DEVELOPING INHIBITORS OF BROMODOMAIN-HISTONE INTERACTIONS

David Stephen Hewings

St John's College

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Supervisors: Dr Stuart Conway and Dr Paul Brennan

Abstract

David Hewings, St John's College

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Lysine acetylation is a widespread protein post-translational modification that influences diverse cellular processes. An association between acetylation of histone N-terminal tails and transcriptional activation has been recognised since the 1960s. However, it has only become apparent since 2000 that many of the effects of histone acetylation are mediated by proteins that bind to acetyl-lysine through a specialised acetyl-lysine recognition domain, the bromodomain. Small-molecule inhibitors of bromodomain-histone interactions can greatly assist studies into the functions of bromodomain-containing proteins, and show promise as treatments for several diseases, including cancers. Herein I describe the discovery and development of a novel chemical series of bromodomain-binding ligands containing the 3,5-dimethyisoxazole moiety. This heterocycle acts as an acetyl-lysine bioisostere, mimicking key interactions formed between acetyl-lysine and the bromodomain. Optimised compounds show sub-micromolar affinities for bromodomains of the BET family, a class of transcriptional co-regulators. Crystallographic and structure-activity relationship studies shed light on the structural requirements for potent and selective BET ligands. Furthermore, the compounds show cellular effects consistent with BET bromodomain inhibition: cytotoxicity studies in a range of cell lines, including the NCI-60 human tumour cell line screen, reveal differential activity, with leukaemias showing particular sensitivity. 3,5-Dimethylisoxazole-containing compounds were also shown to downregulate known BET target genes.

Further studies investigated the effect of modifying or replacing the methyl groups of 3,5-dimethylisoxazole on BET bromodomain affinity, which indicated that the 3-methyl group is necessary for affinity. Finally, three novel isoxazole-containing amino acids were synthesised and incorporated into histone peptides as potential bromodomain-binding, non-hydrolysable, acetyl-lysine mimics. These amino acids might be useful in uncovering the function of individual acetylated lysine residues.

The identification of methyl-isoxazoles as acetyl-lysine-mimetic bromodomain ligands represents a significant advance in our understanding of structure-activity relationships for these important proteins. The confirmed cellular activity of these compounds will enable their use in future biological studies.

Contents

Acknowledgements **1**

Abbreviations **5**

Chapter 1: Introduction **9**

1.1 Post-translational modifications and histone modification **9**

1.1.1 Protein post-translational modification 9

1.1.2 Lysine acetylation 10

1.1.3 An acetyl-lysine recognition domain 12

1.1.4 A histone code? 14

1.2 Bromodomain structure and function **17**

1.2.1 Bromodomain structure 17

1.2.2 Classification of the bromodomains 19

1.2.3 BET bromodomains – function 20

1.2.4 BET bromodomains – roles in disease 24

1.3 BET bromodomain inhibitors **26**

1.3.1 3-Methyl-1,2,4-triazoles 27

1.3.2 3-Methylisoxazoles 35

1.3.3 3-Methyl-dihydroquinazolin-2-ones 40

1.3.4 Thiazol-2-one 42

1.3.5 1-Acetyl-tetrahydroquinolines 43

1.3.6 4-Acylpyrroles 46

1.3.7 2-Methylphenols 47

1.3.8 Other compounds 48

1.3.9 BET bromodomain SAR 53

1.4 Ligands for bromodomains outside the BET family **58**

1.5 Assays for the identification and characterisation of bromodomain ligands **60**

1.5.1 Label-free techniques 61

1.5.2 Displacement assays 62

1.5.3 Phenotypic assays 65

1.6 Applications of BET ligands to the study of biological systems **66**

1.6.1 BET inhibitors and cancer 66

1.6.2 BET inhibitors and inflammation 68

1.6.3 BET inhibitors and heart disease 69

1.6.4 BET inhibitors and viral infection 70

1.7 Conclusion **70**

Chapter 2: 3,5-Dimethylisoxazole as a Novel Acetyl-Lysine Mimic **73**

2.1 Introduction and aims **73**

2.1.1 The need for new bromodomain ligands 73

2.1.2 A novel assay leads to the identification of a 3,5-dimethylisoxazole-containing BET ligand 75

2.1.3 Aims 78

2.2 Design of 3,5-dimethyl-4-phenylisoxazole derivatives **79**

2.3 Synthesis of 3,5-dimethyl-4-phenylisoxazole derivatives **80**

2.3.1 Synthesis of 3′-substituted 3,5-dimethyl-4-phenylisoxazole derivatives 80

2.3.2 Synthesis of 3′,5′-disubstituted 3,5-dimethyl-4-phenylisoxazole derivatives 82

2.3.3 Resynthesis of **45a** 83

2.4 Evaluation of 3,5-dimethyl-4-phenylisoxazole derivatives as BET bromodomain ligands **84**

2.4.1 Peptide displacement and protein stabilisation 84

2.4.2 X-Ray crystal structure 85
2.4.3 Investigating non-specific toxicity 90
2.4.4 Implications for the design of improved isoxazole-containing bromodomain inhibitors....91
2.5 Synthesis of diarylmethanol derivatives 93
2.5.1 Synthesis of halogenated compounds....93
2.5.2 Modifications to the ZA channel substituent 93
2.5.3 Alternatives to the secondary alcohol....95
2.6 Evaluation of diarylmethanol derivatives 58a-e, 61a-g and 69a-b as BET bromodomain ligands 96
2.6.1 Peptide displacement and protein stabilisation 96
2.6.2 X-Ray crystal structure 100
2.7 Discussion and conclusion 103
2.7.1 Binding of 3,5-dimethylisoxazoles to bromodomains 103
2.7.2 Ligand efficiency....108
2.7.3 Conclusion and future work 108

Chapter 3: Cellular Activity of 3,5-Dimethylisoxazole-Containing BET Bromodomain Ligands 112
3.1 Antiproliferative effects of 61a and 61b in cancer cell lines....112
3.1.1 Leukaemia and lung cancer cell lines....112
3.1.2 NCI-60 DTP human tumour cell line screen 114
3.2 Effect of 61a on BRD4-dependent transcriptional coactivation 116
3.2.1 Effect of **61a** on c-Myc levels....116
3.2.2 Effect of **61a** on NF-κB-dependent transcription 117
3.3 Discussion and conclusion 118
3.3.1 Discussion: Cellular effects of **61a** and **61b** 118
3.3.2 Conclusion and future work 124

Chapter 4: Modifications to the Isoxazole at the 3- and 5-Positions 126
4.1 Introduction and aims 126
4.1.1 Can modifications to 3,5-dimethylisoxazole introduce additional interactions with bromodomains?....126
4.1.2 Aims....128
4.1.3 Initial compound design....129
4.2 Synthesis of 3-amino-5-methyl- and 5-amino-3-methylisoxzoles 130
4.3 Aminomethyl-methyl- and hydroxymethyl-methylisoxazoles and derivatives 134
4.3.1 Modifications to the 3-position 136
4.3.2 Modifications to the 5-position 141
4.3.3 Evaluation of aminomethyl-, aminomethyl-methyl- and hydroxymethyl-methylisoxazoles and derivatives 143
4.4 Introducing hydrogen bond donors at the 5-position of 61a....145
4.4.1 Synthesis of **129a**-**e**....145
4.4.2 Evaluation of compounds **129a**-**e** as bromodomain ligands....151
4.5 Discussion and conclusions 151
4.5.1 Discussion 151
4.5.2 Conclusions and future work 158

Chapter 5: Acetyl-Lysine-Mimicking Amino Acids 160
5.1 Introduction and aims 160
5.1.1 Synthesis of post-translationally modified proteins....160
5.1.2 Introduction of modified lysines 162
5.1.3 Acetyl-lysine mimicking amino acids 166
5.1.4 Improving bromodomain assays using KAc mimics 168
5.1.5 Aims....169

5.2 Design of KAc-mimicking amino acids 170
5.3 Synthesis of Fmoc-protected (*S*)-2-amino-3-(3-methylisoxazole-5-carboxamido)propanoic acid 155 172
5.4 Synthesis of Fmoc-protected (*S*)-2-amino-3-((3-methylisoxazol-5-yl)methoxy)propanoic acid 156 175
5.5 Synthesis of Fmoc-protected (*S*)-2-amino-5-(3-methylisoxazol-5-yl)pentanoic acid 157 175
5.5.1 Cycloaddition strategy for the synthesis of **157** 177
5.5.2 Alkylation of 3,5-dimethylisoxazole for the synthesis of **157** 179
5.6 Assessing the enantiopurity of Fmoc-protected amino acids 185
5.7 Peptide synthesis 186
5.8 Preliminary evaluation of peptides 158-161 186
5.9 Conclusion and further work 188

Chapter 6: Conclusion 192

Chapter 7: Experimental Procedures 195
7.1 General experimental procedures for synthetic chemistry 195
7.2 General experimental procedures for biological evaluation 201
7.2.1 Procedures employing recombinant protein 201
7.2.2 Cellular assays 202
7.3 Chapter 2: Synthetic Procedures 205
7.3.1 3′-Substituted 3,5-dimethyl-4-phenylisoxazole derivatives 205
7.3.2 3′,5′-Disubstituted 3,5-dimethyl-4-phenylisoxazole derivatives 211
7.3.3 Resynthesis of **45a** 219
7.3.4 Diarylmethanol derivatives 221
7.4 Chapter 4: Synthetic Procedures 243
7.4.1 3-amino-5-methyl- and 5-amino-3-methylisoxzoles 243
7.4.2 Aminomethyl-methyl- and hydroxymethyl-methylisoxazoles and derivatives 259
7.4.3 Modifications to the 5-position of **61a** 298
7.5 Chapter 5: Synthetic Procedures 319
7.5.1 Fmoc-protected (S)-2-amino-3-(3-methylisoxazole-5-carboxamido)propanoic acid **155** 319
7.5.2 Fmoc-protected (S)-2-amino-3-((3-methylisoxazol-5-yl)methoxy)propanoic acid **156** 322
7.5.3 Fmoc-protected 2-amino-5-(3-methylisoxazol-5-yl)pentanoic acid **157** 327

Appendices 345
Appendix 1: Mass spectra of 45 345
Appendix 2: Preparative chiral HPLC separation of (+)-61a and (–)-61a. 346
Appendix 3: Clonogenic assay for A549 and H1975 cells 347
Appendix 4. Comparison of NCI-60 DTP Human Tumour Cell Line Screen for 61a, PFI-1 and XD14 348

References 351

Selected NMR Spectra 369

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Abbreviations

AlphaScreen®	amplified luminescent proximity homogeneous assay
AML	acute myeloid leukaemia
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ApoA1	apolipoprotein A1
AUC	area under the curve
BAZ2A/B	bromodomain adjacent to zinc finger domain protein 2A/B
BCL2	apoptosis regulator Bcl-2 (alternative name: B-cell CLL/lymphoma 2)
BCP	bromodomain-containing protein
BET	bromodomain and extra terminal domain
BLI	bio-layer interferometry
Boc	*tert*-butoxycarbonyl
Bpin	4,4,5,5-tetramethyl-1,3,2-dioxaborolyl
BRD2/3/4	bromodomain-containing protein 2/3/4
BRDT	bromodomain testis-specific protein
Cbz	carboxybenzyl
CDK	cyclin-dependent kinase
CDK9	cyclin-dependent kinase 9
CREBBP	cAMP-response element binding protein (CREB)-binding protein
CTD	C-terminal domain
CYP	cytochrome P450
Dap	2,3-diaminopropionic acid
Dha	dehydroalanine
DME	1,2-dimethoxyethane
DMAc	*N*,*N*-dimethylacetamide
DMAP	4-dimethylaminopyridine
DMEM	Dulbecco's modified Eagle's medium
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DMTMM	4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methylmorpholinium chloride
DPPF	1,1'-bis(diphenylphosphino)ferrocene
DSF	differential scanning fluorimetry
EBV	Epstein-Barr virus
ELISA	enzyme-linked immunosorbent assay
EPL	expressed protein ligation
ESI	electrospray ionisation
ET	extra terminal
FA	fluorecence anisotropy (alternative name: fluorecence polarisation, FP)
FI	field ionisation
FMOC	9-fluorenylmethyloxycarbonyl
FOSL1	FOS-like antigen 1
FRAP	fluorescence recovery after photobleaching
FRET	Förster resonance energy transfer

GATA1	erythroid transcription factor
GCN5	general control nonderepressible 5 (alternative name: histone acetyltransferase KAT2A)
GFP	green fluorecent protein
GI	growth inhibition
HAT	histone acetyltransferase
HAT1	histone acetyltransferase 1
HBTU	*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HDAC	histone deacetylase
HDAC1/2/8	histone deacetylase 1/2/8
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HPV	human papiloma virus
HTS	high-throughput screening
IL-6	interleukin-6
ISWI	imitation SWI
ITC	isothermal titration calorimetry
JMJD6	bifunctional arginine demethylase and lysyl-hydroxylase JMJD6 (alternative name: JmjC domain-containing protein 6)
KAc	*N*-ε-acetyl-L-lysine
K_SAc	acetyl-thialysine
KSHV	Kaposi's sarcoma-associated herpesvirus
K_SMe	methy thialysine
LC	liquid chromatography
LDA	lithium diisopropylamide
LE	ligand efficiency
LLE	ligand-lipophilic efficiency
LP	lipopolysaccharide
MLL	mixed lineage leukaemia
MM	multiple myeloma
Ms	methanesulfonyl (= mesyl)
MS	mass spectrometry
MTCTK	methythiocarbonyl-thialysine
MTS	(3-(4,5- dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo- phenyl)-2H-tetrazolium)
MTT	3-(4,5-di- methylthiazol-2-yl)-2,5-diphenylltetrazolium bromide
MW	microwave
NBS	*N*-bromosuccinimide
NCL	native chemical ligation
NF-κB	nuclear factor κB
NHS	*N*-hydroxysuccinimide
NSCLC	non-small-cell lung carcinoma
NSD3	histone-lysine N-methyltransferase NSD3 (alternative name: nuclear SET domain-containing protein 3)
NUT	nuclear protein in testis
P-TEFb	positive transcription elongation factor

PAFc	polymerase-associated factor complex
PB1	polybromo-1
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCAF	p300-CREBBP associated factor
PHD	plant homeodomain
PI3K	phosphoinositide 3-kinase
PID	P-TEFb interaction domain
PK	pharmacokinetics
Pol II	RNA polymerase II
PTB	phosphotyrosine-binding domain
PTM	post-translational modification
RBP1	retinol binding protein 1
RELA	transcription factor p65 (alternative name: NF-κB p65 subunit)
RNAi	RNA interference
RPD3	reduced potassium dependency 3
rt	room temperature
RuPhos	2-dicyclohexylphosphino-2′,6′-diisopropoxybiphenyl
SAR	structure-activity relationship
SEC	super elongation complex
SGC	Structural Genomics Consortium
SH2	*Src* homology 2
shRNA	short hairpin RNA
SIR2	silent information regulator 2
siRNA	short interfering RNA
SMARCA2	probable global transcription activator SNF2L2 (alternative name: SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 2)
SMARCA4	transcription activator BRG1 (alternative name: SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 4)
SNP	single-nucleotide polymorphism
SPPS	solid-phase peptide synthsis
SPR	surface plasmon resonance
Su	*N*-succinimidyl
SWI/SNF	switch/sucrose non-fermentable
TAF1	transcription initiation factor $TF_{II}D$ subunit 1 (alternative names: TATA box binding protein (TBP)-associated factor, 250 kDa; $TAF_{II}250$)
TAF3	transcription initiation factor $TF_{II}D$ subunit 3 (alternative names: TATA box binding protein (TBP)-associated factor, 140 kDa; $TAF_{II}140$)
TAT	transactivator of transcription
TAZ	transcription adaptor putative zinc finger
TBAF	tetra-*n*-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
$TF_{II}D$	transcription factor II D

TGI	total growth inhibition
THF	tetrahydrofuran
THP	2-tetrahydropyranyl
TIPS	triisopropylsilyl
TLC	thin later chromatography
TMEDA	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine
Ts	*para*-toluenesulfonyl (= tosyl)
WST-1	water-soluble tetrazolium 1

Chapter 1: Introduction

1.1 Post-translational modifications and histone modification

1.1.1 Protein post-translational modification

Cell type and function are controlled principally by the expression levels of the approximately 21,000 genes in the human genome. However, modulating gene expression provides only coarse-grained control over protein activity, and expression is relatively slow to respond to stimuli, with protein levels typically changing over a timescale of hours. Furthermore, changes in gene expression have little effect on protein degradation or sub-cellular localisation, and cannot modulate the interactions between proteins that are so important for intracellular signalling. Alternative splicing and proteolytic cleavage can change the primary amino acid sequence with profound functional consequences, but other mechanisms are needed to control protein-protein interactions, and to regulate the activity, localisation and lifetime of expressed proteins. These roles are played by post-translational modifications (PTMs), chemical changes to a protein made by enzymes to affect its function.

PTM may involve changing the chemical nature of amino acids (such as arginine deimination to form citrulline), or removal of part of the protein (such as cleavage of terminal methionine residues), but by far the most extensive and intensively-studied class of modifications are additions to the protein. The additions may be other proteins, other amino acids, sugars, cofactors, or small chemical modifications. The last of these often plays a critical role in intracellular signalling and in regulating enzyme activity. Phosphorylation is perhaps the best-studied of these PTMs; since the discovery in the 1950s that reversible phos-

phorylation of glycogen phosphorylase modulates its catalytic activity,[1] it has become apparent that phosphorylation regulates the function of many proteins, and mediates most signal transduction processes in eukaryotes.[2] Proteomic studies suggest that over 8,000 proteins are phosphorylated in humans, giving rise to a dynamic 'phosphoproteome' involved in regulating a vast array of cellular functions.[3] Phosphorylation, primarily of serine, threonine or tyrosine hydroxyl groups, is highly dynamic, and carried out by kinases and reversed by phosphatases. In addition, specialised domains such as the SRC homology 2 (SH2) or phosphotyrosine-binding (PTB) domain, can recognise phosphorylated residues in a sequence-dependent manner and thereby promote certain protein-protein interactions.

1.1.2 Lysine acetylation

Another small chemical modification, the addition of an acetyl group to the terminal nitrogen of lysine, is also exceptionally widespread, although compared to phosphorylation, its regulatory and signalling roles became apparent only recently. Acetyl lysine (KAc), or more precisely *N*-ε-acetyl-L-lysine, was first identified in 1968 as a component of histone proteins by V.G. Allfrey, who demonstrated that the modification occurred post-translationally.[4,5] Allfrey also suggested a connection between lysine acetylation and transcription, proposing that "acetylation, by neutralizing the positive charges associated with the ε-amino groups of lysine residues, would be expected to influence the interactions between histones and DNA … and this may offer a molecular basis for the pronounced changes in histone acetylation and RNA synthesis during the course of gene activation in many cell types."[5] Shortly afterwards, proteins possessing histone acetyltransferase (HAT)[6,7] or histone deacetylase (HDAC)[8,9] activity were

extracted, with varying degrees of purity, but it was not until 1995 that the first acetyltransferase gene, histone acetyltransferase 1 (*HAT1*), was identified and cloned in yeast.[10] The following year, Allis and co-workers identified a nuclear histone acetyltransferase gene in *Teterahymena*.[11] Contemporaneously, in a pioneering chemical biology study, Schreiber and colleagues used an irreversible covalent inhibitor of histone deacetylase activity, trapoxin, to identify and clone the first histone deacetylase gene, histone deacetylase 1 (*HDAC1*), from human cells.[12] These studies demonstrated that KAc was a dynamic mark with characteristic 'writer' and 'eraser' proteins.

The *Tetrahymena* HAT showed significant homology to the yeast gene general control nonderepressible 5 (*GCN5*), a known transcriptional activator, suggesting that HATs link lysine acetylation to gene activation.[11] Likewise, *HDAC1* was homologous to yeast gene reduced potassium dependency 3 (*RPD3*), a transcriptional repressor.[12] By providing a functional link between lysine acetylation and gene expression, the identification of HATs and HDACs appeared to support Allfrey's early hypothesis.

Lysine acetylation is by no means limited to the histone proteins in which it was first detected. In particular, many transcription factors are acetylated, with profound effects on transcriptional activation.[13] p53 was the first non-histone substrate of HATs to be identified;[14] acetylation of p53 by cAMP-response element binding protein (CREB)-binding protein (CREBBP or CBP) and p300 promotes sequence-specific DNA binding and enhances p53-dependent transcription.[15] Indeed, acetylation of transcription factors is usually[16] associated with enhanced DNA binding and transcription, and therefore seems to complement histone acetylation as an activating modification. A recent proteomics study

suggested that acetylation occurs on over 1750 proteins of both nuclear and cytoplasmic origin.[17] The targets of acetylation were often components of large macromolecular complexes, consistent with the importance of KAc in modulating protein-protein interactions. The study found acetylation sites in many proteins involved in nuclear processes, including histone modification and DNA repair, in which the importance of lysine acetylation had already been established. Additionally, the study suggested extensive acetylation of proteins involved in other nuclear processes, including RNA splicing, nuclear transport and cell cycle control, as well as cytoplasmic activities such as cytoskeleton remodelling and chaperone function. Thus, the acetylome, though somewhat smaller than the phosphoproteome, appears to regulate a similarly broad range of functions, and indeed significant cross-talk exists between these two PTMs.[13,17] Such studies appear to bear out the suggestion made by Kouzarides in 2000 that acetylation represents “a new biologically relevant regulatory modification to rival phosphorylation”.[18]

1.1.3 An acetyl-lysine recognition domain

Protein acetylation is carried out by ‘writer’ enzymes, HATs, and removed by ‘erasers’, HDACs, which play a role in gene expression by adding or removing acetyl groups. These parallel the kinase and phosphatase enzymes that phosphorylate and dephosphorylate proteins. But does the analogy between acetylation and phosphorylation extend further? Are there ‘reader’ protein domains that bind specifically to KAc, analogous to the recognition domains for phospho-serine, phospho-threonine and phospho-tyrosine? And could these domains, by interacting with KAc on histone tails, offer a second mechanism through which lysine acetylation affects transcription? Given the known sequence specificity of HATs,

it seemed unlikely that charge neutralisation was the only means by which lysine acetylation affected transcription.

An KAc-binding domain, the bromodomain, was first identified in 1999 by Ming-Ming Zhou and colleagues, who demonstrated an acetylation-dependent interaction between the bromodomain of the nuclear HAT p300-CREBBP associated factor (PCAF) and a histone peptide. They also solved the solution structure of this bromodomain, revealing a left-handed bundle of four alpha helices, with the proposed KAc binding site in a hydrophobic cleft at the end of the bundle.[19] A second solution structure of a HAT bromodomain, this time from GCN5, displayed the same overall fold.[20] Two bromodomain X-ray crystal structures were obtained in 2000: Jacobson *et al.* solved the structure of the two adjacent bromodomains of transcription initiation factor $TF_{II}D$ subunit 1 (TAF1, also known as $TAF_{II}250$), a subunit of the general transcription factor II D ($TF_{II}D$), and demonstrated that they simultaneously recognised two acetylated lysines on multiply-acetylated H4 tails.[21] Owen *et al.* obtained the first peptide-bound structure of a bromodomain, solving the structure of an acetylated H4 peptide bound to yeast GCN5 at 1.9 Å resolution. Significantly, this revealed the exact orientation of KAc in the bromodomain, and the interactions formed between the KAc and the binding pocket (Section 1.2.1).[22] In addition to demonstrating that the bromodomain could bind to KAc, these studies showed that the interaction was strongly dependent on the amino acids flanking KAc. This suggested that, like SH2 and PTB domains for phosphotyrosine, the bromodomain could recognise a PTM in a sequence-dependent manner, and that sequence differences around the binding site would dictate the bromodomain's interacting partners.[23]

The remainder of this chapter will discuss the structure and function of the bromodomain, and the state of current medicinal chemistry efforts to develop bromodomain ligands, but I would like first to place lysine acetylation in the context of two key concepts with which it is often associated: the 'histone code hypothesis', and epigenetics.

1.1.4 A histone code?

The emergence of distinct 'writer', 'eraser' and 'reader' domains for lysine acetylation, along with cellular and biochemical data suggesting specific functions for certain patterns of histone modifications, led Strahl and Allis in 2000 to suggest the existence of a 'histone code', whereby "multiple histone modifications, acting in a combinatorial or sequential fashion on one or multiple histone tails, specify unique downstream functions".[24] At the time, only a handful of reader, writer and eraser domains had been identified, but the subsequent discovery of lysine demethylases,[25] and methyl-lysine and methyl-arginine reader domains[26] lent credence to the idea of a dynamic code with a regulatory function in gene transcription. The number of histone modifications has also expanded greatly, with both new types and new sites of modification identified.[27] A summary of some of these modifications is presented in Figure 1.[28]

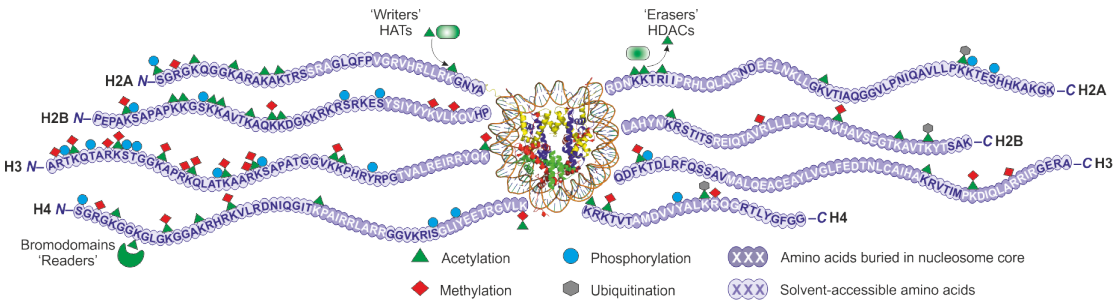


Figure 1. Human histone N-terminal tails and associated PTMs. The N-terminal tails of histones are extensively post-translationally modified in both solvent-exposed and buried regions. Lysine acetylation is one of the most extensively-studied modifications. It is dynamic, being established ('written') by histone acetyltrasferases (HATs) and removed ('erased') by histone

deacetylases (HDACs). Sequence-selective recognition ('reading') is performed by the bromodomain. Reproduced with permission from Jennings, L. E.; Measures, A. R.; Wilson, B. G.; Conway, S. J. *Future Med Chem* **2014**, *6*, 179. Copyright 2014 Future Science Ltd.

The histone code hypothesis, although now a widely-used concept,[29-32] has not been without its critics. Part of this debate has been whether histone modifications can truly be said to constitute a 'code', in the sense of a semiotic system of signs, meanings and codes which link them. Bryan Turner identifies two key requirements for a semiotic system: that it must be arbitrary, and that there is no direct causal relationship between sign and meaning. This, he argues, is clearly true for the genetic code, but not for chromatin modifications. For example, as we have already noted, lysine acetylation increases the accessibility of DNA to the transcriptional machinery through charge neutralisation, and so the modification is directly involved in producing the outcome. Thus 'chromatin context' should be seen as separate from the histone code.[33] But perhaps more fundamentally, the histone code hypothesis implies that specific combinations of modifications *bring about* transcription or repression, rather than being *associated with* a transcriptional state. In some instances, it appears that histone modifications are a prerequisite for (or at least have a powerfully activating effect on) transcription. For example, transcription initiation factor $TF_{II}D$ subunit 3 (TAF3, also known as $TAF_{II}140$), a component of the general transcription factor $TF_{II}D$, contains a plant homeodomain (PHD) finger which binds to trimethylated H3K4; if the recruitment of TAF3 to H3K4Me3 is prevented (for example by mutations in the PHD finger), $TF_{II}D$ recruitment and subsequent transcription are suppressed.[34] On the other hand, certain modifications may constitute part of the mechanism downstream of canonical DNA-binding transcriptional activators or repressors. Histone-modifying enzymes, notably HATs and HDACs, are often

recruited by activators or repressors to specific genes, and are then able to create a more permissive or repressive chromatin environment.[35] Gene expression is thus regulated by transcription factors, and not by chromatin modification, which instead forms part of the transcriptional response. Perhaps, as was argued by Gardner, Allis and Strahl in a reflection on their histone code hypothesis in 2011, "the nature of the histone code may not necessarily be as clear-cut as histone PTMs functioning solely as a consequence of or as a prerequisite for recruitment of the canonical transcriptional machinery."[32] Both scenarios may be operating simultaneously, and perhaps interdependently, within the same genomic region, or even on the same histone tail.

Histone modification is frequently regarded as being an epigenetic process,[36] i.e. one that contributes to "mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence".[37] However, many histone modifications may not be heritable, even through mitosis. The NIH, in its "Roadmap Epigenetics Project", adopts a rather broader definition which encompasses more transient modifications: "epigenetics refers to both heritable changes in gene activity and expression (in the progeny of cells or of individuals) and also stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable."[38] The use of the term 'epigenetic' to refer to non-heritable modifications, in particular histone modifications, is not universally accepted. Mark Ptashne, in particular, has criticised this use of the term, as it is not clear that histone marks are self-perpetuating.[39] Furthermore, he suggests that it is misleading to regard gene expression as being regulated by chromatin modifications, since histone-modifying enzymes have no inherent specificity for one genomic region over another. Rather, he states, "genes are typically regulat-

ed, in the meaningful sense, by specific DNA binding proteins that recruit transcribing or repressing complexes – including nucleosome modifiers."[40] Nonetheless, it is useful to have a term to encompass all of the mechanisms that alter "transcriptional potential" (to use the NIH phrase) that are distinct from classical transcription factor activity alone, and 'epigenetics' has been widely adopted. Like the 'histone code' hypothesis, our current use of the term 'epigenetics' is at once inspirational and controversial.

1.2 Bromodomain structure and function

1.2.1 Bromodomain structure

The bromodomain was first reported by Tamkun *et al.* in 1992 as a structural motif present in the *Drosophila* protein brahma, and was named after this protein by analogy with the chromodomain.[41] This conserved sequence of around 60 amino acids showed significant homology with sequences from human and yeast genes with known roles in transcriptional activation,[41,42] and was predicted to form two alpha-helices, termed A and B.[42] Subsequent alignment and secondary structure prediction suggested a larger domain of around 110 amino acids with four alpha helices, the additional amino- and carboxy-terminal helices being termed Z and C, respectively.[43] These structure predictions were proved correct by the first solution and crystal structures, as discussed in Section 1.1.3.

The structure of the CREBBP bromodomain is illustrated in Figure 2 (PDB ID: 3P1C).[44] As with many bromodomains, it exists as part of a much larger, multi-domain protein (Figure 2A); CREBBP, for example, possesses several other protein interaction domains, as well a HAT domain. Figure 2B illustrates the characteristic four-helix bundle structure. Two loop regions are also present (shown in green, Figure 2B): the short BC loop, and the longer ZA loop, which

often contains short helical regions. These two loops are highly diverse in sequence and allow bromodomains to discriminate between KAc in different contexts.[44] KAc binds in a well-defined pocket at the end of the four-helix bundle. The pocket is largely hydrophobic in nature, but is lined by a network of water molecules that are observed in most bromodomain structures (Figure 2C). A direct hydrogen bond between the carbonyl oxygen and an asparagine residue at the C-terminal end of helix B (N1168 in CREBBP) anchors KAc in the binding site. This residue is highly conserved, present in 48 out the 61 human bromodomains identified by Filippakopoulos *et al.* in their extensive study of bromodomain structure. In 12 bromodomains, it is replaced by other hydrogen-bond donors such as threonine or tyrosine, with KAc-binding ability demonstrably maintained in some cases.[44] One isolated example (MLL) exists of an aspartate at this position, suggesting that the domain may not bind KAc. A second hydrogen bond is formed between the carbonyl group and an even more highly-conserved tyrosine residue in the ZA loop (58/61 human bromodomains; Y1125 in CREBBP), *via* a structured water molecule (Water 1, Figure 2C). The KAc N–H interacts with the protein backbone *via* another structured water molecule (Water 5, Figure 2C), which is located further away from the base of the pocket. A second, hydrophobic, binding mode is observed in some bromodomains, including the Bromodomain and Extra Terminal domain (BET) family, and allows the simultaneous recognition of two KAc residues on one peptide by a single bromodomain (Section 1.2.3, Figure 4). A third binding mode (not shown) has been suggested by a molecular dynamics study, in which the acetamide N–H interacts with a conserved proline carbonyl group in the ZA loop (P1110 in CREBBP, Figure 2C).[45] However no experimental evidence yet exists for this interaction.

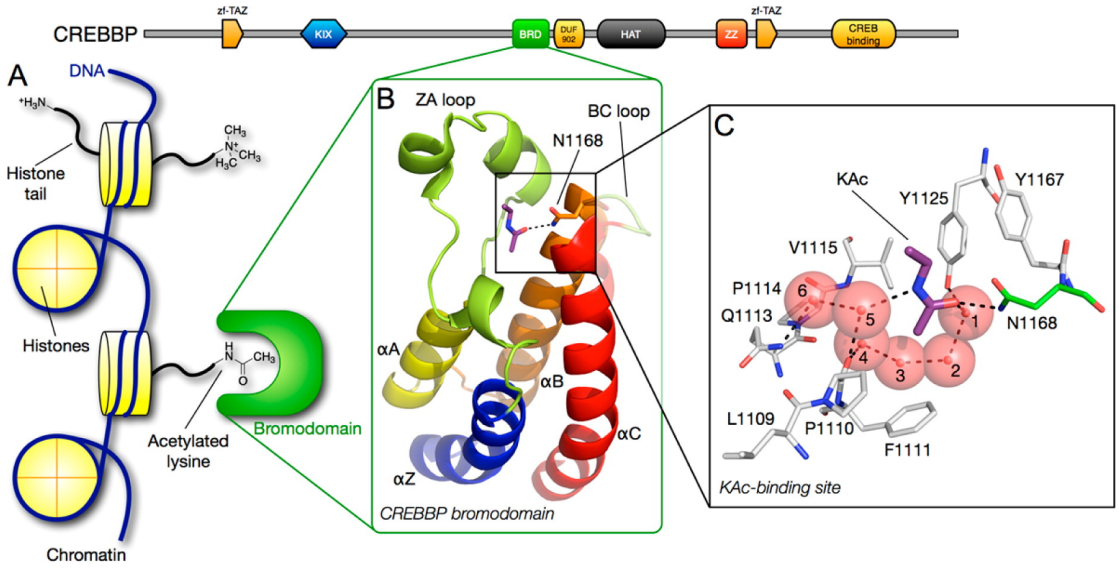


Figure 2. Bromodomains bind to acetylated lysine residues. (A) Histone tails are subjected to multiple PTMs, including lysine acetylation. Lysine acetylation state is "read" by bromodomains, protein modules that invariably exist as part of a more complex protein architecture. The example shown is CREBBP. (B) X-ray crystal structure of the CREBBP bromodomain in complex with KAc (carbon in purple, PDB code 3P1C).[44] The bromodomain fold comprises four helices Z (blue), A (yellow), B (orange), and C (red) and two loop regions known as the ZA loop and the BC loop (light green). (C) The KAc residue binds in a well-defined pocket and, in CREBBP, forms interactions with N1168 and a second interaction with Y1125, via structured water molecule 1 (red sphere). The pocket is substantially hydrophobic, but four structured water molecules (red spheres) form its base (waters 1–4). Many bromodomains have been crystallised with one or two additional water molecules bound in the ZA channel. In the CREBBP above, water molecules 5 and 6 are observed in the ZA channel. Water 5 binds to the NH of the acetylated lysine residue, P1110, and water 6. Water 6 binds to Q1113 and water 5. Reprinted with permission from Hewings, D. S.; Rooney, T. P. C.; Jennings, L. E.; Hay, D. A.; Schofield, C. J.; Brennan, P. E.; Knapp, S.; Conway, S. J. *J. Med. Chem.* **2012**, *55*, 9393. Copyright 2012 American Chemical Society.

1.2.2 Classification of the bromodomains

The human genome contains 61 distinct bromodomains in 46 proteins.[44,46] Most proteins contain only one bromodomain, though ten contain two, and one (polybromo-1 or PB1) contains five. Bromodomain-containing proteins (BCPs) are primarily transcriptional co-regulators or histone-modifying enzymes, and include HATs, ATP-dependent chromatin remodelling complexes, helicases, methyltransferases, transcriptional coregulators and nuclear scaffolding proteins.[47] The bromodomains have been classified into eight families on the basis of three-dimensional structure-based alignment (Figure 3).[44] The precise biologi-

cal roles of many of the proteins are poorly understood, and the role of the bromodomain in protein function frequently less so.

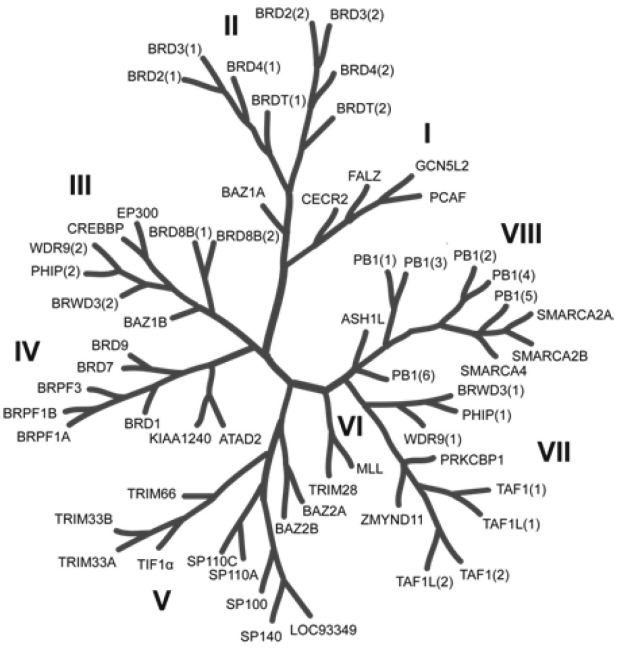


Figure 3. Phylogenetic tree of the human bromodomain family constructed on a structure-based alignment.[44] The eight bromodomain families are highlighted with Roman numerals. Reprinted with permission from Hewings, D. S.; Rooney, T. P. C.; Jennings, L. E.; Hay, D. A.; Schofield, C. J.; Brennan, P. E.; Knapp, S.; Conway, S. J. *J. Med. Chem.* **2012**, *55*, 9393. Copyright 2012 American Chemical Society.

1.2.3 BET bromodomains – function

Arguably the best-characterised family of BCPs are the BET proteins bromodomain containing 2, 3, 4 and testis-specific (BRD2, BRD3, BRD4 and BRDT; Family II, Figure 3). Each contains two N-terminal bromodomains and a long C-terminal region containing the extra terminal (ET) protein-protein interaction domain. The first bromodomains are more closely related to each other than to the second bromodomain in the same protein, as can be seen from Figure 3. BET proteins associate with hyperacetylated histone tails, particularly histone H4, and possibly to a lesser extent H3.[44,48-52] The N-terminal bromodomains can recognise two adjacent acetylation marks simultaneously on the same histone tail, one binding as a 'canonical' KAc, the other interacting primarily through hydro-

phobic contacts (Figure 4).[44,52] The interactions of the N-terminal bromodomains with tetra-acetylated histone H4 (H4K5AcK8AcK12AcK16Ac) are some of the strongest bromodomain-peptide interactions reported, with dissociation constants in the low micromolar range.[44] Interactions between acetylated histone peptides and the second bromodomain are somewhat weaker.

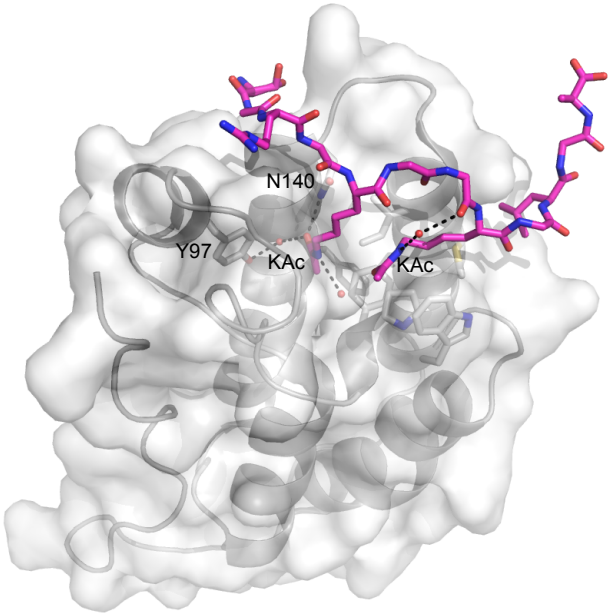


Figure 4. H4K5AcK8Ac bound to BRD4(1). K5Ac binds at the base of the pocket, forming a hydrogen bond to N140, while K8Ac forms only hydrophobic contacts with the protein (PDB ID: 3UVW).[44]

BRD2 was the first BET protein to be studied in human cells. Originally named RING3, it was identified as a nuclear kinase,[53,54] with a role in cell-cycle control through transactivation of E2F transcription factor-responsive genes.[55] Only later was it shown to bind to acetylated chromatin,[48,56] where it interacts with transcription factors (including E2F),[57] HATs,[58] chromatin remodelling proteins[57] and essential components of the transcriptional machinery such as the Mediator complex.[56] Furthermore, BRD2 allows RNA polymerase II (Pol II) to transcribe through acetylated histones which otherwise hinder its passage.[50] Homozygous *BRD2* mutation is embryonic lethal in mice.[59,60] However, engineered mice with

low levels of BRD2 expression show no developmental defects but are obese and overproduce insulin. Surprisingly, they do not develop insulin resistance and have normal blood glucose levels, and have therefore been suggested as models for obese metabolically-healthy humans.[61] Other reports have suggested a role for BRD2 in haematopoiesis: forced overexpression of BRD2 in transgenic mice leads to B-cell lymphomas and leukaemias, in part through upregulation of cyclin A.[62,63]

BRD4 is also a transcriptional co-activator, recruiting general transcription factors,[64] notably the positive transcription elongation factor (P-TEFb), to acetylated chromatin.[65-68] BRD4 binds to the regulatory subunit of P-TEFb, cyclin T, *via* an alpha-helical C-terminal region referred to as the P-TEFb interaction domain (PID).[69] This promotes the phosphorylation of the C-terminal domain (CTD) of the Pol II subunit retinol binding protein 1 (RBP1) on serine 2 by the catalytic P-TEFb subunit cyclin-dependent kinase 9 (CDK9), releasing the polymerase from promoter-proximal pausing and stimulating transcriptional elongation.[65,66] The BRD4-dependent switch from initiation to elongation plays a particularly important role in the induction of primary response genes (i.e. genes that do not require *de novo* protein synthesis for their expression) following stimulation.[70,71] Intriguingly, both the C-terminal PID and the second bromodomain appear to bind to P-TEFb, the latter by interacting with KAc on cyclin T.[69,72] Given the weaker affinity of the second bromodomain for histones, it is possible that the first bromodomain is primarily responsible for interactions with histones, while the second interacts significantly with non-histone substrates. BRD4 may also activate Pol II-dependent transcription independently of P-TEFb. This may occur through the recruitment of other effectors such as the histone methyltransferase NSD3 or the

histone demethylase JMJD6, *via* the ET domain of BRD4.[73] Additionally, the bromodomain-containing region of BRD4 has been reported to poses intrinsic kinase activity and is able to phosphorylate serine 2 of the Pol II CTD, though the relative importance of direct *versus* P-TEFb-mediated phosphorylation of Pol II by BRD4 *in vivo* is unclear.[74]

As suggested above, the bromodomains of BRD4 may form functional associations with acetylated lysines on non-histone proteins. In addition to the aforementioned interaction with cyclin T, it has emerged that the bromodomains interact with acetylated RELA, a component of the NF-κB transcription factor that controls the expression of many pro-inflammatory genes. Acetylation of RELA recruits BRD4 to the promoters of NF-κB-dependent genes where it acts as a co-activator by recruiting P-TEFb and activating Pol II.[75]

Unlike most chromatin-associated proteins, BRD4 remains bound to chromatin during mitosis,[76,77] and is therefore proposed to act as a 'mitotic bookmark' for actively-transcribed genes.[78,79] After the near-shutdown of transcription during mitosis, transcription must be activated on entry into G_1 in order to maintain cell lineage and continued cell-cycle progression.[80,81] BRD4 appears to play a key role in accelerating transcription initiation after mitosis at the genes where it has remained bound, by recruiting P-TEFb and promoting chromatin decompaction after mitosis.[82] Interestingly, BRD2 also binds to mitotic chromosomes, but the functional significance of the interaction remains unclear, as BRD2 lacks the PID necessary for P-TEFb recruitment.[83]

Like *BRD2*, *BRD4* is an essential gene: *BRD4*$^{-/-}$ mouse embryos die shortly after implantation, while the *BRD4*$^{+/-}$ genotype results in severe developmental defects.[84] This suggests that there is insufficient functional redundancy

between the BET proteins to rescue null phenotypes, pointing to distinct, largely non-overlapping roles for the BETs in development.

BRD3 is much less studied than BRD2 or BRD4. It binds to acetylated nucleosomes, and may play a similar role to BRD2 in coupling histone acetylation to transcription.[50] It also binds to erythroid transcription factor (GATA1) in an acetylation-dependent manner, which appears to be critical for erythroid maturation.[85,86]

While BRD2, BRD3 and BRD4 are ubiquitously expressed, BRDT expression is limited to the testes.[87] It is essential for spermatogenesis, as deletion of the first bromodomain in mice leads to abnormal spermatozoa and sterility.[88] During meiosis, BRDT co-regulates the expression of many key meiotic genes. Like BRD4, but not BRD2, BRDT possesses a C-terminal PID, so effects on transcription are likely to occur by recruitment of P-TEFb to acetylated promoter regions.[89] BRDT has also been shown to regulate pre-mRNA splicing during spermatogenesis.[90] BRDT is also required for the massive chromatin compaction in maturing spermatids which occurs after meiosis and culminates in the replacement of histones with protamines.[89,91,92] Consistent with its essential role in multiple stages of spermatogenesis, single-nucleotide polymorphisms (SNPs) in the *BRDT* gene have been associated with impaired fertility.[93] As a result of these findings, BRDT inhibition has been proposed for male contraception.[94]

1.2.4 BET bromodomains – roles in disease

BRD2 and more especially BRD4 have been implicated in various disease states, including cancer and viral infection. Below, I will highlight some of the known roles of BET proteins in disease. Small-molecule approaches to target

these proteins in disease, and their therapeutic potential, will be discussed below (Section 1.6).

In a rare but aggressive form of squamous cell carcinoma known as NUT midline carcinoma, *BRD4*, or more occasionally *BRD3*, are fused in frame to the nuclear protein in testis (*NUT*) gene of unknown function.[95] The resultant fusion is under the control of the *BRD4* promoter and encodes the two bromodomains and ET domain of BRD4, and almost the entire coding region of *NUT*, which includes a binding domain for the HATs CREBBP and p300.[96] As a result, NUT, which is normally expressed only in testes, becomes expressed in cells harbouring the rearrangement. Reynoird *et al.* have proposed an intriguing mechanism for the oncogenic effect of this translocation: the NUT region of the fusion recruits CREBBP/p300 to acetylated chromatin *via* the CREBBP/p300 TAZ2 domain, bringing about further acetylation, binding of BRD4-NUT and recruitment of CREBBP/p300. The resultant sequestration of CREBBP/p300 on chromatin prevents it from activating p53, suppressing the activity of this critical tumour suppressor.[96]

BET proteins, particularly BRD4, have recently been shown to play a critical role in many haematological and solid tumours, by acting as co-activators for the expression of pro-proliferative genes. Notably, BRD4 is required for the expression of *MYC*, an oncogenic driver in many cancers.[97,98] This has attracted considerable attention, as therapeutic inhibition of BRD4-histone interactions represents a novel strategy to target *MYC*-dependent cancers (Section 1.6.1).

BET proteins also have diverse roles in viral infection. Several viruses 'hijack' BET proteins to ensure they are transferred to daughter cells after mitosis.[83] Papillomaviruses, which include the cancer-associated human papillomavirus

(HPV), employ BRD4 to tether the viral genome (episome) to mitotic chromosomes. This association is mediated by the viral protein E2, which binds both to the C-terminal domain of BRD4 and the episome,[99] ensuring faithful segregation of the viral genome in latently-infected cells. E2 also regulates transcription of papillomavirus genes in a BRD4-dependent manner.[100,101] BRD4 plays similar roles in regulating transcription and genome maintenance in other viruses, including Kaposi's sarcoma-associated herpesvirus (KSHV)[102,103] and Epstein-Barr virus (EBV).[104,105] BRD4 also appears to negatively regulate transcription of human immunodeficiency virus (HIV) genes:[106] HIV transactivator of transcription (TAT) protein normally enhances transcription by recruiting P-TEFb, but the BRD4 PID antagonises this interaction by competing with TAT for P-TEFb binding.[69,107]

As we will see in Section 1.6, many of the roles of BET proteins in disease have only become apparent following the development of chemical probes to inhibit the bromodomain-KAc interaction. These discoveries have opened up new experimental approaches to the treatment of various diseases, testifying to the power of chemical probe development.

1.3 BET bromodomain inhibitors[i]

Below I describe current progress in the development of ligands for BET bromodomains, grouped by KAc mimic. The focus is on compounds published in the scientific literature; compounds disclosed in patents will be mentioned only when similar KAc-mimicking chemotypes have not been published. (Several excellent reviews covering compounds in the patent literature have recently been

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published.)[108-110] At the time of starting the work described in this dissertation, very few[111,112] BET bromodomain inhibitors had been described. However, I will present the state of the field as it currently stands, to provide context for discussion in later chapters.

1.3.1 3-Methyl-1,2,4-triazoles

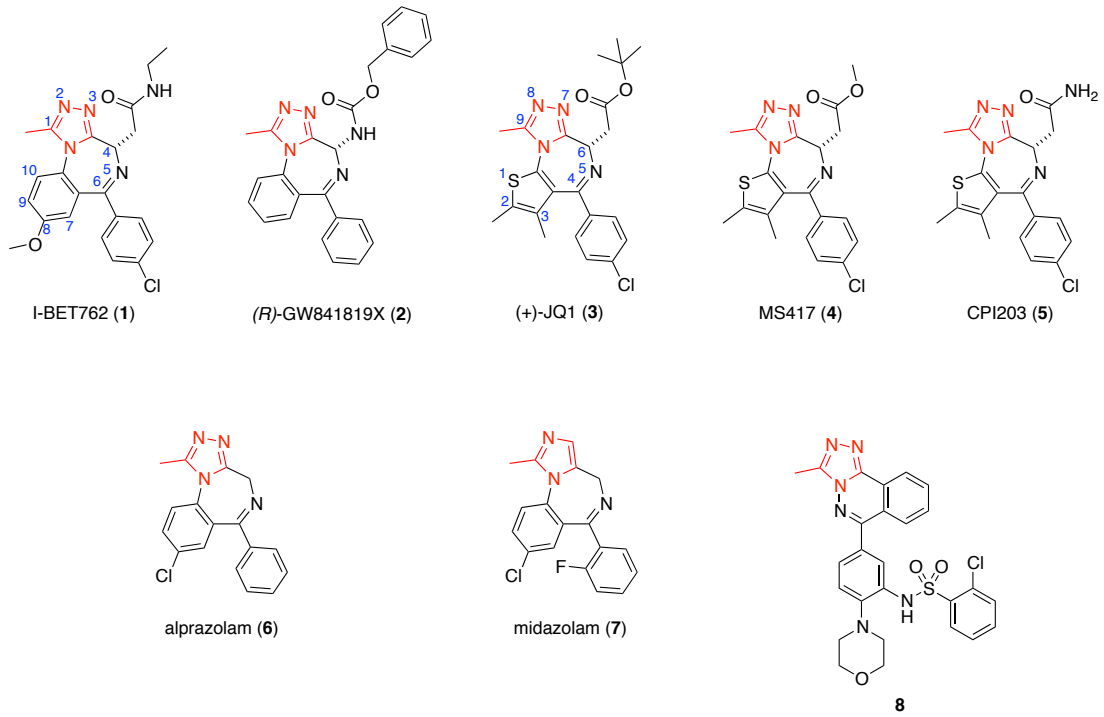


Figure 5. 3-Methyl-1,2,4-triazoles and related compounds discussed in the text. Numbering systems for triazolobenzodiazepines (such as I-BET762) and triazolothienodiazepines (such as (+)-JQ1) are indicated. The KAc mimic is shown in red.

The first potent and selective bromodomain ligands were methyltriazolodiazepines (Figure 5), published simultaneously in late 2010 by Filippakopoulos *et al.*[111] and Nicodeme et al.[112] The studies from GSK[112,113] that eventually led to the discovery of I-BET762 (**1**, Figure 5) were based on a phenotypic screening approach to discover small molecule up-regulators of apolipoprotein A1 (ApoA1). It was not initially known that these compounds functioned by binding to the BET bromodomains. ApoA1 up-regulation is involved in protection from atherosclero-

sis progression and with anti-inflammatory effects; however, when these studies commenced, no method existed to achieve this up-regulation in a therapeutic manner. A luciferase reporter-based screen was used to identify small-molecule enhancers of ApoA1 expression, which included the triazolobenzodiazepine GW841819X (**2**, Figure 5).[113] This compound has an EC_{50} of 440 nM for the induction of the ApoA1 reporter gene. A programme of medicinal chemistry was undertaken to optimise the potency of **2**, in which it was found that the benzodiazepine core was essential for activity, as was the aryl group extending from the 6-position. Additionally, replacing the carbamate of **2** with an acetamide improved compound stability.[114] These studies identified I-BET762 (**1**, Figure 5), which had a higher EC_{50} of 700 nM in the reporter gene assay but improved properties for *in vivo* experiments.[112,114]

It was noted that the C(4) stereochemistry of the molecule had a large influence on its potency, with only the (*S*)-enantiomer showing activity. The dependence of the compound's activity on stereochemistry was interpreted as evidence that its biological effects resulted from defined interactions with specific, but unknown, molecular targets. This observation highlights a significant challenge with phenotypic screens: although this approach is a powerful strategy for the identification of cell-permeant small molecules that effect the desired phenotype, the cellular target or targets that evoke the phenotype must be subsequently identified.[115,116] Since screening against a panel of known drug targets failed to identify a possible mode of action for I-BET762 (**1**), a chemoproteomic approach was employed to identify the target. An affinity matrix of the active compound was generated and exposed to a lysate of HepG2 cells. This technique identified the bromodomains of BRD2, BRD3, and BRD4, members of the BET family, as

potential cellular targets of I-BET762 (**1**); the proposed interaction was confirmed by *in vitro* isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR) experiments. Furthermore, I-BET762 (**1**) inhibited the binding of a tetra-acetylated histone H4 peptide ($H4_{1-18}$K5AcK8AcK12AK16Ac) to the tandem bromodomains of BRD2, BRD3, and BRD4 in a Förster resonance energy transfer (FRET) assay.[113] Similarly, I-BET762 (**1**) bound to the tandem bromodomains of BRD2–4 [K_d (BRD4) = 55 nM by ITC] and displaced a tetra-acetylated histone H4 peptide [IC_{50} (BRD4) = 36 nM by FRET] while showing no affinity for bromodomains outside the BET family.[112,113] Crystal structures of I-BET762 (**1**) with BRD2(1) and BRD4(1)/(2) in addition to **2** with BRD2(1) and BRD4(1) reveal essentially identical binding interactions (*vide infra*).

A second triazolodiazepine-based BET ligand, (+)-JQ1 (**3**, Figure 5), was independently developed by a collaboration between scientists at the Structural Genomics Consortium (SGC), Harvard Medical School and Dana-Farber Cancer Institute[111] based on compounds disclosed in a patent from Mitsubishi Tanabe Pharma Corporation.[117] A previous patent from this company indicates that related thienotriazolodiazepine compounds were identified from phenotypic studies investigating potential therapies for transplant rejection or autoimmune diseases.[118] The authors were encouraged by the synthetic availability of the lead compounds and the fact that they each contain a “privileged” structural core, found in drug molecules such as alprazolam and triazolam. Using docking studies based on the X-ray crystal structure of apo-BRD4(1), **3** was designed as a putative ligand. A *tert*-butyl ester group was attached at the C(6) position to reduce the likelihood of the compound interacting with other known benzodiazepine-binding proteins. Like I-BET762 (**1**), only a single enantiomer was active, provid-

ing a useful negative control for cellular studies. (+)-JQ1 (**3**) bound to BRD4(1) with a K_d of 49 nM (ITC) and showed excellent selectivity for the BET family of bromodomains. (+)-JQ1 (**3**) displaced a tetra-acetylated histone H4 peptide ($H4_{1-21}$K5AcK8AcK12AcK16Ac) from BRD4(1) with an IC_{50} of 77 nM, as determined by a bead-based amplified luminescent proximity homogeneous assay (AlphaScreen®).[119] Furthermore, (+)-JQ1 (**3**) inhibited the interaction of BRD4 with nuclear chromatin in human cells, as demonstrated by fluorescence recovery after photobleaching (FRAP) experiments, which measure the mobility of green fluorescent protein (GFP)-labelled BRD4.[111]

A very similar compound, MS417 (**4**, Figure 5) from Ming-Ming Zhou and colleagues, has recently appeared in the literature. This contains a methy ester in place of the *tert*-butyl ester of (+)-JQ1 (**3**), and binds to BRD4(1) and BRD4(2) with similar affinities to (+)-JQ1.[68] Constellation Pharmaceuticals have also disclosed a (+)-JQ1 analogue, CPI203 (**5**, Figure 5), which has a primary amide in place of the *tert*-butyl ester.[74] The compound has an IC_{50} of 22 nM (BRD4(1) AlphaScreen® assay), and shows IC_{50} values of < 100 nM in cellular assays for *MYC* downregulation and inhibition of interleukin-6 (IL-6) release after lipopolysaccharide (LPS) stimulation. It has been clamed that CPI203 (**5**) has improved bioavailability compared to (+)-JQ1, but no evidence has yet been published to substantiate this.[120]

Crystal structures of I-BET762 (**1**), GW8411819X (**2**, not shown), (+)-JQ1 (**3**), and MS417 (**4**, not shown) in complex with a variety of BET bromodomains have been solved, revealing excellent shape complementarity between ligand and the KAc-binding site (Figure 6A). Analysis of the X-ray crystal structures of (+)-JQ1 (**3**) and I-BET762 (**1**) in complex with BRD4(1) reveals that the ligand–

protein interactions are almost identical for these two molecules (Figure 6). The key interactions of (+)-JQ1 (**3**) and BRD4(1) are shown in Figure 6A-C. The 3-methyl-1,2,4-triazole of (+)-JQ1 (**3**) acts as a KAc mimic, with the methyl group occupying the hydrophobic pocket that recognises the methyl group of KAc. N(2) forms a water-mediated hydrogen bond to a conserved tyrosine residue [Y97 in BRD4(1)], and N(1) accepts a hydrogen bond from the conserved asparagine NH [N140 in BRD4(1)], analogous to the interaction between the carbonyl group of KAc and N140 (Figure 6B). GW8411819X (**2**) additionally donates a hydrogen bond to the carbonyl oxygen atom of N140, while I-BET762 (**3**) forms a water-mediated interaction to the same residue (Figure 6D). A similarly-positioned water molecule appears to bridge the carbonyl groups of the ester group of (+)-JQ1 (**3**) and the asparagine residue, though with somewhat longer interatomic distances than for I-BET762 (**1**). The diazepine is held in a hydrophobic clamp between I146 on one side and L92 and L94 on the other. The isoleucine residue in the first (N-terminal) BET bromodomains, or valine in the second, has been termed the 'gatekeeper' residue, as in non-BET bromodomains a larger residue is often found at this position (e.g. a tyrosine in PCAF or GCN5). This restricts the size of the pocket entrance and is thus thought to contribute to the selectivity of compounds for the BET bromodomains.

The 4-chlorophenyl moieties of I-BET762 (**1**) and (+)-JQ1 (**3**) occupy a hydrophobic part of the peptide-binding groove termed the WPF shelf,[112,113] formed by residues in the BC loop and the ZA loop at the end of helix Z. The WPF motif found at the entrance to this region [W97, P98, F99 in BRD4(1)] is conserved in the BET family of bromodomains, and occupancy of this site is believed to contribute to the selectivity of the above compounds for BET bromodomains.[113] The

fused dimethylthiophene of (+)-JQ1 (**3**) and the methoxybenzo moiety of I-BET762 (**1**) are directed into the pocket formed in part by the ZA loop, which is termed the ZA channel.

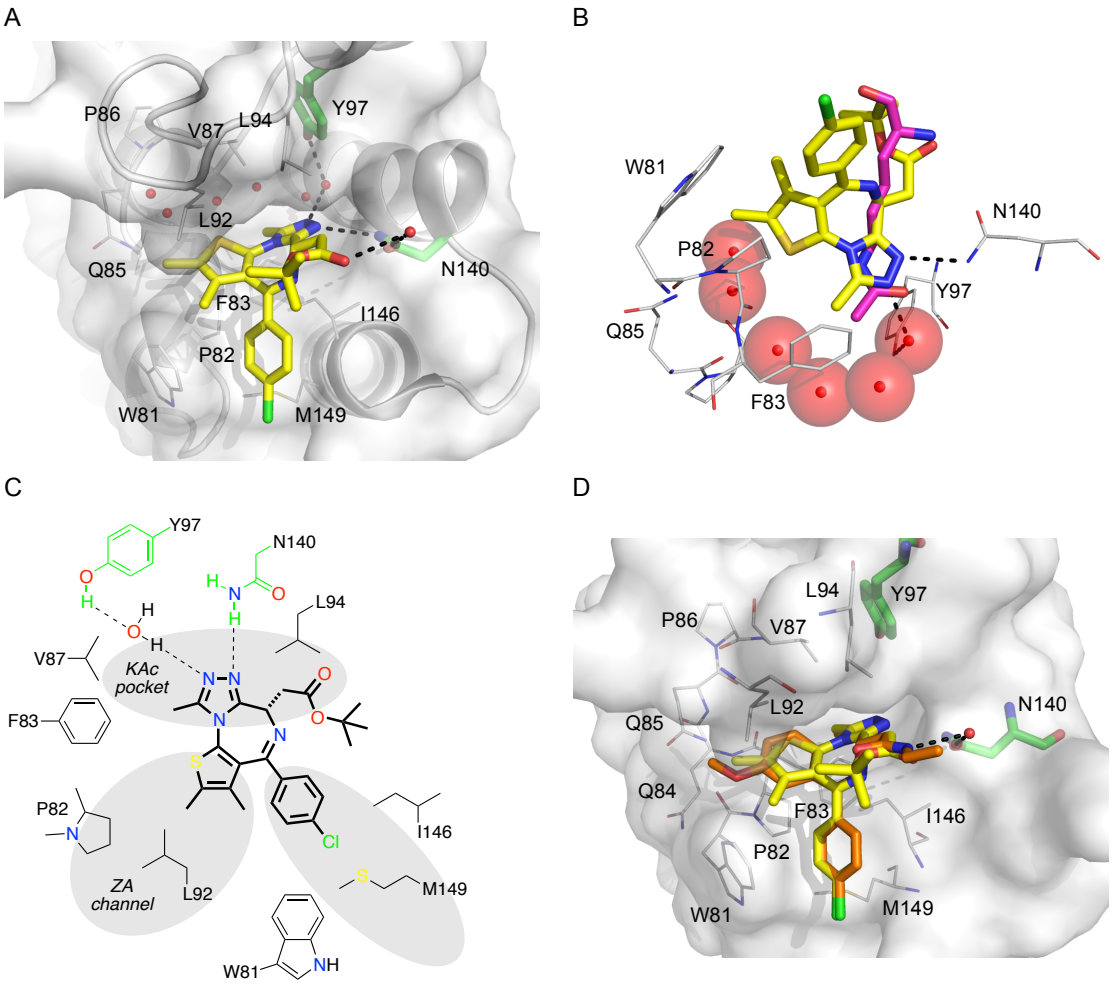


Figure 6. Triazolodiazepines bind to BET bromodomains and mimic KAc. (A) X-ray crystal structure of (+)-JQ1 (**3**) bound to human BRD4(1) (carbon in yellow, PDB ID: 3MXF),[111] showing the excellent shape complementarity between (+)-JQ1 and the KAc-binding pocket. Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (B) The 3-methyl-1,2,4-triazole of (+)-JQ1 acts as an effective KAc mimic. N(2) is positioned to form a water-mediated hydrogen bond to Y97, and N(1) accepts a hydrogen bond from N140. The structure is overlaid with KAc from a histone peptide ($H4_{1-12}$KAc5KAc8, carbon in purple, PDB ID: 3UVW).[44] (C) Schematic representation of the binding interactions between (+)-JQ1 and BRD4(1). (D) Overlay of an X-ray crystal structure of (+)-JQ1 (carbon in yellow, PDB ID: 3MXF) and I-BET762 (**1**, carbon in orange, PDB ID: 3P5O)[121] bound to human BRD4(1) bound to human BRD4(1) (PDB code: 3P5O). (+)-JQ1 and I-BET762 form similar interactions with the bromodomain, but I-BET762 forms a water-mediated hydrogen bond between its amide NH and the carbonyl oxygen atom of N140 (shown). Structures were aligned using the "cealign" command in PyMOL.

A further study by Filippakopoulos *et al*. has provided additional insight into the structure–activity relationships (SARs) in this important class of BET bromodomain inhibitors and has demonstrated that a number of clinically approved benzodiazepines (Figure 5) bind to BET bromodomains with low micromolar affinities.[122] Notably, the widely prescribed anxiolytic alprazolam (**6**) bound to BRD4(1) with a K_d of 2.5 μM [cf. 0.05 μM for (+)-JQ1 (**3**)] (Figure 7A, B); however, the authors note that this affinity means that it is unlikely that alprazolam has any effect on bromodomains at clinically relevant doses.[122] Unlike (+)-JQ1 (**3**) and I-BET762 (**1**), these fused diazepines are achiral, as they are not substituted at the 4-position of the diazepine ring (numbering as for I-BET762, Figure 5), simplifying their synthesis. The sedative midazolam (**7**, Figure 7C) contains an imidazole rather than a triazole ring and therefore cannot form a hydrogen bond to the KAc-binding asparagine residue (Figure 7D). Nonetheless, midazolam still interacts with the BET bromodomains, albeit more weakly than the triazoles. Crystallographic evidence shows that despite this compound binding further out of the KAc-binding pocket, two of the four water molecules found in most apo and ligand-bound BET bromodomain structures are not observed (Figure 7D). Although an imidazole ring is tolerated, a methyl substituent in the KAc-binding site is required for binding affinity, as replacement of the methyl group with H, Et, Ph, NH_2, or carbonyl resulted in a loss of BET bromodomain affinity.[122] This observation is consistent with the work of Vollmuth and Geyer, who showed that BRD2(1) and BRD4(1) bind *N*-propionyl-lysine but to a lesser extent *N*-butyryl-lysine.[123] Interestingly, these larger lysine modifications did not perturb or displace the KAc-binding pocket water molecules.

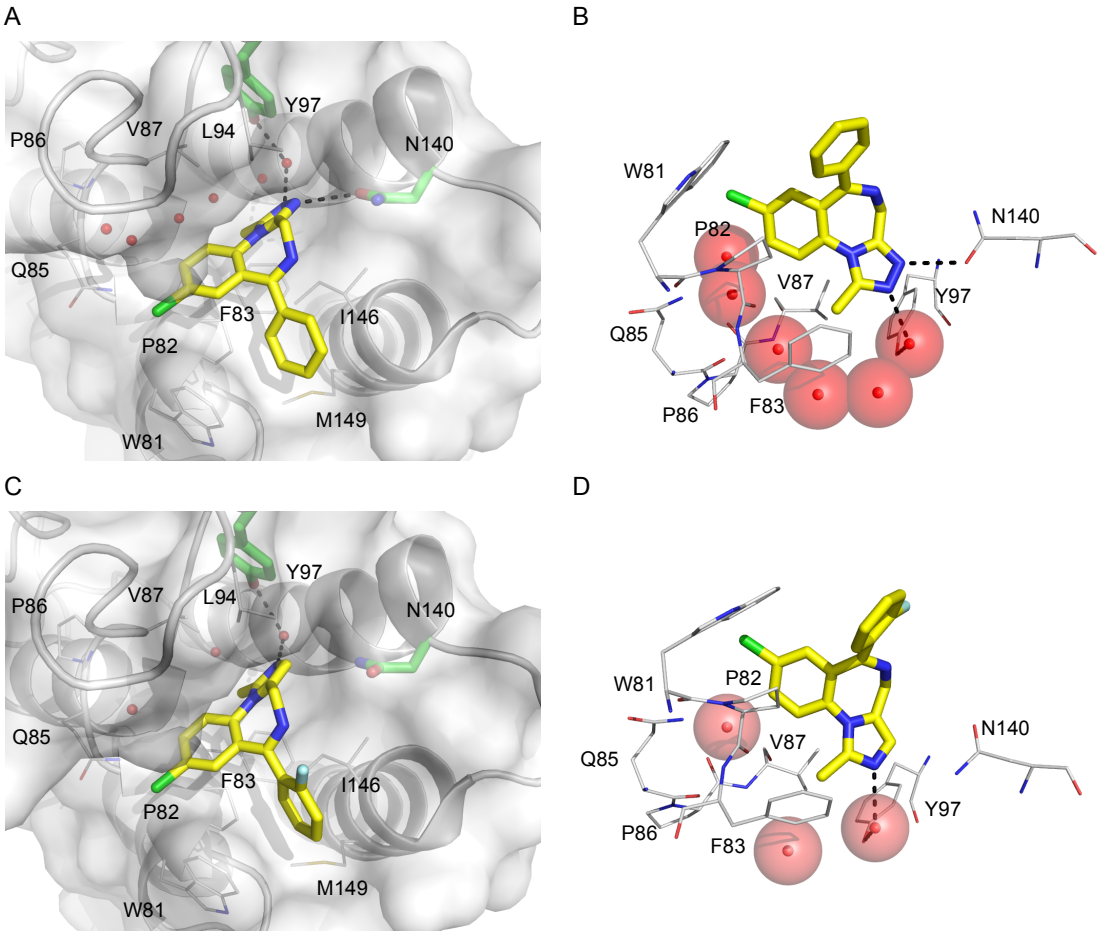


Figure 7. The diazepines alprazolam and midazolam can interact with BET bromodomains.[122] (A) X-ray crystal structure of alprazolam (**6**) in complex with BRD4(1) (PDB ID: 3U5J). Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (B) The triazole moiety of alprazolam resides in the KAc-binding pocket and forms a hydrogen bond with N140 and a water-mediated hydrogen bond with Y97. (C) X-ray crystal structure of midazolam (**7**) in complex with BRD4(1) (PDB ID: 3U5K). Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (C) Midazolam interacts with Y97 *via* a water molecule, but cannot interact with N140 as it lacks an appropriately-positioned heteroatom. Several water molecules normally present at the base of the KAc binding pocket are absent in the crystal structure.

The SGC have recently reported a distinct class of triazoles, the [1,2,4]triazolo[4,3-*a*]phthalazines, which bind to multiple bromodomain classes including the BET bromodomains. For example, compound **8** displaced a tetraacetylated H4 peptide from BRD4(1) with an IC_{50} of 160 nM (by AlphaScreen®), but also showed sub-micromolar IC_{50} values against the BRD9 and CREBBP bromodomains.[124] An X-ray crystal structure of **8** with BRD4(1) showed the methyl-triazole acts as a KAc mimic (Figure 8). The phthalazine core forms

similar hydrophobic interactions in the vicinity of the I146 'gatekeeper' residue to the diazepine ring of compounds such as (+)-JQ1 (**3**), while the 2-chlorophenyl sulfonamide occupies the WPF shelf. The sulfonamide appears to be deprotonated, and forms a previously unreported hydrogen bond with W81, which is flipped in comparison to other ligand-bound and apo structures. The morpholine is solvent-exposed (Figure 8). Structurally-related bicyclic triazolopyridazines had previously been disclosed by Constellation Pharmaceuticals.[125]

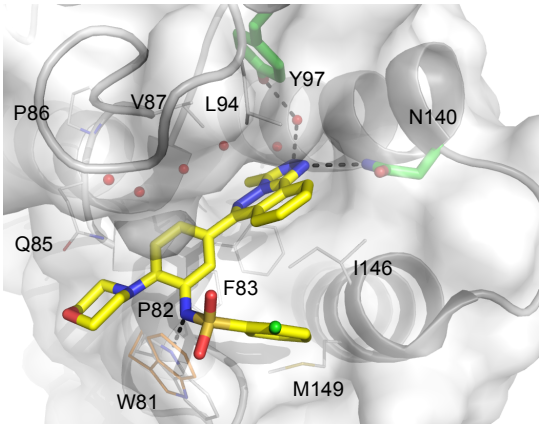


Figure 8. Triazolophthalazines bind to BET bromodomains. X-ray crystal structure of **8** in complex with BRD4(1) (PDB ID: 3NQM).[124] Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. Note that W81 flips from its normal position in order to form a hydrogen bond to the sulfonamide; the position of this residue in the (+)-JQ1-BRD4(1) complex (PDB ID: 3MXF) is shown in orange.

1.3.2 3-Methylisoxazoles

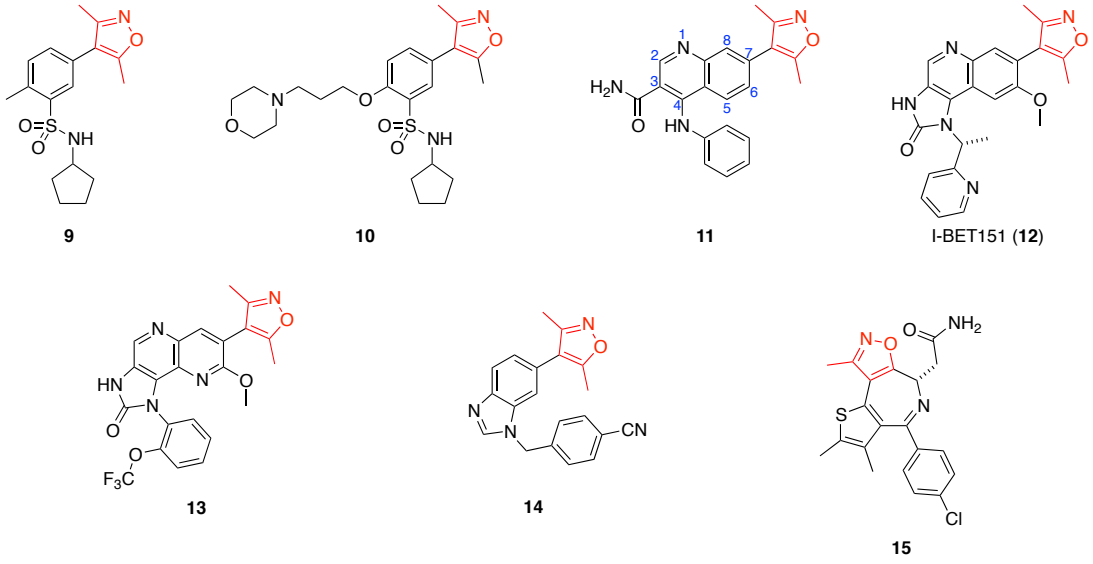


Figure 9. 3-Methylisoxazoles discussed in the text. The KAc mimic is shown in red.

3,5-Dimethylisoxazoles were the second class of KAc-mimicking BET bromodomain ligands to be reported (Figure 9). Their initial discovery[126] and subsequent optimisation[127] form the basis of the work described in Chapter 2, and so will not be discussed further here. The same KAc mimic was independently identified by a fragment screen of potential KAc mimics against bromodomains by workers at GSK.[128] 4-Phenyl-3,5-dimethylisoxazole (not shown) was observed to inhibit the interaction of an I-BET762-based fluorogenic ligand with bromodomains of BRD2, BRD3, and BRD4 in a fluorescence anisotropy (FA) assay. An X-ray crystal structure of the 3,5-dimethylisoxazole in complex with BRD2(1) confirmed this interaction. Synthesis of the 5-ethyl-3-methyl-4-phenylisoxazole and crystallisation with BRD2(1) provided strong evidence that the isoxazole bound with the nitrogen atom forming a water-mediated hydrogen bond to a conserved tyrosine residue [Y113 in BRD2(1), equivalent to Y97 in BRD4(1)] while the oxygen atom forms a direct hydrogen bond to the NH_2 of the asparagine amide [N156 in BRD2(1)].

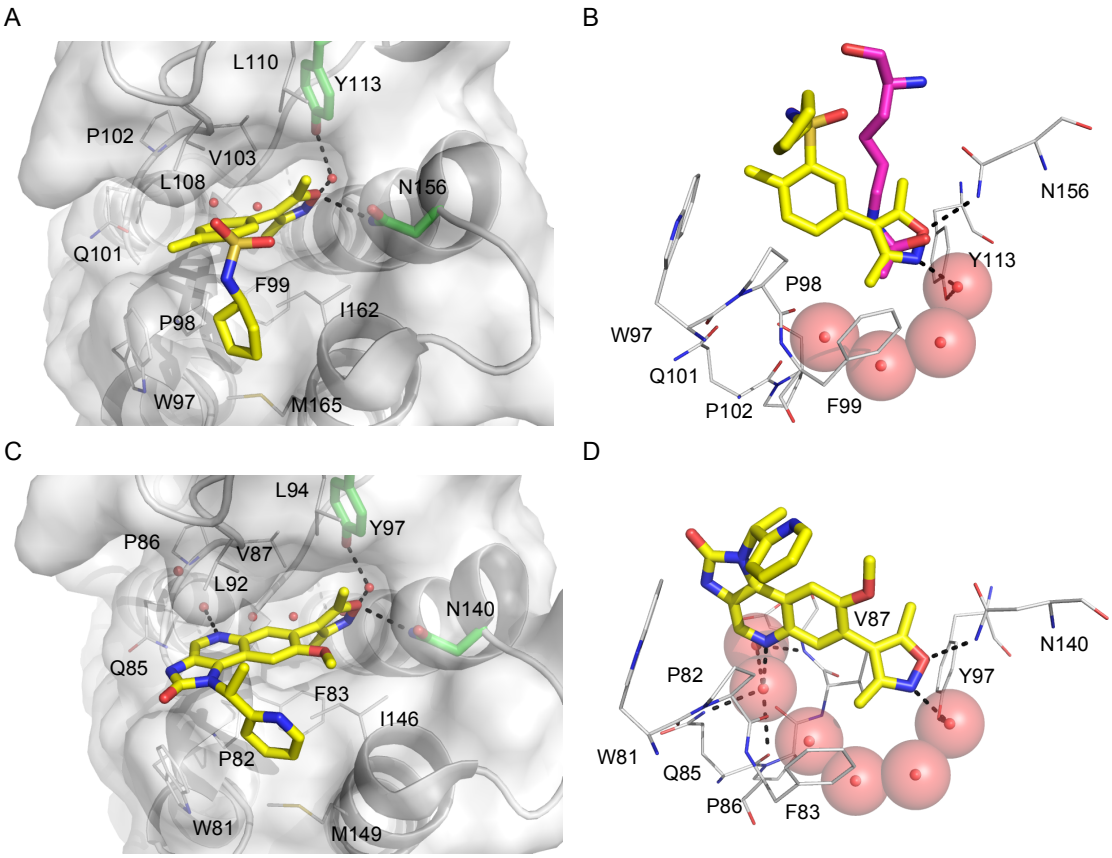
To increase the affinity of the fragment hit, Bamborough *et al*. constructed a 3D pharmacophore model encapsulating the crystallographically-observed hydrogen-bonding and hydrophobic interactions. A search of commercially available compounds using the criteria of this pharmacophore identified a series of 4-phenyl-3,5-dimethylisoxazoles with a sulfonamide appended to the phenyl ring *meta* to the isoxazole. The *meta* substituents are directed toward the WPF shelf, while the sulfonamide moiety itself forms a number of water-mediated hydrogen-bonding interactions. Exploration of the sulfonamide substituent SAR revealed that small, hydrophobic substituents displayed the highest affinity, consistent with the hydrophobic nature of the WPF shelf (Figure 10A, B). The cyclopentyl-

containing compound **9** displayed the greatest activity [IC_{50} 0.5 μM against the tandem bromodomains of BRD4 in a time-resolved FRET (TR-FRET) assay] and showed selectivity for the BET bromodomains in a thermal shift (ΔT_m) binding assay. Furthermore, **9** was active in a cellular assay, inhibiting the release of the proinflammatory cytokine IL-6 from LPS-stimulated peripheral blood mononuclear cells (PBMCs), with an IC_{50} of 3.1 μM. In addition, the compound reduced tumour necrosis factor α (TNFα) release from LPS-stimulated PBMCs. However, the series suffered from limited aqueous solubility; therefore, analogues possessing solubilizing groups attached to the phenyl ring *para* to the isoxazole moiety were investigated. This modification was well tolerated, with compound **10** showing similar BRD4 affinity and cellular activity to compound **9**. Aqueous solubility was increased 1000-fold, albeit at the expense of ligand efficiency.

A distinct 3,5-dimethylisoxazole-containing series, the 7-isoxazolo-quinolines, was also identified by GSK. Like the triazolobenzodiazepine series that gave rise to I-BET762 (**1**), this series arose from a phenotypic high-throughput screening (HTS) campaign, which identified **11** (Figure 9) as a potent up-regulator of ApoA1.[129] SAR studies revealed that a variety of hydrophobic substituents on the amine at position 4 were well tolerated. Cyclisation of the adjacent carboxamide onto this amine to form the urea was also tolerated, but the compounds displayed undesirable cytochrome P450 (CYP) inhibition. Appropriate choice of amine substituent and the addition of a methoxy substituent at position 6 abolished the CYP liability of these ureas. The resultant compound **12** (Figure 9), referred to as I-BET151, showed similar activity to I-BET762 (**1**) in ligand displacement, binding, and cellular assays (inhibition of cytokine release) but with markedly improved pharmacokinetic properties over both I-BET762 (**1**)

and (+)-JQ1 (**3**).[121,130] An X-ray crystal structure of I-BET151 (**12**) with BRD4(1) showed the isoxazole acting as a KAc mimic, while the 2-pyridyl moiety is directed into the WPF shelf. The quinoline nitrogen forms a hydrogen bond to one of the structural water molecules present in the ZA channel (Figure 10C, D).

In order to improve solubility of the I-BET151 series, Mirguet *et al.* explored the effect of an additional nitrogen in the quinoline core to give naphthyridines.[131] 1,5-Naphthyridines, but not the 1,6- or 1,8-regioisomers were tolerated. Compound **13** showed comparable pharmacokinetics and *in vivo* anti-inflammatory pharmacology to I-BET151. A crystal structure of **13** with BRD4(1) revealed similar binding interactions to I-BET151, although hydrophobic contacts in the WPF shelf were formed by the 2-trifluoromethoxy substituent on the pendant aryl ring, rather than by the aryl ring itself (Figure 10E, F).



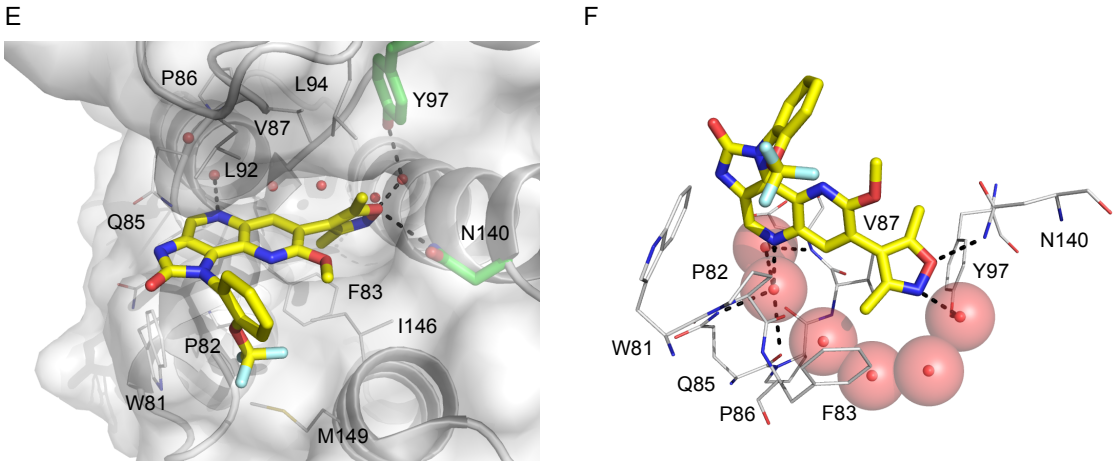


Figure 10. 3,5-Dimethylisoxaxoles bind to BET bromodomains. (A) X-ray crystal structure of **9** in complex with BRD2(1) (carbon in yellow, PDB ID: 4A9M).[128] Conserved Y113 and N156 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (B) The 3,5-dimethylisoxazole moiety of **9** resides in the KAc-binding pocket and forms a hydrogen bond with N156 and a water-mediated hydrogen bond with Y113. The structure is overlaid with KAc from a histone peptide bound to BRD2(1) ($H4_{1-15}$K12Ac, carbon in purple, PDB ID: 2DVQ).[44] (C) X-ray crystal structure of I-BET151 (**12**) in complex with BRD4(1) (PDB ID: 3ZYU).[121] Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (D) The 3,5-dimethylisoxazole moiety of I-BET151 (**12**) resides in the KAc-binding pocket and forms a hydrogen bond with N140 and a water-mediated hydrogen bond with Y97. The quinoline nitrogen forms water-mediated interactions with residues in the ZA channel. (E) X-ray crystal structure of **13** in complex with BRD4(1) (PDB ID: 4BW4).[131] Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (F) The 3,5-dimethylisoxazole moiety of **13** resides in the KAc-binding pocket and forms a hydrogen bond with N140 and a water-mediated hydrogen bond with Y97. The quinoline nitrogen forms water-mediated interactions with residues in the ZA channel.

