Development of Ligands to Target Bromodomain-Histone Interactions



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Abstract

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Title: Development of Ligands to Target Bromodomain-Histone Interactions

Histone acetylation is an epigenetic post-translational modification recognised by the bromodomain, a protein module that forms part of multi-component complexes affecting transcription. This interaction plays fundamental cellular roles, and shows association with particular diseases including inflammation and cancer. The biological roles of bromodomains and the progress of ligands developed so far has been summarised in introductory Chapter 1.

Work within the group has led to the development of a nanomolar ligand for BRD4, a BET bromodomain implicated in cancer and numerous diseases. Evaluation in an NCI-60 cancer cell screen indicated antiproliferative activity in a variety of cancer types. However, metabolic predictions indicate that this compound is unoptimised for use *in vivo*. Chapter 2 describes synthesis of a collection of analogues to improve the physical and pharmacokinetic properties of this series of compounds. This work identified compounds with equivalent affinity but greater predicted metabolic stability, as well as more potent derivatives. This research will direct the design of potent and metabolically stable derivatives that can be used in animal models.

Chapter 3 describes work carried out towards the development of small molecules to target bromodomains for which there are no known ligands, using the FALZ bromodomain as an initial target. A fragment-based approach has identified a number of compounds that bind to different non-BET bromodomains. These fragments will be a useful starting point for the development of more potent and selective non-BET bromodomain ligands.

As well as acetylated lysines, a number of other acylation post-translational modifications occur on lysine residues. Chapter 4 describes work carried out to investigate the interaction of other acylated lysine residues with bromodomains. This work highlighted that other acylated lysines can interact with bromodomains, and selectivity for particular bromodomains can also be achieved. These modified lysines could be incorporated into cognate peptides to improve *in vitro* peptide displacement assays, aiding the development of small molecular bromodomain probes.

The Parts of My Thesis Carried Out By Others

- Thermal shift experiments and an AlphaScreen™ assay for analogues of OXFBD02 against BRD4(1) in Chapter 2 were carried out by O. Fedorov and O. Monteiro at the Structural Genomics Consortium

- An AlphaScreen™ assay for analogues of OXFBD02 against BRD4(1) in Chapter 2 was carried out by A. Sekirnik

- 3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(2-fluorophenyl)(hydroxyl)methyl]phenol and 3-(3,5-dimethyl-1,2-oxazol-4-yl)-5-[(4-fluorophenyl)(hydroxyl)methyl]phenol was synthesised by A. Hicks

- 3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(pyridine-2-yl)methyl]phenol was synthesised by D. Hewings

- Synthesis of [3-(3,5-dimethyl-1,2-oxazol-4-yl)-5-hydroxyphenyl](morpholin-4-yl)methanone and [3-(3,5-dimethyl-1,2-oxazol-4-yl)-5-hydroxyphenyl](piperidin-1-yl)methanone was carried out by P. Guzanov

- Docking of fragments into the FALZ bromodomain binding-pocket was carried out in Molsoft ICM-Pro by the Structural Genomics Consortium

- AlphaScreen™ assays of fragments against a range of bromodomains in Chapter 3 were carried out by O. Fedorov and O. Monteiro at the Structural Genomics Consortium

- STD NMR spectra for Chapter 3 were obtained by B. Odell and T. Claridge

- AlphaScreen™ assays of modified amino acids against a range of bromodomains in Chapter 4 were carried out by O. Fedorov and O. Monteiro at the Structural Genomics Consortium

Author's Declaration

The work presented in this thesis was conducted at the Chemistry Research Laboratory at the University of Oxford under the supervision of Professor Stuart Conway. All the work is my own, except where otherwise stated, and has not been submitted for any other degree at this or any other university.

Laura Jennings

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Abbreviations

2-TY	2 × Typtone-Yeast
#Ar	number of aromatic rings
AlphaScreen	amplified luminescent proximity homogeneous assay
AMU	atomic mass units
ApoA-1	apolipoprotein A-1
APS	ammonium persulfate
ATAD2	AAA domain-containing protein 2
Aβ40	amyloid beta 40
BAZ	bromodomain adjacent to zinc finger protein
BAZ2A/B	bromodomain adjacent to zinc finger protein 2A/B
BCP	bromodomain-containing protein
BET	bromodomain and extra terminal domain
Boc	*tert*-butoxycarbonyl
BPTF	bromodomain and PHD finger transcription factor
br	broad
br s	broad singlet
BRD2/3/4/9	bromodomain-containing protein 2/3/4/9
BRDT	testis-specific bromodomain-containing protein
BRPF	bromodomain and PHD finger
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
Cbz	carboxybenzyl
CDK9	cyclin-dependent kinase
CECR2	cat eye syndrome critical region 2
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
chrom	chromatographic

CREB	cAMP response element-binding protein
CREBBP	CREB binding protein
CV	column volumes
CYP	cytochrome P450
d	doublet
D	distribution coefficient
dd	doublet of doublets
ddd	doublet of doublet of doublets
DIPEA	diisopropylethylamine
DMAc	*N*,*N*-dimethylacetamide
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DSF	differential scanning fluorimetry
EDC	*N*-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide
EI/FI	electron impact/field ionisation
eq	equivalents
ES	electrospray ionisation
FAC1	fetal Alz-50-reactive clone 1
FALZ	fetal Alzheimer antigen
FMOC	9-fluorenylmethyloxycarbonyl
FRAP	fluorescence-recovery after photobleaching
FRET	Förster or fluorescence resonance energy transfer
GI_{50}	half maximal growth inhibitory concentration
GSK	GlaxoSmithKline
HAC	heavy atom count
HAT	histone acetyltransferase

HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxid hexafluorophosphate
HBTU	*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HDAC	histone deacetylase
HDL-C	high-density lipoprotein cholesterol
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
HIV	human immunodeficiency virus
HOBt	1-hydroxybenzotriazole
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
IB	ligand signal intensity in the absence of protein
IC_{50}	half maximal inhibitory concentration
IL-6	interleukin 6
IP	ligand signal intensity in the presence of protein
IPTG	isopropyl β-D-1-thiogalactopyranoside
ISWI	imitation switch
ITC	isothermal titration calorimetry
J	coupling constant
JAK	Janus kinase
KAc	acetylated lysine
K_d	binding constant
LCMS	liquid chromatography mass spectrometry
LE	ligand efficiency
LLE	lipophilic ligand efficiency
$LogD_{pH7.4}$	effective lipophilicity at physiological pH
LPS	lipopolysaccharide
LRMS	low-resolution mass spectrometry

m	multiplet
m	medium
m/z	mass to charge ratio
min	minute(s)
MORF	MOZ-related factor
MOZ	monocytic leukaemia zinc finger protein
mp	melting point
mw	microwave
NBS	*N*-bromosuccinimide
n.d.	not determined
NHS	*N*-hydroxysuccinimide
NMC	NUT midline carcinoma
NURF	nucleosome-remodelling factor
NUT	nuclear protein in testis
P	intrinsic lipophilicity
P-TEFb	positive transcription elongation factor
PAGE	polyacrylamide gel electrophoresis
PB1(5)	fifth bromodomain of polybromo-1
PCAF	p300-CREBBP-associated factor
PFI	property forecast index
PHD	plant homeodomain
PI3K	phosphoinositide 3-kinase
Pol II	RNA polymerase II
PPA	polyphosphoric acid
PPI	protein-protein interaction
ppm	parts per million
PTM	post-translational modification
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate

q	quartet
RelA	transcription factor p65
R_f	retention factor
rt	room temperature
RT	retention time
RuPhos	2-dicyclohexylphosphino-2',6'-diisopropoxybiphenyl
s	singlet
s	strong
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SGC	Structural Genomics Consortium
siRNA	small interfering RNA
SMARCA	SWI/SNF-related, matrix associated, actin-dependent regulator of chromatin
SPR	surface plasmon resonance
STD	saturation transfer difference
SWI/SNF	switch/sucrose non-fermentable
t	triplet
TAF1	TBP-associated factor 1
TAF1L	TAF1-like
TBAF	tetra-*N*-butylammonium fluoride
TBP	TATA-box binding protein
TEMED	*N*,*N*,*N'*,*N'*-tetramethylethylenediamine
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
THP	tetrahydropyran
TIF1α	transcription intermediary factor 1α
TIPS	triisopropylsilyl

TLC	analytical thin layer chromatography
T_m	melting temperature
TMS	trimethylsilane
TPSA	topological polar surface area
TRIS	2-amino-2-hydroxymethyl-propane-1,3-diol
tt	triplet of triplets
UPLC	ultra-performance liquid chromatography
v:v	volume-to-volume ratio
v/v	volume-to-volume
w	weak
w/v	weight-to-volume
WDR9(2)	second bromodomain of WD repeat-containing protein 9
ΔI	relative change in the ligand signal intensity in the presence of protein
ΔT_m	change in melting temperature
v_{max}	absorption maxima

Chapter 1: Introduction

1.1 Bromodomains recognise acetylated lysines

1.1.1 Epigenetics and the histone code

Epigenetics, the rapidly expanding field in biology, has had many different definitions over the past 40 years: the one that is most generally accepted, proposed by Wu & Morris in 2001, describes epigenetics as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence".[1,2] These changes in gene expression can be part of healthy processes, but also link epigenetics with various different diseases, including cancer, inflammation, diabetes, neurodegenerative diseases and psychological diseases.[3] Therefore, epigenetic targets have great therapeutic potential for numerous diseases.

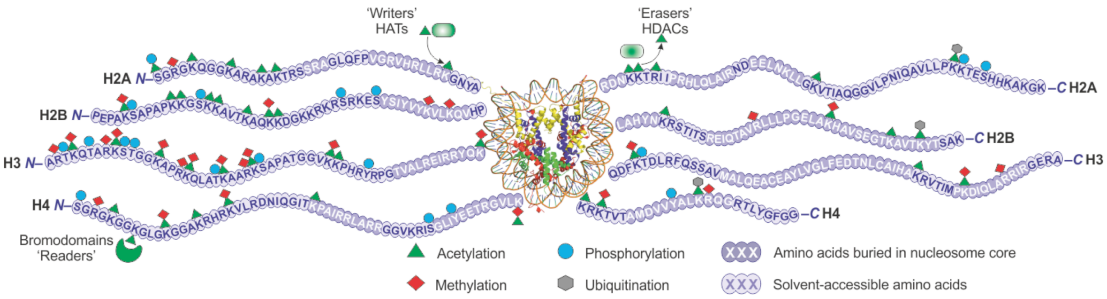


Figure 1.1. Post-translational modifications (PTMs), including acetylation, methylation, phosphorylation and ubiquitination, can occur on histone tails. Specific combinations of these marks can lead to context-dependent affects on transcription. This idea is known as the histone code. The marks are "written" and "erased" by enzymes known as histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively, and are "interpreted" by reader domains including the bromodomain. Reproduced with permission from Jennings, L. E.; Measures, A. M.; Wilson, B. G.; Conway, S. J. Future Medicinal Chemistry 2014, 6, 179. Copyright 2014 Future Science Ltd.

The epigenetic changes in gene expression are most often brought about by chemical modification to DNA and RNA, such as methylation, and to histones, which are the small globular proteins around which DNA is wound, forming nucleosomes, the building blocks of chromatin.[4,5] These post-translational modifications (PTMs) include acetylation of lysine, methylation of arginine, histidine, lysine and glutamic acid, and phosphorylation of aspartic acid, cysteine, histidine, serine, threonine and tyrosine.[6] Specific combinations of these marks and their cross talk is thought to lead to context-dependent effects on transcription, a concept known as the 'histone code' (Figure 1.1).

This code gives medicinal chemists the opportunity to target the cellular machinery that manipulates the PTMs linked with disease rather than the genes themselves. The histone code is dynamic, and can be "written" and "erased" by enzymes and "interpreted" by reader domains. ε-*N*-Acetylation of lysine residues within histone tails is one epigenetic mark that is associated with numerous diseases.[7] The acetyl functionality is appended to the terminal amino group of lysine residues by histone acetyltransferases (HATs) and is removed by histone deacetylases (HDACs).[8-10] Initially, it was regarded that lysine acetylation neutralises the largely positive histone tails, reducing the affinity for the negatively charged backbone of DNA. This change in charge converts densely packed heterochromatin to more open, transcriptionally active euchromatin, which allows access of polymerase enzymes. Upon further study it was realised that acetylated lysines can also form protein-protein interactions (PPIs); the mark is recognised by a non-catalytic unit known as the bromodomain.[11]

1.1.2 Bromodomain structure and interactions

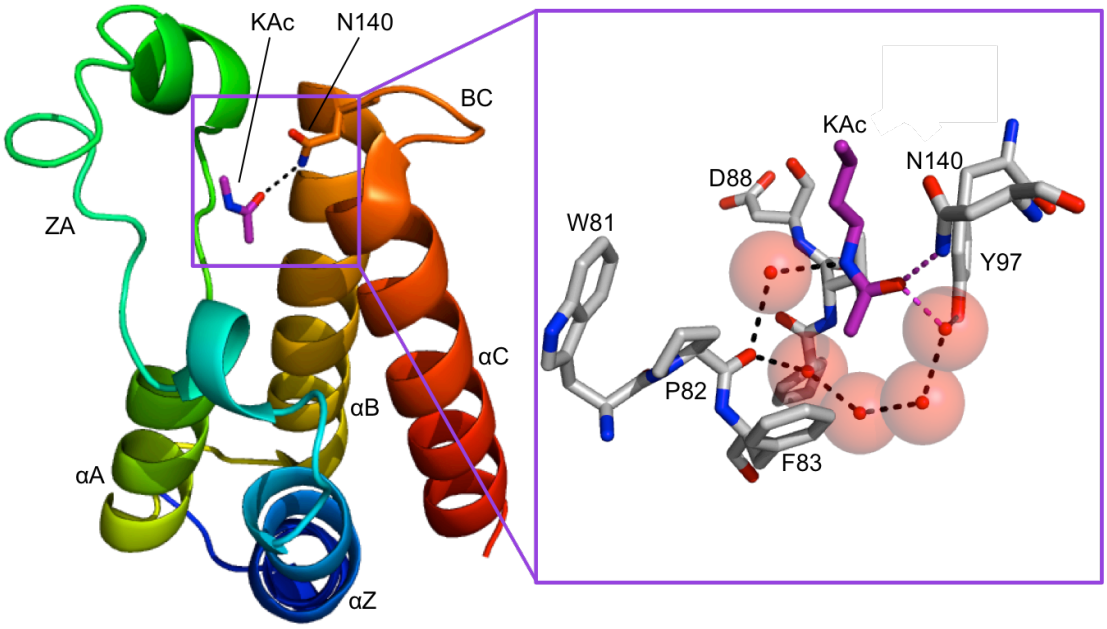


Figure 1.2. The first bromodomain of BRD4 (BRD4(1)), in complex with H4K12AcK16Ac (PDB 3UVX).[12] Bromodomains comprise four alpha-helices (αZ, αA, αB and αC) and two flexible loop regions (ZA and BC) that bundle to form a largely hydrophobic recognition pocket. There is a highly conserved asparagine residue (N140 in BRD4(1)) that hydrogen bonds to the carbonyl moiety of acetylated lysines, and is important to their recognition. Despite the largely hydrophobic nature of the bromodomain pocket, there is a well-structured hydrogen-bonding network of water molecules that allows a water-mediated interaction with a conserved tyrosine residue (Y97 in BRD4(1)).

The bromodomain is a small and highly conserved domain that forms part of numerous protein complexes, including those that modify chromatin. Interaction of the bromodomain with acetylated lysine (KAc) residues mediates PPIs often with components of transcriptional machinery. The bromodomain comprises four alpha-helices and two flexible loop regions that bundle to form a largely hydrophobic pocket with a highly conserved asparagine residue at the top of helix B (αB) that can hydrogen bond to the carbonyl moiety of a KAc residue, fundamental to its recognition (Figure 1.2). Despite the pocket being largely hydrophobic, there are four

conserved water molecules within the base of the recognition pocket, one of which mediates an interaction between KAc and an almost strictly conserved tyrosine residue.

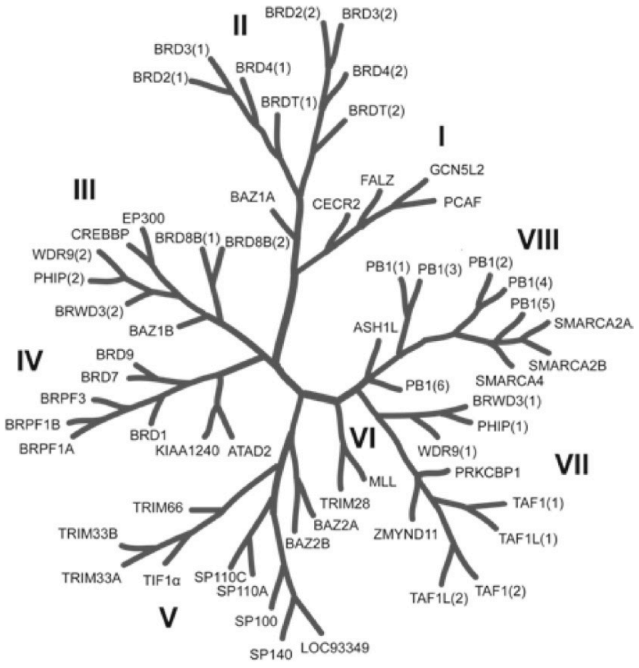


Figure 1.3. A phylogenetic tree of the human bromodomain family, constructed using sequence alignment. Different families are denoted by roman numerals I-VIII. Reproduced with permission from Hewings, D. S.; Rooney, T. P. C.; Jennings, L. E.; Hay, D. A.; Schofield, C. J.; Brennan, P. E; Conway, S. J. *Journal of Medicinal Chemistry* **2012**, *55*, 9393. Copyright 2012 American Chemical Society.

The human genome encodes for 61 unique bromodomains, within 46 different bromodomain-containing proteins (BCPs). Some of these proteins are known to function as transcriptional co-regulators and chromatin-modifying enzymes, including HATs, helicases, methyltransferases and nuclear scaffolding proteins.[13] The bromodomains have been divided into eight sub-families based on sequence homology and structural similarity (Figure 1.3).[14] Work within the Conway group, and elsewhere, has focused heavily on the bromodomain and extra terminal domain (BET) family of bromodomains, denoted family II in the sequence alignment-based

phylogenetic tree. This family comprises bromodomain-containing proteins 2, 3 and 4 (BRD2-4) as well as the testis-specific bromodomain-containing protein (BRDT). These all have two *N*-terminal bromodomains, with stronger resemblance within the set of first bromodomains (BRD2(1), BRD3(1), BRD4(1) and BRDT(1)) and the set of second bromodomains (BRD2(2), BRD3(2), BRD4(2) and BRDT(2)), than between the first and second bromodomains of a particular BET BCP.

In an analysis conducted by Filippakopoulos *et al*., members of the BET family were found to bind to hyperacetylated histone tails, but BRD4(2) bound non-specifically to combinations of di- and tri-acetylated H3 and H4 peptides, while BRD4(1) proved to be more selective for H4, specifically recognising multiple acetylations on this histone.[12] Filippakopoulos *et al*. determined crystal structures of BRD4(1) with diacetylated peptides H4K5$_{Ac}$K8$_{Ac}$, H4K12$_{Ac}$K16$_{Ac}$ and H4K16$_{Ac}$K20$_{Ac}$ to investigate this selectivity. The different pairs of acetylated lysines all bound in the same manner, with *N*-terminal KAc residues forming the hydrogen bond with the highly conserved asparagine. Interestingly, glycine residues in the *N*-terminal region of H4 allow H4$_{1-11}$K5$_{Ac}$K8$_{Ac}$ and H4$_{11-21}$K12$_{Ac}$K16$_{Ac}$ afford flexible conformations, whereas the large side-groups of H4$_{15-25}$K16$_{Ac}$K20$_{Ac}$ fit perfectly in the grooves formed by the ZA and BC loops. Bromodomains bind weakly to KAc residues, but affinity improves upon binding to additional acetylation marks. For example, BRDT requires diacetylation for high affinity binding, while tetra-acetylated H4 binds to BRD2(1) and BRD4(1) with single digit micromolar affinity, much greater than to single acetylation marks.[15] Furthermore, the first bromodomain of BRD4 also binds the tetra-acetylated H4 with greater affinity than the second bromodomain, again suggesting that the first bromodomain selectively recognises H4. At the time of writing this, no published X-ray crystal structures of this interaction exist in the RCSB Protein Data Bank, but Filippakopoulos *et al*. established that the high affinity for tetra-acetylated H4 is the result of an avidity effect. Crystal structures of diacetylated

peptides showed that BRD4(1) can host both acetylation marks, in a bidentate fashion, with the *N*-terminal KAc forming the traditional hydrogen bond with N140.

1.1.3 The biological roles of BET bromodomains

The BET family members are transcriptional co-activators, recruiting transcription factors to regulate transcription. For example, BRD4 is a general regulator that couples chromatin acetylation with RNA polymerase II (Pol II) elongation. Regulatory factors can hold Pol II in a reversibly paused state. Histone acetylation prompts BRD4 to recruit the positive transcription elongation factor (P-TEFb) kinase to phosphorylate these regulatory factors, releasing Pol II to stimulate transcriptional elongation (Figure 1.4).[16-21] As such, aberrant acetylation, and subsequent binding of BRD4 to chromatin, results in dysregulation of transcription, associating the BET bromodomains with cancer and numerous other diseases.

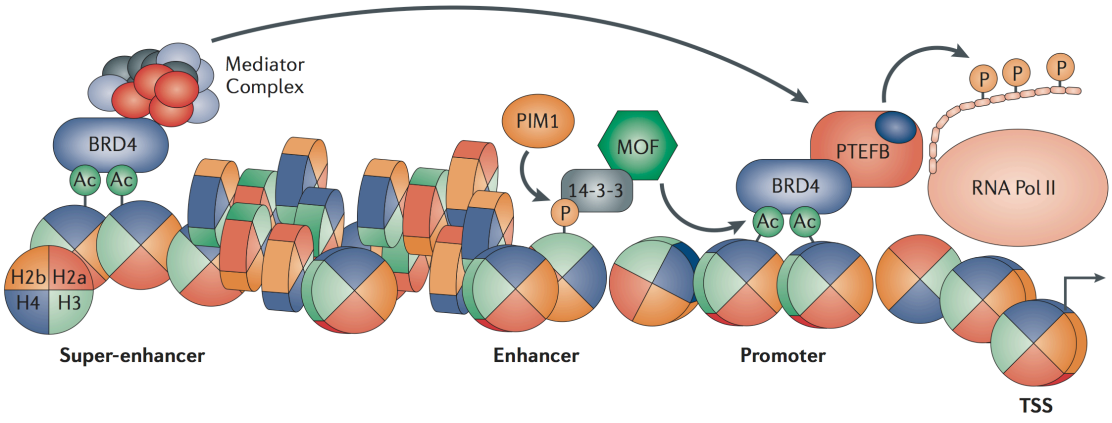


Figure 1.4. Transcriptional regulation by BRD4: Acetylation of super-enhancers leads to recruitment of kinase P-TEFb, releasing Pol II into a transcriptionally active state. Reproduced with permission from Filippakopoulos, P.; Knapp, S. *Nature Reviews Drug Discovery* **2014**, *13*, 337. Copyright 2014 Nature Publishing Group.

As well as recruiting P-TEFb directly, a high density of BRD4 in enhancer regions, comprising multiple binding sites of transcription factors, links bromodomains to the binding of other transcription factors to chromatin, that in turn employ P-TEFb for transcriptional activation.[22] As such, disrupting the interaction of chromatin with transcription factors can be achieved with BET bromodomain ligands.[23]

In addition to binding acetylated chromatin, bromodomains can also bind to non-histone partners. For example, another transcription factor that BRD4 binds to is NF-κB. Binding of BRD4 to acetylated transcription factor p65 (RelA), a subunit of NF-κB, leads to P-TEFb-mediated phosphorylation of Pol II, resulting in the expression of pro-inflammatory genes.[24]

The remaining BET bromodomains are less prevalent in the literature, but still play important biological roles. BRDT is only expressed in the testes, playing an important part in spermatogenesis, and can potentially be targeted as a male contraceptive.[25-28] The biology of BRD3 is less well understood, but BRD2 is linked with metabolic regulation and lymphogenesis, and may also be linked to human immunodeficiency virus (HIV) latency.[29-31]

Due in large part to work of the Structural Genomics Consortium (SGC), there is now a good understanding of the structure of bromodomains, with a wide portfolio of resolved NMR and X-Ray crystal structures. Despite this, the role of many bromodomains is still undefined. However, for those that have been studied in detail, it is clear that they play fundamental cellular roles, and are associated with particular diseases including leukaemia, psychiatric diseases, obesity, inflammation and cancer.[32-34] Elucidation of the roles that bromodomains play in disease has become possible through the use of small molecular probes. Due to the well-defined structure of the bromodomains, some have proved to be ligandable, with a number of weak

inhibitors of the bromodomain-KAc interaction having emerged in the literature. A handful of ligands have even progressed to clinical trials.[35,36] A brief summary of the development of these probes will be discussed here.

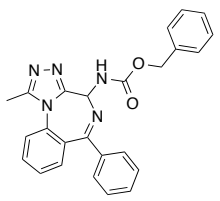
1.2 Inhibitors of BET bromodomain-KAc interactions

1.2.1 Chemical probes

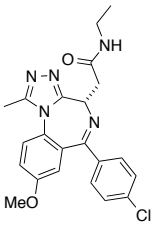
Chemical probes allow investigation of the effects of targeting a biological molecule of interest. According to Stephen Frye, a "high quality probe" will have (i) sufficient *in vitro* potency and selectivity data to unequivocally connect its *in vitro* profile to its cellular or *in vivo* profile; (ii) dose-dependent activity on a target-dependent action, as displayed by assays that mimic a physiologic function of the target of interest; (iii) a defined active species, as evidenced by sufficient chemical and physical property data; (iv) sufficient cellular activity data to reliably approach one or more hypotheses regarding the target's biological roles; and (v) availability to academia to facilitate biological exploration.[37] It is important to bear these principles in mind in the development of chemical probes.

1.2.2 BET bromodomain ligands containing diazepines

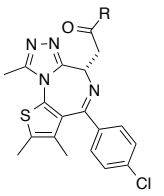
Attempts had been made in the past to target bromodomains with small molecules, but until recently only micromolar-binding ligands had been reported.[38,39] However, in 2010 two high affinity BET bromodomain ligands were published concurrently by Nicodeme *et al*. and Filippakopoulos *et al*.[40,41] These papers sparked an exponentially expanding interest in small molecule-targeting of bromodomains, which has led to the development of a number of bromodomain ligands, including



GSK: (1.1) (GW841819X)
BRD2(1) K_d = 46 nM *(ITC)*



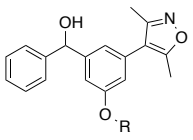
GSK: I-BET762 (1.2) (GSK525762A)
BRD4(1/2) IC_{50} = 36 nM *(FRET)*



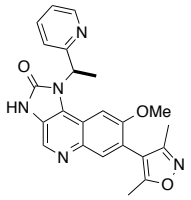
SGC / Broad: R = O^{t}Bu: (+)-JQ1 (1.3)
BRD4(1) IC_{50} = 77 nM *(AlphaScreen)*

Mount Sinai: R = OMe: MS417 (1.4)
BRD4(1) IC_{50} = 30 nM *(Fluorescence Anisotropy)*

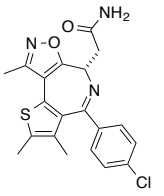
Constellation: R = NH_2: CPI-203 (1.5)
BRD4(1) IC_{50} = 37 nM *(AlphaScreen)*



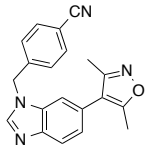
Oxford Chemistry / SGC:
R = H: OXFBD02 (1.6)
BRD4(1) IC_{50} = 382 nM *(AlphaScreen)*
R = Ac: OXFBD03 (1.7)
BRD4(1) IC_{50} = 371 nM *(AlphaScreen)*



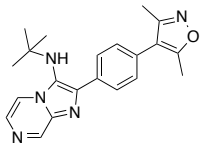
GSK: I-BET151 (1.8)
BRD4(1/2) IC_{50} = 794 nM *(Fluorescence Anisotropy)*
BRD4(1/2) K_d = 100 nM *(SPR)*



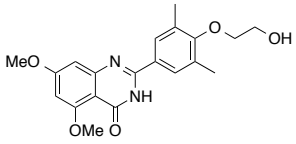
Constellation: (1.9)
BRD4(1) IC_{50} = 236 nM *(AlphaScreen)*



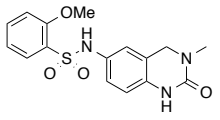
Oxford Chemistry / SGC: (1.10)
BRD4(1) IC_{50} = 180 nM *(AlphaScreen)*



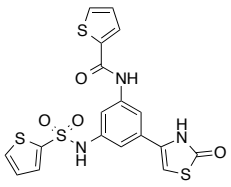
Dana-Farber Cancer Institute: UMB-32 (1.11)
BRD4(1) K_d = 550 nM *(ITC)*



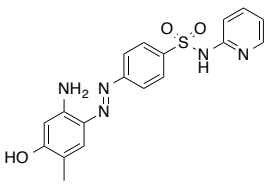
Resverlogix: RVX-208 (1.12)
BRD4(1) IC_{50} = 1800 nM *(FRET)*
BRD4(2) IC_{50} = 40 nM *(FRET)*



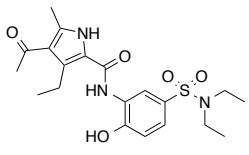
Pfizer: PFI-1 (1.13)
BRD4(1) IC_{50} = 220 nM *(AlphaScreen)*



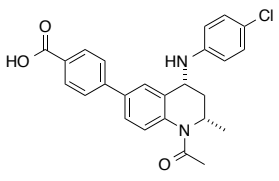
Chinese Academy of Sciences: (1.14)
BRD4(1) IC_{50} = 230 nM *(Fluorescence Anisotropy)*



Mount Sinai: MS436 (1.15)
BRD4(1) K_d < 85 nM *(Fluorescence Anisotropy)*
BRD4(2) K_d = 340 nM *(Fluorescence Anisotropy)*



Freiburg: XD14 (1.16)
BRD4(1) K_d = 160 nM *(Bromoscan)*
BRD4(1) K_d = 237 nM *(ITC)*



GSK: I-BET726 (1.17)
BRD4(1/2) IC_{50} = 22 nM *(FRET)*

Figure 1.5. Nanomolar BET bromodomain ligands, with their IC_{50} or K_d values and the quoted method used to obtain these values. [41-59]

those targeting the BET bromodomains as seen in Figure 1.5.

GlaxoSmithKline (GSK) aimed to increase levels of apolipoprotein A-1 (ApoA-1) in patients for treatment of atherosclerosis and for anti-inflammatory response.[42] They carried out a phenotypic screen and identified a range of benzodiazepines that enhanced ApoA-1 expression. Chemical optimisation led to the development of I-BET762 (**1.2**), a single enantiomer that has a nanomolar EC_{50} and suitable properties for use in *in vivo* experiments, while the opposite enantiomer showed no activity. Affinity chromatography with cell lysates led to the identification of BET bromodomains as the target of interest for the effect of I-BET762 (**1.2**) on ApoA-1 expression levels. Using isothermal titration calorimetry (ITC), considered to be the "gold standard" technique for measuring binding affinities and thermodynamic parameters, and one of the *in vitro* techniques discussed by Brand *et al*., displayed nanomolar affinity of a structurally-related analogue, GW841819X (**1.1**), with K_d = 46 nM and 53 nM for BRD2(1) and BRD2(2).[60] Surface plasmon resonance (SPR), also highlighted by Brand *et al*., gave dissociation constants of this analogue, with K_d = 220 nM and 54 nM for BRD2(1) and BRD2(2). Dose-dependent inhibition of binding of a tetra-acetylated histone peptide to the BET bromodomains by I-BET762 (**1.2**) was observed in a Förster or fluorescence resonance energy transfer (FRET) assay, also mentioned by Brand *et al*., with IC_{50} = 36 nM for the bromodomains of BRD4. X-Ray crystallography demonstrated that **1.1** directly bound in the KAc-recognition pocket of BRD2(1), with two nitrogen atoms of the triazole moiety mimicking the carbonyl functionality of KAc: one formed a water-mediated interaction with the conserved tyrosine residue, while the other accepted a hydrogen bond from the highly conserved asparagine residue. The latter interaction does not exist for I-BET762 (**1.2**), due to the amide functionality, but is replaced by a water-mediated interaction with asparagine N156 instead. siRNA knock-down of the BET bromodomains BRD2 and BRD3 did not affect levels of ApoA-1 expression, however

siRNA knock-down of BRD4 resulted in a significant increase in ApoA-1 expression levels, highlighting different biological roles of the BET family bromodomains.

The SGC learned from a Mitsubishi patent that thienodiazepines bind to BRD4.[41] Using the *apo*-structure of BRD4(1), the Bradner group were able to design a ligand (+)-JQ1 (**1.3**) that was predicted to be selective for the bromodomain over benzodiazepine receptors that bind to the class of structurally-related therapeutics. (+)-JQ1 (**1.3**) showed highly selective binding to the BET bromodomains over a range of other phylogenetically diverse bromodomains, based on differential scanning fluorimetry (DSF), another technique mentioned in the review by Brand *et al*. Its enantiomer, (–)-JQ1, did not bind to any bromodomains tested. Importantly, this selectivity was confirmed with ITC data that gave K_d = 49 nM for BRD4(1) and 90 nM for BRD4(2), but displayed no binding of (+)-JQ1 (**1.3**) to the bromodomain of cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) binding protein (CREBBP), a HAT-containing protein, and the second bromodomain of WD repeat-containing protein 9 (WDR9(2)), a chromatin remodelling protein, two bromodomains that showed little to no stabilisation by DSF. The ability of (+)-JQ1 (**1.3**) to out-compete a tetra-acetylated histone from BRD4(1) was assessed using an amplified luminescence proximity homogeneous assay (AlphaScreen™). This assay gave IC_{50} = 77 nM for BRD4(1) and 33 nM for BRD4(2), with selectivity over CREBBP displayed through an IC_{50} estimated to be > 10 μM. The enantiomer, (–)-JQ1, was confirmed as less active, with an IC_{50} also estimated to be > 10 μM. With these selectivity and affinity data in hand, work was carried out to obtain an X-Ray crystal structure of (+)-JQ1 (**1.3**) in the BRD4 bromodomains. This showed that (+)-JQ1 (**1.3**) bound deep within the bromodomains' KAc recognition pockets, and mimicked KAc by forming a hydrogen bond with the highly conserved N140 in BRD4(1) and N429 in BRD4(2). In addition, the ligand was stabilised by hydrophobic interactions with residues in the ZA and BC loops. With encouraging *in vitro* data,

work was carried out to establish whether this affinity could be translated into cells. Fluorescence-recovery after photobleaching (FRAP) highlighted that (+)-JQ1 (**1.3**) could competitively bind to BRD4 in cell-based assays, including cells transfected with the BRD4-nuclear protein in testis (NUT) oncoprotein responsible for NUT midline carcinoma (NMC). The results of this competition were then assessed by immunohistochemistry. This showed that (+)-JQ1 (**1.3**) induced dose- and time-dependent differentiation then apoptosis of NMC cells, without causing significant cytotoxicity of healthy cells. (+)-JQ1 (**1.3**) treatment of mice with NMC xenografts showed slower tumour growth relative to vehicle treatment.

A structurally-related analogue of (+)-JQ1 (**1.3**), MS417 (**1.4**), was developed by Zhang *et al*. to block the interaction of BRD4 with acetylated NF-κB.[44] This compound contains a methyl carbamate instead of a *tert*-butyl carbamate, and due to its interaction with BRD4, improved inflammation and kidney injury in HIV-1 transgenic mice. MS417 (**1.4**) displayed a 30 nM IC_{50} value against BRD4(1) using fluorescence anisotropy. An ITC K_d value of 36.1 nM verified this strong affinity for BRD4(1). Furthermore, this compound displayed at least 100-fold selectivity over a range of other bromodomains by fluorescence anisotropy. However, orthogonal assays to confirm this potency and selectivity are important before this compound can be used as a BET bromodomain probe.

Another (+)-JQ1 (**1.3**) analogue, CPI-203 (**1.5**), containing a primary amide instead of the *tert*-butyl carbamate, was developed by Constellation.[45] CPI-203 (**1.5**) and (+)-JQ1 (**1.3**) displayed similar binding to BRD4(1) with IC_{50} = 37 nM and 22 nM respectively, as measured by AlphaScreen™. Orthogonal assays are required to validate this potency for BRD4(1). However, CPI-203 (**1.5**) also showed inhibition of cellular MYC and lipopolysaccharide (LPS)-induced interleukin 6 (IL-6) expression with IC_{50} = 99 nM and 30 nM respectively. Furthermore, CPI-203 (**1.5**) enhanced the

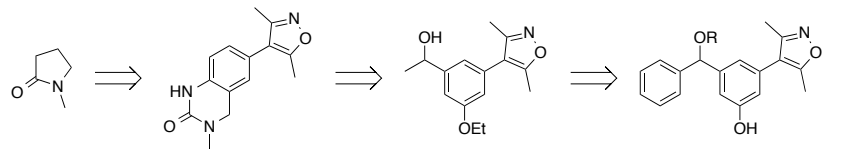
antitumour activity of rapamycin for the treatment of pancreatic neuroendocrine tumour cells.[61]

1.2.3 BET bromodomain ligands containing 3,5-dimethylisoxazoles

Analogous to the triazole moiety mimicking KAc through hydrogen-bonding interactions with highly conserved asparagine and tyrosine residues, the 3,5-dimethylisoxazole moiety was also discovered to be an excellent KAc mimic. Several groups have developed 3,5-dimethylisoxazole compounds that bind to the BET bromodomains and CREBBP.[46,47,49,62-66]

Based on finding that *N*-methyl pyrrolidinone (**1.18**) binds ligand-efficiently with the BET bromodomains, a 3,5-dimethylisoxazole-containing compound **1.19** was discovered to bind to BET bromodomains with single digit micromolar affinity (Figure 1.6).[62,67] Crystallography established that the 3,5-dimethylisoxazole component was the KAc-mimicking moiety of this molecule. Optimisation afforded a BET bromodomain ligand **1.20**, that was 6-fold selective over CREBBP, but replacement of the methyl group with a phenyl ring allowed **1.21** to bind to the BET bromodomains with nanomolar affinity, by interacting with a lipophilic portion of the recognition pocket known as the WPF shelf.[46] Replacing the ethoxy group with the free phenol in OXFBD02 (**1.6**) allowed hydrogen-bonding interactions with a water molecule within the BET bromodomain pocket, and improved the affinity for BET bromodomains, with IC_{50} = 384 nM, however, this derivative was only 2-fold selective over CREBBP. Addition of an acetate group to the phenol gave OXFBD03 (**1.7**) with almost identical affinity for BRD4(1), but improved selectivity over CREBBP to 7-fold. The inhibition of BET bromodomain-KAc interactions has shown antiproliferative activity in the MV4;11 acute myeloid leukaemia cell line.[47,68,69] The *in vitro* nanomolar affinity for the

BET bromodomains translated into these cells, with sub-micromolar cytotoxicity IC_{50} values obtained within the MV4;11 cell line.



1.18 BRD4(1) IC_{50} = 6.0 mM *(AlphaScreen)*
1.19 BRD4(1) IC_{50} ~ 7 μM *(AlphaScreen)*
1.20 BRD4(1) IC_{50} = 4.8 μM *(AlphaScreen)*
R = Et **1.21** BRD4(1) IC_{50} = 640 nM
R = H **1.6** BRD4(1) IC_{50} = 382 nM
R = Ac **1.7** BRD4(1) IC_{50} = 371 nM *(AlphaScreen)*

Figure 1.6. Fragment-based development of BET bromodomain ligands by Hewings *et al*.[46,62] IC_{50} values were measured by AlphaScreen™.

Alongside the optimisation of benzodiazepines that identified I-BET762 (**1.2**) as a BET bromodomain ligand, a phenotypic assay to identify novel chemotypes that upregulate ApoA-1 was performed.[64,65] This identified I-BET151 (**1.8**), a 3,5-dimethylisoxazole with BET bromodomain selectivity and affinity similar to I-BET762 (**1.2**), but with improved pharmacokinetics and a longer half-life. Furthermore, this ligand suppressed LPS-induced inflammation in the same manner as I-BET762 (**1.2**). In addition, I-BET151 displayed potent toxicity of mixed lineage leukaemia cell lines.[47] These results translated into leukaemia-xenograft mice, further highlighting the therapeutic potential of inhibiting the interaction of BET bromodomains with chromatin.

The KAc-mimicking ability of 3,5-dimethylisoxazoles was also discovered by Bamborough *et al*., who used fragment-based screening to develop BET bromodomain ligands.[63] Lead molecule 4-phenyl-3,5-dimethylisoxazole **1.22** guided identification of cyclopropylsulfonamide **1.23** that could interact with the lipophilic WPF shelf of the BET bromodomains, and also form water-mediated interactions (Figure 1.7). In attempts to optimise this series, it was realised that expansion of the ring from cyclopropyl- to cyclobutyl- and cyclopentylsulfonamides improved the

affinity, but larger molecules proved too big, with reduced affinity for the BET bromodomains. Solubility problems with this series of compounds were ameliorated with solubilising groups *para* to the isoxazole, while maintaining most of the BET bromodomain affinity. DSF assays indicated reasonable selectivity of **1.24** for the BET family over a collection of other bromodomains. BRD4 displayed > 5 °C shift in melting temperature, whereas ATD2B, BAZ2B, BRD1, CREBBP, FALZ, PB1(4), PHIP(2) and TRIM33 all showed < 1.5 °C shift. However, it is not possible to directly compare thermal shift results between different proteins, so an orthogonal assay is required to ensure selectivity for the BET bromodomains exists. BET bromodomain ligands can show inhibition of cytokine production. This inhibition was observed for these derivatives, the *in vitro* affinity of which translated into cellular assays, with micromolar inhibition of IL-6 production and nanomolar inhibition of tumour necrosis factor release in LPS-stimulated peripheral blood mononuclear cells.

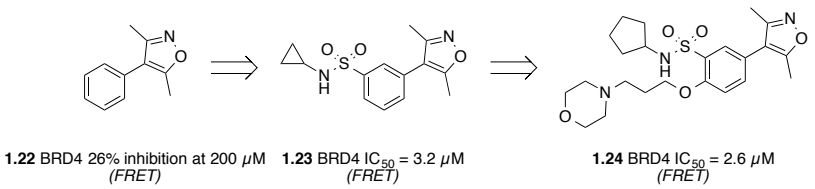
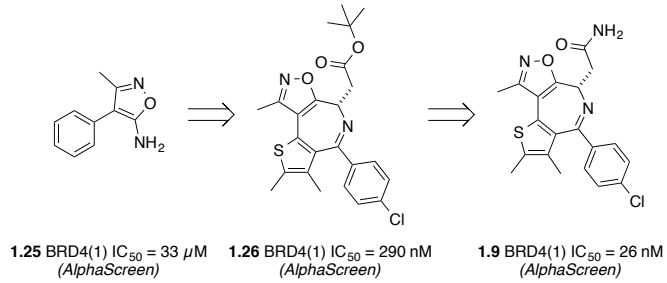


Figure 1.7. Fragment-based development of **1.22** by Bamborough *et al*. gave **1.24**.[63] Percentage inhibition was measured by fluorescence anisotropy, and IC_{50} values were measured by FRET.

In efforts to develop novel small molecular probes for the BET bromodomains, a fragment-based screen by Constellation identified that aminoisoxazole **1.25** also bound with micromolar affinity (Figure 1.8).[48] Comparison of the binding modes of **1.25** and (+)-JQ1 (**1.3**) suggested that an isoxazole could replace the triazole moiety within (+)-JQ1 (**1.3**). Direct substitution of the heterocycles to give **1.26** did not improve the affinity with respect to (+)-JQ1 (**1.3**), however replacement of the *tert*-butyl ester for a primary amide (**1.9**), in the same manner as for CPI-203 (**1.5**),

resulted in affinity equivalent to (+)-JQ1 (**1.3**).[61] Modifications of this amide were not particularly well tolerated. Further optimisation was attempted by changing the groups that lie on the WPF shelf. A range of aromatic groups was well tolerated, but no significant improvement on the parent derivative **1.9** was achieved. *In vitro* ADME screening assays, and subsequent *in vivo* studies, showed that **1.9** was suitable for use in rat and dog experiments. **1.9** also displayed dose-dependent suppression of *MYC* expression.



1.25 BRD4(1) IC_{50} = 33 μM *(AlphaScreen)*

1.26 BRD4(1) IC_{50} = 290 nM *(AlphaScreen)*

1.9 BRD4(1) IC_{50} = 26 nM *(AlphaScreen)*

Figure 1.8. Optimisation of aminoisoxazole **1.25**, by incorporation of 3,5-dimethylisoxazole moiety into the (+)-JQ1 (**1.3**) core scaffold by Gehling *et al*. gave **1.9**.[48] IC_{50} values were measured by AlphaScreen™.

Due to the complex chiral nature of most BET bromodomain ligands published, work by Hay *et al*. was carried out to develop simple ligands with selectivity for BET bromodomains over CREBBP.[49] Indanone **1.27** and its indanol derivatives appeared to be good leads, however, optimisation suffered from decomposition of the indanols (Figure 1.9). Synthesising derivatives of less selective benzimidazole **1.28** led to the development of BET bromodomain ligand **1.10** with IC_{50} = 180 nM, and with 100-fold selectivity over CREBBP as measured by AlphaScreen™, that did not bind to additional bromodomains ATAD2, BRD9, PB1(5), PCAF, PHIP(2), TAF1(2) and TIF1α as confirmed by DSF. As mentioned earlier, it will be important to verify this selectivity with orthogonal assays.

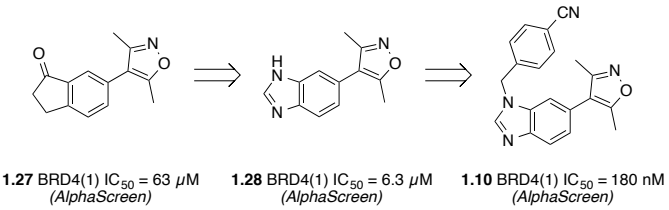


Figure 1.9. Development of BET bromodomain ligand **1.27** by Hay *et al*. gave **1.10**.[49] IC_{50} values were measured by AlphaScreen™.

Recently, McKeown *et al*. have also published a BET bromodomain ligand containing a 3,5-dimethylisoxazole moiety as the KAc mimic.[50] The imidazo[1,2-*a*]pyrazine UMB-32 (**1.11**) binds to BRD4(1) with K_d = 550 nM, as measured by ITC, and has submicromolar BRD4 cellular affinity. In a BROMOscan™ selectivity screen, UMB-32 (**1.11**) also displayed binding to the bromodomains of TATA-box binding protein (TBP)-associated factor 1 (TAF1) and TAF1-like (TAF1L), proteins associated with cancer and Parkinson's disease, albeit with weaker affinity than for the BET bromodomains.[70,71]

1.2.4 RVX-208 (**1.12**)

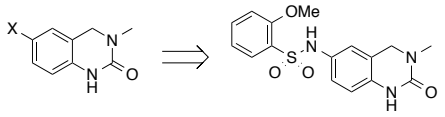
In a similar manner to Nicodeme *et al*., Bailey and co-workers used phenotypic screening with a HepG2 cell-based assay to identify compounds that up-regulated ApoA1 expression.[51] This approach resulted in the identification of RVX-208 (**1.12**), which also showed suitable pharmacological properties for use in *in vivo* models. This activity translated into a pre-clinical model of atherosclerosis, with dose-dependent increases in ApoA1 and high-density lipoprotein cholesterol (HDL-C) levels in RVX-208-treated male African green monkeys. This study allowed progression of RVX-208 (**1.12**) to clinical trials for atherosclerosis. It was subsequently established as a first-in-class quinolinone BET bromodomain ligand,

with preference for the second bromodomain, particularly for BRD3(2) with 170-fold selectivity over BRD3(1) as measured by AlphaScreen™.[52,53]

1.2.5 PFI-1 (**1.13**)

A fragment-based approach to develop novel BET bromodomain ligands was carried out by Fish *et al*.[54] Unlike quinazolinone **1.19** (Figure 1.6) developed by Hewings *et al*., of which the 3,5-dimethyisoxazole proved to be the KAc-mimicking moiety, basic quinazolinones **1.29**, also identified by Chung *et al*., and **1.30** mimicked KAc through its urea functionality, with excellent ligand efficiency (Figure 1.10).[72] The urea carbonyl accepts a hydrogen bond from N140. In attempts to improve affinity for the BET bromodomains, sulfonamidation of the *C*-6 position of these fragments was then pursued. This work led to the identification of significantly more potent derivatives including PFI-1 (**1.13**), with 220 nM affinity for the BET bromodomains as determined by AlphaScreen™, and with selectivity over other bromodomains as seen by DSF. The BET bromodomains displayed moderate shifts in melting temperature of 2-6 °C, whereas BAZ2B, PB1(5) and PCAF stabilisations were < 1 °C. A concern with this molecule is the benzylic carbon, which has the potential to oxidise easily. However, an X-ray crystal structure of PFI-1 (**1.13**) bound to BRD4(1), no oxidation is observed. Importantly, the urea carbonyl hydrogen bond to N140 was maintained, and it also makes the water-mediated interaction with Y97. Furthermore, the urea donates a hydrogen bond to the carbonyl of N140, and the *N*-Me group mimics the methyl group of KAc. The derivative showed single digit micromolar inhibition of IL-6 production following LPS-stimulation of human blood mononuclear cells. It also displayed reasonable rodent pharmacokinetics following *intravenous* administration. However, PFI-1 (**1.13**) suffered from poor bioavailability in rats due to solubility issues following oral administration. PFI-1 (**1.13**) also showed antiproliferative activity

in a number of leukaemia cell lines, although this activity proved to be 5-10-fold weaker than for (+)-JQ1 (**1.3**).



X = H **1.29** BRD4(1) IC_{50} = 39 μM
X = Br **1.30** BRD4(1) IC_{50} = 23 μM
(AlphaScreen)

1.13 BRD4(1) IC_{50} = 220 nM
(AlphaScreen)

Figure 1.10. Fragment-based development of PFI-1 (**1.13**) by Fish *et al*.[54] IC_{50} values were measured by AlphaScreen™.

1.2.6 BET bromodomain ligands containing 2-thiazolidinones

Zhao *et al*. carried out a fragment-based approach to discover novel ligands for BRD4.[56] Docking approximately 500 fragment molecules into the X-Ray crystal structure of (+)-JQ1 (**1.3**) in BRD4(1) allowed the identification of 41 fragment binders with the potential to hydrogen bond with N140. Crystallography confirmed the binding of nine fragments, of which four were revealed in the manuscript. This paper focused on the optimisation of a 2-thiazolidinone (**1.31**), which led to the development of a few nanomolar ligands for BRD4, including **1.14** with IC_{50} = 230 nM, as measured by fluorescence anisotropy, although no bromodomain selectivity data was disclosed (Figure 1.11). Unfortunately, there was poor correlation between *in vitro* AlphaScreen™ and cellular data, presumably as a result of poor cell permeability due to lipophilicity, with smaller compounds having greater cellular affinity. These derivatives showed antiproliferative activity in a human cancer cell line HT-29. **1.32**, which had an *in vitro* IC_{50} value of 2.6 μM, was the most potent derivative, with a half maximal growth inhibitory concentration (GI_{50}) of 37 μM. However, **1.32** was not as potent as 4-phenyl-3,5-dimethylisoxazole **1.22** (Figure 1.7) reported by Hewings *et al*.

and Chung *et al*., with GI_{50} = 23 μM, and was considerably weaker than (+)-JQ1 (**1.3**), with GI_{50} = 2 μM.[62,72]

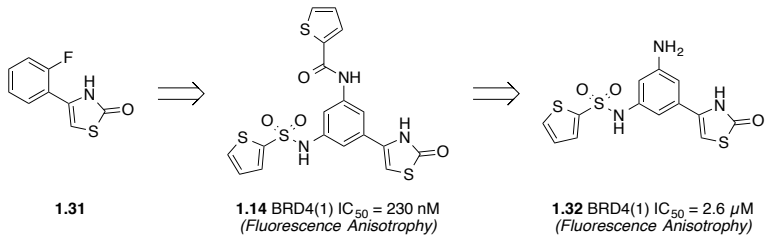


Figure 1.11. Development of 2-thiazolidinones by Zhao *et al*.[56] IC_{50} values were determined by fluorescence anisotropy.

1.2.7 BET bromodomain ligands containing azobenzenes

Based on the identification of azobenzenes as a scaffold with which to target bromodomains, optimisation was carried out in order to develop BET bromodomain selective ligands.[73,74] Synthesis and testing of a range of analogues resulted in the identification of a number of BET bromodomain ligands, including MS436 (**1.15**), a ligand that not only showed selectivity against other bromodomains, but also showed 10-fold selectivity for BRD4(1) over BRD4(2) with K_i < 85 nM for BRD4(1), presumably due to a water-mediated interaction with K91, a residue that is unique to BRD4(1). MS436 (**1.15**) also displays good physicochemical properties, balancing strong affinity with acceptable lipophilicity. These compounds were not cytotoxic, and despite the compound having an electron rich 5-hydroxy-4-methylanilin-2-yl substituent, which could undergo undesirable off-target nucleophilic reactions, their affinity translated into cellular settings, with MS436 (**1.15**) displaying 4 μM blocking of the interaction of BRD4 with NF-κB, and also 5 μM inhibition of IL-6 production. Due to the potential reactivity of this molecule, it is important to generate data in orthogonal assays, and to assess its stability *in vivo*. Interestingly, binding data

disclosed in a patent indicate that substitution of the diazo functionality for an alkene has no detrimental effect on affinity.[75]

1.2.8 XD14 (**1.16**)

In order to develop novel BET bromodomain ligands, a high-throughput virtual screen of more than seven million small molecules was carried out by Lucas *et al*.[58] ITC measurements of 22 candidate molecules gave seven strong binding ligands for BRD4(1), six of which contained novel scaffolds. 4-Acylpyrrole XD14 (**1.16**) was the strongest binder, with K_d = 237 nM for BRD4(1). It also displayed single digit micromolar binding to CREBBP, a significant value for this bromodomain. Otherwise this compound was highly selective for the BET family. As well as mimicking KAc by forming the hydrogen-bonding interaction with N140 and the water-mediated interaction with Y97, it also hydrogen bonds to the backbone carbonyl of P82. Low micromolar GI_{50} values were obtained in the HL-60 leukaemia cell line for this series of compounds, with no cytotoxicity observed in HeLa cells. Screening XD14 (**1.16**) against the NCI-60 human cancer cell lines showed further antiproliferative activity, particularly in leukaemia cell lines. Good ligand efficiency values indicate that this scaffold is an interesting starting point for the development of BET bromodomain ligands.

1.2.9 I-BET726 (**1.17**, GSK1324726A)

As noted, the antiproliferative effects of BET bromodomain ligands in haematological malignancies are well established in the literature.[47,68,69,76] Few papers have described the effect of BET bromodomain ligands on solid tumours, other than NMC. However, downregulation of *MYC* has been recognised. As such,

work was carried out by GSK to determine whether BET bromodomain ligands could have antiproliferative effects on neuroblastoma, an aggressive solid tumour associated with a high frequency of *MYCN* gene amplification.[57] A potent and highly selective BET bromodomain ligand, I-BET726 (**1.17**), with IC_{50} = 22 nM for BRD4 as measured by FRET, was used in this study.[59] This ligand showed antiproliferative activity in a panel of neuroblastoma cell lines, with median GI_{50} = 75 nM. This resulted in growth inhibition, differentiation and apoptosis, and this study showed that BET bromodomain ligands have therapeutic potential for solid tumours.

1.2.10 *In silico* discovery of BET bromodomain ligands

Recently, several papers have emerged that have used computational chemistry to identify BET bromodomain ligands. Vidler *et al*. assessed the "druggability" of bromodomains, based on particular amino acid signatures, by using SiteMap, a readily-available structure-based druggability prediction tool that allows rapid binding site identification, and importantly for water molecules to be included in the analysis, by using parameters including pocket volume, enclosure and hydrophobicity.[77] These signatures allowed classification of the bromodomains into different groups, which could alert medicinal chemists to selectivity issues when designing ligands for particular bromodomains.

A significant amount of *in silico* work has been carried out in the lab of Caflisch. Magno *et al*. carried out the first molecular dynamics simulations of a histone mark binding to epigenetic reader domains by simulating the binding of KAc to TAF1(2).[78] This study identified two binding sites: firstly, that observed in crystal structures, stabilised through hydrogen-bonding with an asparagine residue (Figure 1.12A), complete with six structured water molecules (Figure 1.12C); secondly, a deeper interaction was observed, with three of the water molecules displaced by the KAc

side-chain (Figure 1.12C), and a hydrogen-bonding interaction with the backbone carbonyl of the proline residue in the WPF shelf (Figure 1.12B).

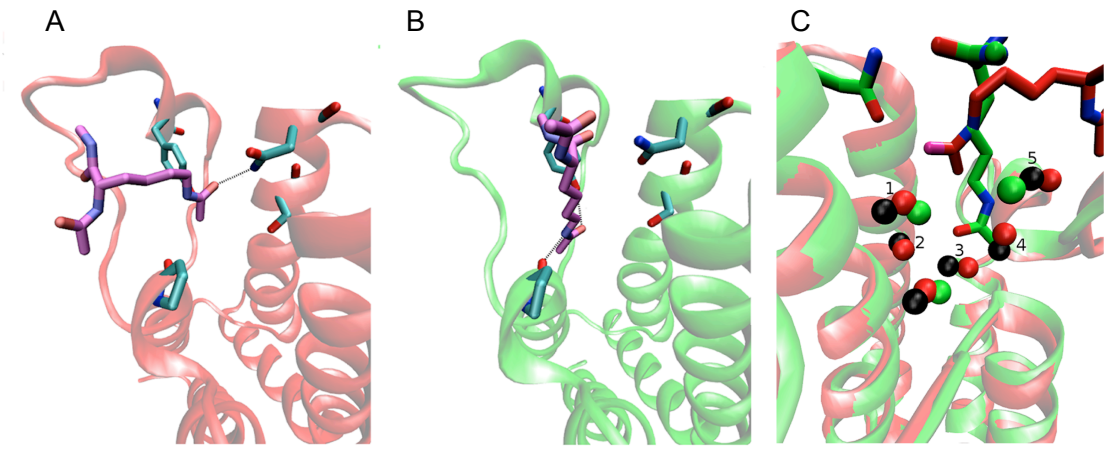
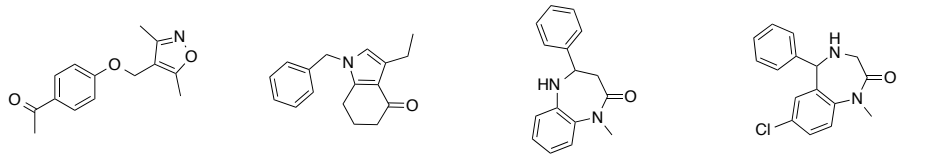


Figure 1.12. The two binding modes of KAc to TAF1(2) identified by Magno *et al*. A: The asparagine-binding mode, whereby the carbonyl moiety of KAc hydrogen bonds to the amino functionality of N1604. B: The proline-binding mode, where KAc binds deeper in the pocket, allowing a hydrogen bond with the backbone carbonyl of P1548 instead. C: Structured waters of apo TAF1(2) (PDB 3UV4, water = black) are conserved in the asparagine-binding mode (water = red), while waters 2-4 are displaced in the proline-binding mode (water = green). Reproduced with permission from Magno, A.; Steiner, S.; Caflisch, A. *Journal of Chemical Theory and Computation* **2013**, *9*, 4225. Copyright 2013 American Chemical Society.

Further analysis of water displaceability was carried out by Huang *et al*., who also carried out molecular dynamics simulations. They identified that most structural waters within the bromodomain recognition pockets of CREBBP and bromodomain adjacent to zinc finger protein (BAZ) 2B (BAZ2B), the biology of which is not yet well understood, are displaceable by solvents (DMSO, methanol and ethanol).[79] Furthermore, work was carried out by Steiner *et al*. to assess the flexibility of 20 bromodomain recognition pockets.[80] Molecular dynamics simulations showed that access to some of the bromodomain pockets could be prevented by displacement of certain residues. They suggest that the presence of metastable states could be exploited when designing selective bromodomain ligands. In the same research

group, Zhao *et al*. used *in silico* screening, combining docking and molecular dynamics simulations, to narrow nine million compounds down to 24 potential KAc mimics, in attempts to discover novel BET bromodomain ligands.[81] Validation of these compounds *in vitro* using DSF, identified four weak BET bromodomain-selective binders: one isoxazole (**1.33**), and three novel KAc mimics (**1.34**-**1.36**), including the drug diazepam (**1.36**) (Figure 1.13). An AlphaScreen™ assay confirmed micromolar binding to BRD4, showing that these molecules are a good starting point for the development of more potent BET bromodomain ligands.



1.33 BRD4(1) 35% inhibition at 50 μM *(AlphaScreen)*

1.34 BRD4(1) 99% inhibition at 50 μM *(AlphaScreen)*

1.35 BRD4(1) 99% inhibition at 50 μM *(AlphaScreen)*

1.36 BRD4(1) 43% inhibition at 50 μM *(AlphaScreen)*

Figure 1.13. Fragments for BRD4(1) identified by Zhao *et al*.[81] Percentage inhibition at 50 μM, as measured by AlphaScreen™.

Vidler *et al*. carried out a similarity search of 2.4 million commercially available compounds, to look for novel KAc mimics based on the triazole moiety of (+)-JQ1 (**1.3**).[82] This search narrowed the collection down to 143 compounds that were tested against BRD4(1). Six ligands were identified, including four novel KAc mimics, although all showed weak binding, as measured by AlphaScreen™, with IC_{50} values only obtained for two. However, with these compounds in hand, co-crystallisation allowed more potent binders to be obtained through structure-based design (**1.37**-**1.42**, Figure 1.14).

Virtual screening was also used by Muvva *et al*. to identify BET bromodomain ligands.[83] This work led to the identification of 11 compounds predicted to bind to BRD4 with higher affinity than (+)-JQ1 (**1.3**). Molecular dynamics suggested that

three of these compounds are stable in the bromodomain recognition pocket, however, validation of these predictions was not reported.

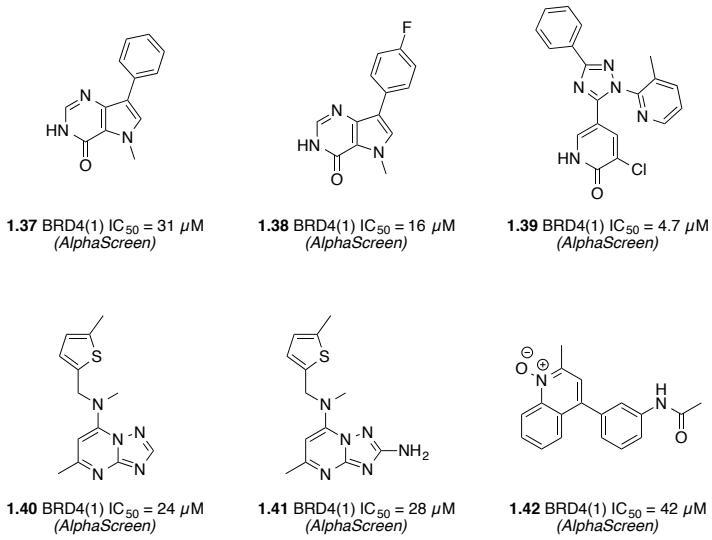


Figure 1.14. Fragments for BRD4(1) identified through fragment screening and optimisation by Vidler *et al*.[82] IC_{50} values were measured by AlphaScreen™.

1.2.11 Dual kinase-bromodomain inhibitors

There is growing evidence suggesting that kinases and bromodomains are associated. For example, BCPs have displayed atypical kinase activity, but are structurally dissimilar from kinases.[45,84] The mechanism of this kinase activity remains unclear. In addition, bromodomains recruit kinases for their downstream effects, such as the recruitment of P-TEFb to elongate Pol II.[22] Furthermore, both kinases and bromodomains are therapeutic targets for cancer and inflammation. As such, a dual kinase-bromodomain inhibitor could be a promising strategy for treating disease. Interestingly, several kinase inhibitors have recently emerged as bromodomain binders.[85-88]

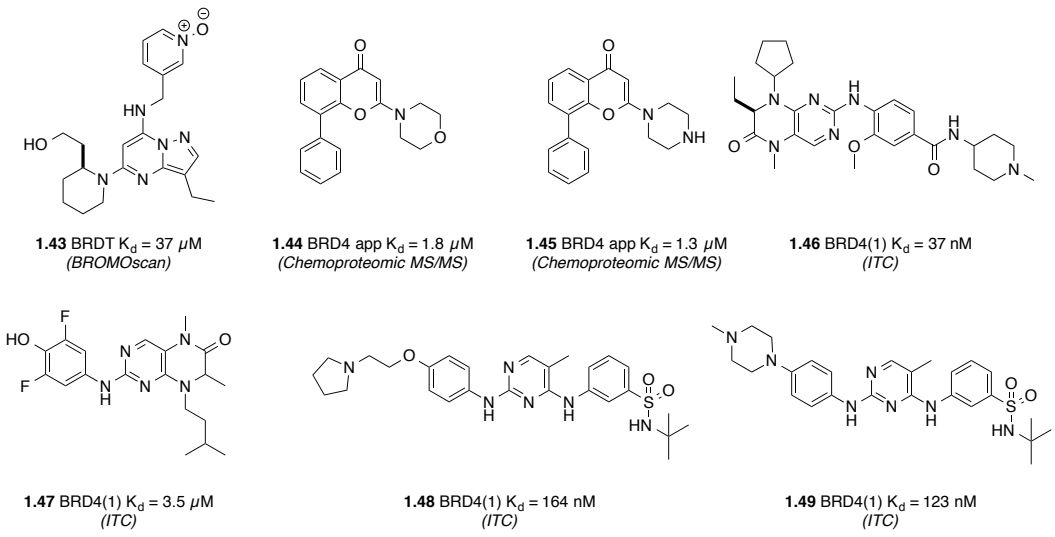


Figure 1.15. Some examples of dual kinase-bromodomain inhibitors, identified by Martin *et al*., Dittmann *et al*., Ciceri *et al*. and Mishra *et al*., with their K_d values and the quoted method used to obtain these values.[85-88]

Interestingly, as highlighted in the review by Brand *et al.*, the interaction of BRD4 with the phosphoinositide 3-kinase (PI3K) inhibitor LY294002 (**1.44**, Figure 1.15) was reported in 2007, following a pull-down assay using a Sepharose-immobilised version of LY294002 (**1.44**), and effects of this inhibitor on NF-κB-dependent transcription now appear to be due to disruption of the interaction of bromodomains with chromatin.[60,89] This activity was confirmed by Dittmann *et al*., who showed that LY294002 (**1.44**) binds to the BET bromodomains, albeit with approximately 10-fold less affinity than to PI3K.[86] In addition, its inactive structural analogue LY303511 (**1.45**) also binds to BET bromodomains, and showed antiproliferative and anti-inflammatory effects observed for published BET bromodomain ligands. The compounds also displayed domain selectivity.

Since then, a number of other dual kinase-bromodomain ligands have emerged in the literature. Martin *et al*. realised that dinaciclib (**1.43**), a cyclin-dependent kinase inhibitor currently in a Phase III clinical trial for chronic lymphocytic leukaemia

(ClinicalTrials.gov identifier: NCT01783171), binds weakly to bromodomains.[85] Crystallography identified that dinaciclib (**1.43**) can make a hydrogen-bonding interaction with the highly conserved asparagine residue. However, the distance between the two molecules is sub-optimal, resulting in weak binding. Lipophilic interactions are also observed on the WPF shelf, as well as hydrogen-bonding interactions to the backbone carbonyls of a proline and a valine residue. Testing against a panel of 24 bromodomains indicated that dinaciclib (**1.43**) preferentially binds to the BET bromodomains, as well as TAF1 and TAF1L, with K_d = 37 μM for BRDT, as determined using BROMOscan™.

Ciceri *et al*. showed that several kinase inhibitors also interact with bromodomains.[87] Screening 628 kinase inhibitors against BRD4 using AlphaScreen™ identified nine examples with > 90% inhibition of peptide binding at 50 μM. Three examples, polo-like kinase inhibitor BI-2536 (**1.46**), ribosomal s6 kinase inhibitor BI-D1870 (**1.47**) and janus kinase (JAK) inhibitor TG-101348 (**1.48**), displayed nanomolar IC_{50} values. Nanomolar to single digit micromolar K_d values as determined by ITC, were also obtained for these compounds and other kinase inhibitors such as JAK inhibitor TG-101209 (**1.49**). These represent more suitable dual kinase-bromodomain inhibitors for therapeutic polypharmacology. This *in vitro* affinity translated into cells, with FRAP assays demonstrating that BI-2536 and TG-101348 can displace BRD4 from chromatin. Interestingly they also suppress *MYC* expression in myelomonocytic leukaemia cells, a mechanism previously reported to be due to JAK kinase inhibition, but which now appears to be due to binding to BET bromodomains instead. Similarly, Ember *et al*. identified 14 dual kinase-bromodomain inhibitors, including BI-2536 and TG-101209 also identified by Ciceri *et al*., in co-crystallisation screening of 581 kinases against BRD4(1) and BRDT(1). These results were validated by DSF and AlphaScreen™, with 25 nM-20 μM IC_{50} values observed. Selectivity of BI-2536 and TG-101209 for the

BET bromodomains was demonstrated in this paper by BROMOscan™. Interestingly, work by Mishra *et al*., who were developing NMR-based assays using ^{19}F-labelled bromodomains to detect novel ligands, identified that BI-2536 in fact binds to the bromodomain of fetal Alzheimer's antigen (FALZ), an interaction undetected by BROMOscan™.[88] In summary, this work highlighted that bromodomains can bind to a wide range of kinase inhibitor scaffolds. Furthermore, different binding modes are often observed within the KAc and ATP binding sites indicating that optimisation can be achieved in the development of more potent and selective dual kinase-bromodomain inhibitors, or even to eradicate binding to kinases resulting in bromodomain-specific binders.

1.3 BET bromodomain ligands in clinical trials

1.3.1 BET bromodomains in disease

Many BET bromodomain ligands have shown antiproliferative activity in a range of cancer cell types, particularly haematological malignancies such as leukaemias, in addition to some solid tumours.[47,68,90] Evidence suggests that down-regulation of the *MYC* gene through inhibition of histone-bromodomain interactions is, at least partly, responsible for these observations.[69,76,91] For most cancers, the role of BET bromodomains, and the selectivity of bromodomain ligands for malignant cells is not yet completely understood.[92] However, in one example, NMC, a rare but lethal form of lung cancer, BET bromodomains play a fundamental role in the emergence of the disease. A chromosomal translocation of BRD4 allows it to form in-frame fusions with the *NUT* gene. The bromodomains of the BRD4-NUT oncoprotein interact with chromatin, responsible for the cancer proliferation. Inhibiting this interaction with a BET bromodomain ligand, (+)-JQ1 (**1.3**), has shown

antiproliferative activity, selective for the cancerous cells, and extended the survival of patient-derived xenograft models of NMC.[41]

The interaction of histones with BET bromodomains has also been linked to the expression of inflammatory genes. Disruption of this interaction with BET bromodomain ligand I-BET762 (**1.2**) suppressed transcription of inflammatory genes that induce an inflammatory response.[40] Furthermore, the link between BET bromodomains and T helper cell differentiation has shown a potential avenue for treatment of autoimmune diseases.[93,94]

In addition to cancer and inflammation, the disruption of interactions with BET bromodomains has prevented the genesis of diseases and other biological processes, including heart failure and spermatogenesis.[28,95,96] Furthermore, BET bromodomains are linked with viral replication. HIV latency prevents the activity of antiretrovirals, but BET bromodomain ligands can be used to trigger HIV transcription in latent cells, allowing antiretroviral activity.[31,97-99] BRD4 competes with the viral transactivator Tat for the transcription factor P-TEFb. Inhibiting the interaction of BRD4 with P-TEFb, increases the levels of the transcription factor available to activate viral replication. In fact, a large number of preclinical studies, for a range of diseases, have shown the diverse therapeutic potential of BET bromodomain ligands.[100] However, with BET bromodomains linked to a vast number of transcriptional pathways, the question remains whether BET bromodomain ligands are safe to use in therapy. Several clinical trials are now underway, or have already reached completion, in attempts to answer this question.

1.3.2 Clinical trials of bromodomain ligands

As discussed previously, a number of groups have established the importance of BET bromodomains, and several potent inhibitors of BRD4 have now made their way into clinical trials (Table 1.1). Interestingly, as mentioned earlier, clinical trials of RVX-208 (**1.12**), established as an ApoA-1 up-regulator, were conducted before it was identified as a BET bromodomain ligand. A small study in healthy humans saw translation of its observed phenotype with a statistically significant increase in ApoA-1 and a trend towards increased HDL-C (ClinicalTrials.gov identifier: NCT00768274).[51] Following success in early stages, three Phase II trials, ASSURE, ASSERT and SUSTAIN, were undertaken to principally investigate dose range, safety and efficacy of RVX-208 (**1.12**) for patients with atherosclerosis or coronary artery disease (ClinicalTrials.gov identifiers: NCT02067820, NCT01058018 and NCT01423188).[101] Both ASSURE and ASSERT failed to meet their primary endpoints, but good results were observed for patients administered RVX-208 (**1.12**) in combination with rosuvastatin. Good results were also observed in patients with established cardiovascular disease, low HDL-C and heightened inflammation, indicating that RVX-208 (**1.12**) has the potential to be used as a personalised medicine. In the ASSERT trial, reversible elevated levels of transaminases, causing potential for liver problems in the long-term, were observed most frequently in patients treated with simvastatin, on high-dose statin treatment or who had elevated enzyme levels at baseline. As such, SUSTAIN was carried out with optimised background statin therapy, and over a longer duration. Pooled data collected from these trials have revealed a statistically significant reduction in major adverse cardiac events following treatment with RVX-208 (**1.12**). This reduction was particularly noticeable for patients with diabetes mellitus, who also showed lower blood glucose levels compared to placebo-treated patients. Based on the hypothesis that increased levels of ApoA-1 and HDL-C would increase insulin secretion, a Phase II trial was

conducted to establish whether RVX-208 (**1.12**) resulted in glucose metabolism changes in people with pre-diabetes (ClinicalTrials.gov identifier: NCT01728467). Unfortunately, preliminary results proved inconsistent with this hypothesis. Another Phase II trial to investigate combination therapy of RVX-208 (**1.12**) with atorvastatin or rosuvastatin for treatment of dyslipidemia has been terminated (ClinicalTrials.gov identifier: NCT01863225). Interestingly, due to the association between HDL-C and protection from developing Alzheimer's disease, the effects of RVX-208 (**1.12**) on amyloid beta 40 ($A\beta_{40}$), a biomarker for the disease, were also assessed.[102] $A\beta_{40}$ binds to ApoA-1, and it is thought that high levels of plasma $A\beta_{40}$ indicate its transport away from the brain. RVX-208 (**1.12**) showed a positive effect on plasma $A\beta_{40}$ levels in a Phase I trial and the ASSERT trial.[103,104]

I-BET762 (**1.2**) is currently in Phase I clinical trials for haematological malignancies, as well as NMC (ClinicalTrials.gov identifiers: NCT01943851 and NCT01587703). NMC is also the focus of clinical trials of the OncoEthix compound OTX015, a triazolodiazepine licensed to OncoEthix by Mitsubishi Tanabe, and the Tensha Therapeutics compound TEN-010, of undisclosed structure, previously named JQ2 (ClinicalTrials.gov identifiers: NCT02259114 and NCT01987362). OTX015 and a Constellation compound CPI-0610 of undisclosed structure are also being trialled for haematological malignancies (ClinicalTrials.gov identifiers: NCT01713582, NCT01949883, NCT02157636 and NCT02158858). The expanding interest in targeting bromodomain interactions, and the launch of new clinical trials, will hopefully better establish the link between bromodomains and disease, and validate bromodomain ligands as potential therapeutics.

Compound	Company	Phase	Conditions	ClinicalTrials.gov identifier (status)
RVX-208 (**1.12**)	Resverlogix	I/II	Dyslipidemia; Atherosclerosis; Acute coronary syndrome; Cardiovascular disease	NCT00768274 (completed)
		II	Coronary artery disease; Atherosclerosis	NCT01058018 (completed)
		II	Coronary artery disease	NCT01067820 (completed)
		II	Coronary artery disease; Dyslipidemia	NCT01423188 (completed)
		II	Pre-diabetes	NCT01728467 (completed)
		II	Coronary artery disease; Dyslipidemia	NCT01863225 (terminated)
I-BET762 (**1.2**, GSK525762)	GSK	I/II	Relapsed, refractory haematologic malignancies	NCT01943851 (recruiting)
		I/II	Nut midline carcinoma	NCT01587703 (recruiting)
OTX015	OncoEthix	I	Acute leukemia; Other haematological malignancies	NCT01713582 (recruiting)
		I	NUT midline carcinoma; Triple negative breast cancer; Non-small cell lung cancer with rearranged ALK gene/fusion protein or KRAS mutation; Castrate-resistant prostate cancer; Pancreatic ductal Adenocarcinoma	NCT02259114 (recruiting)
		II	Glioblastoma multiforme	NCT02296476 (recruiting)
CPI-0610	Constellation	I	Lymphoma	NCT01949883 (recruiting)
		I	Multiple myeloma	NCT02157636 (recruiting)
		I	Acute leukaemia; Myelodysplastic syndrome; Myelodysplastic/ myeloproliferative neoplasms	NCT02158858 (recruiting)
TEN-010	Tensha Therapeutics	I	Nut midline carcinoma; Other solid tumours	NCT01987362 (recruiting)

Table 1.1. Clinical trials of bromodomain ligands correct on 1st November 2014.

1.4 Inhibitors of CREBBP bromodomain-KAc interactions

1.4.1 The biological roles of CREBBP

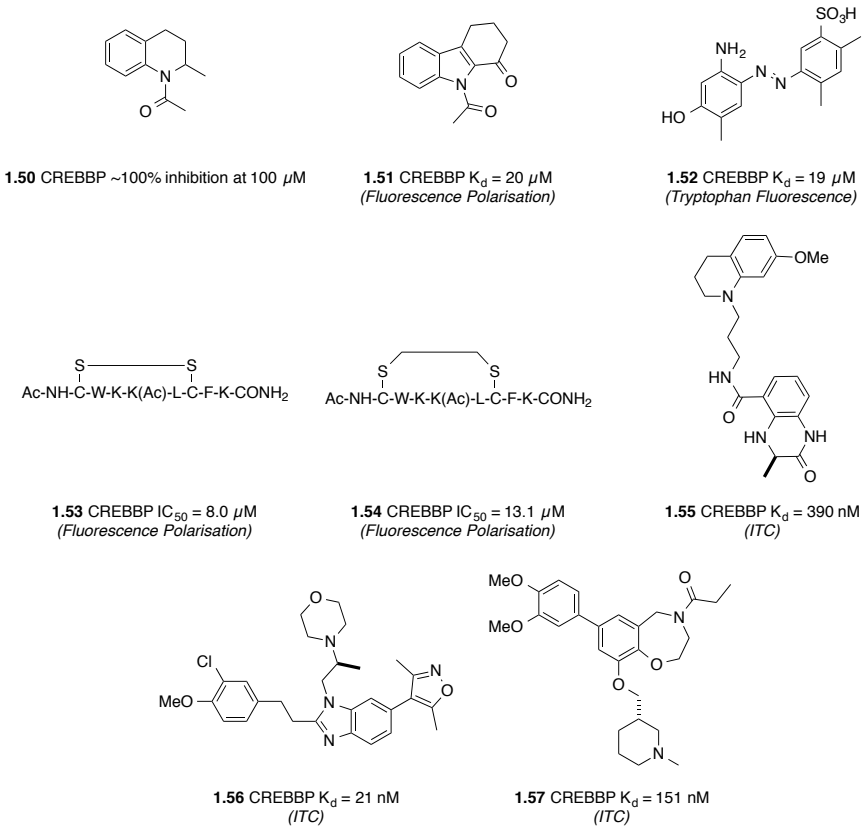


Figure 1.16. CREBBP bromodomain ligands developed by Sachchidanand *et al*., Borah *et al*., Gerona-Navarro *et al*., Rooney *et al*., Hay *et al*. and the SGC, with their percentage inhibition, IC_{50} or K_d values and the quoted method used to obtain these values.[39,66,73,105,106]

Until recently, the development of ligands for bromodomains has focused heavily on the BET family, with potent bromodomain ligands limited to this family only. However, with the success achieved in BET bromodomain ligand development, and the resulting expanded interest in bromodomains, there is now much interest in targeting non-BET bromodomains. One bromodomain in particular, CREBBP, has been studied by a number of groups (Figure 1.16).[39,66,73,105,106] CREBBP, and its paralogue p300, are general transcriptional co-activators that interact with more than 400 binding proteins, including the tumour suppressor p53, following DNA

damage.[11,107] CREBBP and p300 contain nine domains including an acetyltransferase domain as well as a bromodomain. This allows them to not only read the histone code but write it as well. CREBBP is linked with numerous diseases including Rubinstein-Taybi syndrome, amyotrophic lateral sclerosis, Alzheimer's disease and Huntington's disease.[108-110] As such, there is a clear demand for probes with which to gain a better understanding of the CREBBP bromodomain and its link with disease.

1.4.2 *N*-Acetylated fragments bind to the CREBBP bromodomain.

CREBBP was one of the first bromodomains to be targeted with small chemical molecules, when Sachchidanand *et al*. carried out an NMR-based screen of 200 compounds in order to target the interaction of CREBBP with p53.[39] 14000 aromatic acetamides, with drug-like properties according to Lipinski's rule of five, were narrowed down to 200 compounds based on knowledge of the structure and interactions of CREBBP with acetylated lysines, obtained from an NMR structure of acetylated p53 bound to CREBBP produced in the group.[11] NMR screening of these 200 compounds identified 14 compounds that modulate resonances of bromodomain residues, including two compounds, MS2126 (**1.50**) and MS7972 (**1.51**), which almost completely inhibited p53 binding to CREBBP at 100 μM and 50 μM respectively (Figure 1.16). The latter exhibited K_d = 20 μM using a fluorescence assay. The modified NMR resonances corresponded with the flexible ZA and BC loops, indicating that these derivatives bind near the recognition pocket, resulting in inhibition of p53-CREBBP binding. Solving the NMR structure of MS7972 (**1.51**) bound to CREBBP confirmed that this activity is due to binding at the entrance of the recognition pocket, not deep within the pocket as has been described for the BET bromodomains. However, treatment of U2OS cells with these compounds,

before doxorubicin-induced DNA damage, limited doxorubicin-induced p53 levels in cells, demonstrating that their *in vitro* activity translates into cells.

1.4.3 CREBBP bromodomain ligands containing azobenzenes

An NMR-based screen of 3000 compounds against the CREBBP bromodomain, conducted by the same group, highlighted that azobenzenes can bind, and are therefore an interesting scaffold when attempting to block peptide binding to CREBBP.[73] Synthesis of a range of analogues, and a subsequent cellular assay measuring inhibition of p53 activity following DNA damage, led to the identification of ischemin (**1.52**), which blocked p53 binding to CREBBP in a dose-dependent manner. Ischemin (**1.52**) displayed complete inhibition of p53 activity, and bound to CREBBP with K_d = 19 μM in a tryptophan fluorescence binding assay, and IC_{50} = 5 μM in a cellular assay, and displayed up to five-fold selectivity over other bromodomains. However, as mentioned earlier, when discussing BET bromodomain ligand **1.15**, the electron rich 5-hydroxy-4-methylanilin-2-yl substituent of ischemin (**1.52**), could undergo undesirable off-target nucleophilic reactions, and therefore it is important to assess the stability of this compound before it is used as a CREBBP probe.

1.4.4 Cyclic peptides as CREBBP bromodomain ligands

Further work was carried out by Gerona-Navarro *et al*. to disrupt the interaction of acetylated p53 with CREBBP.[105] In the NMR structure previously reported in the group, the acetylated p53 peptide binds to CREBBP in a β-turn-like conformation, with the acetylated lysine residue at the start of the turn.[11] Molecular dynamics simulations performed on these binding partners suggested that the ends of the

peptide were particularly flexible, and that binding could be improved through the use of rigidity, minimising the entropic loss upon binding. As such, various cyclic peptides based on the acetylated p53 peptide were synthesised. By exchanging residues for cysteines, disulfide bridges locked the peptides in a ring. Following their synthesis they were tested against p53-CREBBP binding, in a competition fluorescence polarisation assay. The cyclic peptides competed with p53 in a dose-dependent manner, displaying an approximately 10-fold improvement in affinity, with IC_{50} = 23 μM with respect to the linear peptide that has IC_{50} = 190 μM. Optimisation of this peptide, following further molecular dynamics simulations, gave **1.53** with IC_{50} = 8 μM. This derivative proved selective for CREBBP over BRD4 and p300-CREBBP-associated factor (PCAF), presumably due to the β-turn-like conformation that is characteristic of the interaction of p53 with CREBBP. To improve the stability of the cyclic peptides, thioether linkers were incorporated instead of disulfide bridges. This resulted in **1.54** with an IC_{50} of 13 μM. To establish whether these derivatives would show cellular activity, p53-induced p21 activation following doxorubicin-induced DNA damage was assessed following treatment of colorectal carcinoma HCT116 cells with **1.53** and **1.54**. These derivatives showed dose-response inhibition of p21 activation, whereas the linear p53 peptide did not. Furthermore, thioether **1.54** showed better activity than disulfide **1.53** in a pull-down experiment carried out in HEK298 cells to determine how the cyclic peptides inhibit the interaction of p53 with CREBBP, consistent with the greater stability of this derivative.

1.4.5 CREBBP bromodomain ligands containing dihydroquinoxalinones

The first CREBBP ligands with nanomolar affinity were developed within the Conway group by Rooney *et al*.[106] Based on the finding that NMP binds to CREBBP

as well as the BET bromodomains, investigating the binding of NMP analogues revealed that 3,4-dihydro-3-methyl-2(1*H*)-quinazolinone **1.58** also binds to CREBBP (Figure 1.17).[62,67] Previous work in the Conway group showed that this compound is unstable to benzylic oxidation, but benzoxazinones proved to be a suitable alternative for CREBBP. *In silico* screening of 6000 benzoxazinones identified 50 potential binders, which were synthesised and tested against CREBBP in an AlphaScreen™ assay. The highest affinity binders **1.59** and **1.60** had IC_{50} = 77 μM and 51 μM respectively, potentially due to an unfavourable interaction with the backbone carbonyl of P1110. To overcome this, dihydroquinoxalinones were investigated instead, with IC_{50} = 758 nM obtained for **1.61**. Two hydrogen bonds were formed between the dihydroquinoxalinone amide and N1168, and another also formed a water-mediated interaction between the amide carbonyl and Y1125. An internal hydrogen bond oriented the tetrahydroquinoline moiety into an induced pocket, as evidenced by X-Ray crystallography. This allowed a cation-π interaction with R1173, not previously reported, and as such not predicted in docking studies into a static protein structure of CREBBP. Optimisation of the cation-π interaction was achieved with electron-donating substituents on the aromatic ring, affording **1.62** with IC_{50} = 323 nM and K_d = 390 nM as measured by ITC, with modest selectivity over BRD4(1) with K_d = 1.4 μM. A FRAP assay showed that **1.62** disrupts CREBBP binding with chromatin in a dose-dependent manner. Molecular dynamics simulations of **1.62** and CREBBP suggest that the cation-π interaction spontaneously forms in solution.

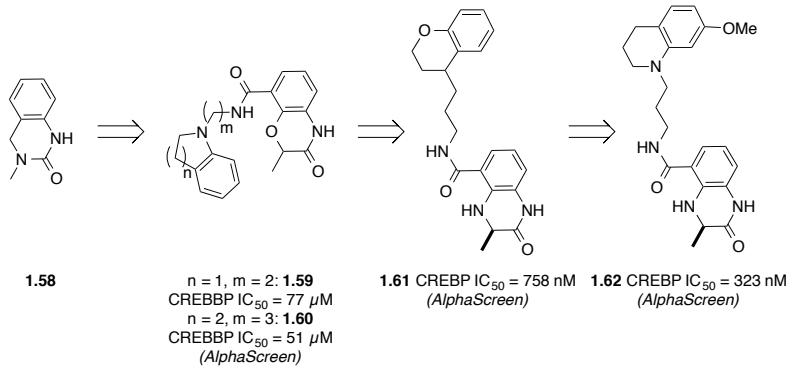
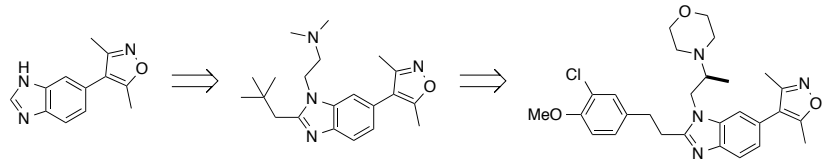


Figure 1.17. Optimisation of CREBBP bromodomain ligand by Rooney *et al*.[106] IC_{50} values were measured by AlphaScreen™.

1.4.6 CREBBP bromodomain ligands containing 3,5-dimethylisoxazoles

Following the work carried out by Hay *et al*. targeting BET bromodomains with simple 3,5-dimethylisoxazoles, work was subsequently carried out to develop the non-selective benzimidazole **1.28** into a probe for CREBBP (Figure 1.18).[49,66] A library of compounds containing different *N*-1 and *C*-2 functionalisations were assessed for binding against CREBBP and BRD4(1) using DSF. Obtaining CREBBP selectivity proved challenging, but optimisation of a lead compound **1.63** led to the discovery of a ligand **1.56** with K_d = 21 nM for CREBBP, which was 40-fold selective over BRD4(1), as measured by ITC, and highly selective over other bromodomains as evidenced by DSF. Melting stabilisations for CREBBP and p300 were > 9 °C, but < 2 °C for the BETs, as well as BRD9, CECR2, PB1(1), TAF1(1), TAF1L(1), TIFIα and TRIM28. X-Ray crystallography of **1.56** in CREBBP showed that the 3,5-dimethylisoxazole moiety binds in a KAc-mimicking capacity, but that the aryl ring forms a cation-π interaction with R1173, in the same induced-fit pocket observed by Rooney *et al*.[106] **1.56** displayed inhibition of CREBBP-mediated p53 activity, with low cytotoxicity. Unfortunately, **1.56** has low microsomal stability and therefore is unsuitable for use in *in vivo* models, but is a good starting point to probe CREBBP.



1.28 CREBBP IC_{50} = 4.0 μM *(AlphaScreen)*

1.63 CREBBP IC_{50} = 4.0 μM *(AlphaScreen)*

1.56 CREBBP K_d = 21 nM *(ITC)*

Figure 1.18. Optimisation of CREBBP bromodomain ligand binding and selectivity by Hay *et al*.[66] IC_{50} values were measured by AlphaScreen™ and K_d value was measured by ITC.

Recently, the SGC have released an additional CREBBP/p300 probe, I-CBP112 (**1.57**, Figure 1.16).[111] This compound has IC_{50} = 170 nM for CREBBP as measured by AlphaScreen™, and K_d = 151 nM and 525 nM for CREBBP and p300 respectively, as measured by ITC. This compound has also shown selectivity against other bromodomains, with only weak binding observed for the BET family. FRAP showed cellular activity of I-CBP112 in U2OS cells. Examination of the deposited X-Ray crystal structure shows that this compound mimics KAc through its propionyl group, which forms a hydrogen bond with N1168 and a water-mediated interaction with Y1125, with the benzoxazepine binding across the entrance of the recognition pocket. Unlike the cation-π interaction observed in the crystal structure of Rooney *et al*. the dimethoxyaromatic lies close to R1173, but does not stack beneath it, indicating that the cation-π interaction was not observed in this crystal structure.[112]

1.5 Inhibitors of other bromodomain-KAc interactions

1.5.1 Targeting non-BET bromodomains

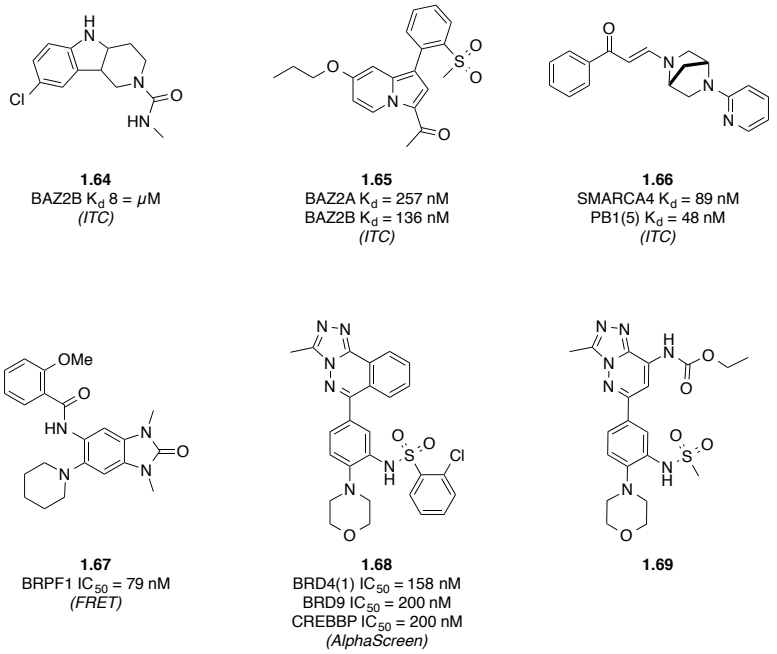


Figure 1.19. Ligands developed for other non-BET bromodomains, by Ferguson *et al*., the SGC, GSK and Pfizer.[113-118]

In addition to targeting the CREBBP bromodomain, recent efforts have begun to focus heavily on other non-BET bromodomains (Figure 1.19). Categorically connecting phenotypic responses to particular non-BET bromodomains is significantly more challenging than for BET bromodomains. BET bromodomain ligands have displayed striking phenotypic responses, across a wide range of indications.[119] This is considered to be due to the ubiquitous expression of BRD2-4, because of their role in cellular state maintenance, rather than activation of only specific pathways.[120] Non-BET bromodomain ligands with high selectivity, profiled, for example, using DiscoveRx's primary human cell-based BioMAP system, have only demonstrated very subtle phenotypic responses, perhaps due to the long time scales over which these changes are observed.[121] Therefore, selectivity is extremely

important for non-BET bromodomain ligands, in order for their biological roles to be conclusively elucidated. The upshot of this is that inhibiting BET bromodomain interactions has the potential to alter scores of undesired biological processes. If non-BET bromodomain ligands are selective enough over other bromodomains, particularly the BET bromodomains, then there is greater potential for more focused pharmacology.

1.5.2 Targeting the BAZ family bromodomains

Although Vidler *et al*. classed BAZ2B as difficult to drug, three groups have targeted the BAZ2A/B bromodomains.[77,113] BAZ2A has been shown to play a fundamental role in the formation of heterochromatin.[122] The biological role of BAZ2B is not yet well understood, although it has been suggested by Jones *et al*. to interact with the imitation switch (ISWI) chromatin-remodelling protein, mediated by the orthologous BAZ1 proteins.[123] Ferguson *et al*. were able to identify starting points for the development of selective ligands for BAZ2B, using a fragment-based approach. Screening 1300 rule-of-three compliant fragments, using AlphaScreen™, followed by crystallography studies identified three micromolar fragments.[124] Optimisation of the most potent fragment afforded a ligand with K_d = 10 μM as determined by ITC, but poor selectivity over CREBBP and BRD2 as suggested by DSF. Further optimisation afforded **1.64**, with little improvement in affinity, with K_d = 8 μM, but good selectivity over CREBBP and BRD2. The SGC and GSK have released GSK2801 (**1.65**), a much more potent BAZ2A/B selective probe with K_d = 136 nM and 257 nM, respectively, as determined by ITC, with cellular activity seen in a BAZ2B FRAP assay.[115] The SGC and the ICR have also announced another probe BAZ2-ICR, of undisclosed structure, which displays K_d = 109 and 170 nM for BAZ2A and BAZ2B respectively, as measured by ITC.[125] This probe also displays 15-fold selectivity over

CECR2, > 100-fold selectivity over other bromodomains, and has also shown cellular activity in a BAZ2B FRAP assay.

1.5.3 PFI-3 binds to bromodomains involved in the SWI/SNF chromatin-remodelling complex

The SGC and Pfizer have released PFI-3, a switch/sucrose non-fermentable (SWI/SNF)-related, matrix-associated, actin-dependent regulator of chromatin (SMARC)A2, SMARCA4 and the fifth bromodomain of polybromo-1 (PB1(5)) ligand, all part of the SWI/SNF chromatin-remodelling complex.[116] PFI-3 (**1.66**) displayed K_d = 89 nM and 48 nM by ITC for SMARCA4 and PB1(5), has shown cellular activity in a SMARCA2 FRAP assay, and has shown selectivity by DSF over a wide range of other bromodomains.

1.5.4 Targeting the ATAD2 bromodomain

Overexpression of AAA domain-containing protein 2 (ATAD2), a co-activator of transcription factors such as MYC, has been linked with a poor prognosis in numerous types of cancer.[126] An NMR screen of fragments carried out by Harner *et al*. identified 65 fragments hits for ATAD2, of which 12 bind with millimolar K_d values.

1.5.5 OF-1 binds to the BRPF bromodomain family

Another non-BET bromodomain probe OF-1, of undisclosed structure, has been released by the SGC, for the bromodomain and plant homeodomain (PHD) finger containing (BRPF) family.[117] The BRPF family plays a pivotal role in the

assembly and HAT activity of monocytic leukaemia zinc finger protein (MOZ)/MOZ-related factor (MORF) complexes.[127] These complexes are essential to development, and are linked with leukaemia.[128] OF-1 binds with good selectivity to BRPF1B and BRPF2 with K_d = 100 nM and 500 nM respectively, as determined by ITC. Cellular activity was observed in a BRPF2 FRAP assay, but modest general cytotoxicity was observed. A potent and selective probe for BRPF1 was also discovered by GSK, who screened 1,3-dimethyl benzimidazolones against BRPF1 and BRD4, looking for selective ligands.[129] **1.67** displayed potent binding to BRPF1 with IC_{50} = 79 nM as measured by FRET, and surprisingly showed at least two logarithmic units of selectivity over BRPF2 and BRPF3, despite their highly conserved KAc-recognition pockets. Furthermore, **1.67** displayed three logarithmic units of selectivity over the BET family, as well as excellent selectivity over other bromodomains, and displayed cellular activity by displacing BRPF1 from chromatin in a dose-dependent manner.

1.5.6 Pan-bromodomain ligands

Work was carried out by the SGC to establish whether the core scaffold of (+)-JQ1 (**1.3**) and I-BET762 (**1.2**) could be manipulated to favour binding to non-BET bromodomains instead.[114] Screening commercially available triazolo compounds against 17 bromodomains using DSF identified that [1,2,4]triazolo[4,3-a]phthalazines can preferentially bind to some bromodomains over others, namely cat eye syndrome critical region 2 (CECR2), a chromatin remodelling protein, BRD4(1), CREBBP, BRD9, a protein linked with cancer, and TAF1L(2). Sub-micromolar IC_{50} values, as determined by AlphaScreen™, were obtained for previously untargeted BRD9 and CECR2. For example, **1.68** showed nanomolar IC_{50} values for BRD4(1), BRD9 and CREBBP. Co-crystallisation in BRD4(1) and BRD9 displayed the expected

hydrogen-bonding interactions with the highly conserved asparagines (N140 and N100 respectively). This derivative, and analogues, displayed cellular disruption of CREBBP binding to chromatin in a FRAP assay. A structurally similar analogue, bromosporin (**1.69**) was announced as a pan-bromodomain ligand by the SGC.[118] Bromosporin (**1.69**) showed > 3 °C melting temperature stabilisation for the BET family, CECR2, TAF1(2), BRD9 and CREBBP as seen by DSF, and has displayed cellular activity for BRD4, CREBBP, transcription intermediary factor 1α (TIF1α), BAZ2A and SMARCA2, but has also displayed cytotoxicity in HeLa cells.

1.6 Summary and conclusions

In summary, in recent years, with the precedented ligandability of bromodomains, and emergence of several bromodomain ligands in clinical trials for numerous diseases, multiple groups have gained an interest in the development of bromodomain ligands. Work has focused mainly on the BET family of bromodomains, primarily BRD4, which plays a pivotal role in elongation of Pol II due to its localisation at super-enhancers. BET bromodomains are associated with a wide range of diseases, and the therapeutic potential of targeting these proteins will be established through the development of novel, selective ligands with suitable pharmacokinetic properties for *in vivo* studies and ultimately with further clinical trials. Research is also focussing on developing non-BET bromodomain ligands, to elucidate their biological mechanisms and therapeutic potential. A handful of non-BET bromodomains have been targeted, but plenty remain to be studied. Importantly, due to the diverse phenotypic responses caused by BET bromodomains, selectivity for non-BET bromodomains needs to be extremely high in order to definitively associate inhibition of non-BET bromodomain interactions with particular phenotypes. What has been made clear, however, is that small chemical molecules are an effective tool for

probing bromodomain biology, and will undoubtedly lead to a better understanding of the interactions and roles of these domains as even more research groups gain interest in this area.

1.7 Aims

The aim of the work described in this dissertation was to develop ligands for bromodomains, to probe their interactions with histones. Specifically, we aimed to optimise a nanomolar BET bromodomain ligand, OXFBD02 (**1.6**), developed by D. Hewings in the Conway group, and also to use a fragment-based approach to develop small molecules to target non-BET bromodomains. Furthermore, we aimed to assess the interactions of bromodomains with acylated lysines other than KAc, by synthesising naturally and unnaturally occurring modified lysines, to better understand the binding and selectivity within the bromodomain recognition pocket.

Chapter 2: Optimising *In Vivo* Probes for the BET Bromodomains

This chapter describes work carried out to optimise a BET bromodomain ligand, developed within the Conway group, for use in in vivo models. This ligand has shown antiproliferative activity in a range of cancer cell lines and disrupts the interaction of BET bromodomains with non-histone partners.

2.1 Introduction and aims

2.1.1 OXFBD02 displays antiproliferative activity

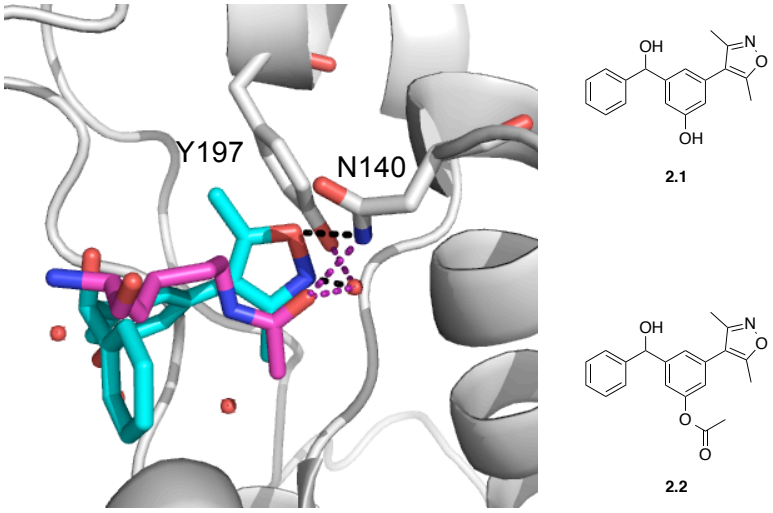
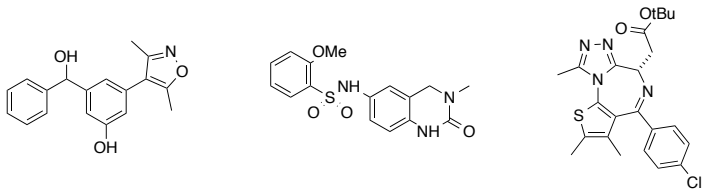


Figure 2.1. BRD4(1) in complex with OXFBD02 (**2.1**) (PDB 4J0R, carbon = cyan) overlaid with BRD4(1) in complex with H4K8AcK12Ac (PDB 3UW9, carbon = magenta).[12,46] The 3,5-dimethylisoxazole moiety of OXFBD02 mimics KAc by forming a direct hydrogen-bonding interaction with N140 and a water-mediated interaction with Y97.

Work carried out by D. Hewings in the Conway group, and by others, has shown that 3,5-dimethylisoxazoles act as an excellent acetyl-lysine mimic, forming hydrogen-bonding interactions with the highly conserved asparagine residue (N140

in BRD4(1)), and a water-mediated interaction with the highly conserved tyrosine residue (Y97 in BRD4(1)) in BET bromodomains (Figure 2.1).[47,62,63]

The series developed within the Conway group displayed nanomolar IC_{50} values (OXFBD02 (**2.1**): 382 nM; OXFBD03 (**2.2**): 371 nM) for the BET family using an *in vitro* peptide-displacement assay, and showed ~3-fold selectivity for BRD4 over CREBBP (IC_{50} = 771-1100 nM).[46] With the encouraging *in vitro* binding data in hand, work was carried out to determine whether the series would show the expected phenotypic response in cells. Inhibitors of the KAc-BET bromodomain interaction have shown antiproliferative activity in haematological malignancies, including acute myeloid leukaemia. Treatment of the MV4;11 acute myeloid leukaemia cell line with OXFBD02 (**2.1**) and OXFBD03 (**2.2**) displayed toxicity, with IC_{50} values of 794 nM and 616 nM, respectively. Conversely, treatment of U2OS and HeLa cell lines, which have been shown to be less sensitive, over the same time frame, to KAc-BET bromodomain interaction inhibition, with the same series showed no cytotoxicity. Furthermore, a weak inhibitor of the KAc-BRD4 interaction also showed a weak cellular IC_{50} (*in vitro* IC_{50} = 2.6 μM; cellular IC_{50} = 2.9 μM). These data suggest that the toxic effects of OXFBD02 (**2.1**) and OXFBD03 (**2.2**) in the MV4;11 cell line might be due to bromodomain-binding.



2.1
BRD4(1) IC_{50} = 382 nM
(*AlphaScreen*)

2.3
BRD4(1) IC_{50} = 220 nM
(AlphaScreen)

2.4
BRD4(1) IC_{50} = 77 nM
(AlphaScreen)

Figure 2.2. OXFBD02 (**2.1**), PFI-1 (**2.3**) and (+)-JQ1 (**2.4**).

With this pleasing data in hand, OXFBD02 (**2.1**) was submitted for testing at a single dose against the NCI-60 human cancer cell line screen, to further evaluate its antiproliferative effects.[130] Greater than 50% reduction in proliferation was observed

for all cell lines, and approximately 50% cell death was observed for two cancer cell lines at this single dose. With these encouraging results in hand, further experiments were carried out at different concentrations of the phenol, in order to calculate GI_{50} values, the concentrations at which growth inhibition is 50%. In accordance with other BET bromodomain ligands, OXFBD02 (**2.1**) showed particular activity in leukaemia cell lines, as well as breast and renal cancer cell lines. Calculation of the GI_{50} values allowed the comparison of OXFBD02 (**2.1**) with PFI-1 (**2.3**, Figure 2.2), a BET-selective ligand that the SGC have NCI-60 panel data for. As well as being more selective than OXFBD02 (**2.1**), PFI-1 (**2.3**) has a very different chemotype, but displays a similar profile in the NCI-60 screen, suggesting that the activity seen by OXFBD02 (**2.1**) is specifically due to binding to the BET bromodomains.

2.1.2 OXFBD02 (**2.1**) disrupts the interaction of BRD4 with non-histone partners

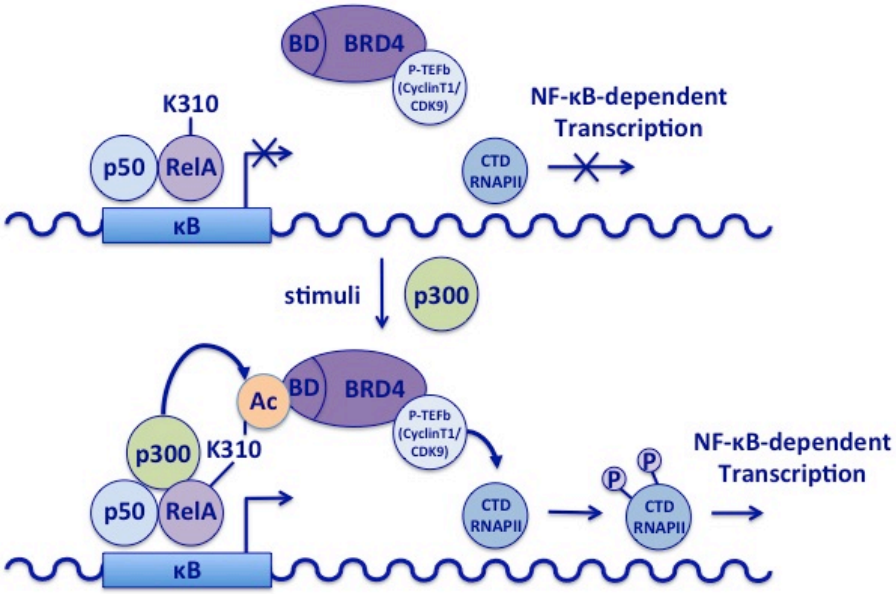


Figure 2.3. Binding of BRD4 to the RelA subunit activates NF-κB-dependent transcription. Figure adapted with permission from Huang, B.; Yang, X.-D.; Zhou, M.-M.; Ozato, K.; Chen, L.-F.; *Molecular and Cellular Biology* **2009**, *29*, 1475. Copyright 2009 American Society for Microbiology.

In addition to binding acetylated histone proteins, BET bromodomains also bind to non-histone partners, including transcription factors. For example, binding of BRD4 to the RelA subunit of NF-κB enhances transcriptional activation of NF-κB-dependent genes (Figure 2.3).[24,131-133] This interaction is brought about by acetylation of K310 of RelA, which allows binding to the bromodomains of BRD4. This in turn permits recruitment of cyclin-dependent kinase 9 (CDK9), which phosphorylates polymerase II, resulting in NF-κB-dependent gene expression.

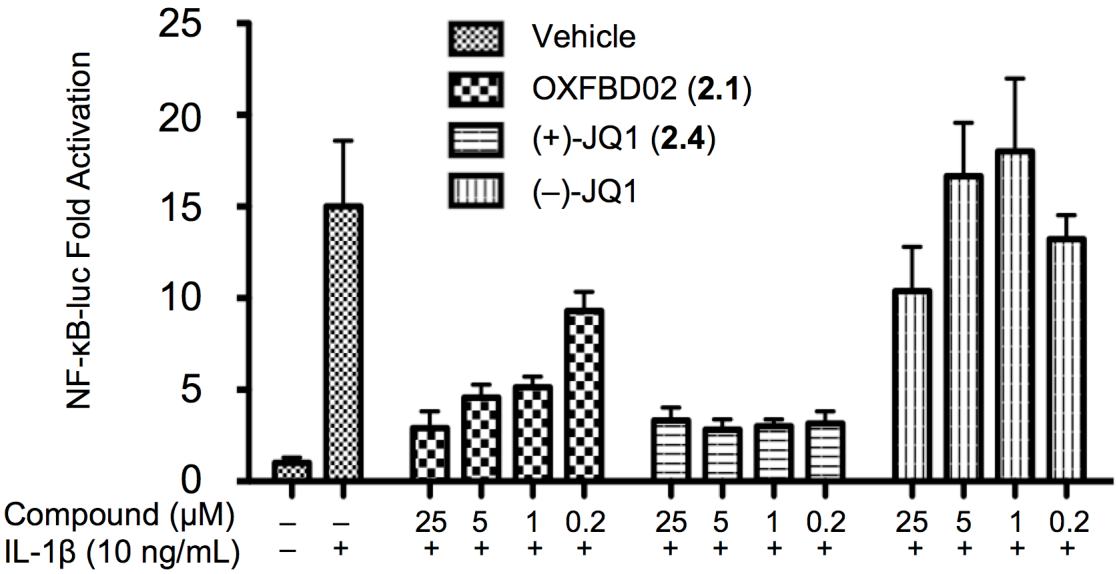


Figure 2.4. Preliminary data from luciferase reporter assay developed in the Professor Anna Mapp lab, to assess the effect of compounds on NF-κB-dependent gene expression. HeLa cells were transiently transfected with a reporter plasmid containing luciferase and five κB binding sites. The cells were treated with a DMSO-treated control (–), or different concentrations (0.2, 1, 5 and 25 μM) of OXFBD02 (2.1), (+)-JQ1 (**2.4**) and (–)-JQ1 for 12 hours before stimulation with IL-1β (+). The gene expression without stimulation of IL-1β (–) was also measured for the DMSO-treated control. Results were obtained in quadruplicate and averaged, with error bars signifying standard deviation. OXFBD02 (**2.1**) shows a dose-dependent affect on transcription.

Disruption of the RelA-BRD4 interaction with the BET ligand (+)-JQ1 (**2.4**) suppresses NF-κB-dependent transcription, as shown by the preliminary results of a luciferase reporter assay developed within the Professor Mapp lab (Figure 2.4). This assay allows the effects of bromodomain binding on NF-κB-dependent transcription following IL-1β stimulation to be assessed. The enantiomer (–)-JQ1 that does not bind to BET bromodomains did not suppress gene expression, whereas OXFBD02 (**2.1**) showed dose-dependent suppression of gene expression. Therefore, OXFBD02 (**2.1**) also disrupts the interaction of BET bromodomains with non-histone partners. As such, we believe this is an extremely interesting series to take forward, ultimately into *in vivo* models.

2.1.3 Predicted Metabolic Liabilities of OXFBD02 (**2.1**)

To assess whether the series was suitable for use in *in vivo* models, the effective lipophilicity at physiological pH, or chromatographic $LogD_{pH7.4}$ ($ChromLogD_{pH7.4}$) for OXFBD02 (**2.1**) was predicted using an in-house calculator at GlaxoSmithKline (GSK) (version 6).[134] Unlike the intrinsic lipophilicity (P) of a molecule, which only refers to the ratio of the unionised form in octanol and water, the distribution coefficient (D) gives the equilibrium of the molecule between octanol and water, under the degree of ionisation corresponding to the specified pH. As such, $ChromLogD_{pH7.4}$ values are a useful metric when considering the lipophilicity of drug molecules with ionisable atoms. Compounds with $ChromLogD_{pH7.4} < 4$ often have more reasonable pharmacokinetic properties than those with $ChromLogD_{pH7.4} > 4$, however compounds that are too hydrophilic are likely to suffer from poor permeability. Therefore, $ChromLogD_{pH7.4}$ can only be used to reliably compare compounds within the same physicochemical space.

In combination with the $ChromLogD_{ph7.4}$ value, the property forecast index (PFI = $ChromLogD_{pH7.4}$ + number of aromatic rings (#Ar)) was also used to indicate

the probability of good outcomes in developability assays (Table 2.1). PFI values < 5 suggest good solubility, weaker binding to human serum albumin, less metabolism by cytochrome p450 enzymes and slower intrinsic clearance, however the more hydrophilic nature of such compounds results in poorer permeation of biological membranes, resulting in less absorption and cell penetration than more lipophilic molecules. On the other hand, PFI > 5 often highlights issues with solubility, binding to human serum albumin, metabolism by cytochrome p450 enzymes, and fast intrinsic clearance, for example, and so a balance needs to be met between strong affinity and optimum drug-like parameters, including PFI values.

Assay/Target Value	< 3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	> 10
	PFI = $ChromLogD_{pH7.4}$ + #Ar								
Solubility > 200 µM	89	83	72	58	33	13	5	3	2
% Human Serum Albumin < 95%	88	80	74	64	50	30	17	8	4
Cytochrome P450 2C9 pIC_{50} < 5	97	90	83	68	48	32	23	22	38
Cytochrome P450 2C19 pIC_{50} < 5	97	95	91	82	67	52	42	42	56
Cytochrome P450 3A4 pIC_{50} < 5	92	83	80	75	67	60	58	61	66
Intrinsic Clearance < 3 mL/min/kg	79	76	68	61	54	42	41	39	52
Permeability > 200 nm/s	20	30	46	65	74	77	65	50	33

Table 2.1. Percentages of compounds achieving defined target values in the various developability assays categorised by PFI bins. Colouring refers to the percentage chance of achieving the benchmark value in that PFI bin: green, > 67%; yellow, 33–67%; and red, < 33%. Table adapted with permission from Young, R.; Green, D.; Luscombe, C.; Hill, A.; *Drug Discovery Today* **2011**, *16*, 822. Copyright 2011 Elsevier.

Commonly highlighted in medicinal chemistry is the importance of developing compounds that are as hydrophilic as possible, without losing affinity or cell

penetration. OXFBD02 (**2.1**) has a sub-optimal predicted ChromLog$D_{pH7.4}$ value of 3.7, and PFI value of 6.7, indicating that it would probably be metabolically unstable in *in vivo* models, and therefore, optimisation of the pharmacokinetic properties of this series is presumably required. To predict sites of metabolic degradation, Metasite 3.1.2 (phase I metabolism) and Meteor 2.0.2 (phase I and II metabolism) were used in order to direct compound design for more suitable *in vivo* probes (Figure 2.5). Metasite is an automated docking model that predicts a compound's most likely sites of reactivity in phase I metabolism, by predicting the interactions of a molecule's individual atoms, with the enzymatic active sites.[135,136] Conversely, Meteor is a rule-based, predictive method for phase I and II metabolism. It compares a molecule's substructures with those in its knowledge base. Using its rules, it then predicts and ranks the likelihood of reactivity for the molecule or a metabolite. The most vulnerable position was predicted to be the *para*-position of the monosubstituted aromatic ring, which could undergo hydroxylation. The next most susceptible positions are the methyl carbons of the isoxazole, which can also undergo hydroxylation followed by oxidation of the resulting primary alcohols. Other, less reactive, metabolic predictions include *ortho*-hydroxylation of the monosubstituted aromatic, oxidation of the secondary alcohol, *N*-oxidation of the isoxazole, and *O*-glucuronidation or *O*-sulfation of the free phenol.

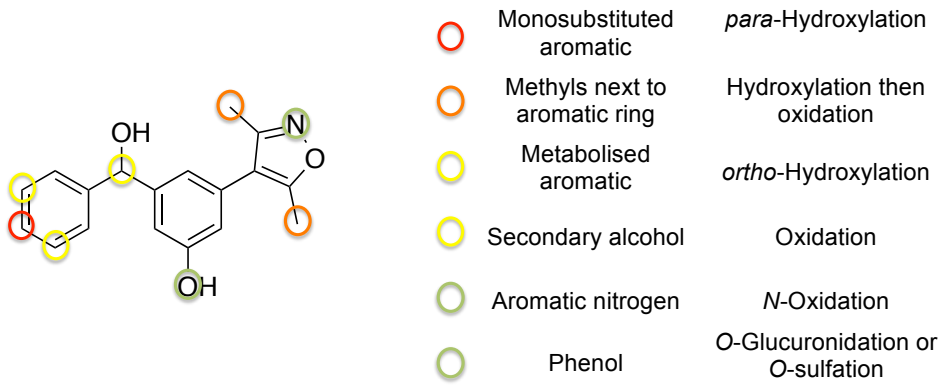


Figure 2.5. Metasite 3.1.2 (phase I) and Meteor 2.0.2 (phase I and II) predictions of metabolic liabilities of OXFBD02 (**2.1**), ranked in order of decreasing reactivity from red to green.

During the optimisation of any series, it is important to analyse various physicochemical parameters in detail, to assess suitability of compounds as probe molecules. The physicochemical parameters that we are particularly interested in, for OXFBD02 (**2.1**) and (+)-JQ1 (**2.4**), are shown in Table 2.2. One definition of ligand efficiency (LE) is the binding affinity per heavy atom (non-hydrogen atoms), which allows a better comparison of affinity between molecules of different size. As we can see, the larger size of (+)-JQ1 (**2.4**) means that its LE is lower than that of OXFBD02 (**2.1**). Maintaining affinity and the small size of this series will be important in optimisation of the series. Another important physicochemical parameter is lipophilic ligand efficiency (LLE), which also takes account of lipophilicity. As medicinal chemists intend to move away from compound "obesity", it is important to develop compounds that are as polar as possible, while maintaining affinity. As such, large LLE values are important, and the LLE value for OXFBD02 (**2.1**) is almost 2-fold that of (+)-JQ1 (**2.4**), due to its larger lipophilicity. Some other important parameters to consider fall within Lipinski's rules of five.[137] These rules allow good absorption and permeation, and suggest that drug-like compounds should have clogP < 5, relative molecular mass < 500, number of hydrogen bond donors < 5 and number of hydrogen bond acceptors < 10. Both OXFBD02 (**2.1**) and (+)-JQ1 (**2.4**) lie within all of Lipinski's rules of 5. In addition, to ensure that compounds will not suffer from permeability issues, they should have topological polar surface area (TPSA) < 40 $Å^2$. Both OXFBD02 (**2.1**) and (+)-JQ1 (**2.4**) have acceptable TPSA values. However, as well as considering these physicochemical properties, it is also crucial to consider other factors, such as avoiding reactive and therefore metabolically liable functionality, when developing drug-like or probe molecules.

Parameters	OXFBD02 **2.1**	(+)-JQ1 **2.4**
IC_{50} *vs* BRD4(1) / nM	97.5	77[C]
pIC_{50}	7.01	7.11
Heavy atom count (HAC)	22	31
cLogP[A]	2.53	4.48
LE	0.45	0.32
LLE	4.5	2.6
RMM	295	456
H-bond donors	2	1
H-bond acceptors	4	6
TPSA[B] / Å^2	66.49	97.09
$ChromLogD_{pH7.4}$	3.7	7.1
#Ar rings	3	3
PFI	6.7	10.1

Table 2.2. Parameters for OXFBD02 (**2.1**) and (+)-JQ1 (**2.4**). (A) cLogP was calculated using ACD/Labs algorithm (version C10E41); LE = (pIC_{50} × 1.4 kcal/mol)/HAC; LLE = pIC_{50} – clogP; PFI = $clogD_{pH7.4}$ + #Ar rings. (B) TPSA values were calculated using ACD/I-Lab (version 5.0.0.184). (C) The published IC_{50} of (+)-JQ1 (**2.4**) was not obtained on the same AlphaScreen™ plate as OXFBD02 (**2.1**), therefore we must be cautious when comparing these values.

