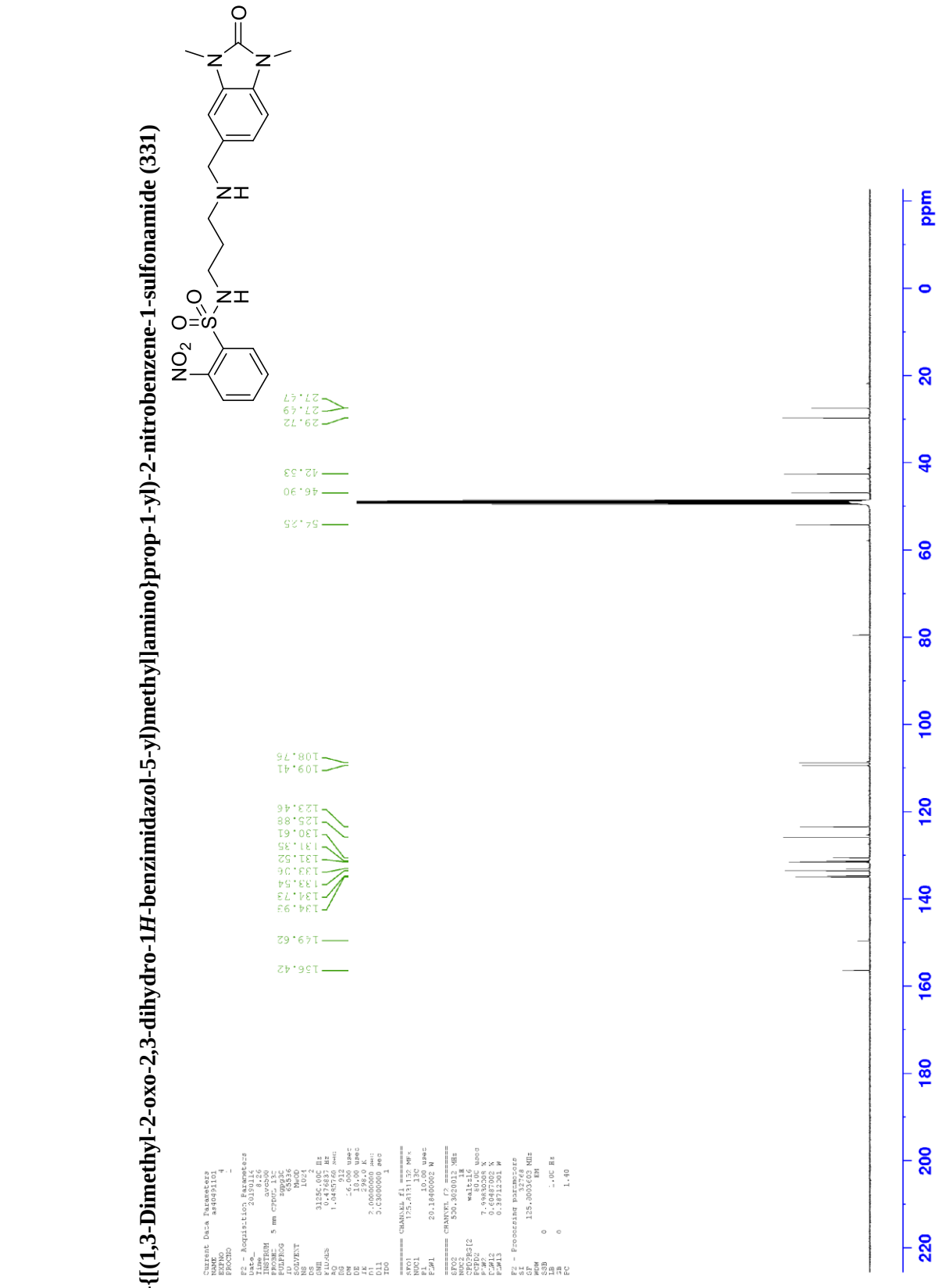
tert-Butyl [3-(benzylamino)propyl]carbamate (325)



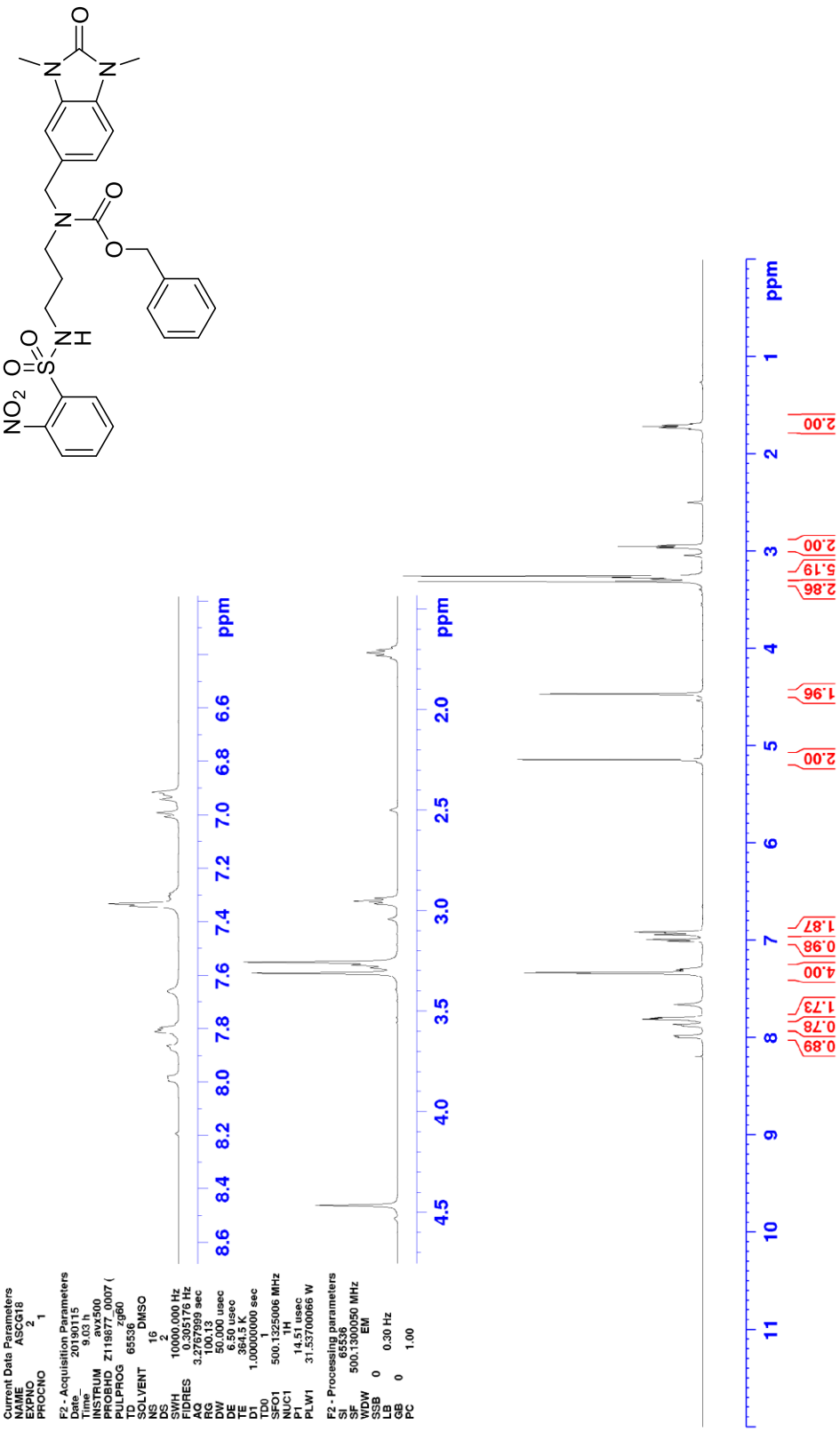
_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}prop-1-yl)-2-nitrobenzene-1-sulfonamide (331)