Hay *et al.*, following on from our first report of 4-phenyl-3,5-dimethylisoxazoles as BET ligands (as described in Chapter 2),[126] synthesised a range of 5- and 6-isoxazolylbenzimidazoles. The 6-isoxazolyl-benzimidazole, **14**, showed an IC_{50} of 180 nM against BRD4(1) and was at least 100-fold selective over the CREBBP bromodomain in an AlphaScreen® peptide displacement assay. It also showed little activity against a diverse panel of bromodomains in a thermal shift (ΔT_m) binding assay.[132] Interestingly, the 5-substituted regioisomers were less active against BRD4(1) but showed appreciable binding to CREBBP, which has been exploited in the design of selective CREBBP ligands (Section 1.4).

Constellation Pharmaceuticals have recently reported the development isoxazolothienoazepines such as **15**, which are isoxazole-containing analogues

of the (+)-JQ1/I-BET762 scaffold.[74,133] 5-Amino-3-methyl-4-phenylisoxazole was identified as a fragment hit against BRD4, with excellent ligand efficiency. Reasoning that incorporation of an isoxazole into an azepine scaffold would lead to increased affinity, the authors synthesised a range of isoxazole azepines with various side-chains in place of the *tert*-butyl acetate of (+)-JQ1 (**3**). The acetamide analogue **15** showed improved BRD4 binding and cellular potency compared to the direct isoxazole-containing analogue of (+)-JQ1. As with (+)-JQ1 and I-BET762, a *para*-chlorophenyl substituent in the WPF shelf was found to be optimal. Compound **15** and (+)-JQ1 showed almost identical binding modes in a BRD4(1) co-crystal structure (not shown), and had similar pharmacokinetic properties. Target engagement *in vivo* was demonstrated by dose-dependent downregulation of *MYC*, a known BRD4-dependent gene,[98,134] in a lymphoma xenograft in mice.[133]

1.3.3 3-Methyl-dihydroquinazolin-2-ones



PFI-1 (**16**)

17a, R = piperidin-1-yl
17b, R = 5-chloro-2-methyoxyphenyl
17c, R = 2-methoxyphenyl

Figure 11. 3-Methyldihydroquinazolin-2-ones and 3-methylquinolin-2-ones discussed in the text. The KAc mimic is shown in red.

A collaboration between the SGC and Pfizer led to the development of BET bromodomain inhibitors based around the 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one scaffold (Figure 11).[135,136] The carbonyl group of the cyclic urea mimics that of KAc, while the *N*-methyl group binds in the same position as the methyl group of KAc (Figure 12A, B). The aromatic core occupies the 'gatekeeper' region, clamped between hydrophobic residues. To direct a substituent into the

WPF shelf, the authors explored sulfonamides at the 6-position. Attaching the sulfonamide nitrogen to this position was well tolerated, while the reverse (S-linked) sulfonamides were not. Introducing aromatic substituents *via* the sulfonamide led to a substantial increase in potency, consistent with a crystal structure of the *ortho*-methoxyphenyl-substituted compound, PFI-1 (**16**), in which the pendant aromatic group was directed into the WPF shelf (Figure 12A). PFI-1 (**16**), showed an IC_{50} of 220 nM against BRD4(1) in the AlphaScreen® peptide displacement assay, and an EC_{50} of 1.89 µM for the inhibition of IL-6 production in LPS-stimulated PBMCs.[135] Additionally the compound displays antileukaemic activity, downregulating *MYC* expression and inducing differentiation.[136] However, pharmacokinetic properties are sub-optimal, with the low oral bioavailability in rats being attributed to poor solubility in the gut.[135]

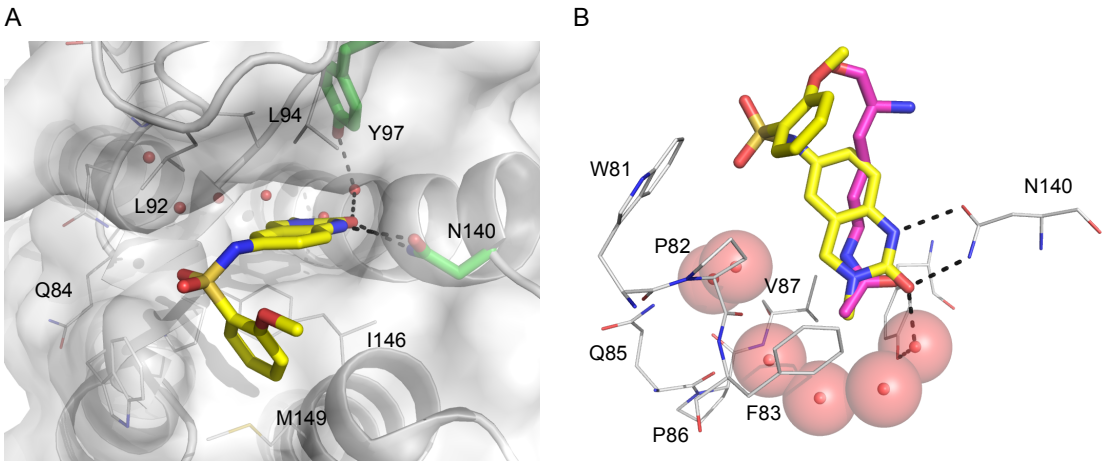
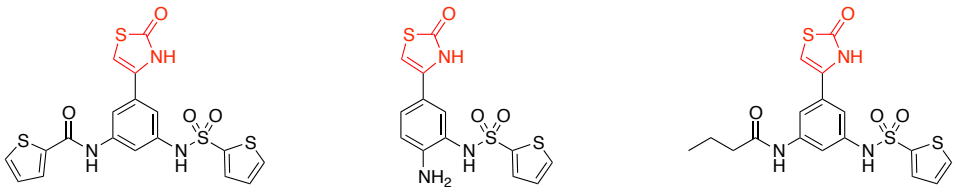


Figure 12. PFI-1 (16), a 3-methyl-dihydroquinazolin-2-one, binds to BET bromodomains. (A) X-ray crystal structure of PFI-1 (**16**) in complex with BRD4(1) (carbon in yellow, PDB ID: 4E96).[135] Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (B) The 3-methyl-dihydroquinazolin-2-one of PFI-1 acts as an effective KAc mimic. The carbonyl is positioned to form a water-mediated hydrogen bond to N140 and, *via* a water molecule, to Y97. N(1) also donates a hydrogen bond to the carbonyl group of N140. The structure is overlaid with KAc from a histone peptide ($H4_{1-12}$K5AcK8Ac, carbon in purple, PDB ID: 3UVW).[44]

A Pfizer patent covers compounds containing the related 3-methylquinolin-2(1*H*)-one KAc mimic.[137] **17a-c** (the quinolinone analogue of PFI-1) all showed

IC_{50} values of 50 μM or less in an AlphaScreen® assay against BRD4 (domain not specified). No cellular or PK data are presented.

1.3.4 Thiazol-2-one



18a **18b** **18c**

Figure 13. Thiazol-2-one BET inhibitors discussed in the text. The KAc mimic is shown in red.

A publication from Zhao *et al.* at the State Key Laboratory of Drug Research in the Chinese Academy of Sciences describes a fragment-based approach to identifying new BET bromodomain inhibitor chemotypes.[138] Docking was used to select 41 putative BRD4-binding fragments, which were progressed to crystallographic studies. Nine fragments were identified, of which four were disclosed in the 2013 paper. Structure-based optimisation of a 4-phenyl-thiazol-2-one hit identified several compounds with sub-micromolar IC_{50} values, including **18a** (Figure 13), which had an IC_{50} of 230 nM in a FA assay for the displacement of fluorescein-tagged (+)-JQ1 from BRD4(1). No data were presented on the selectivity of such compounds over other bromodomains. Activity in cellular assays, including growth inhibition and *MYC* mRNA downregulation in HT-29 colon cancer cells, correlated poorly with inhibitory activity in the FA assay, possibly as a result of limited membrane permeability. The most potent of the compounds in the cellular assay, **18b**, was only moderately active [GI_{50} of 37 μM in HT-29 proliferation assay, cf. 23 μM for **9** and 2.3 μM for (+)-JQ1 (**3**)].[138] An X-ray co-crystal structure of **18c** with BRD4(1) (IC_{50} of 790 nM in FA assay) revealed that the thiazol-2-one bound in the KAc binding site, with the carbonyl

forming the expected interactions with Y97 and N140. N(3) forms a water-mediated interaction with the carbonyl of N140. The sulfur atom of the thiazol-2-one occupies the site of the KAc methyl group, in contrast to all the inhibitors described above, which have a methyl group in this position. The thiophenylsulfonamide occupies the WPF shelf, while the propyl amide is directed into the ZA channel where it forms water-mediated interactions (Figure 14).

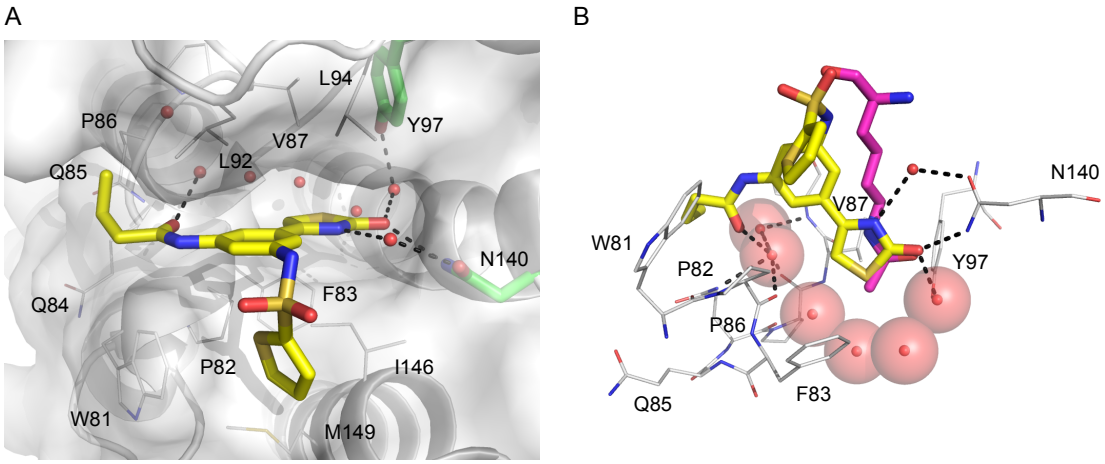
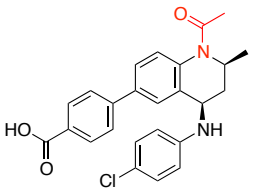


Figure 14. 18c, a thiazol-2-one, bind to BET bromodomains. (A) X-ray crystal structure of **18c** in complex with BRD4(1) (carbon in yellow, PDB ID: 4HXM).[138] Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (B) The thiazol-2-one of **18c** acts as an effective KAc mimic. The carbonyl is positioned to form a water-mediated hydrogen bond to N140 and, *via* a water molecule, to Y97. N(3) also interacts with the carbonyl group of N140 *via* a water molecule. The structure is overlaid with KAc from a histone peptide ($H4_{1-12}$K5AcK8Ac, carbon in purple, PDB ID: 3UVW).[44]

1.3.5 1-Acetyl-tetrahydroquinolines



I-BET726 (**19**)

Figure 15. The structure of the 1-acetyl-tetrahydroquinoline I-BET726 (19). The KAc mimic is shown in red.

This KAc mimic, originally disclosed in a GSK patent filed in 2010,[139] was recently published by Wyce *et al*.[140] Compound **19** (Figure 15), also referred to somewhat confusingly as I-BET726, binds to the tandem bromodomains of BRD4 with a K_d of 4 nM by ITC (IC_{50} of 22 nM in a TR-FRET assay), making it the most potent BET ligand in the published literature to date. It shows excellent selectivity against the closely-related CREBBP bromodomain (K_d of 6.3 µM by ITC) and no appreciable activity against a panel of other bromodomains. The compound has been extensively profiled in cells, and shows potent antiproliferative activity in many, but not all, solid tumour cell lines tested, with IC_{50} values in a 6-day growth inhibition assay of less than 50 nM in some neuroblastoma cell lines.

A co-crystal structure of I-BET726 (**19**) bound to BRD4(1) is shown in Figure 16A. Unsurprisingly, the acetamide closely mimics that of KAc, forming the expected hydrogen bonds to N140 and Y91 (Figure 16B). The nitrogen-containing ring of the tetrahydroquinoline core overlays very closely with the diazepine ring of (+)-JQ1 (**3**) (Figure 16C), and the (*R*) stereochemistry at C(4) directs the *para*-chloroaniline substituent into the WPF shelf. The compound also forms several previously-unreported interactions. The methyl group at C(2) makes contact with the hydrophobic side-chains of V87, L94, Y97 and Y139. Additionally, the terminal nitrogen of K91 moves considerably (4.4 Å in the aligned structures) compared to the (+)-JQ1-bound structure. This brings the positively-charged amine within about 6 Å of the carboxylate of I-BET726 (**19**), suggesting some degree of electrostatic interaction (Figure 16C). (The corresponding residue in BRD4(2) is alanine; however as affinities for the two bromodomains were not reported separately, it is not clear if this imparts selectivity for the first over the second bromodomain.) Along with the excellent shape comple-

mentarity with the pocket, these interactions may account for the exceptional potency of the compound.

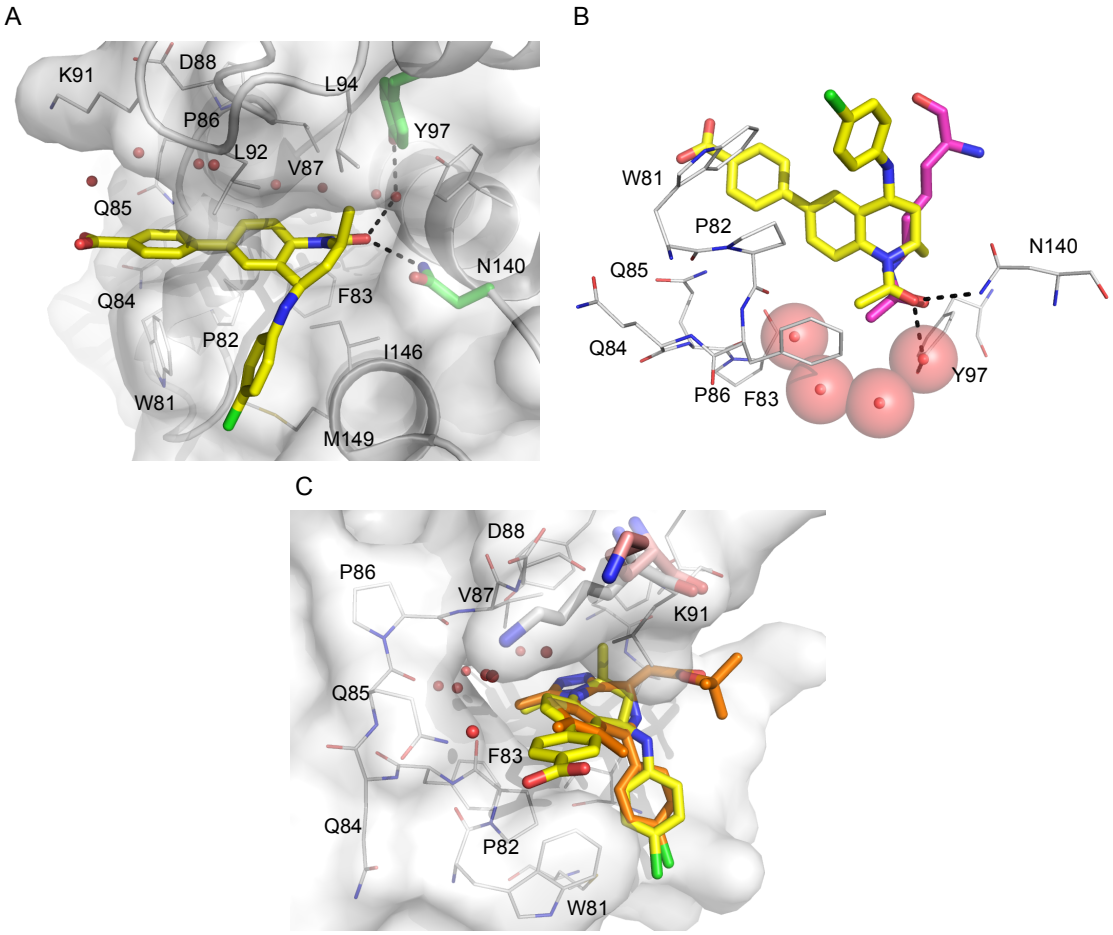
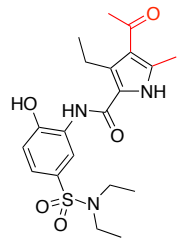


Figure 16. I-BET726 (19), a 1-acetyl-tetrahydroquinoline, binds to BET bromodomains. (A) X-ray crystal structure of I-BET726 (**19**) in complex with BRD4(1) (carbon in yellow, PDB ID: 4BJX).[140] Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (B) The 1-acetyltetrahydroquinoline of I-BET726 acts as an effective KAc mimic. The carbonyl is positioned to form a water-mediated hydrogen bond to N140 and, *via* a water molecule, to Y97. The structure is overlaid with KAc from a histone peptide ($H4_{1-12}$K5AcK8Ac, carbon in purple, PDB ID: 3UVW).[44] (C) Binding of I-BET726 induces movement of the side-chain of K91 (carbon in grey) towards the carboxylate. K91 in the (+)-JQ1-bound structure (carbon in pink) and (+)-JQ1 (**3**, carbon in orange, PDB ID: 3MXF)[111] are shown for comparison.

1.3.6 4-Acylpyrroles



XD14 (**20**)

Figure 17. The structure of XD14 (20). The KAc-mimicking moieties are shown in red.

Lucas *et al.* used virtual screening followed by X-ray crystallography and ITC measurements to identify new inhibitors of BRD4(1).[141] By far the most active hit was **20** (Figure 17), referred to as XD14, which had a K_d of 237 nM against BRD4(1) by ITC (160 nM in BROMOscan ligand displacement assay). It also interacted significantly with the CREBBP bromodomain (1.6 μM in BROMOscan assay), but showed good selectivity over other bromodomains from outside the BET family. The compound was crystallised with BRD4(1), and the X-ray structure revealed that the ketone carbonyl group mimics the carbonyl of KAc in its hydrogen-bonding interactions, while a methyl group at C(5) occupies a similar position as the terminal methyl group of KAc (Figure 18A, B). The amide carbonyl interacts with a water molecule in the ZA channel, as with I-BET151 (**12**), **13** and **18c**, and the pyrrole N–H forms a hydrogen bond to the carbonyl group of P82. The compound does not occupy the WPF shelf, but the phenyl ring forms an edge-face π-stacking interaction with W81 (Figure 18A).[142] Interestingly, an analogue with a methyl group in place of the substituted phenyl ring bound to BRD4(1) with an almost identical pose but significantly reduced affinity (16 μM by ITC), suggesting that this interaction contributes significantly to the binding of XD14.

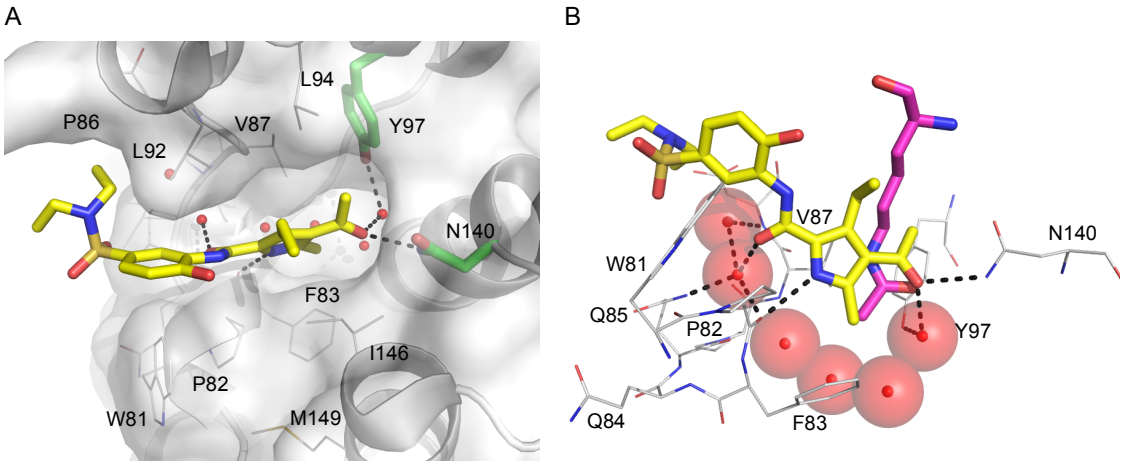
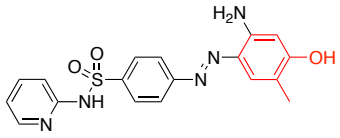


Figure 18. XD14 (20), a 4-acyl pyrrole, binds to BET bromodomains. (A) X-ray crystal structure of XD14 (**20**) in complex with BRD4(1) (carbon in yellow, PDB ID: 4LYW).[141] Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (B) The 4-acylpyrrole of XD14 acts as an effective KAc mimic. The carbonyl is positioned to form a water-mediated hydrogen bond to N140 and, *via* a water molecule, to Y97. The structure is overlaid with KAc from a histone peptide ($H4_{1-12}$KAc5KAc8, carbon in purple, PDB ID: 3UVW).[44]

1.3.7 2-Methylphenols



MS436 (**22**)

Figure 19. The structure of MS436 (22). The KAc mimic is shown in red.

Ming-Ming Zhou and co-workers identified a diazobenzene compound **21**, referred to as ischemin, as a ligand for the CREBBP bromodomain (Figure 24, Section 1.4)[143] and have subsequently used a similar scaffold to develop potent BET bromodomain inhibitors.[144] MS436 (**22**, Figure 19) inhibited the binding of fluorescein-tagged MS417 (**4**) to BRD4(1) in a FA assay with a K_i of < 85 nM (limited by the affinity of the assay probe), with > 4-fold selectivity over BRD4(2). The compound also showed activity against the bromodomains of CREBBP, PCAF, BRD7, BPTF, BAZ2b and SMARCA4, with K_i values between 2 and 8 μM in FA assays. A crystal structure of MS436 (**22**) bound to BRD4(1) was described, but has not been released.[144] The 2-methylphenol mimics the key interactions of

KAc: the methyl group binds at the base of the KAc-binding pocket, and the phenolic hydroxyl interacts with N140 and Y97. The amino group *meta* to the hydroxyl forms a water-mediated hydrogen bond to the carbonyl of N140, in a similar manner to **18c**. The diazo linker interacts *via* a water molecule with residues in the ZA channel, and the sulfonamide forms water-mediated interactions with K91. [The authors do not comment on whether the lysine side-chain has moved to form this interaction, as was observed with I-BET726 (**19**).] The 2-pyridyl substituent is in contact with W81, but unusually is on the solvent-exposed face, not in the WPF shelf. Analogues with substituted phenyl rings in place of the pyridine were less active. The compound also showed activity in several cellular assays, including suppressing the release of pro-inflammatory cytokine IL-6 after LPS stimulation, with an IC_{50} of 4.9 μM in an enzyme-linked immunosorbent (ELISA) assay. Data published in a patent from the same laboratory revealed that the diazo linker can be replaced with an alkene without loss of affinity.[145]

1.3.8 Other compounds

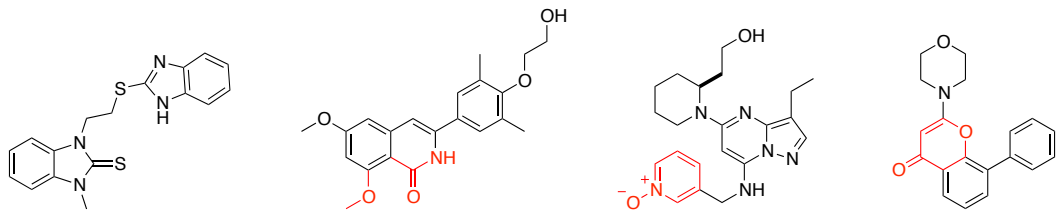


Figure 20. The structures of BET ligands BIC1 (23) and RVX-208 (24), and kinase inhibitors dinaciclib (25) and LY294002 (26). The KAc mimic is shown in red.

A benzimidazole-based BET bromodomain inhibitor BIC1 (**23**, Figure 20), was identified by Ito *et al*.[146] This compound was shown to block the interaction of the BRD2 bromodomains to a H4K12Ac-mimicking peptide in a novel cell-based assay for histone acetylation. The interaction of BIC1 (**23**) with BRD2(1) was

confirmed by SPR, which gave a K_d of 28 μM. X-ray crystallography reveals that the benzene ring of the benzimidazole moiety occupied the KAc-binding pocket while the benzimidazole-2-thione was directed toward the WPF shelf. The compound does not form hydrogen bonds to the conserved asparagine and tyrosine residues, perhaps explaining its lower affinity for BRD2 compared to other reported ligands. No data were presented on the selectivity of this compound for BRD2(1) over other bromodomains.

RXV-208 (**24**, Figure 20) is currently undergoing clinical development as a treatment for atherosclerosis.[147,148] As with I-BET762 (**1**) and I-BET151 (**12**), it was originally identified as an upregulator of ApoA1, and two recent studies have shown that it binds to BET bromodomains.[149,150] In contrast to all previously-described BET inhibitors, RVX-208 (**24**) shows selectivity for the second over the first bromodomain of the BETs. For example, ITC experiments carried out by Picaud *et al.* gave a K_d of 140 nM for BRD4(2), but 1.1 μM for BRD4(1).[149] (McLure *et al.* obtained somewhat higher values but observed the same trend.)[150] Both publications show that the enthalpic contribution to binding is greater (more negative) for the first bromodomain, but the entropic gain on binding is much larger for the second bromodomain. The compound shows no appreciable affinity for bromodomains outside the BET family. X-ray crystal structures with BRD4(1) and BRD2(2) reveal almost identical binding poses (Figure 21). The quinazolone carbonyl mimics that of KAc, and interacts with the conserved asparagine and tyrosine residues. The quinazolone N–H also interacts with the key asparagine, donating a hydrogen bond to the amide side-chain carbonyl. One of the methoxy groups is directed into the base of the water-lined pocket, so that, as with XD14 (**20**), the methyl and carbonyl functionalities of KAc are mimicked by different

parts of the ligand. The other methoxy group is directed into the ZA channel, and in BRD4(1), forms a water-mediated hydrogen bond to Q85 (Figure 21A). This is the only interaction unique to the BRD4(1) structure; in BRD2(2), the similarly-positioned K374 is directed away from the binding site. Only two residues in contact with the ligand differ between BRD4(1) and BRD2(2): D144 and I146 in BRD4(1), which are conserved in all of the N-terminal BET bromodomains, are replaced in the second bromodomains by histidine and valine residues respectively [H433 and V435 in BRD2(2)]. Significantly, H433 is in contact with the tetrasubstituted aromatic ring of RVX-208, while D144 in BRD4(1) is directed towards the solvent. This histidine appears to be relatively flexible, as in a peptide-bound structure of BRD2(2) the residue is flipped away from the pocket (Figure 21B). It is possible that RVX-208 stabilises this side-chain conformation, contributing to the selectivity for the second bromodomain.

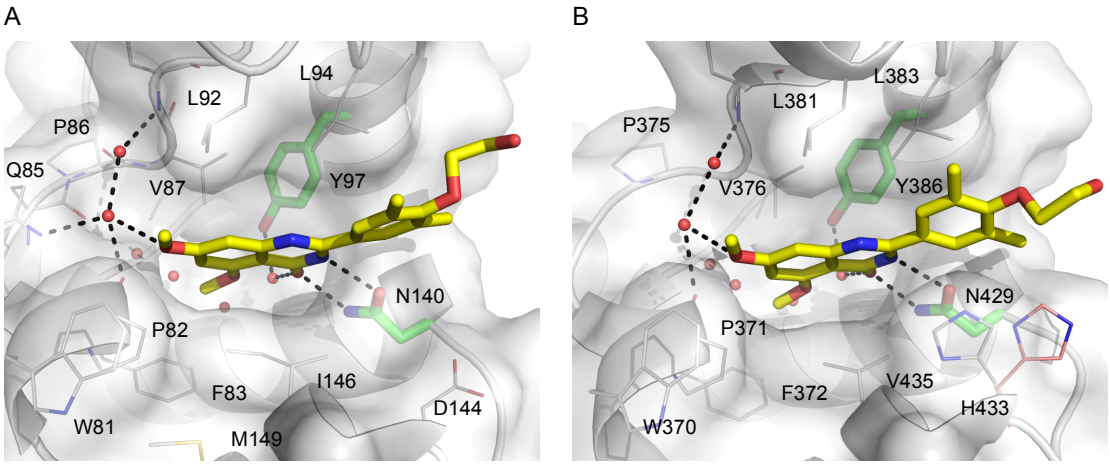


Figure 21. RVX-208 (24) shows selectivity for the second bromodomain of BET proteins. (A) X-ray crystal structure of RVX-208 (**24**) in complex with BRD4(1) (PDB ID: 4MR4).[149] Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. (B) X-ray crystal structure of RVX-208 in complex with BRD2(2) (PDB ID: 4MR6).[149] Conserved Y386 and N429 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. The position of H433 in a peptide-bound structure (PDB ID: 2E3K) is shown in pink.

Two recent publications have shown that widely-used kinase inhibitors can bind to bromodomains (Figure 20).[151,152] Dinaciclib (**25**), a cyclin-dependent

kinase (CDK) inhibitor currently in Phase III trails for chronic lymphocytic leukaemia, was shown crystallographically to bind to BRDT(1) (Figure 22A). The pyridine *N*-oxide forms a rather weak hydrogen bond (3.5 Å) to the conserved asparagine residue [N109 in BRDT(1)], mimicking the KAc interaction. Intriguingly, the hinge-binding 7-aminopyrazolo-pyrimidine motif interacts *via* water molecules with the backbone carbonyl groups of R54 and P55 in the ZA channel, leading Martin *et al.* to suggest that other common kinase inhibitor scaffolds may also interact with the ZA channel of bromodomains.[151] W50 at the edge of the pocket undergoes a large conformational change to accommodate the piperidine ring, which may be very unfavourable for binding. Indeed, the interaction with BET bromodomains is rather weak: the BROMOscan assay gave a modest K_d of 37 μM for BRDT.

The phosphoinositide 3-kinase (PI3K) inhibitor LY294002 (**26**), widely used as a chemical probe, also interacts with BET bromodomains. Dissociation constants were estimated by titrating in LY284002 to compete bromodomains off an immobilised LY294002 derivative, giving a K_d^{app} of 2 μM for BRD4. A crystal structure of LY294002 (**26**) bound to BRD4(1) reveals a very different mode of binding to dinaciclib (**25**) (Figure 22B): the chromen-4-one carbonyl mimics KAc in its interactions with Y97 and N140, while the pyranone and morpholine rings form hydrophobic interactions in the 'gatekeeper' region between I146 and L94. It does not interact with water molecules in the ZA channel, nor significantly occupy the WPF shelf region. LY294002 (**26**) also showed cellular effects consistent with BET bromodomain inhibition, such as suppression of cytokine release after LPS stimulation.[152] Neither study investigated whether these compounds could inhibit the reported kinase activity of BRD4. Remarkably, BRD4 had been identified as a

cellular target of LY294002 (**26**) in 2007 by a similar chemoproteomic approach using immobilised inhibitor, predating all reported BET inhibitors by several years.[153] Furthermore, a study in 2005 showed that LY294002 (**26**) inhibited NF-κB-dependent transcription and nitric oxide production while another PI3K inhibitor, wortmannin, did not.[154] These effects now appear likely to have resulted from BRD4 inhibition.[75,144,155]

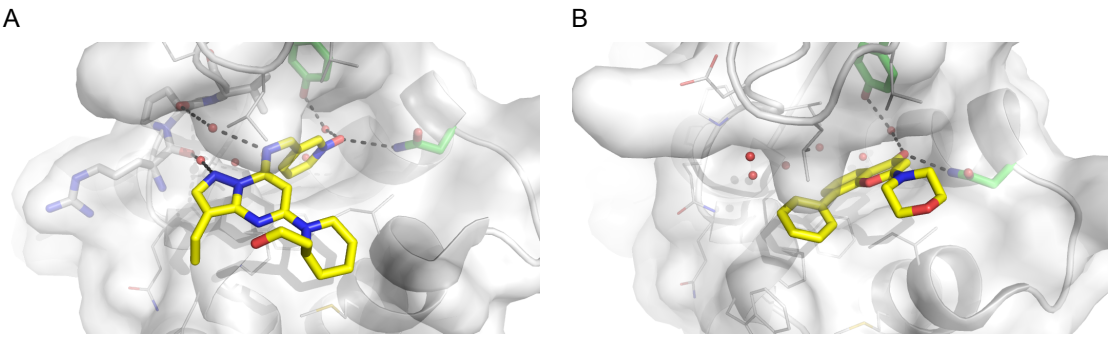


Figure 22. Kinase inhibitors dinaciclib (25) and LY294002 (26) bind to BET bromodomains. (A) X-ray crystal structure of dinaciclib (**25**) in complex with BRDT(1) (PDB ID: 4KCX).[151] Conserved Y97 and N140 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red. The ZA channel residues that interact with dinaciclib are shown as sticks. (B) X-ray crystal structure of LY294002 (**26**) in complex with BRD4(1) (PDB ID: 4CFK).[152] Conserved Y66 and N109 residues shown in green, with hydrogen bonds indicated. Structural water molecules at the base of the pocket shown in red.

Two further papers describe multiple fragment ligands for BET bromodomains. The first, from GSK, describes the assembly of a library of putative KAc-mimicking fragments, screening, and high-throughput crystallisation to give forty structures of fragments bound to bromodomains, of which five are described.[156] Three of these, *N*-acetyl-2-methyltetrahydroquinoline (**27**), 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (**28**), and *N*-methylpyrrolidin-2-one (**29**), have also been described or optimised, by GSK[140] or others.[126,135] The other two, paracetamol (**30**) and the 3-acetyl-indolizine (**31**) are shown in Figure 23. Vidler *et al.* used a virtual screening approach, combining pharmacophore modelling and similarity searching against known bromodomain ligands, followed by dock-

ing.[157] Top-ranked compounds were purchased and tested, and X-ray crystal structures with BRD4(1) were obtained for four compounds **32**-**35** containing novel KAc mimics (Figure 23). The most potent of these was **32**, with an IC_{50} of 4.7 µM against BRD4(1) in an AlphaScreen® peptide displacement assay.

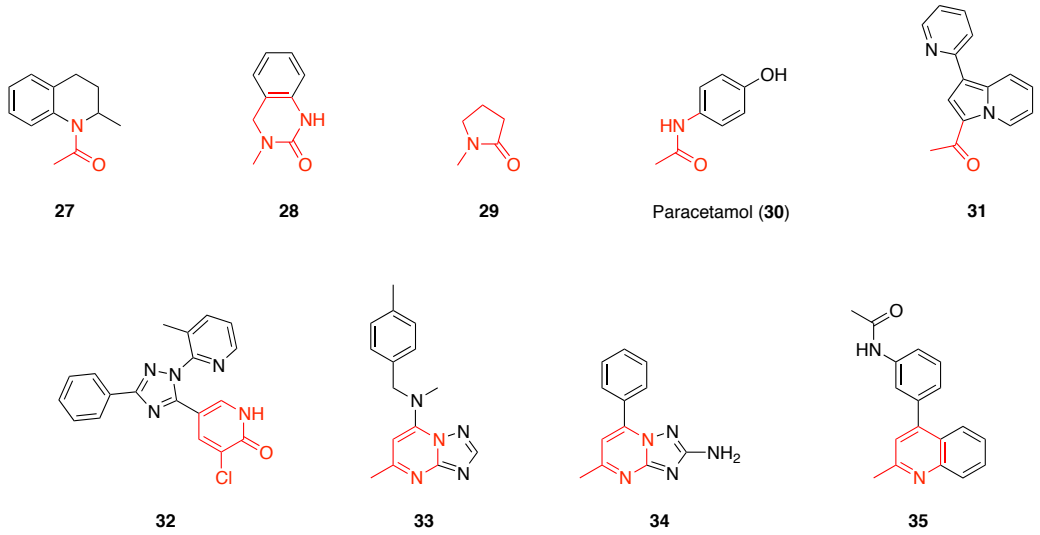


Figure 23. Fragment-derived bromodomain ligands. 27-**31** were described by Chung *et al.*,[156] **32**-**35** by Vidler *et al.*[157] The KAc mimic is indicted in red.

1.3.9 BET bromodomain SAR

With an increasing number of ligands for BET bromodomains being reported, the structure−activity requirements for potent and selective BET ligands are beginning to emerge. The four key areas of interaction with the BET bromodomains are the KAc-binding pocket, the 'gatekeeper' region, the WPF shelf, and the ZA channel.

All of the potent BET inhibitors described to date mimic the hydrogen-bonding interactions formed by the carbonyl group of the KAc side-chain to conserved asparagine and tyrosine residues. [BIC1 (**23**), which does not make these interactions, is a weak ligand.] The KAc-mimicking hydrogen-bond donors in the BET ligands described to date are N- or O-containing heterocycles (tria-

zoles, isoxazoles), carbonyl groups (amides in the 1-acetyltetrahydroquinolines and RVX-208, ureas in the dihydroquinazolinones, ketones in the 4-acylpyrroles, or thiazol-2-ones) or phenols (such as the 2-methylphenols). All potent inhibitors also place a hydrophobic moiety at the base of the pocket, occupied by the methyl group of KAc. In most examples, this moiety is also a methyl group, though it can also be an exocyclic chlorine atom (**32**) or a sulfur atom (**18a**-**c**). There are no high-affinity ligands that displace any of the four water molecules in this hydrophobic pocket. In most compounds, like in KAc itself, the hydrophobic moiety is directly connected to the hydrogen-bond donor, although in the 4-acylpyrrole (**20**) and RVX-208 (**24**) they are separated by several atoms.

The 'gatekeeper' region, a narrow groove between an isoleucine (first bromodomain) or valine (second bromodomain) and two leucines from the ZA loop is invariably occupied by a hydrophobic ring system. This is often a planar aromatic moiety, such as a phenyl ring in **9**, **10** and **18**, a quinoline in I-BET151 (**12**) or a pyrrole in XD-14 (**20**). However, the diazepines such as (+)-JQ1 (**3**) and the tetrahydroquinolines such as I-BET726 (**19**) have a puckered ring in this region. This may provide somewhat better shape complementarity, particularly in forming hydrophobic contact with the gatekeeper residue, and contribute to the exceptional affinities of these series. Indeed, (+)-JQ1 and I-BET726 are among the most potent compounds so far reported.

Many BET inhibitors bear a hydrophobic substituent directed into the WPF shelf. This is often an aromatic ring, and a *para*-chlorophenyl is employed in several compounds, including I-BET762 (**1**), (+)-JQ1 (**3**), MS417 (**4**), CPI203 (**5**), I-BET726 (**19**) and **15**. (An unsubstituted phenyl ring had low microsomal stability, at least in the isoxazoloazepine series.)[133] Saturated rings, such as the cyclopen-

tyl group of **9** and **10**, are also tolerated. No clear SAR trends for substitution patterns on the ring emerge across multiple series; it is likely that a subtle balance of steric and electronic properties, including π-stacking interactions with a tryptophan residue, dictate the substituent effects for each series. The geometry and flexibility of the linker between the ring in the WPF shelf and the core significantly affects the positioning of the ring, and thus the preferred ring size and substituents. In the diazepine compounds, a C–C single bond links the core and the phenyl group, and for naphthyridine **13**, the 2-trifluoromethylphenyl substituent is connected to the cyclic urea *via* a N–C single bond. I-BET151 (**12**) has a one-atom linker, while a number of series use the larger sulfonamide moiety to direct a substituent into the WPF shelf.

Several authors have suggested that the presence of a substituent in the WPF shelf is critical for BET bromodomain affinity and selectivity.[113,128,130,138,158,159] A recent analysis of the druggability of bromodomain KAc-binding sites suggests that the high druggability of the BET family results in large part from the enclosed nature of this region,[160] and interactions in the WPF shelf clearly make a significant contribution to binding. However, recent studies bring this hypothesis into question: XD14 (**20**) and MS436 (**22**) have good affinity for the BET bromodomains without a substituent in the WPF shelf, although neither compound is especially BET-selective. RVX-208 (**24**), however, shows remarkable selectivity for the BET bromodomains without occupying the WPF shelf. Given the very limited published SAR around the RVX-208 scaffold, is not clear why this compound is quite so selective, or whether its affinity would increase with the addition of a substituent directed into the WPF shelf.

sThe third region of the BET bromodomains that can potentially influence ligand binding is the ZA channel. This channel initially appears to form a second pocket that can be exploited to gain further ligand potency and selectivity. In the BET bromodomains, the entrance to the ZA channel is formed by tryptophan and leucine side-chains [W81 and L92 in BRD4(1)], while the channel itself is made up of the backbone of turn-forming residues P82, F83, Q84, Q85 and P86, and the Q85 side-chain. In the apo structures of the BET bromodomains, as well as in most ligand-bound structures, two water molecules are present. One forms interactions with the backbone carbonyl groups of P82 and Q85 and the side chain amide of Q85 in BRD4(1). This water molecule is in contact with one of the KAc-binding pocket water molecules *via* their P82 interactions. The second forms interactions with the backbone NH of D88 and the other water molecule. Several BET ligands form interactions with the first of these water molecules, including I-BET151 (**10**), XD14 (**20**), MS436 (**22**) and RVX-208 (**24**). From the published literature, it is not clear to what extent this interaction contributes to the affinity of the compounds for the BET bromodomains. However, as noted above, the diazo linker in the MS436 series could be replaced with an alkene without loss of affinity. Significant rearrangement of these water molecules is seen only in the I-BET726 (**19**) structure, and is likely to result from the electrostatic influence of the nearby carboxylate and the resulting shift in the K91 side-chain. It is apparent that the significance of interacting with or displacing these water molecules is not yet clear, and that SARs in the ZA channel are underexplored.

Due to the hydrophobic nature of the KAc-binding pocket, developing soluble ligands of low lipophilicity is challenging. Substituents directed past the tryptophan residue [W81 in BRD4(1)] are solvent-exposed, allowing solubilising

groups to be attached at this position. Bamborough *et al*. introduced a morpholine ring to enhance solubility (Figure 9), and **8** has a similarly-positioned morpholine. Likewise, the carboxylate of I-BET726 (**19**) is also largely solvent-exposed. The pyridylsulfonamide of MS436 is also directed out of the pocket, but forms a previously-unreported π-stacking interaction on the outside face of W81, suggesting that affinity, as well as solubility, can be affected by substituents directed past W81.

The bromodomain fold is very rigid, and binding occurs with little change in the position of the protein backbone. However, some of the side-chains around the KAc-binding site appear to be mobile, with effects on ligand binding. Notably, W81 and K91 can move in order to form additional interactions with the ligand, as shown by structures of **8** and I-BET726 (**19**) bound to BRD4(1). The flexibility of a histidine residue in the second bromodomain [H433 in BRD2(2)] may in part account for the greater affinity of RVX-208 (**24**) for the second bromodomain. Computational approaches to identifying new BET ligands have assumed a rigid receptor, with fixed side-chains and immovable water molecules.[138,157] This assumption might limit the novel scaffolds thus identified, and opportunities to develop ligands with unusual selectivity profiles, such as RVX-208 (**24**), may be missed. A recent molecular dynamics study of 20 human bromodomains suggest that, in contrast to the homogeneity of KAc binding sites in crystal structures, there may be significant plasticity in the shape of the KAc binding site in some bromodomains.[161] In particular, the conserved asparagine and tyrosine residues show considerable flexibility. Since multiple metastable states are observed only in a subset of bromodomains, targeting such states may provide a novel ap-

proach to achieving selectivity. Further studies on the dynamic properties of bromodomains are certainly warranted.

1.4 Ligands for bromodomains outside the BET family

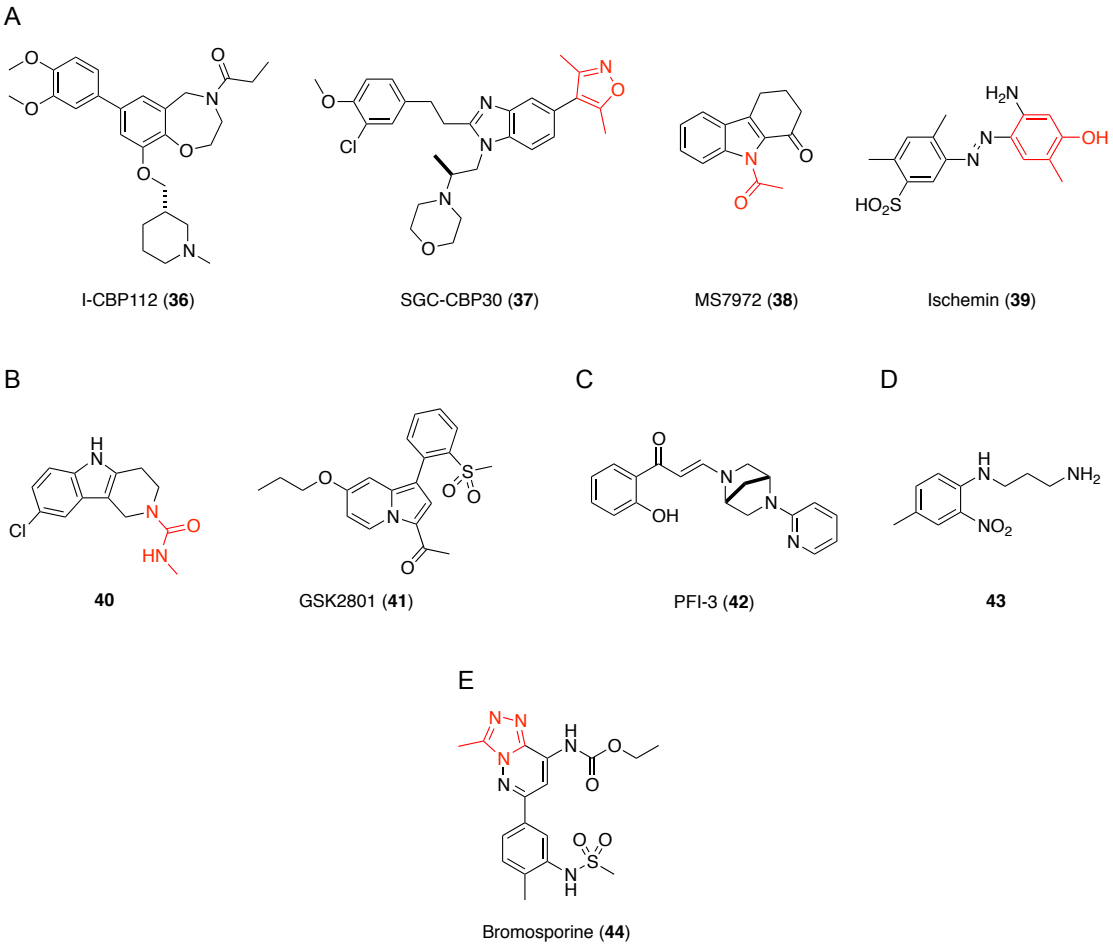


Figure 24. Ligands for non-BET bromodomains. (A) CREBBP bromodomain ligands. (B) BAZ2A/B ligands. (C) A SMARCA2/4 and PB1(5) ligand. (D) PCAF bromodomain fragment. (E) A broad-spectrum bromodomain inhibitor. Where known, the KAc mimic is shown in red.

To date, most progress has been made on developing inhibitors of the BET bromodomains. Nevertheless, some of the earliest bromodomain ligands reported were for proteins outside the BET family, and potent ligands have now been reported for several other bromodomains (Figure 24). Two chemical probes for the CREBBP bromodomain are available from the SGC, I-CBP112 (**36**)[162] and SGC-CBP30 (**37**),[163] with CREBBP dissociation constants of 150 nM and 21 nM

respectively, as measured by ITC (Figure 24A). Both are highly selective against other bromodomains, including BET bromodomains. Previously Ming-Ming Zhou and co-workers identified the 1-acylindole MS7972 (**38**) and the 2-methylphenol-containing diazobenzene **39** as CREBBP ligands, with dissociation constants of around 20 μM in an assay measuring change in tryptophan fluorescence as a function of compound concentration (Figure 24A).[143,164] Both compounds inhibited the interaction between the CREBBP bromodomain and acetylated p53, with resultant suppression of p53-mediated responses. **39** was named ischemin due to its ability to reduce apoptosis in ischemic cardiomyocytes, likely due to reduced p53 activation.[143]

Ferguson *et al*. have reported a tetrahydro-γ-carboline derivative **40** as an inhibitor of the BAZ2B bromodomain, with a K_d of 8 μM from ITC experiments (Figure 24B).[165] The compound also shows significant affinity for the BET and CREBBP bromodomains. An X-ray co-crystal structure with BAZ2B demonstrates that methyl carbamate acts as an KAc mimic; the analogous acetamide was considerably less active. GSK2801 (**41**), a 3-acetylindolozine, was disclosed by the SGC as a ligand for BAZ2A and BAZ2B (K_d of 140 nM against BAZ2B by ITC), with considerable selectivity over other bromodomains including BETs and CREBBP (Figure 24B).[166]

The SGC have also reported a chemical probe, PFI-3 (**42**) for the bromodomains of SMARCA2 and SMARCA4, and the closely-related fifth bromodomain of polybromo-1 [PB1(5)] (Figure 24C). Dissociation constants of 89 nM and 48 nM were reported for SMARCA4 and PB1(5), respectively. No affinity was detected for more distantly-related bromodomains.[167]

Polyamines such as **43** (Figure 24D) were described as ligands for the bromodomain of p300-CREBBP-associated factor (PCAF) as early as 2005, providing the first demonstration that non-peptidic small molecules could act as bromodomain ligands. **43** displaced an acetylated HIV-TAT peptide from PCAF with an IC_{50} of 2 μM in an ELISA assay.[168] Modest improvements to activity were made by replacing the methyl group with 1-chroethyl,[169] or the nitro group with an amine.[170] An NMR structure of the PCAF bromodomain with **43** indicates that it occupies the peptide-binding groove rather than the KAc-binding site.

Compound **44**, related to methyltriazole **8**, showed broad inhibitor activity across many bromodomains, including the BET bromodomains, CREBBP, CECR2, TAF1(2) and BRD9 (Figure 24E).[171] The compound was named 'bromosporine' by analogy to the multi-kinase inhibitor staurosporine. It is anticipated that such a non-selective compound will prove useful in assay development.

1.5 Assays for the identification and characterisation of bromodomain ligands

Developing assay techniques to identify novel ligands, and employing secondary assays to characterise and quantify protein-ligand interactions, is essential in any attempt to develop quality chemical tool compounds. Assays for reader domains present particular challenges, due to the domains' lack of catalytic activity. Therefore the assay must detect the compound-ligand interaction directly ('label-free' techniques), or measure displacement of a known binding partner.

1.5.1 Label-free techniques

Label-free biochemical techniques have been widely used, particularly in earlier studies. These experiments do not require prior knowledge of a suitable peptide or small molecule ligand and so remain the only option for many bromodomains for which no cognate peptide sequence or chemical probe has been identified. The use of NMR was pioneered by Zhou and colleagues, who have identified small molecules including **38**, **39** and **43** which bind to the CREBBP[143,164] and PCAF[168] bromodomains, as well as cyclic peptide inhibitors of the CREBBP bromodomain.[172] Although NMR provides insights into the ligand binding mode, it requires significant quantities of protein and is unlikely to be suitable for large screening campaigns. Dissociation constants for the CREBBP ligands were estimated by a second label-free technique, fluorescence spectroscopy, which exploited ligand-induced changes in the fluorescence spectrum of tryptophan residues near the KAc binding site.[143,164]

Compared with NMR or fluorescence spectroscopy methods, SPR has the advantage of reduced protein consumption, and provides K_d and kinetic data on compound binding. To date, SPR has been used only as a secondary assay to validate hits obtained using other techniques.[113,121,128,135,146] A second technique involving surface-immobilised protein, bio-layer interferometry (BLI), has been used to quantify compound affinity for the CREBBP bromodomain.[136] It will be interesting to see if SPR and BLI, which have the advantages of low protein consumption and potentially high throughput, and provide kinetic binding data, gain more widespread use.

Differential scanning fluorimetry (DSF) or thermal shift indicates the degree of protein stabilisation on ligand binding. It measures the change in protein

unfolding ('melting') temperature on the addition of ligand (ΔT_m), as monitored by a dye that is highly fluorescent in non-polar environments, such as the hydrophobic sites from the core of an unfolded protein, but is quenched in solution.[173] DSF has proved to be especially useful in evaluating compound selectivity over multiple bromodomains.[111-113,121,124,130,132,133,149,150,165] ITC provides a direct measurement of the thermodynamics of binding, and therefore remains the "gold standard" method for evaluating small-molecule–bromodomain interactions, despite its low throughput and high protein consumption.[111-113,122,136,141,149,150]

1.5.2 Displacement assays

The first report of a displacement assay to quantify the interaction of small molecules with the bromodomain emerged from the laboratory of Ming-Ming Zhou. In an ELISA employing immobilised PCAF bromodomain and a GST-tagged KAc-containing peptide, they determined IC_{50} values for a compound series derived from an NMR screening hit.[168] The modest throughput of ELISA limits its use in compound screening, but ELISA and similar formats have been used to identify cognate peptides for bromodomains,[174] including in a detailed analysis of the interactions of 33 bromodomains with acetylated and nonacetylated histone peptides.[44] Identification of cognate peptides and small molecule probes for bromodomains by such studies facilitates the development of biochemical assays for bromodomain-binding compounds.

Because of the low inherent affinity of bromodomains for acetylated peptides, highly sensitive assay techniques such as AlphaScreen® have been employed. This method detects the proximity of streptavidin-coated 'donor' and nickel-coated 'acceptor' beads. The donor bead interacts with a biotin-tagged acetylated histone peptide, while the acceptor binds His-tagged recombinant

bromodomains. The bromodomain-histone interaction brings the two beads into proximity. Laser excitation of a photosensitiser within the donor beads generates singlet oxygen, which can diffuse and initiate a chemiluminescent reaction with a dye in the acceptor bead (Figure 25). Since the lifetime of singlet oxygen in solution is very short, the luminescence signal is very sensitive to the distance between the beads. If an inhibitor prevents the binding of the bromodomain to the acetylated peptide, donor and acceptor beads are no longer in proximity, and the singlet oxygen returns to the ground state before it reaches the acceptor bead and no signal is produced. Researchers at the SGC reported the use of AlphaScreen® technology to screen 13 bromodomains against a library of modified histone peptides. Strongly binding peptides were then used in an AlphaScreen® peptide-displacement assay, which evaluated the effects of various solvents on bromodomain–peptide binding. Interestingly, they demonstrated that several solvents, including DMSO and NMP, inhibited the binding of acetylated peptides to bromodomains, with NMR and X-ray crystallography confirming that these compounds acted as KAc mimics.[119] The AlphaScreen® format has since been used by the SGC to characterise inhibitors from several bromodomain families.[111,124,132,135,136,149,157,165]

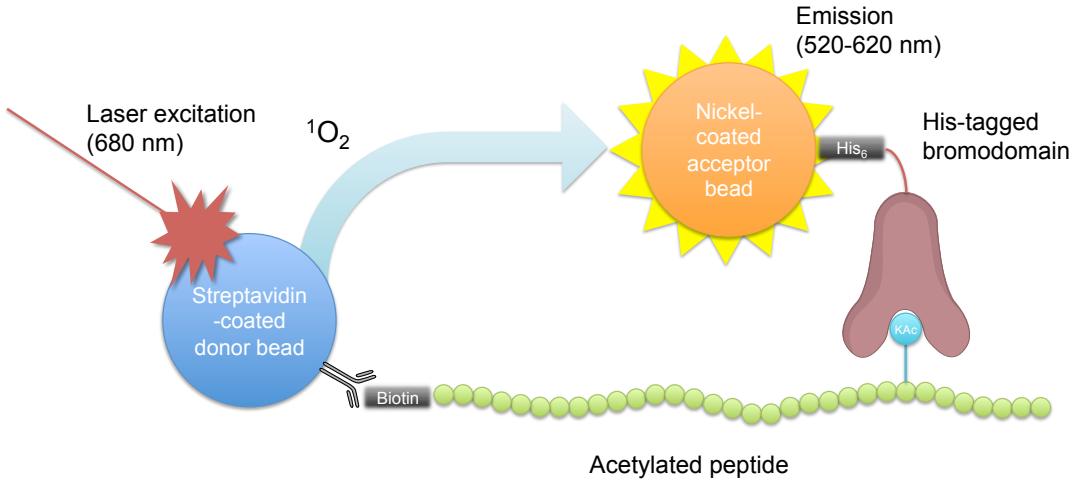


Figure 25. Principle of the AlphaScreen® assay as applied to bromodomain-peptide interactions.[119] In the absence of an inhibitor, streptavidin-coated donor beads and nickel-coated acceptor beads are brought together through their association with biotinylated histone peptides and His-tagged bromodomains, respectively. Laser excitation of the donor bead generates singlet oxygen, which upon interaction with the acceptor bead generates emission at a longer wavelength. Inhibiting the bromodomain-KAc interaction separates donor and acceptor beads, with the result that singlet oxygen is largely quenched before reaching the donor bead and emission is reduced.

TR-FRET assays also require a suitable peptide binding partner for the bromodomain and have been used to validate hits against the BET bromodomains.[112,113,128,150,156] The discovery of tight-binding small molecules for bromodomains has allowed the development of sensitive FA assays (also known as fluorescence polarisation, FP) in which the displacement of a fluorescently-labelled probe molecule is detected. A FA assay employing a fluorescent analogue of I-BET762 (**1**) was developed by Chung and colleagues at GSK.[113,114,121] It was subsequently used in a fragment screen to identify compounds that bind to BET bromodomains,[128,156] and during the optimisation of I-BET151 (**12**) and **13**.[130,131] Related fluorescein-tagged triazolodiazepine ligands have also been employed in FA assays by other groups.[138,144]

A polymerase chain reaction- (PCR)-based displacement assay has been commercialised by DiscoveRX.[175] Named BROMOscan, the assay employs immobilised ligands and DNA-tagged bromodomains. Test ligands compete with

the immobilised ligand for bromodomain binding, so the amount of bromodomain captured on the solid support reduces with increased affinity of the test ligand. Captured bromodomain is quantified by quantitative PCR (qPCR). This is now available for over thirty bromodomains.

Displacement assays have clear advantages over many of the label-free methods in terms of sensitivity and throughput. However, high-affinity ligands suitable for FA assays have only been identified for a handful of bromodomains. Likewise, tightly-binding peptides which can be employed in AlphaScreen® or TR-FRET assays are limited in number.

As noted by Bamborough *et al*.,[128] K_d or IC_{50} values obtained using different assay techniques can vary significantly in part because of the differing protein concentrations employed and differing substrate affinities (in the case of ligand displacement assays), among other factors. Similarly, measured peptide affinities are dependent on the assay format. Consequently care must be taken when comparing affinities from different assay methods.

1.5.3 Phenotypic assays

Cell-based methods have also proved to be important in identifying small-molecule ligands for bromodomains. In particular, the methyltriazolodiazepine and 7-isoxazoloquinoline series of BET inhibitors described by GSK were identified in a phenotypic screen for up-regulators of the *ApoA1* gene (Sections 1.3.1 and 1.3.2), and bromodomains were only later identified as the molecular target.[112-114,129,130] The thienotriazolodiazepine series, including (+)-JQ1, was initially identified by Mitsubishi Tanabe Pharma Corporation as having immunomodulatory activity.[118] Related compounds were later patented as bromodomain inhibitors with anti-cancer properties.[117] An in-cell FRET assay for histone acetylation was

developed by Yoshida and colleagues,[176] who used a similar technique to identify BIC1 (**23**) as a small-molecule inhibitor of BRD2–histone H4K12Ac interaction.[146]

1.6 Applications of BET ligands to the study of biological systems

An important endorsement of the utility of the BET bromodomain ligands has been their application in exploring the roles of BET BCPs. A remarkable number of studies employing BET inhibitors have been published since 2010, and I will not attempt to review them all. Instead, I will cover some of the early studies that used these compounds, and attempt to give an indication of the therapeutic potential of BET inhibition.

1.6.1 BET inhibitors and cancer

Much attention has focused on the use of BET inhibitors in haematological malignancies. In one of the first studies, (+)-JQ1 (**3**) was used to corroborate the findings of an RNAi screen that identified BRD4 as a therapeutic target for acute myeloid leukaemia (AML).[97] In addition to reproducing the antiproliferative and differentiation-inducing effects of BRD4 short hairpin RNA (shRNA) in AML cells, (+)-JQ1 showed single-agent antileukaemic effects in an AML mouse model. Intriguingly, the effect of (+)-JQ1 on leukaemia cells was due, at least in part, to down-regulation of the *MYC* oncogene, an attractive but challenging therapeutic target. A reduction in *MYC* expression was also shown to be partly responsible for the activity of (+)-JQ1 in multiple myeloma (MM).[134] Similar findings have been reported by Mertz *et al*. who independently demonstrated the MYC-dependent antiproliferative effects of (+)-JQ1 in leukaemia and MM cell lines and showed activity in xenograft models of AML and Burkitt's lymphoma.[98] Encouragingly, (+)-JQ1 showed selectivity for malignant cells in all studies; however, the

reason underpinning the observed selectivity for transformed cells remains to be fully elucidated.

I-BET151 (**12**) showed efficacy against leukaemia cell lines and xenograft leukaemia models containing mixed lineage leukaemia (MLL) gene fusions.[121] The authors suggest that BET bromodomain-containing proteins recruit members of the superelongation complex (SEC) or polymerase-associated factor complex (PAFc). SEC and PAFc are regulators of transcriptional elongation, and since components of these complexes form fusions with MLL proteins in leukaemias, they are crucial to the transforming effects of the translocations. Known MLL target genes, including *BCL2*, *CDK6*, and *MYC*, were down-regulated by I-BET151 treatment, strengthening the association between BET inhibition and *MYC* down-regulation.[97,121]

Because of the potential for a MYC-targeted agent to act as a cancer therapy,[134] there has been considerable interest in establishing the effects of a *MYC*-downregulating compound, such as a BET bromodomain inhibitor, in other tumour types.[177] Indeed, the antiproliferative effects of BET bromodomain inhibitors in diverse leukaemias,[120,178-181] lymphomas[182-185] and myelomas[186] are at least in part dependent on MYC. Three BET inhibitors [I-BET762 (**1**) from GSK, OTX015, a triazolodiazepine licensed by OncoEthix from Mitsubishi Tanabe, and CPI-0610, a compound of unknown structure from Constellation Pharmaceuticals] are currently in early-stage clinical trials for haematological malignancies.

Recent work has shown that a range of solid tumours is also sensitive to BET inhibition. In many cases, the mode of action appears to be MYC-dependent. Cancers in which *MYC* or *MYCN* are frequently amplified or overexpressed are often particularly sensitive. These include neuroblastomas,[140,187] medulloblasto-

mas,[188,189] lung adenocarcinomas[190] and prostate cancer.[191,192] However, in some cancers, the effects of BET inhibition are largely *MYC*-independent.[193-197] It will be interesting to see whether *MYC* status is a useful indicator of a patient's responsiveness to BET inhibitors.

In the aforementioned malignancies, BET proteins are required to express key genes involved in cancer growth and progression, but are not themselves oncogenic drivers. There is little correlation between BRD4 expression and sensitivity to BET inhibitors, and in fact high BRD4 levels are associated with reduced invasiveness and improved survival in breast cancers.[198] NMC (Section 1.2.4) is unusual in that BET bromodomains are fundamental to the aetiology of the disease. Consequently BET inhibitors are being investigated as potential therapies. Filippakopoulos *et al*. observed that (+)-JQ1 (**3**) led to differentiation, growth arrest, and apoptosis in patient-derived NMC cell lines, phenocopying the effect of BRD4-NUT siRNA knock-down. (+)-JQ1 treatment also led to the down-regulation of BRD4-dependent genes. In patient-derived NMC mouse xenografts, (+)-JQ1 led to tumour shrinkage and increased overall survival rate.[111] Two clinical trials are now underway to investigate BET inhibitors in NMC and other solid tumours: GSK are studying I-BET726 (**1**), while Tensha therapeutics (a spin-off from the Bradner laboratory at the Dana Faber Cancer Centre) is using an undisclosed compound referred to as TEN-010.

1.6.2 BET inhibitors and inflammation

Inhibitors of BET bromodomains have shown potential as anti-inflammatory agents. **2** and **11** were initially identified as up-regulators of ApoA1, leading to I-BET762 (**1**) and I-BET151 (**12**).[112-114,129,130] ApoA1 is the major component of high-density lipoprotein and is associated with anti-inflammatory effects.

Nicodeme *et al*. found that I-BET762 suppressed the inflammatory response in LPS-stimulated macrophages,[112] down-regulating the expression of LPS-inducible cytokines. The susceptibility of a gene to BET inhibition depended on its pattern of chromatin modifications, suggesting that the anti-inflammatory effects of I-BET762 arose through inhibiting BET-chromatin interactions. I-BET762 and I-BET151 also show anti-inflammatory activity *in vivo*, improving survival and down-regulating proinflammatory genes in mouse models of endotoxic shock and bacterial sepsis.[112,130]

BET inhibitors may exert a second, independent anti-inflammatory effect by inhibiting the BRD4–NF-κB interaction (Section 1.2.3).[75] Several BET inhibitors suppress the expression of NF-κB target genes following stimulation.[114,144,155,199] Treatment with BET inhibitors has therefore been suggested to reduce inflammatory kidney damage in HIV-associated kidney disease,[199] and as a treatment for cancers in which constitutive NF-κB activity drives proliferation.[155]

1.6.3 BET inhibitors and heart disease

RVX-208 (**24**) has shown promising clinical results in atherosclerotic patients, reducing major adverse cardiac events. It is thought that BET inhibition, by upregulating ApoA1 production, increases levels of high-density lipoprotein and reduces atherosclerotic plaques.[147,200] (Note that *ApoA1* regulation presents a rather unusual example of BET proteins acting as transcriptional co-repressors rather than co-activators.) BET proteins may also play a role in controlling gene expression during heart failure, and (+)-JQ1 (**3**) suppressed hypertrophy in cardiac myocytes and reduced heart failure in a mouse model.[201]

1.6.4 BET inhibitors and viral infection

EBV infection can immortalise B-cell lineages, a process associated with the development of several cancers including Burkitt's lymphoma and Hodgkin's lymphoma.[202] BRD4 is essential for the expression of EBV genes required for immortalisation, as it releases stalled Pol II at viral promoters, enhancing elongation (Section 1.2.4). Palermo *et al*. demonstrated that (+)-JQ1 (**3**) treatment of infected B-cells reduced the expression of EBV immortalising genes, suggesting the potential of BET inhibitors as anti-EBV agents.[105]

Several recent publications have highlighted the possibility of using BET inhibitors to enhance the efficacy of antiretroviral therapy for HIV. Integrated but transcriptionally silent (latent) proviruses may remain in infected cells after antiretroviral therapy is stopped, and constitute a major barrier to the eradication of HIV infection. As noted above (Section 1.2.4), the HIV TAT protein is required for the expression of viral genes, and is antagonised by BRD4. (+)-JQ1 treatment reactivates HIV expression, thereby making latent cells susceptible once again to antiretroviral therapy.[107,203,204] It has also been suggested that BRD2 maintains HIV latency independent of TAT.[205] BET inhibition therefore appears to be a promising strategy for reversing HIV latency and eradicating viral reservoirs.

1.7 Conclusion

Lysine acetylation is a widespread PTM, occurring on nuclear and cytosolic proteins with diverse functions. It appears to play a particularly important role in influencing gene expression, most obviously through the acetylation of histone N-terminal tails, but also through the modification of transcription factors. In the 15 years since the bromodomain was identified as an KAc-binding domain, it has become apparent that bromodomain-containing proteins mediate many of

the cellular effects associated with lysine acetylation, analogous to the role of the various phosphotyrosine-binding proteins in mediating cellular signalling networks. Bromodomain-KAc interactions are particularly significant in controlling the sub-cellular localisation of proteins, and the recruitment of BCPs to chromatin regions containing certain patterns of lysine acetylation is an important process in the expression of many genes. There is also some evidence that the KAc-bromodomain interaction may play a signalling role: for example, the acetylation of NF-κB and its recognition by BRD4 appears to couple pro-inflammatory stimuli to gene expression changes (Section 1.2.3).

There has been much progress in understanding the significance of many of these bromodomain-KAc interactions, but as the bromodomain is typically found as a component in much larger multi-domain proteins, genetic approaches or gene knockdown are of limited use in dissecting the role of the bromodomain itself in the overall function of the protein. Chemical tool compounds, which show useful selectivity and affinity for a particular bromodomain or class of bromodomains, have already proved invaluable in the study of bromodomains in health and disease. However, as I will elucidate more fully in Chapter 2, having access to multiple compound classes with similar intended targets is important for validating the findings obtained with these compounds, and provides diverse starting points for further lead optimisation. The work described in Chapters 2, 3 and 4 describes the discovery and optimisation of a new class of compounds as BET bromodomain ligands, the SAR insights thus obtained, and cellular studies performed with these compounds. In Chapter 5, knowledge of this bromodomain-binding chemotype is used to develop synthetic KAc-mimicking amino acids, with

potential applications in studies of KAc function and in bromodomain assay development.

Chapter 2: 3,5-Dimethylisoxazole as a Novel Acetyl-Lysine Mimic[ii]

This chapter describes the identification of 3,5-dimethylisoxazoles as a novel KAc mimic, and the development and optimisation of BET bromodomain ligands containing the 3,5-dimethylisoxazole motif. The interactions of these ligands with bromodomains is analysed using X-ray crystallography. Comparison with other bromodomain ligands highlights some key requirements for BET affinity and selectivity.

2.1 Introduction and aims

2.1.1 The need for new bromodomain ligands

Since the publication in late 2010 of the seminal papers by Filippakopoulos *et al*.[111] and Nicodeme *et al.*[112] reporting the discovery of I-BET762 (**1**) and (+)-JQ1 (**3**), there has been an explosion of interest in BET bromodomains, as indicated by the number of publications on the topic (Figure 26). Such compounds have greatly facilitated functional studies of these proteins, uncovering their roles in pathologic states including haematological cancers, solid tumours, inflammation and viral replication. In addition, BET bromodomain inhibitors have been employed to study processes as diverse as the DNA damage response and spermatogenesis. The availability of open-access chemical probes, notably (+)-JQ1, has greatly accelerated research in the field, assisting the rapid progression of BET bromodomain inhibitors into clinical trials.

[ii] This chapter contains work published in Hewings, D. S.; Wang, M.; Philpott, M.; Fedorov, O.; Uttarkar, S.; Filippakopoulos, P.; Picaud, S.; Vuppusetty, C.; Marsden, B.; Knapp, S.; Conway, S. J.; Heightman, T. D. *J. Med. Chem.* **2011**, *54*, 676, and Hewings, D. S.; Fedorov, O.; Filippakopoulos, P.; Martin, S.; Picaud, S.; Tumber, A.; Wells, C.; Olcina, M. M.; Freeman, K.; Gill, A.; Ritchie, A. J.; Sheppard, D. W.; Russell, A. J.; Hammond, E. M.; Knapp, S.; Brennan, P. E.; Conway, S. J. *J. Med. Chem.* **2013**, *56*, 3217. Reproduced with permission from American Chemical Society, copyright 2011 and 2013.

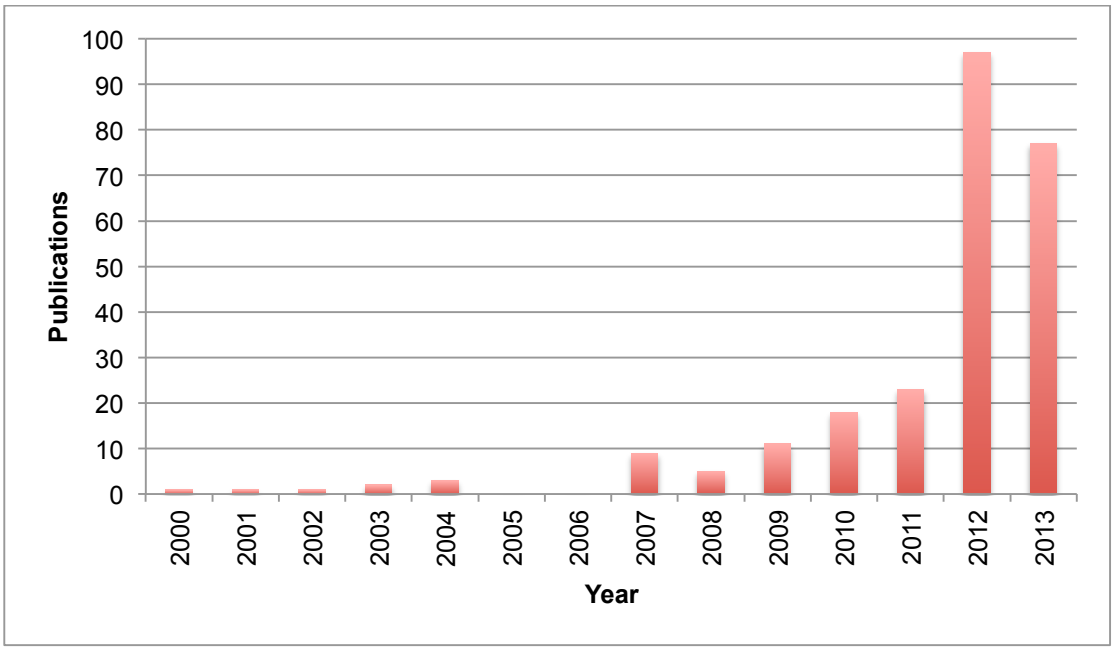


Figure 26. Research into BET bromodomains has greatly increased since the disclosure of the first chemical probes. Number of publications on the topic of BET bromodomains by year. Source: Web of Knowledge, search topic: "BET bromodomain", date 27th January 2014.

Despite the successes of these compounds, there is still a need to generate new potent small-molecule inhibitors of BET bromodomains. Most fundamentally, if several chemically-distinct small molecules with the same molecular target produce similar responses in a biological system, greater confidence can be placed in the conclusion that the response results from modulation of the expected target, and is not an off-target effect. Given the current interest in bromodomains as therapeutic targets, having multiple chemical probes for a given target will lead to more robust target validation studies. Secondly, some of the earliest BET bromodomain probes, including (+)-JQ1 and I-BET762, were derived from phenotypic screens, and the molecular target of this compound class was only later identified. Consequently, at the time of starting this work, no SAR studies had been published for BET bromodomain inhibitors, and only one chemotype, the methyltriazolodiazepines, had been disclosed. There was a

significant need to understand the key factors controlling potency and selectivity for the BET bromodomains, and to integrate this understanding with structural information from X-ray crystallographic studies. Finally, the pharmacokinetic properties of the triazolodiazepine BET inhibitors (+)-JQ1 and I-BET762 are not optimal, with short half-lives *in vivo*.[121] New chemotypes may help to overcome the limitations of these compound series.

2.1.2 A novel assay leads to the identification of a 3,5-dimethylisoxazole-containing BET ligand

The SGC has been at the forefront of developing ligands for chromatin-associated targets, including not only bromodomains[111,122,124,126,127,132,135,136,165] but also methyl-lysine reader domains,[206] methyltransferrases[207,208] and demethylases.[209] The work has been greatly aided by extensive structural characterisation of these protein families, and an detailed analysis of the X-ray structures and binding specificity of over 30 human bromodomains has been published.[44] Central to their efforts in discovering ligands for these targets has been the development of biochemical and biophysical assays to detect and quantify compound binding.

Shortly before the work described herein began, the SGC developed a peptide displacement assay for bromodomains based on AlphaScreen® technology,[119] as described in Chapter 1. The assay was used to identify several fragment inhibitors of bromodomain-histone peptide interactions, including the solvent NMP (**29**). NMP inhibited the binding of a tetraacetylated histone H4 peptide ($H4_{1\text{-}20}KAc_4$) to BRD4(1) with an IC_{50} of 6 mM, and the binding of $H4_{27\text{-}45}$K36Ac to the CREBBP bromodomain with an IC_{50} of 1.9 mM.[119] Despite the weak affinity of NMP, its low molecular weight makes it a highly ligand-efficient fragment (0.54 for

CREBBP), and consequently structurally-related compounds were purchased and tested. One such compound was the dihydroquinazolinone-containing 3,5-dimethylisoxazole **45a** (Figure 27), which showed an unexpectedly low IC_{50} of ~3 μM against BRD2(1) and ~7 μM against BRD4(1). Docking studies carried out by the SGC suggested that two binding modes were possible for this compound, with either the dihydroquinazolinone or 3,5-dimethylisoxazole moiety acting as a KAc bioisostere.[iii] To explore the possibility that the 3,5-dimethylisoxazole might occupy the KAc-binding pocket, an X-ray crystal structure of the complex between human BRD4(1) and **45a** was obtained.

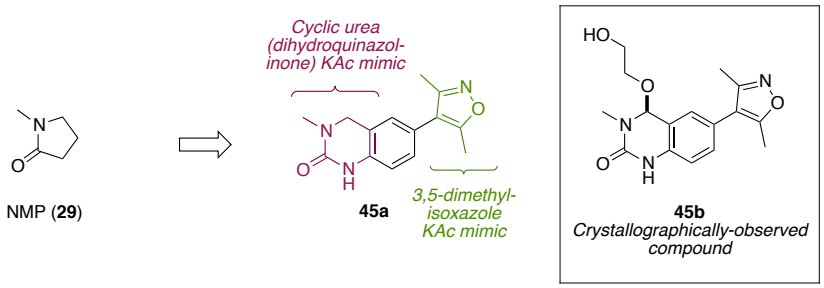


Figure 27. Serendipitous identification of 3,5-dimethylisoxazole as a potential KAc mimic. NMP (**29**) was identified as a fragment hit by AlphaScreen®; **45a** was consequently purchased and showed a low micromolar IC_{50}. Docking studies predict that either heterocycle can act as a KAc mimic. Compound **45b** was observed in the X-ray crystal structure with BRD4(1).

The X-ray crystal structure of the BRD4(1)-**45** complex was solved at 1.98 Å (Figure 28A, PDB ID: 3SVF). It demonstrated that the 3,5-dimethylisoxazole was indeed acting as a KAc bioisostere, accepting a hydrogen bond from the highly-conserved asparagine residue within the binding pocket [N140 in BRD4(1)] and forming a water-bridged hydrogen bond to a conserved tyrosine [Y97 in BRD4(1)], mimicking the key interactions made by the carbonyl oxygen of KAc. Additionally, additional unexplained electron density was observed at the 4-position of the dihydroquinazolinone that was interpreted as a

[iii] Docking study carried out by Minghua Wang, SCG Oxford, using Molsoft ICM-Pro.

substitution of one hydrogen atom by an ethylene glycol unit. As dihydroquinazolinones are liable to benzylic oxidation under laboratory conditions,[210,211] it was hypothesized that **45b** might arise from oxidation at this position to form the corresponding iminium species, followed by addition of ethylene glycol, which is present in the crystallization buffer. (Mass spectrometric data supporting this proposal are presented in Appendix 1). Interestingly, only one enantiomer was observed in the X-ray crystal structure; the ethylene glycol unit attached to the stereogenic centre occupies a hydrophobic groove, referred to as the WPF shelf,[112] formed by W81 and P82 from the ZA loop, and I146 and M149 from helix C. Occupation of this site by the 4-chlorophenyl substituent of (+)-JQ1 and I-BET762 (Figure 28B) is believed to contribute to the affinity and selectivity of the these compounds for the BET subfamily over other bromodomains, since a similar groove is not available in other bromodomains.[111] Compound **45a** displayed significant cytotoxicity in the HeLa human cell line, while (+)-JQ1 showed no cytotoxicity over the same concentration range (see section 2.4.3 and Figure 32).[126]. HeLa cells are insensitive to BET bromodomain inhibition (Section 3.3.1), indicating that **45a** possesses undesirable off-target cytotoxicity. Nonetheless, it appeared that 3,5-dimethylisoxazoles may represent a new class of bromodomain ligands, and a medicinal chemistry effort was required to understand the generality of this potential bioisostere and to develop more potent, stable compounds. This work forms the basis of this chapter.

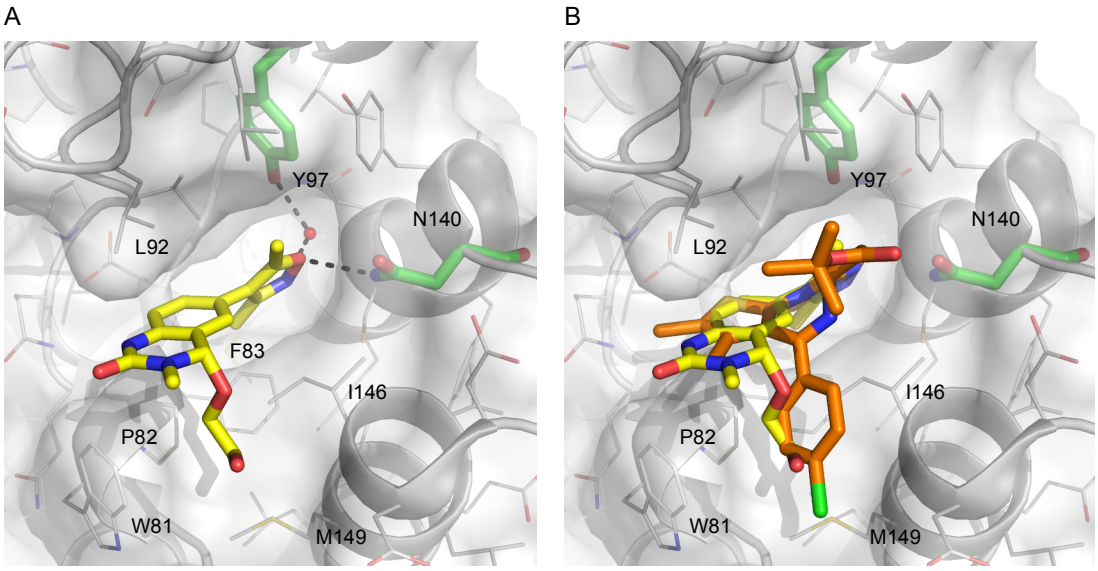


Figure 28. 45b binds with 3,5-dimethylisoxazole mimicking KAc. (A) The X-ray crystal structure of **45b** (carbon in yellow, PDB ID: 3SVF) bound to human BRD4(1). Hydrogen bonds to conserved N140 and to Y97 (via a water molecule) are shown in grey. (B) Overlay of the crystal structures of **45b** and (+)-JQ1 (**3**, carbon in orange, PBD ID: 3MXF)[111] bound to human BRD4(1), showing the pocket from the structure of **45b**.

2.1.3 Aims

The crystallographic and binding data presented above posed several intriguing questions:

- Is the 3,5-dimethylisoxazole an effective KAc mimic, or does the presence of the pendant 2-hydroxyethoxy moiety bias **45b** towards binding with the isoxazole, rather than the cyclic urea, in the KAc binding site?
- Can analogues of **45a** be developed which are not susceptible to oxidation?
- Does the bicyclic urea of **45a** contribute to its affinity for the BET bromodomains?
- Can the selectivity of such compounds for the BET bromodomains be increased?
- Can the cytotoxicity of **45a** be reduced?

Guided by the structural data obtained by the SGC, I wished to confirm and explore the ability of 3,5-dimethylisoxazoles to mimic KAc in binding to bromodomains. More specifically, my aims were as follows:

I. to design and synthesise 3,5-dimethylisoxazole-containing compounds in order to establish whether this moiety was indeed a KAc mimic, and that **45b** was not simply an anomaly resulting from the 2-hydroxyethoxy moiety favouring binding of the isoxazole over the urea.

II. to remove the oxidative liability of the dihydroquinazolinone.

If these aims are successful, then:

III. to improve the potency of these compounds for BET bromodomains, and to improve their selectivity over other bromodomains.

IV. to establish whether the resultant compounds display cellular activity consistent with BET bromodomain inhibition, without the off-target toxicity associated with **45a**. This is further addressed in Chapter 3.