As mentioned earlier, $ChromLogD_{pH7.4}$ values < 4 could have reasonable pharmacokinetic properties. Unlike OXFBD02 (**2.1**), which has a $ChromLogD_{pH7.4}$ value just below this limit, (+)-JQ1 (**2.4**) suffers from a high value of 7.1. As such, the corresponding PFI value of 10.1 is much worse for (+)-JQ1 (**2.4**) than that for OXFBD02 (**2.1**), suggesting that (+)-JQ1 (**2.4**) could have, for example, poorer solubility, faster metabolism by cytochrome p450 enzymes and faster clearance *in vivo*. This prediction is in line with a poor bioavailability and high clearance observed in animal models.[138]

2.1.4 Aims

The aim of the work described in this chapter was to optimise OXFBD02 (**2.1**) to improve its binding affinity for the BET bromodomains, while also making it more

suitable for use *in vivo*. We aimed to explore the effects of different functionality in OXFBD02 (**2.1**) analogues, and to use predictive metabolism software and validation with microsomal stability assays to guide the optimisation of OXFBD02 (**2.1**).

2.1.5 Hypothesis

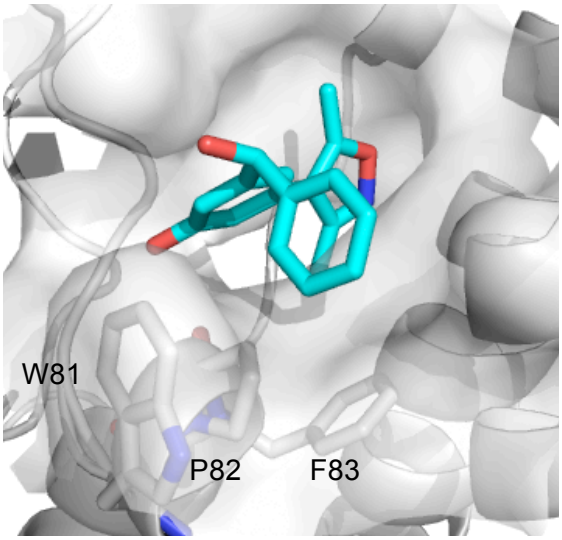
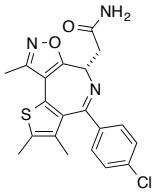


Figure 2.6. The monosubstituted aromatic ring of OXFBD02 (**2.1**, carbon = cyan) lies on the WPF shelf of the BET bromodomains, made up of W81, P82 and F83 in BRD4(1) (PDB 4J0R).[46]

When binding to the BET bromodomains, the monosubstituted aromatic ring lies on a lipophilic portion of the protein, known as the WPF shelf (Figure 2.6). The prediction that the unsubstituted *para*-position of this functionality is the most susceptible to metabolic degradation, led to the hypothesis that the physical and pharmacokinetic properties of this series of compounds could be optimised by including different functionality on the WPF shelf. It was envisaged that a collection of analogues could be easily prepared by functionalisation of the key aldehyde that can be used to prepare OXFBD02 (**2.1**).



2.5

Figure 2.7. Isoxazole (**2.5**) that was developed by Gehling *et al*.[48]

In the development of an isoxazole analogue of (+)-JQ1 (**2.4**), **2.5** (Figure 2.7), by Gehling *et al*., modifications to the 4-chlorophenyl substituent were carried out in attempts to modulate the biophysical and structural properties of their analogues, while maintaining affinity.[48] Their collection of analogues included the corresponding *ortho*- and *meta*-chloro isomers. The *meta*- and *para*-chloro analogues were similarly potent, but the *ortho*-chloro analogue was 10-fold less potent. The authors claimed that this was due to an unfavourable electrostatic interaction, rather than a steric clash, because the affinity was maintained for an *ortho*-methyl-*para*-chloro analogue. A *para*-nitrile analogue was also included in their collection, which displayed very similar affinity to the *para*-chloro analogue. An unsubstituted phenyl analogue, resembling that of OXFBD02 (**2.1**), was also included, which showed very similar affinity to the *para*-chloro analogue. Their collection also included more hydrophilic substituents, with 4-chloro-2-pyridyl and 4-amino-3-pyridyl derivatives, both of which showed similar affinity to their *para*-chloro derivative. An additional hydrophilic analogue in their collection, a pyrimidine derivative, was not tolerated by BRD4(1). Furthermore, they exchanged the *para*-chloro aromatic ring for the saturated systems morpholine, tetrahydropyran and an acyclic propyl group, although none of these analogues were tolerated on the WPF shelf. This portfolio of analogues guided the design of our collection of analogues, in order for us to establish structure-activity relationships that will aid further optimisation of our series of compounds.

2.2 Analogues of OXFBD02 (2.1)

2.2.1 Desired analogues of OXFBD02 (**2.1**)

Primarily, blocking the *para*-position with metabolically stable functionality was predicted to be a facile way to overcome the issue of its oxidation. This hypothesis, in combination with literature precedent suggesting that *para*-electron withdrawing groups are favourable for binding to the BET bromodomains, with the *para*-chloro functionality of I-BET762, (+)-JQ1 (**2.4**), MS417 (**2.6**), CPI-206 (**2.7**) and **2.5** (Figure 2.8), led to the design of a number of analogues with electron-withdrawing functionality in the *para*-position (Figure 2.9, **2.11**-**2.13**).[41,42,44,48]

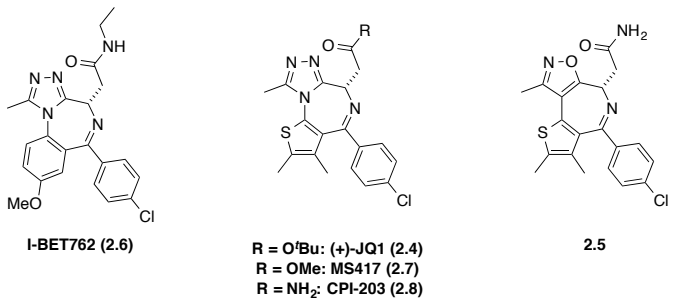


Figure 2.8. Literature precedent for *para*-electron withdrawing groups on the WPF shelf.

To further assess the effect of electronics, on both affinity and metabolic stability, some compounds with a fluorine substituent in the *ortho*- and *meta*-positions were also included (**2.9** & **2.10**), as were electron-donating groups primarily in the *para*-position, but also in the *ortho*- and *meta*-positions for anisole derivatives (**2.14**-**2.18**). The fluoro, anisole and tolyl substituents of **2.9**-**2.11** & **2.14**-**2.17** were not included in the analogue collection of Gehling *et al*. but we felt these derivatives would allow us to draw more conclusions about structure-activity relationships of this series of molecules.[48]

In an attempt to optimise the physical properties of our series of molecules, more hydrophilic and solubilising functionalities were also considered (**2.19**-**2.24**). We

included the 4-chloro-2-pyridyl and 4-amino-3-pyridyl substituents of the analogue collection of Gehling *et al*. as well as unsubstituted pyridyl derivatives.

Furthermore, to assess the importance of having an sp^2 centre on the WPF shelf, and to establish the necessity of the secondary alcohol, the tetrahydropyran derivative included in the analogue collection of Gehling *et al*., as well as cyclohexyl, cyclopropyl and methylene derivatives were also included in our analogue collection (**2.25**-**2.28**).

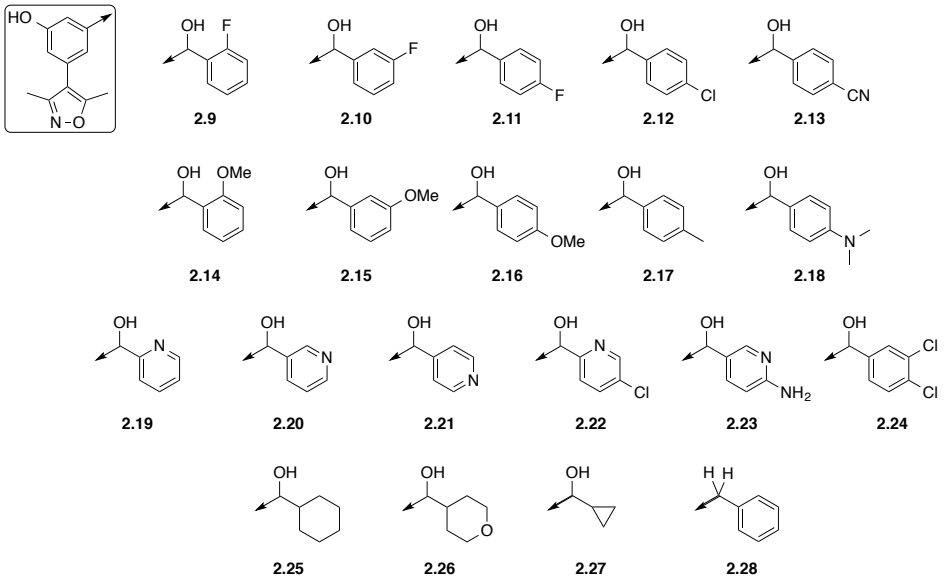
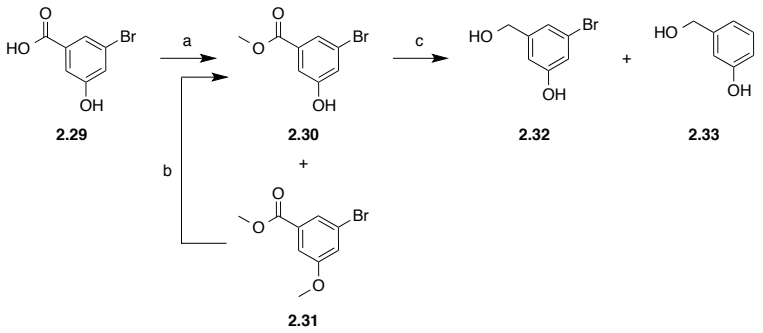


Figure 2.9. Initial target set of derivatives of OXFBD02 (**2.1**).

2.2.2 Synthesis of analogue collection

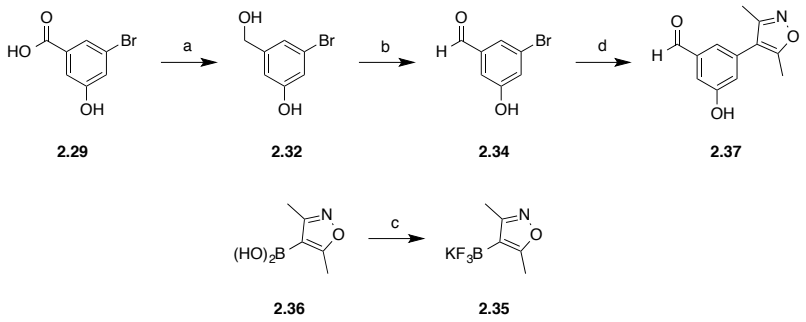
The key aldehyde with which to synthesise this collection was prepared using the protocol of D. Hewings.[139] Initially, the published procedure, whereby commercially available 3-bromo-5-hydroxybenzoic acid **2.29** was converted to the methyl ester, followed by reduction with lithium aluminium hydride, was attempted.[46] Heating **2.29** under reflux in sulfuric acid and methanol afforded the desired intermediate **2.30** in 35% yield, and the side-product methyl ether **2.31** in 26% yield (Scheme 2.1). Fortunately the side-product (**2.31**) could be converted to the desired

intermediate (**2.30**) in 70% yield by dealkylation with BBr_3, however, reduction of **2.30** to the desired alcohol **2.32** also resulted in a debrominated side-product (**2.33**) that could not be separated from the desired brominated product (**2.32**).



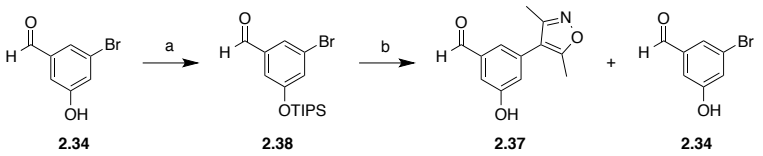
Scheme 2.1. Attempted synthesis of primary alcohol **2.32**. *Reagents and conditions:* (a) H_2SO_4, MeOH, 70 °C, 71 h, 35% **2.30** and 26% **2.31**; (b) BBr_3, CH_2Cl_2, –78 °C-rt, 40 h, 70%; (c) $LiAlH_4$, THF, 80 °C-rt, 18 h.

Fortunately, further work by D. Hewings had simplified the conversion of benzoic acid **2.29** to the desired alcohol (**2.32**) to one step, by reduction of **2.29** with BH_3•THF. Following this protocol, and subsequent oxidation to the aldehyde (**2.34**), using MnO_2, followed by cross-coupling with the stable trifluoroborate derivative (**2.35**) of the commercially available boronic acid **2.36**, afforded desired aldehyde **2.37** (Scheme 2.2). The trifluoroborate (**2.35**) was synthesised using the procedure of Molander *et al*., whereby the boronic acid **2.36** was stirred with potassium hydrogenfluoride and the resulting product was isolated using Soxhlet extraction.[140]



Scheme 2.2. Synthesis of key aldehyde **2.37**. *Reagents and conditions:* (a) BH_3•THF, THF, 0 °C-rt, 44 h, 96%; (b) MnO_2, CH_2Cl_2, acetone, rt, 43 h, 55%; (c) KHF_2, MeOH, H_2O, rt, 10 min, 81%; (d) **2.35**, $Pd(OAc)_2$, RuPhos, Na_2CO_3, EtOH, reflux, 18 h, 55%.

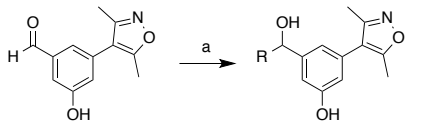
To establish whether the aldehyde (**2.37**) could be synthesised in fewer steps, C–H activation of the commercially available starting material 3-bromo-5-hydroxybenzoic acid (**2.29**) was attempted. Unfortunately, subjecting **2.29** to the conditions of Fall *et al*., who used C–H activation to incorporate isoxazoles into aryl bromides, failed to give the desired product.[141] However, using the same conditions on the TIPS-protected aldehyde (**2.38**) produced the unprotected desired aldehyde (**2.37**), as well as unprotected starting material **2.34** (Scheme 2.3). This suggests that the conditions are unsuitable for TIPS-protection, and that protection of the phenol is unnecessary for the reaction to work.



Scheme 2.3. C–H activation to produce aldehyde **2.37**. *Reagents and conditions:* (a) TIPSCl, DMF, 0 °C-rt, 22 h, 84%; (b) 3,5-Dimethylisoxazole, $PdCl_2$, AcOK, DMAc, 130 °C, 25 h, 52% **3.37** and 15% **2.34**.

With aldehyde **2.37** in hand, a range of functionalisation reactions including addition of alkyl lithium reagents, Grignard reagents, and reductions furnished the desired collection of analogues **2.9**-**2.22**, **2.24**, **2.25**, **2.27** & **2.28**. OXFBD02 (**2.1**) and

a range of its derivatives (**2.12**, **2.14**-**2.17** & **2.25)** could be obtained in one step, by addition of commercially available Grignard reagents phenylmagnesium bromide, 2-, 3- and 4-methoxyphenylmagnesium bromides, *para*-tolylmagnesium bromide, 4-chlorophenylmagnesium bromide and cyclohexylmagnesium bromide to the unprotected aldehyde (**2.37**), and stirring at rt (Scheme 2.4). However, more forcing conditions were required for the addition of 3,4-dichlorophenylmagnesium bromide to aldehyde **2.37**, with heating to 50 °C necessary for complete consumption of the aldehyde to furnish **2.24**. Notably, the oxidised, benzophenone side-products of the 4-chlorophenyl, 4-methoxyphenyl and 3,4-dichlorophenyl derivatives (**2.12**, **2.16** & **2.24**) were observed by LCMS during the reactions.



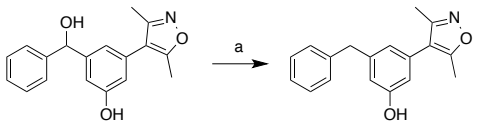
2.37

2.1: R = Phenyl
2.14: R = 2-Methoxyphenyl
2.15: R = 3-Methoxyphenyl
2.16: R = 4-Methoxyphenyl
2.17: R = 4-Tolyl
2.12: R = 4-Chlorophenyl
2.24: R = 3,4-Dichlorophenyl
2.25: R = Cyclohexyl

Scheme 2.4. Addition of commercially available Grignard reagents to unprotected aldehyde **2.37**. *Reagents and conditions:* (a) R = Phenyl: Phenylmagnesium bromide, THF, rt, 4 h, 40%; R = 2-Methoxyphenyl: 2-Methoxyphenylmagnesium bromide, THF, rt, 3 h, 80%; R = 3-Methoxyphenyl: 3-Methoxyphenylmagnesium bromide, THF, rt, 5 h, 68%; R = 4-Methoxyphenyl: 4-Methoxyphenylmagnesium bromide, THF, rt, 3 h, 68%; R = 4-Tolyl: *para*-Tolylmagnesium bromide, THF, rt, 18 h, 56%; R = 4-Chlorophenyl: 4-Chlorophenylmagnesium bromide, Et_2O, THF, rt, 19 h, 51%; R = 3,4-Dichlorophenyl: 3,4-Dichlorophenylmagnesium bromide, THF, rt-50 °C, 29 h, 11%; R = Cyclohexyl: Cyclohexylmagnesium chloride, Et_2O, THF, rt, 26%.

The methylene derivative **2.28** was synthesised by D. Hewings, by treating OXFBD02 (**2.1**) with TFA and Et_3SiH to reduce the secondary alcohol (Scheme 2.5). The desired dimethylaniline derivative (**2.18**) was synthesised using 4-dimethylaminophenylmagnesium bromide, however this product proved to be

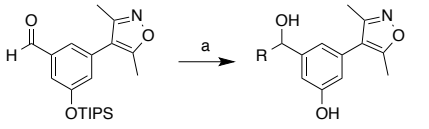
unstable, and could not be isolated, so was removed from the set of analogues to be tested. This instability is perhaps due to the aniline p-orbital lone pair facilitating elimination of the secondary alcohol, however this was not observed for the anisole derivatives, presumably due to the more electronegative nature of the oxygen atom.



2.1 **2.28**

Scheme 2.5. Reduction of OXFBD02 (**2.1**) to methylene derivative **2.28**, carried out by D. Hewings. *Reagents and conditions:* (a) Et_3SiH, TFA, rt, 15 min, 70%.

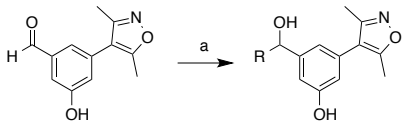
In the absence of appropriate commercially available Grignard reagents, other derivatives could be acquired using metal-halogen exchange. TIPS-protection of the phenol improved the electrophilicity of the aldehyde, allowing organolithium reagents to be used at low temperatures. Lithium-halogen exchange of 1-bromo-2-fluorobenzene, carried out by A. Hicks, using ^{n}BuLi in THF at –78 °C, followed by addition of TIPS-protected aldehyde **2.38** and subsequent TIPS-deprotection with TBAF afforded desired 2-fluorophenyl derivative **2.9** (Scheme 2.6). However, lithium-halogen exchange of 2-bromopyridine using ^{n}BuLi, carried out by D. Hewings, required more forcing conditions. Replacing THF with Et_2O allowed the reaction to be warmed to rt to push the lithium-halogen exchange to occur. With the desired organolithium reagent produced *in situ*, re-cooling to –78 °C allowed addition of the TIPS-protected aldehyde, then TIPS-deprotection with TBAF furnished the 2-pyridyl derivative (**2.19**).



2.38 **2.9:** R = 2-Fluorophenyl
2.19: R = 2-Pyridyl

Scheme 2.6. Organolithium reactions to generate analogues, carried out by A. Hicks and D. Hewings. *Reagents and conditions:* (a) R = 2-Fluorophenyl: i) 1-Bromo-2-fluorobenzene, ^{n}BuLi, THF, –78 °C, 40 min then **2.38**, –78 °C, 3h; ii) TBAF, THF, 0 °C, 2 h, 17%; R = 2-Pyridyl: i) 2-Bromopyridine, ^{n}BuLi, Et_2O, –78 °C-rt, 2 h then **2.38**, –78 °C, 3 h; ii) TBAF, THF, 0 °C, 16 h, 54%.

A number of derivatives could be produced by treating aldehyde **2.37** with the appropriate Grignard reagent generated *in situ*, either by magnesium-insertion into carbon-halogen bonds or by magnesium-halogen exchange. Magnesium-insertion into 1-bromo-4-fluorobenzene, carried out by A. Hicks, and into bromocyclopropane, produced the required Grignard reagents *in situ* to synthesise the desired 4-fluorophenyl and cyclopropyl derivatives (**2.11** & **2.27**, Scheme 2.7).

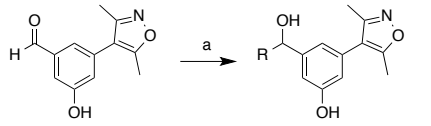


2.37 **2.11:** R = 4-Fluorophenyl
2.27: R = Cyclopropyl

Scheme 2.7. Magnesium-insertion reactions to generate analogues **2.11** and **2.27**. *Reagents and conditions:* (a) R = 2-Fluorophenyl, carried out by A. Hicks: i) 1-Bromo-4-fluorobenzene, Mg, I_2, THF, reflux, 1.5 h; ii) **2.37**, 0 °C-rt, 2 h, 16%; R = Cyclopropyl: i) Bromocyclopropane, Mg, I_2, THF, reflux-rt, 30 min; ii) **2.37**, rt, 18 h, 85%.

Treatment of 4-iodopyridine with ethylmagnesium bromide did not result in the desired magnesium-halogen exchange required to generate the Grignard reagent. However, the desired Grignard reagent could be produced with ^{i}PrMgCl, allowing the 4-pyridyl derivative (**2.21**) to be synthesised (Scheme 2.8). For complete consumption of aldehyde **2.37**, this reaction required heating to 50 °C, and despite

full consumption, the desired product was afforded in a very poor yield of 9%, due to difficult purification. Using the same methodology, the 3-pyridyl derivative **2.20** was produced using 3-iodopyridine and ^{i}PrMgCl. This reaction required heating for 5 days, with a poor yield of the desired product **2.20**. In the same manner, ^{i}PrMgCl magnesium-halogen exchange of 1-fluoro-3-iodobenzene could be used to produce the 3-fluoro derivative (**2.10**) in poor yield, again heating to 50 °C after addition of the aldehyde was required.



2.37

2.20: R = 3-Pyridyl
2.21: R = 4-Pyridyl
2.10: R = 3-Fluorophenyl
2.13: R = 4-Cyanophenyl
2.22: R = 4-Chloro-2-pyridyl

Scheme 2.8. Magnesium-halogen exchange reactions to generate analogues. *Reagents and conditions:* (a) R = 3-Pyridyl: i) 3-Iodopyridine, ^{i}PrMgCl, THF, rt, 1 h; ii) **2.37**, rt-50 °C, 5 days, 8%; R = 4-Pyridyl: i) 4-Iodopyridine, ^{i}PrMgCl, THF, rt, 1 h; ii) **2.37**, rt-50 °C, 18 h, 9%; R = 3-Fluorophenyl: i) 1-Bromo-3-fluorobenzene, ^{i}PrMgCl, THF, –10 °C-rt, 5 h; ii) **2.37**, rt-50 °C, 24 h, 11%; R = 4-Cyanophenyl: i) 4-Iodobenzonitrile, ^{i}PrMgCl•LiCl, THF, –10 °C, 5 h; ii) **2.37**, –10 °C, 3 h, 23%; R = 4-Chloro-2-pyridyl: i) 5-Chloro-2-iodopyridine, ^{i}PrMgCl•LiCl, THF, rt, 2 h; ii) **2.37**, rt-50 °C, 49 h, 15%.

The magnesium-halogen exchange to afford the *para*-nitrile derivative **2.13** did not occur when 4-bromobenzonitrile was treated with ^{i}PrMgCl•LiCl. However, when this Grignard reagent was used with 4-iodobenzonitrile instead, the desired derivative (**2.13**) could be produced. Cold temperatures were maintained in this reaction to prevent any nucleophilic addition to the nitrile functionality (Figure 2.10). Likewise, treatment of 2-bromo-5-chloropyridine with ^{i}PrMgCl and with ^{i}PrMgCl•LiCl failed to afford magnesium-halogen exchange, however, treatment with 5-chloro-2-iodopyridine furnished the desired derivative (**2.22**) upon magnesium-halogen exchange with ^{i}PrMgCl•LiCl.

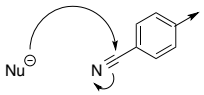
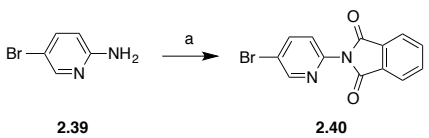


Figure 2.10. Nucleophilic attack at nitrile functionality.

One derivative for which magnesium-halogen exchange could not be achieved was for the attempted synthesis of the 4-amino-3-pyridyl derivative (**2.23**). Postulating that the unprotected aniline of 2-amino-5-bromopyridine poisons the Grignard reagent ^{i}PrMgCl, preventing magnesium-halogen exchange, the aniline (**2.39**) was protected with a phthalimide protecting group, by heating with phthalic anhydride, to give **2.40** (Scheme 2.9). However, magnesium-halogen exchange also failed to occur for protected derivative **2.40**. Lithium-halogen exchange was attempted instead with ^{n}BuLi in THF and the TIPS-protected aldehyde at –78 °C, however, even at this low temperature, TIPS deprotection occurred, to give the less electrophilic phenolate, and hence less-reactive aldehyde, with no desired product formation observed. Warming this reaction up to rt was attempted to force the nucleophilic attack at the aldehyde. Lithiated species are quenched with THF at rt, to yield extremely volatile acetaldehyde and ethene, and therefore Et_2O was used as the solvent instead. Despite the risk of phthalimide deprotection, only TIPS deprotection was observed, with no desired product formation. Despite several attempts, tetrahydropyran derivative **2.26** proved challenging to synthesise, and therefore the synthesis of this derivative was postponed until preliminary affinity data had been obtained.



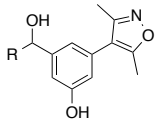
2.39 **2.40**

Scheme 2.9. Phthalimide protection of 2-amino-5-bromopyridine (**2.39**). *Reagents and conditions:* (a) Phthalic anhydride, 160 °C, 3 h, 45%.

2.2.3 *In vitro* affinity for BRD4(1)

Following synthesis of the desired collection of analogues, their affinity for BRD4 was measured using DSF, otherwise known as a thermal shift assay.[142] This assay allows the stabilisation of a protein conformation due to ligand binding to be determined, by measuring the change in melting temperature (ΔT_m) of the unbound protein to that of the ligand-bound protein. A fluorescent dye, such as SYPRO® Orange, is used to detect the melting temperature of the protein. When the protein is folded, the fluorescent dye is largely quenched by the surrounding water. Upon heating the protein unfolds, revealing hydrophobic regions, which allow the dye to bind non-specifically, thereby increasing the fluorescence. This allows a stability curve to be obtained, and its transition mid-point, the melting temperature (T_m), is used to measure the relative binding affinity of a ligand-bound protein compared to the unbound protein. This technique requires minimal protein, gives repeatable results and can be used for high-throughput screening, but the non-uniform effect of ligands upon melting of different proteins means that conclusions about relative binding affinities cannot be made accurately between thermal shift results for different proteins.

For reference, the parent compound OXFBD02 (**2.1**) has a Tm shift of 7.8 °C. In general, all compounds showed some stabilisation of BRD4(1). In corroboration with previous findings in the literature, electron-withdrawing groups, particularly in the *para*-position, were well tolerated (6.7-7.3 °C, Table 2.3). Conversely, electron-donating groups were not as well tolerated (5.3-6.1 °C). The more hydrophilic pyridyl compounds gave a wide range of thermal shifts (5.0-6.9 °C), with the 2-pyridyl compound (**2.19**) a significantly weaker binder than 4-pyridyl **2.21**. The solubilising 3,4-dichlorinated derivative **2.24** showed moderate stabilisation (6.5 °C). The cyclopropyl derivative (**2.27**) showed the lowest stabilisation of the collection of analogues (4.7 °C), but the compound displaying the highest protein stabilisation of



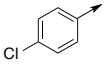
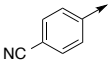
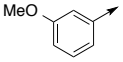
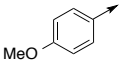
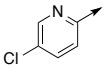
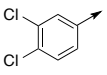
Compound Number	R	BRD4(1) ΔT_m (°C)
2.1		7.8
2.9		6.8
2.10		6.7
2.11		7.3
2.12		7.3
2.13		6.9
2.14		5.9
2.15		6.1
2.16		5.3
2.17		6.0
2.19		5.0
2.20		5.7
2.21		6.9
2.22		5.5
2.24		6.5
2.25		8.8
2.27		4.7

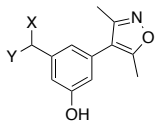
Table 2.3. DSF data obtained (N = 1) by the SGC for OXFBD02 analogues against BRD4(1). Data are displayed as a heatmap, with the highest ΔT_m value in red, and lowest ΔT_m value in green. Compounds and protein concentrations were 10 and 2 μM.

the series was cyclohexyl derivative **2.25** (8.8 °C), which even showed improvement over the parent compound (**2.1**), indicating that saturated systems are well tolerated on the WPF shelf, but that the cyclopropyl substituent is not large enough to make enough favourable interactions with the lipophilic shelf.

When analysing thermal shift data, however, it is important to realise that there can be discrepancies between thermal shift and relative binding affinity. In fact, thermal shift values can be associated with different binding affinities. This is due to the thermodynamic contributions to free energy of binding; different combinations of enthalpic and entropic changes can result in the same energy release on binding. At elevated temperatures, entropic contributions to binding will be magnified. As such, it is common to observe large ΔT_m for hydrophobic binders, the binding of which is entropically driven. Therefore we must be cautious when comparing compounds with different physicochemical properties, i.e. hydrophobic and hydrophilic molecules. Therefore, the stabilisation of BRD4(1) by the cyclohexyl derivative (**2.25**), will in some part have been amplified due to the elevated temperatures in this assay.[143]

To verify this DSF data, a collection of the analogues was also tested in an Amplified Luminescence Proximity Homogeneous Assay (AlphaScreen™).[67] In this assay a protein and its binding partner, in this case its cognate peptide, are bound to donor and acceptor beads. Irradiation of the donor bead releases singlet oxygen, which allows an acceptor bead to fluoresce when it is in close proximity to the donor bead, i.e. when the protein and binding partner are bound. Displacement of the binding partner by ligands can therefore be detected by a reduction in fluorescence. A limitation of this technique is that the beads used in AlphaScreen™ assays have multiple binding sites, giving rise to variability in the batches of beads used in the assay. Furthermore, amplification of signal leads to variability in IC_{50} values between AlphaScreen™ assays, and, therefore, to make accurate comparisons between different ligands, they need to be run on the same plate. This variability was

experienced in the assay, when the parent compound OXFBD02 (**2.1**), with a published IC_{50} of 382 nM, displayed an IC_{50} value of 808 nM (Table 2.4).



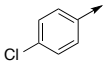
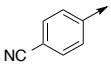
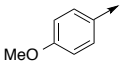
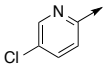
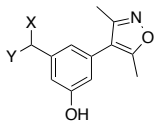
Compound number	X	Y	BRD4(1) IC_{50} (nM) *(upper/lower 95% confidence limits)*
2.1	OH		808 *(862/758)*
2.9	OH		1772 *(1903-1651)*
2.10	OH		1254 *(1334/1178)*
2.11	OH		774 *(829-724)*
2.12	OH		1922 *(2030/1820)*
2.13	OH		1790 *(1899-1686)*
2.14	OH		1774 *(1853-1699)*
2.16	OH		2644 *(2820-2479)*
2.17	OH		1798 *(1930-1674)*
2.19	OH		1833 *(1910-1759)*
2.20	OH		918 *(950-887)*
2.21	OH		1721 *(1863-1590)*
2.22	OH		2810 *(3029-2607)*

Table 2.4(i). AlphaScreen™ IC_{50} data for analogues of OXFBD02 (**2.1**) against BRD4(1), obtained in duplicate, and averaged, by the SGC. Data are displayed as a heatmap, with the lowest IC_{50} value in red, and highest IC_{50} value in green.



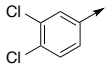
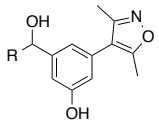
Compound number	X	Y	BRD4(1) IC_{50} (nM) *(upper/lower 95% confidence limits)*
2.24	OH		5020 *(5438-4633)*
2.25	OH		379 *(401-359)*
2.27	OH		3049 *(3175-2928)*
2.28	H		1787 *(2055-1554)*

Table 2.4(ii). AlphaScreen™ IC_{50} data for analogues of OXFBD02 (**2.1**) against BRD4(1), obtained in duplicate, and averaged, by the SGC. Data are displayed as a heatmap, with the lowest IC_{50} value in red, and highest IC_{50} value in green.

In support of the DSF data, electron-withdrawing groups were again well tolerated, particularly for *para*-fluoro derivative **2.11**, which showed a very similar IC_{50} value to the monosubstituted parent compound OXFBD02 (**2.1**). It is important to note that AlphaScreen™ assays are not sensitive enough to rank compounds with only 5-10% difference in values, such as compounds **2.12** and **2.13** but a 1.5-2-fold difference in values, for example, is significant. Control compounds, such as the parent compound OXFBD02 (**2.1**) allow more reliable comparison between assays. But ideally, orthogonal assays should be used to confirm affinity, such as thermal shift in combination with AlphaScreen™ as carried out here. In addition, the *para*-tolyl and *ortho*-methoxyphenyl electron-donating groups (**2.17** & **2.14)** were similarly well tolerated. However, *para*-methoxyphenyl derivative **2.16** was less well tolerated than its *ortho*-analogue (**2.14**), indicating that steric bulk could not be favoured on the WPF shelf. The more hydrophilic pyridyl substitutents were again relatively well tolerated, particularly in the 3-position (**2.20**), with a similar IC_{50} value to the parent compound (**2.1**). However, the 4-chloro-2-pyridyl functionality (**2.22**),

previously shown to aid solubility, was not as well tolerated. In addition, the solubilising 3,4-dichlorinated substituent (**2.24**) was again not well tolerated, with the poorest IC_{50} value of the collection, possibly also due to the increased steric bulk on the WPF shelf. Methylene derivative **2.28** showed that the secondary alcohol is important for binding to the BET bromodomains. Again the cyclopropyl derivative (**2.27**) displayed very weak binding, but the best compound of the entire series was cyclohexyl derivative **2.25**, verifying that saturated systems are well tolerated on the WPF shelf, but that the larger and more hydrophobic nature of the cyclohexyl derivative (**2.27**) binds far better than the smaller cyclopropyl group. With these data in hand, work was then carried out to establish whether any of the more potent derivatives could be taken forward into *in vivo* models.

2.2.4 Metabolic liabilities of the most potent analogues



Parameters	R				
	Compound Number	**2.1**	**2.11**	**2.20**	**2.25**
$ChromLogD_{pH7.4}$		3.7	4.1	2.1	4.4
#Ar rings		3	3	3	2
PFI		6.7	7.1	5.1	6.4

Table 2.5. $ChromLogD_{pH7.4}$ values calculated with a GSK in-house calculator (version 6), number of aromatic rings (#Ar) and PFI values for **2.1**, **2.11**, **2.20** & **2.25**. PFI = $ChromLogD_{pH7.4}$ + #Ar.

To assess the pharmacokinetics of the most potent compounds, the $ChromLogD_{pH7.4}$ values were again predicted using an in-house calculator at GSK (version 6). The more potent cyclohexyl compound **2.25** is predicted to have a large

ChromLogD$_{pH7.4}$ value of 4.4, being slightly higher than the *para*-fluoro (**2.11**) and OXFBD02 (**2.1**), with ChromLogD$_{pH7.4}$ values of 4.1 and 3.7 respectively (Table 2.5). However, the PFI value of 6.4 for cyclohexyl **2.25** is marginally better than 7.1 and 6.7 for *para*-fluoro **2.11** and OXFBD02 (**2.1**) due to **2.25** having fewer aromatic rings. PFI values greater than 5 often highlights issues with developability assays, including solubility, human serum albumin binding, cytochrome P450 IC$_{50}$ inhibition, intrinsic clearance and permeability, as can be seen in Table 2.1. PFI values between 6 and 7 correspond with ≥ 49% of compounds achieving defined target values in the various developability assays, except for only 33% achieving the solubility target value. For PFI values between 7 and 8, ≥ 36% of compounds achieve defined target values for some cytochrome P450 IC$_{50}$ values, intrinsic clearance and permeability, with poorer results for the other developability assays. The 3-pyridyl derivative (**2.20**) could be an interesting compound to take forward, with a low ChromLogD$_{pH7.4}$ = 2.1 and hence better PFI = 5.1. PFI values between 5 and 6 correspond with ≥ 58% of compounds achieving target values in all developability assays mentioned. In addition, hydrophilic aromatic rings, such as pyridyls, are also predicted to have better outcomes in developability assays than more lipophilic rings with the same PFI values, due to their increased polarity.

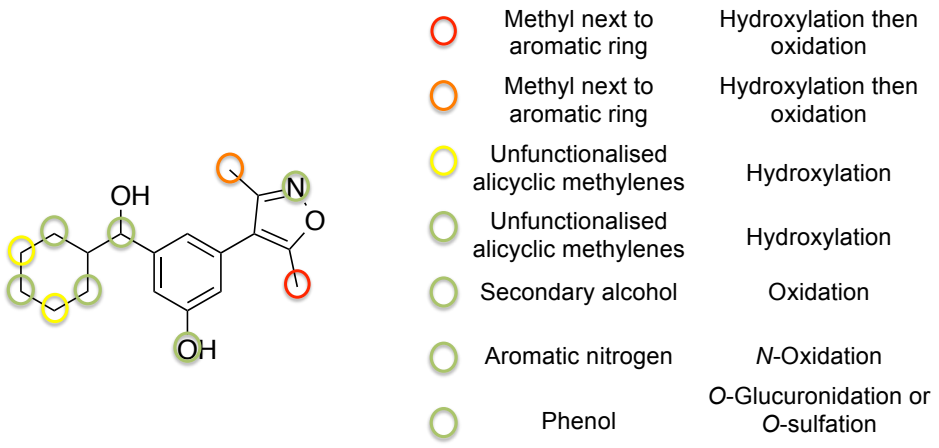
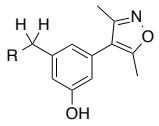


Figure 2.11. Metasite 3.1.2 (phase I) and Meteor 2.0.2 (phase I and II) predictions of metabolic liabilities of **2.25**, ranked in order of decreasing reactivity from red to green.

Metabolic liabilities were also predicted for cyclohexyl derivative **2.25**, using Metasite 3.1.2 (phase I metabolism) and Meteor 2.0.2 (phase I and II metabolism). Hydroxylation of the cyclohexyl functionality was predicted to be a metabolic liability, however, was ranked much lower than *para*-hydroxylation of the monosubstituted phenyl ring of OXFBD02 (**2.1**, Figure 2.11).

To validate these predictions, metabolic stability assays were carried out for OXFBD02 (**2.1**) and the cyclohexyl derivative (**2.25**). Preliminary results from single experiments that involved incubation with mouse microsomes as well as mouse S9 fraction, which contains mouse microsomes, including cytochrome p450 isoforms, and other enzymes, as well as cytosol including transferases, corroborated with the predictions, with rapid metabolism of OXFBD02 (**2.1**) with $t_{1/2}$ = 37 min, and even more rapid metabolism of **2.25** with $t_{1/2}$ = 8 min. In an attempt to optimise the metabolic liability while maintaining the affinity of the cyclohexyl derivative, the ChromLogD$_{pH7.4}$ and PFI values of some alternative saturated systems (**2.41**-**2.44**) were also predicted (Table 2.6).



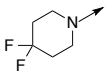
Parameters	R				
	Compound Number	**2.41**	**2.42**	**2.43**	**2.44**
ChromLogD$_{pH7.4}$		2.4	2.5	1.5	4.1
#Ar rings		2	2	2	2
PFI		4.4	4.5	3.5	6.1

Table 2.6. ChromLogD$_{pH7.4}$ values calculated with a GSK in-house calculator (version 6), number of aromatic rings (#Ar) and PFI values for **2.41**-**2.44**. PFI = ChromLogD$_{pH7.4}$ + #Ar.

The tetrahydropyran analogue (**2.26**) would reduce the lipophilicity of the saturated series, and block the 4-position from hydroxylation. Another way to introduce saturated systems is with the use of cyclic amines. The instability of

hemiaminal derivatives to elimination of water required the use of methylamine derivatives **2.41**-**2.46** instead (Figure 2.12).

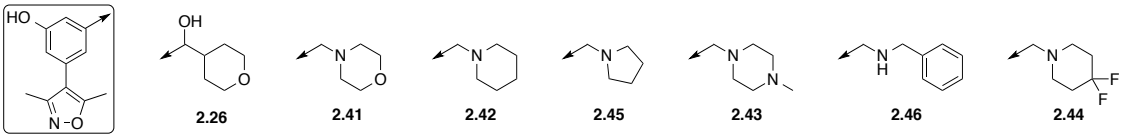
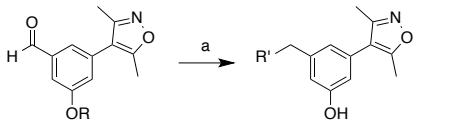


Figure 2.12. New target set of saturated analogues (**2.41**-**2.46**) of OXFBD02 (**2.1**).

Some saturated amines showed particularly good $ChromLogD_{pH7.4}$ and PFI values. PFI values between 3 and 4 correspond with ≥ 76% of compounds achieving defined target values for all developability assays mentioned, except for only 30% meeting the solubility target. PFI values between 4 and 5 correspond with ≥ 68% of compounds achieving defined target values for all developability assays mentioned, except for 46% meeting the solubility target. Geminal difluorinated piperidine **2.44** is predicted to be a more metabolically stable cyclohexyl mimic, and despite the sub-optimal $ChromLogD_{pH7.4}$ value of 4.1, its PFI value of 6.1 suggests it could have slightly better pharmacokinetics than cyclohexyl derivative **2.25** with a PFI value of 6.4. Therefore, these compounds became part of a set of new synthetic targets for this series of compounds, which we could use to probe the importance of the secondary alcohol hydroxyl group.

2.2.5 Synthesis of the second analogue set

The amine derivatives were synthesised *via* reductive amination reactions of key aldehyde **2.37** (Scheme 2.10). Pre-stirring **2.37** with *N*-methylpiperazine at pH 4.0, followed by addition of $NaBH_3CN$ afforded desired amine **2.43** in 69% yield, and the reduced starting material side-product in only 11% yield. However, treatment of the aldehyde with morpholine under the same acidic conditions afforded the desired product (**2.41**) in only 20% yield and the side-product in 57% yield.



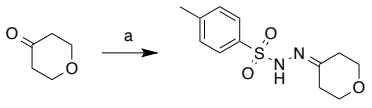
2.37: R = H
2.38: R = TIPS

2.43: R' = *N*-Methylpiperazinyl
2.41: R' = Morpholinyl
2.42: R' = Piperidinyl
2.45: R' = Pyrrolidinyl
2.44: R' = 4,4-Difluoropiperidinyl
2.46: R' = *N*-Benzylamino

Scheme 2.10. Reductive amination reactions to generate amino analogues. *Reagents and conditions:* (a) R = *N*-Methylpiperazinyl: i) **2.37**, 1-methylpiperazine, AcOH (pH 4), EtOH, rt, 20 min; ii) $NaBH_3CN$, rt, 17 h, 69%; R = Morpholinyl: i) **2.37**, morpholine, AcOH (pH 4), EtOH, rt, 40 min; ii) $NaBH_3CN$, rt, 23 h, 20%; R = Piperidinyl: i) **2.38**, piperidine, AcOH (pH 6), EtOH, rt, 1 h; ii) $NaBH_3CN$, rt, 22 h; iii) TBAF, THF, 0 °C-rt, 2 h, 8%; R = Pyrrolidinyl: i) **2.38**, pyrrolidine, AcOH (pH 6), EtOH, rt, 1 h; ii) $NaBH_3CN$, rt, 22 h; iii) TBAF, THF, 0 °C-rt, 1 h, 33%; R = 4,4-Difluoropiperidinyl: i) **2.37**, 4,4-difluoropiperidine hydrochloride, EtOH, rt, 30 min; ii) $NaBH_3CN$, rt, 19 h, 33%; R = Benzylamine: i) **2.37**, benzylamine, AcOH (pH 6), EtOH, rt, 30 min; ii) $NaBH_3CN$, rt, 19 h, 69%.

Less acidic conditions (pH 6) were used to afford the piperidine and pyrollidine derivatives (**2.42** & **2.45**), without formation of the side-product. However, in these instances, boron impurities were observed by ^1H NMR. In case the boron impurity was due to the presence of the unprotected phenol, TIPS protection followed by reductive aminations were carried out, however, these products also displayed boron impurities. As such, an ISOLUTE® SCX-2 amine catch and release column was used for purification, however the 1 M aqueous solution of NH_4OH used to elute the piperidine and pyrrolidine products also cleaved some of the TIPS protection. Treatment with TBAF deprotected the remaining TIPS group, affording the desired piperidine and pyrrolidine derivatives (**2.42** & **2.45**). The TIPS protection proved unnecessary, as the 4,4-difluoropiperidine and benzylamine derivatives (**2.44** & **2.46**) were acquired using the unprotected aldehyde, carrying out the reductive amination at pH 6, and using ISOLUTE® SCX-2 amine catch and release columns for purification.

As mentioned earlier, several efforts were made to synthesise tetrahydropyran analogue **2.26**. Firstly, magnesium-insertion into 4-chlorotetrahydropyran, following the procedure of Holzer *et al*. was attempted multiple times, however, initiation could not be achieved to generate the Grignard reagent.[144] One alternative is to make 4-bromo-3,6-dihydro-2*H*-pyran, which could be reacted with aldehyde **2.37** instead of the saturated 4-bromotetrahydropyran, following a procedure by Härter *et al*.[145] Subsequent reduction of the vinylic species would furnish the desired derivative (**2.26**). Härter *et al*. report using triphenylphosphine dibromide in the presence of triethylamine to convert tetrahydropyran-4-one to the vinyl bromide using the Appel reaction. Despite keeping this reaction in the dark, to avoid degradation of the vinyl bromide, no product could be produced when these reaction conditions were attempted. Similarly, attempts to use the Shapiro reaction to generate a vinyl lithium species that could attack the aldehyde were also made. Facile conversion of tetrahydropyran-4-one (**2.64**) to the tosylhydrazone (**2.65**) was achieved by stirring at rt with *para*-toluenesulfonyl hydrazine (Scheme 2.10). However, the subsequent Shapiro reaction using ^{n}BuLi at –78 °C failed to generate the required vinyl lithium species for reaction with the aldehyde. To establish whether exchanging the aldehyde and organohalide functionality would furnish the desired derivative, TIPS protection of 3,5-dibromophenol, followed by magnesium-halogen exchange with one equivalent of ^{i}PrMgCl•LiCl and addition of 4-formyltetrahydropyran was attempted, however, no reaction was observed. Without any success, and with the poor $ChromLogD_{pH7.4}$ for the tetrahydropyran derivative, this analogue was removed from the new collection of analogues. However, further attempts to synthesise this derivative could include lithium-halogen exchange of 4-bromotetrahydropyran. Unlike THF, which is cleaved to extremely volatile acetaldehyde and ethene by organolithiums at rt, making it an excellent solvent for organolithium reactions at low temperatures due to quenching of the organolithium as the reaction is warmed up, tetrahydropyrans are not cleaved by ^{n}BuLi.[146]

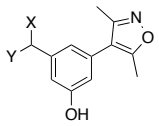


2.64 **2.65**

Scheme 2.10. Conversion of tetrahydropyran-4-one (**2.64**) to tosylhydrazone **2.65**. *Reagents and conditions:* (a) 4-Toluenesulfonyl hydrazine, MeOH, rt, 16 h, 62%.

2.2.6 Affinity for BRD4 of amine-derived saturated systems **2.41**-**2.46**

With the amine-derived saturated systems in hand, they were also tested in an AlphaScreen™ assay. In this instance, the parent compound OXFBD02 (**2.1**) gave an IC_{50} value of 97.5 nM (Table 2.7). However, in corroboration with the previous AlphaScreen™ assay, the *para*-fluorophenyl and 3-pyridyl substituents **2.11** and **2.20** showed very similar IC_{50} values, and the cyclohexyl derivative **2.25** showed an improved IC_{50} value, relative to the IC_{50} value of OXFBD02 (**2.1**). The morpholine, *N*-methylpiperazine and benzylamine derivatives (**2.41**, **2.43** and **2.46**) displayed slightly weaker affinity than the methylene derivative **2.28**, and the piperidine and pyrollidine derivatives (**2.42** & **2.45**) showed lower affinity for the BET bromodomains, signifying the importance of the secondary alcohol. However, the geminal difluorinated piperidine (**2.44**) displayed significantly stronger affinity for BRD4 than the other amine derivatives, presumably due to the reduced basicity suppressing amine protonation, allowing piperidine **2.44** to interact better on the lipophilic WPF shelf. Using an in-house calculator at GSK (version 6), the $ChromLogD_{pH7.4}$ value of **2.44** was predicted to be 4.1, an improvement on cyclohexyl **2.25** with $ChromLogD_{pH7.4}$ = 4.4. The resulting PFI value of 6.1 is also an improvement on OXFBD02 (**2.1**), with PFI = 6.7.



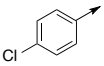
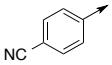
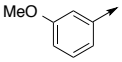
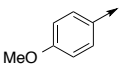
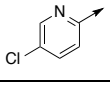
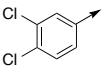
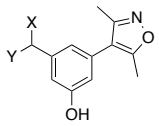
Compound Number	X	Y	BRD4(1) IC_{50} (nM) *(error (nM))*
2.1 OXFBD02	OH		98 *(0.010)*
2.9	OH		242 *(0.022)*
2.10	OH		150 *(0.017)*
2.11	OH		89 *(0.007)*
2.12	OH		204 *(0.016)*
2.13	OH		227 *(0.020)*
2.14	OH		161 *(0.017)*
2.15	OH		216 *(0.021)*
2.16	OH		267 *(0.023)*
2.19	OH		182 *(0.017)*
2.20	OH		94 *(0.009)*
2.21	OH		120 *(0.010)*
2.22	OH		219 *(0.017)*
2.24	OH		766 *(0.130)*
2.25	OH		53 *(0.004)*

Table 2.7(i). AlphaScreen™ IC_{50} data obtained in triplicate, and averaged, by A. Sekirnik for a set of analogues, including the new amino derivatives, of OXFBD02 (**2.1**) against BRD4(1). Data are displayed as a heatmap, with the lowest IC_{50} value in red, and highest IC_{50} value in green.



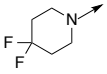
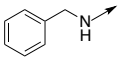
Compound Number	X	Y	BRD4(1) IC_{50} (nM) *(error (nM))*
2.28	H		619 *(0.076)*
2.41	H		756 *(0.067)*
2.42	H		4000 *(2.395)*
2.43	H		1313 *(0.169)*
2.44	H		164 *(0.012)*
2.45	H		6887 *(3.063)*
2.46	H		1952 *(5.745)*

Table 2.7(ii). AlphaScreen™ IC_{50} data obtained in triplicate, and averaged, by A. Sekirnik for a set of analogues, including the new amino derivatives, of OXFBD02 (**2.1**) against BRD4(1). Data are displayed as a heatmap, with the lowest IC_{50} value in red, and highest IC_{50} value in green.

Overall, these results highlight that saturated systems can be well tolerated on the WPF shelf, but that the hydroxyl group is important for binding, and that protonated amines are in general too hydrophilic to have good affinity for the WPF shelf. We feel that it would be interesting to make the corresponding amides **2.47**-**2.52**, which would add a hydrophilic oxygen atom, while reducing the basicity of the nitrogen atom. Furthermore, the BRD4(1) IC_{50} of 544 nM for the benzophenone analogue of OXFBD02 (**2.1**), published by Hewings *et al*., shows that sp^2 functionality is tolerated at this position.[46] Therefore, synthesis of these amide derivatives would allow us to assess whether the affinity can be improved with more lipophilic saturated

systems. As such, compounds **2.47**-**2.52** are the new target compounds in this series (Figure 2.13).

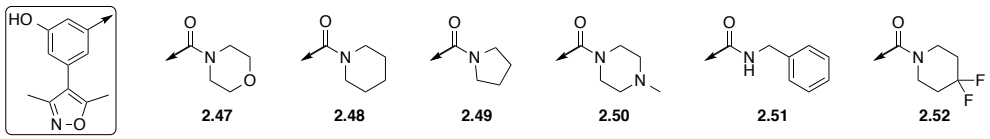


Figure 2.13. New target set of lipophilic saturated analogues of OXFBD02 (**2.1**).

2.2.7 Analysis of the physicochemical parameters of analogues **2.1**, **2.11**, **2.20**, **2.25**, **2.44** and (+)-JQ1 (**2.4**)

With these data in hand it is possible to analyse a variety of physicochemical parameters to assess their suitability as probe molecules (Table 2.8). Such parameters can allow a better comparison of affinity between molecules of different size, such as between the analogues of OXFBD02 (**2.1**) and (+)-JQ1 (**2.4**), a potent but larger BRD4 ligand. For example, ligand efficiency (LE) measures the binding affinity per heavy atom, and due to the larger molecule, the LE of (+)-JQ1 (**2.4**) is considerably smaller than that of the OXFBD02 (**2.1**) analogues. Another important parameter to consider is lipophilic ligand efficiency (LLE), which accounts for lipophilicity. As mentioned earlier, it is vital to develop compounds that are as polar as possible, while maintaining affinity. Therefore, large LLE values are important. Although a number of the OXFBD02 (**2.1**) analogues suffer from low LLE values, the 3-pyridyl and 4,4-difluoropiperidine derivatives **2.20** and **2.44** are still potent but less lipophilic, with larger LLE values. (+)-JQ1 (**2.4**) on the other hand is more lipophilic, meaning it has a considerably poorer LLE in contrast with the OXFBD02 (**2.1**) derivatives. All derivatives, including (+)-JQ1 (**2.4**) have an acceptable amount of lipophilicity with regards to Lipinski's rule of five (clogP < 5), however the analogues of OXFBD02 (**2.1**) are less lipophilic than (+)-JQ1 (**2.4**).[137] Considering Lipinski's other rules of five, all analogues and (+)-JQ1 (**2.4**) have acceptable relative

molecular masses (< 500), numbers of hydrogen bond donors (< 5) and hydrogen bond acceptors (< 10). Breaking these rules predicts poor absorption or permeation. Furthermore, all derivatives have a small enough total polar surface area (TPSA < 140 $Å^2$) to suggest these derivatives will not suffer from permeability issues. As mentioned earlier, PFI > 5 often indicates a poor outcome in developability assays, with issues such as metabolism, clearance and solubility. Unfortunately, all of the most potent OXFBD02 (**2.1**) analogues have PFI > 5, although the 3-pyridyl and 4,4-difluoropiperidine derivatives (**2.20** & **2.44**) have the best PFI values, and all have significantly better PFI values than that of (+)-JQ1 (**2.4**).

Parameters	OXFBD02 (**2.1**)	**2.11**	**2.20**	**2.25**	**2.44**	(+)-JQ1 (**2.4**)
IC_{50} *vs* BRD4(1) / nM	97.5	88.8	93.6	52.9	164	77[C]
pIC_{50}	7.01	7.05	7.03	7.28	6.79	7.11
Heavy atom count (HAC)	22	23	22	22	23	31
cLogP[A]	2.53	2.45	1.04	3.24	1.37	4.48
LE	0.45	0.43	0.45	0.46	0.41	0.32
LLE	4.5	4.6	6.0	4.0	5.4	2.6
RMM	295	313	296	301	322	456
H-bond donors	2	2	2	2	1	1
H-bond acceptors	4	4	5	4	4	6
TPSA[B] / $Å^2$	66.49	66.49	79.38	66.49	49.50	97.09
$ChromLogD_{pH7.4}$	3.7	4.1	2.1	4.4	4.1	7.1
#Ar rings	3	3	3	2	2	3
PFI	6.7	7.1	5.1	6.4	6.1	10.1

Table 2.8. Parameters for **2.1**, **2.11**, **2.20**, **2.25**, **2.44** and (+)-JQ1 (**2.4**). (A) cLogP was calculated using ACD/Labs algorithm (version C10E41); LE = (pIC_{50} × 1.4 kcal/mol)/HAC; LLE = pIC_{50} – clogP; PFI = $clogD_{pH7.4}$ + #Ar rings. (B) TPSA values were calculated using ACD/I-Lab (version 5.0.0.184). (C) The published IC_{50} of (+)-JQ1 (**2.4**) was not obtained on the same AlphaScreen™ plate as the other compounds, therefore we must be cautious when comparing these values.

In summary, 3-pyridyl **2.20** and geminal difluorinated piperidine **2.44** have the best LLE values of the compounds in Table 2.8, and also displayed the best PFI

values, particularly for 3-pyridyl **2.20**. However, improvements are still required in order to maximise the chance of good outcomes in developability assays, aiding the development of *in vivo* probes.

2.2.8 Predicting metabolic stability

One of the limitations when developing compounds for use in *in vivo* models is metabolic stability; if the compound degrades before it has time for any activity, then the affinity of the compound is irrelevant. Establishing metabolic stability experimentally is demanding, but a number of predictive computational tools are now available to assess metabolic liability. One tool that emerged recently is FAME, developed by Kirchmair *et al*.[147] FAME is a simple predictive tool that has been trained on more than 20000 compounds with experimentally defined sites of metabolism. Unlike many other predictive tools, it does not focus on one specific enzyme family or species; FAME provides a global model of metabolism, or specific models of human, rat or dog and phase I and II metabolism. It rapidly generates probability scores for metabolism of each atom in a molecule, and when the output is visualised using Jmol, the most likely sites of metabolism are highlighted as spheres of radius decreasing with probability. We aimed to use FAME to predict whether any of our compounds are suitable for use in *in vivo* models, or to direct the synthesis of more suitable compounds.

FAME's global model was used on the synthesised compounds, and the other target amides, in their dominant protonation state at physiological pH, as predicted using ACD/I-LAB (version 5.0.0.184, Figure 2.14). By far the most susceptible atom to metabolism is the ester carbon of **2.2**. This predicts that **2.2** is even less stable than its phenol derivative OXFBD02 (**2.1**). The acetate functionality also makes the 4-position of the monosubstituted aromatic ring more susceptible to metabolism.

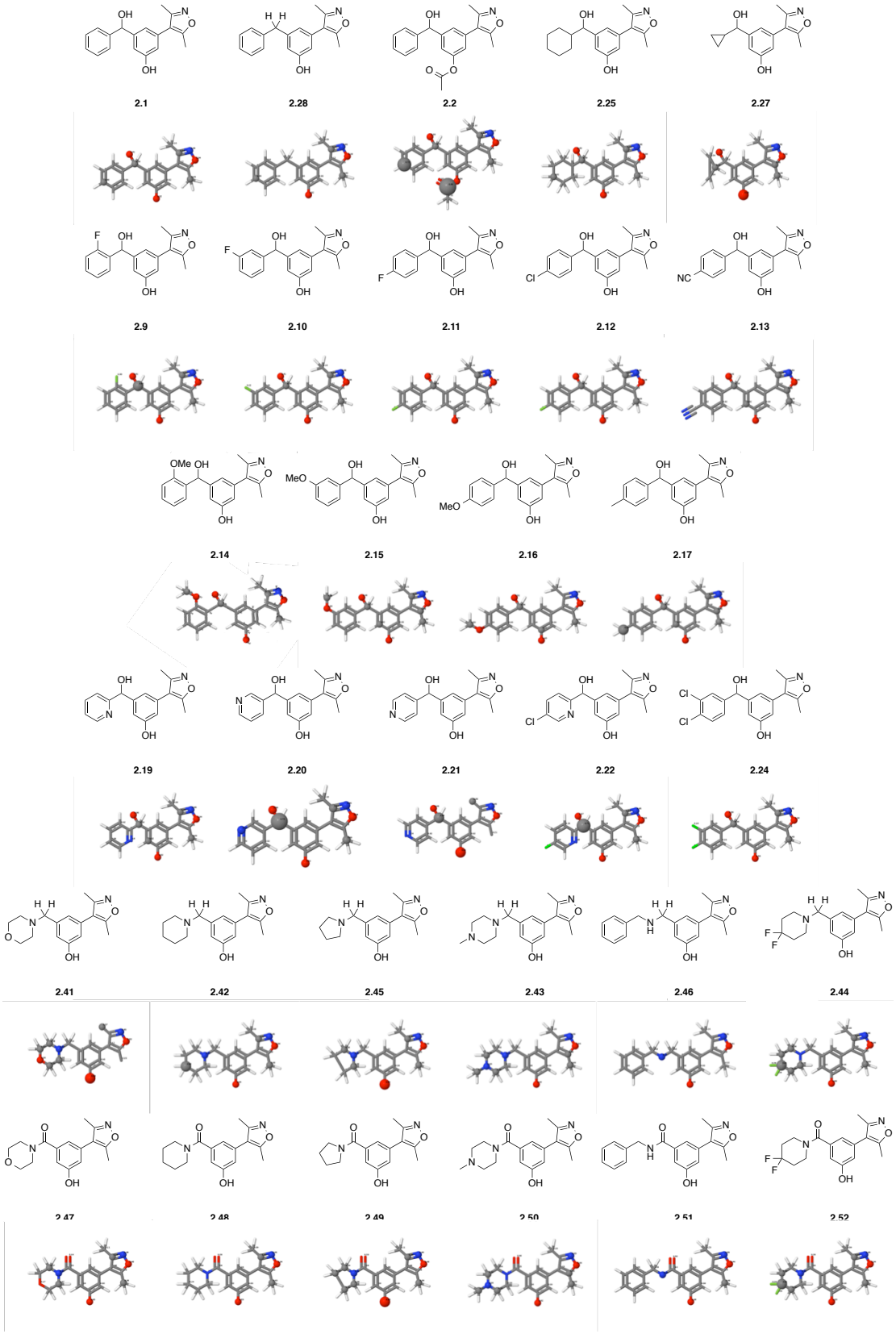


Figure 2.14. Jmol images depicting FAME scores of synthesised compounds and target amide derivatives.[147] Spheres indicate the most likely sites of degradation, with sphere size decreasing with probability of metabolism. Grey atoms = carbon, white atoms = hydrogen, red atoms = nitrogen, blue atoms = oxygen, green atoms = chlorine or fluorine.

Except in the case of the benzylamine (**2.46**) and benzylamide (**2.51**), the 4-position of monosubstituted aromatics was predicted to have much greater reactivity than other aromatic carbons. This reactivity can be ameliorated with simple substitution of the aromatic ring, without necessarily blocking the 4-position itself. For example, 2-fluoro derivative **2.9** displayed almost identical FAME scores to OXFBD02 (**2.1**) for other atoms, but predicted no reactivity at the 4-position. This amelioration was also observed for pyridyl **2.9**. Over the range of compounds assessed, the secondary alcohol carbon was predicted to be fairly reactive. However, when this alcohol functionality was replaced by a methylene or carbonyl, FAME predicted this position to be much less reactive than the alcoholic derivatives. The secondary alcohol and phenol oxygens showed a moderate chance for metabolism, presumably by glucuronidation or sulfation. The isoxazole heteroatoms as well as the 3- and 5-methyl groups consistently showed a weak to moderate probability of metabolism, indicting that the KAc-mimicking functionality also has a moderate chance of metabolic liability. The heteroatoms and methylenes in the saturated amine derivatives **2.41**-**2.46** were in general more reactive than those in the amide derivatives **2.47**-**2.52**. Overall, relative to OXFBD02 (**2.1**), halogenated **2.9**-**2.12** and pyridyls **2.19**-**2.22** were all predicted to be similarly well tolerated, while para-nitrile **2.13**, benzylamine **2.46** and benzylamide **2.51** look to be relatively stable to metabolism. Other saturated systems and the anisole derivatives **2.14**-**2.16** host more sites of weak to modest reactivity than OXFBD02 (**2.1**). Comparing these results with the strongest binders to BRD4, the *meta*- and *para*-fluoro derivatives (**2.9** & **2.11**), and the 2- and 4-pyridyl derivatives (**2.19** & **2.21**) have good activity and are predicted to be more stable to metabolism than OXFBD02 (**2.1**).

Unlike FAME, which provides a global model of metabolism, SMARTCyp is a predictive tool to specifically highlight a molecule's sites of metabolism by cytochrome P450 (CYP). The collection of analogues of OXFBD02 (**2.1**), again, in

their most abundant protonation state at physiological pH as predicted using ACD/I-LAB (version 5.0.0.184), was analysed using SMARTCyp (version 2.4.2).[148] SMARTCyp gives each atom a score, with low scores indicating a high probability of metabolism, and *vice versa*. The three atoms of greatest metabolic liability are highlighted yellow for the greatest, then peach, followed by green (Figure 2.15).

Upon analysis of the SMARTCyp data, a number of patterns emerged. The isoxazole methyl groups, the secondary alcohol carbons, the isoxazole nitrogens and aromatic carbons bearing a proton were consistently predicted to have moderate reactivity. Interestingly, the *para*-position of the monosubstituted aromatic ring of OXFBD02 (**2.1**) is not predicted to be any more susceptible to oxidation than the other aromatic carbons.

Methylenes and methyl groups adjacent to a nitrogen atom in the reductive amination products were predicted to be the most reactive functionality for the entire set of analogues. The pyrrolidine derivative (**2.45**) contained the methylenes most susceptible to degradation. Conversely, the methylenes and methyl groups adjacent to oxygens are predicted to be more stable than those adjacent to nitrogens, and even more stable to metabolism were methylenes next to amide functionality.

The non-isoxazole nitrogens in the amine derivatives were the most susceptible to metabolism, except in the case of the 4,4-difluoropiperidine substituent of **2.44**. Presumably this stability must be due to the highly electron withdrawing ability of the fluorines impeding oxidation of the amino nitrogen. The pyridyl nitrogens of **2.19**-**2.22**, and the amide nitrogens of **2.47**-**2.42** are predicted to be more stable to oxidation than the amine derivatives.

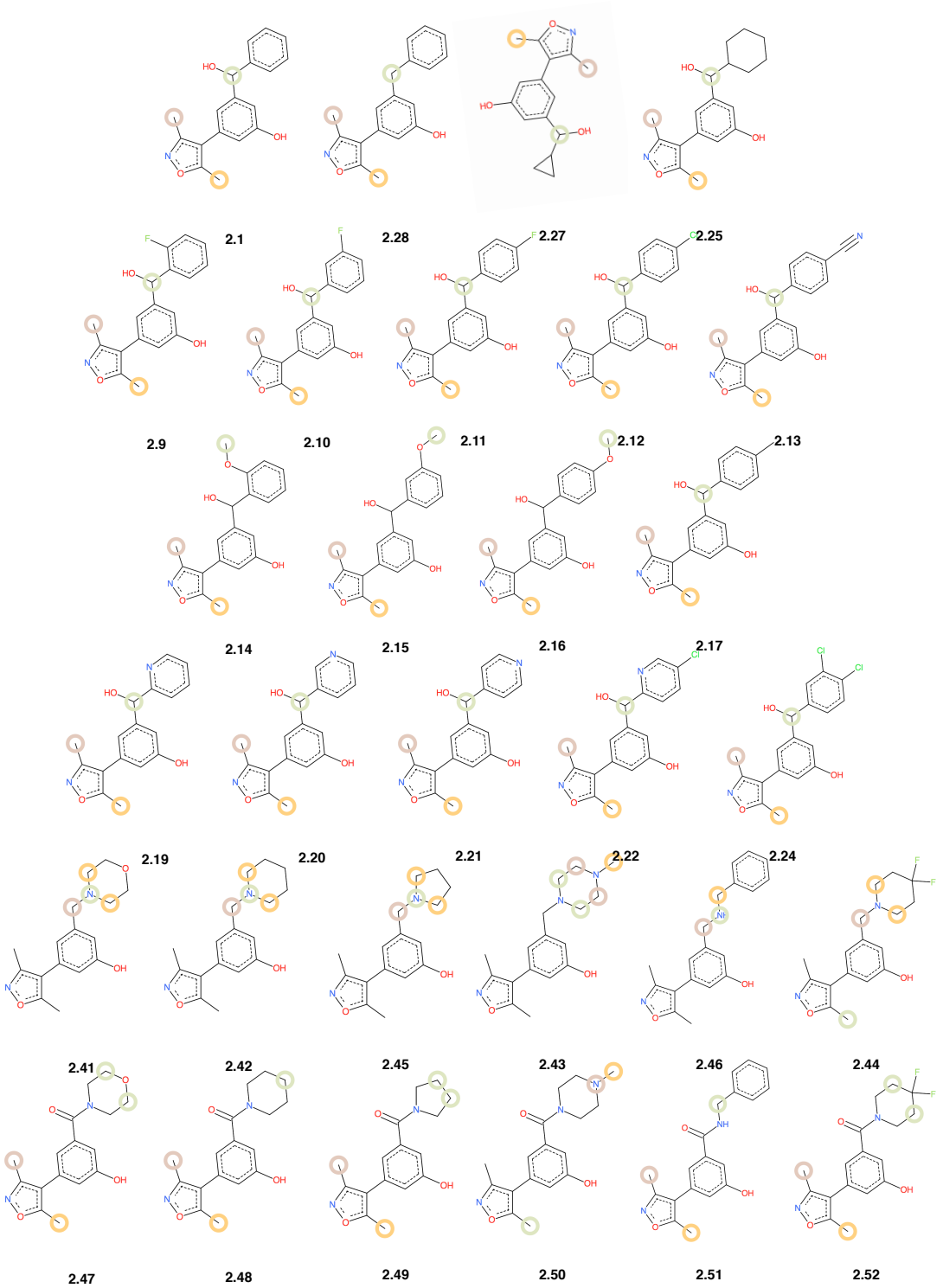


Figure 2.15. SMARTCyp (version 2.4.2) predictions of the three sites most liable to metabolism by CYP for the analogues of OXFBD02 (**2.1**). Yellow indicates the most likely site, peach indicates the second most likely site and green indicates the third most likely site of metabolism.

Therefore in summary, although the amine derivatives benefit from being more hydrophilic than cyclohexyl derivative **2.25**, and therefore are likely to benefit from better aqueous solubility, their methylenes are predicted to be much more susceptible to oxidation by CYP. Furthermore, *N*-oxidation is also suggested to be slightly more likely for these derivatives than oxidation of the secondary alcohol of other derivatives. The amides **2.47**-**2.52** on the other hand are in general predicted to have more stable methylenes and nitrogen atoms, so appear more suitable for use in *in vivo* models. Therefore, it could be interesting to synthesise these derivatives to see whether they show any affinity for the BET bromodomains.

In order to validate these predictions and establish whether any of the OXFBD02 (**2.1**) analogues can be used *in vivo*, attempts were made to assess their metabolism experimentally, using microsomal stability assays with mouse microsomes and mouse S9 fraction. Frustratingly, replicate experiments gave inconsistent data, and the preliminary results for OXFBD02 (**2.1**) and cyclohexyl **2.25** could not be verified either. Therefore, no conclusions could be made regarding the metabolism of the collection of analogues, and this highlights a significant limitation of attempting to measure metabolism experimentally using microsomal stability assays. In order to validate the FAME and SMARTCyp predictions, external specialists will carry out microsomal stability assays on all of the OXFBD02 (**2.1**) analogues. If accurate measurements of metabolism can be acquired, this will highlight any compounds that are suitable for use in animal models, as well as direct the optimisation of this series of compounds.

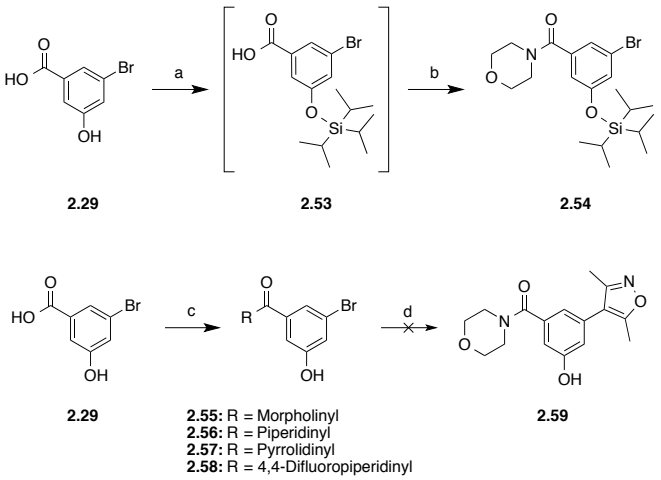
2.2.9 Investigating the binding to BRD4 of more lipophilic saturated systems

We predicted that the weak binding of amines **2.41**-**2.43**, **2.45** & **2.46** is due to their dominant protonation state of +1 at physiological pH, as predicted using ACD/I-LAB (version 5.0.0.184). The ionised molecules are less lipophilic than their

unprotonated analogues, resulting in unfavourable charge interactions with the lipophilic WPF shelf. The 4,4-difluoropiperidinyl derivative (**2.44**) is mostly neutral at physiological pH, due to the electron-withdrawing ability of the geminal fluorines, accounting for its stronger interaction with BRD4 relative to the other amine derivatives. As such, preliminary work has been carried out to determine whether alternative saturated systems that are largely neutral at physiological pH, could bind to BRD4. We hypothesised that the amide analogues **2.47**-**2.52** would be more potent due to their neutral charge. In addition, the FAME and SMARTCyp predictions indicate that these amides are interesting derivatives in terms of metabolic stability. The synthesis of the amide analogues **2.47**-**2.52** was therefore investigated.

It was envisaged that the derivatives could be furnished in two steps from commercially available 3-bromo-5-hydroxybenzoic acid **2.29**: an amide formation from the benzoic acid, and introduction of the isoxazole ring as previously described, either through C–H activation, or *via* a Suzuki-Miyaura cross-coupling reaction. Initially, work was carried out to establish whether C–H activation with 3,5-dimethylisoxazole could furnish the desired products, following a procedure by Fall *et al*.[141] This approach involved first introducing the amide functionality, because, as mentioned previously, C–H activation had not proved successful on the commercially available benzoic acid **2.29**. Initially, amide coupling to give the morpholine intermediate was carried out on TIPS protected **2.53**, in order to prevent side-reactions with the unprotected phenol of benzoic acid **2.29**. *In situ* TIPS protection using TIPSCl was achieved in 46% crude yield, and PyBOP coupling with morpholine occurred in 66% yield (Scheme 2.11). With the poor yield obtained for the TIPS protection, the coupling reaction was also carried out on the unprotected starting material (**2.29**), and the desired unprotected intermediate (**2.55**) was achieved in 64% yield, proving that protection of the phenol is unnecessary for this reaction to occur successfully. As such, the piperidine, pyrrolidine and

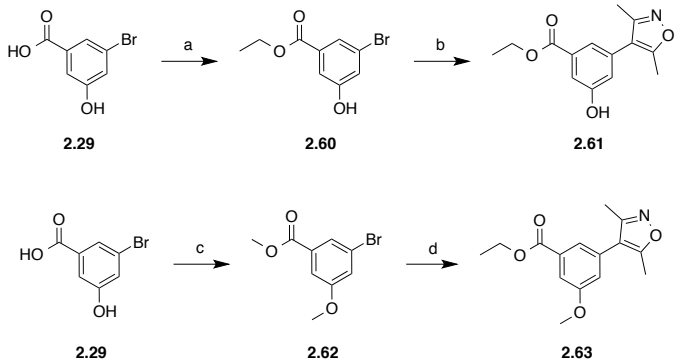
4,4-difluoropiperidine derivatives (**2.56**-**2.58**) were synthesised using a range of coupling agents, chosen purely on availability in the lab at the time of reaction. Unfortunately, however, C–H coupling of morpholinyl derivative **2.55** failed to give desired final product **2.59**.



Scheme 2.11. Attempts to synthesise desired amides. *Reagents and conditions:* (a) TIPSCl, imidazole, DMF, 0 °C-rt, 1 h, 46%; (b) i) PyBOP, NEt_3, DMF, THF, rt, 15 min; ii) Morpholine, rt, 15 h, 66%; (c) R = Morpholinyl: i) PyBOP, NEt_3, DMF, rt, 1 h; ii) Morpholine, rt, 17 h, 64%; R = Piperidinyl: i) HATU, NEt_3, DMF, rt, 40 min; ii) Piperidine, rt, 23 h, 69%; R = Pyrrolidinyl: i) HBTU, NEt_3, DMF, rt, 2 h; ii) Pyrrolidine, rt, 20 h, 70%; R = 4,4-Difluoropiperidinyl: i) PyBOP, NEt_3, DMF, rt, 35 min; ii) 4,4-Difluoropiperidine, rt, 167 h, 67%; (d) 3,5-Dimethylisoxazole, $PdCl_2$, AcOK, DMAc, 130 °C, 46 h, then 130 °C, mw, 150 min.

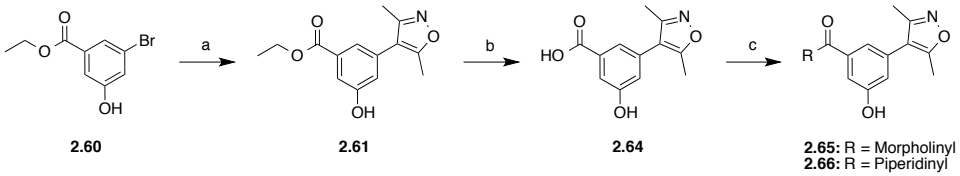
Without success in the C–H activation reaction, preliminary work was then carried out by P. Guzanov to establish a synthetic procedure that could be used to generate the desired derivatives. Reversing the synthetic steps, introducing the isoxazole functionality *via* Suzuki-Miyaura with the trifluoroborate derivative (**2.35**) of isoxazole boronic acid **2.36** (Scheme 2.2), followed by amide couplings was trialled. Unfortunately the Suzuki-Miyaura reaction failed to work on commercially available benzoic acid **2.29**, but this reaction is known to be unsuitable for carboxylic acid-containing derivatives. As such, the acid (**2.29**) was protected as an ethyl ester (**2.60**), which allowed the successful synthesis of intermediate **2.61**, although in a

poor yield (Scheme 2.12). In case the free phenol interfered with this reaction, the methyl ether methyl ester (**2.62**) was also trialled. This did not improve the yield of the required intermediate. Interestingly, transesterification with EtOH in the Suzuki-Miyaura reaction occurred (**2.63**), and therefore the monoprotected ethyl ester was selected for this route.



Scheme 2.12. Trialling protecting groups necessary for Suzuki-Miyaura cross-coupling, carried out by P. Guzanov. *Reagents and conditions:* (a) H_2SO_4, EtOH, 85 °C, 20 h, 88%; (b) **2.35**, $Pd(OAc)_2$, RuPhos, Na_2CO_3, EtOH, 90 °C, 50 h, 21%; (c) MeI, K_2CO_3, DMF, 100 °C, 20 min, 76%; (d) **2.35**, $Pd(OAc)_2$, RuPhos, Na_2CO_3, EtOH, 90 °C, 50 h, 11%.

Fortunately, the use of microwave irradiation improved the yield of this reaction on a larger scale (Scheme 2.13). Ester hydrolysis and subsequent amide couplings were then carried out using PyBOP, HATU and EDC/HOBt, however, in all cases the free phenol reacted with the activated ester functionality. Fortunately, heating for an extended period of time allowed the more thermodynamically stable amides **2.65** and **2.66** to be produced. With this synthetic route in hand, it should be feasible to synthesise the remaining amide derivatives.



Scheme 2.13. Synthesis of amides **2.65** and **2.66**, carried out by P. Guzanov. *Reagents and conditions:* (a) **2.35**, $Pd(OAc)_2$, RuPhos, Na_2CO_3, EtOH, 90 °C, mw, 90 min, 68%; (b) LiOH, THF, rt, 24 h, 96%; (c) R = Morpholinyl: Morpholine, EDC•HCl, HOBt, THF, 55 °C, 3 days, 52%; R = Piperidinyl: Piperidine, EDC•HCl, HOBt, THF, 55 °C, 3 days, 56%.

2.3 Summary and conclusions

2.3.1 Summary

In summary, OXFBD02 (**2.1**) has displayed antiproliferative activity in a range of cancer cell lines, and disrupts the interaction of BET bromodomains with non-histone partners, but is unsuitable for use in *in vivo* models due in large part to *para*-hydroxylation of its monosubstituted aromatic ring. In an attempt to optimise this compound, various analogues were synthesised, aiming to find a balance between affinity and metabolic liability.

A particularly interesting compound developed within this work is 3-pyridyl **2.20**, which has a very similar affinity for BRD4, but is predicted to have much more reasonable pharmacokinetics than OXFBD02 (**2.1**), with a significantly reduced $ChromLogD_{pH7.4}$ value, and reasonable FAME predictions. Another interesting analogue is the *para*-fluoro derivative (**2.11**), which gave very similar affinity to OXFBD02 (**2.1**). The fluorine blocks the metabolically liable 4-position of the monosubstituted aromatic, and this derivative is therefore predicted to have slightly more reasonable pharmacokinetics than OXFBD02 (**2.1**). However, its larger PFI value than OXFBD02 (**2.1**) indicates that this derivative could suffer in developability assays. In an attempt to establish whether an sp^2 centre was necessary on the WPF

shelf, the cyclohexyl derivative (**2.25**) proved that more saturated systems are well tolerated, with the best IC_{50} of the set of analogues. However, due to the very lipophilic nature of this compound, it is predicted to have poor pharmacokinetics with a large $ChromLogD_{pH7.4}$ value. When trying to find a better balance between affinity and metabolic stability, most amine derivatives synthesised showed weak affinity for BRD4, presumably due to their polarity. However, geminal difluorinated piperidine **2.44** showed much greater affinity, presumably due to the reduced basicity of the amine, increasing the lipophilicity of this derivative. This compound also has a large $ChromLogD_{pH7.4}$ value, and is therefore predicted to be unsuitable for use *in vivo*. However, based on the FAME and SMARTCyp metabolism predictions, we hypothesise that more lipophilic amide analogues could balance the affinity and metabolic stability for this series of BRD4 ligands. Future work should focus on synthesis of the remaining amide derivatives and testing against BRD4 to study the hypothesis that they would interact more effectively with the WPF shelf. Furthermore, it would be important to carry out microsomal stability assays, which can validate the FAME and SMARTCyp metabolism predictions, indicating whether any compounds are suitable for use *in vivo*. These results will also direct the optimisation of this series of compounds.

2.3.2 Conclusions

In conclusion, a number of interesting analogues of OXFBD02 (**2.1**) were developed within this work. The structure-activity relationships displayed in this chapter will guide the design of future BET bromodomain ligands. Furthermore, metabolic predictions suggest that a balance can be made between affinity for the BET bromodomains and metabolic stability, but it will be important to validate these predictions, for example using microsomal stability assays, to establish whether any of these compounds can be used *in vivo*.

Chapter 3: Fragment Ligands for Non-BET Bromodomains

This chapter describes work carried out to develop small fragment molecules for bromodomains without known small molecule ligands. The initial focus was the bromodomain of FALZ, a subunit of the NURF complex, which is important for chromatin remodelling, necessary for transcription.

3.1 Introduction and aims

3.1.1 Targeting non-BET bromodomains

When this work commenced, the literature was heavily focused on the BET bromodomains, with very little work being carried out on other bromodomains. As such, we felt it was important to gain a better understanding of the interactions of non-BET bromodomains with acetylated lysines, and believed that the best way to achieve this was with the use of small molecules.

3.1.2 Aims

The aim of the work described in this chapter was to aid in the development of compounds that are potent and selective for non-BET bromodomains, by adopting a fragment-based approach to ligand discovery. We aimed to initially target one particular non-BET bromodomain, but with the intention of discovering fragments that were potentially capable of binding any non-BET bromodomain.

3.1.3 The FALZ bromodomain

At the time of this work, there were no small molecule ligands for the bromodomain of fetal Alzheimer antigen (FALZ), otherwise known as bromodomain plant homeodomain (PHD) finger transcription factor (BPTF) and fetal Alz-50-reactive

clone 1 (FAC1). FALZ is the largest subunit of the nucleosome-remodelling factor (NURF) complex, which is implicated in development and numerous diseases including cancers and neurodegenerative diseases.[149-152] This protein contains a C-terminal bromodomain in close proximity to a PHD zinc finger motif known to bind to trimethylated lysine H3K4Me3.[153] Simultaneous interaction of acetylated lysines H4K16Ac and/or H4K20Ac with the bromodomain, and H3K4Me3 with the PHD finger, synergistically activate transcription.[67,154] The binding of the zinc finger motif to the trimethylated lysine is relatively well understood, however, little is known about the binding of the bromodomain. Therefore, it would be interesting to probe the histone-bromodomain interaction with small molecules, to enhance our understanding of bromodomain biology and potentially validate FALZ as a therapeutic target.

3.2 Initial target fragment molecules for FALZ

3.2.1 Docking studies highlighted potential starting fragment molecules

Initial docking studies carried out by the SGC indicated that **3.1**-**3.4**, incorporating the KAc-mimicking 3,5-dimethylisoxazole chemotype, had the potential to be good starting fragment molecules for FALZ (Figure 3.1). The drawback of using this high-throughput technique is that it only considers the flexibility of ligands, not the protein, making this technique inherently unreliability. Docking studies use X-ray crystal structures that only provide a snap-shot of a flexible protein, and therefore do not account for conformational changes upon binding. On the other hand, simulations that account for protein flexibility require significantly more time and computer power, and therefore are not useful when looking at a large collection of molecules. Importantly, the conformations of the ligands shown in Figure 3.1 are consistent with the X-ray crystal structures of published 3,5-dimethylisoxazoles, discussed previously, mimicking KAc's hydrogen bonding interactions with the highly conserved

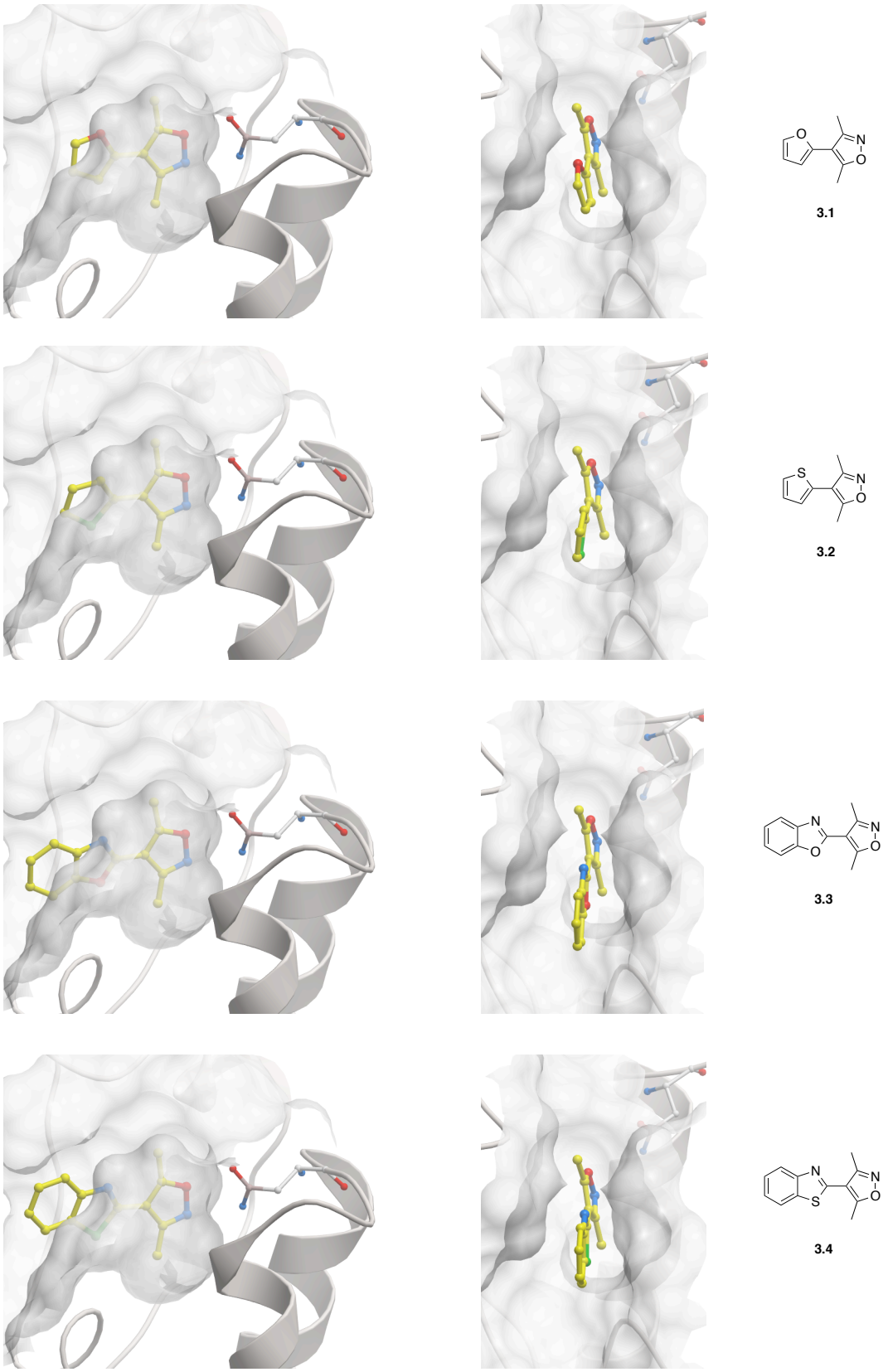
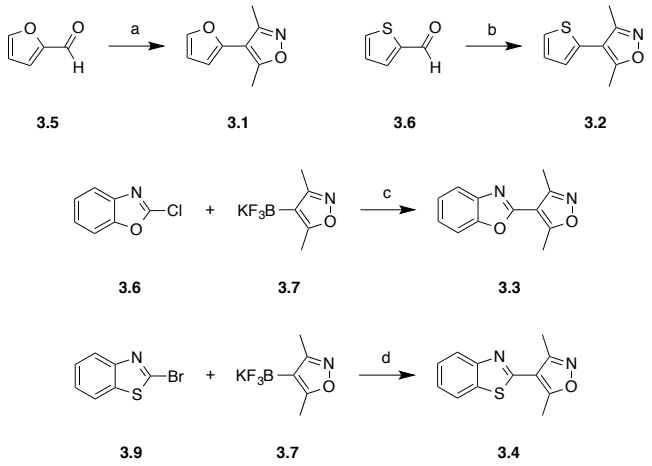


Figure 3.1: Initial fragment molecules **3.1**-**3.4** selected to target FALZ, from docking studies carried out by the SGC. Docking of small molecules into FALZ (PDB 3UV2) carried out using Molsoft ICM-Pro.[12]

asparagine residue. Therefore, we were confident that these ligands were good starting points for targeting the FALZ bromodomain.

3.2.2 Synthesis of initial target molecules

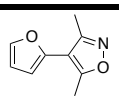
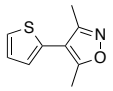
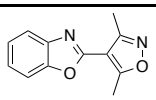
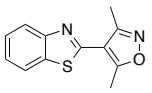


Scheme 3.1: Synthesis of initial targets **3.1**-**3.4**. *Reagents and conditions:* (a) Nitroethane, NaOH, EtOH, 120 °C, mw, 20 min, 18%; (b) Nitroethane, Cs_2CO_3, EtOH, 120 °C, 18 h, 6%; (c) $Pd(OAc)_2$, RuPhos, Na_2CO_3, EtOH, 85 °C, 25 h, 17%; (d) $Pd(OAc)_2$, RuPhos, Na_2CO_3, EtOH, 85 °C, 24 h, 29%.

Based on literature procedures, two possible synthetic routes to access the target molecules were envisaged (Scheme 3.1). One method for the synthesis of isoxazoles, first reported by Heim *et al*. in 1911, was used to generate target molecules **3.1** and **3.2**.[155] This method involved based-catalysed condensation of nitroethane with the aromatic aldehydes furfural (**3.5**) and 2-thiophenecarboxaldehyde (**3.6**) respectively. Following the procedure of Best *et al*., who obtained the desired product in 96% yield, dropwise addition of an aqueous solution of NaOH to furfural and nitroethane gave the desired product (**3.1**) in a poor yield of 18%, due to polymerisation of aromatic aldehyde **3.5**.[156] In an attempt to improve the yield of this reaction, slow, dropwise addition of furfural (**3.5**) to nitroethane and NaOH slowed the formation of the aromatic polymer, but afforded

the desired product (**3.1**) in an even smaller yield of 16%. Poor yields were also obtained when following the procedure of Noronha *et al*. to synthesise **3.2**, by heating thiophene-2-carbaldehyde (**3.6**) with nitroethane and caesium carbonate.[157] Target molecules **3.3** and **3.4** were prepared using a palladium-catalysed Suzuki-Miyaura cross-coupling of potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate (**3.8**) with 2-chlorobenzoxazole (**3.7**) and 2-bromo-1,3-benzothiazole (**3.9**) respectively.[140]

3.2.3 AlphaScreen™ analysis of **3.1**-**3.4** against a range of bromodomains

Compound Number	Compound	BRD4(1) %I[A]	BRPF3 %I[A]	FALZ %I[A]	TIF1 %I[B]	ATAD2 %I[A]	PHIP %I[A]	BAZ2A %I[A]	BRD4(1) IC_{50} (μM) *(upper/lower 95% confidence limits)*
3.1		5.90	3.75	3.95	0.00	0.00	0.00	0.00	n.d.
3.2		50.05	0.00	1.90	0.00	21.20	4.80	0.00	18.8 *(23.5/ 15.0)*
3.3		20.05	4.15	2.60	0.00	16.55	0.00	0.00	n.d.
3.4		43.30	1.50	2.20	0.00	11.85	0.00	0.00	16.5 *(21.3/ 12.8)*

Key: 0 — 100

Table 3.1. Single-shot percentage inhibition (%I), obtained (N = 1) using AlphaScreen™ by the SGC, of **3.1**-**3.2** against various bromodomains at concentrations of (A) 25 μM and (B) 18.75 μM, and IC_{50} values, measured in triplicate and averaged, of **3.2** and **3.4** against BRD4(1). Data are displayed as a heatmap, with 100% inhibition in red, and 0% inhibition in green.

With the target molecules in hand, their peptide displacement activity was tested against a selection of bromodomains, using an AlphaScreen™ assay at a single concentration, at the SGC (Table 3.1). Furan **3.1** showed negligible inhibition of peptide binding to all bromodomains tested. Benzoxazole **3.3** showed improved inhibition of peptide binding of BRD4 and ATAD2 over other bromodomains, while thiophene **3.2** and benzothiazole **3.4** displayed comparable inhibition of peptide binding of ATAD2, but proved to be better inhibitors of peptide binding of BRD4 than FALZ and other tested bromodomains. Measured IC_{50} values of these two compounds showed comparable but weak binding to BRD4.

3.3 New target molecules to exploit differences between FALZ and BRD4

3.3.1 Examination of crystal structures highlight opportunities to favour FALZ binding

With the 4-aryl-3,5-dimethylisoxazole compounds showing greatest affinity for the BET bromodomains, and with the intention of developing fragments for non-BET bromodomains, work was carried out to identify ligands that show preferential binding for non-BET bromodomains, including FALZ.

Upon examination of the crystal structure of FALZ, it is clear that the bromodomain recognition pocket has restricted entry relative to the large and promiscuous recognition pocket of BRD4 (Figure 3.2). This is due to a phenylalanine residue (F2887) that lies beside FALZ's WPF shelf. Predicted minimum energy torsion angles and observed torsion angles for 4-aryl-3,5-dimethylisoxazoles bound to BET and CREBBP bromodomains, calculated within the Conway group, suggest that the original set of fragment molecules would bind to BRD4 with a twist of 40-50 °.[62] With this in mind, we hypothesised that it would be possible to disfavour binding to BRD4, and to encourage entrance into the restricted recognition pocket of FALZ, by synthesising planar 6,5- and 6,5,5-fused systems incorporating

KAc-mimicking chemotypes, that would disrupt this dihedral angle. Consequently, we designed a range of 6,5,5- and 6,5-fused ring systems **3.9**-**3.13** as our new synthetic target set (Figure 3.3).

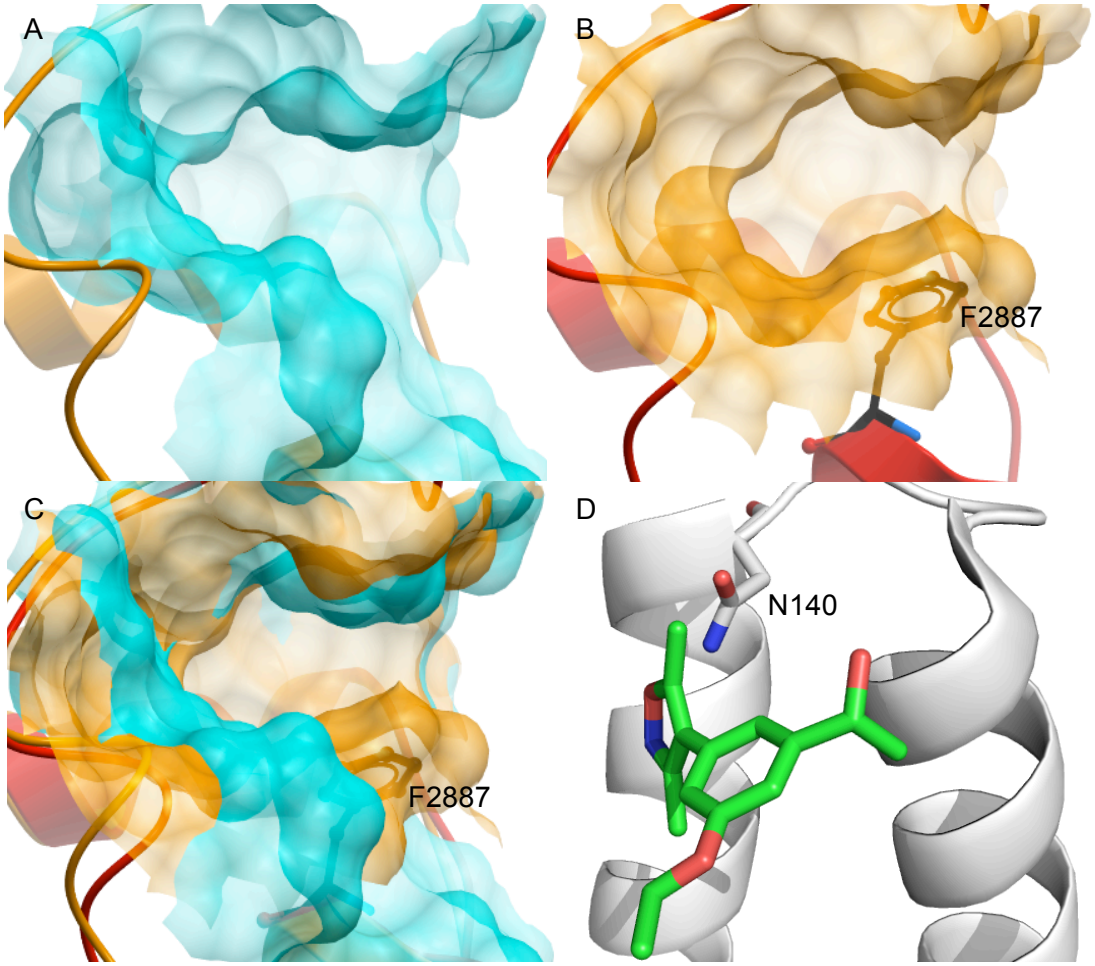
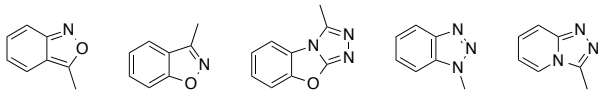


Figure 3.2. Overlay of recognition pockets of BRD4(1) (A, PDB 4GPJ) and FALZ (B, PDB 3UV2) bromodomains, using the *align* command in PyMOL.[12,49] C: Superimposition of the two bromodomains shows that the recognition pocket of FALZ is smaller than that of BRD4, and lacks the WPF shelf. D: 4-Aryl-3,5-dimethylisoxazoles bind to BRD4(1) with a twist of 40-50 °, as shown by the crystal structure of 3-(3,5-dimethyl-1,2-oxazol-4-yl)-5-[(*R*)-hydroxy(phenyl) methyl] phenol in complex with BRD4(1) (PDB 4J0R, carbon = green).[46]

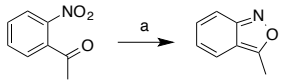


3.9 3.10 3.11 3.12 3.13

Figure 3.3. New target benzo-fused isoxazole and triazole fragments **3.9**-**3.13**.

3.3.2 Synthesis of new target molecules

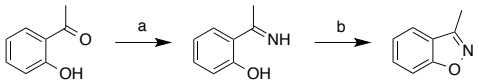
Facile reductive cyclisation of 2-nitroacetophenone **3.14**, following the procedure of Kim *et al*. afforded fused isoxazole **3.9** in one step (Scheme 3.2).[158]



3.14 3.9

Scheme 3.2. Synthesis of new target molecule **3.9**. *Reagents and conditions:* (a) Zn, 2-bromo-2-nitropropane, MeOH, 50 °C, 22 h, 25%.

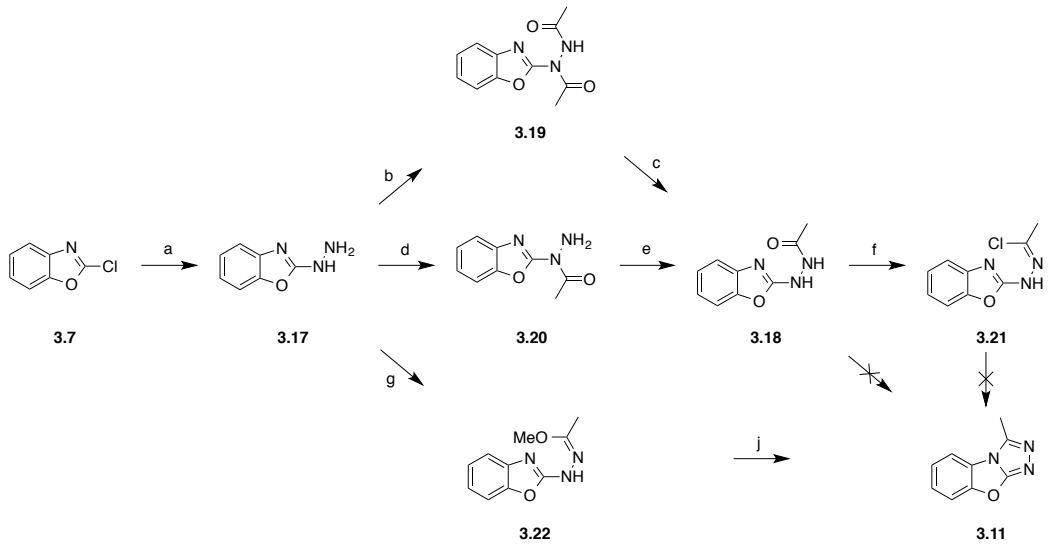
Its isomer **3.10** was obtained following the procedure of Chen *et al*., by forming the imine **3.16** of 2-hydroxyacetophenone by treating with ammonia, followed by NCS-mediated cyclisation, facilitated by generating the *N*-Cl imine intermediate *in situ* (Scheme 3.3).



3.15 3.16 3.10

Scheme 3.3. Synthesis of new target molecule **3.10**. *Reagents and conditions:* (a) NH_3, MeOH, rt, 41 h, 60%; (b) NCS, K_2CO_3, THF, rt, 19 h, 27%.

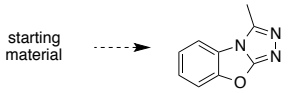
The synthesis of triazolobenzoxazole **3.11** was reported by Badr *et al*., although no yields were provided, by forming 2-hydrazinobenzoxazole, acetylating it with Ac_2O then performing a disfavoured 5-endo-trig cyclisation to give the desired product (**3.11**), by heating the acetohydrazide above its melting point.[159] Rapid synthesis of hydrazinobenzoxazole **3.17** was achieved by treatment of 2-chlorobenzoxazole **3.7** with hydrazine monohydrate in dioxane (Scheme 3.4). However, treating **3.17** with neat Ac_2O following the procedure of Badr *et al*. led to very rapid diacetylation to give **3.19**, but methanolysis afforded the desired monoacetylated hydrazide (**3.18**). Carrying out the acetylation under milder conditions, using Ac_2O and NEt_3 in CH_2Cl_2 in fact afforded acetylation on the undesired nitrogen instead (**3.20**), and still gave the diacetylation side-product (**3.18**). However, heating monoacetylated hydrazide **3.20** at 140 °C, under a gentle stream of nitrogen led to migration of the acetyl group to the desired nitrogen (**3.18**). This migration was evidenced by 500 MHz ^{1}H NMR in DMSO-D_6; the starting material's singlet peak at 5.56 ppm, corresponding to a NH_2 were replaced by two singlet peaks at 9.76 and 10.14 ppm in the product ^{1}H NMR, corresponding to the C(2)N*H* and N*H*$COCH_3$ protons respectively. Due to the synthesis of this compound being less trivial than first imagined, 2-chlorobenzoxazole was instead treated with acetylhydrazide, and was heated to 60 °C, but the desired product was afforded in a yield of 7%.



Scheme 3.4. Synthesis of new target molecule **3.11**. *Reagents and conditions:* (a) $NH_2NH_2•H_2O$, dioxane, 0 °C-rt, 10 min, 44%; (b) Ac_2O, rt, 5 min, 35%; (c) NaOMe, MeOH, 0 °C-rt, 1 h, 82%; (d) Ac_2O, NEt_3, CH_2Cl_2, rt, 90 min, 13%; (e) 140 °C, 1 h, not isolated; (f) i) $POCl_3$, 100 °C, 1 h; ii) H_2O, 100 °C, 2 h, 50%; (g) $CMe(OMe)_3$, MeOH, rt, 16 h, 58%; (h) Table 3.2, Entries 1-6; (i) Table 3.2, Entries 9-11; (j) 200 °C, 20 min, 14%.

With desired monoacetylated hydrazide **3.18** in hand, cyclisation attempts were made (Table 3.2). Heating the solid to 190 °C, indeed above the starting material's melting point as suggested by Badr *et al*., did not produce any of cyclised final product **3.11** (Table 3.2, Entry 1). To encourage the elimination of MeOH, efforts were made to cyclise the acetylated hydrazide (**3.18**) in acid. Dissolving in AcOH, and heating to 180 °C with microwave irradiation, did not fully consume the starting material (**3.18**), and none of the desired cyclised product (**3.11**) could be isolated (Table 3.2, Entry 2). Heating **3.18** at a higher temperature and for longer degraded the starting material to benzoxazole (Table 3.2, Entry 3). To prevent this degradation, acetylhydrazide **3.18** was heated at a lower temperature without microwave irradiation, but starting material (**3.18**) was still present after 49 h and no product (**3.11**) could be isolated (Table 3.2, Entry 4). Switching to tosic acid and stirring at rt gave no consumption of starting material (**3.18**), and when heated at 70 °C for an extended period of time, starting material **3.18** was consumed but no product could

be isolated (Table 3.2, Entry 5). As an alternative to acidic cyclisation, attempts were also made in base, by heating under reflux in an aqueous 2% NaOH solution, using the conditions of Pitucha *et al*., however these conditions also failed to yield any product (Table 3.2, Entry 6).[160]



3.11

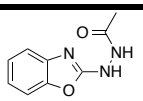
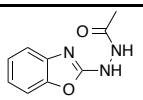
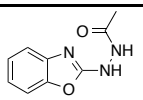
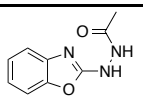
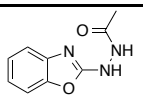
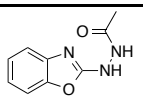
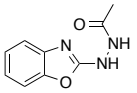
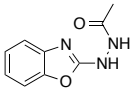
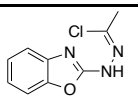
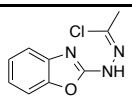
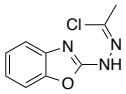
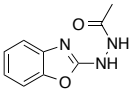
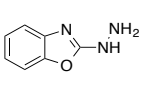
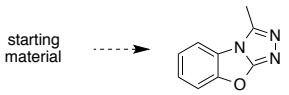
Entry	Starting material	Reagents/ Solvents	T (°C)	Conditions	t (h)	Product observed
1		neat	190	-	1	✗
2		AcOH	180	mw	0.5	✗
3		AcOH	180-210	mw	2	✗
4		AcOH	120	-	49	✗
5		$TsOH•H_2O$/ THF	rt-70	-	117	✗
6		2% NaOH/ H_2O	100	-	20	✗
7		i) $POCl_3$ ii) H_2O	100 100	- -	1 2	✗
8		i) $POCl_3$ ii) H_2O	110 120	- -	2 20	✗
9		AcOH	180	mw	0.5	✗
10		NEt_3/ CH_2Cl_2	180	mw	0.5	✗
11		$AgNO_3$/ DMF	180	mw	0.5	✗
12		PPA	100-140	-	19	✗
13		$CH(OMe)_3$/ MeOH	rt	-	4	✗

Table 3.2(i). Reagents and conditions used in attempts to synthesise triazole **3.11**.



3.11

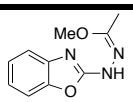
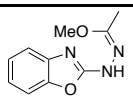
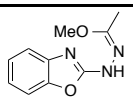
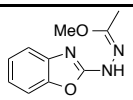
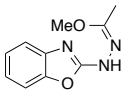
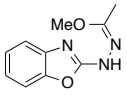
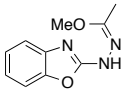
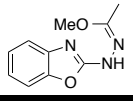
Entry	Starting material	Reagents/ Solvents	T (°C)	Conditions	t (h)	Product observed
14		$TsOH•H_2O$/ THF	rt	-	3	✓
15		AcOH	rt	-	41	✗
16		AcOH	140	-	1.5	✗
17		$BF_3•Et_2O$/ CH_2Cl_2	rt	-	27	✗
18		-	100	*in vacuo*	1	✗
19		-	150	*in vacuo*	19	✓
20		-	150	-	4	✓
21		-	200	-	0.3	✓

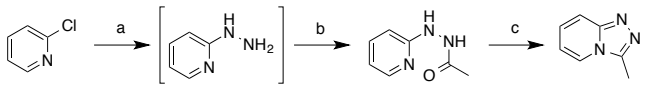
Table 3.2(ii). Reagents and conditions used in attempts to synthesise triazole **3.11**.

In an attempt to encourage the cyclisation by forming the chlorimine *in situ*, following a procedure by Bounaud *et al*., acetylhydrazide **3.18** was heated with $POCl_3$, to which was added H_2O, followed by more heating to cyclise.[161] Despite heating at 100 °C for 2 h under these acidic conditions, no cyclisation was observed, but chlorimine **3.21** was isolated in 50% yield (Table 3.2, Entry 7). However, when heated for 20 h under these acidic conditions, neither chlorimine **3.21** nor cyclised product **3.11** could be isolated (Table 3.2, Entry 8). When the isolated chlorimine **3.21** was subjected to heating at 180 °C with microwave irradiation in the presence of AcOH, NEt_3 or $AgNO_3$, no desired product **3.11** was afforded, but starting material **3.21** was recovered (Table 3.2, Entries 9, 10 and 11). An alternative strategy that was attempted was the use of another dehydrating agent, polyphosphoric acid

(PPA). Following the conditions of Arcus and Prydal, heating acetylhydrazide **3.18** in PPA at 100-140 °C did not afford desired product **3.11** (Table 3.2, Entry 12).[162] Another approach to form desired triazole **3.11** was by treatment of unacetylated hydrazine **3.17** with trimethylorthoacetate, following the procedure of Elworthy *et al*. These conditions did not afford desired cyclised product **3.11**, but did afford methylethanehydrazonoate **3.22** (Table 3.2, Entry 13).[163] Stirring **3.22** in tosic acid gave the first observation of desired triazole product **3.11** (Table 3.2, Entry 14), however, this compound proved inseparable from tosic acid, and so alternative cyclising agents were sought. Unfortunately, stirring methylethanehydrazonoate **3.22** in AcOH at rt did not afford the product, with incomplete consumption of starting material **3.22**, and heating under reflux at 140 °C gave neither returned starting material **3.22** nor afforded desired product **3.11** (Table 3.2, Entries 15 and 16). Using a Lewis acid, $BF_3 \bullet Et_2O$, failed to encourage the cyclisation as well (Table 3.2, Entry 17). Therefore, attempts were made to eliminate MeOH and force the cyclisation without additional reagents. Heating methylethanehydrazonoate **3.22** at 100 °C under vacuum gave no conversion, but heating at 150 °C under vacuum showed evidence of product, as seen by mass spectrometry, but also caused the starting material (**3.22**) to sublime (Table 3.2, Entries 18 and 19). Therefore, the methylethanehydrazonoate **(3.22**) was instead heated at 150 °C for 4 h, at atmospheric pressure, and the desired triazolo compound (**3.11**) was isolated in 7% yield (Table 3.2, Entry 20). Pleasingly, higher temperatures for a shorter length of time afforded the desired compound (**3.11**) in an improved yield of 14% (Table 3.2, Entry 21).

Synthesis of pyridyl derivative (**3.13**) was also approached by forming the hydrazine (**3.23**) from 2-chloropyridine (**3.24**), and acetylating it before cyclisation (Scheme 3.5). Unlike for benzoxazole analogue (**3.11**), forming pyridyl hydrazine **3.23** proved slow, with incomplete consumption of starting material **3.24** after three

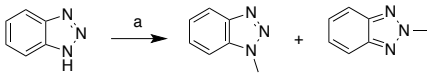
days, and poor yielding, affording the hydrazine (**3.13**) in 10% yield. Due to rapid diacetylation of **3.23** in neat Ac_2O, alongside the more dilute benzoxazole reaction (Scheme 3.4(d)), the pyridyl hydrazine (**3.23**) was concurrently treated with Ac_2O in NEt_3 and CH_2Cl_2. Fortunately, unlike for the benzoxazole derivative that was acetylated on the undesired hydrazine nitrogen under these conditions (**3.20**), desired acetylhydrazide (**3.25**) was produced in 20% yield, but diacetylation was also observed. To generate more material, the hydrazine formation was carried out under much more forcing conditions, by heating 2-chloropyridine (**3.24**) in neat hydrazine monohydrate at 120-130 °C with microwave irradiation. Subsequent acetylation under the previous conditions, followed by heating at 180 °C with microwave irradiation in AcOH, afforded desired triazole **3.13** in 34% yield.



3.24 **3.23** **3.25** **3.13**

Scheme 3.5. Synthesis of new target molecule **3.13**. *Reagents and conditions:* (a) $NH_2NH_2{\bullet}H_2O$, 120-130 °C, 90 min; (b) Ac_2O, NEt_3, CH_2Cl_2, 0 °C-rt, 2 h; (c) AcOH, 180 °C, mw, 30 min, 34%.

The last analogue of the collection was the 1-methyl-1*H*-benzotriazole (**3.12**), which was synthesised in one step from benzotriazole (**3.26**), by stirring with MeI and sodium hyroxide in acetonitrile at rt, following the procedure of Rajakumar *et al*., which also produced its isomer 2-methyl-2*H*-benzotriazole (**3.27**) as a minor side-product (Scheme 3.6).[164]



3.26 **3.12** **3.27**

Scheme 3.6. Synthesis of new target molecule **3.12**. *Reagents and conditions:* (a) MeI, NaOH, H_2O, MeCN, rt, 7 h, 65% **3.12** and 13% **3.27**.

3.3.3 STD NMR and AlphaScreen™ evaluation of compounds **3.1**, **3.2** & **3.9**-**3.13**

With the fused 6,5- and 6,5,5-systems in hand, we carried out work to establish whether these derivatives bind to the FALZ bromodomain. At this point in time, the SGC were having problems with their AlphaScreen™ assay, and therefore an alternative technique was sought until the AlphaScreen™ assay could be used again. As such, the first approach used to assess whether the compounds bound to FALZ, was saturation transfer difference (STD) NMR, a simple technique that could be run in the department, with the help of Tim Claridge and Barbara Odell from the NMR department. In this technique, the protein is in the presence of a large excess of ligand. Due to the nuclear Overhauser effect, magnetic saturation of the protein leads to transfer of saturation to ligands that bind to the protein. An STD spectrum, showing only the saturated resonances, can be obtained by taking the difference between the off-saturation and on-saturation spectra. This technique is able to identify relatively weak-binders, because saturation transfer needs to occur before the bound ligand has transferred to an unbound state, but detection of this saturation transfer is only achieved when it has exchanged to the unbound state. Tightly bound ligands will not exchange as efficiently to the unbound state, meaning weak STD effects are observed. On the other hand, extremely weak binders will not be bound to the protein long enough for saturation transfer to occur. Therefore, this technique relies on ligands with an off-rate that is slow enough to allow saturation transfer, but fast enough for detection in the unbound state before relaxation occurs.[165] Therefore, correlation of binding affinity with STD data is challenging, but, at the very least, an observed STD effect indicates binding.

Compounds **3.9**-**3.13**, as well as the non-fused furan and thiophene compounds **3.1** and **3.2**, were assessed for their STD effect with FALZ. Unfortunately, the non-fused benzo derivatives **3.3** and **3.4** suffered from solubility issues, so their STD effects in FALZ could not be measured. As a representative example, Figure 3.4 shows the STD spectrum, and STD effect, for **3.2**.

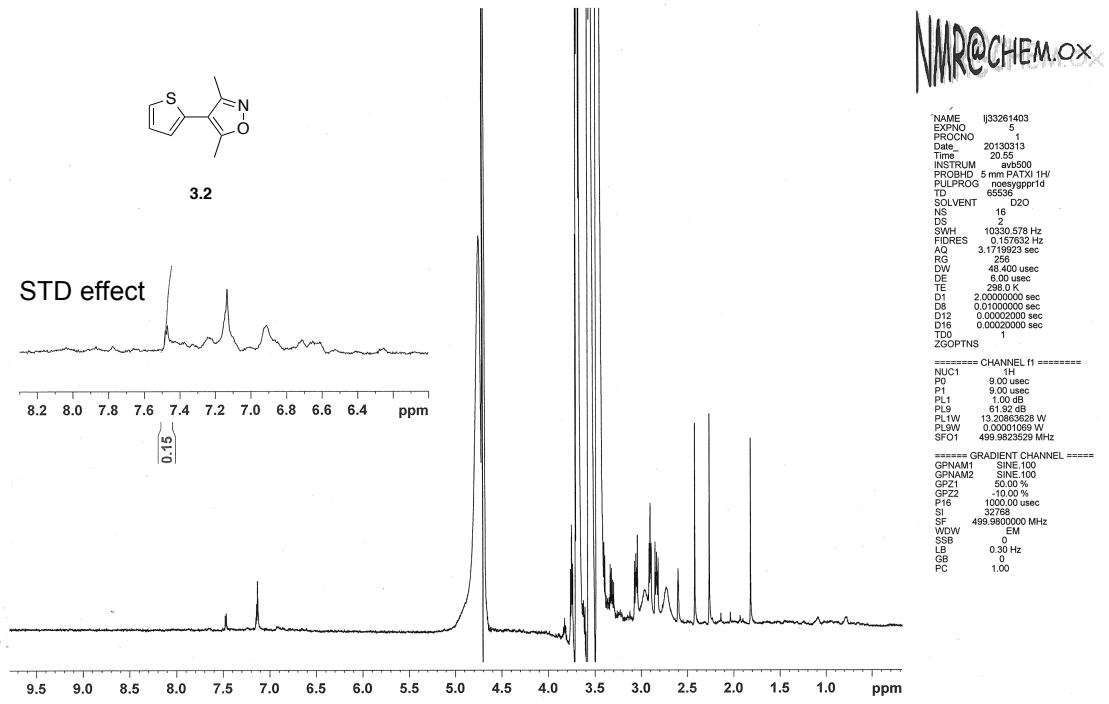


Figure 3.4. STD spectrum and STD effect for **3.2**.

All derivatives tested showed a weak to moderate STD effect in FALZ, indicating that these fragments all bind to the protein (Table 3.3). The isoxazoles showed a greater STD effect than the triazoles, however, as mentioned previously, we cannot rank these compounds in terms of binding affinity, but we can say that they bind weakly to FALZ. In order to get a better idea of the affinity of these compounds, they were subjected to an AlphaScreen™ assay at a single concentration, once the issues with this assay had been resolved by the SGC. In this assay, the only compounds to show any inhibition of peptide binding to FALZ were furan **3.1** and fused isoxazole **3.9**, with moderate percentage inhibition, and triazole **3.11**, with a very small percentage inhibition of binding. Due to the poor accuracy of

AlphaScreen results, we cannot conclude that fused isoxazole **3.9** binds with higher affinity than non-fused isoxazole **3.1**, despite the larger percentage inhibition value obtained. However, based on our previous AlphaScreen™ results, which showed a strong preference of **3.1** for BRD4, along with our hypothesis that fused isoxazoles would not bind as well to BRD4, which binds 4-substituted-3,5-dimethylisxoazoles with a twist of 40-50 °, as discussed previously, we decided to pursue optimisation of **3.9**.

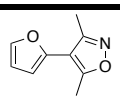
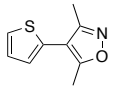
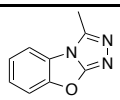
Compound Number	Compound	Approx % STD Effect	FALZ %I
3.1		11	22.9
3.2		15	0.00
3.9		10	27.65
3.10		7	0.00
3.11		1	3.35
3.12		3	0.00
3.13		1	0.00
	Key	0	100

Table 3.3. Approximate percentage STD effect, obtained (N = 1) by T. Claridge and B. Odell, and single-shot percentage inhibition (%I) of FALZ peptide binding of **3.1**, **3.2** & **3.9**-**3.13** at 250 μM concentration, obtained (N = 1) by the SGC. Data are displayed as a heatmap, with 100% STD effect or inhibition in red, and 0% STD effect or inhibition in green.

3.4 Hypothesised optimisation of 3.9

3.4.1 Targeting additional interactions

Upon further examination of the crystal structure of FALZ, it was hypothesised that the carbonyl of WPF shelf proline (P2825) can potentially be exploited to improve binding affinity. Docking of **3.28**, an aniline derivative of fused isoxazole **3.9**, suggests that this compound can hydrogen bond to the proline carbonyl moiety (Figure 3.5). Incorporating the aniline functionality would not only provide a hydrogen-bond donor, but also provides a vector for expansion of the fragment molecule. It was hypothesised that an internal hydrogen bond with a carbonyl in the 5-position would cement this orientation of the amino group, so both the mono- and di-substituted fused isoxazoles **3.28** and **3.29** became the new target molecules.