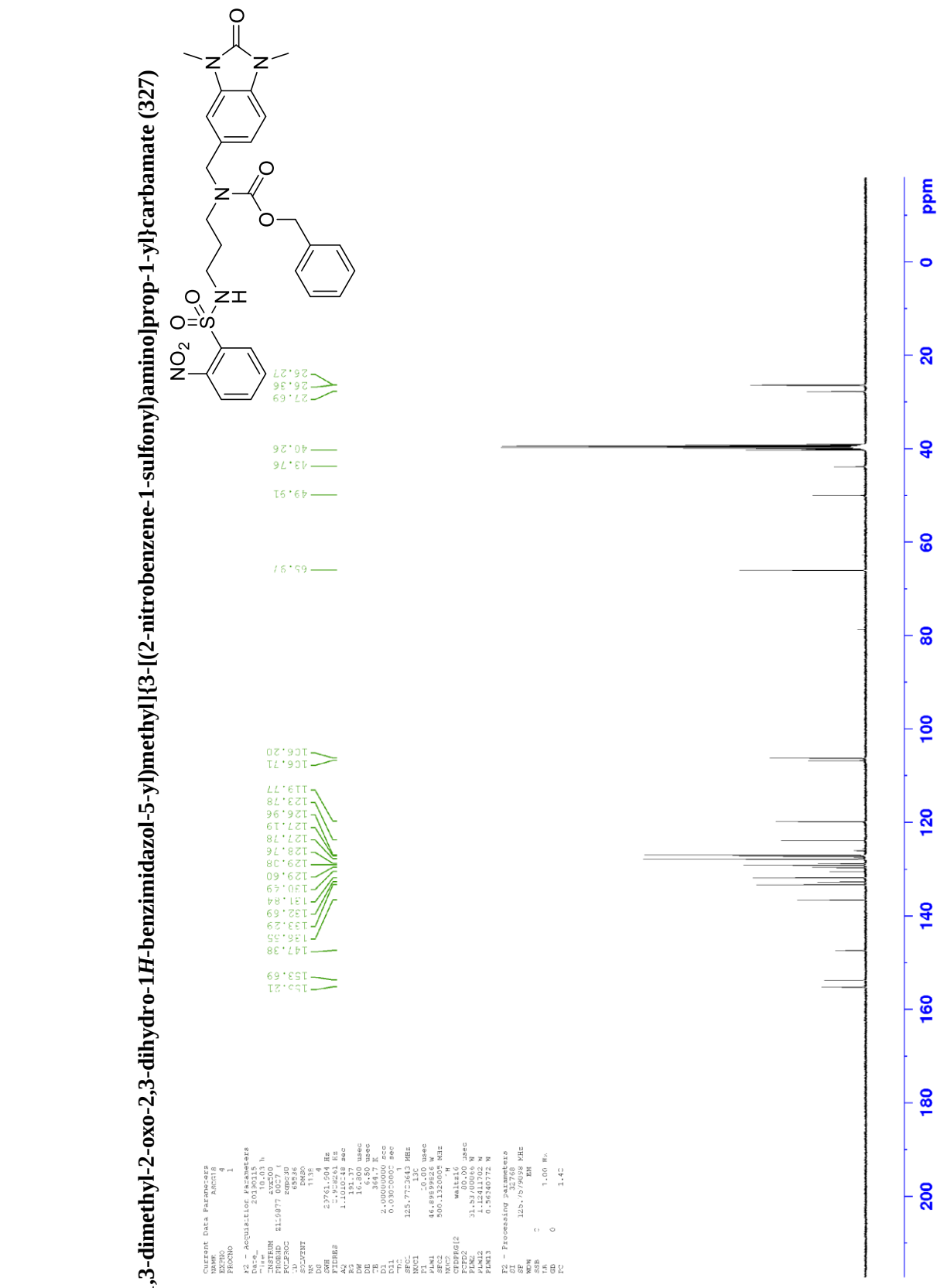
_N_-(3-{[(1,3-Dimethyl-2-oxo-2,3-dihydro-1_H_-benzimidazol-5-yl)methyl]amino}prop-1-yl)-2-nitrobenzene-1-sulfonamide (331)



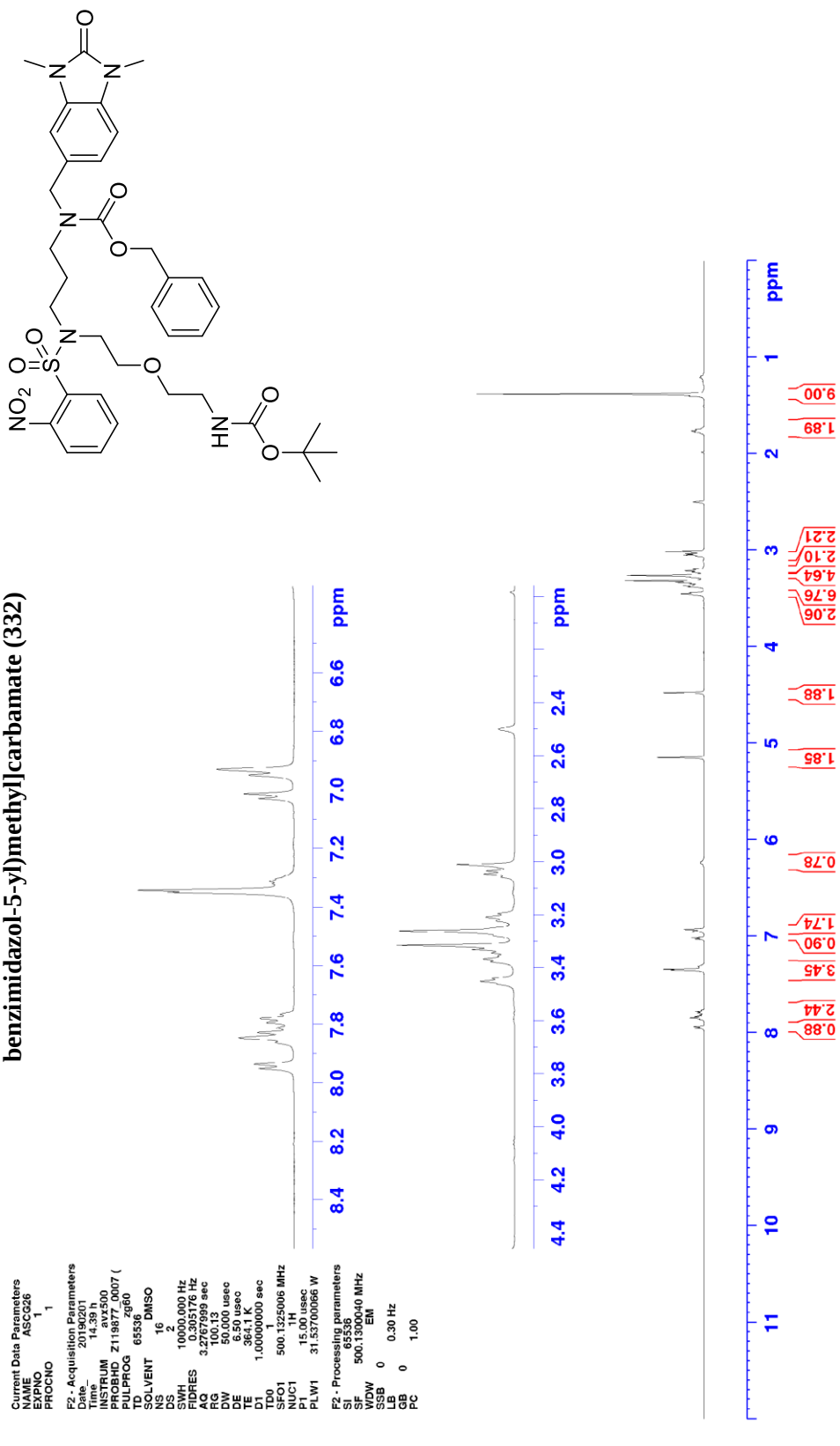
Benzyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{3-[(2-nitrobenzene-1-sulfonyl)amino]prop-1-yl}carbamate (327)