2.2 Design of 3,5-dimethyl-4-phenylisoxazole derivatives

In order to establish whether 3,5-dimethylisoxazoles without the oxidatively-unstable dihydroquinazolinone moiety could bind to BET bromodomains, I chose to synthesise 3,5-dimethyl-4-phenylisoxazoles that did not possess a readily-oxidised benzylic methylene unit. Initial docking studies were carried out using ICM-Pro,[212] with the receptor generated from the BRD4(1)–**45b** complex retaining the four water molecules at the base of the KAc-binding pocket. These studies suggested that a single *meta*-substituent would be directed towards the WPF shelf, occupying a position similar to that of the ethylene glycol moiety of

45b. A second *meta* substituent would be directed into the ZA channel,[112,113] defined by W81 on one side and L92 on the other (Figure 29A). Consequently, the 3,5-dimethylisoxazole derivatives **46a**-**d** and **47a**-**d** were selected as target molecules that contain a range of substituents to explore the WPF shelf and ZA channel (Figure 29B).

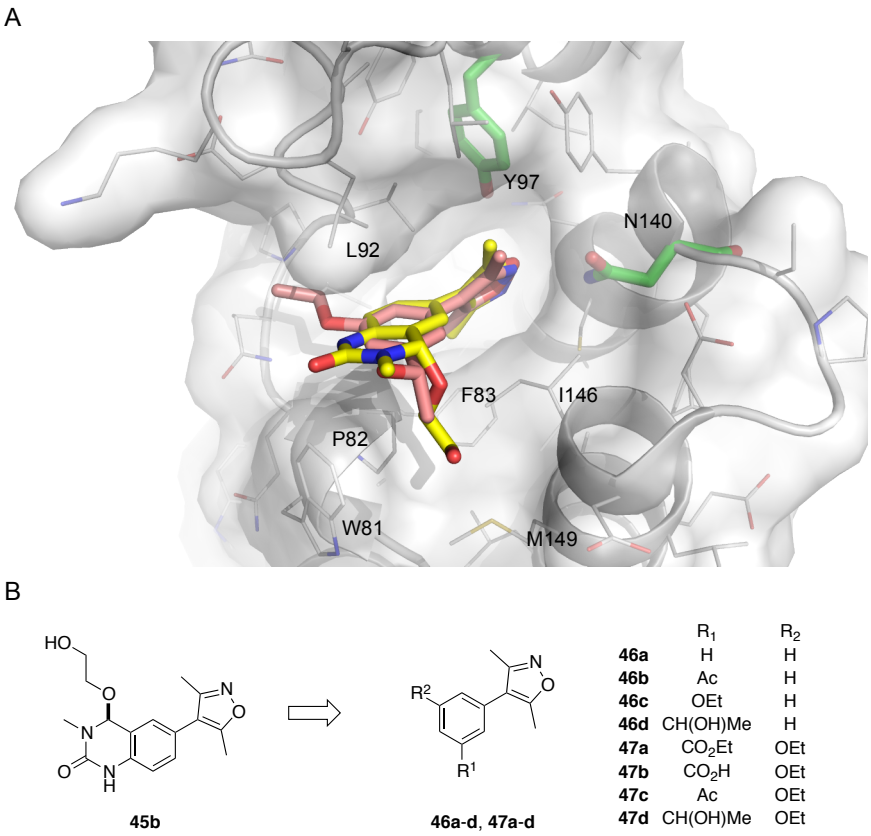


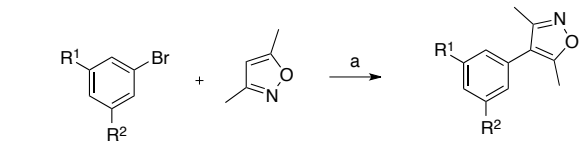
Figure 29. Initial design of 4-phenyl-3,5-dimethylisoxazole derivatives. (A) Predicted binding mode of (*S*)-**47d** (carbon in pink) overlaid with the crystal structure of **45b** (carbon in yellow, PDB ID: 3SVF) demonstrating that *meta* substituents are directed toward the WPF shelf and ZA channel. (B) The concept employed in the design of the 3,5-dimethylisoxazole-based bromodomain inhibitors.

2.3 Synthesis of 3,5-dimethyl-4-phenylisoxazole derivatives

2.3.1 Synthesis of 3'-substituted 3,5-dimethyl-4-phenylisoxazole derivatives

Fall *et al.* recently reported a Pd-catalysed direct arylation of 3,5-dimethylisoxazole using aryl bromides.[213] Given the simplicity of this transformation, initial work focused on applying this protocol for the synthesis of the

target 3,5-dimethyl-4-phenylisoxazole derivatives. While this reaction proceeded well for bromobenzene (**48**), somewhat lower yields were obtained on reaction with **49** and **50** (Scheme 1). (The synthesis of **50** is shown in Scheme 4.) Furthermore, the reaction has been reported to be less successful with electron-rich aryl bromides.[213]

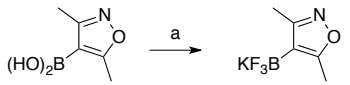


48 R^1 = H, R^2 = H
49 R^1 = Ac, R^2 = H
50 R^1 = CO_2Et, R^2 = OEt

46a R^1 = H, R^2 = H
46b R^1 = Ac, R^2 = H
47a R^1 = CO_2Et, R^2 = OEt

Scheme 1. *Reagents and conditions:* (a)*:* R^1 = H, R^2 = H: $PdCl_2$, KOAc, DMAc, 130 °C, 27 h, 69% (**46a**); R^1 = Ac, R^2 = H: $PdCl_2$, KOAc, DMAc, 130 °C, 20 h, 51% (**46b**); R^1 = CO_2Et, R^2 = OEt: $PdCl_2$, KOAc, DMAc, 130 °C, 44 h, 44% (**47a**).

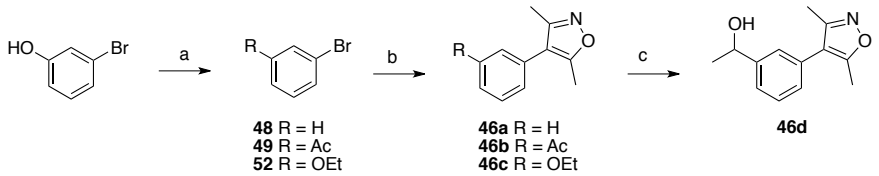
Molander and co-workers have demonstrated that potassium heteroaryltrifluoroborates react efficiently with both electron-rich and electron-deficient aryl halides, with low catalyst loadings and minimal protodeboronation.[214] Therefore potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate (**51**) was prepared from (3,5-dimethylisoxazol-4-yl)boronic acid (Scheme 2) in 84% yield.[214]



51

Scheme 2. *Reagents and conditions:* (a) KHF_2, MeOH, H_2O, rt, 10 min, 84%.

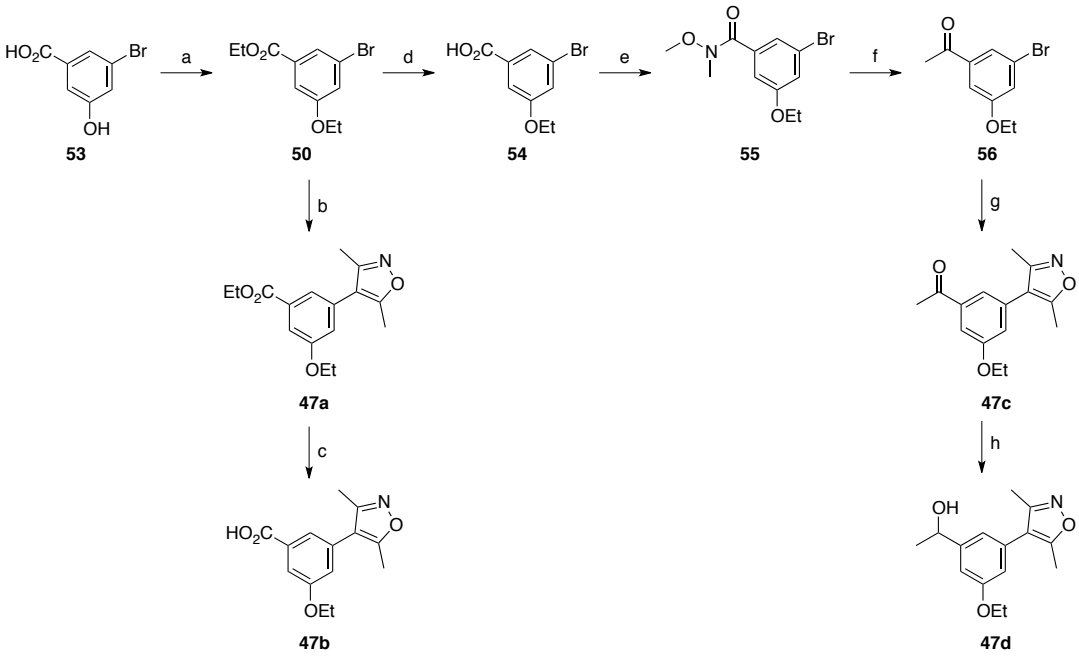
Coupling of **51** to commercially-available aryl bromides **48** and **49**, and aryl bromide **52** (easily prepared from 3-bromophenol following the procedure of Sarju *et al.*)[215] afforded 3,5-dimethyl-4-phenylisoxazole (**46a**) and the 3′-substituted 3,5-dimethyl-4-phenylisoxazoles **46b** and **46c** in excellent yields (≥ 85%). The methyl ketone **46c** was converted to the racemic secondary alcohol **46d** by reduction with sodium borohydride (Scheme 3).



Scheme 3. *Reagents and conditions:* (a) EtBr, K_2CO_3 MeOH, 120 °C (microwave), 20 min, 95% (**52**); (b) **51**, Na_2CO_3, $Pd(OAc)_2$, RuPhos, EtOH, 85 °C; R = H: 4 h, 85% (**46a**); R = Ac: 13 h, 86% (**46b**); R = OEt: 2 h, 87% (**46c**); (c) R = Ac: $NaBH_4$, MeOH, rt, 2 h, 77%.

2.3.2 Synthesis of 3',5'-disubstituted 3,5-dimethyl-4-phenylisoxazole derivatives

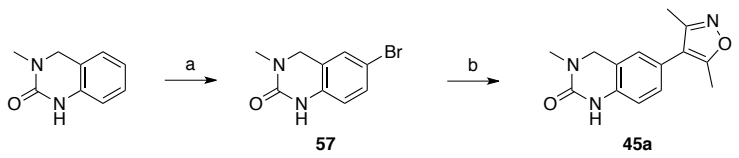
The 3′,5′-disubstituted 3,5-dimethyl-4-phenylisoxazoles **47a**-**d** were synthesised from 3-bromo-5-hydroxybenzoic acid (**53**) (Scheme 4). Alkylation of **53** afforded the ethyl ester **50**, which was coupled to **51**, yielding **47a**. Subsequent lithium hydroxide-mediated hydrolysis furnished the carboxylic acid **47b**. Alternatively, the ethyl ester of **50** was converted to the acetyl group *via* the Weinreb amide **55** in three steps. An initial attempt to convert the ester directly to the ketone with *in situ* formation of the Weinreb amide was unsuccessful.[216] Coupling of **56** to **51** under the same conditions employed above yielded the isoxazole **47c**. A portion of methyl ketone **47c** was then reduced with sodium borohydride to give the alcohol **47d**.



Scheme 4. *Reagents and conditions:* (a) EtBr, K_2CO_3, DMF, 100 ˚C (microwave), 15 min, 96%; (b) **51**, Na_2CO_3, $Pd(OAc)_2$, RuPhos, EtOH, 100 ˚C, 48 h, 70%; (c) LiOH, THF, H_2O, rt, 16 h, 83%; (d) LiOH, THF, H_2O, rt, 23 h, 95%; (e) NHMe(OMe)·HCl, HBTU, $^{i}Pr_2NEt$, DMF, rt, 14 h; 80%; (f) MeMgBr, THF, rt, 15 h, 86%; (g) **51**, Na_2CO_3, $Pd(OAc)_2$, RuPhos, EtOH, 85 ˚C, 64 h, 67%; (h) $NaBH_4$, MeOH, rt, 2 h, 81%.

*2.3.3 Resynthesis of **45a***

To enable accurate comparison with new compounds, hit **45a** was resynthesised (Scheme 5). A sample of 6-bromo-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one **57** had previously been prepared in this laboratory by Tim Rooney, *via* electrophilic bromination of 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one. This was subject to coupling with **51**, giving **45a** in 64% yield.

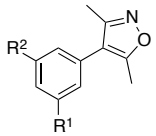


Scheme 5. *Reagents and conditions:* (a) *N*-Bromosuccinimide, DMF, rt, 2 h, 12%; (b) **51,** Na_2CO_3, $Pd(OAc)_2$, RuPhos, EtOH, 110 °C (microwave), 3 h, 64%.

2.4 Evaluation of 3,5-dimethyl-4-phenylisoxazole derivatives as BET bromodomain ligands[iv]

2.4.1 Peptide displacement and protein stabilisation

An assay based on AlphaScreen® technology was used to evaluate whether the compounds **46a**-**d** and **47a**-**d** inhibited bromodomain-histone peptide binding, as described in Chapter 1.[119] For BRD2(1) and BRD4(1), a histone H4 peptide acetylated at K5, 8, 12 and 16 was employed, while for CREBBP a histone H3 peptide acetylated at K56 was used. IC_{50} values for **46a**-**d** and **47a**-**d** are shown in Table 1.



Compound number	R^1	R^2	BRD4(1)[a] IC_{50} (μM)	BRD2(1)[a] IC_{50} (μM)	CREBBP[b] IC_{50} (μM)
45a	-	-	4.8 (4.6-5.1)	n.d.	3.4 (2.3-5.2)
46a	H	H	84.2 (80.0-88.6)	34.3 (29.5-39.8)	>200
46b	Ac	H	24.6 (22.2-27.3)	6.0 (5.6-6.6)	55.7 (43.9-70.7)
46c	OEt	H	23.2 (21.5-25.0)	7.4 (6.7-8.3)	86.9 (82.0-92.0)
46d	CH(OH)Me	H	9.7 (9.3-10.1)	4.2 (3.8-4.6)	45.4 (42.0-49.0)
47a	CO_2Et	OEt	7.7 (7.0-8.5)	3.3 (2.7-4.0)	49.4 (43.5-56.2)
47b	CO_2H	OEt	51.2 (32.6-80.3)	28.2 (26.7-29.8)	32.2 (31.1-33.4)
47c	Ac	OEt	7.5 (7.2-7.8)	2.3 (2.1-2.6)	12.9 (11.7-14.2)
47d	CH(OH)Me	OEt	4.8 (4.5-5.0)	1.6 (1.5-1.6)	28.1 (24.4-32.5)

Table 1. Effect of 3′- and 5′-substituents on competitive displacement of histone peptides from BRD4(1) and BRD2(1), and CREBBP bromodomain. [a] Protein and peptide concentration: 50 nM; [b] Protein and peptide concentration: 100 nM. Heat map shows relative IC_{50} values; red indicates low IC_{50} values and green indicates high IC_{50} values. Ranges in parentheses represent 95% confidence intervals resulting from sigmoidal curve fitting to duplicate data. n.d. = not determined.

With the exception of **47b**, all compounds inhibited BRD4(1) and BRD2(1) with $IC_{50} \leq 50$ μM, the most potent compounds showing low micromolar activity.

[iv] Binding assays and crystallography were performed by The SGC Oxford: AlphaScreen® and ΔT_m^{obs} in this and all subsequent chapters was carried out by Oleg Fedorov and colleagues, X-ray crystallography was carried out by Panagis Filippakopoulos and colleagues.

Introduction of an ethoxy substituent at R^2 increases potency against BRD4(1) and BRD2(1). A variety of substituents at R^1 are well tolerated, with the exception of R^1 = CO_2H in which the positioning of a negatively-charged substituent in a hydrophobic site is likely to disfavour binding. IC_{50} values were generally higher against CREBBP, except for **47b** (R^1 = CO_2H) where the IC_{50} for CREBBP was lower than that for BRD4(1) and similar to that for BRD2(1).

To investigate the selectivity of these compounds against other bromodomains, we employed a binding assay based on a change in protein melting temperature, as described in Chapter 1 (Table 2).[173] Of the compounds that produced substantial changes in melting temperature (ΔT_m^{obs}), most displayed selectivity for BET bromodomains. However, **47b**-**d** also produced an appreciable ΔT_m^{obs} with CREBBP, in agreement with the IC_{50} values. Indeed, **47b** showed selectivity, albeit modest, for CREBBP over the BET bromodomains.

Compound number	R^1	R^2	BAZ2B	BRD2(1)	BRD2(2)	BRD3(1)	BRD3(2)	BRD4(1)	BRD4(2)	BRDT	CREBBP	LOC93349	PB1(5)	PCAF
45a	-	-	0.4	5.4	6.2	7.2	6.8	8.0	5.3	3.2	3.8	-0.2	0.7	-0.1
46a	H	H	-0.1	0.9	2.1	1.6	1.9	2.0	2.6	0.0	0.6	-0.6	0.0	-0.8
46b	Ac	H	-0.4	1.3	2.6	2.0	2.9	2.1	1.9	-0.2	1.2	-0.9	-0.3	-0.8
46c	OEt	H	-0.1	1.3	3.7	2.7	4.2	2.8	4.0	-0.4	0.7	-0.9	-0.2	-0.6
46d	CH(OH)Me	H	0.2	3.3	5.6	5.3	6.5	5.4	6.3	1.6	1.5	-0.8	0.2	0.0
47a	CO_2Et	OEt	0.1	2.3	3.3	3.6	3.8	3.7	3.8	1.1	-0.2	-0.2	-0.1	-1.5
47b	CO_2H	OEt	-0.1	1.1	2.5	2.2	2.6	2.3	2.2	-0.8	2.9	-1.2	-0.1	-0.5
47c	Ac	OEt	0.2	3.2	5.3	4.8	6.0	5.1	6.5	0.2	3.4	-1.0	-0.3	-0.7
47d	CH(OH)Me	OEt	0.3	6.1	8.3	8.5	8.9	8.5	8.0	3.5	4.0	-0.9	0.0	0.0

Table 2. ΔT_m^{obs} of 46a-d and 47a-d against selected bromodomains. Compound concentration: 100 μM; protein concentration: 2 μM. Heat map shows relative ΔT_m^{obs}; red indicates large ΔT_m^{obs} and green indicates small ΔT_m^{obs}. Experiments were carried out in duplicate; average values are reported.

2.4.2 X-Ray crystal structure

The SGC Oxford were able to obtain X-ray co-crystal structures of **47d** with BRD4(1) and **47b** with CREBBP. The co-structure of **47d** bound to BRD4(1)

was obtained at 1.68 Å resolution (PDB ID: 3SVG). Globally, the ligand-bound and apo structure (PDB ID: 2OSS) are very similar, with RMSD of 0.17. This structure confirms that **47d** binds with the dimethylisoxazole oriented into the KAc-binding pocket as anticipated by the aforementioned docking studies. The isoxazole was modelled with the oxygen atom forming the expected hydrogen bond to N–H of the conserved N140, and the nitrogen atom interacting with the phenol group of Y97 *via* a structured water molecule (Figure 30A, B). However, the two heteroatoms cannot be distinguished by electron density alone, so the alternative orientation, with the heterocyclic nitrogen interacting with N140, cannot be ruled out. (Data presented in Chapter 4 suggests that the orientation shown is indeed preferred.)

Although **47d** was synthesised as a racemate, the electron density for this compound implies that the *(R)*-enantiomer crystallizes preferentially with BRD4(1). The secondary alcohol forms a network of hydrogen bonds with water molecules, which interact with D144, D145 and the carbonyl oxygen atom of N140. The methyl group attached to the secondary alcohol is directed towards the hydrophobic residues I146, M149 and W81 that form part of the WPF shelf (Figure 30C). This is the same region that binds the ethylene glycol unit of **45b** and the chlorophenyl moieties of I-BET762 (**1**) and (+)-JQ1 (**3**, Figure 30D). The ethyl ether binds within the ZA channel that accommodates the thiophene moiety of (+)-JQ1 and the methylated phenol of I-BET762, but it does not interact with the water molecules in the channel (Figure 30A, B). Therefore, analysis of the X-ray crystal structure of **47d** in complex with BRD4(1) shows that this compound is forming interactions in many of the same regions as (+)-JQ1 and I-BET, perhaps explaining how a compound with relatively low molecular weight (261) still shows

good affinity for BRD4(1) and reasonable selectivity for the BET subfamily of bromodomains. Indeed, **47d** has ligand efficiencies of 0.39 for BRD4(1) and 0.43 for BRD2(1), which compare favourably with the value of 0.32 for (+)-JQ1 at BRD4(1) (see section 2.7.2 and Table 6 for details of ligand efficiency calculations). These ligand efficiencies suggest that compound **47d** is a promising lead for the development of BET bromodomain ligands.

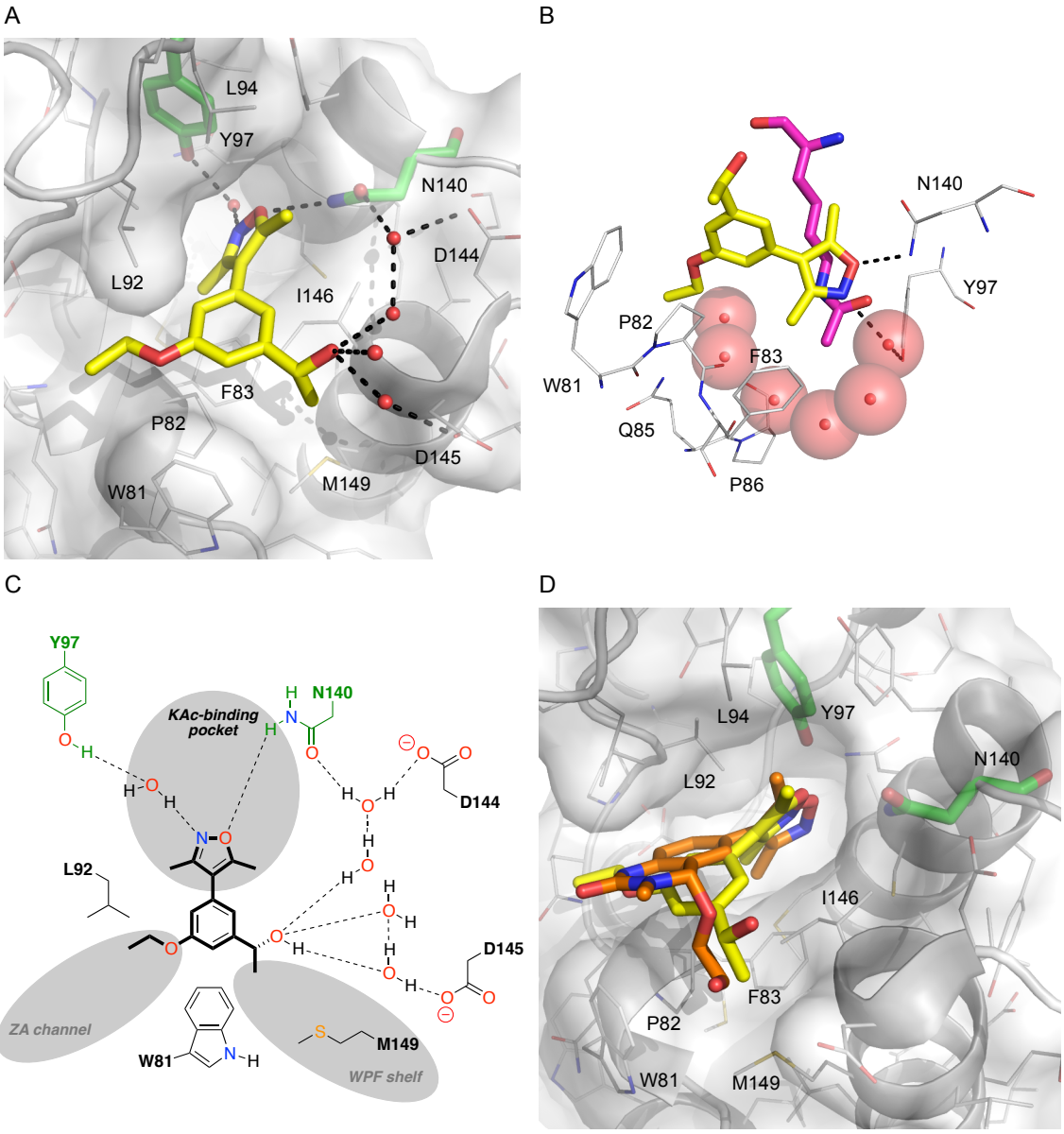


Figure 30. Compound 47d binds to BRD4(1) with 3,5-dimethylisoxazole as a KAc mimic. (A) X-ray crystal structure of **47d** bound to human BRD4(1) (carbon in yellow, PDB ID: 3SVG). The conserved Y97 and N140 residues are highlighted in green. Black lines represent hydrogen bonds between **47d**, structured water molecules (red spheres), and interacting residues. (B) The 3,5-

dimethyisoxazole moiety of **47d** acts as an effective KAc mimic, forming a hydrogen bond with N140 and a water-mediated hydrogen bond with Y97. The structure is overlaid with KAc from a histone peptide ($H4_{1-12}$KAc5KAc8, PDB ID: 3UVW, purple).[44] (C) A schematic representation of the key interactions formed between **47d** and BRD4(1). (D) Overlay of the structures of **47d** and **45b** (carbon in orange, PDB ID: 3SVF) bound to BRD4(1).

At the time of this work, identification of CREBBP bromodomain inhibitors was proving challenging, with only two studies identifying small-molecule ligands for this bromodomain.[14-15] Therefore, given that compound **47b** shows some selectivity for CREBBP bromodomain over other subfamilies, the co-structure of **47b** bound to the CREBBP bromodomain was obtained at 1.80 Å resolution (PDB ID: 3SVH). Compound **47b**, which possesses a carboxylic acid group in place of the secondary alcohol of **47d**, binds to the CREBBP bromodomain in an orientation similar to that adopted by **47d** when binding to BRD4(1). The dimethylisoxazole moiety again acts as a KAc mimic and occupies the KAc-binding pocket. Likewise, the isoxazole oxygen atom forms a hydrogen bond with the amine group of N1168 (equivalent to N140 in BRD4(1), green, Figure 31A). The isoxazole nitrogen atom also interacts with the phenol group of Y1125 [equivalent to Y97 in BRD4(1), green] *via* a structured water molecule. The oxygen atom of the ethoxy group in **47b** forms a hydrogen bond with a structured water molecule, which interacts with the turn-forming residues 1110-1113. Interestingly, this structured water molecule is also present in BRD4(1), but the orientation of the ethoxy group in compound **47d** is such that it does not form a similar hydrogen bond, at least within crystals. Residue W81, which plays a key role in defining the binding site in BRD4(1), is not present in CREBBP. The equivalent amino acid is L1109, which although also hydrophobic, is oriented away from the binding site, providing more space in this region. R1173 forms part of a basic rim to the binding pocket, which is not present in BRD4. The carboxylate functionality of **47b** and guanidinium side-chain of R1173 are separated by around 5 Å (Figure 31A).

While this is too great a distance for direct contact, it is possible that a weak electrostatic interaction might explain, at least in part, the preferential binding of this compound to CREBBP over the BET bromodomains.

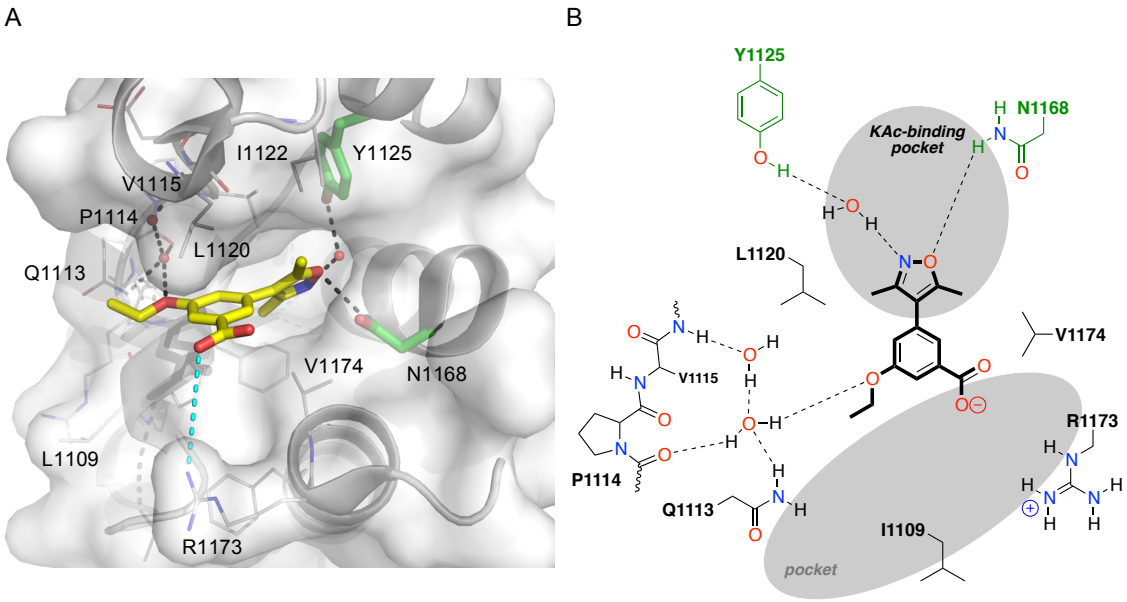


Figure 31: Compound 47b binds to the CREBBP bromodomain with 3,5-dimethylisoxazole as a KAc mimic. (A) X-Ray crystal structure of **47b** bound to the bromodomain of human CREBBP (carbon in yellow, PDB ID: 3SVH). The conserved N1168 and Y1125 residues are highlighted in green. Black dashed lines represent hydrogen bonds between **47b**, structured water molecules (red spheres) and interacting residues. The carboxylate of **47b** and guanidinium group of R1173 are separated by around 5 Å (cyan dashed line). (B) A schematic representation of the key interactions formed between **47b** and the bromodomain of human CREBBP.

It was interesting to note that the dihedral angle between the 3,5-dimethylisoxazole and the phenyl ring of compounds **45b**, **47b** and **47d** differs in the crystal structures obtained. This angle is 50 ˚ in the case of **45a** [BRD4(1)], 45 ˚ in the case of **47d** [BRD4(1)] and 39 ˚ in the case of **47b** (CREBBP). These are all similar to the angle of 40 ˚ in the predicted lowest energy conformation of **47d**,[v] suggesting that these compounds can bind in the KAc-binding pocket without the introduction of significant strain.[126]

[v] Calculation performed by Minghua Wang at The SGC Oxford, in Macromodel (Schrödinger) using OPLS2005 force field.

2.4.3 Investigating non-specific toxicity

As noted above, dihydroquinazolinone **45a** showed appreciable toxicity in HeLa cells, in contrast to (+)-JQ1 (**3**). Since avoiding this off-target toxicity was one of the aforementioned aims, the effect of **47d** on the proliferation of HeLa cells was investigated using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylltetrazolium bromide) assay, which indicates mitochondrial reductase activity (Figure 32).[vi] Pleasingly, **47d** showed no appreciable affect in this cell line at concentrations of up to 10 μM, suggesting that removal of the dihydroquinazolinone moiety mitigated this undesirable toxicity.

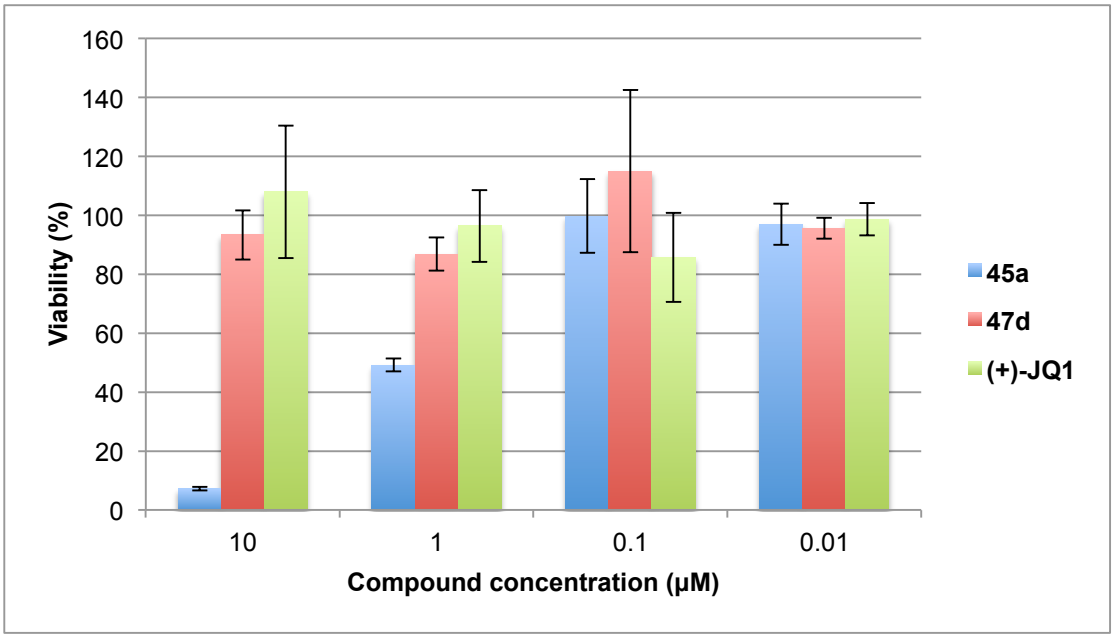


Figure 32. 45a, but not 47d or (+)-JQ1 (3), show significant toxicity in HeLa cells. Cytotoxicity determined by MTT assay, indicating mitochondrial reductase activity. A reduction in viability indicates cytotoxicity in this cell line. Experiments were performed in triplicate. Error bars indicate standard deviation.

[vi] MTT assay performed by Chaitanya Vuppusetty at The SGC Oxford.

2.4.4 Implications for the design of improved isoxazole-containing bromodomain inhibitors

The compounds described above demonstrate that the 3,5-dimethylisoxazoles can act as effective KAc mimics in both the BET and CREBBP bromodomains. Compound **47d** maintains the affinity and ligand efficiency of the initial lead **45a** for the BET bromodomains, but does not contain the oxidatively-unstable dihydroquinazolinone system. It appears from the X-ray crystal structure that the 1,3,5-trisubstituted phenyl core directs substituents into the ZA channel and WPF shelf regions, as predicted by initial docking studies, and represents a useful starting point for the development of more potent BET ligands. Comparison of the structure of **47d** and (+)-JQ1 reveals that the methyl group of the secondary alcohol of **47d** occupies a similar position in the WPF shelf to aromatic substituents of other, more potent, BET bromodomain inhibitors, such as the *para*-chlorophenyl ring of (+)-JQ1. Introducing an aromatic ring to form diarylmethanols in place of the methyl group of **47d** would therefore be expected to increase BET affinity (Figure 33A).

As noted above, the oxygen atom of the ethoxy group of **47b** bound to CREBBP forms water-mediated hydrogen-bonding interactions to the protein, while no such interactions are formed by the ethoxy substituent of **47d** in BRD4(1), despite the presence of a similarly-positioned water molecule. Other BET ligands, such as I-BET151, form hydrogen-bonding interactions with water molecules in the ZA channel. It was therefore of interest to investigate the effect of hydrogen-bond-donating or -accepting substituents on affinity of these BET ligands. Small substituents at this position are likely to interact with the protein *via*

water-mediated interactions, while larger substituents may be able to displace water molecules and interact directly with the protein (Figure 33B).

The X-ray crystal structures suggest that the secondary alcohol is largely solvent-exposed and interacts extensively with water molecules. This may enhance the solubility of these compounds, but it is not clear whether it contributes towards affinity. It is possible that the two enantiomers have different BET bromodomain affinities, given that only one enantiomer was observed in the crystal structure. It would therefore be illuminating to evaluate the affinities of two enantiomeric secondary alcohols. Furthermore, it is not obvious from the structure whether an sp^3 centre at this position is optimal. An sp^2-hybridised carbon would change the orientation of the attached substituent in the WPF shelf and may affect affinity. Removal of the hydroxyl group, or replacement of this sp^3-hybridised carbon with an sp^2 centre, would shed light on the importance of the hydrogen-bonding ability and geometry of this centre (Figure 33C).

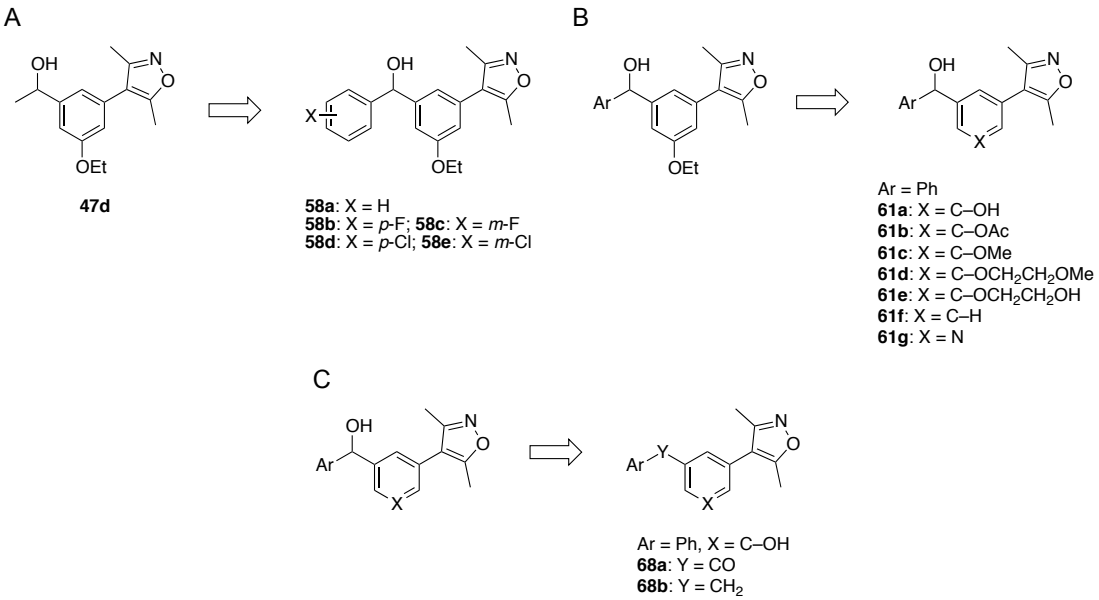


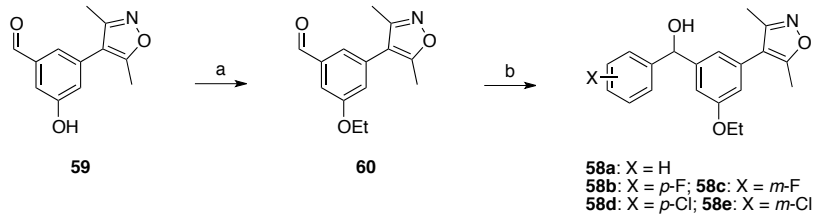
Figure 33: Design of improved BET bromodomain inhibitors. (A) Diarymethanols are expected to increase hydrophobic contact with the WPF shelf region. (B) Hydrogen bond donors and acceptors may interact with or displace water molecules in the ZA channel. (C) Alternatives to

a secondary alcohol between the two rings will affect geometry and hydrogen-bonding ability.

2.5 Synthesis of diarylmethanol derivatives

2.5.1 Synthesis of halogenated compounds

A series of diarylmethanol derivatives **58a**-**e** were designed to enhance interactions in the WPF shelf (Figure 33A). The aldehyde **59** was a common precursor for the synthesis of these compounds, and was synthesised externally, according to a route that I designed.[127] Alkylation of **59** using ethyl bromide and sodium carbonate in methanol under microwave irradiation gave **60**.[215] Addition of the corresponding Grignard reagent, either commercially-available (phenylmagnesium bromide) or formed from the aryl bromide, gave **58a**-**e** in 61-90% yields (Scheme 6).



59 **60**

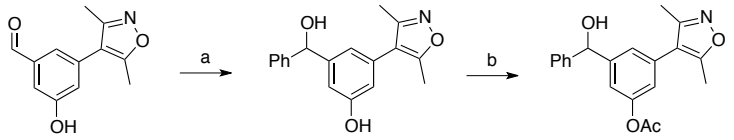
58a: X = H
58b: X = *p*-F; **58c**: X = *m*-F
58d: X = *p*-Cl; **58e**: X = *m*-Cl

Scheme 6. *Reagents and conditions:* (a) EtBr, K_2CO_3, MeOH, 120 °C (microwave), 30 min, 69%; (b) X = H: PhMgBr, THF, rt, 17 h, 80% (**58a**); X = *p*-F: 1-bromo-4-fluorobenzene, Mg, THF, reflux, 3 h then **60**, THF, rt, 61% (**58b**); X = *m*-F: 1-bromo-3-fluorobenzene, Mg, Et_2O, reflux, 2 h then **60**, Et_2O, rt, 2h, 92% (**58c**); X = *p*-Cl: 1-bromo-4-chlorobenzene, Mg, THF, reflux, 2.5 h then **60**, THF, rt, 3 h, 67% (**58c**); X = *m*-Cl: 1-bromo-3-chlorobenzene, Mg, THF, reflux, 1 h then **60**, THF, rt, 2 h, 90% (**58d**).

2.5.2 Modifications to the ZA channel substituent

Evaluation of compounds **58a**-**e** by AlphaScreen® (Section 2.6.1, Table 3) suggested that an unsubstituted phenyl ring was preferred, so this substituent was maintained in a series of compounds designed to probe interactions in the ZA channel (Figure 33B). Phenol **61a** was synthesised from aldehyde **59** by addition of phenylmagnesium bromide and was resolved into (+)-**61a** and (–)-**61a**

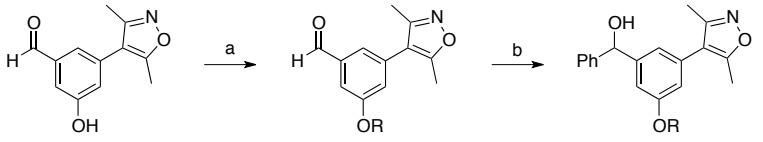
by preparative chiral HPLC.[vii] A sample of (+)-**61a** showed no appreciable racemisation or decomposition after 2 h at room temperature in the aqueous buffer used to perform AlphaScreen® experiments, as indicated by analytical chiral HPLC (Appendix 2). **61a** could be selectively acetylated on the phenol in basic aqueous isopropanol[217] to afford acetate **61b**. Both steps proceeded in excellent yield (Scheme 7).



59 **61a** **61b**

Scheme 7. *Reagents and conditions:* (a) PhMgBr, THF, rt, 1 h, 91%; (b) Ac_2O, NaOH, H_2O, ^{i}PrOH, rt, 1 h, 90%.

To obtain **61c**-**e** (Scheme 8), the phenol moiety of **59** was reacted with either methyl iodide[218] or 1-bromo-2-methoxyethane to form **62** and **63**,[215] followed by addition of phenylmagnesium bromide to afford **61c** and **61d**, respectively. To form **61e**, the phenol **59** was alkylated with 2-bromoethyl acetate in the presence of caesium carbonate, followed by addition of methanol to effect an *in situ* acetate deprotection, affording **64**. Subsequent addition of phenylmagnesium bromide furnished **61e** (Scheme 8).



59

62: R = Me
63: R = CH_2CH_2OMe
64: R = CH_2CH_2OH

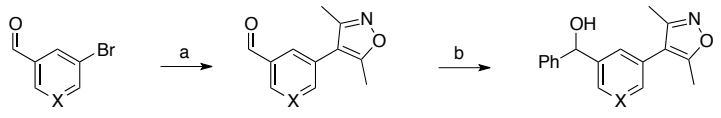
61c: R = Me
61d: R = CH_2CH_2OMe
61e: R = CH_2CH_2OH

Scheme 8. *Reagents and conditions:* R = Me: (a) MeI, Cs_2CO_3, DMF, rt, 2.5 h, 92% (**62**); (b) PhMgBr, THF, 0 °C, 1 h, 76% (**61c**); R = CH_2CH_2OMe: (a) 1-Bromo-2-methoxyethane, K_2CO_3, MeOH, 110 °C (microwave), 30 min, 70% (**63**); (b) PhMgBr, THF, rt, 1.5 h, 83% (**61d**); R = CH_2CH_2OH: (a) 2-Bromoethyl acetate, Cs_2CO_3, DMF, 80 °C, 16 h then MeOH, rt, 1.5 h, 41% (**64**);

[vii] Preparative and analytical chiral HPLC on **61a** carried out by Dr Pavol Jakubec, Department of Chemistry, University of Oxford.

(b) PhMgBr, THF, rt, 3 h, 62% (**61e**).

61f and **61g** were prepared in two steps from 3-bromobenzaldelyde (**65**) or 5-bromo-3-pyridinecarboxaldehyde (**66**) (Scheme 9). Coupling with potassium aryltrifluoroborate **51**[214] to afford **67** and **68,** followed by addition of phenyl magnesium bromide, proceeded in excellent yield in both cases.



65: X = CH
66: X = N

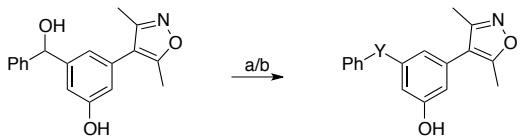
67: X = CH
68: X = N

61f: X = CH
61g: X = N

Scheme 9. *Reagents and conditions:* X = CH: (a) **51**, $Pd(OAc)_2$, RuPhos, Na_2CO_3, EtOH, EtOH, 100 °C, 2.5 h, 82% (**47**); (b) PhMgBr, THF, rt, 45 min, 87% (**61f**); X = N: (a) **51**, $Pd(OAc)_2$, RuPhos, Na_2CO_3, EtOH, EtOH, 100 °C, 2.5 h, 88% (**48**); (b) PhMgBr, THF, rt, 1 h, 75% (**61g**).

2.5.3 Alternatives to the secondary alcohol

The secondary alcohol of **61a** was replaced with a methylene unit or a ketone, in order to investigate the effects of changing the hydrogen-bonding characteristics and geometry at this centre. Alcohol **61a** could be oxidised to ketone **69a** by manganese dioxide. Alternatively, reduction with triethylsilane in trifluoroacetic acid afforded **69b** (Scheme 10).



61a

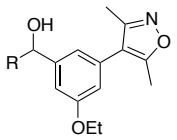
69a: Y = CO
69b: Y = CH_2

Scheme 10. *Reagents and conditions:* (a) Y = CO: MnO_2, 1,4-dioxane, 80 °C, 53 h, 84%; (b) Y = CH_2: Et_3SiH, TFA, rt, 15 min, 70%.

2.6 Evaluation of diarylmethanol derivatives 58a-e, 61a-g and 69a-b as BET bromodomain ligands

2.6.1 Peptide displacement and protein stabilisation

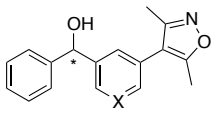
Compounds **58a**-**e** were evaluated by AlphaScreen® for their ability to displace a tetraacetylated histone H4 peptide from BRD4(1) and an acetylated H3 peptide from CREBBP, as described above (Table 3). All compounds showed improved BRD4(1) affinity compared to the lead **47d**. A clear SAR trend is evident for BRD4(1) affinity, and the simple phenyl derivative **58a** is the most potent compound with an IC_{50} value of 640 nM. The fluorophenyl substituents (**58b** and **58c**) are better tolerated than the chlorophenyl substituents (**58d** and **58e**), with *para*-substitution preferred to *meta*-substitution. These data suggest that the increased affinity does result from an interaction with the WPF shelf, but it seems that there is insufficient room for even small ring substitutions to be tolerated. The affinity of all compounds for the CREBBP bromodomain was also increased with compound **58a** again the most potent. However, the selectivity for BRD4(1) over CREBBP was maintained, with compounds **47d** and **58a** both displaying approximately 6-fold selectivity for the bromodomain of BRD4(1). One advantage of compound **47d** was its lead-like ligand efficiency of 0.39 for BRD4(1), and despite an increase in molecular weight, compound **58a** retains a respectable ligand efficiency of 0.41 for BRD4(1).



Compound number	R	BRD4(1)[a] IC_{50} (μM)	CREBBP[b] IC_{50} (μM)
47d	Me	4.8 (4.5-5.0)[c]	28.1 (24.4-32.5)[c]
58a	Ph	0.64 (0.50-0.73)	4.1 (3.6-4.8)
58b	*p*-F-Ph	1.5 (1.4-1.8)	8.6 (7.4-10.0)
58c	*m*-F-Ph	1.7 (1.4-2.0)	6.3 (5.6-7.1)
58d	*p*-Cl-Ph	2.6 (2.5-2.7)	10.8 (8.9-10.1)
58e	*m*-Cl-Ph	3.9 (3.2-4.8)	8.5 (7.2-10.1)

Table 3. IC_{50} values for 58a-e for competitive displacement of histone peptides from BRD4(1) and CREBBP bromodomain. [a] Protein and peptide concentration: 50 nM; [b] Protein and peptide concentration: 200 nM. Heat map shows relative IC_{50} values; red indicates low IC_{50} values and green indicates high IC_{50} values. Ranges in parentheses represent 95% confidence intervals resulting from sigmoidal curve fitting to duplicate data.

Derivatives of **58a** (**61a**-**g**) were prepared to probe interactions with ZA channel water molecules, as discussed above. Their affinity for BRD4(1) and CREBBP was evaluated by AlphaScreen® (Table 4). The phenol **61a** and acetate **61b** showed IC_{50} values of 370-390 nM against BRD4(1), an enhancement in BRD4(1) affinity compared to the ethyl ether **58a**. Conversely, the ethylene glycol, methoxy, methoxyethoxy and pyridine derivatives (**61c-e** and **61g**) had a lower affinity for BRD4(1), with IC_{50} values 3-4–fold higher than phenol **61a**. The un-substituted analogue **61f** gave an IC_{50} about twice that of phenol **61a**. (Note that **61f** and **61g** were tested in AlphaScreen® subsequently to **61a**-**e**; **61a** was also run simultaneously as a control and gave an IC_{50} around twice that shown in the table. Most previous runs gave an IC_{50} of around 0.35-0.40 μM for **61a**.)



Compound number	X	Chirality (*)	BRD4(1)[a] IC_{50} (μM)	CREBBP[b] IC_{50} (μM)	BRD4(1)[c] K_d (μM)
61a	C–OH	*(S)*-(+)	0.382 (0.346-0.420)	0.771 (0.593-1.00)	0.39 (0.37-0.40)
61a	C–OH	*(R)*-(−)	0.386 (0.345-0.420)	1.10 (0.787-1.55)	0.36 (0.30-0.41)
61b	C–OAc	*rac*	0.371 (0.341-0.403)	2.48 (1.95-3.15)	n. d.
61c	C–OMe	*rac*	1.36 (1.18-1.57)	3.96 (3.09-5.07)	n. d.
61d	$C–OCH_2CH_2OMe$	*rac*	1.38 (1.18-1.61)	2.07 (1.58-2.72)	n. d.
61e	$C–OCH_2CH_2OH$	*rac*	1.17 (1.02-1.33)	2.81 (2.33-3.38)	n. d.
61f	C–H	*rac*	1.50 (1.22-1.85)†	n. d.	n. d.
61g	N	*rac*	2.71 (2.53-2.91)†	n. d.	n. d.

Table 4. IC_{50} and K_d values for 61a-g against BRD4(1) and CREBBP bromodomains. [a] Protein and peptide concentration: 50 nM; [b] Protein and peptide concentration: 50 nM. [c] SPR assay. Heat map shows relative IC_{50} values; red indicates low IC_{50} values and green indicates high IC_{50} values. Ranges in parentheses represent 95% confidence intervals resulting from sigmoidal curve fitting to duplicate data. † The IC_{50} for compounds **61f** and **61g** was determined separately; on this occasion, racemic **61a** gave an IC_{50} of 0.808 μM, so these compounds are not included in the heat map.

Both enantiomers of **61a** displayed similar affinity for BRD4(1). This observation was confirmed by SPR using immobilised BRD4(1). K_d values of 0.36 (0.30-0.41) μM for the *(R)*- and 0.39 (0.37-0.40) μM for the *(S)*- enantiomers were in good agreement with those determined by AlphaScreen® and demonstrated 1:1 binding stoichiometry with rapid on and off rates.[127] [viii] Compared to the lead compound **47d**, **61a** maintains the ligand efficiency and improves the lipophilic ligand efficiency (Section 2.7.2, Table 6).

Comparison of IC_{50} values indicates that phenol **61a** is modestly selective (2- to 3-fold) over the bromodomain of CREBBP, while acetate **61b** is somewhat

[viii] SPR carried out by Andrew Gill, Alison J. Ritchie and David W. Sheppard, Biofocus UK.

more selective (~7-fold). The selectivity of **61a** was further evaluated across a phylogenetically-diverse range of bromodomains (Figure 5). AlphaScreen® indicated that compound **61a** displayed less than 25% inhibition of bromodomains contained in this panel at a concentration of 25 μM (Figure 34), with the exception of BRD4(1) and CREBBP. In addition, thermal shift analysis of **61a** against the bromodomains of PCAF and TAF1(1) showed a ΔT_m^{obs} of less than 0.5 ˚C, suggesting very low affinity for these proteins (Figure 34).

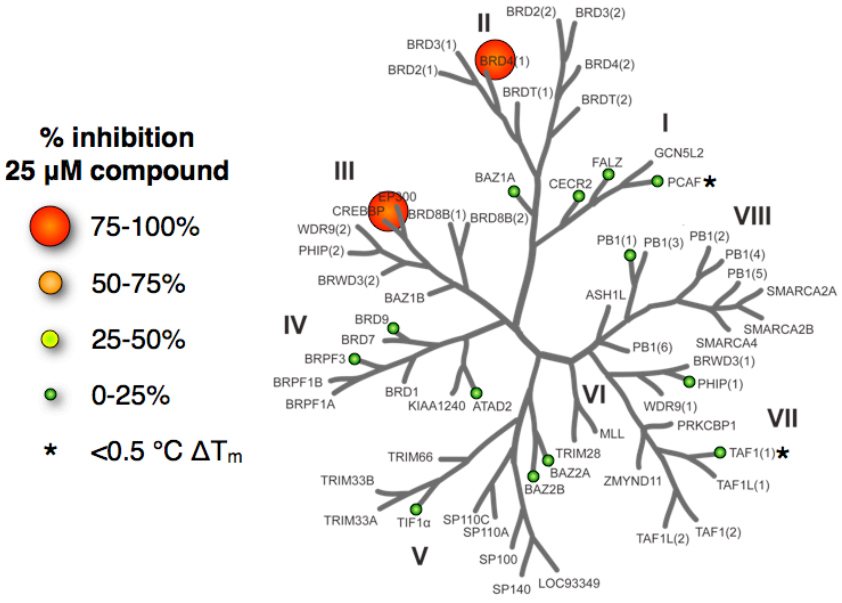
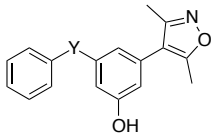


Figure 34. The selectivity of 61a displayed on a phylogenetic tree of bromodomains. Compound **61a** showed ~100% inhibition of the BRD4(1) and CREBBP bromodomains at a concentration of 25 μM. **61a** showed very little inhibition of ATAD2, BAZ1A, BAZ2A, BAZ2B, BRD9, BRPF3, CECR2, GCN5L2, PHIP(1), PB1(1) and TIF1α at a concentration of 25 μM by AlphaScreen®, and little binding to PCAF and TAF(1) by thermal shift analysis. Reprinted with permission from Hewings, D. S.; Fedorov, O.; Filippakopoulos, P.; Martin, S.; Picaud, S.; Tumber, A.; Wells, C.; Olcina, M. M.; Freeman, K.; Gill, A.; Ritchie, A. J.; Sheppard, D. W.; Russell, A. J.; Hammond, E. M.; Knapp, S.; Brennan, P. E.; Conway, S. J. *J. Med. Chem.* **2013**, *56*, 3217. Copyright (2013) American Chemical Society.

Derivatives of **61a**, in which the secondary alcohol was replaced with a carbonyl group (**69a**) or methylene (**69b**), were prepared in order to investigate the effects of hydrogen bonding and geometry at this centre (Table 5). Both

compounds are weaker inhibitors than the alcohol **61a**: ketone **69a** is about 1.4-fold less active while the methylene-containing compound **69b** is about 2-fold less active.



Compound number	Y	BRD4(1)[a] IC_{50} (μM)
61a	CH(OH)	0.808 (0.758-0.861)
69a	C=O	0.544 (0.459-0.645)*
69b	CH_2	1.79 (1.55-2.01)

Table 5: IC_{50} values for 61a, 69a and 69b for competitive displacement of histone peptides from BRD4(1) and CREBBP bromodomain. [a] Protein and peptide concentration: 50 nM; Ranges in parentheses represent 95% confidence intervals resulting from sigmoidal curve fitting to duplicate data. * The IC_{50} for compound **69a** was determined separately; on this occasion, **61a** gave an IC_{50} of 0.38 μM.

2.6.2 X-Ray crystal structure

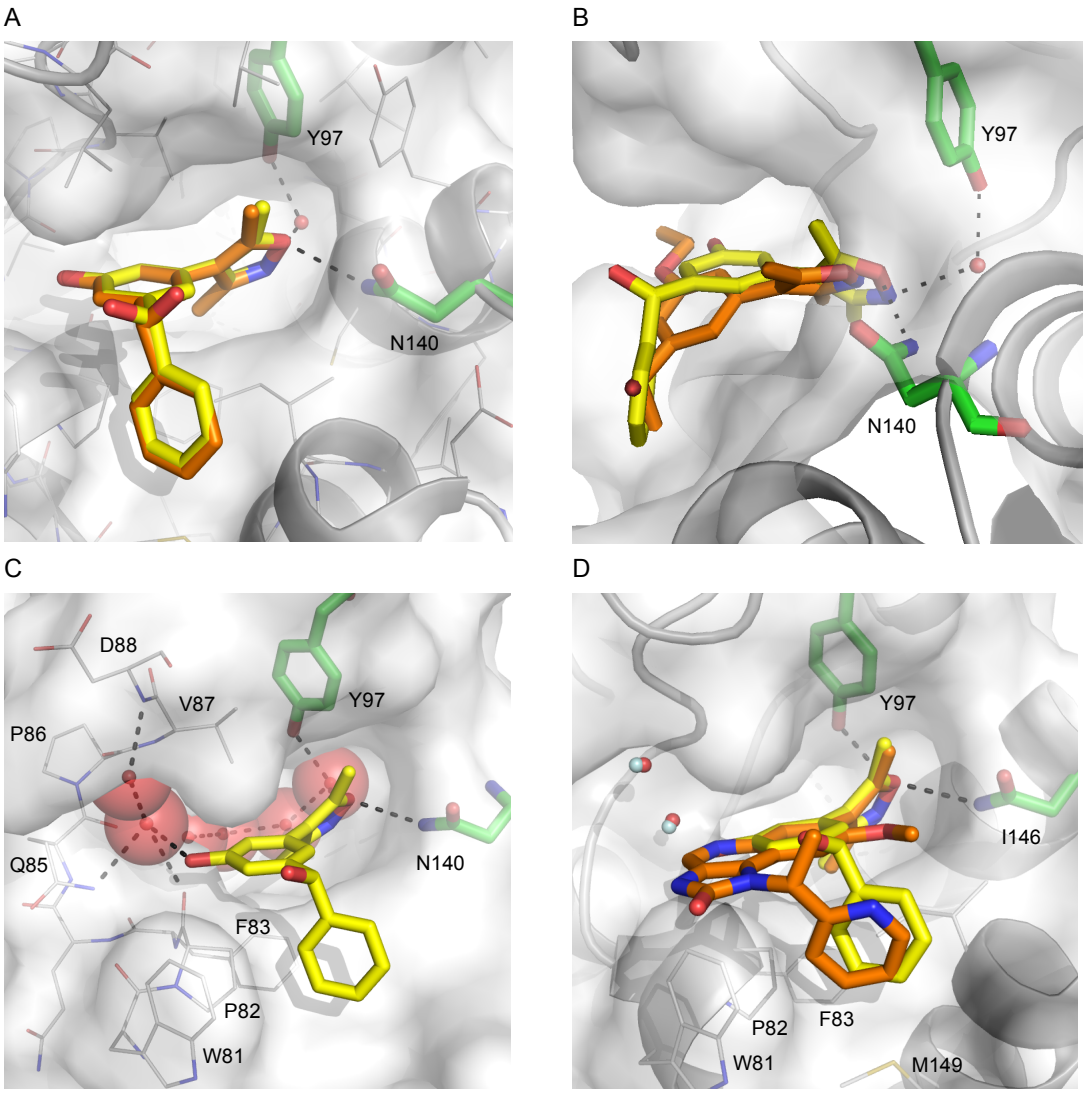
The SGC Oxford were able to obtain X-ray co-crystal structures of (+)-**61a** and (–)-**61a** with BRD4(1). These structures allowed the absolute stereochemistries to be assigned as (*S*)-(+) and (*R*)-(–). The co-structure of (*R*)-**61a** bound to BRD4(1) was obtained at 1.72 Å resolution (PDB ID: 4J0R), and the structure of the (*S*)-**61a**-BRD4(1) complex was obtained at 1.84 Å resolution (PDB ID: 4J0R). The two enantiomers bind in an almost identical manner (Figure 35A), explaining the similar affinity of these compounds for BRD4(1). The isoxazole moiety resides deeper in the KAc-binding pocket than in **47d**, and there is a considerable shift in the position of the core phenyl ring (Figure 35B). In contrast to the ethoxy group of lead **47d**, the phenolic hydroxyl group forms water-mediated hydrogen-bonding interactions to residues in the ZA channel (Figure 35C): the O atom is 2.8 Å from a water molecule that interacts with the backbone C=O of P82 and the side-chain amide of Q85; this molecule is within hydrogen-bonding distance of another water

molecule that in turn interacts with the backbone N–H of D88, all of which form part of the ZA loop. A comparison with the structure of I-BET151 (**12**) shows that the phenol is positioned identically to the quinoline nitrogen, and the water molecules also occupy identical sites (Figure 35D). Overlaying the structure of (*S*)-**61a** with (+)-JQ1 (**3**) reveals that the phenyl ring of (*S*)-**61a** resides in the WPF shelf in a very similar position to the *para*-chlorophenyl ring of (+)-JQ1, although the orientation of the ring is somewhat different (Figure 35E).

The acetate **61b** showed similar, if not greater, affinity for BRD4(1) than phenol **61a**. In an attempt to rationalise its activity, the compound was docked into the crystal structure of (S)-**61a** using AutoDock Vina.[219] [ix] Several possible poses were found with similar predicted affinities, but only those in which the isoxazole binds in the KAc-binding site were considered further. These suggested that either the acetate or the pendant phenyl ring could bind in the WPF shelf. Given the known propensity of the WPF shelf to bind aromatic or hydrophobic substituents, it appears likely that the phenyl ring is directed into that region, although an X-ray structure would be needed to unambiguously demonstrate this. A predicted pose with the pendant phenyl ring in the WPF shelf is shown in Figure 35F. This pose suggests that the phenolic oxygen can form water-mediated hydrogen bonds to the backbone of residues in the ZA channel, as for phenol **61a**. The carbonyl oxygen may also be able to form an additional direct hydrogen bond (predicted distance 2.8 Å) with the main-chain N–H of Q85. The acetate methyl group is directed towards a hydrophobic residue, W81. To form these interactions, the ester is predicted to adopt an otherwise-unfavourable *Z* conformation. Docking therefore appears to suggest a plausible model for the

[ix] Docking in AutoDock Vina carried out by Samuel Grayer, Department of Chemistry, University of Oxford

binding of **61b** to BRD4(1). However, as well as acting as a ligand in its own right, acetate **61b** could serve as a precursor to phenol **61a** in a cellular environment due to esterase activity.



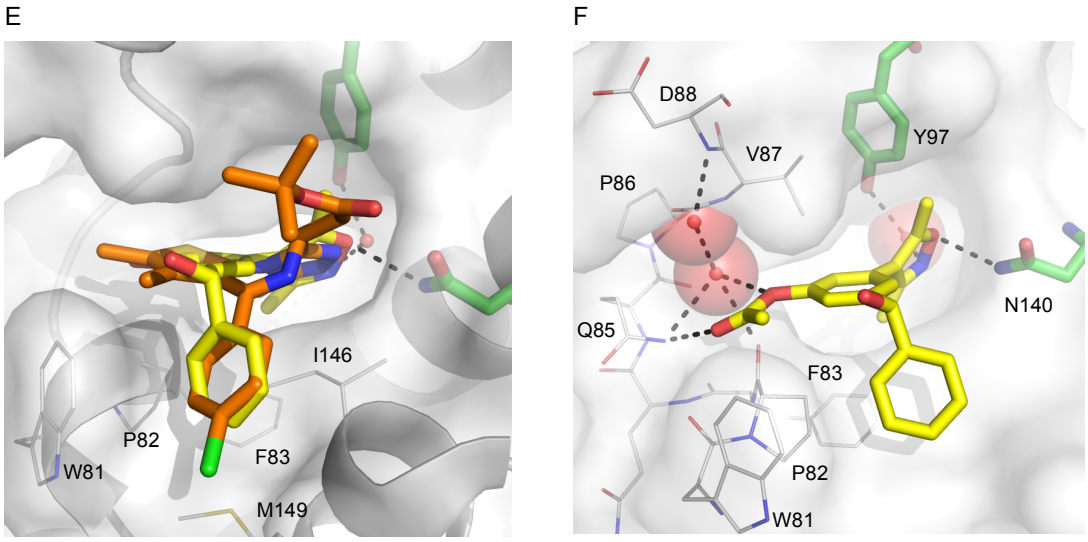


Figure 35. X-ray crystal structures of 61a in complex with BRD4(1). (A) The X-ray crystal structures of (*S*)-**61a** (carbon in yellow, PDB ID: 4J0S) and (*R*)-**61a** (carbon in orange, PDB ID: 4J0R) bound to human BRD4(1) show almost identical binding poses. (B) Overlaying the X-ray crystal structure of (*S*)-**61a** (carbon in yellow) and **47d** (carbon in orange, PDB ID: 3SVG) reveals that the isoxazole of (*S*)-**61a** binds further into the KAc-binding pocket. (C) The phenolic hydroxyl of (*S*)-**61a** forms water-mediated hydrogen-bonding interactions with the ZA loop. (D) Overlaying the X-ray crystal structures of (*S*)-**61a** (carbon in yellow) and I-BET151 (**12**) (carbon in orange, PDB ID: 3ZYU) shows that the phenolic OH and quinoline N occupy identical positions. (E) Overlaying the X-ray crystal structures of (*S*)-**61a** (carbon in yellow) and (+)-JQ1 (**3**, carbon in orange, PDB ID: 3MXF) demonstrates that the pendant aromatic substituents occupy similar positions in the WPF shelf. (F) A predicted binding mode of the acetate **61b**.

2.7 Discussion and conclusion

2.7.1 Binding of 3,5-dimethylisoxazoles to bromodomains

The work presented above constitutes the development of a novel class of BET bromodomain inhibitors. Starting from an unstable, unselective hit **45a**, I initially used structure-guided design, assisted by molecular docking, to develop a lead compound **47d** which employed the 3,5-dimethylisoxazole as a novel KAc mimic.[126] A simple, flexible synthetic route was devised for these compounds, starting from 3,5-dimethylisoxazole-4-boronic acid ad 3-bromo-5-hydroxybenzoic acid, employing the Suzuki coupling of an aryl bromide and a potassium heteroaryltrifluoroborate as the key C–C bond forming reaction. An X-ray crystal structure of **47d** with BRD4(1) confirmed that two *meta* substituents are directed

into the WPF shelf and ZA channel regions. In comparison with **45a**, **47d** showed improved selectivity over the related CREBBP bromodomain, improved stability and reduced toxicity, while maintaining BRD4(1) potency and ligand efficiency. These characteristics suggested that **47d** was an excellent lead for further optimisation into more potent BET ligands. Furthermore, it was demonstrated that 4-phenyl-3,5-dimethylisoxazoles could bind to the CREBBP bromodomain. This finding has prompted investigation of this chemotype as BET and CREBBP ligands by Hay and co-workers,[132] which ultimately led to the development of a potent 3,5-dimethylisoxazole-containing CREBBP probe SGC-CBP30 (**37**).[163]

The work presented in Section 2.2 and 2.3 was published in 2011.[126] At the time, the 4-phenyl-3,5-dimethylisoxazoles were only the second KAc-mimicking chemotype for BET bromodomain ligands, after the methyl-triazolodiazepines.[111,112] Very shortly after this initial work, GSK published a series of 4-phenyl-3,5-dimethylisoxazoles including **9** and **10** which also bore substituents at the *meta* position of the phenyl ring.[128] 3,5-Dimethylisoxazole was identified as an KAc mimic by fragment screening, which was elaborated into the final series through structure-based design assisted by a 3D pharmacophore model. GSK also independently identified the 3,5-dimethylisoxazole motif in a phenotypic screen for ApoA1 upregulators, the same approach that identified the methyl-triazolodiazepine I-BET762 (**1**). Optimisation of potency and pharmacokinetic properties led to the BET bromodomain inhibitor I-BET151 (**12**).[121,129,130] Very recently, Constellation Pharmaceuticals have also described isoxazole azepines such as **15** as BET bromodomain inhibitors.[133,220] It seems that 3-methylisoxazles may constitute a ‘privileged scaffold’ for bromodomain ligands.

Having demonstrated that 3,5-dimethylisoxazoles acted as KAc-mimicking bromodomain ligands, I looked to optimise the potency of **47d** for BET bromodomains. Introducing an aromatic substituent directed towards the WPF shelf improved activity against BRD4. This increased affinity is likely to result from hydrophobic interactions of the phenyl ring with W81, P82, I146, the carbon chain of D145 and M149. Somewhat disappointingly, this change did not increase selectivity over the CREBBP bromodomain. This protein does not possess the enclosed hydrophobic channel of the BET bromodomains, and instead has a more open surface that, it had been hoped, would interact more weakly with an aromatic ring than the corresponding region of BET bromodomains. Instead, the IC_{50} against CREBBP also decreased, remaining about 6-fold higher than for the BRD4(1). Halogen substitution on this ring decreased affinity, with *para* substitution being somewhat better tolerated than *meta*, and fluorine being preferable to chlorine.

Little data on substituent effects on aromatic rings in the WFP shelf had been published at the time of this work,[128] but the above trends are broadly consistent with more recent findings. For example, in Constellation Pharmaceutical's isoxazole azepine series, a *para*-chloro substituent on the pendant phenyl ring is somewhat preferred over *meta* substitution, while *ortho* substitution is poorly tolerated.[133] Similar trends were observed during the development of I-BET762.[114] However, not all series show the same SAR trends, since in several cases, *para* substitution is least favourable, and *ortho* substitution is preferred.[128,129,131,135] A possible explanation is provided by examining the orientation of the pendant aromatic ring in the WPF shelf: in the triazolodiazepines I-BE762 (**1**), (+)-JQ1 (**3**), and the Constellation Pharmaceuticals isoxazoloazepine series

(including **15**), as well as in the crystal structure of **61a**, the substituent is directed 'down' the WPF shelf, with the ring almost parallel to the tyrosine residue, giving sufficient space for *meta* or *para* substation. In the crystal structure of I-BET151 (**12**), however, the substituent is directed 'across' this hydrophobic shelf (Figure 36). Modelling the addition of a *meta*- or *para*-chloro substituent to I-BET151 (**12**, PDB ID: 3ZYU) using PyMOL suggested that the chlorine atom would lie within the van der Waals radius of the Asp145 and Met149 residues. Such substitutions may therefore cause the pendant aromatic ring to twist away from its preferred orientation. This suggests that subtle changes in the orientation of this substituent in the WPF shelf can significantly affect the SAR around the ring.

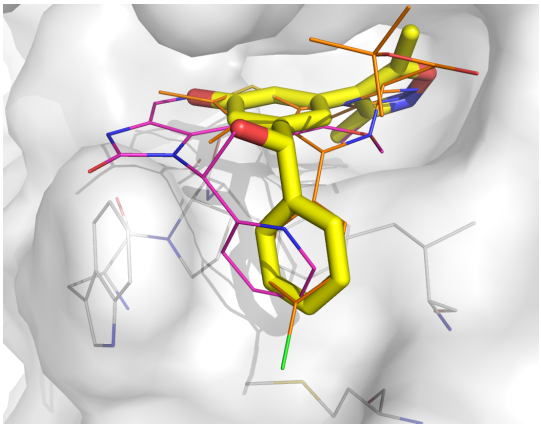


Figure 36. Aromatic substituents in the WPF shelf can occupy different orientations. The structure of (S)-**61a** bound to BRD4(1) (carbon in yellow, PDB ID: 4JOS) is overlaid with the BRD4(1)-bound structures of (+)-JQ1 (**3**, carbon in yellow PDB ID: 3MXF)[111] and I-BET151 (**12**, carbon in purple, PDB ID: 3ZYU).[121]

Replacing the secondary alcohol connecting the two benzene rings with either a carbonyl group or a methylene led only to a slight reduction in potency, with the ketone being the better-tolerated modification. This suggests that stereochemistry and hydrogen bonding ability at this centre does not significantly affect BRD4(1) affinity, consistent with the hydroxyl group of **47d** and **61a** being exposed to solvent.

Varying the second *meta* substituent had only a modest effect on potency. The crystal structure of **47d** with BRD4(1) would suggest that this position is directed towards the water-lined ZA channel, and so substituents bearing hydrogen bond donors and acceptors were investigated. A hydroxyl group or acetate at this position was optimal, and crystal structures of (*R*)- and (*S*)-**61a** suggested that the hydroxyl group could interact with a water molecule in this channel. The pyridine analogue **61g** was less active, suggesting that the nitrogen lone pair is too distant to form the ZA channel water molecule to interact. Removal of any modification at this position (compound **61f**) also led to somewhat reduced activity. Many published BET inhibitors interact with this water molecule. These include I-BET151 (**12**) *via* its quinoline nitrogen,[121,129,130] the related naphthyridine **13** *via* N(1),[131] XD14 (**20**) *via* an amide carbonyl group,[141] the diazobenzenes developed by Zhang *et al.* (e.g. **22**) *via* a diazo nitrogen,[144] and the triazolo-chloropyridone **32** identified by Vidler *et al. via* a triazole nitrogen.[157] None of these studies, however, investigated the contribution of this interaction to binding, or investigated alterative atoms at this position. Based on the above compound series, it appears that the hydrogen bond to a water molecule has a modest but beneficial effect on binding to BET bromodomains. It is intriguing, however, that acetate **61b** is rather more selective than **61a** for BRD4(1) over CREBBP (around 7-fold selective for **61b**, as opposed to around 3-fold for **61a**). There is high sequence homology between BRD4(1) and CREEBP in this region, and all of the residues which interact with water in the ZA channel are the same in both proteins. Docking **61b** into the BRD4(1) structure suggested that the methyl group of the acetate may make hydrophobic contact with the nearby tyrosine residue W91, an interaction not possible in CREBBP where the corresponding residue is L1109

(Figure 35F). It is possible that dynamic structural changes not seen in the crystal structure may also play a role.

2.7.2 Ligand efficiency

Ligand efficiency (LE) indices provide an estimate of binding efficiency with respect to a physiochemical property, such as the compound's weight.[221] LE, which quantifies affinity per heavy atom, and ligand-lipophilicity efficiency (LLE), which takes into account lipophilicity, are shown for **45a**, **47a**, **61a** and **61b** in Table 6. (+)-JQ1 (**3**) is also shown for comparison. From hit **45a** to **47a**, LE was maintained, though LLE decreased somewhat due to an increase in lipophilicity. Phenol **61a** has both an improved LE and LLE compared to **45a** and **47a**. LE and LLE compare favourably to the more lipophilic (+)-JQ1. Overall, these data suggest that compounds such as **61a** show excellent potency with respect to compound size and lipophilicity, and represent high-quality lead compounds for further optimisation.

Compound	BRD4(1) IC_{50} (μM)	pIC_{50}	LE	clogP [a]	LLE
45a (DHQ)	4.8	5.32	0.39	1.5	3.8
47a	4.8	5.32	0.39	2.2	3.1
(*S*)-(+)-**61a**	0.38	6.42	0.41	2.5	3.9
61b	0.37	6.43	0.36	2.6	3.8
(+)-JQ1 (**3**)	0.077	7.11	0.32	4.5	2.6

Table 6: Ligand efficiency (LE) and ligand-lipophilic efficiency (LEE) for 45a, 47a, 61a, 61b and (+)-JQ1 (3). [a] clogP was calculated using ACD/Labs algorithm (version 5.0.0.184).

$pIC_{50} = -\log_{10} IC_{50}$; $LE = \frac{pIC_{50} \times 1.4\ (\text{kcal/mol})}{\text{Heavy atom count}}$; $LLE = pIC_{50} - \text{clogP}$

2.7.3 Conclusion and future work

The work described in this chapter has demonstrated that 3,5-dimethylisoxazoles are able to mimic KAc in their interaction with bromodomains.

Starting from an unstable, unselective and cytotoxic hit **45a**, highly ligand-efficient, stable and non-toxic 4-phenyl-3,5-dimethylisoxazoles were shown to bind to BRD4(1) and CREBBP.[126] The most selective of the compounds was elaborated into BET bromodomain ligands **61a** and **61b** with sub-micromolar IC_{50} values in a peptide displacement assay, and similar K_d values by SPR, while maintaining ligand efficiency.[127] They show some selectivity over the CREBBP bromodomain, and are very selective over other, less closely-related bromodomains (Figure 34). Crystallographic and SAR studies highlighted the importance of hydrophobic, aromatic substituents in the WPF shelf, and substituents capable of forming hydrogen bonds in the ZA channel.

This work, and the work of many others, shows BET bromodomains are highly 'ligandable' targets.[222] In the course of just a few years many diverse chemical series have been disclosed employing several KAc-mimicking moieties, of which the 3,5-dimethylisoxazoles were only the second potent series reported. **61a** and **61b** constitute useful 'tool' compounds for the investigation of BET bromodomain function, especially in the light of cellular data presented in Chapter 3. However, they are not among the most potent or selective BET ligands reported. Based on the SAR and crystallographic studies presented above, several strategies exist for further optimisation:

- The position and orientation of the 3,5-dimethylisoxazole is likely to be close to optimal, judging by comparison to related structures. However, no attempt has been made to alter either methyl group on the isoxazole; such a change may introduce an additional H-bonding interaction to the conserved Asn residue, as seen in the X-ray crystal structures of

ligands including I-BET762 (**1**) and PFI-1 (**16**). Investigating modifications to the methyl groups of the isoxazole form the basis of Chapter 4.

- Substitution on the pendant phenyl ring was only investigated when an ethoxy group was also present on the aromatic core of the molecule (compounds **58a**-**e**). Given that the position of the aromatic core can shift significantly (for example, between **47d** and **61a**), it is possible that substitution may have quite different effects on the free phenol. Furthermore, only *meta*- and *para*-halogen substitution was investigated. *Ortho*-substituents, which are preferred in several series,[128,129,131,135] were not explored, nor were electron-donating or heteroaryl substituents. Likewise, non-aromatic rings, favoured in the compound series investigated by Bamborough *et al*.,[128] were not investigated. I would not anticipate very large gains in affinity from such compounds, but they would provide further useful SAR, helping to dissect out the relative importance of steric, electronic and hydrophobic effects in binding to the WPF shelf, and would test the hypothesis (*vide supra*) that the angle of the aromatic substituent is important for the SAR trends observed.
- Alternatives to the secondary alcohol in **61a** have not been extensively explored. Compounds containing a a carbonyl (**61a**) or methylene unit (**61b**) in this position displayed somewhat reduced potency, but alternative single-atom linkers between the two rings were not investigated. Notably, a tertiary amine or ether linkage may be more rigid than the secondary alcohol, making binding more entropically favoured, although the compounds may be less soluble.

- Of the two most potent compounds developed, acetate **61b** was rather more selective for BRD4(1) over CREBBP. The reason for this apparent selectivity is unclear. Docking suggested that the acetate can form additional hydrogen-bonding and hydrophobic interactions not formed by the phenol, but adopts an unfavourable *E* conformation to do so. Cyclisation to form a 5- or 6-membered lactone (or similar cyclic moiety) would enforce the *E* geometry and may therefore enhance BET potency and selectivity.

No attempt has yet been made to investigate the pharmacokinetics or metabolism of these compounds. It might be anticipated that the unsubstituted phenyl ring of **61a** and **61b** could undergo oxidation by cytochrome P450 enzymes; the methyl groups of the isoxazole may also be liable to oxidation. The phenolic hydroxyl of **61a** may be susceptible to glucoronidation or sulfoconjugation. Before further optimisation is carried out on this compound series, I suggest that it is critical to investigate likely sites of metabolism, initially by determining microsomal stability.

Chapter 3: Cellular Activity of 3,5-Dimethylisoxazole-Containing BET Bromodomain Ligands

*This chapter details the cellular experiments that have been carried out using 3,5-dimethylisoxazole-containing compounds **61a** and **61b**. Their antiproliferative effects were studied in several cancer cell lines. Additionally, **61a** was submitted to the NCI-60 DTP cancer cell line screen; an analysis of the findings and a comparison with screening results for other bromodomain inhibitors is presented. Finally, evidence is presented that **61a** suppresses BRD4-dependent transcription.*

3.1 Antiproliferative effects of 61a and 61b in cancer cell lines

3.1.1 Leukaemia and lung cancer cell lines

As described in Chapter 2, compounds **61a** and **61b** show appreciable inhibition of BET bromodomains in *in vitro* assays. Attention next turned to the cellular effects of these compounds. Previous studies employing small-molecule BET bromodomain inhibitors have shown antiproliferative effects in a number of haematological malignancies, including leukaemias, lymphomas and myelomas.[97,98,121,134] The MV4;11 AML cell line, which harbours an MLL-AF4 fusion, has previously been shown to be sensitive to the BET bromodomain inhibitors (+)-JQ1 (**3**)[97,98] and I-BET151 (**12**).[121] Compounds **61a**, **61b** and **58d** were therefore evaluated for their antiproliferative effects in the MV4;11 cell line using an MTS viability assay (Table 7).[x] **61a** and **61b** had IC_{50} values of 794 and 616 nM, respectively, after 72 h incubation. **58d** was five-fold less active than **61a** and **61b** in this assay (IC_{50} 2.9 μM), consistent with its weaker inhibition of peptide-BRD4(1) interaction by AlphaScreen® (IC_{50} approximately seven-fold higher than

[x] MTS assay carried out with the assistance of Katherine Freeman, Department of Pharmacology, University of Oxford.

61a or **61b**). Similarly, as would be anticipated from its affinity for BRD4, (+)-JQ1 was somewhat more potent.

As discussed in Section 2.4.3, lead **47d** did not posses the non-specific cytotoxicity of dihydroquinazolinone **45a**, with no effect on the proliferation of HeLa cells up to 100 μM. Gratifyingly, **61a**, **61b** and (+)-JQ1 (**3**) also had little effect on the viability of HeLa or U2OS cell lines after 24 h incubation, as indicated by a WST-1 assay (Table 7).[xi] (After 72 h, **61a** and **61b** showed less toxicity than (+)-JQ1 in HeLa and U2OS cells.) These data are therefore consistent with the effects of **61a** and **61b** in MV4;11 cells resulting predominantly from inhibiting BET bromodomains.

The effect of **61a** and **61b** was also investigated in two lung adenocarcinoma cell lines, A549 and H1975 (Table 7).[xii] Both cell lines were much less sensitive than MV4;11 to these compounds, but a considerable decrease in viability was seen at high concentrations (100 μM) in an MTS assay. H1975 appeared to be somewhat more sensitive, a finding confirmed by a clonogenic survival assay (Appendix 3).

Cell line	IC_{50} (μM)			
	61a	**61b**	**58d**	**(+)-JQ1 (3)**
MV4;11 (AML)[a]	0.79	0.62	2.9	0.24
A549 (lung adenocarcinoma)[a]	>10	>10	n.d.	n.d.
H1975 (lung adenocarcinoma)[a]	>10	>10	n.d.	n.d.
U2OS (osteosarcoma)[b]	>100	>100	n.d.	>100
HeLa (cervical adenocarcinoma)[b]	>100	>100	n.d.	>100

Table 7. Effect of 61a, 61b, 58d and (+)-JQ1 (3) in cancer cell lines. [a] Viability was assessed after 72 h incubation using MTS; [b] viability was assessed after 24 h incubation using WST-1. n.d. = not determined.

[xi] WST-1 assay carried out by Christopher Wells, SGC Oxford.

[xii] MTS and clonogenic survival assays carried out by Monica Olcina del Molino, Department of Oncology, University of Oxford.

3.1.2 NCI-60 DTP human tumour cell line screen

61a was also submitted to the NCI-60 DTP human tumour cell line screen.[223] Growth inhibition (GI) was determined after 48 h treatment at 10 μM by sulforhodamine B assay (to indicate cellular protein content). The compound was also evaluated at five concentrations between 10 nM and 100 μM to obtain GI_{50} values, and to indicate the concentration required (if < 100 μM) for total growth inhibition (TGI) (Table 8). A box plot of the data grouped by cancer type is also shown (Figure 37). Leukaemic and renal cancer cell lines are on average most sensitive to **61a**, though particularly sensitive cell lines are also found among NSCLC, CNS and breast cancer cell lines. Activity correlates well between the two assay formats, with only one cell line (NCI-H522) being highly susceptible in the single-concentration assay but not in dose-response format, and one cell line (DU-145) showing negligible activity in the single-point assay but an unexpectedly low GI_{50}.