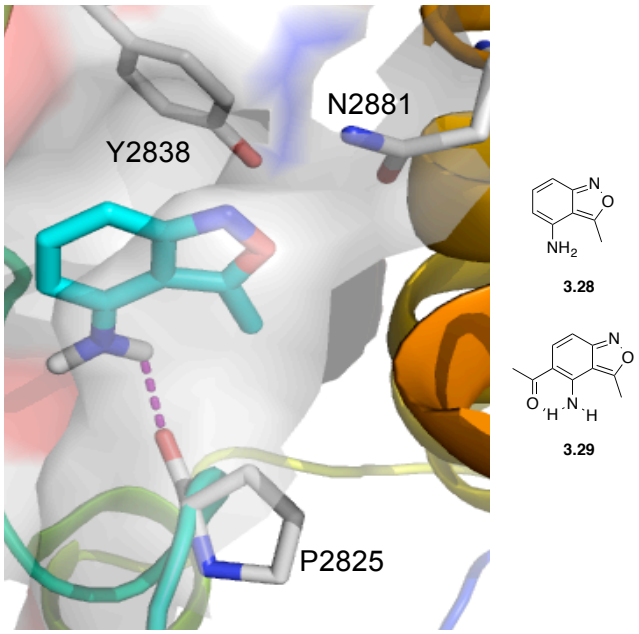
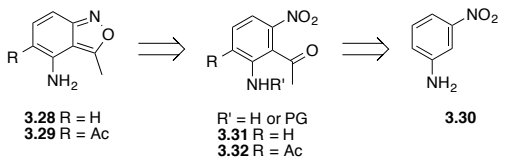


Figure 3.5: Target molecules **3.28** and **3.29**. Docking of **3.28** into FALZ (PDB 3UV2) shows potential interaction with P2825.

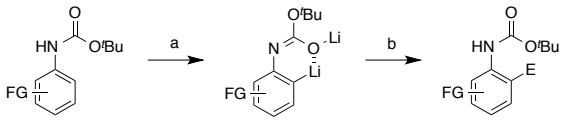
3.4.2 Synthesis of aniline derivatives

Retrosynthetic analysis highlighted that we could synthesise the functionalised, fused, isoxazoles from 2-acetyl-3-nitroaniline derivatives, which in turn could be synthesised from commercially available starting materials (Scheme 3.7). For example, by incorporation of the acetyl functionality into 3-nitroaniline (**3.30**) in the positions *ortho* to the amino group. We envisaged that subsequent reductive cyclisation of the 2-acetyl-3-nitroanilines (**3.31** & **3.32**) could afford the desired final compounds **3.28** and **3.29**.



Scheme 3.7. Proposed retrosynthesis of **3.28** and **3.29** to commercially available 3-nitroaniline **3.30**.

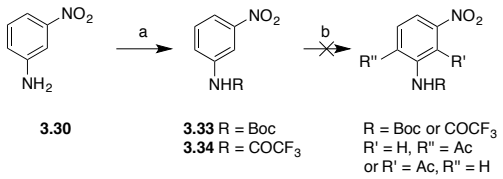
Exploiting the amino functionality of anilines by way of *ortho*-metallation is one technique that has been well explored in the literature. For example, *ortho*-functionalisation of Boc-protected aniline using ^{t}BuLi to form a dilithiated intermediate, followed by trapping with an electrophile such as MeI, $(PhS)_2$, benzaldehyde, carbon dioxide and benzonitrile, has been reported by Muchowski and Venuti (Scheme 3.8).[166]



Scheme 3.8. *ortho*-Lithiation of functionalised anilines, by Muchowski and Venuti.[166] *Reagents and conditions:* (a) ^{t}BuLi; (b) Electrophile (E^{+}) such as CH_3I, $(PhS)_2$, PhCHO, CO_2 and PhCN.

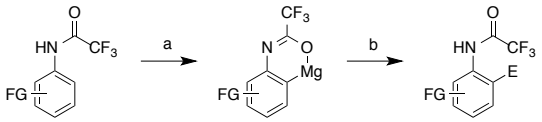
Boc protection of 3-nitroaniline (**3.30**) gave **3.33** in 42% yield, however, *ortho*-lithiation, using the conditions of Muchowski and Venuti, treating **3.33** with ^{t}BuLi

at –78 °C, then with AcCl at –10 °C, failed to afford any of the desired intermediate (Scheme 3.9). Despite carbonyl functionality, including aldehydes and amides, being introduced using their methodology, no cases of *ortho*-acetylation have been reported. Furthermore, no cases of *ortho*-functionalisation in the presence of a nitro group have been reported either. With *ortho*-lithiation proving unsuitable for generation of the desired functionalised fused isoxazole, work was then carried out to establish whether *ortho*-magnesiation could be an appropriate alternative.



Scheme 3.9. Attempts to *ortho*-functionalise proved unsuccessful. *Reagents and conditions:* (a) R = Boc: Boc_2O, DMAP, THF, 0 °C-reflux, 22 h, 42%; R = $COCF_3$: TFAA, $NaHCO_3$, H_2O, CH_2Cl_2, rt, 30 min, 93%; (b) R = Boc: i) ^{t}BuLi, THF, –78- –10 °C, 2.5 h; ii) AcCl, rt, 1 h; R = $COCF_3$: i) MeMgCl, THF, 0 °C, 10 min; ii) TMPMgCl•LiCl, 0 °C-rt, 4 h; iii) CuCN•2LiCl, –40 °C, 10 min; iv) AcCl, –40-0 °C, 2 h.

Work in the group of Knochel has focused on the *ortho*-magnesiation of functionalised anilines using mild conditions.[167] Some *ortho*-functionalisations published by the Knochel group require conversion of the functionalised aniline to the trifluoroacetamide, using TFAA, followed by *ortho*-magnesiation, and reaction with an aryl halide, a heteroaryl halide, an allyl halide, an aldehyde or an acyl chloride, or alternatively first transmetallation with CuCN•2LiCl, to optimise reactivity, then reaction with the electrophile (Scheme 3.10).

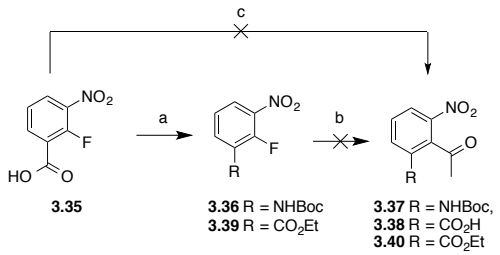


Scheme 3.10. *ortho*-Magnesiation of functionalised anilines developed in the Knochel group.[167] *Reagents and conditions:* (a) i) MeMgCl, THF, 0 °C, 10 min; ii) TMPMgCl•LiCl, rt; (b) E^+ or CuCN•2LiCl then E^+. E^+ are aryl- and heteroaryl halides, allyl halides, aldehydes and acyl chlorides.

Facile trifluoroacetylation of 3-nitroaniline gave **3.34** in 93% yield, but the magnesiation of **3.34**, using MeMgCl followed by TMPMgCl•LiCl, then quenching with CuCN•2LiCl and distilled acetyl chloride, only gave starting material **3.34** (Scheme 3.10). To establish whether the reaction failed due to magnesiation incompletion or due to addition of the acetyl chloride, the magnesiation was monitored using electrospray ionisation gas chromatography with a time-of-flight mass spectrometer. Despite quenching the reaction aliquots with CuCN•2LiCl then allyl bromide or D_2O, only starting material **3.34** could be observed, indicating that no magnesiation was being achieved. Despite publishing compatibility with functionalities including halogens, nitriles and esters, suitability has not been reported for nitro groups, suggesting that this is the reason magnesiation did not occur for this substrate.

With *ortho*-functionalisation of 3-nitroaniline proving challenging, work was then carried out to derive 2-acetyl-3-nitroanilines from trisubstituted aromatics. One common method for acetylating *ortho* to a nitro group is using nucleophilic aromatic substitution. This approach has been used to acetylate 2-fluoroaniline, and other halogenated analogues by Reid and Runge.[168] Their methodology involved substitution of the halogen for a nitroethane group, which could then be oxidised to the acetyl group. It was envisaged that Curtius rearrangement of commercially available 2-fluoro-3-nitrobenzoic acid to *tert*-butyl (2-fluoro-3-nitrophenyl)carbamate provided a protected precursor to the desired 2-acetyl-3-nitroaniline derivatives (**3.31**,

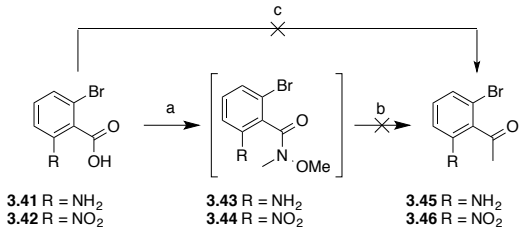
Scheme 3.7). The Curtius rearrangement of **3.35** gave **3.36** in good yield, however, subjecting this product to nitroethane in the presence of DBU, even with heating to 60 °C, did not convert the starting material (**3.36**) to the desired intermediate (**3.37**, Scheme 3.11). To investigate whether the electron-donating amino functionality was hindering this substitution, the same conditions were applied to commercially available 2-fluoro-3-nitrobenzoic acid **3.35**, with the intention that the Curtius rearrangement could be carried out at a later stage. However, despite full consumption of starting material (**3.35**), no product (**3.38**) was obtained after addition of H_2O_2. Reid and Runge had not reported the substitution in the presence of either amines or acids, but they had successfully carried out the reaction in the presence of an ester. As such, protection of the benzoic acid (**3.35**) as an ethyl ester **3.39** was carried out in good yield. However, subjecting **3.39** to nitroethane and DBU at rt consumed the starting material, but did not give any of the required intermediate (**3.40**), consequently the reaction was abandoned.



Scheme 3.11. Attempted conversion of aryl fluorides to acetyls failed to work. *Reagents and conditions:* (a) R = NHBoc: Diphenyl phosphorazidate, NEt_3, ^tBuOH, 105 °C, 19 h, 70%; R = CO_2Et: EtI, $NaHCO_3$, DMF, rt, 43 h, 77%; (b) R = NHBoc: i) Nitroethane, DBU, EtOAc, rt-60 °C, 5 days; ii) H_2O_2, $NaHCO_3$, H_2O, MeOH, rt, 22 h; R = CO_2Et: Nitroethane, DBU, EtOAc, rt, 1 h; (c) i) Nitroethane, DBU, EtOAc, rt-60 °C, 3 days; ii) H_2O_2, $NaHCO_3$, H_2O, MeOH, rt, 20 h.

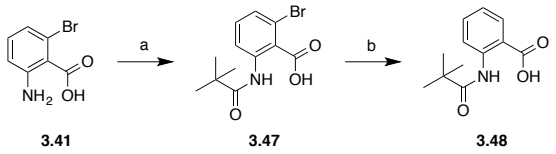
An alternative synthetic strategy was envisaged, in which a benzoic acid is converted to an acetyl group. This approach was attempted on two commercially available substrates, 2-amino-6-bromobenzoic acid (**3.41**) and

2-bromo-6-nitrobenzoic acid (**3.42**), with the intention that the other nitro and amino groups could be added at a later stage. Conversion to the respective Weinreb amides (**3.43** & **3.44**), followed by treatment with MeMgBr did not afford the desired acetyl derivatives (**3.45** & **3.46**, Scheme 3.12). Another attempt at this conversion was made following the work of Lee and Youn, who used MeLi to convert 2-aminobenzoic acids to 2-acetylanilines.[169] However, treatment of 2-amino-6-bromobenzoic acid (**3.41**) with MeLi failed to give any of the desired product (**3.45**), presumably due to faster lithium-halogen exchange than nucleophilic attack.



Scheme 3.12. Attempted conversion of benzoic acids **3.41** to acetylated **3.42**. *Reagents and conditions:* (a) R = NH_2: *N*,*O*-Dimethylhydroxylamine hydrochloride, DIPEA, HATU, DMF, rt, 5 h; R = NO_2: *N*,*O*-Dimethylhydroxylamine hydrochloride, DIPEA, HATU, DMF, rt, 4 h; (b) R = NH_2: MeMgBr, THF, 0 °C-rt, 18 h; R = NO_2: MeMgBr, THF, 0 °C-rt, 73 h; (c) R = NH_2: MeLi, DME, 0 °C-rt, 24 h.

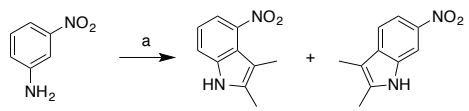
Work by Zhang *et al*. has shown the reactivity of Grignard reagents with pivaloyl protected 2-aminobenzoic acids.[170] Pivaloyl protection of the aniline derivative amino group (**3.41**) gave **3.47** in a moderate yield, but treatment of this with MeMgBr did not produce the desired acetylated aromatic, with the debrominated starting material (**3.48**) isolated instead (Scheme 3.13).



3.41 3.47 3.48

Scheme 3.13. Attempts to make *N*-(2-acetyl-3-bromophenyl)-2,2-dimethylpropanamide failed, producing the debrominated starting material (**3.48**) instead. *Reagents and conditions:* (a) Pivaloyl chloride, NaOH, TBME, H_2O, 0-50 °C, 3 days, 36%; (b) MeMgBr, THF, Et_2O, 0-70 °C, 5 days, 19%.

Success was achieved following the work of Guo *et al*. who synthesised *N*-(2-acetyl-3-nitrophenyl)acetamide **3.49** in two steps from 3-nitroaniline (**3.30**): firstly *via* formation of 2,3-dimethyl-4-nitro-1*H*-indole (**3.50**), and secondly through oxidative opening of the indole.[171] As with the work of Guo *et al*. and Moskalev *et al*. this procedure suffers from poor yields in the first step.[172] On a 600 mg scale, yields for the desired and undesired indoles (**3.50** & **3.51**) were 19 and 11%, and on a 5 g scale the indoles (**3.50** & **3.51**) were obtained in 15 and 12% yields, respectively (Scheme 3.14). Analysis of coupling constants (*J*) enabled the different regioisomers to be identified. The aromatic peaks of **3.50** were two doublet of doublets with *J* = 8.0 and 0.7 Hz, and one doublet of doublets with *J* = 8.0 and 8.0 Hz, corresponding to three adjacent aromatic protons. Conversely, the aromatic peaks of **3.51** were a doublet with *J* = 2.0 Hz, another doublet with *J* = 8.8 Hz, and one doublet of doublets with *J* = 8.8 Hz and 2.0 Hz, corresponding to two adjacent aromatic protons and another aromatic proton in a *meta*- and *para*-position to the adjacent aromatic protons.

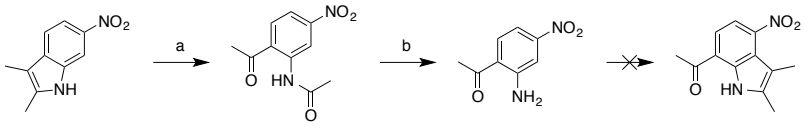


3.30 3.50 3.51

Scheme 3.14. Formation of indoles **3.50** and **3.51**. *Reagents and conditions:* (a) Butan-2-one, KO^{t}Bu, DMSO, rt, 2 h, 19% **3.50** and 11% **3.51**.

Considerable amounts of DMSO were used as a solvent in these reactions, and without the use of a centrifugal evaporator, this made the work-up procedures extremely challenging. Therefore, it was hypothesised that the yields of these reactions could be improved with the use of more volatile solvents. Firstly it was considered that the butanone reagent could also be used as a solvent, however, this gave no conversion of starting material (**3.30**) to either of the indoles (**3.50** & **3.51**). Therefore alternative solvents were considered. In DMSO, the starting material (**3.30**) was consumed within two hours. However in DMF, dioxane or ^{t}BuOH, starting material (**3.30**) was only consumed after 22 h. In the case of DMF, both the desired and undesired indoles (**3.50** & **3.51**) were produced, but pleasingly, in dioxane and ^{t}BuOH only the desired indole (**3.50**) was formed, as seen by thin layer chromatography (TLC). The dioxane reaction appeared cleaner than the ^{t}BuOH reaction, as evidenced by TLC, and so dioxane was chosen as the solvent for subsequent reactions. Disappointingly, even after removing as much of the volatile components as possible *in vacuo*, the work-up for the indole formation carried out in dioxane still proved extremely challenging, and the best yield obtained for the desired indole (**3.50**) was only 12%, with no improvement in yield achieved.

In an attempt to derive the difunctionalised, fused isoxazole **3.29** (Figure 3.5), the undesired indole **3.51** was opened to **3.52** with CrO_3, however, in poor yield (Scheme 3.15). Subsequent deacetylation of the amino group to give **3.53** was achieved by heating under reflux in ethanolic HCl in excellent yield, however, attempts to form the 7-acetyl-4-nitroindole derivative of this substrate (**3.54**) proved unsuccessful, presumably due to the acetyl functionality. As such, attempts were made to protect the acetyl group as an acetal, but no product could be isolated. Therefore, work focused on the formation of the monofunctionalised, fused isoxazole (**3.28**) instead.



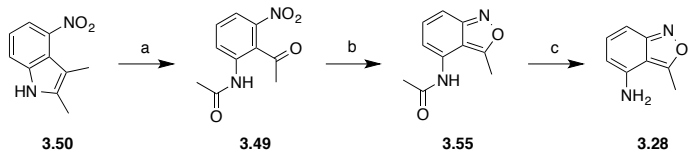
3.51 **3.52** **3.53** **3.54**

Scheme 3.15. Attempts to derive the difunctionalised fused isoxazole **3.29** proved unsuccessful. *Reagents and conditions:* (a) CrO_3, AcOH, H_2O, 0 °C-rt, 4 h, 10%; (b) HCl, H_2O, EtOH, reflux, 2 h, 97%; (c) Butan-2-one, KO^tBu, DMSO, rt.

With the desired indole (**3.50**) in hand, oxidative opening using CrO_3 afforded **3.49** in good yield (Scheme 3.16). Reductive cyclisation to the fused isoxazole acetamide **3.55** was also afforded in good yield, despite taking several days to reach completion. This derivative was selected as a new molecule for testing in comparison with its unacetylated form (**3.28**).

Deacetylation of the amino group of **3.55** proved more challenging. Attempts were made to deacetylate the amino group in the same way as was achieved in good yield for **3.52**, by heating under reflux in HCl and EtOH. These conditions led to significant degradation of **3.55**, with an inseparable mixture of many products. The desired product **3.28** could not be isolated. As such, basic deacetylation was attempted instead. Heating **3.55** at 75 °C with NaOH in H_2O and ^{i}PrOH gave very little conversion of starting material to product (**3.28**). However, using MeOH as a smaller nucleophile instead, by heating to 50 °C for several days with LiOH, gave almost complete consumption of starting material (**3.55**), and a yield of 34% of the desired functionalised fused isoxazole **3.28**. Efforts to improve this yield were attempted. In order to prevent degradation that had been observed in other deacetylation attempts, **3.55** was not heated above 50 °C, but this time use of microwave irradiation cut the length of reaction to 45 h. However, the yield of **3.28** of 23% was not an improvement on the previous attempt. Stopping this reaction after only 16 h in fact yielded 33% of product, so clearly a balance needs to be met

between the consumption of starting material (**3.55**) and degradation of product (**3.28**) to obtain the best yield for this reaction.



Scheme 3.16. Successful synthesis of desired fused isoxazole **3.28**. *Reagents and conditions:* (a) CrO_3, AcOH, H_2O, 0 °C-rt, 3 h, 52%; (b) Zn, 2-bromo-2-nitropropane, MeOH, 50 °C, 8 days, 48%; (c) LiOH, MeOH, 50 °C, mw, 45 h, 23%.

3.4.3 AlphaScreen™ analysis of compounds **3.28** and **3.55**

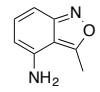
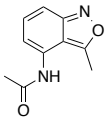
Compound Number	Compound	BRD4(1) %I	CECR2 %I	FALZ %I
3.28		12.2	12.2	13.9
3.55		6.6	10.1	11.5
		Key	0	100

Table 3.4. Single-shot percentage inhibition (%I), obtained (N = 1) by the SGC, of FALZ peptide binding of **3.28** & **3.55** at 250 µM concentration. Data are displayed as a heatmap, with 100% inhibition in red, and 0% inhibition in green.

With the amino fused isoxazole **3.28** and its acetylated precursor **3.55** in hand, an AlphaScreen™ assay was run at a single concentration against three different bromodomains (Table 3.4). The greatest activity was observed for **3.28** against FALZ, however, this derivative showed almost equal affinity for the other two bromodomains evaluated, BRD4(1) and CECR2. The lack of selectivity observed between FALZ and CECR2 can be explained by the sequence similarity between

these bromodomains; they belong to the same bromodomain sub-family. Overlaying the bromodomains of FALZ with CECR2 shows their structural similarity, with the narrow entrance to the KAc recognition pocket maintained in CECR2 (Figure 3.6). Unlike FALZ, where this is caused by a phenylalanine residue (F2887) beside the WPF shelf, a tyrosine residue (Y520) creates this narrow entrance in CECR2. Acetylated **3.55** shows moderate selectivity for this sub-family over BRD4, and displayed almost the same activity as non-acetylated **3.28**.

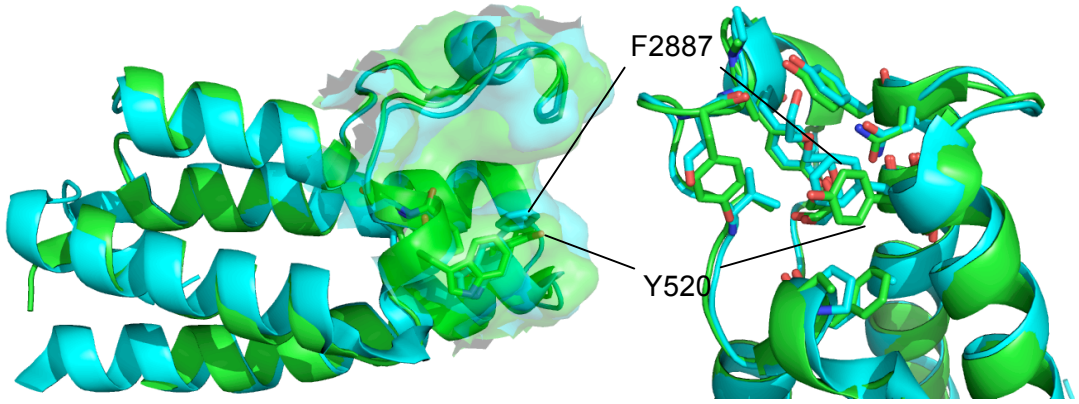


Figure 3.6. Overlay of *apo* crystal structures of CECR2 (green, PDB 3NXB) with FALZ (cyan, PDB 3UV2), using the *align* command in PyMOL.[12]

3.5 Summary and conclusions

3.5.1 Summary

In summary, with the plethora of research papers that have focused on targeting BET bromodomains, a fragment-based approach was used in order to develop new fragment molecules for non-BET bromodomains. This work began by targetting the FALZ bromodomain, with the vision of identifying fragments for other non-BET bromodomains. Initial docking studies carried out by the SGC against FALZ suggested that 4-aryl-3,5-dimethylisoxazoles **3.1**-**3.4** were good starting fragments. These derivatives showed very weak binding to FALZ, as well as BRPF3 and PHIP, but **3.2**-**3.4** displayed modest activity against ATAD2 and good activity for BRD4. In

attempts to disfavour binding to BRD4, examination of the crystal structures revealed that the FALZ bromodomain pocket has a much narrower entrance than BRD4, due to a phenylalanine residue that lies beside the WPF shelf. In addition, work within the Conway group has shown that 4-aryl-3,5-dimethylisoxazoles bind to BRD4(1) with a twist. Therefore, planar, fused isoxazoles **3.9**-**3.13** were synthesised to favour binding to non-BET bromodomains, a number of which have shown weak binding to the FALZ bromodomain. In attempts to optimise the most potent derivative **3.9**, docking identified that hydrogen-bonding interactions could be made to the proline residue of the WPF shelf, with aniline derivatives **3.28** and **3.29**. Fused isoxazole **3.28** and its acetylated form **3.55** both showed weak binding to FALZ, CECR2 and BRD4(1), with **3.55** showing modest selectivity for the group I bromodomains over BRD4(1). Future work in this area should focus on developing these fragments into more potent and selective ligands for non-BET bromodomains.

3.5.2 Conclusions

In conclusion, iterative cycles of docking, synthesis and testing identified a number of compounds that inhibited KAc interactions with initial target FALZ, CECR2 from the same bromodomain sub-family, other non-BET bromodomains BRPF3, ATAD2 and PHIP as well as BET bromodomain BRD4. These fragments present a useful starting point for the development of more potent and selective non-BET bromodomain ligands.

Chapter 4: The Interaction of Bromodomains with Natural and Unnatural Modified Lysines

This chapter describes work carried out to better understand the interaction of modified lysine residues with bromodomains. Work was carried out to develop unnatural modified lysine residues that could potentially interact with, or displace, water molecules within the KAc-recognition pocket. Furthermore, in addition to acetyl lysine, there are multiple naturally modified lysines, and attempts were made to synthesise a range of these to establish whether they could also interact with bromodomains.

4.1 Introduction and aims

4.1.1 Naturally occurring modified lysines

Reversible acetylation of histone lysines was first reported in 1964, when phosphorylation as a post-translational modification was also beginning to be studied.[173] Sites of phosphorylation proved easy to detect, due to a wide availability of sensitive and specific reagents that could act as markers, enabling the field of phosphorylation to rapidly expand. However, identification of acetylation sites was more challenging due to a lack of markers. As such, the first case of non-histone lysine acetylation was only published in 1985, with the second and third cases published in the late 1990s. With significant advancements in mass spectrometry-based proteomics, detection of KAc residues has now flourished to the extent that KAc identifications are almost as prevalent as other post-translational modifications, including phosphorylation. In addition, due to these improvements in proteomics, a number of other naturally occurring acylated lysines have been identified. These modifications include formyl,[174,175] malonyl,[176] succinyl,[177-179]

crotonyl,[180] propionyl,[181-183] butyryl, myristoyl[184] and carbamoyl lysine[185] (Figure 4.1). Interestingly, despite the different lengths, structures and charges, several of these marks can be removed by sirtuins, the NAD^+-dependent lysine deacetylase enzymes. This observation raises the question of whether these marks can also interact with bromodomains. In fact, the interaction of KAc with many bromodomains has been published, although the cognate KAc peptides of only a few are known, and it is not absolutely clear whether all bromodomains bind to KAc residues.[13] Twelve bromodomains lack the asparagine residue that anchors KAc, however, it is most often replaced by another hydrogen-bonding residue threonine (BRWD3(2), PHIP(2), WDR9(2) and TRIM28) or tyrosine (LOC93349, SP140, SP100, SP110A, SP110C, ZMYND11, ASH1L and PB1(1)).[12] However, in the case of MLL, the asparagine is instead replaced by an aspartic acid residue, raising the question of whether this bromodomain can in fact interact with KAc, and if it does what mechanism is involved in KAc recognition for this bromodomain. Interestingly, Vollmuth and Geyer have already demonstrated that propionylated and butyrylated lysines (**4.6** & **4.7**) could bind to bromodomains, albeit with even lower affinity than KAc (**4.1**).[186]

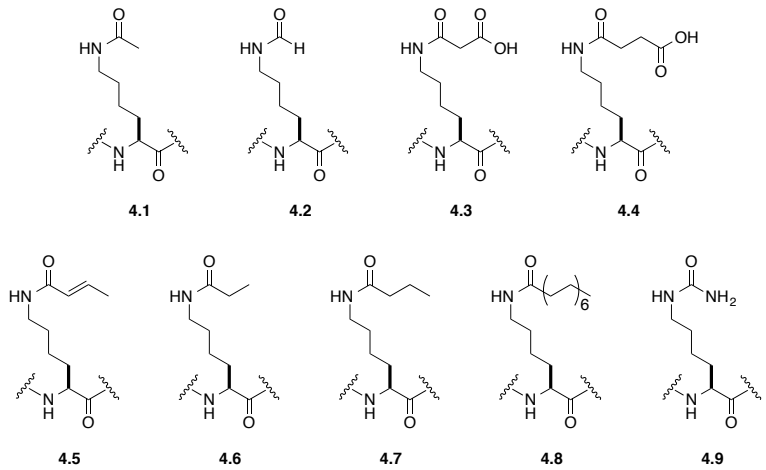


Figure 4.1. Naturally occurring acylated lysines: acetyl lysine (KAc) (**4.1**), formyl lysine (**4.2**),[174,175] malonyl lysine (**4.3**),[176] succinyl lysine (**4.4**),[177-179] crotonyl lysine (**4.5**),[180] propionyl lysine (**4.6**),[181-183] butyryl lysine (**4.7**), myrisotoyl lysine (**4.8**)[184] and carbamoyl lysine (**4.9**).[185]

4.1.2 Unnatural modified lysines

When Vollmuth and Geyer demonstrated that propionylated and butyrylated lysines (**4.6** & **4.7**) can bind to bromodomains, they expected that the longer alkyl chains would displace water molecules from the binding pocket; however, due to their hydrophobic nature, and tight binding of the water molecules, they bound further out of the pocket instead. Because displacement of water molecules in a binding pocket might be entropically favourable, this technique has been used to improve binding affinity.[187-189]

4.1.3 Aims

In order to gain a better understanding of the other naturally occurring acylated lysines, and to further investigate the binding pattern of the bromodomains, we aimed to synthesise the remaining lysine derivatives **4.2**-**4.5**, **4.8** & **4.9** and evaluate their interactions with different bromodomain families. In addition to the naturally occurring acylated lysines mentioned previously, we also aimed to investigate the binding affinity of more hydrophilic KAc mimics to either displace or interact with the water molecules.

4.2 Design and synthesis of unnatural, hydrophilic modified lysines

4.2.1 Docking studies of modified lysines in BRD4(1)

The interactions of different modified lysines with BRD4(1) was modelled using Molsoft ICM-Pro docking software. The crystal structure of BRD4(1), with butyrylated histone H3K14buty (PDB 3MUL) was used as a starting point with which to propose potential KAc mimics (Figure 4.2).[186]

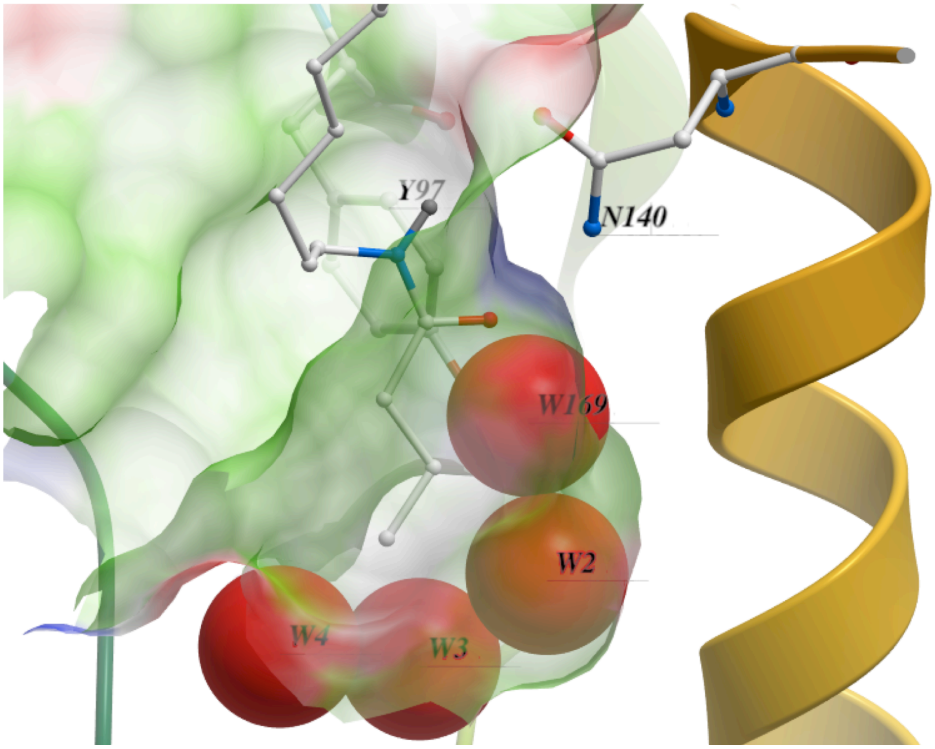


Figure 4.2. X-Ray crystal structure of H3K14buty and BRD4(1) (PDB 3MUL) shows four water molecules in the base of the pocket, forming a hydrogen-bonding network with the conserved tyrosine residue Y97.[186]

Four conserved water molecules (W2-4 and W169) are located deep within the binding pocket. At the time this work was carried out, little was known about the displaceability of these water molecules. Under the assumption that they were all displaceable, removal of these water molecules, and modification of the butyrylated lysine, enabled the optimal conformations of various modified lysines to be simulated. Overlaying each of these models with the removed water molecules enabled the identification of molecules that could potentially either interact in the same way as the water molecules, or interact with the water molecules themselves.

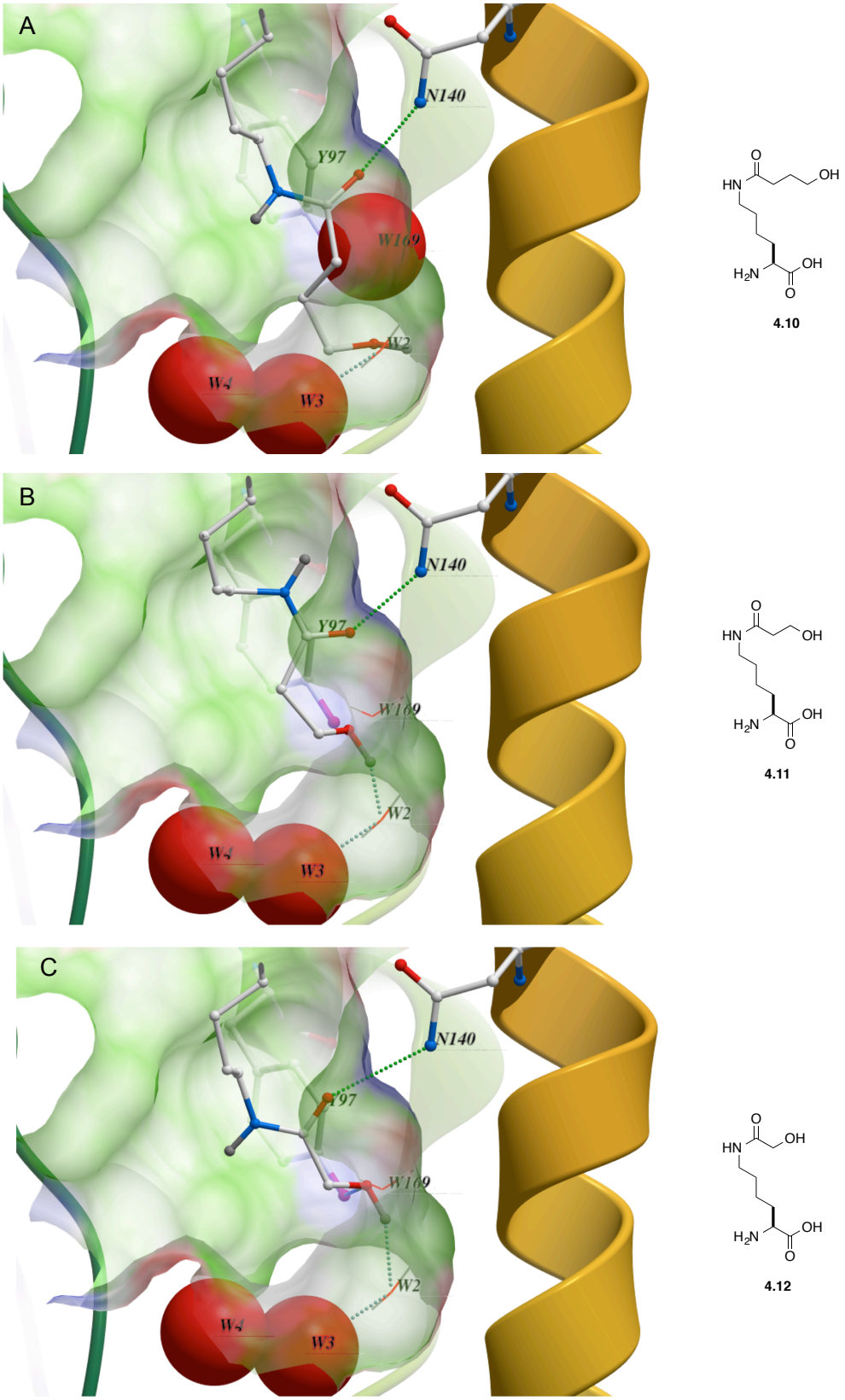


Figure 4.3. Molsoft ICM-Pro docking of **4.10**-**4.12** into BRD4(1) (PDB 3MUL) shows potential displacement of, or interaction with water molecules and the bromodomain.[186]

The four water molecules form a well-structured hydrogen-bonding network with the conserved tyrosine (Y97) residue. As such, a hydroxyl group is an obvious functional group to be introduced into the peptides, because it can either displace one or more water molecules in the pocket, or add to the hydrogen-bonding network. Upon addition of a hydroxyl group to the terminal carbon of butyrylated lysine, the hydroxyl-oxygen of **4.10** is predicted to not only overlay exactly with W2, but also to mimic its hydrogen bond with W3, while conserving the key hydrogen bond of the carbonyl to the N140 residue (Figure 4.3A). Interestingly, upon contraction of the alkyl chain and addition of a hydroxyl group, the hydroxyl-oxygen of **4.11** appears to position itself closely to W169, allowing this ligand to hydrogen bond to W2 and the conserved N140 (Figure 4.3B), while **4.12** also hydrogen bonds to N140, W2, and Y97 (Figure 4.3C).

With these observations in hand, three lysine derivatives (**4.10**-**4.12**) were selected as unnatural target molecules (Figure 4.4). In addition, we thought that it would be interesting to synthesise the amino analogues (**4.13**-**4.15**) for comparison.

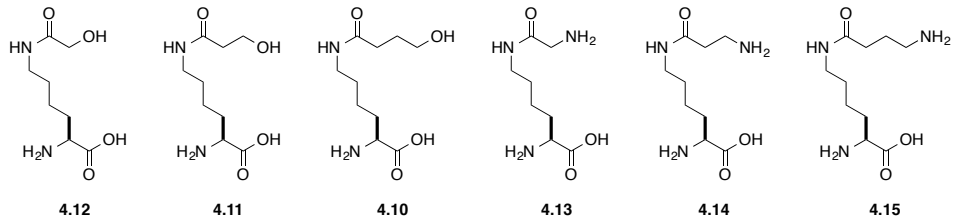
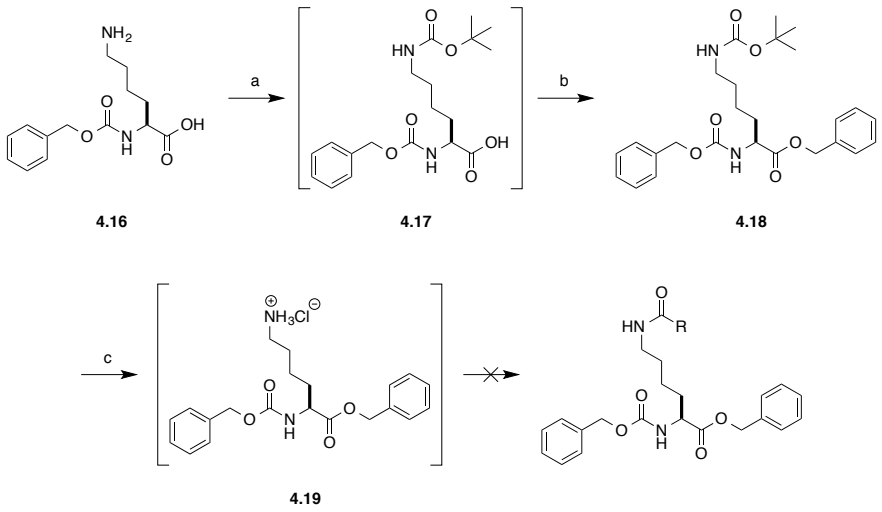


Figure 4.4. Unnatural, hydrophilic modified lysines (**4.10**-**4.15**) selected as target molecules.

4.2.2 Synthesis of unnatural, hydrophilic modified lysines

In order to obtain the desired unnatural amino acids, synthetic strategies involving protecting group manipulations of commercially available lysines were carried out. The carboxybenzyl (Cbz) group was deemed to be a suitable protecting group of lysine's α-amino group, because it could be removed along with benzyl or *tert*-butyl protecting groups on the modified lysine. Orthogonal benzyl protection of

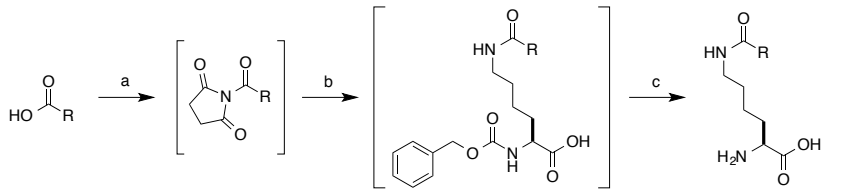
the carboxylic acid of Cbz-Lys-OH (**4.16**) in the presence of the free ε-amino group with thionyl chloride and benzyl alcohol was investigated, but ε-*N*-benzyl and ε-*N,N*-dibenzyl lysine were produced instead. Therefore, alternative protecting group manipulations of Cbz-Lys-OH (**4.16**) were attempted in order to synthesise the target molecules**.** Boc protection of the ε-amino group of Cbz-Lys-OH (**4.16**), using Boc_2O in the presence of $NaHCO_3$, gave **4.17.** Subsequent benzyl protection of the carboxylic acid by stirring **4.17** at rt with Cs_2CO_3 in DMF only gave an 18% yield of **4.18**, and with DIPEA in MeCN gave a 50% yield, but heating under reflux with K_2CO_3 in acetone afforded **4.18** in 88% yield over the 2 steps. However, in attempts to produce **4.19**, subsequent Boc-deprotection using HCl in Et_2O or TFA in CH_2Cl_2 suffered from difficult purification. As acylation of amines is a common method of purification, attempts were made to acylate the crude material anyway, however, this method proved ineffective.



Scheme 4.1. Protecting group manipulations of Cbz-Lys-OH (**4.16**), in an attempt to produce modified lysines. *Reagents and conditions:* (a) Boc_2O, $NaHCO_3$, THF, H_2O, rt, 19 h; (b) BnBr, K_2CO_3, acetone, reflux, 2 h, 88%; (c) HCl, Et_2O, rt or TFA, CH_2Cl_2, rt.

After further investigation of the literature, the use of succinate esters for facile synthesis of the targets was identified as an alternative strategy.[190] Activation of carboxylic acids with *N*-hydroxysuccinimide (NHS) allowed for amide formation in the

presence of a free carboxylic acid, and hence the ε-amino group of Cbz-Lys-OH (**4.16**) could be acylated without additional protecting group manipulations. NHS-activation of acids **4.20**-**4.25**, to give **4.26**-**4.31**, using a procedure by Leonard and Brunckova, followed by subsequent coupling, to give **4.32**-**4.37**, and deprotections afforded targets **4.10**-**4.15** (Scheme 4.2).[191] Straightforward deprotection of the Cbz and *tert*-butyl protecting groups of **4.32** and **4.33** was achieved with HBr in AcOH, although these final compounds (**4.12** & **4.13**) were contaminated with inseparable impurities. On the other hand, under palladium on carbon-catalysed hydrogenolysis conditions the benzyl protecting group of **4.34** proved to be orthogonal to the Cbz protecting group, due to catalyst poisoning, even when AcOH was used to lower the pH. As such, stoichiometric palladium black was required to produce the desired target (**4.10**), however, again this compound was contaminated with inseparable impurities.



4.20: R = CH_2O^tBu
4.21: R = $CH_2CH_2O^tBu$
4.22: R = $CH_2CH_2CH_2OBn$
4.23: R = CH_2NHBoc
4.24: R = CH_2CH_2NHBoc
4.25: R = $CH_2CH_2CH_2NHBoc$

4.26: R = CH_2O^tBu
4.27: R = $CH_2CH_2O^tBu$
4.28: R = $CH_2CH_2CH_2OBn$
4.29: R = CH_2NHBoc
4.30: R = CH_2CH_2NHBoc
4.31: R = $CH_2CH_2CH_2NHBoc$

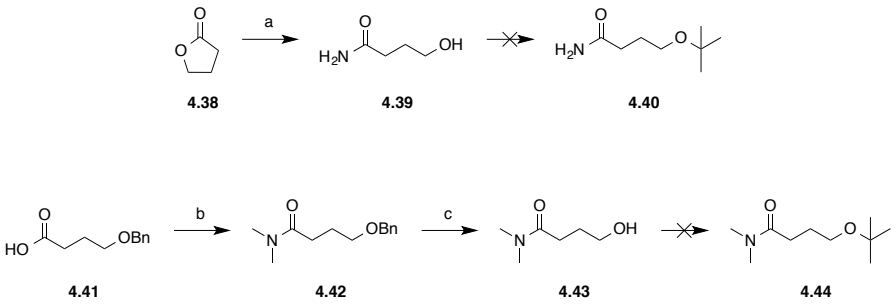
4.32: R = CH_2O^tBu
4.33: R = $CH_2CH_2O^tBu$
4.34: R = $CH_2CH_2CH_2OBn$
4.35: R = CH_2NHBoc
4.36: R = CH_2CH_2NHBoc
4.37: R = $CH_2CH_2CH_2NHBoc$

4.12: R = CH_2OH
4.11: R = CH_2CH_2OH
4.10: R = $CH_2CH_2CH_2OH$
4.13: R = CH_2NH_2
4.14: R = $CH_2CH_2NH_2$
4.15: R = $CH_2CH_2CH_2NH_2$

Scheme 4.2. Synthesis of unnatural modified lysines **4.10**-**4.15**. *Reagents and conditions:* (a) NHS, TFAA, pyridine, CH_2Cl_2, 0 °C-rt, 1 h; (b) Cbz-Lys-OH (**4.16**), DMF, rt; (c) R = CH_2OH, CH_2CH_2OH, CH_2NH_2, $CH_2CH_2NH_2$, $CH_2CH_2CH_2NH_2$: HBr 33% wt in AcOH, rt; R = $CH_2CH_2CH_2OH$: Pd black, H_2 (g), ^tBuOH, H_2O, rt.

In efforts to avoid the use of stoichiometric palladium black, and with the facile deprotection of the *tert*-butyl protecting groups of **4.32** and **4.33** in mind, work was carried out to use the *tert*-butyl protecting group for the synthesis of **4.10**. *tert*-Butoxybutyric acid is not commercially available, so ring-opening of

γ-butyrolactone (**4.38**) to form an amide, which could be hydrolysed after *tert*-butyl protection of the subsequent free hydroxyl group was attempted. Ring-opening of γ-butyrolactone (**4.38**) with dimethylamine suffered from incomplete consumption and difficult purification. Attempts to *tert*-butyl protect the hydroxyl group of the crude mixture with isobutylene, H_3PO_4 and $BF_3 \bullet Et_2O$ at –75 °C, following the work of Micheli *et al*. also proved unsuccessful.[192] On the other hand, ring-opening of γ-butyrolactone (**4.38**) with ammonia in methanol afforded primary amide **4.39** in 86% yield; however, subsequent protection attempts with isobutylene, to give **4.40**, suffered from insolubility of the starting material (**4.39**). In a final attempt to afford the desired target molecule, HBTU-mediated amide coupling of dimethyl amine and benzoxybutyric acid **4.41**, to give **4.42**, followed by hydrogenolysis afforded amide **4.43** in 57% yield over two steps (Scheme 4.3). However, subsequent attempts to *tert*-butyl protect the hydroxyl group, to give **4.44**, using Boc_2O and $HClO_4$ also proved unsuccessful.

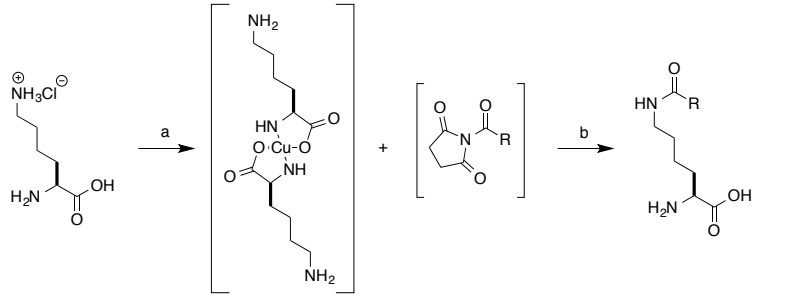


Scheme 4.3. Attempts to synthesise *tert*-butoxybutyric acid. *Reagents and conditions:* (a) NH_3, MeOH, rt, 4 days, 86%; (b) Dimethylamine hydrochloride, HBTU, DIPEA, DMF, rt, 17 h, 58%; (c) 10% Pd-C, H_2 (g), MeOH, rt, 17 h, 99%.

Amino derivatives **4.13**-**4.15** were produced in the same manner as the hydroxyl derivatives **4.10**-**4.12**, first with NHS-activation of the corresponding Boc-protected amino acids (**4.23**-**4.35**), coupling with Cbz-Lys-OH (**4.16**) to give **4.35**-**4.37** and deprotection with HBr in AcOH. However, again these derivatives were difficult to purify and contained inseparable impurities. Before an effective method of

purification was identified, an alternative synthetic route was attempted to see whether these impurities could be avoided.

At this point in time, site-specific incorporation of crotonyl lysine (**4.5**, Figure 4.1) into histones was published by Kim *et al*.[193] Their method involved copper chelation of unprotected lysine, published by Nagaoka *et al*., followed by addition of the NHS-ester of crotonic acid.[194] This protecting group-free method seemed an appropriate alternative route, with the potential to produce cleaner final products.



4.46 **4.45**

4.29: R = CH_2NHBoc **4.13:** R = CH_2NH_2
4.30: R = CH_2CH_2NHBoc **4.14:** R = $CH_2CH_2NH_2$
4.31: R = $CH_2CH_2CH_2NHBoc$ **4.15:** R = $CH_2CH_2CH_2NH_2$

Scheme 4.4. Synthesis of **4.13**-**4.15** using copper chelation of lysine. *Reagents and conditions:* (a) $CuSO_4$•$5H_2O$, $NaHCO_3$, H_2O, rt, 5 min; (b) R = CH_2NH_2: i) Acetone, rt, 6 h; ii) 8-Hydroxyquinoline, H_2O, $CHCl_3$, 10 min; iii) 1 M HCl in dioxane, H_2O, rt, 29 h, 20%; R = $CH_2CH_2NH_2$: i) Acetone, rt, 20 h; ii) 8-Hydroxyquinoline, H_2O, $CHCl_3$, 10 min; iii) 1 M HCl in dioxane, rt, 14 h, 12%; R = $CH_2CH_2CH_2NH_2$: i) Acetone, rt, 8 h; ii) 8-Hydroxyquinoline, H_2O, $CHCl_3$, 10 min; iii) 1 M HCl in dioxane, H_2O, rt, 19 h, 59%.

Formation of the copper chelate **4.45** of lysine **4.46**, then treatment with the NHS-esters **4.29**-**4.31** followed by extraction of copper using 8-hydroxyquinoline as a chelate precipitant, and subsequent Boc-deprotection with 1 M HCl in dioxane afforded the desired compounds **4.13**-**4.15** in poor to moderate yields (Scheme 4.4). These derivatives were much cleaner than those synthesised from Cbz-Lys-OH (**4.16**), but unfortunately attempts to treat copper-chelated lysine with the NHS esters **4.26**-**4.28** failed to give any of the desired coupled intermediates. Therefore, attempts were made to optimise the purification of these products.

4.3 Purification of amino acids

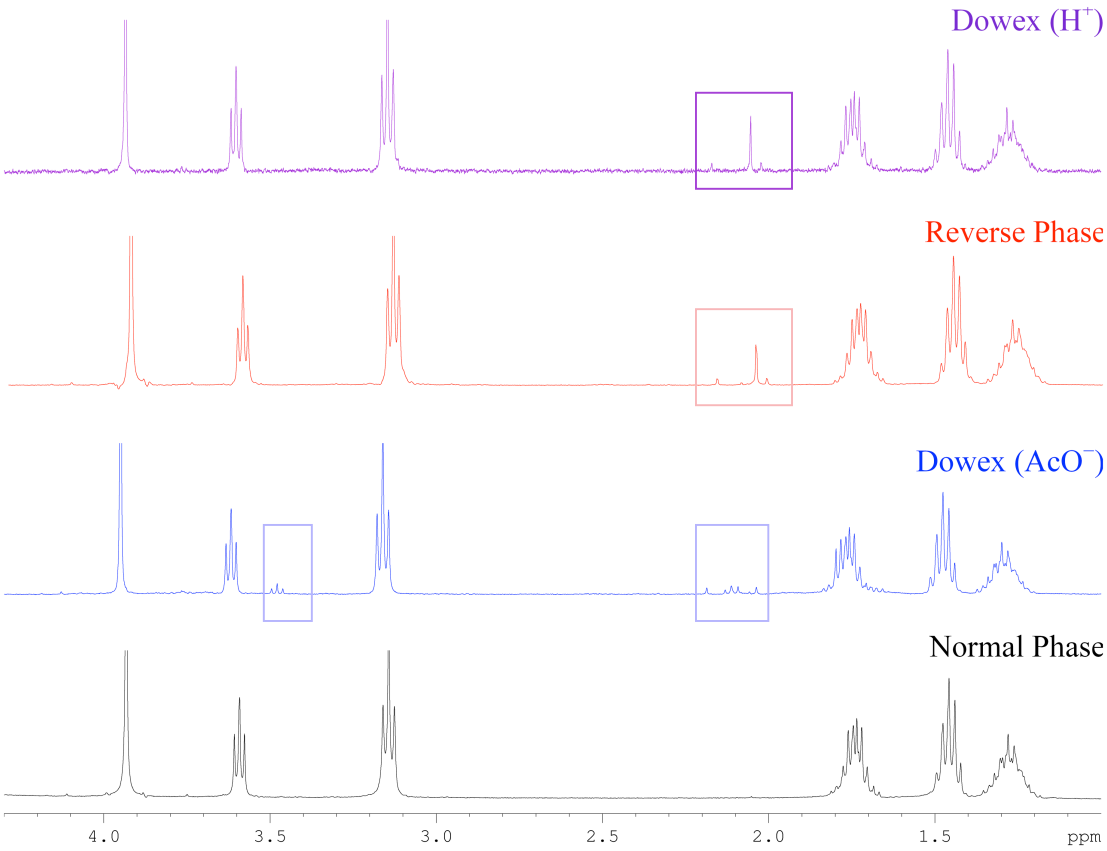


Figure 4.5. An example of purification attempts for modified lysines. Here the partial ^{1}H NMR spectra for **4.12** after a strong cation Dowex® column, reverse phase column chromatography eluting with H_2O, an anionic Dowex® column and normal phase column chromatography eluting with H_2O in MeCN are aligned.

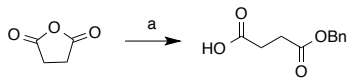
When amino acids are being incorporated into peptides using solid phase synthetic strategies, high purity is not required because the final peptide will be purified at a later stage. However, because the unprotected natural and unnatural modified lysines mentioned here were being tested in *in vitro* assays, it was very important to have clean samples for testing. Unfortunately, purification of the amino acids proved challenging. Purification of these unprotected amino acids was first attempted through a column of strong cation Dowex® resin. However, in all cases, small impurities were detected by ^{1}H NMR, as can be seen in NMRs of **4.12**, a representative example, (Figure 4.5). As such, reverse phase chromatography was

attempted, but the significant polarity of the free amino acids caused the product to elute in 100% H_2O, and the impurities were brought with it.

Frustratingly, a subsequent column of anionic Dowex® resin removed the largest contaminant, but introduced new impurities. Pleasingly, normal phase column chromatography, eluting with H_2O in MeCN allowed successful purification of the impure amino acids, and is therefore an appropriate purification technique for such polar molecules. With this optimised purification method, the hydroxyl derivatives **4.10**-**4.12** were isolated in 19, 28 and 41% yields respectively.

4.4 Synthesis of natural modified lysines

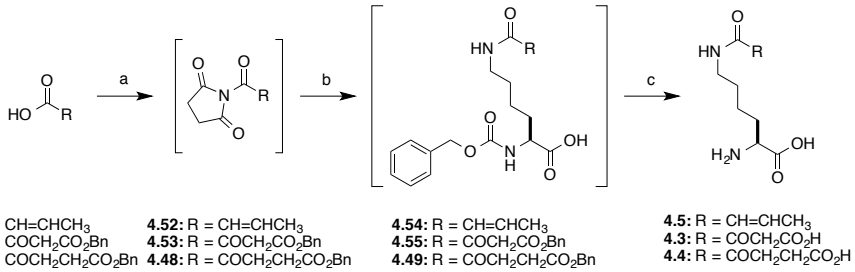
With the unnatural modified lysines in hand, work was then carried out to furnish the desired natural modified lysines. Ring-opening of succinic anhydride (**4.46**) with Cbz-Lys-OH (**4.16**) proved unsuccessful, and so opening with benzyl alcohol was used to synthesise **4.47** instead (Scheme 4.5).



4.46 **4.47**

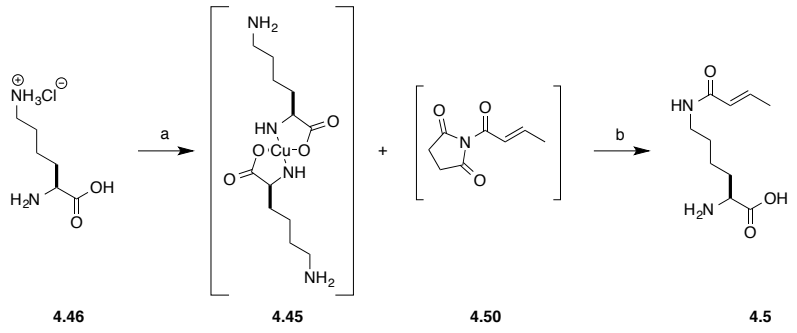
Scheme 4.5. Conversion of succinic anhydride **4.46** to **4.47**. *Reagents and conditions:* (a) BnOH, NEt_3, DMAP, CH_2Cl_2, 0 °C-rt, 25 h, 82%.

Coupling of the succinate ester (**4.48**) of **4.47** with Cbz-Lys-OH (**4.16**), to give **4.49**, as was used for synthesis of the unnatural modified lysines, followed by hydrogenolysis afforded the desired succinyl lysine (**4.4**) in 90% yield. This route could also afford malonyl lysine (**4.3**) and crotonyl lysine (**4.5**) in 41% and 69% yields. These derivatives used acids **4.50** and **4.51** to make succinate esters **4.52** and **4.53**, which were coupled with Cbz-Lys-OH (**4.16**) to give **4.54** and **4.55**, which were subsequently deprotected to afford the final products **4.3** and **4.5** (Scheme 4.6).



Scheme 4.6. Synthesis of modified lysines **4.3**-**4.5**. *Reagents and conditions:* R = CH=CHCH$_3$: (a) NHS, TFAA, pyridine, CH_2Cl_2, 0 °C-rt, 1 h; (b) Cbz-Lys-OH (**4.16**), DMF, rt, 112 h; (c) HBr 33% wt in AcOH, rt, 41%; R = $COCH_2CO_2H$: (a) NHS, TFAA, pyridine, CH_2Cl_2, 0 °C-rt, 1 h; (b) Cbz-Lys-OH (**4.16**), DMF, rt, 14 h; (c) Pd black, H_2 (g), ^{t}BuOH, H_2O, rt, 16 h, 69%; R = $COCH_2CH_2CO_2H$: (a) NHS, TFAA, pyridine, CH_2Cl_2, 0 °C-rt, 1 h; (b) Cbz-Lys-OH (**4.16**), DMF, rt, 23 h; (c) Pd black, H_2 (g), ^{t}BuOH, H_2O, rt, 15 h, 90%.

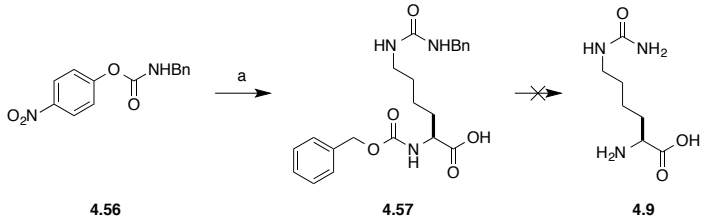
Repeating the work of Kim *et al*., crotonyl lysine (**4.5**) could also be synthesised *via* copper chelation of free lysine (**4.46**, Scheme 4.7). Unlike the amino derivatives **4.13**-**4.15**, this compound required purification by silica gel chromatography, eluting with H_2O in MeCN to isolate the desired product for testing, and much lower yields were obtained using this method rather than coupling with Cbz-Lys-OH (**4.16**).



Scheme 4.7. Protecting group-free synthesis of crotonyl lysine (**4.5**). *Reagents and conditions:* (a) $CuSO_4$•$5H_2O$, $NaHCO_3$, H_2O, rt, 5 min; (b) i) Acetone, rt, 5 h; ii) 8-Hydroxyquinoline, H_2O, $CHCl_3$, 10 min, 26%.

Carbamylation of Cbz-Lys-OH (**4.16**) with isocyanate suffered from poor solubility in water, and so an alternative synthesis of this primary urea in organic solvents was sought. Treatment of Cbz-Lys-OH (**4.16**) with 4-nitrophenyl

N-benzylcarbamate (**4.56**), using a procedure by Liu *et al*. afforded benzylurea **4.57** (Scheme 4.8).[195] Hydrogenolysis was attempted, but primary urea **4.9** proved insoluble in multiple solvents and solvent mixtures (H_2O, MeOH, DMSO, acetone, $CHCl_3$, H_2O/MeOH, H_2O/AcOH, H_2O/NaOH), and therefore could not be isolated.

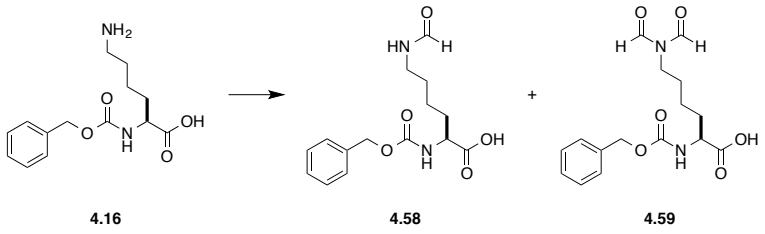


Scheme 4.8. An attempt to produce carbamyl lysine (**4.9**). *Reagents and conditions:* (a) Cbz-Lys-OH (**4.16**), NEt_3, CH_2Cl_2, rt, 5 h, 36%.

Attempts to synthesise myristoyl lysine (**4.8**) also proved unsuccessful. Stirring myristoyl chloride with Cbz-Lys-OH (**4.16**) gave insoluble crude material that could not be purified. Attempts to deprotect this crude mixture with HBr in AcOH, or by hydrogenolysis with stoichiometric palladium black, gave crude samples that again could not be purified due to insolubility in organic and aqueous solvents and solvent mixtures (MeOH, $CDCl_3$, DMSO, benzene, H_2O, aqueous 1 M solution of NaOH, aqueous 1 M solution of NH_4OH, H_2O/$CHCl_3$/MeOH and H_2O/acetone).

Several attempts were made to formylate Cbz-Lys-OH (**4.16**). Firstly, poor solubility when stirring in neat ethylformate prevented any reaction of the starting material. However, solubility was improved when using MeOH as a solvent, and maintaining a pH of 8-9 with the addition of NaOH, allowed the formylated lysine (**4.58**, Scheme 4.9) to be observed, although this product proved inseparable from diformylated lysine imide **4.59**. An alternative approach, following the work of Hughes and Walters, involved gently heating acetic anhydride and formic acid to generate the mixed anhydride *in situ*.[196] Addition of Cbz-Lys-OH (**4.16**) and stirring at rt produced the desired formylated lysine **4.58**, however, again this compound could not be separated from the contaminating imide. Attempts to reduce the number of

equivalents of the mixed anhydride did not prevent formation of the imide. Efforts were made to convert the imide contaminant (**4.59**) to the desired formyl lysine (**4.58**), including the use of methanolysis, aqueous ammonia, and ammonia in MeOH. These attempts gave marginal improvements in purity, but never fully consumed the imide. Stirring with NaOEt and EtOH degraded the crude mixture, with no formyl protons observed by ^{1}H NMR. Due to the challenges in isolation of the formyl lysine (**4.58**), Cbz-deprotection using HBr in AcOH was attempted on an impure mixture, however, the desired product (**4.2**) could not be isolated from impurities at this later stage either, and therefore formyl lysine (**4.2**) was omitted from the collection of acylated lysines to be tested. In addition, insolubility issues would result in unreliable *in vitro* peptide displacement data, and therefore myristoyl lysine (**4.8**) and carbamoyl lysine (**4.9**) were also omitted from the testing set.

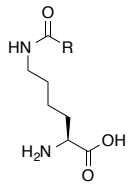


Scheme 4.9. Attempts to formylate Cbz-Lys-OH (**4.16**) gave an inseparable mixture of formylated **4.58** and diformylated **4.59**.

4.5 *In vitro* affinity of the natural and unnatural modified lysines

Initially, a small subset of the target compounds (**4.3**, **4.10** & **4.12**) was tested against the BAZ2A and BRD9 bromodomains at the SGC. Although malonyl lysine **4.3** (Table 4.1) showed negligible binding to either bromodomain, and is therefore not a suitable lead, **4.12** showed some degree of selectivity for BAZ2A over BRD9, showing that lysine derivatives selectively bind to different bromodomain families. Interestingly, **4.10** showed excellent binding to both bromodomains at 250 μM

concentrations. This is an improvement in affinity for BRD4 over KAc, which only inhibits BRD4 at concentrations greater than 500 μM.



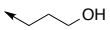
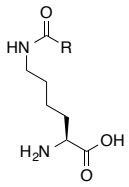
Compound Number	R	BAZ2A %I at 25 μM	BAZ2A %I at 250 μM	BRD9 %I at 25 μM	BRD9 %I at 250 μM
4.3		3.10	2.60	0.00	0.00
4.10		28.90	83.10	20.50	68.30
4.12		11.50	21.75	0.00	0.00
			Key	0	100

Table 4.1. Single-shot percentage inhibition (%I) of **4.3**, **4.10** and **4.12** against BAZ2A and BRD9 at concentrations of 25 μM and 250 μM, obtained (N = 1) by the SGC. Data are displayed as a heatmap, with 100% inhibition in red, and 0% inhibition in green.

The complete set of amino acids was tested against FALZ at two concentrations in an AlphaScreen™ assay at the SGC (Table 4.2). The alcohol **4.12** showed no inhibition of peptide binding to FALZ, as with BRD9, showing further selectivity for BAZ2A. The amines **4.13** and **4.14** also displayed no inhibition of peptide binding, but amine **4.15**, malonyl lysine (**4.3**) and succinyl lysine (**4.4**) showed weak inhibition of peptide binding. Interestingly, alcohols **4.10** and **4.11** as well as crotonyl lysine (**4.5**) showed moderate inhibition of peptide binding at 250 μM, showing ~2-fold greater inhibition than the fragment molecules discussed in Section 3.2.3, run on the same assay plate.



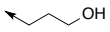
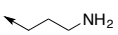
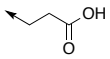
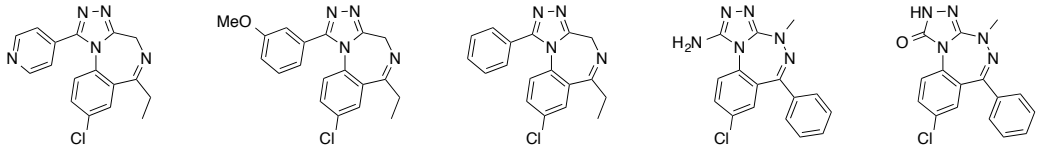
Compound Number	Compound	FALZA %I at 25 μM	FALZA %I at 250 μM
4.12		0.00	0.00
4.11		13.10	43.35
4.10		0.00	38.80
4.13		0.00	0.00
4.14		0.00	0.00
4.15		0.00	10.05
4.3		1.70	2.40
4.4		0.80	1.65
4.5		6.45	45.20
	Key	0	100

Table 4.2. Single-shot percentage inhibition (%I) of **4.3**-**4.5** and **4.10**-**4.15** against FALZA at concentrations of 25 μM and 250 μM, obtained (N = 1) by the SGC. Data are displayed as a heatmap, with 100% inhibition in red, and 0% inhibition in green.

4.6 Displacement of KAc-binding pocket water molecules

Following this work, it was realised that bromodomain waters are not particularly easy to displace. In 2012, Vidler *et al*. noted that no published ligand displaced the KAc-binding pocket waters.[77] Importantly, work by Filippakopoulos *et al*. was carried out to determine whether the waters could be displaced by larger substituents on the triazole moiety of a benzodiazepine that resembles I-BET762 (**1.2**, Figure 1.5), **4.60**-**4.62** (Figure 4.6), that would protrude further into the

KAc-binding pocket.[197] However, these larger groups were not tolerated by the BET bromodomains, with thermal shift values less than 2 °C at 100 μM. In addition, amino- and carbonyl-substitution of this analogous position in a benzotriazepine also resembling I-BET762 (**1.2**), **4.63** and **4.64**, were also not tolerated.



4.60 **4.61** **4.62** **4.63** **4.64**

Figure 4.6. Compounds **4.60**-**4.64**, synthesised by Filippakopoulos *et al*. in attempts to displace KAc-binding pocket water molecules. These derivatives were not tolerated by the BET bromodomains, with thermal shift values smaller than 2 °C at 100 μM for the entire family.[197]

Similarly, work by D. Hewings within the Conway group, explored whether hydrophilic substituents on the 3-position of isoxazoles **4.65**-**4.77** could interact with, or displace, the water molecules within the binding pocket of BRD4(1) (Figure 4.7).[139] Amine and alcohol derivatives were not tolerated, suggesting that these functionalities do not actually interact favourably with, or displace, the water molecules. It appears, however, that some bromodomain water molecules are easily displaced. Molecular dynamics simulations have been carried out in the BAZ2B and CREBBP bromodomains, to establish whether solvent molecules can displace the binding pocket water molecules.[79] These simulations suggested that two of the water molecules are easily displaced, and the other waters can be transiently displaced. As such, there is potential to develop compounds that abuse the water molecules within the KAc-binding pocket of bromodomains.

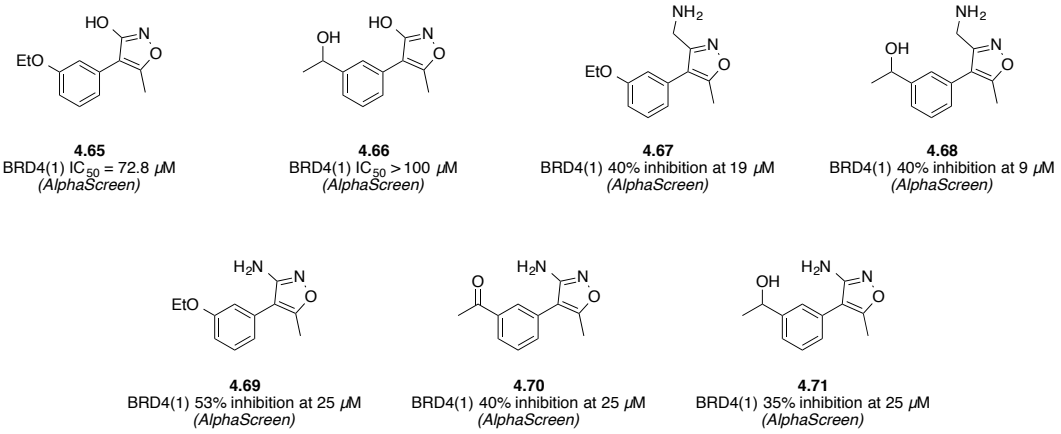


Figure 4.7. Amines and alcohols **4.65**-**4.71**, synthesised by D. Hewings, were also poorly tolerated by BRD4(1), with weak IC_{50} values and poor inhibition of peptide binding in an AlphaScreen assay.[139]

4.7 Summary and conclusions

4.7.1 Summary

In summary, a collection of natural and unnatural modified lysines were synthesised in order to gain a better understanding of bromodomain interactions. Work by Vollmuth and Geyer showed that propionylated and butyrylated lysines (**4.6** & **4.7**, Figure 4.1) bind to bromodomains, albeit with lower affinity than acetylated lysines. In addition, sirtuins have been shown to remove functionality other than acetylation of lysines, such as malonylation, succinylation and myristoylation. As such, it was hypothesised that bromodomains could interact with other naturally occurring acylated lysines. Work was therefore carried out to synthesise other modified lysines, although with problematic syntheses, due to insolubility or inseparable impurities, only malonyl lysine (**4.3**), succinyl lysine (**4.4**) and crotonyl lysine (**4.5**) were produced. Malonyl lysine (**4.3**) and succinyl lysine (**4.4**) displayed weak to no inhibition of peptide binding to bromodomains, however crotonyl lysine (**4.5**) displayed good inhibition of peptide binding to the FALZ bromodomain at a high concentration. In fact, it showed a higher percentage inhibition at 250 μM against the

FALZ bromodomain than any other fragment molecule developed in the Conway group. Perhaps this derivative could be incorporated into the cognate peptide of the FALZ bromodomain, in order to improve *in vitro* peptide displacement assays, aiding the development of small molecule probes for the FALZ bromodomain.

Further attempts to synthesise remaining acylated lysine analogues, as well as other modified lysines including hypusine, would allow better exploration of the interactions of naturally occurring modified lysines. Preparation of soluble salts could perhaps overcome the solubility issues found in this work, furnishing appropriate derivatives for testing. In addition, testing these naturally occurring compounds against a wider range of bromodomains would allow the affinity and selectivity of these derivatives to be more thoroughly assessed.

In addition to this work, some hydrophilic acylated lysines, built on the foundations of work produced by Vollmuth and Geyer, were selected as target molecules. Docking studies suggested that these had potential to interact with or displace water molecules in the recognition pocket. Hydroxyl derivative **4.12** and amino derivatives **4.13** and **4.14** displayed no binding to the FALZ bromodomain, however, amino derivative **4.15** displayed weak binding to the FALZ bromodomain at a high concentration, and hydroxyl derivatives **4.10** and **4.11** displayed moderate binding affinity at a high concentration. **4.10** also showed tight binding to BRD9 and BAZ2A, while **4.12** displayed selectivity between these bromodomains, indicating that selectivity between bromodomains can be achieved with modified lysines. Expanding the number of bromodomains these compounds are tested against would improve our understanding of bromodomain interactions, and allow us to further investigate selectivity between bromodomains. With more data in hand, it would be interesting to investigate the water displaceability within bromodomains that show the tightest binding to the unnatural modified lysines. These data would give an indication as to

whether these hydrophilic derivatives could displace waters within the recognition pocket.

From a synthetic standpoint, the synthesis of the polar molecules described in this chapter suffered from difficult purification, however successful isolation of the final compounds was achieved with the use of normal phase silica gel chromatography, eluting with H_2O in MeCN. With this purification method in hand, these derivatives could be produced in very few steps, using NHS-activation of synthesised or commercially available carboxylic acids, coupling with copper-chelated or α-amino protected lysines, and subsequent deprotection.

Overall it is clear that modified lysines other than KAc can bind to bromodomains, albeit very weakly. The weak binding of these amino acids to bromodomains is unsurprising, given the fact that acetyl lysine binds very weakly to bromodomains, not inhibiting BRD4 until its concentrations are greater than 500 μM, and that selectivity, and also some affinity, is achieved from the peptide instead. Therefore, site-specific incorporation of these modifications into peptides could allow improved affinity for bromodomains, aiding assay development, particularly for those that have already shown inhibition of bromodomains at 250 μM. Alternatively, these modifications could be incorporated into cyclic peptide derivatives of cognate peptides, the binding of which can benefit from rigidity preventing loss of entropy, in the same manner as work carried out by Gerona-Nevarro *et al.* when targeting CREBBP.[105]

4.7.2 Conclusions

In conclusion, naturally and unnaturally occurring acylated lysines have shown weak binding to, and some selectivity between, different bromodomains. Therefore,

incorporation of these amino acids into peptides that bind to particular bromodomains could be used for assay development, for example to aid in the development of ligands for the FALZ bromodomain.

Chapter 5: Conclusion

This dissertation describes work carried out on the development of ligands to target different bromodomain-histone interactions.

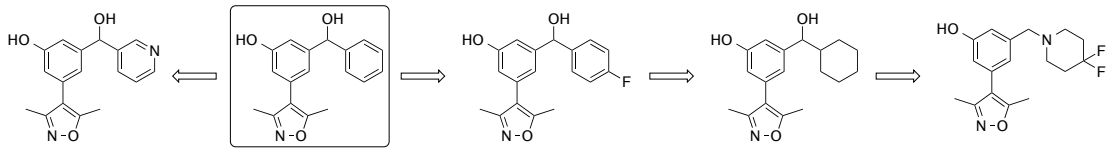
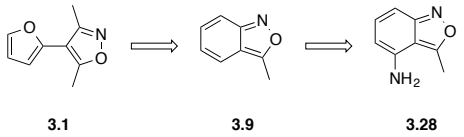


Figure 5.1. Derivatives of OXFBD02

Work carried out in attempts to optimise OXFBD02 (**2.1**, Figure 5.1), a BRD4 ligand developed by D. Hewings in the Conway group, is described. OXFBD02 (**2.1**) showed nanomolar affinity for BRD4 in peptide displacement assays, and, importantly, showed antiproliferative activity in a range of cancer cell lines. However, primarily due to 4-hydroxylation of the monosubstituted aromatic substituent, was unoptimised for use in *in vivo* models, as predicted by metabolic prediction software. As such, work was carried out to optimise the WPF shelf-binding group, in an attempt to better balance affinity and metabolic stability for the OXFBD02 series. A particularly interesting analogue that was identified was 3-pyridyl **2.20**, which displayed similar affinity to OXFBD02, but is predicted to have much better metabolic stability. Microsomal stability assays will help to determine whether this derivative can be used *in vivo*. *para*-Fluoro **2.11** also showed similar affinity to OXFBD02. The fluorine blocks the metabolically liable *para* position of the monosubstituted aromatic ring, but its high lipophilicity means that optimisation is required before this compound can be taken forward into *in vivo* models. Interestingly, cyclohexyl **2.25** was particularly potent for BRD4(1), but again requires optimisation due to its large lipophilicity. Basic methylamine derivatives of OXFBD02 were not well tolerated, due to disfavoured interaction of the WPF shelf with the protonated amines. However,

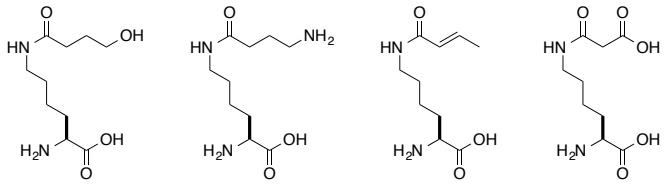
geminal difluorinated **2.44** is less basic, due to the electron-withdrawing fluorine atoms, allowing it to better interact with the WPF shelf. The observations made for these compounds and the other analogues of OXFBD02 will direct the synthesis of more metabolically stable and potent derivatives, for use in animal models.



3.1 **3.9** **3.28**

Figure 5.2. Some examples of bromodomain-binding fragments identified through iterative cycles of docking, synthesis and testing.

As well as working on ligands for a bromodomain that was known to be ligandable, work was also carried out on the development of ligands to target bromodomains for which there are no known ligands. This began by primarily targeting the bromodomain of FALZ. A fragment-based approach, using iterative cycles of docking, synthesis and testing, identified several fragments with weak affinity for different bromodomains (Figure 5.2). Further cycles can develop these fragments into more potent and selective ligands.



4.10 **4.15** **4.5** **4.3**

Figure 5.3. Some examples of modified amino acids that bind to bromodomains.

Finally, work was carried out to investigate whether other natural and unnatural modified lysines interact with bromodomains (Figure 5.3). Some of the modified lysines showed weak affinity for a range of bromodomains, and a few of these displayed selectivity. The interaction of KAc with bromodomains is very weak, and the affinity of some of the synthesised derivatives was an improvement on KAc. As such, these derivatives could be incorporated into peptides for enhanced activity, and

to be used in peptide-displacement assays to aid the development of bromodomain ligands.

In summary, this dissertation describes varied approaches to developing ligands to target bromodomain-histone interactions, and the information gleaned here can be used to further optimise inhibitors of this interaction.

Chapter 6: Experimental Methods

6.1 General Experimental Procedures for Synthetic Chemistry

^{1}H NMR spectra were recorded on a Bruker AVII400 (400 MHz) spectrometer or a Bruker AVII500 (500 MHz) spectrometer using $CDCl_3$ (unless indicated otherwise) as a reference for internal deuterium lock. The chemical shift data for each signal are given as δ_H in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_H (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); q (quartet); dd (doublet of doublets); ddd (doublet of doublet of doublets) etc. or m (multiplet). The number of protons (n) for a given resonance signal is indicated by nH. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. Identical proton coupling constants (*J*) are averaged in each spectrum and reported to the nearest 0.1 Hz. The coupling constants are determined by analysis using Bruker TopSpin software.

^{13}C NMR spectra were recorded on a Bruker AVII400 (100 MHz) spectrometer or a Bruker AVII500 (126 MHz) spectrometer with broadband proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ_C in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_C (TMS) = 0.0 ppm. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 1 Hz. Identical coupling constants (*J*) are averaged in each spectrum and reported to the nearest 1 Hz. The coupling constants are determined by analysis using Bruker TopSpin software.

^{19}F NMR spectra were recorded on a Bruker AVII500 (470 MHz) spectrometer using a broadband proton decoupling pulse sequence and deuterium internal lock.

The chemical shift data for each signal are given as δ_F in units of parts per million (ppm).

^{11}B NMR spectra were recorded on a Bruker DRX500 (160 MHz) spectrometer. The chemical shift data for each signal are given as δ_B in units of parts per million (ppm). Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 1 Hz. Identical coupling constants (*J*) are averaged in each spectrum and reported to the nearest 1 Hz. The coupling constants are determined by analysis using Bruker TopSpin software.

Note: in all isoxazole-containing compounds, positions on the central aromatic ring are numbered first, the isoxazole ring with primes (´) and any additional rings with double primes (´´) etc.

Electrospray Ionisation (ES) High-Resolution Mass Spectrometry (HRMS) spectra were acquired on either a Bruker MicroTOF spectrometer or a Thermo Exactive mass spectrometer, equipped with Agilent 1100 liquid chromatography systems for flow injection analysis, from solutions of MeOH, H_2O or MeCN as stated. MicroTOF data were processed using Bruker Hystar software, while Exactive data were analysed using Thermo Xcalibur software.

Electron Impact/Field Ionisation (EI/FI) High-Resolution Mass Spectrometry (HRMS) spectra were acquired on a Micromass Gas Chromatography Time-of-Flight mass spectrometer, equipped with a direct insertion probe. Data were processed using Masslynx software.

Low-Resolution Mass Spectrometry (LRMS) spectra were recorded on a Waters Liquid Chromatography Time-of-Flight Premier spectrometer or Agilent 6120 Quadrupole LCMS spectrometer (ES). *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses.

Liquid Chromatography Mass Spectrometry (LCMS) Ultra-performance liquid chromatography (UPLC) analysis was conducted on an Acquity UPLC BEH C_{18} column (50 mm x 2.1 mm, i.d. 1.7 µm packing diameter) at 40 ˚C. When using a formic acid modifier, the solvents employed were A = 0.1% *v/v* solution of formic acid in H_2O; B = 0.1% *v/v* solution of formic acid in MeCN, and the gradient (A:B) employed was from 97:3 to 3:97 over 2 min. When using an ammonium bicarbonate modifier, the solvents employed were A = ammonium bicarbonate in H_2O adjusted to pH 10 with ammonia solution; B = MeCN. The gradient (A:B) employed was from 99:1 to 0:100 over 2 min. The UV detection was a summed signal from wavelength of 210 nm to 350 nm. Mass spectrometry was conducted on a Waters ZQ mass spectrometer, with an ionisation mode of alternate-scan positive and negative electrospray. The scan range was 100 to 1000 atomic mass units (AMU), the scan time was 0.27 seconds and the inter-scan delay was 0.10 seconds.

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

Melting points on crystallised samples were determined using a Kofler hot stage microscope and are uncorrected. The solvent of crystallisation is shown in parentheses.

Microanalyses were obtained at the Elemental Analysis Service, London Metropolitan University, London.

Analytical Thin Layer Chromatography (TLC) was carried out on Merck silica gel 60 F_{254} or RP-18 $F_{254}S$ aluminium-supported thin layer chromatography sheets. Visualisation was by absorption of UV light (λ_{max} 254 nm), or thermal development after dipping in one of: (a) ethanolic solution of phosphomolybdic acid;

(b) aqueous solution of potassium permanganate, potassium carbonate and sodium hydroxide; (c) ethanolic solution of ninhydrin.

Column chromatography was performed on a Biotage SP1 or SP4 system using KP-Sil™ cartridges (gradient elution) or on Merk Geduran® silica gel 60 (40-63 μm), with a gradient as supplied, under a positive pressure of compressed air.

Mass-directed autopurification (preparative LCMS) high-performance liquid chromatography (HPLC) analysis was conducted on either a Sunfire C_{18} column (100 mm × 19 mm, i.d 5 μm packing diameter) or a Sunfire C_{18} column (150 mm × 30 mm, i.d. 5 μm packing diameter) at ambient temperature. The solvents employed were: A = 0.1% *v/v* solution of formic acid in H_2O; B = 0.1% *v/v* solution of formic acid in MeCN. The purification was run as a gradient (A:B) over 15 minutes, with a flow rate of 20 mL/min (100 mm × 19 mm, i.d 5 μm packing diameter) or 40 mL/min (150 mm × 30 mm, i.d. 5 μm packing diameter). The UV detection was a summed signal from wavelength of 210 nm to 350 nm. The mass spectrometry was conducted on a Waters ZQ mass spectrometer, with an ionisation mode of alternate-scan positive and negative electrospray. The scan range was 100 to 1000 AMU, the scan time was 0.50 seconds and the inter-scan delay was 0.20 seconds.

Acidic ion exchange column refers to the use of a column of strong cation Dowex® resin that has been washed with H_2O, MeOH, a 1 M aqueous solution of HCl and a 1 M aqueous solution of NH_4OH, and acidified with a 1 M aqueous solution of HCl then neutralised with H_2O prior to loading the sample in H_2O. The loaded sample is then washed on the column with H_2O and eluted with a 1 M aqueous solution of NH_4OH.

Analytical HPLC was carried out on a PerkinElmer Flexar system with a Binary LC Pump and U*V/V*IS LC Detector. For determination of compound purity, a

Dionex Acclaim® 120 column (C18, 5 μm, 120 Å, 4.6 × 150 mm) was used and the solvents employed were A = 0.1% *v/v* solution of formic acid in 95% H_2O/5% MeCN; B = 0.1% *v/v* solution of formic acid in 95% MeCN/5% H_2O, and the gradient (A:B). A 10-minute gradient of 0-100% B was run with a flow rate of 1 mL/min and detection at 254 nm. Samples were injected in DMSO, MeOH, DMSO/MeOH or DMSO/chloroform mixtures as indicated.

Specific optical rotations were measured using a Perkin-Elmer 241 polarimeter, in cells with a 1 dm path-length. The light source was maintained at 589 nm and the concentration (*c*) is expressed in g/100 mL. Specific rotations are denoted $[\alpha]_D^T$, and are given in implied units of 10^{-1} deg cm^2 g^{-1} (T = ambient temperature in °C).

Anhydrous solvents were obtained under the following conditions: anhydrous DMF, anhydrous MeOH and anhydrous EtOH were purchased from Sigma-Aldrich UK in SureSeal™ bottles and used without further purification; anhydrous THF and CH_2Cl_2 were dried over activated 3 Å molecular sieves under an argon or nitrogen atmosphere; where stated, THF was distilled from sodium and benzophenone and pyridine was distilled from CaH_2; anhydrous DMAc (Sigma) was degassed by repeated freeze-thaw cycles and stored over activated 3 Å molecular sieves under an argon atmosphere. EtOH was degassed by repeated freeze-thaw cycles and stored under an argon atmosphere, but was not dried.

Chemicals were purchased from Acros, Sigma-Aldrich, Alfa Aesar, Fisher, Apollo Scientific or Fluorochem. Where appropriate and if not stated otherwise, all non-aqueous reactions were performed in a flame-dried flask under an inert atmosphere of nitrogen or argon. MnO_2 was activated at 250 °C overnight prior to use. Organolithium reagents were titrated against diphenylacetic acid,[198] and commercially-available organomagnesium compounds were titrated against

salicaldehyde phenylhydrazone.[56,199] Where stated, NEt_3 was dried with KOH and distilled onto KOH pellets.

In vacuo refers to the use of a rotary evaporator attached to a diaphragm pump.

Brine refers to a saturated aqueous solution of sodium chloride.

Petroleum ether refers to the fraction boiling between 40-60 °C unless otherwise stated.

Microwave irradiation refers to heating reactions in a Discover CEM or Biotage Initiator microwave.

Lyophilisation refers to removal of H_2O from aqueous solutions by freeze drying using a Christ Alpha 2-4-LD lyophiliser.

6.2 General Experimental Procedures for Biological Chemistry

Protein expression and purification (SGC Oxford): Proteins were cloned, expressed and purified as previously described.[41]

Protein expression and purification (by A. Sekirnik, CRL Oxford): Standard sterile practices were followed thoroughly, using the Heracleus laminar flow hood when necessary. Media and equipment were sterilised by autoclaving at 121 ^{o}C for 20 min. Solutions of isopropyl β-D-1-thiogalactopyranoside (IPTG) and kanamycin were sterilised by filtering through 0.2 mm filters (Minisart®, Sartorius Stedim). The final concentration of kanamycin was 30 μg/mL. 2 × Typtone-Yeast (2-TY) extract media was made up of bactotryptone (16.0 g/L), yeast extract (10.0 g/L) and NaCl (5.0 g/L), then was autoclaved (*Thermo Live Sciences* MAT 490 LEI) at 121 °C for

45 min before use, and left to rest overnight. Optical density readings were taken in 1.6 mL cuvettes with 5 × dilution in Milli-Q water against a reference sample of the growth media at zero time. The absorbance was measured at 600 nM using a Novaspec® II spectrophotometer. BRD4-containing constructs in pNIC28-Bsa4 vector backbones were transformed in competent *E. coli* BL21 (DE3) Gold cells. The competent cells were thawed on ice for 20 min before 1-5 µL of plasmid DNA was added to 20 µL of the competent cells in pre-chilled 50 mL Falcon tubes. The tubes were left on ice for 30 min before being 'heat shocked' by placement into a water bath at 42 °C for 30 sec. The tubes were returned to ice for 5 min, then 300 µL 2-TY media was added gently to each tube, which was then incubated at 37 °C for 1 h. Subsequently, 100 µL of the transformation mixture was plated onto agar plates containing kanamycin. All agar plates were poured and streaked in a laminar flow hood (HereSafe® Model KS12 Class II Biosafety Cabinet) using sterile media and equipment. These bacterial plate cultures were inverted and grown overnight at 37 °C in a Heraeus® TypB 6030 incubator. Small scale growths for starter cultures were 100 mL (in 500 mL Duran flasks) of 2-TY media containing kanamycin, inoculated from a single colony on an agar plate and grown overnight under foil, in a New Brunswick Scientific G25 environmental shaker at 37 °C and 250 rpm shaking. Flasks containing 600 mL 2-TY media with kanamycin were inoculated with 1% of the bacterial starter culture. Flasks (2000 mL PYREX® narrow-mouth graduated Erlenmeyer flasks or plastic baffled flasks) were incubated at 37 °C and 180 rpm in an Innova® 44 shaker (New Brunswick Scientific). When OD_{600} reached 0.8-1.0, the temperature was lowered to 18 °C and IPTG was added to a final concentration of 0.25 mM to induce protein expression. Cultures were incubated at 18°C overnight, after which time cultures were harvested by centrifugation in a JA10 rotor in an Avanti J25 centrifuge (Beckman Coulter™) at 8000 rpm for 15 min at 4 °C. The resulting pellet was weighed and stored in a sealable plastic bag at –80 °C. Buffers

for purification: charge buffer (100 mM $NiSO_4$); binding buffer (50 mM HEPES, 500 mM NaCl, 5 mM imidazole); wash buffer (50 mM HEPES, 500 mM NaCl, 30 mM imidazole); elution buffer (50 mM HEPES, 500 mM NaCl, 500 mM imidazole); strip buffer (50 mM HEPES, 500 mM NaCl, 100 mM EDTA). To purify the BRD4 protein, a cell pellet was dissolved in binding buffer (approximately 5-times *v/w* of cell pellet) and lysed by sonication (10 second bursts at 10 second intervals for a total sonication time of 10 min). The lysate was clarified by centrifugation in an Avanti J25 centrifuge (Beckman Coulter™) using a JLA-25.50 rotor at 23,000 rpm for 20 min at 4 °C. The supernatant was decanted off, filtered through a 0.45 µm filter and applied onto a 3-6 mL HisTrap™ chromatography column (GE Healthcare), which had been charged with 4 column volumes (CV) of 100 mM $NiSO_4$ and equilibrated with 5 CV of binding buffer prior to loading the cell lysate at 1 mL/min. The column was then washed with approximately 100 mL wash buffer. When all residual products of bacterial fermentation were eluted from the column by binding buffer, the protein of interest was batch-eluted with 20 mL of elution buffer containing 500 mM imidazole. Eluted protein was collected and fractionated; fractions containing the highest levels of pure protein (as determined by SDS-PAGE gel electrophoresis) were loaded on a pre-equilibrated 150 mL Superdex 75 size exclusion chromatography column, and further purified by gel filtration chromatography. After elution with 150 mL of gel filtration buffer, fractions identified by UV traces as containing protein were analysed by SDS-PAGE gel electrophoresis.

SDS-PAGE gel electrophoresis: The purity of proteins was analysed by PAGE. Gels were run on a mini-PROTEAN Tetra Electrophoresis System (Bio-Rad) at a constant potential of 200 V. Following electrophoresis, the gels were stained for 20 min with InstantBlue™ Coomasie® stain (Expedeon). For denaturing PAGE analysis, samples were prepared by mixing with sample loading buffer (2 ×) and incubation at 100 °C for 3 min. All gels were loaded with one of the following

molecular weight ladders: SeeBlue® prestained standard (Thermo Fisher Scientific); BLUEstain™ protein ladder, 11 245 kDa (Gold Biotechnology); PageRuler Plus prestained protein Ladder (Thermo Fisher Scientific); blue protein standard, broad range (New England Biolabs); colour protein standard, broad range (New England Biolabs). Resolving 12.5%, resolving 15% and stacking 4% gels were made up with 1.5 M pH 8.8 TRIS-HCl (1.25, 1.25 and 0 mL respectively), 0.5 M pH 6.8 TRIS-HCl (0, 0 and 680 µL respectively), Milli-Q water (1.57, 1.15 and 1.57 mL respectively), 30% (*w*/*v*) acrylamide (2.08, 2.50 and 0.25 mL respectively), 10% (*w*/*v*) SDS (50, 50 and 25 µL respectively), 10% (*w*/*v*) APS (50, 50 and 25 µL respectively) and TEMED (4.0, 4.0, 2.5 µL respectively). SDS-PAGE running buffer was made up of Tris-HCl (30 g), glycine (144 g) and SDS (10 g) per litre. pH 6.8 SDS-PAGE sample loading buffer was made up of 0.5 M Tris-HCl (10 mL), 0.2% (*w*/*v*) bromophenol blue (200 mg), 10% (*w*/*v*) SDS (20 mL), glycerol (12 mL) and β-mercaptoethanol (5 mL) per litre. SDS-PAGE stain was made up of 0.25% Coomassie® brilliant blue R (2.5 mL), 10% AcOH (100 mL) and 30% MeOH (300 mL) per litre. SDS-PAGE destain was made up of 10% AcOH (100 mL) and 30% MeOH (300 mL) per litre.

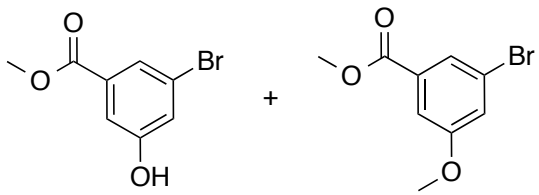
Thermal stability shift assay (SGC Oxford): Thermal melting experiments were carried out using an Mx3005p real-time PCR machine (Agilent) and employing a protein concentration of 2 µM and inhibitor concentration of 10 µM. Buffer conditions were 10 mM HEPES, pH 7.5, 500 mM NaCl and 1:1000 dilution of SyproOrange (Invitrogen, CA). The assay and data evaluation were carried out as described previously.[200] Experiments were performed in duplicate; average values are reported.

Bromodomain AlphaScreen™ assays (SGC Oxford) were carried out as previously described using the following peptides: H4KAc4 peptide (H_2N-SGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RHRK(Biotin)-COOH).[41,198] All experiments were carried out in duplicate on the same plate.

Bromodomain AlphaScreen™ assays (by A. Sekirnik, CRL Oxford): AlphaScreen™ buffer (25 mM HEPES, 100 mM NaCl, 0.05% *w/v* CHAPS, 0.1% *w/v* BSA; pH 7.6) was prepared fresh each day by supplementing HEPES base with BSA, filter sterilisation through a 0.22 µm filter, and storage at 4 °C, with equilibration to room temperature before use. Biotinylated peptides employed and final assay concentrations were: his_6BRD4(1) 10 nM, H4[1-20](KAc)$_4$ 4 nM; donor beads 5 µg/mL; acceptor beads 5 µg/mL; DMSO < 0.5%. Compounds were prepared as 50 mM DMSO stocks with a 1:100 pre-dilution step into buffer before addition to the assay. Inhibition was reported as a reduction in signal arising from peptide-bromodomain interaction, with all plates including buffer and DMSO controls. Concentration-response curves against BRD4 were performed in triplicate on a ProxiPlate-384 Plus (Perkin Elmer), which was read using a Synergy™ 2 MultiMode Microplate Reader. For incubation steps, the plate was sealed, shaken for 10 seconds at 600 rpm, and incubated at room temperature in the dark for 1 h.

6.3 Experimental Procedures for Chapter 2

Methyl 3-bromo-5-hydroxybenzoate (2.30) & methyl 3-bromo-5-methoxybenzoate[201]

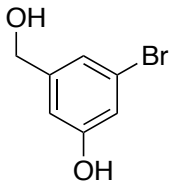


To a solution of 3-bromo-5-hydroxybenzoic acid (5.03 g, 23.2 mmol, 1.0 eq) in MeOH (70 mL) was added 18 M aqueous sulfuric acid (60.0 mL, 106 g, 1.08 mol, 47 eq), and the reaction solution was heated under reflux for 71 h. After cooling to rt, H_2O (200 mL) was added, and the resultant precipitate was filtered and washed with

cold H_2O (500 mL). The solid was dried by azeotropic distillation with toluene. Purification by silica gel chromatography, eluting with EtOAc in cyclohexane (gradient elution 5 → 30%) afforded **2.30** (1.91 g, 35%) as a colourless solid, and methyl 3-bromo-5-methoxybenzoate (1.50 g, 26%) as a clear and colourless liquid. For conversion of methyl 3-bromo-5-methoxybenzoate to methyl 3-bromo-5-hydroxybenzoate (**2.30**): To a solution of methyl 3-bromo-5-methoxybenzoate (1.36 g, 5.55 mmol, 1.0 eq) in CH_2Cl_2 (30 mL) was added 1 M boron tribromide (2.30 mL, 6.10 g, 2.30 mmol, 0.4 eq) at –78 °C. The reaction solution was stirred at rt for 16 h and additional 1 M boron tribromide (4.00 mL, 10.6 g, 4.00 mmol, 0.7 eq) in CH_2Cl_2 was added at –78 °C, then the reaction solution was stirred at rt for 7 h. Additional 1 M boron tribromide (5.00 mL, 13.3 g, 5.00 mmol, 0.9 eq) was added at –78 °C and the reaction solution was stirred at rt for 17 h, then the reaction was quenched with MeOH (50 mL) and concentrated *in vacuo*. The residue was redissolved in H_2O (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$, passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel column chromatography, eluting with EtOAc in cyclohexane (gradient elution 5 → 30%) afforded **2.30** (900 mg, 70%) as a colourless solid. Characterisation for methyl 3-bromo-5-hydroxybenzoate (**2.30**): R_f (10% EtOAc/cyclohexane) 0.16; mp (from EtOAc) 165-166 °C [lit. mp 124-126 °C[201]]; ^{1}H NMR (400 MHz, CD_3OD) δ 7.61-7.56 (1H, m, $C_{Ar}H$), 7.40-7.36 (1H, m, $C_{Ar}H$), 7.21-7.16 (1H, m, $C_{Ar}H$), 3.90 (3H, s, CH_3); LCMS (formic acid) RT = 0.91, $[M(^{79}Br) + H]^+$ 231, $[M(^{81}Br) + H]^+$ 233, $[M(^{79}Br) – H]^–$ 229, $[M(^{81}Br) – H]^–$ 231. Data are in good agreement with literature values.[201] Characterisation for methyl 3-bromo-5-methoxybenzoate: R_f (10% EtOAc/cyclohexane) 0.50; ^{1}H NMR (400 MHz, CD_3OD) δ 7.72-7.66 (1H, m, $C_{Ar}H$), 7.51-7.45 (1H, m, $C_{Ar}H$), 7.35-7.29 (1H, m, $C_{Ar}H$), 3.92 (3H, s, CO_2CH_3), 3.86 (3H, s, C(5)OCH_3); LCMS (formic acid) RT = 1.13, (did

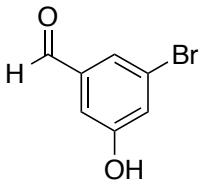
not ionise to give relevant mass). Data are in good agreement with literature values.[201]

3-Bromo-5-hydroxybenzylalcohol (2.32)[62,202]



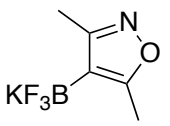
Following the procedure of Hewings *et al*., to a solution of 3-bromo-5-hydroxybenzoic acid (5.15 g, 23.7 mmol, 1.0 eq) in distilled THF (200 mL) was added a 1 M solution of borane (70.0 mL, 6.02 g, 70.0 mmol, 3.0 eq) in THF dropwise at 0 °C. The reaction was warmed to rt and stirred for 44 h, then was cooled to 0 °C. This was followed by slow addition of MeOH (200 mL), then an aqueous 1 M solution of HCl (100 mL), and then the volatile components were removed *in vacuo*. The resulting residue was resuspended in H_2O (150 mL) and extracted with EtOAc (3 × 150 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (450 mL) and brine (450 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give **2.32** (4.65 g, 22.9 mmol, 96%) as an oil that was deemed pure enough for use in the next step: R_f (30% EtOAc/petroleum ether) 0.22; ^{1}H NMR (400 MHz, DMSO-D_6) δ 9.81 (1H, s, C(5)O*H*), 6.93-6.88 (1H, m, C(2)*H*), 6.79 (1H, dd, *J* = 1.9, 1.9 Hz, C(4)*H*), 6.74-6.70 (1H, m, C(6)*H*), 5.25 (1H, t, *J* = 5.7 Hz, CH_2O*H*), 4.40 (2H, d, *J* = 5.7 Hz, CH_2OH); LRMS *m/z* (ES^-) 201 & 203 ([M(^{79}Br) – H]$^-$ & [M(^{81}Br) – H]$^-$, 79%), 403 & 405 & 407 ([M(^{79}Br)M(^{79}Br) – H]$^-$ & [M(^{79}Br)M(^{81}Br) – H]$^-$ & [M(^{81}Br)M(^{81}Br) – H]$^-$, 100%). Data are in good agreement with literature values.[62]

3-Bromo-5-hydroxybenzaldehyde (2.34)[46]



Following the procedure of Hewings *et al*., to a solution of 3-bromo-5-hydroxybenzylalcohol **2.32** (4.65 g, 22.9 mmol, 1.0 eq) in CH_2Cl_2 (100 mL) and acetone (10 mL) was added activated MnO_2 (12.9 g, 148 mmol, 6.5 eq). The reaction was stirred at rt for 43 h, then was filtered through Celite®, eluting with MeOH. The volatile components were removed *in vacuo*, and the resulting solid was purified by silica gel chromatography, eluting with Et_2O and petroleum ether (gradient elution 30 → 40%) yielded **2.34** (2.54 g, 55%) as an off-white solid: R_f (30% EtOAc/petroleum ether) 0.50; mp (from $CHCl_3$) 137-139 °C [lit. mp 137-140 °C[46]]; ^{1}H NMR (400 MHz, DMSO-D_6) δ 10.45 (1H, s, C*H*O), 9.86 (1H, s, C(5)O*H*), 7.52-7.45 (1H, m, $C_{Ar}H$), 7.30-7.19 (2H, m, 2 × $C_{Ar}H$); LRMS *m/z* (ES^-) 199 & 201 ([M(^{79}Br) – H]$^-$ & [M(^{81}Br) – H]$^-$, 31%), 399 & 401 & 403 ([M(^{79}Br)M(^{79}Br) – H]$^-$ & [M(^{79}Br)M(^{81}Br) – H]$^-$ & [M(^{81}Br)M(^{81}Br) – H]$^-$, 100%). Data are in good agreement with literature values.[46]

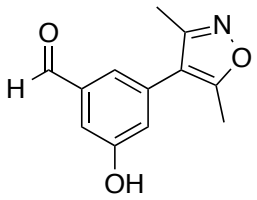
Potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate (2.35)[140]



Following the procedure of Molander *et al*., to a suspension of 3,5-dimethylisoxazol-4-boronic acid (4.92 g, 34.9 mmol, 1.0 eq) in MeOH (15 mL) at 0 °C was added KHF_2 (8.84 g, 113 mmol, 3.2 eq) then H_2O (24 mL) dropwise. The

reaction was warmed to rt and stirred for 10 min, before the volatile components were removed *in vacuo*. Purification was achieved by Soxhlet extraction (17 h) with acetone (200 mL). The collected suspension was dissolved with additional boiling acetone (200 mL), and the product was isolated by precipitation with Et_2O (300 mL) followed by filtration. The filtrate was concentrated *in vacuo*, redissolved in boiling acetone (100 mL) and further product was isolated by precipitation with Et_2O (200 mL) and filtration. The combined solids were dried *in vacuo* to give **2.35** (5.73 g, 81%) as a powdery, colourless solid: mp from (from $CHCl_3$) > 250 °C [lit. mp > 200 °C[140]]; ^{1}H NMR (400 MHz, DMSO-D_6) δ 2.19 (3H, s, C(5')CH_3), 2.04 (3H, s, C(3')CH_3); ^{11}B NMR (160 MHz, DMSO-D_6) δ 2.33 (q, *J* = 49 Hz); ^{19}F NMR (377 MHz, DMSO-D_6) δ –134.1 - –134.9 (m); LRMS *m/z* (ES^-) 164 ($[M – K]^-$, 82%), 367 ($[2 × M – K]^-$, 100%). Data are in good agreement with literature values.[140]

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-hydroxybenzaldehyde (2.37)[46,203]



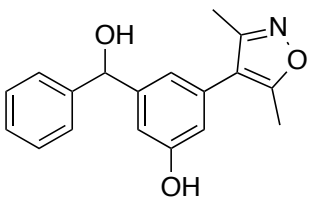
Following the procedure of Hewings *et al*., to a solution of **2.34** (5.00 g, 24.9 mmol, 1.0 eq), **2.35** (5.62 g, 27.5 mmol, 1.1 eq), RuPhos (1.36 g, 2.91 mmol, 0.12 eq) and Na_2CO_3 (5.49 g, 51.8 mmol, 2.1 eq) in degassed EtOH (100 mL) was added $Pd(OAc)_2$ (338 mg, 1.51 mmol, 0.06 eq). The reaction was heated under reflux for 18 h, then was allowed to cool to rt and filtered through a plug of Celite®, and the filtrate was concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 20 → 30%) yielded **2.37** (2.95 g,

55%) as a yellow solid. The characterisation data were identical to those shown below.

Alternative procedure:

To a solution of **2.38** (56 mg, 157 μmol, 1.0 eq) in DMAc (2 mL) was added AcOK (50 mg, 509 μmol, 3.2 eq). The reaction solution was degassed by bubbling with argon for 10 min, then $PdCl_2$ (a single grain) and 3,5-dimethylisoxazole (20.0 μL, 20 mg, 210 μmol, 1.3 eq) were added. The reaction solution was heated at 130 °C for 25 h, then was filtered through Celite®, eluting with CH_2Cl_2 (100 mL). The organic phase was washed with H_2O (3 × 100 mL) and brine (100 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 10 → 40%) yielded **2.37** (19 mg, 52%) as a yellow solid: R_f (20% EtOAc/petroleum ether) 0.13; mp (from EtOAc) 184-186 °C [lit. mp 184-187 °C[46]]; ^{1}H NMR (400 MHz, acetone-D_6) δ 10.02 (1H, s, C*H*O), 9.11 (1H, br s, C(5)O*H*), 7.42 (1H, dd, *J* = 1.4, 1.4 Hz, C(6)*H*), 7.36 (1H, dd, *J* = 2.3, 1.4 Hz, C(2)*H*), 7.15 (1H, dd, *J* = 2.3, 1.4 Hz, C(4)*H*), 2.44 (3H, s, C(5')CH_3), 2.26 (3H, s, C(3')CH_3); LRMS *m/z* (ES^-) 216 ($[M - H]^-$, 100%). Data are in good agreement with literature values.[46]

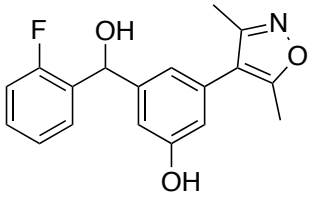
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxyl(phenyl)methyl]phenol (2.1)[46,204]



Following the procedure of Hewings *et al*., to a solution of **2.37** (2.63 g, 12.1 mmol, 1.0 eq) in distilled THF at 0 °C was added dropwise a 1 M solution of PhMgBr (34.0 mL, 34.0 mmol, 2.8 eq) in THF. The reaction solution was warmed to rt

and stirred for 4 h. After this time, the volatile components were removed *in vacuo*. The resulting solid was crystallised from hot MeCN and the mother liquor was purified by silica gel chromatography, eluting with 1% AcOH and EtOAc in petroleum ether (gradient elution 15 → 40%). The combined solids were crystallised from hot MeCN yielding **2.1** (1.43 g, 40%) as an off-white crystalline solid: R_f (50% EtOAc/petroleum ether) 0.38; mp (from MeCN) 187-190 °C [lit. mp 187-188 °C[46,205]]; ^{1}H NMR (400 MHz, acetone-D_6) δ 8.44 (1H, br s, C(1)O*H*), 7.49-7.43 (2H, m, C(2'')*H* and C(6'')*H*), 7.34-7.28 (2H, m, C(3'')*H* and C(5'')*H*), 7.24-7.19 (1H, m, C(4'')*H*), 6.94-6.91 (1H, m, C(6)*H*), 6.91-6.88 (1H, m, C(4)*H*), 6.67 (1H, dd, *J* = 1.9, 1.9 Hz, C(2)*H*), 5.83-5.79 (1H, m, C*H*OH), 4.90 (1H, d, *J* = 3.9 Hz, CHO*H*), 2.36 (3H, s, C(5')CH_3), 2.18 (3H, s, C(3')CH_3); LRMS *m/z* (ES^+) 296 ($[M + H]^+$, 100%). Data are in good agreement with literature values.[46]

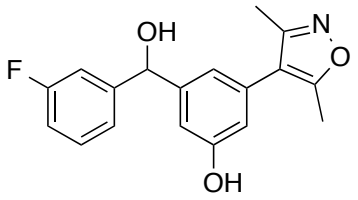
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(2-fluorophenyl)(hydroxyl)methyl]phenol (2.9) synthesised by A. Hicks



To a solution of 1-bromo-2-fluorobenzene (27.0 µL, 43 mg, 247 µmol, 1.2 eq) in distilled THF (2 mL) at –78 °C was added a 2.3 M solution of ^{n}BuLi in THF (12.0 µL, 28 mg, 431 µmol, 2.1 eq). The reaction solution was stirred at –78 °C for 40 min before a solution of **2.38** (75 mg, 201 µmol, 1.0 eq) in distilled THF (2 mL) was added. The reaction solution was stirred at –78 °C for 3 h, then was warmed to rt and quenched with H_2O (5 mL) and neutralised with an aqueous 1 M solution of HCl. The THF was removed *in vacuo*, and the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (30 mL) and brine

(30 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. To a solution of the resulting residue in distilled THF (5 mL) at 0 °C was added a 1 M solution of TBAF (65.0 µL, 59 mg, 224 µmol, 1.1 eq) in THF. The reaction was stirred at 0 °C for 2 h, then the volatile components were removed *in vacuo*. The resulting residue was partitioned between H_2O (10 mL) and EtOAc (10 mL). The phases were separated, the aqueous layer was extracted with EtOAc (2 × 10 mL) and the combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 20 → 100%) afforded **2.9** (11 mg, 17%) as a clear and colourless oil, which was precipitated from $CHCl_3$ with hexane to give a colourless, amorphous solid: R_f (50% EtOAc/petroleum ether) 0.45; ν_{max} (thin film)/cm^{-1}: 2970 (w), 2866 (br), 2844 (br), 1739 (s), 1435 (w), 1371 (w); ^{1}H NMR (500 MHz, CD_3OD) δ 7.56 (1H, ddd, *J* = 7.6, 7.6, 1.6 Hz, C(6'')*H*), 7.30-7.24 (1H, m, C(4'')*H*), 7.17 (1H, ddd, *J* = 7.6, 7.6, 1.0 Hz, C(5'')*H*), 7.04 (1H, ddd, *J* = 10.5, 8.1, 1.0 Hz, C(3'')*H*), 6.84-6.82 (1H, m, C(6)*H*), 6.78-6.76 (1H, m, C(4)*H*), 6.62-6.60 (1H, m, C(2)*H*), 6.03 (1H, s, C*H*OH), 2.35 (3H, s, C(5')CH_3), 2.19 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 166.8 (*C*(5')), 161.2 (d, *J* = 244 Hz, C(2'')), 159.9 (*C*(3')), 159.0 (*C*(1)), 147.3 (*C*(5)), 132.9 (d, *J* = 13 Hz, *C*(1'')), 132.4 (*C*(3)), 130.1 (d, *J* = 8 Hz, *C*(5'')), 128.8 (d, *J* = 4 Hz, *C*(3'')), 125.4 (d, *J* = 3 Hz, *C*(4'')), 119.5 (*C*(4)), 117.9 (*C*(4')), 116.1 (d, *J* = 22 Hz, *C*(6')), 115.8 (*C*(2)), 113.9 (*C*(6)), 69.9 (d, *J* = 3 Hz, *C*HOH), 11.4 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); ^{19}F NMR (377 MHz, CD_3OD) δ –120.6; HRMS *m/z* (ES^+) Found: 336.1013. $C_{18}H_{16}FNNaO_3^+$ requires M^+ 336.1006; LRMS *m/z* (ES^+) 314 ($[M + H]^+$, 100%), 333 ($[M + Na]^+$, 81%); HPLC RT = 11.00 min, purity 95.2%.

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(3-fluorophenyl)(hydroxyl)methyl]phenol (2.10)

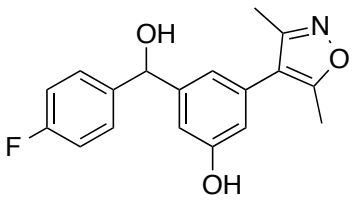


Formation of Grignard solution: To a solution of 1-fluoro-3-iodobenzene (270 µL, 511 mg, 2.30 mmol, 5.3 eq) in anhydrous THF (8 mL) was added a 1.3 M solution of isopropylmagnesium chloride lithium chloride (2.00 mL, 378 mg, 2.60 mmol, 5.8 eq) in THF dropwise at –10 °C. The reaction solution was warmed to rt and stirred for 5 h.

To a solution of **2.37** (94 mg, 433 µmol, 1.0 eq) in anhydrous THF (8 mL) was added dropwise a portion of the above Grignard solution (4.00 mL, 909 µmol, 2.1 eq). The reaction was stirred at rt for 3 h, then was heated at 50 °C for 18 h. Additional Grignard solution (1.00 mL, 227 µmol, 0.5 eq) was added and the reaction solution was heated at 50 °C for 2 h, before addition of the remaining Grignard solution (5.00 mL, 1.15 mmol, 2.7 eq). The reaction solution was stirred at 50 °C for 1 h, then the reaction was quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL), passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in cyclohexane (gradient elution 5 → 100%) followed by mass-directed autopurification (0.1% formic acid, gradient elution MeCN/H_2O 15 → 55%) afforded **2.10** (15 mg, 11%) as a clear and colourless oil that solidified under vacuum to give a colourless, amorphous solid: R_f (50% EtOAc/petroleum ether) 0.49; ν_{max} (thin film)/cm^{-1}: 3291 (br) (O–H), 2470 (br), 1593 (s); ^{1}H NMR (400 MHz, CD_3OD) δ 7.37-7.29 (1H, m, C(5'')*H*), 7.24-7.19 (1H, m, C(6)*H*), 7.19-7.13

(1H, m, C(2'')*H* or C(4'')*H*), 7.01-7.93 (1H, m, C(4'')*H* or C(2'')*H*), 6.88-8.83 (1H, m, C(6)*H*), 6.82-6.78 (1H, m, C(4)*H*), 6.67-6.62 (1H, m, C(2)*H*), 5.76 (1H, s, C*H*OH), 2.38 (3H, s, C(5')CH_3), 2.22 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 168.8 (*C*(5')), 164.3 (d, *J* = 245 Hz, *C*(3'')), 159.9 (*C*(3')), 159.1 (*C*(1)), 148.9 (d, *J* = 7 Hz, *C*(1'')), 147.9 (*C*(5)), 132.5 (*C*(3)), 131.0 (d, *J* = 8 Hz, *C*(5'')), 123.4 (d, *J* = 3 Hz, *C*(6'')), 119.7 (*C*(4)), 117.9 (*C*(4')), 115.9 (*C*(2)), 114.8 (d, *J* = 21 Hz, *C*(2'') or *C*(4'')), 114.1 (d, *J* = 22 Hz, *C*(4'') or *C*(2'')), 114.0 (*C*(6)), 75.9 (*C*HOH), 11.4 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); ^{19}F NMR (377 MHz, CD_3OD) δ –115.4; HRMS *m/z* (ES^-) Found: 312.1043. $C_{18}H_{15}FNO_3^-$ requires M^- 312.1041; LRMS *m/z* (ES^-) 312 ([M – H]$^-$, 100%); HPLC RT = 11.08 min, purity 95.4%.

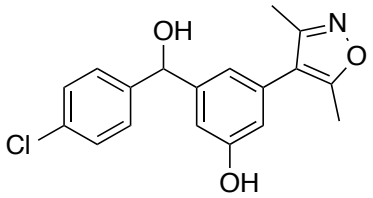
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-fluorophenyl)(hydroxyl)methyl]phenol (2.11) synthesised by A. Hicks



To a 2-necked flask containing magnesium turnings (60 mg, 2.45 mmol, 6.0 eq) and a crystal of iodine in distilled THF (10 mL) was added 1-bromo-4-fluorobenzene (296 µL, 472 mg, 2.69 mmol, 6.6 eq). The reaction was heated under reflux for 1.5 h, then cooled to 0 °C and added dropwise to a solution of **2.37** (89 mg, 410 µmol, 1.0 eq) in distilled THF (10 mL) at 0 °C. The reaction solution was warmed to rt and stirred for 2 h, then the reaction mixture quenched with H_2O (20 mL) and neutralised with an aqueous 1 M solution of HCl. The THF was removed *in vacuo*, and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with H_2O (60 mL) and brine (60 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting

with EtOAc in petroleum ether (gradient elution 10 → 100%) afforded **2.11** (21 mg, 16%) as a clear and colourless oil, which was precipitated from $CHCl_3$ with hexane to give a colourless, amorphous solid: R_f (50% EtOAc/petroleum ether) 0.24; ν_{max} (thin film)/cm^{-1}: 3285 (br) (O–H), 2985 (m), 2972 (m), 2939 (m), 2923 (m), 2866 (m), 2844 (m), 2826 (m), 1597 (w); ^{1}H NMR (400 MHz, CD_3OD) δ 7.34-7.25 (2H, m, C(2'')*H* & C(6'')*H*), 6.98-6.89 (2H, m, C(3'')*H* & C(5'')*H*), 6.74-6.70 (1H, m, C(6)*H*), 6.69-6.64 (1H, m, C(4)*H*), 6.52-6.50 (1H, m, C(2)*H*), 5.63 (1H, s, C*H*OH), 2.25 (3H, s, C(5')CH_3), 2.10 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4 (*C*(5')), 163.1 (d, *J* = 243.8 Hz, *C*(4'')), 159.6 (*C*(3')), 158.7 (*C*(1)), 147.8 (*C*(5)), 141.6 (d, *J* = 2.9 Hz, *C*(1'')), 132.1 (*C*(3)), 129.2 (d, *J* = 8.2 Hz, *C*(2'') & *C*(6'')), 119.2 (*C*(4)H), 117.6 (*C*(4')), 115.51 (d, *J* = 29.3 Hz, *C*(3'') & *C*(5'')), 115.45 (*C*(2)H), 113.6 (*C*(6)H), 75.5 (*C*HOH), 11.1 (C(5')*C*H_3), 10.4 (C(3')*C*H_3); ^{19}F NMR (377 MHz, CD_3OD) δ –117.7; HRMS *m/z* (ES^+) Found: 336.1011. $C_{18}H_{16}FNNaO_3^+$ requires M^+ 336.1006; LRMS *m/z* (ES^-) 312 ($[M - H]^-$, 100%); HPLC RT = 11.03 min, purity 95.7%.

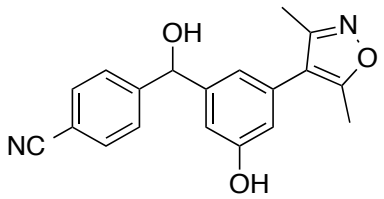
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-chlorophenyl)(hydroxy)methyl]phenol (2.12)



To a solution of **2.37** (96 mg, 442 mmol, 1.0 eq) in anhydrous THF (2 mL) was added a 1 M solution of 4-chlorophenylmagnesium bromide (1.00 mL, 216 mg, 1.00 mmol, 2.3 eq) in Et_2O at rt, and the reaction solution was stirred for 19 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL), passed through an anhydrous frit and concentrated

in vacuo. Purification by silica gel chromatography, eluting with EtOAc in cyclohexane (gradient elution 5 → 100%) followed by mass-directed autopurification (0.1% formic acid, gradient elution MeCN/H_2O 30 → 85%) afforded the desired product **2.12** (74 mg, 51%) as a clear and colourless oil that solidified under vacuum to give a colourless amorphous solid: R_f (50% EtOAc/cyclohexane) 0.35; v_{max} (thin film)/cm^{-1}: 3457 (br) (O–H), 3015 (br), 2970 (w), 2949 (br); ^{1}H NMR (400 MHz, CD_3OD) δ 7.42-7.36 (2H, m, C(2'')*H* & C(6'')*H*), 7.34-7.28 (2H, m, C(3'')*H* & C(5'')*H*), 6.88-6.83 (1H, m, C(6)*H*), 6.82-6.77 (1H, m, C(4)*H*), 6.64 (1H, dd, *J* = 2.2, 1.6 Hz, C(2)*H*), 5.75 (1H, s, C*H*OH), 2.36 (3H, s, C(5')CH_3), 2.21 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 166.9 (*C*(5')), 160.0 (*C*(3')), 159.0 (*C*(1)), 148.0 (*C*(5)), 144.7 (*C*(4'')), 134.0 (*C*(1'')), 132.6 (*C*(3)), 129.4 (*C*(3'') & *C*(5'')), 129.3 (*C*(2'') & *C*(6'')), 119.7 (*C*(4)), 117.9 (*C*(4')), 115.9 (*C*(2)), 114.0 (*C*(6)), 75.9 (*C*HOH), 11.5 (C(5')*C*H_3), 10.8 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 352.0715 & 354.0698. $C_{18}H_{16}ClNNaO_3^+$ requires $[M(^{35}Cl)]^+$ & $[M(^{37}Cl)]^+$ 352.0711 & 354.0683; LRMS *m/z* (ES^-) 328 ($[M(^{35}Cl) – H]^-$, 100%), 330 ($[M(^{37}Cl) – H^-$, 39%); HPLC RT = 11.55 min, purity 95.5%.