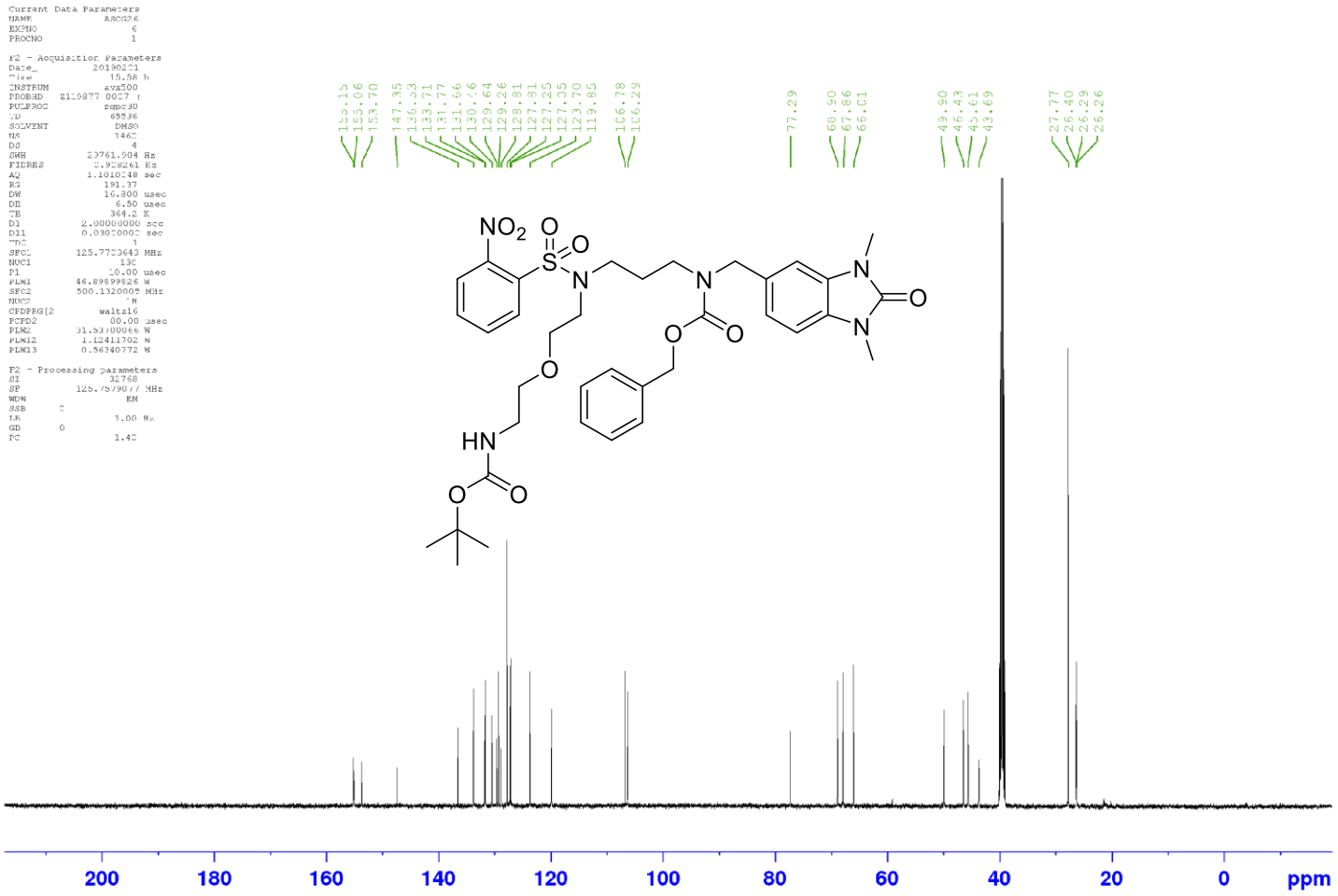
Benzyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{3-[(2-nitrobenzene-1-sulfonyl)amino]prop-1-yl}carbamate (327)



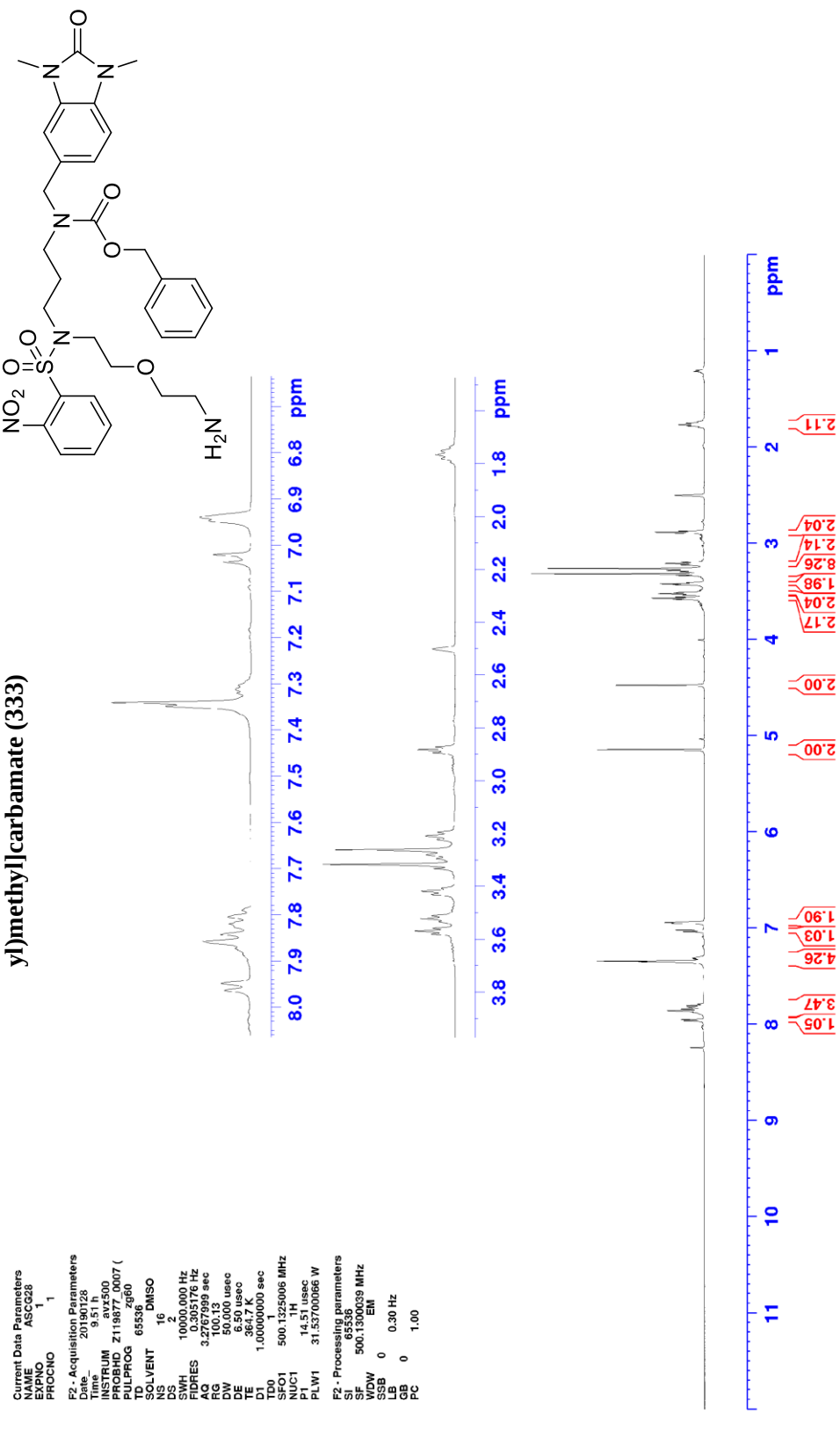
Benzyl [2,2-dimethyl-11-(2-nitrobenzene-1-sulfonyl)-4-oxo-3,8-dioxa-5,11-diazatetradecan-14-yl][(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (332)