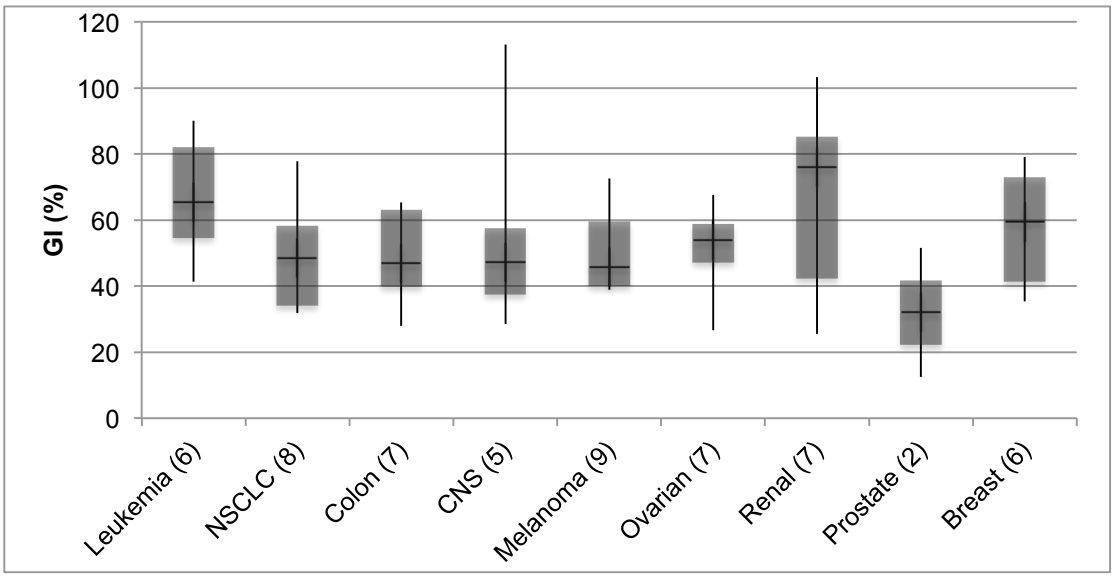


Figure 37. Box plot of growth inhibition by 61a in NCI-60 panel. Plot indicates median, range and quartiles of GI by **61a** after 48 h treatment at 10 μM in the NCI-60 DTP Human Tumour Cell Screen, grouped by cancer type. The number in brackets is the number of cell lines of each type in the NCI-60 panel. (Graphical representation in analogy to Lucas *et al.*)[141]

	Cell line	GI (%)	GI_{50} (μM)	TGI (μM)
Leukaemia	CCRF-CEM	53.3	14.8	
	HL-60(TB)	90.0	2.17	22.7
	K-562	57.9	8.69	
	MOLT-4	41.3	17.9	80.7
	RPMI-8226	85.1	1.94	9.67
	SR	72.9	1.01	
NSCLC	A549/ATCC	44.6	22.4	
	HOP-62	52.8	4.89	
	HOP-92	74.4	1.02	72.9
	NCI-H226	34.5	21.3	
	NCI-H23	31.9	–	
	NCI-H322M	52.4	8.41	
	NCI-H460	32.8	25.1	
	NCI-H522	77.9	27	
Colon	COLO 205	62.9	5.25	
	HCC-2998	37.8	8.55	
	HCT-116	42.0	7.08	
	HCT-15	63.3	3.52	
	HT29	65.4	4.54	
	KM12	46.9	8.2	
	SW-620	27.9	24.6	
CNS	SF-268	28.5	39.3	
	SF-295	57.5	7.31	
	SF-539	37.5	4.56	
	SNB-19	47.3	5.69	
	SNB-75	113.2	0.423	
	U251	–	15	
Melanoma	LOX IMVI	72.7	3.6	80.5
	MALME-3M	40.0	28.9	
	M14	44.0	4.48	
	MDA-MB-435	56.1	3.48	
	SK-MEL-2	39.6	23.5	
	SK-MEL-28	38.8	9.15	
	SK-MEL-5	45.8	5.95	29.5
	UACC-257	59.6	4.66	
	UACC-62	68.5	–	
Ovarian	IGROV1	54.0	23.2	
	OVCAR-3	53.3	6.64	
	OVCAR-4	67.5	6.35	
	OVCAR-5	26.7	31.8	
	OVCAR-8	61.4	8.33	
	NCI/ADR-RES	40.8	6.32	
	SK-OV-3	56.3	7.01	
Renal	786-0	45.2	27.1	
	A498	103.3	2.6	20.3
	ACHN	39.4	4.76	
	CAKI-1	76.2	4.58	
	SN12C	25.5	17.2	
	TK-10	87.4	3.99	
	UO-31	83.1	1.08	
	RXF 393	–	4.58	
Pro-stat-e	PC-3	51.6	–	
	DU-145	12.5	6.95	
Breast	MCF7	79.1	1.7	
	MDA-MB-231/ATCC	37.6	3.92	
	HS 578T	66.3	1.93	90.7
	BT-549	35.3	28.2	
	T-47D	52.9	7.7	
	MDA-MB-468	75.1	1.89	40.6

Table 8. Results of NCI-60 DTP Human Tumour Cell Screen. Growth is determined by sulforhodamine B fluorescence after 48 h incubation with **61a**. GI = 100 – growth (%); growth is measured following treatment at 10 μM and is expressed as a reduction in protein level relative to control (DMSO). GI_{50} is the drug concentration resulting in a 50% reduction in the net protein increase in control cells during the drug incubation. TGI is the concentration resulting in total growth inhibition. The maximum concentration tested was 100 μM; if total growth inhibition was not achieved at this concentration, it can be inferred that TGI > 100 μM. Red indicates greatest activity (high GI, low IC_{50}), green indicates least activity.

3.2 Effect of 61a on BRD4-dependent transcriptional coactivation

*3.2.1 Effect of **61a** on c-Myc levels*

A series of publications in 2011 demonstrated that BET bromodomain inhibitors could suppress *MYC* expression in a variety of cell types.[97,98,121,134] Preliminary data obtained with **61a** suggests that it is also capable of reducing c-Myc levels (Figure 38).[xiii] HCT116 wild-type or p53-null colon cancer cells were treated with **61a** or (+)-JQ1 (**3**). Using an In-Cell Western™ assay, the proportion of viable cells and c-Myc protein levels (normalised to cell number) were determined relative to an untreated control (Figure 38A, B). **61a** displays modest cytotoxicity in HCT116 cells, both p53-null and wild-type (Table 9), and down-regulates c-Myc in a dose-dependent manner (Figure 38A). It also appears that c-Myc is reduced more effectively in p53-null cells than in the p53 wild-type, though further replicates would be needed to confirm this. (+)-JQ1 shows a similar trend (Figure 38B).

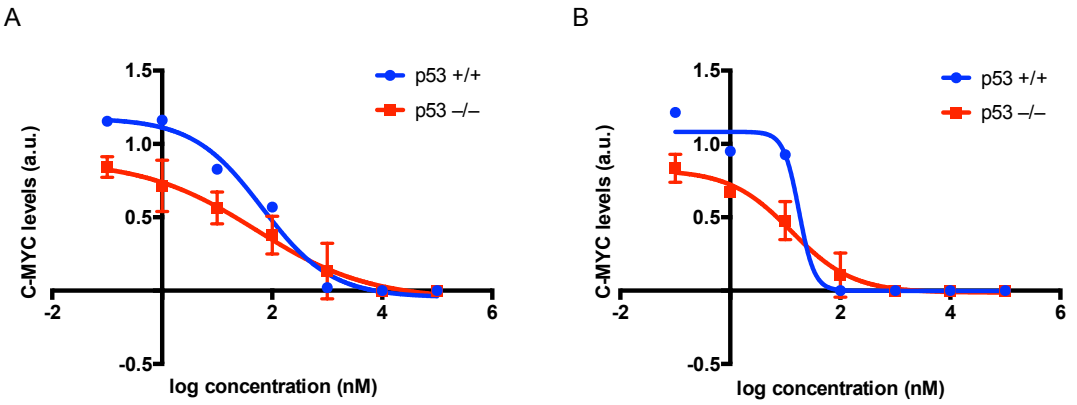


Figure 38. 61a and (+)-JQ1 (3) reduce the cellular levels of c-Myc. 61a (A) and (+)-JQ1 (B) dose-dependently reduce c-Myc levels in both $p53^{-/-}$ and $p53^{+/+}$ cells as determined by In-Cell Western™. Except for the determination of c-Myc levels in $p53^{+/+}$ cells, the data shown are the result of two separate experiments each run in triplicate (n = 2), while for the determination of c-Myc levels in $p53^{+/+}$ cells, the experiment was performed only once (n = 1).

[xiii] In-Cell Western™ carried out by Joana Senra, Department of Oncology, University of Oxford.

	Viability IC_{50} (μM)		c-Myc levels IC_{50} (μM)	
	$p53^{+/+}$ (n = 2)	**$p53^{-/-}$** (n = 2)	**$p53^{+/+}$** (n = 1)	**$p53^{-/-}$** (n = 2)
61a	3.5	3.8	0.086	0.020
(+)-JQ1 (**3**)	0.16	0.22	0.014	0.006

Table 9. IC_{50} values for viability and c-Myc levels in HCT116 cells after 61a or (+)-JQ1 (3) treatment.

*3.2.2 Effect of **61a** on NF-κB-dependent transcription*

In addition to activating transcription through binding to acetylated histones, BRD4 co-activates other transcriptional programmes by binding to KAc on transcription factors. The most studied of these is NF-κB-dependent transcription, where BRD4 binds to acetylated K310 of the RELA subunit and enhances transcriptional activation.[75] Blocking the RELA-BRD4 interaction with (+)-JQ1 (**3**) suppresses TNF-α-mediated NF-κB activation and NF-κB-dependent gene expression.[155] Bruno and Mapp at the University of Michigan have developed a luciferase reporter gene assay to examine the effect of BET bromodomain inhibitors on NF-κB-dependent transcription following IL-1β stimulation (Paul Bruno, personal communication). With the luciferase gene downstream of NF-κB transcriptional response elements, **61a** dose-dependently reduced luciferase activity following treatment with IL-1β (Figure 39). (+)-JQ1 also strongly suppressed luciferase activity while (–)-JQ1, which does not bind to BRD4, showed no effect.[xiv]

[xiv] Luciferase reporter assay carried out by Paul Bruno, University of Michigan.

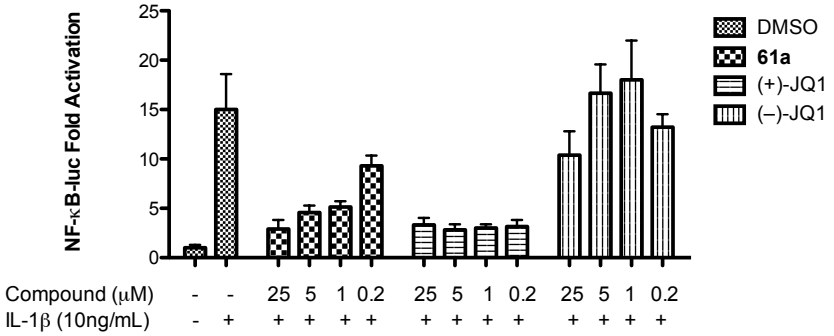


Figure 39. 61a and (+)-JQ1 (3) suppress IL-1β-induced NF-κB-dependent transcription. HeLa cells were transiently transfected with a reporter plasmid containing luciferase and five κB binding sites. Treatment with (+)-JQ1 (**3**) or **61a** for 12 h prior to stimulation with IL-1β suppressed luciferase activity relative to a DMSO-treated control.

3.3 Discussion and conclusion

*3.3.1 Discussion: Cellular effects of **61a** and **61b***

Cellular studies were undertaken by collaborators to establish whether **61a** and **61b** produced the expected phenotypic responses. Both compounds showed little toxicity towards HeLa (cervical adenocarcinoma) or U2OS (osteosarcoma) cells. Likewise, (+)-JQ1 (**3**) shows little effect on HeLa[97,98] or U2OS[97] cells, as demonstrated by our studies and those of others. Other BET bromodomain inhibitor chemotypes have little cytotoxicity in the HeLa cell line.[141] The insensitivity of HeLa cells may be in part due to their overexpression of *MYC* due to viral insertion;[98] HPV is inserted in the proximity of the *MYC* gene,[224] and its expression is therefore likely to be under the control of the viral, rather than the endogenous, promoter. As discussed in Section 1.6.1, the antiproliferative effects of BET inhibition arise, at least in part, due to a reduction in BRD4-dependent *MYC* expression. In HeLa cells, *MYC* expression is not likely to depend on BRD4 binding to promoter regions, and consequently BET inhibition has little effect on c-Myc levels and proliferation. The low cytotoxicity of **61a** and **61b** in HeLa and

U2OS cells therefore suggests that these compounds display little off-target toxicity, in contrast with the initial hit **45a**.

In the MV4;11 leukaemic cell line, by contrast, **61a** and **61b** display sub-micromolar antiproliferative IC_{50} values. A number of BET inhibitors have been evaluated in these cells, both prior to[97,98,121] and after[112,136] these studies. IC_{50} values for growth inhibition range from 26 nM after 72 h of I-BET151 treatment[121] to 1.5 μM after 48 h exposure to PFI-1.[136] Dawson *et al.* have suggested that the efficacy of BET inhibitors in MV4;11 arises from downregulation of key anti-apoptotic/pro-proliferative genes, including *MYC*, *BCL2* and *CDK6*.[121] Others have likewise linked the antiproliferative effects of such compounds in leukaemias to downregulation of the *Myc* pathway.[97,98] MLL fusions, such as the MLL-AF4 fusion found in MV4;11, co-opt the super elongation complex (SEC) and the polymerase-associated factor complex (PAFc) to activate the expression of genes including *MYC*.[225] BRD4 appears to be involved in recruiting these complexes to *MYC* and other key genes involved in leukaemic progression.[121] **61a** and **61b** are therefore likely to exert their antiproliferative effects (at least in part) by displacing BRD4 from chromatin, suppressing the recruitment of the SEC and PAFc to genes such as *MYC*, and consequently inhibiting transcriptional elongation at these genes. Indeed, studies in a colon cancer cell line which shows only modest sensitivity to **61a** suggested that **61a** could downregulate cellular c-Myc levels (Figure 38).

61a and **61b** showed modest antiproliferative activity in two lung adenocarcinoma cell lines, A549 and H1975, with H1975 being somewhat more sensitive. After these studies were carried out, two publications have appeared which investigate the effect of (+)-JQ1 (**3**) treatment on these cell lines. Lockwood *et al.*

report IC_{50} values of 1.5 and 4.2 μM for A549 and H1975 cells, respectively, following 72 h of treatment with (+)-JQ1 and assessment by a viability assay (Resazurin).[197] Contrariwise, Shimamura *et al.* report that H1975 is more sensitive to (+)-JQ1; IC_{50} values of >10 and ~2 μM were determined for A549 and H1975 cells after 72 h treatment, as assessed by the CellTiter-Glo® assay, which detects ATP levels as a surrogate for viability.[190] Lockwood *et al.* claim that growth inhibition in lung adenocarcinoma in response to (+)-JQ1 is Myc independent. Indeed, in H1975 and A549, c-Myc protein and RNA levels remain stable or even increase following (+)-JQ1 treatment. Instead, they suggest that BRD4 inhibition downregulates the transcription factor FOSL1, and that the reduction in FOSL1 target genes is (in part) responsible for the effect of (+)-JQ1 in sensitive cell lines including A549 and H1975.[197] Shimamura *et al.* find A549 to be *unresponsive* to (+)-JQ1.[190] They observe that non-small cell lung cancers harbouring mutations in both the *KRAS* and *LKB1* genes, such as A549, are very unresponsive, whereas mutant *KRAS*/wild-type *LKB1* or cells without *KRAS* mutations are responsive in a *MYC*-dependent manner. Like Lockwood *et al.*, Shimanura *et al.* find that Myc levels in A549 are unaffected by (+)-JQ1, and furthermore they observe that *MYC* knockdown has no significant effect on viability in A549. Shimamura *et al.* also confirm that (+)-JQ1 depletes FOSL1 levels in A549. Taken together, these studies would suggest that any effects seen from BET bromodomain inhibition in A549 or H1975 cells are likely to be *Myc*-independent.

61a was further evaluated by submission to the NCI-60 human tumour cell line screen, and its effects on growth, as indicated by cellular protein levels, was determined both at a single concentration (100 μM) and over a five-point concen-

tration range. The compound showed greatest growth inhibition in leukaemia cell lines, consistent with the known susceptibility of leukaemias to BET bromodomain inhibitors,[98] and also in renal cancers. HL-60(TB) (promyelocytic leukaemia), RPMI-8226 (myeloma), SR (large cell immunoblastic lymphoma), and A498 and UO-31 (renal carcinomas) were particularly sensitive. One NSCLC (HOP-92, large-cell carcinoma), once CNS cancer (SNB-75, glioblastoma), and three breast cancers (MCF7 and MDA-MB-468, adenocarcinomas, and HS 578T, carcinoma) were also sensitive (GI_{50} < 3 μM).

Two other NCI-60 screens using a BET bromodomain inhibitor were available for comparison: 4-acylpyrrole XD14 (**20**, K_d = 240 nM against BRD4(1) by ITC) developed by Lucas *et al.* was profiled in the single-point growth inhibition assay,[141] while for PFI-1 (**16**, IC_{50} = 220 μM against BRD4(1) by AlphaScreen®),[135,136] both single-point growth inhibition and dose-response data were available through the SGC. A comparison of single-point GI and GI_{50} values for the three compounds are shown in Appendix 4, along with box plots for GI with PFI-1 and XD14. PFI-1 and **61a** had similar average activity value across the whole panel (median GI: **61a** = 52.9%, PFI-1 = 59.2%; median GI_{50}: **61a** = 6.5 μM, PFI-1 = 5.5 μM) while XD14 was much less active (median GI: 9.9%). PFI-1 shows a similar GI profile to **61a** when grouped by cancer type (Figure 37 and Appendix 4) with renal cancers, leukaemias and breast cancers being particularly sensitive to PFI-1, and the two prostate cell lines being most resistant. There is a very good correlation (Pearson's product-moment correlation coefficient, r = 0.82) in pGI_{50} values between **61a** and PFI-1 (Figure 40). All of the cell lines which were particularly sensitive to **61a** in the dose-response assay (GI_{50} < 3 μM) are similarly sensitive to PFI-1 (GI_{50} < 4 μM). XD14 showed greatest activity in leu-

kaemias, notably HL-60(TB) and SR (GI > 50%, Appendix 4). However, many cell lines that were sensitive to **61a** and PFI-1 (RPMI-8226, HOP-92, SNB-75, A498, TK-10, U031, MCF-7, HS 578T and MDA-MB-468) were insensitive to XD14 (GI < 30%).

61a, PFI-1 and XD14 have similar *in vitro* affinities for BET bromodomains, perhaps suggesting that XD14 is less cell-permeable or undergoes rapid cellular efflux. Interestingly, **61a** and XD14 show some affinity for the CREBBP bromodomain, while PFI-1 is much more selective for BET bromodomains. The similar profiles of **61a** and PFI-1 in the NCI-60 panel therefore suggest that the effects of **61a** arise from BET, and not CREBBP, inhibition. Overall, it appears that **61a** and PFI-1 have very similar effects in this cell line panel, pointing to a similar mode or modes of action.

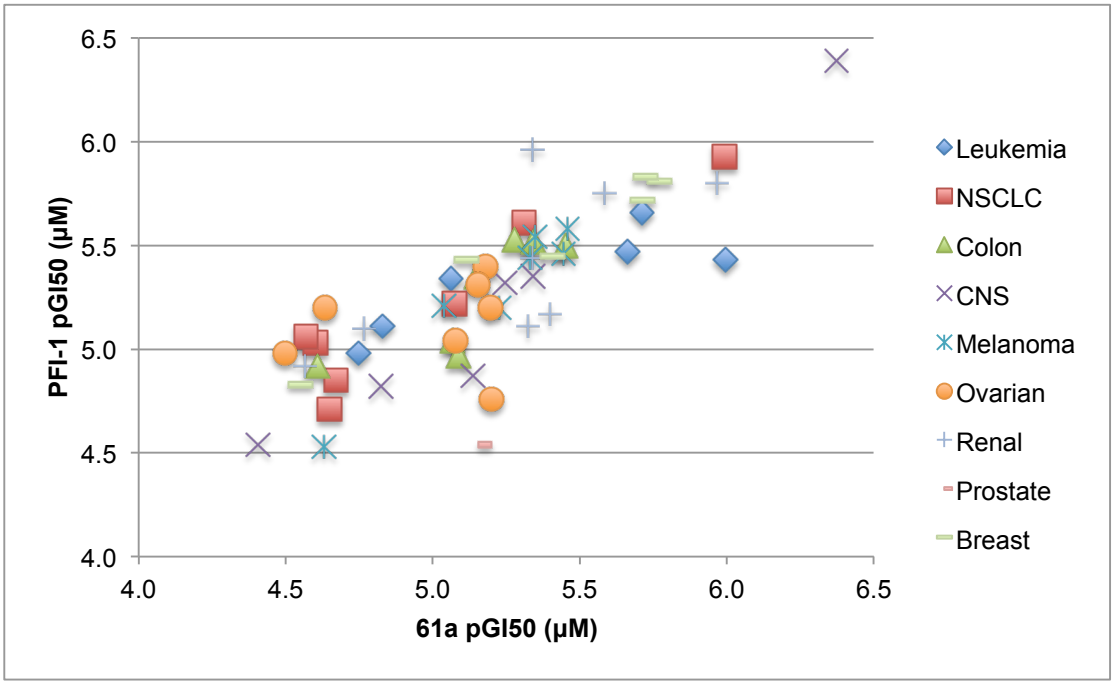


Figure 40. Growth inhibition in the NCI-60 screen by 61a and PFI-1 (16) is strongly correlated. Scatter plot of pGI_{50} values from the NCI-60 screen for **61a** and PFI-1 grouped by tumour type, indicating a good correlation between the two compounds.

The effects of **61a** on viability are consistent with the compound inhibiting BET bromodomain-KAc interactions, but further evidence that **61** can engage BET BCPs in a cellular context was provided by two studies indicating that **61a** downregulates BRD4 target genes. Extensive recent studies have linked BRD4 with c-Myc expression in many cell types, including hematopoietic cancers,[97,98,120,134,136,180,183,184] solid tumours[138,179,188-192,196,197,226] and (non-transformed) T-cells.[227] Treatment of HCT116 cells with **61a** led to dose-dependent suppression of c-Myc levels (Figure 38), consistent with **61a** inhibiting the interaction between BRD4 and acetylated chromatin in the *MYC* promoter region and reducing transcription of the *MYC* gene.[98] Intriguingly, preliminary evidence suggested that the effects of **61a** and (+)-JQ1 (**3**) on c-Myc appeared to be more pronounced in p53-null than p53-wild type cells, although p53 status did not significantly affect viability. Two studies have examined the effect of p53 status on the efficacy of BET bromodomain inhibition: Cheng *et al.* found no significant change in viability following (+)-JQ1 treatment of a primary glioblastoma xenograft line when p53 was knocked down using shRNA;[179] however, Henssen *et al.* in their studies on BET inhibition in medulloblastoma, noted that cell lines harbouring inactivating p53 mutations were less sensitive to (+)-JQ1 than those with wild-type p53. They further demonstrated that an engineered medullobastoma cell line expressing dominant-negative p53 showed an increase in viability and a reduction in apoptosis following (+)-JQ1 treatment, compared to the parental cell line with wild-type p53.[188] Neither studied the effect of p53 status on gene expression following treatment with a BET bromodomain inhibitor. It is quite likely that the effect of p53 status on BET inhibitor sensitivity will be cell-line

dependent, and only a subset of the cellular effects of BET bromodomain inhibition will be dependent on p53 status.

A reduction in NF-κB-dependent transcription in a reporter gene assay following treatment with **61a** suggests that **61a** can also inhibit the interaction of BET bromodomains with acetylated non-histone proteins (Figure 39). In a model developed by Huang *et al*., stimulus-dependent acetylation of RELA at K310 recruits BRD4 to κB enhancers. This in turn recruits pTEF-B, which phosphorylates the CTD of Pol II and activates transcription of NF-κB target genes.[75] BRD4 also suppresses the ubiquitination and subsequent degradation of RELA.[155] Therefore treatment with BET bromodomain inhibitors suppresses NF-κB-dependent transcription.

3.3.2 Conclusion and future work

61a and **61b** are cell-permeable compounds with phenotypic effects including selective antiproliferative activity in sensitive cell lines, and downregulation of genes requiring BRD4 transactivation, as expected for BET bromodomain inhibitors. The cellular data show activity *consistent with*, indeed *strongly suggestive of*, BET bromodomain inhibition, but do not *demonstrate* that the phenotypic effects result from this mode of action. Further evidence could be provided by looking at other cellular responses to compounds such as **61a** or **61b**. Readouts could include the expression of known BRD4 targets by qRT-PCR or Western blot, changes in BRD4 occupancy at promoter regions, cellular engagement of BRD4 through a FRAP assay,[111,124,136,149] or other phenotypic changes such as inhibiting IL-6 production after LPC stimulation (an assay much used by GSK).[97,98,112,114,121,128-131,134,144,156] Rescue of a normal phenotype by overexpres-

sion of BRD4 or its targets alongside **61a** treatment would further support an on-target mode of action.

It would be informative to profile the cellular targets of **61a**, especially given the differences in the profile of **61a**/PFI-1 and XD14 in the NCI-60 screen discussed above. Our laboratory is currently exploring a photoaffinity labelling/pulldown approach to identify the cellular binding partners of **61a**, thus establishing with which bromodomain-containing proteins (and indeed proteins without bromodomains) such compounds interact. Particularly attractive for this approach is ketone **69a**, which although slightly less potent than **61a**, contains a potentially photoreactive benzophenone moiety within the structure.

Chapter 4: Modifications to the Isoxazole at the 3- and 5-Positions

Building on the work described in Chapter 2, this chapter presents the synthesis and evaluation of methyl-isoxazoles designed to introduce additional interactions in the KAc-binding site, and to confirm the preferred orientation of the isoxazole. An initial series of low molecular weight 4-phenylisoxazoles containing modifications to either the 3- or 5-position indicated that substitution at these positions significantly affected BRD4(1) affinity. This series informed the design of analogues of a more potent BET ligand.

4.1 Introduction and aims

4.1.1 Can modifications to 3,5-dimethylisoxazole introduce additional interactions with bromodomains?

The findings presented in Chapter 2 demonstrated that 3,5-dimethylisoxazoles can mimic the interactions of KAc with bromodomains, and that modification of a substituent at the 4-position significantly enhanced potency and selectivity for the BET bromodomains. However, no attempt was made to modify the 3,5-dimethylisoxazole itself in order to develop an improved KAc bioisostere.

The X-ray crystal structures discussed in Section 2.4.2 and Section 2.6.2 indicate that one methyl group of the isoxazole mimics that of KAc. This methyl group resides within a hydrophobic pocket lined by a network of water molecules, which are observed in most published BET bromodomain crystal structures, both *apo* and ligand-bound. At the time of starting this work, no published attempts had been made to interact with or displace these water molecules, although propionyl-lysine and butyryl-lysine were shown bind to BRD4(1), albeit more

weakly than KAc, without displacing these water molecules.[123] It is now well established that displacement of water molecules can contribute to the affinity of a ligand,[228] and this has been exploited in the design of inhibitors.[229-231] Therefore I wished to investigate whether modifying one isoxazole methyl group could increase affinity by displacing these water molecules or by introducing additional interaction with them.

The second methyl group on the isoxazole is directed away from this pocket, and does not seem to form any specific interactions. At the time of starting this work, several ligand-bound bromodomain crystal structures suggested that the conserved KAc-binding asparagine residue could accept a hydrogen bond *via* its carbonyl oxygen, as well as donate a hydrogen bond to the ligand *via* its terminal $–NH_2$. These structures included 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (**28**) bound to the CREBBP bromodomain (PDB ID: 3P1F, d(N···O) = 3.1 Å, Figure 41A) and GW841819X (**2**) bound to BRD4(1) (PDB ID: 3P5O, d(N···O) = 2.9 Å, Figure 41B). The additional possibility of a water-mediated interaction was demonstrated by the structure of I-BET762 (**1**) bound to BRD(1) [Section 1.3.1, Figure 6D, d(N···O) = 3.0 Å, d(O···O) = 2.6 Å]. The introduction of an additional hydrogen bond donor on the isoxazole core may therefore enhance the affinity of isoxazole-containing ligands.

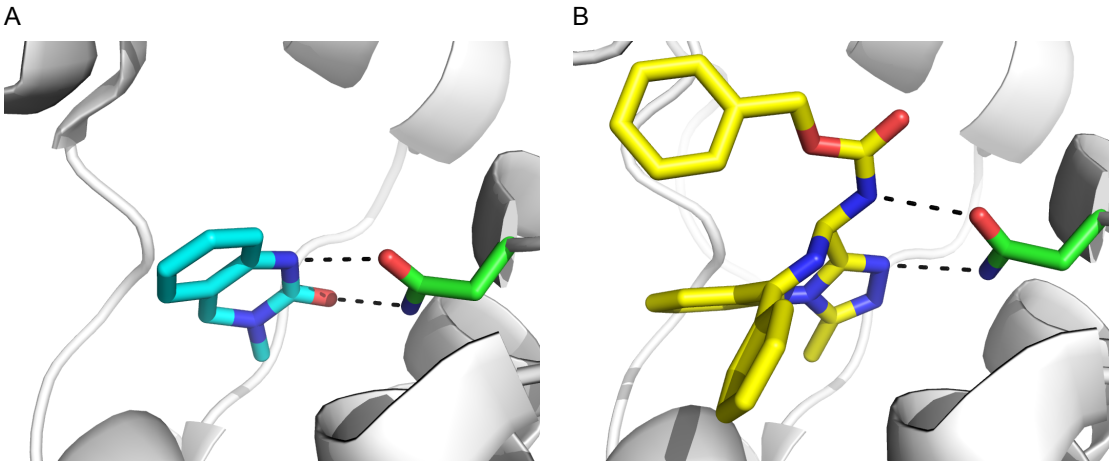


Figure 41. Ligands forming a second hydrogen-bonding interaction to the conserved asparagine residue. (A) 3-Methyl-3,4-dihydroquinazolin-2(1*H*)-one (**28**) bound to the bromodomain of CREBBP (PDB ID: 3P1F, carbon in cyan). (B) GW841819X (**2**) bound to BRD4(1) (carbon in yellow, PDB ID: 2YEL).[113] Hydrogen bonds to conserved asparagine shown in black.

As discussed in Section 2.4.2, and as noted by Bamborough and colleagues at GSK in their work on 4-phenyl-3,5-dimethylisoxazoles,[128] it is difficult to unambiguously distinguish the two heteroatoms of the isoxazole in the crystal structure due to very similar electron densities on N and O. In their crystal structures of 3,5-dimethylisoxazoles bound to bromodomains, the SGC Oxford modelled the isoxazole such that the O atom interacts with the conserved asparagine, and N, the better H-bond acceptor, interacts with tyrosine *via* a water molecule. The methyl group on position 3 mimics the methyl group of KAc. An alternative orientation, however, could not be ruled out; indeed it is also possible that both binding modes are of very similar energy and can bind to bromodomains. Investigating the effect of modifications to the methyl groups may therefore help to establish which of the two binding modes is preferred.

4.1.2 Aims

By synthesising a range of 4-aryl-3,5-dimethylisoxazole analogues in which either the 3- or the 5-methyl group is modified with heteroatoms, I aim to investigate the following questions:

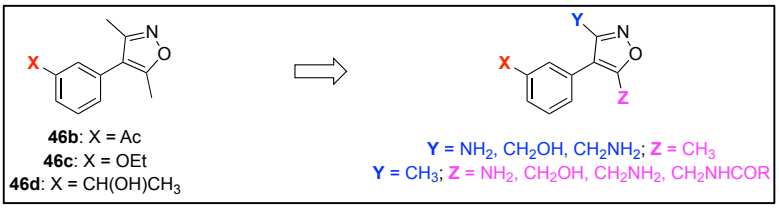
I. can the substituent which mimics the methyl group of KAc be modified so as to interact with or displace water molecules at the base of the KAc binding site?

II. can an additional hydrogen bond donor be introduced to interact with the carbonyl group of the conserved asparagine?

III. what is the most likely binding mode of an isoxazole in the KAc-binding site, i.e. which of the two heteroatoms (N or O) interacts with the conserved asparagine?

4.1.3 Initial compound design

To allow a short synthetic route to a range of modified isoxazoles, I chose initially to prepare 4-phenylisoxazoles containing only a 3′-substituent, analogous to **46b**-**d**. 3-Amino-5-methyl- and 5-amino-3-methylisoxzoles **70**-**71** would place a hydrogen-bond donor adjacent to the isoxazole core, while **72**-**82**, including aminomethyl-methyl- and hydroxymethyl-methylisoxazoles **72**, **74**, **76** and **78**, contain an additional atom between the isoxazole and the heteroatom (Figure 42).



	X	Y	Z
70a	OEt	NH_2	Me
70b	Ac	NH_2	Me
70c	CH(OH)Me	NH_2	Me
71a	OEt	Me	NH_2
71b	Ac	Me	NH_2
71c	CH(OH)Me	Me	NH_2

	X	Y	Z
72a	OEt	CH_2OH	Me
72b	Ac	CH_2OH	Me
72c	CH(OH)Me	CH_2OH	Me
73	OEt	$CONH_2$	Me
74a	OEt	CH_2NH_2	Me
74b	CH(OH)Me	CH_2NH_2	Me

	X	Y	Z
75a	OEt	Me	CO_2Et
75b	Ac	Me	CO_2Et
75a	OEt	Me	CO_2Et
76a	OEt	Me	CH_2OH
76b	Ac	Me	CH_2OH
76c	CH(OH)Me	Me	CH_2OH
77	OEt	Me	$CONH_2$
78a	OEt	Me	CH_2NH_2
78b	CH(OH)Me	Me	CH_2NH_2
79a	OEt	Me	$CH_2NHCO_2{}^tBu$
79b	CH(OH)Me	Me	$CH_2NHCO_2{}^tBu$
80	OEt	Me	CH_2NHCO_2Et
81	OEt	Me	CH_2NHAc
82	OEt	Me	$CH_2NHCONHMe$

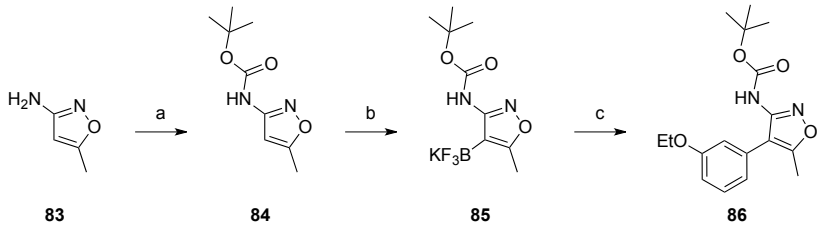
Figure 42. The concept employed in the design of isoxazole-containing bromodomain ligands bearing hydrogen-bond donors at either the 3- or 5-position.

4.2 Synthesis of 3-amino-5-methyl- and 5-amino-3-methylisoxzoles

Inspired by the previous success in forming 4-aryl-3,5-dimethylisoxazoles from potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate **51** and the corresponding aryl bromide, initial efforts focused on developing a synthesis of potassium isoxazolyltrifluoroborate salts containing an appropriately-protected nitrogen at the 3- or 5-position. I was also encouraged by a report from Konoike *et al.* that *N*-Boc-aminoisoxazoles could be selectively lithiated at the 4-position and reacted with a variety of electrophiles.[232] Therefore, I envisioned that the lithiated isoxazoles could be treated with a borate ester to afford a boronic ester, which could

be converted to the potassium trifluoroborate salt *in situ*. Subsequent Suzuki coupling under previously-employed conditions would furnish the desired aminoisoxazoles after deprotection.

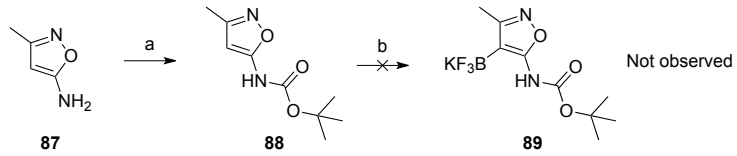
Following the procedure of Konoike *et al.*,[232] 3-amino-5-methylisoxazole (**83**) was Boc-protected in good yield; as they report, this reaction initially gives a mixture of mono-protected and di-protected amine, but the imide is easily cleaved to the carbamate **84** with methanol and sodium hydroxide solution. **84** underwent directed lithiation with 2.2 equivalents of *n*-butyllithium to form the presumed dianion, which was treated with trimethyl borate. Addition of aqueous potassium hydrogen difluoride *in situ* followed by Soxhlet extraction with acetone afforded the desired trifluoroborate **85** in 56% yield, which was then coupled to bromide **52** to form **86** (Scheme 11).



Scheme 11. *Reagents and conditions:* (a) (i) Boc_2O, DMAP, THF, reflux, 3 h; (ii) aq. NaOH (1 M), MeOH, rt, 23 h, 85%; (b) ^{n}BuLi, THF, −78 °C, 1 h, then rt, 30 min, then $B(OMe)_3$, −78 °C, 1 h, then rt, 30 min, then KHF_2, H_2O, rt, 56%; (c) **52**, $Pd(OAc)_2$, RuPhos, Na_2CO_3, EtOH, 80 ˚C, 18 h, 46%.

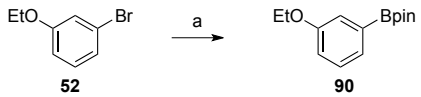
Regioisomeric amine **88** was Boc-protected, and lithiation of the resultant carbamate **87** was attempted. Konoike *et al.* report that *n*-butyllithium does not effectively lithiate **87**, and instead *sec*-butylllithium in THF/TMEDA at −50 °C is required.[232] However, employing these conditions, followed by sequential treatment with trimethyl borate and potassium hydrogen difluoride as before, did not yield any of the desired compound **89** (Scheme 12). In order to identify whether the lithiation was proceeding as expected, alternative electrophiles were used. A

quench with allyl bromide followed by an aqueous work-up gave none of the expected product. Finally, the TMEDA was freshly distilled, and the *sec*-butyllithium/TMEDA complex was preformed and titrated against diphenylacetic acid to verify its concentration. Methanol-d_4 was then used as an electrophilic quench. Disappointingly, ^{1}H NMR analysis of the crude reaction mixture revealed only partial lithiation of C(4), with C(4)*H* integrating to ~0.6.



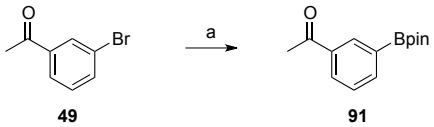
Scheme 12. *Reagents and conditions:* (a) (i) Boc_2O, DMAP, THF, reflux, 14 h; (ii) aq. NaOH (1 M), MeOH, rt, 19 h, 91%; (b) ^{s}BuLi, THF, TMEDA, −78 °C, 1.5 h, then rt, 30 min, then $B(OMe)_3$, −78 °C, 1 h, then rt, 30 min, then KHF_2, H_2O, rt.

Given the difficulties in preparing the desired potassium isoxazolyltrifluoroborates, I next explored using the isoxazole as the halogenated component in the Suzuki coupling, and the substituted benzene derivative as the organoboron component. Bromide **52** (prepared as described in Scheme 3) was lithiated and treated with isopropoxyboronic acid pinacol ester to afford boronic ester **90** (Scheme 13).



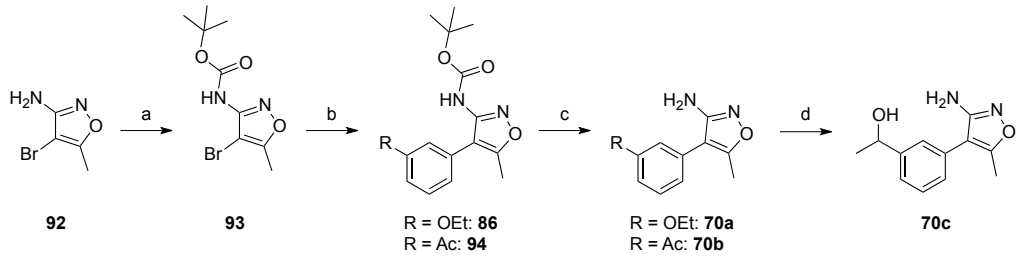
Scheme 13. *Reagents and conditions:* (a) ^{n}BuLi, THF, –78 ˚C, 1.5 h then ^{i}PrOBpin, –78 ˚C, 1 h then rt, 1h, 79%.

Boronic ester **91** was prepared by Pd-catalysed borylation of 3′-bromoacetophenone **49** following the procedure of Ishiyama (Scheme **14**).[233]



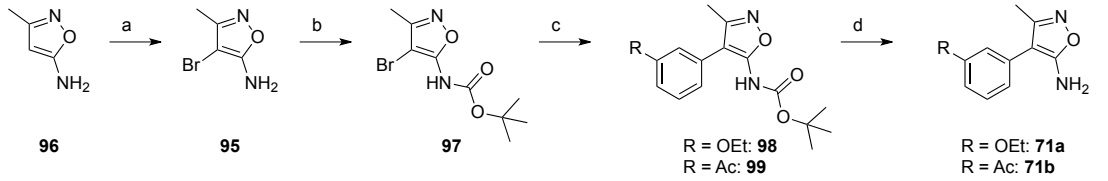
Scheme 14. *Reagents and conditions:* (a) B_2pin_2, KOAc, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, DMSO, 90 ˚C, 15 h, 78%.

Commercially-available 4-bromo-5-methylisoxazol-3-amine **92** was Boc-protected, and **93** was subsequently coupled to boronic esters **90** and **91**. The products **86** and **94** were deprotected with TFA in dichloromethane to afford the free amines **70a** and **70b**. Free amine **70b** was also obtained after the coupling of **91** and **93**; this material was combined with the product of the TFA deprotection, giving **70b** in a combined yield of 32% over two steps, a portion of which was reduced to secondary alcohol **70c**. The ethoxy-substituted analogue **70a** was obtained in a 31% yield over two steps (Scheme 15).



Scheme 15. *Reagents and conditions:* (a) (i) Boc_2O, DMAP, THF, reflux, 4 h; (ii) aq. NaOH (2 M), MeOH, rt, 18 h, 84%; (b) R = OEt: **90**, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, Na_2CO_3 (2 M aq.), DME, 90 °C, 24 h, 50% (**86**); R = Ac: **91**, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, Na_2CO_3 (2 M aq.), DME, 90 °C, 37 h, 52% (**94**), 5% (**71**); (c) R = OEt: TFA, CH_2Cl_2, Et_3SiH, rt, 2 h, 61% (**70a**); R = Ac: TFA, CH_2Cl_2, rt, 1 h, 38% (**70b**, over 2 steps); (d) R = Ac: $NaBH_4$, MeOH, rt, 2.5 h, 76% (**70c**).

With 3-amino-substituted compounds **70a**-**c** in hand, attention was now turned to the 5-amino analogues. 4-Bromo-3-methylisoxazol-5-amine **95** is not commercially available, and so was prepared from 3-methylisoxazol-5-amine **96** by electrophilic bromination with NBS according to a known procedure,[234] then converted to the *tert*-butyl carbamate **97**. Coupling with boronic esters **90** and **91** gave **98** and **99** as expected, along with deprotected compounds **71a** and **71b** which were combined with the products from TFA deprotection of **98** and **99** (Scheme 16).



Scheme 16. *Reagents and conditions:* (a) NBS, $CHCl_3$, 50 ˚C, 6 h, 84%; (b) (i) Boc_2O, DMAP, THF, reflux, 19 h; (ii) NaOH (2 M aq.), MeOH, rt, 19 h, 71%; (c) R = OEt: **90**, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, Na_2CO_3 (2 M aq.), DME, 90 °C, 24 h, 9% (**98**), 7% (**71a**); R = Ac: **91**, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, Na_2CO_3 (2 M aq.), DME, 90 °C, 24 h, 12% (**99**), 12% (**71b**); (d) TFA, CH_2Cl_2, rt, 1 h, R = OEt: 8% (**71a**, over 2 steps), R = Ac: 18% (**71b**, over 2 steps).

The yields of the coupling were considerably poorer for the 5-amino isoxazole than for the 3-amino regioisomer, and in the former case both debrominated isoxazole and the product of homocoupling of the boronic ester were observed in the crude reaction mixture by mass spectrometry. Presumably this is because the 5-amino-substituted bromide **97** is considerably more electron-rich than the 3-amino-substituted regioisomer **93** (Section 4.5.1).

4.3 Aminomethyl-methyl- and hydroxymethyl-methylisoxazoles and derivatives

The compounds described in the previous section contain a hydrogen-bond donor immediately adjacent to the isoxazole ring. I was also interested in investigating compounds containing more remote hydrogen-bond donors.

At the time of undertaking this work, only one synthesis of aminomethyl-methylisoxazoles bearing aryl substituents at the 4-position (general structures **100** or **101**, Figure 43) had been reported. This compound **102** was formed by a late-stage cycloaddition of acetonitrile-*N*-oxide to the corresponding alkyne **103**, and proceeded in only 12% yield (Figure 43).[235] This did not therefore appear to be a suitable route for the preparation of a range of such compounds, so I investigated alternative approaches.

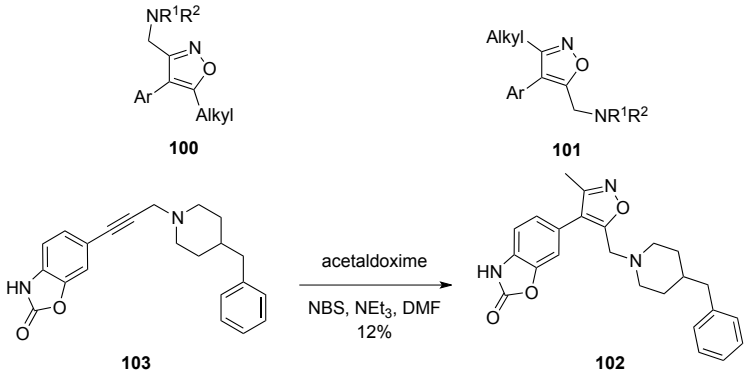


Figure 43. General structures of aminomethyl-methylisoxazoles bearing aryl substituents at the 4-position (R = alkyl), and the one reported preparation of such a compound.[235]

Analogues without substituents at the 4-position have been investigated as muscarinic acetylcholine receptor ligands, due to their structural similarity to muscarine (**104**) and furtrethonium (**105**, Figure 44A).[236] Dannhardt *et al*. synthesised both 3- and 5-aminomethyl regioisomers by borane reduction of the corresponding tertiary amides, themselves obtained from the esters (Figure 44B).

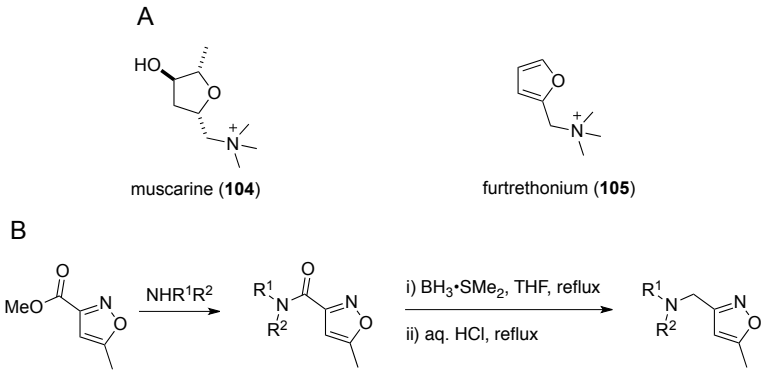
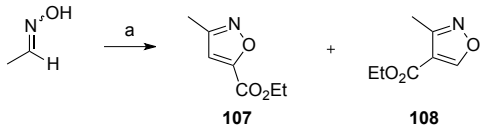


Figure 44. A: Aminomethyl-isoxazoles have previously been investigated due to their structural similarity to muscarine (**104**) and furtrethonium (**105**). B: Preparation of 3-aminomethyl-5-methylisoxazoles reported by Dannhardt *et al.* in their investigations of isoxazole-containing muscarinic receptor ligands. (The 5-aminomethyl-3-methylisoxazoles were prepared in a similar manner).[236]

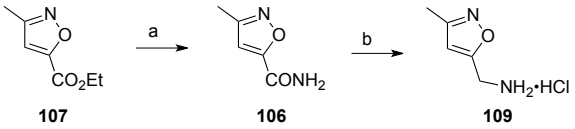
To investigate the applicability of this procedure to primary amides, 3-methylisoxazole-5-carboxamide (**106**) was synthesised as a test substrate. Ethyl 3-methylisoxazole-5-carboxylate (**107**) was prepared by the 1,3-dipolar cycloaddition of ethyl propiolate and acetonitrile-*N*-oxide (formed *in situ* from *N*-

hydroxyacetimidoyl chloride), using a modification of a reported procedure.[237] The crude reaction mixture contained a 3:1 ratio of **107** to the undesired regioisomer **108**, somewhat poorer than the reported 5:1 ratio. The regioisomers could be separated by repeated chromatographic purification (Scheme 17).



Scheme 17. *Reagents and conditions:* (a) (i) NCS, DMF, rt, 3 h; (ii) ethyl propiolate, $KHCO_3$, H_2O, CH_2Cl_2, rt, 75 min, 52% (**107**).

Aminolysis of the ester **107** with ammonia in methanol afforded the primary amide **106** in excellent yield, which was then reduced under the conditions reported by Dannhardt *et al*.[236] The amine (**109**) was precipitated as its hydrochloride salt from a solution in diethyl ether (Scheme 18). Although the yield obtained in the reduction was only moderate (41%), it was apparent that these borane reduction conditions were suitable for primary amides, and so were attempted on more complex systems bearing an aromatic ring at the 4-position. These substrates could be prepared by Suzuki couplings between bromoisoxazoles and boronic esters.



Scheme 18. *Reagents and conditions:* (a) NH_3, MeOH, rt, 16 h, 92%; (b) $BH_3 \cdot SMe_2$, THF, reflux, 6 h, then aq. HCl, reflux, 1 h, 41%.

4.3.1 Modifications to the 3-position

To brominate the isoxazole **107** and its regioisomer **110** at the 4-position prior to Suzuki coupling, we explored conditions recently reported by Li *et al*. for the bromination of electron-poor isoxazoles and pyrazoles with microwave irra-

diation.[238] Due to the strongly deactivated character of the C(4) position towards electrophiles, they found that acidic solvents were necessary to effect bromination of isoxazoles with esters or amides at the 3-position. However, all reported examples contain a phenyl group at the 5-position (Figure 45).

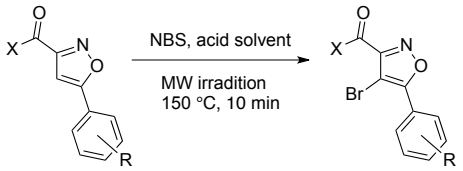
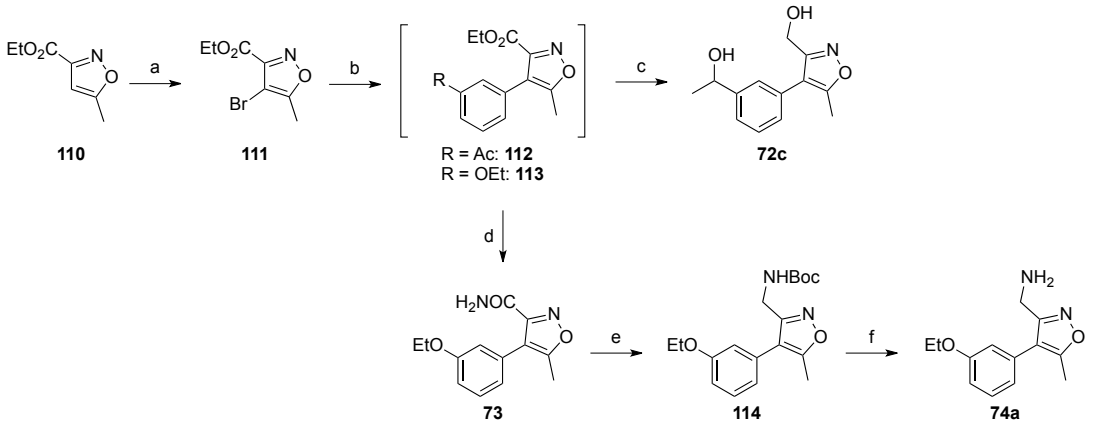


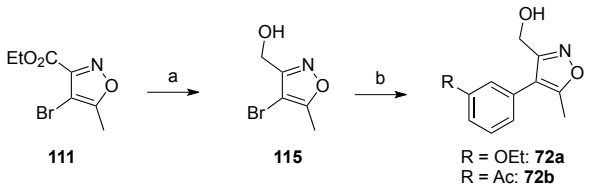
Figure 45. Bromination of electron-poor isoxazoles reported by Li *et al.* X = OMe or NMe_2; acid solvent is AcOH, 5% fuming nitric acid in AcOH, or TFA.

Despite having a methyl rather than a phenyl group at the 5-position, **110** underwent efficient bromination under these conditions (73% yield), using 5% fuming nitric acid in acetic acid as a solvent. Performing the reaction under reflux (18 h) gave a significantly lower yield of **111** (41%). Suzuki coupling to boronic esters **90** and **91** was next attempted, but the desired esters could not be isolated cleanly. The reaction mixtures from the Suzuki coupling also contained significant quantities of homocoupled boronic ester, as well as small amounts of unreacted bromide **111** and boronic esters. A portion of impure **112** was reduced to diol **72c** and subsequently purified, giving a 9% yield over two steps from bromide **111**, while impure **113** was converted to the carboxamide **73** in 20% yield over two steps. Reduction of amide **73** with borane, isolation of basic components of the mixture using a cation exchange resin, and Boc protection afforded **114** in 27% yield after purification from amide **73**. The Boc group could then be removed to give primary amine **74a** (Scheme 19).



Scheme 19. *Reagents and conditions:* (a) NBS, 5% fuming HNO_3/AcOH, 150 ˚C (MW), 12 min, 73%; (b) **91** (R = Ac)/**90** (R = OEt), Pd(dppf)Cl_2·CH_2Cl_2, $NaHCO_3$ (sat. aq.), DME, 100 °C, 2 h, then 110 ˚C, 7 h; (c) R= Ac: $NaBH_4$, EtOH, 8% (2 steps from **111**); (d) R = OEt: NH_3, MeOH, rt, 15 h, 20% (2 steps from **111**); (e) (i) BH_3·THF, THF, reflux, 14 h; (ii) Boc_2O, MeCN, rt, 18 h, 27%; (f) HCl, Et_2O, rt, 22 h, 99%.

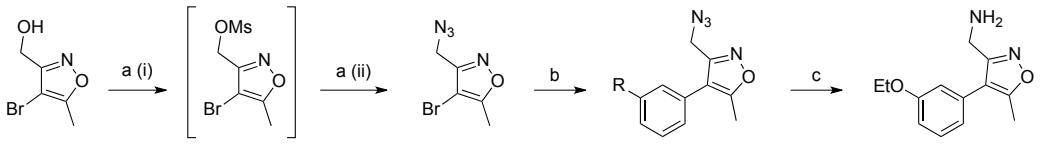
Given the poor yields obtained in the coupling and reduction steps, alternative routes were sought for the preparation of other compounds modified at the 3-position of the isoxazole. I hypothesised that the 3-carboxyethyl group may be detrimental to the Suzuki coupling, so the ester **111** was reduced to the primary alcohol **115** prior to coupling. Reaction with boronic esters **90** and **91** gave alcohols **72a** and **72b** in 46% and 49% yields respectively (Scheme 20).



Scheme 20. *Reagents and conditions:* (a) $NaBH_4$, EtOH, rt, 3 h, 85%; (b) R = OEt: **90**, Pd(dppf)Cl_2·CH_2Cl_2, $NaHCO_3$ (sat. aq.), DME, 100 °C, 46% (**72a**); R = Ac: **91**, Pd(dppf)Cl_2·CH_2Cl_2, $NaHCO_3$ (sat. aq.), DME, 100 °C, 49% (**72b**).

As an alternative route to amine **74a**, alcohol **115** was activated as the mesylate **116** and converted to the azide **117**. This was then coupled to boronic esters **50** and **51** to give azides **118** and **119** in 50% and 32% yields, respectively. (In the latter case, the particularly low yield was a result of the difficulty in sepa-

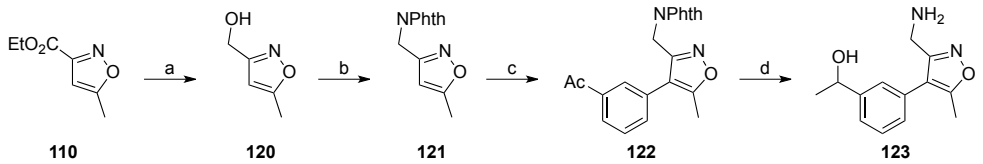
rating **119** from the product of homocoupling of the boronic ester.) Initial attempts to reduce azide **118** to the amine using Staudinger conditions (triphenylphosphine in THF or dioxane, or polymer-bound triphenylphosphine in dioxane, then water) were unsuccessful, giving complex mixtures of products. Pei and Wickham have previously noted the difficulty in reducing azido-isoxazoles; the relative ease of reduction of the isoxazole N—O bond renders many commonly-applied methods, such as catalytic hydrogenolysis or zinc in hydrochloric acid, unsuitable.[239] They therefore developed a very mild reaction that did not affect the isoxazole ring. Specifically, catalytic 1,3-propanedithiol is used as the active reducing agent, with stoichiometric sodium borohydride reducing the cyclic disulfide back to active dithiol. Bayley, Knowles and colleagues had previously reported that 1,3-propanedithiol reduced azides,[240,241] but by employing only catalytic quantities of the foul-smelling liquid, the method of Pei and Wickham is much more suitable for preparative use. Gratifyingly, these conditions were found to be effective for azide **118**, though the product was only obtained in moderate yield (Scheme 21). (Reduction of ketone-containing **119** was not attempted due to likely formation of imine-containing products.)



Scheme 21. *Reagents and conditions:* (a) (i) MsCl, NEt_3, CH_2Cl_2, 0 ˚C, 1 h; (ii) NaN_3, DMF, rt, 15 h, 91%; (b) R = OEt: **90**, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, Na_2CO_3 (2 M aq.), DME, 100 °C, 4 h, 50% (**118**); R = Ac: **91**, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, Na_2CO_3 (2 M aq.), DME, 100 °C, 4 h, 32% (**119**); (c) R = OEt: 1,3-propanedithiol, $NaBH_4$, NEt_3, $^{i}PrOH$, rt, 11 h, 41%.

Finally, in order to prepare a 3-aminomethylisoxazole with an alternative to the ethoxy group on the phenyl ring, I returned to the C—H activation procedure

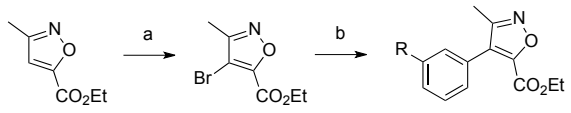
reported by Fall *et al.*, and attempted in Chapter 2.[213] The nitrogen was introduced *via* a Mitsunobu reaction between phthalimide and alcohol **120** to give **121**, which was likely to be stable to the forcing reaction conditions required for C—H activation. Performing the reaction using palladium(II) chloride, as previously attempted, gave only 26% of **122**, with significant amounts of isoxazole **121** and 3′-bromoacetophenone (**49**) remaining, as indicated by TLC analysis of the crude reaction. Fall *et al.* reported that allylpalladium(II) chloride dimer was suitable for the arylation of functionalized isoxazoles, and a modest increase in yield to 35% was obtained by using this catalyst. In this case, unreacted isoxazole **121** was recovered by chromatography (1.5 equivalents of **121** were used relative to bromide, 56% was recovered). Finally, the phthalimide group was cleaved to release the free amine **74b**. It is known that isoxazoles can react with hydrazine to form the pyrazole,[242] therefore hydrazine is unlikely to be a suitable reagent for this deprotection. Instead I turned to a mild reductive deprotection developed by Osby *et al*.[243] In this procedure, the phthalimide is reduced to the *ortho*-hydroxymethyl benzamide using sodium borohydride in aqueous isopropanol, followed by acidolysis with acetic acid to liberate the amine and phthalide. This procedure afforded amine **74b** in excellent yield (Scheme 22).



Scheme 22. *Reagents and conditions:* (a) $NaBH_4$, EtOH, rt, 2 days, 93%; (b) phthalimide, PPh_3, DIAD, THF, rt, 16 h, 71%; (c) **49**, $[Pd(C_3H_5)Cl]_2$, KOAc, DMAc, 80 ˚C, 18 h, 130 ˚C, 5 h, 35%; (d) $NaBH_4$, $^{i}PrOH$, H_2O, CH_2Cl_2, rt, 23 h then AcOH, 80 °C, 2.5 h, 92%.

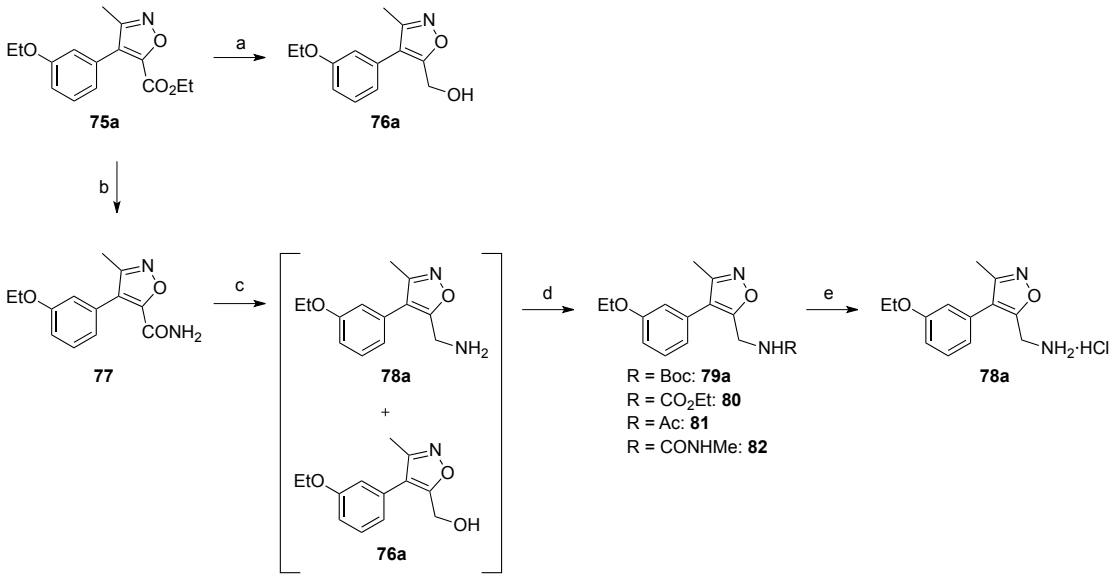
4.3.2 Modifications to the 5-position

As with the compounds modified at the 3-position, the synthesis began with the preparation of the required bromo-isoxazole prior to a Suzuki coupling. Using the aforementioned microwave-assisted electrophilic bromination,[238] ester **107** was efficiently brominated. A stronger acid (TFA) and a longer reaction time was required than for **110**, consistent with the more electron-deficient nature of C(4) in **107**. However, the coupling of **107** to boronic esters **90** and **91** proceeded more rapidly and in much higher yield than coupling of regioisomeric **111**, and unlike the regioisomeric compounds **112** and **113**, **75a** and **75b** could be isolated cleanly by chromatography (Scheme 23).



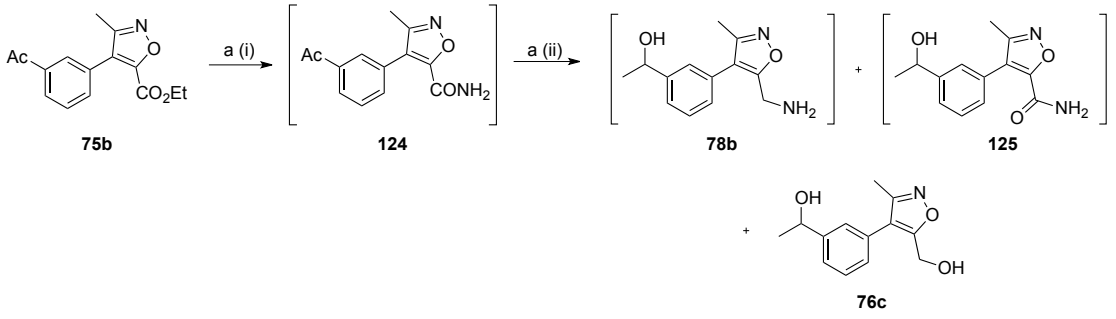
Scheme 23. *Reagents and conditions:* (a) NBS, TFA, 150 °C (MW), 50 min, 85%; (b) R = OEt: **90**, Pd(dppf)$Cl_2 \cdot CH_2Cl_2$, $NaHCO_3$ (sat. aq.), DME, 100 °C, 1.5 h, 77% (**75a**); R = Ac: **91**, Pd(dppf)$Cl_2 \cdot CH_2Cl_2$, $NaHCO_3$ (sat. aq.), DME, 100 °C, 1.5 h, 77% (**75b**).

The ester **75a** was reduced to the alcohol **76a** with sodium borohydride. To obtain the corresponding amine **78a** and derivatives, **75a** was converted to amide **77** in high yield, which was then reduced with borane. Perhaps surprisingly, alcohol **76a** was also isolated, alongside unreacted starting material **77**. Crude amine **76a** was then converted to *tert*-butyl carbamate **79a**, ethyl carbamate **86** and acetamide **81** under standard conditions. The methyl urea **82** was formed using *N*-succimidyl-*N*-methylcarbamate, a safer alternative to the highly toxic methyl isocyanate.[244,245]



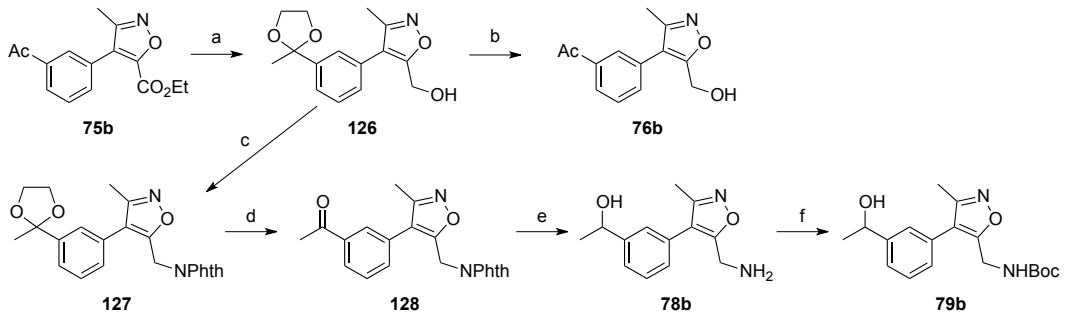
Scheme 24. *Reagents and conditions:* (a) $NaBH_4$, EtOH, rt, 24 h, 84%; (b) NH_3, MeOH, rt, 39 h, 100%; (c) $BH_3 \cdot SMe_2$, THF, reflux, 13 h then aq. HCl, reflux, 45 min, 5% (**76a**), 7% (**77**); (d) R = Boc: Boc_2O, THF, rt, 24 h, 35% (**79a**); R = COOEt: ethyl chloroformate, NEt_3, CH_2Cl_2, rt, 2 h, 36% (**80**); R = Ac: Ac_2O, NEt_3, CH_2Cl_2, rt, 2 h, 48% (**81**); R = CONHMe: *N*-succimidyl-*N*-methylcarbamate, THF, rt 33% (**82**); (d) R= Boc: HCl, Et_2O, rt, 17 h, 89% (**78a**).

To obtain alternative substituents on the phenyl ring, ester **75b** was converted to amide **124**, which was then reduced with borane. Although amine **78b** appeared to be the major product from the reaction, it could not be isolated cleanly. **125** was observed by mass spectrometry, suggesting that the ketone was reduced more rapidly than the amide. Alcohol **76c** was also isolated in 6% yield (Scheme 25).



Scheme 25. *Reagents and conditions:* (a) (i) NH_3, MeOH, rt, 36 h, then NH_3, ^{i}PrOH, rt, 24 h; (ii) $BH_3 \cdot SMe_2$, THF, reflux, 8 h then aq. HCl, reflux, 1 h, 6% (**76c**).

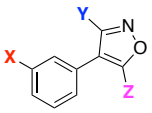
To synthesise the keto-analogue of diol **76a**, ketone **75b** was protected as the dioxolane, then reduced with sodium borohydride to give **126**. Deprotection under mildly acidic conditions gave the desired ketone **76b**. Given the difficulties encountered in preparing primary amine **78b** by borane reduction of **124**, an alternative route to **78b** employing **126** was investigated. Protected keto-alcohol **126** was converted to the phthalimide **127** in 76% yield; efficient cleavage of the acetal gave **128**. The phthalimide protecting group was removed under the aforementioned reductive conditions to give amino-alcohol **78b**.[243] A portion of this was then converted to the *tert*-butyl carbamate derivative **79b** (Scheme 26).



Scheme 26. *Reagents and conditions:* (a) (i) ethylene glycol, $(EtO)_3CH$, *p*-TsOH·H_2O, 60 °C, 3.5 h; (ii) $NaBH_4$ EtOH, rt, 22 h, 80%; (b) HCl (aq), dioxane, rt, 2 h, 73%; (c) phthalimide, PPh_3, DIAD, THF, rt, 11 h, 76%; (d) HCl (aq), dioxane, rt, 2.5 h, 96%; (e) $NaBH_4$, ^{i}PrOH, H_2O, CH_2Cl_2, rt, 23 h then AcOH, 80 °C, 2.5 h, 92%; (f) Boc_2O, THF, rt, 5 h, 65%.

4.3.3 Evaluation of aminomethyl-, aminomethyl-methyl- and hydroxymethyl-methylisoxazoles and derivatives

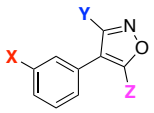
Compounds **70**-**71** were evaluated as BET bromodomain ligands for their ability to bind to BRD4(1) by AlphaScreen® and a protein stability shift (ΔT_m^{obs}) assay (Table 10). Compounds **70a**-**c** interact weakly with BRD4(1), indicating that an amino substituent at position 3 is not tolerated. Replacing the C(5) methyl group with an amine is tolerated, in the case of Z = OEt (**71a**), but offers little improvement on the parent compound **46c** (IC_{50} = 23.2 μM, ΔT_m^{obs} = 2.8 °C).



Compound number	X	Y	Z	BRD4(1)[a] IC_{50} (μM)	BRD4(1)[b] ΔT_m^{obs} (°C)
70a	OEt	NH_2	Me	72.8 (59.37-89.23)	0.5
70b	Ac	NH_2	Me	n. d.	0.0
70c	CH(OH)Me	NH_2	Me	>100	0.7
71a	OEt	Me	NH_2	14.9 (14.2-15.7)	2.5
71b	Ac	Me	NH_2	n. d.	0.2

Table 10. IC_{50} and ΔT_m^{obs} of 70-71 against BRD4(1). [a] Protein and peptide concentration: 50 nM; [b] compound concentration: 10 μM; protein concentration: 2 μM. 95% confidence intervals for curve fitting to duplicate data are shown in brackets.

Compounds **72-82** were evaluated by AlphaScreen® for their ability to inhibit the binding of a tetraacetylated H4 peptide ($H4_{1-20}KAc_4$) to BRD4(1) at a single concentration of 25 μM, close to the IC_{50} values of the parent compounds **46b**-**d** (Table 11). Generally, modification at the 3-position is not well tolerated, with all compounds (**72**-**74**) giving modest inhibition. In the 5-position, an ethyl ester (**75a**, **75b**) or primary amine (**78a**, **78b**) is poorly-tolerated. Primary amide **77**, *tert*-butyl carbamates **79a** and **79b**, and acetamide **81** all show > 60% inhibition of peptide binding. The primary alcohol-containing compounds **76a**-**c**, ethyl carbamate **80** and methyl urea **82** show greater potency, **76a** (Z = CH_2OH) and **80** (Z = CH_2NHCO_2Et) in particular almost completely inhibiting peptide binding to BRD4(1). These data suggests that these compounds are substantially more active than their 3,5-dimethylisoxazole-containing analogue **46c**, consistent with the formation of an additional hydrogen-bonding interaction between N140 and the 5-position substituent.



Compound number	X	Y	Z	BRD4(1) inhibition (%)
72a	OEt	CH_2OH	Me	53
72b	Ac	CH_2OH	Me	40
72c	CH(OH)Me	CH_2OH	Me	35
73	OEt	$CONH_2$	Me	28
74a	OEt	CH_2NH_2	Me	19
74b	CH(OH)Me	CH_2NH_2	Me	9
75a	OEt	Me	COOEt	37
75b	Ac	Me	COOEt	47
76a	OEt	Me	CH_2OH	97
76b	Ac	Me	CH_2OH	75
76c	CH(OH)Me	Me	CH_2OH	79
77	OEt	Me	$CONH_2$	63
78a	OEt	Me	CH_2NH_2	37
78b	CH(OH)Me	Me	CH_2NH_2	31
79a	OEt	Me	$CH_2NHCO_2{}^tBu$	71
79b	CH(OH)Me	Me	$CH_2NHCO_2{}^tBu$	21
80	OEt	Me	CH_2NHCO_2Et	92
81	OEt	Me	CH_2NHAc	78
82	OEt	Me	$CH_2NHCONHMe$	84

Table 11. Inhibition of H4KAc4 peptide binding to BRD4(1) by 72-82. Protein concentration: 50 nM; Compound concentration: 25 μM. Heat map shows relative inhibition; red indicates high inhibition and green indicates low inhibition.

4.4 Introducing hydrogen bond donors at the 5-position of 61a

*4.4.1 Synthesis of **129a-e***

Given the results presented in Section 4.3.3, I hypothesised that introducing hydrogen-bond donors at the 5-position of the more potent isoxazole **61a** would lead to the formation of an additional hydrogen bond to the conserved asparagine residue, and may therefore increase the affinity of these compounds for BET bromodomains. Furthermore, comparison of the affinities of compounds

able to form this additional interaction with 3,5-dimethylisoxazole **61a** might provide insight into the strength of this hydrogen bonding interaction.

Of the compounds presented in Table 11, the primary alcohol **76a** and carbamates, such as **80**, were the most potent against BRD4(1). Therefore, I wished to synthesise the alcohol **129a** and nitrogen-containing derivatives such as carbamates and sulfonamides **129b**-**e**. I envisaged that such compounds could be prepared from **130**, where the phenol is protected to allow selective transformations of the primary alcohol. I had previously shown that ethyl 4-bromo-3-methylisoxazole-5-carboxylate (**123**) undergoes Suzuki couplings to aryl boronic acid pinacol esters in good yields (Scheme 23), and so an appropriate boronic ester **131** was required. This could be prepared from a bromoaldehyde **132** by reaction with phenylmagnesium bromide followed by palladium-catalysed borylation. This intermediate in turn could be synthesised from 3-bromo-5-hydroxybenzoic acid (**53**), as employed for the synthesis of compounds in Chapter 2 (Figure 46).

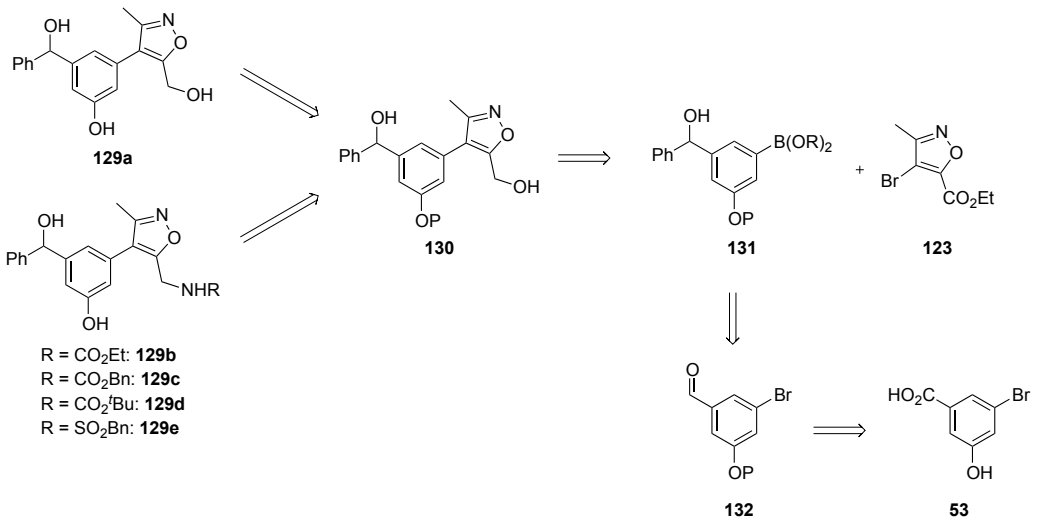
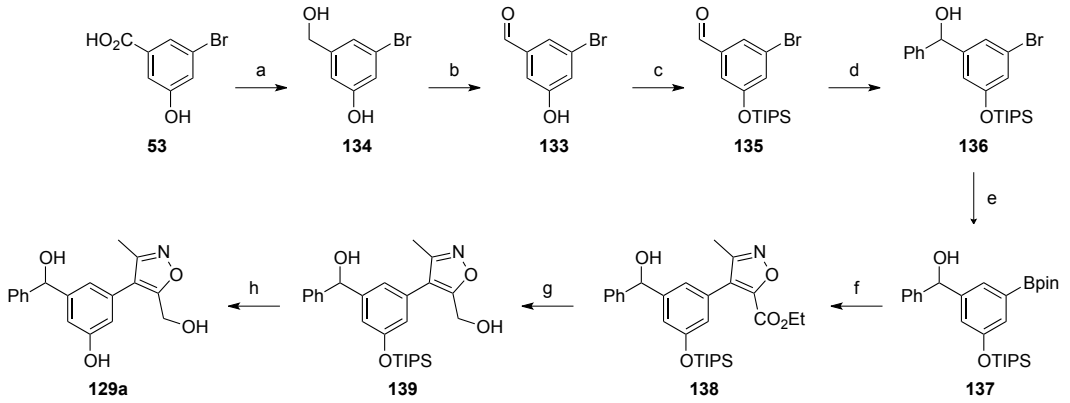


Figure 46. Retrosynthetic approach to the synthesis of **129a**-**e**, derivatives of **61a** modified at the 5-position of the isoxazole. P represents a protecting group.

Aldehyde **133** had already been employed by contractors in their preparation of **59** (as reported in Chapter 2).[127] It was synthesised from **53** by formation of the methyl ester, reduction with lithium aluminium hydride and oxidation to the aldehyde with pyridinium chlorochromate. I wished to avoid the use of a toxic chromium (VI) oxidant, and if possible to remove one step from this synthesis by reducing the acid directly to the alcohol, without prior formation of the ester. Therefore acid **53** was treated with borane in THF, which afforded alcohol **134** in excellent yield without the need for chromatographic purification. Oxidation with manganese(IV) oxide, while very slow, gave **133** (Scheme 27). The yield of **133** over two steps was 79%, which compares very favourably to 39% over three steps as previously reported.[127]

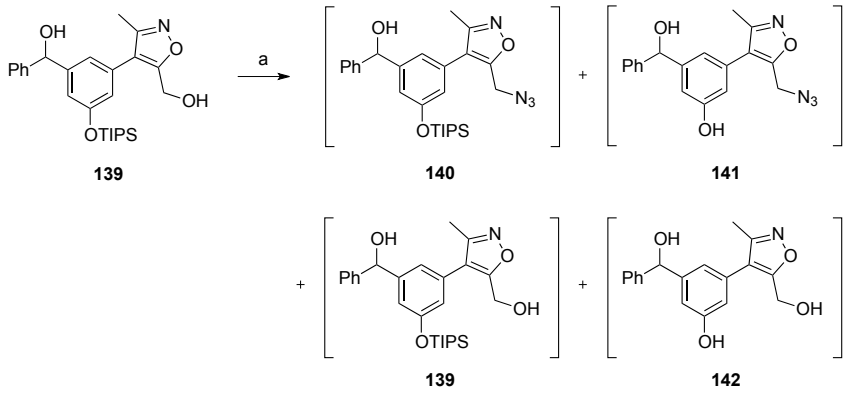
The phenol was then protected as a TIPS ether **135** in high yield. The TIPS group was chosen because of its stability towards the hot aqueous basic conditions required for a Suzuki coupling later in the synthesis. Addition of phenylmagnesium bromide gave secondary alcohol **136**. An attempted borylation in DMSO, as employed previously, gave only 13% of desired pinacol ester **137**. However, switching to dioxane increased the isolated yield to 97%. Suzuki coupling to bromide **123** afforded ester **138** in 74% yield, which was subsequently reduced to the alcohol **139**. Hence this key intermediate was prepared in an overall yield of 36% over seven steps from **53**. Deprotection of the TIPS group afforded target triol **129a** (Scheme 27).



Scheme 27. *Reagents and conditions:* (a) $BH_3 \cdot THF$, THF, rt, 16 h, 96%; (b) MnO_2, CH_2Cl_2, acetone, rt, 7 days, 82%; (c) TIPSCl, imidazole, DMF, rt, 13 h, 96%; (d) PhMgBr, THF, rt, 7 h, 73%; (e) B_2pin_2, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, KOAc, dioxane, 80 °C, 3 h, 97%; (f) **123**, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, $NaHCO_3$ (sat. aq.), DME, 90 °C, 2.5 h, 74%; (g) $NaBH_4$, EtOH, rt, 16 h, 89%; (h) TBAF, THF, 0 ˚C, 40 min, 91%.

A method was next sought to convert alcohol **139** into an amine. A Mitsunobu reaction with phthalimide had been successfully employed on alcohols **120** and **126**, but only starting material was recovered with **139**. This may be a result of the presence of the large triisopropylsilyloxy group, which could slow the reaction with either the electrophilic azodicarboxylate intermediate or with the phthalimide nucleophile. Therefore less sterically-demanding activating agents and nucleophiles were investigated. **139** was treated with mesyl chloride at −60 ˚C and the solution was then warmed to 0 ˚C. After work-up, the residue was immediately dissolved in DMF, sodium azide was added and the reaction mixture was stirred at rt overnight. Analysis of the reaction by TLC, MS and ^{1}H NMR indicated a complex mixture, including desired product **140**, TIPS-deprotected product **141**, and both TIPS-protected and deprotected (**142**) starting material (Scheme 28). A similar reaction using potassium phthalimide as a nucleophile also gave TIPS-deprotected product and starting material. Sodium azide has been reported as a reagent for the removal of methyldiphenylsilyl protecting groups,[246] but there are only two examples of sodium azide cleaving a TIPS

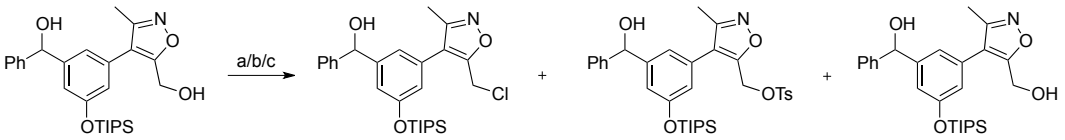
ether,[247,248] both under more forcing conditions (> 90 ˚C). No examples could be found of silyl ether cleavage by phthalimide. Given the lack of precedent for such transformations, it was somewhat surprising that TIPS deprotection was observed under these conditions, but it suggested that careful control of reaction stoichiometry and conditions would allow displacement of a leaving group without removal of the protecting group.



Scheme 28. *Reagents and conditions:* (a) (i) MsCl, CH_2Cl_2, NEt_3, −60 ˚C → 0 ˚C, 3 h; (ii) NaN_3, DMF, rt, 16 h.

Activation of the hydroxyl group was next attempted with tosyl chloride (Scheme 29). Treatment of **139** with 1.1 equivalents of tosyl chloride over 16 h gave chloride **143** (48%) and starting material **139** (20%) after chromatographic purification. Using 1.25 equivalents of tosyl chloride and a shorter reaction time (2 h) improved mass return, and both chloride **143**, tosylate **144** and starting material **139** were isolated. A longer reaction time (16 h) was used to try and force complete conversion of the starting material to the chloride, but the maximum yield of chloride thus obtained was 63%, and attempts to repeat or improve on this procedure led to lower yields. The mass spectra of the crude reaction mixtures prior to work-up always contained the product formed by alkylation of triethylamine by the chloride or tosylate as the predominant peak. The moderate

to low yields obtained in this step may therefore result from consumption of product by reaction with the base, particularly at longer reaction times.