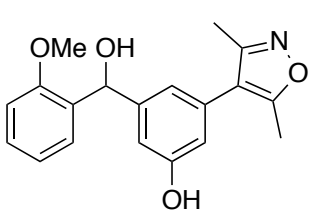
3-{[3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-hydroxyphenyl](hydroxyl)methyl} benzonitrile (2.13)



Formation of Grignard solution: To a solution of 4-iodobenzonitrile (484 mg, 2.11 mmol, 4.4 eq) in distilled THF (2 mL) was added a 1.3 M solution of isopropylmagnesium chloride lithium chloride (2.00 mL, 378 mg, 2.60 mmol, 5.4 eq) in THF dropwise at –10 °C. The reaction solution was stirred for 4 h.

To a solution of **2.37** (105 mg, 483 μmol, 1.0 eq) in distilled THF (8 mL) at –10 °C was added dropwise the above Grignard solution. The reaction was stirred for 3 h, then was warmed to rt and quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL) and brine (45 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 10 → 60%) afforded **2.13** (34 mg, 23%) as a clear and colourless oil: R_f (50% EtOAc/petroleum ether) 0.32; v_{max} (thin film)/cm^{-1}: 3319 (br) (O–H), 2972 (w), 2230 (w) (C≡N), 1632 (w), 1595 (m); ^1H NMR (400 MHz, CD_3OD) δ 7.71-7.66 (2H, m, C(3'')*H* & C(5'')*H*), 7.62-7.57 (2H, m, C(2'')*H* & C(6'')*H*), 6.83-6.80 (1H, m, C(6)*H*), 6.79-6.77 (1H, m, C(4)*H*), 6.79-6.77 (1H, m, C(2)*H*), 5.79 (1H, s, C*H*OH), 2.36 (3H, s, C(5')CH_3), 2.20 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 165.5 (*C*(5')), 158.5 (*C*(3')), 157.8 (*C*(1)), 150.3 (*C*(5)), 146.0 (*C*≡N), 131.9 (*C*(3'') and *C*(5'')), 131.3 (*C*(3)), 127.0 (*C*(2'') and *C*(6'')), 118.4 (*C*(4'')), 118.3 (*C*(4)), 116.4 (*C*(4')), 114.7 (*C*(2)), 112.6 (*C*(6)), 110.5 (*C*(1'')), 74.5 (*C*HOH), 10.0 (C(5')*C*H_3), 9.3 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 343.1061. $C_{19}H_{16}N_2NaO_3^+$ requires M^+ 343.1053; LRMS *m/z* (ES^-) 319 ([M – H]$^-$, 52%), 639 ([2M – H]$^-$, 100%); HPLC RT = 10.83 min, purity 95.7%.

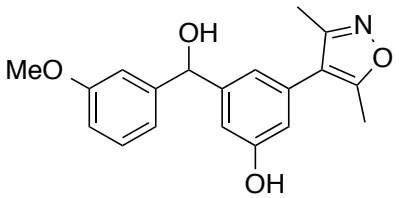
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(2-methyoxyphenyl)methyl]phenol (2.14)



To a solution of **2.37** (94 mg, 433 μmol, 1.0 eq) in anhydrous THF (2 mL) was added a 1 M solution 2-methoxyphenylmagnesium bromide (1.00 mL, 211 mg,

1.00 mmol, 2.3 eq) in THF at rt, and the reaction solution was stirred for 3 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL), passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in cyclohexane (gradient elution 10 → 60%) afforded **2.14** (113 mg, 80%) as a colourless solid: R_f (50% EtOAc/cyclohexane) 0.36; v_{max} (thin film)/cm^{-1}: 3196 (br) (O–H), 2993 (w), 2837 (w), 1642 (m) 1596 (s); mp (from EtOAc) 167-169 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.49 (1H, dd, *J* = 7.5, 1.5 Hz, C(6'')*H*), 7.25 (1H, ddd, *J* = 7.8, 7.8, 1.5 Hz, C(3'') *H*), 7.01-6.92 (2H, m, C(4'')*H* & C(5'')*H*), 6.88-6.84 (1H, m, C(6)*H*), 6.80-6.76 (1H, m, C(4)*H*), 6.60 (1H, dd, *J* = 1.9, 1.9 Hz, C(2)*H*), 6.12 (1H, s, C*H*OH), 3.81 (3H, s, OCH_3), 2.37 (3H, s, C(5')CH_3), 2.21 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 166.7 (*C*(5')), 160.0 (*C*(3')), 158.8 (*C*(1)), 157.7 (*C*(2'')), 148.1 (*C*(5)), 133.9 (*C*(1'')), 132.0 (*C*(3)), 129.5 (*C*(3'')), 127.9 (*C*(6'')), 121.6 (*C*(4'') or *C*(5'')), 119.9 (*C*(4)), 118.0 (*C*(4')), 115.4 (*C*(2)), 114.2 (*C*(6)), 111.7 (*C*(5'') or *C*(4'')), 70.5 (*C*HOH), 55.9 (O*C*H_3), 11.4 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 326.1386. $C_{19}H_{20}NO_4^+$ requires M^+ 326.1387; LCMS (formic acid) RT = 0.88, $[M + H]^+$ = 326; HPLC RT = 10.95 min, purity 98.0%.

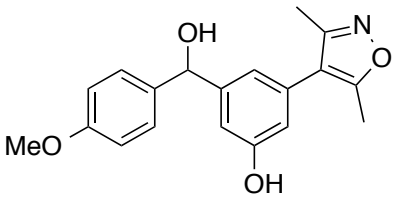
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(3-methyoxyphenyl)methyl]phenol (2.15)



To a solution of **2.37** (103 mg, 474 μmol, 1.0 eq) in anhydrous THF (2 mL) was added a 1 M solution of 3-methoxyphenylmagnesium bromide (1.00 mL, 211 mg,

1.00 mmol, 2.1 eq) in THF at rt, and the reaction solution was stirred for 5 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL), passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in cyclohexane (gradient elution 10 → 60%) afforded **2.15** (106 mg, 68%) as a colourless solid: R_f (50% EtOAc/cyclohexane) 0.34; v_{max} (thin film)/cm^{-1}: 3176 (br) (O–H), 2933 (w), 1640 (w), 1596 (w); mp (from EtOAc) 94-96 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (1H, dd, *J* = 7.8, 7.8 Hz, C(2'')*H*), 7.05-6.93 (2H, m, C(4'')*H* & C(5'')*H* or C(4'')*H* & C(6'')*H* or C(5'')*H* & C(6'')*H*), 6.89-6.84 (1H, m, C(6)*H*), 6.84-6.76 (2H, m, C(4)*H* & C(4'')*H,* C(5'')*H or* C(6'')*H*), 6.67-6.59 (1H, m, C(2)*H*), 5.72 (1H, s, C*H*OH), 3.78 (3H, s, OCH_3), 2.73 (3H, s, C(5')CH_3), 2.22 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 166.8 (*C*(5')), 161.2 (*C*(3'')), 160.0 (*C*(3')), 158.9 (*C*(1)), 148.33 (*C*(1'')), 147.4 (*C*(5)), 132.4 (*C*(3)), 130.3 (*C*(2'')), 120.0 (*C*(4''), *C*(5'') or C(6'')), 119.8 (*C*(4''), *C*(5'') or C(6'')), 118.0 (*C*(4')), 115.7 (*C*(2)), 114.1 (*C*(6)), 113.7 (*C*(4)), 113.2 (*C*(4''), *C*(5'') or C(6'')), 76.6 (*C*HOH), 55.7 (O*C*H_3), 11.4 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 326.1386. $C_{19}H_{20}NO_4^+$ requires M^+ 326.1387; LCMS (formic acid) RT = 0.87, $[M + H]^+$ = 326; HPLC RT = 10.84 min, purity 98.5%.

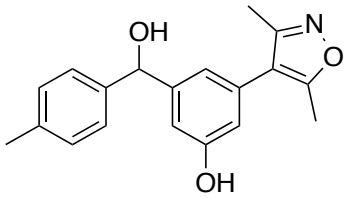
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(4-methyoxyphenyl)methyl]phenol (2.16)



To a solution of **2.37** (102 mg, 470 μmol, 1.0 eq) in anhydrous THF (2 mL) was added a 0.5 M solution of 4-methoxyphenylmagnesium bromide (2.00 mL, 211 mg,

1.00 mmol, 2.1 eq) in THF at rt, and the reaction solution was stirred for 3 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL), passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in cyclohexane (gradient elution 10 → 60%) afforded **2.16** (104 mg, 68%) as a clear and colourless oil. Precipitation from Et_2O and hexane afforded a colourless amorphous solid: R_f (50% EtOAc/cyclohexane) 0.44; ν_{max} (thin film)/cm^{-1}: 3016 (w) (O–H), 2970 (w), 1739 (s); ^1H NMR (400 MHz, CD_3OD) δ 7.34-7.26 (2H, m, C(3'')*H* & C(5'')*H*), 6.91-6.84 (3H, m, 2 × C(2'')*H*, C(6'')*H* & C(6)*H*), 6.81-6.78 (1H, m, C(4)*H*), 6.65-6.60 (1H, m C(2)*H*), 5.72 (1H, s, C*H*OH), 3.74 (3H, s, OCH_3), 2.35 (3H, s, C(5')CH_3), 2.20 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 166.8 (*C*(5')), 160.4 (*C*(4'')), 160.0 (*C*(3')), 158.9 (*C*(1)), 148.6 (*C*(5) or *C*(1'')), 137.9 (*C*(1'') or *C*(5)), 132.3 (*C*(3)), 129.0 (*C*(3'') & *C*(5'')), 119.6 (*C*(4)), 118.0 (*C*(4')), 115.5 (*C*(2)), 114.7 (*C*(2'') & *C*(6'')), 113.9 (*C*(6)), 76.2 (*C*HOH), 55.7 (O*C*H_3), 11.4 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); HRMS *m/z* (ES^-) Found: 324.1243. $C_{19}H_{18}NO_4^-$ requires M^-, 324.1241; LRMS *m/z* (ES^-) 324 ($[M - H]^-$, 100%); HPLC RT = 10.74 min, purity 97.1%.

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(4-methylphenyl)methyl]phenol (2.17)

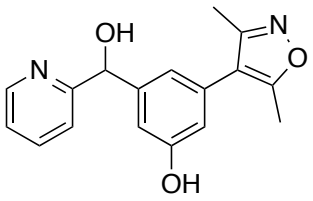


To a solution of **2.37** (93 mg, 428 μmol, 1.0 eq) in anhydrous THF (2 mL) was added a 1 M solution of 4-tolylmagnesium bromide (1.00 mL, 195 mg, 1.00 mmol, 2.3 eq) in THF at rt, and the reaction solution was stirred for 1 h. To aid solubility, additional THF (2 mL) was added and the reaction solution was stirred at rt for 17 h.

The reaction was quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL), passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with acetone in petroleum ether (gradient elution 10 → 50%) afforded **2.17** (75 mg, 56%) as a clear and colourless oil. Precipitation from Et_2O and hexane gave a colourless, amorphous solid: R_f (20% EtOAc/cyclohexane) 0.08; ν_{max} (thin film)/cm^{-1}: 3280 (br) (O–H), 1596 (s), 1421 (s); ^{1}H NMR (400 MHz, CD_3OD) δ 7.27 (2H, d, *J* = 7.9 Hz, C(3'')*H* & C(5'')*H*), 7.12 (2H, d, *J* = 7.9 Hz, C(2'')*H* & C(6'')*H*), 6.90-6.85 (1H, m, C(6)*H*), 6.82-6.77 (1H, m, C(4)*H*), 6.65-6.60 (1H, m, C(2)*H*), 5.72 (1H, s, C*H*OH), 2.33 (3H, s, C(5')CH_3), 2.28 (3H, s, C(4'')CH_3), 2.19 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 166.4 (*C*(5')), 159.9 (*C*(3')), 158.5 (*C*(1)), 148.1 (*C*(5)), 142.4 (*C*(1'')), 137.7 (*C*(4'')), 131.9 (*C*(3)), 129.5 (*C*(2'') & *C*(6'')), 127.3 (*C*(3'') & *C*(5'')), 119.3 (*C*(4)), 117.6 (*C*(4')), 115.2 (*C*(2)), 113.6 (*C*(6)), 76.1 (*C*HOH), 20.7 (*C*(4'')), 11.1 (*C*(5')), 10.4 (*C*(3')); HRMS *m/z* (ES^+) Found: 310.1433. $C_{19}H_{20}NO_3^+$ requires M^+ 310.1438; LRMS (formic acid) RT = 0.92, $[M + H]^+$ = 310; HPLC RT = 11.29 min, purity 98.5%.

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(pyridine-2-yl)methyl]phenol (2.19)

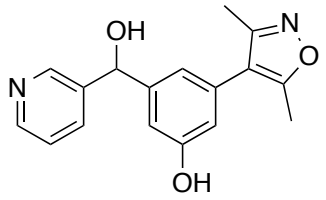
synthesised by D. Hewings



To a solution of 2-bromopyridine (38.0 µL, 64 mg, 402 µmol, 1.5 eq) in diethyl ether (3 mL) at –78 °C was added a 2.3 M solution of ^{n}BuLi in hexanes (180 µL, 420 mg, 414 µmol, 1.5 eq). The reaction solution was stirred at –78 °C for 40 min,

then was warmed to rt and stirred for a further 40 min before recooling to –78 °C. To this was added a solution of **2.38** (100 mg, 268 μmol, 1.0 eq) in diethyl ether (5 mL). The reaction solution was allowed to slowly warm to rt, over 16 h then the reaction was quenched with H_2O (5 mL) and neutralised with an aqueous 20% (*w/v*) solution of NH_4Cl. The aqueous phase was extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with brine (40 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in 30-40 °C petroleum ether (gradient elution 4 → 50%) afforded the TIPS-protected intermediate (65 mg, 54%) as a yellow oil. To a solution of this intermediate (50 mg, 110 μmol, 1.0 eq) in distilled THF (2 mL) at 0 °C was added a 1 M solution of TBAF (121 μL, 110 mg, 121 μmol, 1.1 eq). The reaction was stirred at 0 °C for 30 min, then the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with acetone in 30-40 °C petroleum ether (gradient elution 20 → 60%) afforded **2.19** (29 mg, 89%) as a colourless solid: R_f (40% EtOAc/petroleum ether) 0.26; mp (from acetone) 221-223 °C; ν_{max} (thin film)/cm^{-1}: 3389 (br), 2925 (w), 1593 (s); ^{1}H NMR (400 MHz, DMSO-D_6) δ 9.53 (1H, s, C(1)O*H*), 8.48-8.43 (1H, m, C(6'')*H*), 7.78 (1H, ddd, *J* = 9.5, 7.6, 1.8 Hz, C(4'')*H*), 7.59-7.54 (1H, m, C(3'')*H*), 7.24 (1H, ddd, *J* = 7.6, 4.9, 1.1 Hz, C(5'')*H*), 6.84-6.79 (2H, m, C(4)*H* & C(6)*H*), 6.59-6.56 (1H, m, C(2)*H*), 6.08 (1H, d, *J* = 4.1 Hz, C*H*OH), 5.66 (1H, d, *J* = 4.1, CHO*H*), 2.35 (3H, s, C(5')CH_3), 2.17 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, DMSO-D_6) δ 164.9 (*C*(5')), 163.9 (*C*(3')), 157.9 (*C*(1)), 157.4 (*C*(2'')), 148.3 (*C*(6'')), 146.4 (*C*(5)), 136.8 (*C*(4'')), 130.5 (*C*(3)), 122.2 (*C*(5'')), 120.0 (*C*(3'')), 117.7 (*C*(4)), 115.9 (*C*(4')), 114.1 (*C*(2)), 112.4 (*C*(6)), 75.4 (*C*HOH), 11.3 (C(5')*C*H_3), 10.5 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 319.1048. $C_{17}H_{17}N_2NaO_3^+$ requires M^+ 319.1053; LRMS *m/z* (ES^+) 297 ($[M + H]^+$, 100%), 319 ($[M + Na]^+$, 20%); HPLC RT = 8.42, purity 98.9%.

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(pyridine-3-yl)methyl]phenol (2.20)

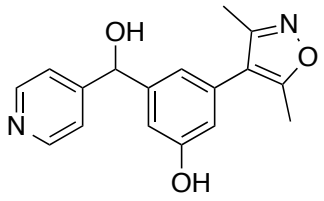


Formation of Grignard solution: To a solution of 3-iodopyridine (914 mg, 4.46 mmol, 9.7 eq) in anhydrous THF (16 mL) was added a 2 M solution of isopropylmagnesium chloride (2.50 mL, 514 mg, 5.00 mmol) in THF dropwise at rt. The reaction solution was stirred for 1 h.

To a solution of **2.37** (100 mg, 460 µmol, 10.9 eq) in anhydrous THF (8 mL) was added dropwise a portion of the above Grignard solution (5 mL, 1.21 mmol, 2.6 eq), and the reaction was stirred at rt for 3 h, then it was heated at 50 °C for 18 h. The reaction solution was cooled to rt, additional Grignard solution (5.00 mL, 1.21 mmol, 2.6 eq) was added and the reaction solution was heated at 50 °C for 26 h. The remaining Grignard solution (8.50 mL, 2.05 mmol, 4.5 eq) was added to the reaction solution at 50 °C. The reaction solution was heated at 50 °C for 70 h. The reaction solution was then allowed to cool to rt, was quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL), passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with MeOH in CH_2Cl_2 (gradient elution 0 → 20%) followed by mass-directed autopurification (0.1% formic acid, gradient elution MeCN/H_2O 15 → 55%) afforded **2.20** (11 mg, 8%) as a clear and colourless oil that solidified under vacuum to form a colourless, amorphous solid: R_f (10% MeOH/CH_2Cl_2) 0.46; ν_{max} (thin film)/cm^{-1}: 2970 (br), 2949 (w), 2923 (br), 2866 (br), 2844 (w); ^{1}H NMR (400 MHz, DMSO-D_6) δ 9.65 (1H, br s, C(1)O*H*), 8.63 (1H, d, *J* = 1.5 Hz, C(2'')*H*), 8.44 (1H, dd, *J* = 4.7, 1.5 Hz, C(6'')*H*), 7.80-7.74 (1H, m, C(4'')*H*), 7.39-7.32 (1H, m,

C(5'')*H*), 6.87-6.80 (2H, m, C(4)*H* & C(6)*H*), 6.62 (1H, dd, *J* = 1.8, 1.8 Hz, C(2)*H*), 6.10 (1H, d, *J* = 3.3 Hz, C*H*OH), 5.75 (1H, d, *J* = 3.3 Hz, CHO*H*), 2.38 (3H, s, C(5')CH_3), 2.20 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 166.9 (*C*(5')), 159.9 (*C*(3')), 159.3 (*C*(1)), 148.8 (*C*(6'')), 148.6 (*C*(2'')), 147.4 (*C*(3'') or *C*(5)), 142.4 (*C*(5) or *C*(3'')), 136.4 (*C*(4'')), 132.8 (*C*(3)), 125.1 (*C*(5'')), 119.5 (*C*(4)), 117.8 (*C*(4')), 116.2 (*C*(2)), 113.9 (*C*(6)), 74.3 (*C*HOH), 11.5 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); HRMS *m/z* (ES^-) Found: 295.1092. $C_{17}H_{17}N_2O_3^-$ requires M^- 295.1088; LRMS *m/z* (ES^-) 295 ($[M - H]^-$, 100%); HPLC RT = 8.29 min, purity 97.8%.

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(pyridine-4-yl)methyl]phenol (2.21)

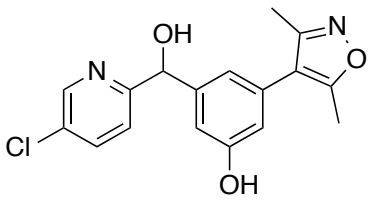


Formation of Grignard solution: To a solution of 4-iodopyridine (242 mg, 1.18 mmol, 5.1 eq) in anhydrous THF (4 mL) was added a 2 M solution of isopropylmagnesium chloride (1.20 mL, 247 mg, 2.40 mmol, 10.3 eq) in THF dropwise at rt. The reaction solution was stirred for 1 h.

To a solution of **2.37** (50 mg, 230 μmol, 1.0 eq) in anhydrous THF (4 mL) was added a portion of the above Grignard solution (2.60 mL, 590 μmol, 2.6 eq) and the reaction was stirred at rt for 4 h, then was heated at 50 °C for 18 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL), passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with MeOH in CH_2Cl_2 (gradient elution 0 → 20%) afforded **2.21** (6 mg, 9%) as a yellow oil: R_f (10%

EtOAc/cyclohexane) 0.40; v_{max} (thin film)/cm^{-1}: 3356 (br) (O–H), 2482 (br), 2244 (w), 2072 (m), 1598 (m); ^{1}H NMR (400 MHz, CD_3OD) δ 8.56-8.43 (2H, m, C(2'')*H* & C(6'')*H*), 7.53-7.48 (2H, m, C(3'')*H* & C(5'')*H*), 6.87-6.84 (1H, m, (C(6)*H*), 6.84-6.81 (1H, m, C(4)*H*), 6.67 (1H, dd, *J* = 2.2, 1.6 Hz, (C(2)*H*)), 5.78 (1H, s, C*H*OH), 2.38 (3H, s, C(5')CH_3), 2.22 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 166.9 (*C*(5')), 159.9 (*C*(3')), 159.3 (*C*(1)), 156.4 (*C*(5) or *C*(4'')), 150.0 (*C*(2'')H & *C*(6'')H), 147.0 (*C*(4'') or *C*(5)), 132.9 (*C*(3)), 123.0 (*C*(3'')H & *C*(5'')H), 119.8 (*C*(4)), 117.8 (*C*(4')), 116.4 (*C*(2)), 114.1 (*C*(6)), 75.2 (*C*HOH), 11.4 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); HRMS *m/z* (ES$^+$) Found: 297.1238. $C_{17}H_{17}N_2O_3^+$ requires M^+ 297.11234; LRMS *m/z* (ES$^+$) 297 ([M + H]$^+$, 100%); HPLC RT = 8.27 min, purity 95.4%.

3-[(5-Chloropyridin-2-yl)(hydroxyl)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.22)

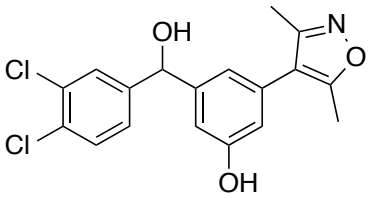


Formation of Grignard solution: To a solution of 5-chloro-2-iodopyridine (532 mg, 2.22 mmol, 5.1 eq) in distilled THF (2 mL) was added a 1.3 M solution of isopropylmagnesium chloride lithium chloride (2.00 mL, 378 mg, 2.60 mmol, 6.0 eq) in THF dropwise at rt. The reaction solution was stirred for 2 h.

To a solution of **2.37** (94 mg, 433 μmol, 1.0 eq) in distilled THF (2 mL) was added dropwise a portion of the above Grignard solution (2.00 mL, 1.11 mmol, 2.6 eq). The reaction was stirred at rt for 23 h, then was heated at 50 °C for 21 h, before the remaining Grignard solution (2.00 mL, 1.11 mmol, 2.6 eq) was added. The reaction was stirred for a further 5 h at 50 °C, then the reaction was quenched with a

saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL) and brine (45 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with acetone in petroleum ether (gradient elution 20 → 80%) afforded **2.22** (22 mg, 15%) as a clear and colourless oil. Precipitation from $CHCl_3$ and hexane gave a colourless amorphous solid: R_f (50% EtOAc/cyclohexane) 0.35; ν_{max} (thin film)/cm^{-1}: 3457 (br), 3016 (br) (O–H), 2970 (w), 2837 (w), 1631 (m), 1369 (s); ^{1}H NMR (400 MHz, CD_3OD) δ 8.45 (1H, d, *J* = 2.4 Hz, C(6'')*H*), 7.85 (1H, dd, *J* = 8.5, 2.4 Hz, C(4'')*H*), 7.61 (1H, d, *J* = 8.5 Hz, C(3'')*H*), 6.87-6.80 (2H, m, C(4)*H* and C(6)*H*), 6.61 (1H, dd, *J* = 1.8, 1.8 Hz, C(2)*H*), 5.76 (1H, s, C*H*OH), 2.36 (3H, s, C(5')CH_3), 2.20 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 166.9 (*C*(5')), 163.1 (*C*(2'')), 159.9 (*C*(3')), 159.2 (*C*(1)), 148.1 (*C*(6'')), 146.6 (*C*(5)), 138.3 (*C*(4'')), 132.6 (*C*(3)), 131.9 (*C*(5'')), 123.1 (*C*(3'')), 119.6 (*C*(6)), 117.8 (*C*(4')), 116.1 (*C*(2)), 114.0 (*C*(4)), 76.7 (*C*HOH), 11.4 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 353.0659. $C_{17}H_{15}ClN_2NaO_3^+$ requires M^+ 353.0663; LRMS *m/z* (ES^-) 329 ($[M - H]^-$, 100%); HPLC RT = 9.93 min, purity 97.2%.

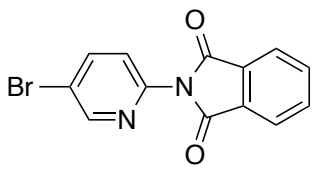
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(3,4-dichlorophenyl)(hydroxy)methyl]phenol (2.24)



To a solution of **2.37** (110 mg, 506 μmol, 1.0 eq) in anhydrous THF (2 mL) was added a 0.5 M solution of 3,4-dichlorophenylmagnesium bromide (2.50 mL, 313 mg, 1.25 mmol, 2.5 eq) in THF at rt, and the reaction solution was stirred for 7 h, then

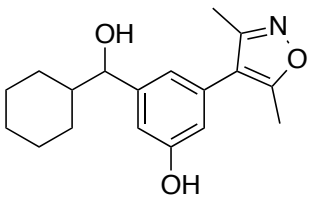
was heated at 50 °C for 15 h. Additional 3,4-dichlorophenylmagnesium bromide (1.00 mL, 125 mg, 500 μmol, 1.0 eq) was added and the reaction solution was stirred at 50 °C for 7 h. The reaction was quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL), passed through an anhydrous frit and concentrated *in vacuo*. The residue was resuspended in MeOH, filtered and the filtrate was concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in cylcohexane (gradient elution 5 → 55%) followed by mass-directed autopurification (0.1% formic acid, gradient elution MeCN/H_2O 30 → 85%) afforded **2.24** (20 mg, 11%) as a colourless solid: R_f (50% EtOAc/cyclohexane) 0.49; ν_{max} (thin film)/cm^{-1}: 3198 (br) (O–H), 1637 (m), 1596 (s); ^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (1H, d, *J* = 1.8 Hz, C(2'')*H*), 7.47 (1H, d, *J* = 8.3 Hz, C(5'')*H*), 7.31 (1H, dd, *J* = 8.3, 1.8 Hz, C(6'')*H*), 6.86-6.82 (1H, m, C(6)H), 6.82-6.78 (1H, m, C(4)*H*), 6.69-6.63 (1H, m, C(2)*H*), 5.74 (1H, s, C*H*OH), 2.38 (3H, s, C(5')CH_3), 2.23 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, CD_3OD) δ 166.9 (*C*(5')), 160.0 (*C*(3')), 159.2 (*C*(1)), 147.5 (*C*(3'')), 146.9 (*C*(4'')), 133.2 (*C*(3)), 132.7 (*C*(5)), 131.9 (*C*(1'')), 131.4 (*C*(5'')), 129.6 (*C*(2'')), 127.5 (*C*(6'')), 119.7 (*C*(4)), 117.9 (*C*(4')), 116.2 (*C*(2)), 113.9 (*C*(6)), 75.3 (*C*HOH), 11.5 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 386.0322, $C_{18}H_{15}Cl_2NNaO_3^+$ requires M^+ 386.0321; LCMS (formic acid) RT = 1.05, $[M + H]^+$ 364; HPLC RT = 13.52 min, purity 99.2%.

2-(5-Bromopyridin-2-yl)-1*H*-isoindole-1,3-(2*H*)-dione (2.40)[206]



A mixture of 5-bromopyridin-2-amine (2.34 g, 13.5 mmol, 1.0 eq) and phthalic anhydride (5.97 g, 40.3 mmol, 3.0 eq) was heated at 160 °C for 3 h. The reaction solution was allowed to cool to rt, and the crude material was crystallised from hot MeOH, affording **2.40** (1.97 g, 45%) as a colourless crystalline solid: R_f (20% EtOAc/petroleum ether) 0.30; ν_{max} (thin film)/cm^{-1}: 1712 (s) (C=O), 1421 (s); mp (from MeOH) 160-162 °C [lit. mp 162-164 °C[206]]; ^{1}H NMR (400 MHz, acetone-D_6) δ 8.74 (1H, dd, *J* = 2.5, 0.5 Hz, C(6)*H*), 8.24 (1H, dd, *J* = 8.5, 2.5 Hz, C(4)*H*), 8.01-7.91 (4H, m, C_6H_4), 7.57 (1H, dd, *J* = 8.5, 0.5 Hz, C(3)*H*); ^{13}C NMR (101 MHz, acetone-D_6) δ 166.9 (2 × *C*=O), 151.2 (*C*(6)), 146.3 (*C*(5)), 141.8 (*C*(4)), 135.7 (2 × *C*(2') or 2 × *C*(3')), 132.8 (2 × *C*(1')), 125.1 (*C*(3)), 124.5 (2 × *C*(3') or 2 × *C*(2')), 120.8 (*C*(2)); HRMS *m/z* (ES$^+$) Found: 302.9755 & 304.9735. $C_{13}H_8N_2O_2Br^+$ requires [M(^{79}Br)]$^+$ & [M(^{81}Br)]$^+$ 302.9764 & 304.9743; LRMS *m/z* (ES$^+$) 303 & 305 ([M(^{79}Br) + H]$^+$ & [M(^{81}Br) + H]$^+$, 56%), 325 & 327 ([M(^{79}Br) + Na]$^+$ & [M(^{81}Br) + Na]$^+$, 42%), 627 & 629 & 631 ([M(^{79}Br)M(^{79}Br) – H]$^-$ & [M(^{79}Br)M(^{81}Br) – H]$^-$ & [M(^{81}Br)M(^{81}Br) – H]$^-$, 100%); HPLC RT = 12.1 min, purity 100.0%. Data are in good agreement with literature values.[206]

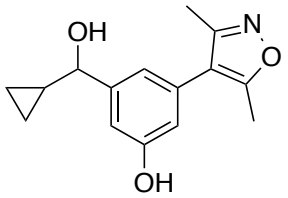
3-[Cyclohexyl(hydroxyl)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.25)



To a solution of **2.37** (104 mg, 479 μmol, 1.0 eq) in distilled THF (2 mL) was added a 2 M solution of cyclohexylmagnesium chloride (600 μL, 171 mg, 1.20 mmol, 2.5 eq) in Et_2O, dropwise at rt. The reaction solution was stirred for 3.5 h before further addition of cyclohexylmagnesium chloride (600 μL, 171 mg, 1.20 mmol, 2.5 eq) in Et_2O. The reaction was stirred for a further 23 h before it was quenched

with a saturated aqueous solution of NH_4Cl (15 mL). The aqueous phase was extracted with EtOAc (3 × 15 mL) and the combined organic layers were washed with H_2O (45 mL) and brine (45 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 10 → 40%) yielded **2.25** (38 mg, 26%) as a yellow oil: R_f (50% EtOAc/petroleum ether) 0.51; ν_{max} (thin film)/cm^{-1}: 3245 (br) (O–H), 2925 (m), 2852 (w), 2426 (br), 1739 (w), 1632 (m), 1595 (s); ^{1}H NMR (500 MHz, CD_3OD) δ 6.77-6.74 (1H, m, C(4)*H*), 6.71-6.68 (1H, m, C(6)*H*), 6.53-6.50 (1H, m, C(2)*H*), 4.26 (1H, d, *J* = 7.2 Hz, C*H*OH), 2.39 (3H, s, C(5')CH_3), 2.24 (3H, s, C(3')CH_3), 2.03-1.96 (1H, m, C*H*), 1.80-1.73 (1H, m, C*H*), 1.72-1.61 (2H, m, 2 × C*H*), 1.61-1.52 (1H, m, C*H*), 1.45-1.37 (1H, m, C*H*), 1.31-0.84 (5H, m, 5 × C*H*); ^{13}C NMR (126 MHz, CD_3OD) δ 166.7 (*C*(5')), 160.0 (*C*(3')), 158.8 (*C*(1)), 147.7 (*C*(5)), 132.0 (*C*(3)), 119.9 (*C*(4)), 118.1 (*C*(4')), 115.6 (*C*(2)), 114.2 (*C*(6)), 79.8 (*C*HOH), 46.4 (*C*HCHOH), 30.6 (*C*H_2), 30.2 (*C*H_2), 27.6 (*C*H_2), 27.3 (*C*H_2), 27.2 (*C*H_2), 11.5 (C(5')*C*H_3), 10.8 (C(3')*C*H_3); HRMS *m/z* (ES^-) Found: 300.1602. $C_{18}H_{22}NO_3^-$ requires M^- 300.1605; LRMS *m/z* (ES^-) 300 ($[M - H]^-$, 100%); HPLC RT = 11.83 min, purity 98.1%.

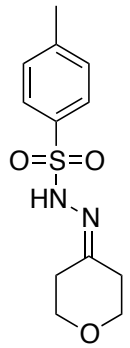
3-[Cyclopropyl(hydroxyl)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.27)



To a flask containing magnesium turnings (55 mg, 2.26 mmol, 6.1 eq) and a crystal of iodine was added dropwise bromocyclopropane (200 µL, 302 mg, 2.50 mmol, 6.8 eq) in distilled THF (2 mL). Following initiation, the reaction solution was stirred for 30 min, then **2.37** (80 mg, 368 µmol, 1.0 eq) and distilled THF (2 mL)

were added. The reaction solution was stirred at rt for 18 h then the reaction was quenched with a saturated aqueous solution of NH_4Cl (15 mL), and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (45 mL) and brine (45 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 20 → 80%) afforded **2.27** (81 mg, 85%) as a clear and colourless oil: R_f (50% EtOAc/petroleum ether) 0.24; ν_{max} (thin film)/cm^{-1}: 3240 (br) (O–H), 3001 (w), 2361 (w), 2343 (w), 1638 (w), 1597 (m); ^{1}H NMR (400 MHz, CD_3OD) δ 6.80-6.76 (1H, m, C(6)*H*), 6.74-6.70 (1H, m, C(4)*H*), 6.53 (1H, dd, *J* = 1.8. 1.8 Hz, C(2)*H*), 3.82 (1H, d, *J* = 8.3 Hz, C*H*OH), 2.30 (3H, s, C(5')CH_3), 2.15 (3H, s, C(3')CH_3), 1.09-0.98 (1H, m, C*H*CHOH), 0.55-0.46 (1H, m, C*H*), 0.45-0.31 (2H, m, 2 × C*H*), 0.29-0.22 (1H, m, C*H*); ^{13}C NMR (101 MHz, CD_3OD) δ 166.8 (*C*(5')), 160.0 (*C*(3')), 158.9 (*C*(1)), 148.3 (*C*(5)), 132.2 (*C*(3)), 119.3 (*C*(4)), 118.1 (*C*(4')), 115.7 (*C*(2)), 113.6 (*C*(6)), 78.9 (*C*HOH), 19.8 (*C*HCHOH), 11.5 (C(5')*C*H_3), 10.8 (C(3')*C*H_3), 4.4 (*C*H_2), 3.1 (*C*H_2); HRMS *m/z* (ES^+) Found: 282.1110. $C_{15}H_{17}NaO_3^+$ requires M^+ 282.1101; LRMS *m/z* (ES^+) 260 ($[M + H]^+$, 97%), 282 ($[M + Na]^+$, 100%); HPLC RT = 9.98 min, purity 98.7%.

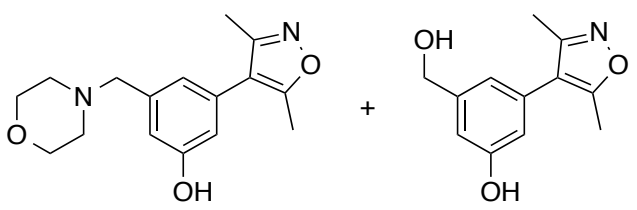
4-Methyl-*N*'-(tetrahydro-4*H*-pyran-4-ylidene)benzenesulfonohydrazide (2.65)



To a solution of tetrahydropyran-4-one (200 μL, 217 mg, 2.17 mmol, 1.0 eq) in MeOH (10 mL) over 3 Å sieves was added 4-toluenesulfonyl hydrazine (750 mg, 4.03 mmol, 1.9 eq). The reaction was stirred at rt for 16 h then the reaction was quenched with an aqueous 10% (*w/v*) solution of citric acid (25 mL) and the aqueous phase was extracted with EtOAc (3 × 25 mL). The aqueous layer was basified with a saturated aqueous solution of $NaHCO_3$ and re-extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine (300 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 20 → 80%) yielded **2.53** (362 mg, 62%) as a colourless, amorphous solid: R_f (50% EtOAc/petroleum ether) 0.26; ν_{max} (thin film)/cm^{-1}: 3231 (m), 2971 (w), 2951 (w), 2921 (w), 2865 (w), 1648 (m) (C=N), 1328 (S=O); ^{1}H NMR (400 MHz, acetone-D_6) δ 9.12 (1H, br s, N*H*), 7.79-7.74 (2H, m, 2 × $C_{Ar}H$), 7.40-7.35 (2H, m, 2 × $C_{Ar}H$), 3.69 (2H, t, *J* = 5.7 Hz, $OC_AH_2C_BH_2N$), 3.64 (2H, t, *J* = 5.8 Hz, $OC_CH_2C_DH_2N$), 2.45 (2H, t, *J* = 5.8 Hz, $OC_CH_2C_DH_2N$), 2.41 (3H, s, CH_3), 2.27 (2H, t, *J* = 5.7 Hz, $OC_AH_2C_BH_2N$); ^{13}C NMR (126 MHz, acetone-D_6) δ 156.1 (*C*=N), 143.3 (*C*(1)), 136.9 (*C*(4)), 129.2 (*C*(3) & *C*(5)), 128.0 (*C*(2) & *C*(6)), 67.4 ($OCH_2C_BH_2N$), 67.3 ($OCH_2C_DH_2$), 67.3 ($OC_CH_2CH_2$), 42.5 ($OC_AH_2CH_2N$), 20.5 (CH_3); HRMS *m/z* (ES^+) Found: 269.0949. $C_{12}H_{17}N_2O_3S^+$ requires M^+ 269.0954; LRMS *m/z* (ES^+) 269 ($[M + H]^+$, 100%), 291 ($[M + Na]^+$, 78%), 559 ($[2M + Na]^+$, 70%); HPLC RT = 10.46 min, purity 92.2%.

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(morpholin-4-ylmethyl)phenol (2.41) &

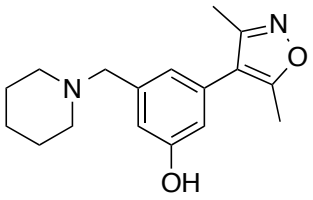
3-(3,5-dimethyl-1,2-oxazol-4-yl)-5-(hydroxymethyl)phenol (2.41a)



To a solution of **2.37** (100 mg, 460 μmol, 1.0 eq) and morpholine (50.0 μL, 50 mg, 572 μmol, 1.2 eq) in EtOH (5 mL) was added AcOH dropwise until the solution was pH 4. The reaction was stirred at rt for 40 min before addition of $NaBH_3CN$ (23 mg, 366 μmol, 0.8 eq). The reaction was stirred for a further 23 h and the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 40 → 100%) yielded **2.41** (27 mg, 20%) as a yellow oil, and **2.41a** (57 mg, 57%) as an off-white solid. A small sample of the 1.0:0.32 amine:TFA salt was purified by semi-preparative HPLC for biological testing. The degree of protonation was quantified using 1,4-difluorobenzene as an internal standard in ^{19}F NMR. Characterisation for **2.41**: R_f (80% EtOAc/petroleum ether) 0.10; ν_{max} (thin film)/cm^{-1}: 2982 (m), 2886 (w), 2359 (m), 2344 (m), 1673 (m); mp TFA salt (from MeCN) > 250 °C; ^1H NMR (400 MHz, CD_3OD) δ 6.97 (1H, dd, *J* = 1.9, 1.9 Hz, C(4)*H*), 6.94 (1H, dd, *J* = 1.4, 1.4 Hz, C(6)*H*), 6.88 (1H, dd, *J* = 1.9, 1.4 Hz, C(2)*H*), 4.34 (2H, s, CH_2Ar), 4.19-3.94 (2H, m, 2 × C$H_A$$H_B$), 3.87-3.63 (2H, m, 2 × C$H_A$$H_B$), 3.50-3.07 (4H, m, 2 × CH_2), 2.42 (3H, s, C(5')CH_3), 2.27 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 167.2 (*C*(5')), 160.0 (*C*(3')), 159.8 (*C*(1)), 133.9 (*C*(3)), 131.7 (*C*(5)), 123.8 (*C*(4)), 118.7 (*C*(2)H), 118.4 (*C*(6)), 117.1 (*C*(4')), 64.9 (2 × *C*H_2O), 61.7 (*C*H_2Ar), 52.9 (2 × *C*H_2N), 11.5 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); ^{19}F NMR (377 MHz, CD_3OD) δ –77.1; HRMS *m/z* (ES^+) Found: 289.1547. $C_{16}H_{21}N_2O_3^+$ requires M^+ 289.1547; LRMS *m/z* (ES^+) 289 ($[M + H]^+$, 100%); HPLC RT = 8.58 min, purity 99.5%. Characterisation for **2.41a**: R_f (80% EtOAc/petroleum ether) 0.50; mp (from EtOAc) 116-118 °C; ν_{max} (thin film)/cm^{-1}: 3204 (br) (O–H), 2363 (m), 1622 (m), 1595 (m), 1422 (s); ^1H NMR (500 MHz, CD_3OD) δ 6.83-6.80 (1H, m, C(4)*H*), 6.78-6.80 (1H, m, C(6)*H*), 6.64-6.62 (1H, m, C(2)*H*), 4.58 (2H, s, CH_2Ar), 2.40 (3H, s, C(5')CH_3), 2.24 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 165.4 (*C*(5')), 158.6 (*C*(3')), 157.7 (*C*(1)), 143.7 (*C*(5)), 131.1 (*C*(3)), 118.4 (*C*(4)), 116.6 (*C*(4')), 114.4 (*C*(2)), 112.8 (*C*(6)), 63.5 (*C*H_2Ar), 10.1 (C(5')*C*H_3), 9.3 (C(3')*C*H_3); HRMS *m/z* (ES^-) Found: 242.0786. $C_{12}H_{13}NNaO_3^+$

requires M^+ 242.0788; LRMS *m/z* (ES^-) 218 ($[M - H]^-$, 100%), 437 ($[2M - H]^-$, 95%); HPLC RT = 9.46 min, purity 99.1%.

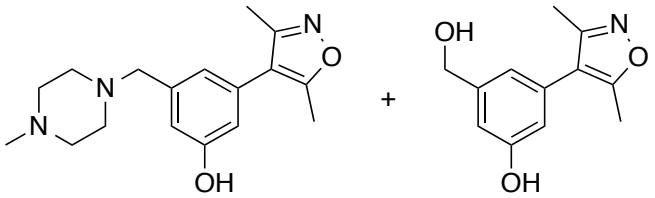
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(piperidin-1-ylmethyl)phenol (2.42)



To a solution of piperidine (50.0 µL, 43 mg, 506 µmol, 1.5 eq) and AcOH (50.0 µL, 52 mg, 873 µmol, 2.7 eq) in EtOH (5 mL) was added **2.38** (123 mg, 329 µmol, 1.0 eq). The reaction was stirred at rt for 1 h, then $NaBH_3CN$ (11 mg, 175 µmol, 0.5 eq) was added. The reaction solution was stirred for a further 22 h, then the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 0 → 50%), followed by ISOLUTE® SCX-2 amine catch and release column yielded an oil that contained a mixture of **2.42** (1 mg, 3.49 µmol) and the TIPS-protected intermediate (14 mg, 31.6 µmol). To a solution of the oil resuspended in distilled THF (1 mL) was added a 1 M solution of TBAF (35.0 µL, 32 mg, 35.0 µmol, 1.1 eq) in THF dropwise at 0 °C. The reaction was warmed to rt and stirred for 2 h, then the reaction was quenched with H_2O (5 mL). The aqueous phase was extracted with EtOAc (3 × 5 mL), and the combined organic layers were washed with brine (15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOH in EtOAc (gradient elution 0 → 50%) yielded **2.42** (11 mg, 8%) as a clear and colourless oil: R_f (20% MeOH/CH_2Cl_2) 0.56; ν_{max} (thin film)/cm^{-1}: 2982 (w), 2938 (w), 2361 (m), 2334 (m); ^1H NMR (500 MHz, CD_3OD) δ 6.79 (1H, dd, *J* = 1.8, 1.8 Hz, C(4)*H*), 6.77-6.64 (1H, m, C(6)*H*), 6.65 (1H, dd, *J* = 1.8, 1.8 Hz, C(2)*H*), 3.48 (2H, s, CH_2Ar), 2.52-2.40 (4H, m, 2 × CH_2N), 2.40 (3H,

s, C(5')CH_3), 2.25 (3H, s, C(3')CH_3), 1.61 (4H, tt, J = 5.7, 5.7 Hz, 2 × CH_2CH_2N), 1.53-1.32 (2H, m, CH_2CH_2CH_2N); ^{13}C NMR (126 MHz, CD_3OD) δ 166.8 (*C*(5')), 160.0 (*C*(3')), 159.1 (*C*(1)), 140.5 (*C*(5)), 132.4 (*C*(3)), 122.6 (*C*(4)), 117.9 (*C*(4')), 117.2 (*C*(6)), 116.0 (*C*(2)), 64.5 (*C*H_2Ar), 55.4 (2 × *C*H_2N), 26.5 (2 × *C*H_2CH_2N), 25.1 (*C*H_2CH_2CH_2N), 11.5 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 287.1759. $C_{17}H_{23}N_2O_2^+$ requires M^+ 287.1754; LRMS *m/z* (ES^+) 287 ($[M + H]^+$, 100%); HPLC RT = 9.24 min, purity 99.2%.

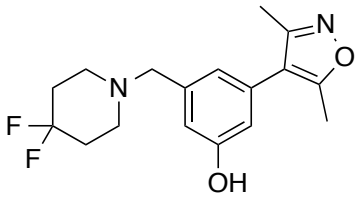
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-methylpiperazin-1-yl)methyl]phenol (2.43) & 3-(3,5-dimethyl-1,2-oxazol-4-yl)-5-(hydroxymethyl)phenol (2.41a)



To a solution of **2.37** (106 mg, 488 μmol, 1.0 eq) and 1-methylpiperazine (70.0 μL, 63 mg, 631 μmol, 1.3 eq) in EtOH (5 mL) was added AcOH dropwise until the solution was pH 4. The reaction was stirred at rt for 20 min before addition of $NaBH_3CN$ (20 mg, 318 μmol, 0.7 eq). The reaction was stirred for a further 17 h and the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with MeOH in CH_2Cl_2 (gradient elution 0 → 20%) yielded **2.43** (101 mg, 69%) as an oil that crystallised under vacuum and **2.41a** (12 mg, 11%) as an off-white solid. A small sample of the 1.0:0.59 amine:TFA salt was purified by semi-preparative HPLC for biological testing. The degree of protonation was quantified using 1,4-difluorobenzene as an internal standard in ^{19}F NMR. R_f (20% MeOH/CH_2Cl_2) 0.43; mp free amine (from MeOH) 144-146 °C, TFA salt (from MeCN) > 250 °C; ν_{max} (thin film)/cm^{-1}: 2965 (w), 2813 (w), 1589 (m); ^{1}H NMR (400 MHz, CD_3OD) δ 6.91-6.84 (2H, m, C(4)*H* & C(6)*H*), 6.79-6.74 (1H, m, C(2)*H*), 3.99 (2H, s,

C*H*$_2$Ar), 3.53-3.36 (4H, m, 4 × NC*H*$_2$), 3.28-3.04 (4H, m, 4 × NC*H*$_2$), 2.92 (3H, s, NC*H*$_3$), 2.41 (3H, s, C(5')C*H*$_3$), 2.25 (3H, s, C(3')C*H*$_3$); ^{13}C NMR (126 MHz, CD_3OD) δ 167.0 (*C*(5')H), 159.9 (*C*(3')H), 159.5 (*C*(1)), 137.6 (*C*(3)), 132.5 (*C*(5)), 122.5 (*C*(4)), 117.6 (*C*(2)), 115.8 (*C*(6)), 114.1 (*C*(4')), 62.0 (*C*H$_2$Ar), 53.8 (2 × N*C*H$_2$), 50.5 (2 × N*C*H$_2$), 43.5 (*C*H$_3$), 11.4 (C(5')*C*H$_3$), 10.7 (C(3')*C*H$_3$); ^{19}F NMR (377 MHz, CD_3OD) δ –77.3; HRMS *m/z* (ES$^+$) Found: 302.1858. $C_{17}H_{24}N_3O_2^+$ requires M^+ 302.1863; LRMS *m/z* (ES$^+$) 302 ($[M + H]^+$, 100%); HPLC RT = 8.14 min, purity 99.7%. The characterisation data for **2.41a** were identical to those shown above.

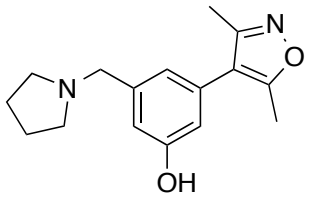
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(4,4-difluoropiperidin-1-ylmethyl)phenol (2.44)



To a solution of 4,4-difluoropiperidine hydrochloride (156 mg, 987 μmol, 2.1 eq) in EtOH (5 mL) was added **2.37** (100 mg, 460 μmol, 1.0 eq). The reaction was stirred at rt for 30 min, then $NaBH_3CN$ (49 mg, 780 μmol, 1.6 eq) was added. The reaction solution was stirred for a further 19 h, then the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 15 → 100%), followed by ISOLUTE® SCX-2 amine catch and release column yielded **2.44** (49 mg, 33%) as a clear and colourless oil. A small sample of the 1.00:1.33 amine:TFA salt was purified by semi-preparative HPLC for biological testing. The degree of protonation was quantified by ^{19}F NMR: R_f free amine (100% EtOAc) 0.69; ν_{max} (thin film)/cm^{-1}: 2982 (m), 2886 (w), 2359 (m), 2344 (m), 2160 (w); mp TFA salt (from MeCN) > 250 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 6.99-6.96 (1H, m, C(6)*H*), 6.94 (1H, dd, *J* = 1.4, 1.4 Hz, C(4)*H*), 6.90-6.87 (1H, m, C(2)*H*), 4.37 (2H, s, C*H*$_2$Ar), 3.71-3.22 (4H, m, 2 × NC*H*$_2$), 2.48-2.23 (4H, m,

2 × CF_2CH_2), 2.42 (3H, s, C(5')CH_3), 2.27 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2 (*C*(5')), 160.0 (*C*(3')), 159.8 (*C*(1)), 134.0 (*C*(3)), 132.5 (*C*(5)), 123.5 (*C*(4)), 120.2 (dd, *J* = 242, 242 Hz, CF_2), 118.7 (*C*(6)), 118.2 (*C*(2)), 117.1 (*C*(4')), 60.7 (CH_2Ar), 50.4 (dd, *J* = 5, 5 Hz, 2 × NCH_2), 32.1 (dd, *J* = 26, 26 Hz, 2 × CF_2CH_2), 11.5 (C(5')CH_3), 10.7 (C(3')CH_3); ^{19}F NMR (377 MHz, CD_3OD) δ –77.0 (CF_3CO_2), –98.7 (1F, d, *J* = 195 Hz, CF_AF_B), –104.9 (1F, d, *J* = 195 Hz, CF_AF_B); HRMS *m/z* (ES^+) Found: 323.1560. $C_{17}H_{21}O_2N_2F_2^+$ requires M^+ 323.1566; LRMS *m/z* (ES^+) 323 ($[M + H]^+$, 100%); HPLC RT = 9.29 min, purity 99.6%.

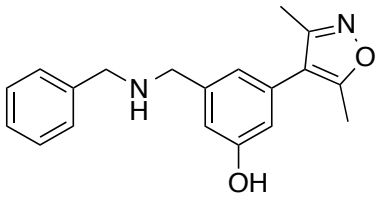
3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(pyrrolidin-1-ylmethyl)phenol (2.45)



To a solution of pyrrolidine (50.0 µL, 43 mg, 493 µmol, 1.3 eq) and AcOH (50.0 µL, 52 mg, 873 µmol, 2.4 eq) in EtOH (5 mL) was added **2.38** (138 mg, 369 µmol, 1.0 eq). The reaction was stirred at rt for 1 h, then $NaBH_3CN$ (13 mg, 207 µmol, 0.6 eq) was added. The reaction solution was stirred for a further 22 h, then the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 0 → 50%), followed by ISOLUTE® SCX-2 amine catch and release column yielded an oil that contained a mixture of **2.45** (10 mg, 3.67 µmol) and the TIPS-protected intermediate (54 mg, 126 µmol). To a solution of the oil resuspended in distilled THF (2 mL) was added a 1 M solution of TBAF (200 µL, 181 mg, 200 µmol, 1.6 eq) in THF dropwise at 0 °C. The reaction was warmed to rt and stirred for 1 h, then the reaction was quenched with H_2O (5 mL). The aqueous phase was extracted with EtOAc (3 × 5 mL), and the combined organic layers were washed with brine (15 mL), dried

over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOH in EtOAc (gradient elution 0 → 40%) yielded **2.45** (33 mg, 33%) as a clear and colourless oil: R_f (20% MeOH/CH_2Cl_2) 0.44; ν_{max} (thin film)/cm^{-1}: 2961 (m), 2930 (m), 1632 (w), 1593 (s); ^1H NMR (500 MHz, CD_3OD) δ 6.81 (1H, dd, *J* = 1.7, 1.7 Hz, C(4)*H*), 6.79-6.77 (1H, m, C(6)*H*), 6.66 (1H, dd, *J* = 2.2, 1.7 Hz, C(2)*H*), 3.66 (2H, s, CH_2Ar), 2.67-2.60 (4H, m, 2 × CH_2N), 2.39 (3H, s, C(5')CH_3), 2.24 (3H, s, C(3')CH_3), 1.87-1.80 (4H, m, 2 × CH_2CH_2N); ^{13}C NMR (126 MHz, CD_3OD) δ 166.8 (*C*(5')), 159.9 (*C*(3')), 159.2 (*C*(1)), 141.0 (*C*(5)), 132.6 (*C*(3)), 122.3 (*C*(4)), 117.8 (*C*(4')), 116.8 (*C*(6)), 116.2 (*C*(2)), 61.1 (*C*H_2Ar), 54.9 (2 × *C*H_2N), 24.1 (2 × *C*H_2CH_2N), 11.5 (C(5')*C*H_3), 10.7 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 273.1597. $C_{16}H_{21}N_2O_2^+$ requires M^+ 273.1598; LRMS *m/z* (ES^+) 273 ($[M + H]^+$, 100%); HPLC RT = 8.98 min, purity 95.9%.

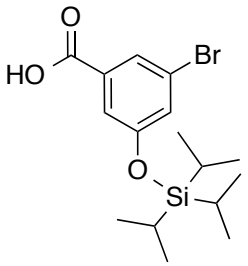
3-[(Benzylamino)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.46)



To a solution of benzylamine (100 µL, 98 mg, 916 µmol, 1.9 eq) and AcOH (100 µL, 104 mg, 1.75 mmol, 3.6 eq) in EtOH (5 mL) was added **2.37** (105 mg, 483 µmol, 1.0 eq). The reaction was stirred at rt for 30 min, then $NaBH_3CN$ (39 mg, 621 µmol, 1.3 eq) was added. The reaction solution was stirred for a further 19 h, then the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with EtOH in EtOAc (gradient elution 0 → 50%), followed by ISOLUTE® SCX-2 amine catch and release column yielded **2.46** (103 mg, 69%) as a clear and colourless oil. A small sample of the 1.00:0.14 amine:TFA salt was purified by semi-preparative HPLC for biological testing. The degree of protonation was

quantified using 1,4-difluorobenzene as an internal standard in ^{19}F NMR: R_f free amine (70% EtOAc/petroleum ether) 0.18; v_{max} (thin film)/cm^{-1}: 2982 (w), 2361 (m), 2344 (m), 1670 (m); mp TFA salt (from MeCN) > 250 °C; ^1H NMR (500 MHz, CD_3OD) δ 7.52-7.44 (5H, m, C_6H_5), 6.95-6.92 (1H, m, C(6)*H*), 6.91-6.88 (1H, m, C(4)*H*), 6.84-6.81 (1H, m, C(2)*H*), 4.26 (2H, s, CH_2Ph), 4.22 (2H, s, CH_2Ar), 2.41 (3H, s, C(5')CH_3), 2.25 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2 (*C*(5')), 159.8 (*C*(3')), 159.8 (*C*(1)), 134.5 (*C*(5)), 133.8 (*C*(3)), 132.4 (*C*(1'')), 131.1 (*C*(2'') & *C*(6'') or *C*(3'') & *C*(5'')), 130.8 (*C*(4'')), 130.3 (*C*(3'') & *C*(5'') or *C*(2'') & *C*(6'')), 122.4 (*C*(4)), 118.1 (*C*(2)), 117.4 (*C*(4')), 117.2 (*C*(6)), 52.2 (*C*H$_2$Ph), 51.8 (*C*H$_2$Ar), 11.5 (C(5')*C*H$_3$), 10.7 (C(3')*C*H$_3$); ^{19}F NMR (377 MHz, CD_3OD) δ –77.1; HRMS *m/z* (ES$^+$) Found: 309.1592. $C_{19}H_{21}O_2N_2^+$ requires M^+ 309.1598; LRMS *m/z* (ES$^+$) 309 ([M + H]$^+$, 100%); HPLC RT = 9.67 min, purity 97.7%.

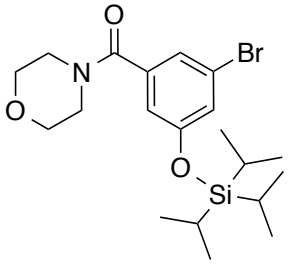
3-(3,5-Dimethylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)benzoic acid (2.53)



To a solution of **2.37** (475 mg, 2.19 mmol, 1.0 eq) and imidazole (420 mg, 6.17 mmol, 2.8 eq) in DMF (2 mL) at 0 °C was added TIPSCl (500 µL, 451 mg, 2.34 mmol, 1.1 eq) dropwise. The reaction was warmed to rt, and stirred for 22 h, then was diluted with H_2O (25 mL). The aqueous phase was extracted with EtOAc (3 × 25 mL) and the combined organic layers were washed with brine (75 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, to yield **2.53** (683 mg, 84%) as a volatile liquid that was used without purification: R_f (20% EtOAc/petroleum ether) 0.69; v_{max} (thin film)/cm^{-1}: 2946 (s), 2868 (s), 1702 (s) (C=O), 1590 (s);

^{1}H NMR (500 MHz, acetone-D_6) δ 10.06 (1H, s, C*H*O), 7.56-7.54 (1H, m, C(6)*H*), 7.45-7.42 (1H, m, C(2)*H*), 7.23-7.20 (1H, m, C(4)*H*), 2.44 (3H, s, C(5')CH_3), 2.26 (3H, s, C(3')CH_3), 1.42-1.31 (3H, m, 3 × C*H*$(CH_2)_2$), 1.16 (9H, s, 3 × CH(CH_3)$_A$$(CH_3)_B$), 1.14 (9H, s, 3 × CH$(CH_3)_A$(C$H_3$)$_B$); ^{13}C NMR (126 MHz, acetone-D_6) δ 192.6 (*C*HO), 166.5 (*C*(5')), 158.8 (*C*(5)), 157.9 (*C*(3')), 139.7 (*C*(1)), 134.0 (*C*(3)), 127.0 (*C*(2)), 124.6 (*C*(4)), 119.2 (*C*(6)), 116.1 (*C*(4')), 18.2 (3 × *C*H$(CH_3)_2$), 13.4 (3 × CH(*C*$H_3)_2$), 11.6 (C(5')*C*H_3), 10.8 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 396.1956. $C_{21}H_{31}NNaO_3Si^+$ requires M^+ 396.1965; LRMS *m/z* (ES^+) 374 ($[M + H]^+$, 100%); Anal. Calcd for $C_{21}H_{31}NO_3Si$: C, 67.5; H, 8.4; N, 3.8. Found: C, 67.7; H, 8.4; N, 3.9.

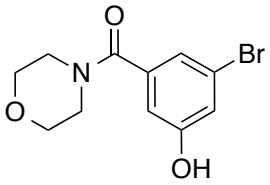
(3-Bromo-5-(triisopropylsilyl)oxy)phenyl)(morpholin-4-yl)methanone (2.54)



To a solution of 3-bromo-5-hydroxybenzoic acid (1.10 g, 5.07 mmol, 1.0 eq) and imidazole (810 mg, 11.9 mmol, 2.3 eq) in DMF (5 mL) at 0 °C was added TIPSCl (1.50 mL, 1.35 g, 7.01 mmol, 1.4 eq) dropwise. The reaction was warmed to rt, and stirred for 1 h, then diluted with H_2O (15 mL). The aqueous phase was extracted with EtOAc (3 × 15 mL) and brine (15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, to yield **2.53** (879 mg, 46%) as a clear liquid, which was used without purification. To a solution of **2.53** (154 mg, 268 μmol, 1.0 eq) and NEt_3 (150 μL, 109 mg, 1.08 mmol, 4.0 eq) in DMF (1.0 mL) and distilled THF (2.0 mL) was added PyBOP (221 mg, 425 mmol, 1.6 eq). The reaction was stirred at rt for 15 min before addition of morpholine (30.0 μL, 30 mg, 343 mmol, 1.3 eq). The reaction solution was stirred at rt for a further 15 h, then was concentrated *in vacuo*.

Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 20 → 80%) yielded **2.54** (88 mg, 66%) as a clear and colourless, volatile oil: R_f (20% EtOAc/petroleum ether) 0.38; ν_{max} (thin film)/cm^{-1}: 3066 (m), 2956 (w), 2862 (m), 1643 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.11-7.09 (1H, m, C(4)*H*), 7.08 (1H, dd, *J* = 2.0, 2.0 Hz, C(2)*H*), 6.81-6.78 (1H, m, C(6)*H*), 3.85-3.52 (6H, m, 2 × CH_2O & 2 × C$_A H_2$N), 3.46-3.31 (2H, m, 2 × C$_B H_2$N), 1.30-1.19 (3H, m, CH(CH_3)$_2$), 1.10 (9H, s, 3 × CH_3), 1.08 (9H, s, 3 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.7 (*C*=O), 157.1 (*C*(1)), 138.0 (*C*(5)), 124.6 (*C*(4)), 123.0 (*C*(3)), 122.7 (*C*(2)), 117.3 (*C*(6)), 66.9 (2 × *C*H_2O), 49.2 (*C*$_A$H$_2$N), 42.7 (*C*$_B$H$_2$N), 18.0 (3 × CH(*C*H$_3$)$_2$), 12.7 (3 × *C*H(CH$_3$)$_2$); HRMS *m/z* (ES$^+$) Found: 442.1400 & 444.1379. $C_{20}H_{33}O_3NBrSi^+$ requires [M(^{79}Br)]$^+$ & [M(^{81}Br)]$^+$ 442.1408 & 444.1387; LRMS *m/z* (ES$^+$) 442 & 444 ([M(^{79}Br) + H]$^+$ & [M(^{81}Br) + H]$^+$, 56%), 464 & 466 ([M(^{79}Br) + Na]$^+$ & [M(^{81}Br) + Na]$^+$, 100%); Unstable due to hydrolysis during HPLC.

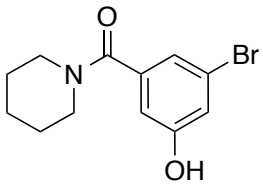
(3-Bromo-5-hydroxyphenyl)(morpholin-4-yl)methanone (2.55)



To a solution of 3-bromo-5-hydroxybenzoic acid (112 mg, 516 μmol, 1.0 eq) and NEt_3 (250 μL, 182 mg, 1.78 mmol, 3.5 eq) in DMF (1.0 mL) was added PyBOP (372 mg, 715 μmol, 1.4 eq). The reaction was stirred at rt for 1 h before addition of morpholine (60.0 μL, 60 mg, 686 mmol, 1.3 eq). The reaction solution was stirred at rt for a further 16 h, then an aqueous 1 M solution of HCl (5 mL) and EtOAc (5 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (15 mL) and brine (3 × 15 mL), dried over anhydrous

$MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 20 → 80%) yielded **2.55** (94 mg, 64%) as a colourless solid: R_f (100% EtOAc) 0.43; ν_{max} (thin film)/cm^{-1}: 2982 (s), 2889 (m), 2361 (m), 2344 (m), 1611 (m); mp (from EtOAc) 209-212 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (1H, dd, *J* = 2.0, 2.0 Hz, C(2)*H*), 7.02-7.00 (1H, m, C(4)*H* or C(6)*H*), 6.88-6.83 (1H, dd, *J* = 2.0, 1.4 Hz, C(6)*H* or C(4)*H*), 3.82-3.55 (6H, m, 2 × CH_2O, C_AH_2N), 3.50-3.37 (2H, m, C_BH_2N); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8 (*C*=O), 160.2 (*C*(1)), 139.3 (*C*(5)), 123.9 (*C*(3)), 121.5 (*C*(4) or *C*(6)), 121.0 (*C*(2)), 113.9 (*C*(6) or *C*(4)), 67.7 (2 × *C*H_2O), 49.2 (C_AH_2N), 43.8 (C_BH_2N); HRMS *m/z* (ES^+) Found: 289.0069 & 288.0084. $C_{11}H_{13}NO_3Br^+$ requires $[M(^{79}Br)]^+$ & $[M(^{81}Br)]^+$ 286.0073 & 288.0053; LRMS *m/z* (ES^+) 286 & 288 ($[^{79}M + H]^+$ & $[^{81}M + H]^+$, 31%), 308 & 310 ($[^{79}M + Na]^+$ & $[^{81}M + Na]^+$, 100%); HPLC RT = 9.63 min, purity 98.5%.

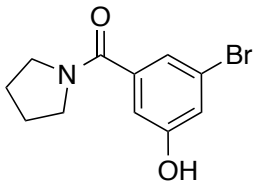
(3-Bromo-5-hydroxyphenyl)(piperidin-1-yl)methanone (2.56)



To a solution of 3-bromo-5-hydroxybenzoic acid (202 mg, 931 μmol, 1.0 eq) and NEt_3 (400 μL, 290 mg, 2.85 mmol, 3.1 eq) in DMF (2 mL) was added HATU (445 mg, 1.17 mmol, 1.3 eq). The reaction was stirred at rt for 40 min before addition of piperidine (100 μL, 86 mg, 1.01 mmol, 1.1 eq). The reaction solution was stirred at rt for a further 23 h, then an aqueous 1 M solution of HCl (5 mL) and EtOAc (5 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (15 mL) and brine (3 × 15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography,

eluting with EtOAc in petroleum ether (gradient elution 50 → 100%) yielded **2.56** (183 mg, 69%) as an off-white solid: R_f (100% EtOAc) 0.66; ν_{max} (thin film)/cm^{-1}: 3005 (br), 2970 (w), 2942 (br), 1739 (s) (C=O); mp (from EtOAc) 165-167 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.03 (1H, dd, *J* = 1.9, 1.9 Hz, C(2)*H*), 6.96-6.94 (1H, m, C(4)*H*), 6.72 (1H, dd, *J* = 1.9, 1.4 Hz, C(6)*H*), 3.72-3.62 (2H, m, C_AH_2N), 3.41-3.28 (2H, m, C_BH_2N), 1.76-1.49 (6H, m, $CH_2CH_2CH_2$); ^{13}C NMR (101 MHz, CD_3OD) δ 170.5 (*C*=O), 160.1 (*C*(1)), 140.2 (*C*(5)), 123.8 (*C*(3)), 121.1 (*C*(2)), 120.7 (*C*(4)), 113.5 (*C*(6)), 44.2 (C_AH_2N), 27.5 (C_BH_2N), 26.6 ($N(CH_2CH_2CH_2CH_2CH_2)$), 25.4 ($N(CH_2CH_2CH_2CH_2CH_2)$); HRMS *m/z* (ES^-) Found: 282.0138 & 284.0117. $C_{12}H_{13}O_2NBr^-$ requires $[M(^{79}Br)]^-$ & $[M(^{81}Br)]^-$ 282.0135 & 284.0115; LRMS *m/z* (ES^+) 284 & 286 ($[M(^{79}Br) + H]^+$ & $[M(^{81}Br) + H]^+$, 61%), 306 & 308 ($[M(^{79}Br) + Na]^+$ & $[M(^{81}Br) + Na]^+$, 100%); HPLC RT = 11.43 min, purity 99.7%.

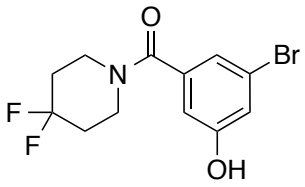
(3-Bromo-5-hydroxyphenyl)(pyrrolidin-1-yl)methanone (2.57)



To a solution of 3-bromo-5-hydroxybenzoic acid (207 mg, 954 μmol, 1.0 eq) and NEt_3 (300 μL, 218 mg, 2.14 mmol, 2.2 eq) in DMF (2 mL) was added HBTU (435 mg, 1.15 mmol, 1.2 eq). The reaction was stirred at rt for 2 h before addition of pyrrolidine (100 μL, 85 mg, 1.20 mmol, 1.3 eq). The reaction solution was stirred at rt for a further 20 h, then an aqueous 1 M solution of HCl (15 mL) and EtOAc (15 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (45 mL) and brine (45 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography,

eluting with EtOAc in petroleum ether (gradient elution 50 → 100%), followed by precipitation from EtOAc yielded **2.57** (180 mg, 70%) as a colourless solid: R_f (100% EtOAc) 0.48; ν_{max} (thin film)/cm^{-1}: 3016 (br), 2971 (w), 2947 (br), 1743 (s) (C=O), 1736 (s) (C=O), 1584 (br); mp (from EtOAc) 168-170 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.09-7.06 (1H, m, C(4)*H*), 7.05-7.02 (1H, m, C(2)*H*), 6.85-6.83 (1H, m, C(6)*H*), 3.56 (2H, t, *J* = 6.8 Hz, C_AH_2N), 3.43 (2H, t, *J* = 6.5 Hz, C_BH_2N), 2.02-1.86 (4H, m, CH_2CH_2); ^{13}C NMR (101 MHz, CD_3OD) δ 170.1 (*C*=O), 160.0 (*C*(1)), 140.8 (*C*(5)), 123.6 (*C*(3)), 121.6 (*C*(4)), 121.0 (*C*(2)), 113.9 (*C*(6)), 50.8 (C_AH_2N), 47.4 (C_BH_2N), 27.2, 25.3 ($N(CH_2CH_2CH_2CH_2)$); HRMS *m/z* (ES$^-$) Found: 267.9981 & 269.9960. $C_{11}H_{11}O_2NBr^-$ requires [M(^{79}Br)]$^-$ & [M(^{81}Br)]$^-$ 267.9979 & 269.9958; LRMS *m/z* (ES$^+$) 270 & 272 ([M(^{79}Br) + H]$^+$ & [M(^{81}Br) + H]$^+$, 48%), 292 & 294 ([M(^{79}Br) + Na]$^+$ & [M(^{81}Br) + Na]$^+$, 100%); HPLC RT = 10.43 min, purity 99.5%.

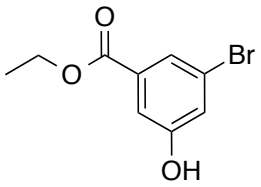
(3-Bromo-5-hydroxyphenyl)(4,4-difluoropiperidin-1-yl)methanone (2.58)



To a solution of 3-bromo-5-hydroxybenzoic acid (203 mg, 935 μmol, 1.0 eq) and NEt_3 (450 μL, 327 mg, 3.21 mmol, 3.4 eq) in DMF (4 mL) was added PyBOP (622 mg, 1.20 mmol, 1.3 eq). The reaction was stirred at rt for 35 min before addition of 4,4-difluoropiperidine (216 mg, 1.37 mmol, 1.5 eq). The reaction solution was stirred at rt for a further 167 h, then an aqueous 1 M solution of HCl (5 mL) and EtOAc (5 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (15 mL) and brine (3 × 15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel

chromatography, eluting with EtOAc in petroleum ether (gradient elution 20 → 100%) yielded **2.58** (202 mg, 67%) as an off-white solid: R_f (100% EtOAc) 0.77; ν_{max} (thin film)/cm^{-1}: 3016 (br), 2970 (w), 1738 (s) (C=O); mp (from EtOAc) 201-203 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.07-7.04 (1H, m, C(2)*H* or C(4)*H*), 7.01 (1H, dd, *J* = 1.4, 1.4 Hz, C(4)*H* or C(2)*H*), 6.78 (1H, dd, *J* = 2.1, 1.4 Hz, C(6)*H*), 4.00-3.72 (2H, m, C_AH_2N), 3.64-3.46 (2H, m, C_BH_2N), 2.19-1.90 (4H, m, 2 × CH_2CF_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.9 (*C*=O), 168.1 (*C*(1)), 148.2 (*C*(5)), 132.3 (t, *J* = 241 Hz, CF_2), 131.4 (*C*(3)), 129.1 (*C*(2) or *C*(4)), 128.7 (*C*(4) or *C*(2)), 122.4 (*C*(6)), 53.3 (NC_AH_2), 47.9 (NC_BH_2), 43.4-41.8 (m, 2 × CH_2CF_2); ^{19}F NMR (377 MHz, CD_3OD) δ –99.5; HRMS *m/z* (ES^+) Found: 320.0087 & 322.0066. $C_{12}H_{13}O_2NBrF_2^+$ requires [M(^{79}Br)]$^+$ & [M(^{81}Br)]$^+$ 320.0092 & 322.0072; LRMS *m/z* (ES^+) 320 & 322 ([^{79}M + H]$^+$ & [^{81}M + H]$^+$, 87%), 342 & 344 ([^{79}M + Na]$^+$ & [^{81}M + Na]$^+$, 100%); HPLC RT = 11.57 min, purity 96.5%.

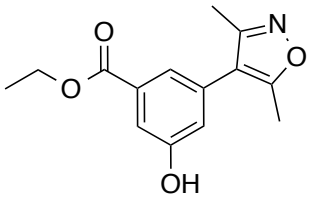
Ethyl 3-bromo-5-hydroxybenzoate (2.60)[207] synthesised by P. Guzanov



To a solution of 3-bromo-5-hydroxybenzoic acid (500 mg, 2.30 mmol, 1.0 eq) in ethanol (5 mL) was added an aqueous 18.4 M H_2SO_4 solution (615 µL, 11.3 mmol, 4.9 eq). The reaction vessel was sealed, and the reaction mixture was heated at 88 °C for 24 h. The volatile components were removed *in vacuo* and the resulting residue was redissolved in H_2O (10 ml). The pH was adjusted to 9 with a 2 M aqueous solution of NaOH, then the product was extracted with CH_2Cl_2 (5 × 10 mL). The combined organic layers were dried over anhydrous MgSO4, filtered and

concentrated *in vacuo*, yielding **2.60** (800 mg, 88%) as a yellow solid that was deemed pure enough to proceed without purification: R_f (1% AcOH/EtOAc) 0.83; ^{1}H NMR (400 MHz, MeOD) δ 7.56 (1H, dd, *J* = 1.6, 1.6 Hz, C(2)*H*), 7.36 (1H, dd, *J* = 2.3, 1.6 Hz, C(6)*H*), 7.16 (1H, dd, *J* = 2.3, 1.6 Hz, C(4)*H*), 4.34 (2H, q, *J* = 6.7 Hz, CH_2CH$_3$), 1.37 (3H, t, *J* = 6.7 Hz, CH$_2$CH_3); LRMS m/z (ES$^-$) 243 & 245 ([M(^{79}Br) – H]$^-$ & [M(^{81}Br) – H]$^-$, 100%). Data are in agreement with literature values.[207]

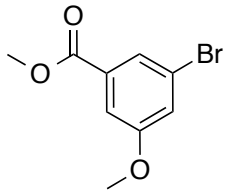
Ethyl 3-(3,5-dimethylisoxazol-4-yl)-5-hydroxybenzoate (2.61) synthesised by P. Guzanov



To a dry 10-20 mL microwave vial were added **2.60** (500 mg, 2.04 mmol, 1.0 eq), **2.35** (435 mg, 2.14 mmol, 1.05 eq), Pd(OAc)$_2$ (5 mg, 20.0 µmol, 0.01 eq), RuPhos (29 mg, 61.1 µmol, 0.03 eq) and anhydrous Na$_2$CO$_3$ (649 mg, 6.12 mmol, 3.0 eq). The vial was sealed and purged with nitrogen, before addition of ethanol (10 mL). The reaction solution was degassed, by bubbling with nitrogen for 40 min, then heated at 90 °C with microwave irradiation for 90 min. The mixture was allowed to cool to rt and filtered through a thin pad of silica gel, eluting with CH$_2$Cl$_2$, and the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with Et$_2$O in petroleum ether (gradient elution 30 → 80%) yielded **2.61** (224 mg, 68%) as a yellow solid: R_f (1% AcOH/EtOAc) 0.53; ν_{max} (thin film)/cm^{-1}: 3214 (br), 2983 (w), 2935 (w), 1718 (s), 1633 (m), 1421 (m), 1329 (s), 1265 (s), 1235 (s), 1108 (w), 1023 (w); ^{1}H NMR (400 MHz, MeOD) δ 7.43 (1H, dd,

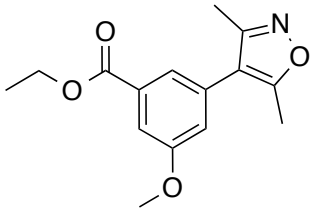
J = 2.4, 1.5 Hz, C(2)*H*), 7.41 (1H, dd, *J* = 1.5, 1.5 Hz, C(6)*H*), 6.97 (1 H, dd, *J* = 2.4, 1.5 Hz, C(4)*H*), 4.36 (2H, q, *J* = 7.1 Hz, CH_2CH$_3$), 2.42 (3H, s, C(5')CH$_3$), 2.26 (3H, s, C(3')CH_3), 1.39 (3H, t, *J* = 7.1 Hz, CH$_2$CH$_3$); ^{13}C NMR (101 MHz, CDCl$_3$) δ 167.6 (*C*=O), 167.1 (*C*(5')), 159.7 (*C*(5)), 159.3 (*C*(3')), 133.5 (*C*(3)), 132.9 (*C*(1)), 122.0 (*C*(2)), 121.5 (*C*(4)), 117.1 (*C*(6)), 116.2 (*C*(4')), 62.2 (*C*H$_2$CH$_3$), 14.5 (CH$_2$*C*H$_3$), 11.3 (C(5')*C*H$_3$), 10.6 (C(3')*C*H$_3$); HRMS *m/z* (ES$^-$) Found: 260.0927. $C_{14}H_{14}NO_4^-$ requires M$^-$ 260.0928; LRMS *m/z* (ES$^-$) 260 ([M – H]$^-$, 100%).

Methyl 3-bromo-5-methoxybenzoate (2.62)[208] synthesised by P. Guzanov



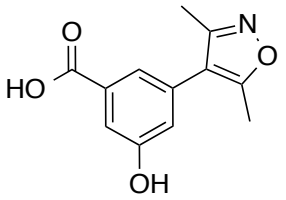
To a solution of 3-bromo-5-hydroxybenzoic acid (100 mg, 460 µmol, 1.0 eq) in DMF (500 µL) was added K_2CO_3 (138 mg, 1.00 mmol, 2.2 eq) and MeI (86 µL, 196 mg, 1.38 mmol, 3 eq). The reaction vessel was sealed, and the reaction mixture was heated at 100 °C for 20 min. The reaction mixture was then diluted with H_2O (10 mL) and extracted with EtOAc (3 × 8 ml). The combined organic layers were washed with H_2O (5 × 10 ml) and brine (4 **x** 10 ml), dried over anhydrous MgSO4, filtered and concentrated *in vacuo*, yielding **2.62** (86 mg, 76%) as a yellow oil that was deemed pure enough to proceed without purification: R_f (10% EtOAc/petroleum ether) 0.54; ^{1}H NMR (400 MHz, MeOD) δ 7.71 (1H, dd, *J* = 1.8, 1.8 Hz, C(2)*H*), 7.51 (1H, dd, *J* = 2.3, 1.8 Hz, C(4)*H*), 7.35 (1H, dd, *J* = 2.3, 1.8 Hz, C(6)*H*), 3.91 (3H, s, C(5)OCH_3), 3.85 (3H, s, $CO_2C H_3$); LRMS m/z (ES$^-$) 229 & 231 ([M(^{79}Br) – CH$_3$]$^-$ & [M(^{81}Br) – CH$_3$]$^-$, 100%). Data are in good agreement with literature values.[208]

Ethyl 3-(3,5-dimethylisoxazol-4-yl)-5-methoxybenzoate (2.63)[209] synthesised by P. Guzanov



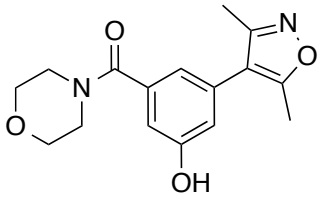
To a dry 2-5 mL microwave vial were added **2.62** (134 mg, 547 μmol, 1.0 eq), **2.35** (116 mg, 571 μmol, 1.04 eq), $Pd(OAc)_2$ (5 mg, 36.6 μmol, 0.07 eq), RuPhos (11 mg, 23.6 μmol, 0.04 eq) and anhydrous Na_2CO_3 (174 mg, 1.64 mmol, 3.0 eq). The vial was sealed and purged with nitrogen, before addition of ethanol (3 mL). The reaction solution was degassed, by bubbling with nitrogen for 40 min, then heated at 90 °C with microwave irradiation for 50 h. The mixture was allowed to cool to rt and filtered through a thin pad of silica gel, eluting with CH_2Cl_2, and the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with Et_2O in petroleum ether (gradient elution 50 → 100%) yielded **2.63** (17 mg, 11%) as a yellow solid: R_f (50% Et_2O/petroleum ether) 0.17; ^{1}H NMR (400 MHz, MeOD) δ 7.48 (1H, dd, *J* = 2.5, 1.4 Hz, C(2)*H*), 7.40 (1H, dd, *J* = 1.4, 1.4 Hz, C(6)*H*), 6.99 (1 H, dd, *J* = 2.5, 1.4 Hz, C(4)*H*), 4.36 (2H, q, *J* = 7.1 Hz, CH_2CH$_3$), 3.85 (3H, s, OCH_3), 1.78 (3H, s, C(5')CH$_3$), 1.72 (3H, s, C(3')CH_3), 1.39 (3H, t, *J* = 7.1 Hz, CH$_2$CH$_3$). Data are in good agreement with literature values.[209]

3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxybenzoic acid (2.64) synthesised by P. Guzanov



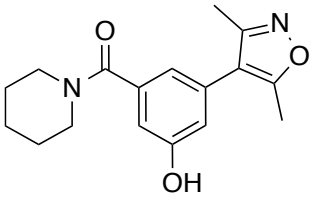
To a solution of **2.61** (295 mg, 1.13 mmol, 1.0 eq) in THF (5 mL) and water (2.5 mL) was added LiOH (81 mg, 3.38 mmol, 3.0 eq). The reaction mixture was stirred for 25 h, then Et_2O (10 mL) and an aqueous 2 M LiOH solution (10 mL) was added. The phases were separated and the aqueous phase was washed with Et_2O (2 × 10 mL), then acidified to pH 3 with an aqeous 1 M HCl solution. The aqueous suspension was then extracted with EtOAc (5 × 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, yielding **2.64** (260 mg, 98%) as a white solid: R_f (1% AcOH/EtOAc) 0.53; ν_{max} (thin film)/cm^{-1}: 3169 (br), 2661 (w), 1695 (s), 1595 (s), 1489 (s), 1325 (s), 1231 (s), 1205 (s), 1078 (w); ^{1}H NMR (400 MHz, MeOD) δ 7.44 (1H, dd, *J* = 2.3, 1.5 Hz, C(2)*H*), 7.43 (1H, dd, *J* = 1.5, 1.5 Hz, C(6)*H*), 6.96 (1 H, dd, *J* = 2.3, 1.5 Hz, C(4)*H*), 2.42 (3H, s, C(5')CH_3), 2.26 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.3 (*C*=O), 167.1 (*C*(5')), 159.8 (*C*(5)), 159.2 (*C*(3')), 133.8 (*C*(3)), 123.8 (*C*(1)), 122.3 (*C*(2)), 121.4 (*C*(4)), 117.2 (*C*(6)), 116.6 (*C*(4')), 11.4 (C(5')*C*H_3), 10.6 (C(3')*C*H_3); HRMS *m/z* (ES^-) Found: 232.0615. $C_{12}H_{12}NO_4^-$ requires M^- 232.0615; LRMS *m/z* (ES^-) 232 ($[M - H]^-$, 100%).

[3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxyphenyl](morpholin-4-yl)methanone (2.65) synthesised by P. Guzanov



To a solution of **2.64** (50 mg, 214 μmol, 1.0 eq) in THF was added EDC•HCl (62 mg, 323 μmol, 1.5 eq.) and HOBt hydrate (15 mg, 111 μmol, 0.5 eq). The reaction was stirred at rt for 20 min, then morpholine (56 μL, 56 mg, 640 μmol, 3.0 eq). The reaction mixture was then heated at 55 °C for 3 days, then diluted with EtOAc (5 mL), and washed with H_2O (4 × 5 mL) and brine (3 × 5 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with MeOH in CH_2Cl_2 (gradient elution 0 → 10%) yielded **2.65** (34 mg, 52%) as a white solid: R_f (10% MeOH/CH_2Cl_2) 0.28; ν_{max} (thin film)/cm^{-1}: 3175 (br) 2999 (med), 2937 (w), 2857 (w), 1585 (s), 1473 (m), 1416 (m), 1324 (med), 1253 (m), 1231 (w), 1206 (w), 1116 (w), 1026 (w); ^{1}H NMR (400 MHz, MeOD) δ 6.85-6.81 (3H, m, C(2)*H,* C(4)*H* and C(6)*H*), 3.84-3.58 (6H, m, 2 × OCH_2 and C_AH_2N), 3.56-3.44 (2H, m, C_AH_2N), 2.40 (3H, s, C(5')CH_3), 2.25 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8 (*C*=O), 167.2 (*C*(5')), 159.8 (*C*(5)), 159.4 (*C*(3')), 133.4 (*C*(3)), 133.4 (*C*(1)), 119.3 (*C*(2)), 118.5 (*C*(4)), 117.1 (*C*(6)), 114.1 (*C*(4')), 67.7 (2 × *C*H_2O), 49.5 (C_AH_2N), 43.7 (C_BH_2N), 11.4 (C(5')*C*H_3), 10.6 (C(3')*C*H_3); HRMS *m/z* (ES^-) Found: 301.1193. $C_{16}H_{17}N_2O_4^-$ requires M^- 301.1194; LRMS *m/z* (ES^-) 301 ($[M - H]^-$, 100%).

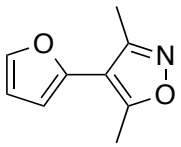
[3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxyphenyl](piperidin-1-yl)methanone (2.66) synthesised by P. Guzanov



To a solution of **2.64** (50 mg, 214 μmol, 1.0 eq) in THF was added EDC•HCl (62 mg, 323 μmol, 1.5 eq.) and HOBt hydrate (15 mg, 111 μmol, 0.5 eq). The reaction was stirred at rt for 20 min, then piperidine (64 μL, 55 mg, 646 μmol, 3.0 eq). The reaction mixture was then heated at 55 °C for 3 days, then diluted with EtOAc (5 mL), and washed with H_2O (4 × 5 mL) and brine (3 × 5 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with MeOH in CH_2Cl_2 (gradient elution 0 → 10%) yielded **2.66** (36 mg, 56%) as a white solid: R_f (1% AcOH/EtOAc) 0.48; ν_{max} (thin film)/cm^{-1}: 3188 (br), 2969 (w), 2925 (w), 2857 (w), 1587 (s), 1432 (s), 1363 (w), 1324 (s), 1303 (med), 1244 (s), 1113 (s), 1071 (w), 1030 (m); ^{1}H NMR (400 MHz, MeOD) δ 6.82 (1H, dd, *J* = 2.1, 1.5 Hz, C(2)*H*), 6.79 (1H, dd, *J* = 2.1, 1.5 Hz, C(6)*H*), 6.77 (1 H, dd, *J* = 1.5, 1.5 Hz, C(4)*H*), 3.77-3.63 (2H, m, C_AH_2N), 3.50-3.37 (2H, m, C_BH_2N), 2.42 (3H, s, C(5')CH_3), 2.26 (3H, s, C(3')CH_3), 1.77-1.62 (4H, m, $CH_2CH_2CH_2CH_2CH_2$), 1.62-1.51 (2H, m, $CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7 (*C*=O), 167.2 (*C*(5')), 159.8 (*C*(5)), 159.4 (*C*(3')), 139.3 (*C*(3)), 133.3 (*C*(1)), 119.1 (*C*(2)), 118.3 (*C*(4)), 117.3 (*C*(6)), 113.8 (*C*(4')), 50.0 (C_AH_2N), 44.3 (C_BH_2N), 27.6, 26.7, 25.4 ($CH_2CH_2CH_2CH_2CH_2$), 11.4 (C(5')CH_3), 10.6 (C(3')CH_3); HRMS *m/z* (ES^-) Found: 299.14015. $C_{17}H_{19}N_2O_3^-$ requires M^- 299.1401; LRMS *m/z* (ES^+) 299 ($[M - H]^-$, 100%).

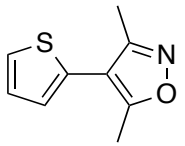
6.4 Experimental Procedures for Chapter 3

4-(Furan-2-yl)-3,5-dimethyl-1,2-oxazole (3.1)[156]



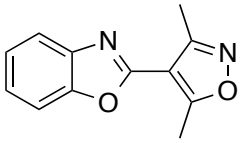
To a solution of furfural (430 µL, 499 mg, 5.20 mmol, 1.0 eq), nitroethane (765 µL, 803 mg, 10.7 mmol, 2.1 eq) and ethanol (4 mL) in a 2-5 mL microwave vial, a 6.4 M aqueous solution of NaOH (1.10 mL, 282 mg, 7.04 mmol, 1.4 eq) was added dropwise. The vial was sealed and the mixture stirred at 120 °C for 20 min with microwave irradiation. The mixture was allowed to cool to rt and the volatile components were removed *in vacuo* giving a residue, which was partitioned between H_2O (5 mL) and petroleum ether (5 mL). The layers were separated and the aqueous layer was extracted with petroleum ether (2 × 5 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with Et_2O in petroleum ether (2%), yielded **3.1** (151 mg, 18%) as a beige, volatile solid: R_f (15% Et_2O/petroleum ether) 0.41; ν_{max} (thin film)/cm^{-1}: 2926 (w), 2854 (w), 1656 (m), 1429 (m), 1250 (m); mp (from Et_2O) 38-40 °C [lit. mp 42-44 °C[156]]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (1H, d, *J* = 1.7 Hz, C(5)*H*), 6.49 (1H, dd, *J* = 3.1, 1.7 Hz, C(4)*H*), 6.36 (1H, d, *J* = 3.1 Hz, C(3)*H*), 2.58 (3H, s, C(5')CH_3), 2.41 (3H, s, C(3')CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.5 (*C*(5')), 157.8 (*C*(3')), 145.7 (*C*(2)), 141.9 (*C*(5)), 111.2 (*C*(2)), 108.4 (*C*(4')), 106.7 (*C*(3)), 12.5 (C(5')*C*H_3), 11.7 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 186.0534. $C_9H_9NNaO_2^+$ requires M^+ 186.0525; LRMS *m/z* (ES^+) 164 ($[M + H]^+$, 43%), 186 ($[M + Na]^+$, 42%), 218 ($[M + Na + MeOH]^+$, 23%), 381 ($[2M + Na + MeOH]^+$, 70%), 422 ($[2M + Na + MeOH + MeCN]^+$, 100%); HPLC RT = 12.18 min, purity 100.0%. Data are in good agreement with literature values.[156]

3,5-Dimethyl-4-(thiophen-2-yl)-1,2-oxazole (3.2)[210]



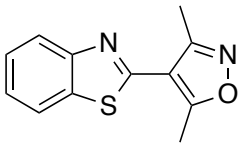
To a solution of 2-thiophenecarboxaldehyde (830 µL, 996 mg, 8.88 mmol, 1.0 eq) in ethanol (9 mL), were added nitroethane (1.40 mL, 1.47 g, 19.6 mmol, 2.2 eq) and caesium carbonate (5.80 g, 17.8 mmol, 2.0 eq), and the mixture was heated under reflux for 18 h. The mixture was allowed to cool to rt, and the volatile components were removed *in vacuo* producing a residue, which was partitioned between H_2O (5 mL), and petroleum ether (5 mL). The layers were separated and the aqueous layer was extracted with petroleum ether (2 × 5 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by vacuum distillation (124 °C, 4 mbar) and silica gel chromatography, eluting with Et_2O and petroleum ether (5%) yielded **3.2** (94 mg, 6%) as a beige solid: R_f (15% Et_2O/petroleum ether) 0.28; ν_{max} (thin film)/cm^{-1}: 3107 (w) (O–H), 2928 (w), 2858 (w), 1629 (m), 1411 (m), 1239 (s); mp (from Et_2O) 49-51 ° [lit. mp 49-51 °C[210]]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.35 (1H, m, C(3)*H*), 7.16-7.11 (1H, m, C(4)*H*), 7.05-7.00 (1H, m, C(5)*H*), 2.50 (3H, s, C(5')CH_3), 2.36 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.7 (*C*(5')), 158.6 (*C*(3')), 131.3 (*C*(2)), 127.5 (*C*(4)), 126.2 (*C*(5)), 125.4 (*C*(3)), 110.6 (*C*(4')), 12.0 (C(5')*C*H_3), 11.1 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 202.0296. $C_9H_9NNaOS^+$ requires M^+ 202.0297; LRMS *m/z* (ES^+) 180 ($[M + H]^+$, 64%), 259 ($[M + K + MeCN]^+$, 74%), 381 ($[2M + Na]^+$, 100%); Anal. Calcd for C_9H_9NOS: C, 60.3; H, 5.1; N, 7.8. Found: C, 60.3; H, 5.2; N, 7.8. Data are in good agreement with literature values.[210]

2-(3,5-Dimethyl-1,2-oxazol-4-yl)-1,3-benzoxazole (3.3)



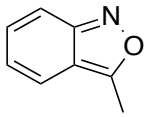
To a dry 2-5 mL microwave vial were added 2-chlorobenzoxazole (75.0 µL, 101 mg, 660 µmol, 1.0 eq), **2.35** (144 mg, 709 µmol, 1.1 eq), $Pd(OAc)_2$ (2 mg, 8.90 µmol, 0.015 eq), RuPhos (16 mg, 20.0 µmol, 0.030 eq) and anhydrous Na_2CO_3 (138 mg, 1.30 mmol, 2.0 eq). The vial was sealed and purged with argon, before addition of ethanol (4 mL). The reaction solution was degassed, by bubbling with argon for 5 min, and heated at 85 °C for 24 h. The mixture was allowed to cool to rt and filtered through a thin pad of silica gel, eluting with CH_2Cl_2, and the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with Et_2O in petroleum ether (gradient elution 0 → 5%) yielded **3.3** (26 mg, 17%) as a colourless solid: R_f (15% Et_2O/petroleum ether) 0.30; ν_{max} (thin film)/cm^{-1}: 2931 (C–H) (w), 1662 (s), 1568 (m), 1456 (s), 1250 (s); mp (from Et_2O) 113-114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80-7.73 (1H, m, C(4)*H* or C(7)*H*), 7.61-7.52 (1H, m, C(7)*H* or C(4)*H*), 7.42-7.33 (2H, m, C(5)*H* & C(6)*H*), 2.87 (3H, s, C(5')CH_3), 2.68 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.9 (*C*(5')), 159.2 (*C*(3')), 157.0 (*C*(2)), 150.0 (*C*(3a) or *C*(7a)), 141.6 (*C*(7a) or *C*(3a)), 125.1 (*C*(4) or *C*(7)), 124.8 (*C*(7) or *C*(4)), 120.9 (*C*(5) or *C*(6)), 110.5 (*C*(6) or *C*(5)), 106.0 (*C*(4')), 13.4 (C(5')*C*H_3), 12.1 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 237.0636. $C_{12}H_{10}N_2NaO_2^+$ requires M^+ 237.0634; LRMS *m/z* (ES^+) 215 ($[M + H]^+$, 100%); Anal. Calcd for $C_{12}H_{10}N_2O_2$: C, 67.3; H, 4.7; N, 13.1. Found: C, 67.2; H, 4.8; N, 13.0.

2-(3,5-Dimethyl-1,2-oxazol-4-yl)-1,3-benzothiazole (3.4)



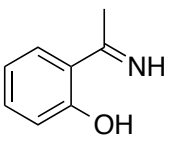
To a dry 2-5 mL microwave vial were added 2-bromo-1,3-benzothiazole (100 mg, 467 μmol, 1.0 eq), **2.35** (105 mg, 517 μmol, 1.1 eq), $Pd(OAc)_2$ (2 mg, 8.90 μmol, 0.019 eq), RuPhos (12 mg, 14.7 μmol, 0.031 eq) and anhydrous Na_2CO_3 (100 mg, 943 μmol, 2.0 eq). The vial was sealed and purged with argon, before addition of ethanol (4 mL). The reaction solution was degassed, by bubbling with argon for 5 min, and heated at 85 °C for 24 h. The mixture was allowed to cool to rt and filtered through a thin pad of silica gel, eluting with CH_2Cl_2, and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with Et_2O in petroleum ether (gradient elution 0 → 1%) afforded **3.4** (35 mg, 29%) as a colourless solid: R_f (15% Et_2O/petroleum ether) 0.27; ν_{max} (thin film)/cm^{-1}: 3060 (w), 2927 (w), 2851 (w), 1612 (m), 1436 (s), 1415 (m), 1218 (m); mp (from Et_2O) 69-71 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (1H, d, *J* = 8.2 Hz, C(4)*H* or C(7)*H*), 7.93 (1H, d, *J* = 8.0 Hz, C(7)*H* or C(4)*H*), 7.56-7.49 (1H, m, C(5)*H* or C(6)*H*), 7.45-7.39 (1H, m, C(6)*H* or C(5)*H*), 2.81 (3H, s, C(5')CH_3), 2.64 (3H, s, C(3')CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.8 (*C*(5')), 158.8 (*C*(3')), 157.8 (*C*(2)), 153.4 (*C*(3a) or *C*(7a)), 134.3 (*C*(7a) or *C*(3a)), 126.6 (*C*(5) or *C*(6)), 125.3 (*C*(6) or *C*(5)), 123.2 (*C*(4) or *C*(7)), 121.5 (*C*(7) or *C*(4)), 111.8 (*C*(4')), 13.3 (C(5')*C*H_3), 12.1 (C(3')*C*H_3); HRMS *m/z* (ES^+) Found: 253.0410. $C_{12}H_{10}N_2NaO_5^+$ requires M^+ 253.0406; LRMS *m/z* (ES^+) 231 ($[M + H]^+$, 100%), 253 ($[M + Na]^+$, 15%); Anal. Calcd for $C_{12}H_{10}N_2OS$: C, 62.6; H, 4.4; N, 12.2. Found: C, 62.7; H, 4.5; N, 12.1.

3-Methyl-2,1-benzoxazole (3.9)[200,211,212]



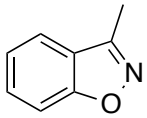
To a dry 10-20 mL microwave vial were added 2'-nitroacetophenone (246 mg, 200 µL, 1.49 mmol, 1.0 eq), 2-bromo-2-nitropropane (300 mg, 190 µL, 1.79 mmol, 1.2 eq) and zinc powder (490 mg, 7.49 mmol, 5.0 eq). The vial was sealed and purged with argon, before addition of freeze-thaw degassed MeOH (5 mL). The reaction solution was heated at 50 °C for 22 h. The mixture was allowed to cool to rt and filtered through Celite®, eluting with CH_2Cl_2. The volatile components were removed *in vacuo*, giving a colourless residue, which was partitioned between CH_2Cl_2 (40 mL) and a 10% aqueous solution of NH_4Cl (40 mL). The layers were separated and the organic layer was washed with a 10% aqueous NH_4Cl solution (2 × 40 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 1 → 10%) afforded **3.9** (50 mg, 25%) as a clear and colourless liquid: R_f (10% EtOAc/petroleum ether) 0.55; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58-7.49 (1H, m, C(4)*H* or C(7)*H*), 7.49-7.40 (1H, m, C(5)*H* or C(6)*H*), 7.35-7.20 (1H, m, C(6)*H* or C(5)*H*), 7.00-6.90 (1H, m, C(7)*H* or C(4)*H*), 2.80 (3H, s, CH_3); LRMS *m/z* (ES^+) 134 ($[M + H]^+$, 100%), 156 ($[M + Na]^+$, 56%); HPLC RT = 11.12 min, purity 99.9%. Data are in good agreement with literature values.[211,212]

2-Ethanimidoylphenol (3.16)[213,214]



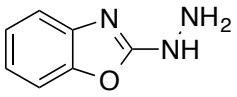
To a sealed 2-5 mL microwave vial were added 2'-hydroxyacetophenone (1.13 g, 1.00 mL, 8.31 mmol, 1.0 eq) and a 7 M methanolic ammonia solution (890 µL, 693 mg, 40.7 mmol, 4.9 eq). The reaction solution was stirred at rt for 41 h, and concentrated *in vacuo*, yielding **3.16** (673 mg, 60%) as a yellow solid that was deemed pure enough to proceed without purification: R_f (50% EtOAc/petroleum ether) 0.22; mp (from EtOAc) 140-141 °C [lit. mp 139 °C[214]]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (1H, br s, N*H*), 7.55-7.49 (1H, m, C(3)*H* or C(6)*H*), 7.39-7.31 (1H, m, C(4)*H* or C(5)*H*), 7.03-6.95 (1H, m, C(6)*H* or C(3)*H*), 6.87-6.79 (1H, m, C(5)*H* or C(4)*H*), 2.56-2.45 (3H, m, CH_3); LRMS *m/z* (ES^+) 136 ($[M + H]^+$, 100%). Data are in good agreement with literature values.[213,214]

3-Methyl-1,2-benzoxazole (3.10)[215,216]



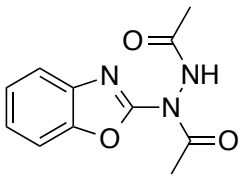
A solution of **3.16** (620 mg, 4.59 mmol, 1.0 eq), NCS (920 mg, 6.89 mmol, 1.5 eq), K_2CO_3 (1.27 g, 9.19 mmol, 2.0 eq) in distilled THF (15 mL) was stirred at rt for 19 h, before addition of Et_2O (15 mL) and H_2O (30 mL). The layers were separated, and the organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give a brown liquid. Purification by vacuum distillation (160 °C, 10 mbar) yielded **3.10** (168 mg, 27%) as a clear and colourless liquid: R_f (15% Et_2O/petroleum ether) 0.27; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67-7.62 (1H, m, C(4)*H* or C(7)*H*), 7.58-7.52 (2H, m, C(7)*H* or C(4)*H* & C(5)*H* or C(6)*H*), 7.35-7.28 (1H, m, C(6)*H* or C(5)*H*), 2.60 (3H, s, CH_3); LRMS *m/z* (ES^+) 134 ($[M + H]^+$, 100%); HPLC RT = 11.72 min, purity 100.0%. Data are in good agreement with literature values.[215,216]

2-Hydrazinyl-1,3-benzoxazole (3.17)[217,218]



To hydrazine monohydrate (2.40 mL, 2.47 g, 49.4 mmol, 5.6 eq) at 0 °C was added dropwise a solution of 2-chlorobenzoxazole (1.00 mL, 1.35 g, 8.82 mmol, 1.0 eq) in dioxane (1 mL). The reaction solution was warmed to rt and stirred for 10 min, before addition of H_2O (50 mL) and EtOAc (50 mL). The layers were separated and the aqueous phase was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with H_2O (150 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Crystallisation from hot EtOH yielded **3.17** (601 mg, 44%) as a colourless, crystalline solid: R_f (1% NEt_3/EtOAc) 0.78; mp (from EtOH) 149-151 °C [lit. mp 150-152 °C[217]]; ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.83-8.77 (1H, m, N*H*), 7.38-7.33 (1H, m, C(7)*H*), 7.29-7.23 (1H, m, C(4)*H*), 7.13 (1H, ddd, *J* = 7.7, 7.7, 0.9 Hz, C(6)*H*), 6.99 (1H, ddd, *J* = 7.7, 7.7, 1.1 Hz, C(5)*H*), 4.52 (2H, d, *J* = 3.2 Hz, NH_2); HRMS *m/z* (EI/FI) Found: 149.0593. $C_7H_7N_3O^+$ requires M^+ 149.0589; LRMS *m/z* (ES^+) 191 ($[M + H + MeCN]^+$, 100%). Data are in good agreement with literature values.[217,218]

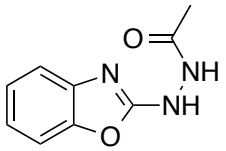
***N*'-Acetyl-*N*-(1,3-benzoxazol-2-yl)acetohydrazide (3.19)**



A solution of **3.17** (1.32 g, 8.82 mmol, 1.0 eq) and Ac_2O (5.00 mL, 5.40 g, 52.9 mmol, 6.0 eq) was stirred at rt for 5 min. The resultant solid was filtered and washed with MeOH, yielding **3.19** (716 mg, 35%) as a colourless solid: R_f (1:75:24

v:v:v AcOH/EtOAc/petroleum ether) 0.38; v_{max} (thin film)/cm^{-1}: 3241 (m), 3032 (C=H) (w), 1734 (C=O) (s), 1668 (C=O) (s), 1623 (C=O) (s), 1572 (C=O) (s); mp 140-141 °C (MeOH); ^{1}H NMR (400 MHz, DMSO-D_6) δ 11.04 (1H, s, N*H*), 7.71-7.64 (2H, m, C(4)*H* & C(7)*H*), 7.42-7.30 (2H, m, C(5)*H* & C(6)*H*), 2.43 (3H, s, NHCOCH_3), 2.04 (3H, s, NCOCH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.1 (NH*C*OCH$_3$), 168.9 (N*C*OCH$_3$), 156.4 (*C*(1)), 148.4 (*C*(7a)), 140.2 (*C*(3a)), 124.9 (*C*(6)), 124.5 (*C*(5)), 118.9 (*C*(7)), 110.5 (*C*(4)), 22.7 (NHCO*C*H$_3$), 20.4 (NCO*C*H$_3$); HRMS *m/z* (EI/FI) Found: 233.0797. $C_{11}H_{11}N_3O_3^+$ requires M^+ 233.0800; LRMS *m/z* (ES^-) 232 ([M − H]$^-$, 100%); Anal. Calcd for $C_{11}H_{11}N_3O_3$: C, 56.7; H, 4.8; N, 18.0. Found: C, 56.5; H, 4.8; N, 17.9.

_N_'-(1,3-Benzoxazol-2-yl)acetohydrazide (3.18)[159,219]

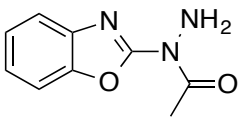


To a solution of **3.19** (99 mg, 424 μmol, 1.0 eq) in MeOH (1.5 mL) was added NaOMe (59 mmol, 1.09 mmol, 2.5 eq) in MeOH (1 mL) at 0 °C. The reaction solution was warmed to rt and stirred for 1 h, before addition of Et_2O (5 mL) and a 1 M aqueous solution of HCl (2.5 mL). The aqueous and organic layers were separated and the aqueous phase was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (15 mL), H_2O (15 mL) then brine (15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, yielding **3.18** (15 mg, 82%) as an off-white solid. The characterisation data were identical to those shown below.

Alternative procedure:

To a solution of acetyl hydrazide (1.46 g, 19.7 mmol, 2.2 eq) in DIPEA (1.70 mL, 1.25 g, 9.67 mmol, 1.1 eq) at 0 °C was added dropwise 2-chlorobenzoxazole (1.35 g, 1.00 mL, 8.82 mmol, 1.0 eq). The reaction solution was warmed to rt and stirred for 4 h, before heating to 60 °C for 4 h. The reaction solution was allowed to cool to rt before addition of CH_2Cl_2 (10 mL) and a 10% aqueous solution of citric acid (10 mL). The layers were separated and the organic phase was washed with the citric acid solution (2 × 10 mL), H_2O (10 mL) and brind (10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Precipitation from EtOAc, followed by filtration and trituration of the filter with H_2O, as well as precipitation of the filtrate with petroleum ether afforded **3.18** (231 mg, 7%) as a colourless solid. R_f (1:75:24 *v:v*:v AcOH/EtOAc/petroleum ether) 0.09; mp (from EtOAc) 175-176 °C [lit. 140-142 °C[219]]; ^{1}H NMR (500 MHz, DMSO-D_6) δ 10.14 (1H, s, N*H*COCH$_3$), 9.76 (1H, s, C(2)N*H*), 7.50-7.38 (1H, m, C(4)*H* or C(7)*H*), 7.38-7.28 (1H, m, C(7)*H* or C(4)*H*), 7.24-7.13 (1H, m, C(5)*H* or C(6)*H*), 7.13-7.02 (1H, m, C(6)*H* or C(5)*H*), 1.93 (3H, s, C*H*$_3$); ^{13}C NMR (126 MHz, DMSO-D_6) δ 170.0 (*C*=O), 163.3 (*C*(2)), 149.0 (*C*(3a) or *C*(7a)), 143.2 (*C*(7a) or *C*(3a)), 124.8 (*C*(5) or *C*(6)), 122.0 (*C*(6) or *C*(5)), 117.2 (*C*(4) or *C*(7)), 110.0 (*C*(7) or *C*(4)), 21.3 (*C*H$_3$); HRMS *m/z* (ES$^+$) Found: 214.0588. $C_9H_9N_3NaO_2^+$ requires M^+ 214.0587; LRMS *m/z* (ES$^-$) 190 ([M – H]$^-$, 100%); Anal. Calcd for $C_9H_9N_3O_2$: C, 56.5; H, 4.7; N, 22.0. Found: C, 56.4; H, 4.8; N, 21.8. Data are in good agreement with literature values.[159]

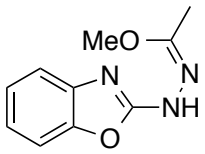
_N_-(1,3-Benzoxazol-2-yl)acetohydrazide (3.20)



A solution of **3.17** (400 mg, 2.68 mmol, 1.0 eq), Ac_2O (302 mg, 280 µL, 2.96 mmol, 1.1 eq) and NEt_3 (298 mg, 410 µL, 2.94 mmol, 1.1 eq) in CH_2Cl_2 (4 mL)

was stirred at rt for 90 min, before addition of CH_2Cl_2 (50 mL) and an aqueous 1 M HCl solution (50 mL). The organic and aqueous layers were separated and the organic phase was washed with H_2O (50 mL), then brine (50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 12 → 100%) afforded diacetylated **3.19** (83 mg, 13%) and monoacetylated **3.20** (101 mg, 20%) as a colourless solid: R_f (50% EtOAc/petroleum ether) 0.64; ν_{max} (thin film)/cm^{-1}: 3333 (w) (N–H), 1708 (m) (C=O), 1636 (m), 1610 (m), 1551 (m), 1483 (m); mp (from Et_2O) 125-126 °C; ^{1}H NMR (500 MHz, DMSO-D_6) δ 7.70-7.64 (2H, m, C(4)*H* & C(7)*H*), 7.38-7.32 (2H, m, C(5)*H* & C(6)*H*), 5.56 (2H, s, NH_2), 3.42 (3H, s, CH_3); ^{13}C NMR (126 MHz, DMSO-D_6) δ 171.3 (*C*=O), 158.5 (*C*(2)), 148.6 (*C*(7a)), 140.3 (*C*(3a)), 124.7 (*C*(6)), 124.2 (*C*(5)), 118.7 (*C*(7)), 110.4 (*C*(4)), 23.2 (*C*H_3); HRMS *m/z* (ES^+) Found: 192.0769. $C_9H_{10}N_3O_2^+$ requires M^+ 192.0768; LRMS *m/z* (ES^+) 192 ($[M + H]^+$, 17%), 214 ($[M + Na]^+$, 100%); HPLC RT = 9.48 min, purity 98.8%.

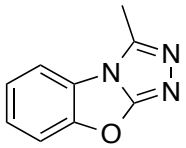
Methyl (1*E*)-*N*-(1,3-benzoxazol-2-yl)ethanehydrazonoate (3.22)



To a solution of **3.17** (1.20 g, 8.05 mmol, 1.0 eq) in MeOH (10 mL) was added trimethyl orthoacetate (2.13 g, 2.25 mL, 17.7 mmol, 2.2 eq). The reaction solution was stirred at rt for 16 h, and then the volatile components were removed *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (1:2) afforded **3.22** (953 mg, 58%) as an off-white solid: R_f 0.40 (50% EtOAc/petroleum ether); ν_{max} (thin film)/cm^{-1}: 2948 (br), 1632 (s), 1581 (s); mp (from EtOAc) 94-96 °C; ^{1}H NMR (500 MHz, DMSO-D_6) δ 8.84 (1H, br s, N*H*), 7.38-7.17 (1H, m, $C_{Ar}H$),

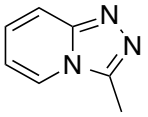
7.17-7.07 (1H, m, $C_{Ar}H$), 7.07-6.95 (2H, m, $C_{Ar}H$), 3.82 (3H, s, OCH_3), 2.11 (3H, s, CCH_3); HRMS *m/z* (ES^+) Found: 228.0741. $C_{10}H_{11}N_3NaO_2^+$ requires M^+ 228.0743; LRMS *m/z* (ES^+) 206 ($[M + H]^+$, 100%); Anal. Calcd for $C_{10}H_{11}N_3O_2$: C, 58.5; H, 5.4; N, 20.5. Found: C, 58.5; H, 5.4; N, 20.6. Compound shows cis and trans hydrazone in ^1H and ^{13}C NMR.

3-Methyl[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole (3.11)[159,220]



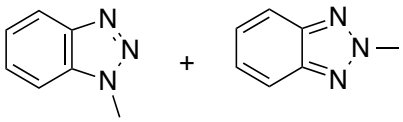
Solid **3.22** (682 mg, 3.32 mmol) was heated at 200 °C for 20 min to give a brown solid. Purification through a short plug of silica gel, eluting with EtOAc, followed by crystallisation from propan-2-ol afforded **3.11** (83 mg, 14%) as colourless crystals: R_f (20% $MeOH/CH_2Cl_2$) 0.35; v_{max} (thin film)/cm^{-1}: 3653 (w), 3070 (w), 2979 (m), 2889 (w), 1630 (m), 1574 (m); mp (from CH_2Cl_2) 169-172 °C [lit. 170 °C[159,221]]; ^1H NMR (500 MHz, CD_3OD) δ 7.89-7.83 (1H, m, C(7)*H*), 7.71-7.64 (1H, m, C(4)*H*), 7.55-7.47 (2H, m, C(5)*H* & C(6)*H*), 2.79 (3H, s, CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 163.9 (*C*(2)), 154.8 (*C*(7a)), 144.4 (*C*CH_3), 127.7 (*C*(6)), 126.2 (*C*(5)), 125.9 (*C*(3a)), 114.1 (*C*(7)), 113.9 (*C*(4)), 11.5 (CH_3); HRMS *m/z* (ES^+) Found: 196.0482. $C_9H_7N_3NaO^+$ requires M^+ 196.0481; LRMS *m/z* (ES^+) 174 ($[M + H]^+$, 100%); HPLC RT = 8.97 min, purity 97.6%. Data are in good agreement with literature values.[159]

3-Methyl[1,2,4]triazolo[4,3-*a*]pyridine (3.13)[210,222,223]



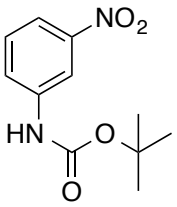
To a 2-5 mL microwave vial was added 2-chloropyridine (1.00 mL, 1.20 g, 10.6 mmol, 1.0 eq) and hydrazine monohydrate (2.90 mL, 2.96 g, 59.2 mmol, 5.6 eq). The vial was sealed and the mixture was stirred at 120 °C for 60 min then at 130 °C for 30 min. The mixture was allowed to cool to rt, and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to give a residue (860 mg, 74%) that was used without further purification. To a solution of the residue (860 mg, 7.88 mmol, 1.0 eq) and NEt_3 (798 mg, 1.10 mL, 7.89 mmol, 1.0 eq) in CH_2Cl_2 (5 mL) was added dropwise a solution of Ac_2O (810 mg, 750 µL, 7.93 mmol, 1.0 eq) in CH_2Cl_2 (5 mL) at 0 °C. The reaction solution was warmed to rt, and stirred for 2 h, then the volatile components were concentrated *in vacuo* into a 10-20 mL microwave vial. The residue was dissolved in AcOH (18 mL). The vial was sealed and the mixture stirred at 180 °C for 30 min with microwave irradiation. The mixture was allowed to cool to rt and the volatile components were removed *in vacuo* giving a residue that was partitioned between CH_2Cl_2 (50 mL) and a saturated aqueous solution of $NaHCO_3$ (50 mL). The organic and aqueous layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo* yielding **3.13** (475 mg, 34%) as a colourless solid: R_f (50% EtOAc/MeOH) 0.44; mp (from Et_2O) 125-127 °C [lit. mp 126-128 °C[221,223]]; ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.38-8.33 (1H, m, C(4)*H* or C(7)*H*), 7.73-7.63 (1H, m, C(7)*H* or C(4)*H*), 7.37-7.30 (1H, m, C(5)*H* or C(6)*H*), 7.00-6.93 (1H, m, C(6)*H* or C(5)*H*), 2.68 (3H, s, CH_3); LRMS *m/z* (ES^+) 134 ($[M + H]^+$, 40%), 156 ($[M + Na]^+$, 20%), 289 ($[2M + Na]^+$, 100%); Anal. Calcd for $C_7H_7N_3$: C, 63.1; H, 5.3; N, 31.6. Found: C, 63.0; H, 5.5; N, 31.4. Data are in good agreement with literature values.[222,223]

1-Methyl-1*H*-benzotriazole (3.12)[56] and 2-methyl-2*H*-benzotriazole (3.27)[214,224,225]



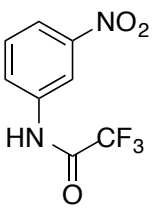
To a solution of benzotriazole (119 mg, 1.00 mmol, 1.0 eq) and an aqueous 25% solution of NaOH (200 µL, 50 mg, 1.25 mmol, 1.3 eq) in MeCN (2 mL) was added MeI (100 µL, 228 mg, 1.60 mmol, 1.6 eq) in MeCN (200 µL). The reaction was stirred at rt for 7 h then diluted with H_2O. The aqueous phase was extracted with $CHCl_3$ (3 × 20 mL) and the combined organic layers were washed with brine (60 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 15 → 25%) afforded **3.12** (86 mg, 65%) as a colourless solid and **3.27** (17 mg, 13%) as a clear and colourless oil. Characterisation for **3.12**: R_f (20% EtOAc/MeOH) 0.08; mp (from EtOAc) 61-62 °C [lit. mp 60-62 °C[56,226]]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10-8.05 (1H, m, C(4)*H* or C(7)*H*), 7.57-7.49 (2H, m, C(7)*H* or C(4)*H* & C(5)*H* or C(6)*H*), 7.39 (1H, ddd, *J* = 8.0, 6.0, 2.0 Hz, C(6)*H* or C(5)*H*), 4.33 (3H, s, CH_3); LRMS *m/z* (ES^+) 134 ($[M + H]^+$, 100%); HPLC RT = 9.56 min, purity 100.0%. Data are in good agreement with literature values.[56,227] Characterisation for **3.27**: R_f (20% EtOAc/MeOH) 0.15; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88-7.82 (2H, m, C(4)*H* & C(7)*H* or C(5)*H* & C(6)*H*), 7.41-7.35 (2H, m, C(5)*H* & C(6)*H* or C(4)*H* & C(7)*H*), 4.52 (3H, s, CH_3); LRMS *m/z* (ES^+) 134 ($[M + H]^+$, 100%). Data are in good agreement with literature values.[224]

tert-Butyl (3-nitrophenyl)carbamate (3.33)[202,228]



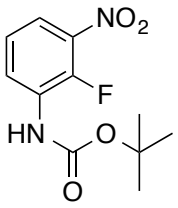
To a solution of 3-nitroaniline (1.15 g, 8.44 mmol, 1.0 eq) in anhydrous THF (10 mL) was added di-*tert*-butyl dicarbonate (2.00 g, 9.16 mmol, 1.1 eq). The solution was cooled to 0 °C, and DMAP (83 mg, 679 μmol, 0.08 eq) was added portionwise. The solution was warmed to rt and stirred for 21 h, then heated under reflux for 1 h, before addition of EtOAc (20 mL) and a saturated aqueous solution of $NaHCO_3$ (20 mL). The layers were separated, and the organic phase was washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography eluting with EtOAc in petroleum ether (gradient elution 2 → 9%), followed by precipitation from CH_2Cl_2/hexane (1:1) yielded **3.33** (732 mg, 42%) as a colourless solid: R_f (5% EtOAc/petroleum ether) 0.40; mp (from CH_2Cl_2) 88-89 °C [lit. mp 96-97 °C[202,229]]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (1H, dd, *J* = 2.1, 2.1 Hz, C(2)*H*), 7.90 (1H, ddd, *J* = 8.2, 2.1, 0.8 Hz, C(6)*H*), 7.75-7.66 (1H, m, C(4)*H*), 7.46 (1H, dd, *J* = 8.2, 8.2 Hz, C(5)*H*), 6.81 (1H, br s, N*H*), 1.56 (9H, s, C(CH_3)$_3$); LRMS *m/z* (ES^-) 237 ($[M - H]^-$, 100%). Data are in good agreement with literature values.[228]

2,2,2-Trifluoro-*N*-(3-nitrophenyl)acetamide (3.34)[217]



To a solution of 3-nitroaniline (993 mg, 7.19 mmol, 1.0 eq) in CH_2Cl_2 (15 mL) cooled to 0 °C, was added dropwise TFAA (2.00 mL, 3.04 g, 14.5 mmol, 2.0 eq) then a saturated aqueous solution of $NaHCO_3$ (8 mL). The reaction solution was warmed to rt, and stirred for 30 min. The layers were separated, the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, yielding **3.34** (1.58 g, 93%) as a tan-brown solid: R_f (20% EtOAc/petroleum ether) 0.32; mp 88-89 °C (CH_2Cl_2) [lit. mp 87-88 °C[203]]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52-8.45 (1H, m, C(2)*H*), 8.20-7.98 (3H, m, C(4)*H* & C(6)*H*, N*H*), 7.69-7.59 (1H, m, C(5)*H*); LRMS *m/z* (ES^-) 233 ($[M - H]^-$, 100%). Data are in good agreement with literature values.[203,230]

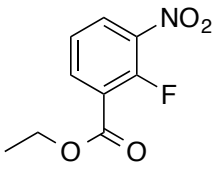
***tert*-Butyl (2-fluoro-3-nitrophenyl)carbamate (3.36)**[204]



To a solution of 2-fluoro-3-nitrobenzoic acid (113 mg, 610 μmol, 1.0 eq), and NEt_3 (100 μL, 73 mg, 717 μmol, 1.2 eq) in *tert*-butanol (5 mL) was added diphenyl phosphorazidate (145 μL, 185 mg, 671 μmol, 1.1 eq). The reaction solution was heated at 105 °C for 19 h, and allowed to cool to rt before dilution with EtOAc (40 mL). The organic phase was washed with H_2O (3 × 50 mL) and brine (50 mL), passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel column chromatography, eluting with EtOAc in cyclohexane (gradient elution 0 → 8%) afforded **3.36** (110 mg, 70%) as an off-white solid that was deemed pure enough for use in the next step: R_f (10% EtOAc/cyclohexane) 0.38; ν_{max} (thin

film)/cm^{-1}: 3232 (w), 2980 (s), 2887 (m), 1727 (w), 1695 (m); mp (from EtOAc) 78-81 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (1H, dd, *J* = 7.5, 7.5 Hz, $C_{Ar}H$), 7.73-7.63 (1H, m, $C_{Ar}H$), 7.31-7.20 (1H, m, $C_{Ar}H$), 6.86 (1H, br s, N*H*), 1.56 (9H, s, $C(CH_3)_3$); LRMS *m/z* (ES^+) 279 ($[M + Na]^+$, 100%). Data are in good agreement with literature values.[204]

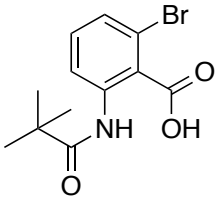
Ethyl 2-fluoro-3-nitrobenzoate (3.39)



To a solution of 2-fluoro-3-nitrobenzoic acid (97 mg, 524 μmol, 1.0 eq) and sodium bicarbonate (230 mg, 2.74 mmol, 5.2 eq) in DMF (1 mL) was added iodoethane (85.0 μL, 165 mg, 1.05 mmol, 2.0 eq) and the reaction solution was stirred at rt for 43 h. The volatile components were removed *in vacuo* and the residue was redissolved in EtOAc (15 mL). The organic phase was washed with H_2O (15 mL), passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel column chromatography, eluting with EtOAc in cyclohexane (gradient elution 1 → 10%) afforded **3.39** (85 mg, 77%) as a colourless solid: R_f (10% EtOAc/cyclohexane) 0.33; ν_{max} (thin film)/cm^{-1}: 1716 (s) (C=O), 1528 (s) (N–O); mp (from EtOAc) <40 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25-8.15 (2H, m, C(4)*H* & C(6)*H*), 7.38 (1H, ddd, *J* = 8.0, 8.0, 1.1 Hz, C(5)*H*), 4.45 (2H, q, *J* = 7.2 Hz, CH_2), 1.43 (3H, t, *J* = 7.2 Hz, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.8 (d, *J* = 3 Hz, CO_2), 154.8 (d, *J* = 278 Hz, *C*(2)), 138.9-138.6 (m, *C*(3)), 137.2 (d, *J* = 1 Hz, *C*(4) or *C*(6)), 129.7 (d, 1 Hz, *C*(6) or *C*(4)), 123.9 (d, *J* = 6 Hz, *C*(5)), 122.1 (d, *J* = 10 Hz, *C*(1)), 62.4 (CH_2), 29.7 (CH_3); ^{19}F NMR (377 MHz, $CDCl_3$) δ –116.5; LRMS *m/z* (ES^+) 236

([M + Na]$^+$, 100%); HPLC RT = 12.50 min, purity 99.9%. Could not obtain HRMS by ES or EI/FI.