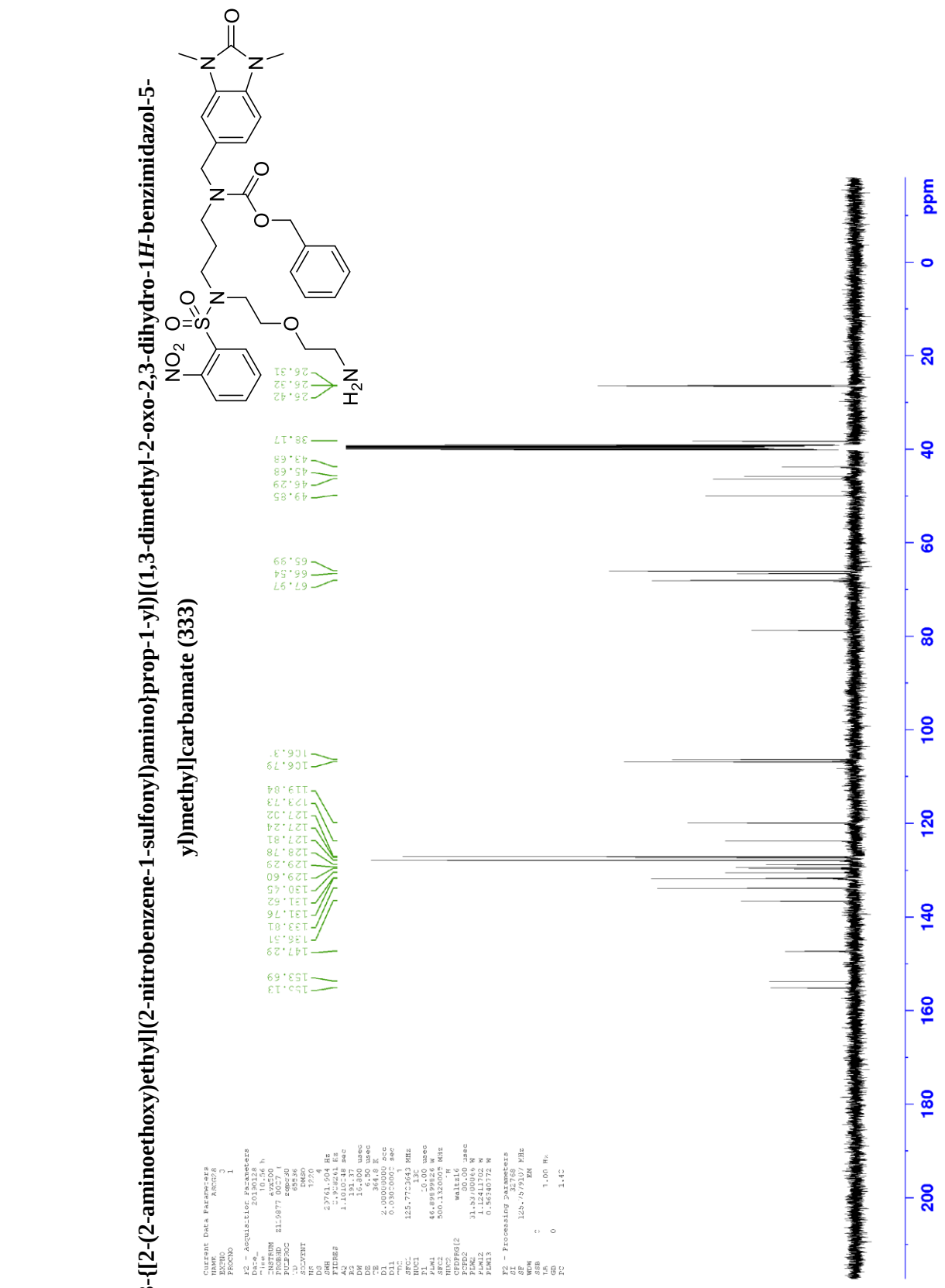
Benzyl [2,2-dimethyl-11-(2-nitrobenzene-1-sulfonyl)-4-oxo-3,8-dioxa-5,11-diazatetradecan-14-yl][(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (332)



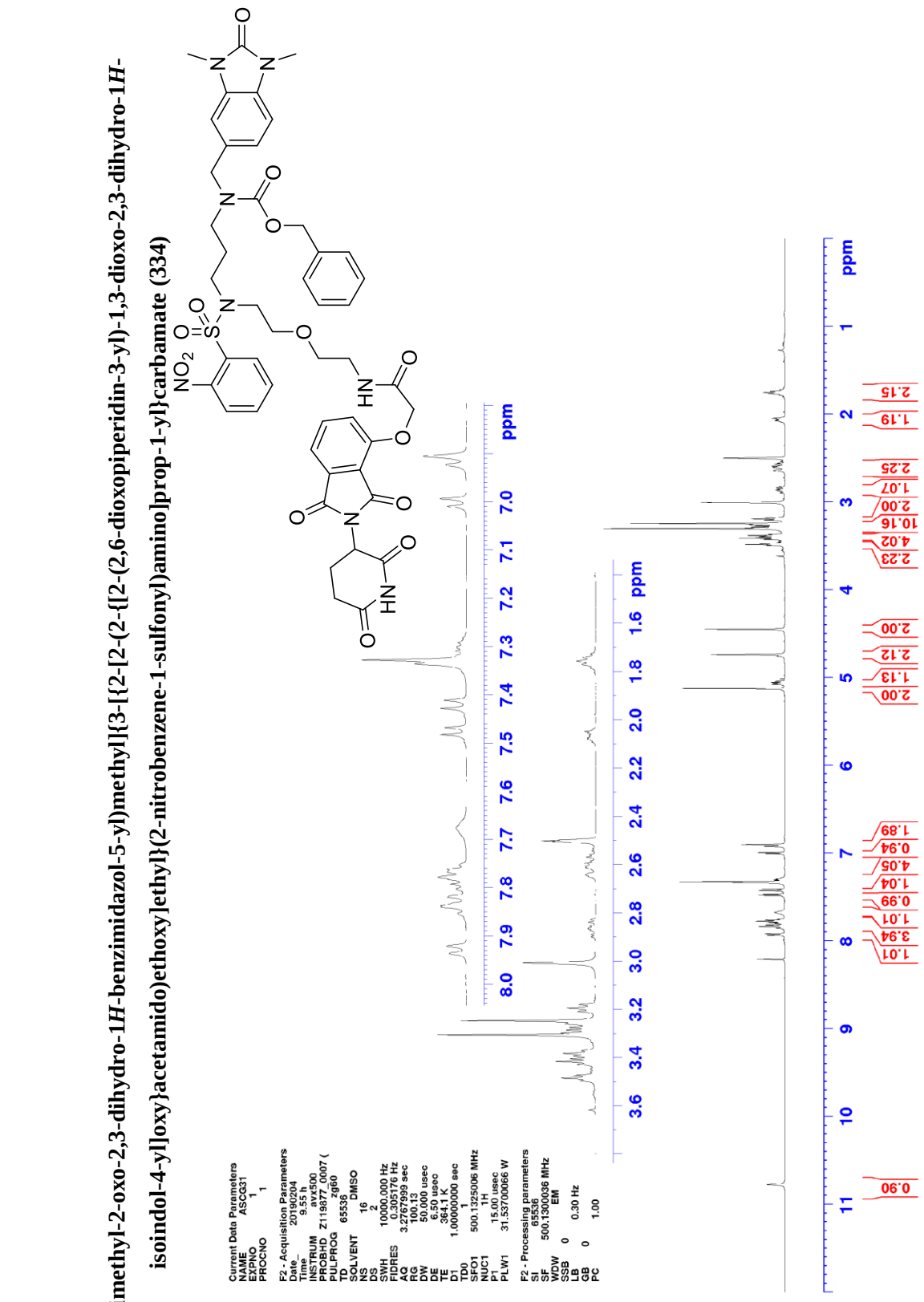
Benzyl (3-{[2-(2-aminoethoxy)ethyl](2-nitrobenzene-1-sulfonyl)amino}prop-1-yl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (333)