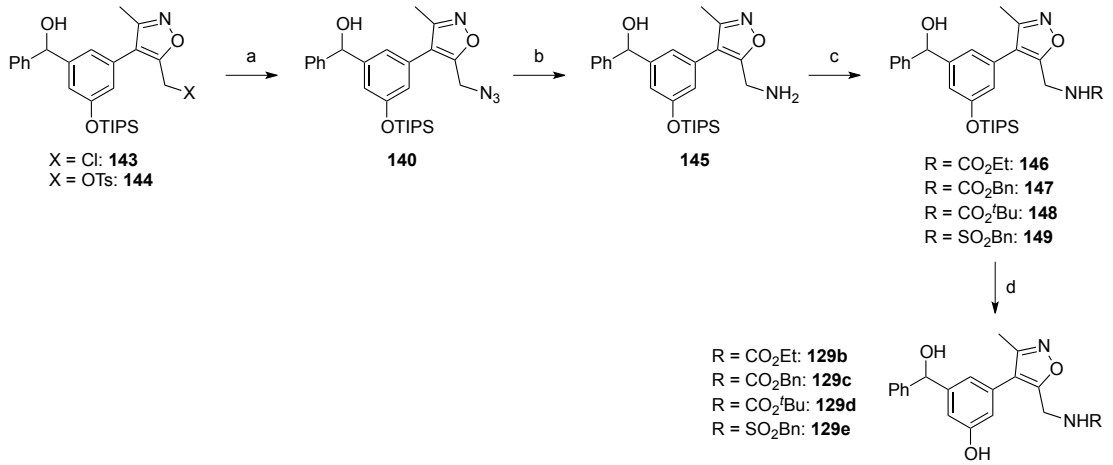


139 **143** **144** **139**

Entry	*Reagents and conditions:*	Isolated yield (%)		
		143	**144**	**139**
a	TsCl (1.1 eq), NEt_3 (1.1 eq) , CH_2Cl_2, rt, 19 h	48	0	20
b	TsCl (1.25 eq), NEt_3 (1.5 eq), CH_2Cl_2, rt, 2 h	17	55	18
c	TsCl (1.25 eq), NEt_3 (1.5 eq), CH_2Cl_2, rt, 16 h	63	0	4

Scheme 29. Activation of **139** as the chloride or tosylate.

Both chloride **143** and tosylate **144** could be converted to azide **140** in excellent yields with 1.05-1.1 equivalents of sodium azide and short reaction times (1-2 h), with no apparent deprotection of the TIPS group. Using the aforementioned 1,3-propanedithiol/sodium borohydride system,[239] **140** was smoothly reduced to amine **145**, which was subsequently treated with a range of electrophiles to give carbamates **146**-**148** and sulfonamide **149**. Final deprotection with TBAF gave the target compounds **129b**-**e** (Scheme 30).

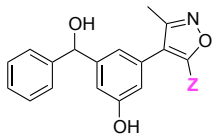


Scheme 30. *Reagents and* conditions: (a) X = Cl: NaN_3, DMF, rt, 1 h, 99%; X = OTs: NaN_3, DMF, 0 °C, 1 h, 82%; (b) 1,3-propanedithiol, $NaBH_4$, NEt_3, ^iPrOH, rt, 16 h, 86%; (c) R = CO_2Et: ethyl chloroformate, NEt_3, CH_2Cl_2, rt, 4 h, 74% (**146**); R = CO_2Bn: benzyl chloroformate, NEt_3, CH_2Cl_2, rt, 3.5 h, 62% (**147**); R = $CO_2{}^tBu$: Boc_2O, NEt_3, CH_2Cl_2, rt, 2 h, 94% (**148**); R = SO_2Bn: phenylme-

thanesulfonyl chloride, NEt_3, DMAP, CH_2Cl_2, rt, 32 h, 65% (**149**); (d) R = CO_2Et: TBAF, THF, 0 ˚C, 30 min, 52% (**129b**); R = CO_2Bn: TBAF, THF, 0 ˚C, 1 h, 66% (**129c**); R = $CO_2{}^tBu$: TBAF, THF, 0 ˚C, 1 h, 97% (**129d**); R = SO_2Bn: TBAF, THF, 0 ˚C, 1 h, 66% (**129e**).

*4.4.2 Evaluation of compounds **129a-e** as bromodomain ligands*

The IC_{50} for displacement of a tetraacetylated histone H4 peptide from BRD4(1) was determined by AlphaScreen® (Table 12). The parent compound **61a** and primary alcohol **129a** bind with comparable affinity. All other modifications result in reduced activity, IC_{50} increasing with the size of the substituent.



Compound number	Z	BRD4(1) IC_{50} (μM)
61a	CH_3	0.808 (0.758-0.861)
129a	CH_2OH	0.972 (0.748-1.26)
129b	$CH_2NHCOOEt$	1.39 (1.06-1.83)
129c	$CH_2NHCOOBn$	3.07 (2.73-3.46)
129d	$CH_2NHCOO{}^tBu$	2.61 (2.31-2.95)
129e	CH_2NHSO_2Bn	3.27 (2.98-3.59)

Table 12. IC_{50} of 61a and 129a-e against BRD4(1). Protein and peptide concentration: 50 nM. Heat map shows relative IC_{50} values; red indicates low IC_{50} values and green indicates high IC_{50} values. Ranges in parentheses represent 95% confidence intervals resulting from sigmoidal curve fitting to duplicate data. (Note the unusually high IC_{50} value for **61a**, which is typically 0.35-0.40 μM.)

4.5 Discussion and conclusions

4.5.1 Discussion

A series of 4-aryl isoxazoles were synthesised in order to investigate the possibility of interacting with water molecules at the base of the KAc-binding pocket or forming an additional interaction with the conserved asparagine residue, and to confirm the orientation of the isoxazole in the binding site. Very few syntheses of 4-arylisoxazoles containing modifications to the 3- or 5-positions have been reported. I demonstrated that such compounds could be prepared by Suzu-

ki coupling reactions between 4-bromoisoxazoles and aryl boronic acid pinacol esters. The success of this reaction was heavily dependent of the nature of the substituents on the isoxazole: coupling of 5-aminoisoxazole **97** was less successful than for the 3-amino analogue **93**, while **123**, bearing an ester at the 5-position, coupled more smoothly to the boronic ester than regioisomeric **111** with an ester at the 3-position. A comparison of the ^{13}C NMR chemical shift of C(4), which is attached to bromine, is illuminating: the large chemical shift for compounds **93** and **123** is indicative of low electron density on the carbon atom. Such compounds are thus likely to undergo rapid oxidative addition, which is usually the rate-determining step. Inversely, the higher electron density of **97** and **111**, as indicated by a lower chemical shift, will slow oxidative addition (Table 13).

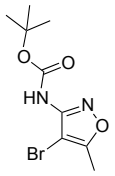
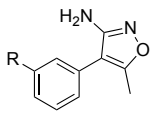
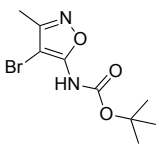
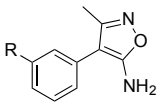
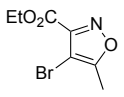
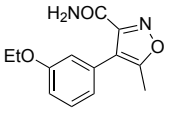
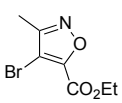
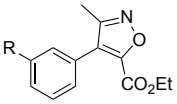
Compound number	Structure	C(4) ^{13}C NMR δ (ppm)	Representatiove products and yields
93		95.5	 **70a** (R = OEt): 31% (2 steps) **70b** (R = Ac): 38% (2 steps)
97		87.4	 **71a** (R = OEt): 8% (2 steps) **71b** (R = Ac): 18% (2 steps)
111		91.6	 **73**: 20% (2 steps)
123		101.3	 **75a** (R = OEt): 77% **75b** (R = Ac): 77%

Table 13. ^{13}C NMR chemical shifts of C(4) in brominated isoxazoles 93, 97, 111 and 123.

Modifications to the 3-position of the isoxazole were poorly tolerated and completely abolished binding to BRD4(1) in some instances, such as with com-

pound **70b**. 5-Position modifications were better tolerated. This would suggest that the 3-methyl group does indeed preferentially bind at the base of the pocket, especially as groups at the 5-position such as ethyl carbamate (**20**), which are too large to be accommodated in this region of the protein, are well tolerated. Consequently, nitrogen, the better hydrogen acceptor of the two heteroatoms in isoxazole, would appear to interact preferentially with tyrosine *via* a bridging water molecule, suggesting that this interaction might be more important for ligand binding than the interaction with the conserved asparagine residue. This observation is consistent with the observation that compounds incapable of interacting with asparagine can still bind to BET bromodomains,[122] and with a crystallographic study of KAc-mimicking fragments, which found that the heteroatom-water hydrogen bond was typically shorter than the heteroatom-asparagine interaction.[156] The weak interaction of **70**, **72** and **74** with BRD4(1) indicates that $–NH_2$, $–CH_2OH$ or $–CH_2NH_2$ moieties at the 3-position do not favourably interact with or displace the water molecules that line the base of the pocket. This observation is in agreement with a study from Filippakoupolos *et al*., in which replacement of a methyl group with $–NH_2$ or –Et in 3-methyltriazole-containing BET inhibitors markedly reduced affinity.[122] While we can confidently predict that 5-position-modified compounds bind with the modification directed away from the base of the pocket, it cannot be assumed that modifications at the 3-position bind in the base of the pocket. It is possible that the isoxazole flips so that the 5-methyl group mimics that of KAc, and the heterocyclic nitrogen interacts directly with the conserved asparagine. This reversed hydrogen-bonding pattern is likely to be less favourable, since the weaker hydrogen bond acceptor would interact with the bridging water molecule.

The nature of the substituent at the 5-position has a significant effect on binding (Table 11). A primary amine markedly reduces affinity (**78a** and **78b**), perhaps because the nitrogen will be protonated under the assay conditions, and positioning a positively-charged substituent in this hydrophobic region of the protein will be unfavourable. Esters are also poorly tolerated, which may result from repulsion between the ester oxygen atoms and the carbonyl group of the asparagine residue. Uncharged groups linked *via* a methylene unit to the isoxazole were better tolerated; notably, the alcohol **76a** almost completely inhibited the binding of an acetylated peptide to BRD4(1) at 25 μM. The carbamate **80** and the urea **82** also showed significant inhibition but were not as active as the corresponding alcohol; since these groups are usually better hydrogen bond donors, these results suggests that the observed trends do not arise from hydrogen bonding alone.

In an attempt to apply the knowledge gained from these weakly-binding inhibitors to a more potent compound series, analogues of **61a** were designed to introduce methylene-linked hydrogen bond donor at the 5-position. These compounds required a considerably longer synthesis than **70**-**82**, with a longest linear sequence of 12 steps for **129b**-**e** from 3-bromo-5-hydroxybenzoic acid, as opposed to 4 steps for **76a**. Gratifyingly, most steps proceeded in good to excellent yield. Most problematic was the activation of alcohol **139**; a Mitsunobu reaction was unsuccessful, and reactions with tosyl chloride and triethylamine afforded mixtures of starting material, tosylate and chloride. In addition, the activated chloride or tosylate appeared to react with the base. A more hindered, less nucleophilic base may therefore increase yields. Nonetheless, sufficient quantities of **129a**-**e** were synthesised to determine IC_{50} values for peptide displacement

from BRD4(1) by AlphaScreen® (Table 12). These compounds showed a similar activity trend to the simpler analogues, i.e. the alcohol **129a** was most active, and the ethyl carbamate **12b** and *tert*-butyl carbamate **129d** were progressively weaker inhibitors. Benzyl carbamate **129c** and benzyl sulfonamide **129e** were weaker still, though the difference in IC_{50} between the most and least active compounds was only about 3-fold. However, alcohol **129a** was equipotent with the 3,5-dimethylisoxazole **61a**, suggesting that either the compound is unable to form an additional hydrogen bond to asparagine, or that such a hydrogen bond contributes little to affinity. Without structural information, it is not clear which of these explanations is most likely.

Since starting this work, two publications have explored the effect of modifying substituents in this region of the BET bromodomains. Mirguet *et al.*, in a report on the lead optimisation effort that led to I-BET762 (**1**), investigated a range of alternative substituents at the 4-position of triazolobenzodiazepine GW841819X (**2**, Figure 5).[114] The original hit (IC_{50} = 0.50 μM against BRD4 in an FP assay) bore a benzyl carbamate at this position, which was demonstrated crystallographically to hydrogen bond to the asparagine carbonyl group *via* the N–H attached to the diazepine ring (Figure 41). Analogous sulfonamides and amides, as well as the unsubstituted amine, were less active; only an ethyl carbamate or a benzyl urea maintained activity. All would be expected to be able to form the same hydrogen bond to asparagine. The authors suggest that this may be due to differences in the preferred geometry of carbamates and ureas, as compared to amides and sulfonamides, but do not expand on this. Due to the instability of this compound series, the authors explored replacing the nitrogen attached to the diazepine with a methylene unit, eliminating the possibility of a

direct hydrogen bond. This did have a deleterious effect on activity, which could be regained by modifications to other sites in the compound. Interestingly, the ester analogue of I-BET762 possessed even greater *in vitro* affinity without having any hydrogen-bond donors.

In the development of CPI203 (**5**), researchers at Constellation Pharmaceuticals began with the isoxazole analogue of (+)-JQ1, in which a *tert*-butyl ester is linked to the diazepine *via* a methylene group. Replacing the ester with a primary carboxamide or ethyl amide improved BRD4(1) activity by around an order of magnitude, and a crystal structure of **5** with BRD4(1) indicates a water-mediated hydrogen bond between the primary amide and the asparagine carbonyl (PDB ID: 4LRG). Replacing the $–CH_2CONH_2$ group with –NHCOOMe resulted in only a small reduction in biochemical potency. Although no crystal structure is presented, it is likely that the carbamate analogue can form a direct hydrogen bond to asparagine.[133] Together, the GSK and Constellation series suggest that hydrogen bonding to asparagine is significant, but this can be direct hydrogen bond donation from an atom adjacent to the core (in GW841819X), a water-mediated interaction from a hydrogen-bond donor (in I-BET762 or CPI203), or a water-mediated interaction from a hydrogen-bond acceptor (as is likely to occur for the ester analogue of I-BET762).

In the light of these findings, it is perhaps unsurprising that compounds **129a**-**e** show rather small differences in affinity. Why, though, is there no enhancement compared to 3,5-dimethylisoxazole **61a**? After all, in other series, it is clear that a substituent at this position is important for affinity.[114,122] One possibility is that the additional contribution to the enthalpy of solvation from a polar group such as hydroxyl is not recouped upon binding, as the hydrogen bonding

interaction may be rather weak. Another possibility is suggested by overlaying the structures of **61a** and compounds that do form a second interaction with asparagine (Figure 47A). The side-chains of I-BET762 (**1**), GW841819X (**2**) and CPI203 (**5**) adopt a very similar position, due to the rigidity of the fused triazolobenzodiazepine ring system. The 5-methyl group of **61a** adopts a different orientation, and is directed towards the side-chain of L94, present in all BET bromodomains (Figure 47B). A larger substituent may be forced to adopt an orientation closer to that of the benzodiazepines in order to avoid unfavourable interactions with L94. The resultant conformational change may reduce the dihedral angle between the isoxazole and the phenyl ring, raising the energy of the bound conformation. This would be consistent with the observation that small substituents (–CH_2OH, –$CH_2NHCOOEt$) are better tolerated than large ones (–$CH_2NHCOOBn$, –CH_2NHSO_2Bn).

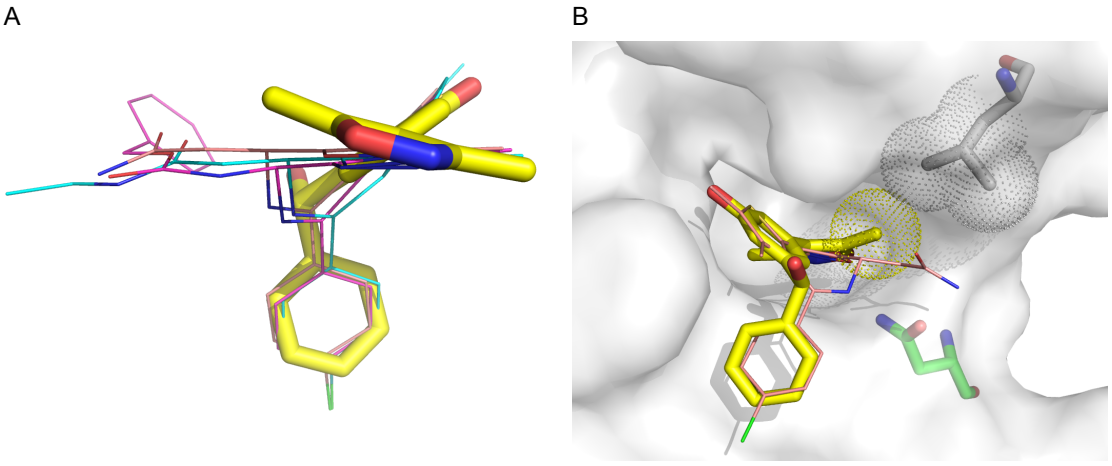


Figure 47. The 5-methyl group of 61a and the C(4) substituents on triazolobenzodiazepines adopt different orientations. (A) Overlay of ligands **61a** (carbon in yellow, PDB ID: 4J0S), GW841819X (**2**, carbon in magenta, PDB ID: 2YEL),[113] I-BET762 (**1**, carbon in cyan, PDB ID: 3P5O)[112] and CPI203 (**5**, carbon in salmon, PDB ID: 4LRG),[133] bound to BRD4(1). (B) The methyl group on C(5) of **61a** is directed towards L94 in BRD4(1) (PDB ID: 4J0S). Van der Waal's radii of C(5)Me and L94 are indicated with dots. The structure of CPI203 (carbon in salmon, PDB ID: 4LRG) is shown for comparison.

4.5.2 Conclusions and future work

This chapter described the synthesis of a range of 4-aryl-methylisoxazoles containing a range of substituents at the 3- and 5-positions, designed to probe interactions at the base of the KAc binding site and with the conserved KAc-binding asparagine residue, and to confirm the preferred orientation of the isoxazole. With regards to the latter objective, the results clearly indicate that the C(3) methyl group is preferentially directed into the base of the KAc binding site, since large and small modifications at C(5) were tolerated, while any modification at C(3) was unfavourable. Hydrophilic modification to the 3-position reduced activity, suggesting that such functionality is unable to interact favourably at the base of the pocket, either through displacing or forming hydrogen bonds to water molecules.

Exploring modifications to the 5-position, replacement of the methyl group with an amine, or with a methylene-linked primary alcohol, carbamate or urea is tolerated, but does not significantly increase affinity. I suggest this is because the preferred orientation of the isoxazole does not put a heteroatom in an optimum position to form a hydrogen bond to the conserved asparagine side-chain, and instead directs these substituents towards a nearby hydrophobic residue. Structural information on the binding of compounds such as **129a** or **129b** would help to test this hypothesis, by indicating whether the heteroatom is positioned to interact with the asparagine carbonyl group, and whether this has introduced an unfavourable conformational change in the rest of the molecule. A co-crystal structure with BRD4(1) would be particularly informative, but docking studies could shed some light on the consequences of 5-position modifications on ligand conformation and position in the binding site. If this hypothesis is correct, it would

only be possible to introduce favourable modifications at the 5-position in a more rigid system, such as the fused diazepines, and so further exploration of 5-position modifications in this series is unlikely to be worthwhile.

Chapter 5: Acetyl-Lysine-Mimicking Amino Acids

Site-specific incorporation of post-translationally modified amino acids and their mimics is an important approach for studying the functional significance of PTMs. It is envisaged that isoxazole-containing amino acids could bind to bromodomains with similar or greater affinity than KAc. Such amino acids would be valuable as non-hydrolysable KAc analogues and may assist in the development of improved bromodomain assays. Herein I review current techniques to produce proteins with homogeneous lysine modifications, focusing in particular on strategies to introduce KAc-mimicking amino acids. I then present the synthesis of three novel 3-methylisoxazole-containing amino acids containing different linkers between the heterocycle and α-carbon, and their incorporation into histone peptides.

5.1 Introduction and aims

5.1.1 Synthesis of post-translationally modified proteins

Post-translational protein modification plays a crucial role in regulating cellular processes, affecting the activity, stability and localisation of proteins, as described in Chapter 1. In a generalisation of the histone code hypothesis, it has been suggested that protein PTMs constitute a code that controls protein-protein interactions and regulates downstream function.[249] A given protein can often undergo many types of modification on many sites, giving rise to a heterogeneous population of modified proteins ('modiforms') which may differ in function.[250] Understanding the functions of particular PTMs represents a major challenge in modern biology, made all the more difficult by the dynamic nature of many PTMs and the complex relationships between them.

Access to homogeneously modified proteins is essential to understand the role of particular modifications. To that end, a number of chemical strategies have

been developed to synthesise single modiforms, either containing the native PTM or an analogue which functions in a similar manner. Three broad approaches have been introduced: native chemical ligation (NCL)/expressed protein ligation (EPL), tag-and-modify, and amber codon suppression.

In NCL and EPL, two peptidic fragments are joined by the reaction between an N-terminal cysteine residue and a C-terminal thioester. While these methods have been used for the total synthesis of proteins, for modified proteins, semisynthesis is often employed: one fragment containing the desired PTMs is produced synthetically, while the other fragment is an expressed protein. In NCL, the reaction between the C-terminal thioester of a synthetic peptide and the N-terminal cysteine of an expressed protein allows PTMs to be selectively introduced at the N-terminal region.[251] In EPL, the peptide is synthesised with an N-terminal cysteine, while the protein contains a C-terminal thioester (introduced by intein rearrangement), allowing the introduction of specific PTMs in the C-terminal region. A major advantage of this technique is that many PTMs can be introduced simultaneously and homogeneously into the protein, with a cysteine residue at the site of ligation being the only trace of the reaction. However, the sites are limited to residues close to the N- or C-terminus since this segment is synthesised to incorporate the desired PTMs. NCL and EPL can be combined to introduce PTMs at both the N- and C-termini.[252]

Amber codon suppression allows a 21st amino acid to be introduced co-translationally at a genetically-encoded site.[253] While extremely powerful, this methodology can be challenging to implement, due in part to the need to optimise the tRNA synthase to load the amino acid of choice onto the tRNA. Additionally, protein yields are often poor due to premature termination at the amber stop

codon rather than incorporation of the amino acid, and it is difficult to introduce more than one unnatural amino acid into a protein (although techniques are being developed to overcome this).[254,255]

A tag-and-modify approach uses an amino acid 'handle', often a cysteine residue, which is introduced co-translationally at the desired site. A selective reaction then converts the cysteine to a mimic of the desired modified amino acid. Cysteine can react directly with electrophiles or with other thiols through disulfide formation.[256] Alternatively cysteine can be converted chemically to dehydroalanine (Dha), followed by conjugate addition,[257] or enzymatically to formylglycine, followed by reaction with a hydroxylamine-containing compound to form an oxime.[258] Such techniques have been used extensively to synthesise analogues of post-translationally modified amino acids,[259] including methylated,[257,260,261] acetylated,[257,261-263] phosphorylated[257,264] and glycosylated[257,265] residues, and have been combined with amber codon by genetically incorporating an unnatural amino acid with bioorthogonal reactivity.[266]

5.1.2 Introduction of modified lysines

EPL/ECL, amber codon suppression and tag-and-modify approaches have all been used to introduce modified lysine residues or mimics thereof. These techniques have proved particularly valuable in uncovering the mechanistic roles of histone modifications.[267,268] Below I highlight examples of each strategy to illustrate their utility. Approaches to mimic KAc are discussed more fully in the next section.

NCL has been used by Shogren-Knaak and co-workers to investigate the effects of histone acetylation on chromatin structure. Using nucleosomes containing homogeneously-modified H4K16Ac (prepared by NCL), they demonstrated

that the H4K16Ac mark prevented chromatin compaction and impaired ATP-dependent nucleosome remodelling by the ISWI complex.[269] By contrast, acetylation of the histone H3 tail enhances remodelling, in part through bromodomain-dependent recruitment of the SWI/SNF complex.[270]

Introducing modified lysines by amber codon suppression has been pioneered by Chin, who first reported the incorporation of KAc into full-length recombinant proteins in 2008 using an engineered pyrrolysine tRNA synthetase/$tRNA_{CUA}$ pair.[271] The following year the same group described the production of homogeneously-acetylated histones which were incorporated into nucleosomal arrays. Their experiments with nucleosomes containing histone H3K36Ac suggested that this modification does not affect chromatin structure or chromatin remodelling by SWI/SNF, but does enhance 'DNA breathing', the partial unwrapping of DNA from histones which increases its accessibility.[272] Chin has subsequently reported the genetic incorporation *N*-Boc-*N*-methyl-lysine into histones, which undergoes mild acidolysis to reveal *N*-methyl-lysine.[273] Alternative approaches using the allyloxycarbonyl[274] or the photolabile *ortho*-nitrobenzyloxycarbonyl[275] protecting groups in place of Boc have also been developed. Chin has additionally reported an indirect method for the incorporation of *N*,*N*-dimethyl-lysine into histones.[276] Similar approaches have been used to incorporate the newly-discovered lysine derivatives *N*-propionyl-, *N*-butyryl- and *N*-crotonyl-lysine into histones.[277,278] Since very little is known about the importance of these modifications, it will be interesting to see if the technology facilitates studies into their function, as has been the case with KAc.

Tag-and-modify approaches employing cysteine modification are particularly attractive for the study of histone PTMs, as the nucleosome contains only a

single cysteine residue (H3C110) that can be mutated to alanine without affecting nucleosome function.[260] Cysteine can then be introduced at the desired location, either by site-directed mutagenesis and expression of the recombinant protein, or by NCL/EPL strategies.[279]. Shokat and co-workers were the first to employ a cysteine alkylation strategy for the synthesis of methyl-lysine analogues.[260,280] In this approach, a thioether-containing analogue of the methyl-lysine (methyl thialysine, K_SMe) is formed by reaction of the nucleophilic cysteine thiol with an appropriately-methylated 2-haloethyamine (Figure 48A). The methyl-lysine analogues behaved similarly to the methylated lysine in binding and enzymatic assays. As the chemistry required to introduce these mimics is relatively straightforward, they have been used in numerous studies investigating the functional significance of histone methylation, particularly the recruitment of proteins containing methyl-lysine recognition domains.[281-284] Similar cysteine alkylation strategies have been employed to incorporate KAc[262] and methyl-arginine[285] mimics. (The former are discussed more fully below.)

Rather than exploit the exceptional nucleophilicity of cysteine residue to allow selective reactions with electrophiles, Davis and co-workers have developed an elegant strategy employing cysteine as a masked electrophile: cysteine residues are converted to Dha rapidly and under ambient conditions,[286] then reacted with a variety of nucleophilic thiols to introduce PTM mimics. An initial report described the introduction of methylated thialysine on a model protein.[287] A more recent study extended the technique to form thioether analogues of methyl-lysine and KAc on histone proteins, as well as phosphorylated and glycosylated serine analogues (Figure 48B).[257]

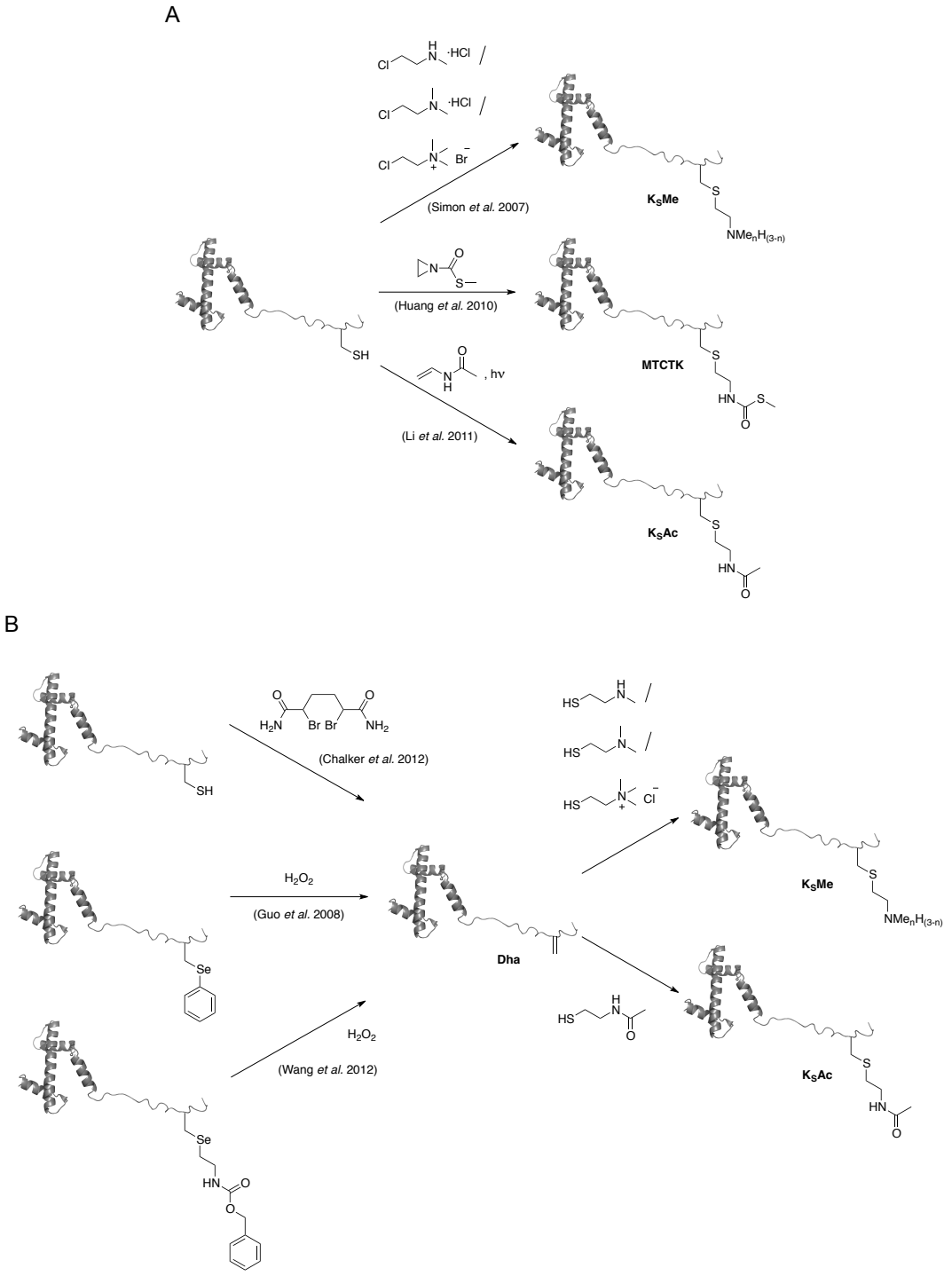


Figure 48. 'Tag-and-modify' approaches to methyl- and acetyl-lysine mimics on histones. A: Methyl- [260] and acetyl-[262,263] lysine mimics accessed directly from cysteine. B: Methyl- and acetyl-lysine mimics formed by addition to dehydroalanine (Dha).[257,261,288]

5.1.3 Acetyl-lysine mimicking amino acids

Traditionally, KAc has been mimicked by a lysine → glutamine mutation, which, like acetylation, constitutively neutralises the positive charge of lysine.[289] However, several lines of evidence suggest that glutamine is often a poor KAc mimic,[279,290] and is apparently unable to bind to bromodomains.[291] This is perhaps unsurprising, given that glutamine lacks the methyl group of KAc, and has a shorter side-chain. Therefore two alternative KAc-mimicking amino acids, methythiocarbonyl-thialysine (MTCTK, **150**) and acetyl thialysine (K_SAc, **151**), have been developed (Figure 49). These can be introduced into proteins using a tag-and-modify strategy.



Figure 49. Existing KAc-mimicking amino acids.

Cole and co-workers used cysteine alkylation to form thioether analogues of KAc,[262] in a similar manner to the aforementioned methyl-lysine analogues. Since *N*-(2-bromoethyl)acetamide and *N*-acetylaziridine were unreactive towards cysteine, the authors used methylthiocarbonyl-aziridine to create a thiocarbamate analogue of KAc (MTCTK, **150**).[262] The mimic was recognised by anti-KAc antibodies, and could partially recapitulate the regulatory function of lysine acetylation on the function of two enzymes. Significantly, histone H4 with MTCTK at K5 and K8 was recognised by the BET protein BRDT, albeit less effectively than native H4K5AcK8Ac. The authors did not investigate the binding of MTCTK to other bromodomains, or quantify the strength of the interaction. A p53 peptide

containing MTCTK was not a substrate for the HDACs HDAC8 or Sir2, in contrast to the KAc-containing peptide. The authors note that this could be useful in studies employing cell lysate where deacetylases are present, allowing the function of a 'constitutive' KAc to be investigated.[262]

K_SAc (**151**), while not accessible *via* cysteine alkylation, has been formed by other means, notably from Dha. Indeed, the first report of an KAc-mimicking amino acid came from the laboratory of Schultz, who introduced phenylselenocysteine using amber stop codon suppression. Oxidation and elimination using basic hydrogen peroxide afforded Dha-containing histones, which reacted with *N*-acetyl cysteamine to form K_SAc.[261] K_SAc was incorporated in place of K9 in histone H3 by an improved procedure employing Cbz-selenolysine, and the modification was recognized by an anti-H3K9Ac antibody.[288] A drawback to this method is the unavoidable oxidation of methionine residues under the conditions used to form Dha.

Two approaches have been developed to introduce K_SAc from cysteine residues. As noted above, Davis and co-workers were able to convert cysteine residues to Dha, which underwent conjugate addition with *N*-acetyl cysteamine.[286] This does not require the use of oxidising agents, and avoids many of the experimental challenges in using amber codon suppression techniques, notably the need to evolve appropriate tRNA synthetase-$tRNA_{CUA}$ pairs, and the low expression yields. K_SAc was a substrate for HDACs, in contrast to MTCTK, as a full-length histone H3K_S9Ac was partially deacetylated by HDAC1 and HDAC2.[257] It is important to note that PTM mimics introduced by addition to Dha are likely to be formed as epimeric mixtures, and it appeared that the anti-KAc antibody employed in their study preferentially recognised the natural L epimer. While the

interaction of K_SAc with bromodomains was not investigated, it seems likely that the two epimers will have different affinities for bromodomains.

An alternative approach for the introduction of K_SAc to histones has been developed by Chuan-Fa Liu and co-workers, who employed a free-radical thiol-ene reaction between cysteine and *N*-vinyl-acetamide.[263] Unlike the addition to Dha, this method is unlikely to cause significant epimerisation at the site of interest. The authors demonstrated that H4K_S16Ac was recognised by an anti-H3K16Ac antibody, and that an K_SAc-containing peptide was deacetylated by the HDAC Sir2. Furthermore, they reconstituted nucleosome arrays containing H4K_S16Ac and H4K_S16, as well as the natural KAc- and lysine-containing analogues. Impressively, K_S16Ac inhibited folding of the nucleosome array relative to both K16 and K_S16,[263] consistent with earlier studies employing H4K16Ac prepared by NCL, as discussed above.[269]

In summary, current KAc mimics, while undoubtedly useful, suffer from several disadvantages, including harsh oxidising conditions for their preparation or formation of epimeric mixtures. Perhaps most significantly, there has only been one reported attempt to assess the binding of these KAc mimics to bromodomains, which showed that MTCTK interacted more weakly with BRDT than KAc itself.[262] Well-characterised mimics with comparable bromodomain affinities to KAc would be valuable for the studying the functional significance of bromodomain-KAc interactions.

5.1.4 Improving bromodomain assays using KAc mimics

In addition to the potential utility of KAc mimics in studying the function of lysine acetylation, they may also prove useful in developing assays for bromodomain ligands. As has previously been noted (Section 1.5.2), a major challenge

in the development of robust peptide displacement assays for bromodomains, such as AlphaScreen®, is the inherently weak affinity of KAc for bromodomains, and the low peptide affinities which result.[119,292] Increasing peptide affinity may improve the reliability of existing assays, and allow new assays to be developed for bromodomains for which a cognate peptide of sufficient affinity was not known. Incorporating an KAc-mimicking amino acid in the peptide in place of KAc is a hitherto unexplored strategy for enhancing affinity.

5.1.5 Aims

Work in the preceding chapters has demonstrated that 3-methylisoxazoles can mimic KAc in their interaction with bromodomains. Therefore I wish to explore whether 3-methylisoxazole-containing amino acids can also bind to bromodomains. Specifically, my aims are as follows:

I. using X-ray crystal structure of acetylated peptides bound to bromodomains, to design 3-methylisoxazole-containing amino acids as KAc mimics.

II. to develop scalable synthetic routes to these Fmoc-protected amino acids.

III. to incorporate the amino acids into histone peptides, and to evaluate their affinity for bromodomains.

If these novel amino acid-containing peptides bind with greater affinity than KAc itself, they may prove useful for the development of more robust peptide displacement assays. Additionally, they could be incorporated into proteins as constitutive, non-hydrolysable, KAc mimics in order to discern the function of particular acetylation events. As with the MTCTK mimic, this would remove the

potential complication of deacetylation in cell extracts, but without the loss in bromodomain affinity.

5.2 Design of KAc-mimicking amino acids

Previous work undertaken in this group had showed that unprotected isoxazole-containing amino acids could displace KAc-containing peptides from some bromodomains at very high concentrations. A number of free amino acids were prepared and evaluated by AlphaScreen® against three bromodomains.[293] Among the most promising amino acids was **152** (Figure 50). The free amino acid completely inhibited the interaction of a $H3_{5-24}$K14Ac peptide with the bromodomain of BAZ2A at 250 μM in an AlphaScreen® assay, and additionally showed > 70% inhibition of the binding of tetraacetylated $H4_{1-20}$ to BRD9 at the same concentration. KAc, by contrast, has IC_{50} values in the millimolar range for various bromodomains.[119] These results suggested that it is most favourable to attach the isoxazole *via* the 5-position, consistent with the preferred orientation of the isoxazole in the KAc binding site. Furthermore, it suggested that a three-atom linker between the isoxazole and the Cα of the amino acid is preferred. To confirm this, I docked amino acid **152** into the structure of BRD4(1) bound to H4K5AcK8Ac (PDB ID: 3UVW) using Molsoft ICM-Pro. Comparing the predicted binding pose of **152** with the KAc residue suggested that the 3-methyl group could mimic the methyl group of KAc, and that a three-atom linker provided the optimum distance between the peptide backbone and the base of the pocket (not shown). Therefore I wished to incorporate **152** into a histone peptide to determine its affinity for bromodomains relative to KAc.

To explore whether alternative linkers would be preferred, I chose to synthesise amino acids **153** and **154**, which have ether or hydrocarbon linkages in

place of the amide of **152** (Figure 50). The greater flexibility of these linkers may permit the amino acid to adopt a conformation closer to that of KAc, but a greater entropic penalty may be encountered upon binding compared to **152**. In addition, the increased hydrophobicity of the hydrocarbon and ether linkers more closely resembles KAc and may therefore be favoured.

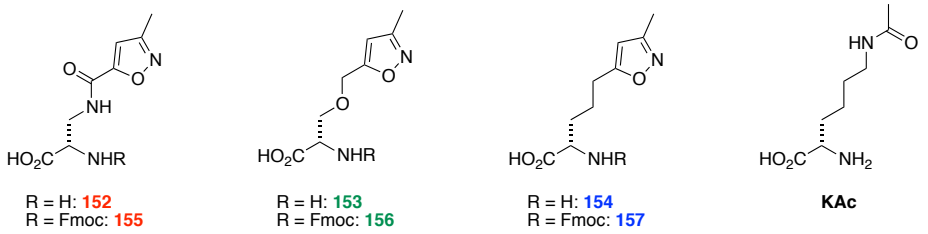


Figure 50. Putative KAc-mimicking amino acids 152-154 and their Fmoc derivatives 155-157. KAc is shown for comparison.

Since we, and others, have demonstrated that methyl-isoxazoles bind to BET bromodomains, I chose to incorporate **152**-**154** into BET bromodomain-binding peptides. BRD4(1) binds strongly to multiply-acetylated H4 tails both *in vitro*[44,119] and in live cells,[294] and a tetraacetylated $H4_{1-20}$ peptide is used in the BRD4(1) AlphaScreen®.[119] Furthermore, the dissociation constant for $H4_{1-20}KAc_4$ with BRD4(1) has been determined by ITC (K_d = 2.8 μM).[44] Therefore the histone $H4_{1-20}$ peptide was chosen as a model peptide to investigate the effects of incorporation of KAc-mimicking amino acids.

It has been established by Filipakopoulos *et al.*[44] and others[52] that a single BET bromodomain recognises two adjacent KAc residues on the same peptide. X-Ray crystal structures of H4K5AcK8Ac, H4K12AcK16Ac, and H4K16AcK20Ac with BRD4(1) revealed that the N-terminal KAc forms the hydrogen-bonding interaction with the conserved asparagine residue.[44] It is not clear whether an isoxazole-containing amino acid would be able to mimic the second (C-terminal) KAc. Consequently I chose to replace each of the four KAc individually with **152**,

153 or **154**. A control peptide, $H4_{1\text{-}20}KAc_4$ (**158**), was also required, in order to determine whether the proposed KAc mimics could interact more strongly with bromodomains than KAc itself (Table 14). In order to accurately quantify peptide concentrations by UV absorbance, an N-terminal tyrosine residue was included in the sequence. (This additional residue was also present in the $H4_{1\text{-}20}KAc_4$ sequence employed by Filippakopoulos *et al*. in ITC studies, and had little apparent effect on affinity.)[44]

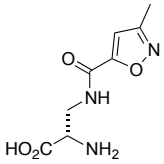
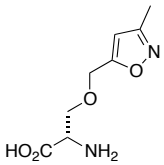
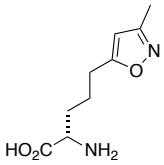
Amino acid	Sequence	Compound number
KAc	H-YSGRG-KAc-GG-KAc-GLG-KAc-GGA-KAc-RHRK-NH_2	**158**
 152	H-YSGRG-**152**-GG-KAc-GLG-KAc-GGA-KAc-RHRK-NH_2	**159a**
	H-YSGRG-KAc-GG-**152**-GLG-KAc-GGA-KAc-RHRK-NH_2	**159b**
	H-YSGRG-KAc-GG-KAc-GLG-**152**-GGA-KAc-RHRK-NH_2	**159c**
	H-YSGRG-KAc-GG-KAc-GLG-KAc-GGA-**152**-RHRK-NH_2	**159d**
 153	H-YSGRG-**153**-GG-KAc-GLG-KAc-GGA-KAc-RHRK-NH_2	**160a**
	H-YSGRG-KAc-GG-**153**-GLG-KAc-GGA-KAc-RHRK-NH_2	**160b**
	H-YSGRG-KAc-GG-KAc-GLG-**153**-GGA-KAc-RHRK-NH_2	**160c**
	H-YSGRG-KAc-GG-KAc-GLG-KAc-GGA-**153**-RHRK-NH_2	**160d**
 154	H-YSGRG-**154**-GG-KAc-GLG-KAc-GGA-KAc-RHRK-NH_2	**161a**
	H-YSGRG-KAc-GG-**154**-GLG-KAc-GGA-KAc-RHRK-NH_2	**161b**
	H-YSGRG-KAc-GG-KAc-GLG-**154**-GGA-KAc-RHRK-NH_2	**161c**
	H-YSGRG-KAc-GG-KAc-GLG-KAc-GGA-**154**-RHRK-NH_2	**161d**

Table 14. H4 peptide sequences synthesised in this work.

5.3 Synthesis of Fmoc-protected (*S*)-2-amino-3-(3-methylisoxazole-5-carboxamido)propanoic acid 155

Free amino acid **152** had previously been prepared in this group by the reaction of Boc-protected 2,3-diaminopropionic acid (Boc-Dap-OH) with 3-methylisoxazole-5-carboxylic acid (**162**), activated as its *N*-hydroxysuccimide

ester (**163**), followed by Boc deprotection (Figure 51).[293] Therefore to prepare the desired Fmoc amino acid for solid-phase peptide synthesis (SPPS), I envisaged reacting **163** with Fmoc-Dap-OH.

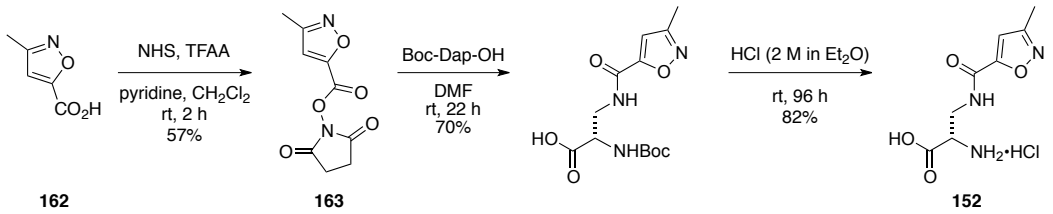


Figure 51. Previous preparation of amino acid 152 carried out within this group.[293]

107, the ethyl ester of acid **162**, had been previously prepared by a 1,3-dipolar cycloaddition, as described in Chapter 4 (Scheme 17). Separation of the regioisomers required repeated rounds of column chromatography, so an alternative route was investigated. Several reports detail the synthesis of ethyl 3-methylisoxazole-5-carboxylate **107** from ethyl 2,4-dioxopentanoate and hydroxylamine. As first noted by Sumimoto,[295] and also more recently by Lepage *et al*.[296] and Dannhardt *et al.*,[236] the regioisomer formed in the reaction depends on the conditions used. Under basic or neutral conditions, the 4-oxime can be isolated. This regioisomer is believed to predominate due to preferential enolisation of the α-ketone.[297-299] Subsequent treatment with acid gives ethyl 3-methylisoxazole-5-carboxylate (**107**). However, if ethyl 2,4-dioxopentanoate and hydroxylamine are heated together under acidic conditions, the alternative regioisomer, ethyl 5-methylisoxazole-3-carboxylate (**110**), is formed (Figure 52).

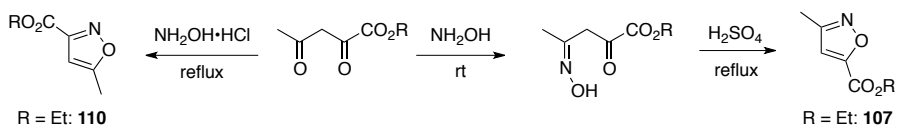
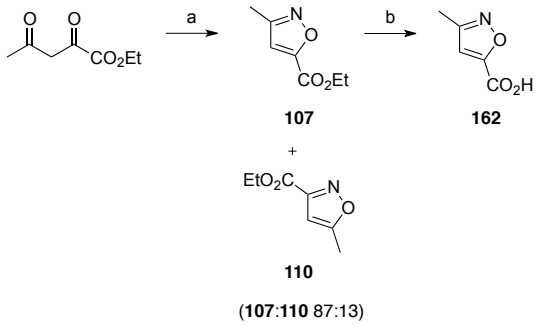


Figure 52. The regiochemistry of the condensation of alkyl 2,4-dioxopentanoates and hydroxylamine depends on the conditions used. Prior formation of the 4-oxime gives the 5-carboxyalkyl regioisomer after refluxing in acid; heating hydroxylamine hydrochloride with the alkyl 2,4-dioxopentanoate gives the 3-carboxyalkyl regioisomer. (Adapted from Dannhardt *et*

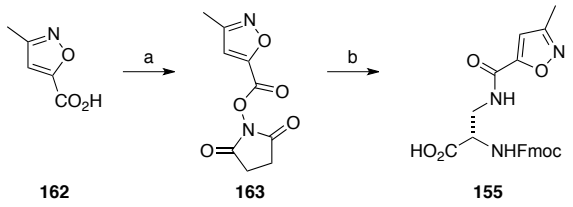
al.[236])

Following the procedure of Lepage *et al.*,[296] a 87:13 mixture of **107**:**110** (as determined by ^{1}H NMR analysis) was formed in a combined yield of 69% after distillation. After hydrolysis, **162** crystallised selectively as a single regioisomer (Scheme 31). (Lepage *et al.*[296] report a 98:2 mixture of acid **162** to the unwanted regioisomer.)



Scheme 31. *Reagents and conditions:* (a) (i) NaOH (20% w/v aq.), $NH_2OH \cdot HCl$, 1 h; (ii) conc. H_2SO_4, EtOH, reflux, 4.5 h, 69% (87:13 mixture of regioisomers); (b) LiOH, THF, H_2O, rt, 16 h, 54%.

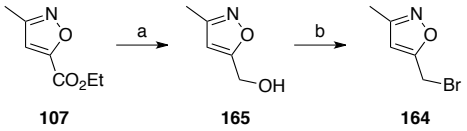
162 was then activated as the *N*-Hydroxysuccinimide ester **163** following the procedure of Leonard and Brunckova,[300] and coupled to Fmoc-Dap-OH. 915 mg of the desired Fmoc-protected amino acid **155** was obtained by crystallisation from boiling chloroform in 84% yield (Scheme 32).



Scheme 32. *Reagents and conditions:* (a) *N*-hydroxysuccinimide, trifluoroacetic anhydride, pyridine, CH_2Cl_2, rt, 1 h, 88%; (b) Fmoc-Dap-OH, rt, 17 h, 84%.

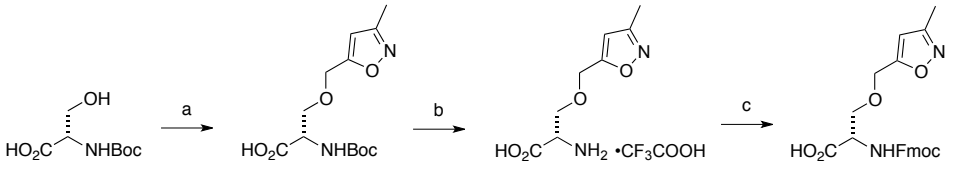
5.4 Synthesis of Fmoc-protected (*S*)-2-amino-3-((3-methylisoxazol-5-yl)methoxy)propanoic acid 156

Like **155**, **156** could be prepared by reaction of an isoxazole-containing electrophile with a commercially-available amino acid, in this case serine. The required electrophile **164** was prepared by Appel-type bromination of alcohol **165**, itself obtained by reduction of previously-prepared ester **107** (Scheme 33).



Scheme 33. *Reagents and conditions:* (a) $NaBH_4$, EtOH, rt, 7 h, 68%; (b) PPh_3Br_2, CH_2Cl_2, 0 ˚C, 1.5 h, then rt, 1.5 h, 80%.

Deprotonation of Boc-Ser-OH with 2.5 equivalents of sodium hydride followed by addition of bromide **164** gave **166** in excellent yield without the need for purification. Boc deprotection with TFA afforded the free amino acid **153** as its trifluoroacetate salt, which was then derivatised with Fmoc *N*-hydroxysuccinimide ester to afford 1.1 g of the desired Fmoc-protected amino acid **156** in 56% yield from Boc-Ser-OH (Scheme 34).



Scheme 34. *Reagents and conditions:* (a) NaH, DMF, 0 ˚C, 30 min, then **164**, 0 ˚C → rt, 13 h, 85%; (b) TFA, Et_3SiH, rt, 3.5 h, 83%; (c) $NaHCO_3$, H_2O, 1,4-dioxane, rt, 30 min, then Fmoc-OSu, rt, 3 h, 80%.

5.5 Synthesis of Fmoc-protected (*S*)-2-amino-5-(3-methylisoxazol-5-yl)pentanoic acid 157

Two alternative disconnection strategies were proposed for amino acid **157**. One strategy, indicated in red ('cycloaddition strategy'), involves the con-

struction of the isoxazole by a 1,3-dipolar cycloaddition to a terminal alkyne-containing amino acid, itself derived from stereoselective alkylation of a glycine equivalent. An alternative strategy, indicated in blue ('alkylation strategy'), starts with the isoxazole already in place, and uses an electrophile derived from 3,5-dimethylisoxazole itself (Figure 53).

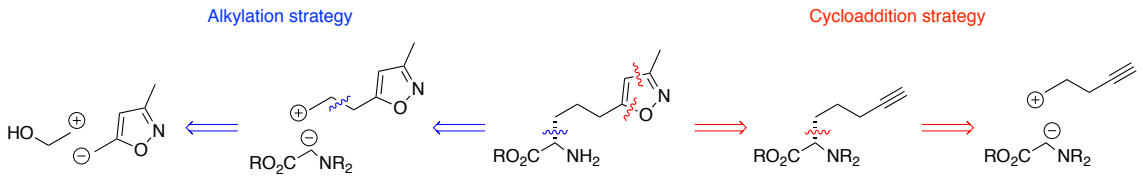


Figure 53. Disconnection strategies for the synthesis of **157**.

Several isoxazole-containing amino acids have been reported (Figure 54). The synthetic compound AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) mimics the binding of glutamate to a sub-class of glutamate receptors (AMPA receptors), where it acts as an agonist. Ibotenic acid is a neurotoxin, isolated from various mushrooms of the genus *Amantia*. It is decarboxylated to muscimol, the major psychoactive component of these mushrooms; muscimol is a $GABA_A$ agonist and mimics the binding of this amino acid to its receptors (Figure 54). Some of the earliest examples of isoxazoles with α-amino acid functionality linked at the 5-position were prepared as analogues of the AMPA.[301] However the achiral starting material employed resulted in racemic amino acids. In more recent examples, enantioenriched amino acids have been prepared. For example, Falorni *et al*., inspired by the discovery of isoxazole-containing mimics of neurotransmitters, used an alkyne derived from L-serine to synthesise isoxazol-5-yl-substituted amino acids.[302] Isoxazoles with α-amino acid functionality linked at the 5-position have also been used as synthetic intermediates: Caplan *et al*. used isoxazole-containing amino acids derived from propargyl

glycine as precursors to vinylogous amides.[303] Interestingly, in all cases the isoxazole is synthesised by a 1,3-dipolar cycloaddition, rather than being present in the starting materials or being introduced by condensation with hydroxylamine.

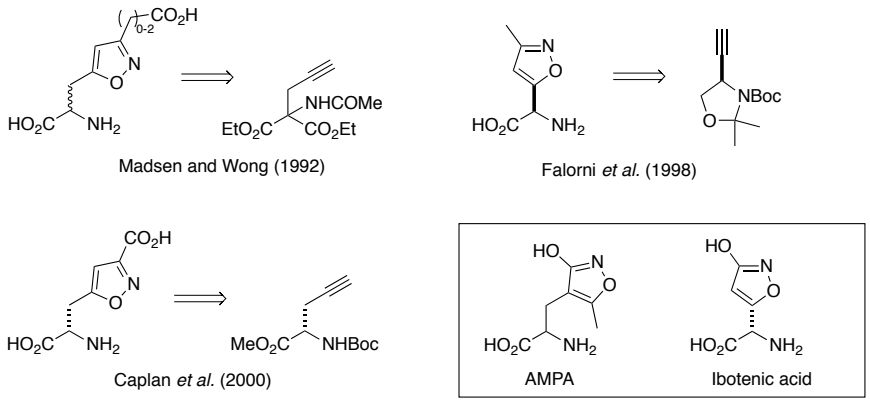


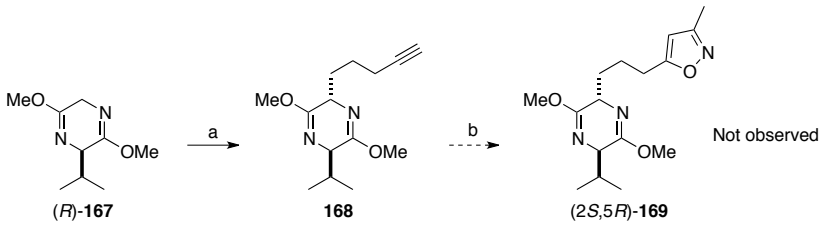
Figure 54. Some reported isoxazole-containing amino acids connected through the 5-position; the glutamate receptor agonist AMPA; and ibotenic acid, a prodrug for $GABA_A$ agonist muscimol.

Despite the interest in this class of compounds, there are no reported syntheses of analogues with longer chains (> 1 carbon) between isoxazole C(5) and the amino acid. Given the utility of the cycloaddition strategy in preparing the compounds described above, this approach was explored first for the synthesis of **157**.

*5.5.1 Cycloaddition strategy for the synthesis of **157***

(*S*)-2-Amino-6-heptynoic acid and protected derivatives, required for the cycloaddition, have previously been prepared by various multi-step strategies, including enzymatic resolutions by enantioselective hydrolysis of the corresponding *N*-acyl amino acid[304] or amino acid amide,[305] or by alkylation of a Ni^{II}-containing chiral auxiliary.[306] Seeking a shorter route to the protected alkyne-containing amino acid, I turned to the well-established chiral auxiliary developed by Schöllkopf.[307] The commercially-available dihydropyrazine (*R*)-**167**, derived from D-valine and glycine, was lithiated with ^{n}BuLi at –78 °C and treated with 5-

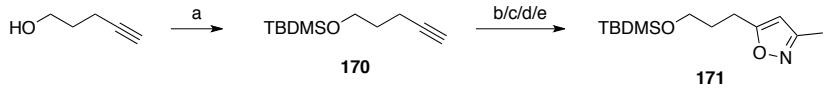
iodopent-1-yne to give **168** in 77% yield. ^{1}H NMR analysis suggested the formation of only a single diastereomer. Nitroethane, DMAP and the dehydrating agent DMTMM (4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methylmorpholinium chloride) with microwave irradiation have previously been employed for the formation of isoxazoles from terminal alkynes.[308] However, no product (2*S*,5*R*)-**169** was observed when using these conditions with alkyne **168** (Scheme 35).



Scheme 35. *Reagents and conditions:* (a) ^{n}BuLi, THF, –78 ˚C, 15 min then 5-iodopent-1-yne, THF, –78 ˚C, 2 h, then rt, 2 h, 77%; (b) DMTMM, DMAP, MeCN, rt, 10 min, then $EtNO_2$, 80 ˚C (microwave irradiation), 18 min.

To explore alternative methods of generating the nitrile oxide, TBDMS-protected 4-pentyn-1-ol **170** was subject to a range of cycloaddition conditions. This substrate was chosen because the steric environment of the alkyne is likely to be similar to that of **168**, and if the cycloaddition is successful, the alcohol could be potentially be activated and used to alkylate the lithiated Schöllkopf auxiliary, reversing the order of disconnections shown in Scheme 36. Repeating the above conditions using nitroethane, DMTMM and DMAP failed to generate any of the desired isoxazole **171**, and only starting alkyne was observed in the crude ^{1}H NMR spectrum. (These same conditions were also attempted, again unsuccessfully, on phenylacetylene.) Using di-*tert*-butyl dicarbonate, DMAP and nitroethane[309] gave only a trace of product (<5% after chromatography). Conditions to generate the nitrile oxide from *N*-hydroxyacetimidoyl chloride were also investigated. Using sodium hypochlorite to generate *N*-hydroxyacetimidoyl chloride from acetaldoxime *in situ*[310] gave 19% of **171**. Finally, *N*-hydroxyacetimidoyl

chloride was isolated from the reaction of acetaldoxime and *N*-chlorosuccinimide, then added in several portions to **170** and heated at reflux. This gave a modest 46% yield of isoxazole **171** (Scheme 36).



Scheme 36. *Reagents and conditions:* (a) Imidazole, DMF, rt, then TBDMSCl, rt, 26 h, 81%; (b) DMTMM, DMAP, MeCN, rt, 10 min, then $EtNO_2$, 80 °C (microwave irradiation), 3 min [no product observed]; (c) Boc_2O, DMAP, $EtNO_2$, MeCN, rt, 4 h, <5%; (d) acetaldoxime, NEt_3, NaOCl, $CHCl_3$, 0 °C → rt, 30 h, 19%; (e) *N*-hydroxyacetimidoyl chloride, NEt_3, CH_2Cl_2, reflux, 22 h, 46%.

*5.5.2 Alkylation of 3,5-dimethylisoxazole for the synthesis of **157***

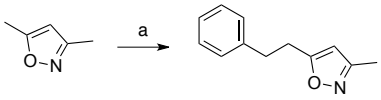
Given the consistently low yields obtained in the cycloadditions described above, I next investigated the alternative strategy outlined above, namely the selective alkylation of the 5-methyl group of 3,5-dimethylisoxazole with a two-carbon electrophile, followed by activation of this fragment as an electrophile and its stereoselective reaction with a glycine equivalent.

The selective deprotonation of 3,5-dimethylisoxazole on the C(5) methyl group is well-established, and is discussed in a review on isoxazole metalation reactions.[311] Briefly, Micetich first observed that 3,5-dimethylisoxazole could be selectively deprotonated with one equivalent of ^{n}BuLi on the C(5) methyl group, and showed that the resulting anion reacted with a variety of electrophiles.[312] This is consistent with the study of Kashima *et al.*, who observed that the rate of H–D exchange in sodium methoxide-d_3/methanol-d_4 is 280 times faster at the C(5) methyl group than at the C(3) methyl group.[313] On the basis of theoretical calculations, they attributed this selectivity to the greater stability of the anion formed at the C(5) methyl group. Interestingly, if lithiation is performed at –105 °C rather than the typical –78 °C, significant amounts of C(3) alkylation are observed,[314]

suggesting that C(3) deprotonation can occur under kinetic control, but C(5) deprotonation is thermodynamically favoured.

I envisaged that reaction of the isoxazole anion with ethylene oxide, a two-carbon electrophile, would furnish 3-(3-methylisoxazol-5-yl)propan-1-ol **172**. This could subsequently be activated and used to alkylate the lithiated Schöllkopf auxiliary, as described above. Deprotonation of 3,5-dimethylisoxzole and reaction with ethylene oxide has previously been reported,[236,315] but yields were moderate (45-58%) and the procedure required the use of an extremely toxic epoxide. Instead, I explored two ethylene oxide equivalents, 2-(2-bromoethoxy)tetrahydro-2*H*-pyran (**173**) and ethylene sulfate.[316,317] The former has previously been used to prepare **172**.[318]

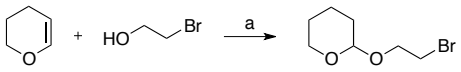
3,5-Dimethylisoxazole in THF was thus treated with 1.0 equivalents of ^{n}BuLi at –78 °C. After 1 h at this temperature, 1.05 equivalents of **173** (Sigma-Aldrich) were added and the reaction was warmed to rt over 15 h. Following an aqueous work-up and chromatographic purification, only 19% of **174** was obtained. In order to confirm that lithiation was proceeding as expected, benzyl bromide was used as the electrophile, but **175** was isolated in only 49% yield. An almost identical yield (48%) was obtained when the temperature was carefully monitored and maintained at < –70 °C during addition of ^{n}BuLi and benzyl bromide. However, when both THF and benzyl bromide were freshly distilled, the yield of **175** improved markedly to 79%, highlighting the importance of rigorously anhydrous conditions for this reaction (Scheme 37).



175

Scheme 37. *Reagents and conditions:* (a) ^{n}BuLi, THF, –78 ˚C, 1.5 h, then BnBr, –78 ˚C → rt, 15 h, 79%.

Repeating the reaction with **173** using distilled THF still gave a poor yield (14%). Addition of 12-crown-4 to chelate the lithium cation and increase the nucleophilicity of the anion led only to a modest improvement in yield (25%), and with an alternative base (KHMDS), no product at all was obtained. Reasoning that the consistently low yield might be a result of wet or otherwise impure commercial **173**, the bromide was prepared by the reaction of 3,4-dihydro-2*H*-pyran and 2-bromoethanol,[319] distilled and stored over 3 Å molecular sieves (Scheme 38).



173

Scheme 38. *Reagents and conditions:* (a) conc. HCl, rt, 3 h then $NaHCO_3$, 1 h, 86%.

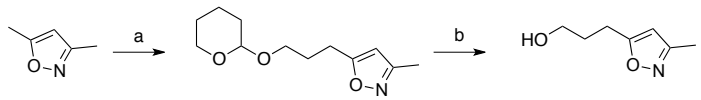
Gratifyingly, the yield of the alkylation improved to 58%. Additives such as 12-crown-4 and DMPU did not increase the yield further. Shorter times at –78 ˚C after the addition of **173** only decreased the yield. On a larger scale, this yield was improved to 70%. This optimisation is summarised in Table 15.

Source of **173**	Additive	Temperature after addition of **173**	Ratio bromide **173**: product **174**	Isolated yield
Commercial	–	–78 ˚C → rt, 15 h	75:25	19%[a]
Commercial	–	–78 ˚C → rt, 20 h	85:15	14%
Commercial	12-crown-4	–78 ˚C → rt, 16 h	69:31	25%
Commercial	–	–78 ˚C → rt, 16 h	Only **173**	0%[b]
Distilled	–	–78 ˚C → rt, 15 h	29:71	58%
Distilled	DMPU	–78 ˚C → rt, 15 h	35:65	54%

Distilled	12-crown-4	–78 ˚C → rt, 20 h	77:22	21%
Distilled	–	–78 ˚C, 2 h, then rt, 13 h	46:54	49%
Distilled	–	–78 ˚C → rt immediately, then rt, 5 h	86:14	10%
Distilled	–	–78 ˚C → rt, 17 h	11:89	70%[c]

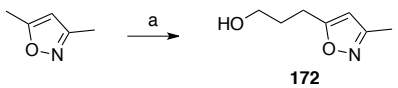
Table 15: Optimising the alkylation of lithiated 3,5-dimethylisoxazole with **173**. All reactions were performed on 1.02 mmol (100 µL) of 3,5-dimethylisoxazole with 1.05 eq (162 µL) **173** (unless stated). The ratio of **173**:**174** was determined by integration of the acetal OC*H*O proton in the ^{1}H NMR spectrum of the crude reaction mixture. Yields refer to isolated **174**; [a] THF dried using alumina drying column; [b] KHMDS used as base; [c] 51 mmol of both 3,5-dimethylisoxazole and **173**.

Cleavage of the THP ether with *para*-toluenesulfonic acid in methanol[318] took place cleanly and rapidly, giving alcohol **172** in 94% yield, or 66% from 3,5-dimethylisoxazole (Scheme 39).



Scheme 39. *Reagents and conditions:* (a) ^{n}BuLi, THF, –78 ˚C, 1.5 h, then **173**, –78 ˚C → rt, 17 h, 70%; (b) TsOH·H_2O, MeOH, rt, 2 h, 94%.

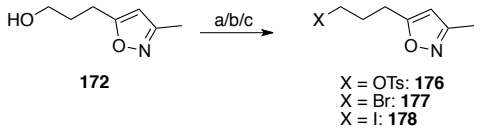
An alternative one-step route to **172** was also investigated, using ethylene sulfate (1,3,2-dioxathiolane-2,2-dioxide) as a C_2 building block. Reaction of the lithiated isoxazole with this cyclic sulfate in distilled THF, followed by acid hydrolysis of the resultant sulfate ester, gave **172** in 43% yield after chromatographic purification (Scheme 40). As significantly higher yields were obtained in the above two-step procedure, no further optimisation was undertaken.



Scheme 40. *Reagents and conditions:* (a) (i) ^{n}BuLi, THF, –78 ˚C, 2 h, then ethylene sulfate, –78 ˚C → rt, 16 h; (ii) conc. H_2SO_4, H_2O, THF, reflux, 14 h, 43%.

Attention was next turned to methods for activating the hydroxyl of **172** as a leaving group. Tosylate ester **176** was prepared under standard conditions.

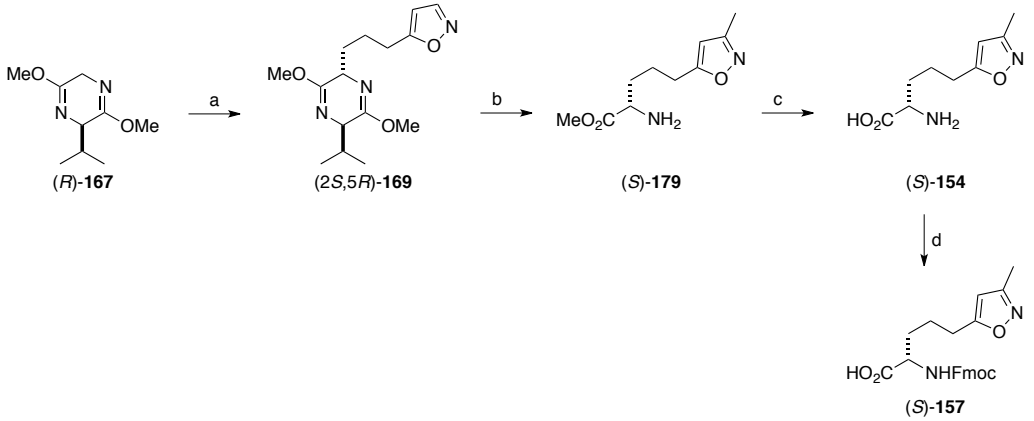
Both bromide **177**[315] and iodide **178** were synthesised by an Appel reaction in excellent yield (Scheme 41).



Scheme 41. *Reagents and conditions:* (a) X = OTs: TsCl, NEt_3, DMAP, CH_2Cl_2, rt, 25 h, 68% (**176**); (b) X = Br: Br_2, pyridine, PPh_3, CH_2Cl_2, 0 ˚C, 30 min, 92% (**177**); (c) X = I: I_2, imidazole, PPh_3, CH_2Cl_2, 0 ˚C, 2 h, 90% (**178**).

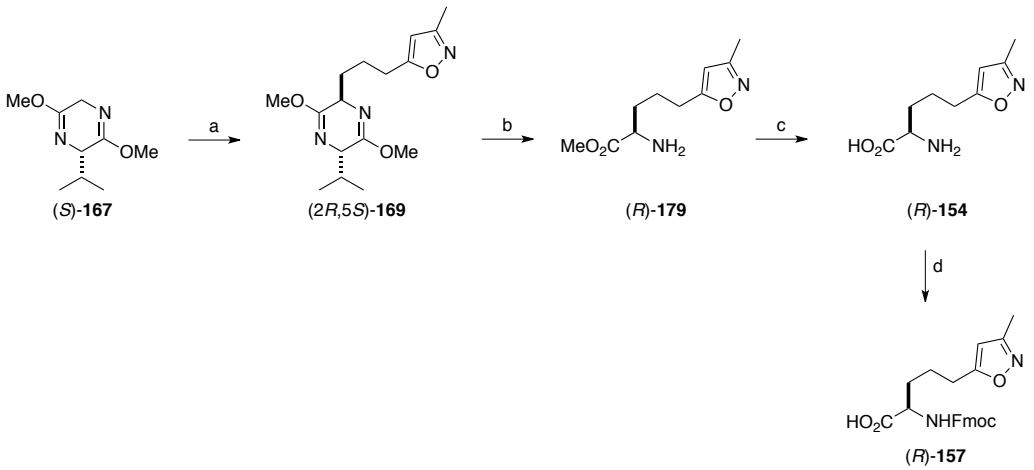
The ability of these electrophiles to react with the lithiated Schöllkopf auxiliary (*R*)-**167** was next examined. No product was observed by ^{1}H NMR or MS analysis in the reaction of the lithiated Schöllkopf auxiliary with tosylate **176**. In reactions with bromide **177**, small amounts of impure **169** (< 15%) could be isolated after the reagents were stirred together at –78 ˚C for 4 h then warmed to rt and stirred for a further 1 h. When LDA was used as a base for deprotonation of the Schöllkopf auxiliary,[320] no product was observed. Initial attempts to use iodide **178** also gave small amounts of impure **169**. However, if the reaction was warmed to –50 ˚C, stirred for 5 h then allowed to warm slowly rt overnight, the desired product was obtained in much higher yields (91% on a 280 µmol scale, 70% on a 10.6 mmol scale). ^{1}H NMR analysis suggested the isolation of a single diastereoisomer. The dihydropyrazine was then cleaved using TFA in aqueous acetonitrile; following column chromatography, any remaining valine methyl ester was removed *in vacuo*. Basic hydrolysis of the methyl ester (*S*)-**179** gave the free amino acid (*S*)-**154**. Purifying the amino acid using an ion exchange resin (Dowex 50WX8) gave moderate yields (29-56%). However, careful acidification of the reaction mixture to pH 5 caused the free amino acid to precipitate, and it was isolated by filtration in improved yield (75%). Reaction with Fmoc *N*-

hydroxysuccinimide ester afforded 1.7 g of the desired Fmoc-protected amino acid (*S*)-**157** in 91% yield after recrystallisation (Scheme 42).



Scheme 42. *Reagents and conditions:* (a) ^{n}BuLi, THF, –78 ˚C, 15 min, then **178**, –78 ˚C, 2 h, then –40 °C - –60 °C, 5 h, then –40 °C → rt, 14 h, 70%; (b) TFA, MeCN, H_2O, rt, 1.5 h, 81%; (c) LiOH, H_2O, THF, rt, 2 h, 75%; (d) $NaHCO_3$, H_2O, 1,4-dioxane, rt, 30 min, then Fmoc-OSu, rt, 2.5 h, 91%.

To allow chiral HPLC analysis (*vide infra*), a sample of the opposite enantiomer of **157** was also prepared, starting from the (*S*)-enantiomer of the Schöllkopf auxiliary (Scheme 43). Only a 25% yield of (*R*)-**154** was obtained after hydrolysis of the ester (*R*)-**179** using an ion exchange resin.



Scheme 43. *Reagents and conditions:* (a) ^{n}BuLi, THF, –78 ˚C, 15 min, then **178**, –78 ˚C, 2 h, then –40 °C - –60 °C, 6 h, then –40 °C → rt, 15 h, 76%; (b) TFA, MeCN, H_2O, rt, 3 h, 81%; (c) LiOH, H_2O, THF, rt, 2 h, 25%; (d) $NaHCO_3$, H_2O, 1,4-dioxane, rt, 30 min, then Fmoc-OSu, rt, 3.5 h, 56%.

5.6 Assessing the enantiopurity of Fmoc-protected amino acids

Prior to incorporation of these amino acids into peptides, I wished to establish their enantiomeric excess by chiral HPLC. Since **157** had been prepared from both enantiomers of the Schöllkopf auxiliary, optimisation was undertaken using these samples. No separation was observed on a Chiralpack® AD-H column with an ^{i}PrOH/heptane/0.1% TFA mobile phase.[321] However, using an Astec CHIROBIOTIC™ T column (5 µm, 25 cm × 4.6 mm) and a mobile phase of 50:50 MeOH + 0.1 % triethylamine + 0.1 % acetic acid / 0.1 M ammonium acetate buffer (pH 4.1), gave excellent separation (Figure 55). The same conditions were used for **155** and **156**. **155** gave one major and one minor peak, which were both confirmed to be **155** by LC-MS. Only one peak was observed with **156**, suggesting that no racemisation had occurred. However, it cannot be ruled out that both enantiomers co-eluted. The enantiomeric excesses are summarised in Table 16.

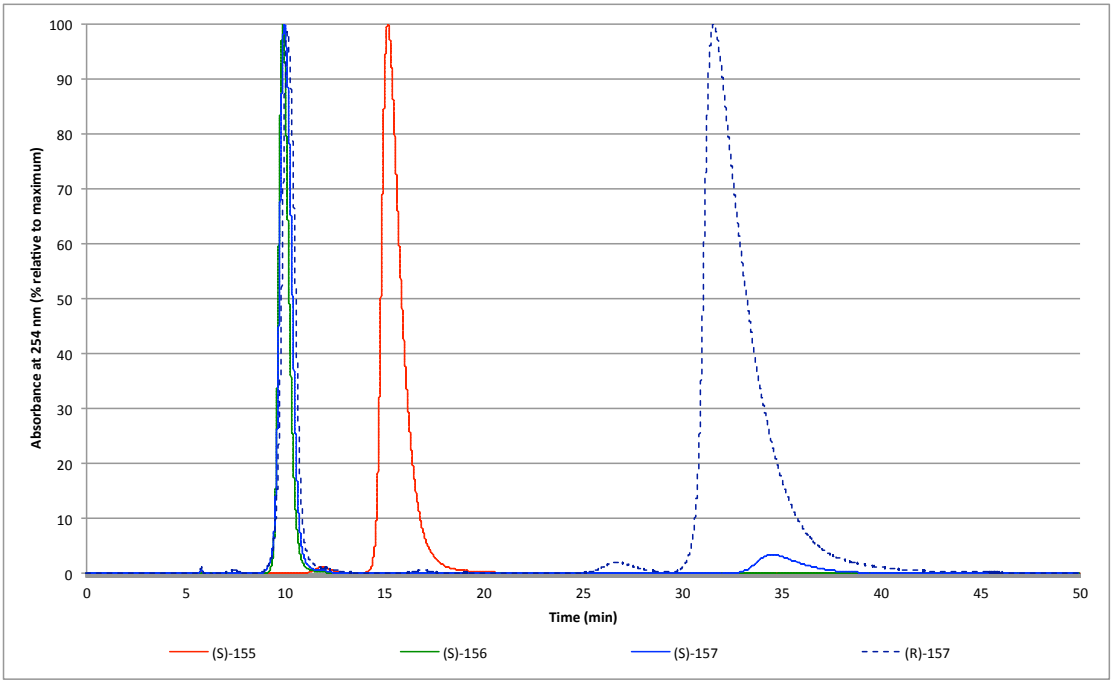


Figure 56. HPLC traces of (*S*)-**155**, (*S*)-**156**, (*S*)-**157** and (*R*)-**157** on an Astec CHIROBIOTIC™ T column.

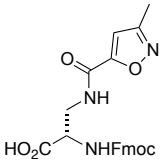
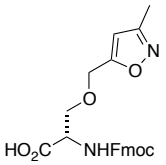
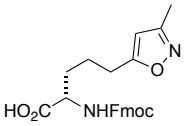
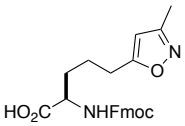
Compound number	Structure	Enantiomeric excess (%)
(*S*)-**155**		98
(*S*)-**157**		(100) [a]
(*S*)-**157**		76
(*R*)-**157**		54

Table 16. Enantiomeric excesses of Fmoc amino acids 155-157. [a] Only one peak observed in chiral HPLC trace.

5.7 Peptide synthesis

Histone H4 peptides (Table 14) were synthesised by standard Fmoc SPPS techniques as C-terminal carboxamides, and purified by reverse-phase HPLC to > 95% purity. I synthesised and purified peptides **158** (control), **159a** and **159b**, while **159c**, **159d**, **160a**-**d** and **161a**-**d** were synthesised and purified externally (ProteoGenix, France).

5.8 Preliminary evaluation of peptides 158-161

A preliminarily evaluation of the H4 peptides containing putative KAc mimics has been carried out by the SGC Oxford using AlphaScreen®. IC_{50} values were determined for **158**-**161** against BRD4(1) for the displacement of $H4_{1\text{-}20}KAc_4$ (Table 17). However, the values found for all peptides, including the control **158**,

were unexpectedly high. **158** gave an IC_{50} of 32 μM; by contrast, an almost identical peptide differing only in its C-terminus (free acid rather than carboxamide) in an identical assay gave an IC_{50} of 5.4 μM, and interacted with BRD4(1) with a K_d of 3.1 μM by ITC.[119] The fold change in IC_{50} relative to the control peptide **158** is shown in Figure 56.

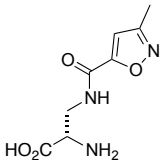
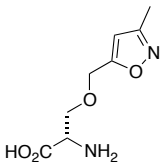
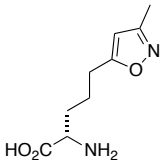
Amino acid	Position of modification	Compound number	BRD4(1) IC_{50} (μM)
KAc	–	**158**	32
 152	5	**159a**	>90
	8	**159b**	43
	12	**159c**	31
	16	**159d**	>90
 153	5	**160a**	39
	8	**160b**	>90
	12	**160c**	59
	16	**160d**	66
 154	5	**161a**	46
	8	**161b**	22
	12	**161c**	54
	16	**161d**	>90

Table 17. Preliminary IC_{50} values for displacement of $H4_{1\text{-}20}KAc_4$ peptide from BRD4(1) by **158**-**161** by AlphaScreen®. Protein and peptide concentration: 50 nM.

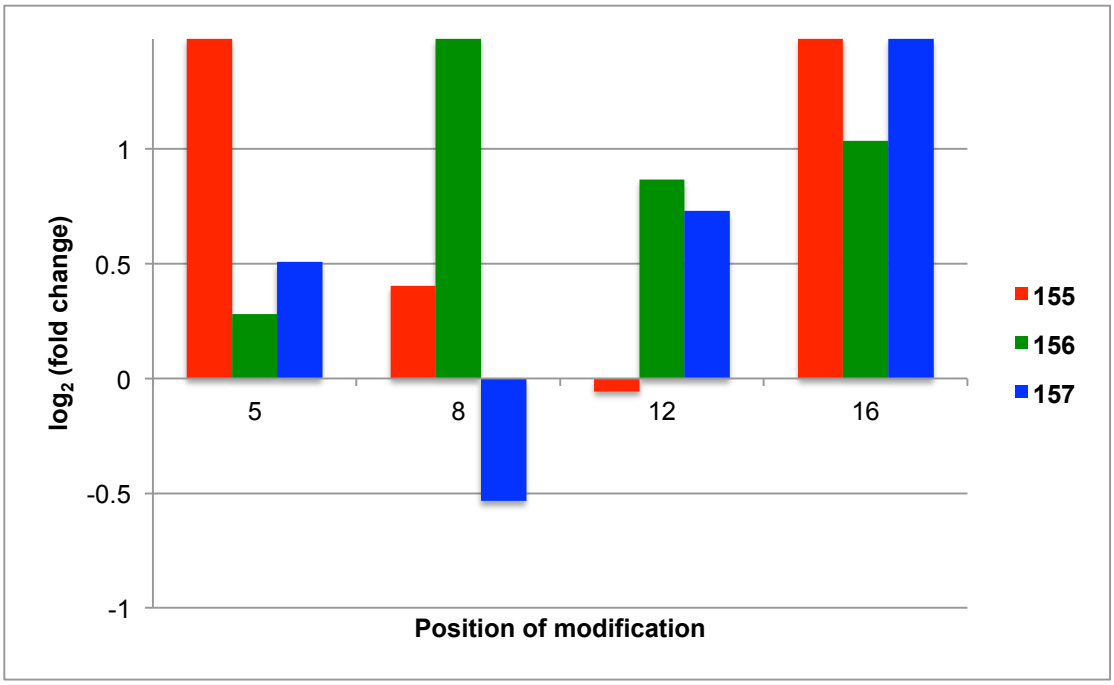


Figure 56. Fold change in IC_{50} relative to control peptide 158. The incorporated unnatural amino acid is indicated in the legend. The most weakly-interacting peptides gave IC_{50} values > 90 μM, implying $\log_2$ (fold change) > 1.5.

Due to the unexpected differences in IC_{50} for the control peptide between this assay and previously-reported values, it is difficult to draw conclusions about the affinity of these unnatural amino acids for BET bromodomains. However, the affinity seems to be strongly dependent on the position of the unnatural residue. Additionally, it appears that certain amino acids in some positions (e.g. **161** at position 8, and **159** at position 12) bind at least as strongly as the control.

5.9 Conclusion and further work

I have developed synthetic routes to three putative KAc-mimicking amino acids, **155**-**157**, containing the 3-methylisoxazole moiety. Fmoc-protected amino acids **155**, containing an amide linkage, and **156**, containing an ether, were synthesised in high enantiomeric excess from commercially-available L-amino acids. **155** was prepared directly from the NHS ester of 3-methylisoxazole-5-carboxylic acid (**163**) and Fmoc-Dap-OH in 88% yield. **156** was prepared from

Boc-Ser-OH and 5-(bromomethyl)-3-methylisoxazole (**164**) in 56% yield over 3 steps (from Boc-Ser-OH). Several strategies were attempted for the synthesis of Fmoc-protected amino acid **157**, containing a 3-carbon alkyl linker between the α-carbon and the isoxazole. Formation of the isoxazole by 1,3-dipolar cycloaddition between a terminal alkyne and acetonitrile-*N*-oxide met with limited success. However, construction of the carbon linker was more successful: lithiation of 3,5-dimethylisoxazole and alkylation with an ethylene oxide equivalent, **173**, followed by deprotection and Appel reaction gave iodide **178** in 59% yield from 3,5-dimethylisoxazole. **178** was then used to alkylate Schöllkopf's auxiliary (*R*)-**167**, with cleavage of the auxiliary and Fmoc protection affording (*S*)-**157** in 39% yield from the auxiliary over four steps. The ee of (*S*)-**157** was rather low, possibly due to racemisation during the lithium hydroxide-mediated ester hydrolysis. To improve the ee, alternative chiral auxiliaries, such as Myers's pseudoephedrine-based auxiliary,[322] or a enzymatic resolution strategy,[304] could be investigated.

All three amino acids were successfully incorporated into 21-mer histone H4 peptides by SPPS. However, preliminary evaluation of the interaction between the modified histone peptides and BRD4(1) was rather inconclusive, as the control peptide ($H4_{1\text{-}20}KAc_4$) gave an unexpectedly high IC_{50}. Due to time constraints, the assay has not yet been repeated. It would be extremely useful to use alternative assay techniques to evaluate the affinity of these peptides for bromodomains; SPR or ITC, both label-free methods, would be suitable.