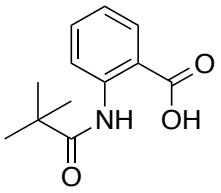
2-Bromo-6-[(2,2-dimethylpropanoyl)amino]benzoic acid (3.47)



To a solution of 2-amino-6-bromobenzoic acid (371 mg, 1.72 mmol, 1.0 eq) in *tert*-butylmethyl ether (2 mL) was added sodium hydroxide (200 mg, 5.00 mmol, 2.9 eq) in H_2O (200 µL) at 0 °C. Pivaloyl chloride (232 µL, 227 mg, 1.89 mmol, 1.1 eq) was added, dropwise at 0 °C, and the suspension was warmed to rt then stir at 50 °C for 3 h. To aid solubility, MeOH (3 mL) was added and the reaction solution was stirred at 50 °C for 45 min. Additional pivaloyl chloride (232 µL, 227 mg, 1.89 mmol, 1.1 eq) was added in an attempt to force the reaction to go to completion, and the reaction solution was stirred at 50 °C for 45 min, before being cooled to rt and stirring for 66 h. Additional pivaloyl chloride (232 µL, 227 mg, 1.89 mmol, 1.1 eq) was added and the reaction solution was stirred at rt for 1h, then was warmed to 50 °C and stirred for 3 h. Despite incomplete consumption of starting material, the reaction solution was concentrated *in vacuo* and the residue was redissolved in H_2O (50 mL). pH indicator paper showed the suspension was acidic (pH 1), and the suspension was extracted with EtOAc (3 × 50 mL), and the combined organic layers were washed with an aqueous 1 M HCl solution (150 mL), passed through an anhydrous frit, and concentrated *in vacuo*. Purification by silica gel column chromatography, eluting with EtOAc in cyclohexane (gradient elution 1 → 100%) afforded **3.47** (185 mg, 36%) as a brown solid: R_f (20% MeOH/CH_2Cl_2) 0.46;

ν_{max} (thin film)/cm^{-1}: 3330 (m) (N–H), 2975 (m), 2893 (m), 2618 (w), 1721 (s) (C=O), 1645 (s); mp (from EtOAc) 232-235 °C; ^{1}H NMR (400 MHz, DMSO-D_6) δ 13.82 (1H, br s, CO_2H), 9.55 (1H, br, s, N*H*), 7.66 (1H, d, *J* = 8.0 Hz, C(3)*H* or C(5)*H*), 7.45 (1H, d, *J* = 8.0 Hz, C(5)*H* or C(3)*H*), 7.30 (1H, dd, *J* = 8.0, 8.0 Hz, C(4)*H*), 1.19 (9H, s, C(CH_3)$_3$); ^{13}C NMR (101 MHz, DMSO-D_6) δ 176.4 (NH*C*O), 167.1 (*C*O_2H), 137.2 (*C*(6)), 130.7 (*C*(1)), 130.6 (*C*(4)), 129.1 (*C*(3) or *C*(5)), 124.0 (*C*(5) or *C*(3)), 119.0 (*C*(2)), 38.9 (*C*(CH_3)$_3$), 27.1 (C(*C*H_3)$_3$); HRMS *m/z* (ES$^+$) Found: 322.0048 and 324.0027. $C_{12}H_{14}O_3NBrNa^+$ requires [M(^{79}Br)]$^+$ & [M(^{81}Br)]$^+$ 322.0049 and 324.0029; LRMS *m/z* (ES$^+$) 300 & 302 ([M(^{79}Br) + H]$^+$ & [M(^{81}Br) + H]$^+$, 100%); HPLC RT = 11.11 min, purity 99.6%.

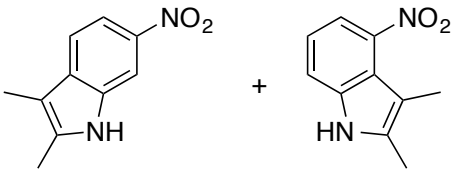
2-[(2,2-Dimethylpropanoyl)amino]benzoic acid (3.48)[205]



To a solution of **3.47** (79 mg, 263 μmol, 1.0 eq) in distilled THF (1 mL) was added a 3 M solution of methylmagnesium bromide (300 μL, 107 mg, 900 μmol, 3.4 eq) in Et_2O dropwise at 0 °C. The reaction solution was warmed to rt and stirred for 90 min, then heated at 50 °C for 1 h. Additional methylmagnesium bromide solution (300 μL, 107 mg, 900 μmol, 3.4 eq) was added at 0 °C and the reaction solution was heated at 50 °C for a further 4 h, then at 70 °C for 1 h. To prevent the reaction running dry overnight, additional distilled THF (2 mL) was added, and the reaction solution was heated at 70 °C for 16 h. In attempts to push the reaction to completion, additional methylmagnesium bromide solution (300 μL, 107 mg, 900 μmol, 3.4 eq) was added at 0 °C, and the reaction solution was heated at 70 °C

for 96 h. The mixture was then cooled to rt, and quenched with a saturated aqueous NH_4Cl solution (50 mL). The aqueous layer was extracted with EtOAc (3 × 50 mL), and the combined organic layers were passed through an anhydrous frit and concentrated *in vacuo*. The desired product was not afforded, but purification by silica gel column chromatography, eluting with EtOAc in cyclohexane (gradient elution 5 → 100%) isolated the side-product 2-pivalamidobenzoic acid **3.48** (11 mg, 19%) as a brown oil: R_f (50% EtOAc/cyclohexane) 0.61; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.24 (1H, br s, CO_2H), 8.81 (1H, dd, *J* = 8.5, 0.8 Hz, C(5)*H*), 8.16 (1H, dd, *J* = 8.0, 1.4 Hz, C(2)*H*), 7.65-7.56 (1H, m, C(4)*H*), 7.17-7.08 (1H, m, C(3)*H*), 1.37 (9H, s, $C(CH_3)_3$); LCMS (formic acid) RT = 0.90 min, $[M + H]^+$ 222, $[M – H]^–$ 220. Data are in good agreement with literature values.[205]

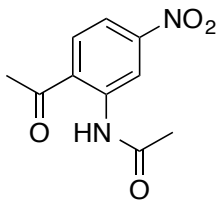
2,3-Dimethyl-4-nitro-1*H*-indole (3.50)[221] **and 2,3-dimethyl-6-nitro-1*H*-indole (3.51)**[159,231,232]



To a stirred solution of 3-nitroaniline (620 mg, 4.49 mmol, 1.0 eq) and butan-2-one (6.00 mL, 67.0 mmol, 14.9 eq) in DMSO (15 mL) was added KO^{t}Bu (1.21 g, 10.8 mmol, 2.4 eq). The reaction solution was stirred at rt in an open flask for 2 h, then the reaction was quenched with a saturated aqueous NH_4Cl solution (50 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL), and the combined organic layers were passed through a hydrophobic frit and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with toluene in cyclohexane (gradient elution 50 → 100%) afforded 2,3-dimethyl-4-nitro-1*H*-indole **3.50** (164 mg, 19%) and 2,3-dimethyl-6-nitro-1*H*-indole **3.51** (91 mg, 11%) as orange solids:

Characterisation for 2,3-dimethyl-4-nitro-1*H*-indole (**3.50**): R_f (100% toluene) 0.32; mp (from toluene) 165-168 °C [lit.[221] mp 172-173 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (1H, br s, N*H*), 7.75 (1H, dd, *J* = 8.0, 0.7 Hz, C(5)*H*), 7.51 (1H, dd, *J* = 8.0, 0.7 Hz, C(7)*H*), 7.13 (1H, dd, *J* = 8.0, 8.0 Hz, C(6)*H*), 2.46 (3H, s, CH_3), 2.30 (3H, s, CH_3); LCMS (formic acid) RT = 1.00, $[M - H]^-$ 189. Data are in good agreement with literature values.[159] Characterisation for 2,3-dimethyl-6-nitro-1*H*-indole (**3.51**): R_f (100% toluene) 0.21; mp (from toluene) 136-137 °C [lit.[233] mp 141.5-142.5 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (1H, d, *J* = 2.0 Hz, C(7)*H*), 8.21 (1H, br s, N*H*), 8.02 (1H, dd, *J* = 8.8, 2.0 Hz, C(5)*H*), 7.49 (1H, d, *J* = 8.8 Hz, C(4)*H*), 2.46 (3H, s, CH_3), 2.27 (3H, s, CH_3); LCMS (formic acid) RT = 1.06, $[M - H]^-$ 189. Data are in good agreement with literature values.[159,231,232]

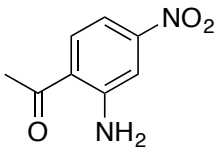
_N_-(2-Acetyl-5-nitrophenyl)acetamide (3.52)[233]



To a solution of 2,3-dimethyl-6-nitro-1*H*-indole (**3.51**) (90 mg, 473 μmol, 1.0 eq) in AcOH (1.5 mL) was slowly added chromium trioxide (76 mg, 757 μmol, 1.6 eq) in H_2O (350 μL) at 0 °C. The reaction solution was warmed to rt and stirred for 4 h, then was diluted with H_2O (25 mL) and the aqueous phase was extracted with $CHCl_3$ (3 × 25 mL) and the combined organic layers were passed through an anhydrous frit and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 25 → 100%) afforded **3.52** (11 mg, 10%) as an off-white solid: R_f (50% EtOAc/petroleum ether) 0.41; mp (from EtOAc) 116-119 °C [lit.[220,234] mp 126-127 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54-8.43 (1H, m,

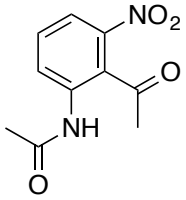
C(6)*H*), 8.22 (1H, br s, N*H*), 7.96-7.88 (1H, m, C(3)*H*), 7.59 (1H, dd, *J* = 8.3, 8.3 Hz, C(4)*H*), 2.44 (3H, s, C(2)COCH_3), 2.20 (3H, s, NHCOCH_3); LRMS *m/z* (ES^+) 245 ($[M + Na]^+$, 100%). Data are in good agreement with literature values.[233]

1-(2-Amino-4-nitrophenyl)ethanone (3.53)[221]



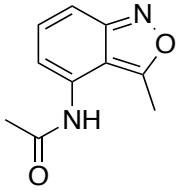
To a solution of **3.52** (44 mg, 198 μmol, 1.0 eq) in EtOH (2 mL) was added a 36% (*v/v*) aqueous solution of HCl (750 μL, 270 mg, 7.41 mmol, 37 eq). The reaction solution was heated under reflux for 2 h, then the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL), the combined organic layers were washed with H_2O (30 mL) and brine (30 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to yield **3.53** (35 mg, 97%) as a brown oil: R_f (50% EtOAc/petroleum ether) 0.60; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (1H, d, *J* = 8.8 Hz, C(6)*H*), 7.49 (1H, d, *J* = 2.2 Hz, C(3)*H*), 7.42 (1H, dd, *J* = 8.8, 2.2 Hz, C(5)*H*), 2.64 (3H, s, CH_3); LRMS *m/z* (ES^+) 235 ($[M + Na + MeOH]^+$, 100%). Data are in good agreement with literature values.[221]

***N*-(2-Acetyl-3-nitrophenyl)acetamide (3.49)**[221]



To a solution of **3.50** (126 mg, 662 μmol, 1.0 eq) in AcOH (3 mL) was slowly added chromium trioxide (110 mg, 1.10 mmol, 1.7 eq) in H_2O (300 μL) at 0 °C. The reaction solution was warmed to rt and stirred for 160 min, then was diluted with H_2O (25 mL), the aqueous phase was extracted with $CHCl_3$ (3 × 25 mL), the combined organic layers were passed through an anhydrous frit, and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in cyclohexane (gradient elution 25 → 100%) afforded **3.49** (76 mg, 52%) as a clear and colourless oil: R_f (50% EtOAc/cyclohexane) 0.16; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (1H, d, *J* = 8.3 Hz, C(4)*H*), 8.24 (1H, br s, N*H*), 7.93 (1H, dd, *J* = 8.3, 0.8 Hz, C(6)*H*), 7.62 (1H, dd, *J* = 8.0, 8.0 Hz, C(5)*H*), 2.46 (3H, s, CH_3), 2.21 (3H, s, CH_3); LCMS (formic acid) RT = 0.58, $[M + H]^+$ 223. Data are in good agreement with literature values.[221]

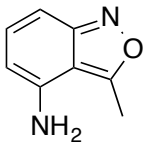
***N*-(3-Methyl-2,1-benzoxazol-4-yl)acetamide (3.55)**



To a solution of **3.49** (542 mg, 2.44 mmol, 1.0 eq) and zinc powder (1.24 g, 19.0 mmol, 7.8 eq) in MeOH (10 mL) was added 2-bromo-2-nitropropane (350 μL, 554 mg, 3.30 mmol, 1.4 eq). The suspension was degassed by bubbling with argon for 5 min, then heated at 50 °C for 194 h. The reaction solution was filtered through a pad of Celite®, and eluted with CH_2Cl_2 (50 mL), then washed with a saturated aqueous solution of NH_4Cl (50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 25 → 100%) afforded **3.55** (223 mg, 48%) as an off-white solid: R_f (100% EtOAc) 0.38; v_{max} (thin film)/cm^{-1}: 3457 (w) (N–H), 3277 (w), 3016 (br), 2970 (w) (C-H), 1739 (s) (C=O); mp (from EtOAc) 149-152 °C; ^{1}H NMR

(400 MHz, CD_3OD) δ 7.26-7.16 (2H, m, C(5)*H* & C(6)*H*), 6.77 (1H, dd, *J* = 6.1, 1.1 Hz, C(7)*H*), 2.70 (3H, s, C(3)CH_3), 2.10 (3H, s, COCH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0 (*C*=O), 167.5 (*C*(3)), 159.4 (*C*(4) or *C*(7a)), 132.8 (*C*(5) or *C*(6)), 131.7 (*C*(7a) or *C*(4)), 119.4 (*C*(7)), 114.0 (*C*(3a)), 113.5 (*C*(6) or *C*(5)), 23.1 (CO*C*H_3), 13.3 (C(3)*C*H_3); HRMS *m/z* (ES^+) Found: 213.0639. $C_{10}H_{10}N_2NaO_2^+$ requires M^+ 213.0634; LRMS *m/z* (ES^-) 189 ($[M - H]^-$, 100%); HPLC RT = 8.57 min, purity 99.3%. Data are in good agreement with literature values.[221]

3-Methyl-2,1-benzoxazol-4-amine (3.28)

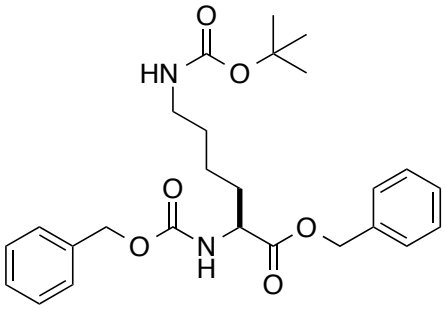


To a 2-5 mL microwave vial containing a solution of **3.55** (50 mg, 263 μmol, 1.0 eq) in MeOH (2 mL) was added LiOH (64 mg, 2.67 mmol, 10 eq). The reaction vessel was sealed and heated at 50 °C with microwave irradiation for 45 h, then was diluted with H_2O (5 mL). The aqueous phase was extracted with EtOAc (3 × 5 mL), and the combined organic layers were washed with H_2O (15 mL) and brine (15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography, eluting with EtOAc in petroleum ether (gradient elution 10 → 60%) yielded **3.28** (9 mg, 23%) as a yellow oil: R_f (100% EtOAc) 0.62; v_{max} (thin film)/cm^{-1}: 3229 (w) (N-H), 3016 (br), 2946 (w), 1739 (s); ^{1}H NMR (400 MHz, CD_3OD) δ 7.07 (1H, dd, *J* = 8.8, 7.2 Hz, C(1)*H*), 6.66 (1H, d, *J* = 8.8 Hz, C(2)*H*), 6.03 (1H, d, *J* = 7.2 Hz, C(6)*H*), 2.91 (3H, s, CH_3); ^{13}C NMR (126 MHz, CD_3OD) δ 166.9 (*C*(3)), 159.9 (*C*(7a)), 143.4 (*C*(4)), 135.1 (*C*(6)), 111.4 (*C*(3a)), 102.7 (*C*(5)), 102.5 (*C*(7)), 13.6 (*C*H_3); HRMS *m/z* (ES^+) Found: 149.0708. $C_8H_9N_2O^+$ requires M^+ 149.0709;

LRMS *m/z* (ES^+) 149 ($[M + H]^+$, 100%), 171 ($[M + Na]^+$, 95%); HPLC RT = 8.84 min, purity 98.1%.

6.5 Experimental Procedures for Chapter 4

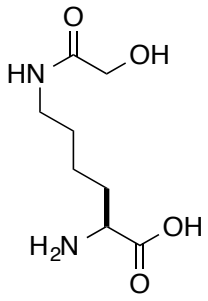
Benzyl (2*S*)-6-[(*tert*-butoxycarbonyl)amino-2-{[(benzyloxy)carbonyl]amino} hexanoate (4.18)[226]



To a solution of Cbz-Lys-OH (1.00 g, 3.57 mmol, 1.0 eq) and $NaHCO_3$ (600 mg, 7.14 mmol, 2.0 eq) in THF/H_2O (20 mL, *v:v* 1:1), was added a solution of Boc_2O (1.81 g, 8.29 mmol, 2.3 eq) in THF (10 mL). The reaction solution was stirred at rt for 19 h. The THF was removed *in vacuo*, giving an aqueous solution that was acidified to ~pH 1.5 with a 1 M aqueous solution of HCl. The aqueous phase was extracted with EtOAc (3 × 50 mL), the combined organic layers were washed with H_2O (150 mL), then brine (150 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. To a solution of the residue dissolved in acetone (15 mL), was added K_2CO_3 (1.48 g, 10.7 mmol, 3.0 eq) and BnBr (470 µL, 676 mg, 3.95 mmol, 1.1 eq). The reaction solution was heated under reflux for 2 h, and then allowed to cool to rt. The mixture was filtered through Celite®, eluting with acetone. The volatile components were removed *in vacuo*, producing a residue that was crystallised from Et_2O/hexane (5:3) yielding **4.18** (1.47 g, 88%) as a colourless solid: R_f (30% EtOAc/petroleum ether) 0.44; $[\alpha]_D^{25}$ = +4.4 (*c* = 1.00, $CHCl_3$); v_{max} (thin

film)/cm^{-1}: 3359 (m), 2936 (w), 1745 (s) (C=O), 1658 (s), 1528 (s); mp (from Et_2O) 75-76 °C [lit. mp 76-79 °C[229]]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.29 (10H, m, 2 × C_6H_5), 5.38 (1H, d, *J* = 6.7 Hz, N*H*C$_\alpha$), 5.20 (1H, d, *J* = 12.3 Hz, C$_\alpha$CO$_2$C*H*$_A$H$_B$Ph), 5.15 (1H, d, *J* = 12.3 Hz, C$_\alpha$CO$_2$CH$_A$*H*$_B$Ph); 5.11 (2H, s, NHCO$_2$C*H*$_2$Ph), 4.60-4.45 (1H, m, C$_\varepsilon$N*H*), 4.45-4.36 (1H, m, C$_\alpha$*H*), 3.13-2.95 (2H, m, C$_\varepsilon$*H*$_2$), 1.92-1.77 (1H, m, C$_\beta$*H*$_A$), 1.75-1.62 (1H, m, C$_\beta$*H*$_B$), 1.53-1.22 (4H, m, C$_\gamma$*H*$_2$ & C$_\delta$*H*$_2$), 1.43 (9H, s, C(C*H*$_3$)$_3$); HRMS *m/z* (ES$^+$) Found: 493.2308. $C_{26}H_{34}N_2NaO_6^+$ requires M$^+$ 493.2309; LRMS *m/z* (ES$^+$) 471 ([M + H]$^+$, 23%), 493 ([M + Na]$^+$, 71%), 963 ([2M + Na]$^+$, 100%). Data are in good agreement with literature values.[229]

(2*S*)-2-Amino-6-(carbamoylamino)hexanoic acid (4.12)

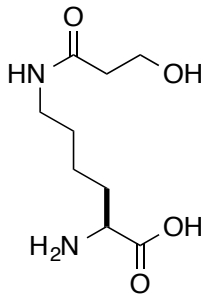


To a solution of *tert*-butoxyacetic acid (250 µL, 257 mg, 1.94 mmol, 1.0 eq), *N*-hydroxysuccinimide (460 mg, 4.00 mmol, 2.1 eq) and distilled pyridine (610 µL, 597 mg, 7.56 mmol, 4.0 eq) in CH_2Cl_2 (25 mL), TFAA (550 µL, 834 mg, 3.97 mmol, 2.1 eq) was added dropwise at 0 °C. The solution was warmed to rt and stirred for 1 h before addition of a 1 M aqueous solution of HCl (25 mL). The layers were separated, and the organic phase was washed with a 1 M aqueous solution of HCl (25 mL), a saturated aqueous solution of $NaHCO_3$ (25 mL) then brine (25 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*, yielding succinate ester **4.26** (336 mg, 78%) as a colourless oil, which was used without purification. A solution of **4.26** (330 mg, 1.44 mmol, 1.0 eq) and Cbz-Lys-OH (525 mg, 1.87 mmol,

1.3 eq) in DMF (5 mL) was stirred at rt for 15 h. The volatile components were removed *in vacuo*, giving a colourless residue that was partitioned between H_2O (10 mL) and EtOAc (10 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic phases were washed with H_2O (5 × 30 mL) then brine (30 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding protected lysine **4.32** (483 mg, 85%), as a colourless oil, which was used without purification. A solution of **4.32** (350 mg, 887 μmol, 1.0 eq) in a 33% (*w/v*) solution of hydrobromic acid in acetic acid (5.00 mL, 7.00 g, 86.5 mmol, 98 eq) was stirred at rt for 2 h, before addition of H_2O (10 mL). The aqueous phase was washed with EtOAc (5 × 15 mL), concentrated *in vacuo* and lyophilised producing an orange residue. Purification using an acidic Dowex ion exchange column, washing with H_2O and eluting with 1 M NH_4OH, followed by lyophilisation yielded **4.12** (163 mg, 90%), as a colourless, hygroscopic solid with minor impurities. Purification of a small sample for testing, by silica gel chromatography, eluting with H_2O in MeCN (gradient elution 10 → 20%), followed by concentration *in vacuo* and lyophilisation afforded **4.12** (87 mg, 29%) as a colourless, fluffy, hygroscopic solid: R_f (RP, 100% H_2O) 0.93; $[\alpha]_D^{24}$ = +3.4 (*c* = 0.50, H_2O); ν_{max} (thin film)/cm^{-1}: 3421 (w) (N–H), 3016 (m), 2864 (m), 1739 (s) (C=O); ^{1}H NMR (400 MHz, D_2O) δ 3.95 (2H, s, CH_2OH), 3.62 (1H, dd, *J* = 6.0, 6.0 Hz, $C_\alpha H$), 3.16 (2H, dd, *J* = 6.7, 6.7 Hz, $C_\varepsilon H_2$), 1.85-1.68 (2H, m, $C_\beta H_2$), 1.55-1.42 (2H, m, $C_\delta H_2$), 1.39-1.20 (2H, m, $C_\gamma H_2$); ^{13}C NMR (126 MHz, D_2O) δ 175.1 (NH*C*O or $C_\alpha CO_2$*), 175.1 ($C_\alpha CO_2$* or NH*C*O), 61.2 (CH_2OH), 55.0 (C_α), 38.8 (C_ε), 30.4 (C_β), 28.4 (C_δ), 22.0 (C_γ); HRMS *m/z* (ES^+) Found: 227.1008. $C_8H_{16}N_2NaO_4^+$ requires M^+ 227.1002; LRMS *m/z* (ES^-) 203 ($[M - H]^-$, 100%).

* Carbonyl peaks could not be assigned (despite use of HMBC spectra) because the peaks are too close together.

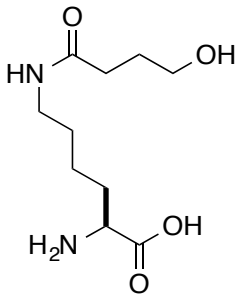
(2*S*)-2-Amino-6-[(3-hydroxypropanoyl)amino]hexanoic acid (4.11)



To a solution of 3-(*tert*-butoxy)propionic acid (20.0 µL, 20 mg, 137 µmol, 1.0 eq), *N*-hydroxysuccinimide (33 mg, 287 µmol, 2.1 eq) and distilled pyridine (45.0 µL, 44 mg, 287 µmol, 4.0 eq) in CH_2Cl_2 (1 mL), TFAA (60 mg, 40.0 µL, 287 µmol, 2.1 eq) was added dropwise at 0 °C. The solution was warmed to rt and stirred for 1 h before addition of a 1 M aqueous solution of HCl (1 mL) and separation of the layers. The organic phase was washed with a 1 M aqueous solution of HCl (1 mL), a saturated aqueous solution of $NaHCO_3$ (1 mL) then brine (1 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding succinate ester **4.27** (22 mg, 67%), as a colourless solid that was used without purification. A solution of **4.27** (20 mg, 82.2 µmol, 1.0 eq) and Cbz-Lys-OH (30 mg, 107 µmol, 1.3 eq) in DMF (1 mL) was stirred at rt for 15 h. The volatile components were removed *in vacuo*, giving a colourless residue that was partitioned between H_2O (5 mL) and EtOAc (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with H_2O (5 × 15 mL) then brine (15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding protected lysine **4.33** (26 mg, 78%), as a colourless oil, which was used without purification. A solution of **4.33** (25 mg, 61.2 µmol, 1.0 eq) in a 33% (*w/v*) solution of hydrobromic acid in acetic acid (3.00 mL, 4.20 g, 51.9 mmol, 59 eq) was stirred at rt for 3 h, before addition of H_2O (7 mL). The aqueous phase was washed with EtOAc (5 × 10 mL), concentrated *in vacuo* and lyophilised producing an orange residue. Purification using an acidic Dowex ion exchange column, washing with H_2O

and eluting with 1 M NH_4OH, followed by lyophilisation yielded **4.11** (7 mg, 54%) as a colourless, hygroscopic solid: R_f (RP, 100% H_2O) 0.80; $[\alpha]_D^{24}$= + 2.6 (*c* = 0.50, H_2O); v_{max} (thin film)/cm^{-1}: 3456 (w) (N–H), 3307 (br), 2971 (m) (C–H), 1739 (s) (C=O), 1634 (m) (C=O); ^{1}H NMR (400 MHz, D_2O) δ 3.72 (2H, t, *J* = 5.7 Hz, C*H*$_2$OH), 3.62 (1H, dd, *J* = 5.7, 5.7 Hz, C$_\alpha$*H*), 3.11 (2H, dd, *J* = 6.4, 6.4 Hz, C$_\varepsilon$*H*$_2$), 2.35 (2H, t, *J* = 5.7 Hz, COC*H*$_2$), 1.85-1.67 (2H, m, C$_\beta$*H*$_2$), 1.53-1.40 (2H, m, C$_\delta$*H*$_2$), 1.40-1.20 (2H, m, C$_\gamma$*H*$_2$); ^{13}C NMR (101 MHz, D_2O) δ 175.2 (*C*O_2H), 174.6 (*C*OCH$_2$), 58.4 (*C*H_2OH), 55.0 (*C*$_\alpha$), 39.3 (*C*$_\varepsilon$), 38.9 (CO*C*H$_2$), 30.4 (*C*$_\beta$), 28.3 (*C*$_\delta$), 22.1 (*C*$_\gamma$); HRMS *m/z* (ES^-) Found: 217.1203. $C_9H_{17}N_2O_4^-$ requires M^- 217.1194; LRMS *m/z* (ES^-) 217 ($[M - H]^-$, 100%).

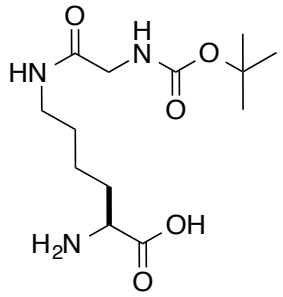
(2*S*)-2-Amino-6-[(4-hydroxybutanoyl)amino]hexanoic acid (4.10)



To a solution of 4-benzyloxybutyric acid (250 µL, 274 mg, 1.41 mmol, 1.0 eq), *N*-hydroxysuccinimide (337 mg, 2.92 mmol, 2.1 eq) and distilled pyridine (450 µL, 440 mg, 5.57 mmol, 3.9 eq) in CH_2Cl_2 (10 mL), was added TFAA (420 µL, 635 mg, 3.03 mmol, 2.1 eq) dropwise at 0 °C. The solution was warmed to rt, and stirred for 1 h before addition of a 1 M aqueous solution of HCl (10 mL), and separation of the layers. The organic phase was washed with a 1 M aqueous solution of HCl (10 mL), a saturated aqueous solution of $NaHCO_3$ (10 mL) then brine (10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding succinate ester **4.28** (350 mg, 85%) as a colourless solid that was used without purification. A solution of

4.28 (350 mg, 1.20 mmol, 1.0 eq) and Cbz-Lys-OH (437 mg, 1.56 mmol, 1.3 eq) in DMF (2 mL) was stirred at rt for 18 h. The volatile components were removed *in vacuo*, producing a clear residue that was partitioned between brine (30 mL) and EtOAc (30 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with H_2O (4 × 100 mL) then brine (100 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The resulting residue was redissolved in *tert*-butanol and H_2O (7 mL, *v:v* 6:1), was degassed for 5 min, and purged with nitrogen, before addition of palladium black (500 mg, 4.70 mmol, 3.9 eq). The mixture was purged with hydrogen (3 ×) and, after the hydrogen balloon was reattached, stirred at rt for 19 h. The mixture was filtered through Celite® and washed with Et_2O (100 mL). The filtrate was discarded, and the product was eluted from the filter cake with H_2O (100 mL). The aqueous phase was concentrated *in vacuo* and lyophilised. Purification by silica gel chromatography, eluting with H_2O in MeCN (gradient elution 20 → 50%), followed by concentration *in vacuo* and lyophilisation afforded **4.10** (133 mg, 48%) as a colourless, fluffy, hygroscopic solid: R_f (RP, 100% H_2O) 0.78; $[\alpha]_D^{25}$ = +1.7 (*c* = 0.71, H_2O); ν_{max} (thin film)/cm^{-1}: 3321 (w), 3088 (br) (O–H), 2947 (w), 2868 (w), 1635 (s) (C=O); ^{1}H NMR (400 MHz, D_2O) δ 3.51 (2H, t, *J* = 1 Hz, C*H*$_2$OH), 3.55-3.35 (1H, m, C$_\alpha$*H*), 3.11 (2H, dd, *J* = 6.8, 6.8 Hz, C$_\varepsilon$*H*$_2$), 2.21 (2H, t, *J* = 7.5 Hz, NHCOC*H*$_2$), 1.73 (2H, tt, *J* = 7.5, 6.6 Hz, C*H*$_2$CH$_2$OH), 1.75-1.60 (2H, m, C$_\beta$*H*$_2$), 1.52-1.40 (2H, m, C$_\delta$*H*$_2$), 1.36-1.22 (2H, m, C$_\gamma$*H*$_2$); ^{13}C NMR (101 MHz, D_2O) δ 178.7 (*C*O$_2$H), 176.2 (*C*OCH$_2$), 60.8 (*C*H$_2$OH), 55.2 (*C*$_\alpha$), 39.0 (*C*$_\varepsilon$), 32.4 (CO*C*H$_2$), 32.0 (*C*$_\beta$), 28.1 (*C*$_\delta$), 27.9 (*C*H$_2$CH$_2$OH), 22.1 (*C*$_\gamma$); HRMS *m/z* (ES$^+$) Found: 255.1312. $C_{10}H_{20}N_2NaO_4^+$ requires M^+ 255.1315; LRMS *m/z* (ES$^+$) 233 ($[M + H]^+$, 27%), 255 ($[M + Na]^+$, 100%).

(2*S*)-2-Amino-6-({[(*tert*-butoxycarbonyl)amino]acetyl}amino)hexanoic acid (4.78)



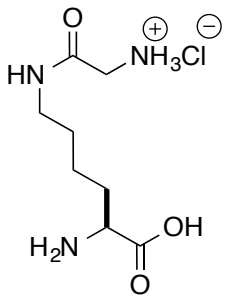
Solution A: To a solution of Boc-Gly-OH (172 mg, 984 µmol, 1.0 eq), *N*-hydroxysuccinimide (238 mg, 2.06 mmol, 2.1 eq) and distilled pyridine (320 µL, 312 mg, 3.94 mmol, 4.0 eq) in CH_2Cl_2 (2 mL), was added TFAA (190 µL, 287 mg, 1.37 mmol, 1.4 eq) dropwise at 0 °C. The solution was warmed to rt and stirred for 1 h before addition of a 1 M aqueous solution of HCl (2 mL), and separation of the layers. The organic phase was washed with a 1 M aqueous solution of HCl (2 mL), a saturated aqueous solution of $NaHCO_3$ (2 mL) then brine (2 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give a residue that was redissolved in acetone (1 mL).

Solution B: To a solution of L-Lys-OH•HCl (183 mg, 1.00 mmol, 1.0 eq) and $NaHCO_3$ (404 mg, 4.81 mmol, 4.9 eq) in H_2O (2 mL) was added copper(II) sulfate pentahydrate (126 mg, 505 µmol, 0.51 eq) in H_2O (1 mL).

Solution A was added to Solution B, the reaction solution was stirred for 6 h and then the milky blue suspension was filtered. The blue precipitate was washed with H_2O/acetone (10 mL, *v:v* 1:1), was resuspended in $H_2O/CHCl_3$ (10 mL, *v:v* 1:1) and following addition of 8-hydroxyquinoline (270 mg, 1.07 mmol, 1.9 eq), the reaction solution was stirred for 10 min. The green suspension was filtered and the filtrate layers were separated. The aqueous layer was washed with $CHCl_3$ (4 × 5 mL), and concentrated *in vacuo*, then lyophilised. Purification by silica gel chromatography, eluting with H_2O in MeCN (gradient elution 10 → 20%), followed by

concentration *in vacuo* and lyophilisation afforded **4.78** (87 mg, 29%) as a colourless, fluffy, hygroscopic solid: R_f (20% H_2O/MeCN) 0.28; $[\alpha]_D^{24}$ = +1.2 (*c* = 0.50, H_2O); ν_{max} (thin film)/cm^{-1}: 3458 (w), 3298 (w), 2971 (br), 1739 (s) (C=O), 1652 (w) (C=O); ^{1}H NMR (400 MHz, D_2O) δ 3.66-3.56 (3H, m, $C_\alpha H$ and CH_2NHCO_2), 3.12 (2H, dd, *J* = 6.8, 6.8 Hz, $C_\varepsilon H_2$), 1.84-1.66 (2H, m, $C_\beta H_2$), 1.53-1.38 (2H, m, $C_\delta H_2$), 1.38-1.22 (11H, m, $C_\gamma H_2$ and $C(CH_3)_3$); ^{13}C NMR (126 MHz, D_2O) δ 174.8 (CO_2H), 172.4 ($NHCOCH_2$), 158.0 ($CO_2C(CH_3)_3$), 81.6 ($C(CH_3)_3$), 54.7 (C_α), 43.4 (CH_2NHCO_2), 38.8 (C_ε), 30.1 (C_β), 28.0 (C_δ), 27.5 ($C(CH_3)_3$), 21.7 (C_γ); HRMS *m/z* (ES^+) Found: 326.1678. $C_{13}H_{25}N_3NaO_5^+$ requires M^+ 326.1686; LRMS *m/z* (ES^+) 304 ($[M + H]^+$, 100%).

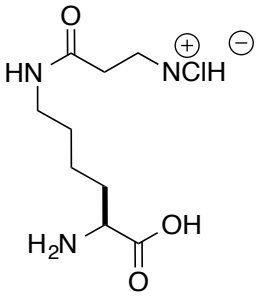
2-{[(5*S*)-5-Amino-5-carboxypentyl]amino}-2-oxoethanaminium chloride (4.13)



A solution of **4.78** (65 mg, 214 μmol, 1.0 eq) in a 4 M solution of HCl (500 μL, 2.10 g, 2.00 mmol, 9.3 eq) in dioxane was stirred at rt for 14 h, forming a colourless precipitate. The dioxane was decanted, the precipitate was washed with dioxane then resuspended in a 1 M aqueous solution of HCl (2.00 mL, 2.10 g, 2.00 mmol, 9.3 eq), and the reaction solution was stirred at rt for 15 h. The volatile components were removed *in vacuo* to give a colourless solid that was lyophilised to afford **4.13** (36 mg, 70%) as a colourless, fluffy, hygroscopic solid: R_f (100% H_2O) 0.10; $[\alpha]_D^{24}$ = +4.2 (*c* = 0.52, H_2O); ν_{max} (thin film)/cm^{-1}: 3321 (m), 2971 (br), 1739 (s) (C=O), 1666 (s) (C=O); ^{1}H NMR (500 MHz, D_2O) δ 3.89 (1H, dd, *J* = 5.8, 5.8 Hz,

$C_\alpha H$), 3.66 (2H, s, CH_2NH_2), 3.14 (2H, dd, J = 6.6, 6.6 Hz, $C_\varepsilon H_2$), 1.91-1.74 (2H, m, $C_\beta H_2$), 1.53-1.41 (2H, m, $C_\delta H_2$), 1.41-1.23 (2H, m, $C_\gamma H_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.6 ($C_\alpha HCO$), 166.7 ($C_\varepsilon NHCO$), 53.1 (C_α), 40.3 (CH_2NH_2), 38.9 (C_ε), 29.4 (C_β), 27.7 (C_δ), 21.5 (C_γ); HRMS *m/z* (ES^+) Found: free amine 204.1343. $C_8H_{18}N_3O_3^+$ requires M^+ 204.1343; LRMS *m/z* (ES^+) 226 (free amine, $[M + Na]^+$, 100%), 242 (free amine, $[M + K]^+$, 94%).

3-{[(5*S*)-5-Amino-5-carboxypentyl]amino}-3-oxopropan-1-aminium chloride (4.14)

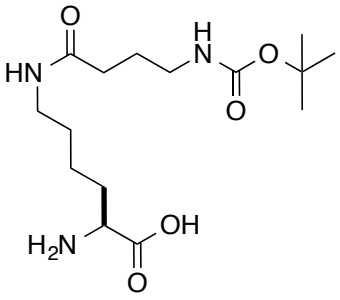


Solution A: To a solution of Boc-β-Ala-OH (113 mg, 597 µmol, 1.2 eq), *N*-hydroxysuccinimide (163 mg, 1.41 mmol, 3.0 eq) and distilled pyridine (160 µL, 156 mg, 1.97 mmol, 4.1 eq) in CH_2Cl_2 (2 mL), TFAA (150 µL, 227 mg, 1.08 mmol, 2.2 eq) was added dropwise at 0 °C. The solution was warmed to rt and stirred for 1 h before addition of a 1 M aqueous solution of HCl (2 mL), and separation of the layers. The organic phase was washed with a 1 M aqueous solution of HCl (2 mL), a saturated aqueous solution of $NaHCO_3$ (2 mL) then brine (2 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give a residue that was redissolved in acetone (500 µL).

Solution B: To a solution of L-Lys-OH•HCl (88 mg, 482 µmol, 1.0 eq) and $NaHCO_3$ (204 mg, 2.42 mmol, 5.0 eq) in H_2O (1 mL) was added copper(II) sulfate pentahydrate (74 mg, 296 µmol, 0.62 eq) in H_2O (500 µL).

Solution A was added to Solution B, the reaction solution was stirred for 20 h and then the milky blue suspension was filtered. The blue precipitate was washed with H_2O/acetone (10 mL, *v:v* 1:1), and resuspended in $H_2O/CHCl_3$ (10 mL, *v:v* 1:1) and, following addition of 8-hydroxyquinoline (155 mg, 1.07 mmol, 2.2 eq), the reaction solution was stirred for 10 min. The green suspension was filtered and the filtrate layers were separated. The aqueous layer was washed with $CHCl_3$ (4 × 5 mL) and was concentrated *in vacuo* then lyophilised. Purification by silica gel chromatography, eluting with H_2O in MeCN (15%), followed by concentration *in vacuo* and lyophilisation afforded the intermediate (20 mg, 14%) as a colourless, fluffy, hygroscopic solid. A solution of the solid (20 mg, 63.0 μmol, 1.0 eq) in 1 M HCl in dioxane (2 mL) was stirred at rt for 14 h. The volatile components were removed *in vacuo* to give a colourless solid that was lyophilised to afford **4.14** (14 mg, 88%) as a colourless, fluffy, hygroscopic solid: R_f (100% H_2O) 0.09; $[\alpha]_D^{24}$ = +2.3 (*c* = 0.53, H_2O); ν_{max} (thin film)/cm^{-1}: 3294 (w), 2938 (br), 1635 (m); ^{1}H NMR (500 MHz, D_2O) δ 3.95 (1H, dd, *J* = 6.2, 6.2 Hz, $C_\alpha H$), 3.17 (2H, t, *J* = 6.7 Hz, CH_2NH_2), 3.13 (2H, dd, *J* = 6.9, 6.9 Hz, $C_\varepsilon H_2$), 2.58 (2H, t, *J* = 6.7 Hz, $CH_2CH_2NH_2$), 1.95-1.79 (2H, m, $C_\beta H_2$), 1.54-1.45 (2H, m, $C_\delta H_2$), 1.45-1.28 (2H, m, $C_\gamma H_2$); ^{13}C NMR (126 MHz, D_2O) δ 172.5 ($C_\alpha HCO$), 172.0 ($C_\varepsilon NHCO$), 53.0 (C_α), 38.8 (C_ε), 35.8 (CH_2NH_2), 32.0 ($CH_2CH_2NH_2$), 29.4 (C_β), 27.7 (C_δ), 21.5 (C_γ); HRMS *m/z* (ES^+) Found: free amine 218.1504. $C_9H_{20}N_3O_3^+$ requires M^+ 218.1499; LRMS *m/z* (ES^+) 218 (free amine, $[M + H]^+$, 100%).

(2*S*)-2-Amino-6-({4-[(*tert*-butoxycarbonyl)amino]butanoyl}amino)hexanoic acid (4.79)



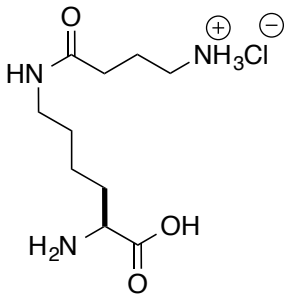
Solution A: To a solution of Boc-γ-aminobutyric acid (200 mg, 984 μmol, 1.1 eq), *N*-hydroxysuccinimide (238 mg, 2.06 mmol, 2.4 eq) and distilled pyridine (320 μL, 312 mg, 3.94 mmol, 4.5 eq) in CH_2Cl_2 (2 mL), was added TFAA (190 μL, 287 mg, 1.37 mmol, 1.6 eq) dropwise at 0 °C. The solution was warmed to rt, and stirred for 1 h before addition of a 1 M aqueous solution of HCl (2 mL) and separation of the layers. The organic phase was washed with a 1 M aqueous solution of HCl (2 mL), a saturated aqueous solution of $NaHCO_3$ (2 mL) then brine (2 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give a residue that was redissolved in acetone (1 mL).

Solution B: To a solution of L-Lys-OH•HCl (160 mg, 876 μmol, 1.0 eq) and $NaHCO_3$ (260 mg, 3.09 mmol, 3.5 eq) in H_2O (1 mL) was added copper(II) sulfate pentahydrate (126 mg, 505 μmol, 0.58 eq) in H_2O (1 mL).

Solution A was added to Solution B, the reaction solution was stirred for 8 h, and the milky blue suspension was filtered. The blue precipitate was washed with H_2O/acetone (10 mL, *v:v* 1:1), resuspended in $H_2O/CHCl_3$ (10 mL, *v:v* 1:1), and, following addition of 8-hydroxyquinoline (238 mg, 1.64 mmol, 1.9 eq), the reaction solution was stirred for 10 min. The green suspension was filtered, and the filtrate layers were separated. The aqueous layer was washed with $CHCl_3$ (4 × 5 mL), concentrated *in vacuo*, then lyophilised. Purification by silica gel chromatography,

eluting with H_2O in MeCN (gradient elution 10 → 30%), followed by concentration *in vacuo* and lyophilisation afforded **4.79** (173 mg, 60%) as a colourless, fluffy, hygroscopic solid: R_f (20% H_2O/MeCN) 0.32; $[\alpha]_D^{24}$ = –1.0 (*c* = 0.49, H_2O); ν_{max} (thin film)/cm^{-1}: 3310 (br), 3067 (br), 2867 (w), 1685 (m), 1637 (m); ^{1}H NMR (400 MHz, D_2O) δ 3.61 (1H, dd, *J* = 6.0, 6.0 Hz, $C_\alpha H$), 3.08 (2H, dd, *J* = 6.7, 6.7 Hz, $C_\varepsilon H_2$), 2.96 (2H, t, *J* = 6.6, 6.6 Hz, $CH_2CH_2NHCO_2$), 2.14 (2H, t, *J* = 7.4 Hz, CH_2NHCO_2), 1.83-1.68 (2H, m, $C_\beta H_2$), 1.69-1.59 (2H, m, $CH_2CH_2CH_2$), 1.51-1.38 (2H, m, $C_\delta H_2$), 1.38-1.20 (11H, m, $C_\gamma H_2$ and $C(CH_3)_3$); ^{13}C NMR (126 MHz, D_2O) δ 176.0 ($NHCOCH_2$), 174.8 (CO_2H), 158.2 ($CO_2C(CH_3)_3$), 80.9 ($C(CH_3)_3$), 54.6 (C_α), 39.3 (CH_2NHCO_2), 38.9 (C_ε), 33.1 ($NHCOCH_2$), 30.1 (C_β), 28.0 (C_δ), 27.6 ($C(CH_3)_3$), 25.4 ($CH_2CH_2CH_2$), 22.7 (C_γ); HRMS *m/z* (ES^+) Found: free amine 354.1991. $C_{15}H_{29}N_3NaO_5^+$ requires M^+ 354.1999; LRMS *m/z* (ES^+) 323 ($[M + H]^+$, 100%).

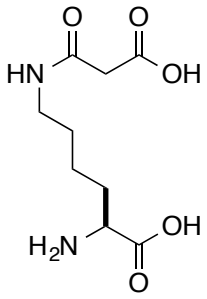
4-{[(5*S*)-5-Amino-5-carboxypentyl]amino}-4-oxobutan-1-aminium chloride (4.15)



A solution of **4.79** (127 mg, 383 μmol, 1.0 eq) in 1 M solution of HCl in dioxane (2.00 mL, 2.10 g, 2.00 mmol, 5.2 eq) was stirred at rt for 62 h. The volatile components were removed *in vacuo* to give a colourless solid that was lyophilised to afford **4.15** (100 mg, 98%) as a colourless, fluffy, hygroscopic solid: R_f (100% H_2O) 0.07; $[\alpha]_D^{24}$ = +8.3 (*c* = 0.52, H_2O); ν_{max} (thin film)/cm^{-1}: 3308 (w), 2947 (br), 1738 (m) (C=O), 1635 (s) (C=O); ^{1}H NMR (500 MHz, D_2O) δ 3.90 (1H, dd, *J* = 6.2, 6.2 Hz, $C_\alpha H$), 3.12 (2H, dd, *J* = 6.8, 6.8 Hz, $C_\varepsilon H_2$), 2.92 (2H, t, *J* = 7.7 Hz, CH_2NH_2), 2.27 (2H,

t, J = 7.5 Hz, CH_2CH$_2$CH$_2$NH$_2$), 1.92-1.77 (4H, m, CH_2CH$_2$NH$_2$ & C$_\beta H_B$), 1.53-1.43 (2H, m, C$_\delta H_2$), 1.43-1.25 (2H, m, C$_\gamma H_2$); ^{13}C NMR (126 MHz, $CDCl_3$): 174.7 (C$_\varepsilon$NH*C*O), 172.8 (C$_\alpha$H*C*O), 53.0 (*C*$_\alpha$), 38.8 (*C*$_\varepsilon$), 38.8 (*C*H$_2$NH$_2$), 32.5 (*C*H$_2$CH$_2$CH$_2$NH$_2$), 29.5 (*C*$_\beta$), 27.8 (*C*$_\delta$), 23.1 (*C*H$_2$CH$_2$NH$_2$), 21.5 (*C*$_\gamma$); HRMS *m/z* (ES$^+$) Found: free amine 232.1646. $C_{10}H_{22}N_3O_3^+$ requires M^+ 232.1656; LRMS *m/z* (ES$^+$) 232 (free amine, $[M + H]^+$, 100%).

(2*S*)-2-Amino-6-[(carboxyacetyl)amino]hexanoic acid (4.3)

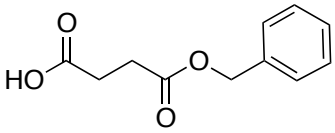


To a solution of monobenzyl malonate (245 mg, 1.26 mmol, 1.0 eq), *N*-hydroxysuccinimide (200 mg, 1.74 mmol, 1.4 eq) and distilled pyridine (330 µL, 322 mg, 4.08 mmol, 3.2 eq) in CH_2Cl_2 (10 mL), was added TFAA (300 µL, 454 mg, 2.16 mmol, 1.7 eq) dropwise at 0 °C. The solution was warmed to rt, and stirred for 1 h before addition of a 1 M aqueous solution of HCl (10 mL), and separation of the layers. The organic phase was washed with a 1 M aqueous solution of HCl (10 mL), a saturated aqueous solution of $NaHCO_3$ (10 mL) then brine (10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding succinate ester **4.53** (233 mg, 80%) as a colourless solid that was used without purification. A solution of **4.53** (233 mg, 800 µmol, 1.0 eq) and Cbz-Lys-OH (291 mg, 1.04 mmol, 1.0 eq) in DMF (2 mL) was stirred at rt for 14 h. The volatile components were removed *in vacuo*, producing a colourless residue that was partitioned between H_2O (10 mL) and EtOAc (10 mL). The layers were separated and the aqueous layer was extracted with

EtOAc (2 × 10 mL). The combined organic layers were washed with H_2O (5 × 30 mL) then brine (30 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding protected lysine **4.55** (327 mg, 72%) as a clear and colourless oil, which was used without purification. A solution of **4.55** (160 mg, 351 μmol, 1.0 eq) in *tert*-butanol and H_2O (30 mL, *v:v* 5:1) was degassed for 5 min, then purged with nitrogen, before addition of palladium black (150 mg, 1.41 mmol, 4.0 eq). The mixture was purged with hydrogen (3 ×) and, after the hydrogen balloon was reattached, was stirred at rt for 16 h. The mixture was filtered through Celite® and washed with Et_2O (100 mL). The filtrate was discarded, and the product was eluted from the filter cake with H_2O (100 mL). The aqueous phase was concentrated *in vacuo* and lyophilised. Purification by silica gel chromatography, eluting with H_2O in MeCN (gradient elution 20 → 30%), followed by treatment with Chelex®, filtration and lyophilisation afforded **4.3** (58 mg, 71%) as a colourless, hygroscopic solid: R_f (RP, 100% H_2O) 0.90; $[\alpha]_D^{25}$ = –4.4 (*c* = 0.75, H_2O); ν_{max} (thin film)/cm^{-1}: 3281 (br), 2935 (w), 1570 (s), 1379 (s); ^{1}H NMR (400 MHz, D_2O) δ 3.58 (1H, dd, *J* = 6.0, 6.0 Hz, $C_\alpha H$), 3.12 (2H, dd, *J* = 6.6, 6.6 Hz, $C_\varepsilon H_2$), 3.07-3.02 (2H, m, CH_2CO_2), 1.88-1.64 (2H, m, $C_\beta H_2$), 1.52-1.37 (2H, m, $C_\delta H_2$), 1.36-1.19 (2H, m, $C_\gamma H_2$); ^{13}C NMR (126 MHz, D_2O) δ 176.5 (NH*C*O or CH_2CO_2*), 175.3 (CH_2CO_2 or NH*C*O*), 171.1 ($C_\alpha CO_2$), 54.9 (C_α), 44.7 (CH_2CO_2), 39.0 (C_ε), 30.8 (C_β), 27.9 (C_δ), 21.8 (C_γ); HRMS *m/z* (ES^-) Found: 231.0989. $C_9H_{15}N_2O_5^-$ requires M^- 231.0986; LRMS *m/z* (ES^-) 113 (plasticiser, 100%), 231 ($[M - H]^-$, 80%).

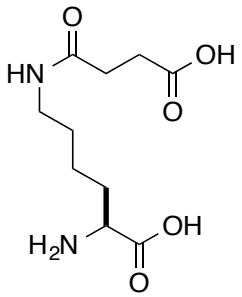
* Could not distinguish between carbonyl carbon peaks despite the use of HMBC.

4-(Benzyloxy)-4-oxobutanoic acid (4.47)[223,234,235]



A solution of succinic anhydride (100 mg, 1.00 mmol, 1.0 eq) in CH_2Cl_2 (1 mL) was added dropwise at 0 °C to a solution of benzyl alcohol (125 µL, 131 mg, 1.21 mmol, 1.2 eq), distilled NEt_3 (250 µL, 182 mg, 1.80 mmol, 1.8 eq) and DMAP (6 mg, 50 µmol, 0.05 eq) in CH_2Cl_2 (2 mL). The solution was warmed to rt, stirred for 25 h, was washed with a 1 M aqueous solution of HCl (2 × 5 mL), then extracted with a saturated aqueous solution of $NaHCO_3$ (3 × 5 mL). The combined basic aqueous layers were reacidified to pH 1-2 with conc. HCl and extracted with CH_2Cl_2 (4 × 20 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding **4.47** (170 mg, 82%) as a colourless solid: R_f (1% AcOH/EtOAc) 0.67; mp 56-57 °C (from CH_2Cl_2) [lit. mp 56-57 °C[234]]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.30 (5H, m, C_6H_5), 5.16 (2H, s, CH_2Ph), 2.75-2.66 (4H, m, CH_2CH_2); LRMS *m/z* (ES^+) 231 ($[M + Na]^+$, 100%), 461 ($[2M + Na]^+$, 90%), 691 ($[3M + Na]^+$, 47%). Data are in good agreement with literature values.[234,235]

(2*S*)-2-Amino-6-[(3-carboxypropanyl)amino]hexanoic acid (4.4)

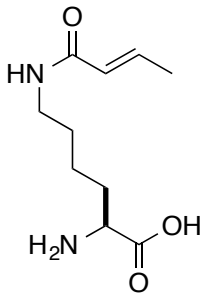


To a solution of **4.47** (100 mg, 480 µmol, 1.0 eq), *N*-hydroxysuccinimide (116 mg, 1.01 mmol, 2.1 eq) and distilled pyridine (155 µL, 152 mg, 1.92 mmol,

4.0 eq) in CH_2Cl_2 (3 mL), was added TFAA (140 µL, 212 mg, 1.01 mmol, 2.1 eq) dropwise at 0 °C. The solution was warmed to rt and stirred for 1 h before addition of a 1 M aqueous solution of HCl (3 mL), and separation of the layers. The organic phase was washed with a 1 M aqueous solution of HCl (3 mL), a saturated aqueous solution of $NaHCO_3$ (3 mL) then brine (3 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding succinate ester **4.48** (136 mg, 93%) as a colourless solid that was used without purification. A solution of **4.48** (116 mg, 380 µmol, 1.0 eq) and Cbz-Lys-OH (138 mg, 494 µmol, 1.3 eq) in DMF (3 mL) was stirred at rt for 23 h. The volatile components were removed *in vacuo* producing a colourless residue that was partitioned between H_2O (5 mL) and EtOAc (5 mL). The aqueous and organic layers were separated and the aqueous layer was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with H_2O (5 × 15 mL) then brine (15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding protected lysine **4.49** (175 mg, 98%), as a colourless oil, which was used without purification. A solution of **4.49** (43 mg, 90.3 µmol, 1.0 eq) in *tert*-butanol and H_2O (7.0 mL, *v:v* 6:1) was degassed for 5 min, then purged with nitrogen, before addition of palladium black (46 mg, 432 µmol, 4.8 eq). The mixture was purged with hydrogen (3 ×) and, after the hydrogen balloon was reattached, was stirred at rt for 15 h. The mixture was filtered through Celite® and washed with Et_2O (200 mL). The filtrate was discarded, and the product was eluted from the filter cake with H_2O (150 mL). The aqueous phase was concentrated *in vacuo* and lyophilised to yield **4.4** (22 mg, 99%) as a colourless, fluffy, hygroscopic solid: R_f (RP, 100% H_2O) 0.97; $[\alpha]_D^{25}$ = –4.2 (*c* = 1.13, H_2O); ν_{max} (thin film)/cm^{-1}: 2987 (s), 2888 (m), 1465 (w), 1387 (m); ^{1}H NMR (400 MHz, D_2O) δ 3.55-3.45 (1H, m, $C_\alpha H$), 3.07 (2H, dd, *J* = 6.7, 6.7 Hz, $C_\varepsilon H_2$), 2.34-2.30 (4H, m, $CO_2CH_2CH_2CO_2$), 1.78-1.59 (2H, m, $C_\beta H_2$), 1.48-1.36 (2H, m, $C_\delta H_2$), 1.35-1.18 (2H, m, $C_\gamma H_2$); ^{13}C NMR (126 MHz, D_2O) δ 181.2 ($NHCOCH_2CH_2CO_2H$), 176.8 ($C_\alpha CO_2H$), 175.8 ($C_\varepsilon NHCO$), 54.9 (C_α), 38.9 (C_ε), 33.1

(CO*C*H$_2$CH$_2$*C*O$_2$H or COCH$_2$*C*H$_2$*C*O$_2$H), 32.4 (COCH$_2$*C*H$_2$*C*O$_2$H or CO*C*H$_2$CH$_2$*C*O$_2$H), 30.9 (C_β), 28.0 (C_δ), 21.8 (C_γ); HRMS *m/z* (ES$^+$) Found: 269.1097. $C_{10}H_{18}N_2NaO_5$ requires M^+ 269.1108; LRMS *m/z* (ES$^+$) 245 ([M – Na]$^-$, 100%).

(2*S*)-2-Amino-6-[(2*E*)-but-2-enoylamino]hexanoic acid (4.15)[193,236]



To a solution of crotonic acid (100 mg, 1.16 mmol, 1.0 eq), *N*-hydroxysuccinimide (280 mg, 2.44 mmol, 2.1 eq) and distilled pyridine (375 µL, 367 mg, 4.65 mmol, 4.0 eq) in CH_2Cl_2 (5 mL), TFAA (340 µL, 514 mg, 2.45 mmol, 2.1 eq) was added dropwise at 0 °C. The solution was warmed to rt and stirred for 1 h before addition of a 1 M aqueous solution of HCl (5 mL), and separation of the layers. The organic phase was washed with a 1 M aqueous solution of HCl (5 mL), a saturated aqueous solution of $NaHCO_3$ (5 mL) then brine (5 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding succinate ester **4.52** (202 mg, 95%) as a colourless solid that was used without purification. A solution of **4.52** (170 mg, 923 µmol, 1.0 eq) and Cbz-Lys-OH (336 mg, 1.20 mmol, 1.3 eq) in DMF (3 mL) was stirred at rt for 112 h. The volatile components were removed *in vacuo*, producing a colourless residue that was partitioned between H_2O (10 mL) and EtOAc (10 mL). The aqueous and organic layers were separated and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with H_2O (5 × 30 mL) then brine (30 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* yielding protected lysine **4.54** (238 mg, 74%), as a

colourless oil, which was used without purification. A solution of **4.54** (25 mg, 71.8 µmol, 1.0 eq) in a 33% (*w/v*) solution of hydrobromic acid in acetic acid (2.5mL, 3.50 g, 43.3 mmol, 49 eq) was stirred at rt for 3 h, before addition of H_2O (10 mL). The aqueous phase was washed with EtOAc (5 × 15 mL), concentrated *in vacuo* and lyophilised producing an orange residue. Purification using an acidic Dowex ion exchange column, washing with H_2O and eluting with 1 M NH_4OH, followed by lyophilisation yielded **4.5** (15 mg, 98%) as a colourless, hygroscopic solid. The characterisation data were identical to those shown below.

Alternative procedure:

Solution A: To a solution of crotonic acid (89 mg, 1.03 mmol, 1.0 eq), *N*-hydroxysuccinimide (248 mg, 2.15 mmol, 2.1 eq) and distilled pyridine (330 µL, 323 mg, 4.08 mmol, 4.0 eq) in CH_2Cl_2 (2 mL), was added TFAA (300 µL, 453 mg, 2.16 mmol, 2.1 eq) dropwise at 0 °C. The solution was warmed to rt and stirred for 3 h before addition of a 1 M aqueous solution of HCl (2 mL), and separation of the layers. The organic phase was washed with a 1 M aqueous solution of HCl (2 mL), a saturated aqueous solution of $NaHCO_3$ (2 mL) then brine (2 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give a residue that was redissolved in acetone (500 µL).

Solution B: To a solution of L-Lys-OH•HCl (195 mg, 1.07 mmol, 1.0 eq) and $NaHCO_3$ (320 mg, 3.81 mmol, 3.7 eq) in H_2O (1 mL) was added copper(II) sulfate pentahydrate (125 mg, 501 µmol, 0.5 eq) in H_2O (500 µL).

Solution A was added to Solution B, the reaction solution was stirred for 5 h and the milky blue suspension was filtered. The blue precipitate was washed with a mixture of H_2O and acetone (5 mL, *v:v* 1:1), was resuspended in a mixture of H_2O and $CHCl_3$ (5 mL, *v:v* 1:1) and, following addition of 8-hydroxyquinoline (245 mg, 1.69 mmol, 1.6 eq), the reaction solution was stirred for 10 min. The green

suspension was filtered and the filtrate layers were separated. The aqueous layer was washed with $CHCl_3$ (4 × 5 mL) and was concentrated *in vacuo* then lyophilised. Purification by silica gel chromatography, eluting with 20% H_2O in MeCN, followed by concentration *in vacuo* and lyophilisation afforded **4.5** (57 mg, 26%) as a colourless, hygroscopic solid: R_f (RP, 100% H_2O) 0.71; $[\alpha]_D^{25}$ = –10.3 (*c* = 0.34, H_2O); ν_{max} (thin film)/cm^{-1}: 3312 (m) (N–H), 3066 (br) (O–H), 2947 (m), 2872 (m), 1675 (m) (C=C), 1626 (s) (C=O); ^{1}H NMR (400 MHz, D_2O) δ 6.65 (1H, dt, *J* = 15.4, 6.8 Hz, C*H*CH_3), 5.85 (1H, dd, *J* = 15.4, 1.1 Hz, C*H*CON), 3.61 (1H, dd, *J* = 5.9, 5.9 Hz, $C_\alpha H$), 3.14 (2H, dd, *J* = 6.4, 6.4 Hz, $C_\varepsilon H_2$), 1.85-1.68 (2H, m, $C_\beta H_2$), 1.73 (3H, dd, *J* = 6.8, 1.1 Hz, CH_3), 1.52-1.41 (2H, m, $C_\delta H_2$), 1.38-1.20 (2H, m, $C_\gamma H_2$); ^{13}C NMR (101 MHz, D_2O) δ 175.1 (*C*O_2H or NH*C*O*), 169.3 (NH*C*O or *C*O_2H*), 142.0 (*C*HCON), 124.1 (*C*HCH_3), 55.0 (C_α), 39.2 (C_ε), 30.4 (C_β), 28.4 (C_δ), 22.1 (C_γ), 17.5 (*C*H_3); HRMS *m/z* (ES^+) Found: 237.1209. $C_{10}H_{18}N_2NaO_3^+$ requires M^+ 237.1210; LRMS *m/z* (ES^+) 215 ($[M + H]^+$, 21%), 237 ($[M + Na]^+$, 100%). Data are in good agreement with literature values.[193,236]

* Could not distinguish between carbonyl carbon peaks despite the use of HMBC.

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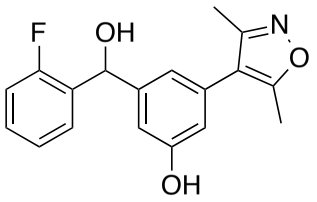
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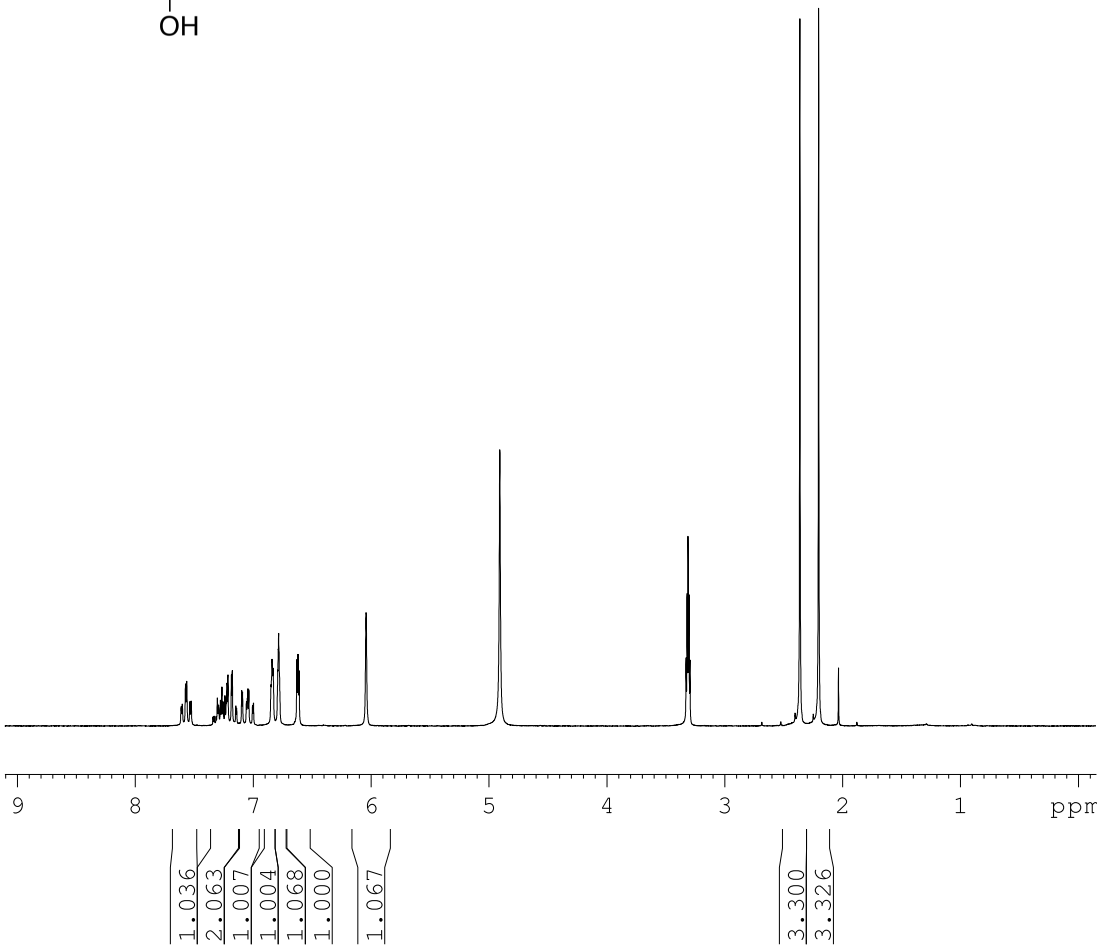
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Selected NMR Spectra

^{1}H, ^{13}C and (where appropriate) ^{19}F NMR spectra are reported for all novel compounds. Spectra are presented in the order in which the compounds appear in Chapter 6: Experimental Methods.

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(2-fluorophenyl)(hydroxyl)methyl]phenol (2.9)





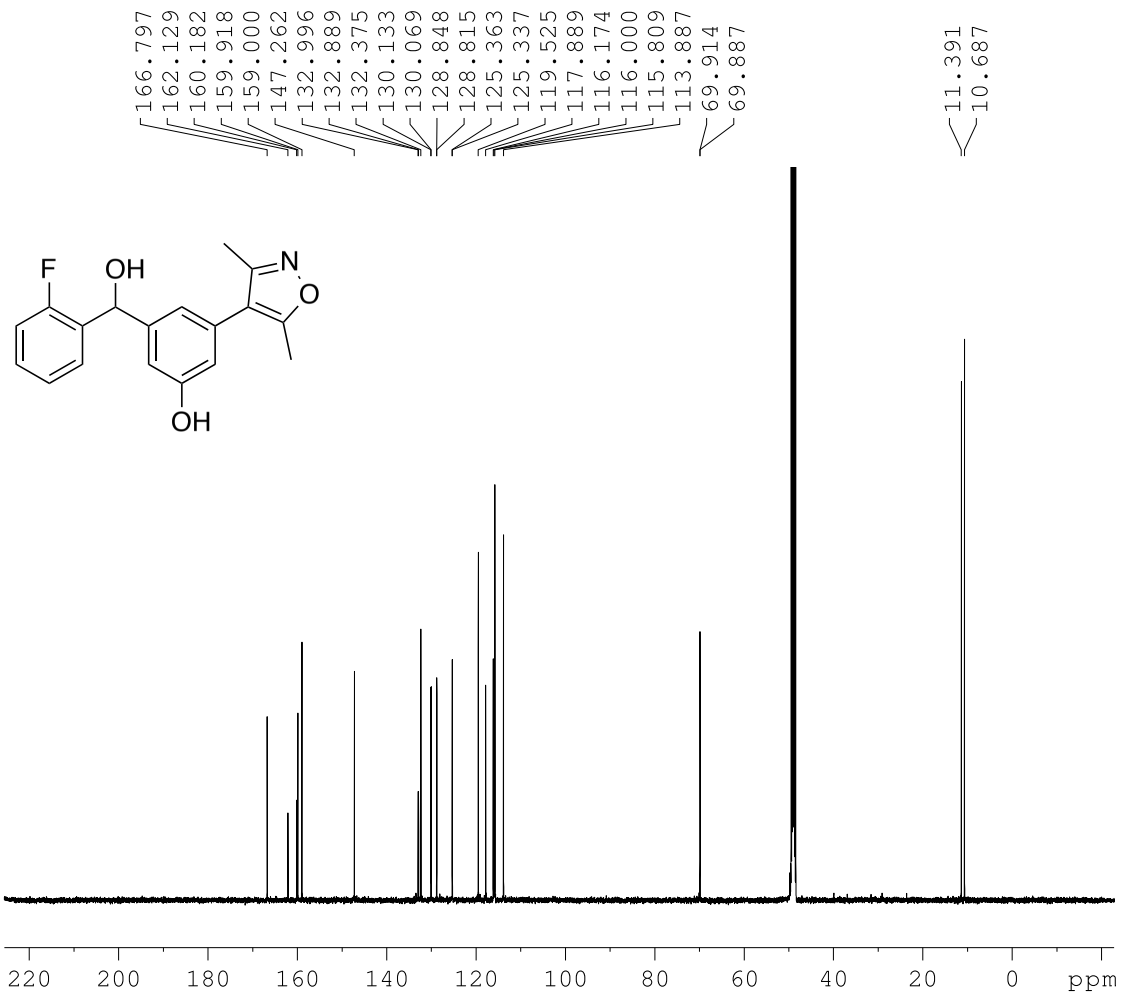

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3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(2-fluorophenyl)(hydroxyl)methyl]phenol (2.9)




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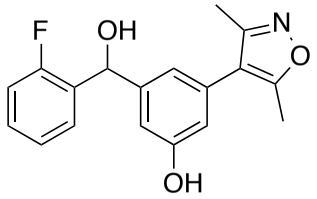
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3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(2-fluorophenyl)(hydroxyl)methyl]phenol (2.9)




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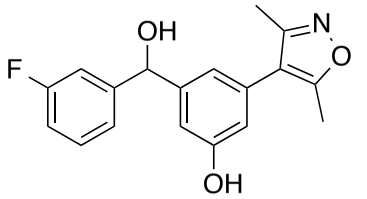
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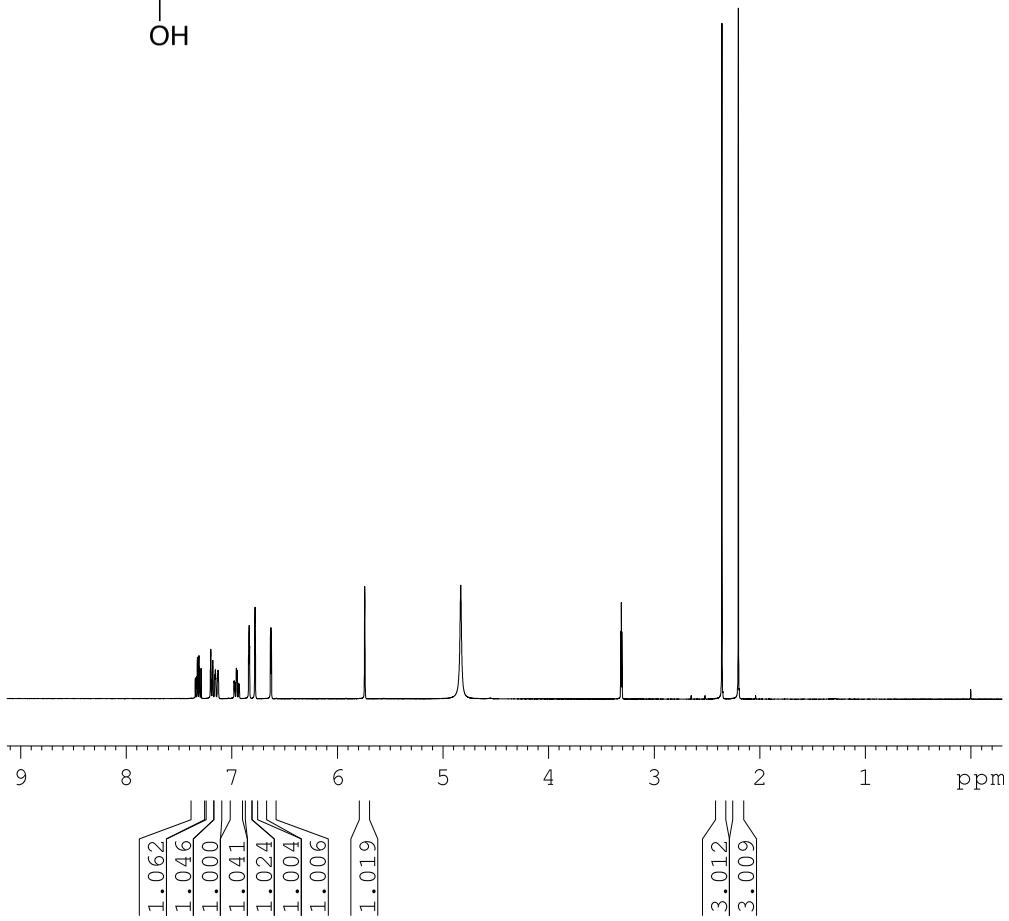
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3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(3-fluorophenyl)(hydroxyl)methyl]phenol (2.10)





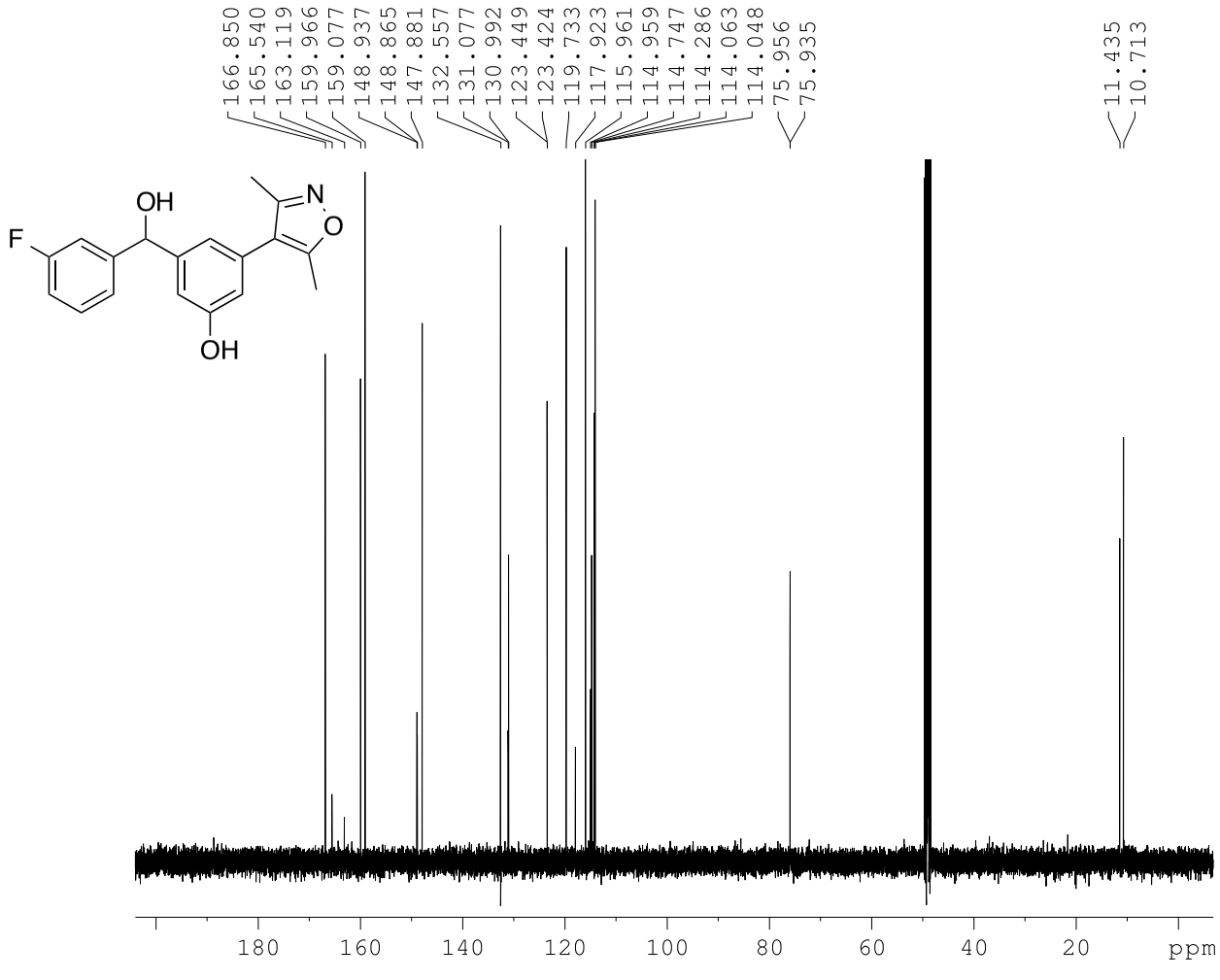

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3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(3-fluorophenyl)(hydroxyl)methyl]phenol (2.10)




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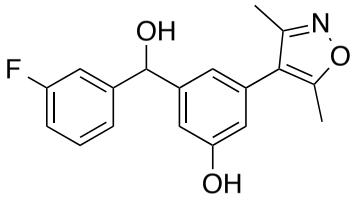
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D11          0.03000000 sec
TD0                   1

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P1                  8.65 usec
PL1                -2.00 dB
PL1W        24.80207634 W
SFO1        100.6248425 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             75.00 usec
PL2               -6.00 dB
PL12               6.50 dB
PL13               8.50 dB
PL2W        49.59999847 W
PL12W        2.78921294 W
PL13W        1.75987446 W
SFO2        400.1316005 MHz

F2 - Processing parameters
SI                32768
SF          100.6126261 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(3-fluorophenyl)(hydroxyl)methyl]phenol (2.10)




```
Current Data Parameters
NAME          N28992-52
EXPNO                15
PROCNO                1

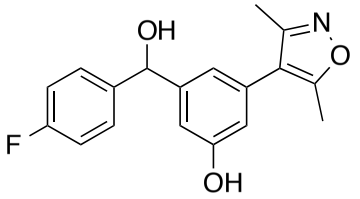
F2 - Acquisition Parameters
Date_          20130828
Time              10.15
INSTRUM            S400
PROBHD   5 mm PABBO BB/
PULPROG          zgdc30
TD               131072
SOLVENT             MeOD
NS                    32
DS                     2
SWH          113636.367 Hz
FIDRES         0.866977 Hz
AQ            0.5767168 sec
RG                191.6
DW                4.400 usec
DE                6.50 usec
TE                297.4 K
D1           3.00000000 sec
D11          0.03000000 sec
TD0                   1

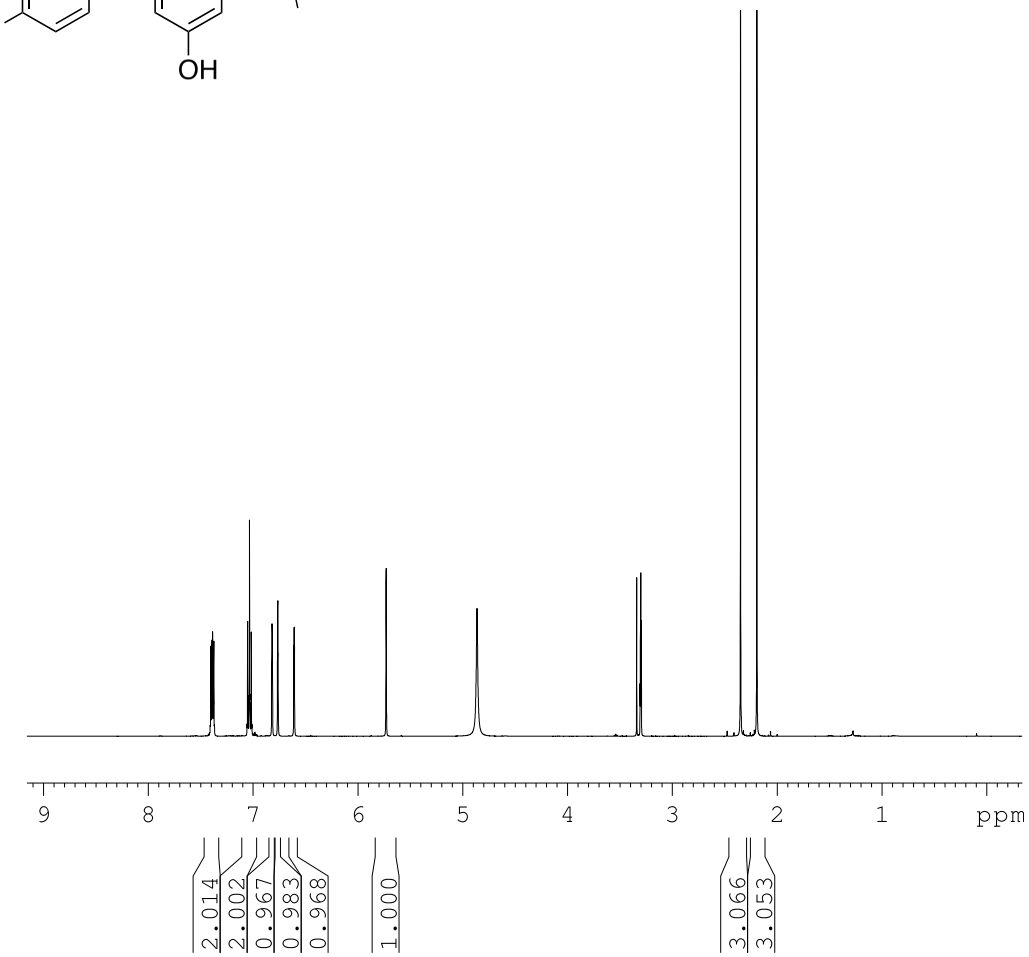
======== CHANNEL f1 ========
NUC1                 19F
P1                12.40 usec
PLW1         30.97400093 W
SFO1         376.4889442 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PLW2         16.98200035 W
PLW12         0.31623000 W
SFO2         400.1632010 MHz

F2 - Processing parameters
SI                131072
SF           376.5265940 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                 1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-fluorophenyl)(hydroxyl)methyl]phenol (2.11)





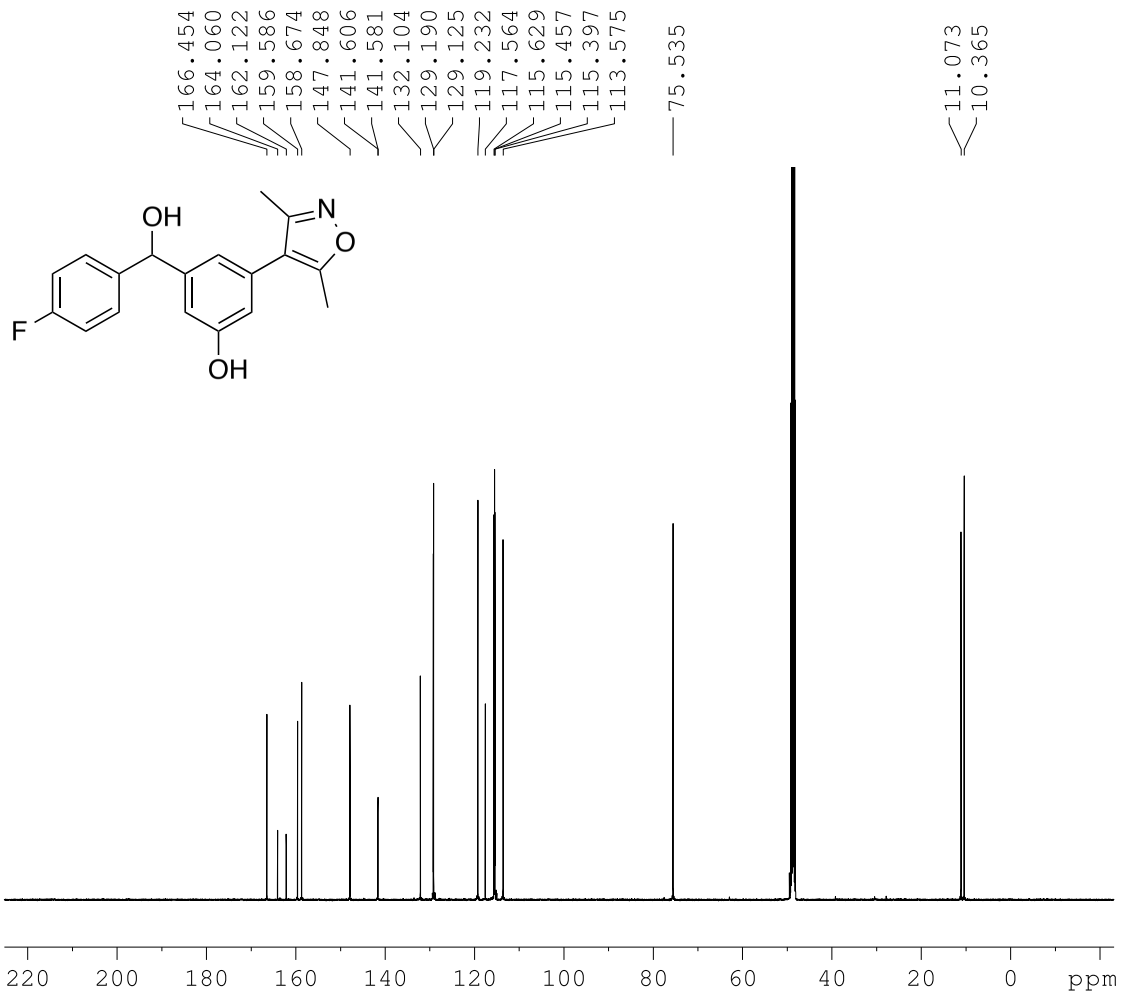

```
Current Data Parameters
NAME      AH043 service
EXPNO                1
PROCNO               1

F2 - Acquisition Parameters
Date_          20131002
Time              20.25
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT             MeOD
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                   4.5
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1                1H
P1                15.00 usec
PLW1         7.99830008 W

F2 - Processing parameters
SI                 32768
SF          500.3000151 MHz
WDW                   EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-fluorophenyl)(hydroxyl)methyl]phenol (2.11)




```
Current Data Parameters
NAME       AH043 service
EXPNO                 4
PROCNO                1

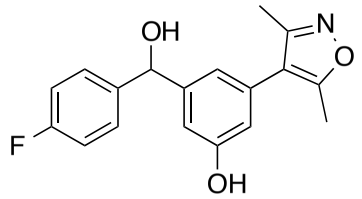
F2 - Acquisition Parameters
Date_          20131002
Time              22.33
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 2048
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                  912
DW               16.000 usec
DE               18.00 usec
TE               298.1 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        125.8131151 MHz
NUC1                13C
P1                10.00 usec
PLW1        20.18400002 W

======== CHANNEL f2 ========
SFO2        500.3020012 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         7.99830008 W
PLW12        0.28119001 W
PLW13        0.17996000 W

F2 - Processing parameters
SI                 32768
SF          125.8004050 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-fluorophenyl)(hydroxyl)methyl]phenol (2.11)




```
Current Data Parameters
NAME       AH043 AVF400
EXPNO                 5
PROCNO                1

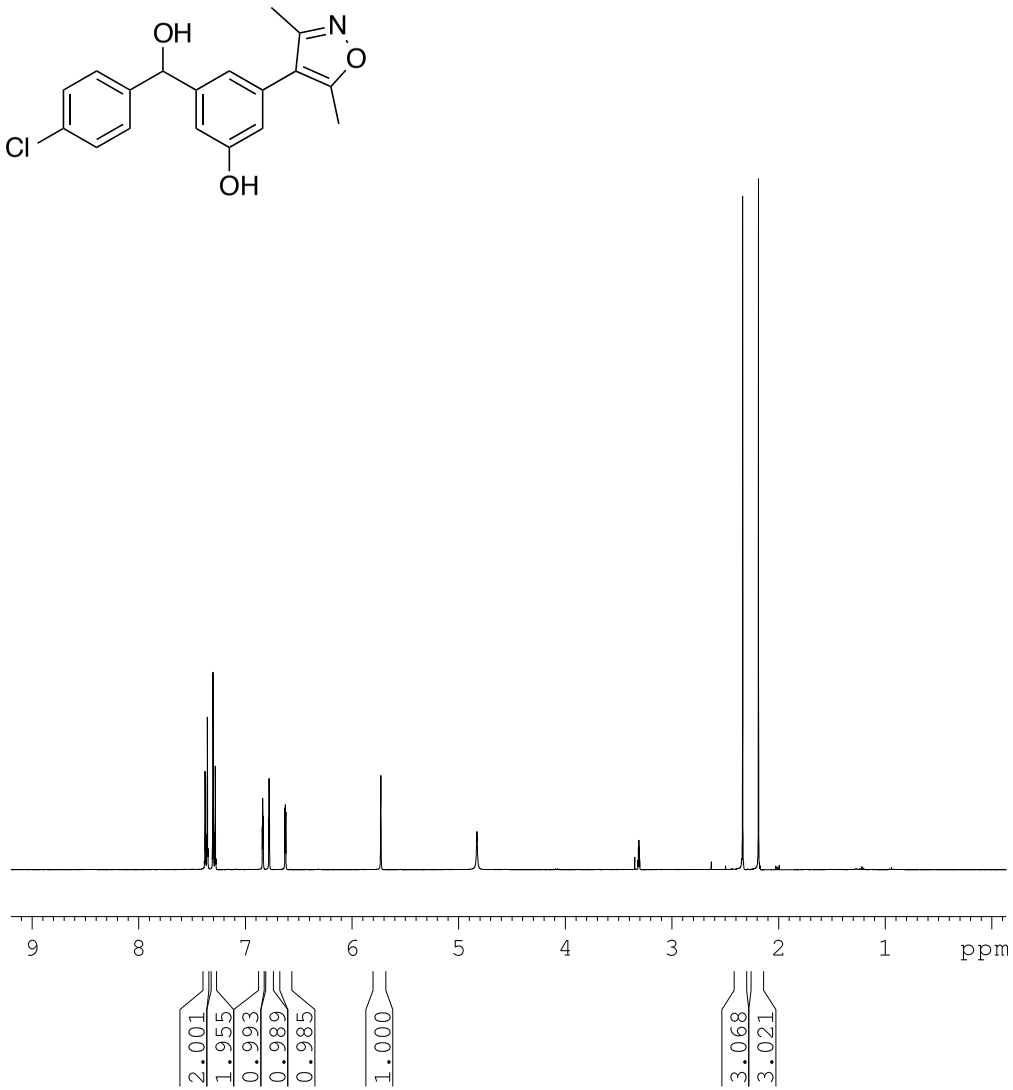
F2 - Acquisition Parameters
Date_          20130930
Time              22.26
INSTRUM          avn400
PROBHD   5 mm PABBO BB/
PULPROG        zgfhigqn
TD               131072
SOLVENT             MeOD
NS                   256
DS                     4
SWH           75000.000 Hz
FIDRES         0.572205 Hz
AQ            0.8738133 sec
RG               205.43
DW                6.667 usec
DE                 6.50 usec
TE                295.2 K
D1           1.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        376.5547873 MHz
NUC1                  19F
P1                 13.50 usec
PLW1        19.00000000 W

======== CHANNEL f2 ========
SFO2        400.2516010 MHz
NUC2                  1H
CPDPRG[2         waltz16
PCPD2              90.00 usec
PLW2        16.70000076 W
PLW12        0.32991999 W

F2 - Processing parameters
SI                 65536
SF          376.6112790 MHz
WDW                   EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-chlorophenyl)(hydroxy)methyl]phenol (2.12)



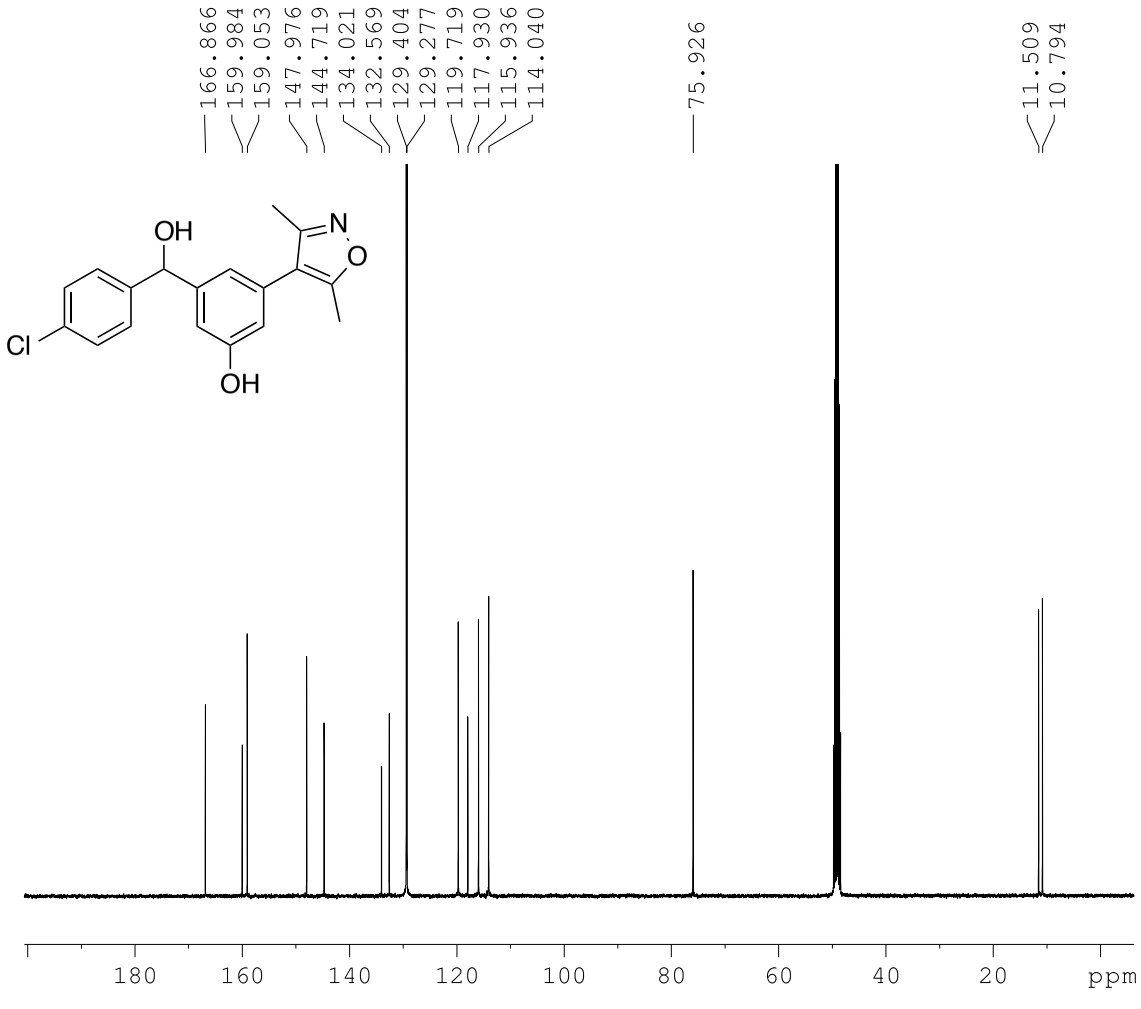

```
Current Data Parameters
NAME           N28992-54
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130823
Time                1.01
INSTRUM             w400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH              8278.146 Hz
FIDRES           0.126314 Hz
AQ               3.9583745 sec
RG                  71.8
DW                60.400 usec
DE                 6.50 usec
TE                303.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.10 usec
PL1                -6.00 dB
PL1W         49.59999847 W
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300075 MHz
WDW                   no
SSB       0
LB        0 Hz
GB        0
PC                  1.50
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-chlorophenyl)(hydroxy)methyl]phenol (2.12)




```
Current Data Parameters
NAME          N28992-54
EXPNO                11
PROCNO                1

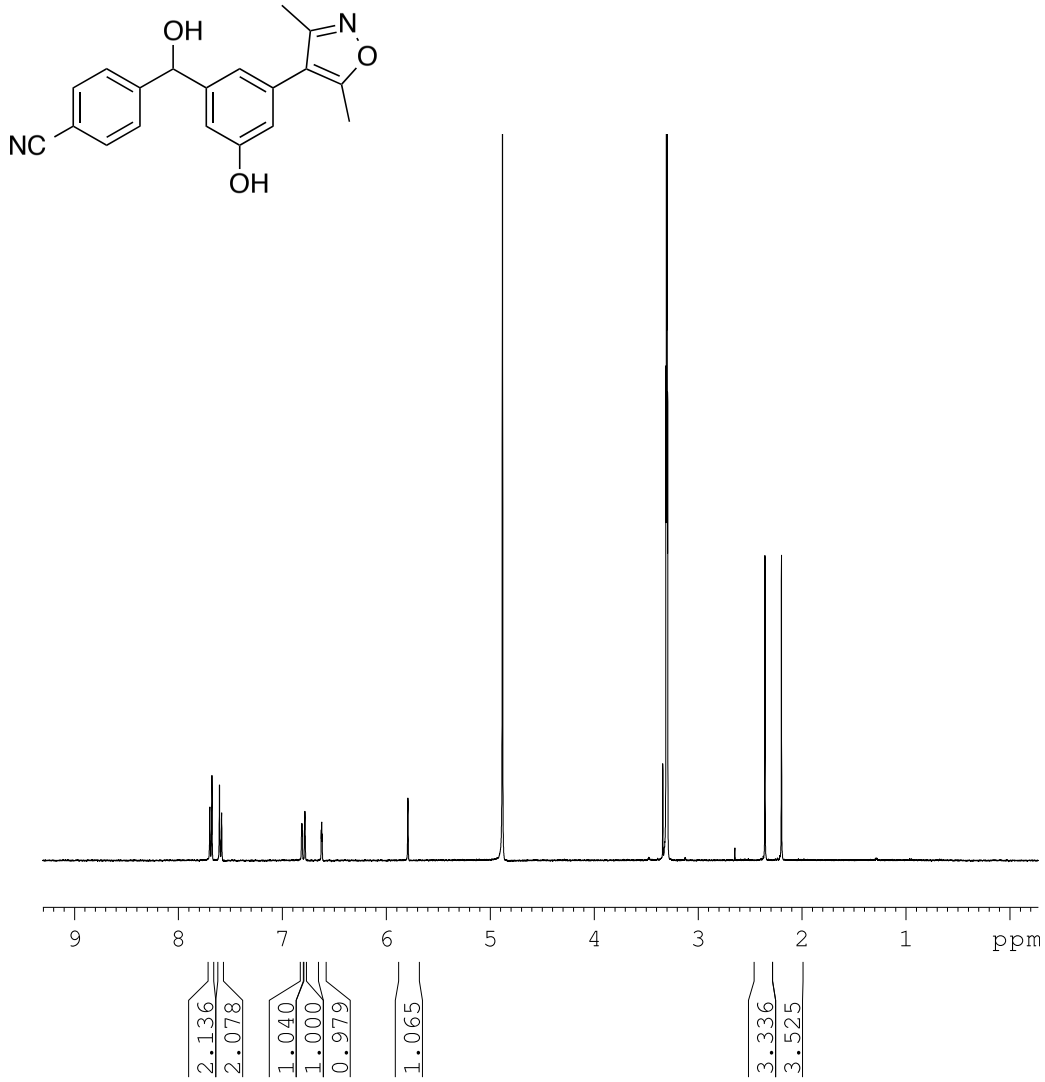
F2 - Acquisition Parameters
Date_          20130823
Time               2.21
INSTRUM            w400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                65536
SOLVENT            MeOD
NS                 2048
DS                    4
SWH           26178.010 Hz
FIDRES         0.399445 Hz
AQ            1.2517376 sec
RG                 4096
DW               19.100 usec
DE                6.50 usec
TE                303.0 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  8.65 usec
PL1                -2.00 dB
PL1W        24.80207634 W
SFO1        100.6248425 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             75.00 usec
PL2                -6.00 dB
PL12                6.50 dB
PL13                8.50 dB
PL2W        49.59999847 W
PL12W        2.78921294 W
PL13W        1.75987446 W
SFO2        400.1316005 MHz

F2 - Processing parameters
SI                 32768
SF          100.6126261 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

3-{[3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-hydroxyphenyl](hydroxyl)methyl}benzonitrile (2.13)



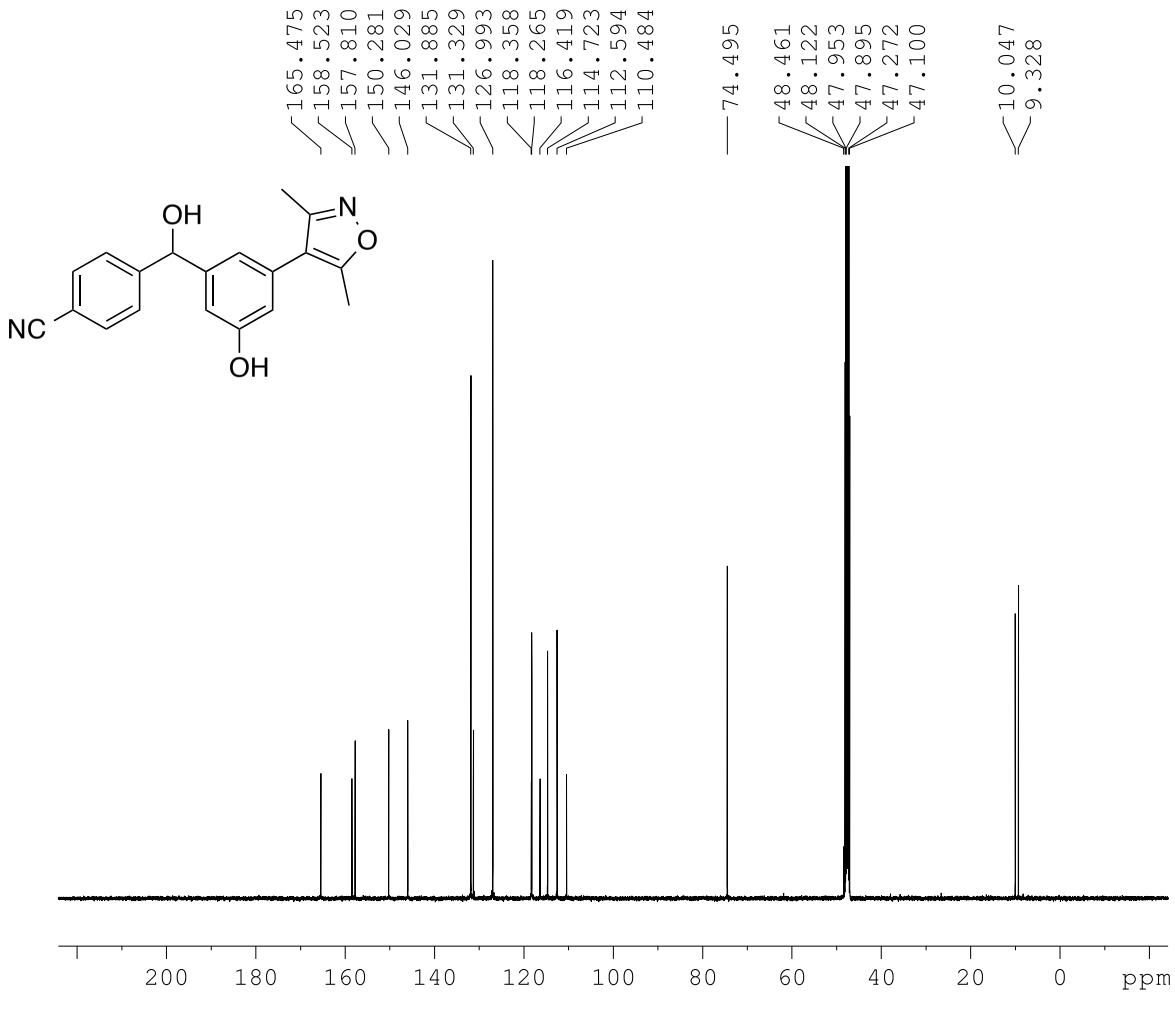

```
Current Data Parameters
NAME      LJ363 fra 1-4
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20131111
Time              10.06
INSTRUM          avg400
PROBHD   5 mm QNP 1H/13
PULPROG            zg30
TD                65536
SOLVENT            MeOD
NS                   16
DS                    2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ            3.2767999 sec
RG               647.17
DW               50.000 usec
DE                6.50 usec
TE                294.3 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        400.2024714 MHz
NUC1                1H
P1                12.23 usec
PLW1        11.30000019 W

F2 - Processing parameters
SI                65536
SF          400.2000151 MHz
WDW                  EM
SSB      0
LB                0.30 Hz
GB       0
PC                1.00
```

3-{[3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-hydroxyphenyl](hydroxyl)methyl}benzonitrile (2.13)




```
Current Data Parameters
NAME       LJ363 service
EXPNO                  3
PROCNO                 1

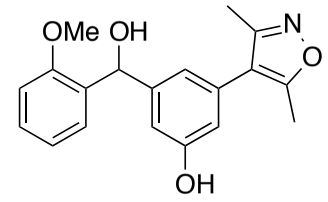
F2 - Acquisition Parameters
Date_           20131113
Time               15.02
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                   512
DS                     2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                   912
DW              16.000 usec
DE              18.00 usec
TE                298.1 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1

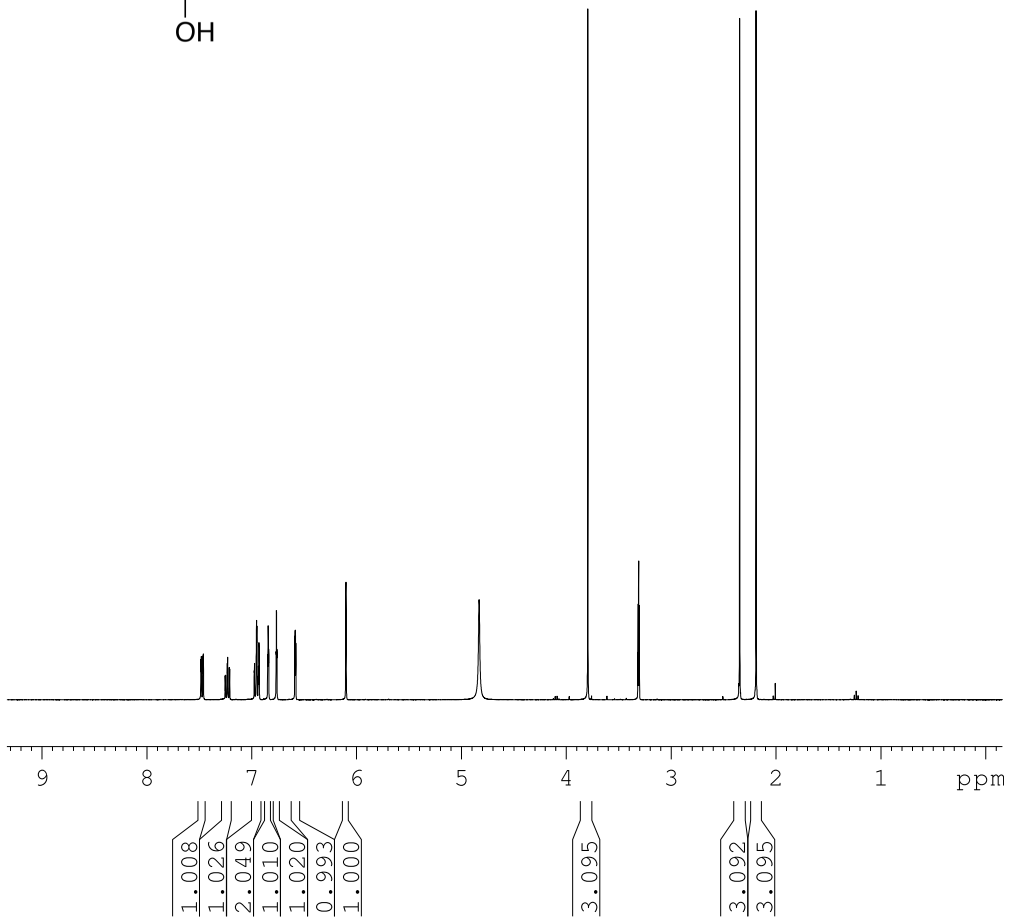
======== CHANNEL f1 ========
SFO1        125.8131151 MHz
NUC1                  13C
P1                10.00 usec
PLW1        20.18400002 W

======== CHANNEL f2 ========
SFO2        500.3020012 MHz
NUC2                  1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                 32768
SF          125.8005350 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(2-methyoxyphenyl)methyl]phenol (2.14)





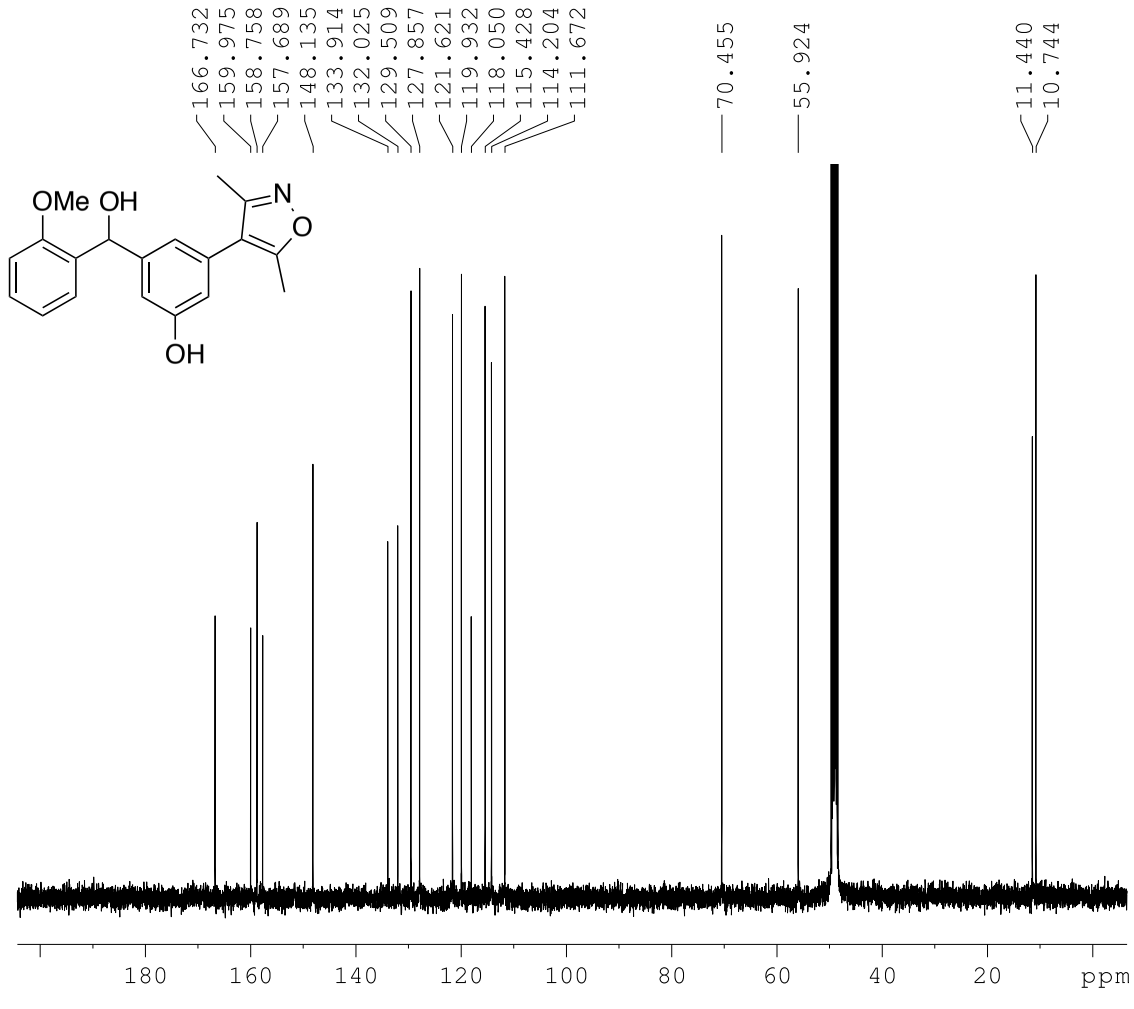

```
Current Data Parameters
NAME          N28992-53
EXPNO                10
PROCNO                1