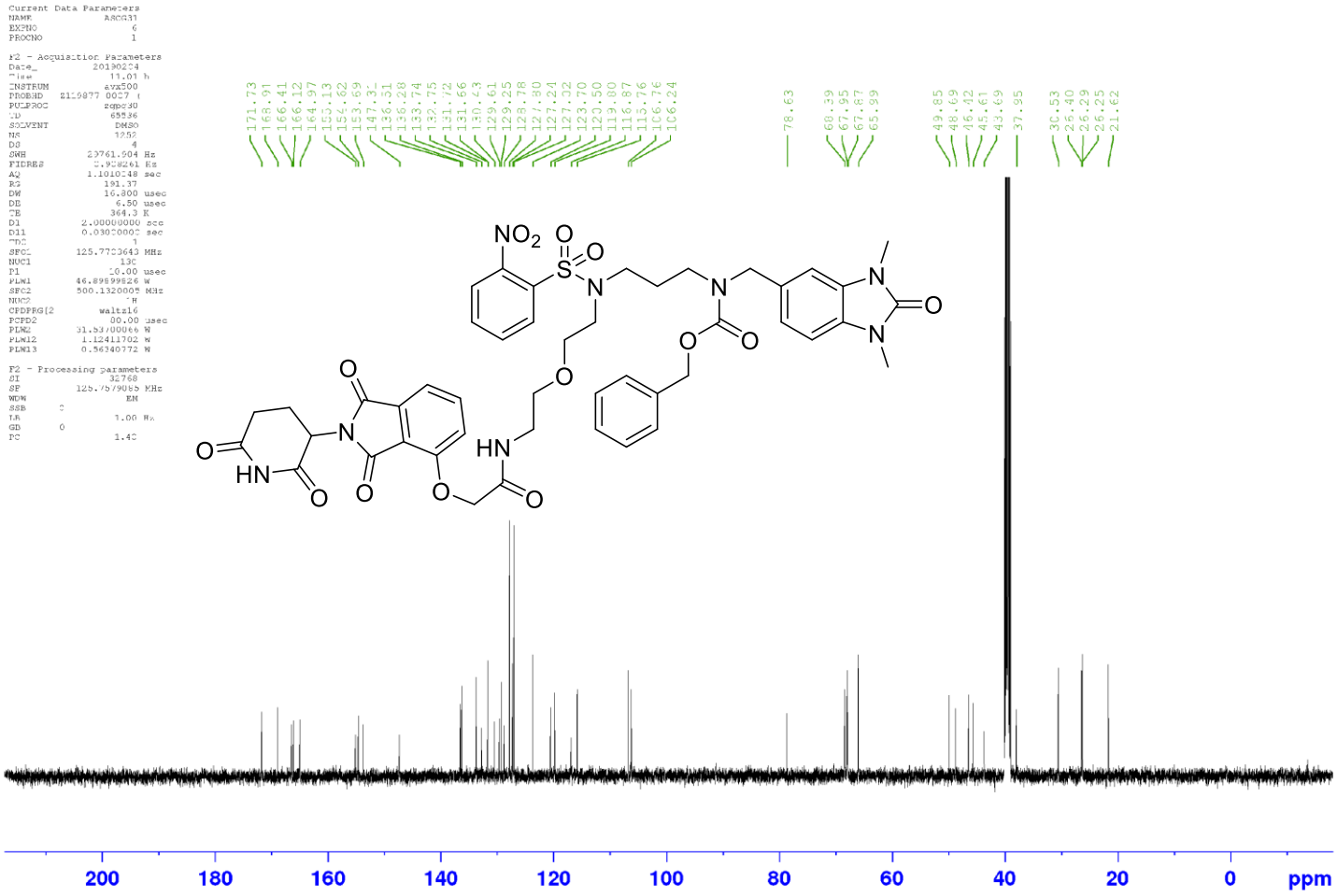
Benzyl (3-{[2-(2-aminoethoxy)ethyl](2-nitrobenzene-1-sulfonyl)amino}prop-1-yl)[(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]carbamate (333)



Benzyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{3-[{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]ethyl}(2-nitrobenzene-1-sulfonyl)amino]prop-1-yl}carbamate (334)



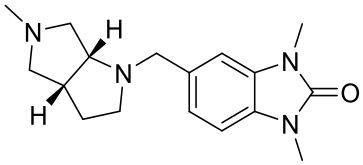
Benzyl [(1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-5-yl)methyl]{3-[{2-[2-(2-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}acetamido)ethoxy]ethyl}(2-nitrobenzene-1-sulfonyl)amino]prop-1-yl}carbamate (334)



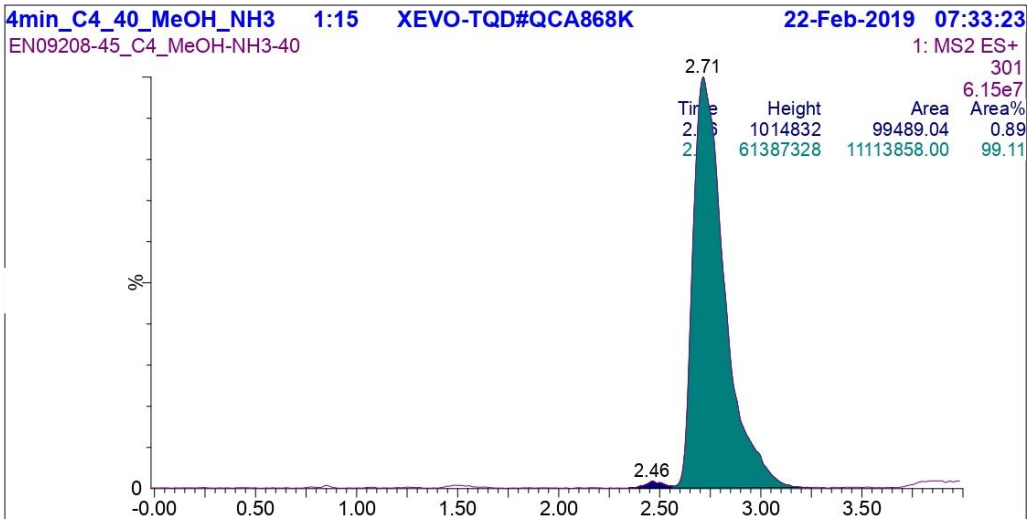
F. Chiral HPLC Traces

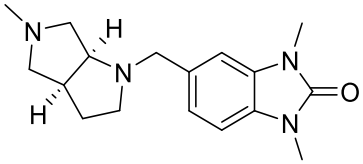
Chiral HPLC traces are presented for all final compounds (where relevant). For details about the HPLC method and column used, see Chapter 6.