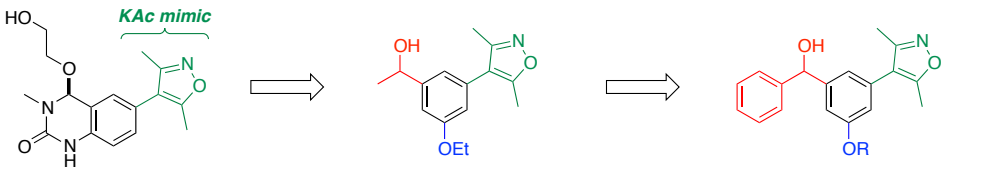
If it can be reliably shown that one or more of the amino acids **152**-**154** can bind to bromodomains with similar or greater affinity to KAc in the context of a histone peptide, I envisage several strategies for further extension of this work:

- an X-ray crystal structure or NMR structure of the peptide bound to a bromodomain would confirm that the modified amino acid mimics KAc. If subsequent data reinforces preliminary indications that affinity is strongly dependent on the position of the unnatural residue, structures might provide a possible rationale for this selectivity.
- the stability of these amino acids towards HDACs should be investigated. If they are neither substrates nor high-affinity competitive inhibitors of HDACs, such KAc mimics would be useful for the study of lysine acetylation in cell extracts without the complicating effects of deacetylation.
- the utility of these amino acids would be significantly enhanced if they bind to anti-KAc antibodies, as has been demonstrated for MTCTK and K_SAc. Therefore the recognition of **152**-, **153**- and **154**-containing peptides by appropriate antibodies should be investigated.
- in order to enhance existing bromodomain assays, peptides containing the modified amino acids could be profiled against a range of bromodomains for which suitable peptides have not been identified. Any enhancement in affinity over the analogous KAc-containing peptide may provide a starting point for developing an improved assay.
- incorporating KAc mimics into full-length proteins by amber codon suppression could be investigated, allowing site-specific installation of a bromodomain-binding constitutively-acetylated lysine residue into a protein for functional studies. As an alternative, a tag-and-

modify strategy to introduce isoxazole KAc mimics into peptides by cysteine modification may be suitable, and is currently under investigation in our laboratory.

Chapter 6: Conclusion

The work presented in the preceding chapters concerns the design, synthesis and evaluation of isoxazole-containing small-molecule and peptidic ligands for bromodomains. These compounds constituted only the second reported class of potent inhibitors of bromodomain-histone interactions. Starting from an unstable, unselective and cytotoxic hit **45**, highly ligand-efficient, stable and non-toxic 4-phenyl-3,5-dimethylisoxazoles were shown to bind to BRD4(1) and CREBBP and inhibit the binding of acetylated histone peptides.[126] Using a structure-guided design approach, these compounds were elaborated into BET bromodomain ligands **61a** and **61b** with sub-micromolar IC_{50} and K_d values while maintaining ligand efficiency (Figure 57).[127] They show some selectivity over the CREBBP bromodomain, and are very selective over other, less closely-related bromodomains. Crystallographic and SAR studies highlighted the importance for BET bromodomain affinity of hydrophobic substituents in the WPF shelf and substituents capable of forming hydrogen bonds in the ZA channel. Based on these findings, several possible strategies were presented for further optimisation.



hit (45)
IC_{50} = **~7 μM** *vs* BRD4(1)

47d
IC_{50} = **4.8 μM** *vs* BRD4(1)
LE = 0.39

R = H (**61a**): IC_{50} = **384 nM** *vs* BRD4(1)
LE = 0.41
R = Ac (**61b**): IC_{50} = **371 nM** *vs* BRD4(1)
LE = 0.36

Figure 57. Development of 3.5-dimethylisoxazole-containing BET ligands from an unstable, unselective hit.

Compounds **61a** and **61b** show cellular effects consistent with BET bromodomain inhibition. Notably, they possess appreciable cytotoxicity in the MV4;11 AML cell line, but have little effect on HeLa or U2OS cells. Evaluation of

61a in the NCI-60 human tumour cell line screen revealed large differences in sensitivity between cell lines, and a similar overall profile to the BET inhibitor PFI-1 (**16**), though a rather different profile to XD-14 (**20**). Preliminary studies suggest that **61a** downregulates *MYC* expression and suppresses NF-κB-dependent transcription, consistent with inhibiting BRD4-KAc interactions.

Modification or replacement of the methyl groups of 4-phenyl-3,5-dimethyisoxazoles indicated that the 3-methyl group was required for affinity, consistent with its location at the base of the KAc-binding site of the bromodomain. Modifications of the 5-methyl group were tolerated, but did not lead to enhanced affinity.

The knowledge that 3-methylisoxazole-containing small molecules could mimic the interactions of KAc with bromodomains suggested that isoxazole-containing amino acids might also bind to bromodomains. Such amino acids would prove useful in studying the function of lysine acetylation events, and in developing improved assays for bromodomain ligand discovery. Three amino acids were synthesised and incorporated into histone peptides for evaluation of their bromodomain affinities. Initial evaluation of their affinity was inconclusive, but further studies are ongoing.

In summary, this dissertation describes the identification of a novel class of KAc-mimetic bromodomain ligands containing the isoxazole moiety. These compounds constitute valuable leads for further optimisation, as well as useful tool compounds for the study of bromodomain biology.

Chapter 7: Experimental Procedures

7.1 General experimental procedures for synthetic chemistry

^{1}H NMR spectra were recorded on Bruker AV400 (400 MHz) or Bruker AVII 500 (500 MHz) 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 Bruker AV400 (100 MHz) or Bruker AVII 500 (126 MHz) spectrometers 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) = 0.00 ppm.

^{19}F NMR spectra were recorded on a Bruker AVII 500 (470 MHz) 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). The chemical shift data for each signal are given as δ_B in units of parts per million (ppm).

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

High-resolution mass spectra were acquired on a Bruker MicroTOF spectrometer from solutions of methanol, water or acetonitrile (ESI), or a Waters GCT TOF spectrometer with a temperature-programmed solids probe inlet (FI), operating in positive or negative mode.

Low-resolution mass spectra were recorded on a Waters LCT Premier spectrometer or Agilent 6120 Quadrupole LC/MS spectrometer (ESI). *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses.

Mass spectra of purchased 45a (Appendix 1) were obtained using an Agilent MSD-ToF electrospray ionisation orthogonal time-of-flight mass spectrometer. The sample was diluted 1:100 (v/v) in LC-MS grade acetonitrile and infused directly into the ion source at a flow rate of 3 µL per minute using a syringe pump. The instrument was configured with the standard ESI source and operated in positive ion mode. Data analysis was performed using Quantitative Analysis software (Agilent Technologies Inc). LCMS of resynthesized **45a** (Appendix 1 was obtained using a Waters Nano Acquity nano-LC system interfaced to a Waters Synapt mass spectrometer *via* an electrospray source. LC conditions were: Waters 1.7 µM BEH C18 75 µM × 150 mm column with a binary solvent

system using water + 0.1% formic acid and MeCN. Data analysis was performed with using Synapt software (Waters Corporation).

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

Specific optical rotations were measured using a Perkin Elmer 341 polarimeter, in cells with a path length of 1 dm. The light source was maintained at 589 nm. The concentration (*c*) is expressed in g/100 mL (equivalent to g/0.1 dm^3). Specific rotations are denoted $[\alpha]_D^T$ and are given in implied units of 10^{-1} deg cm^2 g^{-1} (where T = ambient temperature in ˚C).

Melting points on recrystallised 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. Elemental analysis was carried out in duplicate; average values are reported.

Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} aluminium-supported thin layer chromatography sheets. Visualisation was by absorption of UV light (λ_{max} 254 nm), or thermal development after dipping in an aqueous solution of potassium permanganate, potassium carbonate and sodium hydroxide.

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).

Analytical HPLC was carried out on a PerkinElmer Flexar system with a Binary LC Pump and UV/VIS LC Detector. For determination of compound purity, a Dionex Acclaim® 120 column (C18, 5 μm, 120 Å, 4.6 × 150 mm) was employed, with a 10-minute gradient of 95%H_2O/5%MeCN + 0.1% TFA (Solvent A) to 95% MeCN/5% H_2O + 0.1% TFA (Solvent B), flow rate 1 mL/min and detection at 254 nm (unless stated otherwise). Samples were injected in DMSO, MeOH, or DMSO/acetone or DMSO/chloroform mixtures. The method was as described below:

Step length (min)	Elapsed time (min)	%A	%B
1	1	100	0
10	11	0	100
3	14	0	100
1	15	100	0
5	20	100	0

For determination of the enantiomeric excess of **155**, **156** and **157**, an Astec CHIROBIOTIC™ T column (5 μm, 25 cm × 4.6 mm) was employed, with isocratic elution of 50:50 MeOH/0.1% NEt_3/0.1% AcOH and pH 4.1 0.1 M aqueous triethylammonium acetate buffer, flow rate 0.6 mL/min and detection at 254 nm. Samples were injected in MeOH.

Preparative chiral HPLC for the separation of (–)-**61a** and (+)-**61a** was carried out on a CHIRALCEL OG column (2 × 25 cm) using an Agilent 1260 Infinity Series instrument, eluent hexane:2-propanol 85:15, flow rate 18 mL/min. UV absorbance was monitored at 230 nM. Retention times for (–) and (+) enantiomers were 18.7 and 24.4 min respectively. Analytical chiral HPLC on these

samples was carried out on a CHIRALCEL OG column (4.6 × 250 mm) using an Agilent 1200 Series instrument, eluent hexane:2-propanol 80:20, flow rate 0.80 mL/min. UV absorbance was monitored at 230 nM. Retention times for (–)-8 and (+)-8 enantiomers were 12.8 and 15.4 min respectively. [Enantiomeric excess of both enantiomers was >99%.]

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 Et_2O were dried over activated 3 Å molecular sieves under an argon or nitrogen atmosphere (where stated, THF was distilled from sodium and benzophenone); anhydrous DMAc (Sigma) was degassed by repeated freeze-thaw cycles and stored over activated 3 Å molecular sieves under an argon atmosphere. DME was distilled prior to use. DMSO, DME and aqueous Na_2CO_3 were degassed by bubbling a stream of nitrogen gas through the solution for several hours with sonication before use. EtOH was degassed by repeated freeze-thaw cycles and stored under an argon atmosphere, but was not dried.

Chemicals were purchased from Acros UK, Sigma-Aldrich UK, Alfa Aesar UK or Fisher UK. 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. Where necessary, K_2CO_3, Cs_2CO_3 and Na_2CO_3 were dried in an oven prior to use. MnO_2 was activated at 250 °C overnight prior to use. *N*,*O*-Dimethylhydroxylamine hydrochloride and KOAc were dried in a vacuum desiccator prior to use. BnBr was distilled under vacuum and stored over 3 Å molecular sieves under an argon atmosphere. TsCl was crystallised from boiling 40-60 °C petroleum ether. Organolithium reagents were titrated against diphenylacetic

acid,[323] and commercially-available organomagnesium compounds were titrated against salicaldehyde phenylhydrazone.[324]

Reactions with microwave irradiation were carried out in a Biotage Initiator microwave synthesizer.

Peptide synthesis: **158**, **159a** and **159b** were prepared on a CS Bio CS336X synthesiser using standard Fmoc SPPS techniques with double coupling, then purified by semi-preparative reverse phase HPLC to > 95% purity (analytical HPLC at 220 nm). **159c**, **159d**, **160a**-**d** and **161a**-**d** were synthesised by Proteo-Genix and purified to > 95 % purity.

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 30-40 °C unless otherwise stated.

General procedure for Grignard reaction of aryl bromide and 60: To a dry two-necked flask equipped with a condenser, containing Mg turnings (66 mg, 2.45 mmol, 3.0 eq) and a crystal of iodine, was added anhydrous Et_2O or anhydrous THF (20 mL) under a nitrogen atmosphere. Aryl bromide (2.69 mmol, 3.3 eq) was then added, either neat (if liquid) or as a solution in THF (269 mM, 10 mL). The reaction was heated gently to initiate Grignard reagent formation, then heated under reflux until all Mg had reacted. The reaction was then cooled to 0 °C and added slowly to a solution of **15** (200 mg, 815 μmol, 1.0 eq) in Et_2O or THF (81.5 mM, 10 mL) at 0 °C under a nitrogen atmosphere. The solution was warmed to rt. On consumption of aldehyde (as indicated by TLC analysis), the reaction was quenched with H_2O and aq. HCl (1 M). The phases were separated, and the aqueous layer extracted with Et_2O. The combined organic layers were washed with H_2O and brine, dried ($MgSO_4$), filtered and concentrated *in vacuo*.

The crude residues were purified by silica gel column chromatography (gradient elution, Et_2O/petroleum ether).

7.2 General experimental procedures for biological evaluation

7.2.1 Procedures employing recombinant protein

Protein expression and purification (SGC Oxford): Proteins were cloned, expressed and purified as previously described.[111]

Thermal stability shift assay (SGC Oxford): Thermal melting experiments were carried out using the Mx3005p real-time PCR machine (Agilent) and employing a protein concentration of 2 µM and inhibitor concentration of 100 mM. 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.[29] Experiments were performed in duplicate; average values are reported.

Bromodomain AlphaScreen® assays (SGC Oxford) were carried out as previously described[111,119] using the following peptides: H4KAc4 peptide (BRD2 and BRD4 assays, H_2N-SGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RHRK(Biotin)-COOH) and H3K56Ac peptide (CREBBP assay, H_2N-ALREIRRYQK(Ac)STELLIRKLK(Biotin)-COOH). All experiments were carried out in duplicate on the same plate.

Surface plasmon resonance methods and analysis of compound binding to BRD4(1) (Andrew Gill, Alison J. Ritchie, David W. Sheppard, BioFocus UK) was carried out as previously described.[127]. Sensorgrams and binding curves were analyzed with BIAevaluation (GE Healthcare) software using a 1: 1 binding model. All compounds tested were selective for binding to BRD4(1) over control protein (carbonic anhydrase). Good agreement was observed in the

K_d values derived from kinetic and equilibrium analysis (within 2- fold). The kinetic parameters k_a and k_d could not be accurately determined in this experiment due to the rapid rates of association and dissociation observed under these conditions. Reported data were generated using steady state analysis with a 1:1 binding model.

7.2.2 Cellular assays

Cytotoxicity of 45a, 47d and (+)-JQ1 in HeLa cells (Chaitanya Vuppusetty, SGC Oxford):[126] HeLa cells were grown and maintained in DMEM supplemented with L-glutamine and 10% fetal calf serum. 20000 cells per well were seeded into a 96 well plate. After 24 h incubation at 37 ˚C, the medium was changed and compounds were applied at final concentrations of 10, 1, 0.1 and 0.01 μM. The cells were incubated for 24 or 48 h, after which time the medium was changed and MTT solution (5 mg/mL) was added to plate. After 2 h incubation at 37 ˚C, the medium was replaced with 200 μL DMSO to solubilize the formazan crystals. After a further 15 minutes, absorbance was measured at 550 nm using a spectrophotometer. Experiments were performed in triplicate on the same plate.

Cytotoxcity in HeLa and U2OS cells (Christopher Wells, SGC Oxford):[127] HeLa cells were grown and maintained in DMEM supplemented with L-glutamine and 10% fetal calf serum; U2OS cells were grown and maintained in McCoy's medium supplemented with 10% fetal calf serum. Cells were seeded in a 96-well plate at a density of 5×10^3 cells per well in 100 μL of media. After 24 h, cells were treated with DMSO (negative control), staurosporine (positive control) or test compounds. After 24 h or 72 h, 10 μL of WST-1 (Roche Diagnostics) was added to the culture medium and cell viability was determined by colorimetric

WST-1 conversion assay. Formazan dye formation was measured at 450 nm using a Molecular devices Spectramax Plus384 microplate reader. Relative WST-1 conversion in treated cells compared with untreated control cells was calculated after subtraction of a WST-1 conversion in the absence of cells. Dose response graphs were analysed using the GraphPad Prism 5 program (GraphPad Software, Inc.).

Cytotoxicity in MV4;11 cells (Katherine Freeman, Department of Chemistry, University of Oxford):[127] MV4;11 cells were grown and maintained in RPMI-1640 medium with L-glutamine supplemented with 10% fetal calf serum and 1% (v/v) penicillin/streptomycin antibiotics. MV4;11 cells were seeded in a 96-well plate at a density of 1×10^4 cells per well in 100 µL of media. Cells were treated with **58d, 61a, 61b,** DMSO (negative control), (+)-JQ1 or SGI-1776 (SuperGen) (positive controls) in 100 µL of media. After 72 h at 37 ˚C, 25 µL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) coupled with phenazine methosulfate was added to each well, according to the manufacturers' instructions. Plates were incubated for 2 h at 37 ˚C, 5% CO_2 and the absorbance read at 490 nm. Positive and negative controls behaved as expected (IC_{50} SGI-1176: 53 nM, (+)-JQ1: 242 nM). The same protocol was followed for the MTS viability assay A549 and H1975 cells (Monica Olcina del Molino, Department of Oncology, University of Oxford).

Clonogenic survival assay (Monica Olcina del Molino, Department of Oncology, University of Oxford):[127] H1975 or A549 cells were plated in a 6-well plate at a density of 300 cells/well, and allowed to adhere for 5 h before treatment. The cells were treated with either **61a, 61b** or DMSO (as a control). Colonies (of

at least 50 cells) were allowed to form for 10-14 days. The media was removed, and colonies were stained with crystal violet and counted.

In-Cell Western for c-Myc (Joana Senra, Department of Oncology, University of Oxford): HCT116 cells (p53 wild-type or null) were plated in a 96-well plate at a density of 50,000-75,000 cells/well in 180 µL of medium (DMEM supplemented with 10% **foetal bovine serum)**, and allowed to adhere for 3 h before treatment. The cells were treated with **61a**, (+)-JQ1 or DMSO (as a control) to give an overall volume of 200 µL. After 96 h incubation at 37 ˚C, the medium was removed and the cells were washed with PBS. The cells were fixed with 4% paraformaldehyde solution (50 µL/well) for 15 min, and then washed twice with PBS. Cells were permeabilised four times by application of 0.1% Triton X-100 detergent (100 µL/well) for 5 min, then blocked with 100 µL Odyssey™ blocking buffer (Li-Cor®) for 1.5 h at rt. The buffer was removed, 25 µL anti-c-MYC antibody (clone: D84C12, Cell Signalling Technology®) diluted 1:500 in Odyssey™ blocking buffer was applied to each well, and the plate was incubated overnight at 4 ˚C with gentle shaking. The plate was washed four times with 100 µL PBS + 0.1% Tween 20 for 5 min at rt with gentle shaking. The buffer was removed, and 25 µL of fluorescently-labelled IRDye® 800CW donkey anti-rabbit (Li-Cor®) secondary antibody (diluted 1:800 in Odyssey™ blocking buffer) and DRAQ5® (Biostatus) stain (diluted 1:10,000 in Odyssey™ blocking buffer) was applied to each well, and the plate was incubated for 1 h at rt protected from light. The plate was washed four times with 100 µL PBS + 0.1% Tween 20 for 5 min at rt with gentle shaking, protected from light, then with 100 µL PBS alone for 5 min at rt with gentle shaking, protected from light. The buffer was removed, and the

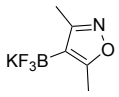
plates were visualised using an Odyssey™ system, with detection at 700 and 800 nm.

NF-κB reporter gene assay (Paul Bruno, Department of Chemistry, University of Michigan): HeLa cells were plated in a 6-well plate at a density of 400,000 cells/well, and allowed to adhere overnight. The cells were transfected with 5×κB-luc plasmid (final concentration 200 ng/mL), pCMV-β-Gal plasmid (100 ng/mL) and pBSSK (non-coding plasmid to normalize plasmid amounts, 700 ng/mL) using Lipofectamine 2000® (10 μL) in Opti-MEM® medium (overall volume 2 mL). After 4.5 h incubation, the transfection mix was replaced with 2 mL DMEM + 10% FBS and the cells were incubated for a further 18 h. The cells were trypsinised and plated in a 96-well plate at a density of 8,000 cells/well, then allowed to adhere overnight. The cells were treated with **61a**, (+)-JQ1 or (–)-JQ1 at final concentrations of 25, 5, 1 and 0.2 μM and incubated for 12 h. IL-1β was added to a final concentration of 10 ng/mL. (Two controls, with DMSO and +/– IL-1β, were included.) After 3 h stimulation, the cells were lysed using passive lysis buffer, and cellular lysates were assayed for β-gal and luciferase activity.

7.3 Chapter 2: Synthetic Procedures

7.3.1 3'-Substituted 3,5-dimethyl-4-phenylisoxazole derivatives

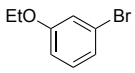
Potassium (3,5-dimethylisoxazol-4-yl)trifluoroborate **51**[214]



Following the procedure of Molander *et al.*,[214] to a suspension of 3,5-dimethylisoxazol-4-ylboronic acid (2.50 g, 17.7 mmol, 1.0 eq) in MeOH (5 mL) at 0 °C was added KHF_2 (4.16 g, 53.2 mmol, 3.0 eq). H_2O (11.8 mL) was then added dropwise. The solution was warmed to rt and stirred for 10 min, then

concentrated and dried overnight *in vacuo*. The crude solid was purified by Soxhlet extraction (16 h) with acetone (100 mL). The collected solvent was concentrated *in vacuo*, and the residues were redissolved in the minimum amount of boiling acetone (400 mL). The product was precipitated by the addition of Et_2O (600 mL) and collected by filtration. The filtrate was concentrated *in vacuo*, redissolved in acetone (100 mL) and further product was precipitated by the addition of Et_2O (200 mL) and collected by filtration. The filtrate was again concentrated *in vacuo*, redissolved in acetone (30 mL) and further product was precipitated by the addition of Et_2O (60 mL) and collected by filtration. The combined solids were dried *in vacuo* to give **51** as a powdery colourless solid (3.02 g, 84%); mp >275 °C (lit. >200 °C);[214] ^{1}H NMR (400 MHz, DMSO-d_6) 2.05 (3H, s, CH_3), 2.20 (3H, s, CH_3); ^{11}B NMR (160 MHz, DMSO-d_6) 2.33 (q, J = 49 Hz); ^{19}F NMR (470 MHz, DMSO-d_6) −134.8-−134.2; LRMS *m*/*z* (ES^-) 164 ($[M\text{-}K]^-$, 100), 351 ($[2M-2K+Na]^-$, 43), 367 ($[2M-K]^-$, 22). Anal. Calcd for $C_5H_6BF_3KNO$: C, 29.6; H, 3.0; N, 6.9. Found: C, 29.7; H, 2.9; N, 6.8. These data are in good agreement with the literature values.[214]

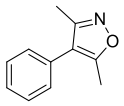
1-Bromo-3-ethoxybenzene **52**



Following the procedure of Sarju *et al.*,[215] anhydrous K_2CO_3 (829 mg, 6.00 mmol, 1.0 eq), 3-bromophenol (637 µL, 1.04 g, 6.00 mmol, 1.0 eq), EtBr (672 µL, 981 mg, 9.00 mmol, 3.0 eq) and anhydrous MeOH (1.8 mL) were added to a dry 2-5 mL microwave vial under a nitrogen atmosphere. The vial was sealed, and the mixture stirred at 120 °C for 20 min with microwave irradiation then concentrated *in vacuo*. The residues were extracted with 40-60 °C petroleum

ether (3 × 15 mL) and concentrated *in vacuo* to give **52** as a pale yellow oil (1.14 g, 95%); R_f 0.21 (40-60 °C petroleum ether); ν_{max} (NaCl plate)/cm^{-1}: 3066 (Ar C–H) (w), 2982 (C–H) (m), 1591, 1573 (Ar C=C) (s), 1246, 1229 (C–O) (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.42 (3H, t, *J* = 7.0 Hz, C*H*$_3$), 4.02 (2H, q, *J* = 7.0 Hz, C*H*$_2$), 6.83 (1H, ddd, *J* = 8.1, 2.3, 1.0 Hz, C(6)*H*), 7.05-7.08 (2H, m, C(2)*H*, C(4)*H*), 7.14 (1H, dd, *J* = 8.1, 8.1 Hz, C(5)*H*); ^{13}C NMR (100 MHz, $CDCl_3$) 14.7 (*C*H$_3$), 63.7 (*C*H$_2$), 113.6 (*C*(6)), 117.7 (*C*(2)), 122.8 (*C*(1)), 123.6 (*C*(4)), 130.5 (*C*(5)), 159.7 (*C*(3)); HRMS *m*/z (FI$^+$) found M$^+$ 199.9838, 201.9817; $C_8H_9{}^{79}BrO$ requires M$^+$ 199.9837, $C_8H_9{}^{81}BrO$ requires M$^+$ 201.9811. Anal. Calcd for C_8H_9BrO: C, 47.8; H, 4.5. Found: C, 47.7; H, 4.4.

3,5-Dimethyl-4-phenylisoxazole **46a**[325,326]

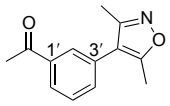


Method A: To a 2-5 mL microwave vial were added **51** (108 mg, 532 µmol, 1.06 eq), $Pd(OAc)_2$ (2 mg, 8 µmol, 0.015 eq), RuPhos (7 mg, 15 µmol, 0.03 eq) and Na_2CO_3 (106 mg, 1.00 mmol, 1.9 eq). The vial was sealed and purged with argon (3 × evacuate/fill). Bromobenzene (**48**, 79 mg, 53 µL, 503 µmol, 1.0 eq) and degassed EtOH (2.8 mL) were added by syringe, and the mixture was heated at 85 °C for 4 h. The mixture was cooled to rt, filtered through a thin pad of silica (eluent CH_2Cl_2) and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 6 → 30% Et_2O/petroleum ether) gave **46a** as a colourless oil which crystallised slowly on standing (73 mg, 85%); R_f 0.26 (15% Et_2O/40-60 °C petroleum ether); mp 37-38 °C (lit. 42-43 °C,[325] 40-42 °C)[326]; ^{1}H NMR (400 MHz, $CDCl_3$) 2.29 (3H, s, C(3)C*H*$_3$), 2.42 (3H, s, C(5)C*H*$_3$), 7.24-7.29 (2H, m, *Ar*), 7.34-7.40 (1H, m, *Ar*), 7.42-7.48 (2H, m, *Ar*); LRMS *m*/*z* (ES$^+$) 174

([M+H]$^+$, 73), 196 ([M+Na]$^+$, 100), 369 ([2M+Na]$^+$, 19). Anal. Calcd. for $C_{11}H_{11}NO$: C, 76.3; H, 6.4; N, 8.1. Found: C, 76.4; H, 6.5; N, 8.0. These data are in good agreement with the literature values.[325,326]

Method B: To a dry 2-5 mL microwave vial were added $PdCl_2$ (0.5 mg, 3 µmol, 0.01 eq) and KOAc (98 mg, 1.0 mmol, 2.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). Anhydrous degassed DMAc (2.5 mL), bromobenzene (**48**, 79 mg, 53 µL, 503 µmol, 1.0 eq) and 3,5-dimethylisoxazole (73 mg, 74 µL, 752 µmol, 1.5 eq) were added, and the reaction was heated at 130 °C for 27 h. The mixture was filtered through Celite® (eluent Et_2O), and the filtrate was washed with water (40 mL) and brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 3 → 30% Et_2O/petroleum ether) gave **46a** as a pale yellow solid (60 mg, 69%). The data are identical to those reported above.

1-(3-(3,5-Dimethylisoxazol-4-yl)phenyl)ethanone **46b**[213]

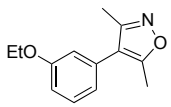


Method A: To a 2-5 mL microwave vial were added **51** (108 mg, 532 µmol, 1.06 eq), $Pd(OAc)_2$ (2 mg, 8 µmol, 0.015 eq), RuPhos (7 mg, 15 µmol, 0.03 eq) and K_2CO_3 (106 mg, 1.00 mmol, 2.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). 3´-Bromoacetophenone (**49**, 100 mg, 66 µL, 500 µmol, 1.0 eq) and degassed EtOH (3.8 mL) were added by syringe, and the mixture was heated at 85 °C for 13 h. The mixture was cooled to rt, filtered through a thin pad of silica (eluent 25% MeOH/EtOAc) and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 6 → 50% EtOAc/petroleum ether) gave **46b** as a pale yellow solid (93 mg, 86%); R_f 0.24

(40% EtOAc/40-60 °C petroleum ether); mp 107-110 °C (50% Et_2O/40-60 °C petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) 2.29 (3H, s, C(3)C*H*$_3$), 2.43 (3H, s, C(5)C*H*$_3$), 2.65 (3H, s, COC*H*$_3$), 7.44-7.49 (1H, m, *Ar*), 7.53-7.60 (1H, m, *Ar*), 7.85-7.89 (1H, m, *Ar*), 7.93-7.98 (1H, m, *Ar*); LRMS *m*/*z* (ES^+) 216 ($[M+H]^+$, 54), 238 ($[M+Na]^+$, 100), 453 ($[2M+Na]^+$, 65). These data are in good agreement with the literature values.[213]

Method B: To a dry 2-5 mL microwave vial were added $PdCl_2$ (0.5 mg, 3 µmol, 0.01 eq) and KOAc (98 mg, 1.0 mmol, 2.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). Anhydrous degassed DMAc (2.5 mL), 3´-bromoacetophenone (**49**, 100 mg, 66 µL, 502 µmol, 1.0 eq) and 3,5-dimethylisoxazole (73 mg, 74 µL, 752 µmol, 1.5 eq) were added, and the reaction was heated at 130 °C for 20 h. The mixture was filtered through Celite® (eluent EtOAc), and the filtrate was washed with water (2 × 25 mL) and brine (25 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 6 → 50% EtOAc/petroleum ether) gave **46b** as a pale yellow solid (55 mg, 51%). The data are identical to those reported above.

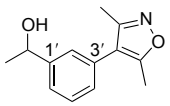
4-(3-Ethoxyphenyl)-3,5-dimethylisoxazole **46c**



To a 2-5 mL microwave vial was added **51** (108 mg, 532 µmol, 1.06 eq), $Pd(OAc)_2$ (2 mg, 8 µmol, 0.015 eq), RuPhos (7 mg, 15 µmol, 0.03 eq) and anhydrous Na_2CO_3 (106 mg, 1.00 mmol, 2.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). Compound **52** in degassed EtOH (2.8 mL, 178 µM, 499 µmol, 1.0 eq) was added by syringe, and the mixture was heated at 85 °C for

2 h. The mixture was filtered through a thin pad of silica (eluent CH_2Cl_2) and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 40% Et_2O/petroleum ether) gave **46c** as a pale yellow solid (94 mg, 87%); R_f 0.34 (20% Et_2O/40-60 °C petroleum ether); mp 51-52 °C (15% Et_2O/petroleum ether); ν_{max} (NaCl plate)/cm^{-1}: 3063 (Ar C–H) (w), 2980, 2931 (C–H) (m), 1632 (m), 1602 (m), 1577 (s), 1298 (s), 1249 (s), 1206 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.44 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.27 (3H, s, C(3)CH_3), 2.41 (3H, s, C(5)CH_3), 4.06 (2H, q, *J* = 7.0 Hz, CH_2), 6.78 (1H, dd, *J* = 2.4, 1.6 Hz, C(2′)*H*), 6.82 (1H, ddd, *J* = 7.7, 1.6, 0.9 Hz, C(6′)*H*), 6.89 (1H, ddd, *J* = 8.2, 2.4, 0.9 Hz, C(4′)*H*), 7.33 (1H, dd, *J* = 8.2, 7.7 Hz, C(5′)*H*); ^{13}C NMR (100 MHz, $CDCl_3$) 10.8 (C(3)CH_3), 11.6 (C(5)CH_3), 14.8 (CH_2CH_3), 63.5 (CH_2), 113.2 (*C*(4′)), 115.6 (*C*(2′)), 116.6 (*C*(4)), 121.3 (*C*(6′)), 129.8 (*C*(5′)), 131.7 (*C*(1′)), 158.6 (*C*(3)), 159.2 (*C*(3′)), 165.2 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 240.0994; $C_{13}H_{15}NNaO_2$ requires M^+ 240.0995; LRMS *m*/*z* (ES^+) 218 ($[M+H]^+$, 47), 240 ($[M+Na]^+$, 100), 457 ($[2M+Na]^+$, 99). Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.9; H, 7.0; N, 6.5. Found: C, 72.0; H, 7.1; N, 6.4.

(*RS*)-1-(3-(3,5-Dimethylisoxazol-4-yl)phenyl)ethanol **46d**

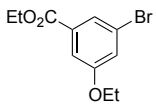


To a solution of **46c** (100 mg, 465 μmol, 1.0 eq) in anhydrous MeOH (2 mL) at 0 °C under a nitrogen atmosphere was added $NaBH_4$ (18 mg, 476 μmol, 1.02 eq). The mixture was warmed to rt and stirred for 2 h, after which time TLC analysis indicated complete consumption of **46c**, then quenched with sat. aq. NH_4Cl (10 mL). The solution was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers were washed with H_2O (30 mL) and brine (30 mL), then

dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 8 → 70% EtOAc/40-60 °C petroleum ether) gave **46d** as a colourless solid (78 mg, 77%); R_f 0.19 (35% EtOAc/40-60 °C petroleum ether); mp 72-73 °C (40% EtOAc/40-60 °C petroleum ether); ν_{max} (NaCl plate)/cm^{-1}: 3389 (O–H) (br), 3053 (Ar C–H) (w), 2971, 2927 (C–H) (m), 1632 (m), 1422 (s), 1242 (m), 1185 (m); ^{1}H NMR (400 MHz, $CDCl_3$) 1.53 (3H, d, *J* = 6.4 Hz, CH(OH)C*H*$_3$), 2.26 (3H, s, C(3)C*H*$_3$), 2.30 (1H, s, O*H*), 2.40 (3H, s, C(5)C*H*$_3$), 4.95 (1H, q, *J* = 6.4 Hz, C*H*(OH)), 7.13-7.17 (1H, m, *Ar*), 7.26-7.29 (1H, m, C(2′)*H*), 7.34-7.44 (2H, m, *Ar*); ^{13}C NMR (100 MHz, $CDCl_3$) 10.8 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 25.4 (CH(OH)*C*H$_3$), 70.1 (*C*H(OH)), 116.6 (*C*(4)), 124.6 (*C*(4′)/*C*(6′)), 126.1 (*C*(2′)), 128.0 (*C*(6′)/*C*(4′)), 128.9 (*C*(5′)), 130.6 (*C*(3′)), 146.6 (*C*(1′)), 158.7 (*C*(3)), 165.2 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 240.1002; $C_{13}H_{15}NNaO_2$ requires M^+ 240.0995; LRMS *m*/*z* (ES^+) 218 ($[M+H]^+$, 26), 240 ($[M+Na]^+$, 81), 457 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.9; H, 7.0; N, 6.5. Found: C, 72.0; H, 7.0; N; 6.3.

7.3.2 3',5'-Disubstituted 3,5-dimethyl-4-phenylisoxazole derivatives

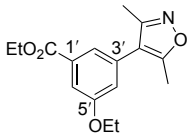
Ethyl 3-bromo-5-ethoxybenzoate **50**



To a dry 10-20 mL microwave vial were added 3-bromo-5-hydroxybenzoic acid (**53**, 1.30 g, 5.99 mmol, 1.0 eq), anhydrous K_2CO_3 (2.07 g, 15.0 mmol, 2.5 eq), anhydrous DMF (5 mL) and EtBr (1.96 g, 1.34 mL, 18.0 mmol) under a nitrogen atmosphere. The vial was sealed, and the mixture stirred at 100 °C for 15 min with microwave irradiation then concentrated *in vacuo*. The mixture was diluted with H_2O (40 mL) and extracted with EtOAc (3 × 40 mL). The combined

organic layers were washed with H_2O (2 × 120 mL) and brine (120 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **50** as an orange solid (1.57 g, 96%); R_f 0.65 (40% Et_2O/40-60 °C petroleum ether); mp 43-44 °C (50% Et_2O/40-60 °C petroleum ether); ν_{max} (NaCl plate)/cm^{-1}: 2982 (C–H) (m), 1722 (C=O) (s), 1575 (Ar C=C) (m), 1281 (C–O) (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.39 (3H, t, *J* = 7.2 Hz, $COOCH_2CH_3$), 1.42 (3H, t, *J* = 7.0 Hz, OCH_2CH_3), 4.06 (2H, q, *J* = 7.0 Hz, OCH_2), 4.37 (2H, q, *J* = 7.2 Hz, $COOCH_2$), 7.22 (1H, dd, *J* = 2.4, 1.8 Hz, C(4)*H*), 7.49 (1H, dd, *J* = 2.4, 1.5 Hz, C(6)*H*), 7.75 (1H, dd, *J* = 1.8, 1.5 Hz, C(2)*H*); ^{13}C NMR (100 MHz, $CDCl_3$) 14.3 (CH_3), 14.6 (CH_3), 61.4 ($COOCH_2$), 64.1 (OCH_2), 114.1 (*C*(6)), 122.4 (*C*(4)), 122.6 (*C*(3)), 124.7 (*C*(2)), 133.0 (*C*(1)), 159.6 (*C*(5)), 165.3 (*C*=O); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 294.9931, 296.9925; $C_{11}H_{13}{}^{79}BrNaNO_3$ requires M^+ 294.9940, $C_{11}H_{13}{}^{81}BrNaNO_3$ requires M^+ 296.9920; LRMS *m*/*z* (ES^+) 295 ($[^{79}M+Na]^+$, 100), 297 ($[^{81}M+Na]^+$, 97), 567 ($[2^{79}M+Na]^+$, 42), 569 ($[^{79}M+^{81}M+Na]^+$, 78), 571 ($[2^{81}M+Na]^+$, 37). Anal. Calcd for $C_{11}H_{13}BrNO_3$: C, 48.4; H, 4.8. Found: C, 48.3; H, 4.8.

Ethyl 3-(3,5-dimethylisoxazol-4-yl)-5-ethoxybenzoate **47a**



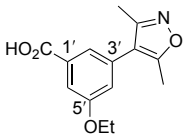
Method A: To a 2-5 mL microwave vial were added **51** (408 mg, 2.01 mmol, 1.1 eq), **50** (500 mg, 1.83 mmol, 1.0 eq), $Pd(OAc)_2$ (4 mg, 16 μmol, 0.01 eq), RuPhos (26 mg, 56 μmol, 0.03 eq) and anhydrous Na_2CO_3 (388 mg, 3.66 mmol, 2.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). Degassed EtOH (10 mL) was added by syringe, and the mixture was heated at 100 °C for 48 h. The mixture was cooled to rt and filtered through a thin pad of

silica (eluent CH_2Cl_2) and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 6 → 50% Et_2O/petroleum ether) gave **47a** as a colourless solid (370 mg, 70%); R_f 0.23 (25% Et_2O/40-60 °C petroleum ether); mp 102-105 °C (CH_2Cl_2); ν_{max} (NaCl plate)/cm^{-1}: 3081 (Ar C–H) (w), 2981, 2933 (C–H) (m), 1719 (C=O) (s), 1589 (Ar C=C) (m), 1232 (C–O) (m); ^{1}H NMR (400 MHz, $CDCl_3$) 1.40 (3H, t, *J* = 7.1 Hz, $COOCH_2CH_3$), 1.46 (3H, t, *J* = 7.0 Hz, $COCH_2CH_3$), 2.28 (3H, s, C(3)CH_3), 2.42 (3H, s, C(5)CH_3), 4.11 (2H, q, *J* = 7.0 Hz, $COCH_2$), 4.40 (2H, q, *J* = 7.1 Hz, $COOCH_2$), 6.96 (1H, dd, *J* = 2.3, 1.7 Hz, C(4´)*H*), 7.52 (1H, dd, *J* = 1.7, 1.4 Hz, C(2´)*H*), 7.55 (1H, dd, *J* = 2.3, 1.4 Hz, C(6´)*H*); ^{13}C NMR (100 MHz, $CDCl_3$) 10.8 (C(3)CH_3), 11.6 (C(5)CH_3), 14.3 ($COOCH_2CH_3$), 14.7 ($COCH_2CH_3$), 61.3 ($COOCH_2$), 63.9 (C(5´)OCH_2), 113.5 (*C*(6´)), 115.9 (*C*(4)), 120.5 (*C*(4´)), 122.5 (*C*(2´)), 131.9, 132.3 ((*C*(1´), *C*(3´)), 158.5 (*C*(3)), 159.2 (*C*(5´)), 165.6 (*C*(5)), 166.1 (*C*=O); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 312.1204; $C_{16}H_{19}NNaO_4$ requires M^+ 312.1206; LRMS *m*/*z* (ES^+) 290 ($[M+H]^+$, 29), 312 ($[M+Na]^+$, 50), 602 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.4; H, 6.6; N, 4.8. Found: C, 66.6; H, 6.5; N, 4.7.

Method B: To a dry 0.5-2 mL microwave vial were added $PdCl_2$ (0.5 mg, 3 μmol, 0.01 eq), KOAc (36 mg, 367 μmol, 3.6 eq) and **50** (28 mg, 103 μmol, 1.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). Anhydrous degassed DMAc (1 mL) and 3,5-dimethylisoxazole (15 mg, 15 μL, 154 μmol, 1.5 eq) were added, and the reaction was heated at 130 °C for 44 h. The mixture was filtered through Celite® (eluent EtOAc), and the filtrate was washed with water (2 × 30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 →

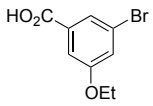
60% EtOAc/petroleum ether) gave **47a** as a colourless solid (25 mg, 44%). The data are identical to those reported above.

3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxybenzoic acid **47b**



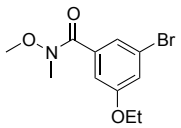
To a solution of **47a** (300 mg, 1.04 mmol, 1.0 eq) in THF (5 mL) were added H_2O (2.5 mL) and LiOH (37 mg, 1.56 µmol, 1.5 eq), and the mixture was stirred at rt for 16 h. Aq. NaOH (1 M, 10 mL) was then added, and the mixture was washed with Et_2O (3 × 10 mL). The solution was acidified to pH 2 with aq. HCl (1 M) and extracted with CH_2Cl_2 (3 × 40 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Repeated recrystallisation from cyclohexane/$CHCl_3$ (2:1) gave **47b** as a colourless crystalline solid (225 mg, 83%); mp 191-193 °C (cyclohexane/$CHCl_3$, 2:1); ν_{max} (solid)/cm^{-1}: 3000 (O–H) (br), 2982, 2857 (C–H) (m), 1694 (C=O) (s), 1590, 1419 1329, 1206; ^{1}H NMR (500 MHz, $CDCl_3$) 1.48 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.31 (3H, s, C(3)CH_3), 2.45 (3H, s, C(5)CH_3), 4.14 (2H, q, *J* = 7.0 Hz, CH_2), 7.03-7.05 (1H, m, C(4′)*H*), 7.60-7.63 (2H, m, C(2′)*H*, C(6′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)CH_3), 11.6 (C(5)CH_3), 14.7 (CH_2CH_3), 64.0 (CH_2), 113.8 (*C*(6′)), 115.7 (*C*(4)), 121.6 (*C*(4′)), 123.1 (*C*(2′)), 131.0, 132.1 (*C*(1′), *C*(3′)), 158.5 (*C*(3)), 159.3 (*C*(5′)), 165.7 (*C*(5)), 171.1 (*C*=O); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 284.0886; $C_{14}H_{15}NNaO_4$ requires M^+ 284.0893; LRMS *m*/*z* (ES^+) 262 ($[M+H]^+$, 32), 284 ($[M+Na]^+$, 100), 545 ($[2M+Na]^+$, 75). Anal. Calcd for $C_{14}H_{15}NO_4$: C, 64.4; H, 5.8; N, 5.4. Found: C, 64.2; H, 5.9; N, 5.3.

3-Bromo-5-ethoxybenzoic acid **54**



To a solution of **50** (500 mg, 1.83 mmol, 1.0 eq) in THF (2 mL) were added H_2O (1 mL) and LiOH (66 mg, 2.75 mmol, 1.5 eq), and the mixture was stirred for 23 h at rt. Aq. HCl (1 M, 10 mL) was then added, and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **54** as a pale yellow solid (425 mg, 95%); mp 139-143 °C (EtOAc); ν_{max} (NaCl plate)/cm^{-1}: 2984 (C–H) (m), 2900 (O–H) (br), 1686 (C=O) (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.44 (3H, t, *J* = 7.0 Hz, C*H*$_3$), 4.09 (q, 2H, *J* = 7.0 Hz, C*H*$_2$), 7.30 (1H, dd, *J* = 2.5, 1.8 Hz, C(4)*H*), 7.55 (1H, dd, *J* = 2.5, 1.4 Hz, C(6)*H*), 7.83 (1H, dd, *J* = 1.8, 1.4 Hz, C(2)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 14.6 (*C*H_3), 64.2 (*C*H_2), 114.5 (*C*(6)), 122.8 (*C*(3)), 123.5 (*C*(4)), 125.3 (*C*(2)), 131.6 (*C*(1)), 159.7 (*C*(5)), 170.3 (*C*=O); HRMS *m*/*z* (ES^-) found $[M-H]^-$ 242.9962, 244.9642; $C_9H_9{}^{79}BrO_3$ requires M^- 242.9962, $C_9H_9{}^{81}BrO_3$ requires M^- 244.9642; LRMS *m*/*z* (ES^-) 243 ($[^{79}M]^-$, 96), 245 ($[^{81}M]^-$, 100), 487 ($[2^{79}M]^-$, 6) 489 ($[^{79}M+^{81}M]^-$, 13), 491 ($[2^{81}M]^-$, 6), 509 ($[2^{79}M–2H+Na]^-$, 45), 511 ($[^{79}M+^{81}M-2H+Na]^-$, 80), 513 ($[2^{81}M–2H+Na]^-$, 37). Anal. Calcd for $C_9H_9BrO_3$: C, 44.1; H, 3.7. Found: C, 44.0; H, 3.6.

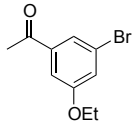
3-Bromo-5-ethoxy-*N*-methoxy-*N*-methylbenzamide **55**



To a dry flask containing **54** (353 mg, 1.44 mmol, 1.0 eq), *N*,*O*-dimethylhydroxylamine hydrochloride (285 mg, 2.92 mmol, 2.0 eq) and HBTU

(576 mg, 1.52 mmol, 1.06 eq) at 0 °C under a nitrogen atmosphere were added anhydrous DMF (3.5 mL) and diisopropylethylamine (1.3 mL, 1.0 g, 7.46 mmol, 5.2 eq). The mixture was warmed to rt and stirred for 14 h, then concentrated *in vacuo*. The residues were redissolved in EtOAc (50 mL), washed with aq. citric acid (10% w/v, 2 × 50 mL), sat. aq. $NaHCO_3$ (2 × 50 mL), H_2O (2 × 50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 6 → 40% EtOAc/petroleum ether) gave **55** as a colourless oil (331 mg, 80%); R_f 0.30 (30% EtOAc/40-60 °C petroleum ether); ν_{max} (NaCl plate)/cm^{-1}: 3078 (Ar C–H) (w), 2980, 2935 (C–H) (m), 1650 (C=O) (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.41 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 3.34 (3H, s, NCH_3), 3.57 (3H, s, OCH_3), 4.03 (2H, q, *J* = 7.0 Hz, CH_2), 7.10-7.14 (2H, m, C(4)*H*, C(6)*H*), 7.35-7.38 (1H, m, C(2)*H*); ^{13}C NMR (100 MHz, $CDCl_3$) 14.6 (CH_2CH_3), 33.7 (NCH_3), 61.2 (OCH_3), 64.0 (CH_2), 113.2 (*C*(6)), 119.9 (*C*(4)), 122.3 (*C*(3)), 123.1 (*C*(2)), 136.6 (*C*(1)), 159.3 (*C*(5)), 168.1 (*C*=O); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 310.0055, 312.0034; $C_{11}H_{14}{}^{79}BrNNaO_3$ requires M^+ 310.0049, $C_{11}H_{14}{}^{81}BrNNaO_3$ requires M^+ 312.0029; LRMS *m*/*z* (ES^+) 288 ($[^{79}M+H]^+$, 25), 290 ($[^{81}M+H]^+$, 24), 310 ($[^{79}M+Na]^+$, 57), 312 ($[^{81}M+Na]^+$, 55), 597 ($[2^{79}M+Na]^+$, 96), 599 ($[^{79}M+^{81}M+Na]^+$, 100), 601 ($[2^{81}M+Na]^+$, 95). Anal. Calcd for $C_{11}H_{14}BrNO_3$: C, 45.9; H, 4.9; N, 4.9. Found: C, 46.0; H, 4.8; N, 4.7.

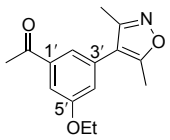
1-(3-Bromo-5-ethoxyphenyl)ethanone **56**



To a solution of **55** (250 mg, 868 μmol, 1.0 eq) in anhydrous THF (8 mL) under an argon atmosphere was added MeMgBr solution (1.0 M in Bu_2O, 2.6 mL, 2.6 mmol, 3.0 eq) dropwise at 0 °C. The solution was warmed to rt and stirred for

15 h, then quenched with aq. HCl (1 M, 15 mL). The mixture was extracted with EtOAc (3 × 15 mL), and the combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 3 → 30% Et_2O/petroleum ether) gave **56** as a colourless solid (181 mg, 86%); R_f 0.28 (15% Et_2O/40–60 °C petroleum ether); mp 40-41 °C (15% Et_2O/petroleum ether); ν_{max} (NaCl plate)/cm^{-1}: 3078 (Ar C–H) (w), 2980, 2935 (C–H) (m), 1650 (C=O) (s), 1595, 1566 (Ar C=C) (m), 1268 (C–O) (m); ^{1}H NMR (400 MHz, $CDCl_3$) 1.43 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.57 (3H, s, $COCH_3$), 4.07 (2H, q, *J* = 7.0 Hz, CH_2), 7.23-7.25 (1H, m, C(4)*H*), 7.38-7.41 (1H, m, C(6)*H*), 7.63-7.65 (1H, m, C(2)*H*); ^{13}C NMR (100 MHz, $CDCl_3$) 14.6 (CH_2CH_3), 26.7 ($COCH_3$), 64.2 (CH_2), 112.7 (*C*(6)), 122.5 (*C*(4)), 123.0 (*C*(3)), 123.8 (*C*(2)), 139.4 (*C*(1)), 159.8 (*C*(5)), 196.5 (*C*=O); HRMS *m*/z (ES^+) found $[M+Na]^+$ 264.9833, 266.9814; $C_{10}H_{11}{}^{79}BrNaO_2$ requires M^+ 264.9835, $C_{10}H_{11}{}^{81}BrNaO_2$ requires M^+ 266.9814; LRMS *m*/z (ES^+) 265 ($[^{79}M+Na]^+$, 100), 267 ($[^{81}M+Na]^+$, 97). Anal. Calcd for $C_{10}H_{11}BrO_2$: C, 49.4; H, 4.6. Found: C, 49.6; H, 4.4.

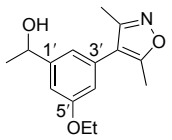
1-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)ethanone **47c**



To a 10-20 mL microwave vial were added **51** (88 mg, 433 μmol, 1.05 eq), **56** (100 mg, 411 μmol, 1.0 eq), $Pd(OAc)_2$ (1 mg, 4 μmol, 0.01 eq), RuPhos (6 mg, 13 μmol, 0.03 eq) and Na_2CO_3 (87 mg, 821 μmol, 2.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). Degassed EtOH (2.3 mL) was added by syringe, and the mixture was heated at 85 °C for 64 h. The mixture was cooled

to rt and filtered through a thin pad of silica (eluent CH_2Cl_2) and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 6 → 50% EtOAc/petroleum ether) gave **47c** as a pale yellow solid (71 mg, 67%); R_f 0.21 (25% EtOAc/40-60 °C petroleum ether); mp 104-106 °C (CH_2Cl_2); ν_{max} (NaCl plate)/cm^{-1}: 3079 (Ar C–H) (w), 2978, 2932 (C–H) (m), 1686 (C=O) (s), 1632 (m), 1584 (m), 1328 (m), 1222 (s), 1201 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.47 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.29 (3H, s, C(3)CH_3), 2.43 (3H, s, C(5)CH_3), 2.62 (3H, s, $COCH_3$), 4.13 (2H, q, *J* = 7.0 Hz, CH_2), 6.98 (1H, dd, *J* = 2.2, 1.5 Hz, C(4′)*H*), 7.41 (1H, dd, *J* = 1.5, 1.5 Hz, C(2′)*H*), 7.46 (1H, dd, *J* = 2.2, 1.5 Hz, C(6′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)CH_3), 11.6 (C(5)CH_3), 14.7 (CH_2CH_3), 26.7 ($COCH_3$), 63.9 (CH_2), 112.3 (*C*(6′)), 115.9 (*C*(4)), 120.5 (*C*(4′)), 121.5 (*C*(2′)), 132.2 (*C*(3′)), 138.9 (*C*(1′)), 158.5 (*C*(3)), 159.5 (*C*(5′)), 165.6 (*C*(5)), 197.5 (*C*=O); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 282.1098; $C_{15}H_{17}NNaO_3$ requires M^+ 282.1101; LRMS *m*/*z* (ES^+) 260 ($[M+H]^+$, 35), 282 ($[M+Na]^+$, 62), 541 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.5; H, 6.6; N, 5.4. Found: C, 69.6; H, 6.7; N, 5.3.

(*RS*)-1-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)ethanol **47d**

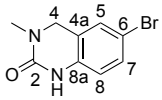


To a solution of **47c** (302 mg, 123 μmol, 1.0 eq) in anhydrous MeOH (1 mL) at 0 °C under a nitrogen atmosphere was added $NaBH_4$ (6 mg, 159 μmol, 1.3 eq). The mixture was warmed to rt and stirred for 2 h, then quenched with sat. aq. NH_4Cl (10 mL). The solution was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers were washed with H_2O (30 mL) and brine (30 mL), then

dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 10 → 100% EtOAc/40-60 °C petroleum ether) gave **47d** as a pale yellow viscous oil (26 mg, 81%); R_f 0.28 (50% EtOAc/40-60 °C petroleum ether); ν_{max} (NaCl plate)/cm^{-1}: 3397 (O–H) (br), 2977, 2930 (C–H) (m), 1633 (m), 1592 (s), 1419 (s), 1326 (m), 1257 (m), 1205 (m), 1053 (m); ^{1}H NMR (400 MHz, $CDCl_3$) 1.44 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 1.52 (3H, d, *J* = 6.4 Hz, CH(OH)C*H*$_3$), 2.14 (1H, s, O*H*), 2.27 (3H, s, C(3)C*H*$_3$), 2.41 (3H, s, C(5)C*H*$_3$), 4.07 (2H, q, *J* = 7.0 Hz, C*H*$_2$), 4.91 (1H, q, *J* = 6.4 Hz, C*H*(OH)), 6.66-6.69 (1H, m, *Ar*), 6.81-6.84 (1H, m, *Ar*), 6.92-6.95 (1H, m, *Ar*); ^{13}C NMR (100 MHz, $CDCl_3$) 10.8 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 14.8 (CH_2CH_3), 25.4 (CH(OH)*C*H$_3$), 63.6 (*C*H$_2$), 70.1 (*C*H(OH)), 110.3 (Ar *C*H), 114.4 (Ar *C*H), 116.6 (*C*(4)), 118.3 (Ar *C*H), 131.8 (*C*(3´)), 148.1 (*C*(1´)), 158.6 (*C*(3)), 159.4 (*C*(5´)), 165.2 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 284.1246; $C_{15}H_{15}NNaO_3$ requires M$^+$ 284.1257; LRMS *m*/*z* (ES$^+$) 262 ([M+H]$^+$, 21), 284 ([M+Na]$^+$, 47), 545 ([2M+Na]$^+$, 100). Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.9; H, 7.3; N, 5.3. Found: C, 69.1; H, 7.1; N, 5.5.

*7.3.3 Resynthesis of **45a***

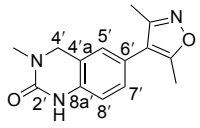
6-Bromo-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one **57** (prepared by Tim Rooney, SJC group)



To a solution of 3-methyl-3,4-dihydroquinazolin-2(1*H*)-one (5.00 g, 31.0 mmol, 1.0 eq) in DMF (120 mL) was added NBS (6.61 g, 37.1 mmol, 1.2 eq). The reaction was stirred at rt for 2 h in the dark, then concentrated *in vacuo*. The resulting residue was resuspended in EtOAc, washed with H_2O (3 × 100 mL),

dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (5:1 CH_2Cl_2:EtOAc) gave **57** as a colourless solid (916 mg, 12%); mp 195-197 °C (EtOH); ^{1}H NMR (500 MHz, DMSO-d_6) 2.85 (3H, s, NC*H*$_3$) 4.39 (2H, s, C(4)*H*$_2$), 6.70-6.74 (1H, m, C(8)*H*), 7.28-7.32 (2H, m, C(5)*H*, C(7)*H*), 9.33 (1H, s, N*H*); ^{13}C NMR (126 MHz, DMSO-d_6) 33.8 (N*C*H$_3$), 49.2 (*C*H$_2$), 112.0 (*C*(6)), 115.2 (*C*(8)), 120.3 (*C*(4a)/*C*(8a)), 128.1, 130.4 (*C*(5), *C*(7)), 137.3 (*C*(8a)/*C*(4a)), 153.3 (*C*=O); HRMS *m/z* (ES$^+$) found [M+H]$^+$ 240.9969; $C_9H_{10}{}^{79}BrN_2O^+$ requires 240.9971; LRMS *m/z* (ES$^+$) 263 ([^{79}M+Na]$^+$, 56), 265 ([^{81}M+Na]$^+$, 55), 295 ([^{79}M+MeOH+Na]$^+$, 12), 297 ([^{81}M+MeOH+Na]$^+$, 12), 503 ([2^{79}M+Na]$^+$, 53), 505 ([^{79}M+^{81}M+Na]$^+$, 100), 507 ([2^{81}M+Na]$^+$, 52). Anal. Calcd for $C_9H_9BrN_2O$: C, 44.8; H, 3.8; N, 11.6. Found: C, 44.8; H, 3.7; N, 11.6.

6-(3,5-Dimethylisoxazol-4-yl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one **45a**

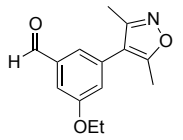


To a 2-5 mL microwave vial were added **51** (93 mg, 456 μmol, 1.1 eq), **57** (100 mg, 415 μmol, 1.0 eq), $Pd(OAc)_2$ (1 mg, 4 μmol, 0.01 eq), RuPhos (6 mg, 13 μmol, 0.03 eq) and Na_2CO_3 (88 mg, 830 μmmol, 2.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). Degassed EtOH (2.3 mL) was added by syringe, and the mixture was heated at 110 °C for 3 h with microwave irradiation. Purification of the crude reaction mixture by silica gel column chromatography (gradient elution, 60 → 100% EtOAc/petroleum ether) gave **45a** as a colourless solid (68 mg, 64%); R_f 0.19 (80% EtOAc/40-60 °C petroleum ether); mp 224-226 °C (MeOH); ν_{max} (solid)/cm^{-1}: 3211 (N–H) (w), 2970, 2929 (C–H) (w), 1739 (m), 1669 (C=O) (s), 1616 (m), 1511 (m), 1445 (m), 1398 (m), 1316 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 2.24 (3H, s, C(3)C*H*$_3$), 2.38 (3H, s, C(5)C*H*$_3$), 3.07 (3H, s,

NCH_3), 4.50 (2H, s, CH_2), 6.83 (1H, d, *J* = 8.1 Hz, C(8´)*H*), 6.91 (1H, s, C(5´)*H*), 7.05 (1H, dd, *J* = 8.1, 1.8 Hz, C(7´)*H*), 8.15 (1H, s, N*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)*C*H_3), 11.5 (C(5)*C*H_3), 34.6 (N*C*H_3), 50.8 (*C*H_2), 114.2 (*C*(8´)), 116.1 (*C*(4)), 117.8, 123.8 (*C*(4´a), *C*(8´a)), 126.0 (*C*(5´)), 129.1 (*C*(7´)), 136.6 (*C*(6´)), 154.5 (*C*=O), 158.7 (*C*(3)), 165.0 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 280.1055; $C_{14}H_{15}N_3NaO_2$ requires M^+ 280.1056; LRMS *m*/*z* (ES^+) 258 ($[M+H]^+$, 28), 280 ($[M+Na]^+$, 86), 312 ($[M+Na+MeOH]^+$, 85), 537 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{14}H_{15}N_3O_2$: C, 65.4; H, 5.9; N, 16.3. Found: C, 65.2; H, 5.9; N, 16.2.

7.3.4 Diarylmethanol derivatives

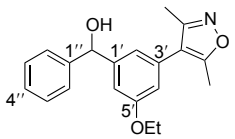
3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxybenzaldehyde **60**



Following the procedure of Sarju *et al.*,[215] anhydrous K_2CO_3 (1.91 g, 13.8 mmol, 1.0 eq) and **59** (3.00 g, 13.8 mmol, 1.0 eq) were added to a dry 10-20 mL microwave vial. The vial was sealed and purged with nitrogen (3 × evacuate/fill). EtBr (3.00 g, 2.06 mL, 27.6 mmol) and anhydrous MeOH (15 mL) were added, and the mixture was stirred at 120 °C for 30 min with microwave irradiation, then concentrated *in vacuo*. The residues were extracted with EtOAc (5 × 50 mL), and the combined organic extracts washed with H_2O (2 × 250 mL) and brine (250 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 20 → 40% Et_2O/petroleum ether) gave **60** as a colourless solid (2.32 g, 69 %); R_f 0.43 (60% Et_2O/40-60 °C petroleum ether); mp 99-100 °C (EtOAc); ν_{max} (solid)/cm^{-1} 2982, 2933 (C–H) (w), 2730 (C–HO) (w), 1701 (C=O) (s), 1587 (Ar C=C) (m), 1330 (m), 1207 (C–O) (m),

1052 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.48 (3H, q, *J* = 7.0 Hz, CH_2CH_3), 2.30 (3H, s, C(3)CH_3), 2.44 (3H, s, C(5)CH_3), 4.14 (2H, q, *J* = 7.0 Hz, CH_2), 7.05 (1H, dd, *J* = 2.5, 1.5 Hz, C(4´)*H*), 7.33 (1H, dd, *J* = 1.5, 1.3 Hz, C(2´)*H*), 7.38 (1H, dd, *J* = 2.5, 1.3 Hz, C(6´)*H*), 10.01 (1H, s, C*H*O); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)CH_3), 11.6 (C(5)CH_3), 14.7 (CH_2CH_3), 64.0 (CH_2CH_3), 111.9 (*C*(6´)), 115.5 (*C*(4)), 122.4 (*C*(4´)), 123.5 (*C*(2´)), 132.8 (*C*(3´)), 138.2 (*C*(1´)), 158.4 (*C*(3)), 159.8 (*C*(5´)), 165.7 (*C*(5)), 191.7 (*C*HO); HMRS *m*/*z* (ES^+) found $[M+Na]^+$ 268.0940; $C_{14}H_{15}NNaO_3$ M^+ requires 268.0944; LRMS *m*/*z* (ES^+) 246 ($[M+H]^+$, 4), 268 ($[M+Na]^+$, 10), 300 ($[M+MeOH+Na]^+$, 40), 513 ($[2M+Na]^+$, 9), 577 ($[2M+2MeOH+Na]^+$, 100). Anal. Calcd. for $C_{14}H_{15}NO_3$: C, 68.6; H, 6.2; N, 5.7. Found: C, 68.6; H, 6.2; N, 5.6.

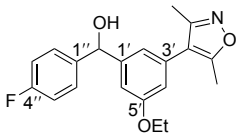
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(phenyl)methanol **58a**



To a solution of **60** (79 mg, 322 μmol, 1.0 eq) in anhydrous THF (5 mL) under a nitrogen atmosphere was added PhMgBr (0.9 M in THF, 716 μL, 644 μmol, 2.0 eq) dropwise at 0 °C. The solution was warmed to rt over 17 h, then quenched with aq. HCl (1M, 10 mL) and extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with H_2O (2 × 30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 10 → 60% Et_2O/petroleum ether) gave **58a** as a colourless oil (83 mg, 80%); R_f 0.31 (60% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3377 (O–H) (br), 3062, 3029 (Ar C–H) (w), 2980, 2930 (C–H) (m), 1591 (Ar C=C) (s), 1419 (s), 1326 (s), 1255 (s), 1204 (s), 1054

(s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.42 (3 H, t, *J* = 7.0 Hz, CH_2CH_3), 2.23 (3H, s, C(3)CH_3), 2.29 (1H, d, *J* = 3.3 Hz, O*H*), 2.37 (3H, s, C(5)CH_3), 4.04 (2H, q, *J* = 7.0 CH_2CH_3), 5.85 (1H, d, *J* = 3.3 Hz, C*H*OH), 6.67 (1H, dd, *J* = 2.2, 1.6 Hz, C(4′)*H*), 6.81-6.83 (1H, m, C(2′)*H*), 6.96 (1H, dd, *J* = 2.2, 1.1 Hz, C(6′)*H*), 7.29 (1H, dddd, *J* = 7.2, 7.2, 1.5, 1.5 Hz, C(4′′)*H*), 7.34-7.43 (4H, m, C(2′′)*H*, C(3′′)*H*, C(5′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (*C*(3)), 11.6 (*C*(5)), 14.8 (CH_2CH_3), 63.6 (CH_2CH_3), 76.1 (*C*HOH), 111.4 (*C*(6′)), 114.4 (*C*(4′)), 116.5 (*C*(4)), 119.5 (*C*(2′)), 126.5 (*C*(2′′), *C*(6′′)/*C*(3′′), *C*(5′′)), 127.8 (*C*(4′′)), 128.6 (*C*(3′′), *C*(5′′)/*C*(2′′), *C*(6′′)), 131.7 (*C*(3′)), 143.5 (*C*(1′′)), 145.9 (*C*(1′)), 158.6 (*C*(3)), 159.3 (*C*(5′)), 165.2 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 346.1406; $C_{20}H_{21}NNaO_3$ requires M^+ 346.1414; LRMS *m*/*z* (ES^+) 324 ($[M+H]^+$, 24), 346 ($[M+Na]^+$, 33), 669 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{20}H_{21}NO_3$: C, 74.3; H, 6.6; N, 4.3. Found: C, 74.2; H, 6.7; N, 4.4.

(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(4-fluorophenyl)methanol **58b**

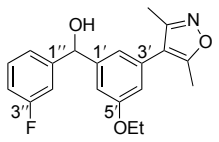


Following the general procedure, 1-bromo-4-fluorobenzene (471 mg, 296 µL) and Mg turnings in anhydrous THF were heated under reflux for 3 h. Following addition of the resultant cloudy yellow suspension to **60** in THF, the reaction was stirred for 4.5 h, then quenched with H_2O (15 mL) and aq. HCl (1 M, 15 mL). The phases were separated, and the aqueous layer extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with H_2O (90 mL) and brine (90 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 20 → 80% Et_2O/petroleum

ether) gave **58b** as a pale yellow viscous gum (170 mg, 61%); R_f 0.21 (50% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3368 (br, O–H), 2981, 2931, 2882 (w, C–H), 1590 (s), 1507 (s), 1419 (m), 1221 (s), 1204 (s), 1156 (s), 1045 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.41 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.19 (3H, s, C(3)CH_3), 2.35 (3H, s, C(5)CH_3), 3.0 (1H, br s, O*H*), 4.03 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 5.80 (1H, s, C*H*(OH)), 6.66 (1H, dd, *J* = 2.3, 1.5 Hz, C(4´)*H*), 6.77-6.80 (1H, m, C(2´)*H*), 6.91-6.94 (1H, m, C(6´)*H*), 6.98-7.05 (2H, m C(3´´)*H*, C(5´´)*H*), 7.32-7.38 (2H, m, C(2´´)*H*, C(6´´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)CH_3), 11.5 (C(5)CH_3), 14.7 (CH_2CH_3), 63.6 (CH_2CH_3), 75.2 (*C*H(OH)), 111.4 (*C*(6´)), 114.4 (*C*(4´)), 115.3 (d, *J* = 21 Hz, *C*(3´´), *C*(5´´)), 116.5 (*C*(4)), 119.4 (*C*(2´)), 128.2 (d, *J* = 8.1 Hz, *C*(2´´), *C*(6´´)), 131.7 (*C*(3´)), 139.5 (d, *J* = 3.5 Hz, *C*(1´´)), 145.9 (*C*(1´)), 158.6 (*C*(3)), 159.3 (*C*(5´)), 162.2 (d, *J* = 246 Hz, *C*(4´´)), 165.3 (*C*(5)); ^{19}F NMR (470 MHz, $CDCl_3$) –114.7; HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 364.1318; $C_{20}H_{20}FNNaO_3$ requires M^+ 364.1319; LRMS *m*/*z* (ES^+) 342 ($[M+H]^+$, 12), 364 ($[M+Na]^+$, 19), 396 ($[M+Na+MeOH]^+$, 8), 705 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{20}H_{20}FNO_3$: C, 70.4; H, 5.9; N, 4.1. Found: C, 70.2; H, 6.0; N, 4.0.

(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(3-fluorophenyl)methanol

58c

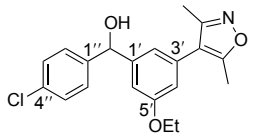


Following the general procedure, 1-bromo-3-fluorobenzene (471 mg, 300 µL) and Mg turnings in Et_2O were heated under reflux for 2 h. Following addition of the resultant cloudy yellow suspension to **60** in Et_2O, the reaction was stirred for 2 h, then quenched with H_2O (10 mL) and aq. HCl (1 M, 10 mL). The

phases were separated, and the aqueous layer extracted with Et_2O (2 × 20 mL). The combined organic layers were washed with H_2O (60 mL) and brine (60 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 20 → 80% Et_2O/petroleum ether) gave **58c** as a pale yellow viscous gum (255 mg, 92%); R_f 0.21 (50% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3365 (O–H) (br), 2981, 2931 (C–H) (w), 1590 (Ar C=C) (s), 1447 (m), 1419 (m), 1253 (s), 1203 (s), 1046 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.42 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.21 (3H, s, C(3)C*H*$_3$), 2.36 (3H, s, C(5)C*H*$_3$), 2.87 (1H, d, *J* = 3.4 Hz, O*H*), 4.03 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 5.81 (1H, d, *J* = 3.4 Hz, C*H*OH), 6.68 (1H, dd, *J* = 2.1, 1.3 Hz, C(4´)*H*), 6.80 (1H, dd, *J* = 1.4, 1.3 Hz, C(2´)*H*), 6.92 (1H, dd, *J* = 2.1, 1.4 Hz, C(6´)*H*), 6.93-6.99 (1H, m, *Ar*), 7.10-7.14 (1H, m, *Ar*), 7.14-7.19 (1H, m, *Ar*), 7.27-7.33 (1H, m, *Ar*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H_3), 11.6 (C(5)*C*H_3), 14.7 (CH_2CH_3), 63.6 (CH_2CH_3), 75.3 (*C*H(OH)), 111.5 (*C*(6´)), 113.3 (d, *J* = 23 Hz, Ar *C*H), 114.4 (Ar *C*H), 114.6 (*C*(4´)), 116.4 (*C*(4)), 119.4 (*C*(2´)), 122.0 (d, *J* = 2.8 Hz, Ar *C*H), 130.0 (d, *J* = 8.1 Hz, Ar *C*H), 131.8 (*C*(3´)), 145.5 (*C*(1´)), 146.2 (d, *J* = 6.8 Hz, *C*(1´´)), 158.6 (*C*(3)), 159.4 (*C*(5´)), 162.9 (d, *J* = 246 Hz, *C*(3´´)), 165.3 (*C*(5)); ^{19}F NMR (470 MHz, $CDCl_3$) –112.5; HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 364.1312; $C_{20}H_{20}FNNaO_3$ requires M^+ 364.1319; LRMS *m*/*z* (ES^+) 342 ($[M+H]^+$, 4), 364 ($[M+Na]^+$, 14), 396 ($[M+Na+MeOH]^+$, 10), 705 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{20}H_{20}FNO_3$: C, 70.4; H, 5.9; N, 4.1. Found: C, 70.2; H, 5.8; N, 4.1.

(*RS*)-(4-Chlorophenyl)(3-(3,5-dimethylisoxazol-4-yl)-5-ethoxyphenyl)methanol

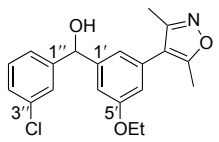
58d



Following the general procedure, 1-bromo-4-chlorobenzene (515 mg) and Mg turnings in THF were heated under reflux for 2.5 h. Following addition of the resultant cloudy yellow suspension to **60** in THF, the reaction was stirred for 3 h, then quenched with H_2O (10 mL) and aq. HCl (1 M, 20 mL). The phases were separated, and the aqueous layer extracted with Et_2O (3 × 40 mL). The combined organic layers were washed with H_2O (120 mL) and brine (120 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 20 → 80% Et_2O/petroleum ether) gave **58d** as a colourless viscous gum which slowly crystallised to a colourless solid under vacuum (195 mg, 67%); R_f 0.22 (50% Et_2O/40-60 °C petroleum ether); mp 103-104 °C; ν_{max} (thin film)/cm^{-1}: 3372 (O–H) (br), 3064, 2980, 2930 (C–H) (w), 1590 (s), 1490 (m), 1419 (s), 1327 (m), 1256 (m), 1204 (s), 1089 (m), 1048 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.42 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.22 (3H, s, C(3)C*H*$_3$), 2.37 (3H, s, C(5)C*H*$_3$), 2.62 (1H, br s, O*H*), 4.03 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 5.81 (1H, s, C*H*(OH)), 6.68 (1H, s, C(4′)*H*), 6.80 (1H, s, C(2′)*H*), 6.91 (1H, s, C(6′)*H*), 7.29-7.37 (4H, m, C(2′′)*H*, C(3′′)*H*, C(5′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.9 (C(3)*C*H$_3$), 11.7 (C(5)*C*H$_3$), 14.8 (CH_2CH_3), 63.7 (CH_2CH_3), 75.4 (*C*H(OH)), 111.5 (*C*(6′)), 114.6 (*C*(4′)), 116.5 (*C*(4)), 119.4 (*C*(2′)), 127.9, 128.7 (*C*(2′′), *C*(6′′), *C*(3′′), *C*(5′′)), 131.9 (*C*(3′)), 133.5 (*C*(4′′), 142.1 (*C*(1′′)), 145.7 (*C*(1′)), 158.6 (*C*(3)), 159.5 (*C*(5′)), 165.3 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 380.1018; $C_{20}H_{20}ClNNaO_3$ requires M$^+$ 380.1024; LRMS

m/*z* (ES^+) 358 ($[M+H]^+$, 7), 380 ($[M+Na]^+$, 7), 412 ($[M+Na+MeOH]^+$, 9), 737 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{20}H_{20}ClNO_3$: C, 67.1; H, 5.6; N, 3.9. Found: C, 67.1; H, 5.5; N, 3.8.

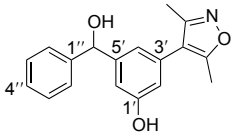
(*RS*)-(3-Chlorophenyl)(3-(3,5-dimethylisoxazol-4-yl)-5-ethoxyphenyl)methanol **58e**



Following the general procedure, 1-bromo-3-chlorobenzene (515 mg, 316 µL) and Mg turnings in THF were heated under reflux for 1 h. Following addition of the resultant yellow solution to **60** in THF, the reaction was stirred for 2 h, then quenched with H_2O (10 mL) and aq. HCl (1 M, 10 mL). The phases were separated, and the aqueous layer extracted with Et_2O (3 × 40 mL). The combined organic layers were washed with H_2O (120 mL) and brine (120 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 20 → 80% Et_2O/petroleum ether) gave **58e** as a colourless viscous gum (262 mg, 90%); R_f 0.23 (50% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3361 (O–H) (br), 3066, 2980, 2930, 2881 (C–H) (w), 1731 (m), 1590 (s), 1419 (s), 1326 (m), 1255 (m), 1203 (s), 1047 (s); ^1H NMR (500 MHz, $CDCl_3$) 1.42 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.20 (3H, s, C(3)CH_3), 2.36 (3H, s, C(5)CH_3), 2.9 (1H, br s, O*H*), 4.04 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 5.78 (1H, s, C*H*(OH)), 6.67 (1H, s, C(4′)*H*), 6.79 (1H, s, C(2′)*H*), 6.92 (1H, s, C(6′)*H*), 7.21-7.29 (3H, m, C(4′′)*H*, C(5′′)*H*, C(6′′)*H*), 7.40 (1H, s, C(2′′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)CH_3), 11.6 (C(5)CH_3), 14.8 (CH_2CH_3), 63.7 (CH_2CH_3), 75.2 (*C*H(OH)), 111.6 (*C*(6′)), 114.6 (*C*(4′)), 116.5

(*C*(4)), 119.5 (*C*(2′)), 124.7 (*C*(4′′)/*C*(5′′)/*C*(6′′)), 126.7 (*C*(2′′), 127.8 (*C*(5′′)/*C*(6′′)/*C*(4′′)), 129.8 (*C*(6′′)/*C*(4′′)/*C*(5′′)), 131.9 (*C*(3′)), 134.5 (*C*(3′′)), 145.5 (*C*(1′)), 145.8 (*C*(1′′)), 158.6 (*C*(3)), 159.5 (*C*(5′′)), 165.4 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 380.1018; $C_{20}H_{20}ClNNaO_3$ requires M^+ 380.1024; LRMS *m*/*z* (ES^+) 380 ($[M+Na]^+$, 4), 737 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{20}H_{20}ClNO_3$: C, 67.1; H, 5.6; N, 3.9. Found: C, 67.2; H, 5.6; N, 3.9.

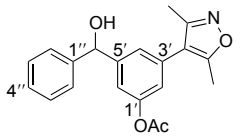
(*RS*)-3-(3,5-Dimethylisoxazol-4-yl)-5-(hydroxy(phenyl)methyl)phenol **61a**



To a solution of aldehyde **59** (250 mg, 1.02 mmol, 1.0 eq) in anhydrous THF (20 mL) under a nitrogen atmosphere was added PhMgBr (0.9 M in THF, 3.4 mL, 3.06 mmol, 3.0 eq) dropwise at 0 °C. The solution was warmed to rt over 1 h, then quenched with aq. HCl (1 M, 10 mL) and concentrated *in vacuo* to remove THF. The residues were extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with H_2O (2 × 40 mL) and brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 10 → 50% EtOAc/petroleum ether) gave **61a** as a pale yellow solid (273 mg, 91%); R_f 0.37 (50% EtOAc/40-60 °C petroleum ether); mp 187-188 °C ($CHCl_3$); ν_{max} (thin film)/cm^{-1}: 3268 (O–H) (br), 2928 (C–H) (w), 1692 (m), 1594 (s), 1421 (s), 1322 (m), 1256 (s), 1201 (m); ^1H NMR (500 MHz, acetone-d_6) 2.21 (3H, s, C(3)C*H*$_3$), 2.38 (3H, s, C(5)C*H*$_3$), 4.95 (1H, d, *J* = 3.1 Hz, CH(O*H*)), 5.84 (1H, d, *J* = 3.1 Hz, C*H*(OH)), 6.71 (1H, dd, *J* = 2.3, 1.6 Hz, C(2′)*H*), 6.91-6.94 (1H, m, C(4′)*H*), 6.96 (1H, dd, *J* = 2.3, 1.4 Hz, C(6′)*H*), 7.24 (1H, dddd, *J* = 7.3, 7.3, 1.3, 1.3 Hz, C(4′′)*H*), 7.31-7.36 (2H, m, C(3′′)*H*,

C(5´´)*H*), 7.46-7.50 (2H, m, C(2´´)*H*, C(6´´)*H*), 8.50 (1H, s, C(1´)O*H*); ^{13}C NMR (126 MHz, acetone-d_6) 10.9 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 75.9 (*C*H(OH)), 113.5 (*C*(6´)), 115.2 (*C*(2´)), 117.2 (*C*(4)), 119.3 (*C*(4´)), 127.3 (*C*(2´´), *C*(6´´)), 127.8 (*C*(4´´)), 129.0 (*C*(3´´), *C*(5´´)), 132.3 (*C*(3´)), 146.3 (*C*(1´´)), 148.8 (*C*(5´)), 158.5 (*C*(1´)), 158.9 (*C*(3)), 165.8 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 318.1102; $C_{18}H_{17}NNaO_3$ requires M$^+$ 318.1101; LRMS *m*/*z* (ES$^-$) 294 ([M-H]$^-$, 48), 330 [M+Cl]$^-$, 14), 408 ([M+TFA–H]$^-$, 9), 589 ([2M–H]$^-$, 100), 884 ([3M–H]$^-$, 18). Anal. Calcd for $C_{18}H_{17}NO_3$: C, 73.2; H, 5.8; N, 4.7. Found: C, 73.1; H, 5.8; N, 4.8.

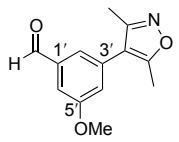
(*RS*)-3-(3,5-Dimethylisoxazol-4-yl)-5-(hydroxy(phenyl)methyl)phenyl acetate **61b**



Following the procedure of Srivastava *et al.*,[217] NaOH dissolved in a minimum amount of H_2O (37 mg, 915 µmol, 2.7 eq) was added to a stirred suspension of **61a** (100 mg, 339 µmol, 1.0 eq) in ^{i}PrOH (5 mL). Ac_2O (86 µl, 93 mg, 915 µmol, 2.7 eq) was added dropwise and the mixture was stirred at rt for 1 h. The mixture was then concentrated *in vacuo* to remove ^{i}PrOH and diluted with 10 mL H_2O, then extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 10 → 50% EtOAc/petroleum ether) gave **61b** as a colourless oil (104 mg, 90%); R_f 0.11 (25% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1} 3394 (O–H) (br), 3028, 2970 (C–H) (w), 1765 (s), 1740 (C=O) (s), 1416 (m), 1368 (m), 1205 (C–O) (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.22 (3H, s, C(3)C*H*$_3$), 2.30 (3H, s, COC*H*$_3$), 2.37 (3H, s, C(5)C*H*$_3$), 2.63 (1H, d, *J* = 2.9 Hz, O*H*), 5.86 (1H, d, *J* =

2.9 Hz, C*H*(OH)), 6.89-6.91 (1H, m, C(2′)*H*), 7.10-7.13 (1H, m, C(4′)*H*), 7.13-7.16 (1H, m, C(6′)*H*), 7.28-7.32 (1H, m, C(4′′)*H*), 7.33-7.42 (4H, m, C(2′′)*H*, C(3′′)*H*, C(5′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H_3), 11.6 (C(5)*C*H_3), 21.3 (CO*C*H_3), 75.5 (*C*H(OH)), 115.8 (*C*(4)), 118.7 (*C*(6′)), 121.1 (*C*(2′)), 124.4 (*C*(4′)), 126.6 (*C*(2′′), *C*(6′′)), 128.0 (*C*(4′′)), 128.7 (*C*(3′′), *C*(5′′)), 131.7 (*C*(3′)), 143.2 (*C*(1′′)), 146.1 (*C*(5′)), 150.9 (*C*(1′)), 158.4 (*C*(3)), 165.6 (*C*(5)), 169.3 (*C*=O); HRMS *m*/*z* (ES$^+$) found $[M+Na]^+$ 360.1206; $C_{20}H_{19}NNaO_4$ requires M^+ 360.1206; *m*/*z* (ES$^+$) 338 ($[M+H]^+$, 35), 360 ($[M+Na]^+$, 100), 697 ($[2M+Na]^+$, 95). Anal. Calcd for $C_{20}H_{19}NO_4$: C, 71.2; H, 5.7; N, 4.2. Found: C, 71.1; H, 5.6; N, 4.1.

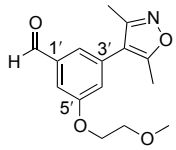
3-(3,5-Dimethylisoxazol-4-yl)-5-methoxybenzaldehyde **62**



Following the procedure of Parrish *et al.*,[218] anhydrous Cs_2CO_3 (225 mg, 690 μmol, 1.5 eq) was added to a dry 2-5 mL microwave vial containing **59** (100 mg, 460 μmol, 1.0 eq) and anhydrous DMF (2.3 mL) under a nitrogen atmosphere. The vial was sealed and MeI (57 μL, 131 mg, 920 μmol, 2.0 eq) was added. The suspension was stirred at rt for 2.5 h, then quenched with aq. HCl (1 M, 10 mL). The resultant precipitate was collected by filtration, washed with H_2O (20 mL) and dried under vacuum to give **62** as a brown solid (68 mg). The filtrate was extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with H_2O (2 × 50 mL), brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **62** as a yellow solid (30 mg, combined yield 92%); R_f 0.55 (40% EtOAc/40-60 °C petroleum ether); mp 90-92 °C ($CHCl_3$); ν_{max} (solid)/cm^{-1}: 2970, 2937, 2843 (C–H) (w), 1699 (C=O) (s), 1587 (m), 1416 (m), 1331

(s), 1259 (m), 1213 (s), 1186 (m), 1150 (m), 1082 (m), 1051 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 2.30 (3H, s, C(3)CH_3), 2.44 (3H, s, C(5)CH_3), 3.92 (3H, s, OCH_3), 7.06 (1H, dd, *J* = 2.3, 1.4 Hz, C(4′)*H*), 7.35 (1H, dd, *J* = 1.4, 1.1 Hz, C(2′)*H*), 7.40 (1H, dd, *J* = 2.3, 1.1 Hz, C(6′)*H*), 10.02 (1H, s, C*H*O); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)*C*H_3), 11.6 (C(5)*C*H_3), 55.7 (O*C*H_3), 111.2 (*C*(6′)), 115.5 (*C*(4)), 122.1 (*C*(4′)), 123.8 (*C*(2′)), 132.8 (*C*(3′)), 138.2 (*C*(1′)), 158.4 (*C*(3)), 160.5 (*C*(5′)), 165.8 (*C*(5)), 191.6 (*C*=O); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 254.0789; $C_{13}H_{13}NNaO_3$, requires M^+ 254.0788; LRMS *m*/*z* (ES^+) 232 ($[M+H]^+$, 14), 254 ($[M+Na]^+$, 15), 286 ($[M+Na+MeOH]^+$, 83), 318 ($[M+Na+2MeOH]^+$, 33), 517 ($[2M+Na+MeOH]^+$, 9), 549 ($[2M+Na+2MeOH]^+$, 100). Anal. Calcd for $C_{13}H_{13}NO_3$: C, 67.5; H, 5.7; N, 6.1. Found: C, 67.4; H, 5.6; N, 6.0.