F2 - Acquisition Parameters
Date_          20130819
Time              19.06
INSTRUM            w400
PROBHD   5 mm QNP 1H/13
PULPROG            zg30
TD                65536
SOLVENT            MeOD
NS                   16
DS                    2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ              3.9583745 sec
RG                  128
DW                60.400 usec
DE                 6.50 usec
TE                303.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                11.10 usec
PL1               -6.00 dB
PL1W        49.59999847 W
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                32768
SF          400.1300075 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                 1.50
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(2-methyoxyphenyl)methyl]phenol (2.14)




```
Current Data Parameters
NAME          N28992-53
EXPNO                11
PROCNO                1

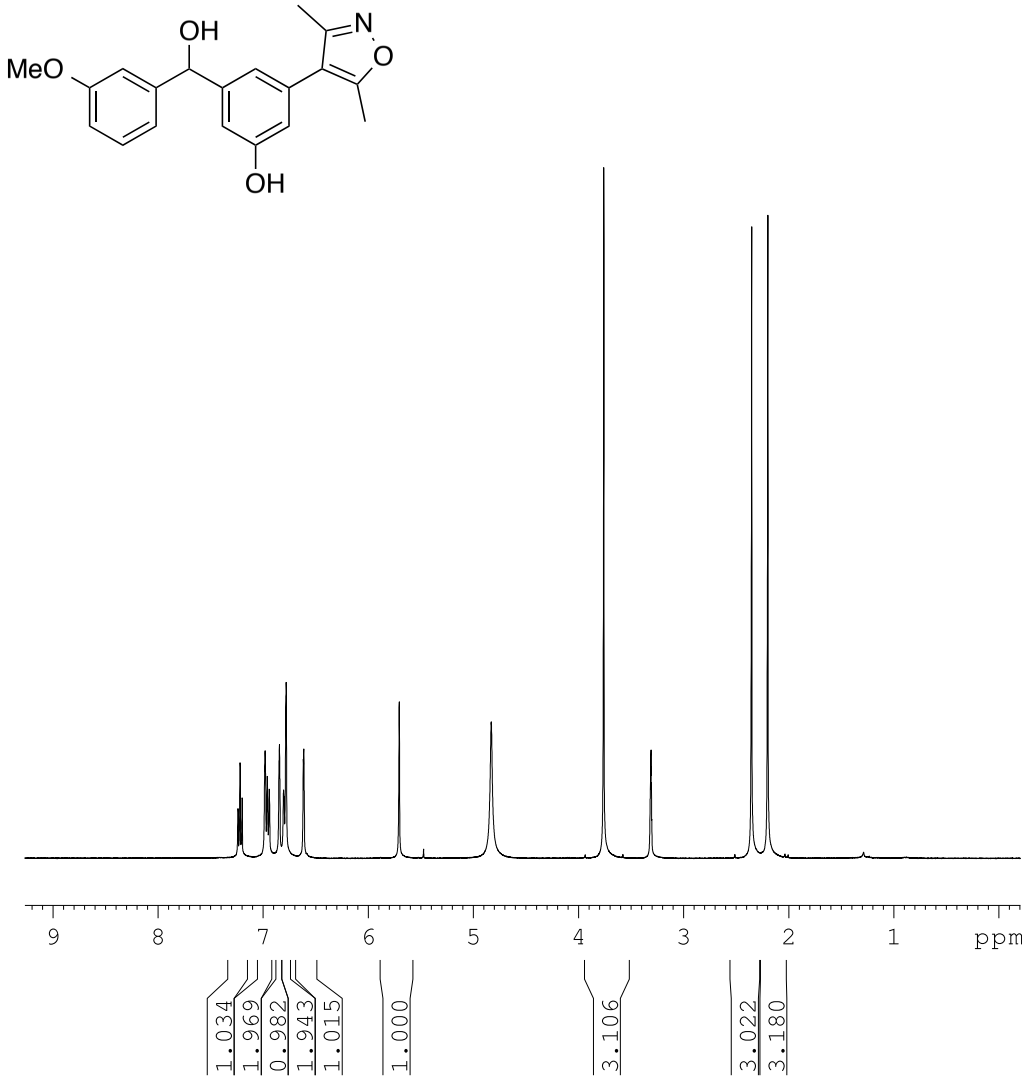
F2 - Acquisition Parameters
Date_          20130819
Time              20.27
INSTRUM            w400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                65536
SOLVENT            MeOD
NS                 2048
DS                    4
SWH           26178.010 Hz
FIDRES         0.399445 Hz
AQ            1.2517376 sec
RG                 4096
DW               19.100 usec
DE                6.50 usec
TE                303.0 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  8.65 usec
PL1                -2.00 dB
PL1W        24.80207634 W
SFO1        100.6248425 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             75.00 usec
PL2                -6.00 dB
PL12                6.50 dB
PL13                8.50 dB
PL2W        49.59999847 W
PL12W        2.78921294 W
PL13W        1.75987446 W
SFO2        400.1316005 MHz

F2 - Processing parameters
SI                 32768
SF          100.6126261 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(3-methyoxyphenyl)methyl]phenol (2.15)



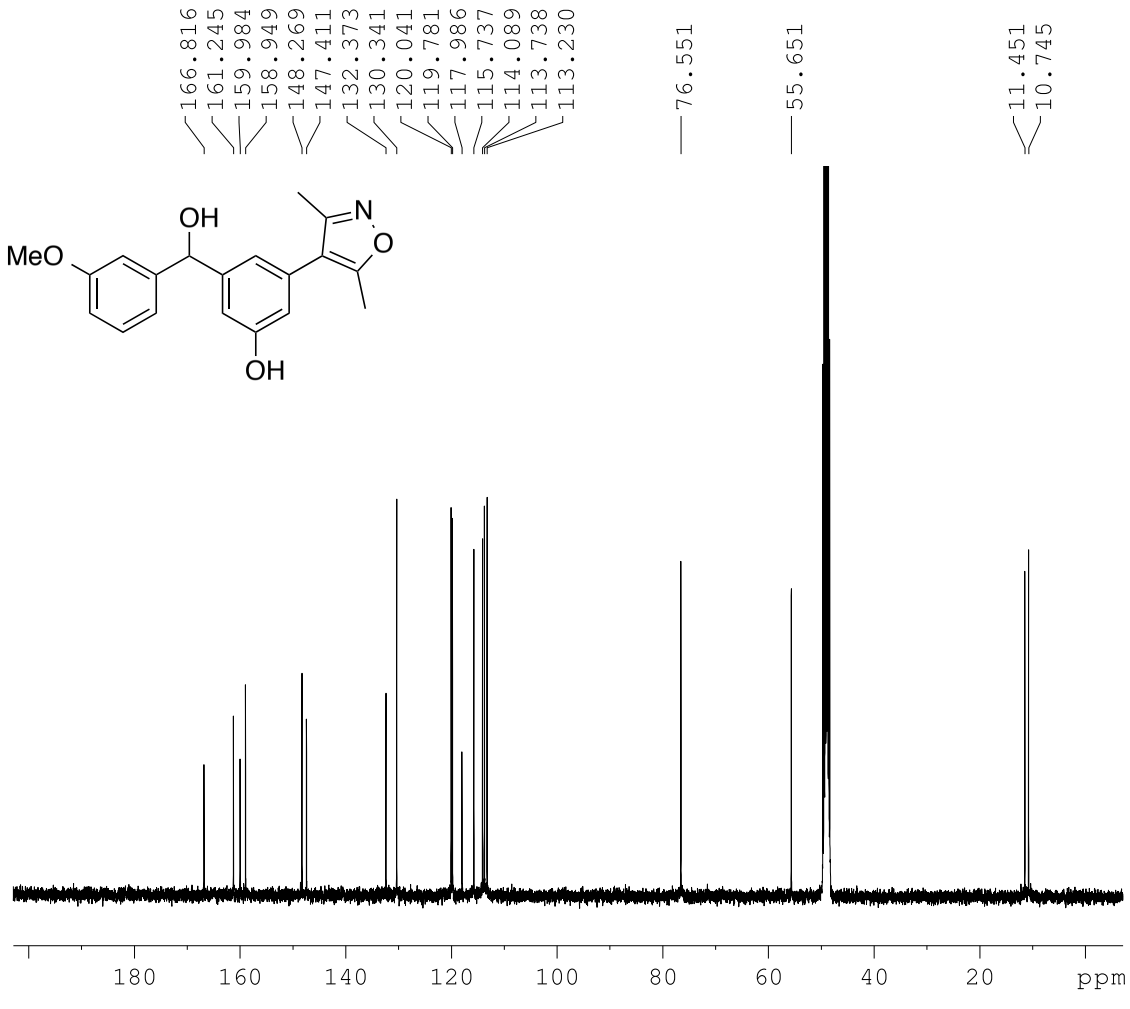

```
Current Data Parameters
NAME          N28992-55
EXPNO                10
PROCNO                1

F2 - Acquisition Parameters
Date_          20130821
Time              20.37
INSTRUM            w400
PROBHD   5 mm QNP 1H/13
PULPROG            zg30
TD                65536
SOLVENT            MeOD
NS                   16
DS                    2
SWH            8278.146 Hz
FIDRES         0.126314 Hz
AQ            3.9583745 sec
RG                  128
DW                60.400 usec
DE                 6.50 usec
TE                303.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                11.10 usec
PL1               -6.00 dB
PL1W        49.59999847 W
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                32768
SF          400.1300075 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                 1.50
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(3-methyoxyphenyl)methyl]phenol (2.15)




```
Current Data Parameters
NAME          N28992-55
EXPNO                11
PROCNO                1

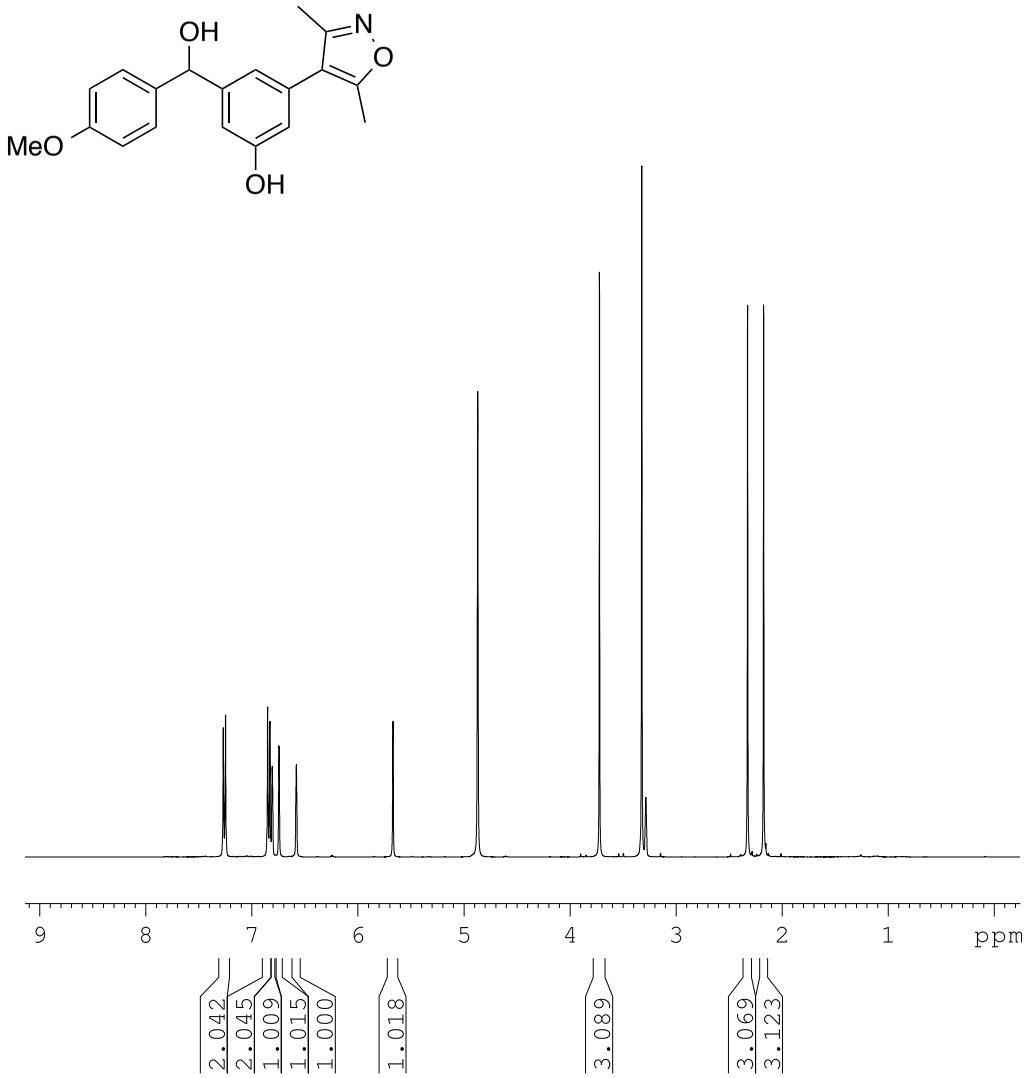
F2 - Acquisition Parameters
Date_          20130821
Time              21.57
INSTRUM             w400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 2048
DS                    4
SWH           26178.010 Hz
FIDRES         0.399445 Hz
AQ            1.2517376 sec
RG                 4096
DW               19.100 usec
DE                6.50 usec
TE                303.0 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  8.65 usec
PL1                -2.00 dB
PL1W        24.80207634 W
SFO1        100.6248425 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             75.00 usec
PL2                -6.00 dB
PL12                6.50 dB
PL13                8.50 dB
PL2W        49.59999847 W
PL12W        2.78921294 W
PL13W        1.75987446 W
SFO2        400.1316005 MHz

F2 - Processing parameters
SI                 32768
SF          100.6126261 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(4-methyoxyphenyl)methyl]phenol (2.16)



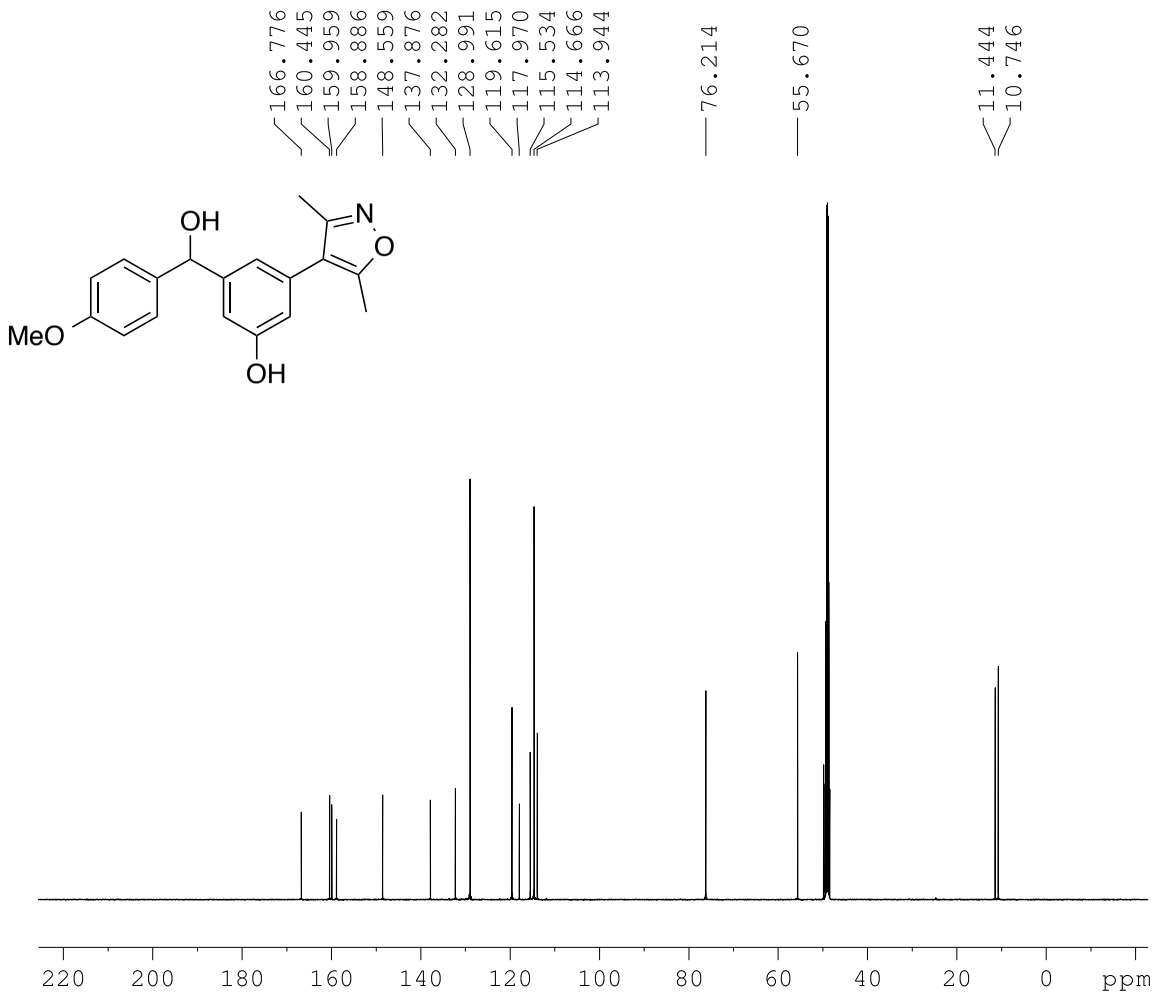

```
Current Data Parameters
NAME     N28992-56 grease wash
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20131118
Time               12.00
INSTRUM          avg400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ             3.2767999 sec
RG                141.58
DW                50.000 usec
DE                 6.50 usec
TE                294.3 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                  1H
P1                 12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                 65536
SF           400.2000217 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(4-methyoxyphenyl)methyl]phenol (2.16)




```
Current Data Parameters
NAME      N28992-56 service
EXPNO                    3
PROCNO                   1

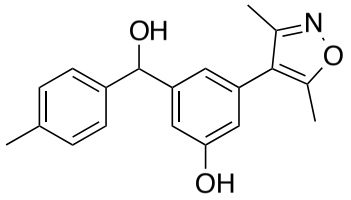
F2 - Acquisition Parameters
Date_           20131119
Time               15.08
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                   512
DS                     2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                   912
DW              16.000 usec
DE              18.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1

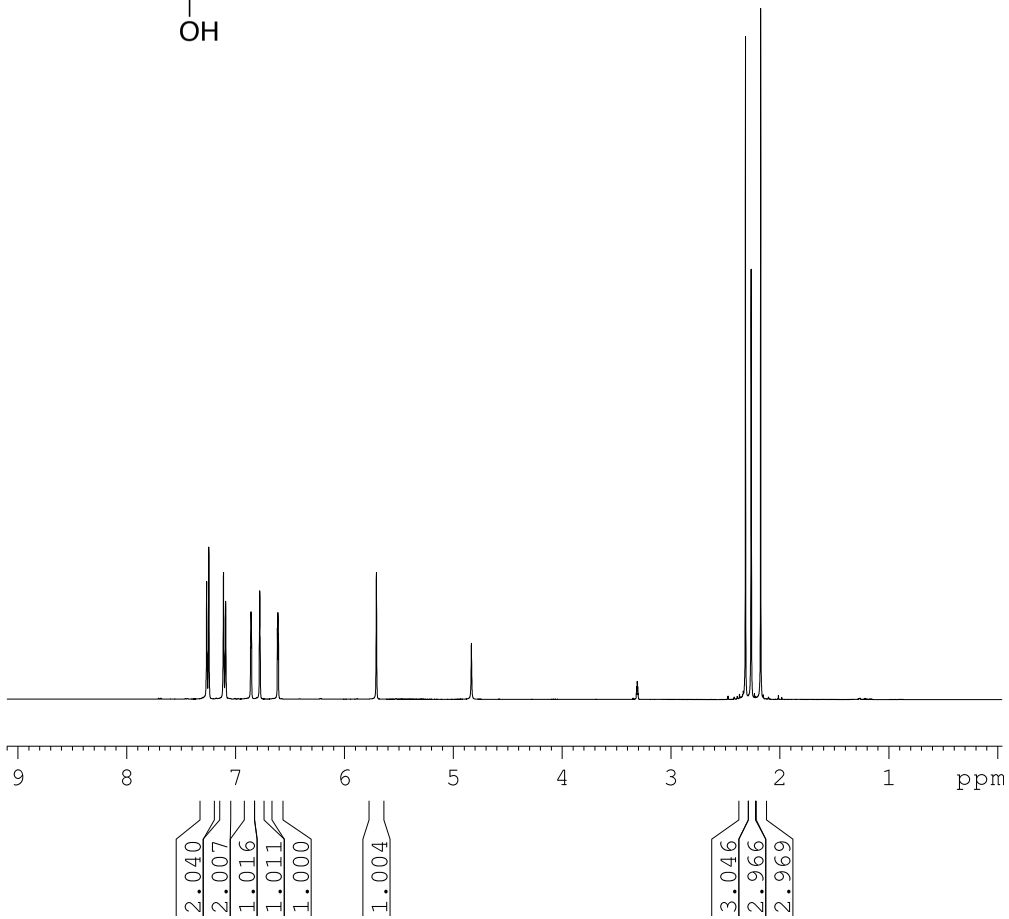
======== CHANNEL f1 ========
SFO1         125.8131151 MHz
NUC1                 13C
P1                10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                   32768
SF           125.8003617 MHz
WDW                    EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(4-methylphenyl)methyl]phenol (2.17)





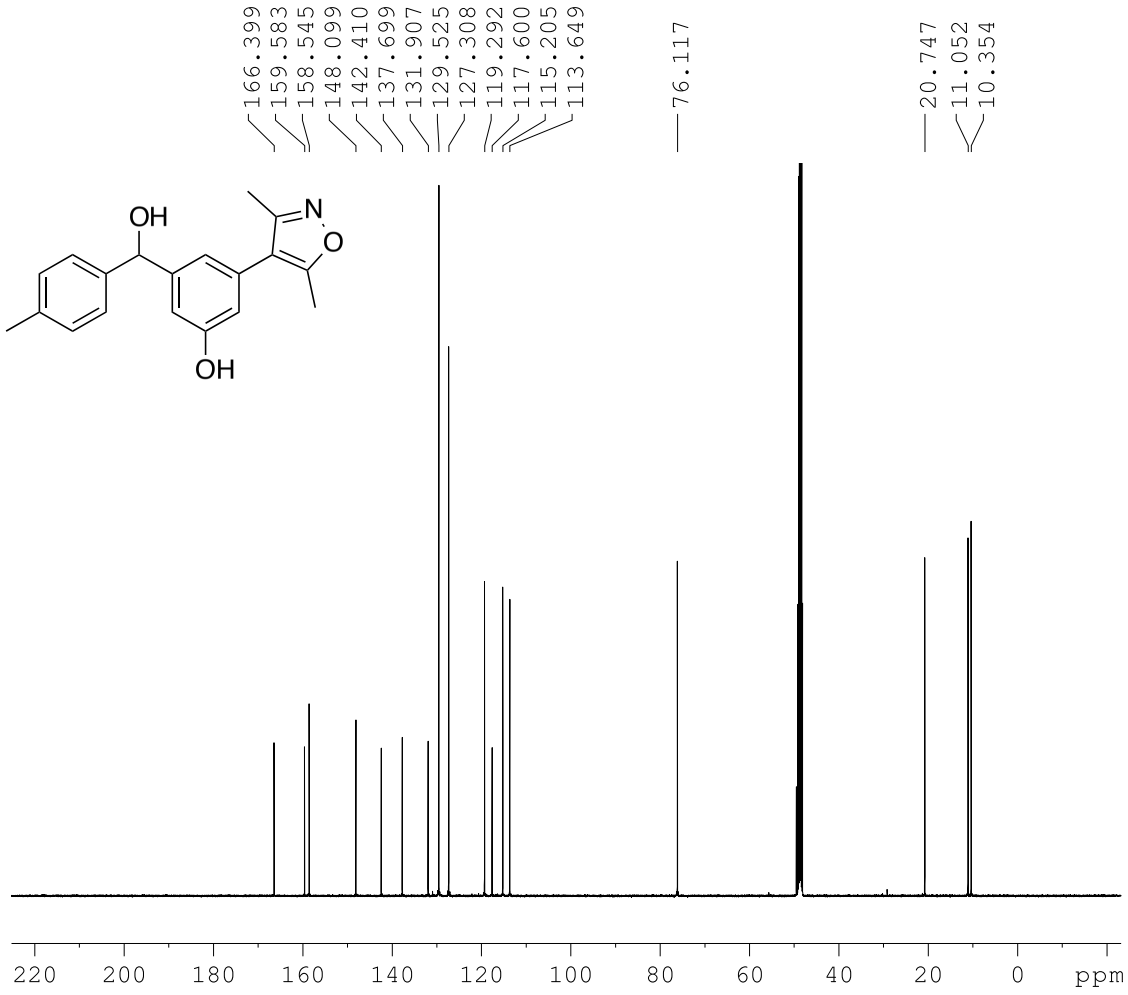

```
Current Data Parameters
NAME       N28992-44 GSK
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130820
Time               19.06
INSTRUM             w400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH              8278.146 Hz
FIDRES           0.126314 Hz
AQ               3.9583745 sec
RG                  35.9
DW                 60.400 usec
DE                  6.50 usec
TE                 303.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.10 usec
PL1                -6.00 dB
PL1W         49.59999847 W
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300075 MHz
WDW                   no
SSB       0
LB        0 Hz
GB        0
PC                  1.50
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(4-methylphenyl)methyl]phenol (2.17)




```
Current Data Parameters
NAME      N28992-44 service
EXPNO                   3
PROCNO                  1

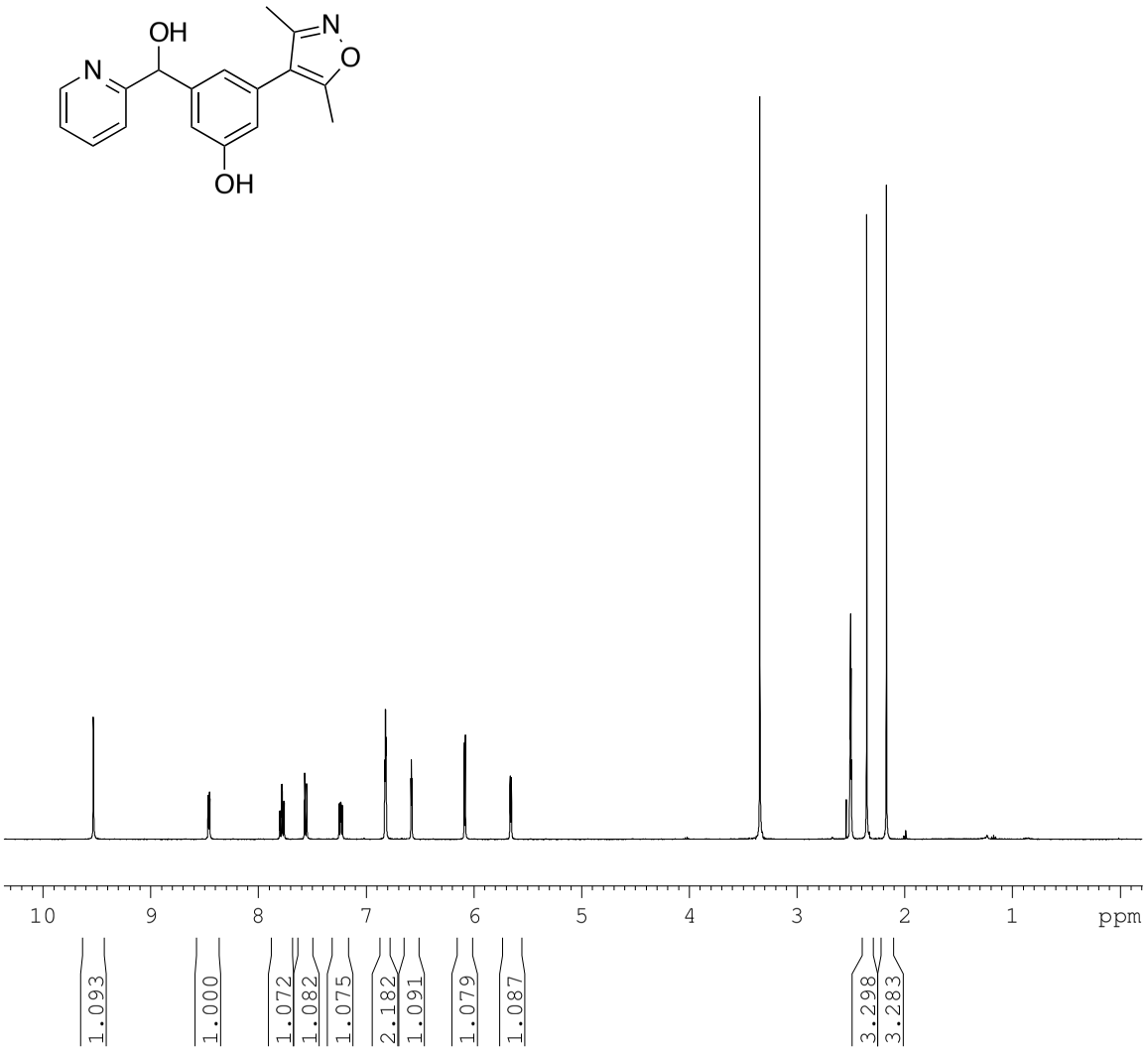
F2 - Acquisition Parameters
Date_           20131117
Time                4.28
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                65536
SOLVENT             MeOD
NS                    512
DS                      2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                    912
DW               16.000 usec
DE                18.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1         125.8131151 MHz
NUC1                  13C
P1                 10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                  32768
SF           125.8004081 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(pyridine-2-yl)methyl]phenol (2.19) synthesised by D. Hewings



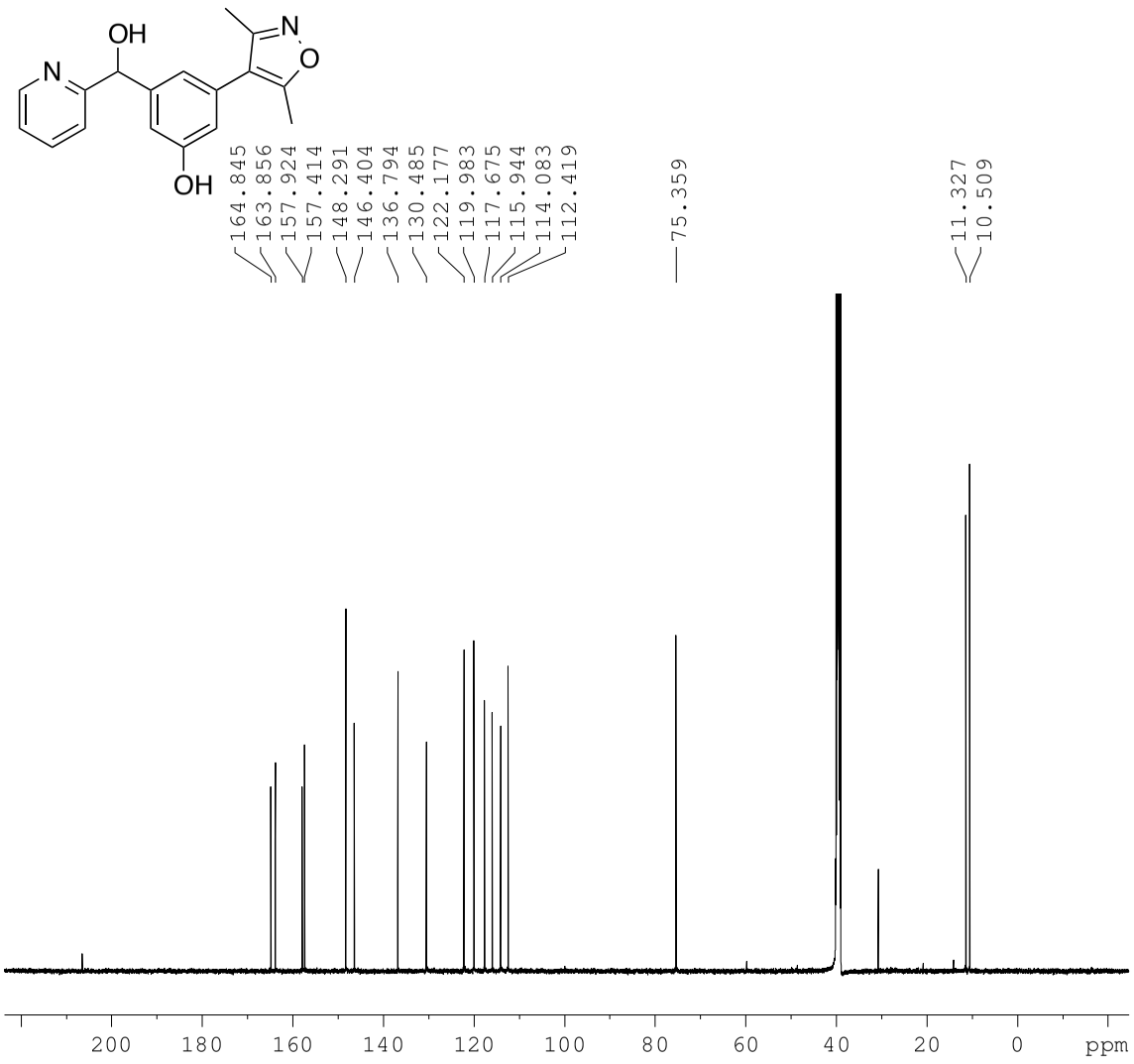

```
Current Data Parameters
NAME                  DSH361
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_               20130614
Time                   12.35
INSTRUM                av400
PROBHD   5 mm QNP 1H/13
PULPROG                 zg60
TD                     65536
SOLVENT                 DMSO
NS                        16
DS                         2
SWH                 8278.146 Hz
FIDRES              0.126314 Hz
AQ                  3.9583745 sec
RG                       256
DW                    60.400 usec
DE                     7.50 usec
TE                     300.0 K
D1                 1.00000000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                    10.10 usec
PL1       0 dB
SFO1           400.2024714 MHz

F2 - Processing parameters
SI                     32768
SF               400.1999990 MHz
WDW                       no
SSB       0
LB        0 Hz
GB        0
PC                      1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(pyridine-2-yl)methyl]phenol (2.19) synthesised by D. Hewings




```
Current Data Parameters
NAME      DSH361 service
EXPNO                 4
PROCNO                1

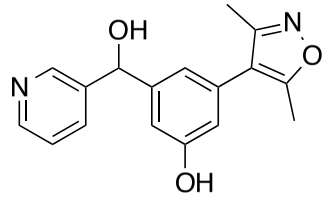
F2 - Acquisition Parameters
Date_          20130605
Time              13.57
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                 1015
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                  912
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

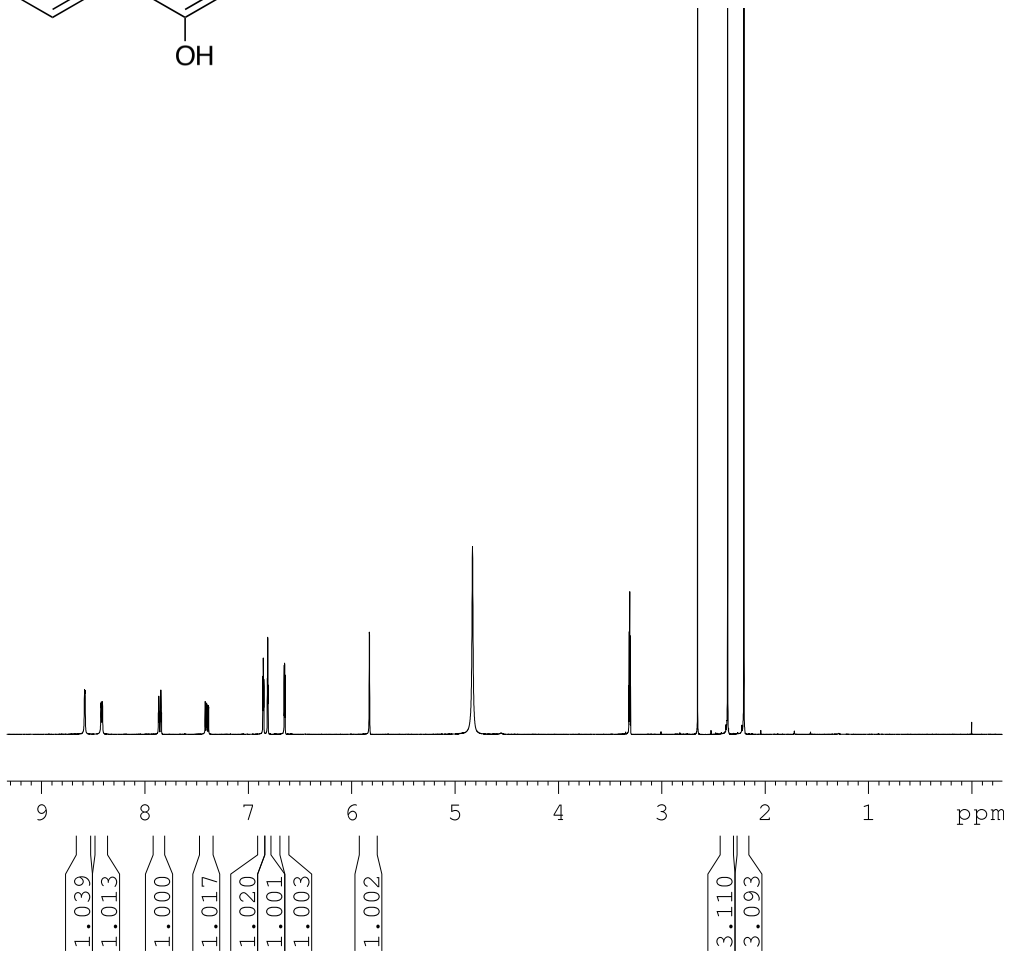
======== CHANNEL f1 ========
NUC1                 13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005954 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(pyridine-3-yl)methyl]phenol (2.20)





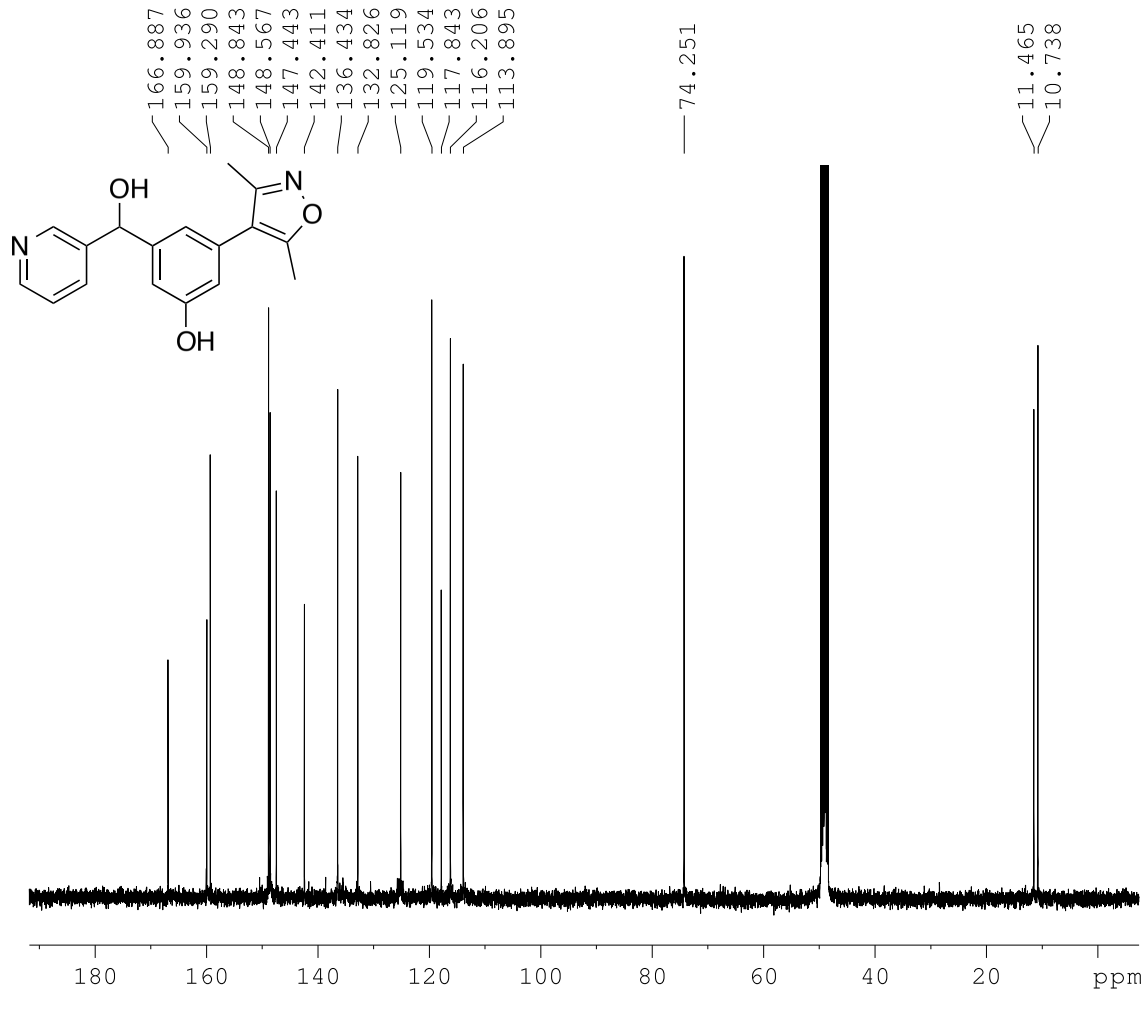

```
Current Data Parameters
NAME          N28992-43
EXPNO               106
PROCNO                1

F2 - Acquisition Parameters
Date_          20130828
Time              19.05
INSTRUM            w400
PROBHD   5 mm QNP 1H/13
PULPROG            zg30
TD                65536
SOLVENT            MeOD
NS                   16
DS                    2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ              3.9583745 sec
RG                  181
DW                60.400 usec
DE                 6.50 usec
TE                303.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.10 usec
PL1                -6.00 dB
PL1W        49.59999847 W
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300075 MHz
WDW                   no
SSB       0
LB        0 Hz
GB        0
PC                  1.50
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(pyridine-3-yl)methyl]phenol (2.20)




```
Current Data Parameters
NAME          N28992-43
EXPNO                11
PROCNO                1

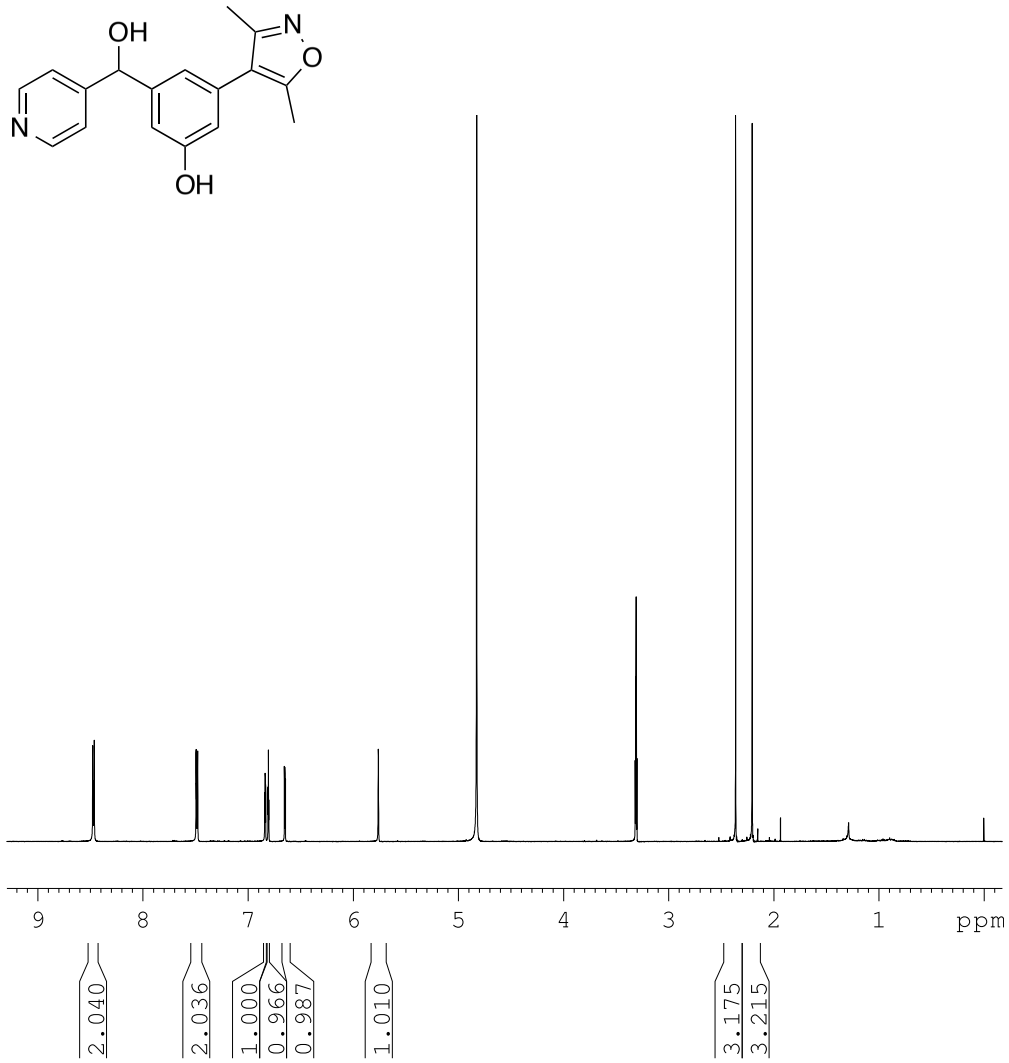
F2 - Acquisition Parameters
Date_          20130822
Time               2.39
INSTRUM            w400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                65536
SOLVENT            MeOD
NS                 2048
DS                    4
SWH           26178.010 Hz
FIDRES         0.399445 Hz
AQ            1.2517376 sec
RG                 4096
DW               19.100 usec
DE                6.50 usec
TE                303.0 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                 8.65 usec
PL1                -2.00 dB
PL1W       24.80207634 W
SFO1       100.6248425 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             75.00 usec
PL2               -6.00 dB
PL12               6.50 dB
PL13               8.50 dB
PL2W       49.59999847 W
PL12W       2.78921294 W
PL13W       1.75987446 W
SFO2       400.1316005 MHz

F2 - Processing parameters
SI                32768
SF         100.6126261 MHz
WDW                  EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(pyridine-4-yl)methyl]phenol (2.21)



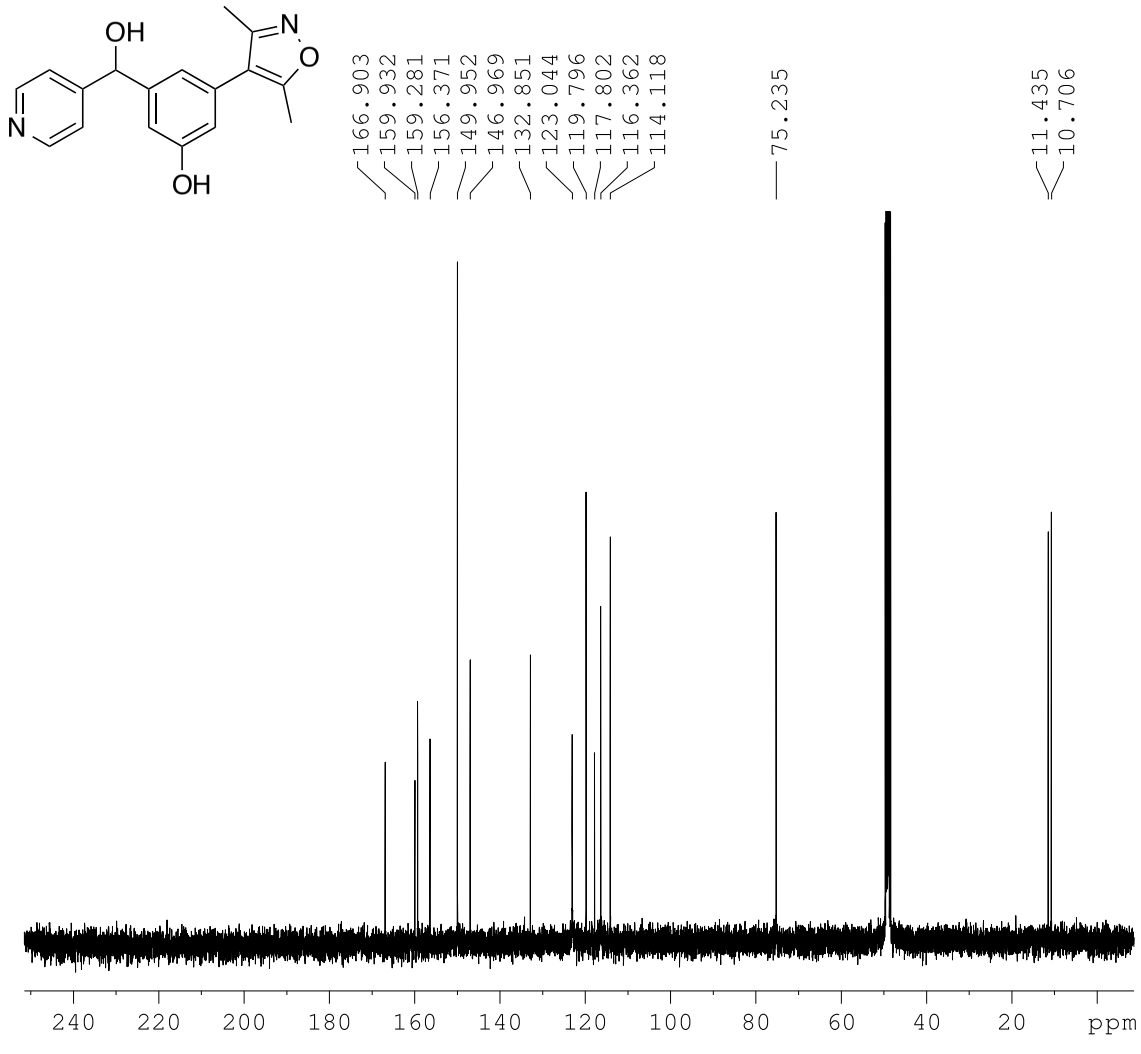

```
Current Data Parameters
NAME          N28992-42
EXPNO                10
PROCNO                1

F2 - Acquisition Parameters
Date_          20130827
Time              10.11
INSTRUM            w400
PROBHD   5 mm QNP 1H/13
PULPROG            zg30
TD                65536
SOLVENT            MeOD
NS                   16
DS                    2
SWH            8278.146 Hz
FIDRES         0.126314 Hz
AQ             3.9583745 sec
RG                  181
DW                60.400 usec
DE                 6.50 usec
TE                303.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                11.10 usec
PL1               -6.00 dB
PL1W        49.59999847 W
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                32768
SF          400.1300075 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                 1.50
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[hydroxy(pyridine-4-yl)methyl]phenol (2.21)




```
Current Data Parameters
NAME          N28992-42
EXPNO                11
PROCNO                1

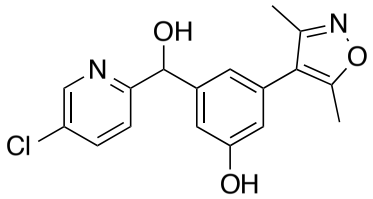
F2 - Acquisition Parameters
Date_          20130828
Time               1.08
INSTRUM            w400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                  2048
DS                     4
SWH          26178.010 Hz
FIDRES         0.399445 Hz
AQ           1.2517376 sec
RG                  4096
DW                19.100 usec
DE                 6.50 usec
TE                303.0 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                    1

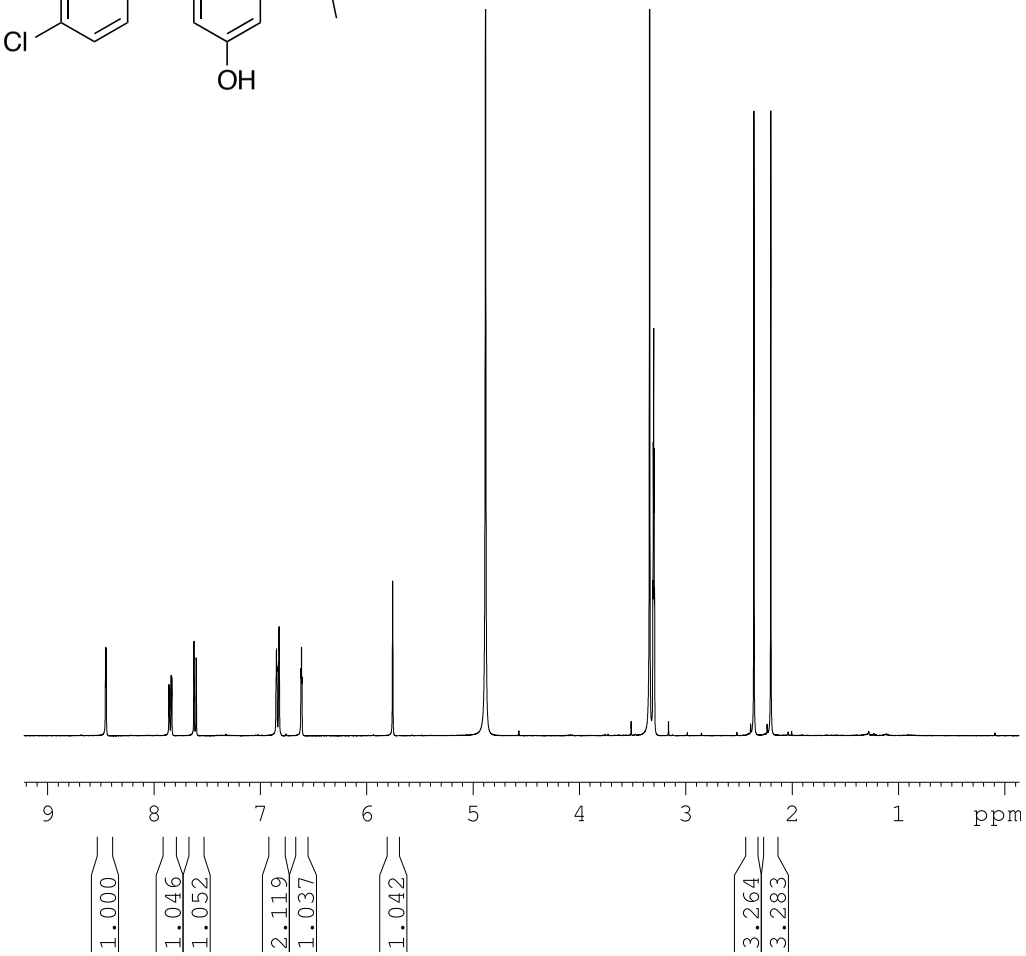
======== CHANNEL f1 ========
NUC1                 13C
P1                  8.65 usec
PL1                -2.00 dB
PL1W        24.80207634 W
SFO1        100.6248425 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             75.00 usec
PL2                -6.00 dB
PL12                6.50 dB
PL13                8.50 dB
PL2W        49.59999847 W
PL12W        2.78921294 W
PL13W        1.75987446 W
SFO2        400.1316005 MHz

F2 - Processing parameters
SI                 32768
SF          100.6126261 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

3-[(5-Chloropyridin-2-yl)(hydroxyl)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.22)





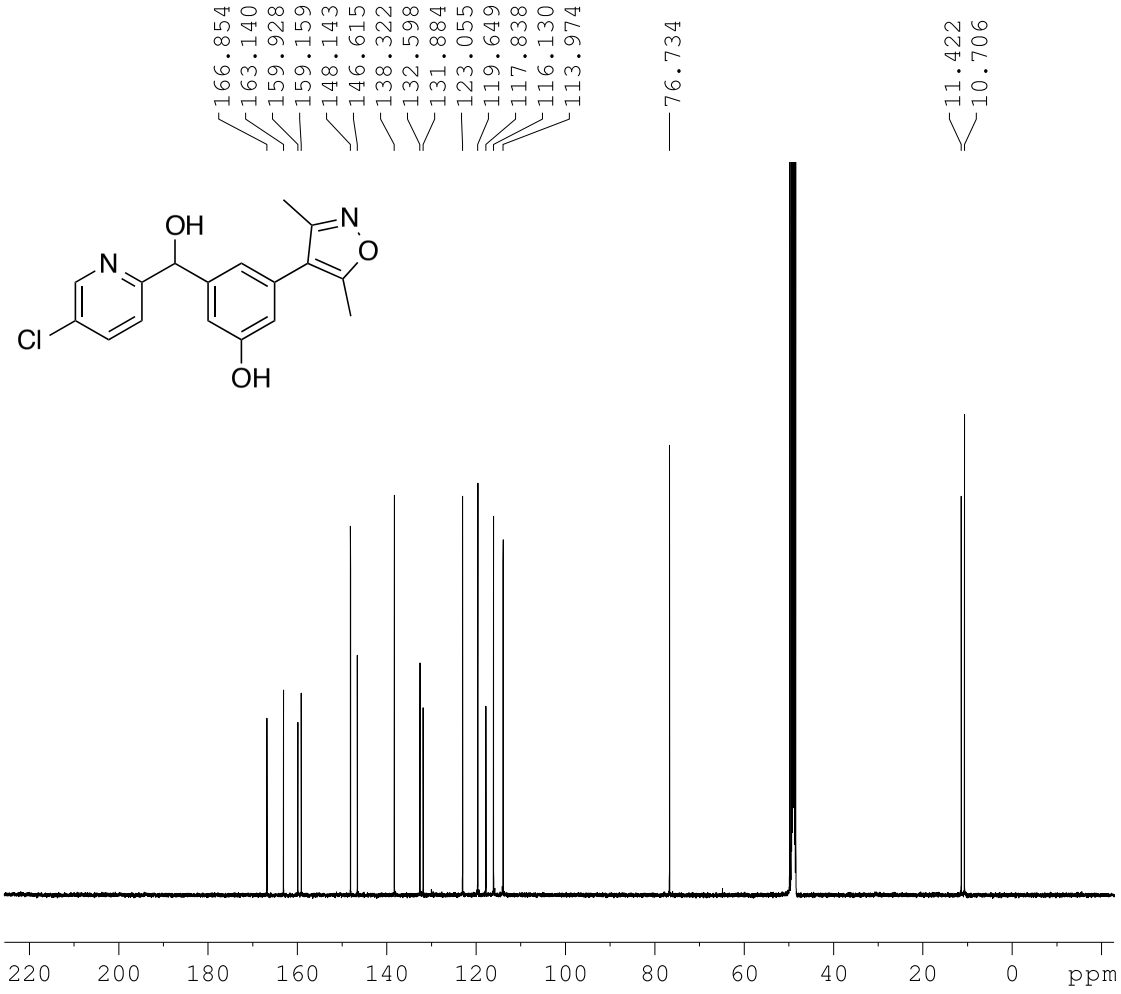

```
Current Data Parameters
NAME      LJ360 precipitate
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20131120
Time               13.28
INSTRUM          avg400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ            3.2767999 sec
RG               359.87
DW               50.000 usec
DE                6.50 usec
TE                294.8 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                   1H
P1                 12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                  65536
SF           400.2000149 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

3-[(5-Chloropyridin-2-yl)(hydroxyl)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.22)




```
Current Data Parameters
NAME       LJ360 service
EXPNO                  4
PROCNO                 1

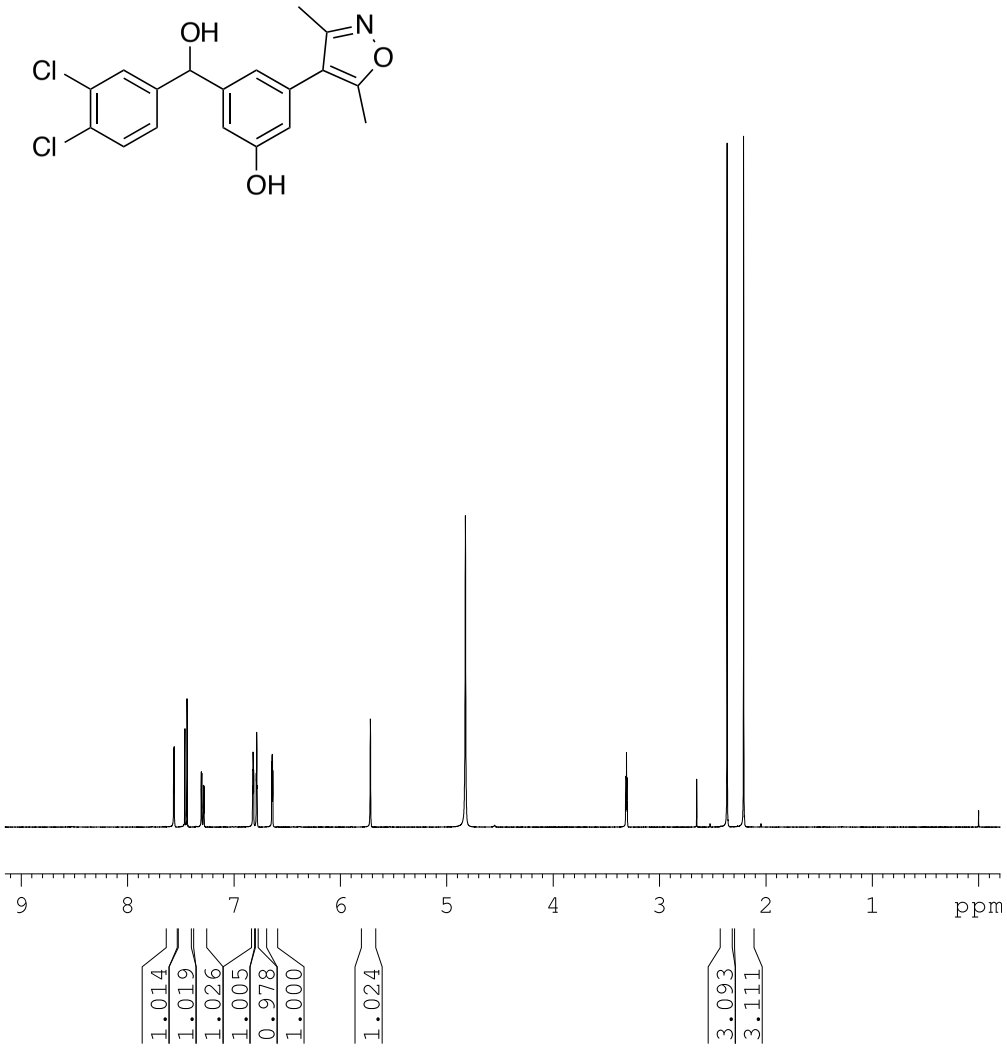
F2 - Acquisition Parameters
Date_           20131122
Time              21.54
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 3072
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                  912
DW               16.000 usec
DE               18.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         125.8131151 MHz
NUC1                13C
P1                10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                 32768
SF           125.8003601 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(3,4-dichlorophenyl)(hydroxy)methyl]phenol (2.24)



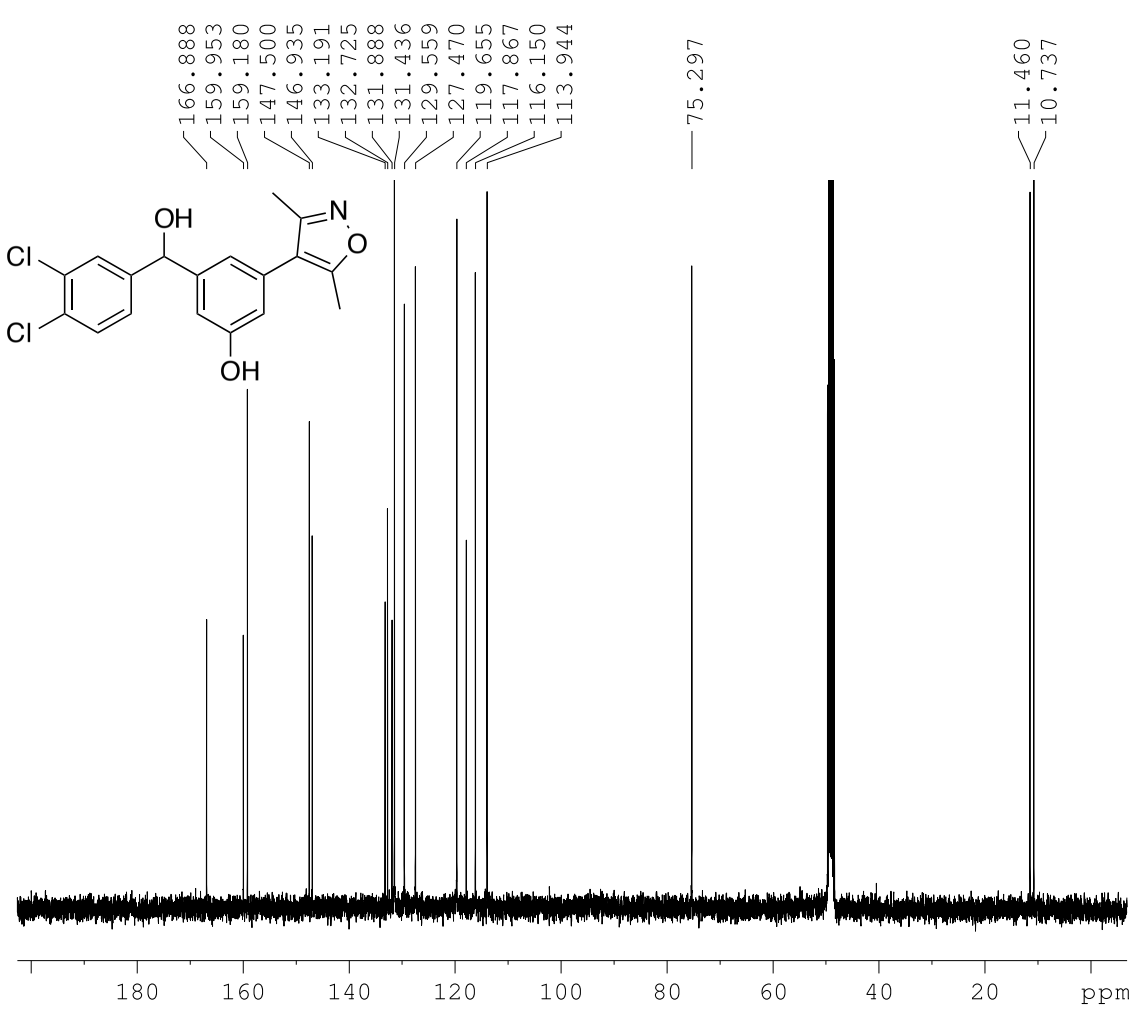

```
Current Data Parameters
NAME           N28992-57
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130829
Time               19.06
INSTRUM             w400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ              3.9583745 sec
RG                   181
DW                60.400 usec
DE                 6.50 usec
TE                303.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.10 usec
PL1                -6.00 dB
PL1W         49.59999847 W
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300075 MHz
WDW                   no
SSB       0
LB        0 Hz
GB        0
PC                  1.50
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(3,4-dichlorophenyl)(hydroxy)methyl]phenol (2.24)




```
Current Data Parameters
NAME          N28992-57
EXPNO                11
PROCNO                1

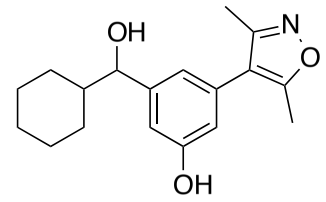
F2 - Acquisition Parameters
Date_          20130829
Time              20.27
INSTRUM             w400
PROBHD   5 mm QNP 1H/13
PULPROG           zgpg30
TD                65536
SOLVENT             MeOD
NS                 2048
DS                    4
SWH           26178.010 Hz
FIDRES         0.399445 Hz
AQ            1.2517376 sec
RG                 4096
DW               19.100 usec
DE                6.50 usec
TE                303.0 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  8.65 usec
PL1                -2.00 dB
PL1W        24.80207634 W
SFO1        100.6248425 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             75.00 usec
PL2                -6.00 dB
PL12                6.50 dB
PL13                8.50 dB
PL2W        49.59999847 W
PL12W        2.78921294 W
PL13W        1.75987446 W
SFO2        400.1316005 MHz

F2 - Processing parameters
SI                  32768
SF          100.6126261 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

3-[Cyclohexyl(hydroxyl)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.25)



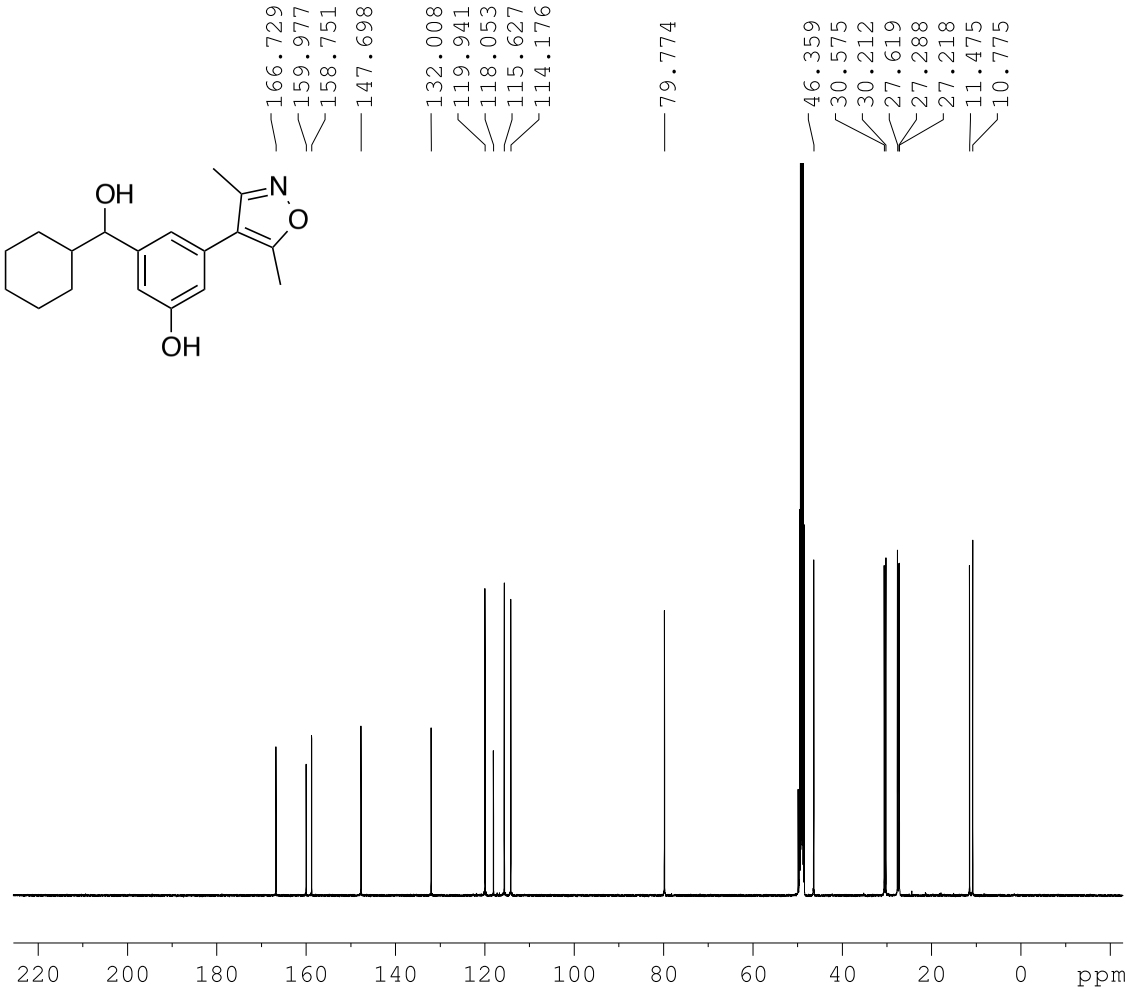

```
Current Data Parameters
NAME      LJ372 service
EXPNO                1
PROCNO               1

F2 - Acquisition Parameters
Date_          20131203
Time              12.57
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                65536
SOLVENT             MeOD
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                   5.6
DW                48.400 usec
DE                10.00 usec
TE                300.0 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          500.3000122 MHz
WDW                    EM
SSB      0
LB                  0.30 Hz
GB       0
PC                  1.00
```

3-[Cyclohexyl(hydroxyl)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.25)




```
Current Data Parameters
NAME      LJ372 service
EXPNO                 3
PROCNO                1

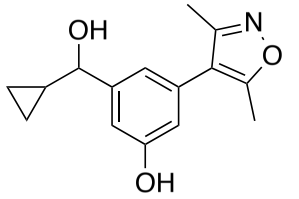
F2 - Acquisition Parameters
Date_          20131203
Time               13.55
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                  912
DW               16.000 usec
DE               18.00 usec
TE               300.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

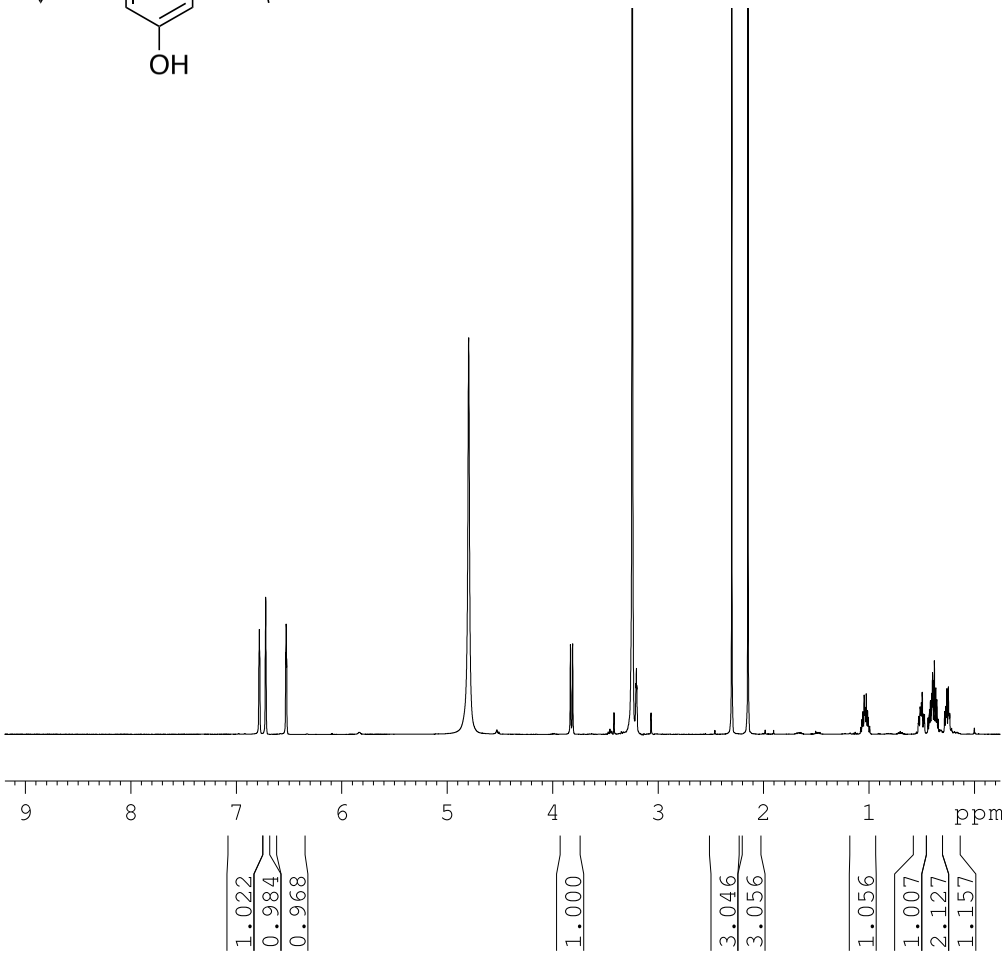
======== CHANNEL f1 ========
SFO1         125.8131151 MHz
NUC1                13C
P1                10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                 32768
SF           125.8003673 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

3-[Cyclopropyl(hydroxyl)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.27)





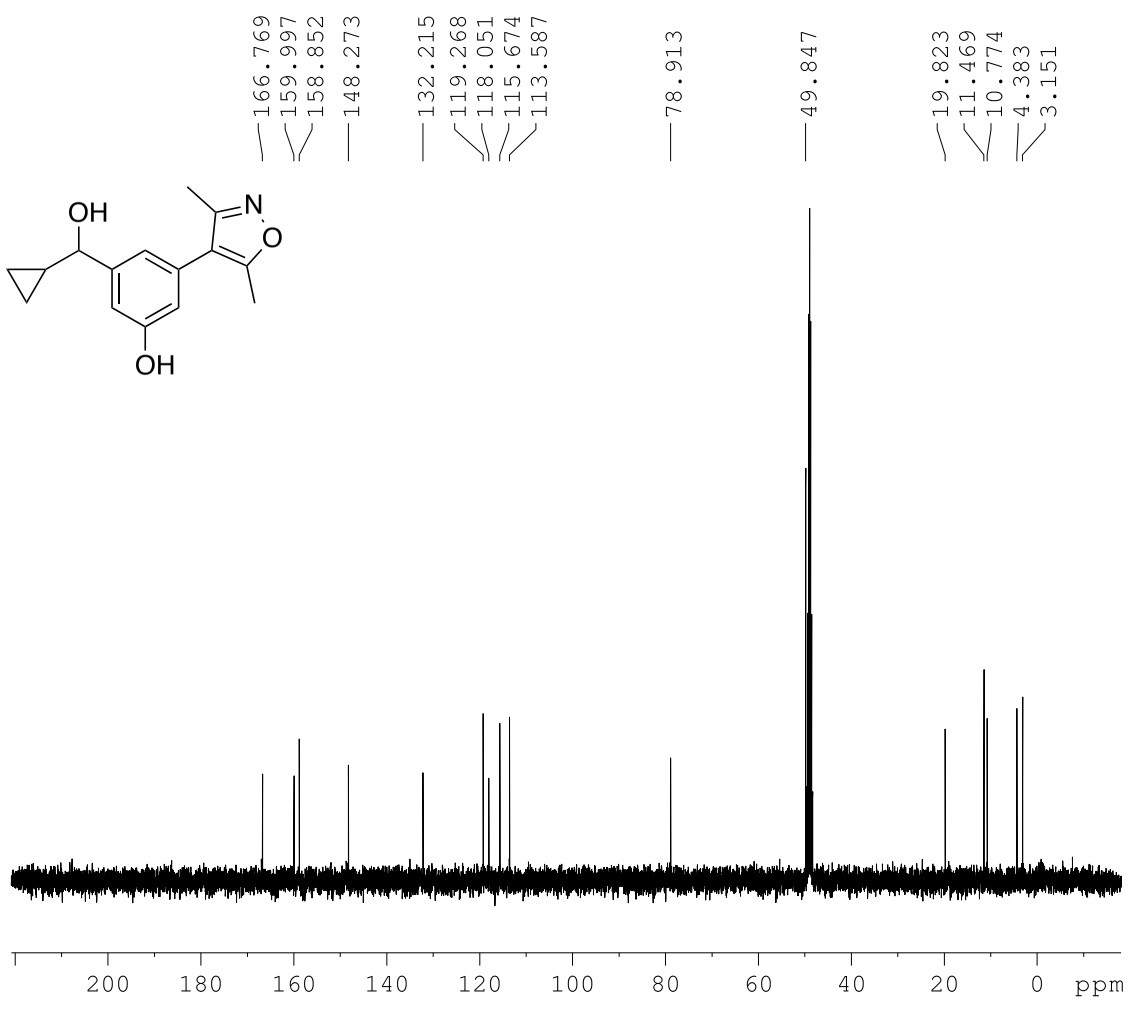

```
Current Data Parameters
NAME      LJ366 fra 1-4 AVG400
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20131107
Time               12.40
INSTRUM           avg400
PROBHD    5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH            10000.000 Hz
FIDRES          0.152588 Hz
AQ             3.2767999 sec
RG                 93.53
DW                50.000 usec
DE                 6.50 usec
TE                294.1 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                   1H
P1                12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                 65536
SF           400.2000532 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

3-[Cyclopropyl(hydroxyl)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.27)




```
Current Data Parameters
NAME      LJ366 fra 1-4 AVG40(
EXPNO                    4
PROCNO                   1

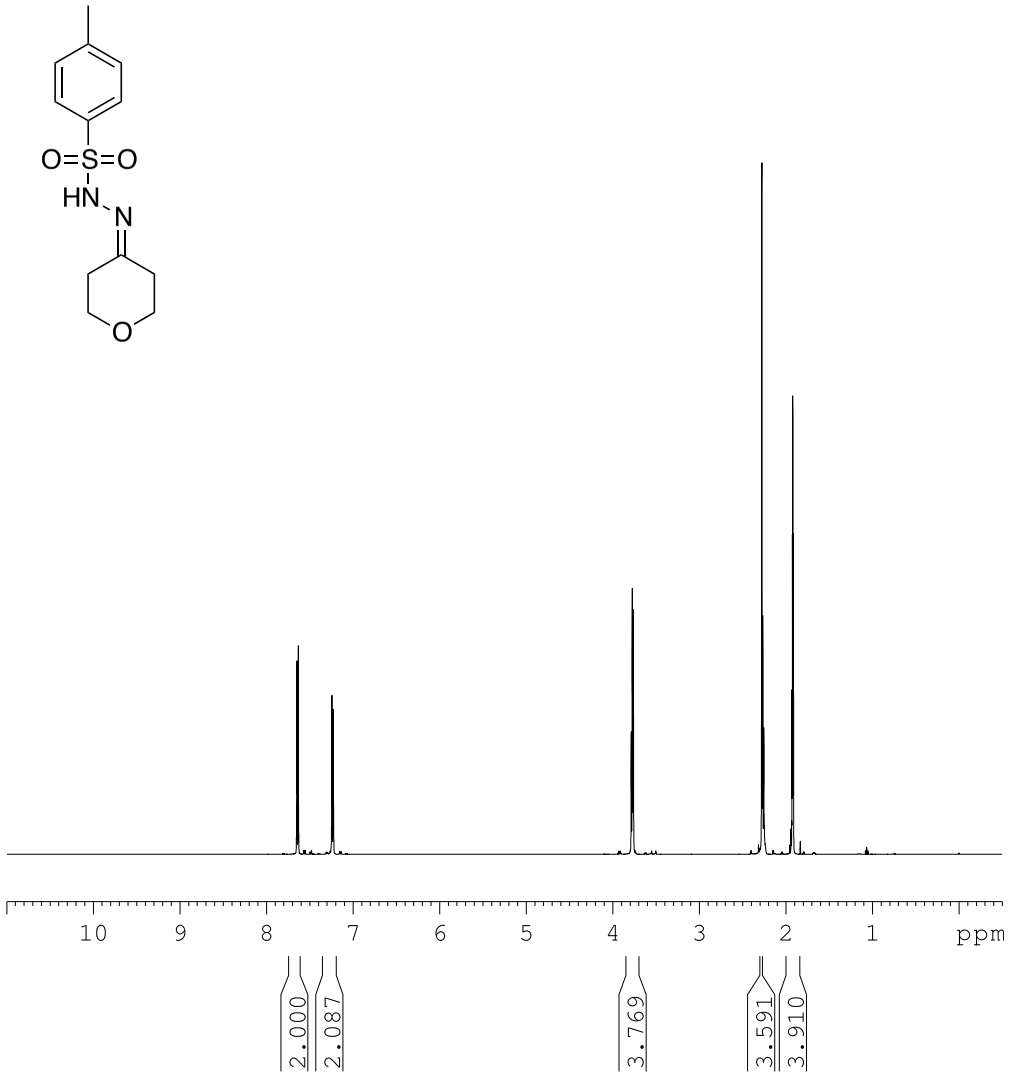
F2 - Acquisition Parameters
Date_             20131107
Time                 22.47
INSTRUM              avg400
PROBHD    5 mm QNP 1H/13
PULPROG              zgpg30
TD                    65536
SOLVENT                MeOD
NS                      256
DS                        4
SWH              24038.461 Hz
FIDRES            0.366798 Hz
AQ               1.3631488 sec
RG                     2050
DW                  20.800 usec
DE                   6.50 usec
TE                   295.3 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1          100.6404326 MHz
NUC1                    13C
P1                   10.00 usec
PLW1          40.00000000 W

======== CHANNEL f2 ========
SFO2          400.2016008 MHz
NUC2                    1H
CPDPRG[2           waltz16
PCPD2                90.00 usec
PLW2          11.29800034 W
PLW12          0.20863000 W
PLW13          0.16899000 W

F2 - Processing parameters
SI                    32768
SF              100.6302322 MHz
WDW                      EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```

4-Methyl-*N*'-(tetrahydro-4*H*-pyran-4-ylidene)benzenesulfonohydrazide (2.65)



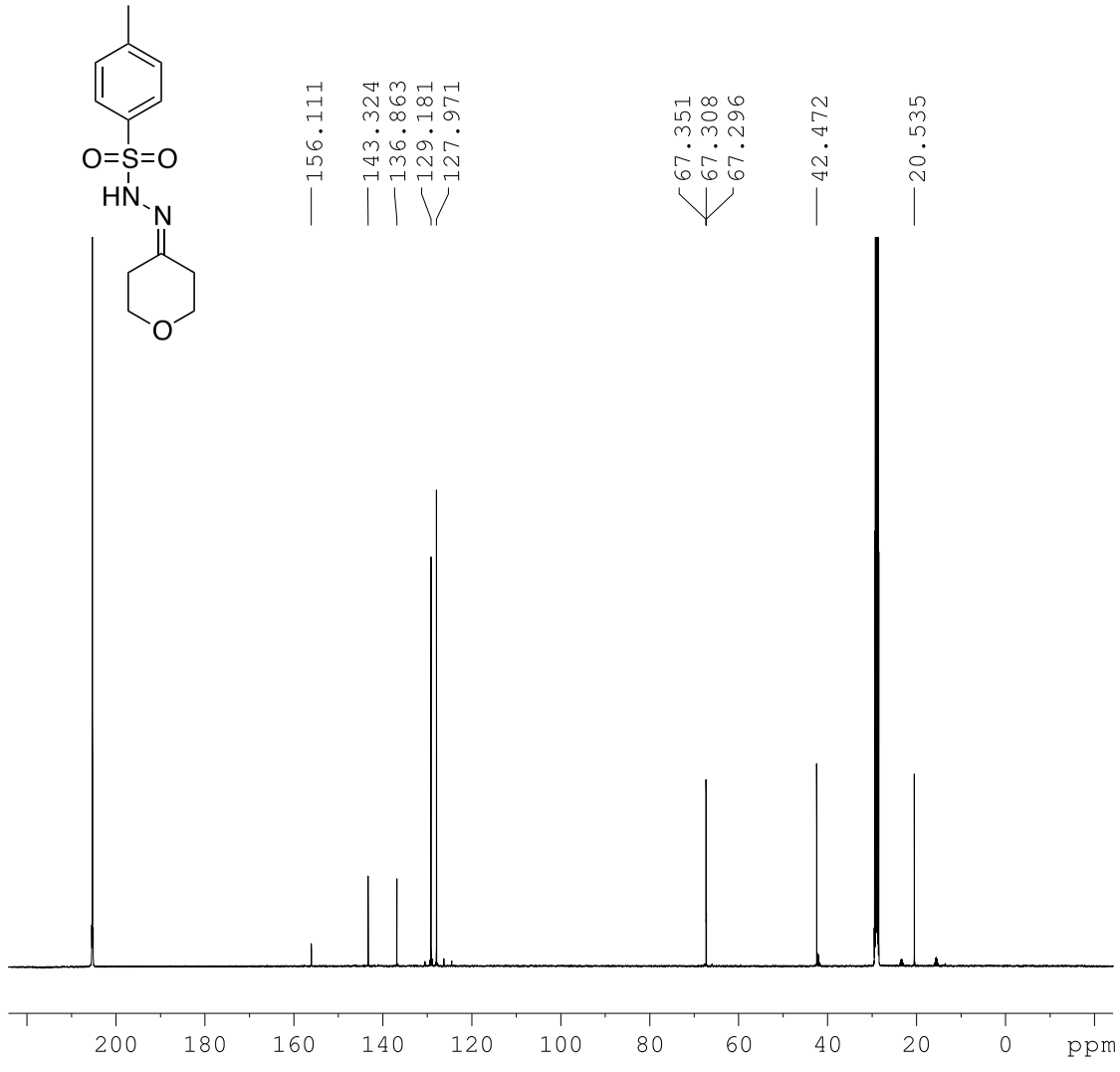

```
Current Data Parameters
NAME      LJ369 service
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20131117
Time               2.24
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT          Acetone
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  3.56
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1                  1H
P1                15.00 usec
PLW1         7.99830008 W

F2 - Processing parameters
SI                 65536
SF          500.3000748 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

4-Methyl-*N*'-(tetrahydro-4*H*-pyran-4-ylidene)benzenesulfonohydrazide (2.65)




```
Current Data Parameters
NAME      LJ369 service
EXPNO                 3
PROCNO                1

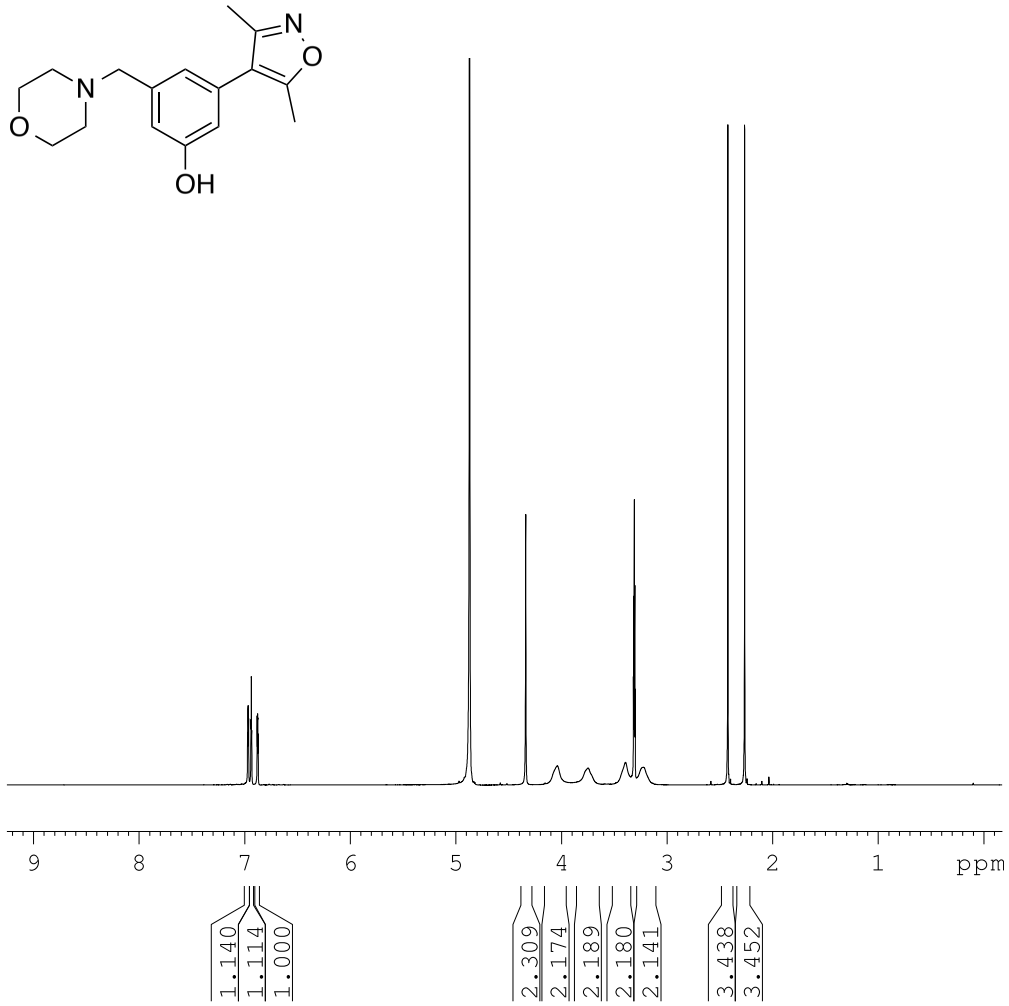
F2 - Acquisition Parameters
Date_          20131117
Time               3.22
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT         Acetone
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                  912
DW               16.000 usec
DE               18.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        125.8131151 MHz
NUC1                13C
P1                10.00 usec
PLW1        20.18400002 W

======== CHANNEL f2 ========
SFO2        500.3020012 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                 32768
SF           125.8005350 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(morpholin-4-ylmethyl)phenol (2.41)



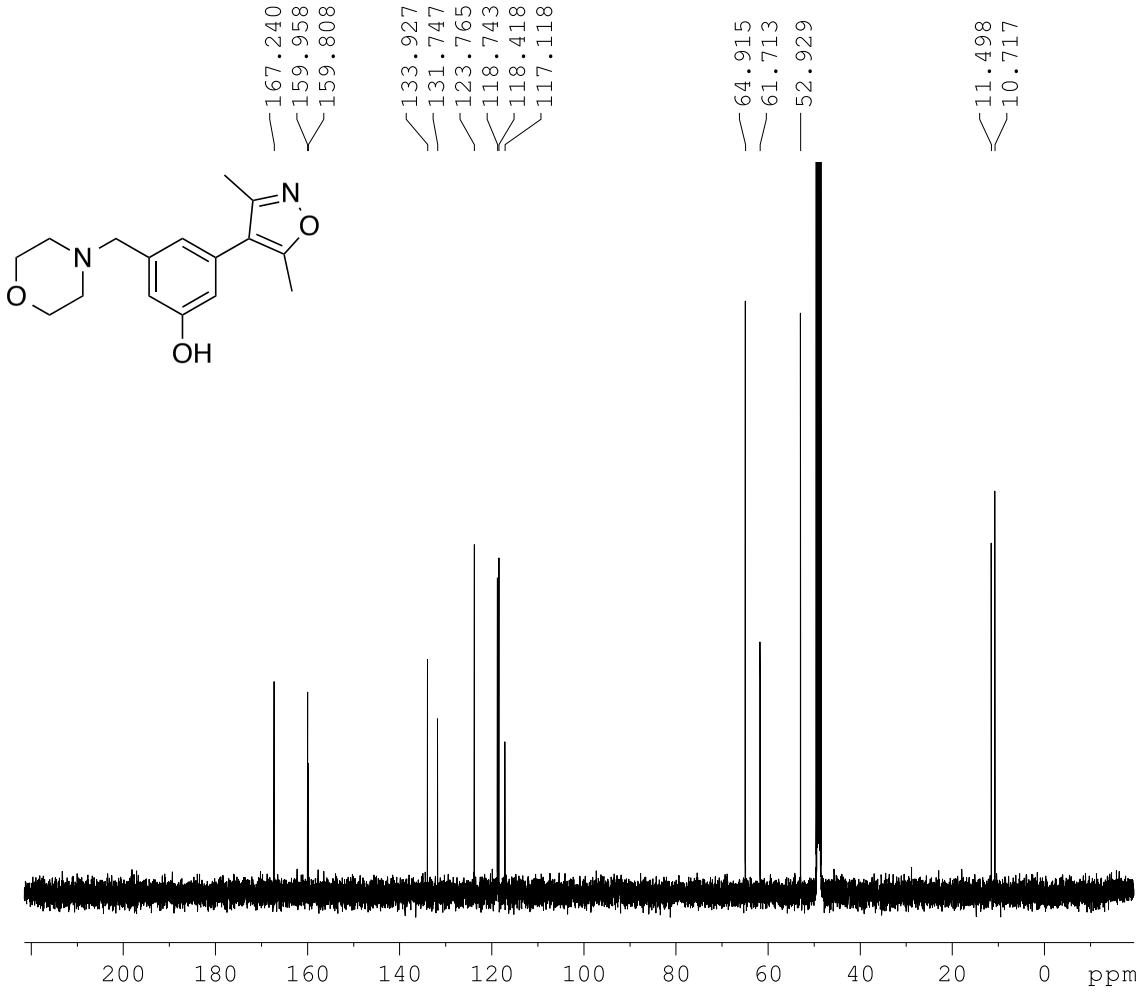

```
Current Data Parameters
NAME      LJ382 semi-prep
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20140725
Time               13.10
INSTRUM          avf400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES           0.122266 Hz
AQ              4.0894465 sec
RG                    93
DW                 62.400 usec
DE                  6.50 usec
TE                 297.5 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                   1H
P1                 12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                  32768
SF           400.2500098 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(morpholin-4-ylmethyl)phenol (2.41)




```
Current Data Parameters
NAME     LJ382 semi-prep service
EXPNO                 3
PROCNO                1

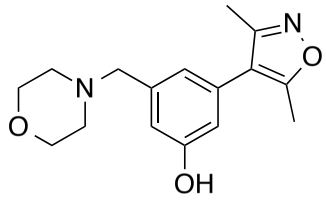
F2 - Acquisition Parameters
Date_          20140729
Time              11.13
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                65536
SOLVENT             MeOD
NS                  2048
DS                     4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG                  6502
DW               16.500 usec
DE               11.00 usec
TE                297.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 13C
P1                6.80 usec
PL1               5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2            100.00 usec
PL2      0 dB
PL12              19.00 dB
PL13              23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                 32768
SF          125.7576131 MHz
WDW                  EM
SSB      0
LB                1.00 Hz
GB       0
PC                1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(morpholin-4-ylmethyl)phenol (2.41)



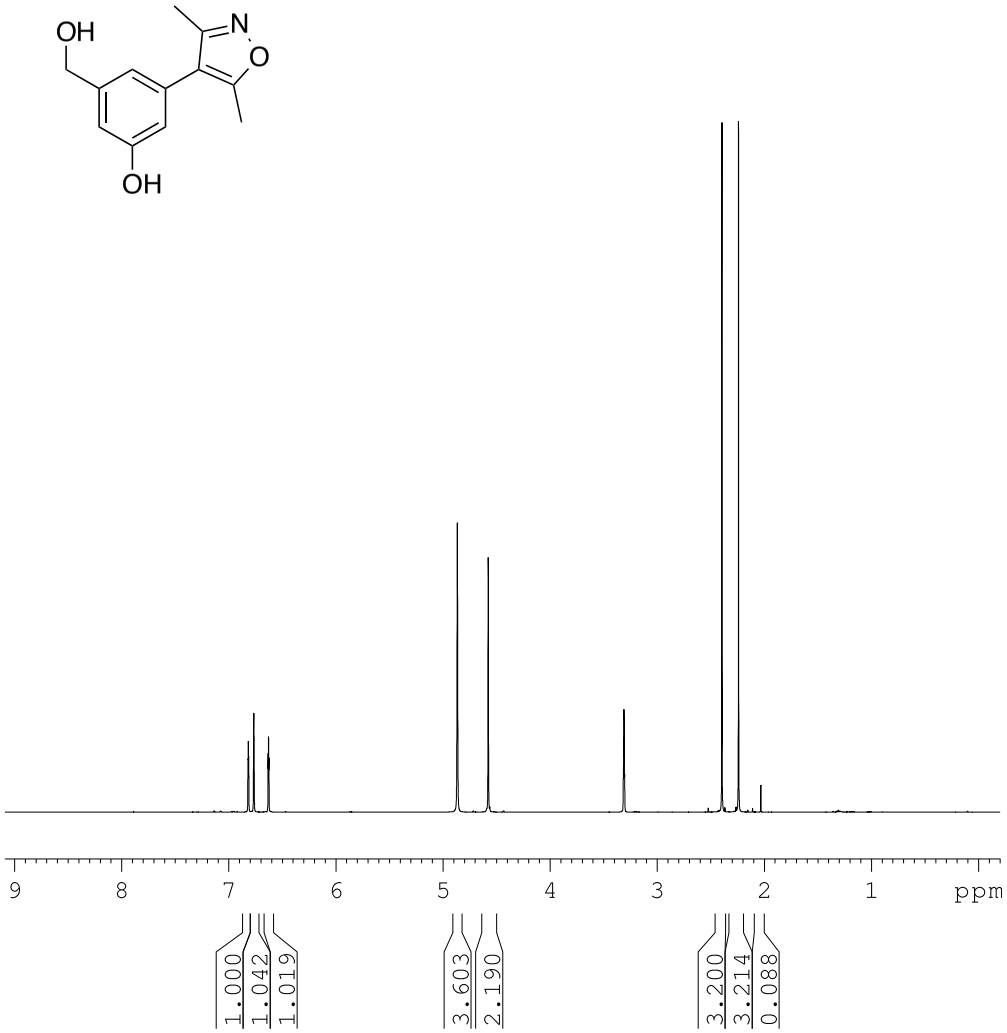

```
Current Data Parameters
NAME      LJ382 semi-prep AVG
EXPNO                   6
PROCNO                  1

F2 - Acquisition Parameters
Date_           20140726
Time                1.34
INSTRUM           avf400
PROBHD   5 mm PABBO BB/
PULPROG           zgflqn
TD                131072
SOLVENT             MeOD
NS                   256
DS                     4
SWH          113636.367 Hz
FIDRES         0.866977 Hz
AQ            0.5767168 sec
RG               205.43
DW                4.400 usec
DE                6.50 usec
TE                297.3 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         376.5924484 MHz
NUC1                  19F
P1                  13.50 usec
PLW1         19.00000000 W

F2 - Processing parameters
SI                 65536
SF           376.6112790 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(hydroxymethyl)phenol (2.41a)



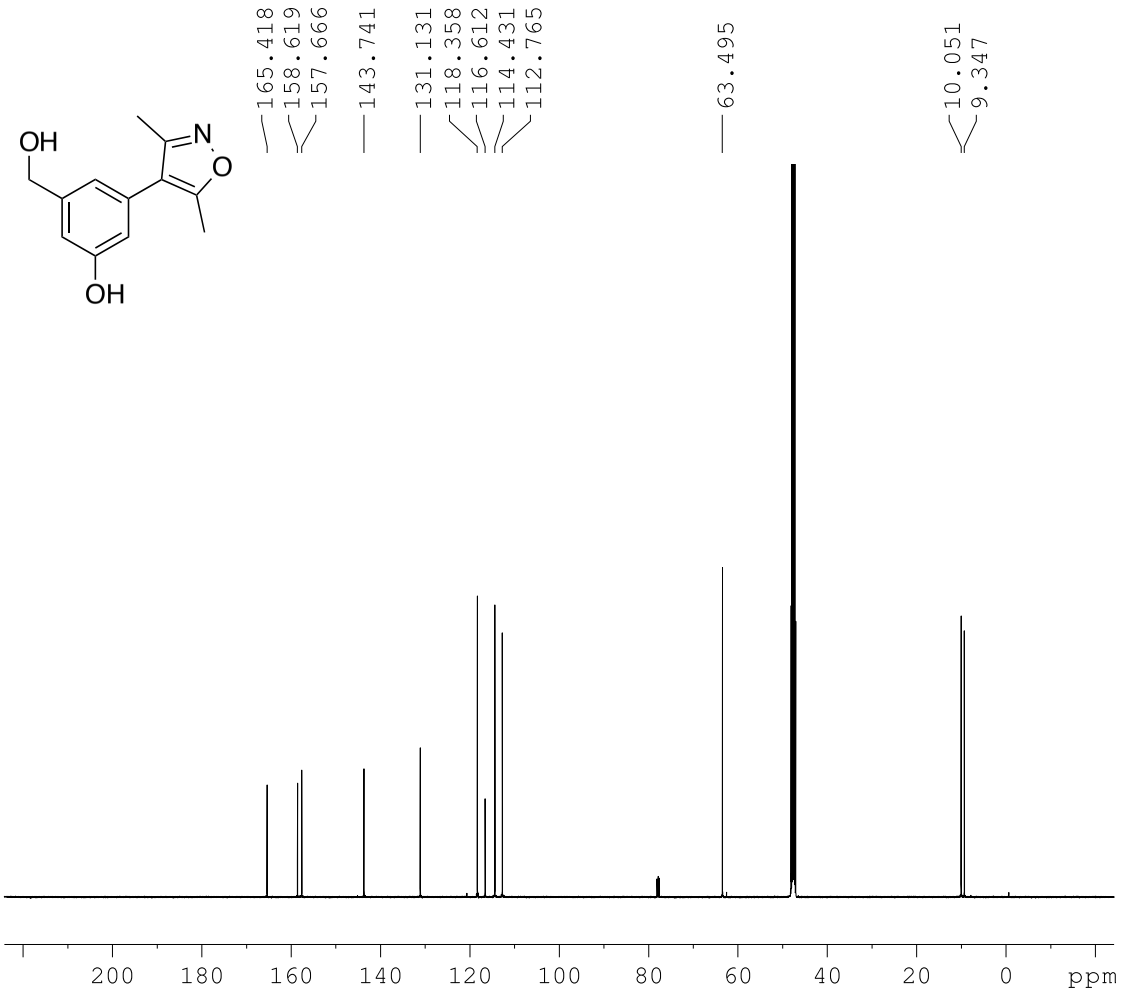

```
Current Data Parameters
NAME      LJ387 fra 11-19 servic
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20140303
Time               10.51
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  2.25
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         500.3030896 MHz
NUC1                   1H
P1                 15.00 usec
PLW1          7.99830008 W

F2 - Processing parameters
SI                 65536
SF           500.3000110 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(hydroxymethyl)phenol (2.41a)




```
Current Data Parameters
NAME     LJ387 fra 11-19 service
EXPNO                 3
PROCNO                1

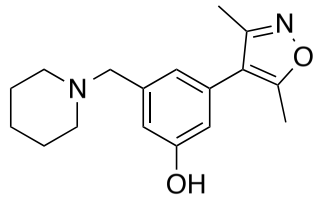
F2 - Acquisition Parameters
Date_          20140303
Time               12.16
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                  912
DW               16.000 usec
DE               18.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

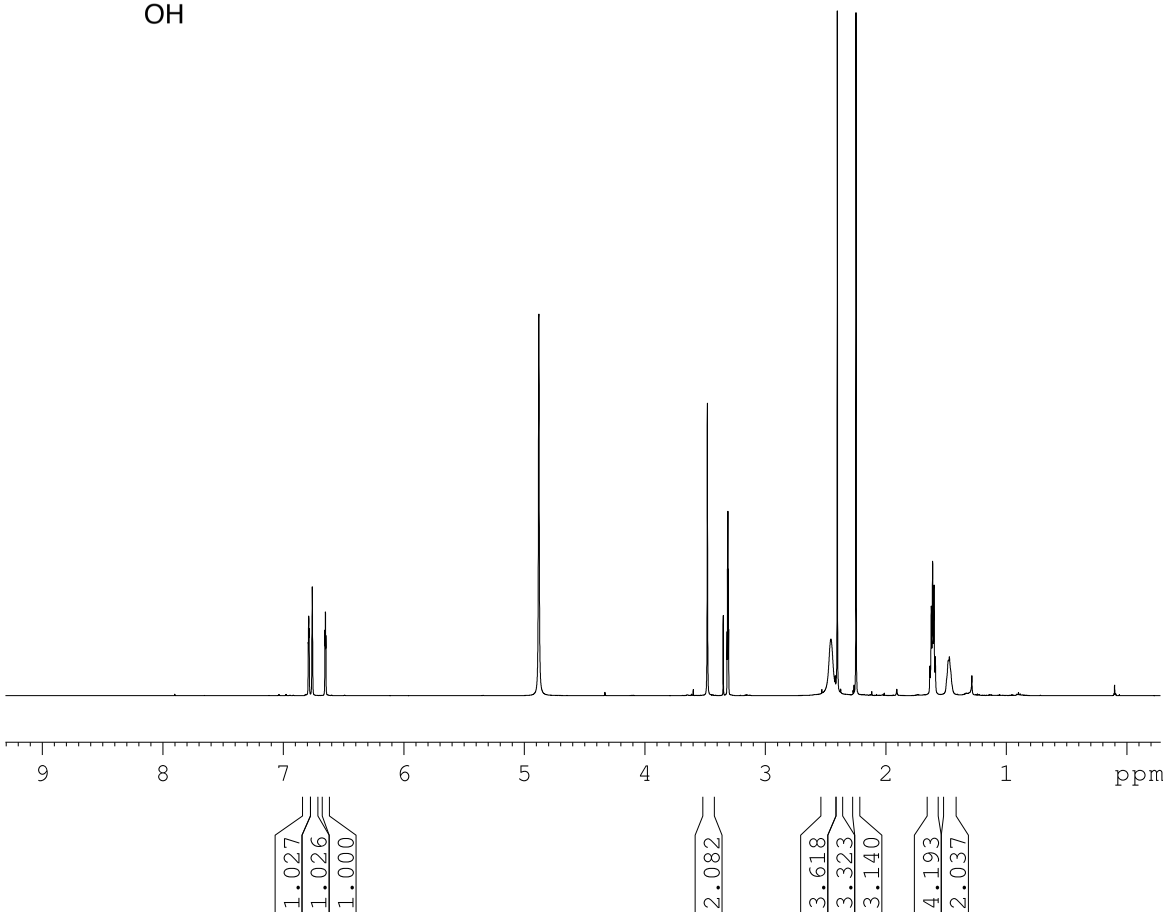
======== CHANNEL f1 ========
SFO1        125.8131151 MHz
NUC1               13C
P1               10.00 usec
PLW1        20.18400002 W

======== CHANNEL f2 ========
SFO2        500.3020012 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         7.99830008 W
PLW12        0.28119001 W
PLW13        0.17996000 W

F2 - Processing parameters
SI                32768
SF          125.8005350 MHz
WDW                EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(piperidin-1-ylmethyl)phenol (2.42)





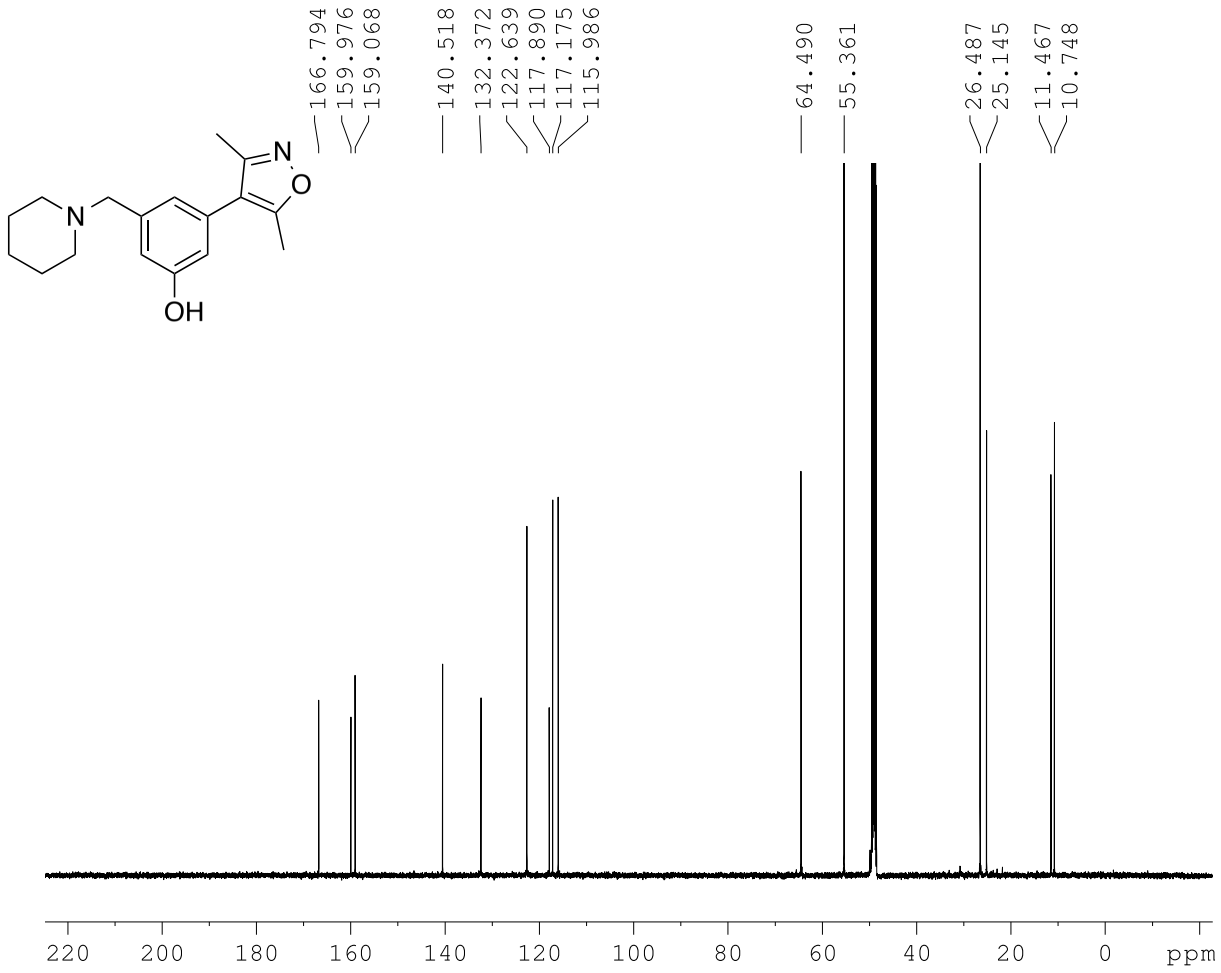

```
Current Data Parameters
NAME       LJ405 service
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameter
Date_           20140314
Time                5.45
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 se
RG                  3.56
DW                48.400 use
DE                10.00 us
TE                298.0 K
D1           1.00000000 se
TD0                    1

======== CHANNEL f1 ======
SFO1         500.3030896 MH
NUC1                1H
P1                15.00 use
PLW1          7.99830008 W

F2 - Processing parameters
SI                 65536
SF           500.3000108 MH
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(piperidin-1-ylmethyl)phenol (2.42)




```
Current Data Parameters
NAME       LJ405 service
EXPNO                  3
PROCNO                 1

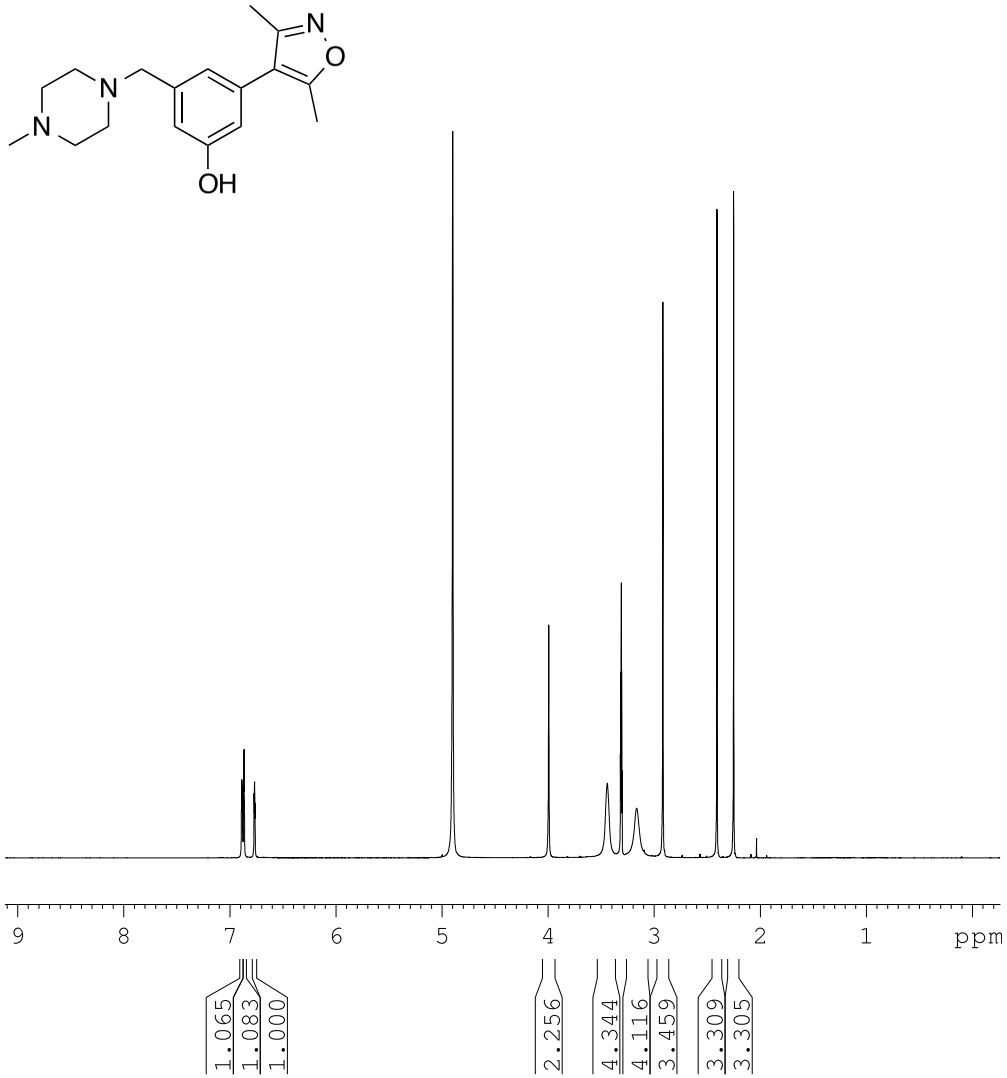
F2 - Acquisition Paramet
Date_           20140314
Time                6.43
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                   512
DS                     2
SWH            31250.000
FIDRES          0.476837
AQ             1.0485760
RG                   912
DW                16.000
DE                18.00
TE                298.0
D1            2.00000000
D11           0.03000000
TD0                    1

======== CHANNEL f1 ====
SFO1         125.8131151
NUC1                 13C
P1                 10.00
PLW1         20.18400002

======== CHANNEL f2 ====
SFO2         500.3020012
NUC2                  1H
CPDPRG[2          waltz16
PCPD2               80.00
PLW2           7.99830008
PLW12          0.28119001
PLW13          0.17996000

F2 - Processing paramete
SI                 32768
SF           125.8003594
WDW                   EM
SSB       0
LB                  1.00
GB        0
PC                  1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-methylpiperazin-1-yl)methyl]phenol (2.43)



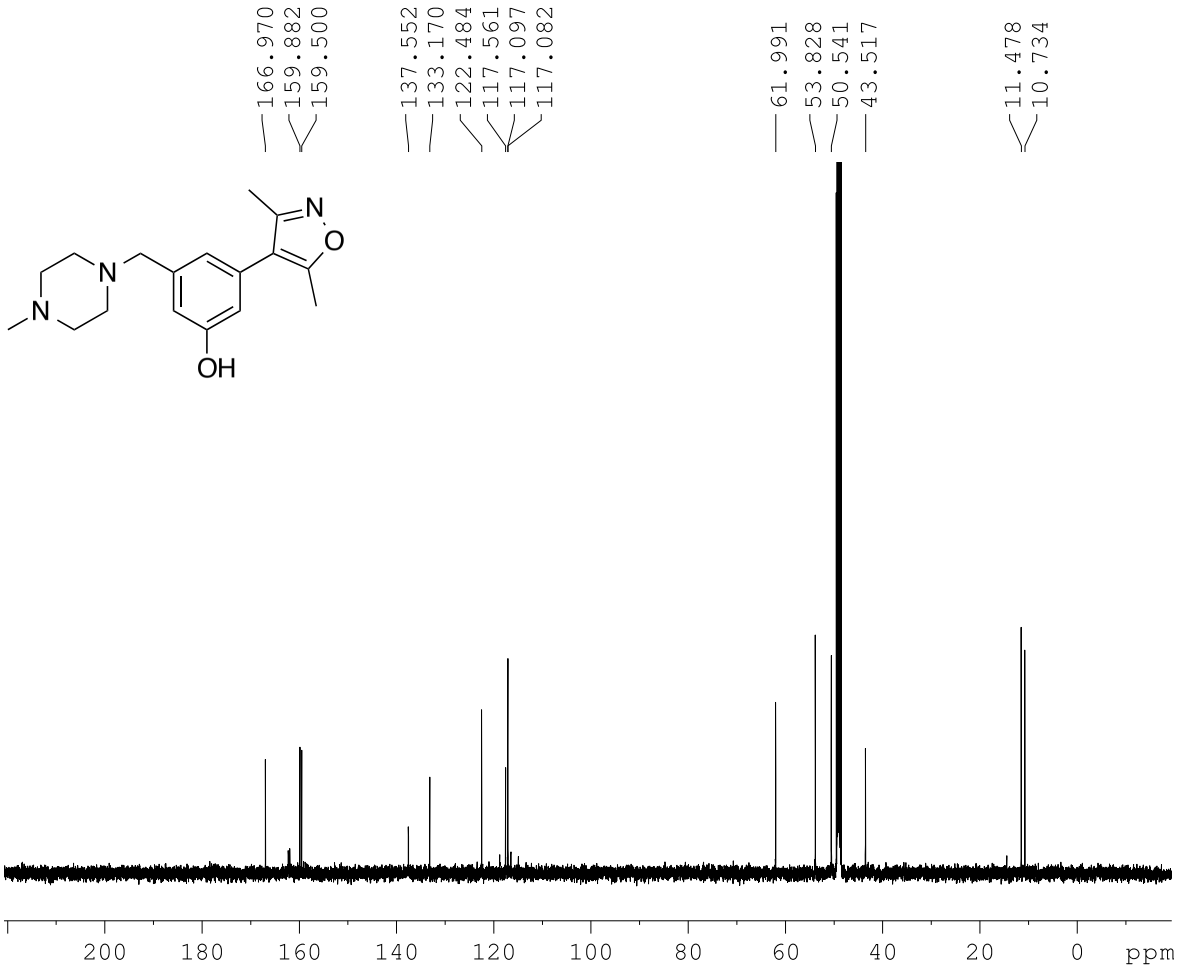

```
Current Data Parameters
NAME      LJ387 semi-prep
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20140724
Time               10.43
INSTRUM          avf400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ             4.0894465 sec
RG                    93
DW                62.400 usec
DE                 6.50 usec
TE                297.2 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                   1H
P1                 12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                 32768
SF           400.2500097 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-methylpiperazin-1-yl)methyl]phenol (2.43)




```
Current Data Parameters
NAME     LJ387 semi-prep servi
EXPNO                 2
PROCNO                1

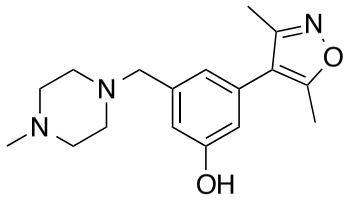
F2 - Acquisition Parameters
Date_          20140728
Time               19.10
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 3072
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               3649.1
DW              16.500 usec
DE              11.00 usec
TE                297.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                6.80 usec
PL1               5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2            100.00 usec
PL2      0 dB
PL12              19.00 dB
PL13              23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                32768
SF          125.7576126 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-[(4-methylpiperazin-1-yl)methyl]phenol (2.43)



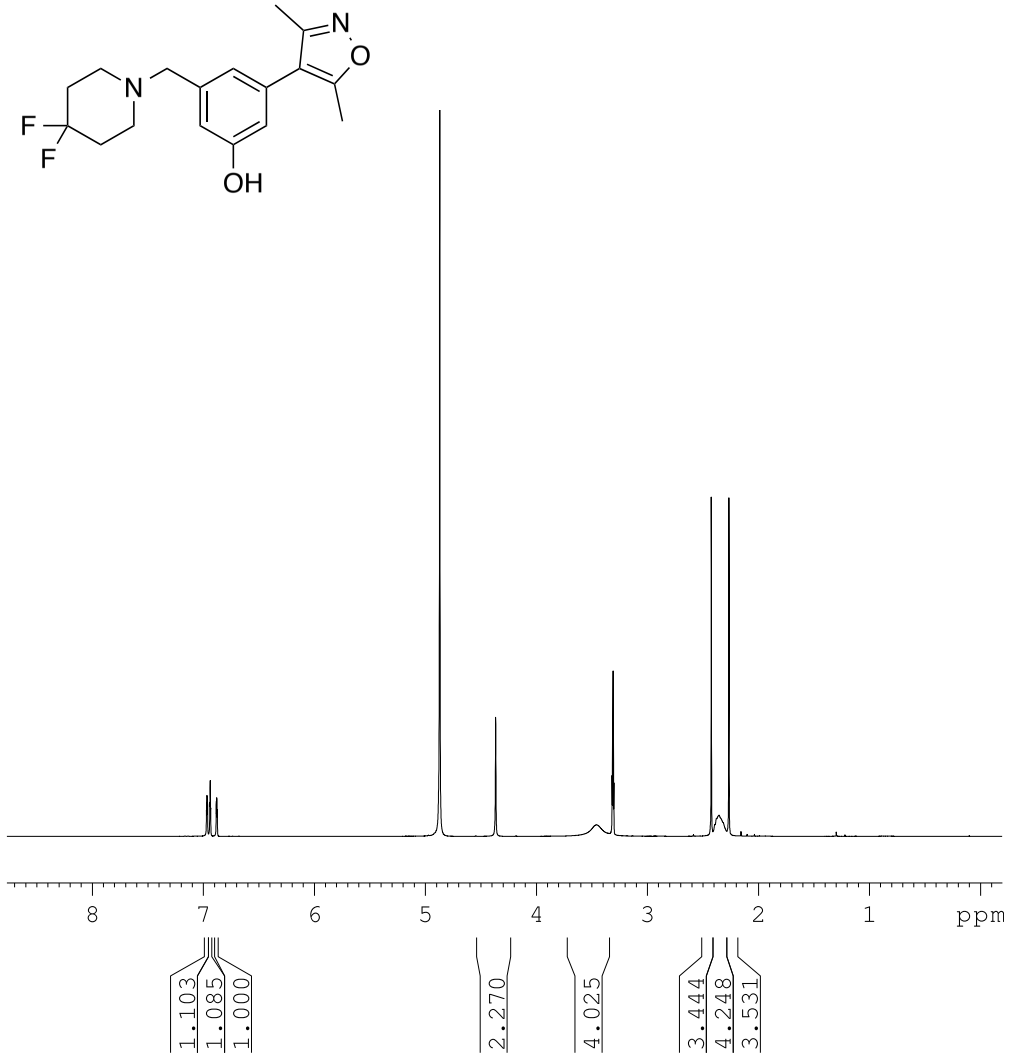

```
Current Data Parameters
NAME      LJ387 semi-prep
EXPNO                   4
PROCNO                  1

F2 - Acquisition Parameter
Date_           20140724
Time               11.03
INSTRUM          avf400
PROBHD   5 mm PABBO BB/
PULPROG          zgflqn
TD              131072
SOLVENT            MeOD
NS                  256
DS                    4
SWH          113636.367 Hz
FIDRES         0.866977 Hz
AQ            0.5767168 se
RG               205.43
DW                4.400 us
DE                6.50 us
TE               297.0 K
D1           1.00000000 se
TD0                   1

======== CHANNEL f1 ======
SFO1        376.5924484 MH
NUC1               19F
P1               13.50 us
PLW1        19.00000000 W