Chiral HPLC traces of **(*S*)-161**, **(*R*)-161**, **(*R*)-167**, **(*S*)-167**, **(*S*)-179** and **(*R*)-179** were obtained in collaboration with AstraZeneca.

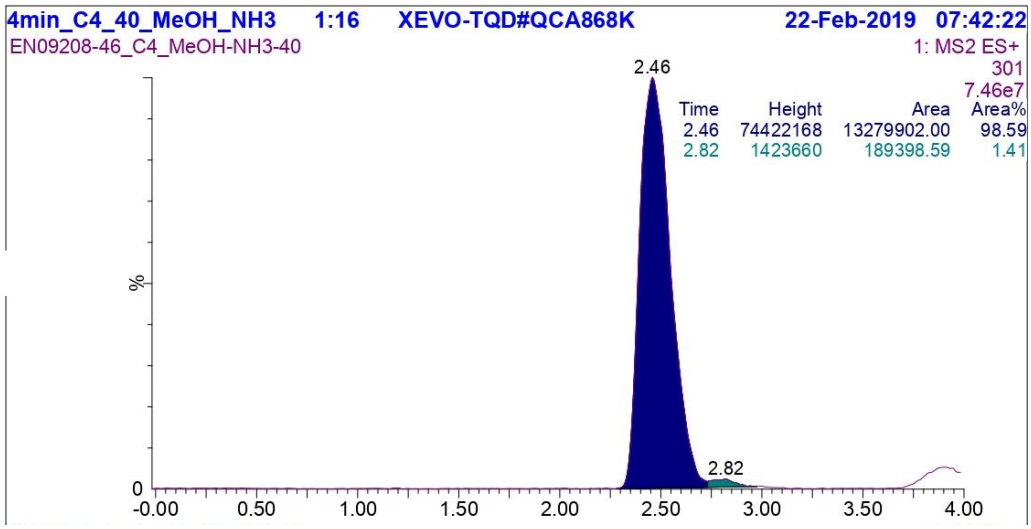


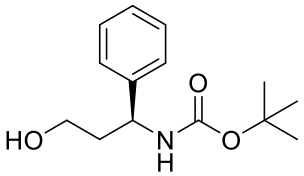
(*R*)-167


4min_C4_40_MeOH_NH3 1:15 XEVO-TQD#QCA868K 22-Feb-2019 07:33:23
EN09208-45_C4_MeOH-NH3-40
1: MS2 ES+
301
6.15e7
Time Height Area Area%
2.46 1014832 99489.04 0.89
2.71 61387328 11113858.00 99.11
2.71
2.46
%
0
-0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50

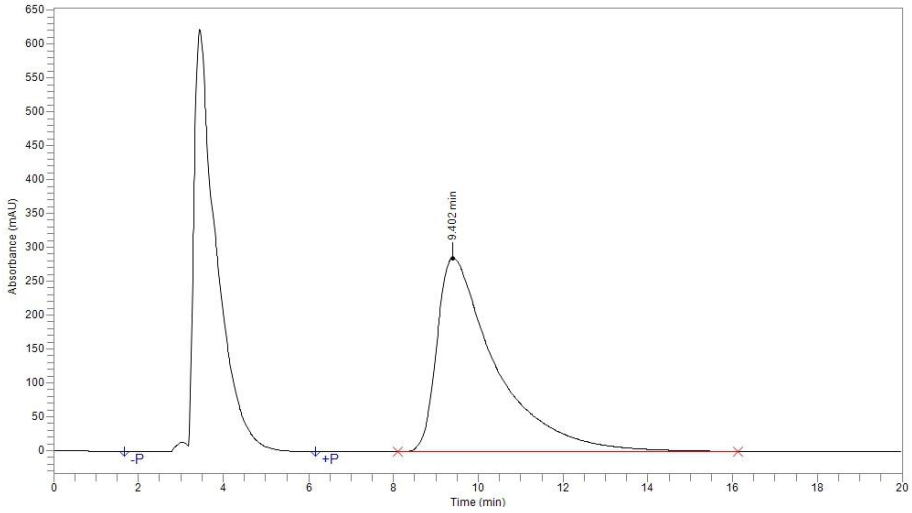


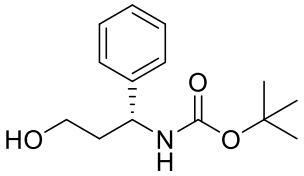
(*S*)-167


4min_C4_40_MeOH_NH3 1:16 XEVO-TQD#QCA868K 22-Feb-2019 07:42:22
EN09208-46_C4_MeOH-NH3-40
1: MS2 ES+
301
7.46e7
Time Height Area Area%
2.46 74422168 13279902.00 98.59
2.82 1423660 189398.59 1.41
2.46
2.82
%
0
-0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00