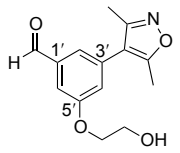
3-(3,5-Dimethylisoxazol-4-yl)-5-(2-methoxyethoxy)benzaldehyde **63**



Following the procedure of Sarju *et al.*,[215] **59** (250 mg, 1.15 mmol, 1.0 eq) and anhydrous K_2CO_3 (159 mg, 1.15 mmol, 1.0 eq) were added to a dry 2-5 mL microwave vial. The vial was sealed, and anhydrous MeOH (0.5 mL) and 2-bromoethyl methyl ether (130 µL, 192 mg, 1.38 mmol, 1.2 eq) were added under a nitrogen atmosphere. The mixture was stirred at 110 °C for 30 min with microwave irradiation, then concentrated *in vacuo*. The residues were extracted with EtOAc (40 mL), and the organic extracts were washed with H_2O (2 × 40 mL), aq. HCl (1 M, 40 mL) and brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 15 → 60% EtOAc/petroleum ether) gave **63** as a pale yellow oil (221 mg, 70%); R_f 0.23

(40% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film) 2930, 2884, 2822 (C–H) (w), 1698 (C=O) (s), 1587 (m), 1420 (m), 1330 (m), 1307 (m), 1259 (m), 1208 (m), 1126 (m), 1058 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 2.29 (3H, s, C(3)C*H*$_3$), 2.43 (3H, s, C(5)C*H*$_3$), 3.47 (3H, s, OC*H*$_3$), 3.80 (2H, d, *J* = 4.5 Hz, C*H*$_2$OCH$_3$), 4.23 (2H, d, *J* = 4.5 Hz, C*H*$_2$CH$_2$OCH$_3$), 7.12 (1H, dd, *J* = 2.5, 1.5 Hz, C(4′)*H*), 7.35 (1H, dd, *J* = 1.5, 1.3 Hz, C(2′)*H*), 7.41 (1H, dd, *J* = 2.5, 1.3 Hz, C(6′)*H*), 10.00 (1H, s, C*H*O); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 59.3 (O*C*H$_3$), 67.8 (*C*H$_2$CH$_2$OCH$_3$), 70.8 (*C*H$_2$OCH$_3$), 112.0 (*C*(6′)), 115.5 (*C*(4)), 122.7 (*C*(4′)), 123.9 (*C*(2′)), 132.8 (*C*(3′)), 138.2 (*C*(1′)), 158.4 (*C*(3)), 159.7 (*C*(5′)), 165.8 (*C*(5)), 191.5 (*C*=O); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 298.1055; $C_{15}H_{17}NNaO_4$ requires M$^+$ 298.1050; LRMS *m*/*z* (ES$^+$) 298 ([M+Na]$^+$, 11), 330 ([M+Na+MeOH]$^+$, 70), 573 ([2M+Na]$^+$, 14), 605 ([2M+Na+MeOH]$^+$, 32), 637 ([2M+Na+2MeOH]$^+$, 100). Anal. Calcd for $C_{15}H_{17}NO_4$: C, 65.4; H, 6.2; N, 5.1. Found: C, 65.5; H, 6.1; N, 5.0.

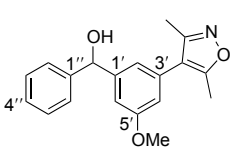
3-(3,5-Dimethylisoxazol-4-yl)-5-(2-hydroxyethoxy)benzaldehyde **64**



To a dry 10-20 mL microwave vial under a nitrogen atmosphere were added **59** (200 mg, 921 µmol, 1.0 eq) and anhydrous DMF (5 mL). Anhydrous Cs_2CO_3 (450 mg, 1.38 mmol, 1.5 eq) was then added and the vial was sealed. 2-Bromoethyl acetate (150 µL, 230 mg, 1.38 mmol, 1.5 eq) was added by syringe, and the mixture was stirred at 80 °C for 16 h, then concentrated *in vacuo*. The residues were resuspended in MeOH (10 mL) and stirred at rt for 90 min, then concentrated *in vacuo*. Aq. HCl (1 M, 10 mL) was added, and the solution was

extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 30 → 70% EtOAc/petroleum ether) gave **64** as a colourless solid (98 mg, 41%); R_f 0.24 (60% EtOAc/40-60 °C petroleum ether); mp 96-98 °C ($CHCl_3$); ν_{max} (solid) 3528 (O–H) (w), 3059 (Ar C–H) (w), 2937, 2872, 2735 (C–H) (w), 1693 (C=O) (s), 1601 (m), 1586 (m), 1423 (m), 1327 (s), 1309 (s), 1260 (s), 1202 (s), 1078 (s), 1050 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.06-2.18 (1H, m, O*H*), 2.30 (3H, s, C(3)C*H*$_3$), 2.44 (3H, s, C(5)C*H*$_3$), 4.00-4.07 (2H, m, C*H*$_2$OH), 4.20 (2H, t, *J* = 4.5 Hz, C*H*$_2$CH$_2$OH), 7.10 (1H, dd, *J* = 2.5, 1.5 Hz, C(4´)*H*), 7.37 (1H, dd, *J* = 1.5, 1.3 Hz, C(2´)*H*), 7.41 (1H, dd, *J* = 2.5, 1.3 Hz, C(6´)*H*), 10.01 (1H, s, C*H*O); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 61.2 (*C*H$_2$OH), 69.7 (*C*H$_2$CH$_2$OH), 111.9 (*C*(6´)), 115.4 (*C*(4)), 122.4 (*C*(4´)), 124.1 (*C*(2´)), 133.0 (*C*(3´)), 138.2 (*C*(1´)), 158.4 (*C*(3)), 159.6 (*C*(5´)), 165.8 (*C*(5)), 191.5 (*C*HO); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 284.0893; $C_{14}H_{15}NNaO_4$ requires M$^+$ 284.0893; LRMS *m*/*z* (ES$^+$) 284 ([M+Na]$^+$, 7), 316 ([M+Na+MeOH]$^+$, 56), 545 ([2M+Na]$^+$, 9), 577 ([2M+Na+MeOH]$^+$, 11), 609 ([2M+Na+2MeOH]$^+$, 100), 902 ([3M+Na+3MeOH]$^+$, 18). Anal. Calcd for $C_{14}H_{15}NO_4$: C, 64.4; H, 5.8; N, 5.4. Found: C, 64.5; H, 5.7; N, 5.2.

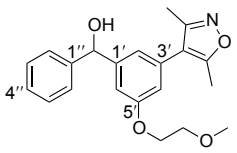
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-methoxyphenyl)(phenyl)methanol **61c**



To a solution of **62** (80 mg, 346 μmol, 1.0 eq) in anhydrous THF (5 mL) under a nitrogen atmosphere was added PhMgBr (0.9 M in THF, 769 μL,

692 μmol, 2.0 eq) dropwise at 0 °C. The solution was stirred for 1 h, then quenched with aq. HCl (1 M, 5 mL) and concentrated *in vacuo* to remove THF. The residues were extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 30 → 80% Et_2O/30-40 °C petroleum ether) gave **61c** as a colourless oil (66 mg, 76%); R_f 0.25 (60% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film) 3372 (O–H) (br), 3028, 3002, 2963, 2935 (C–H) (w), 1632 (m), 1591 (s), 1453 (m), 1415 (s), 1326 (m), 1255 (s), 1209 (s), 1057 (m), 1036 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.21 (3H, s, C(3)C*H*$_3$), 2.36 (3H, s, C(5)C*H*$_3$), 2.68 (1H, s, O*H*), 3.82 (3H, s, OC*H*$_3$), 5.84 (1H, s, C*H*(OH)), 6.68 (1H, dd, *J* = 2.3, 1.3 Hz, C(4′)*H*), 6.83 (1H, dd, *J* = 1.3, 1.3 Hz, C(2′)*H*), 6.98 (1H, dd, *J* = 2.3, 1.3 Hz, C(6′)*H*), 7.26-7.31 (1H, m, C(4′′)*H*), 7.33-7.38 (2H, m, C(3′′)*H*, C(5′′)*H*), 7.38-7.42 (2H, m, C(2′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 55.3 (O*C*H$_3$), 75.9 (*C*H(OH)), 110.8 (*C*(6′)), 114.0 (*C*(4′)), 116.5 (*C*(4)), 119.7 (*C*(2′)), 126.5 (*C*(2′′), *C*(6′′)), 127.8 (*C*(4′′)), 128.6 (*C*(3′′), *C*(5′′)), 131.7 (*C*(3′)), 143.6 (*C*(1′′)), 146.0 (*C*(1′)), 158.6 (*C*(3)), 159.9 (*C*(5′)), 165.3 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 332.1257; $C_{19}H_{19}NNaO_3$ requires M^+ 332.1257; LRMS *m*/*z* (ES^+) 332 ($[M+Na]^+$, 12), 364 ($[M+Na+MeOH]^+$, 6), 641 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{19}H_{19}NO_3$: C, 73.8; H, 6.2; N, 4.5. Found: C, 74.0; H, 6.0; N, 4.4.

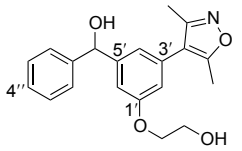
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-(2-methoxyethoxy)phenyl)(phenyl)methanol **61d**



To a solution of **63** (150 mg, 545 μmol, 1.0 eq) in anhydrous THF (10 mL) under a nitrogen atmosphere was added PhMgBr (0.9 M in THF, 1.2 mL, 1.08 mmol, 2.0 eq) dropwise at 0 °C. The solution was warmed to rt and stirred for 90 min, then quenched with aq. HCl (1 M, 5 mL) and concentrated *in vacuo* to remove THF. The residues were extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with H_2O (15 mL) and brine (15 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 6 → 60% EtOAc/petroleum ether) gave **61d** as a colourless oil which crystallised slowly to a colourless solid under vacuum (159 mg, 83%); R_f 0.15 (30% EtOAc/40-60 °C petroleum ether); mp 70-73 °C; ν_{max} (thin film) 3390 (O–H) (br), 3062, 3028 (Ar C–C) (w), 2929, 2881 (C–H) (w), 1739 (m), 1632 (m), 1590 (s), 1450 (m), 1419 (s), 1326 (m), 1225 (m), 1204 (s), 1125 (m), 1062 (m), 1033 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 2.20 (3H, s, C(3)C*H*$_3$), 2.35 (3H, s, C(5)C*H*$_3$), 2.74 (1H, d, *J* = 3.2 Hz, O*H*), 3.44 (3H, s, OC*H*$_3$), 3.74 (2H, t, *J* = 4.6 Hz, C*H*$_2$OCH$_3$), 4.11 (2H, t, *J* = 4.6 Hz, C*H*$_2$CH$_2$OCH$_3$), 5.82 (1H, d, *J* = 3.2 Hz, C*H*(OH)), 6.71 (1H, dd, *J* = 1.9, 1.5 Hz, C(4´)*H*), 6.83 (1H, dd, *J* = 1.6, 1.5 Hz, C(2´)*H*), 6.98 (1H, dd, *J* = 1.9, 1.6 Hz, C(6´)*H*), 7.25-7.30 (1H, m, C(4´´)*H*), 7.31-7.40 (4H, m, C(2´´)*H*, C(3´´)*H*, C(5´´)*H*, C(6´´)*H*)); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 59.2 (O*C*H$_3$), 67.3 (*C*H$_2$CH$_2$OCH$_3$), 71.0 (*C*H$_2$OCH$_3$), 75.9 (*C*H(OH)), 111.6 (*C*(6´)), 114.5 (*C*(4´)), 116.5 (*C*(4)), 119.9 (*C*(2´)), 126.5 (*C*(2´´), *C*(6´´)), 127.8 (*C*(4´´)), 128.5 (*C*(3´´), *C*(5´´)), 131.6 (*C*(3´)), 143.6 (*C*(1´´)), 146.0 (*C*(1´)), 158.6 (*C*(3)), 159.2 (*C*(5´)), 165.3 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 376.1515; $C_{21}H_{23}NNaO_4$ requires M$^+$ 376.1519; LRMS *m*/*z* (ES$^+$) 376 ([M+Na]$^+$, 23), 729 ([2M+Na]$^+$, 100). Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.4; H, 6.6; N, 4.0. Found: C, 71.5; H, 6.3; N, 3.8.

(*RS*)-2-(3-(3,5-Dimethylisoxazol-4-yl)-5-(hydroxy(phenyl)methyl)phenoxy)ethanol

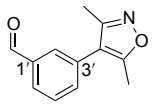
61e



To a solution of **64** (125 mg, 478 µmol, 1.0 eq) in anhydrous THF (8 mL) under a nitrogen atmosphere was added PhMgBr (0.9 M in THF, 1.6 mL, 1.44 mmol, 3.0 eq) dropwise at 0 °C. The solution was warmed to rt and stirred for 3 h, then quenched with aq. HCl (1 M, 10 mL) and concentrated *in vacuo* to remove THF. The residues were extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residues were purified by silica gel column chromatography (gradient elution, 40 → 80% EtOAc/petroleum ether) to give a colourless gum that crystallised slowly to a colourless solid under vacuum. Repeated recrystallisation from boiling $CHCl_3$/cyclohexane (1:1) gave **61e** as a colourless solid (100 mg, 62%); R_f 0.50 (EtOAc); mp 133-134 °C (1:1 $CHCl_3$/cyclohexane); ν_{max} (solid) 3348 (O–H) (br), 2933, 2871 (C–H) (w), 1637 (m), 1589 (m), 1471 (m), 1324 (m), 1253 (m), 1074 (m), 1058 (s); ^{1}H NMR (500 MHz, DMSO-d_6) 2.20 (3H, s, C(3)C*H*$_3$), 2.38 (3H, s, C(5)C*H*$_3$), 3.71 (2H, dt, *J* = 5.5, 5.0 Hz, C*H*$_2$OH), 4.00 (2H, t, *J* = 5.0 Hz, C*H*$_2$CH$_2$OH), 4.86 (1H, t, *J* = 5.5 Hz, CH$_2$O*H*) 5.73 (1H, d, *J* = 3.9 Hz, C*H*(OH)), 5.96 (1H, d, *J* = 3.9, CH(O*H*)), 6.76 (1H, dd, *J* = 2.2, 1.4 Hz, C(2′)*H*), 6.93-6.96 (1H, m, C(4′)*H*), 6.96-6.98 (1H, m, C(6′)*H*), 7.21 (1H, dd, *J* = 7.5, 7.5 Hz, C(4′′)*H*), 7.31 (2H, dd, *J* = 7.5, 7.5 Hz, C(3′′)*H*, C(5′′)*H*), 7.43 (2H, d, *J* = 7.5, Hz, C(2′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, DMSO-d_6) 10.5 (C(3)*C*H$_3$), 11.3 (C(5)*C*H$_3$), 59.5 (*C*H$_2$OH), 69.6 (*C*H$_2$CH$_2$OH),

73.9 (*C*H(OH)), 111.5 (*C*(6′)), 112.9 (*C*(2′)), 115.9 (*C*(4)), 119.0 (*C*(4′)), 126.1 (*C*(2′′), *C*(6′′)), 126.8 (*C*(4′′)), 128.1 (*C*(3′′), *C*(5′′)), 130.7 (*C*(3′)), 145.5 (*C*(1′′)) 148.0 (*C*(5′)), 158.0 (*C*(3)), 158.9 (*C*(1′)), 165.1 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 362.1353; $C_{20}H_{21}NNaO_4$ requires M^+ 362.1363; LRMS *m*/*z* (ES^+) 362 ($[M+Na]^+$, 7), 701 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{20}H_{21}NO_4$: C: 70.8; H, 6.2; N, 4.1. Found: C, 70.7; H, 6.4; N, 4.1.

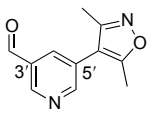
3-(3,5-Dimethylisoxazol-4-yl)benzaldehyde **67**



To a 2-5 mL microwave vial were added **51** (89 mg, 440 μmol, 1.1 eq), $Pd(OAc)_2$ (2 mg, 8 μmol, 0.02 eq), RuPhos (11 mg, 24 μmol, 0.06 eq) and Na_2CO_3 (85 mg, 800 μmol, 2.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). 3-Bromobenzaldehyde (**65**, 47 μL, 74 mg, 400 μmol, 1.0 eq) and degassed EtOH (2 mL) were added by syringe, and the mixture was heated at 100 °C for 2.5 h, after which time TLC analysis indicated complete consumption of **65**. The mixture was concentrated *in vacuo* onto Celite®, and purified by silica gel column chromatography (gradient elution, 10 → 40% EtOAc/40-60 °C petroleum ether) to give **67** as a colourless solid (66 mg, 82%); R_f 0.30 (30% EtOAc/40-60 °C petroleum ether); mp 92-93 °C (Et_2O); ν_{max} (thin film)/cm^{-1}: 2930, 2832, 2730 (C–H) (w), 1691 (C=O) (s), 1416 (m), 1179 (s); ^1H NMR (500 MHz, $CDCl_3$) 2.30 (3H, s, C(3)C*H*$_3$), 2.44 (3H, s, C(5)C*H*$_3$), 7.55 (1H, ddd, *J* = 7.7, 1.6, 1.3 Hz, C(4′)*H*/C(6′)*H*), 7.65 (1H, dd, *J* = 7.7, 7.7 Hz, C(5′)*H*), 7.79 (1H, dd, *J* = 1.6, 1.4 Hz, C(2′)*H*), 7.89 (1H, ddd, *J* = 7.7, 1.4, 1.3 Hz, C(6′)*H*/C(4′)*H*), 10.08 (1H, s, C*H*O); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 115.6 (*C*(4)), 129.2 (*C*(6′)/*C*(4′)), 129.6 (*C*(5′)), 129.7 (*C*(2′)), 131.7 (*C*(1′)/*C*(3′)), 134.9

(*C*(4′)/*C*(6′)), 136.9 (*C*(3′)/*C*(1′)), 158.4 (*C*(3)), 165.8 (*C*(5)), 191.9 (*C*=O); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 224.0680; $C_{12}H_{11}NNaO_2$ requires M^+ 224.0682; LRMS *m*/*z* (ES^+) 202 ($[M+H]^+$, 100), 224 ($[M+Na]^+$, 30), 234 ($[M+MeOH+H]^+$, 56), 256 ($[M+MeOH+Na]^+$, 100); HPLC: retention time 11.43 min, purity 100.0%.

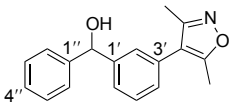
5-(3,5-Dimethylisoxazol-4-yl)-3-pyridinecarboxaldehyde **68**



To a 2-5 mL microwave vial were added **51** (89 mg, 440 μmol, 1.1 eq), 5-bromo-3-pyridinecarboxaldehyde (**66**, 174 mg, 400 μmol, 1.0 eq), $Pd(OAc)_2$ (2 mg, 8 μmol, 0.02 eq), RuPhos (11 mg, 24 μmol, 0.06 eq) and Na_2CO_3 (85 mg, 800 μmol, 2.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). Degassed EtOH (2 mL) were added by syringe, and the mixture was heated at 100 °C for 2.5 h, after which time TLC analysis indicated complete consumption of **66**. The mixture was concentrated *in vacuo* onto Celite®, and purified by silica gel column chromatography (gradient elution, 40 → 70% EtOAc/40-60 °C petroleum ether) to give **68** as a yellow solid (71 mg, 88%). An analytical sample was prepared by crystallisation from Et_2O; R_f 0.25 (60% EtOAc/40-60 °C petroleum ether); mp 98-99 °C (Et_2O); ν_{max} (thin film)/cm^{-1}: 3060, 2989, 2970, 2972 (C–H) (w), 2858, 2759 (CO–H) (w), 1695 (C=O) (s), 1411 (m), 1397 (m), 1189 (s); ^1H NMR (500 MHz, $CDCl_3$) 2.33 (3H, s, C(3)C*H*$_3$), 2.48 (3H, s, C(5)C*H*$_3$), 8.09 (1H, dd, *J* = 2.1, 2.1 Hz, C(4′)*H*), 8.80 (1H, d, *J* = 2.1 Hz, C(6′)*H*), 9.09 (1H, d, *J* = 2.1 Hz, C(2′)*H*), 10.21 (1H, s, C*H*O); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)*C*H$_3$), 11.7 (C(5)*C*H$_3$), 112.5 (*C*(4)), 127.6 (*C*(5′)), 131.4 (*C*(3′)), 135.3 (*C*(4′)), 151.0 (*C*(2′)), 154.6 (*C*(6′)), 158.3 (*C*(3)), 166.7 (*C*(5)), 196.3 (*C*HO); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 225.0635; $C_{11}H_{10}N_2NaO_2$ requires M^+ 225.0634; LRMS *m*/z (ES^+)

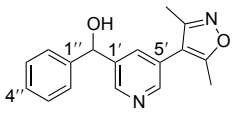
203 ([M+H]$^+$, 4), 235 ([M+MeOH+H]$^+$, 100), 257 ([M+MeOH+Na]$^+$, 5); HPLC: retention time 7.80 min, purity 99.9%.

(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)phenyl)(phenyl)methanol **61f**



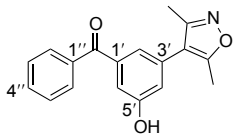
To a solution of **67** (62 mg, 308 μmol, 1.0 eq) in anhydrous THF (1.2 mL) at 0°C under an argon atmosphere was added PhMgBr (0.9 M in THF, 410 μL, 370 μmol, 1.2 eq), and the solution warmed to rt. After 45 min, TLC analysis indicated complete consumption of **67**, and the reaction was quenched with H_2O (5 mL) and sat. aq. NH_4Cl (5 mL), then concentrated *in vacuo* to remove THF. The mixture was extracted with Et_2O (3 × 10 mL), and the combined organic layers were washed with H_2O (30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 30% EtOAc/40-60 °C petroleum ether) gave **61f** as a colourless gum (75 mg, 87%); R_f 0.32 (30% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3374 (O–H) (br), 3061, 3028 2927 (C–H) (s), 1631 (m), 1452 (s), 1423 (s), 1242 (s), 1172 (m), 1022 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.11 (3H, s, C(3)C*H*$_3$), 2.26 (3H, s, C(5)C*H*$_3$), 2.69 (1H, d, *J* = 2.6 Hz, CH(O*H*)), 5.78 (1H, d, *J* = 2.6 Hz, C*H*(OH)), 7.05 (1H, ddd, *J* = 6.7, 1.9, 1.9 Hz, C(4′′)*H*), 7.15-7.35 (8H, m, Ar C*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 76.0 (*C*H(OH)), 116.6 (*C*(4)), 125.8, 126.5, 127.2, 127.8, 128.6, 128.9 (Ar C*H*), 128.0 (*C*(4′′)), 130.5 (*C*(3′)), 143.8 (*C*(1′)), 144.6 (*C*(1′′)), 158.7 (*C*(3)), 165.3 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 302.1151; $C_{18}H_{17}NNaO_2$ requires M$^+$ 302.1151; LRMS *m*/*z* (ES$^+$) 280 ([M+H]$^+$, 100), 302 ([M+Na]$^+$, 97); HPLC: retention time 12.13 min, purity 95.9%.

(*RS*)-(5-(3,5-Dimethylisoxazol-4-yl)pyridin-3-yl)(phenyl)methanol **61g**



To a solution of **68** (57 mg, 281 μmol, 1.0 eq) in anhydrous THF (1.2 mL) at 0°C under an argon atmosphere was added PhMgBr (0.9 M in THF, 375 μL, 338 μmol, 1.2 eq), and the solution warmed to rt. After 1 h, TLC analysis indicated complete consumption of **68**, and the reaction was quenched with H_2O (10 mL), then concentrated *in vacuo* to remove THF. The mixture was extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with H_2O (30 mL) and brine (30 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 30 → 80% EtOAc/40-60 °C petroleum ether) gave **61g** as a yellow solid (59 mg, 75%); R_f 0.25 (70% EtOAc/40-60 °C petroleum ether); mp 132-133 °C ($CHCl_3$); ν_{max} (thin film)/cm^{-1}: 3203 (O–H) (br), 3062, 3029, 2929 (C–H) (w), 1632 (m), 1454 (s), 1410 (s), 1250 (m), 1024 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.08 (3H, s, C(3)C*H*$_3$), 2.25 (3H, s, C(3)C*H*$_3$), 4.44 (1H, s, CH(O*H*)), 5.82 (1H, s, C*H*(OH)), 7.18-7.32 (5H, m, Ph C*H*), 7.54 (1H, dd, *J* = 1.7, 1.7 Hz, C(6′)*H*), 8.17 (1H, d, *J* = 1.7 Hz, C(4′)*H*), 8.46 (1H, d, *J* = 1.7 Hz, C(2′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 73.6 (*C*H(OH)), 113.3 (*C*(4)), 126.4 (*C*(5′)), 126.5 (*C*(2′′), *C*(6′′)), 128.1 (*C*(4′′)), 128.8 (*C*(3′′), *C*(5′′)), 134.5 (*C*(6′)), 140.0 (*C*(1′)), 143.2 (*C*(1′′)), 147.0 (*C*(2′)), 148.0 (*C*(4′)), 158.4 (*C*(3)), 166.1 (*C*(5)); HRMS *m*/*z* (ES$^+$) found $[M+H]^+$ 281.1289; $C_{17}H_{17}N_2O_2$ requires M^+ 281.1285; LRMS *m*/*z* (ES$^+$) 281 ($[M+H]^+$, 100), 303 ($[M+Na]^+$, 96). HPLC: retention time 9.09 min, purity 95.2%.

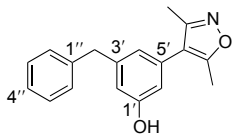
(3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxyphenyl)(phenyl)methanone **69a**



To a solution of alcohol **61a** (207 mg, 701 μmol) in anhydrous 1,4-dioxane (10 mL) was added activated MnO_2 (305 mg, 3.50 mmol, 5 eq). The reaction was stirred under a nitrogen atmosphere at 80 °C for 15 h, after which time further MnO_2 (152 mg, 1.75 mmol, 2.5 eq) was added, and stirring was continued at 80 °C for 9 h. Further MnO_2 (152 mg, 1.75 mmol, 2.5 eq) was then added, and stirring was continued at 80 °C for 29 h. The reaction was cooled to rt and filtered through Celite® (eluent CH_2Cl_2), then concentrated *in vacuo*. Purification by repeated silica gel column chromatography (gradient elution, 20 → 50% EtOAc/40-60 °C petroleum ether, then further purification of mixed fractions, 20→35% EtOAc/40-60 °C petroleum ether) gave **69a** as a cream solid (172 mg, 84%); R_f 0.43 (50% EtOAc/40-60 °C petroleum ether); mp 188-190 °C (acetone); ν_{max} (solid)/cm^{-1}: 3155 (O–H) (br), 1655 (m), 1638 (m), 1591 (m), 1421 (m), 1333 (m), 1268 (m), 1241 (s); ^{1}H NMR (500 MHz, acetone-d_6) 2.27 (3H, s, C(3)C*H*$_3$), 2.45 (3H, s, C(5)C*H*$_3$), 7.13 (1H, dd, *J* = 2.4, 1.5 Hz, C(4′)*H*), 7.20 (1H, dd, *J* = 1.5, 1.5 Hz, C(2′)*H*), 7.27 (1H, dd, *J* = 2.4, 1.5 Hz, C(6′)*H*), 7.55-7.62 (2H, m, C(3′′)*H*, C(5′′)*H*), 7.66-7.71 (1H, m, C(4′′)*H*), 7.82-7.88 (2H, m, C(2′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, acetone-d_6) 10.9 (C(3)*C*H$_3$), 11.6 (C(5)*C*H$_3$), 116.2 (*C*(6′)), 116.5 (*C*(4)), 120.6 (*C*(4′)), 122.6 (*C*(2′)), 129.3 (*C*(3′′), *C*(5′′)), 130.6 (*C*(2′′), *C*(6′′), 133.0 (*C*(3′′)), 133.4 (*C*(4′′)), 138.5 (*C*(1′′)), 140.5 (*C*(1′)), 158.7 (*C*(5′)), 158.9 (*C*(3)), 166.3 (*C*(5)), 196.1 (*C*=O); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 316.0938; $C_{18}H_{15}NNaO_3$ requires M^+ 316.0944; LRMS *m*/*z* (ES$^-$) 292 ([M-H]$^-$,

100), 328 ([M+Cl]$^-$, 13). Anal. Calcd for $C_{18}H_{15}NO_3$: C, 73.7; H. 5.2; N, 4.8. Found: C, 73.6; H, 5.3; N, 4.9.

3-Benzyl-5-(3,5-dimethylisoxazol-4-yl)phenol **69b**



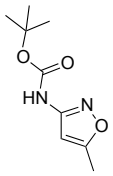
To a mixture of **61a** (30 mg, 102 μmol, 1.0 eq) and Et_3SiH (81 μL, 59 mg, 510 μmol, 5.0 eq) was added TFA (0.5 mL). The solution was stirred for 15 min, after which time TLC analysis indicated complete consumption of **61a**. The reaction was concentrated *in vacuo* and the residues were dissolved in CH_2Cl_2 (15 mL), washed with sat. aq. $NaHCO_3$ (15 mL), H_2O (15 mL) and brine (15 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 3 → 40% EtOAc/40-60 °C petroleum ether) gave **69b** as a pale brown solid (20 mg, 70%); R_f 0.24 (30% EtOAc/40-60 °C petroleum ether); mp 100-102 °C (acetone); ν_{max} (thin film)/cm^{-1}: 3217 (O–H) (br), 3026, 2929, 2852 (C–H) (w), 1632 (m), 1593 (s), 1420 (s), 1325 (s), 1258 (m); ^{1}H NMR (500 MHz, acetone-d_6) 2.21 (3H, s, C(3)C*H*$_3$), 2.38 (3H, s, C(5)C*H*$_3$), 3.98 (2H, s, C*H*$_2$), 6.66-6.69 (1H, m, C(6′)*H*), 6.71-6.73 (1H, m, C(2′)*H*), 6.73-6.76 (1H, m, C(4′)*H*), 7.18-7.24 (1H, m, C(4′′)*H*), 7.28-7.35 (4H, m, C(2′′)*H*, C(6′′)*H*, C(3′′)*H*, C(5′′)*H*), 8.44 (1H, s, O*H*); ^{13}C NMR (126 MHz, acetone-d_6) 10.0 (C(3)*C*H$_3$), 10.7 (C(5)*C*H$_3$), 41.3 (*C*H$_2$), 113.5 (*C*(6′)), 114.9 (*C*(2′)), 116.2 (*C*(4)), 120.7 (*C*(4′)), 126.0 (*C*(4′′)), 128.4 (*C*(3′′), *C*(5′′)), 128.9 (*C*(2′′), *C*(6′′)), 131.7 (*C*(5′)), 141.2 (*C*(1′′)), 143.7 (*C*(3′)), 157.8, 158.0 (*C*(1′), *C*(3)), 164,8 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 302.1151; $C_{18}H_{17}NNaO_2$ requires M$^+$

302.1151; LRMS *m*/*z* (ES^+) 280 ($[M+H]^+$, 100), 302 ($[M+Na]^+$, 88); HPLC: retention time 12.59 min, purity 97.5%.

7.4 Chapter 4: Synthetic Procedures

7.4.1 3-amino-5-methyl- and 5-amino-3-methylisoxzoles

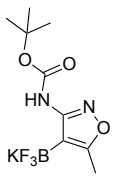
tert-Butyl (5-methylisoxazol-3-yl)carbamate **84**[232]



To a solution of 3-amino-5-methylisoxazole (**83**, 1.50 g, 15.3 mmol, 1.0 eq) in anhydrous THF (30 mL) were added Boc_2O (3.50 g, 16.0 mmol, 1.05 eq) and DMAP (93 mg, 761 µmol, 0.05 eq), and the reaction was heated under reflux for 3 h, after which time TLC analysis indicated complete consumption of starting material. The mixture was then concentrated *in vacuo* and redissolved in MeOH (15 mL). NaOH (1 M, 1.6 mL, 1.6 mmol, 0.10 eq) was added, and the mixture stirred at rt for 23 h. The solution was neutralised to pH 7 with sat. aq. NH_4Cl, and 100 mL H_2O were added. The resulting precipitate was collected by filtration, and dried *in vacuo* to give **84** as a pale yellow solid (2.60 g, 85%) which was used without further purification. An analytically pure sample was prepared by crystallisation from boiling toluene; R_f 0.55 (40% EtOAc/40-60 °C petroleum ether); mp 102-105 °C (toluene) [lit. 108-109 °C (EtOAc-hexane)[232]]; ν_{max} (thin film)/cm^{-1}: 3457 (N−H) (br), 2971, 2946 (C−H) (m), 1729 (C=O) (s), 1630 (m), 1423 (m), 1366 (s), 1231 (s); ^1H NMR (400 MHz, $CDCl_3$) 1.51 (9H, s, C(C*H*$_3$)$_3$), 2.37 (3H, d, *J* = 0.7 Hz, C(5)C*H*$_3$), 6.50 (1H, s, C(4)*H*), 7.97 (1H, s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) 12.6 (C(5)*C*H$_3$), 28.1 (C(*C*H$_3$)$_3$), 81.6 (*C*(CH$_3$)$_3$), 95.5 (*C*(4)), 151.9 (*C*=O),

158.7, 169.6 (*C*(3), *C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 221.0892; $C_9H_{14}NaN_2O_3$ requires M^+ 221.0897; LRMS *m*/*z* (ES^+) 221 ($[M+Na]^+$, 83), 419 ($[2M+Na]^+$, 100). The data are in good agreement with the literature values.[232]

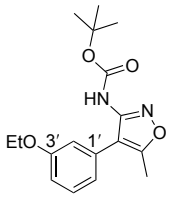
Potassium (3-((*tert*-butoxycarbonyl)amino)-5-methylisoxazol-4-yl)trifluoroborate **85**



To a solution of **84** (200 mg, 1.01 mmol, 1.0 eq) in anhydrous THF (2 mL) under a nitrogen atmosphere was added ^{n}BuLi (1.4 M in hexanes, 1.6 mL, 2.24 mmol, 2.2 eq) dropwise at −78 °C. The solution was stirred at −78 °C for 1 h, then for 30 min at rt. The solution was cooled to −78 °C and a solution of $B(OMe)_3$ in anhydrous THF (4.44 mL, 0.5 M, 2.22 mmol, 2.2 eq) was added dropwise. The mixture was stirred for 1 h at −78 °C then for 30 min at rt. A sat. aq. solution of KHF_2 (394 mg, 5.04 mmol, 4.0 eq) was then added dropwise, and the solvent was removed *in vacuo*. The residues were extracted with boiling acetone (4 × 10 mL) and concentrated *in vacuo*, then redissolved in a minimal amount of boiling acetone (10 mL). The product was precipitated by the addition of Et_2O (50 mL) and collected by filtration. The filtrate was concentrated *in vacuo*, redissolved in acetone (5 mL) and further product was precipitated by the addition of Et_2O (20 mL) and collected by filtration. The combined solids were dried *in vacuo* to give **85** as a powdery colourless solid (172 mg, 56%); mp > 250 °C; ν_{max} (solid)/cm^{-1} 3418 (N–H) (br), 2979, 2936 (C–H) (w), 1726 (C=O) (s), 1530 (s), 1274 (m), 1241 (m), 1159 (s); ^{1}H NMR (500 MHz, DMSO-d_6) 1.44 (9H, s, C(C*H*$_3$)$_3$), 2.17 (3H, s, C(5)C*H*$_3$), 7.40 (1H, q, *J* = 4.0 Hz, N*H*); ^{13}C NMR (126 MHz, DMSO-

d_6) 11.9 (C(5)*C*H_3), 27.8 (C(*C*H_3)$_3$), 79.7 (*C*(CH_3)$_3$), 150.1 (*C*=O), 160.0, 161.8 ((*C*(3), *C*(5)). The ^{13}C signal of C(4) was obscured due to coupling with the quadrupolar ^{11}B nucleus. ^{11}B NMR (160 MHz, DMSO-d_6) 2.13 (br s); ^{19}F NMR (470 MHz, DMSO-d_6) –134.8- –134.2; HRMS *m*/*z* (ES^-) found $[M-K]^-$ 265.0974; $C_9H_{13}BF_3N_2O_3$ requires M^- 265.0977; LRMS *m*/*z* (ES^-) 265 ($[M-K]^-$, 100), 553 ($[2M-2K+Na]^-$, 98), 569 ($[2M-K]^-$, 9). Anal. Calcd for $C_9H_{13}BF_3N_2O_3$: C, 35.5; H, 4.3; N, 9.2. Found: C; 35.7; H, 4.2; N, 9.1.

tert-Butyl (4-(3-ethoxyphenyl)-5-methylisoxazol-3-yl)carbamate **86**

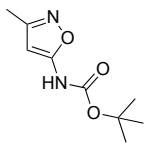


Method A: To a 0.5-2 mL microwave vial were added **85** (50 mg, 164 µmol, 1.05 eq), $Pd(OAc)_2$ (1 mg, 4 µmol, 0.03 eq), RuPhos (2 mg, 4 µmol, 0.03 eq) and Na_2CO_3 (32 mg, 298 µmol, 2.0 eq). The vial was sealed and purged with argon (3 × evacuate/fill). A solution of **52** in degassed EtOH (0.15 M, 1.0 mL, 149 µmol, 1.0 eq) was added, and the mixture was heated at 85 °C for 18 h. The mixture was cooled to rt and filtered through a thin pad of silica (eluent 10% MeOH/CH_2Cl_2) and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 4 → 40% EtOAc/petroleum ether) gave **86** as a pale yellow solid (22 mg, 46%); R_f 0.25 (20% EtOAc/40-60 °C petroleum ether); mp 89-90 °C ($CHCl_3$); ν_{max} (solid)/cm^{-1} 3232 (w), 3192 (w), 3102 (w), 2987 (C–H) (w), 2931 (C–H) (w), 1701 (C=O) (s), 1639 (m), 1358 (m), 1240 (s), 1155 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.42 (9H, s, C(C*H*$_3$)$_3$), 1.44 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.39 (3H, s, C(5)C*H*$_3$), 4.06 (2H, q, *J* = 7.0 Hz, C*H*$_2$CH$_3$), 6.45 (1H, s, N*H*), 6.80 (1H, dd, *J* = 2.4, 1.3 Hz, C(2′)*H*), 6.85 (1H, ddd, *J* = 7.5, 1.3, 0.9 Hz, C(6′)*H*),

6.93 (1H, ddd, *J* = 8.4, 2.4, 0.9 Hz, C(4´)*H*), 7.38 (1H, dd, *J* = 8.4, 7.5 Hz, C(5´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.7 (C(5)*C*H_3), 14.8 (CH_2*C*H_3), 28.0 (C(*C*H_3)$_3$), 63.6 (*C*H_2CH$_3$), 81.9 (*C*(CH$_3$)$_3$), 110.1 (*C*(4)), 113.9 (*C*(4´)), 115.2 (*C*(2´)), 121.0 (*C*(6´)), 130.1 (*C*(1´)), 130.4 (*C*(5´)), 150.9 (*C*=O), 155.6 (*C*(3)), 159.5 (*C*(3´)), 165.9 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 341.1459; $C_{17}H_{22}N_2NaO_4$ requires M$^+$ 341.1472; LRMS *m*/*z* (ES$^+$) 341 ([M+Na]$^+$, 16), 659 ([2M+Na]$^+$, 100).

Method B: To a 2-5 mL microwave vial were added **90** (134 mg, 540 µmol, 1.25 eq), **94** (120 mg, 433 µmol, 1.0 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (18 mg, 22 µmol, 0.05 eq). The vial was sealed and purged with argon (5 × evacuate/fill). Degassed aq. Na_2CO_3 (2 M, 1.1 mL, 2.2 mmol, 5 eq) and degassed DME (2.2 mL) were added, and the reaction was heated at 90 °C for 24 h. The reaction was cooled, diluted with H_2O (30 mL) and extracted with EtOAc (4 × 40 mL). The combined organic layers were washed with H_2O (120 mL) and brine (120 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 50% EtOAc/petroleum ether) gave **86** as a pale yellow solid (69 mg, 50%). The data are identical to those reported above.

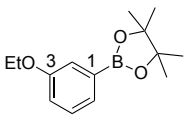
tert-Butyl (5-methylisoxazol-3-yl)carbamate **88**



To a solution of 5-amino-3-methylisoxazole (**87**, 500 mg, 5.10 mmol, 1.0 eq) in anhydrous THF (10 mL) was added Boc_2O (2.33 g, 10.7 mmol, 2.1 eq) and DMAP (31 mg, 255 µmol, 0.05 eq). The reaction was heated under reflux for

14 h, after which time TLC analysis indicated complete consumption of **87**. The mixture was then concentrated *in vacuo* and redissolved in MeOH (5 mL). NaOH (2 M, 3.05 mL, 6.10 mmol, 1.2 eq) was added, and the mixture stirred at rt for 19 h. The solution was neutralised with sat. aq. NH_4Cl and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with H_2O (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 15 → 25 % EtOAc/petroleum ether) gave **88** as a colourless solid (923 mg, 91%); R_f 0.23 (20% EtOAc/40-60 °C petroleum ether); mp 78-82 °C ($CHCl_3$); ν_{max} (thin film)/cm^{-1}: 3196 (N−H) (br), 2980, 2934 (C−H) (m), 1738 (C=O) (m), 1615 (s), 1547 (s), 1282 (s), 1155 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.52 (9H, s, C(C*H*$_3$)$_3$) 2.24 (3H, s, C(3)C*H*$_3$), 5.91 (1H, s, C(4)*H*), 7.71 (1H, s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) 11.8 (C(3)*C*H_3), 28.1 (C(*C*H_3)$_3$) 82.5 (*C*(CH$_3$)$_3$), 87.4 (*C*(4)), 150.1 (*C*=O), 161.1, 161.4 (*C*(3), *C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 221.0898; $C_9H_{14}NaN_2O_3$ requires M$^+$ 221.0897; LRMS *m*/*z* (ES$^+$) 221 ([M+Na]$^+$, 81), 419 ([2M+Na]$^+$, 100). Anal. Calcd for $C_9H_{14}N_2O_3$: C, 54.5; H, 7.1; N, 14.1. Found: C, 54.5; H, 7.0; N, 13.9.

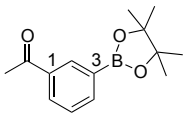
2-(3-Ethoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **90**



To a solution of **52** (3.14 g, 15.7 mmol, 1.0 eq) in anhydrous THF (50 mL) at −78 °C under a nitrogen atmosphere was added ^{n}BuLi (2.2 M in hexanes, 8.0 mL, 17.6 mmol, 1.1 eq) dropwise. After stirring for 2 h at −78 °C, ^{i}PrOBpin (5.0 mL, 4.6 g, 24.5 mmol, 1.6 eq) was added dropwise. The mixture was stirred at –78 °C for 1 h, then warmed to rt and stirred for 1 h. The reaction was quenched with H_2O (100 mL) and concentrated *in vacuo*, acidified to pH 1 with aq.

HCl (1 M) and extracted with Et_2O (3 × 100 mL). The combined organic layers were washed with H_2O (200 mL) and brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by Kugelrohr distillation (bp 140 °C, 1 mbar) gave **90** as a colourless oil (3.08 g, 79%); R_f 0.48 (20% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1} 2979, 2932 (C–H) (m), 1575 (Ar C=C) (m), 1428 (m), 1353 (B–O) (s), 1315 (m), 1225 (m), 1145 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.35 (12H, s, OC(CH_3)$_2$C(CH_3)$_2$O), 1.41 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 4.08 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 7.01 (1H, ddd, *J* = 8.0, 2.7, 0.9 Hz, C(4)*H*), 7.29 (1H, dd, *J* = 8.0, 7.5 Hz, C(5)*H*), 7.32-7.35 (1H, m, C(2)*H*), 7.37-7.41 (1H, m, C(6)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 14.9 (CH_2CH_3), 24.8 (OC(CH_2)$_2$C(CH_2)$_2$O), 63.3 (CH_2CH_3), 83.8 (OC(CH_2)$_2$C(CH_2)$_2$O), 118.4 (*C*(4)), 119.5 (*C*(2)), 127.0 (*C*(6)), 128.9 (*C*(5)), 158.4 (*C*(3)). The ^{13}C signal of C(1) was obscured due to coupling to the quadrupolar ^{11}B nucleus. ^{11}B NMR (160 MHz, $CDCl_3$) 30.8 (br s); HRMS *m*/*z* (ES$^+$) found $[M+Na]^+$ 271.1471; $C_{14}H_{21}BNaO_3$ requires M^+ 271.1478; LRMS *m*/*z* (ES$^+$) 249 ($[M+H]^+$, 49), 271 ($[M+Na]^+$, 100). Anal. Calcd. for $C_{14}H_{21}BO_3$: C, 67.8; H; 8.5. Found: C, 67.9; H, 8.6.

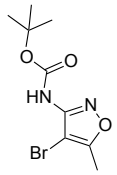
1-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethanone **91**[327,328]



Following the procedure of Ishiyama *et al.*,[233] B_2pin_2 (1.12 g, 4.42 mmol, 1.1 eq), anhydrous KOAc (1.19 mg, 12.1 mmol, 3.0 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (164 mg, 210 μM, 0.05 eq) were added to a dry 10-20 mL microwave vial. The vial was sealed and purged with Ar (3 × evacuate/fill). Anhydrous degassed DMSO (17 mL) and 3′-bromoacetophenone (**49**, 532 μL, 800 mg, 4.02 mmol, 1.0 eq) were added, and the reaction was stirred at 90 °C for 15 h, after which

time TLC analysis indicated complete consumption of bromide. The mixture was then diluted with H_2O (100 mL) and extracted with EtOAc (4 × 60 mL). The combined organic layers were washed with H_2O (2 × 200 mL) and brine (200 mL), then dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 0 → 20% EtOAc/petroleum ether) gave a green oil which was redissolved in CH_2Cl_2 (6 mL) and stirred with activated charcoal for 3 h at rt. The mixture was filtered through Celite® (eluent CH_2Cl_2) to give **91** as a colourless solid (776 mg, 78%); R_f 0.23 (20% EtOAc/40-60 °C petroleum ether); mp 47-48 °C (CH_2Cl_2) (lit. 47-48 °C,[327] 50.7-52.5 °C);[328] ^{1}H NMR (400 MHz, $CDCl_3$) 1.37 (12H, s, OC(C*H*$_3$)$_2$C(C*H*$_3$)$_2$O), 2.64 (3H, s, COC*H*$_3$), 7.48 (1H, dd, *J* = 7.8, 7.4 Hz, C(5)*H*), 8.00 (1H, d, *J* = 7.4 Hz, C(4)*H*, C(6)*H*)), 8.07 (1H, ddd, *J* = 7.8, 7.4, 1.2 Hz, C(6)*H*, C(4)*H*), 8.37 (1H, s, C(2)*H*); LRMS *m*/*z* (ES$^+$) 247 ([M+H]$^+$, 4), 269 ([M+Na]$^+$, 45), 301 ([M+MeOH+Na]$^+$, 50), 515 ([2M+Na]$^+$, 100). The data are in good agreement with the literature values.[327,328]

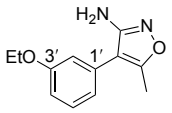
tert-Butyl (4-bromo-5-methylisoxazol-3-yl)carbamate **93**



To a solution of 4-bromo-5-methylisoxazol-3-amine (**92**, 500 mg, 2.82 mmol, 1.0 eq) in anhydrous THF (15 mL) were added Boc_2O (1.36 g, 6.21 mmol, 2.2 eq) and DMAP (17 mg, 141 µmol, 0.05 eq), and the reaction was heated under reflux for 4 h. The mixture was then concentrated *in vacuo* and redissolved in MeOH (15 mL). NaOH (2 M, 1.41 mL, 2.82 mmol, 1.0 eq) was added, and the mixture was stirred at rt for 18 h. The solution was neutralised with sat. aq. NH_4Cl (10 mL) and extracted with EtOAc (3 × 75 mL). The combined organic layers

were washed with H_2O (2 × 150 mL) and brine (150 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 30 % Et_2O/petroleum ether) gave **93** as a colourless solid (657 mg, 84%); R_f 0.26 (30% Et_2O/40-60 °C petroleum ether); mp 73-74 °C (EtOAc); ν_{max} (solid)/cm^{-1} 3259 (N–H) (br), 2980, 2932 (C–H) (w), 1710 (C=O) (s), 1542 (s), 1367 (m), 1234 (m), 1155 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.52 (9H, s, C(C*H*$_3$)$_3$), 2.39 (3H, s, C(5)C*H*$_3$), 6.54 (1H, s, N*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.7 (C(5)*C*H$_3$), 28.1 (C(C*H*$_3$)$_3$), 82.6, 85.3 (*C*(4), *C*(CH$_3$)$_3$), 150.3 (*C*=O), 155.7, 166.9 (*C*(3), *C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 298.9999, 300.9982; $C_9H_{13}{}^{79}BrN_2NaO_3$ requires M^+ 299.0002, $C_9H_{13}{}^{81}BrN_2NaO_3$ requires M^+ 300.9982; LRMS *m*/*z* (ES$^+$) 299 ([^{79}M+Na]$^+$, 23), 301 ([^{81}M+Na]$^+$, 23), 575 ([2^{79}M+Na]$^+$, 92), 577 ([^{79}M+^{81}M+Na]$^+$, 100), 579 ([2^{81}M+Na]$^+$, 92). Anal. Calcd. for $C_9H_{13}BrN_2O_3$: C, 39.0; H, 4.7; N, 10.1. Found: C, 39.2; H, 4.7; N, 10.0.

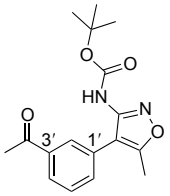
4-(3-Ethoxyphenyl)-5-methylisoxazol-3-amine **70a**

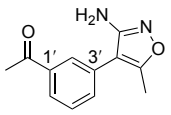


To a solution of **86** (65 mg, 204 μmol, 1.0 eq) in CH_2Cl_2 (1 mL) were added Et_3SiH (130 μL, 95 mg, 817 μmol. 4.0 eq) and TFA (0.5 mL). After 2 h, TFA was removed by azeotropic distillation with cyclohexane. Sat. aq. $NaHCO_3$ (10 mL) was then added, and the solution was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with H_2O (30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 30% EtOAc/petroleum ether) gave **70a** as a colourless solid (27 mg, 61%); R_f 0.28 (30% EtOAc/40-60 °C petroleum

ether); mp 126-127 °C ($CHCl_3$); ν_{max} (solid)/cm^{-1} 3411, 3293, 3194 (N–H) (m), 2981, 2926, 2879 (C–H), (w), 1642 (s), 1583 (s), 1465 (s), 1424 (s), 1256 (s), 1212 (s), 1048 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.44 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.35 (3H, s, C(5)CH_3), 4.04 (2H, br s, NH_2) overlapping 4.06 (2H, q, *J* = 7.0 Hz, CH_2CH_3) 6.85-6.87 (1H, m, C(2′)*H*), 6.87-6.92 (2H, m, C(4′)*H*, C(6′)*H*), 7.35 (1H, dd, *J* = 7.9, 7.9 Hz, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.9 (C(5)CH_3), 14.8 (CH_2CH_3), 63.5 (CH_2CH_3), 108.2 (*C*(4)), 113.5 (*C*(4′)), 115.0 (*C*(2′)), 120.8 (*C*(6′)), 130.2 (*C*(5′)), 131.0 (*C*(1′)), 159.5 (*C*(3′)), 161.6 (*C*(3)), 164.9 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 241.0940; $C_{12}H_{14}N_2NaO_2$ requires M^+ 241.0947; LRMS *m*/*z* (ES^+) 219 ($[M+H]^+$, 25), 241 ($[M+Na]^+$, 92), 273 ($[M+Na+MeOH]^+$, 46), 459 ($[2M+Na]^+$, 100). HPLC: Retention time 11.50 min, purity 98.7%.

tert-Butyl (4-(3-acetylphenyl)-5-methylisoxazol-3-yl)carbamate **94** and 1-(3-(3-amino-5-methylisoxazol-4-yl)phenyl)ethanone **70b**





To a 2-5 mL microwave vial were added **91** (133 mg, 540 µmol, 1.25 eq), **93** (120 mg, 433 µmol, 1.0 eq) and $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (18 mg, 22 µmol, 0.05 eq). The vial was sealed and purged with argon (5 × evacuate/fill). Degassed aq. Na_2CO_3 (2 M, 1.1 mL, 2.2 mmol, 5 eq) and degassed DME (2.2 mL) were added, and the reaction was heated at 90 °C for 37 h. The reaction was cooled, diluted with H_2O (30 mL) and extracted with EtOAc (4 × 40 mL). The combined organic layers were washed with H_2O (120 mL) and brine (120 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 7 → 45% EtOAc/petroleum ether) gave **94** as a pale

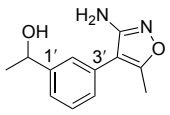
yellow solid (71 mg, 52%); R_f 0.49 (50% EtOAc/40-60 °C petroleum ether); mp 142-144 °C (EtOAc); ν_{max} (solid)/cm^{-1} 3270 (N–H) (br), 2971, 2929 (C–H) (w), 1727 (C=O) (s), 1684 (C=O) (s), 1535 (m), 1367 (m), 1226 (s), 1157 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.35 (9H, s, C(C*H*$_3$)$_3$), 2.42 (3H, s, C(5)C*H*$_3$), 2.63 (3H, s, COC*H*$_3$), 6.56 (1H, s, N*H*), 7.52 (1H, ddd, *J* = 7.7, 1.4, 1.2 Hz, C(6′)*H*), 7.57 (1H, dd, *J* = 7.7, 7.7 Hz, C(5′)*H*), 7.90 (1H, dd, *J* = 1.6, 1.4 Hz, C(2′)*H*), 7.96 (1H, ddd, *J* = 7.7, 1.6, 1.2 Hz, C(4′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.9 (C(5)*C*H$_3$), 26.7 (CO*C*H$_3$), 27.9 (C(*C*H$_3$)$_3$), 82.0 (*C*(CH$_3$)), 110.4 (*C*(4)), 127.8 (*C*(4′)), 128.4 (*C*(2′)), 129.4 (*C*(5′)), 130.0 (*C*(1′)), 133.2 (*C*(6′)), 137.8 (*C*(3′)), 151.2 (*C*O^{t}Bu), 155.8 (*C*(3)), 166.7 (*C*(5)), 197.5 (*C*OCH$_3$); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 339.1303; $C_{17}H_{20}N_2NaO_4$ requires M$^+$ 339.1315; LRMS *m*/*z* (ES$^+$) 317 ([M+H]$^+$, 4), 339 ([M+Na]$^+$, 6), 655 ([2M+Na]$^+$, 100). Anal. Calcd. for $C_{17}H_{20}N_2O_4$: C, 64.5; H, 6.4; N, 8.9. Found: C, 64.7; H, 6.2; N, 8.8.

Further elution gave **70b** (5 mg, 5%).

A solution of **94** (71 mg, 224 μmol) in TFA (0.5 mL) and CH_2Cl_2 (1 mL) was stirred at rt for 1 h. Sat. aq. Na_2CO_3 (5 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residues were combined with the deprotected product **70b** (5 mg) isolated above, and passed through a short plug of silica (eluent EtOAc). The combined residues were dissolved in MeCN (4 mL) and washed with hexane (3 × 4 mL), then concentrated *in vacuo* to give **70b** as a colourless solid (45 mg, 38% over two steps); mp 152-153 °C (MeCN); ν_{max} (solid)/cm^{-1}: 3429, 3340 (N–H) (br), 3211 (C–H) (m), 1682 (C=O) (s), 1645 (m), 1521 (m), 1462 (m), 1359 (m), 1263 (m), 1231 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.37 (3H, s, C(5)C*H*$_3$), 2.67 (3H, s,

COC*H*$_3$), 3.98 (2H, s, N*H*$_2$), 7.52-7.61 (2H, m, C(4´)*H*, C(5´)*H*), 7.90-7.97 (2H, m, C(2´)*H*, C(6´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.9 (C(5)*C*H$_3$), 26.7 (CO*C*H$_3$), 107.6 (*C*(4)), 127.6 (*C*(6´)), 128.3 (*C*(2´)), 129.5 (*C*(5´)), 130.4 (*C*(3´)), 133.2 (*C*(4´)), 138.0 (*C*(1´)), 161.4 (*C*(3)), 165.5 (*C*(5)), 197.6 (*C*OCH$_3$); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 239.0787; $C_{12}H_{12}N_2NaO_2$ requires M$^+$ 239.0791; LRMS *m*/*z* (ES$^+$) 217 ([M+H]$^+$, 10), 239 ([M+Na]$^+$, 56), 271 ([M+MeOH+Na]$^+$, 15), 455 ([2M+Na]$^+$, 100). HPLC: Retention time 10.10 min, purity 98.6%.

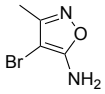
(*RS*)-1-(3-(3-Amino-5-methylisoxazol-4-yl)phenyl)ethanol **70c**



To a solution of **70b** (30 mg, 140 μmol, 1.0 eq) in anhydrous MeOH (1 mL) at 0 °C under a nitrogen atmosphere was added $NaBH_4$ (5 mg, 130 μmol, 0.9 eq). The mixture was warmed to rt and stirred for 2.5 h, after which time TLC analysis indicated complete consumption of **70b**. The reaction was quenched with sat. aq. NH_4Cl (10 mL), and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with H_2O (30 mL) and brine (30 mL), then dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 30 → 80% EtOAc/petroleum ether) gave **70c** as a colourless oil (23 mg, 76%); R_f 0.15 (50% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3330, 3212 (br), 2971, 2926 (C–H) (w), 1646 (s), 1613 (m), 1523 (m), 1462 (s), 1423 (m), 1248 (m), 1188 (m), 1076 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.52 (3H, d, *J* = 6.4 Hz, CH(OH)C*H*$_3$), 2.34 (3H, s, C(5)C*H*$_3$), 2.46 (1H, s, O*H*), 4.02 (2H, s, N*H*$_2$), 4.94 (1H, q, *J* = 6.4 Hz, C*H*(OH)), 7.22 (1H, ddd, *J* = 7.5, 1.4, 1.4 Hz, *Ar*), 7.33-7.38 (2H, m, *Ar*), 7.43 (1H, ddd, *J* = 7.5, 7.5, 1.4 Hz, C(5´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.8 (C(5)*C*H$_3$), 23.4 (CH(OH)*C*H$_3$), 70.0 (*C*H(OH)),

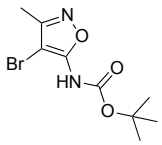
108.2 (*C*(4)), 124.8, 125.7, 127.6 (*C*(2´), *C*(4´), *C*(6´)), 129.3 (*C*(5´)), 129.8 (*C*(3´)), 147.0 (*C*(1´)), 161.6 (*C*(3)), 165.0 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 241.0945; $C_{12}H_{14}N_2NaO_2$ requires M^+ 241.0947; LRMS *m*/*z* (ES^+) 219 ($[M+H]^+$, 31), 241 ($[M+Na]^+$, 71), 257 ($[M+K]^+$, 33), 273 ($[M+Na+MeOH]^+$, 19), 459 ($[2M+Na]^+$, 100).

4-Bromo-3-methylisoxazol-5-amine **95**[234,329]



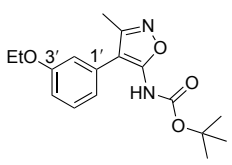
Following the procedure of Gibson *et al.*,[234] NBS (454 mg, 2.55 mmol, 1.0 eq) was added to a solution of 3-methylisoxazol-5-amine (**96**, 250 mg, 2.55 mmol, 1.0 eq) in $CHCl_3$ (12 mL). The mixture was stirred in the dark at 50 °C for 6 h, then concentrated *in vacuo*. The residues were resuspended in H_2O (10 mL) and extracted with Et_2O (3 × 10 mL). The combined organic layers were purified by silica gel column chromatography (eluent CH_2Cl_2) to give **95** as a pale yellow solid (379 mg, 84 %); R_f 0.27 (CH_2Cl_2); mp 58-60 °C ($CHCl_3$) (lit. 67-69 °C);[329] ν_{max} (solid)/cm^{-1} 3438 (N–H) (s), 3310, 3265, 3208, 3160 (C–H) (m), 1648 (s), 1455 (s), 1023 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 2.17 (3H, s, C*H*$_3$), 4.61 (2H, br s, N*H*$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (*C*H$_3$), 68.6 (*C*(4)), 160.0, 164.9 (*C*(3), *C*(5)); HRMS *m*/*z* (FI^+) found M^+ 175.9589, 177.9557; $C_4H_5{}^{79}BrN_2O$ requires M^+ 175.9585, $C_4H_5{}^{81}BrN_2O$ requires M^+ 177.9565. Anal. Calcd for $C_4H_5BrN_2O$: C, 27.1; H, 2.9; N, 15.8. Found: C, 27.3; H, 2.7; N, 15.8.

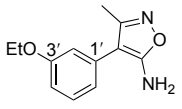
tert-Butyl (4-bromo-3-methylisoxazol-5-yl)carbamate **97**



To a solution of **95** (350 mg, 1.98 mmol, 1.0 eq) in anhydrous THF (10 mL) were added Boc_2O (907 mg, 4.16 mmol, 2.1 eq) and DMAP (12 mg, 99 μmol, 0.05 eq), and the reaction was heated under reflux for 19 h. The mixture was then concentrated *in vacuo* and redissolved in MeOH (10 mL). NaOH (2 M aq, 1.0 mL, 2.0 mmol, 1.0 eq) was added, and the mixture was stirred at rt for 19 h. The solution was neutralised with sat. aq. NH_4Cl (10 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (2 × 15 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 30 % Et_2O/petroleum ether) gave **97** as a colourless solid (391 mg, 71%); R_f 0.31 (30% Et_2O/petroleum ether); mp 74-75 °C (EtOAc); ν_{max} (solid)/cm^{-1} 3213 (N–H) (br), 2981, 2935 (C–H), 1720 (C=O) (s), 1636 (s), 1369 (m), 1232 (m), 1155 (s); ^1H NMR (400 MHz, $CDCl_3$) 1.53 (9H, s, C(CH_3)$_3$), 2.26 (3H, s, C(3)CH_3), 6.74 (1H, br s, N*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.0 (C(3)*C*H_3), 28.0 (C(*C*H_3)$_3$), 81.6 (*C*$(CH_3)_3$), 83.2 (*C*(4)), 149.9 (*C*=O), 158.0, 160.3 (*C*(3), *C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 299.0002, 300.9983; $C_9H_{13}{}^{79}BrN_2NaO_3$ requires M^+ 299.0002, $C_9H_{13}{}^{81}BrN_2NaO_3$ requires M^+ 300.9982; LRMS *m*/*z* (ES^+) 299 ($[^{79}M+Na]^+$, 52), 301 ($[^{81}M+Na]^+$, 52), 575 ($[2^{79}M+Na]^+$, 55), 577 ($[^{79}M+^{81}M+Na]^+$, 100), 579 ($[2^{81}M+Na]^+$, 54). Anal. Calcd. for $C_9H_{13}BrN_2O_3$: C, 39.0; H, 4.7; N, 10.1. Found: C, 39.1; H, 4.7; N, 10.0.

tert-Butyl (4-(3-ethoxyphenyl)-3-methylisoxazol-5-yl)carbamate **98** and 4-(3-ethoxyphenyl)-3-methylisoxazol-5-amine **71a**





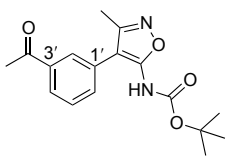
To a 2-5 mL microwave vial were added **90** (134 mg, 540 μmol, 1.25 eq), **97** (120 mg, 433 μmol, 1.0 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (18 mg, 22 μmol, 0.05 eq). The vial was sealed and purged with argon (5 × evacuate/fill). Degassed aq. Na_2CO_3 (2 M, 1.1 mL, 2.2 mmol, 5 eq) and degassed DME (2.2 mL) were added, and the reaction was heated at 100 °C for 24 h. The reaction was cooled, diluted with H_2O (30 mL) and extracted with EtOAc (4 × 40 mL). The combined organic layers were washed with H_2O (120 mL) and brine (120 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Repeated purification by silica gel column chromatography (gradient elution, 0 → 40% EtOAc/petroleum ether then 18 → 25% EtOAc/petroleum ether) gave **98** as a colourless oil (12 mg, 9%); R_f 0.36 (50% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1} 3247 (N–H) (br), 2980, 2933 (C–H) (m), 1711 (C=O) (m), 1646 (m), 1501 (m), 1369 (m), 1320 (m), 1238 (m), 1157 (s), 1050 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.40 (9H, s, C(C*H*$_3$)$_3$), 1.43 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.30 (3H, s, C(3)C*H*$_3$), 4.05 (2H, q, *J* = 7.0 Hz, C*H*$_2$$CH_3$), 6.64 (1H, s, N*H*), 6.83-6.85 (1H, m, C(2′)*H*), 6.86-6.91 (2H, m, C(4′)*H*, C(6′)*H*), 7.34 (1H, dd, *J* = 7.9, 7.9 Hz, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.4 (C(3)*C*H_3), 14.8 (CH_2*C*H_3), 27.9 (C(*C*H_3)$_3$), 63.5, (*C*H_2CH_3), 82.5 (*C*(CH_3)$_3$), 106.8 (*C*(4)), 113.7 (*C*(4′)), 115.0 (*C*(2′)), 120.8 (*C*(6′)), 130.0 (*C*(5′)), 130.6 (*C*(1′)), 150.8 (*C*=O), 156.5 (*C*(3)/*C*(5)), 159.3 (*C*(3′)), 159.8 (*C*(5)/*C*(3)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 341.1475; $C_{17}H_{22}N_2NaO_4$ requires M$^+$ 341.1472; LRMS *m*/*z* (ES$^+$) 319 ([M+H]$^+$, 19), 341 ([M+Na]$^+$, 20), 659 ([2M+Na]$^+$, 100).

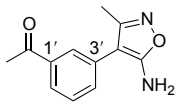
Further elution gave **71a** (7 mg, 7%).

A solution of **98** (10 mg, 31 μmol) in CH_2Cl_2 (0.5 mL) and TFA (0.25 mL) was stirred at rt for 1 h. Sat. aq. Na_2CO_3 (3 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 3 mL). The combined organic layers were washed with

H_2O (10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residues were combined with the deprotected product **71a** (7 mg) isolated above, and passed through a short plug of silica (eluent EtOAc). The combined residues were dissolved in MeCN (3 mL) and washed with hexane (3 × 3 mL), then concentrated *in vacuo* to give **71a** as a yellow solid (10 mg, 8% over two steps); R_f 0.65 (50% EtOAc/40-60 °C petroleum ether); mp 124-127 °C ($CHCl_3$); ν_{max} (solid)/cm^{-1}: 3438, 3318, 3177 (N–H) (br), 2980, 2930 (C–H) (w), 1639 (s), 1609 (s), 1578 (m), 1505 (m), 1439 (m), 1300 (m), 1213 (m), 1046 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.33 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.25 (3H, s, C(3)CH_3), 4.06 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 4.55 (2H, s, NH_2), 6.80-6.85 (2H, m, C(2′)*H*, C(4′)*H*), 6.85-6.88 (1H, m, C(6′)*H*), 7.31-7.36 (1H, m, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.2 (C(3)CH_3), 14.8 (CH_2CH_3), 63.5 (CH_2CH_3), 95.1 (*C*(4)), 112.4, 114.5 (*C*(2′), C(4′)), 120.3 (*C*(6′)), 130.1 (*C*(5′)), 132.2 (*C*(1′)), 159.5 (*C*(3′)), 159.7 (*C*(3)), 164.6 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 241.0941; $C_{12}H_{14}N_2NaO_2$ requires M^+ 241.0947; LRMS *m*/*z* (ES^+) 219 ($[M+H]^+$, 35), 241 ($[M+Na]^+$, 100), 273 ($[M+Na+MeOH]^+$, 33), 459 ($[2M+Na]^+$, 63). HPLC: Retention time 11.44 min, purity 95.0%

tert-Butyl (4-(3-acetylphenyl)-3-methylisoxazol-5-yl)carbamate **99** and 1-(3-(5-amino-3-methylisoxazol-4-yl)phenyl)ethanone **71b**





To a 2-5 mL microwave vial were added **91** (133 mg, 541 µmol, 1.25 eq), **97** (120 mg, 433 µmol, 1.0 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (18 mg, 22 µmol, 0.05 eq). The vial was sealed and purged with argon (5 × evacuate/fill). Degassed aq.

Na_2CO_3 (2 M, 1.1 mL, 2.2 mmol, 5 eq) and degassed DME (2.2 mL) were added, and the reaction was heated at 100 °C for 23 h. The reaction was cooled, diluted with H_2O (30 mL) and extracted with EtOAc (4 × 40 mL). The combined organic layers were washed with H_2O (120 mL) and brine (120 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 7 → 50% EtOAc/petroleum ether) gave **99** as a pale yellow oil (16 mg, 12%); R_f 0.41 (50% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1} 3248 (N–H) (br), 2980, 2932 (C–H) (w), 1730 (C=O) (s), 1684 (C=O) (s), 1645 (m), 1513 (m), 1496 (m), 1368 (m), 1244 (s), 1154 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.36 (9H, s, C(C*H*$_3$)$_3$), 2.30 (3H, s, C(3)C*H*$_3$), 2.62 (3H, s, COC*H*$_3$), 6.82 (1H, s, N*H*), 7.51-7.57 (2H, m, C(5´)*H*, C(6´)*H*), 7.91-7.95 (2H, m, C(2´)*H*, C(4´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.5 (C(3)*C*H$_3$), 26.6 (CO*C*H$_3$), 27.8 (C(*C*H$_3$)$_3$), 82.7 (*C*(CH$_3$)$_3$), 107.0 (*C*(4)), 127.6 (*C*(4´)), 128.3 (*C*(2´)), 129.1 (*C*(5´)), 130.2 (*C*(1´)), 133.1 (*C*(6´)), 136.7 (*C*(3´)), 150.7 (*C*O^{t}Bu), 156.8, 159.9 (*C*(3), *C*(5)), 197.6 (*C*OCH$_3$); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 339.1310; $C_{17}H_{20}N_2NaO_4$ requires M$^+$ 339.1315; LRMS *m*/*z* (ES$^+$) 339 ([M+Na]$^+$, 20). 655 ([2M+Na]$^+$, 100).

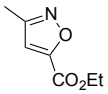
Further elution gave **71b** (11 mg).

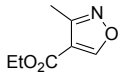
A solution of **99** (15 mg, 47 μmol) in CH_2Cl_2 (0.5 mL) and TFA (0.25 mL) was stirred at rt for 1 h. Sat. aq. $NaHCO_3$ (5 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with H_2O (15 mL) and brine (15 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residues were combined with the deprotected product **71b** (11 mg) isolated above and passed through a short plug of silica (eluent EtOAc). The combined residues were dissolved in MeCN (5 mL) and washed with hexane (4 × 5 mL), then concentrated *in vacuo* to give **71b** as a pale yellow solid (17 mg, 18%

over two steps); R_f 0.13 (30% EtOAc/40-60 °C petroleum ether); mp 155-157 °C (MeCN); ν_{max} (thin film)/cm^{-1} 3327, 3179 (N–H) (br), 2924, 2852 (C–H) (w), 1678 (C=O) (s), 1642 (C=O) (s), 1610 (m), 1509 (m), 1243 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.25 (3H, s, C(3)C*H*$_3$), 2.63 (3H, s, COC*H*$_3$), 4.62 (2H, s, N*H*$_2$), 7.50 (1H, ddd, *J* = 7.6, 1.5, 1.5 Hz, C(4´)*H*), 7.54 (1H, dd, *J* = 7.6, 7.6 Hz, C(5´)*H*), 7.86 (1H, ddd, *J* = 7.6, 1.5, 1.5 Hz, C(6´)*H*), 7.89 (1H, dd, *J* = 1.5, 1.5 Hz, C(2´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.2 (C(3)*C*H$_3$), 26.7 (CO*C*H$_3$), 94.4 (*C*(4)), 126.7 (*C*(6´)), 127.6 (*C*(2´)), 129.4 (*C*(5´)), 131.6 (*C*(3´)), 132.7 (*C*(4´)), 137.9 (*C*(1´)), 159.6 (*C*(3)), 164.8 (*C*(5)), 197.8 (*C*O); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 239.0791; $C_{12}H_{12}N_2NaO_2$ requires M$^+$ 239.0791; LRMS *m*/*z* (ES$^+$) 217 ([M+H]$^+$, 12), 239 ([M+Na]$^+$, 45), 271 ([M+Na+MeOH]$^+$, 18), 455 ([2M+Na]$^+$, 100). HPLC: Retention time 10.09 min, purity 99.4%.

7.4.2 Aminomethyl-methyl- and hydroxymethyl-methylisoxazoles and derivatives

Ethyl 3-methylisoxazole-5-carboxylate **107**[308] and ethyl 3-methylisoxazole-4-carboxylate **108**





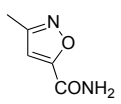
To a solution of acetaldoxime (1.0 mL, 1.0 g, 17 mmol) in DMF (18 mL) at 0 °C was added *N*-chlorosuccinimide (2.94 g, 22.0 mmol, 1.3 eq). The solution was warmed to rt and stirred for 3 h, then diluted with brine (120 mL) and extracted with Et_2O (5 × 100 mL). The combined organic layers were washed with H_2O (200 mL) and brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The residues were dissolved in CH_2Cl_2 (10 mL) and added to a suspension of ethyl propiolate (1.9 mL, 1.8 g, 19 mmol, 1.1 eq), $KHCO_3$ (4.40 g, 43.9 mmol, 2.6 eq) and H_2O (170 µL) in CH_2Cl_2 (70 mL). After 75 min, H_2O (13 mL) was

added and the mixture stirred vigorously to dissolve the $KHCO_3$. The phases were separated, and the organic layer was washed with H_2O (3 × 50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give 2.21 g of pale yellow oil containing a 3:1 ratio of **107** to **108**. Purification by silica gel column chromatography (gradient elution, 5 → 40% Et_2O/petroleum ether) gave **108** as a pale yellow oil (107 mg, 4%) and **107** as a pale yellow oil which crystallised slowly at 4 °C (1.08 g). Further purification of the mixed fractions by silica gel column chromatography (gradient elution, 5 → 12% Et_2O/petroleum ether) gave a further 290 mg of **107** as a pale yellow solid (overall 1.37 g, 52%).

Data for **107**:[308] R_f 0.14 (10% Et_2O/40-60 °C petroleum ether); mp 26–27 °C; (lit. 27–28 °C)[330]; ^{1}H NMR (400 MHz, $CDCl_3$) 1.40 (3H, t, *J* = 7.1 Hz, CH_2CH_3), 2.37 (3H, s, C(3)CH_3), 4.42 (2H, q, *J* = 7.1 Hz, CH_2CH_3), 6.78 (1H, s, C(4)*H*); LRMS *m*/*z* (ES^+) 156 ($[M+H]^+$, 45), 178 ($[M+Na]^+$, 100). These data are in good agreement with the literature values.[308]

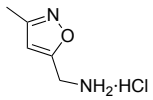
Data for **108**: R_f 0.21 (10% Et_2O/40-60 °C petroleum ether); ν_{max} (solid)/cm^{-1}: 2986, 2971, 2941 (C–H) (w), 1725 (C=O) (s), 1592 (m), 1405 (m), 1377 (m), 1295 (m), 1231 (m), 1131 (m), 1131 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.36 (3H, t, *J* = 7.1 Hz, CH_2CH_3), 2.98 (3H, s, C(3)CH_3), 4.33 (2H, q, *J* = 7.1 Hz, CH_2CH_3), 8.84 (1H, s, C(5)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)CH_3), 14.2 (CH_2CH_3), 60.9 (CH_2CH_3), 113.5 (*C*(4)), 158.8 (*C*(3)), 161.4 (*C*=O), 162.7 (*C*(5)); HRMS *m*/*z* (FI^+) found M^+ 155.0581; $C_7H_9NO_3$ requires M^+ 155.0582.

3-Methylisoxazole-5-carboxamide **106**



107 (120 mg, 773 μmol) was stirred with NH_3 in MeOH (2 M, 2 mL) at rt for 16 h, then concentrated *in vacuo* to give **106** as a colourless solid (90 mg, 92%); R_f 0.49 (EtOAc); mp 140 °C (dec); ν_{max} (solid)/cm^{-1}: 3396, 3172 (N–H) (s), 1660 (s) (C=O), 1627 (s), 1607 (s), 1450 (m), 1369 (s), 1237 (m), 1093 (m), 1024 (m); ^{1}H NMR (500 MHz, DMSO-d_6) 2.29 (3H, s, C(3)C*H*$_3$), 6.89 (1H, s, C(4)*H*), 7.89 (1H, s, N*H*$_A$), 8.26 (1H, s, N*H*$_B$); ^{13}C NMR (126 MHz, DMSO-d_6) 11.0 (C(3)*C*H$_3$), 107.1 (*C*(4)), 157.3 (*C*=O), 160.4 (*C*(3)), 163.3 (*C*(5)); HRMS *m*/*z* (FI^+) found $[M+Na]^+$ 126.0426; $C_5H_6N_2O_2$ requires M^+ 126.0429. Anal. Calcd for $C_5H_6N_2O_2$: C, 47.6; H, 4.8; N, 22.2. Found: C, 47.5; H, 4.8; N, 22.2.

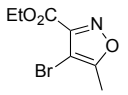
(3-Methylisoxazol-5-yl)methanamine hydrochloride **109**



Following the procedure of Dannhardt *et al*.,[236] to a solution of **106** (81 mg, 640 μmol, 1.0 eq) in anhydrous THF (5 mL) in a 10–20 mL microwave vial under a nitrogen atmosphere was added $BH_3{\cdot}SMe_2$ solution (2 M in THF, 1.3 mL, 2.6 mmol, 4.0 eq). The solution was heated under reflux for 6 h, then cooled to 0 °C. The vial was opened and MeOH (2 mL) was carefully added dropwise with vigorous stirring. The solution was then stirred overnight and concentrated *in vacuo*. Aq. HCl (1 M, 2 mL) was added, the vial was sealed and the reaction was heated under reflux for 1 h. The solution was cooled to 0 °C and basified (pH 10) with aq. NaOH (1 M) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated *in vacuo* at rt. The resultant yellow oil was dissolved in Et_2O (2 mL) and HCl (2 M in Et_2O, 0.5 mL) was added. The solid was collected by filtration and dried *in vacuo* to give **109** as an amorphous colourless solid (39 mg, 41%); mp 175 °C

(dec.); ν_{max} (solid)/cm^{-1}: 2888 (N–H) (br), 2663 (w), 2583 (w), 1618 (m), 1585 (m), 1515 (s), 1417 (m), 1252 (m), 1143 (m), 1082 (m), 1009 (m); ^{1}H NMR (500 MHz, DMSO-d_6) 2.53 (3H, s, C(3)CH_3), 4.20 (2H, s, CH_2NH$_3$), 6.53 (1H, s, C(4)*H*), 8.87 (3H, br s, NH_3); ^{13}C NMR (126 MHz, DMSO-d_6) 1.09 (C(3)*C*H$_3$), 33.8 (*C*H$_2$), 105.1 (*C*(4)), 159.8, 165.1 (*C*(3), *C*(5)); HRMS (FI$^+$) found M$^+$ 1133.0709; $C_5H_8N_2O$ requires M$^+$ 113.0709. Anal. Calcd for $C_5H_9ClN_2O$: C, 40.4; H, 6.1; N, 18.9. Found: C, 40.6; H, 6.2; N, 18.7.

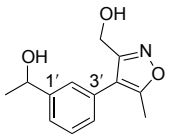
Ethyl 4-bromo-5-methylisoxazole-3-carboxylate **111**



Following the procedure of Li *et al.*,[238] ethyl 5-methylisoxazole-3-carboxylate (**110**, 500 mg, 3.22 mmol, 1.0 eq), NBS (688 mg, 3.87 mmol, 1.2 eq) and 5% fuming nitric acid in acetic acid (12.5 mL) were heated with microwave irradiation in a sealed 10–20 mL microwave vial at 150 °C for 12 min, after which time TLC analysis indicated complete consumption of **110**. The procedure was repeated three further times, and the reaction mixtures were combined. The acidic solvent was removed by azeotropic distillation with toluene, and the residue was filtered through Celite® (eluent Et_2O). Purification by silica gel column chromatography (gradient elution, 2 → 20% Et_2O/petroleum ether) gave **111** as a pale yellow oil (2.19 g, 73%); R_f 0.30 (10% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 2986, 2971, 2941 (C–H) (w), 1737 (C=O) (s), 1458 (m), 1264 (m), 1206 (s), 1089 (s), 1025 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.43 (3H, t, *J* = 7.1 Hz, CH_2CH_3), 2.50 (3H, s, C(5)CH_3), 4.47 (2H, q, *J* = 7.1 Hz, CH_2CH$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) 11.5 (C(5)*C*H$_3$), 14.0 (CH_2CH_3), 62.4 (*C*H$_2$CH$_3$), 91.6 (*C*(4)), 153.5 (*C*(3)), 158.9 (*C*=O), 169.8 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$

255.9580, 257.9562; $C_7H_8{}^{79}BrNNaO_3$ requires M^+ 255.9580, $C_7H_8{}^{81}BrNNaO_3$ requires M^+ 257.9560; LRMS *m*/*z* (ES^+) 234 ($[^{79}M+H]^+$, 13), 236 ($[^{81}M+H]^+$, 14), 256 ($[^{79}M+Na]^+$, 99), 258 ($[^{81}M+Na]^+$, 100), 288 ($[^{79}M+Na+MeOH]^+$, 14), 290 ($[^{81}M+Na+MeOH]^+$, 14).

(*RS*)-1-(3-(3-(Hydroxymethyl)-5-methylisoxazol-4-yl)phenyl)ethanol **72c**

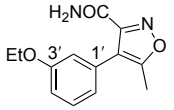


To a sealable tube were added **111** (758 mg, 3.24 mmol, 1.0 eq), **91** (884 mg, 3.56 mmol, 1.1 eq) and $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (75 mg, 92 µmol, 0.03 eq). The tube was sealed and purged with argon (5 × evacuate/fill). Degassed DME (48 mL) and degassed sat. aq. $NaHCO_3$ (9 mL) were added by syringe, and the mixture was heated at 100 °C for 2 h then at 110 °C for 7 h. The mixture was cooled and filtered through Celite® (eluent EtOAc) and concentrated *in vacuo*. The residue was redissolved in EtOAc (50 mL), washed with H_2O (3 × 50 mL) and brine (2 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (2%, 5% then 10 % EtOAc/40-60 °C petroleum ether) gave an impure sample of presumed ethyl 4-(3-ethoxyphenyl)-5-methylisoxazole-3-carboxylate (**112**) as a pale yellow oil (228 mg) which was used without further purification; *m*/*z* (ES^+) 276 ($[M+H]^+$, 62), 298 ($[M+Na]^+$, 100).

To a solution of impure presumed ethyl 4-(3-acetylphenyl)-5-methylisoxazole-3-carboxylate (**112**, 94 mg) in anhydrous EtOH (2 mL) was added $NaBH_4$ (25 mg, 660 µmol)) under a nitrogen atmosphere. The reaction was stirred for 8 h, then further $NaBH_4$ (10 mg, 260 µmol) was added. After a further 12 h, TLC analysis indicated complete consumption of starting material

112, and the reaction was quenched with aq. NH_4Cl (10% w/v, 5 mL) and concentrated *in vacuo* to remove EtOH. The mixture was extracted with EtOAc (10 mL), and the organic layer was washed with H_2O (2 × 10 mL), brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 30 → 70% EtOAc/petroleum ether) gave **72c** as a colourless oil (59 mg, 8% from **111**); R_f 0.38 (75% EtOAc/40-60 °C petroleum ether); ν_{max} (solid)/cm^{-1}: 3346 (O–H) (br), 2972, 2928, 2877 (C–H) (w), 1627 (m), 1481 (m), 1241 (m), 1186 (m), 1076 (s), 1054 (s), 1012 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.50 (3H, d, *J* = 6.4 Hz, CH(OH)C*H*$_3$), 2.43 (3H, s, C(5)C*H*$_3$), 3.20 (2H, br s, O*H*), 4.62 (2H, s, C*H*$_2$OH), 4.90 (1H, q, *J* = 6.4 Hz, C*H*(OH)CH_3), 7.22 (1H, ddd, *J* = 7.6, 1.6, 1.0 Hz, C(4′)*H*), 7.30-7.34 (1H, m, C(6′)*H*), 7.36-7.42 (2H, m, C(2′)*H*, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.6 (C(5)*C*H_3), 25.1 (CH(OH)*C*H_3), 55.5 (*C*H_2OH), 69.9 (*C*H(OH)CH_3), 116.1 (*C*(4)), 125.0 (*C*(6′)), 126.1 (*C*(2′)), 127.9 (*C*(4′)), 128.9 (*C*(5′)), 129.7 (*C*(3′)), 146.6 (*C*(1′)), 161.4 (*C*(3)), 166.1 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 256.0944; $C_{13}H_{15}NNaO_3$ requires M^+ 256.0944; LRMS *m*/*z* (ES^+) 234 ($[M+H]^+$, 21), 256 ($[M+Na]^+$, 100). HPLC: Retention time 9.31 min, purity 96.3%.