F2 - Processing parameters
SI                65536
SF          376.6112790 MH
WDW                EM
SSB       0
LB               0.30 Hz
GB        0
PC               1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(4,4-difluoropiperidin-1-ylmethyl)phenol (2.44)



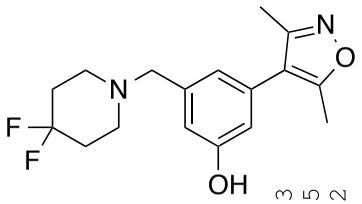

```
Current Data Parameters
NAME      LJ448 semi-prep
EXPNO                   1
PROCNO                  1

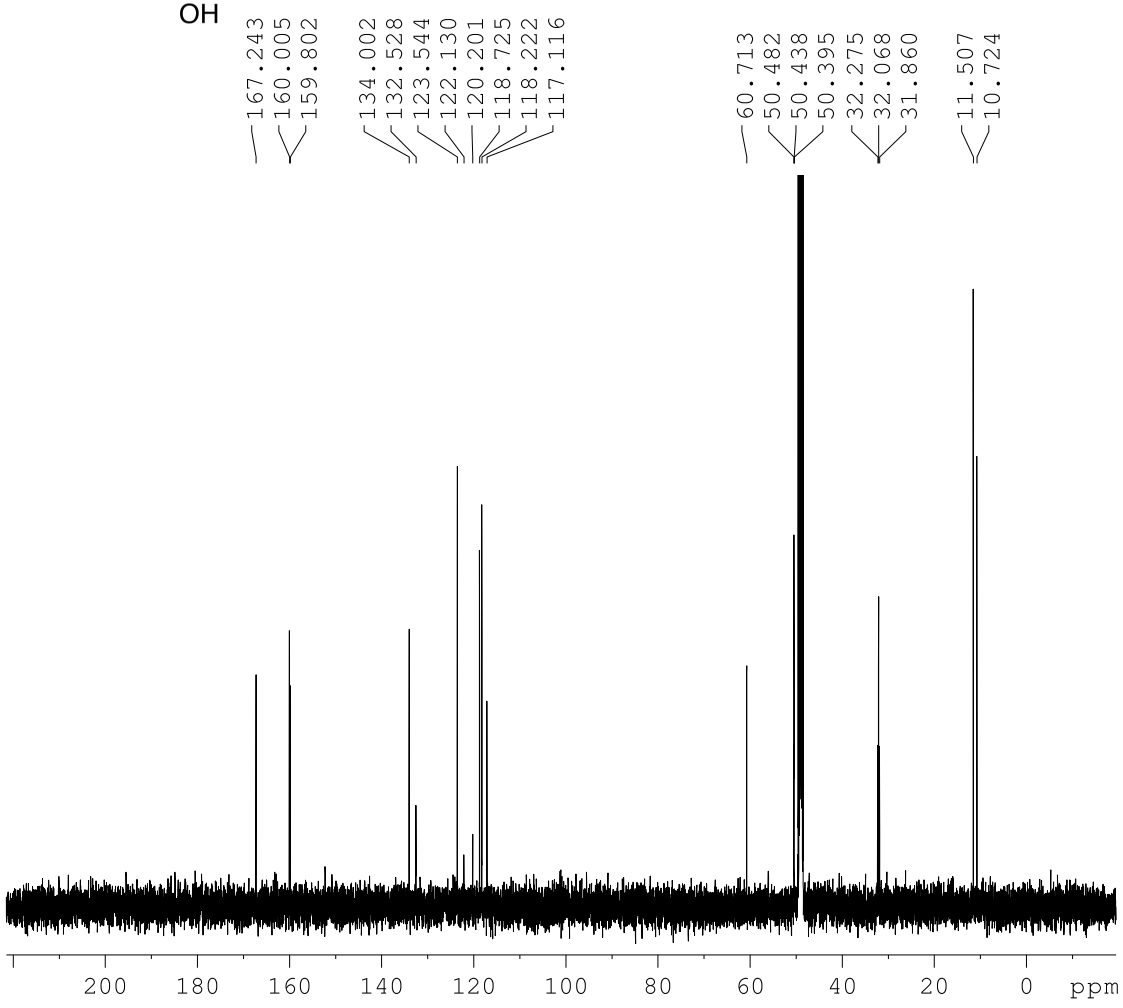
F2 - Acquisition Parameters
Date_           20140724
Time               11.31
INSTRUM          avf400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES           0.122266 Hz
AQ              4.0894465 sec
RG                    93
DW                 62.400 usec
DE                  6.50 usec
TE                 297.1 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                   1H
P1                 12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                 32768
SF           400.2500098 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(4,4-difluoropiperidin-1-ylmethyl)phenol (2.44)






```
Current Data Parameters
NAME     LJ448 semi-prep service
EXPNO                 2
PROCNO                1

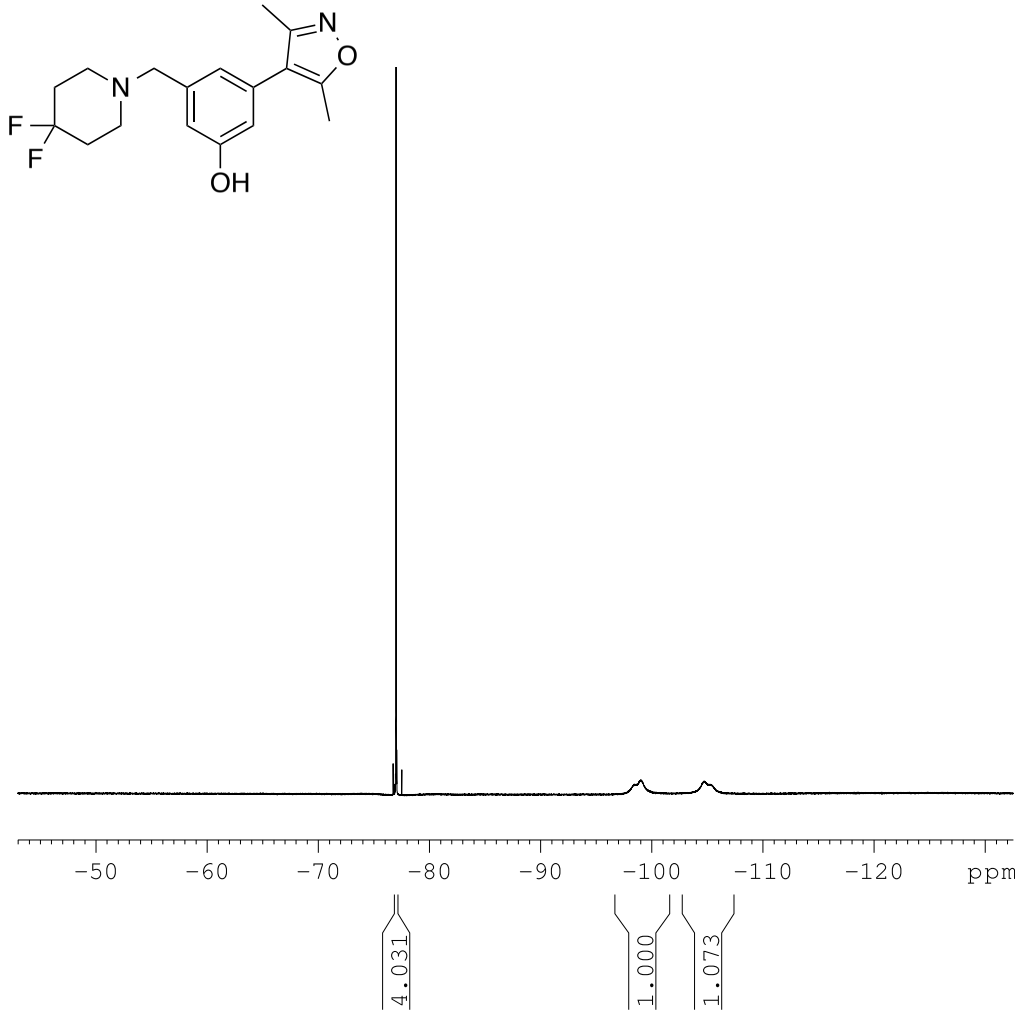
F2 - Acquisition Parameters
Date_          20140728
Time               9.55
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 3146
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG               3649.1
DW              16.500 usec
DE              11.00 usec
TE              297.7 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  6.80 usec
PL1                 5.00 dB
SFO1         125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                 1H
PCPD2             100.00 usec
PL2      0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2         500.1320005 MHz

F2 - Processing parameters
SI                  32768
SF           125.7576128 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(4,4-difluoropiperidin-1-ylmethyl)phenol (2.44)



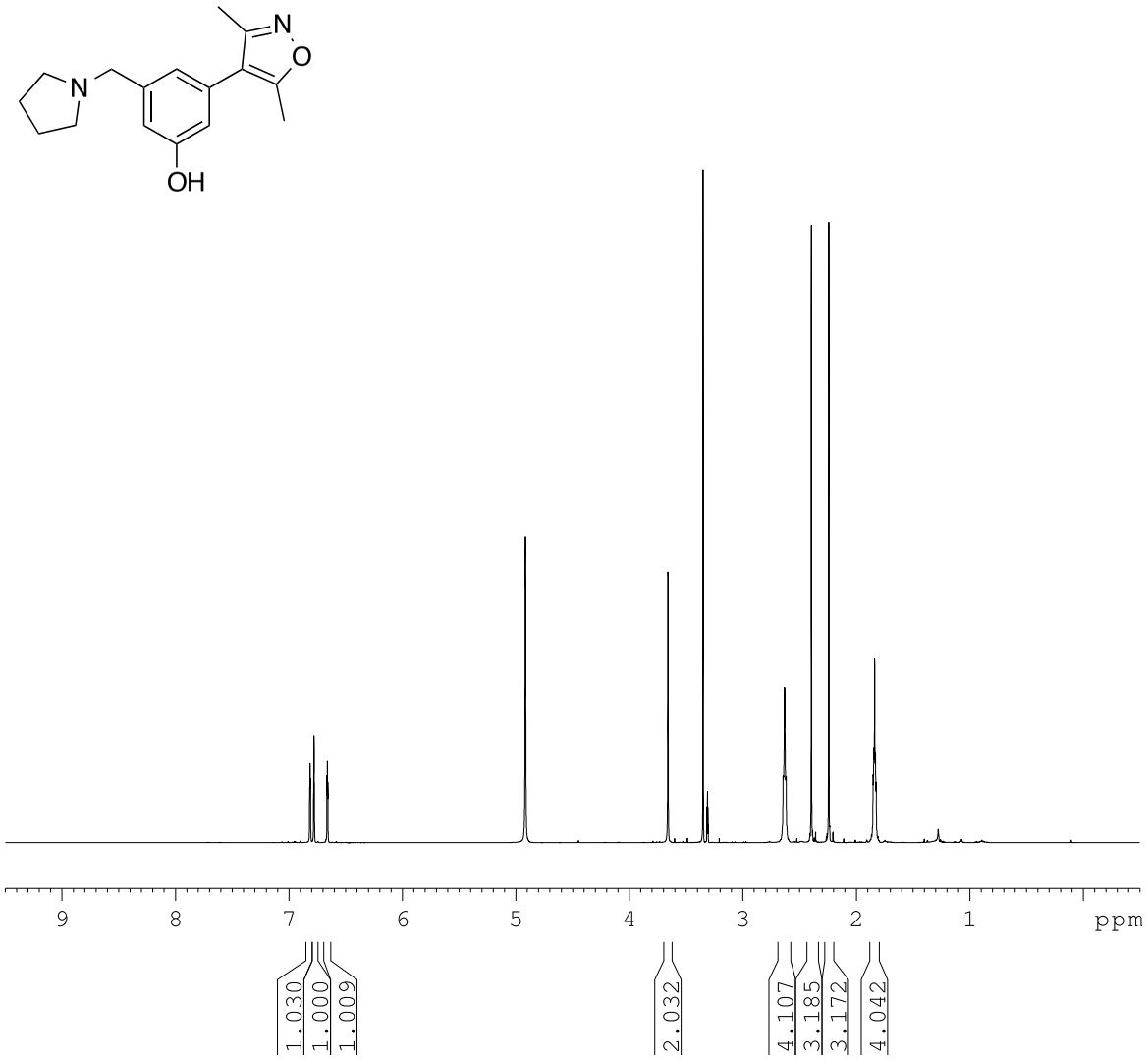

```
Current Data Parameters
NAME      LJ448 semi-prep
EXPNO                   6
PROCNO                  1

F2 - Acquisition Parameters
Date_           20140724
Time               12.07
INSTRUM          avf400
PROBHD   5 mm PABBO BB/
PULPROG           zgflqn
TD                131072
SOLVENT             MeOD
NS                    256
DS                      4
SWH          113636.367 Hz
FIDRES         0.866977 Hz
AQ            0.5767168 sec
RG               205.43
DW                4.400 usec
DE                6.50 usec
TE               297.2 K
D1           1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1         376.5924484 MHz
NUC1                19F
P1                13.50 usec
PLW1         19.00000000 W

F2 - Processing parameters
SI                  65536
SF           376.6112790 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(pyrrolidin-1-ylmethyl)phenol (2.45)



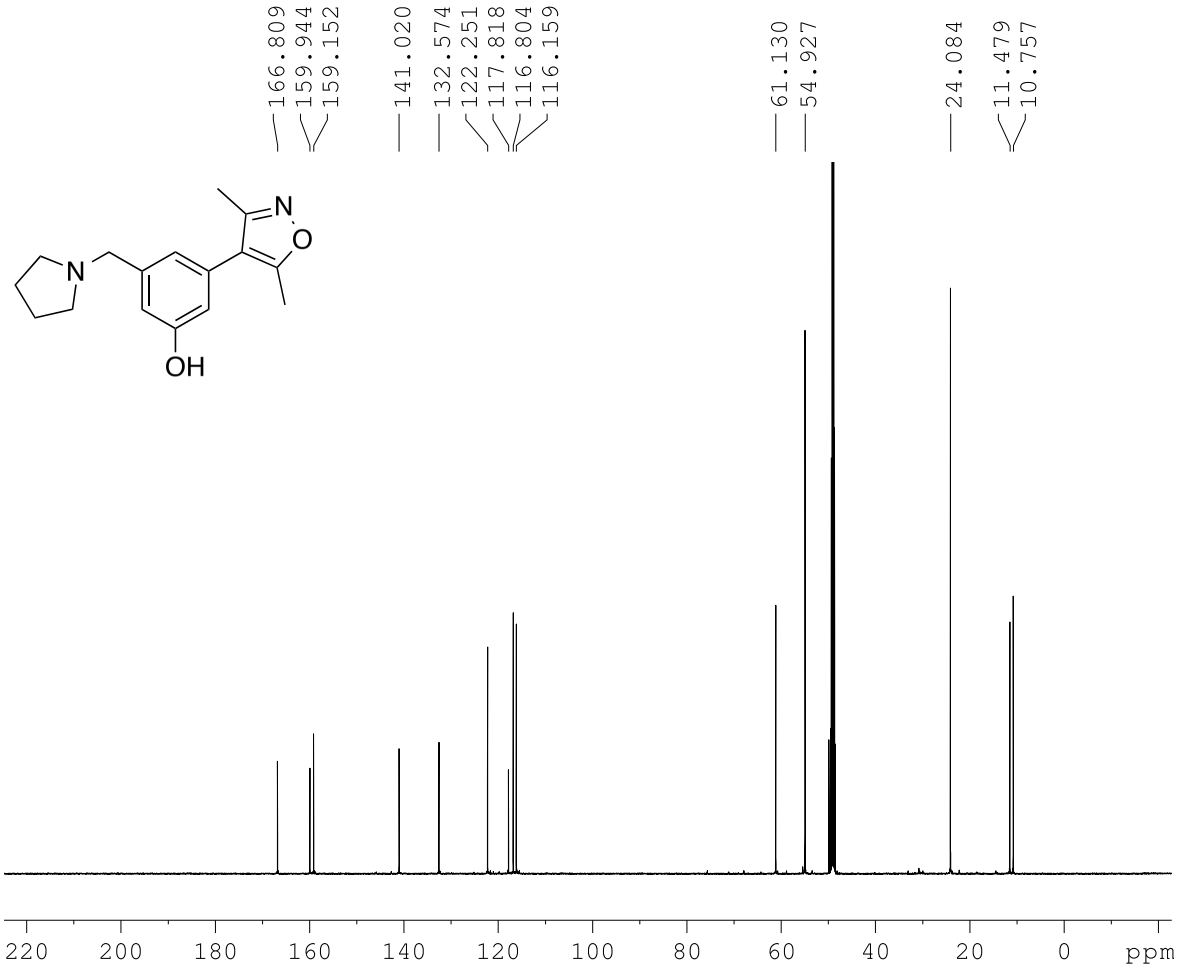

```
Current Data Parameters
NAME       LJ403 service
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameter
Date_           20140314
Time                4.39
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 se
RG                  3.56
DW                48.400 use
DE                10.00 us
TE                298.0 K
D1           1.00000000 se
TD0                    1

======== CHANNEL f1 ======
SFO1         500.3030896 MH
NUC1                1H
P1                15.00 use
PLW1          7.99830008 W

F2 - Processing parameters
SI                 65536
SF           500.3000106 MH
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

3-(3,5-Dimethyl-1,2-oxazol-4-yl)-5-(pyrrolidin-1-ylmethyl)phenol (2.45)




```
Current Data Parameters
NAME       LJ403 service
EXPNO                  3
PROCNO                 1

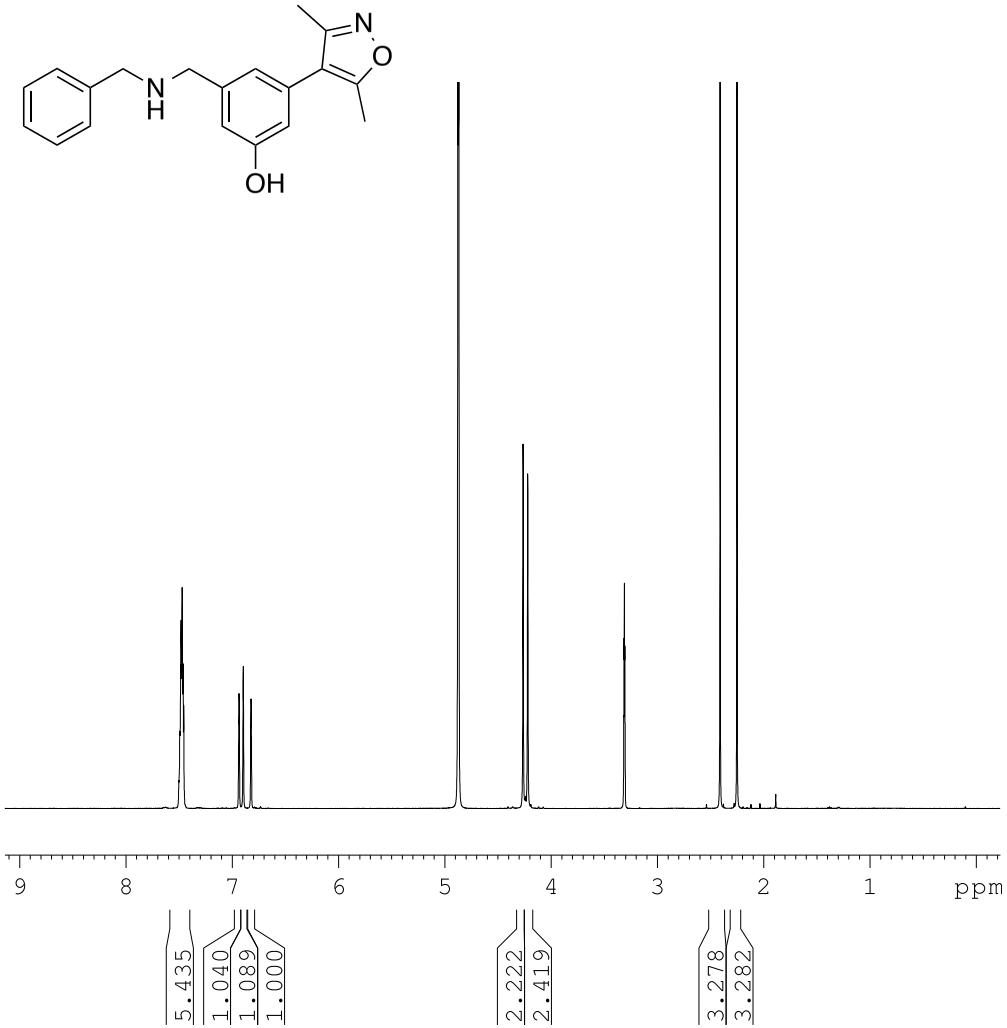
F2 - Acquisition Paramet
Date_           20140314
Time                5.36
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT            MeOD
NS                   512
DS                     2
SWH            31250.000
FIDRES          0.476837
AQ              1.0485760
RG                   912
DW                16.000
DE                18.00
TE                298.0
D1           2.00000000
D11          0.03000000
TD0                    1

======== CHANNEL f1 ====
SFO1         125.8131151
NUC1                13C
P1                10.00
PLW1         20.18400002

======== CHANNEL f2 ====
SFO2         500.3020012
NUC2                1H
CPDPRG[2         waltz16
PCPD2              80.00
PLW2          7.99830008
PLW12         0.28119001
PLW13         0.17996000

F2 - Processing paramete
SI                 32768
SF           125.8003623
WDW                   EM
SSB       0
LB                 1.00
GB        0
PC                 1.40
```

3-[(Benzylamino)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.46)



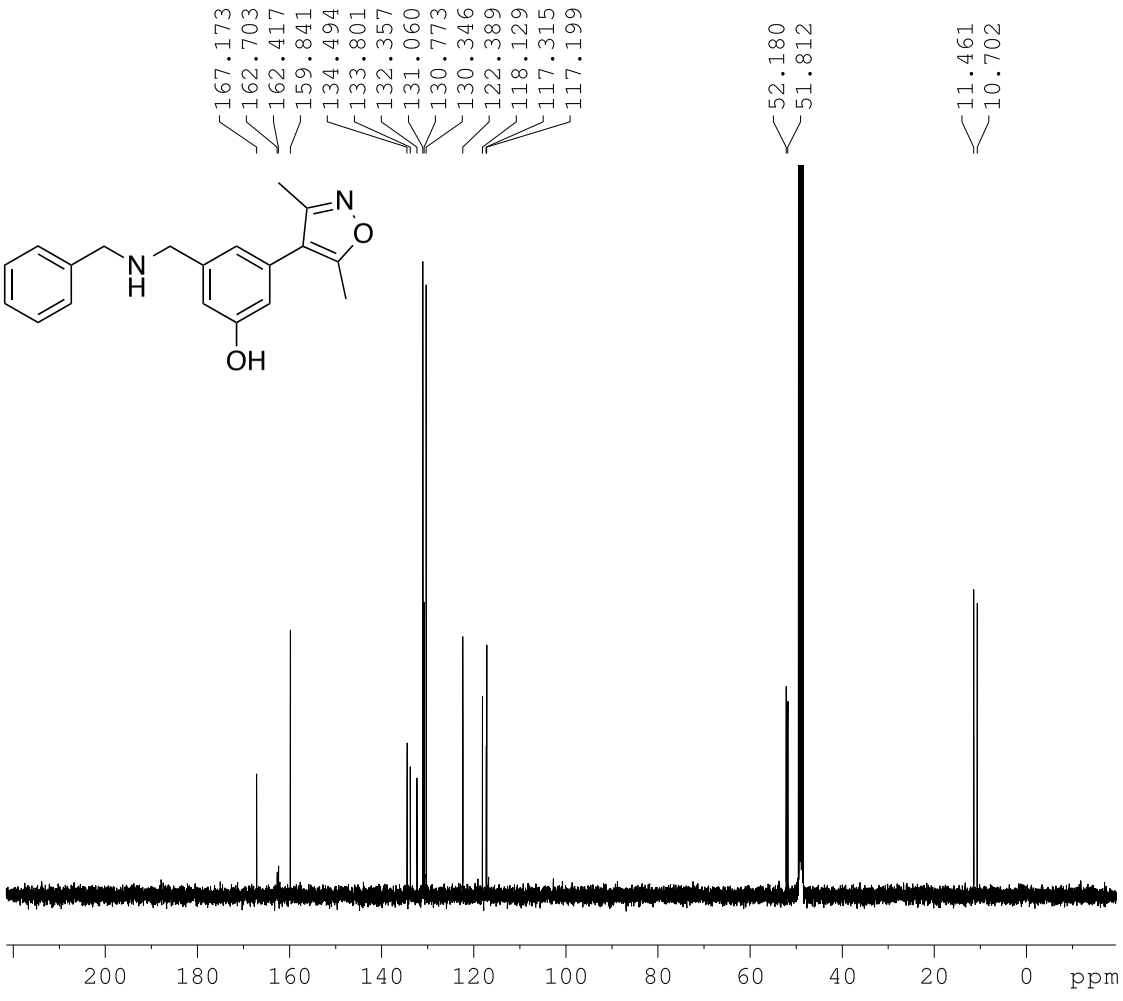

```
Current Data Parameters
NAME      LJ455 semi-prep servic
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20140729
Time               12.35
INSTRUM          drx500
PROBHD    5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                 143.7
DW                48.400 usec
DE                17.00 usec
TE                 298.7 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1         500.1325006 MHz

F2 - Processing parameters
SI                  32768
SF           500.1300113 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

3-[(Benzylamino)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.46)




```
Current Data Parameters
NAME     LJ455 semi-prep service
EXPNO                 3
PROCNO                1

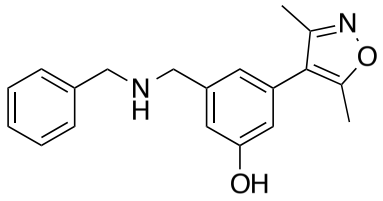
F2 - Acquisition Parameters
Date_          20140729
Time               13.43
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 2048
DS                    4
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813440 sec
RG                 4096
DW               16.500 usec
DE               11.00 usec
TE                297.7 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

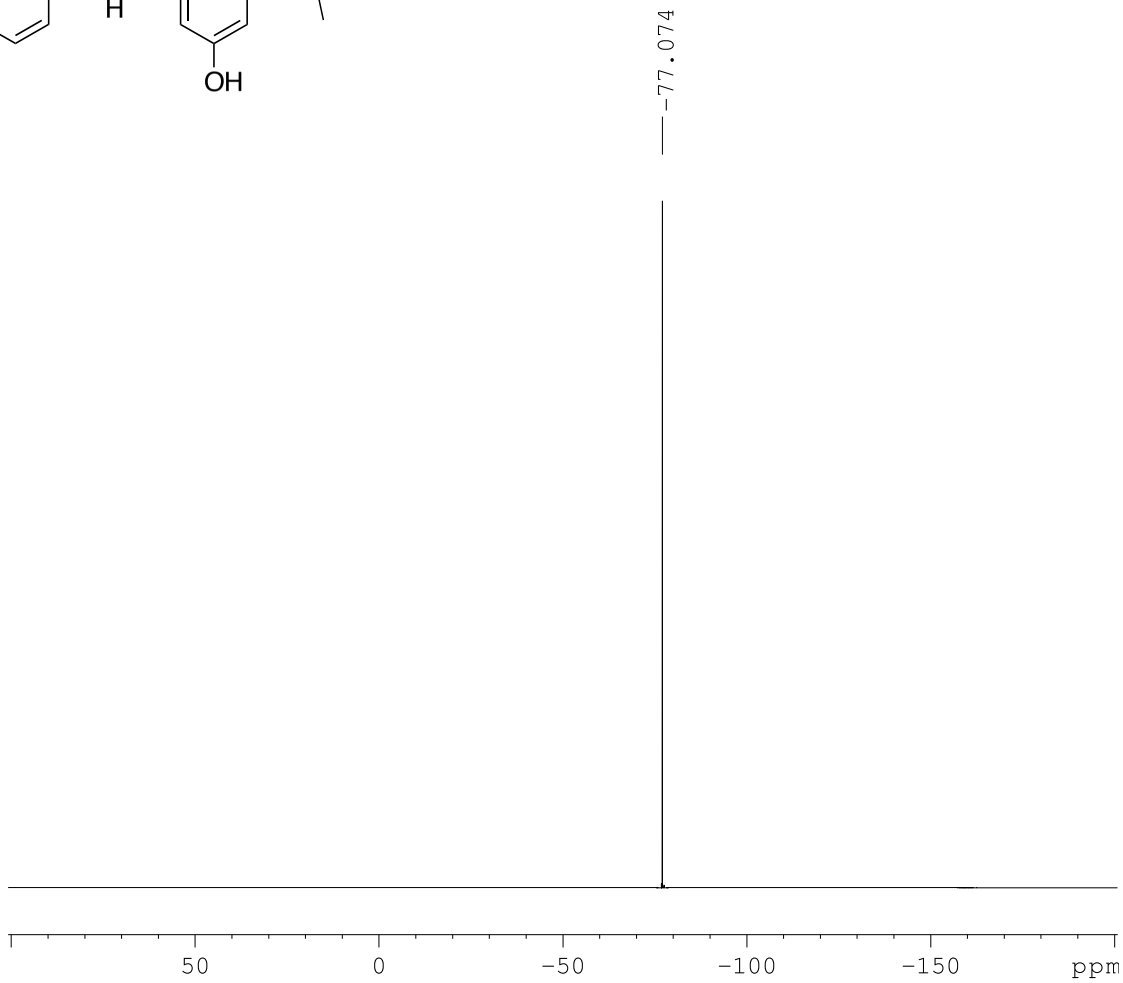
======== CHANNEL f1 ========
NUC1                 13C
P1                 6.80 usec
PL1                5.00 dB
SFO1        125.7703148 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2            100.00 usec
PL2      0 dB
PL12               19.00 dB
PL13               23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                 32768
SF          125.7576130 MHz
WDW                  EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

3-[(Benzylamino)methyl]-5-(3,5-dimethyl-1,2-oxazol-4-yl)phenol (2.46)





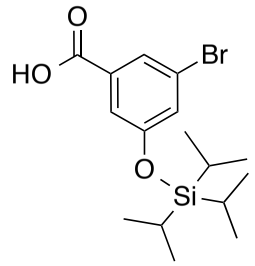

```
Current Data Parameters
NAME      LJ455 semi-prep
EXPNO                  12
PROCNO                  1

F2 - Acquisition Parameter
Date_           20140726
Time                0.48
INSTRUM           avf400
PROBHD   5 mm PABBO BB/
PULPROG           zgflqn
TD                131072
SOLVENT             MeOD
NS                    256
DS                      4
SWH          113636.367 Hz
FIDRES         0.866977 Hz
AQ             0.5767168 se
RG                205.43
DW                 4.400 us
DE                 6.50 us
TE                297.1 K
D1           1.00000000 se
TD0                    1

======== CHANNEL f1 ======
SFO1         376.5924484 MH
NUC1                19F
P1                13.50 us
PLW1         19.00000000 W

F2 - Processing parameters
SI                 65536
SF           376.6112790 MH
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

3-(3,5-Dimethylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)benzoic acid (2.53)



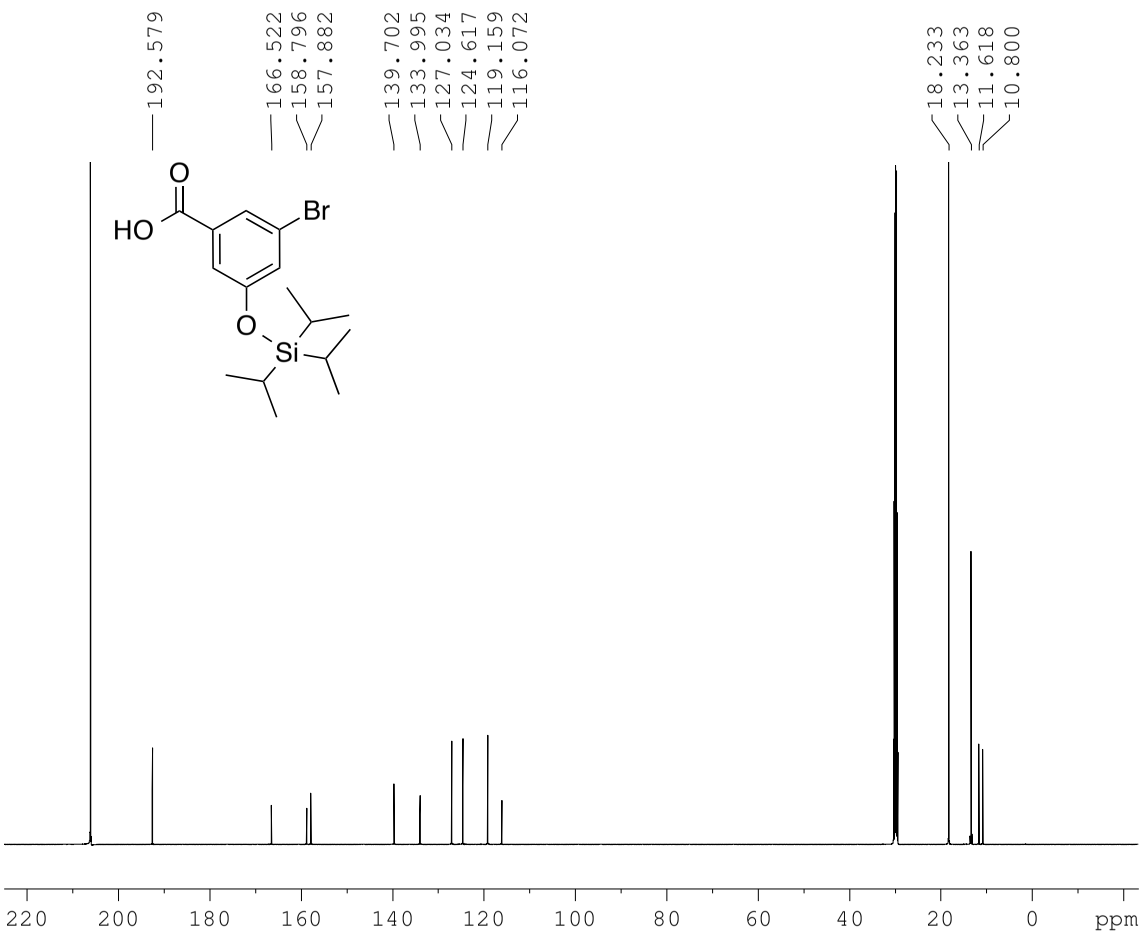

```
Current Data Parameters
NAME      TIPS Protected Aldehyde
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20130317
Time              10.17
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                65536
SOLVENT          Acetone
NS                   16
DS                    4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                 2.25
DW               48.400 usec
DE                6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 1H
P1                10.60 usec
PL1                6.00 dB
PL1W         0.95905519 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                32768
SF           500.3000097 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

3-(3,5-Dimethylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)benzoic acid (2.53)




```
Current Data Parameters
NAME     TIPS Protected Aldehyde
EXPNO                 4
PROCNO                1

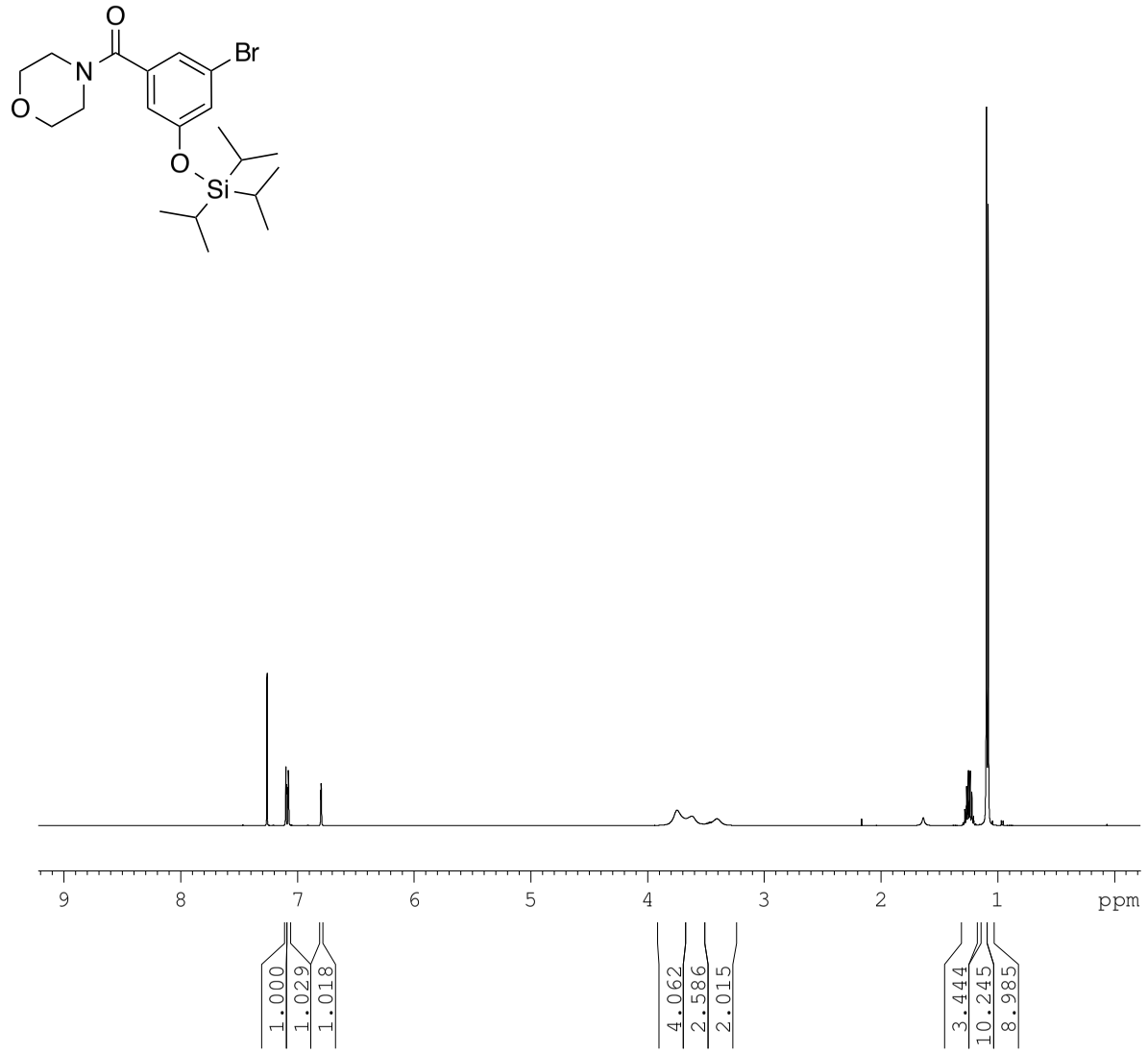
F2 - Acquisition Parameters
Date_           20130317
Time              10.53
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                65536
SOLVENT          Acetone
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                  912
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF           125.8004257 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(3-Bromo-5-(triisopropylsilyl)oxy)phenyl)(morpholin-4-yl)methanone (2.54)



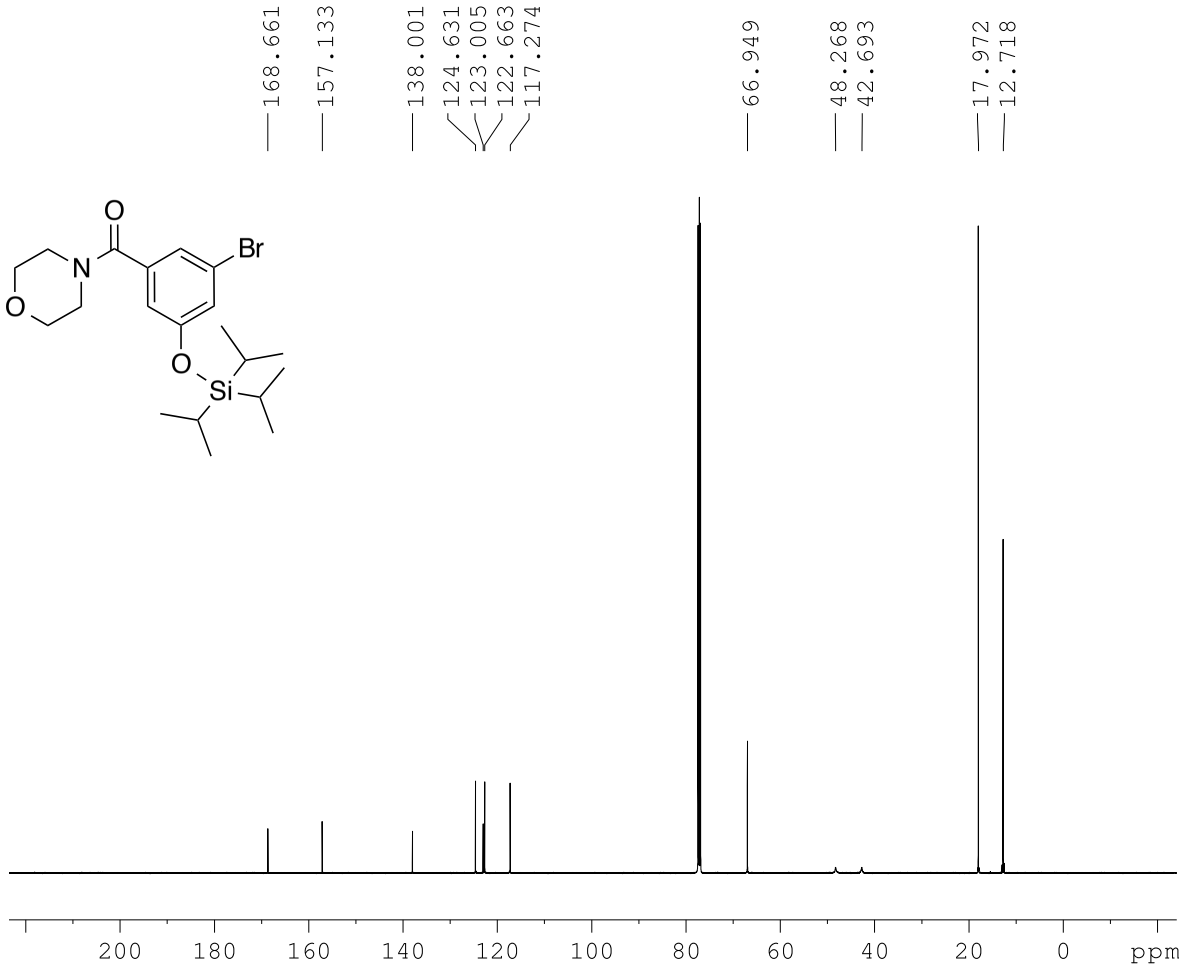

```
Current Data Parameters
NAME      LJ412 service
EXPNO                1
PROCNO               1

F2 - Acquisition Parameters
Date_          20140411
Time               5.30
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT           CDC13
NS                   16
DS                    4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                 3.56
DW               48.400 usec
DE                10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1         500.3030896 MHz
NUC1                1H
P1                15.00 usec
PLW1          7.99830008 W

F2 - Processing parameters
SI                65536
SF           500.3000133 MHz
WDW                  EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

(3-Bromo-5-(triisopropylsilyl)oxy)phenyl)(morpholin-4-yl)methanone (2.54)




```
Current Data Parameters
NAME       LJ412 service
EXPNO                  4
PROCNO                 1

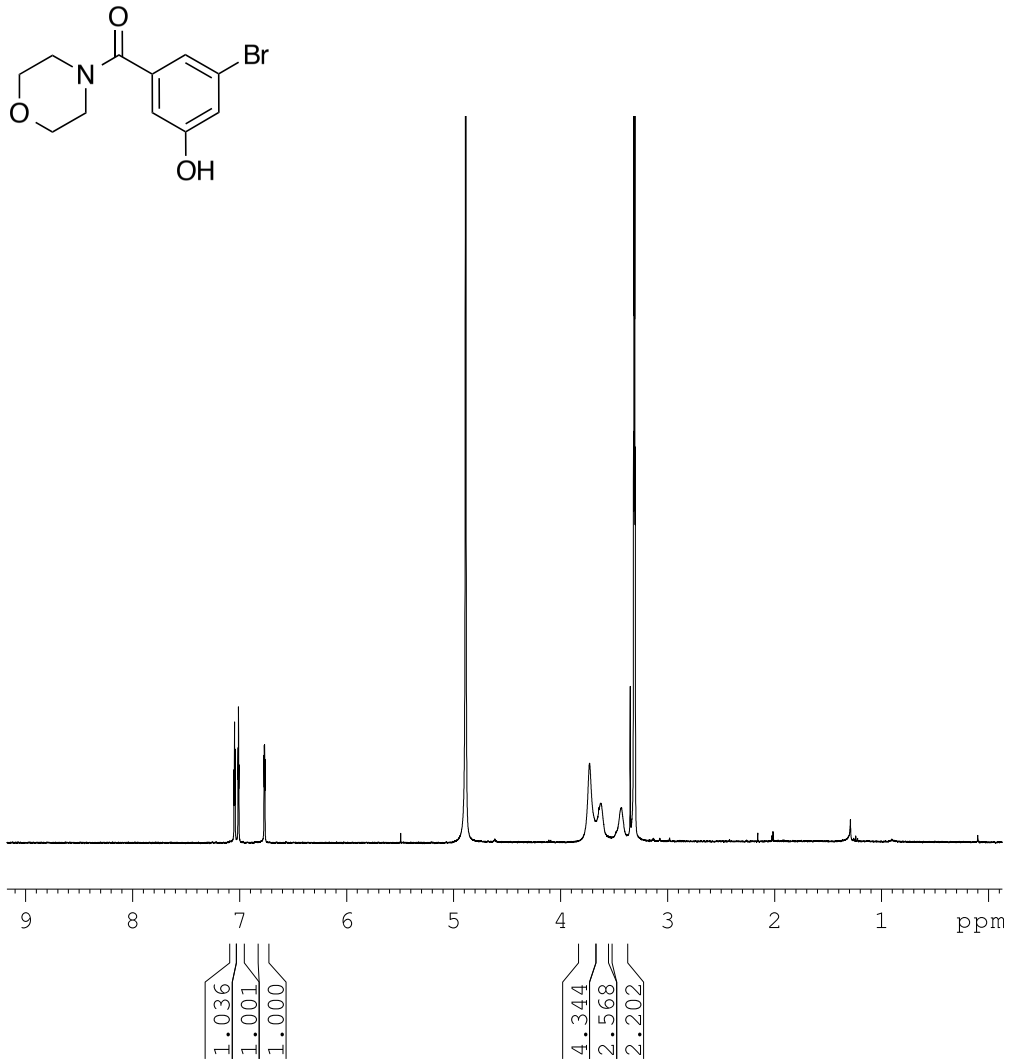
F2 - Acquisition Paramet
Date_           20140411
Time                9.05
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000
FIDRES         0.476837
AQ            1.0485760
RG                  912
DW               16.000
DE                18.00
TE                298.0
D1           2.00000000
D11          0.03000000
TD0                   1

======== CHANNEL f1 ====
SFO1        125.8131152
NUC1                13C
P1                10.00
PLW1        20.18400002

======== CHANNEL f2 ====
SFO2        500.3020012
NUC2                1H
CPDPRG[2         waltz16
PCPD2              80.00
PLW2          7.99830008
PLW12         0.28119001
PLW13         0.17996000

F2 - Processing paramete
SI                 32768
SF          125.8005200
WDW                   EM
SSB       0
LB                 1.00
GB        0
PC                 1.40
```

(3-Bromo-5-hydroxyphenyl)(morpholin-4-yl)methanone (2.55)



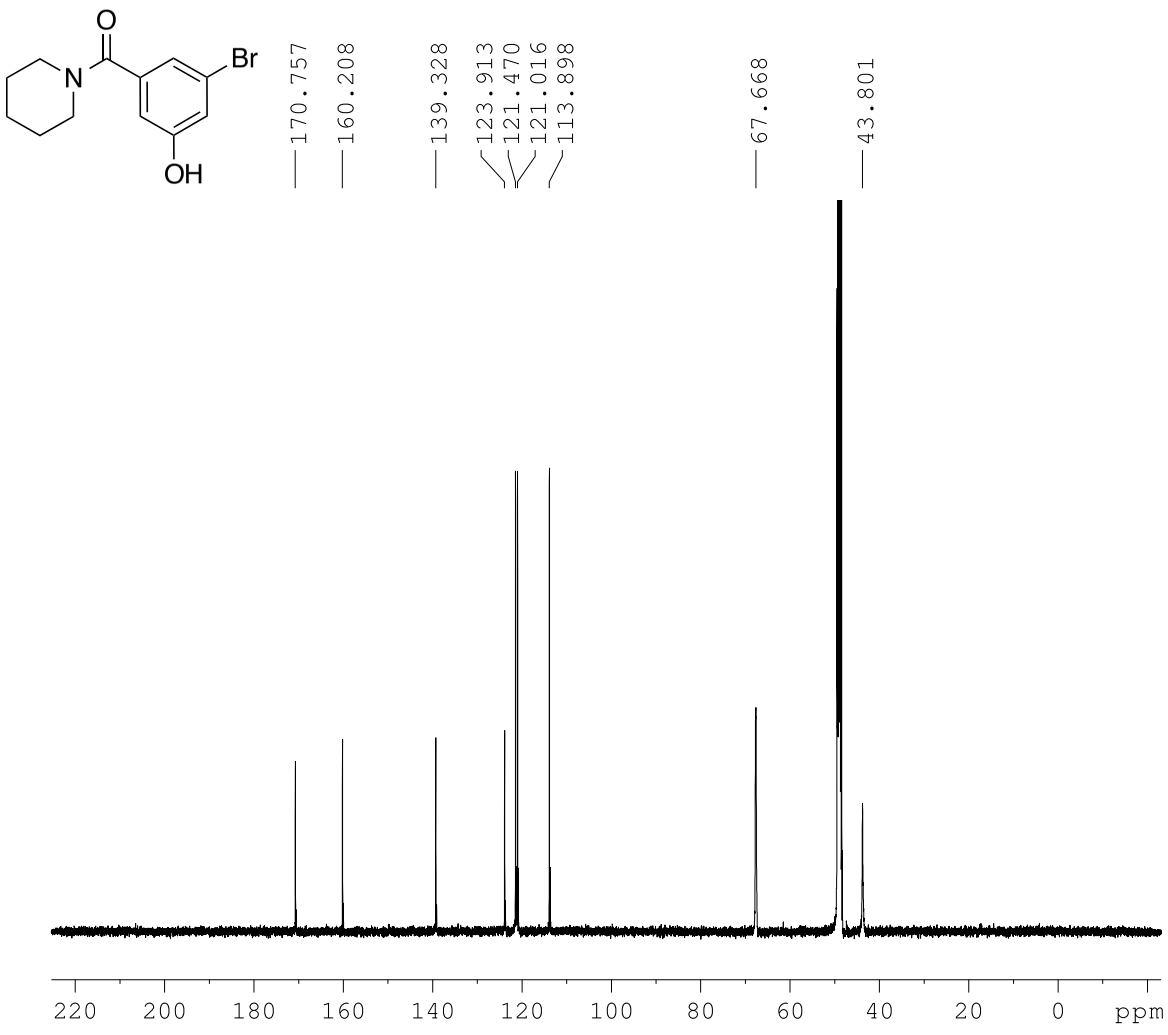

```
Current Data Parameters
NAME      LJ429 concentrated
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20140801
Time               18.17
INSTRUM          avg400
PROBHD    5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH            10000.000 Hz
FIDRES          0.152588 Hz
AQ             3.2767999 sec
RG                467.29
DW                50.000 usec
DE                 6.50 usec
TE                294.8 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                  1H
P1                 12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                 65536
SF           400.2000116 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

(3-Bromo-5-hydroxyphenyl)(morpholin-4-yl)methanone (2.55)




```
Current Data Parameters
NAME      LJ429 service
EXPNO                3
PROCNO               1

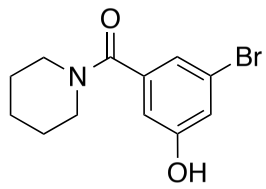
F2 - Acquisition Parameters
Date_          20140708
Time              23.50
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                  912
DW               16.000 usec
DE               18.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

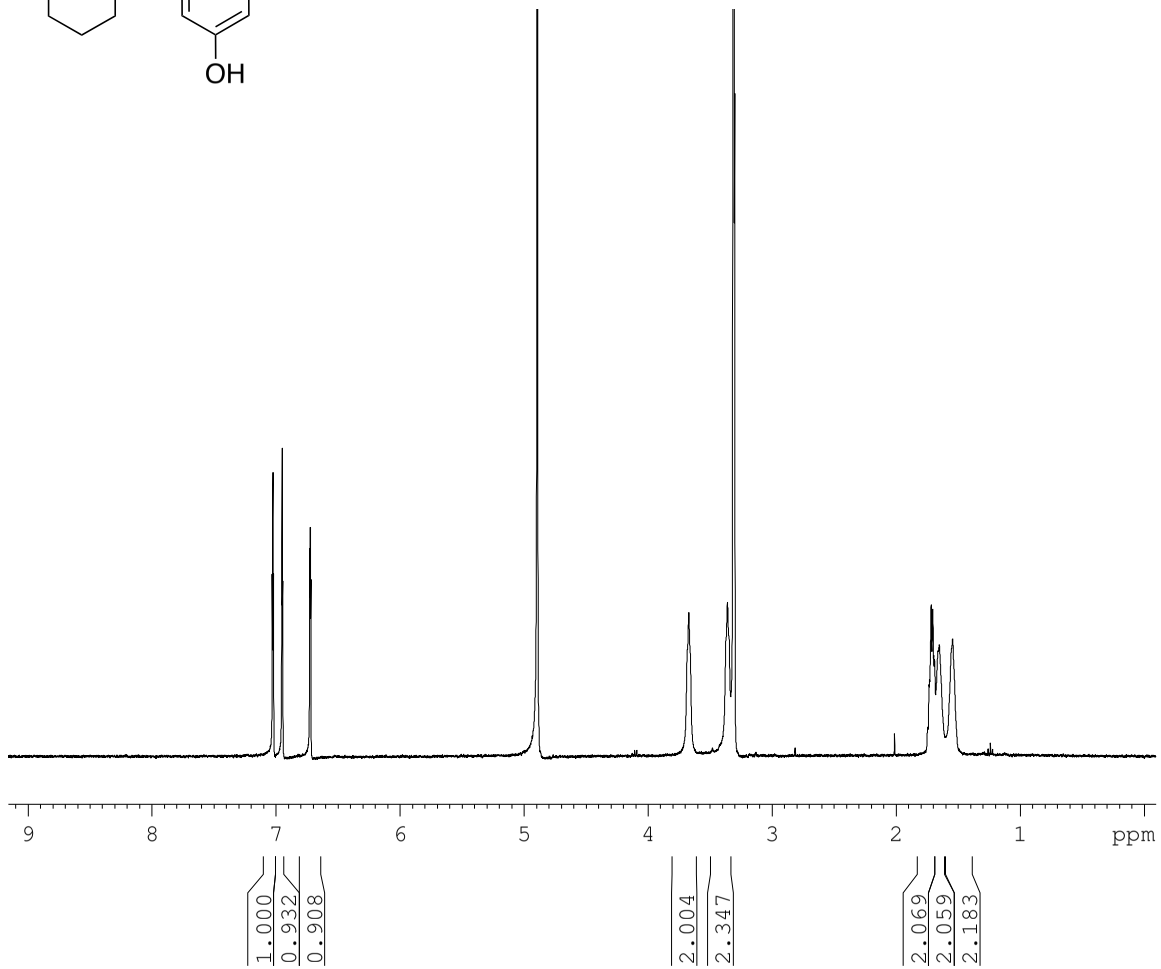
======== CHANNEL f1 ========
SFO1         125.8131152 MHz
NUC1                13C
P1                10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                 32768
SF           125.8003915 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(3-Bromo-5-hydroxyphenyl)(piperidin-1-yl)methanone (2.56)





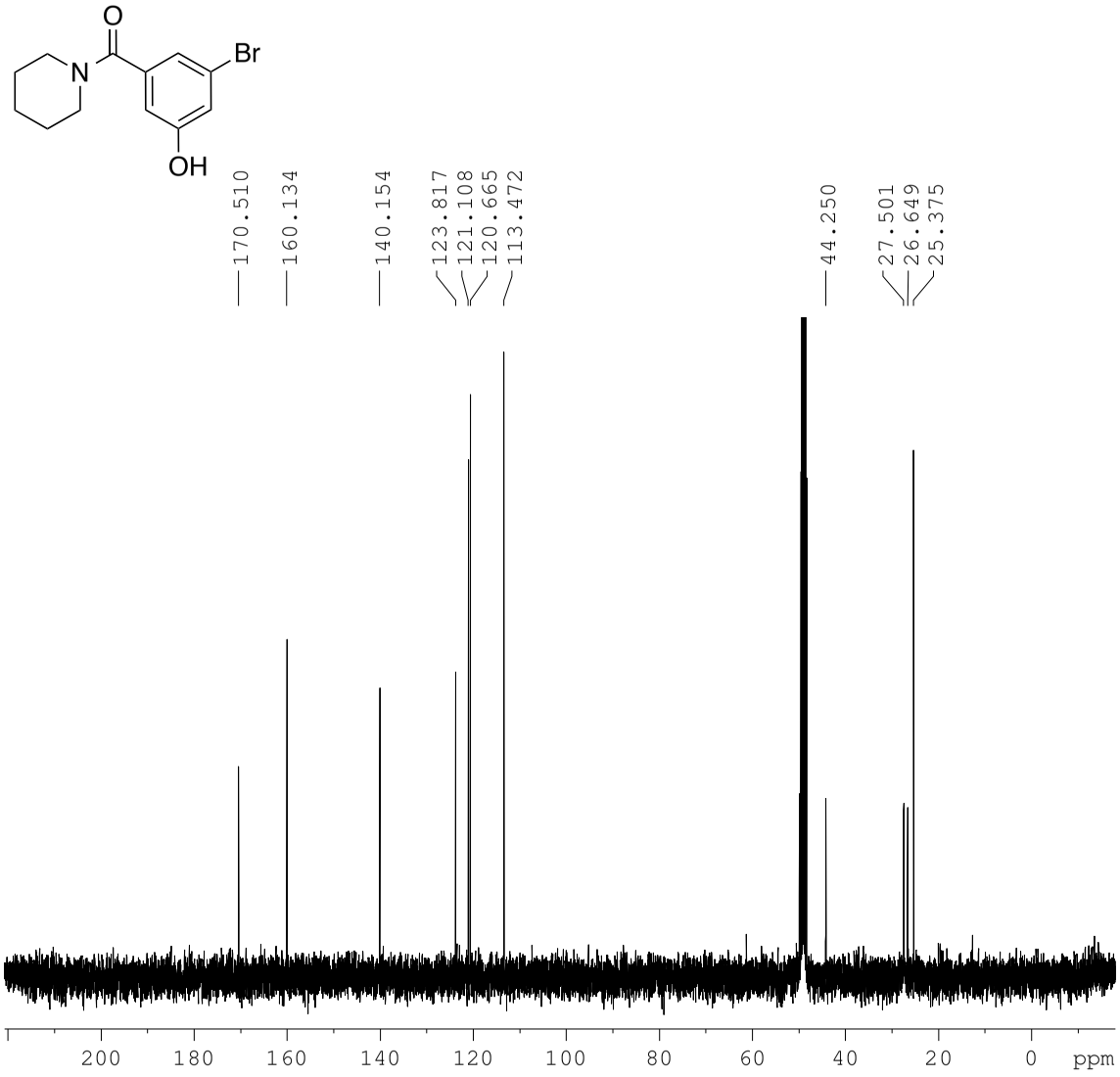

```
Current Data Parameters
NAME     LJ445 precipitate
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20140627
Time               11.01
INSTRUM          avg400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH            10000.000 Hz
FIDRES          0.152588 Hz
AQ             3.2767999 sec
RG                512.86
DW                50.000 usec
DE                 6.50 usec
TE                294.1 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                   1H
P1                 12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                 65536
SF           400.2000115 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

(3-Bromo-5-hydroxyphenyl)(piperidin-1-yl)methanone (2.56)




```
Current Data Parameters
NAME     LJ445 precipitate AVG
EXPNO                 2
PROCNO                1

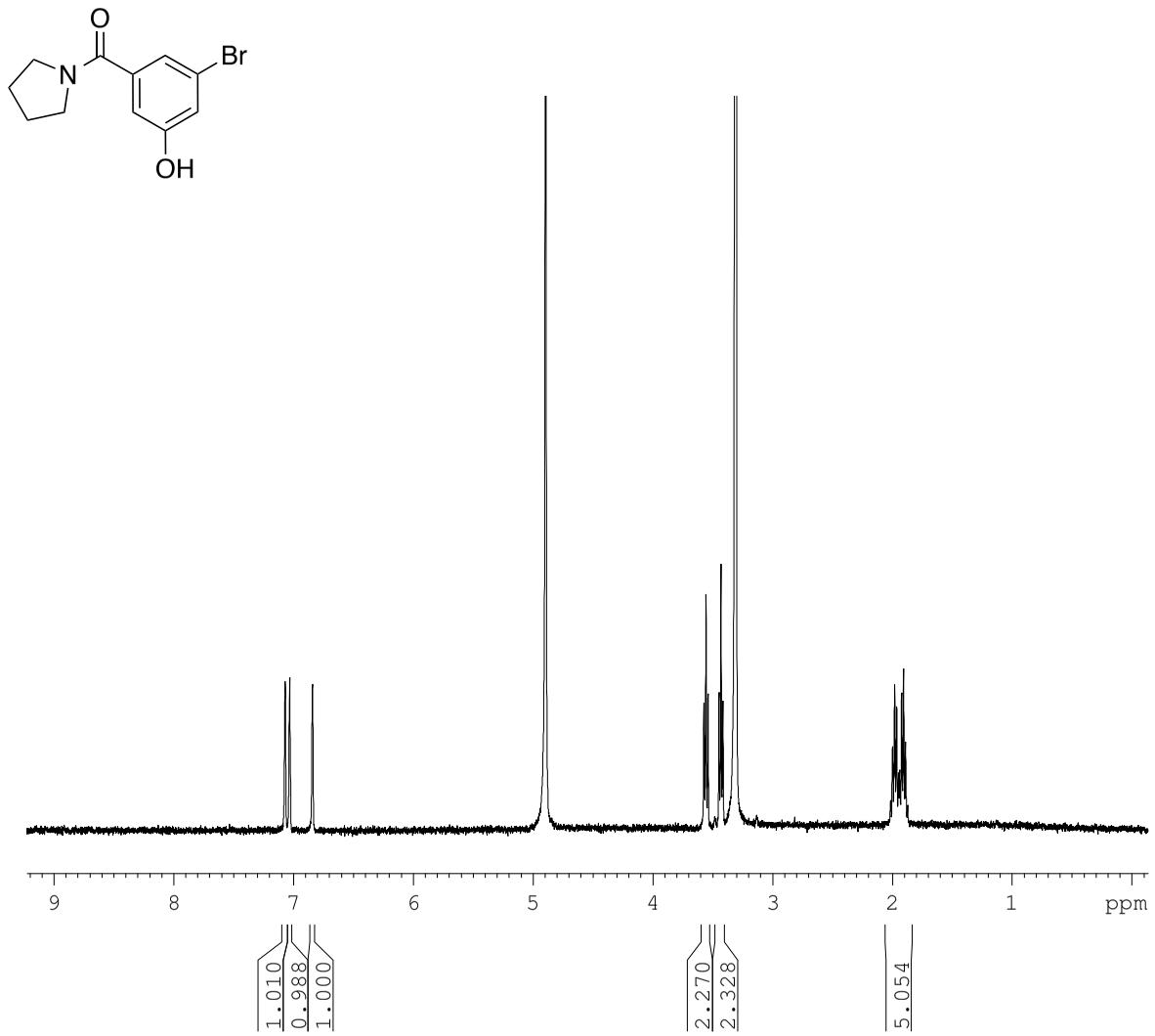
F2 - Acquisition Parameters
Date_           20140702
Time              20.20
INSTRUM           avg400
PROBHD   5 mm QNP 1H/13
PULPROG           zgpg30
TD                65536
SOLVENT             MeOD
NS                  256
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631488 sec
RG                 2050
DW               20.800 usec
DE                6.50 usec
TE               294.2 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         100.6404326 MHz
NUC1                13C
P1                10.00 usec
PLW1         40.00000000 W

======== CHANNEL f2 ========
SFO2         400.2016008 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         11.29800034 W
PLW12         0.20863000 W
PLW13         0.16899000 W

F2 - Processing parameters
SI                32768
SF           100.6302329 MHz
WDW                 EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(3-Bromo-5-hydroxyphenyl)(pyrrolidin-1-yl)methanone (2.57)



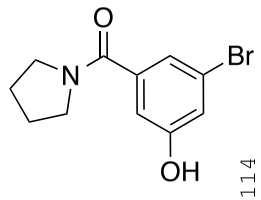

```
Current Data Parameters
NAME      LJ447 precipitate
EXPNO                   1
PROCNO                  1

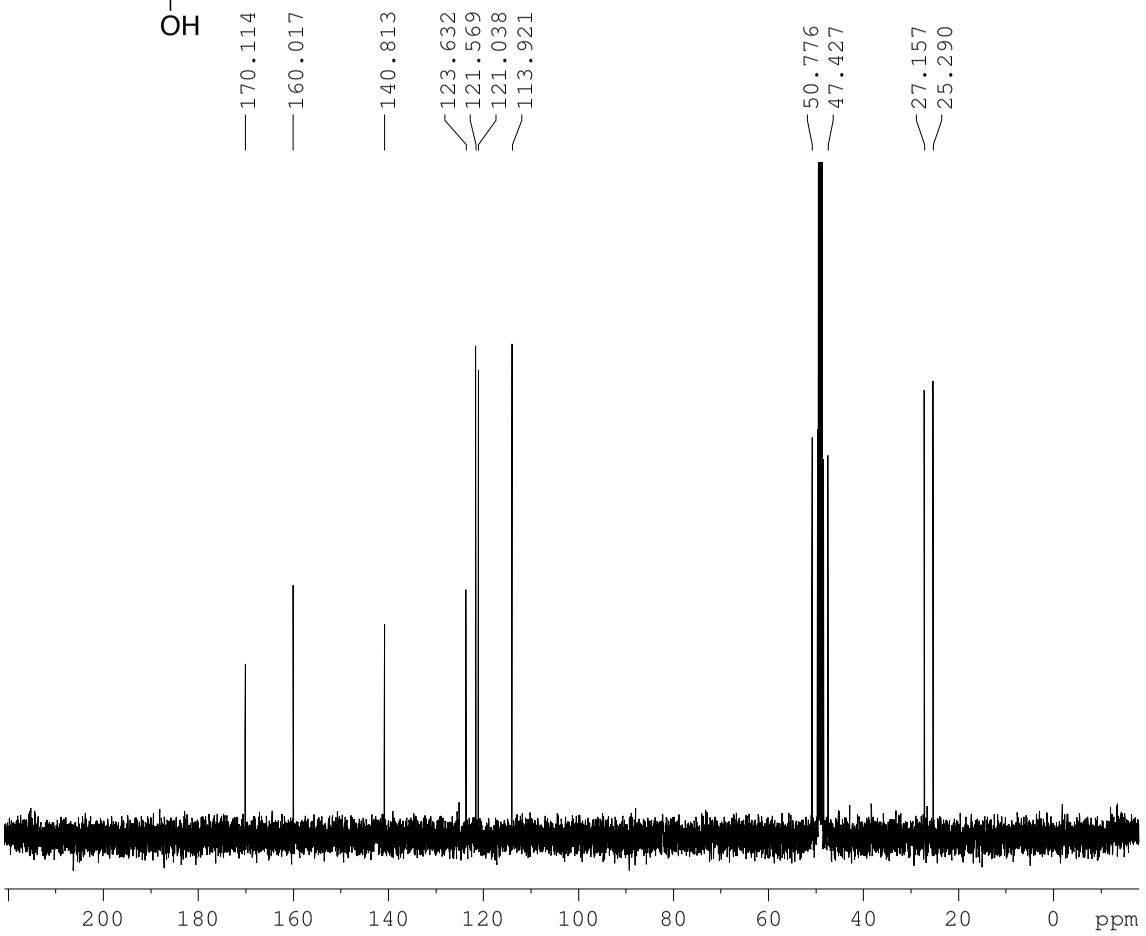
F2 - Acquisition Parameters
Date_           20140625
Time              11.47
INSTRUM          avg400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ            3.2767999 sec
RG               788.56
DW               50.000 usec
DE                6.50 usec
TE                293.9 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                   1H
P1                 12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                 65536
SF           400.2000114 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

(3-Bromo-5-hydroxyphenyl)(pyrrolidin-1-yl)methanone (2.57)






```
Current Data Parameters
NAME      LJ447 precipitate AVG
EXPNO                   2
PROCNO                  1

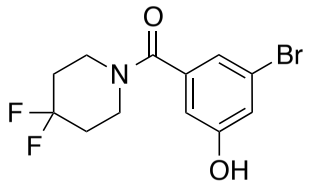
F2 - Acquisition Parameters
Date_           20140702
Time              21.10
INSTRUM           avg400
PROBHD   5 mm QNP 1H/13
PULPROG           zgpg30
TD                65536
SOLVENT             MeOD
NS                  256
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631488 sec
RG                 2050
DW               20.800 usec
DE                6.50 usec
TE                294.4 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

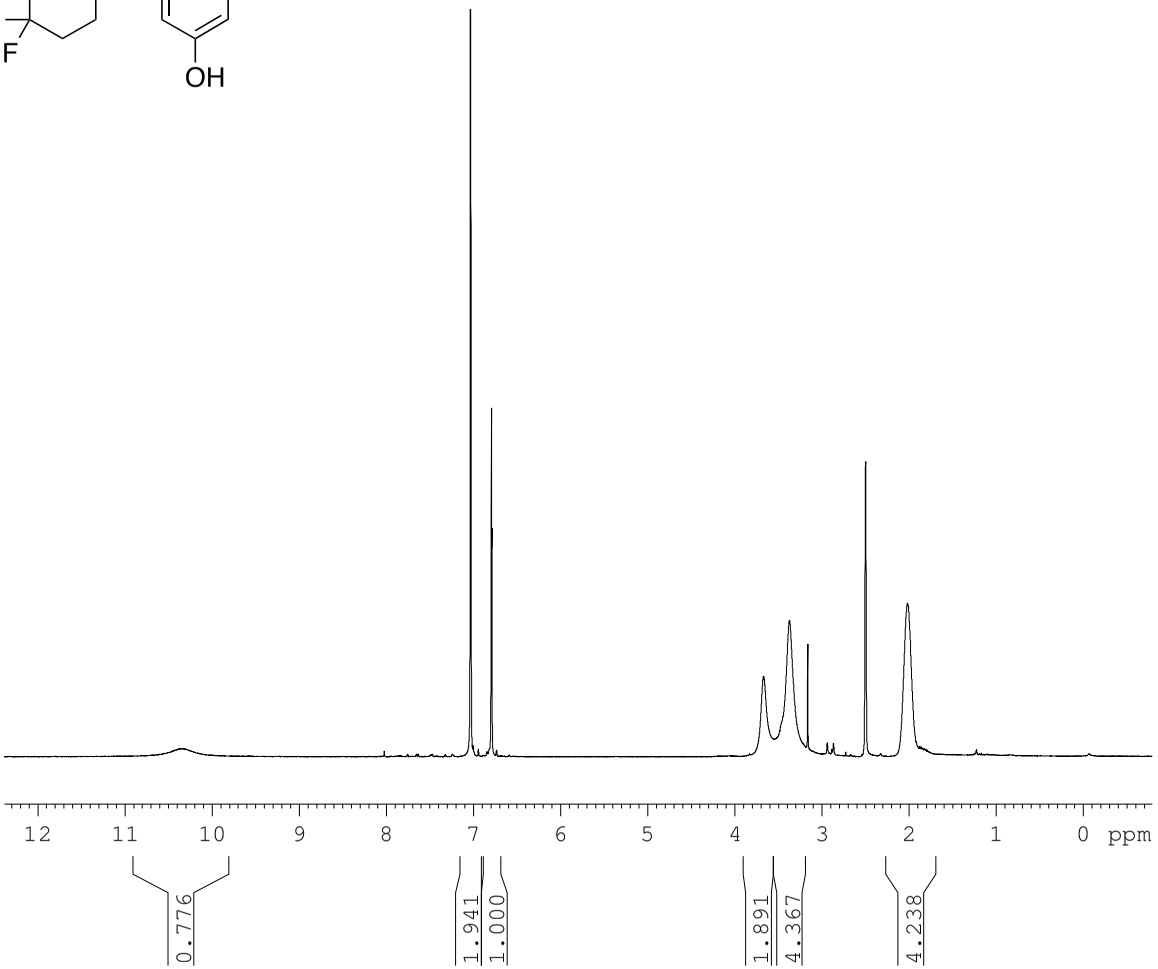
======== CHANNEL f1 ========
SFO1         100.6404326 MHz
NUC1                13C
P1                10.00 usec
PLW1         40.00000000 W

======== CHANNEL f2 ========
SFO2         400.2016008 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         11.29800034 W
PLW12         0.20863000 W
PLW13         0.16899000 W

F2 - Processing parameters
SI                 32768
SF           100.6302308 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(3-Bromo-5-hydroxyphenyl)(4,4-difluoropiperidin-1-yl)methanone (2.58)





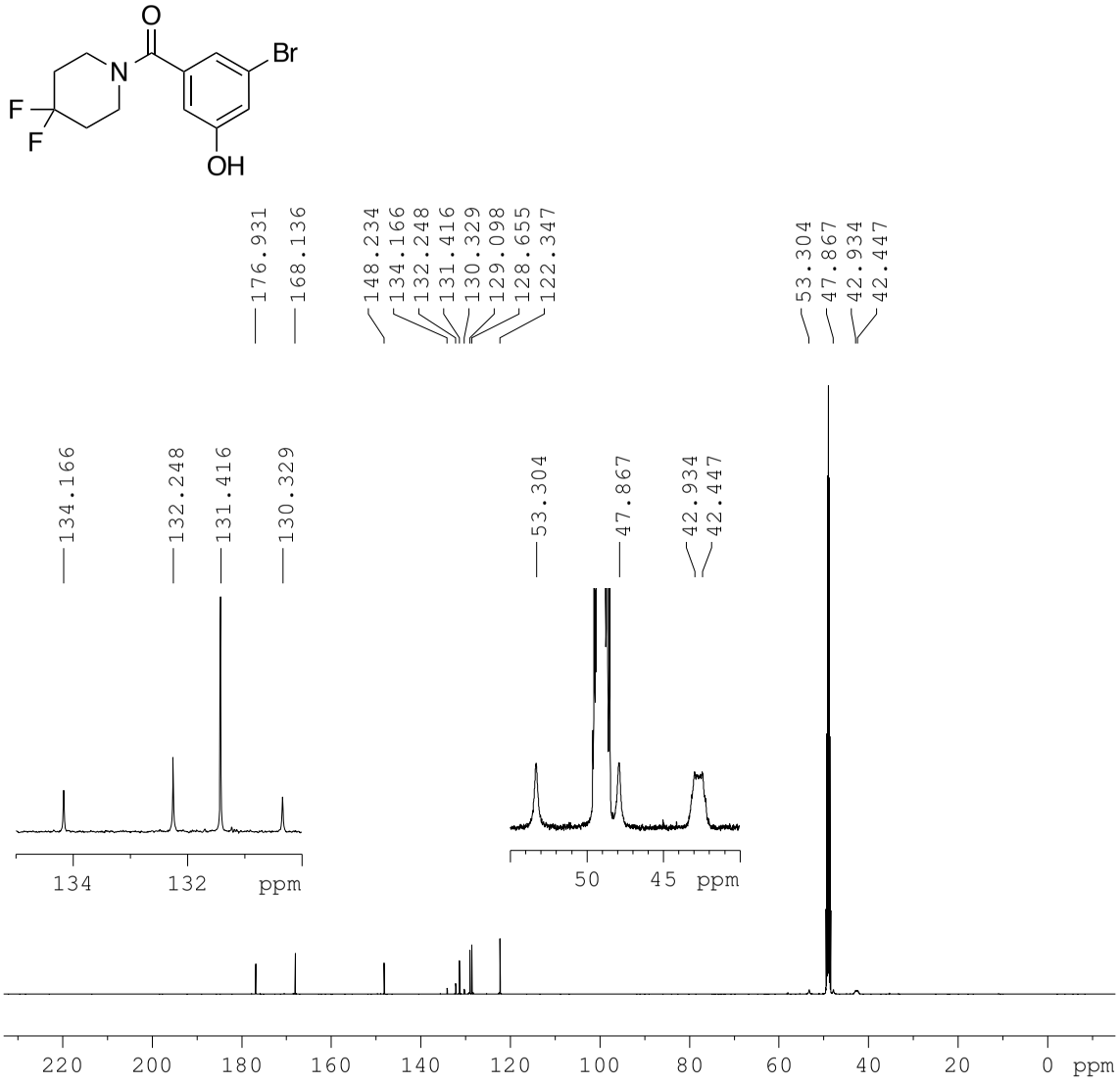

```
Current Data Parameters
NAME      LJ444 fra 13-37 AVG
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20140618
Time               20.19
INSTRUM          avg400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ            3.2767999 sec
RG               279.35
DW               50.000 usec
DE                6.50 usec
TE                294.1 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                   1H
P1                 12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                 65536
SF           400.2000072 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

(3-Bromo-5-hydroxyphenyl)(4,4-difluoropiperidin-1-yl)methanone (2.58)




```
Current Data Parameters
NAME       LJ444 sevice
EXPNO                 2
PROCNO                1

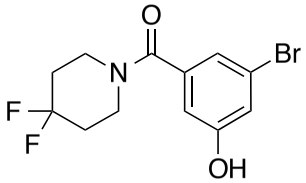
F2 - Acquisition Parameters
Date_          20140619
Time              23.18
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                  912
DW               16.000 usec
DE               18.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

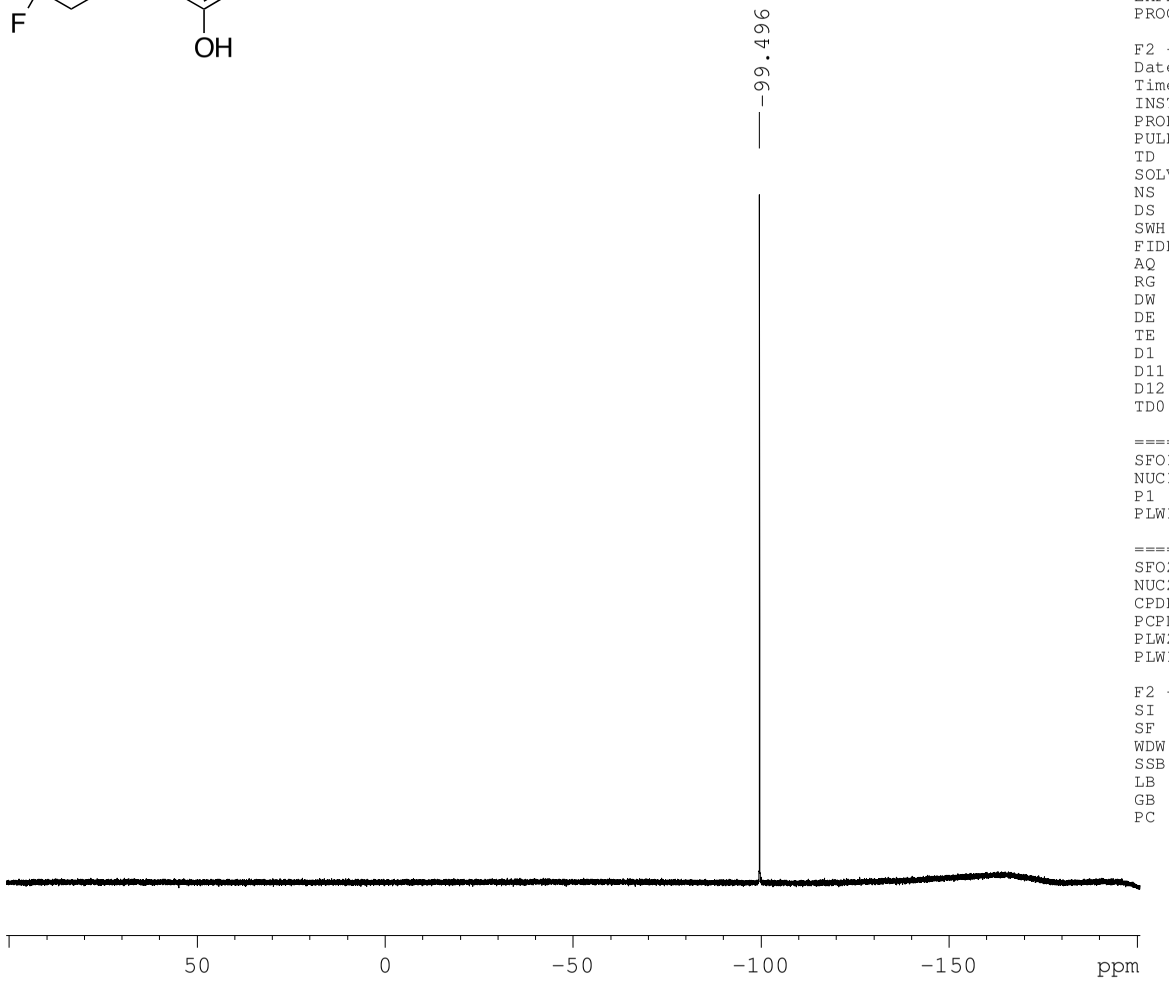
======== CHANNEL f1 ========
SFO1        125.8131152 MHz
NUC1                13C
P1                10.00 usec
PLW1        20.18400002 W

======== CHANNEL f2 ========
SFO2        500.3020012 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2           7.99830008 W
PLW12          0.28119001 W
PLW13          0.17996000 W

F2 - Processing parameters
SI                 32768
SF          125.7994001 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

(3-Bromo-5-hydroxyphenyl)(4,4-difluoropiperidin-1-yl)methanone (2.58)






```
Current Data Parameters
NAME      LJ444 fra 13-37 in MeOD
EXPNO                    3
PROCNO                   1

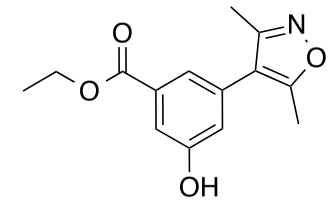
F2 - Acquisition Parameters
Date_           20140618
Time                9.51
INSTRUM           avf400
PROBHD   5 mm PABBO BB/
PULPROG         zgfhigqn
TD                131072
SOLVENT             MeOD
NS                    256
DS                      4
SWH           113636.367 Hz
FIDRES          0.866977 Hz
AQ             0.5767168 sec
RG                205.43
DW                 4.400 usec
DE                 6.50 usec
TE                295.7 K
D1            1.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1         376.5924484 MHz
NUC1                19F
P1                13.50 usec
PLW1         19.00000000 W

======== CHANNEL f2 ========
SFO2         400.2524717 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         16.70000076 W
PLW12         0.32991999 W

F2 - Processing parameters
SI                  65536
SF           376.6112790 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

Ethyl 3-(3,5-dimethylisoxazol-4-yl)-5-hydroxybenzoate (2.61) synthesised by P. Guzanov



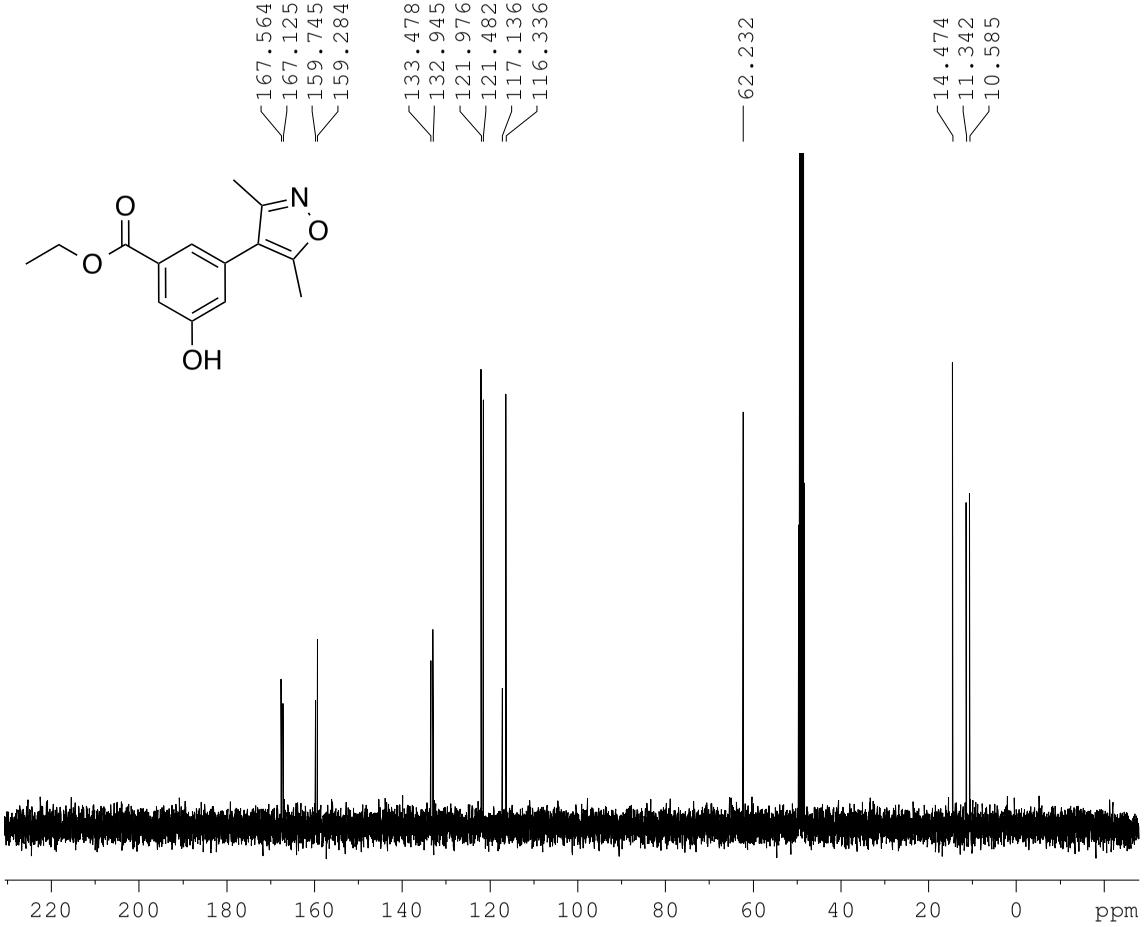

```
Current Data Parameters
NAME      Sep23-2014-16_psg037product
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_           20140923
Time              13.50
INSTRUM           avf400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH            8012.820 Hz
FIDRES         0.122266 Hz
AQ             4.0894465 sec
RG                205.43
DW                62.400 usec
DE                 6.50 usec
TE                295.7 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                1H
P1                12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                32768
SF           400.2500107 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

Ethyl 3-(3,5-dimethylisoxazol-4-yl)-5-hydroxybenzoate (2.61) synthesised by P. Guzanov




```
Current Data Parameters
NAME      Aug12-2014-7_psg014_13C
EXPNO                           3
PROCNO                          1

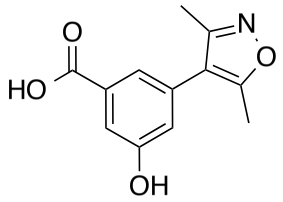
F2 - Acquisition Parameters
Date_              20140812
Time                  20.51
INSTRUM              avf400
PROBHD   5 mm PABBO BB/
PULPROG              zgpg30
TD                    32768
SOLVENT                MeOD
NS                      256
DS                        4
SWH               26041.666 Hz
FIDRES             0.794729 Hz
AQ                 0.6291456 sec
RG                    205.43
DW                    19.200 usec
DE                     6.50 usec
TE                    297.1 K
D1              1.00000000 sec
D11             0.03000000 sec
TD0                       1

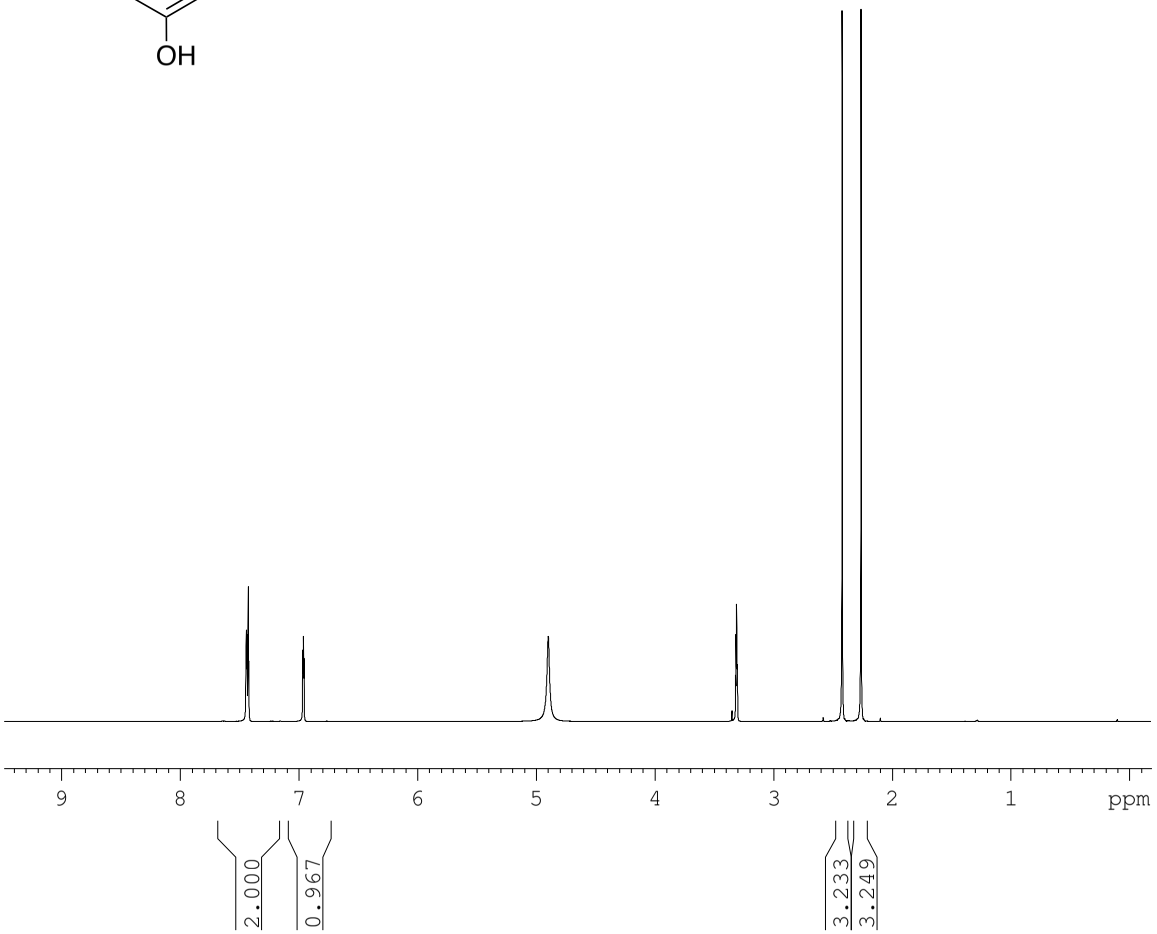
======== CHANNEL f1 ========
SFO1           100.6530073 MHz
NUC1                    13C
P1                     9.00 usec
PLW1           58.70000076 W

======== CHANNEL f2 ========
SFO2           400.2516010 MHz
NUC2                    1H
CPDPRG[2            waltz16
PCPD2                 90.00 usec
PLW2           16.70000076 W
PLW12           0.32991999 W
PLW13           0.26723999 W

F2 - Processing parameters
SI                    32768
SF             100.6428106 MHz
WDW                     EM
SSB       0
LB                     1.00 Hz
GB        0
PC                     1.40
```

3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxybenzoic acid (2.64) synthesised by P. Guzanov





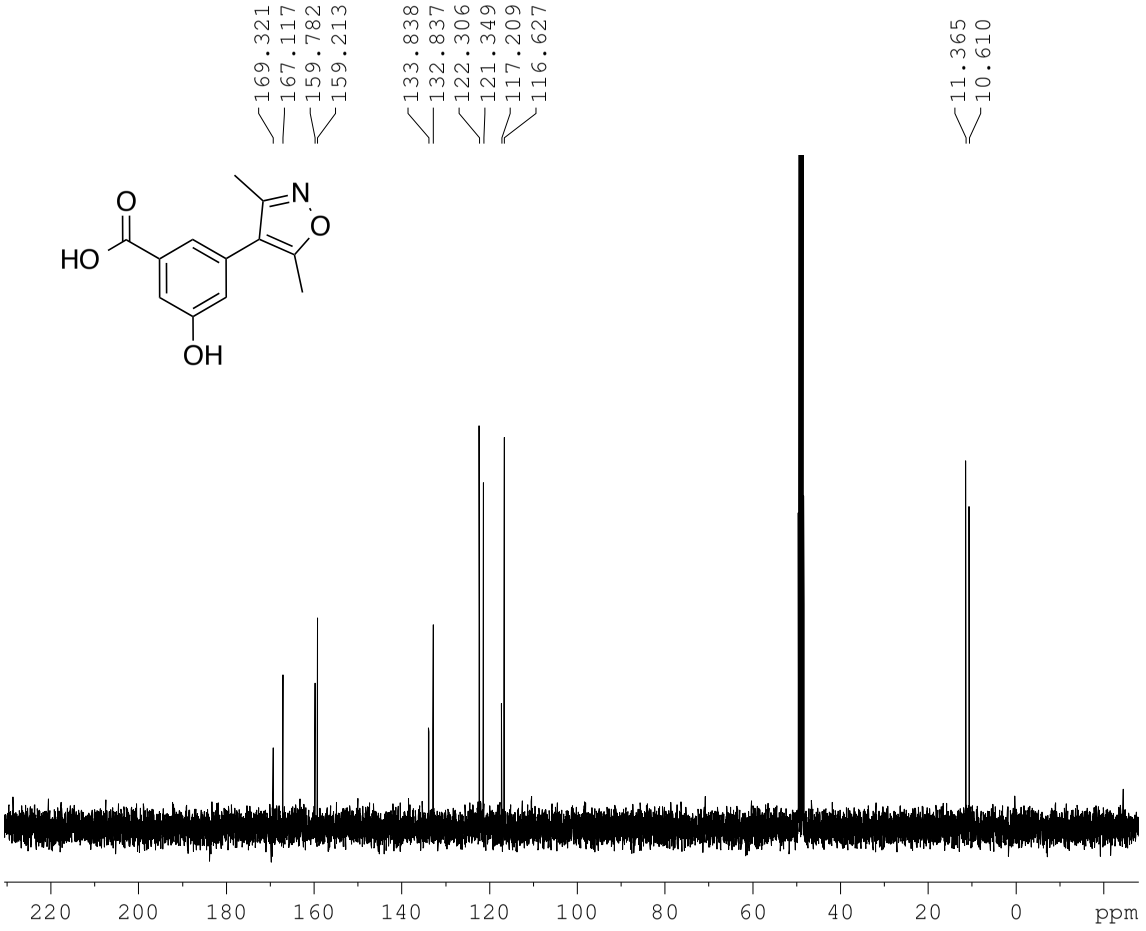

```
Current Data Parameters
NAME      Aug25-2014-1_psg026_productCharacter
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140825
Time               15.56
INSTRUM           avf400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT              MeOD
NS                     16
DS                      2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ              4.0894465 sec
RG                     93
DW                62.400 usec
DE                 6.50 usec
TE                295.6 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          400.2524015 MHz
NUC1                  1H
P1                 12.65 usec
PLW1          16.70000076 W

F2 - Processing parameters
SI                 32768
SF            400.2500100 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxybenzoic acid (2.64) synthesised by P. Guzanov




```
Current Data Parameters
NAME      Aug25-2014-1_psg026_productCharacter
EXPNO                  2
PROCNO                 1

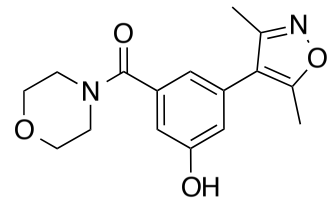
F2 - Acquisition Parameters
Date_               20140825
Time                  16.04
INSTRUM             avf400
PROBHD   5 mm PABBO BB/
PULPROG             zgpg30
TD                   32768
SOLVENT               MeOD
NS                     256
DS                       4
SWH                26041.666 Hz
FIDRES             0.794729 Hz
AQ                 0.6291456 sec
RG                    205.43
DW                   19.200 usec
DE                    6.50 usec
TE                   296.7 K
D1                1.00000000 sec
D11               0.03000000 sec
TD0                      1

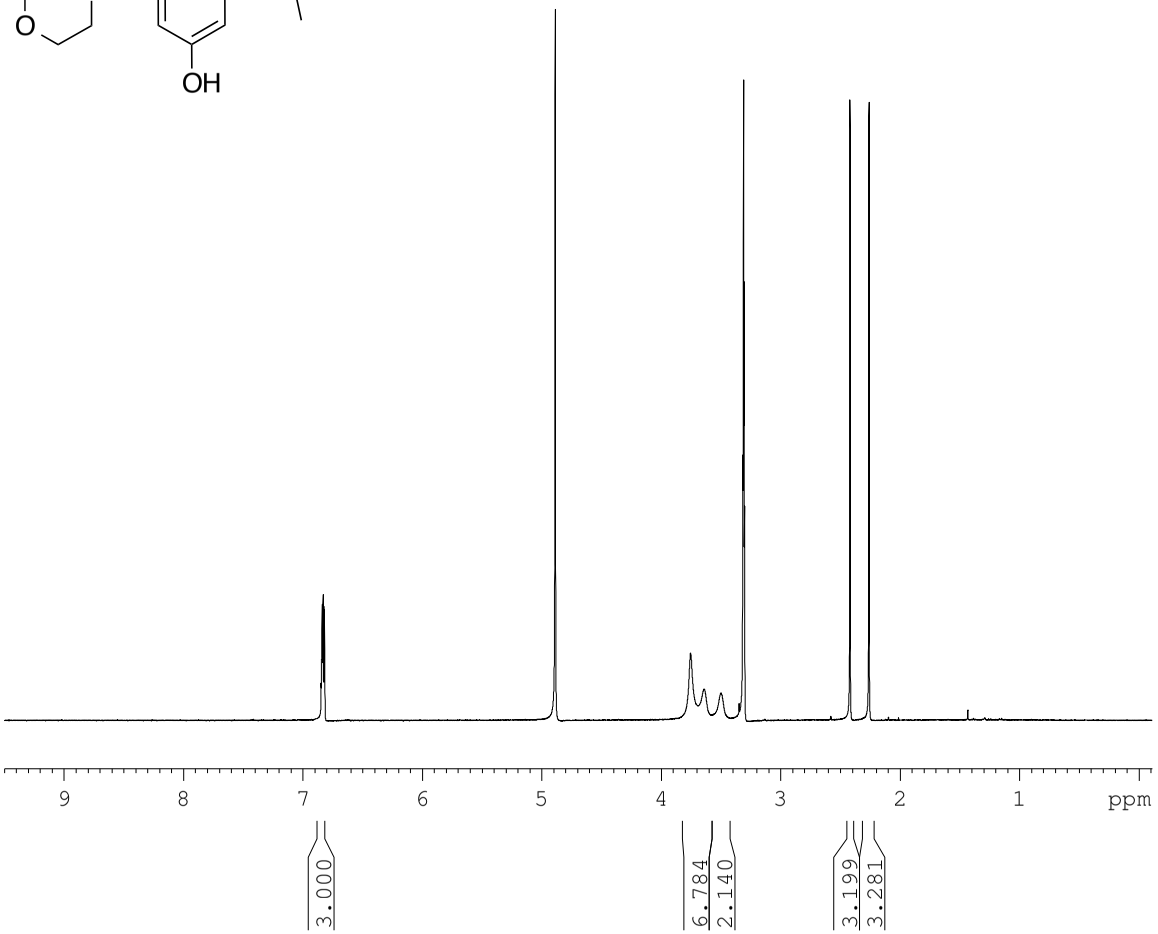
======== CHANNEL f1 ========
SFO1            100.6530073 MHz
NUC1                    13C
P1                    9.00 usec
PLW1           58.70000076 W

======== CHANNEL f2 ========
SFO2            400.2516010 MHz
NUC2                    1H
CPDPRG[2             waltz16
PCPD2                 90.00 usec
PLW2           16.70000076 W
PLW12           0.32991999 W
PLW13           0.26723999 W

F2 - Processing parameters
SI                   32768
SF              100.6428095 MHz
WDW                     EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```

[3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxyphenyl](morpholin-4-yl)methanone (2.65) synthesised by P. Guzanov





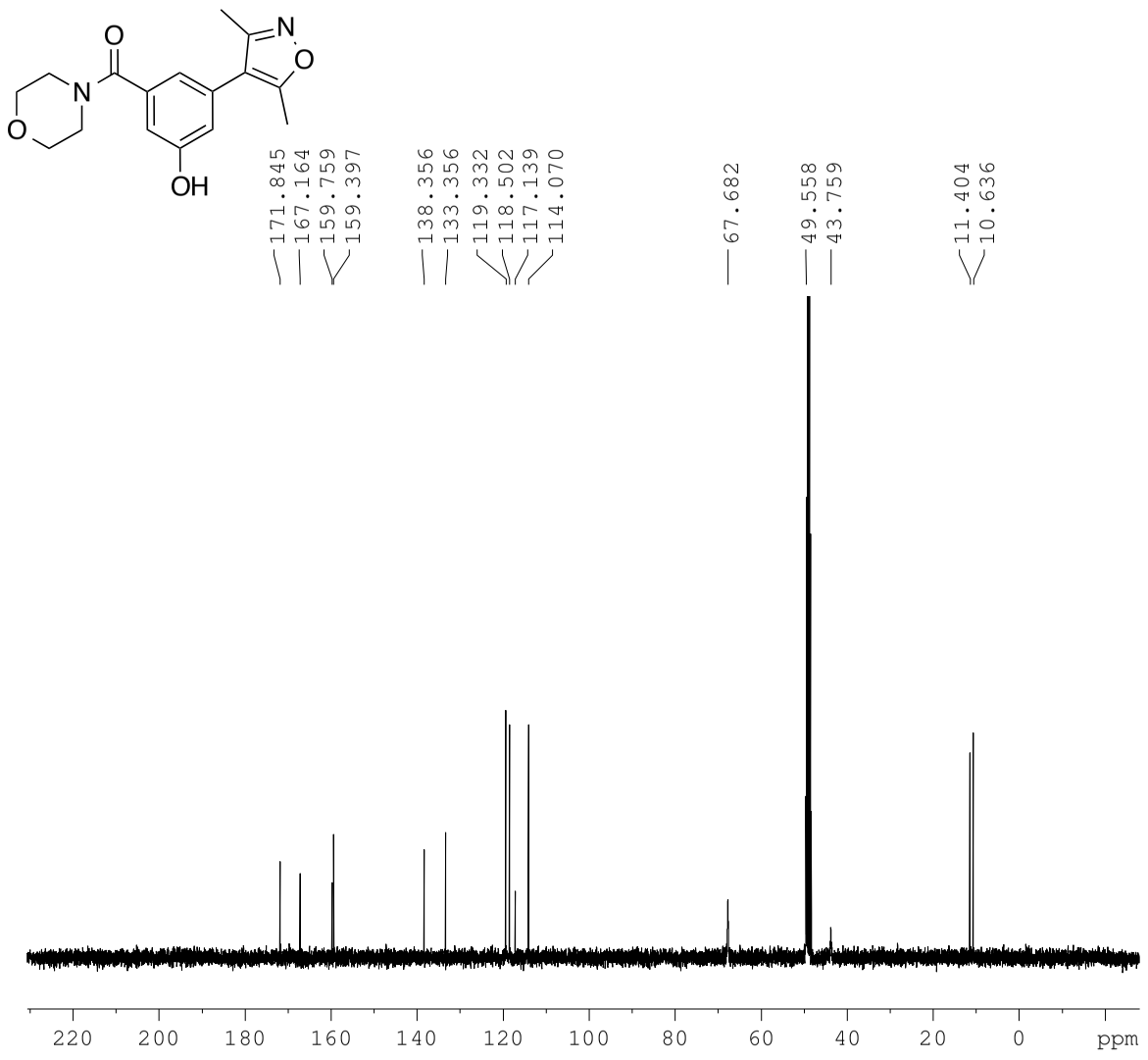

```
Current Data Parameters
NAME      Oct01-2014-56_psg041_clean
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20141001
Time              16.56
INSTRUM          avg400
PROBHD   5 mm QNP 1H/13
PULPROG            zg30
TD                65536
SOLVENT            MeOD
NS                   16
DS                    2
SWH           10000.000 Hz
FIDRES            0.152588 Hz
AQ             3.2767999 sec
RG                467.29
DW                50.000 usec
DE                 6.50 usec
TE                294.5 K
D1             1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                 1H
P1                12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                65536
SF           400.2000115 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

[3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxyphenyl](morpholin-4-yl)methanone (2.65) synthesised by P. Guzanov




```
Current Data Parameters
NAME      Sep29-2014-15_psg041product_chromatography
EXPNO                  2
PROCNO                 1

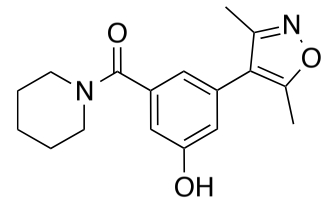
F2 - Acquisition Parameters
Date_           20140930
Time                1.14
INSTRUM           avf400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                 32768
SOLVENT              MeOD
NS                    256
DS                      4
SWH             26041.666 Hz
FIDRES          0.794729 Hz
AQ              0.6291456 sec
RG                 205.43
DW                19.200 usec
DE                 6.50 usec
TE                296.8 K
D1             1.00000000 sec
D11            0.03000000 sec
TD0                    1

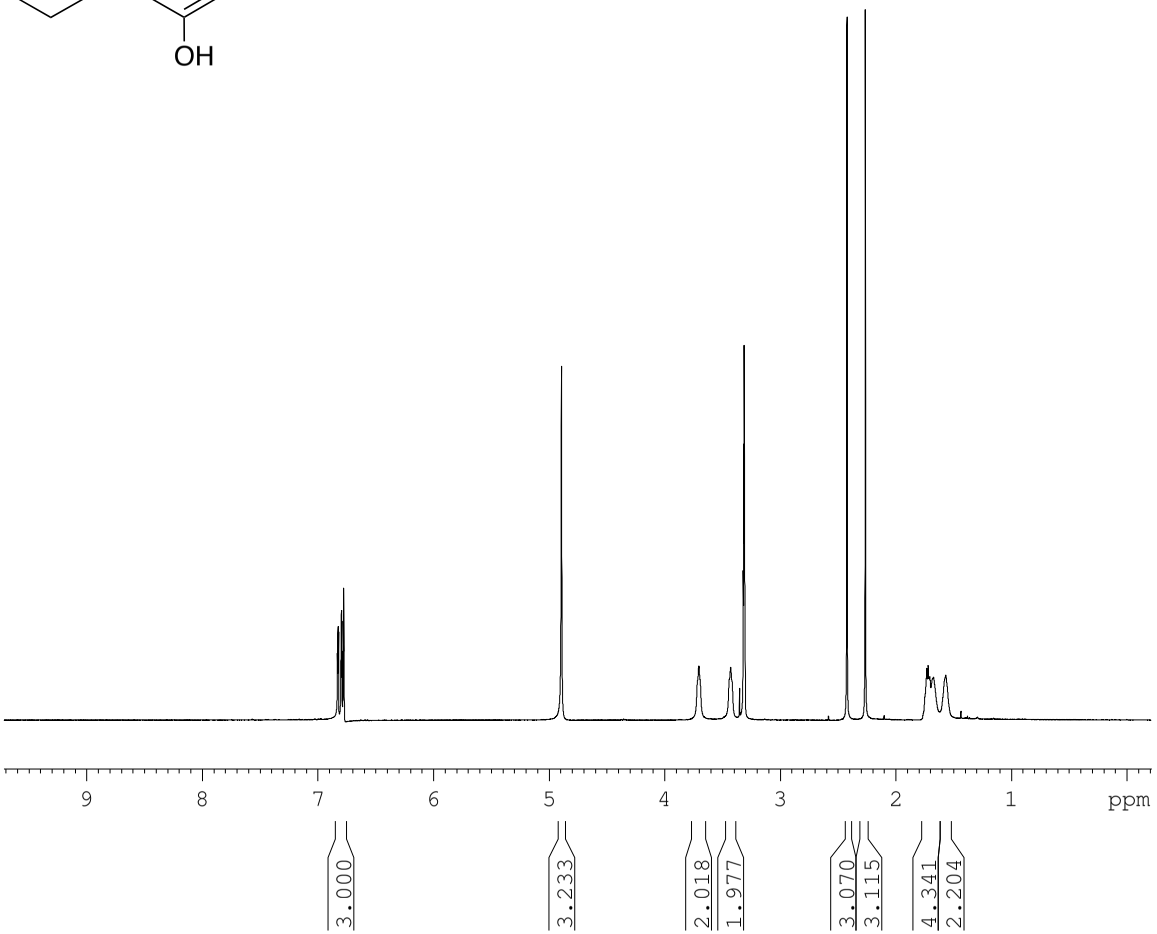
======== CHANNEL f1 ========
SFO1          100.6530073 MHz
NUC1                    13C
P1                    9.00 usec
PLW1          58.70000076 W

======== CHANNEL f2 ========
SFO2          400.2516010 MHz
NUC2                    1H
CPDPRG[2           waltz16
PCPD2                90.00 usec
PLW2          16.70000076 W
PLW12          0.32991999 W
PLW13          0.26723999 W

F2 - Processing parameters
SI                    32768
SF            100.6428106 MHz
WDW                     EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```

[3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxyphenyl](piperidin-1-yl)methanone (2.66) synthesised by P. Guzanov





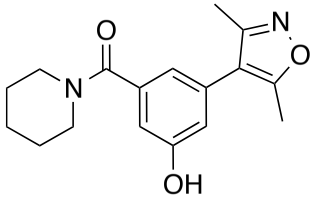

```
Current Data Parameters
NAME      Oct01-2014-48_psg040_clean
EXPNO                  1
PROCNO                 1

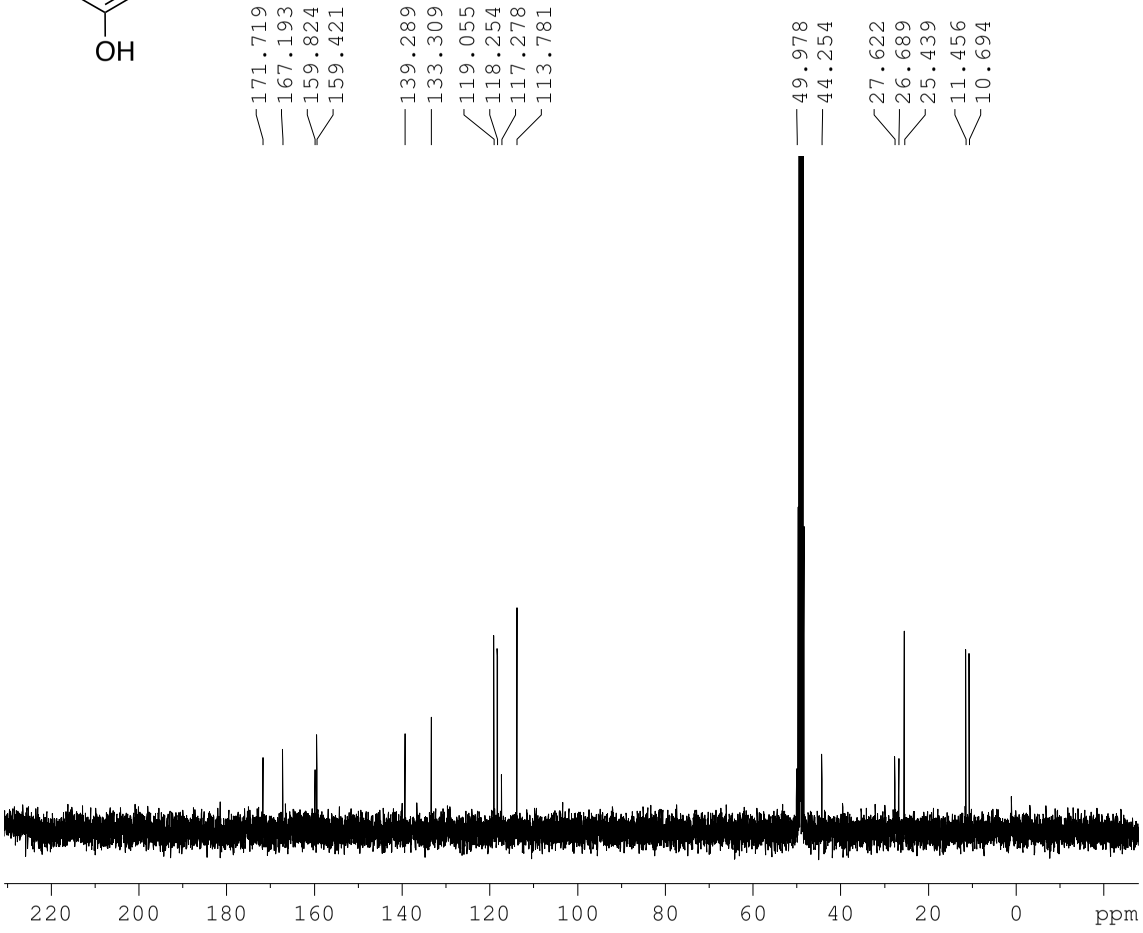
F2 - Acquisition Parameters
Date_           20141001
Time              12.43
INSTRUM          avg400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ             3.2767999 sec
RG                411.88
DW                50.000 usec
DE                 6.50 usec
TE                294.3 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                1H
P1                12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                 65536
SF           400.2000115 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

[3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxyphenyl](piperidin-1-yl)methanone (2.66) synthesised by P. Guzanov






```
Current Data Parameters
NAME      Sep29-2014-13_psg040product_chromatography
EXPNO                  2
PROCNO                 1

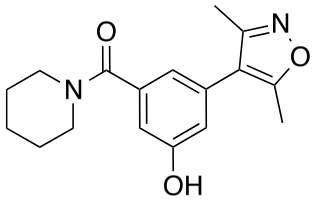
F2 - Acquisition Parameters
Date_           20140930
Time                0.29
INSTRUM           avf400
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                 32768
SOLVENT             MeOD
NS                   256
DS                     4
SWH            26041.666 Hz
FIDRES         0.794729 Hz
AQ             0.6291456 sec
RG                205.43
DW                19.200 usec
DE                 6.50 usec
TE                296.5 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                    1

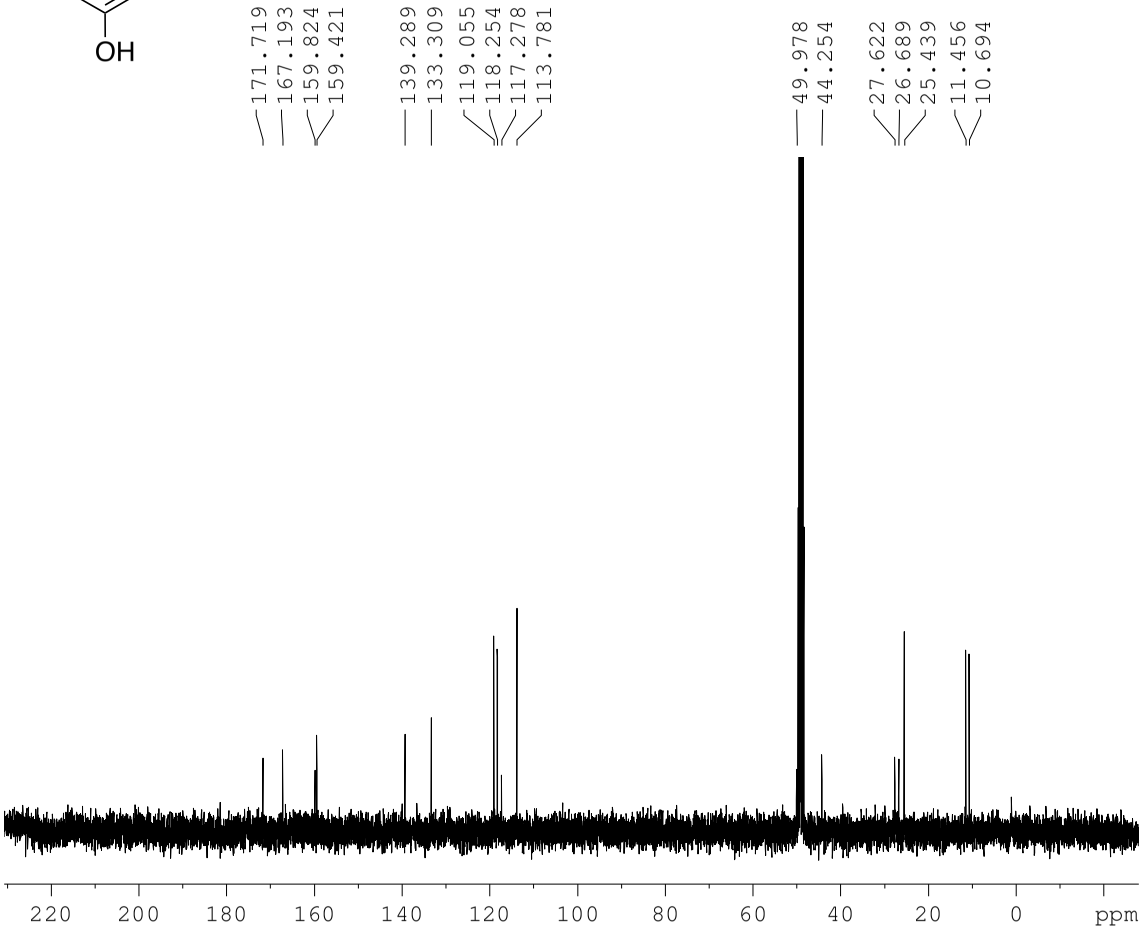
======== CHANNEL f1 ========
SFO1         100.6530073 MHz
NUC1                  13C
P1                   9.00 usec
PLW1         58.70000076 W

======== CHANNEL f2 ========
SFO2         400.2516010 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2               90.00 usec
PLW2         16.70000076 W
PLW12         0.32991999 W
PLW13         0.26723999 W

F2 - Processing parameters
SI                 32768
SF           100.6428034 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

[3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxyphenyl](piperidin-1-yl)methanone (2.66) synthesised by P. Guzanov






```
Current Data Parameters
NAME      Sep29-2014-13_psg040product_chromatography
EXPNO                  2
PROCNO                 1

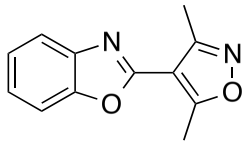
F2 - Acquisition Parameters
Date_           20140930
Time                0.29
INSTRUM           avf400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                32768
SOLVENT             MeOD
NS                   256
DS                     4
SWH            26041.666 Hz
FIDRES         0.794729 Hz
AQ             0.6291456 sec
RG                205.43
DW                19.200 usec
DE                 6.50 usec
TE                296.5 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                    1

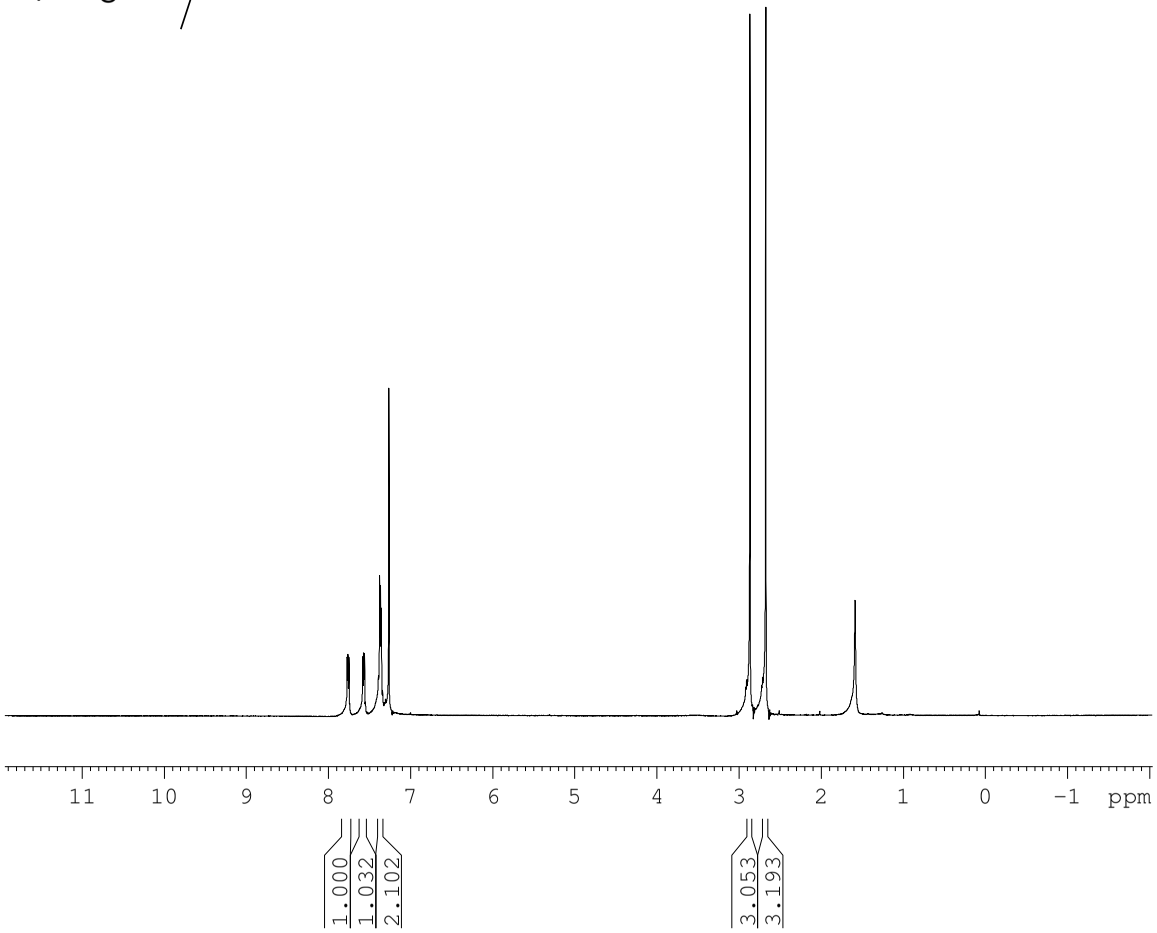
======== CHANNEL f1 ========
SFO1         100.6530073 MHz
NUC1                 13C
P1                  9.00 usec
PLW1         58.70000076 W

======== CHANNEL f2 ========
SFO2         400.2516010 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2               90.00 usec
PLW2         16.70000076 W
PLW12         0.32991999 W
PLW13         0.26723999 W

F2 - Processing parameters
SI                  32768
SF           100.6428034 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

2-(3,5-Dimethyl-1,2-oxazol-4-yl)-1,3-benzoxazole (3.3)





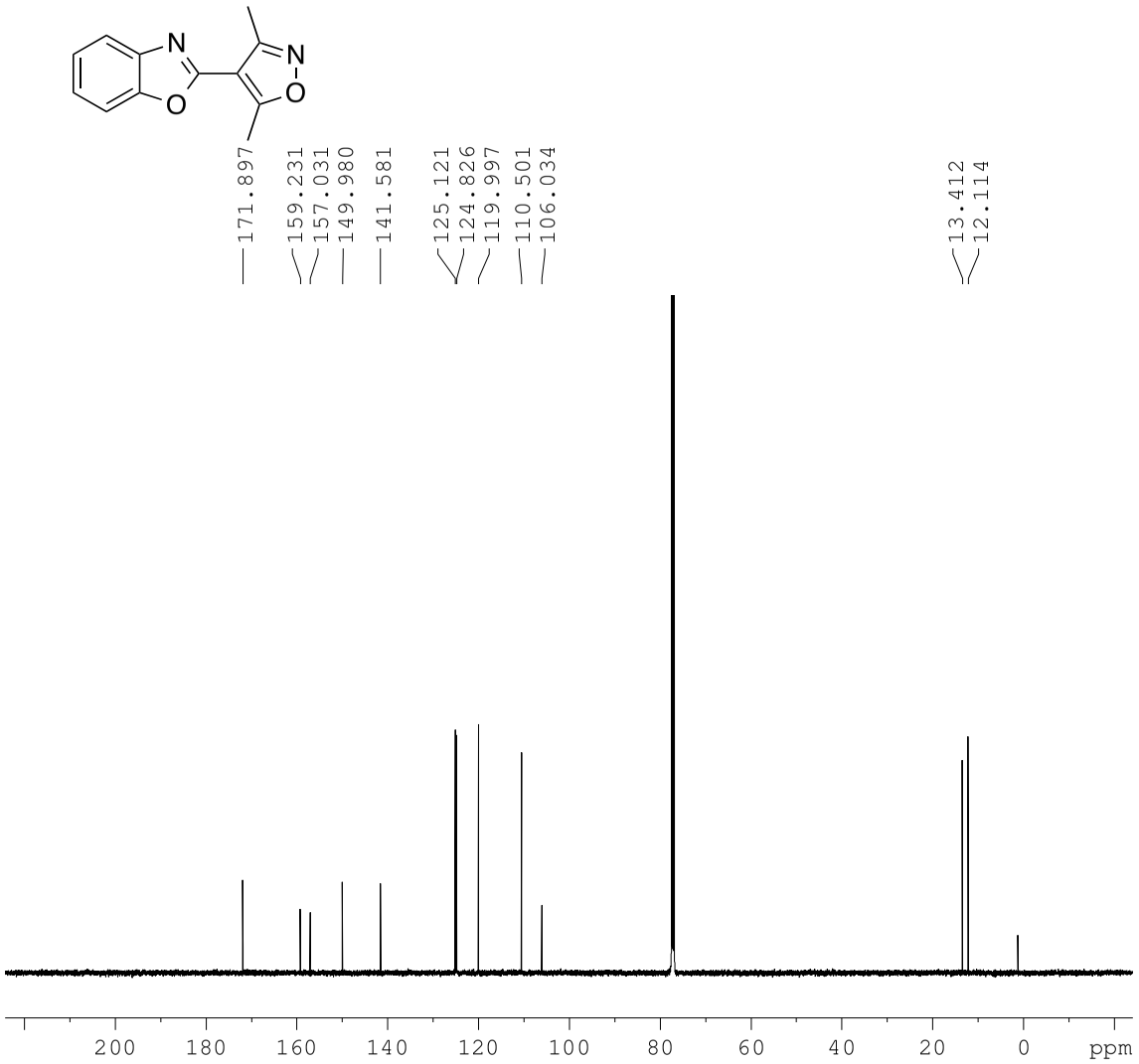