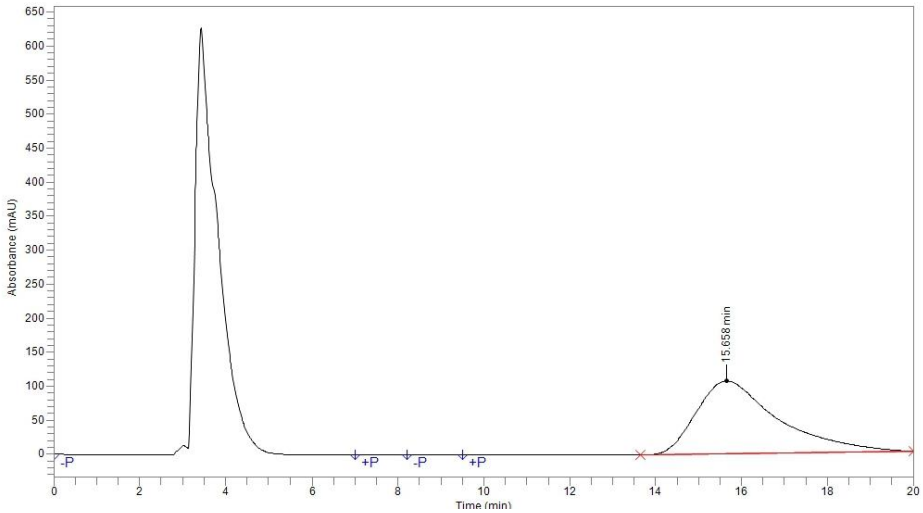

HO
N
H
O
O

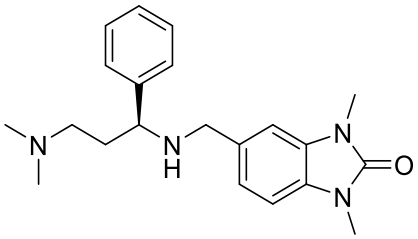
(*S*)-194


Absorbance (mAU)
Time (min)
9.402 min
-P
+P

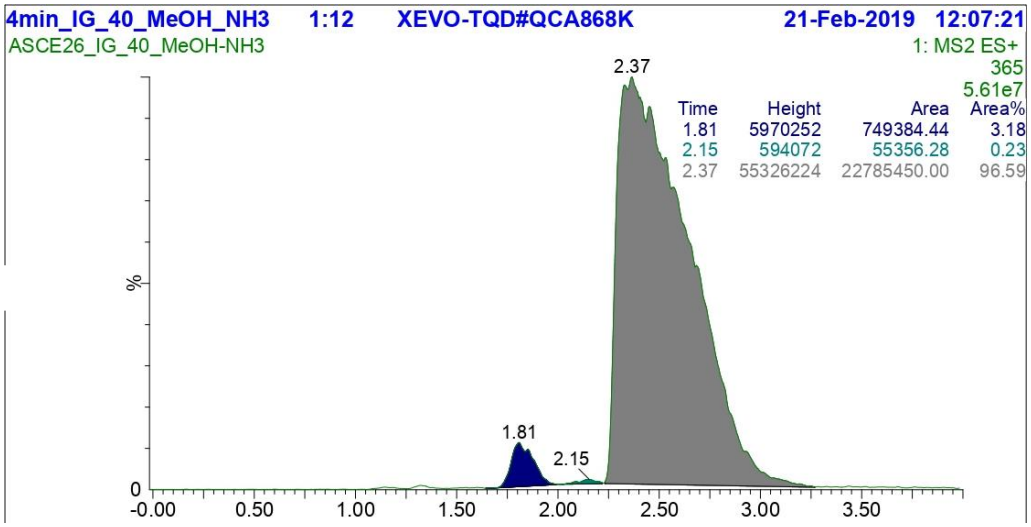

HO
N
H
O
O

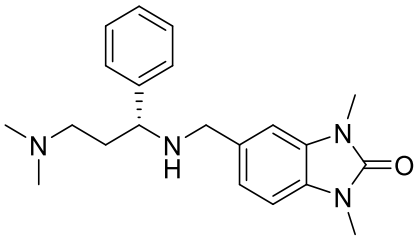
(*R*)-194


Absorbance (mAU)
Time (min)
15.658 min
-P
+P
-P
+P

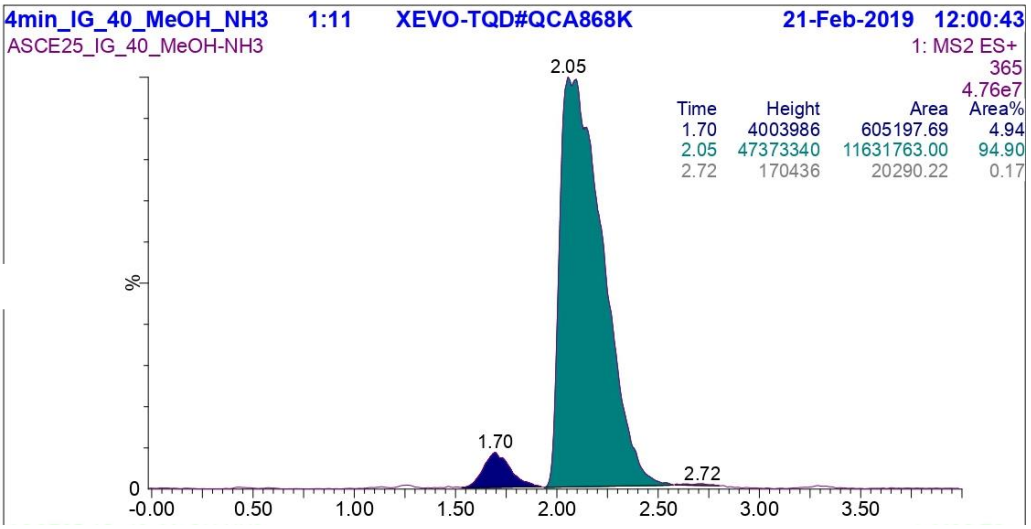


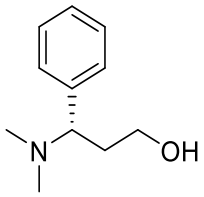
(*S*)-161


4min_IG_40_MeOH_NH3 1:12 XEVO-TQD#QCA868K 21-Feb-2019 12:07:21
ASCE26_IG_40_MeOH-NH3
1: MS2 ES+
365
5.61e7
Time Height Area Area%
1.81 5970252 749384.44 3.18
2.15 594072 55356.28 0.23
2.37 55326224 22785450.00 96.59
2.37
1.81
2.15
%
0
-0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50

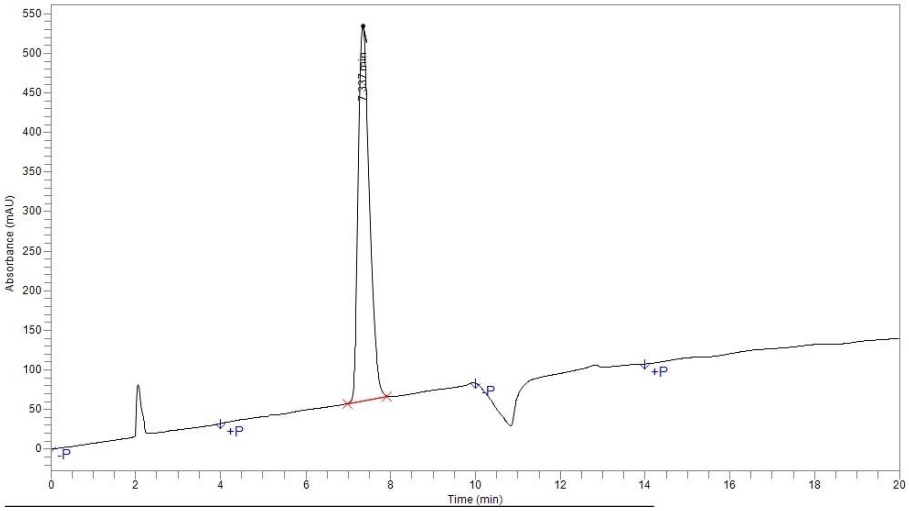


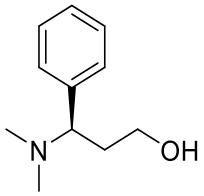
(*R*)-161


4min_IG_40_MeOH_NH3 1:11 XEVO-TQD#QCA868K 21-Feb-2019 12:00:43
ASCE25_IG_40_MeOH-NH3
1: MS2 ES+
365
4.76e7
Time Height Area Area%
1.70 4003986 605197.69 4.94
2.05 47373340 11631763.00 94.90
2.72 170436 20290.22 0.17
2.05
1.70
2.72
%
0
-0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50

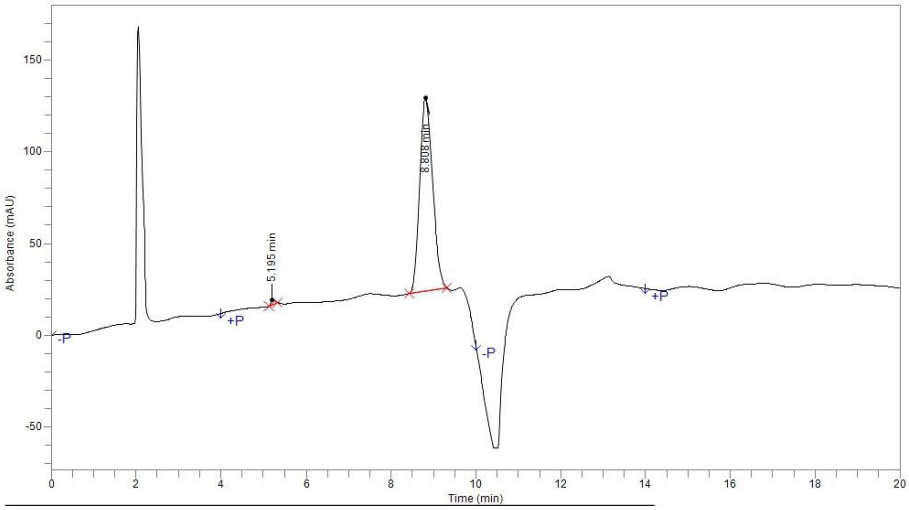

N
OH

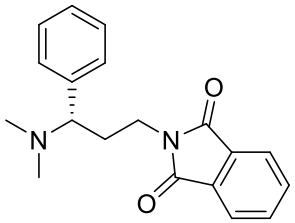
(*S*)-198


Absorbance (mAU)
Time (min)
7.337 min
-P
+P
+P
-P

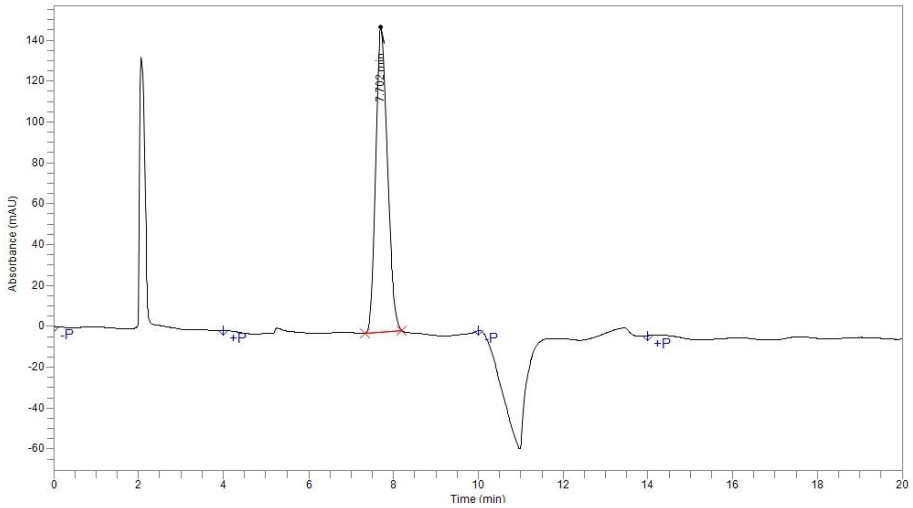

N
OH

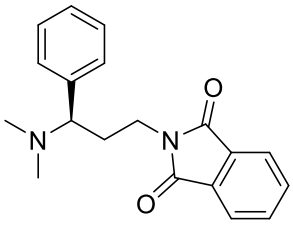
(*R*)-198


Absorbance (mAU)
Time (min)
5.195 min
8.808 min
-P
+P
+P
-P

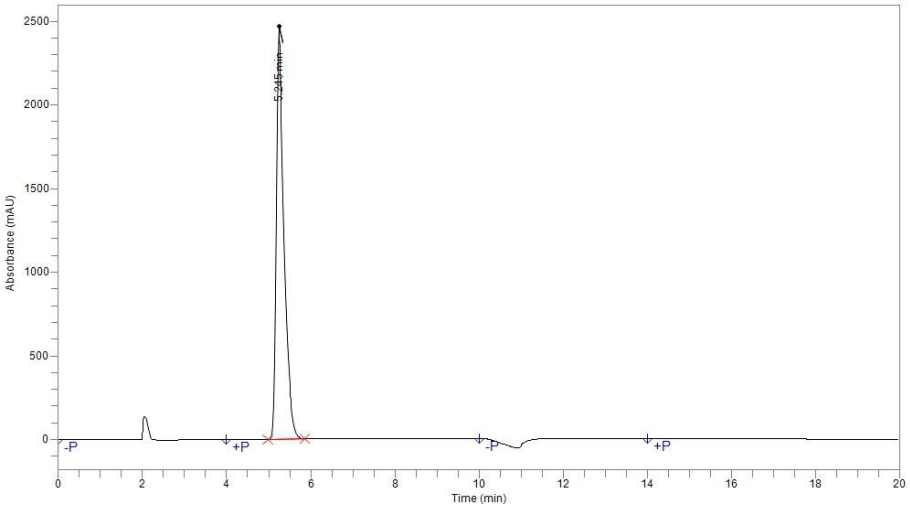


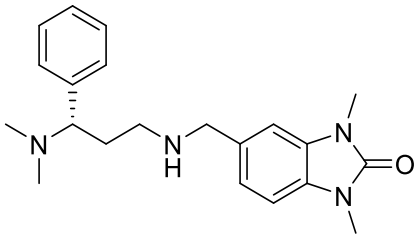
(*S*)-199


7.702 min
Absorbance (mAU)
Time (min)

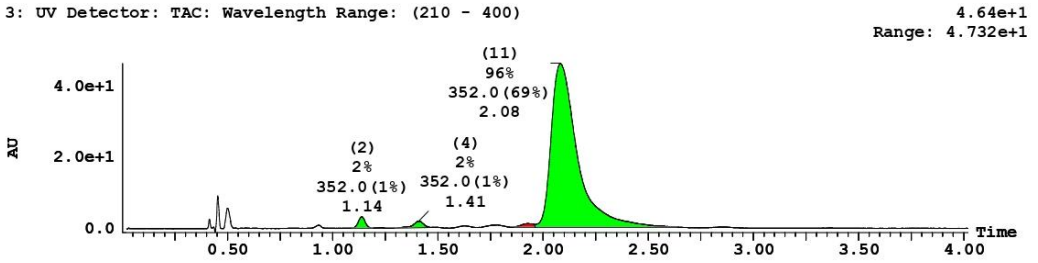


(*R*)-199


5.345 min
Absorbance (mAU)
Time (min)



(*S*)-179


3: UV Detector: TAC: Wavelength Range: (210 - 400)
4.64e+1
Range: 4.732e+1
AU
4.0e+1
2.0e+1
0.0
(11)
96%
352.0(69%)
2.08
(2)
2%
352.0(1%)
1.14
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Time
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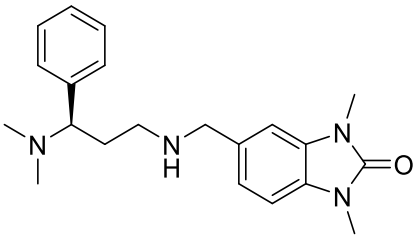
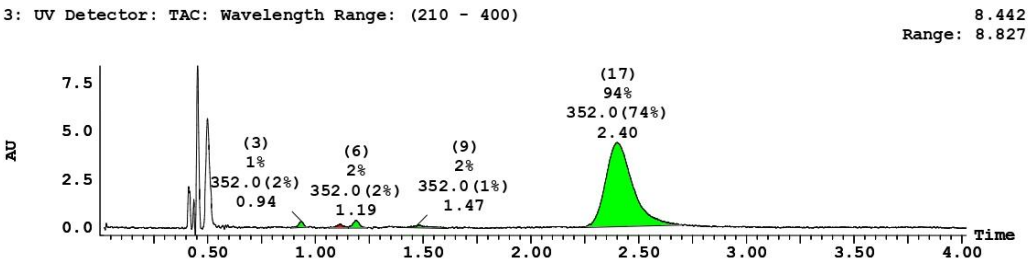


(*R*)-179


3: UV Detector: TAC: Wavelength Range: (210 - 400)
8.442
Range: 8.827
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7.5
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(3)
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(9)
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352.0(1%)
1.47
(17)
94%
352.0(74%)
2.40
Time
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