```
Current Data Parameters
NAME      LJ031 grease wash
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20120822
Time               10.01
INSTRUM           dpx400
PROBHD   5 mm Dual 1H/1
PULPROG             zg60
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             5592.841 Hz
FIDRES          0.170680 Hz
AQ             2.9294591 sec
RG                 287.4
DW                89.400 usec
DE                17.00 usec
TE                 300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                   7.30 usec
PL1       0 dB
SFO1         400.1320007 MHz

F2 - Processing parameters
SI                 32768
SF          400.1300182 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 0.60
```

2-(3,5-Dimethyl-1,2-oxazol-4-yl)-1,3-benzoxazole (3.3)




```
Current Data Parameters
NAME      LJ031 recolumn fra 72-79
EXPNO                  9
PROCNO                 1

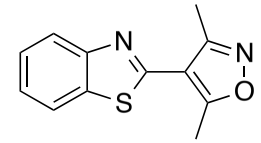
F2 - Acquisition Parameters
Date_           20120121
Time                4.11
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                 1820
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1

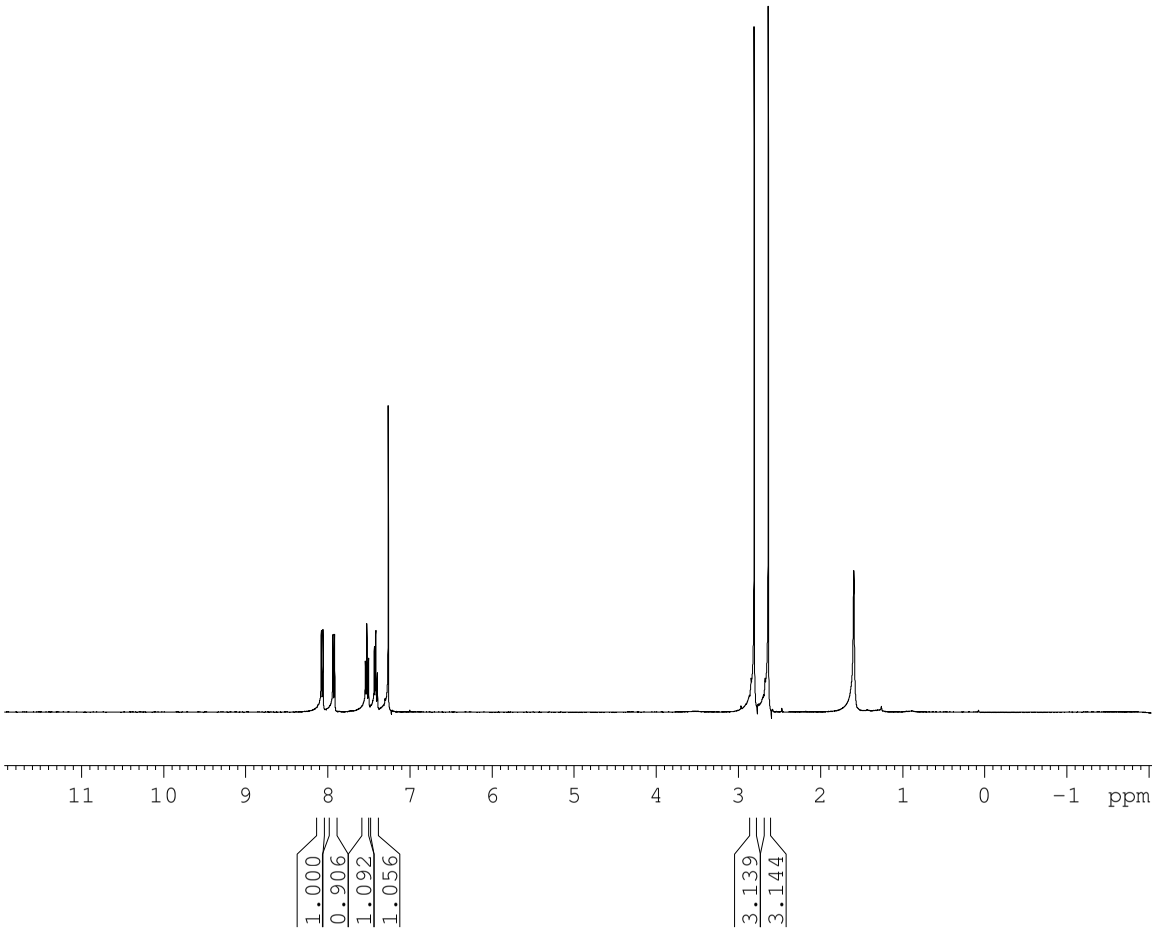
======== CHANNEL f1 ========
NUC1                 13C
P1                 10.00 usec
PL1                -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               12.42 dB
PL13               18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                 32768
SF          125.8005232 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

2-(3,5-Dimethyl-1,2-oxazol-4-yl)-1,3-benzothiazole (3.4)





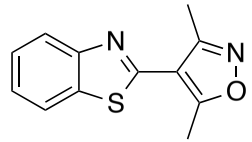

```
Current Data Parameters
NAME      LJ033 grease wash
EXPNO                   1
PROCNO                  1

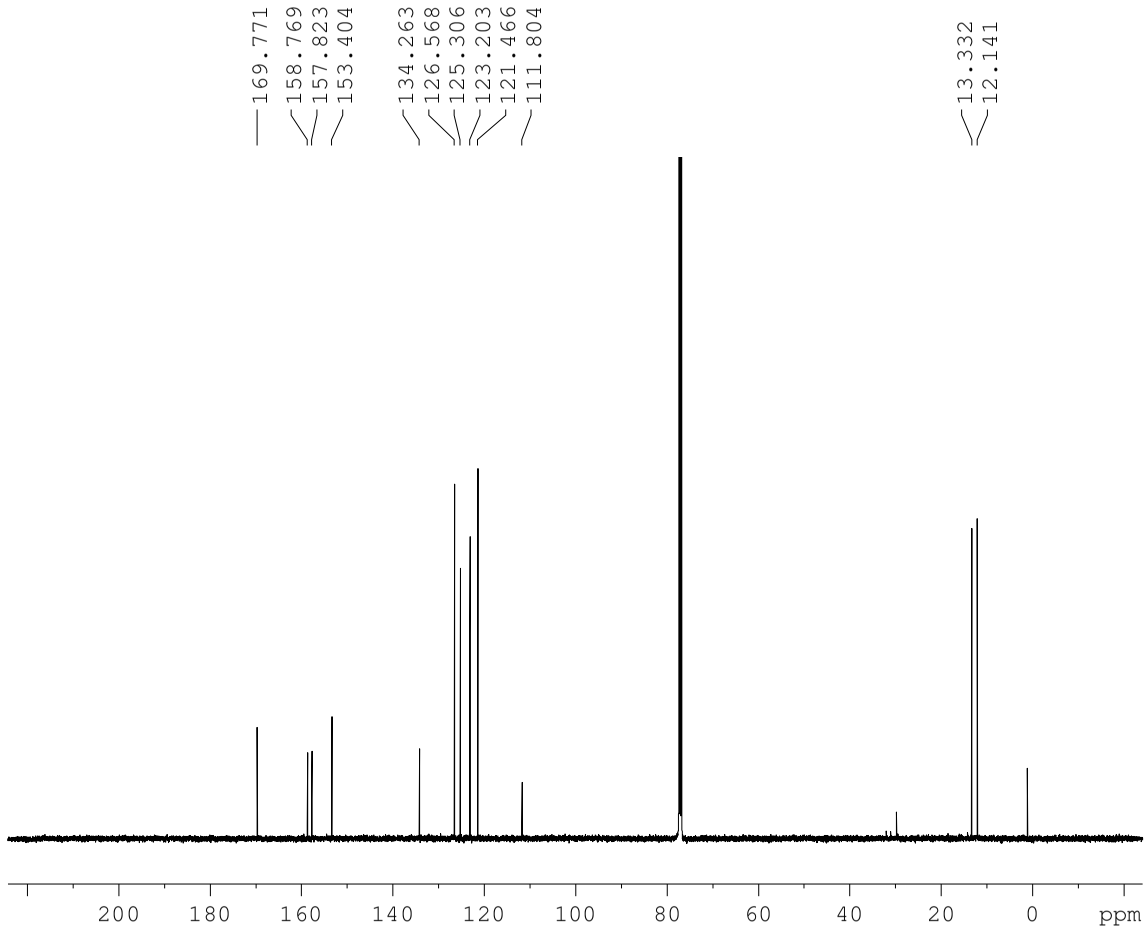
F2 - Acquisition Parameters
Date_           20120822
Time               10.07
INSTRUM           dpx400
PROBHD   5 mm Dual 1H/1
PULPROG             zg60
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             5592.841 Hz
FIDRES          0.170680 Hz
AQ             2.9294591 sec
RG                 287.4
DW                89.400 usec
DE                17.00 usec
TE                 300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  7.30 usec
PL1       0 dB
SFO1         400.1320007 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300182 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 0.60
```

2-(3,5-Dimethyl-1,2-oxazol-4-yl)-1,3-benzothiazole (3.4)






```
Current Data Parameters
NAME      LJ033 fra 168-192
EXPNO                     9
PROCNO                    1

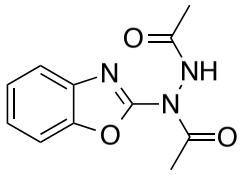
F2 - Acquisition Parameters
Date_           20120121
Time                5.02
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                 1820
DW               16.000 usec
DE               20.00 usec
TE                298.1 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

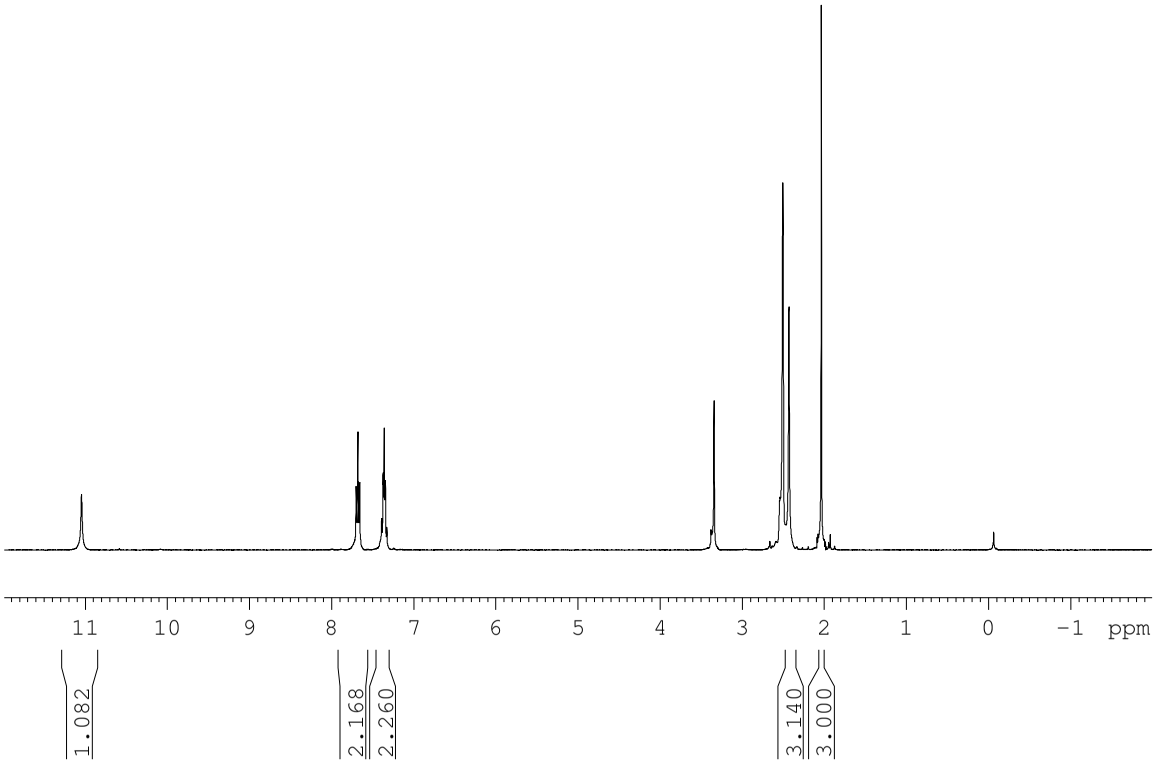
======== CHANNEL f1 ========
NUC1                 13C
P1                 10.00 usec
PL1                -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               12.42 dB
PL13               18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                 32768
SF          125.8005236 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

_N'_-Acetyl-_N_-(1,3-benzoxazol-2-yl)acetohydrazide (3.19)





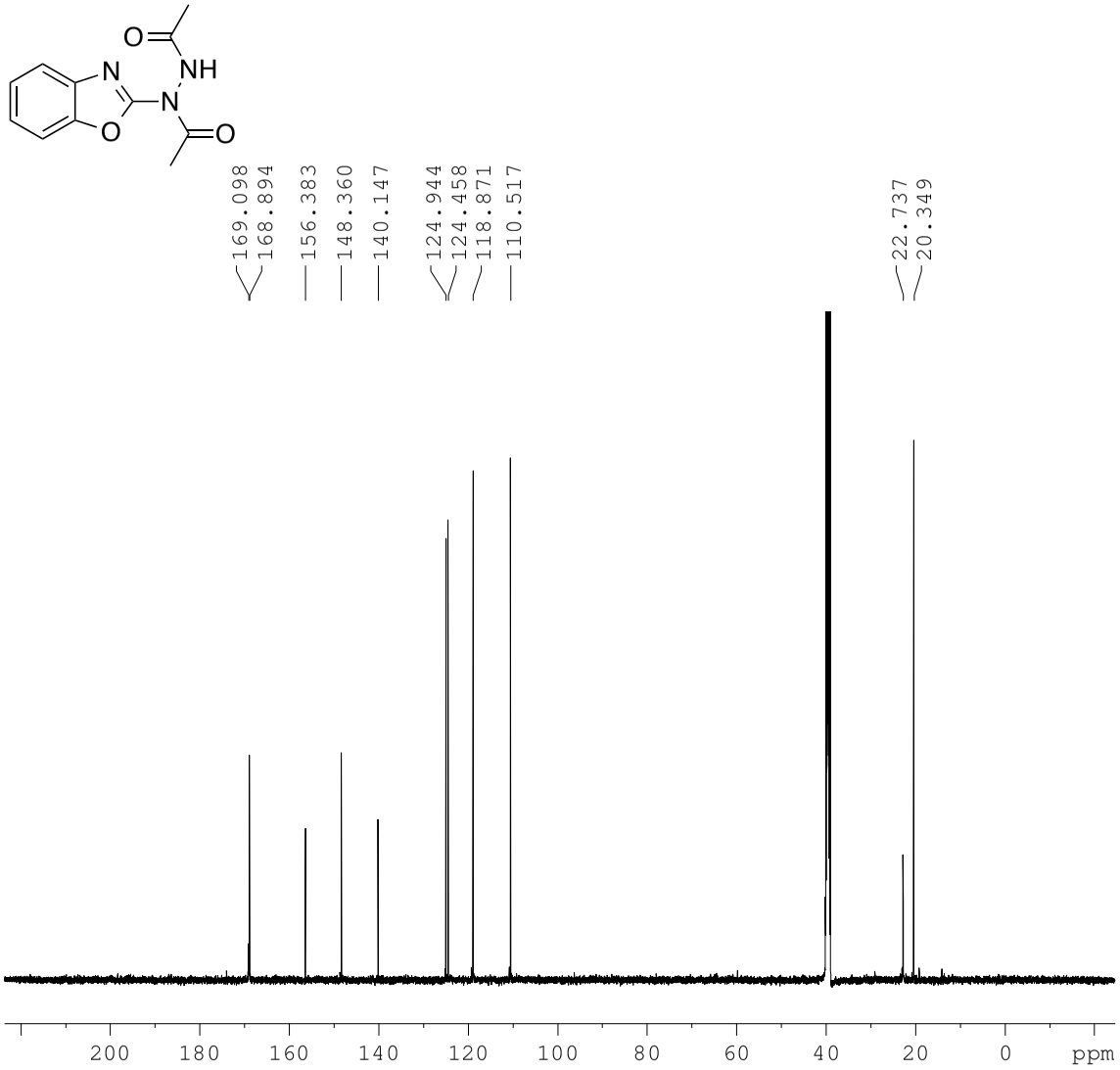

```
Current Data Parameters
NAME                  LJ175
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_              20120724
Time                  10.20
INSTRUM               dpx400
PROBHD   5 mm Dual 1H/1
PULPROG                zg60
TD                    32768
SOLVENT                DMSO
NS                       16
DS                        2
SWH                5592.841 Hz
FIDRES             0.170680 Hz
AQ                 2.9294591 sec
RG                     645.1
DW                   89.400 usec
DE                   17.00 usec
TE                   300.0 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                     7.30 usec
PL1       0 dB
SFO1          400.1320007 MHz

F2 - Processing parameters
SI                    32768
SF            400.1300000 MHz
WDW                      EM
SSB       0
LB                     0.30 Hz
GB        0
PC                     0.60
```

N'-Acetyl-*N*-(1,3-benzoxazol-2-yl)acetohydrazide (3.19)




```
Current Data Parameters
NAME      LJ172 service
EXPNO                 3
PROCNO                1

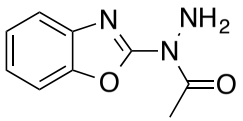
F2 - Acquisition Parameters
Date_          20120727
Time               4.02
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                   512
DS                     2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                 1820
DW               16.000 usec
DE                20.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1

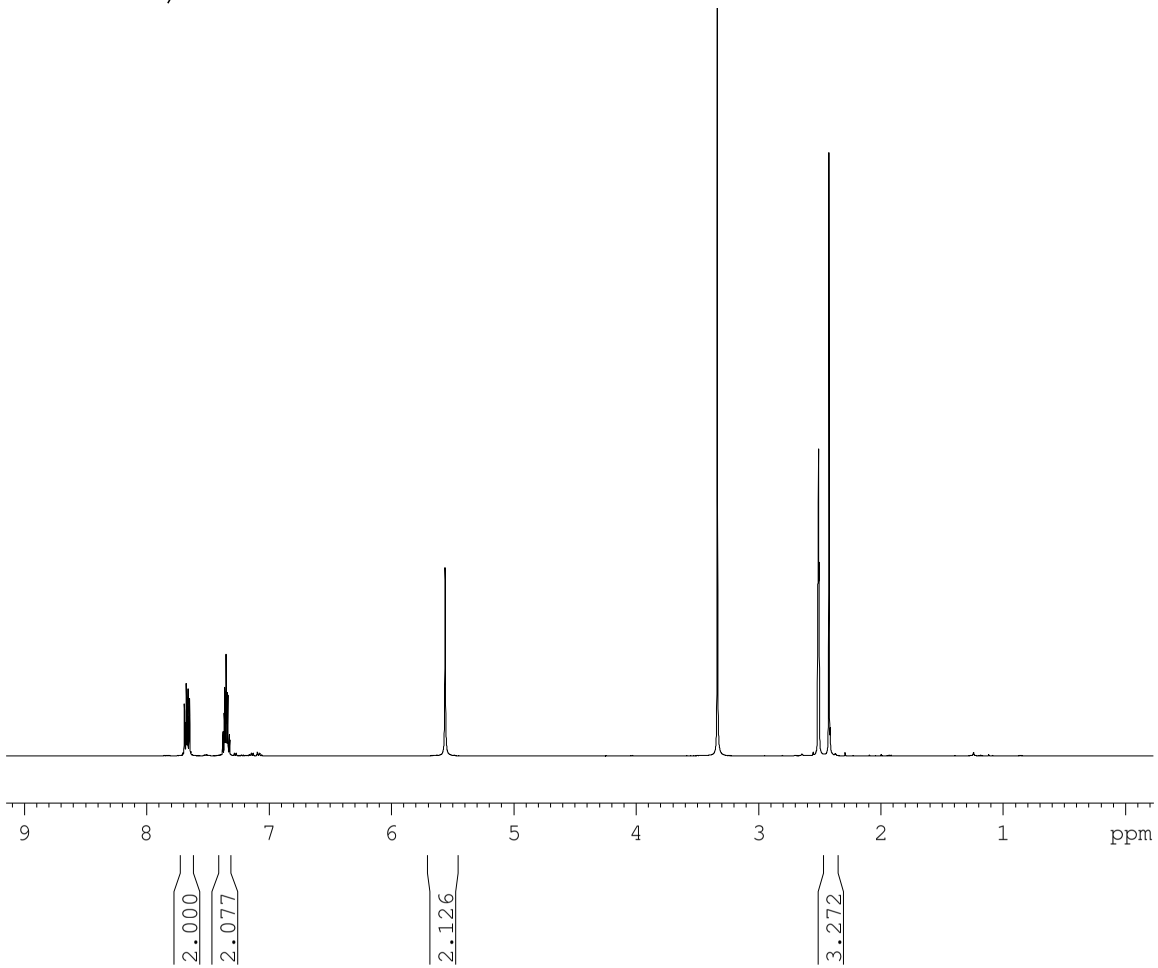
======== CHANNEL f1 ========
NUC1                 13C
P1                 10.00 usec
PL1                -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               12.42 dB
PL13               18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                 32768
SF          125.8005954 MHz
WDW                   EM
SSB      0
LB                 1.00 Hz
GB       0
PC                 1.40
```

_N_-(1,3-Benzoxazol-2-yl)acetohydrazide (3.20)





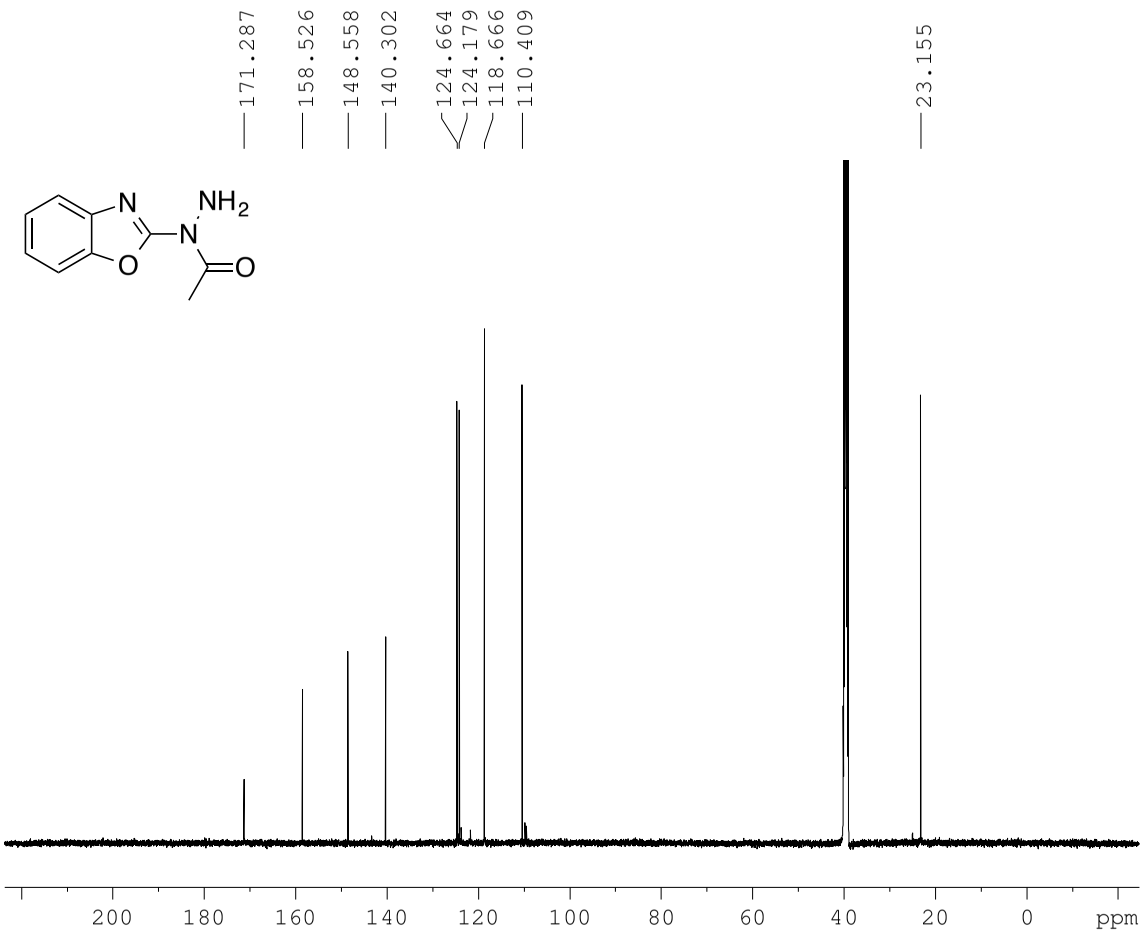

```
Current Data Parameters
NAME      LJ187 service
EXPNO                1
PROCNO               1

F2 - Acquisition Parameters
Date_          20120819
Time              10.52
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719425 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                 32768
SF          500.3000000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

N-(1,3-Benzoxazol-2-yl)acetohydrazide (3.20)




```
Current Data Parameters
NAME      LJ187 service
EXPNO                 4
PROCNO                1

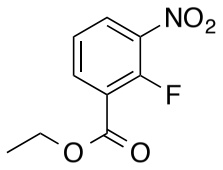
F2 - Acquisition Parameters
Date_          20120819
Time              14.18
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                 2048
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                 1820
DW               16.000 usec
DE               20.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

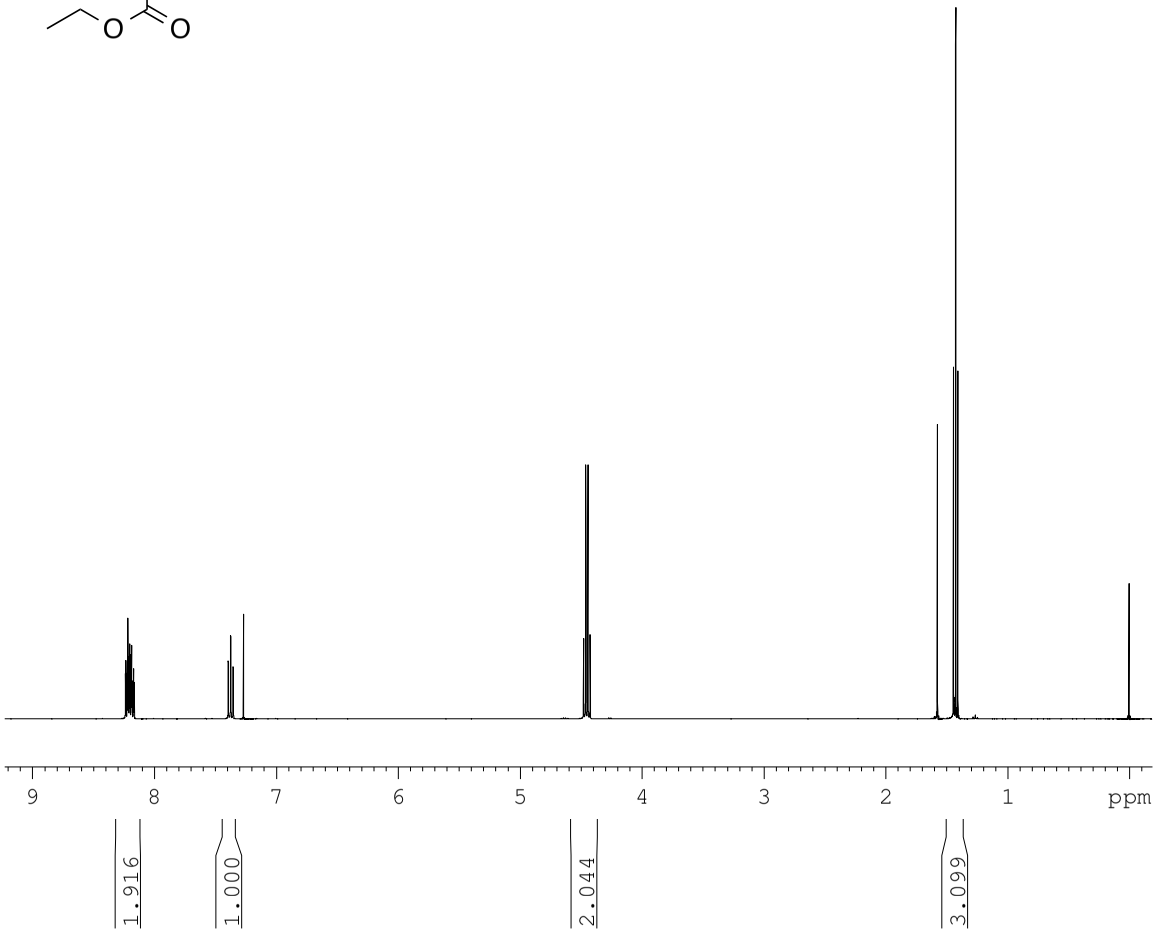
======== CHANNEL f1 ========
NUC1                 13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005954 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

Ethyl 2-fluoro-3-nitrobenzoate (3.39)





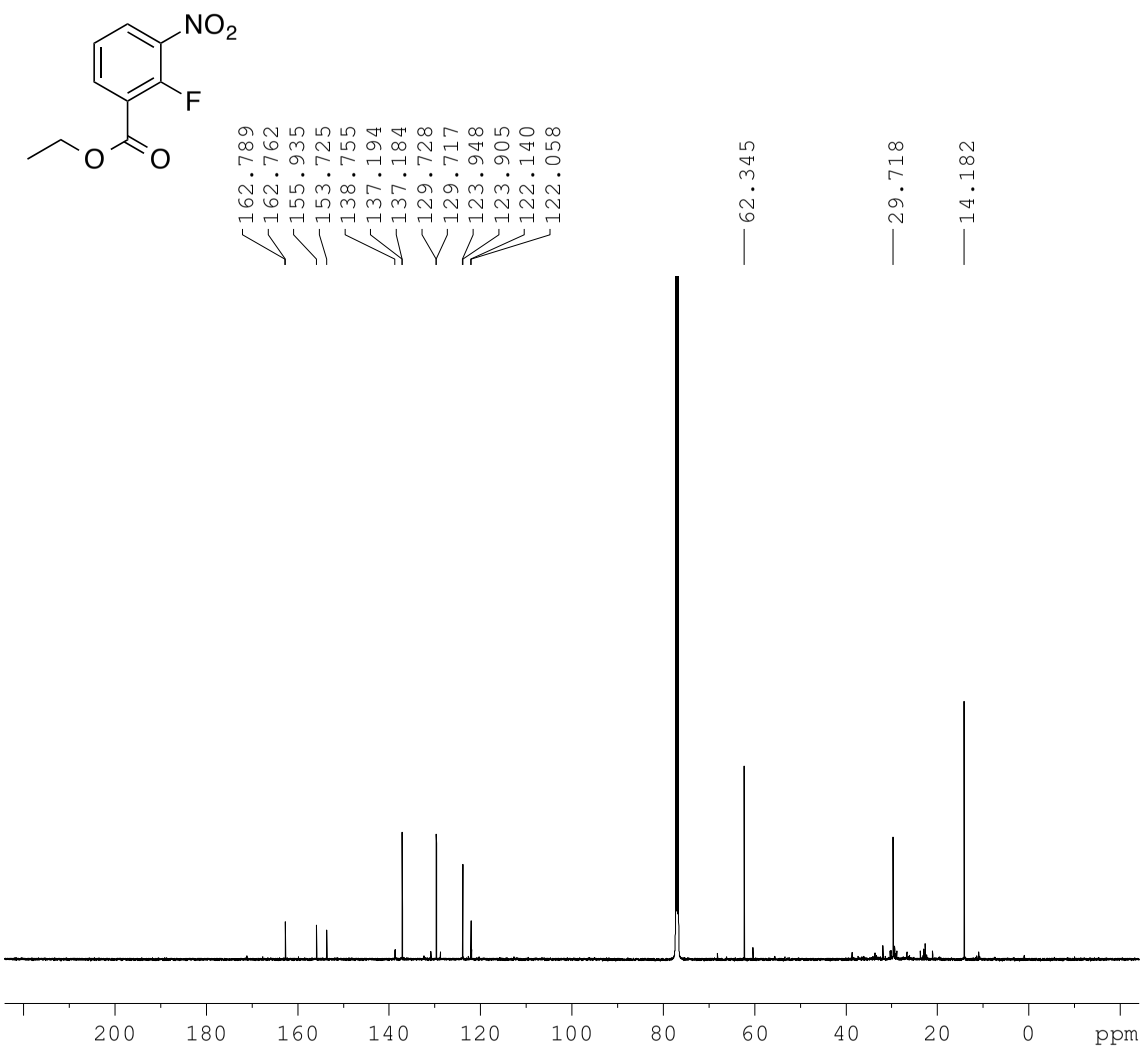

```
Current Data Parameters
NAME          N28992-18
EXPNO                10
PROCNO                1

F2 - Acquisition Parameters
Date_          20130628
Time               8.23
INSTRUM            S400
PROBHD   5 mm PABBO BB/
PULPROG            zg30
TD                32768
SOLVENT           CDC13
NS                   16
DS                    2
SWH            8012.820 Hz
FIDRES         0.244532 Hz
AQ            2.0447233 sec
RG               120.97
DW               62.400 usec
DE                6.50 usec
TE               300.0 K
D1            1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 1H
P1                10.70 usec
PLW1        15.84899998 W
SFO1        400.1632010 MHz

F2 - Processing parameters
SI                65536
SF          400.1600044 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                1.50
```

Ethyl 2-fluoro-3-nitrobenzoate (3.39)




```
Current Data Parameters
NAME      N28992-18 service
EXPNO                   3
PROCNO                  1

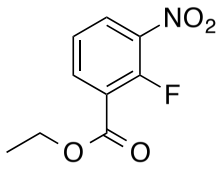
F2 - Acquisition Parameters
Date_            20140924
Time                2.01
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  2048
DS                     2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                   912
DW                16.000 usec
DE                18.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1

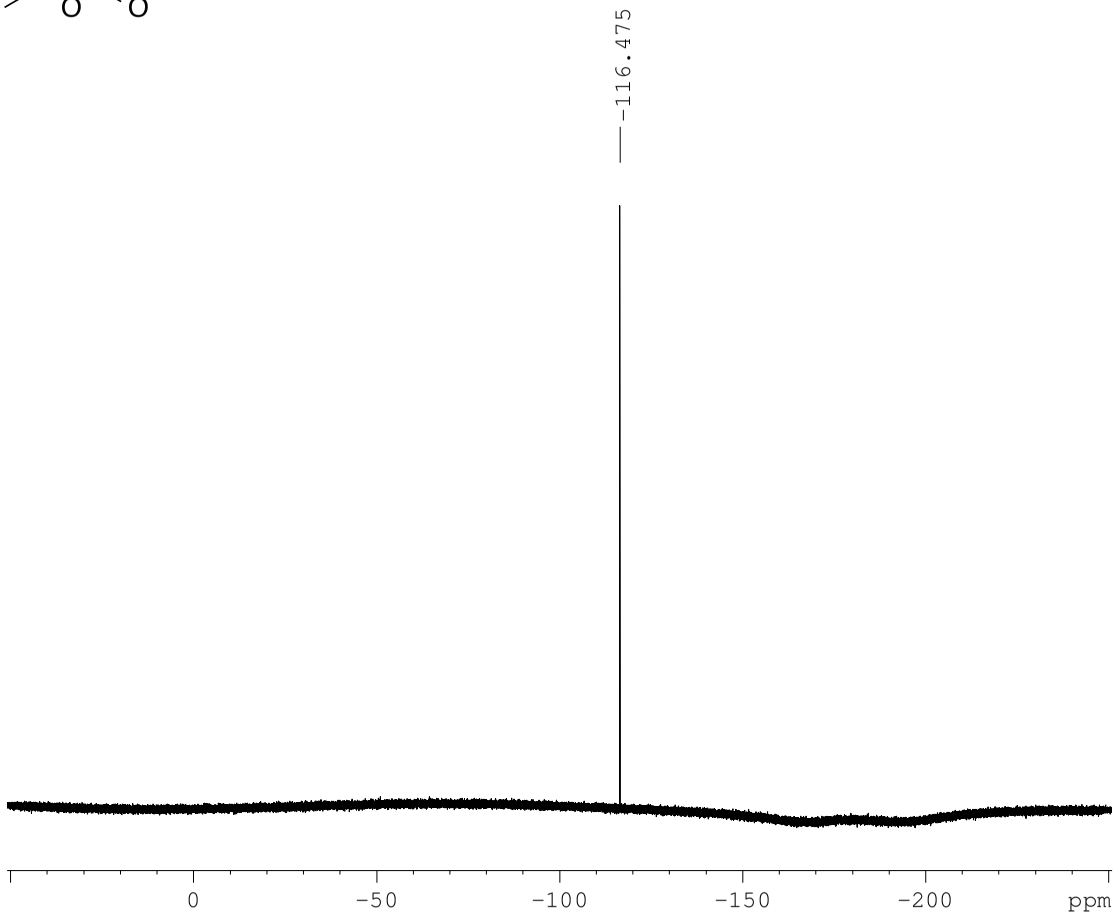
======== CHANNEL f1 ========
SFO1         125.8131152 MHz
NUC1                13C
P1                10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2           7.99830008 W
PLW12          0.28119001 W
PLW13          0.17996000 W

F2 - Processing parameters
SI                 32768
SF           125.8005351 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

Ethyl 2-fluoro-3-nitrobenzoate (3.39)






```
Current Data Parameters
NAME           N28992-18
EXPNO                 12
PROCNO                 1

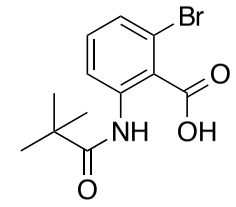
F2 - Acquisition Parameters
Date_           20130717
Time               15.21
INSTRUM             S400
PROBHD   5 mm PABBO BB/
PULPROG          zgdc30
TD               131072
SOLVENT            CDCl3
NS                    32
DS                     2
SWH           113636.367 Hz
FIDRES          0.866977 Hz
AQ             0.5767168 sec
RG                 191.6
DW                 4.400 usec
DE                  6.50 usec
TE                 300.0 K
D1            3.00000000 sec
D11           0.03000000 sec
TD0                    1

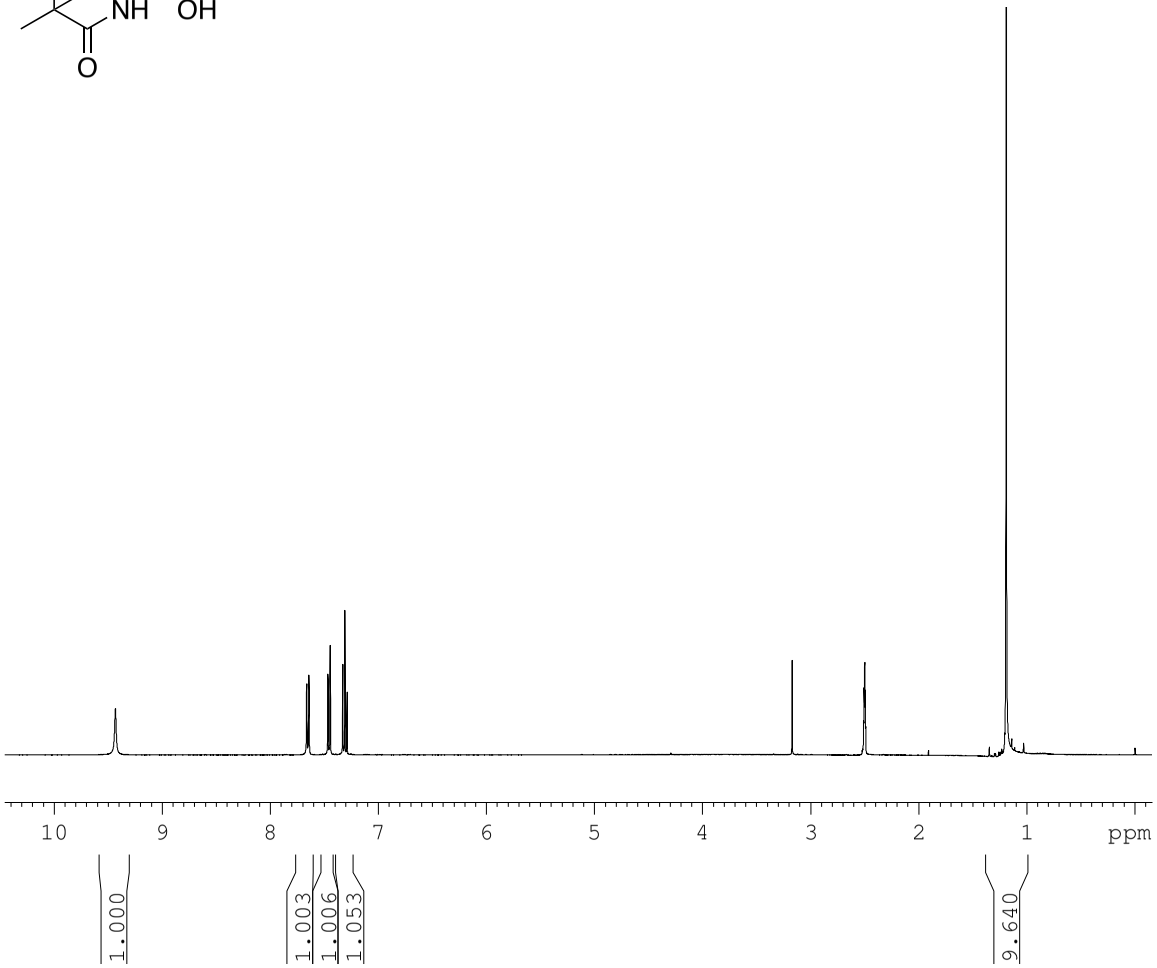
======== CHANNEL f1 ========
NUC1                 19F
P1                 12.40 usec
PLW1         30.97400093 W
SFO1        376.4889442 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PLW2         16.98200035 W
PLW12         0.31623000 W
SFO2        400.1632010 MHz

F2 - Processing parameters
SI                131072
SF           376.5265940 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                 1.00
```

2-Bromo-6-[(2,2-dimethylpropanoyl)amino]benzoic acid (3.47)





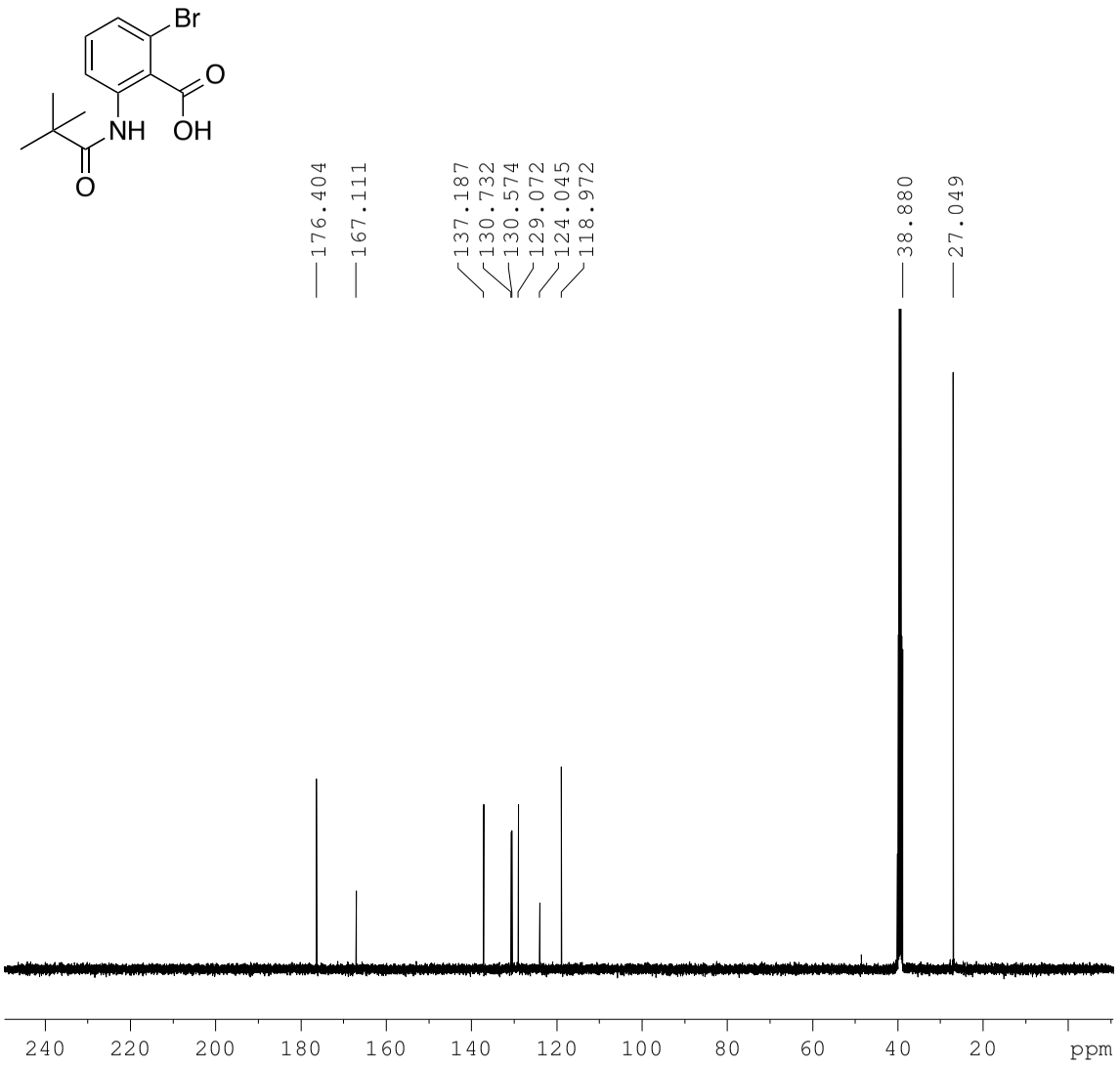

```
Current Data Parameters
NAME          N28992-32
EXPNO                10
PROCNO                1

F2 - Acquisition Parameters
Date_          20130718
Time              19.08
INSTRUM             w400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH            8278.146 Hz
FIDRES        0.126314 Hz
AQ            3.9583745 sec
RG                   128
DW               60.400 usec
DE                6.50 usec
TE               303.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 1H
P1                11.10 usec
PL1               -6.00 dB
PL1W        49.59999847 W
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                32768
SF          400.1300032 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                 1.50
```

2-Bromo-6-[(2,2-dimethylpropanoyl)amino]benzoic acid (3.47)




```
Current Data Parameters
NAME          N28992-32
EXPNO                11
PROCNO                1

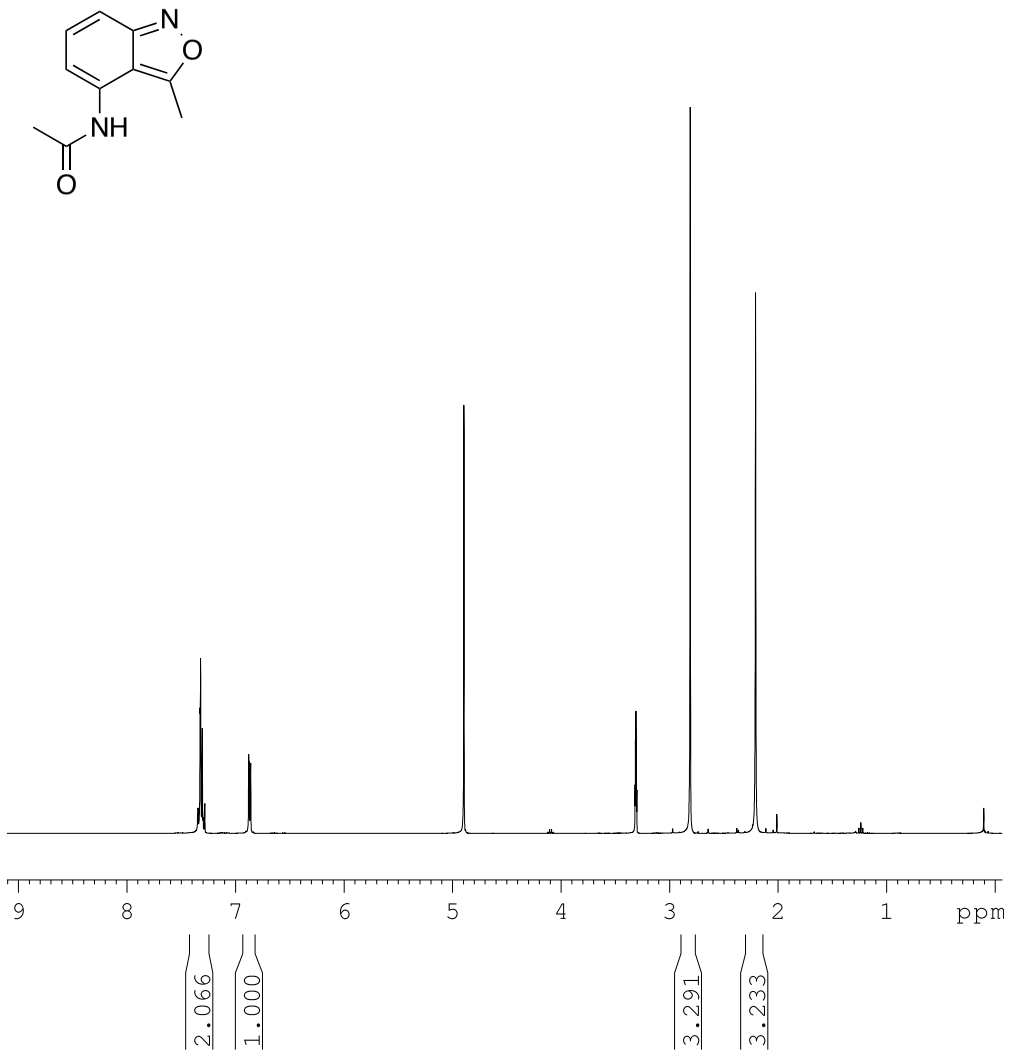
F2 - Acquisition Parameters
Date_          20130718
Time              20.28
INSTRUM            w400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                  2048
DS                     4
SWH           26178.010 Hz
FIDRES         0.399445 Hz
AQ            1.2517376 sec
RG                  4096
DW               19.100 usec
DE                6.50 usec
TE               303.0 K
D1            1.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 13C
P1                  8.65 usec
PL1                -2.00 dB
PL1W        24.80207634 W
SFO1        100.6248425 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             75.00 usec
PL2               -6.00 dB
PL12               6.50 dB
PL13               8.50 dB
PL2W        49.59999847 W
PL12W        2.78921294 W
PL13W        1.75987446 W
SFO2        400.1316005 MHz

F2 - Processing parameters
SI                32768
SF          100.6128193 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                 1.40
```

_N_-(3-Methyl-2,1-benzoxazol-4-yl)acetamide (3.55)



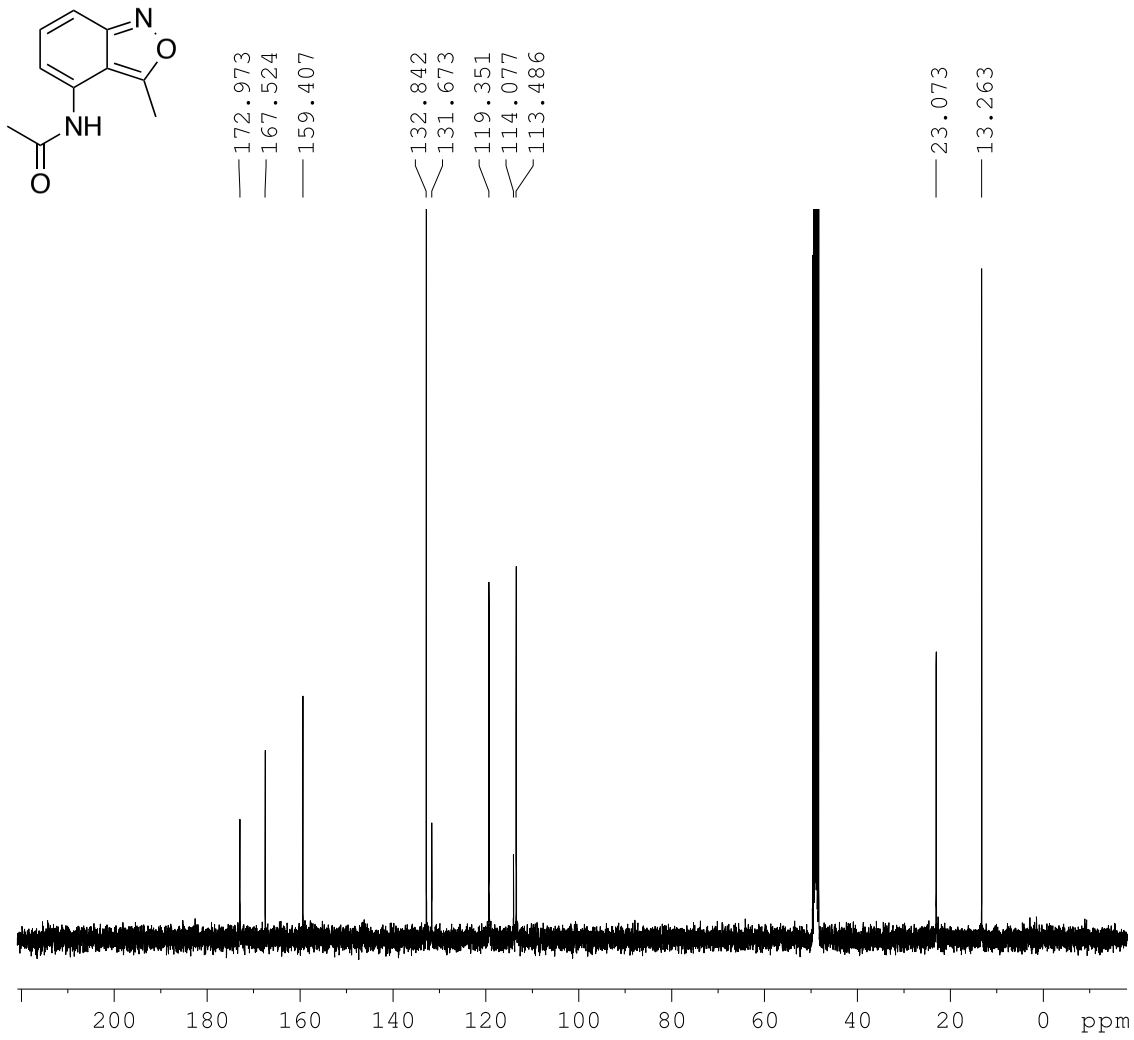

```
Current Data Parameters
NAME      LJ352 fra 29-32 AVF400
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20131007
Time               15.25
INSTRUM          avf400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ             4.0894465 sec
RG                    93
DW                62.400 usec
DE                 6.50 usec
TE                294.1 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                   1H
P1                 12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                 32768
SF           400.2500097 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

_N_-(3-Methyl-2,1-benzoxazol-4-yl)acetamide (3.55)




```
Current Data Parameters
NAME      LJ352 fra 29-32 service
EXPNO                3
PROCNO               1

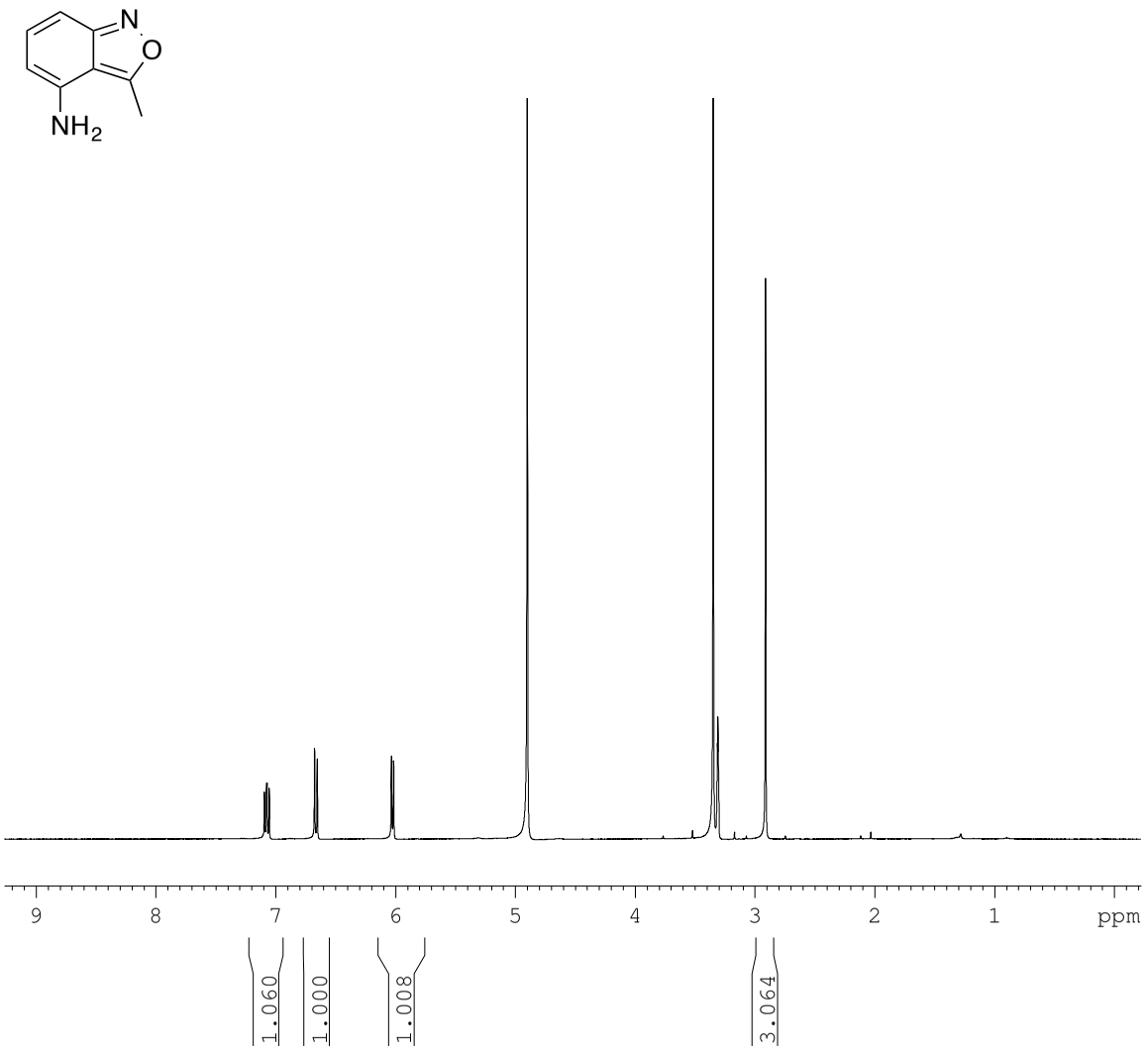
F2 - Acquisition Parameters
Date_          20131012
Time               8.36
INSTRUM          avb400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 1024
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631488 sec
RG               197.74
DW              20.800 usec
DE               6.50 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1        100.6228303 MHz
NUC1               13C
P1               10.00 usec
PLW1        60.95399857 W

======== CHANNEL f2 ========
SFO2        400.1316005 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2        14.58800030 W
PLW12        0.29771000 W
PLW13        0.14588000 W

F2 - Processing parameters
SI                32768
SF          100.6126284 MHz
WDW                EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

3-Methyl-2,1-benzoxazol-4-amine (3.28)



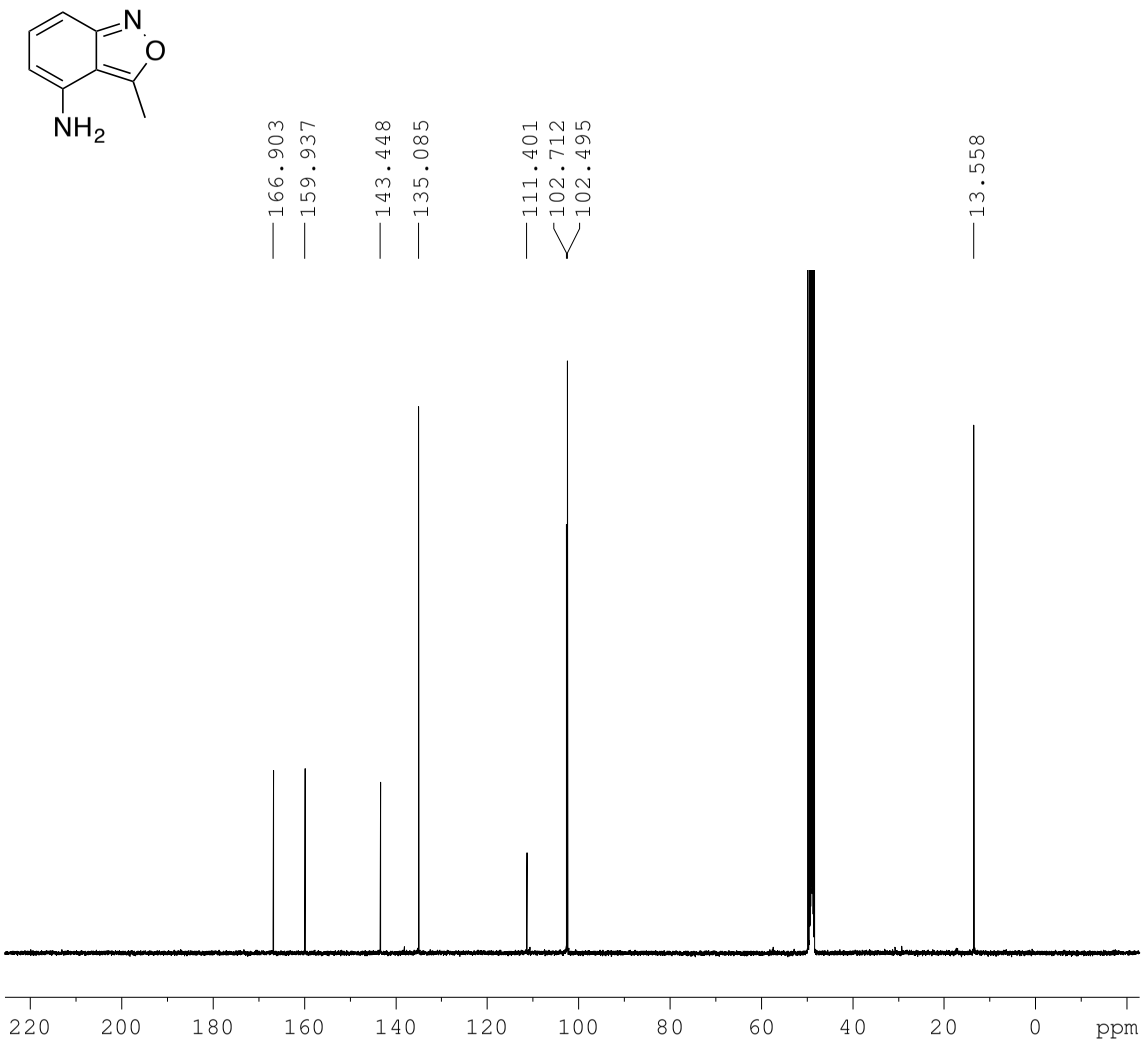

```
Current Data Parameters
NAME      LJ421 fra 8-13 grease wash
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140507
Time              18.03
INSTRUM           avg400
PROBHD   5 mm QNP 1H/13
PULPROG             zg30
TD                65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ            3.2767999 sec
RG                255.2
DW               50.000 usec
DE                6.50 usec
TE                293.5 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2024714 MHz
NUC1                 1H
P1                12.23 usec
PLW1         11.30000019 W

F2 - Processing parameters
SI                 65536
SF           400.2000113 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

3-Methyl-2,1-benzoxazol-4-amine (3.28)




```
Current Data Parameters
NAME      LJ421 service
EXPNO                 3
PROCNO                1

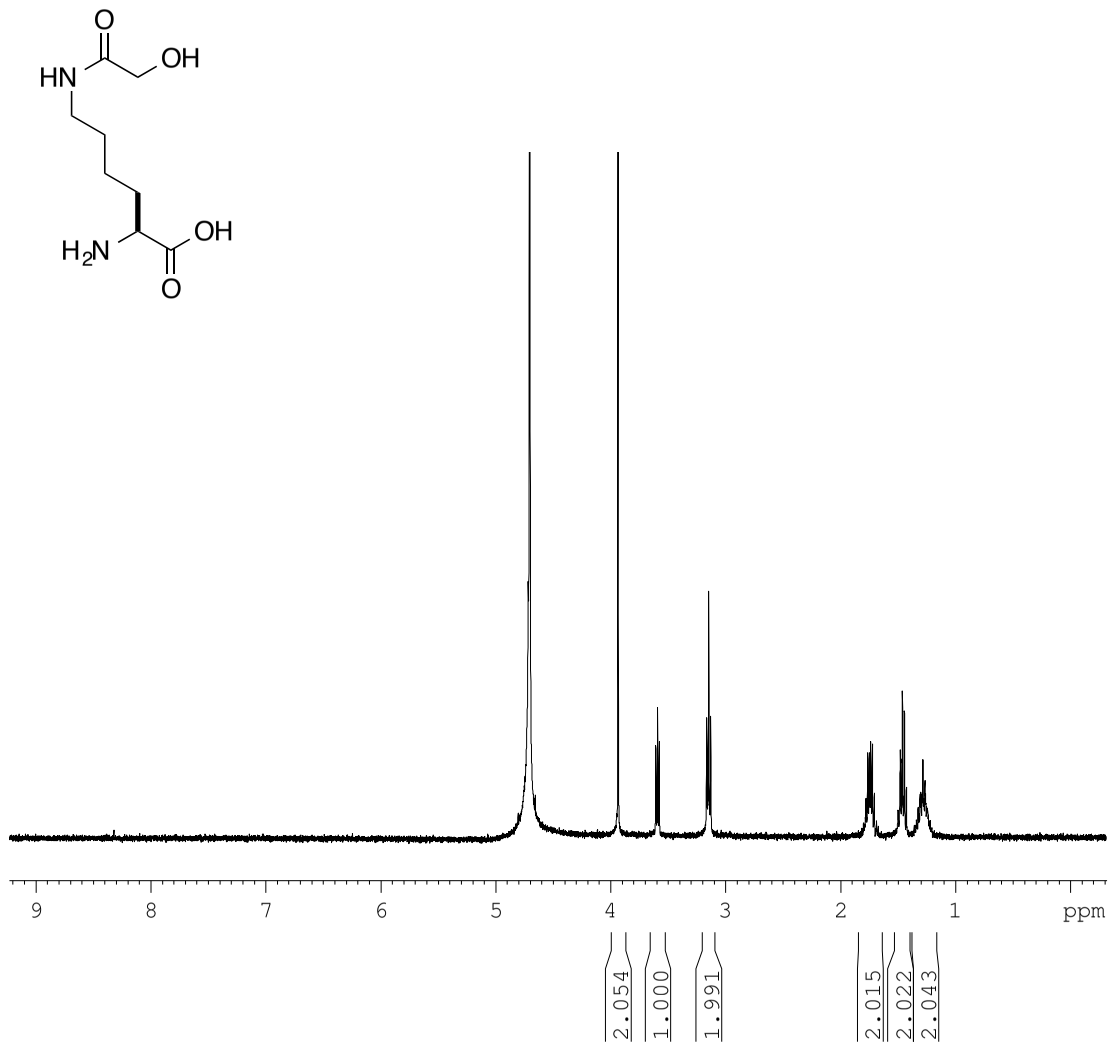
F2 - Acquisition Parameters
Date_          20140510
Time              19.19
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                  912
DW               16.000 usec
DE               18.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         125.8131152 MHz
NUC1                13C
P1                10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2           7.99830008 W
PLW12          0.28119001 W
PLW13          0.17996000 W

F2 - Processing parameters
SI                 32768
SF           125.8003589 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(2*S*)-2-Amino-6-(carbamoylamino)hexanoic acid (4.12)



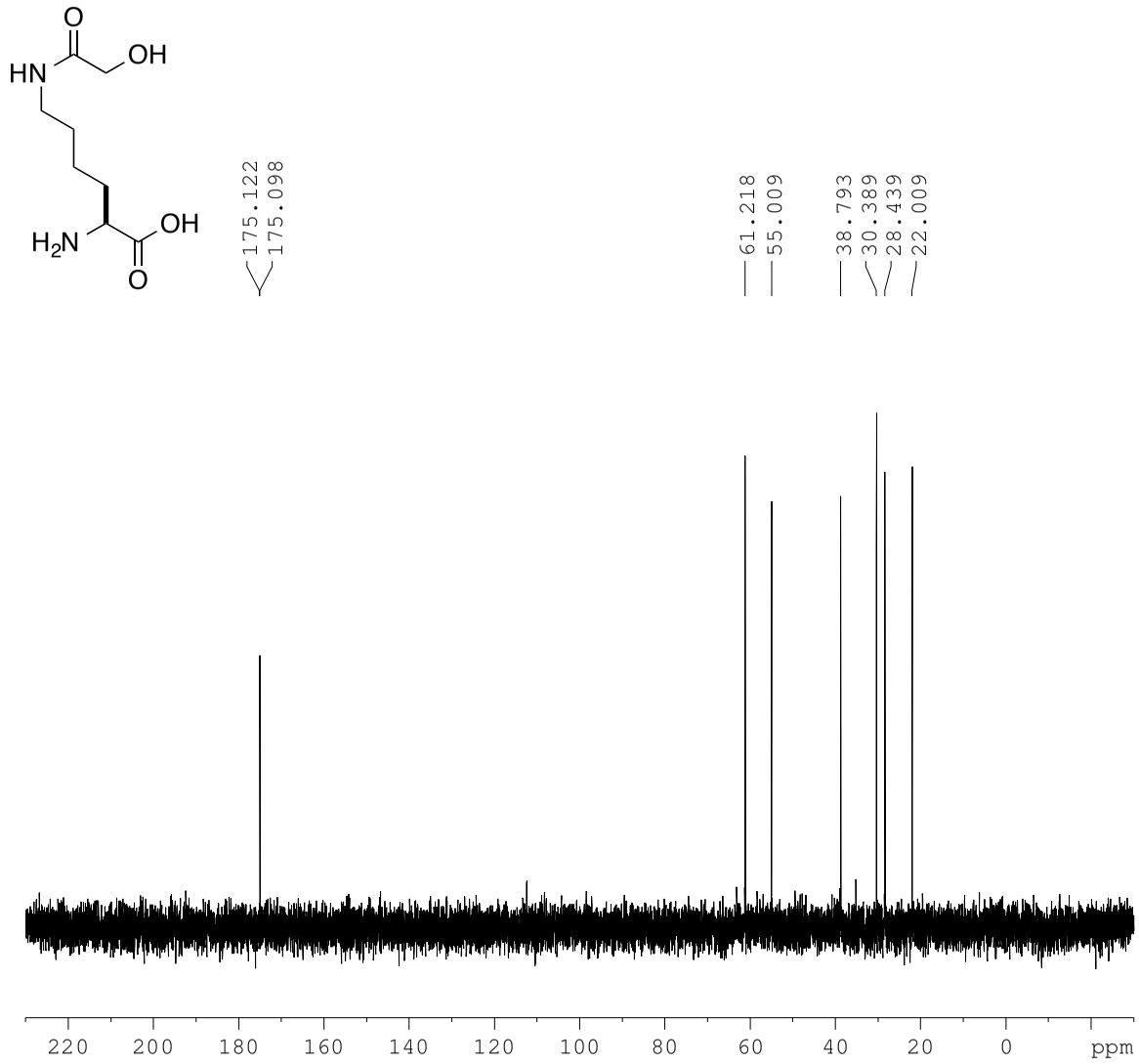

```
Current Data Parameters
NAME      LJ078 fra 25-39
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20121203
Time               11.25
INSTRUM          avn400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT              D2O
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ             4.0894465 sec
RG                149.73
DW                62.400 usec
DE                 6.50 usec
TE                294.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                   1H
P1                 12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                 32768
SF           400.2500000 MHz
WDW                   no
SSB       0
LB        0 Hz
GB        0
PC                 1.00
```

(2*S*)-2-Amino-6-(carbamoylamino)hexanoic acid (4.12)




```
Current Data Parameters
NAME       LJ078 dqx400
EXPNO                 2
PROCNO                1

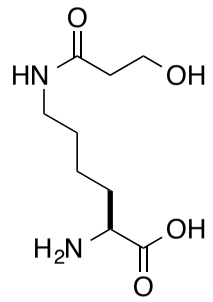
F2 - Acquisition Parameters
Date_          20120510
Time               2.22
INSTRUM          av400
PROBHD   5 mm QNP 1H/13
PULPROG         zgpg30
TD              32768
SOLVENT           D2O
NS                256
DS                  4
SWH         26178.010 Hz
FIDRES       0.798889 Hz
AQ           0.6258688 sec
RG              32768
DW             19.100 usec
DE              7.50 usec
TE              300.0 K
D1          1.00000000 sec
D11         0.03000000 sec
TD0                 1

======== CHANNEL f1 ========
NUC1               13C
P1                9.50 usec
PL1      0 dB
SFO1       100.6403931 MHz

======== CHANNEL f2 ========
CPDPRG[2        waltz16
NUC2                1H
PCPD2           80.00 usec
PL2      0 dB
PL12            19.00 dB
PL13            25.00 dB
SFO2       400.2016008 MHz

F2 - Processing parameters
SI              32768
SF         100.6303300 MHz
WDW                EM
SSB      0
LB              1.00 Hz
GB       0
PC              1.40
```

(2*S*)-2-Amino-6-[(3-hydroxypropanoyl)amino]hexanoic acid (4.11)



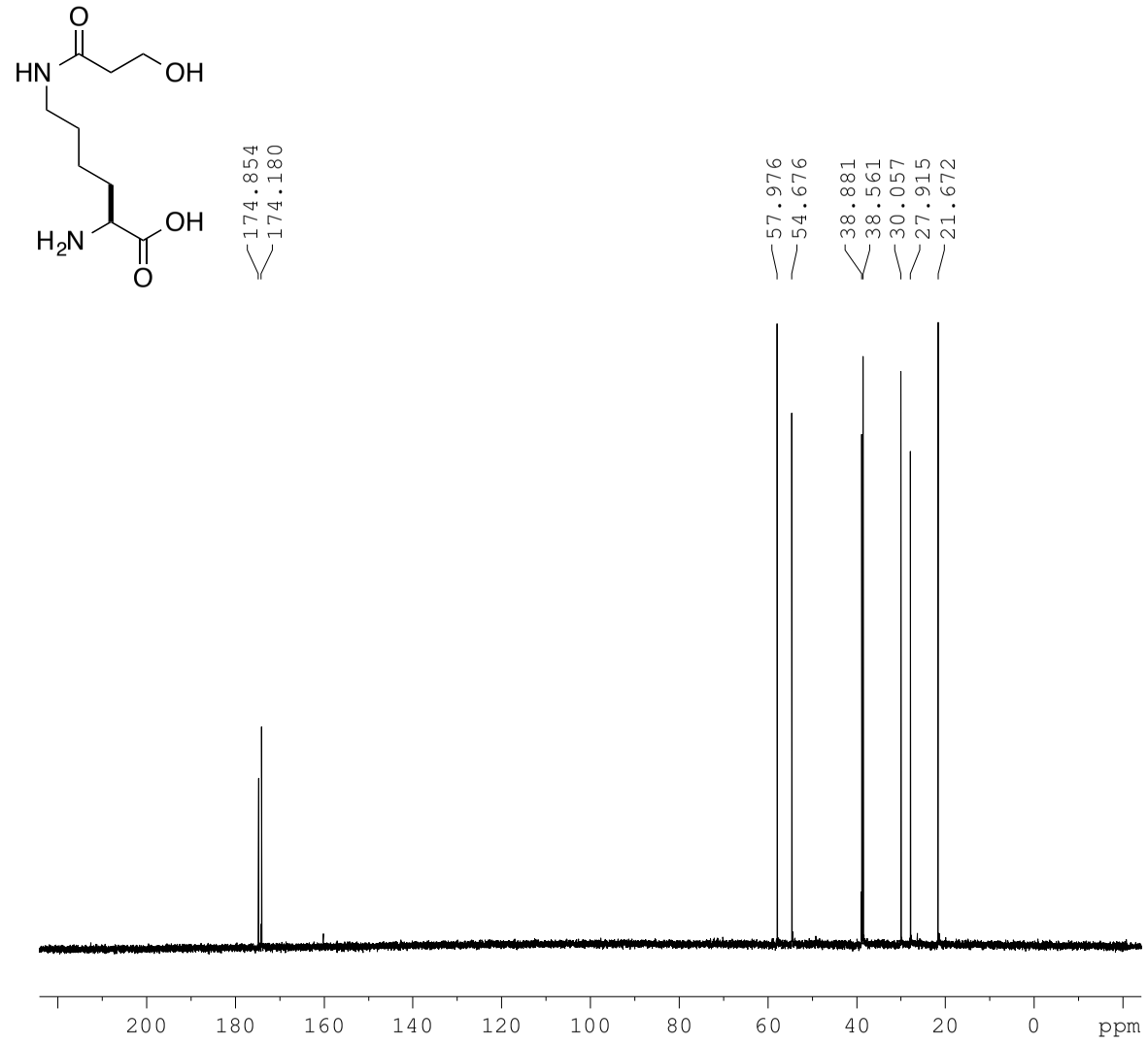

```
Current Data Parameters
NAME      LJ219 service
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20121205
Time              20.51
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT              D2O
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                 2.25
DW               48.400 usec
DE                6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                10.60 usec
PL1                6.00 dB
PL1W         0.95905519 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                 32768
SF          500.3000000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

(2*S*)-2-Amino-6-[(3-hydroxypropanoyl)amino]hexanoic acid (4.11)




```
Current Data Parameters
NAME      LJ219 service
EXPNO                 3
PROCNO                1

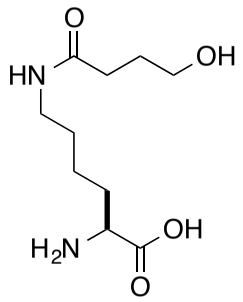
F2 - Acquisition Parameters
Date_          20121205
Time              22.49
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             D2O
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                  912
DW               16.000 usec
DE               20.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005350 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(2*S*)-2-Amino-6-[(4-hydroxybutanoyl)amino]hexanoic acid (4.10)



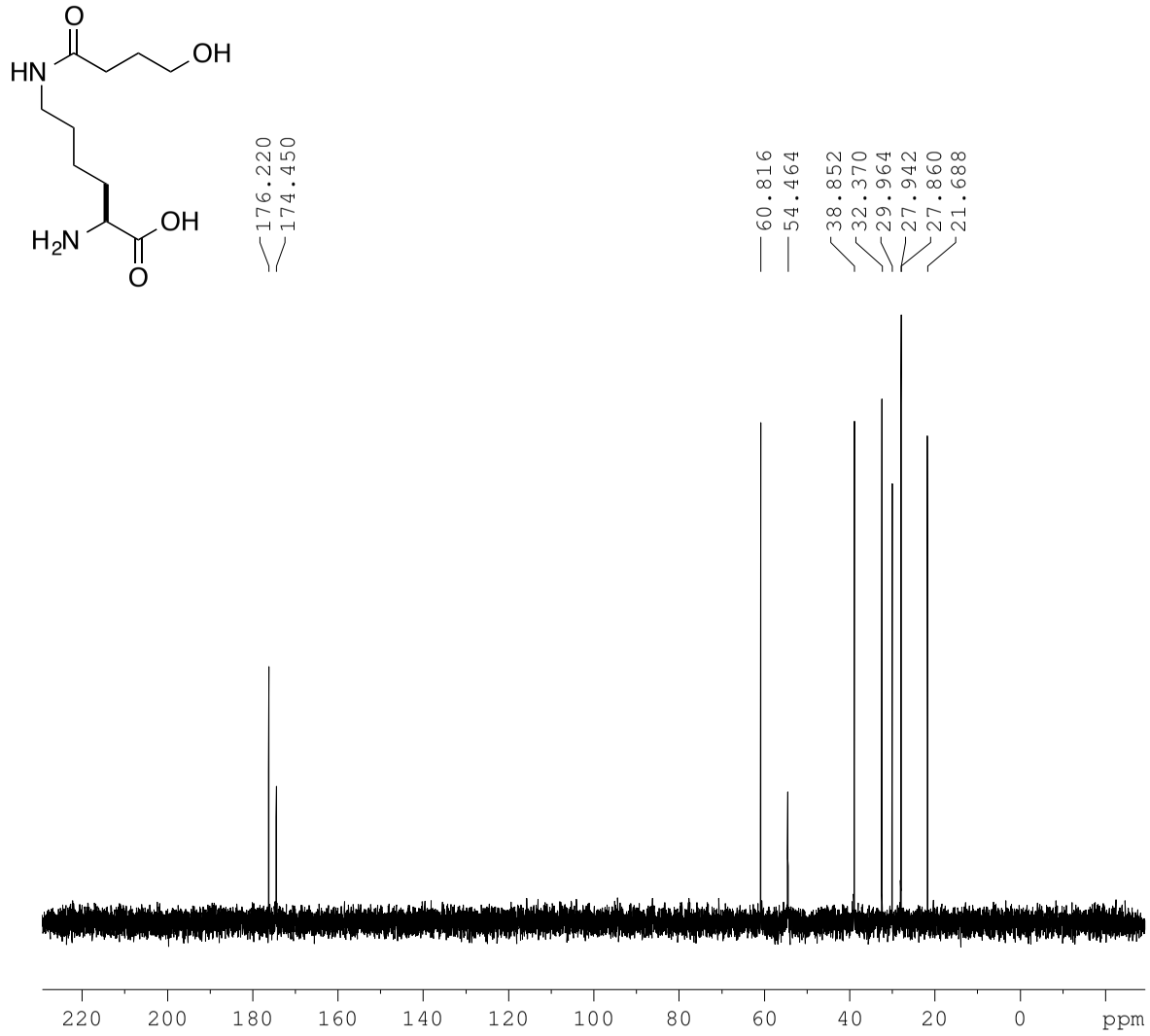

```
Current Data Parameters
NAME      LJ275 fra 21-36 AVN400
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_           20121221
Time              15.06
INSTRUM           avn400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT              D2O
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ              4.0894465 sec
RG                 23.04
DW                62.400 usec
DE                 6.50 usec
TE                295.4 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                   1H
P1                 12.65 usec
PLW1        16.70000076 W

F2 - Processing parameters
SI                 32768
SF           400.2500000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

(2*S*)-2-Amino-6-[(4-hydroxybutanoyl)amino]hexanoic acid (4.10)




```
Current Data Parameters
NAME     LJ275 fra 21-36 AVN400
EXPNO                 2
PROCNO                1

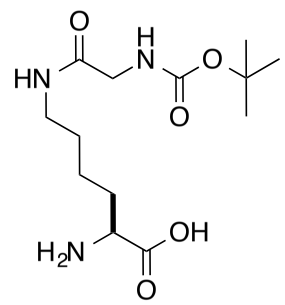
F2 - Acquisition Parameters
Date_          20121221
Time             15.14
INSTRUM          avn400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD               32768
SOLVENT            D2O
NS                   256
DS                     4
SWH          26041.666 Hz
FIDRES        0.794729 Hz
AQ            0.6291456 sec
RG               205.43
DW               19.200 usec
DE                6.50 usec
TE               296.5 K
D1           1.00000000 sec
D11          0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        100.6530073 MHz
NUC1                13C
P1                 9.00 usec
PLW1        58.70000076 W

======== CHANNEL f2 ========
SFO2        400.2516010 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2        16.70000076 W
PLW12        0.32991999 W
PLW13        0.26723999 W

F2 - Processing parameters
SI                 32768
SF          100.6429430 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(2*S*)-2-Amino-6-({[(*tert*-butoxycarbonyl)amino]acetyl}amino)hexanoic acid (4.78)



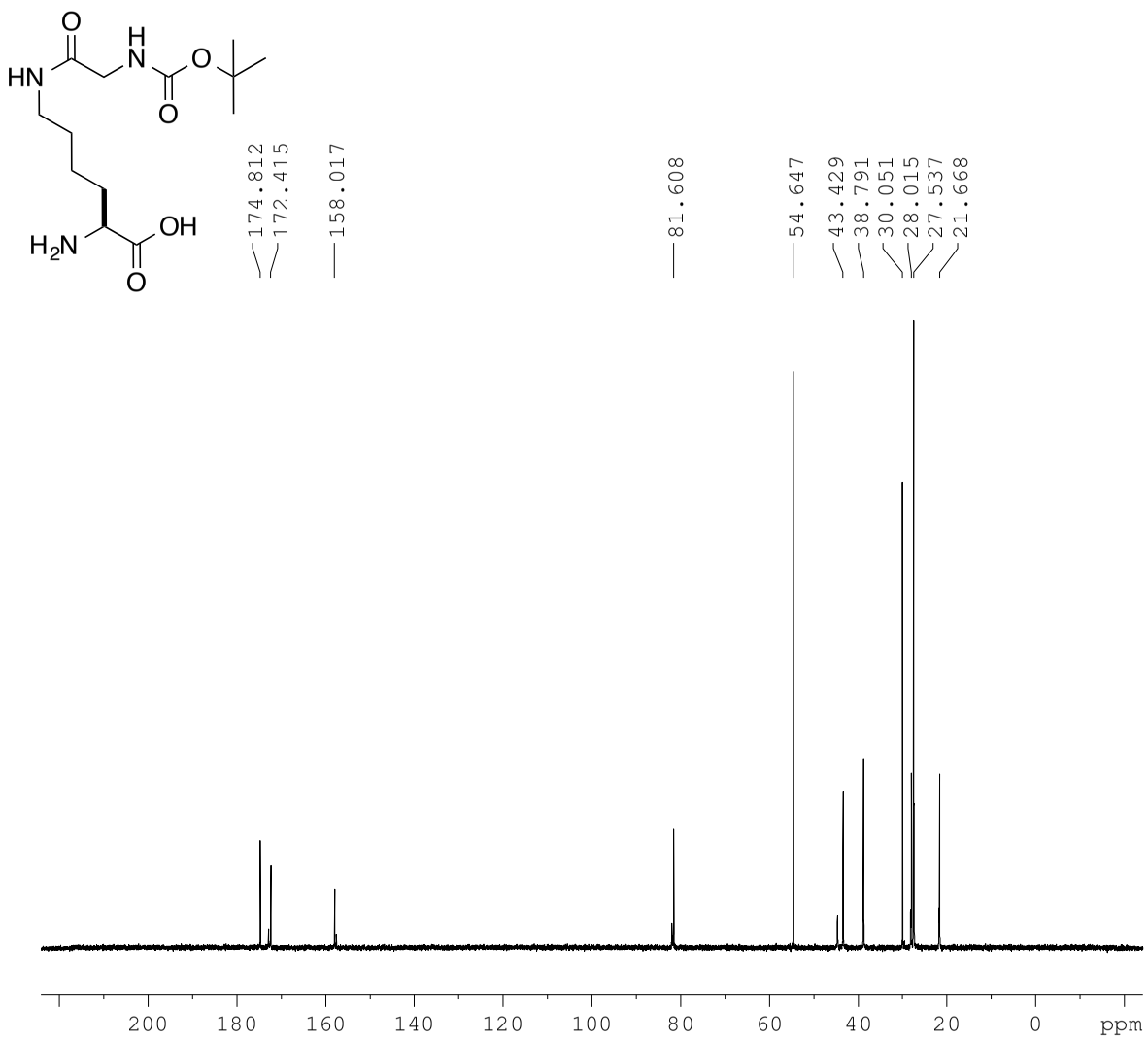

```
Current Data Parameters
NAME        LJ270 AVN400
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20121217
Time               21.24
INSTRUM           avn400
PROBHD   5 mm PABBO BB/
PULPROG             zg60
TD                 65536
SOLVENT              D2O
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ             4.0894465 sec
RG                 74.23
DW                62.400 usec
DE                 6.50 usec
TE                295.4 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.2524015 MHz
NUC1                   1H
P1                 12.65 usec
PLW1         16.70000076 W

F2 - Processing parameters
SI                 32768
SF           400.2500000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

(2*S*)-2-Amino-6-({[(*tert*-butoxycarbonyl)amino]acetyl}amino)hexanoic acid (4.78)




```
Current Data Parameters
NAME      LJ270 service
EXPNO                 3
PROCNO                1

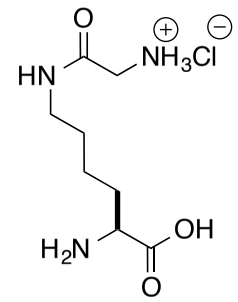
F2 - Acquisition Parameters
Date_          20121220
Time               6.45
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             D2O
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                  912
DW               16.000 usec
DE               20.00 usec
TE               298.1 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                 10.25 usec
PL1                 8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                 6.00 dB
PL12               23.56 dB
PL13               29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                 32768
SF          125.8005350 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

2-{[(5*S*)-5-Amino-5-carboxypentyl]amino}-2-oxoethanaminium chloride (4.13)



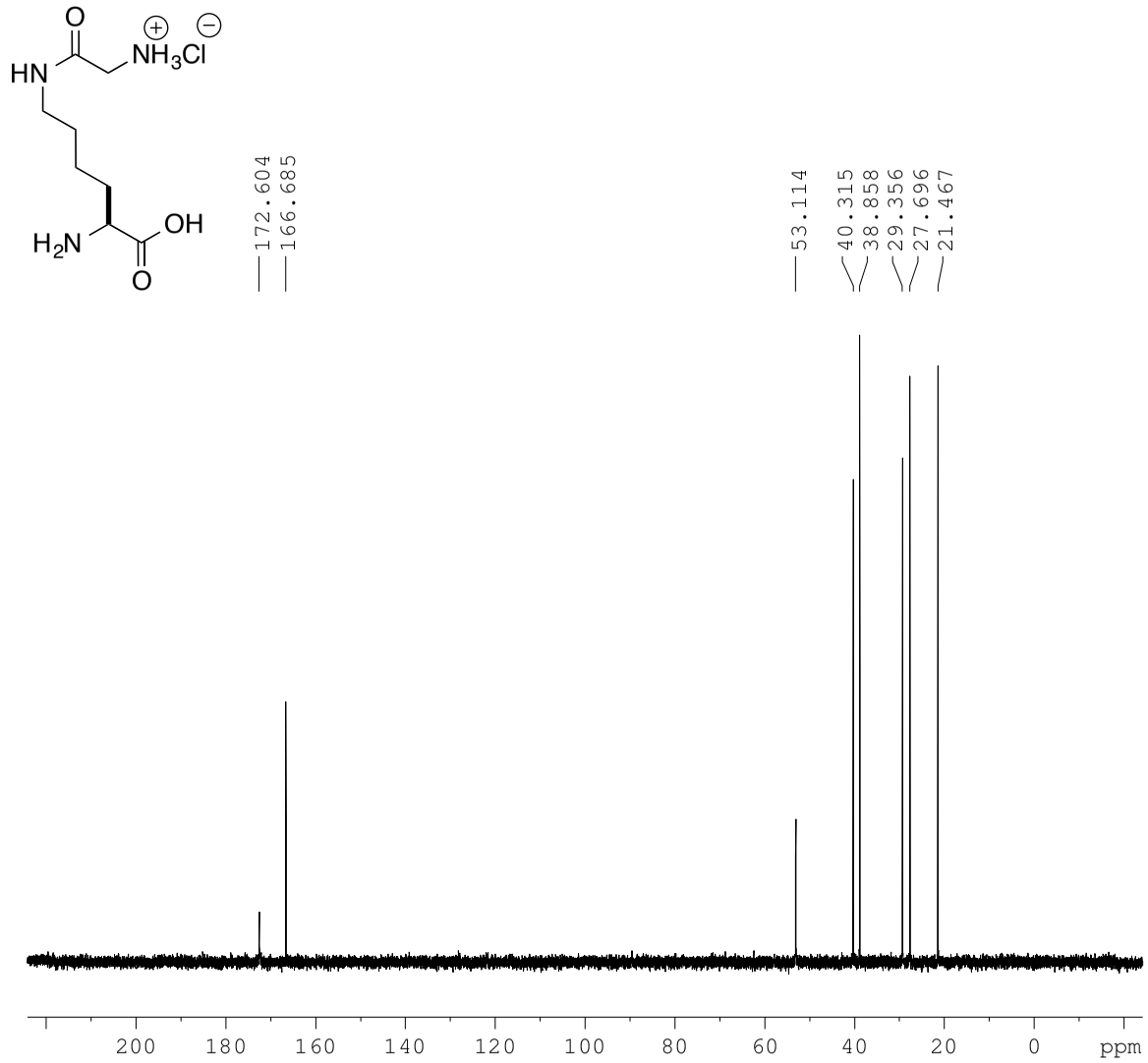

```
Current Data Parameters
NAME      LJ276 service
EXPNO                1
PROCNO               1

F2 - Acquisition Parameters
Date_          20130109
Time               4.19
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT             D2O
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719425 sec
RG                   3.2
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                 1H
P1                10.60 usec
PL1                6.00 dB
PL1W         0.95905519 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                  32768
SF          500.3000000 MHz
WDW                  EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

2-{[(5*S*)-5-Amino-5-carboxypentyl]amino}-2-oxoethanaminium chloride (4.13)




```
Current Data Parameters
NAME      LJ276 service
EXPNO                 3
PROCNO                1

F2 - Acquisition Parameters
Date_          20130109
Time               5.17
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             D2O
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0485760 sec
RG                  912
DW               16.000 usec
DE               20.00 usec
TE                298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

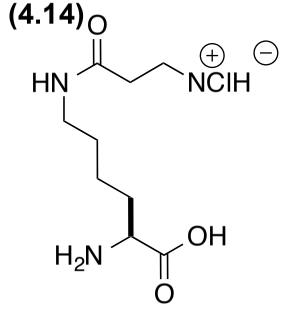
======== CHANNEL f1 ========
NUC1                 13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W          0.95905519 W
PL12W         0.01682068 W
PL13W         0.00422516 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005350 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

3-{[(5*S*)-5-Amino-5-carboxypentyl]amino}-3-oxopropan-1-aminium chloride

(4.14)




```
Current Data Parameters
NAME      LJ277 service
EXPNO                1
PROCNO               1

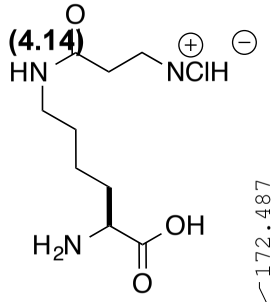
F2 - Acquisition Parameters
Date_          20130109
Time               5.24
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                65536
SOLVENT             D2O
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                 3.56
DW               48.400 usec
DE                6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                  1

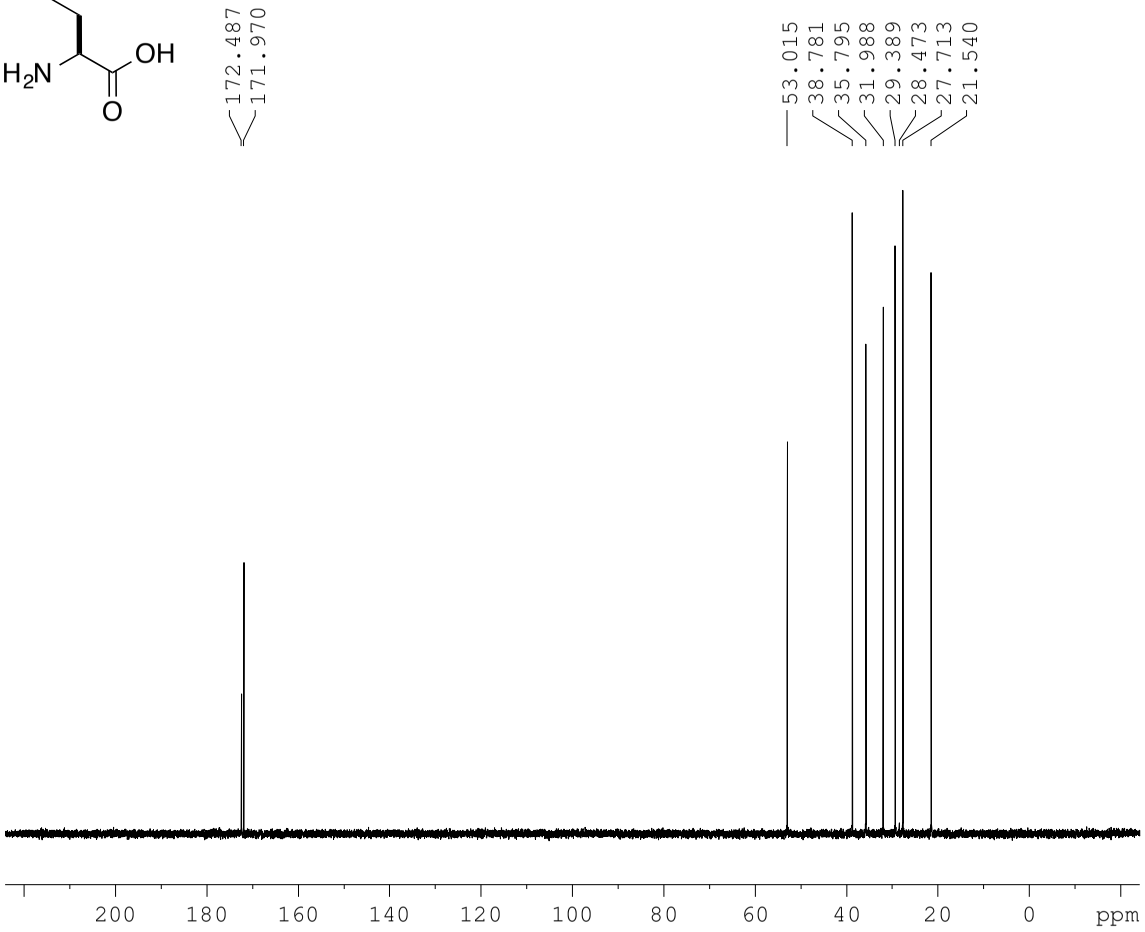
======== CHANNEL f1 ========
NUC1                 1H
P1                10.60 usec
PL1                6.00 dB
PL1W         0.95905519 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                32768
SF          500.3000000 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

3-{[(5*S*)-5-Amino-5-carboxypentyl]amino}-3-oxopropan-1-aminium chloride

(4.14)






```
Current Data Parameters
NAME      LJ277 service
EXPNO                 3
PROCNO                1

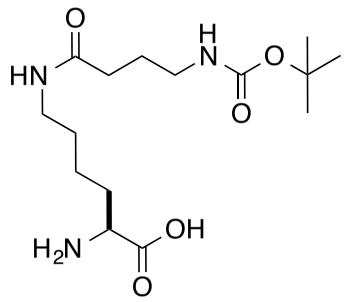
F2 - Acquisition Parameters
Date_          20130109
Time               6.22
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             D2O
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                  912
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12               23.56 dB
PL13               29.56 dB
PL2W          0.95905519 W
PL12W         0.01682068 W
PL13W         0.00422516 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005350 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(2*S*)-2-Amino-6-({4-[(*tert*-butoxycarbonyl)amino]butanoyl}amino)hexanoic acid (4.79)



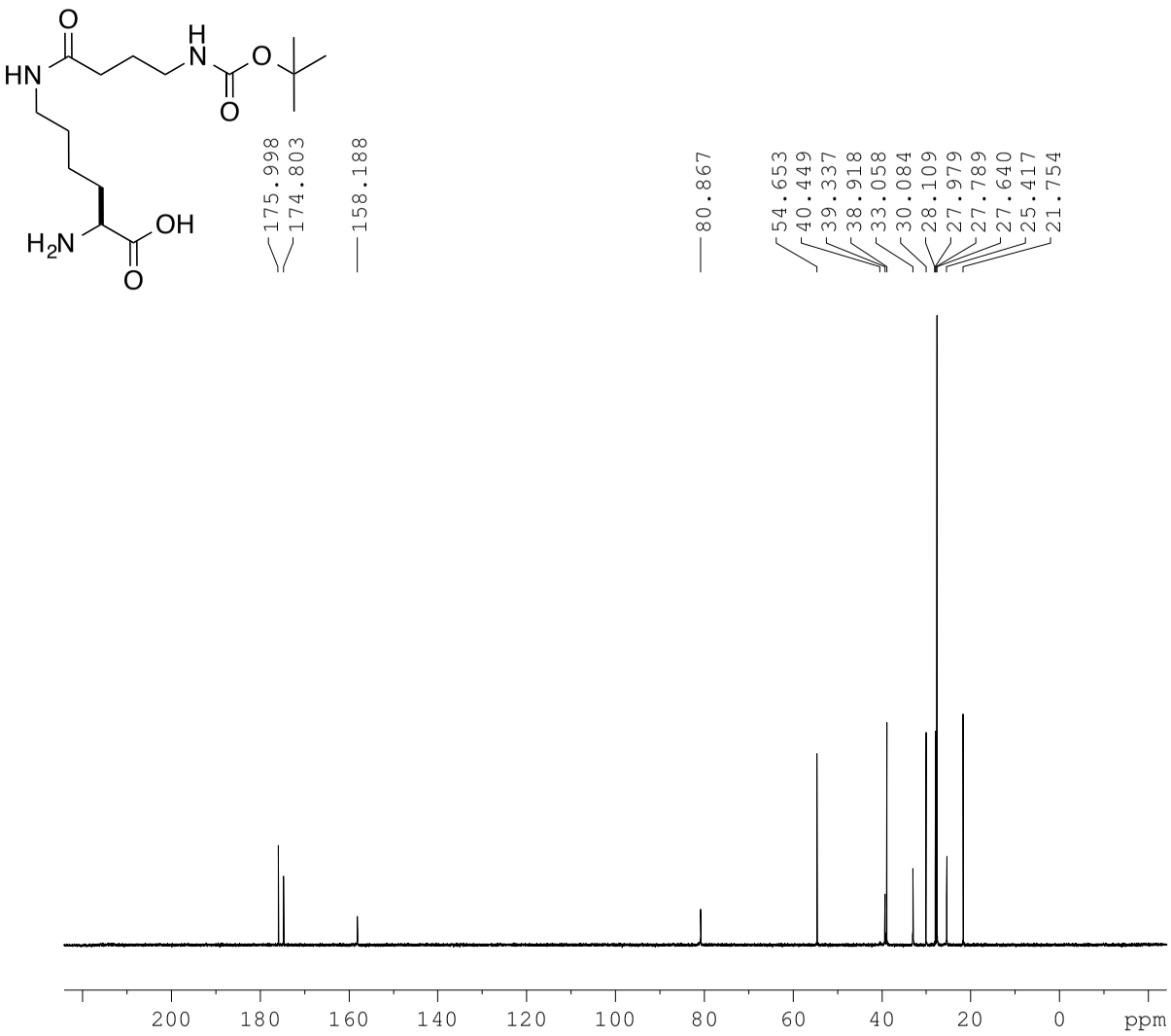

```
Current Data Parameters
NAME      LJ271 service
EXPNO                1
PROCNO               1

F2 - Acquisition Parameters
Date_          20121220
Time               6.52
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG            zg30
TD                65536
SOLVENT             D2O
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  3.2
DW               48.400 usec
DE                6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                 1H
P1                10.60 usec
PL1                6.00 dB
PL1W         0.95905519 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                32768
SF          500.3000000 MHz
WDW                  EM
SSB      0
LB                0.30 Hz
GB       0
PC                1.00
```

(2*S*)-2-Amino-6-({4-[(*tert*-butoxycarbonyl)amino]butanoyl}amino)hexanoic acid (4.79)




```
Current Data Parameters
NAME      LJ271 service
EXPNO                 3
PROCNO                1

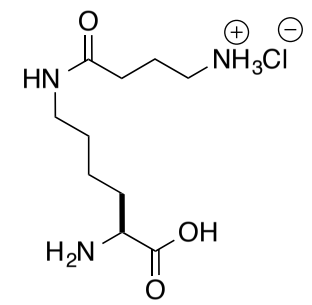
F2 - Acquisition Parameters
Date_          20121220
Time               7.50
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             D2O
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                  912
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                1H
PCPD2              80.00 usec
PL2                 6.00 dB
PL12               23.56 dB
PL13               29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                 32768
SF          125.8005350 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

4-{[(5*S*)-5-Amino-5-carboxypentyl]amino}-4-oxobutan-1-aminium chloride (4.15)



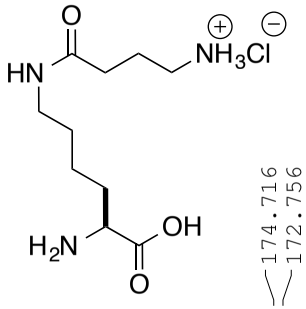

```
Current Data Parameters
NAME      LJ278 service
EXPNO                1
PROCNO               1

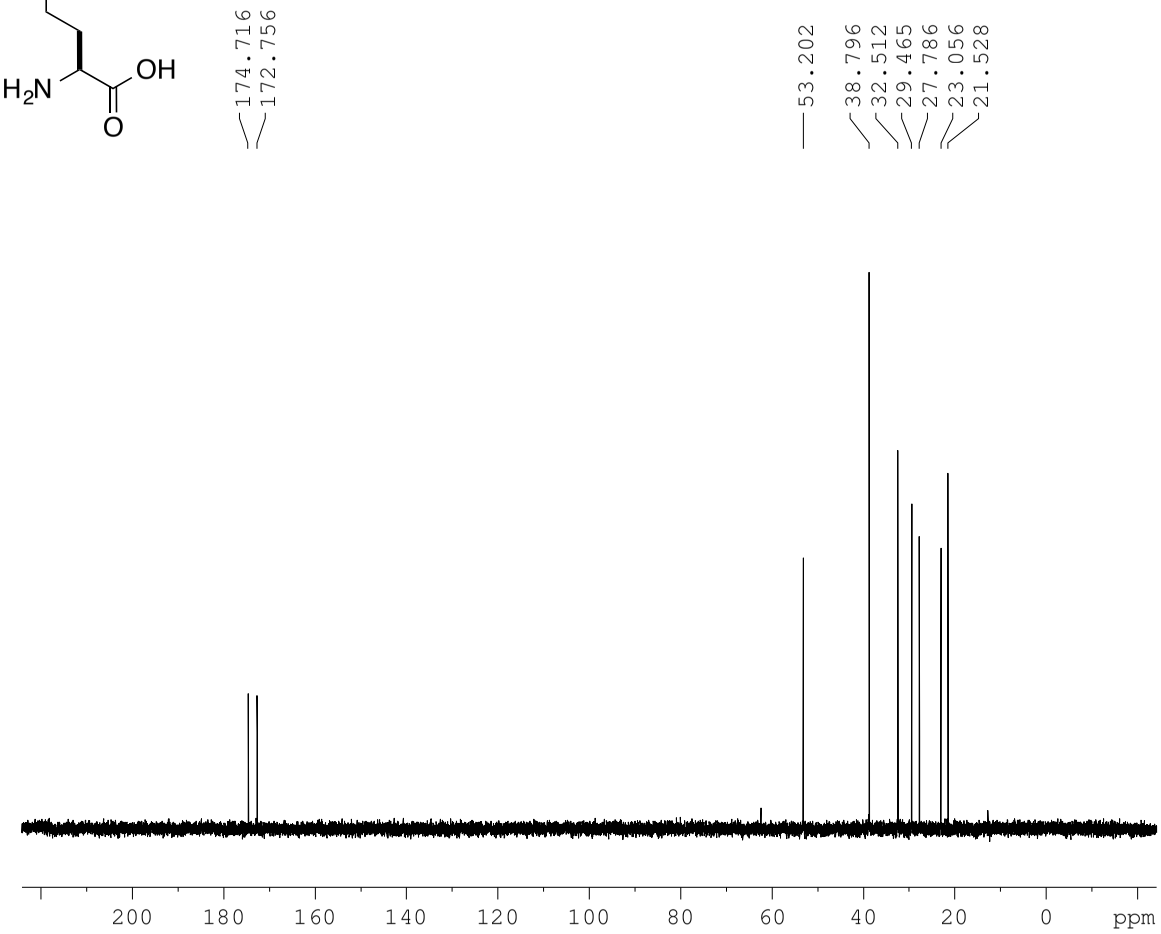
F2 - Acquisition Parameters
Date_           20130109
Time               6.29
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                65536
SOLVENT             D2O
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  3.2
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                1H
P1                10.60 usec
PL1                6.00 dB
PL1W         0.95905519 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                32768
SF          500.3000000 MHz
WDW                  EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

4-{[(5*S*)-5-Amino-5-carboxypentyl]amino}-4-oxobutan-1-aminium chloride (4.15)






```
Current Data Parameters
NAME      LJ278 service
EXPNO                3
PROCNO               1

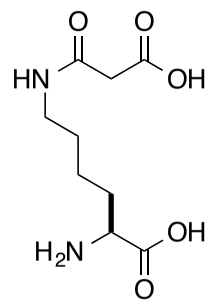
F2 - Acquisition Parameters
Date_          20130109
Time               7.27
INSTRUM         avc500
PROBHD   5 mm CPDUL 13C
PULPROG         zgpg30
TD               65536
SOLVENT            D2O
NS                 512
DS                   2
SWH          31250.000 Hz
FIDRES        0.476837 Hz
AQ            1.0485760 sec
RG                 912
DW              16.000 usec
DE              20.00 usec
TE              298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1               13C
P1              10.25 usec
PL1              8.00 dB
PL1W       1.62029624 W
SFO1     125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2        waltz16
NUC2               1H
PCPD2           80.00 usec
PL2              6.00 dB
PL12            23.56 dB
PL13            29.56 dB
PL2W       0.95905519 W
PL12W      0.01682068 W
PL13W      0.00422516 W
SFO2     500.3020012 MHz

F2 - Processing parameters
SI               32768
SF         125.8005350 MHz
WDW                EM
SSB      0
LB               1.00 Hz
GB       0
PC               1.40
```

(2*S*)-2-Amino-6-[(carboxyacetyl)amino]hexanoic acid (4.3)



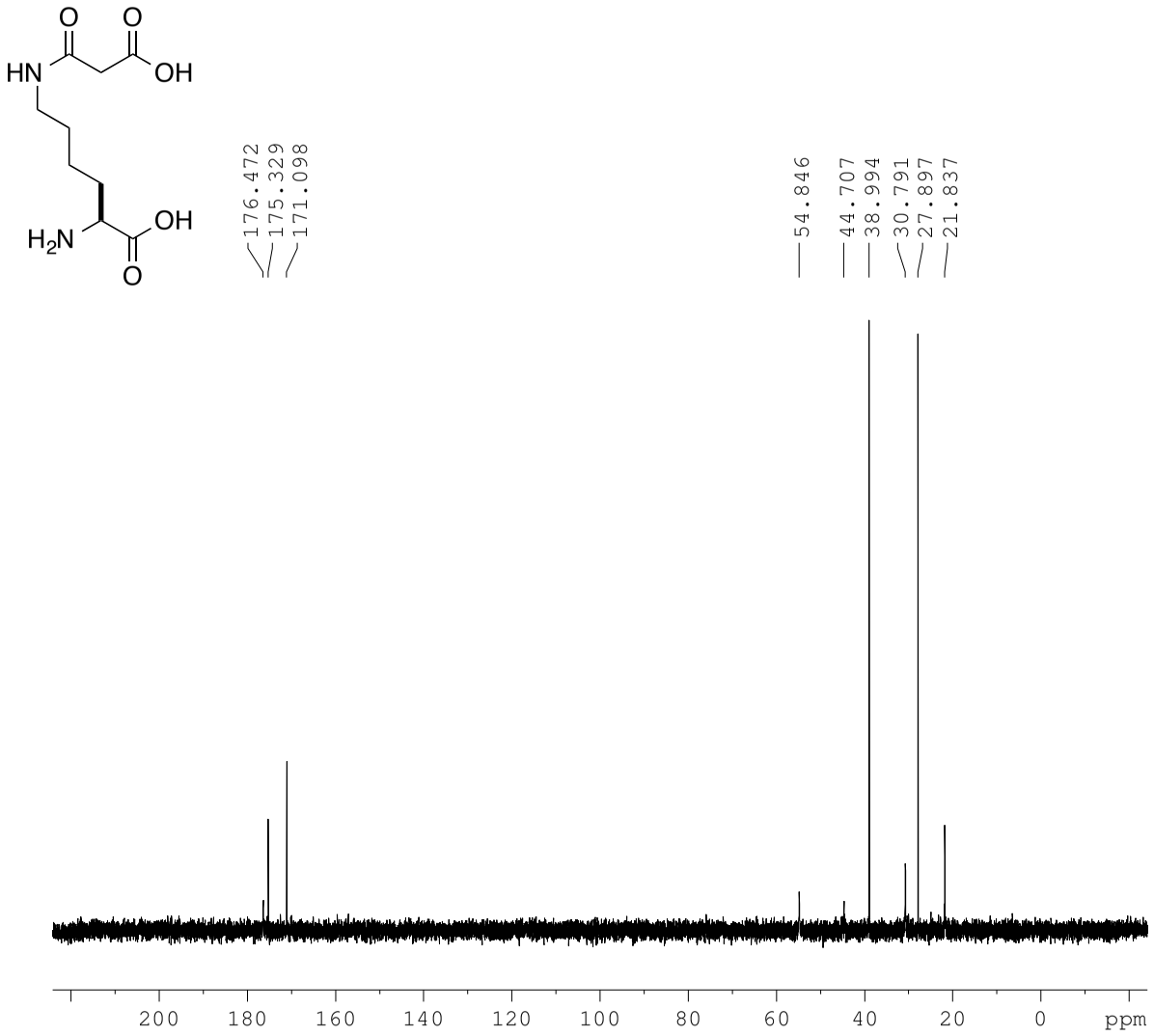

```
Current Data Parameters
NAME       LJ117 dqx400
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20120507
Time              17.00
INSTRUM           av400
PROBHD   5 mm QNP 1H/13
PULPROG            zg60
TD                65536
SOLVENT             D2O
NS                   16
DS                    2
SWH            8278.146 Hz
FIDRES         0.126314 Hz
AQ            3.9583745 sec
RG                  181
DW                60.400 usec
DE                 7.50 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 9.00 usec
PL1       0 dB
SFO1         400.2024714 MHz

F2 - Processing parameters
SI                 32768
SF          400.2000000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

(2*S*)-2-Amino-6-[(carboxyacetyl)amino]hexanoic acid (4.3)




```
Current Data Parameters
NAME      LJ117 service
EXPNO                 4
PROCNO                1

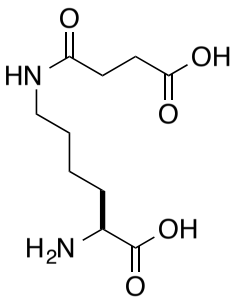
F2 - Acquisition Parameters
Date_          20120509
Time              18.46
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             D2O
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                 1820
DW               16.000 usec
DE               20.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005350 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```

(2*S*)-2-Amino-6-[(3-carboxypropanyl)amino]hexanoic acid (4.4)



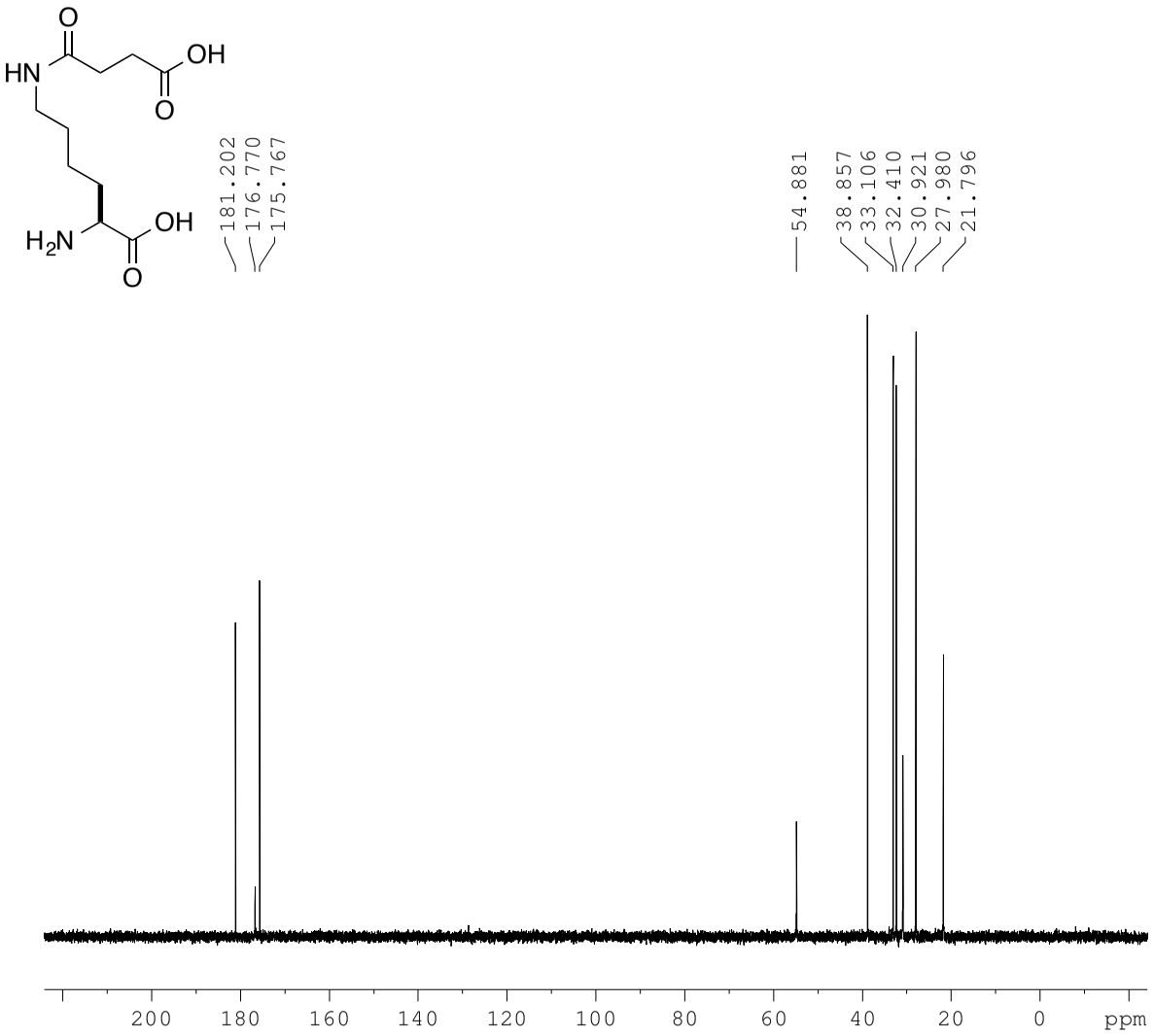

```
Current Data Parameters
NAME      LJ137 service
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20120830
Time              12.00
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                65536
SOLVENT             D2O
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                    4
DW               48.400 usec
DE                6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 1H
P1                 9.60 usec
PL1               -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                32768
SF          500.3000000 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

(2*S*)-2-Amino-6-[(3-carboxypropanyl)amino]hexanoic acid (4.4)




```
Current Data Parameters
NAME      LJ137 service
EXPNO                 3
PROCNO                1

F2 - Acquisition Parameters
Date_          20120830
Time              12.58
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             D2O
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                 1820
DW               16.000 usec
DE               20.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005350 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```