4-(3-Ethoxyphenyl)-5-methylisoxazole-3-carboxamide **73**

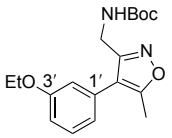


To a sealable tube were added **111** (758 mg, 3.24 mmol, 1.0 eq), **90** (884 mg, 3.56 mmol, 1.1 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (75 mg, 92 μmol, 0.03 eq). The tube was sealed and purged with argon (5 × evacuate/fill). Degassed DME (48 mL) and degassed sat. aq. $NaHCO_3$ (9 mL) were added by syringe, and the mixture was heated at 100 °C for 2 h then at 110 °C for 7 h. The mixture was

cooled and filtered through Celite® (eluent EtOAc) and concentrated *in vacuo*. The residue was redissolved in EtOAc (50 mL), washed with H_2O (3 × 50 mL) and brine (2 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (2%, 5% then 10 % EtOAc/40-60 °C petroleum ether) gave an impure sample of presumed ethyl 4-(3-ethoxyphenyl)-5-methylisoxazole-3-carboxylate (**113**) as a pale yellow oil (228 mg) which was used without further purification; LRMS *m*/*z* (ES^+) 276 ($[M+H]^+$, 62), 298 ($[M+Na]^+$, 100).

A solution of impure presumed ethyl 4-(3-ethoxyphenyl)-5-methylisoxazole-3-carboxylate (**113**) was stirred in 7 M NH_3 in MeOH at rt for 15 h, then concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 12 → 70% EtOAc/40-60 °C petroleum ether) gave **73** as a colourless solid (147 mg, 20% over 2 steps); mp 102-103 °C ($CHCl_3$); R_f 0.34 (50% EtOAc/40-60 °C petroleum ether); ν_{max} (solid)/cm^{-1}: 3460, 3330, 3183 (N–H) (br), 2980, 2932 (C–H) (w), 1693 (C=O) (s), 1599 (m), 1579 (m), 1450 (m), 1258 (s), 1203 (m); ^1H NMR (500 MHz, $CDCl_3$) 1.42 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.46 (3H, s, C(5)CH_3), 4.06 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 5.72 (1H, s, NH), 6.60 (1H, s, NH), 6.89-6.94 (3H, m, C(2´)H, C(4´)H, C(6´)H), 7.29-7.34 (1H, m, C(5´)H); ^{13}C NMR (126 MHz, $CDCl_3$) 11.5 (C(5)CH_3), 14.8 (CH_2CH_3), 63.4 (CH_2CH_3), 114.2, 116.3 (C(2´), C(4´)), 117.0 (C(4)), 122.1 (C(6´)), 129.3 (C(5´)), 129.8 (C(1´)), 155.1 (C(3)), 158.8 (C(3´)), 160.9 (C=O), 168.3 (C(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 269.0897; $C_{13}H_{14}N_2NaO_3$ requires M^+ 269.0897; LRMS *m*/*z* (ES^+) 247 ($[M+H]^+$, 37), 269 ($[M+Na]^+$, 100). Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.4; H, 5.7; N, 11.4. Found: C, 63.4; H, 5.7; N, 11.2. HPLC: Retention time 11.10 min, purity 98.5%.

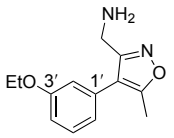
tert-Butyl ((4-(3-ethoxyphenyl)-5-methylisoxazol-3-yl)methyl)carbamate **114**



To a solution of **73** (30 mg, 120 μmol, 1.0 eq) in anhydrous THF (0.3 mL) in a 0.5-2 mL microwave vial under a nitrogen atmosphere was added BH_3·THF solution (1 M in THF, 0.37 mL, 0.37 mmol, 3.0 eq). The reaction was heated under reflux for 5 h, then further BH_3·THF solution (0.37 mL, 0.37 mmol, 3.0 eq) was added. The mixture was heated under reflux for a further 9 h, after which time TLC analysis indicated complete consumption of amide. MeOH (2 mL) was carefully added dropwise at 0 °C with vigorous stirring. The solution was warmed to rt and stirred until evolution of gas had stopped, then concentrated *in vacuo*. Amine-containing compounds were isolated using an Isolute SCX-2 column (load in CH_2Cl_2, wash with 25% MeOH/CH_2Cl_2, elute with 2 M NH_3 in MeOH, then concentrate *in vacuo*). The resulting residues were dissolved in anhydrous MeCN (2 mL), and Boc_2O (53 mg, 240 μmol, 2.0 eq) was added. After 18 h, the reaction was concentrated *in vacuo*, redissolved in EtOAc (10 mL), washed with H_2O (10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 40% Et_2O/40-60 °C petroleum ether) gave **114** as a colourless oil (11 mg, 27%); R_f 0.20 (30% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3340 (N–H) (br), 2978, 2931 (C–H) (m), 1712 (C=O) (s), 1511 (m), 1248 (s), 1164 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.42 (9H, s, C(C*H*$_3$)$_3$) overlapping 1.43 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.43 (3H, s, C(5)C*H*$_3$), 4.06 (2H, q, *J* = 7.0 Hz, C*H*$_2$CH$_3$), 4.41 (2H, d, *J* = 5.3 Hz, C*H*$_2$NH), 5.09 (1H, s, N*H*), 6.77 (1H, dd, *J* = 2.2, 1.6 Hz, C(2′)*H*), 6.81 (1H, ddd, *J* = 7.8, 1.6, 1.0 Hz, C(4′)*H*/C(6′)*H*), 6.89 (1H, dd, *J* = 8.1, 2.2 Hz,

C(6′)*H*/C(4′)*H*), 7.33 (1H, dd, *J* = 8.1, 7.8 Hz, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.5 (C(5)*C*H_3), 14.8 (CH_2*C*H_3), 28.3 (C(*C*H_3)$_3$), 36.7 (*C*H_2NH), 63.5 (O*C*H_2), 79.7 (*C*(CH$_3$)$_3$), 113.7 (*C*(4′)/*C*(6′)), 115.3 (*C*(2′)), 115.6 (*C*(4)), 121.2 (*C*(6′)/*C*(4′)), 129.9 (*C*(5′)), 130.5 (*C*(1′)), 155.5 (*C*=O), 159.2 (*C*(3′), *C*(3)), 166.2 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 355.1630; $C_{18}H_{24}N_2NaO_4$ requires M$^+$ 355.1628; LRMS *m*/*z* (ES$^+$) 355 ([M+Na]$^+$, 100).

(4-(3-Ethoxyphenyl)-5-methylisoxazol-3-yl)methanamine **74a**

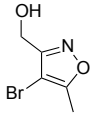


Method A: A solution of **114** (16 mg, 48 μmol) in HCl (1 M in Et_2O, 1 mL) was stirred at rt for 22 h, then concentrated under a stream of nitrogen. The residues were dissolved in aq. HCl (1 M, 3 mL) and washed with CH_2Cl_2 (2 × 3 mL). The aqueous layer was basified with 25% aq. NH_4OH (2 mL), saturated with NaCl and extracted with CH_2Cl_2 (3 × 5 ml). The combined organic layers were washed with brine (15 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The resulting oil was loaded onto a small plug of silica gel as a solution in CH_2Cl_2, followed by washing with hexane then eluting with 5% MeOH and 1% NEt_3 in CH_2Cl_2 to give **74a** as a pale yellow oil (11 mg, 99%); R_f 0.26 (5% MeOH, 1% NEt_3 in CH_2Cl_2); ν_{max} (thin film)/cm^{-1}: 3384 (N–H) (br), 2980, 2928 (C–H) (m), 1628 (m), 1600 (m), 1578 (s), 1478 (m), 1297 (m), 1246 (s), 1206 (s), 1040 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.44 (3H, t, *J* = 7.0 Hz, CH_2C*H*$_3$), 1.63 (3.7 H, s, N*H*$_2$ and residual H_2O), 2.42 (3H, s, C(5)C*H*$_3$), 3.94 (2H, s, C*H*$_2$NH$_2$), 4.06 (2H, q, *J* = 7.0 Hz, C*H*$_2$CH$_3$), 6.82-6.87 (2H, m, C(2′)*H*, C(6′)*H*/C(4′)*H*), 6.90 (1H, ddd, *J* = 8.3, 2.5, 0.7 Hz, C(4′)*H*/C(6′)*H*), 7.35 (1H, dd, *J* = 8.3, 7.6 Hz, C(5′)*H*); ^{13}C NMR

(126 MHz, $CDCl_3$) 11.5 (C(5)C*H*$_3$), 14.8 (CH_2CH_3), 37.6 (CH_2NH_2), 63.5 (CH_2CH_3), 113.5 (*C*(4´)/*C*(6´)), 115.4 (*C*(4)), 115.4 (*C*(2´)), 121.2 (*C*(6´)/*C*(4´)), 130.0 (*C*(5´)), 131.2 (*C*(1´)), 159.3 (*C*(3´)), 163.1 (*C*(3)), 165.9 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+H]^+$ 233.1283; $C_{13}H_{17}N_2O_2$ requires M^+ 233.1285; LRMS *m*/*z* (ES^+) 233 ($[M+H]^+$, 100), 255 ($[M+Na]^+$, 12). HPLC: Retention time 9.37 min, purity 97.8%.

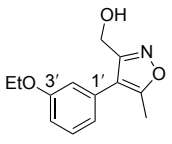
Method B: Following the procedure of Pei and Wickham,[239] to a solution of **118** (35 mg, 140 µmol, 1.0 eq) in ^{i}PrOH (0.7 mL) was added NEt_3 (38 µL, 28 mg, 270 µmol, 2.0 eq), 1,3-propanedithiol (0.1 M in ^{i}PrOH, 140 µL, 14 µmol, 0.1 eq) and $NaBH_4$, (8 mg, 200 µmol, 1.5 eq). After 11 h, TLC analysis indicated complete consumption of **118**, and the reaction was dried under a stream of nitrogen. The residues were diluted with aq. citric acid (10% w/v, 10 mL) and washed with Et_2O (2 × 10 mL). The aqueous layer was basified with NaOH solution (2 M, 10 mL), saturated with NaCl, filtered, and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (2 × 50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residues were then taken up in aq. HCl (1 M, 5 mL) and washed with CH_2Cl_2, then basified with NaOH solution (2 M, 5 mL), saturated with NaCl, filtered, and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*, then taken up in MeCN (5 mL) and washed with hexane (4 × 5 mL) and concentrated *in vacuo* to give **74a** as a pale brown oil (13 mg, 41%). The data are identical to those reported above.

(4-Bromo-5-methylisoxazol-3-yl)methanol **115**



To a solution of **111** (1.54 g, 6.58 mmol, 1.0 eq) in anhydrous EtOH (15 mL) at 0 °C under a nitrogen atmosphere was added $NaBH_4$ (430 mg, 11.4 mmol, 1.7 eq). The solution was warmed to rt over 4.5 h, after which time TLC analysis indicated complete consumption of **111**. The mixture was quenched with H_2O (15 mL) and aq. HCl (1 M, 15 mL), then concentrated *in vacuo* to remove EtOH. The residues were extracted with CH_2Cl_2 (4 × 30 mL), and the combined organic layers were washed with H_2O (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **115** as a colourless solid (1.08 g, 85%); R_f 0.14 (20% EtOAc/40-60 °C petroleum ether); mp 60–61 °C (CH_2Cl_2); ν_{max} (solid)/cm^{-1}: 3348 (O–H) (br), 2968, 2936 (C–H) (w), 1601 (m), 1483 (m), 1426 (m), 1099 (s), 1046 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.42 (3H, s, C*H*$_3$), 2.65 (1H, t, *J* = 6.5 Hz, O*H*), 4.71 (2H, d, *J* = 6.5 Hz, C*H*$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) 11.2 (C(5)*C*H$_3$), 56.2 (*C*H$_2$), 90.5 (*C*(4)), 161.4 (*C*(3)), 167.5 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 213.9475, 215.9456; $C_5H_6{}^{79}BrNNaO_2$ requires M^+ 213.9474, $C_5H_6{}^{81}BrNNaO_2$ requires M^+ 215.9454; LRMS *m*/*z* (ES^+) 214 ($[^{79}M+Na]^+$, 95), 216 ($[^{81}M+Na]^+$, 100), 246 ($[^{79}M+Na+MeOH]^+$, 29), 248 ($[^{81}M+Na+MeOH]^+$, 29). Anal. Calcd for $C_5H_6BrNO_2$: C, 31.3; H, 3.2; N, 7.3. Found: C, 31.4; H, 3.2; N, 7.2.

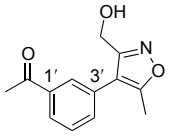
(4-(3-Ethoxyphenyl)-5-methylisoxazol-3-yl)methanol **72a**



To a 2-5 mL microwave vial were added **90** (131 mg, 528 μmol, 1.35 eq), **115** (75 mg, 391 μmol, 1.0 eq) and $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (16 mg, 20 μmol, 0.05 eq). The vial was sealed and purged with argon (5 × evacuate/fill). Degassed aq.

Na_2CO_3 (2 M, 1.0 mL, 2.0 mmol, 5 eq) and degassed DME (2.0 mL) were added, and the reaction was heated at 100 °C for 15 h. The reaction was cooled, and filtered through a pad of Celite® (eluent EtOAc), then concentrated *in vacuo*. The residues were diluted with H_2O (10 mL) and extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with H_2O (40 mL) and brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 10 → 50% EtOAc/petroleum ether) gave **72a** as a pale yellow solid (42 mg, 46%); R_f 0.31 (30% EtOAc/40-60 °C petroleum ether); mp 72–73 °C (MeCN); ν_{max} (solid)/cm^{-1}: 3383 (O–H) (br), 2980, 2931, 2881 (C–H) (w), 1578 (m), 1478 (m), 1301 (m), 1245 (s), 1204 (s), 1042 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.44 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.46 (3H, s, C(5)C*H*$_3$), 2.59 (1H, t, *J* = 6.3 Hz, O*H*), 4.06 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 4.72 (2H, d, *J* = 6.3 Hz, CH_2OH), 6.88–6.94 (3H, m, C(2′)*H*, C(4′)*H*, C(6′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.6 (C(5)*C*H_3), 14.8 (CH_2CH_3), 56.0 (CH_2OH), 63.5 (CH_2CH_3), 113.7 (*C*(4′)), 115.3 (*C*(2′)), 115.8 (*C*(4)), 121.2 (*C*(6′)), 129.9 (*C*(5′)), 130.8 (*C*(1′)), 159.3 (*C*(3′)), 161.4 (*C*(3)), 166.1 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 256.0951; $C_{13}H_{15}NNaO_3$ requires M^+ 256.0944; LRMS *m*/*z* (ES^+) 234 ($[M+H]^+$, 33), 256 ($[M+Na]^+$, 92), 288 ($[M+Na+MeOH]^+$, 48), 490 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.9; H, 6.5; N, 6.0. Found: C, 67.0; H, 6.4; N, 6.0.

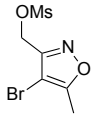
1-(3-(3-(Hydroxymethyl)-5-methylisoxazol-4-yl)phenyl)ethanone **72b**

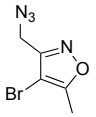


To a 2-5 mL microwave vial were added **91** (121 mg, 492 μmol, 1.25 eq), **115** (75 mg, 390 μmol, 1.0 eq) and $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (16 mg, 20 μmol, 0.05 eq).

The vial was sealed and purged with argon (5 × evacuate/fill). Degassed aq. Na_2CO_3 (2 M, 1.0 mL, 2.0 mmol, 5 eq) and degassed DME (2.0 mL) were added, and the reaction was heated at 100 °C for 6 h. The reaction was cooled and filtered through a plug of Celite® (eluent EtOAc) then concentrated *in vacuo*. The residues were diluted with H_2O (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 50% EtOAc/petroleum ether) gave **72b** as a pale yellow solid (41 mg, 45%); R_f 0.23 (40% EtOAc/40-60 °C petroleum ether); mp 90–92 °C (MeCN); ν_{max} (solid)/cm^{-1}: 3394 (O–H) (br), 2929, 2880 (C–H) (w); 1682 (C=O) (s), 1359 (m), 1259 (m), 1225 (s), 1051 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 2.48 (3H, s, C(5)C*H*$_3$) overlapping 2.49 (1H, t, *J* = 6.0 Hz, O*H*), 2.64 (3H, s, COC*H*$_3$), 4.72 (2H, d, *J* = 6.0 Hz, C*H*$_2$OH), 7.57 (1H, dd, *J* = 7.6, 7.6 Hz, C(5´)*H*), 7.61 (1H, ddd, *J* = 7.6, 1.5, 1.5 Hz, C(4´)*H*), 7.96 (1H, ddd, *J* = 7.6, 1.5, 1.5 Hz, C(6´)*H*), 8.01 (1H, dd, *J* = 1.5, 1.5 Hz, C(2´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.6 (C(5)*C*H$_3$), 26.7 (CO*C*H$_3$), 55.9 (*C*H$_2$OH), 115.3 (*C*(4)), 127.7 (*C*(6´)), 128.9 (*C*(2´)), 129.2 (*C*(5´)), 130.3 (*C*(3´)), 133.6 (*C*(4´)), 137.7 (*C*(1´)), 161.2 (*C*(3)), 166.5 (*C*(5)), 197.8 (*C*=O); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 254.0784; $C_{13}H_{13}NNaO_3$ requires M$^+$ 254.0788; LRMS *m*/*z* (ES$^+$) 232 ([M+H]$^+$, 47), 254 ([M+Na]$^+$, 89), 286 ([M+Na+MeOH]$^+$, 33), 485 ([2M+Na]$^+$, 100). Anal. Calcd for $C_{13}H_{13}NO_3$: C, 67.5; H, 5.6; N, 6.1. Found: C 67.6; H, 5.6; N, 6.0.

(4-Bromo-5-methylisoxazol-3-yl)methyl methanesulfonate **116** and 3-(azidomethyl)-4-bromo-5-methylisoxazole **117**



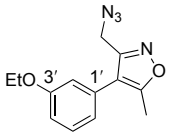


To a solution of **115** (603 mg, 3.14 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (15 mL) at 0°C under a nitrogen atmosphere were added NEt_3 (660 µL, 480 mg, 4.7 mmol, 1.5 eq) and MsCl (300 µL, 440 mg, 3.8 mmol, 1.2 eq) dropwise. The solution was stirred at 0 °C for 1 h, after which time TLC analysis indicated complete consumption of **115**, then quenched with aq. HCl (1 M, 20 mL). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (20 mL). The combined organic layers were washed with brine (40 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give **116** as a yellow oil (848 mg) that was used immediately in the next step without further purification; R_f 0.53 (40% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3028, 2940 (C–H) (w), 1356 (S=O) (s), 1175 (s), 1100 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 2.47 (3H, s, C(5)CH_3), 3.10 (3H, s, SO_2CH_3), 5.27 (2H, s, CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) 11.4 (C(5)CH$_3$), 38.2 (SO_2CH_3), 60.5 (CH_2), 91.5 (C(4)), 156.4 (C(3)), 168.7 (C(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 291.9241, 293.9223; $C_6H_8{}^{79}BrNNaO_4S$ requires M^+ 291.9250; $C_6H_8{}^{81}BrNNaO_4S$ requires M^+ 293.9229; LRMS *m*/*z* (ES^+) 270 ($[^{79}M+H]^+$, 14), 272 ($[^{81}M+H]^+$, 15), 292 ($[^{70}M+Na]^+$, 84), 294 ($[^{81}M+Na]^+$, 100), 561 ($[2^{79}M+Na]^+$, 46), 563 ($[^{79}M+^{81}M+Na]^+$, 87), 565 ($[2^{81}M+Na]^+$, 50).

To a solution of **116** (848 mg) in anhydrous DMF (13 mL) under a nitrogen atmosphere was added NaN_3 (225 mg, 3.46 mmol, 1.1 eq with respect to alcohol **115**) and the reaction was stirred for 15 h, after which time TLC analysis indicated complete consumption of **116**, then concentrated *in vacuo*. The residues were diluted with H_2O (40 mL) and extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with H_2O (40 mL) and brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **117** as a pale yellow oil (619 mg, 91% over two steps); R_f 0.72 (40% EtOAc/40-60 °C petroleum ether); ν_{max} (thin

film)/cm^{-1}: 2929 (C–H) (w), 2100 (N_3) (s), 1607 (m), 1277 (m), 1250 (m), 1095 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.45 (3H, s, C(5)C*H*$_3$), 4.38 (2H, s, C*H*$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) 11.4 (C(5)*C*H$_3$), 44.6 (*C*H$_2$), 91.2 (*C*(4)), 157.8 (*C*(3)), 168.1 (*C*(5)); HRMS *m*/*z* (FI^+) found 215.9645, 217.9620; $C_5H_5{}^{79}BrN_4O$ requires M^+ 215.9647, $C_5H_5{}^{81}BrN_4O$ requires M^+ 217.9626. Anal. Calcd for $C_5H_5BrN_4O$: C, 27.7; H, 2.3; N, 25.8. Found: C, 27.6; H, 2.2; N, 25.7.

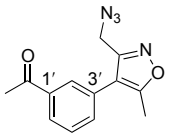
3-(Azidomethyl)-4-(3-ethoxyphenyl)-5-methylisoxazole **118**



To a 10–20 mL microwave vial were added **117** (150 mg, 691 μmol, 1.0 eq), **90** (238 mg, 959 μmol, 1.4 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (28 mg, 34 μmol, 0.05 eq). The vial was sealed and purged with argon (5 × evacuate/fill). Degassed DME (3.5 mL) and degassed aq. Na_2CO_3 (2 M, 1.7 mL, 5 eq) were added by syringe, and the mixture was heated at 100 °C for 4 h. The mixture was then cooled and filtered through Celite® (eluent CH_2Cl_2). Purification by silica gel chromatography (gradient elution, 0 → 20% Et_2O/petroleum ether) gave **118** as a pale yellow oil (89 mg, 50%); R_f 0.61 (50% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 2981, 2931 (C–H) (w), 2100 (N_3) (s), 1578 (m), 1477 (m), 1300 (m), 1245 (s), 1205 (m), 1042 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.45 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.48 (3H, s, C(5)CH_3), 4.07 (2H, q, CH_2CH_3), 4.36 (2H, s, CH_2N_3), 6.84–6.90 (2H, m, C(2′)H, C(6′)H), 6.90–6.95 (1H, m, C(4′)H), 7.36 (1H, dd, *J* = 8.0, 8.0 Hz, C(5′)H); ^{13}C NMR (126 MHz, $CDCl_3$) 11.7 (C(5)CH_3), 14.8 (CH_2CH_3), 44.5 (CH_2N_3), 113.9 (C(4′)), 115.3 (C(2′)), 116.3 (C(4)), 121.2 (C(6′)), 130.1 (C(5′)), 130.3 (C(1′)), 157.4 (C(3)), 159.3 (C(3′)), 166.8 (C(5)); HRMS m/z

(ES$^+$) found [M+Na]$^+$ 281.1015; $C_{13}H_{14}N_4NaO_2$ requires M$^+$ 281.1009; LRMS m/z (ES$^+$) 259 ([M+H]$^+$, 16), 281 ([M+Na]$^+$, 76), 313 ([M+Na+MeOH]$^+$, 57), 539 ([2M+Na]$^+$, 100).

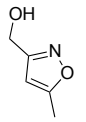
1-(3-(3-(Azidomethyl)-5-methylisoxazol-4-yl)phenyl)ethanone **119**



To a 10–20 mL microwave vial were added **117** (150 mg, 691 μmol, 1.0 eq), **91** (213 mg, 864 μmol, 1.25 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (28 mg, 34 μmol, 0.05 eq). The vial was sealed and purged with argon (5 × evacuate/fill). Degassed DME (3.5 mL) and degassed aq. Na_2CO_3 (2 M, 1.7 mL, 5 eq) were added by syringe, and the mixture was heated at 100 °C for 4 h. The mixture was then cooled and filtered through Celite® (eluent CH_2Cl_2). Repeated purification by silica gel chromatography (gradient elution, 0 → 40% Et_2O/petroleum ether then 10 → 15% Et_2O/petroleum ether then 10 → 13% Et_2O/petroleum ether) gave **119** as a pale yellow oil (57 mg, 32%); R_f 0.61 (50% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3004, 2929 (C–H) (w), 2103 (N_3) (s), 1686 (C=O) (s), 1358 (m), 1256 (m), 1224 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.49 (3H, s, C(5)C*H*$_3$), 2.65 (3H, s, COC*H*$_3$), 4.36 (2H, s, C*H*$_2$N$_3$), 7.54 (1H, dd, *J* = 7.6, 1.5, 1.5 Hz, C(4′)*H*), 7.59 (1H, dd, *J* = 7.6, 7.6 Hz, C(5′)*H*), 7.94 (1H, dd, *J* = 1.5, 1.5 Hz, C(2′)*H*), 7.98 (1H, ddd, *J* = 7.6, 1.5, 1.5 Hz, C(6′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.6 (C(5)*C*H$_3$), 26.7 (CO*C*H$_3$), 44.5 (*C*H$_2$N$_3$), 115.6 (*C*(4)), 128.0 (*C*(6′)), 128.8 (*C*(2′)), 129.4 (*C*(5′)), 129.8 (*C*(4)), 133.5 (*C*(4′)), 137.8 (*C*(1′)), 157.3 (*C*(3)), 167.2 (*C*(5)), 197.5 (*C*=O); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 279.0854; $C_{13}H_{12}N_4NaO_2$ requires

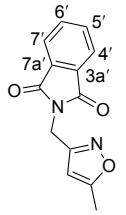
M^+ 279.0852; LRMS *m*/*z* (ES^+) 257 ($[M+H]^+$, 13), 279 ($[M+Na]^+$, 100). HPLC: Retention time 12.19 min, purity 95.2%

(5-Methylisoxazol-3-yl)methanol **120**[331]



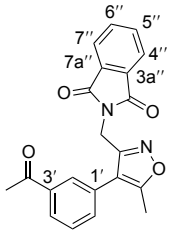
To a solution of ethyl 3-methylisoxazole-5-carboxylate (**110**, 1.00 g, 6.45 mmol, 1.0 eq) in anhydrous EtOH at 0 °C under a nitrogen atmosphere was added $NaBH_4$ (366 mg, 9.68 mmol, 1.5 eq). The solution was warmed to rt and stirred for 25 h, after which time further $NaBH_4$ (49 mg, 1.30 mmol, 0.2 eq) was added. After a further 22 h, TLC analysis indicated complete consumption of **110**. The reaction was quenched with 5 mL H_2O and 10 mL aq. HCl (1 M, 10 mL), then concentrated *in vacuo* to remove EtOH. The residues were diluted with water (30 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (40% Et_2O/30-40 °C petroleum ether then Et_2O) gave **120** as a colourless oil (269 mg, 93%); R_f 0.43 (Et_2O); ν_{max} (thin film)/cm^{-1}: 3364 (O–H) (br), 2932, 2879 (C–H) (w), 1607 (s), 1482 (m), 1450 (m), 1422 (m), 1258 (m), 1049 (s); ^1H NMR (500 MHz, $CDCl_3$) 2.41 (3H, d, *J* = 0.5 Hz, C*H*$_3$) overlapping 2.20 (1H, br s, O*H*), 4.69 (2H, s, C*H*$_2$), 6.03 (1H, d, *J* = 0.5 Hz, C*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 12.2 (*C*H_3), 56.9 (*C*H_2), 100.4 (*C*(4)), 163.7, 169.9 (*C*(3), *C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 136.0363; $C_5H_7NNaO_2$ requires M^+ 136.0369; LRMS *m*/*z* (ES^+) 114 ($[M+H]^+$, 18), 136 ($[M+Na]^+$, 100). These data are in good agreement with the literature values.[331]

2-((5-Methylisoxazol-3-yl)methyl)isoindoline-1,3-dione **121**



To a suspension of **120** (200 mg, 1.77 mmol, 1.0 eq), PPh_3 (556 mg, 2.12 mmol, 1.2 eq) and phthalimide (312 mg, 2.12 mg, 12 eq) in anhydrous THF (2.5 mL) under a nitrogen atmosphere at –10 °C was added DIAD (417 µL, 429 mg, 2.12 mmol, 1.2 eq) in anhydrous THF (2.5 mL) dropwise. The reaction was warmed to rt over 16 h. The resulting precipitate was filtered off and washed with ice cold THF, then dried *in vacuo* to give a colourless solid (136 mg). The filtrate was concentrated *in vacuo* and resuspended in Et_2O. The precipitate was collected and washed with Et_2O, then dried *in vacuo* to give **121** as a colourless solid (139 mg). Further concentration of the filtrate, precipitation with Et_2O and filtration gave an additional 30 mg of **121** as a colourless solid (overall 305 mg, 71%); mp 151-153 °C (Et_2O); R_f 0.48 (50% EtOAc/40-60 °C petroleum ether); ν_{max} (solid)/cm^{-1}: 3031, 2970 (C–H) (w), 1769 (m), 1738 (m), 1708 (s), 1604 (m), 1387 (s), 1344 (s), 1314 (m), 1099 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 2.37 (3H, d, *J* = 0.6 Hz, C*H*$_3$), 4.91 (2H, s, C*H*$_2$), 6.00 (1H, d, *J* = 0.6 Hz, C(4)*H*), 7.72-7.76 (2H, m, C(5′)*H*, C(6′)*H*), 7.86-7.90 (2H, m, C(4′)*H*, C(7′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$), 12.3 (*C*H$_3$), 33.4 (*C*H$_2$), 101.0 (*C*(4)), 123.6 (*C*(4′), *C*(7′)), 131.9 (*C*(3a′), *C*(7a′)), 134.2 (*C*(5′), *C*(6′)), 159.2 (*C*(3)), 167.5, 170.3 (*C*=O, *C*(5)); HRMS *m*/*z* (ES$^+$) found $[M+Na]^+$ 265.0585; $C_{13}H_{10}N_2NaO_3$ requires M^+ 265.0584; LRMS *m*/*z* (ES$^+$) 243 ($[M+H]^+$, 61), 265 ($[M+Na]^+$, 100). Anal. Calcd. for $C_{13}H_{10}N_2O_3$: C, 64.5; H, 4.2; N, 11.6. Found: C, 64.3; H, 4.2; N, 11.5.

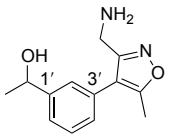
2-((4-(3-Acetylphenyl)-5-methylisoxazol-3-yl)methyl)isoindoline-1,3-dione **122**



To a dry 2-5 mL microwave vial under an argon atmosphere was added **121** (121 mg, 500 µmol, 1.5 eq), KOAc (65 mg, 670 µmol, 2.0 eq) and allylpalladium chloride dimer (2.5 mg, 7 µmol, 0.02 eq). The vial was sealed and purged with argon (5 × evacuate/fill). Degassed anhydrous DMAc (1.7 mL) and 3´-bromoacetophenone (**49**, 44 µL, 66 mg, 330 µmol, 1.0 eq) were added by syringe, and the mixture was heated at 80 °C for 18 h, then at 130 °C for 5 h. The mixture was cooled to rt, filtered through a thin pad of silica (eluent 10% MeOH/EtOAc) and concentrated *in vacuo*. The residues were dissolved in EtOAc (50 mL), washed with H_2O (3 × 50 mL), brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 10 → 40% EtOAc/40-60 °C petroleum ether) gave starting material **121** (68 mg), with further elution giving **122** as a beige solid (42 mg, 35%); mp 158-160 °C ($CHCl_3$); R_f 0.44 (50% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3059, 2924 (C–H) (w), 1771 (m), 1709 (C=O) (s) 1682 (C=O) (s), 1624 (m), 1467 (m), 1424 (m), 1119 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.38 (3H, s, C(5)C*H*$_3$), 2.60 (3H, s, COC*H*$_3$), 4.93 (2H, s, C*H*$_2$), 7.44-7.51 (2H, m, C(5´)*H*, C(6´)*H*), 7.66-7.72 (2H, m, C(5´´)*H*, C(6´´)*H*), 7.75-7.80 (2H, m, C(4´´)*H*, C(7´´)*H*), 7.84-7.88 (2H, m, C(2´)*H*, C(4´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.3 (C(5)*C*H$_3$), 26.6 (CO*C*H$_3$), 33.2 (*C*H$_2$), 115.0 (*C*(4)), 123.4 (*C*(4´´), *C*(7´´)), 127.7, 129.2, 129.2 (*C*(2´), *C*(4´), *C*(5´)), 129.8 (*C*(1´)), 131.8 (C(3a´´), *C*(7a´´)), 133.9 (*C*(6´)), 134.1 (*C*(5´´), *C*(6´´)),

137.6 (*C*(3′)), 157.2 (*C*(3)), 167.0 (*C*(5)), 167.4 (*C*ON*C*O), 197.4 (*C*OCH$_3$); HRMS (ES$^+$) *m*/*z* found [M+Na]$^+$ 383.0985; $C_{21}H_{16}N_2NaO_4$ requires M$^+$ 383.1002; LRMS *m*/*z* (ES$^+$) 361.1 ([M+H]$^+$, 7), 383 ([M+Na]$^+$, 100). Anal. Calcd. for $C_{21}H_{16}N_2O_4$: C, 70.0; H, 4.5; H, 7.8. Found: C, 69.9; H, 4.6; N, 7.7.

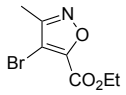
1-(3-(3-(Aminomethyl)-5-methylisoxazol-4-yl)phenyl)ethanol **74b**



Following the procedure of Osby *et al.*,[243] **122** (56 mg, 160 µmol, 1.0 eq) was dissolved in CH_2Cl_2 (1 mL), ^{i}PrOH (1.55 mL) and H_2O (0.25 ml). $NaBH_4$ (41 mg, 1.09 mmol, 7.0 eq) was added, and the reaction mixture was stirred for 23 h, after which time TLC analysis showed complete consumption of **122**. The reaction was quenched with dropwise addition of AcOH (0.5 mL). When evolution of gas had stopped, the reaction was heated to 80 °C for 2.5 h, then concentrated *in vacuo*. The residues were dissolved in aq. HCl (10 mL, 1 M) and washed with CH_2Cl_2 (3 × 10 mL). The aqueous layer was basified to pH 12 with 25% aq. NH_4OH, saturated with NaCl, filtered, and extracted with CH_2Cl_2 (3 × 30 mL). These combined organic extracts were washed with brine (30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by passage through a short plug of silica gel (eluent hexane then 5% MeOH/1% NEt_3 in CH_2Cl_2) to give **74b** as a pale yellow oil (33 mg, 92%); R_f 0.21 (5% MeOH/1% NEt_3 in CH_2Cl_2); ν_{max} (thin film)/cm^{-1}: 3365, 3301 (N–H, O–H) (br), 2970, 2926 (C–H) (m), 1629 (m), 1604 (m), 1480 (m), 1446 (m), 1240 (m), 1186 (m), 1111 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.49 (3H, d, *J* = 6.5 Hz, CH(OH)C*H*$_3$), 2.40 (3H, s, C(5)C*H*$_3$), 2.57 (3H, br s, O*H*, N*H*$_2$), 3.88 (2H, s, C*H*$_2$NH$_2$), 4.91 (1H, q, *J* = 6.5 Hz,

C*H*(OH)CH_3), 7.15 (1H, ddd, *J* = 7.5, 1.3, 1.3 Hz, C(4´)*H*), 7.30-7.35 (2H, m, C(2´)*H*, C(6´)*H*), 7.39 (1H, dd, *J* = 7.5, 7.5 Hz, C(5´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.5 (C(5)*C*H_3), 25.4 (CH(OH)*C*H_3), 37.2 (*C*H_2NH_2), 69.7 (*C*H(OH)CH_3), 115.4 (*C*(4)), 124.9 (*C*(6´)), 125.9 (*C*(2´)), 127.8 (*C*(4´)), 129.0 (*C*(5´)), 129.9 (*C*(3´)), 147.1 (*C*(1´)), 162.5 (*C*(3)), 165.9 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+H]^+$ 233.1281; $C_{13}H_{17}N_2O_2$ requires M^+ 233.1285; LRMS *m*/*z* (ES^+) 233 ($[M+H]^+$, 100), 255 ($[M+Na]^+$, 18). HPLC: Retention time 8.14 min, purity 96.5%.

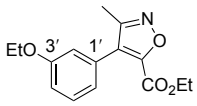
Ethyl 4-bromo-3-methylisoxazole-5-carboxylate **123**



Following the procedure of Li *et al.*,[238] **107** (2.08 g, 13.4 mmol, 1.0 eq), NBS (2.87 mg, 16.1 mmol, 1.2 eq) and TFA (60 mL) were heated with microwave irradiation in a sealed vial at 150 °C for 50 min. The acidic solvent was removed by azeotropic distillation with toluene, and the residue was filtered through Celite® (eluent Et_2O). Purification by silica gel column chromatography (gradient elution, 1 → 10% Et_2O/petroleum ether) gave **123** as a pale yellow oil which solidified at 4 °C (2.67 g, 85%); R_f 0.20 (5% Et_2O/40–60 °C petroleum ether); ν_{max} (solid)/cm^{-1}: 2986, 2939 (C–H) (w), 1737 (C=O) (s), 1278 (s), 1078 (s), 1022 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.44 (3H, t, *J* = 7.1 Hz, CH_2C*H*$_3$), 2.37 (3H, s, C(3)C*H*$_3$), 4.46 (2H, q, *J* = 7.1 Hz, C*H*$_2CH_3$); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H_3), 14.1 (CH_2*C*H_3), 62.4 (*C*H_2CH_3), 101.3 (*C*(4)), 155.8 (*C*(5)), 156.2 (*C*=O), 161.2 (*C*(3)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 255.9587, 257.9568; $C_7H_8{}^{79}BrNNaO_3$ requires M^+ 255.9580, $C_7H_8{}^{81}BrNNaO_3$ requires M^+ 257.9560; LRMS *m*/*z* (ES^+) 234 ($[^{79}M+H]^+$, 19), 236 ($[^{81}M+H]^+$, 19), 256 ($[^{79}M+Na]^+$, 94), 258 ($[^{81}M+Na]^+$, 100), 288

([^{79}M+Na+MeOH]$^+$, 57), 290 ([^{81}M+Na+MeOH]$^+$, 55). Anal. Calcd for $C_7H_8BrNO_3$: C, 35.9; H, 3.5; N, 6.0. Found: C, 35.8; H, 3.3; N, 5.8.

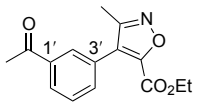
Ethyl 4-(3-ethoxyphenyl)-3-methylisoxazole-5-carboxylate **75a**



To a sealable tube were added **123** (800 mg, 3.42 mmol, 1.0 eq), **90** (933 mg, 3.76 mmol, 1.1 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (84 mg, 100 μmol, 0.03 eq). The tube was sealed and purged with argon (5 × evacuate/fill). Degassed DME (50 mL) and degassed sat. aq. $NaHCO_3$ (15 mL) were added by syringe, and the mixture was heated at 100 °C for 1.5 h. The mixture was then cooled and filtered through Celite® (eluent EtOAc) and concentrated *in vacuo*. The residue was resuspended in Et_2O (150 mL) and filtered through Celite®, then washed with H_2O (2 × 150 mL) and brine (150 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 20% Et_2O/petroleum ether) gave **75a** as a colourless solid (725 mg, 77%); R_f 0.20 (15% Et_2O/40–60 °C petroleum ether); mp 59–61 °C ($CHCl_3$); ν_{max} (solid)/cm^{-1}: 2982, 2935 (C–H) (w), 1732 (C=O) (s), 1578 (m), 1302 (s), 1249 (s), 1188 (s), 1027 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.28 (3H, t, *J* = 7.1 Hz, $CO_2CH_2CH_3$), 1.43 (3H, t, *J* = 7.0 Hz, C(3′)OCH_2CH_3), 2.29 (3H, s, C(3)CH_3), 4.06 (2H, q, *J* = 7.0 Hz, C(3′)OCH_2CH_3), 4.32 (2H, q, *J* = 7.1 Hz, $CO_2CH_2CH_3$), 6.87 (1H, dd, *J* = 2.5, 0.7 Hz, C(2′)*H*), 6.90 (ddd, *J* = 8.0, 0.7, 0.7 Hz, C(6′)*H*), 6.95 (ddd, *J* = 8.0, 2.5, 0.7 Hz, C(4′)*H*), 7.34 (dd, *J* = 8.0, 8.0 Hz, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.6 (C(3)CH_3), 13.9 ($CO_2CH_2CH_3$), 14.8 ($ArOCH_2CH_3$), 61.8 ($CO_2CH_2CH_3$), 63.5 ($ArOCH_2CH_3$), 114.6 (*C*(4′)), 116.1 (*C*(2′)), 121.9 (*C*(6′)), 125.0 (*C*(4)), 129.3 (*C*(5′)), 129.4 (*C*(1′)), 154.6 (*C*(5)), 157.2 (*C*=O), 158.7

(*C*(3′)), 160.3 (*C*(3)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 276.1230; $C_{15}H_{18}NO_4$ requires M^+ 276.1237; LRMS *m*/*z* (ES^+) 276 ($[M+H]^+$, 62), 298 ($[M+Na]^+$, 96), 330 ($[M+Na+MeOH]^+$, 77), 573 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{15}H_{17}NO_4$: C, 65.4; H, 6.2; N, 5.1. Found: C, 65.4; H, 6.1; N, 5.0. HPLC: Retention time 13.54 min, purity 98.9%.

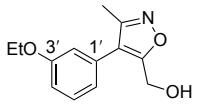
Ethyl 4-(3-acetylphenyl)-3-methylisoxazole-5-carboxylate **75b**



To a sealable tube were added **123** (793 mg, 3.39 mol, 1.0 eq), **91** (918 mg, 3.73 mmol, 1.1 eq) and $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (83 mg, 100 μmol, 0.03 eq). The tube was sealed and purged with argon (5 × evacuate/fill). Degassed DME (50 mL) and degassed sat. aq. $NaHCO_3$ (15 mL) were added by syringe, and the mixture was heated at 100 °C for 1.5 h. The mixture was then cooled and filtered through Celite® (eluent EtOAc) and concentrated *in vacuo*. The residue was resuspended in Et_2O (150 mL) and filtered through Celite®, then washed with H_2O (2 × 150 mL) and brine (150 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 6 → 30% Et_2O/petroleum ether) gave **75b** as a colourless solid (712 mg, 77%). An analytical sample was prepared by recrystallisation from boiling 30% EtOAc/hexane; R_f 0.59 (50% EtOAc/40–60 °C petroleum ether); mp 77-78 °C (30% EtOAc/hexane); ν_{max} (solid)/cm^{-1}: 2987, 2971, 2390 (C–H) (w), 1731 (C=OOEt) (s), 1685 ($C{=}OCH_3$) (s), 1378 (m), 1359 (m), 1302 (m), 1266 (m), 1219 (s), 1190 (s); ^{1}H NMR ($CDCl_3$) 1.28 (3H, t, *J* = 7.1 Hz, CH_2CH_3), 2.31 (3H, s, C(3)CH_3), 2.64 (3H, s, $COCH_3$), 4.33 (2H, q, *J* = 7.1 Hz, CH_2CH_3), 7.55-7.59 (2H, m, C(6′)*H*, C(5′)*H*), 7.96-7.98 (1H, m, C(2′)*H*), 7.99-8.03 (1H, m, C(4′)*H*); ^{13}C

NMR (126 MHz, $CDCl_3$) 10.6 (C(3)*C*H_3), 13.9 (CH_2*C*H_3), 26.6 (CO*C*H_3), 62.1 (*C*H_2CH_3), 124.2 (*C*(4)), 128.6, 128.7 (*C*(4´), *C*(5´)), 128.9 (*C*(1´)), 129.6 (*C*(2´)), 134.3 (*C*(6´)), 137.2 (*C*(3´)), 155.0 (*C*(5)), 157.0 (*C*O_2Et), 160.1 (*C*(3)), 197.4 (*C*OCH_3); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 296.0877; $C_{15}H_{15}NNaO_4$ requires M^+ 296.0893; LRMS *m*/*z* (ES^+) 274 ($[M+H]^+$, 53), 296 ($[M+Na]^+$, 97), 569 ($[2M+Na]^+$, 100). HPLC: Retention time 12.18 min, purity 97.4%.

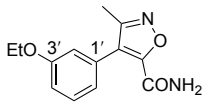
(4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methanol **76a**



To a solution of **75a** (45 mg, 163 μmol, 1.0 eq) in anhydrous EtOH (1 mL) at 0 ˚C under a nitrogen atmosphere was added $NaBH_4$ (12 mg, 320 μmol, 2.0 eq). The reaction was stirred for 1 h at 0 ˚C then for 24 h at rt. The reaction was quenched with H_2O (2 mL) and aq. HCl (1 M, 2 mL), and concentrated *in vacuo* to remove EtOH. The residues were diluted with H_2O (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (40%, 60% Et_2O/petroleum ether) gave **76a** as a pale yellow oil (32 mg, 84%); R_f 0.25 (60% Et_2O/40-60 ˚C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3372 (O–H) (br), 2380, 2933, 2878 (C–H) (w), 1600 (m), 1578 (s), 1395 (m), 1297 (s), 1219 (s), 1047 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.44 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.24 (1H, s, O*H*), 2.33 (3H, s, C(3)C*H*$_3$), 4.07 (2 H, q, *J* = 7.0 Hz, C*H*$_2$CH$_3$), 4.72 (2H, s, C*H*$_2$OH), 6.87 (1H, dd, *J* = 2.4, 1.6 Hz, C(2´)*H*), 6.89 (1H, ddd, *J* = 7.7, 1.6, 0.8 Hz, C(6´)*H*), 6.92 (1H, ddd, *J* = 8.0, 2.4, 0.8 Hz, C(4´)*H*), 7.35 (1H, dd, *J* = 8.0, 7.7 Hz, C(5´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)*C*H_3), 14.8 (CH_2*C*H_3), 54.9 (*C*H_2OH), 63.5 (*C*H_2CH_3),

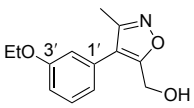
113.9 (*C*(4′)), 115.5 (*C*(2′)), 118.3 (*C*(4)), 121.3 (*C*(6′)), 129.9 (*C*(5′)), 130.6 (*C*(1′)), 158.9 (*C*(3)), 159.2 (*C*(3′)), 165.8 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 256.0945; $C_{13}H_{15}NNaO_3$ requires M^+ 256.0944; LRMS *m*/*z* (ES^+) 234 ($[M+H]^+$, 11), 256 ($[M+Na]^+$, 46), 489 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.9; H, 6.5; N, 6.0. Found: C, 67.1; H, 6.5; N, 6.1. HPLC: Retention time 11.27 min, purity 97.8%.

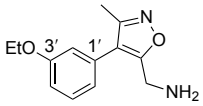
4-(3-Ethoxyphenyl)-3-methylisoxazole-5-carboxamide **77**



75a (208 mg, 756 μmol) was stirred with NH_3 in MeOH (2 M, 5 mL) under a nitrogen atmosphere for 39 h, then concentrated *in vacuo* to give **77** as an off-white solid (186 mg, 100%). An analytically pure sample was prepared by crystallisation from boiling EtOH; R_f 0.42 (50% EtOAc/40–60 °C petroleum ether); mp 117–118 °C (EtOH); ν_{max} (solid)/cm^{-1}: 3455, 3326, 3183 (N–H) (br), 2980, 2934 (C–H) (w), 1691 (C=O) (s), 1601 (m), 1579 (m), 1384 (m), 1343 (m), 1288 (m), 1270 (m), 1216 (m), 1199 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.43 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.29 (3H, s, C(3)CH_3), 4.06 (2H, q, *J* = 7.0 Hz, CH_2CH_3) 5.99 (1H, s, NH_A), 6.30 (1H, s, NH_B), 6.91–6.98 (3H, m, C(2′)*H*, C(4′)*H*, C(6′)*H*), 7.36 (1H, dd, *J* = 7.8, 7.8 Hz, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.6 (C(3)CH_3), 14.7 (CH_2CH_3), 63.5 (CH_2CH_3), 115.0 (*C*(4′)), 116.0 (*C*(2′)), 121.9 (*C*(6′)), 123.0 (*C*(4)), 129.2 (*C*(1′)), 129.7 (*C*(5′)), 156.1, 157.8 (*C*(3), *C*(5)), 159.0 (*C*(3′)), 160.7 (*C*=O); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 269.0897; $C_{13}H_{14}N_2NaO_3$ requires M^+ 269.0897; LRMS *m*/*z* (ES^+) 247 ($[M+H]^+$, 47), 269 ($[M+Na]^+$, 100), 301 ($[M+Na+MeOH]^+$, 18). Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.4; H, 5.7; N, 11.4. Found: C, 63.3; H, 5.5; N, 11.2. HPLC: Retention time 10.91 min, purity 98.5%.

(4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methanol **76a** and (4-(3-ethoxyphenyl)-3-methylisoxazol-5-yl)methanamine **78a**



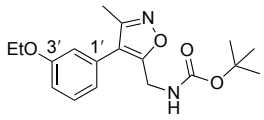


To a solution of **77** (420 mg, 1.71 mmol, 1.0 eq) in anhydrous THF under an argon atmosphere was added $BH_3{\cdot}Me_2S$ (2 M in THF, 3.5 mL, 7.0 mmol, 4.1 eq), and the reaction was heated under reflux for 13 h, then cooled to 0 °C. The reaction was quenched with careful addition of MeOH, then warmed to rt overnight. The mixture was concentrated *in vacuo*, and acidified with aq. HCl (1 M, 10 mL), then heated under reflux for 45 min. After cooling to rt, the mixture was washed with Et_2O (3 × 10 mL). The organic layers were washed with H_2O (30 mL) and brine (30 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by silica gel column chromatography (20%, 30% EtOAc/40-60 ˚C petroleum ether) gave **76a** as a brown oil (18 mg, 5%). The data are identical to those reported above. Further elution gave starting material **77** (30 mg, 7%).

The aqueous layer was basified with aq. NaOH (2 M, 10 mL), saturated with NaCl, filtered and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with H_2O (100 mL) and brine (100 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give an impure sample of **78a** as a brown oil (283 mg) which was used without further purification; R_f 0.12 (EtOAc); ν_{max} (thin film)/cm^{-1}: 3382, 3311 (N–H) (w), 2971, 2932 (C–H) (m), 1739 (s), 1600 (m), 1577 (m), 1366 (m), 1295 (m), 1216 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.44 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 1.62 (2H, br s, NH_2), 2.29 (3H, s, C(3)CH_3), 3.95 (2H, s, CH_2NH_2), 4.06 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 6.80 (1H, dd, *J* = 2.5, 1.6 Hz, C(2´)*H*), 6.83 (1H, ddd, *J* = 7.6, 1.6, 0.8 Hz, C(4´)*H*/C(6´)*H*), 6.91 (1H, ddd, *J* = 8.3, 2.5,

0.8 Hz, C(6′)*H*/C(4′)*H*), 7.34 (1H, dd, *J* = 8.3, 7.6 Hz, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)CH_3), 14.8 (CH_2CH_3), 37.1 (CH_2NH_2), 63.5 (CH_2CH_3), 113.5 (*C*(4′)/*C*(6′)), 115.6 (*C*(2′)), 116.3 (*C*(4)), 121.4 (*C*(6′)/*C*(4′)), 129.9 (*C*(5′)), 131.0 (*C*(1′)), 158.9 (*C*(3)), 159.2 (*C*(3′)), 168.3 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+H]^+$ 233.1282; $C_{13}H_{17}N_2O_2$ requires M^+ 233.1285; LRMS *m*/*z* (ES^+) 233 ($[M+H]^+$, 44), 465 ($[2M+H]^+$, 100).

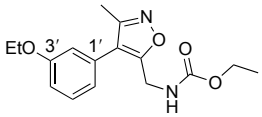
tert-Butyl ((4-(3-ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)carbamate **79a**



To a solution of impure **78a** (100 mg) in anhydrous THF (2 mL) under a nitrogen atmosphere was added Boc_2O (103 mg, 470 μmol). The reaction was stirred at rt for 3 h, then further Boc_2O (50 mg, 230 μmol) was added. After a further 21 h, TLC analysis indicated complete consumption of **78a**, and the reaction was concentrated *in vacuo* to remove THF. The residues were taken up in EtOAc (15 mL), washed with H_2O (2 × 15 mL), brine (15 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (10% EtOAc/40–60 °C petroleum ether) gave **79a** as a colourless viscous oil (104 mg, 35% over 2 steps from **77**); R_f 0.33 (20% EtOAc/40–60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3343 (N–H) (br), 2979, 2933 (C–H) (w), 1702 (m), 1507 (m),1294 (m), 1246 (m), 1162 (s), 1048 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.35-1.46 (12H, m, C(C*H*$_3$)$_3$, CH_2CH_3), 2.27 (3H, s, C(3)C*H*$_3$), 4.06 (2H, q, *J* = 6.9 Hz, C*H*$_2$CH$_3$), 4.43 (2H, d, *J* = 5.4 Hz, C*H*$_2$NH$_2$), 5.02 (1H, br s, N*H*), 6.78-6.87 (2H, m, C(2′)*H*, C(6′)*H*), 6.87-6.92 (1H, m, C(4′)*H*), 7.32 (1H, dd, *J* = 8.0, 8.0 Hz, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)CH_3), 14.8 (CH_2CH_3), 28.3 (C(CH_3)$_3$), 35.8 (CH_2NH_2), 63.5 (CH_2CH_3), 79.9 (*C*(CH_3)$_3$), 114.0 (*C*(4′)),

115.4 (*C*(2´)), 117.4 (*C*(4)), 121.3 (*C*(6´)), 129.9 (*C*(5´)), 130.4 (*C*(1´)), 155.3 (*C*OC(CH$_3$)$_3$), 159.0 (*C*(3)), 159.2 (*C*(3´)), 164.0 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 355.1634; $C_{18}H_{24}N_2NaO_4$ requires M$^+$ 355.1628; LRMS *m*/*z* (ES$^+$) 333 ([M+H]$^+$, 69), 355 ([M+Na]$^+$, 100), 665 ([2M+H]$^+$, 63), 687 ([2M+Na]$^+$, 99). Anal. Calcd for $C_{18}H_{24}N_2O_4$: C, 65.0; H, 7.3; N, 8.4. Found: C, 65.2; H, 7.3; N, 8.3. HPLC: Retention time 13.48 min, purity 97.7%.

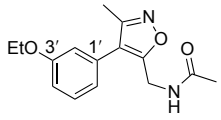
Ethyl ((4-(3-ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)carbamate **80**



To a solution of impure **78a** (38 mg) in anhydrous CH_2Cl_2 (2 mL) under a nitrogen atmosphere was added NEt_3 (50 µL, 36 mg, 360 µmol) and ethyl chloroformate (40 µL, 45 mg, 420 µmol). The reaction was stirred at rt for 2 h, after which time TLC analysis indicated complete consumption of **78a**, then quenched with 5 mL sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with 3% aq. $CuSO_4$ (15 mL), H_2O (15 mL) and brine (15 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (20% EtOAc/40-60 °C petroleum ether) gave **80** as a pale yellow oil which slowly solidified at –20 °C to a pale yellow solid (25 mg, 35% over 2 steps from **77**); mp 47-48 °C; R_f 0.28 (30% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3319 (N–H) (br), 2981, 2934 (C–H) (w), 1704 (C=O) (s), 1577 (m), 1529 (m), 1295 (m), 1240 (s); ^{1}HMR (500 MHz, $CDCl_3$) 1.22 (3H, t, *J* = 7.1 Hz, COOCH$_2$C*H*$_3$), 1.43 (3H, t, *J* = 7.0 Hz, C(3´)OCH$_2$C*H*$_3$), 2.28 (3H, s, C(3)C*H*$_3$), 4.06 (2H, q, *J* = 7.0 Hz, C(3´)OC*H*$_2$CH$_3$) overlapping 4.10 (2H, q, *J* = 7.1 Hz, COOC*H*$_2$CH$_3$), 4.49 (2H, d, *J* = 5.6 Hz, C*H*$_2$NH), 5.14 (1H, s, N*H*), 6.81-6.89 (2H, m, C(2´)*H*, C(6´)*H*), 6.91 (1H, dd, *J* = 8.2, 2.1 Hz, C(4´)*H*), 7.34

(1H, dd, *J* = 8.2, 8.2 Hz, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$), 10.7 (C(3)*C*H_3), 14.5 (COOCH_2*C*H_3), 14.8 (C(3′)OCH_2*C*H_3), 36.1 (*C*H_2NH_2), 61.3 (COO*C*H_2CH_3), 63.5 (C(3′)O*C*H_2CH_3), 114.0 (*C*(4′)), 115.4 (*C*(2′)), 117.6 (*C*(4)), 121.4 (*C*(6′)), 129.9 (*C*(5′)), 130.4 (*C*(1′)), 156.2 (*C*=O), 159.0 (*C*(3)), 159.2 (*C*(3′)), 163.7 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 327.1313; $C_{16}H_{20}N_2NaO_4$ requires M^+ 327.1315; LRMS *m*/*z* (ES^+) 327 ($[M+Na]^+$, 100). HPLC: Retention time 12.35 min, purity 96.9%.

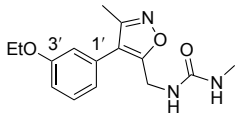
N-((4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)acetamide **81**



A solution of impure **78a** (20 mg), Ac_2O (9 µL, 9 mg, 90 µmol) and NEt_3 (23 µL, 17 mg, 170 µmol) in anhydrous CH_2Cl_2 (0.75 mL) was stirred at rt under a nitrogen atmosphere for 2 h, after which time TLC analysis indicated complete consumption of **78a**. The reaction was diluted with CH_2Cl_2 (5 mL) and washed with aq. HCl (1 M, 5 mL), H_2O (5 mL) and brine (5 mL), then dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 30 → 80% EtOAc/40-60 °C petroleum ether) gave **81** as a colourless oil which slowly solidified under vacuum (16 mg, 48% over two steps from **77**); mp 73-74 °C; R_f 0.24 (70% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3280 (N–H) (br), 2980, 2932 (C–H) (w), 1655 (C=O) (s), 1600 (m), 1577 (m), 1428 (m), 1293 (s), 1225 (s), 1038 (m); ^{1}H NMR (500 MHz, acetone-d_6) 1.38 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 1.87 (3H, s, COCH_3), 2.22 (3H, s, C(3)CH_3), 4.11 (2H, q, *J* = 7.0 Hz, CH_2CH_3), 4.50 (2H, d, *J* = 5.7 Hz, CH_2NH), 6.94 (1H, ddd, *J* = 8.2, 2.5, 1.0 Hz, C(4′)*H*), 6.98 (1H, ddd, *J* = 7.8, 1.4, 1.0 Hz, C(6′)*H*), 7.04 (1H, dd, *J* = 2.5, 1.4 Hz, C(2′)*H*), 7.36 (1H, dd, *J* = 8.2, 7.8 Hz, C(5′)*H*), 7.67 (1H,

br s, N*H*); ^{13}C NMR (126 MHz, acetone-d_6) 10.8 (C(3)*C*H$_3$), 15.2 (CH$_2$*C*H$_3$), 22.6 (CO*C*H$_3$), 35.0 (*C*H$_2$NH), 64.1 (*C*H$_2$CH$_3$), 115.0 (*C*(4′)), 116.1 (*C*(2′)), 118.0 (*C*(4)), 122.3 (*C*(6′)), 130.7 (*C*(5′)), 132.0 (*C*(1′)), 159.3 (*C*(3)), 160.3 (*C*(3′)), 165.5 (*C*=O), 170.1 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 297.1204; $C_{15}H_{18}N_2NaO_3$ requires M$^+$ 297.1210; LRMS *m*/*z* (ES$^+$) 275 ([M+H]$^+$, 83), 297 ([M+Na]$^+$, 100). HPLC: Retention time 10.76 min, purity 97.5%.

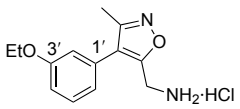
1-((4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)-3-methylurea **82**



To a solution of impure **78a** (37 mg) in anhydrous THF (1 mL) under a nitrogen atmosphere was added *N*-succinimidyl-*N*-methylcarbamate (41 mg, 239 mmol). The reaction was stirred at rt for 20 h, after which time further *N*-succinimidyl-*N*-methylcarbamate was added (27 mg, 157 mmol). After stirring for a further 3 h, TLC analysis indicated complete consumption of **78a**, and the reaction was concentrated *in vacuo*. The residues were dissolved in CH_2Cl_2 (15 mL) and washed with H_2O (15 mL), aq. $NaHCO_3$ (10% w/v, 15 mL) and brine (15 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 40 → 100% EtOAc/40-60 °C petroleum ether) gave **82** as a colourless solid (21 mg, 33% over two steps from **77**); R_f 0.55 (EtOAc); mp 102-104 °C ($CHCl_3$); ν_{max} (solid)/cm^{-1}: 3359, 3314 (N–H) (m), 2981, 2937 (C–H) (w), 1637 (C=O) (s), 1579 (s), 1298 (m), 1259 (m), 1259 (m), 1051 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.47 (3H, t, *J* = 7.0 Hz, CH$_2$C*H*$_3$), 2.26 (3H, s, C(3)C*H*$_3$), 2.70 (3H, d, *J* = 4.8 Hz, NHC*H*$_3$), 4.05 (2H, q, *J* = 7.0 Hz, C*H*$_2$CH$_3$), 4.49 (2H, d, *J* = 5.4 Hz, C*H*$_2$NH), 4.95 (1H, q, *J* = 4.8 Hz, N*H*CH$_3$), 5.36 (1H, t, *J* = 5.4 Hz, CH$_2$N*H*), 6.85 (1H, dd, *J* = 2.0, 2.0 Hz, C(2′(*H*)), 6.87-6.91 (2H, m, C(4′)*H*,

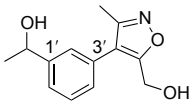
C(6′)*H*), 7.32 (1H, dd, *J* = 7.9, 7.9 Hz, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H_3), 14.8 (CH_2*C*H_3), 27.0 (NH*C*H_3), 35.6 (*C*H_2NH), 63.5 (O*C*H_2), 113.9 (*C*(4′)/*C*(6′)), 115.5 (*C*(2′)), 117.1 (*C*(4)), 121.4 (*C*(6′)/*C*(4′)), 129.9 (*C*(2′)), 130.5 (*C*(1′)), 158.5 (*C*=O), 159.0 (*C*(3)), 159.2 (*C*(3′)), 164.8 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 312.1316; $C_{15}N_{19}N_3NaO_3$ requires M^+ 312.1319; LRMS *m*/*z* (ES^+) 312 ($[M+Na]^+$, 20), 601 ($[2M+Na]^+$, 100). Anal. Calcd for $C_{15}H_{19}N_3O_3$: C, 62.3; H, 6.6; N, 14.5. Found: C, 62.2; H, 6.6; N, 14.5. HPLC: Retention time 10.50 min, purity 97.3%.

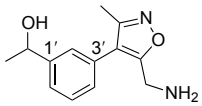
(4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methanamine hydrochloride **78a**



79a (50 mg, 150 µmol) in a solution of HCl in Et_2O (2 M, 2 mL) was stirred at rt for 17 h, then dried under a stream of nitrogen gas. The resultant solid was triturated with Et_2O, then dried *in vacuo* to give **78a** as a colourless solid (23 mg, 89%); mp 153-155 °C (Et_2O); ν_{max} (solid)/cm^{-1}: 2970, 2943 (N–H) (br), 2616 (w), 1739 (s), 1366 (s), 1229 (m), 1212 (s); ^{1}H NMR (500 MHz, DMSO-d_6) 1.35 (3H, t, *J* = 7.0 Hz, CH_2C*H*$_3$), 2.28 (3H, s, C(3)C*H*$_3$), 4.09 (2H, q, *J* = 7.0 Hz, C*H*$_2$$CH_3$), 4.17 (2H, s, C*H*$_2$$NH_3$), 6.99-7.04 (3H, m, C(2′)*H*, C(4′)*H*, C(6′)*H*), 7.38-7.44 (1H, m, C(5′*H*)), 8.86 (3H, s, N*H*$_3$); ^{13}C NMR (126 MHz, DMSO-d_6) 10.3 (C(3)*C*H_3), 14.6 (CH_2*C*H_3), 33.0 (*C*$H_2$$NH_2$), 63.2 (*C*$H_2$$CH_3$), 114.2, 115.2, 121.3 (*C*(2′), *C*(4′), *C*(6′), 118.8 (*C*(4)), 129.5 (*C*(1′)), 130.1 (*C*(5′)), 158.8, 158.9 (*C*(3), *C*(3′)), 160.3 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M–Cl]^+$ 233.1289; $C_{13}H_{17}N_2O_2$ requires M^+ 233.1285; LRMS *m*/*z* (ES^+) 233 ($[M–Cl]^+$, 100), 255 ($[M–Cl+Na]^+$, 17). Anal. Calcd for $C_{13}H_{17}ClN_2O_2$: C, 58.1; H, 6.4; N, 10.4. Found: C, 57.9; H, 6.3; N, 10.5. HPLC: Retention time 9.04 min, purity 96.2%.

(*RS*)-1-(3-(5-(Hydroxymethyl)-3-methylisoxazol-4-yl)phenyl)ethanol **76c** and (*RS*)-1-(3-(5-(aminomethyl)-3-methylisoxazol-4-yl)phenyl)ethanol **78b**





Method A: **75b** (140 mg, 620 μmol) was stirred with NH_3 in MeOH (2 M, 4 mL) for 36 h then concentrated *in vacuo*. ^{1}H NMR and MS analysis indicated a mixture of methyl ester and amide, and the crude mixture was stirred with NH_3 in ^{i}PrOH (2 M, 20 mL) for 24 h, then concentrated *in vacuo* to give crude presumed amide **124** (140 mg) which was used without further purification.

To a solution of presumed **124** (140 mg, 573 μmol, 1.0 eq) in anhydrous THF under an argon atmosphere was added $BH_3.Me_2S$ (2 M in THF, 1.4 mL, 2.8 mmol, 5 eq), and the reaction was heated under reflux for 8 h, then cooled to 0 °C. The reaction was quenched with careful addition of MeOH, then warmed to rt overnight. The mixture was concentrated *in vacuo*, and acidified with aq. HCl (1 M, 5 mL), then heated under reflux for 1 h. After cooling to rt, the mixture was washed with CH_2Cl_2 (3 × 10 mL). The organic layers were washed with brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 3 → 100% EtOAc/30–40 °C petroleum ether) gave **76c** as a yellow oil (8 mg, 6%); R_f 0.14 (50% EtOAc/40–60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3336 (O–H, N–H) (br), 2981, 2929 (C–H) (m), 1738 (s), 1367 (m), 1217 (m), 1010 (m); ^{1}H NMR 1.52 (3H, d, *J* = 6.4 Hz, CH(OH)C*H*$_3$), 2.30 (3H, s, C(3)C*H*$_3$), 2.64 (1H, s, O*H*), 3.16 (1H, s, O*H*), 4.66 (2H, s, C*H*$_2$OH), 4.94 (1H, q, *J* = 6.4 Hz, C*H*OH), 7.20-7.24 (1H, m, C(4′)*H*/C(6′)*H*), 7.34-7.38 (2H, m, C(2′)*H*, C(6′)*H*/C(4′)*H*), 7.40-7.44 (1H, m, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H$_3$), 25.3 (CH(OH)*C*H$_3$), 54.7 (*C*H$_2$OH), 70.0

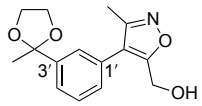
(*C*HOH), 118.3 (*C*(4)), 125.2, 126.2, 128.0 (*C*(2´), *C*(4´), *C*(6´)), 128.9 (*C*(5´)), 129.5 (*C*(3´)), 146.6 (*C*(1´)), 158.8 (*C*(3)), 166.0 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 256.0944; $C_{13}N_{15}NNaO_3$ requires M^+ 256.0944; LRMS *m*/*z* (ES^+) 234 ($[M+H]^+$, 16), 256 ($[M+Na]^+$, 71), 489 ($[2M+Na]^+$, 100), 722 ($[3M+Na]^+$, 26). Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.9; H, 6.5; H, 6.0. Found: C, 67.0; H, 6.6; N, 6.1. HPLC: Retention time 9.21 min, purity 96.2%.

The aqueous layer was basified with aq. NaOH (2 M, 10 mL), saturated with NaCl, filtered and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 0 → 4% MeOH in CH_2Cl_2 with 1% NEt_3) gave a brown solid (87 mg) which was dissolved in aq. HCl (1 M, 5 mL) and washed with CH_2Cl_2 (3 × 5 mL). The aqueous layer was basified with aq. NaOH (2 M, 5 mL), saturated with NaCl, filtered and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give an impure sample of **78b** as a brown oil (35 mg). IR, NMR and MS data are identical to those reported below.

Method B (for the synthesis of **78b** only): Following the procedure of Osby *et al.*,[243] **128** (54 mg, 150 μmol, 1.0 eq) was dissolved in CH_2Cl_2 (1 mL), ^{i}PrOH (1.5 mL) and H_2O (0.25 mL). $NaBH_4$ (40 mg, 1.05 mmol, 7.0 eq) was added, and the reaction mixture was stirred for 23 h, after which time TLC analysis showed complete consumption of **78b**. The reaction was quenched with dropwise addition of AcOH (0.5 mL). When evolution of gas had stopped, the reaction was heated to 80 °C for 2.5 h, then concentrated *in vacuo*. The residues were dissolved in aq. HCl (1 M, 10 mL) and washed with CH_2Cl_2 (3 × 10 mL). The aqueous layer was

basified to pH 12 with 25% aq. NH_4OH, saturated with NaCl, filtered, and extracted with CH_2Cl_2 (3 × 30 mL). These combined organic extracts were washed with brine (30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The residue was purified by passage through a short plug of silica gel (eluent hexane then 5% MeOH/1% NEt_3 in CH_2Cl_2) to give **78b** as a pale yellow oil (32 mg, 92%); R_f 0.16 (5% MeOH/1% NEt_3 in CH_2Cl_2); ν_{max} (thin film)/cm^{-1}: 3365, 3300 (N–H, O–H) (br), 2971, 2928 (C–H) (w), 1739 (s), 1434 (m), 1367 (s), 1217 (m), 1078 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.50 (3H, d, *J* = 6.5 Hz, CH(OH)C*H*$_3$), 2.27 (3H, s, C(3)C*H*$_3$), 2.40 (3H, br s, N*H*$_2$, O*H*), 3.92 (2H, s, C*H*$_2$NH$_2$), 4.92 (1H, q, *J* = 6.5 Hz, C*H*(OH)CH$_3$), 7.14 (1H, ddd, *J* = 7.5, 1.5, 1.3 Hz, C(4´)*H*), 7.30 (1H, dd, *J* = 1.5, 1.3 Hz, C(2´)*H*), 7.36 (1H, ddd, *J* = 7.7, 1.3, 1.3 Hz, C(6´)*H*), 7.41 (1H, dd, *J* = 7.7, 7.5 Hz, C(5´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H$_3$), 25.4 (CH(OH)*C*H$_3$), 36.7 (*C*H$_2$NH$_2$), 68.4 (*C*H(OH)CH$_3$), 116.7 (*C*(4)), 125.0 (*C*(6´)), 126.2 (*C*(2´)), 128.0 (*C*(4´)), 129.0 (*C*(5´)), 129.7 (*C*(3´)), 146.9 (*C*(1´)), 158.9 (*C*(3)), 167.6 (*C*(5)); HRMS *m*/*z* (ES$^+$) found $[M+H]^+$ 233.1283, $C_{13}H_{17}N_2O_2$ requires M^+ 233.1285; LRMS *m*/*z* (ES$^+$) 233 ($[M+H]^+$, 100), 255 ($[M+Na]^+$, 55). HPLC: Retention time 8.06 min, purity 98.6%.

(3-Methyl-4-(3-(2-methyl-1,3-dioxolan-2-yl)phenyl)isoxazol-5-yl)methanol **126**



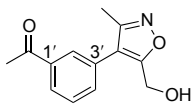
To a solution of **75b** (579 mg, 2.12 mmol, 1.0 eq) in ethylene glycol (1.2 mL, 1.3 g, 22 mmol, 10 eq) and triethyl orthoformate (1.4 mL, 1.2 g, 8 mmol, 4 eq) was added 4-toluenesulfonic acid monohydrate (20 mg, 110 µmol, 0.05 eq), The solution was heated at 60 °C for 3.5 h, then cooled to rt. The reaction was quenched with 10 mL sat. aq. $NaHCO_3$ and extracted with EtOAc (3 × 10 mL).

The combined organic layers were washed with 30 mL H_2O, 30 mL brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give presumed ethyl 3-methyl-4-(3-(2-methyl-1,3-dioxolan-2-yl)phenyl)isoxazole-5-carboxylate (680 mg) as a yellow oil which was used without further purification.

To a solution of crude presumed 3-methyl-4-(3-(2-methyl-1,3-dioxolan-2-yl)phenyl)isoxazole-5-carboxylate (420 mg, 1.32 mmol, 1.0 eq) in anhydrous EtOH (5 mL) at 0 °C under a nitrogen atmosphere was added $NaBH_4$ (75 mg, 2.0 mmol, 1.5 eq). The solution was warmed to rt and stirred for 5 h, after which time further $NaBH_4$ (25 mg, 660 μmol, 0.5 eq) was added. After stirring for a further 17 h, TLC analysis indicated complete consumption of presumed 3-methyl-4-(3-(2-methyl-1,3-dioxolan-2-yl)phenyl)isoxazole-5-carboxylate, and the reaction was quenched with 15 mL H_2O and concentrated *in vacuo*. The residues were extracted with EtOAc (3 × 15 mL), and the combined organic layers were washed with H_2O (45 mL), brine (45 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 10 → 50% EtOAc/40-60 °C petroleum ether containing 1% NEt_3) gave **126** as a colourless solid (287 mg, 80% over 2 steps); R_f 0.27 (40% EtOAc, 1% NEt_3, 40-60 °C petroleum ether); mp 89-91 °C (CH_2Cl_2); ν_{max} (solid)/cm^{-1}: 3350, 3311 (O–H) (m), 2986, 2953, 2934, 2890 (C–H) (w), 1620 (m), 1401 (m), 1224 (m), 1196 (s), 1178 (m), 1038 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.67 (3H, s, C(OCH_2CH_2O)CH_3), 2.32 (3H, s, C(3)CH_3), 3.10 (1H, s, O*H*), 3.76-4.11 (4H, m, OCH_2CH_2O), 4.70 (2H, s, CH_2OH) 7.25-7.30 (1H, m, C(6′)*H*), 7.39-7.47 (2H, m, C(2′)*H*, C(5′)*H*), 7.48-7.52 (1H, m, C(4′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.8 (C(3)CH_3), 27.6 (C(OCH_2CH_2O)CH_3), 55.0 (CH_2OH), 64.5 (OCH_2CH_2O), 108.6 (C(OCH_2CH_2O)CH_3), 118.2 (C(4)), 125.0 (C(4′)), 125.9 (C(2′)), 128.6 (C(6′)),

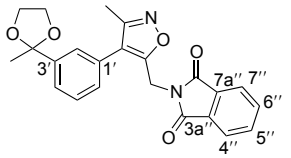
128.9 (*C*(5′)), 129.3 (*C*(1′)), 144.2 (*C*(3′)), 158.8 (*C*(3)), 165.8 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 298.1044; $C_{15}H_{17}NNaO_4$ requires M^+ 298.1050; LRMS *m*/*z* (ES^+) 276 ($[M+H]^+$, 11), 298 ($[M+Na]^+$, 100). Anal. Calcd for $C_{15}H_{17}NO_4$: C, 65.4; H, 6.2; N, 5.1. Found: C, 65.4; H, 6.2; N, 5.2.

1-(3-(5-(Hydroxymethyl)-3-methylisoxazol-4-yl)phenyl)ethanone **76b**



A solution of **126** (34 mg, 120 μmol) in dioxane (0.5 mL) and aq. HCl (1 M, 0.25 mL) was stirred at rt for 2 h, then concentrated *in vacuo*. The residues were dissolved in CH_2Cl_2 (3 mL) and washed with H_2O (3 mL), brine (3 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **76b** as a colourless oil which slowly solidified at –20 °C to a colourless solid (21 mg, 73%); mp 87-89 °C; R_f 0.53 (50% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3386 (O–H) (br), 2931, 2872 (C–H) (w), 1683 (C=O) (s), 1427 (m), 1359 (m), 1241 (s), 1051 (m), 1014 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 2.31 (3H, s, C(3)C*H*$_3$), 2.64 (3H, s, COC*H*$_3$), 3.15 (1H, br s, O*H*), 4.70 (C*H*$_2$OH), 7.53-7.60 (2H, m, *Ar*), 7.91-7.99 (2H, m, *Ar*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H$_3$), 26.7 (CO*C*H$_3$), 54.7 (*C*H$_2$OH), 117.6 (*C*(4)), 128.0, 128.9, 129.2 (*C*(2′), *C*(5′), *C*(6′)), 130.1 (*C*(3′)), 133.7 (*C*(4′)), 137.6 (*C*(1′)), 158.7 (*C*(3)), 166.5 (*C*(5)), 198.0 (*C*=O); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 254.0783; $C_{13}H_{13}NNaO_3$ requires M^+ 254.0788; LRMS *m*/*z* (ES^+) 232 ($[M+H]^+$, 17), 254 ($[M+Na]^+$, 100). Anal. Calcd. for $C_{13}H_{13}NO_3$: C, 67.5; H, 5.7; N, 6.1. Found: C, 67.5; H, 5.7; N, 6.0. HPLC: Retention time 9.91 min, purity 98.3%.

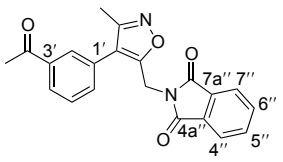
2-((3-Methyl-4-(3-(2-methyl-1,3-dioxolan-2-yl)phenyl)isoxazol-5-yl)methyl)isoindoline-1,3-dione **127**



To a solution of phthalimide (160 mg, 1.09 mmol, 2.0 eq), PPh_3 (286 mg, 1.09 mmol, 2.0 eq) and DIAD (215 µL, 224 mg, 1.09 mmol, 2.0 eq) in anhydrous THF (1.5 mL) at –10 °C under a nitrogen atmosphere was added a solution of **126** (150 mg, 545 µmol, 1.0 eq) in anhydrous THF (2 mL) dropwise. The resultant suspension was warmed to rt over 11 h, then quenched with MeOH (10 mL) and concentrated *in vacuo*. The residue was redissolved in Et_2O (15 mL), and kept at 4 °C for 6 h. The resulting precipitate was filtered off and washed with ice cold Et_2O, then dried *in vacuo* to give **127** as an off-white solid (146 mg). The filtrate was concentrated *in vacuo* and redissolved in boiling Et_2O (5 mL), then kept at 4 °C overnight. The resulting precipitate was filtered off and washed with ice cold Et_2O, then dried *in vacuo* to give a further 22 mg of **127** as an off-white solid (overall 168 mg, 76%); R_f 0.38 (50% EtOAc/40-60 °C petroleum ether); mp 152-153 °C (Et_2O); ν_{max} (solid)/cm^{-1}: 2989, 2896 (C–H) (w), 1772 (m), 1720 (C=O) (s), 1413 (m), 1389 (m), 1342 (m), 1325 (m), 1200 (m), 1031 (s); ^{1}H NMR (500 MHz, acetone-d_6) 1.59 (3H, s, C(OCH$_2$CH$_2$O)C*H*$_3$), 2.20 (3H, s, C(3)C*H*$_3$), 3.72-4.06 (4H, m, OC*H*$_2$C*H*$_2$O) 5.04 (2H, s, C*H*$_2$N), 7.34-7.43 (3H, m, C(4′)*H*, C(5′)*H*, C(6′)*H*), 7.50-7.52 (1H, m, C(2′)*H*), 7.82-7.89 (4H, m, C(4′′)*H*, C(5′′)*H*, C(6′′)*H*, C(7′′)*H*); ^{13}C NMR (126 MHz, acetone-d_6) 10.5 (C(3)*C*H$_3$), 27.8 (C(OCH$_2$CH$_2$O)*C*H$_3$), 33.8 (*C*H$_2$N), 65.2 (O*C*H$_2$*C*H$_2$O), 109.1 (*C*(OCH$_2$CH$_2$O)CH$_3$), 118.5 (*C*(4)), 124.0 (*C*(4′′), *C*(7′′)), 125.8 (*C*(4′)), 127.1 (*C*(2′)), 129.4, 129.7

(*C*(5′), *C*(6′)), 129.9 (*C*(1′)), 132.7 (*C*(3a′′), *C*(7a′′)), 135.3 (*C*(5′′), *C*(6′′)), 145.4 (*C*(3′)), 159.7 (*C*(3)), 162.7 (*C*(5)), 167.8 (*C*=O); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 427.1257; $C_{23}H_{20}N_2NaO_5$ requires M^+ 427.1264; LRMS *m*/*z* (ES^+) 405 ($[M+H]^+$, 62), 427 ($[M+Na]^+$, 100), 443 ($[M+K]^+$, 19), 459 ($[M+Na+MeOH]^+$, 35). Anal. Calcd for $C_{23}H_{20}N_2O_5$: C, 68.3; H, 5.0; N, 6.9. Found: C, 68.2; H, 4.9; N, 7.0.

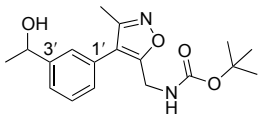
2-((4-(3-Acetylphenyl)-3-methylisoxazol-5-yl)methyl)isoindoline-1,3-dione **128**



A solution of **127** (94 mg, 230 μmol) in dioxane (3 mL) and aq. HCl (1 M, 1 mL) was stirred at rt for 2.5 h, then concentrated *in vacuo*. The residues were dissolved in CH_2Cl_2 (5 mL) and washed with H_2O (2 × 5 mL), brine (5 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Repeated concentration from toluene (to remove residual ethylene glycol) gave **128** as a yellow oil (81 mg, 96%); R_f 0.50 (50% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3033, 2874 (C–H) (w), 1716 (C=O) (s), 1684 (s), 1420 (m), 1392 (s), 1347 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 2.20 (3H, s, C(3)C*H*$_3$), 2.61 (3H, s, COC*H*$_3$), 4.97 (2H, s, C*H*$_2$), 7.45 (1H, dd, *J* = 7.8, 7.6 Hz, C(5′)*H*), 7.54 (1H, ddd, *J* = 7.6, 1.5, 1.5 Hz, C(6′)*H*), 7.67-7.72 (2H, m, C(5′′)*H*, C(6′′)*H*), 7.74-7.78 (2H, m, C(4′′)*H*, C(7′′)*H*), 7.83 (1H, ddd, *J* = 7.8, 1.5, 1.5 Hz, C(4′)*H*), 7.95 (1H, dd, *J* = 1.5, 1.5 Hz, C(2′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.4 (C(3)*C*H$_3$), 26.7 (CO*C*H$_3$), 33.0 (*C*H$_2$), 117.4 (*C*(4)), 123.4 (*C*(4′′), *C*(7′′)), 127.7 (*C*(4′)), 129.1 (*C*(5′)), 129.5 (*C*(1′)), 129.5 (*C*(2′)), 131.6 (*C*(4a′′), *C*(7a′′)), 134.0 (*C*(6′)), 134.2 (*C*(5′′), *C*(6′′)), 137.5 (*C*(3′)), 159.0 (*C*(3)), 161.5 (*C*(5)), 167.1 (*C*ON*C*O), 197.4 (*C*OCH$_3$); HRMS (ES^+) *m*/*z* found

$[M+Na]^+$ 383.0998; $C_{21}H_{16}N_2NaO_4$ requires M^+ 383.1002; LRMS *m*/*z* (ES^+) 361 ($[M+H]^+$, 38), 383 ($[M+Na]^+$, 100), 399 ($[M+K]^+$, 12), 415 ($[M+Na+MeOH]^+$, 77).

(*RS*)-*tert*-Butyl ((4-(3-(1-hydroxyethyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate **79b**

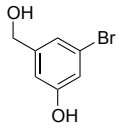


A solution of **78b** (15 mg, 65 μmol) and Boc_2O (17 mg, 77 μmol, 1.2 eq) in anhydrous THF (1 mL) was stirred under a nitrogen atmosphere for 5 h, after which time TLC analysis indicated complete consumption of starting material. The reaction mixture was concentrated *in vacuo* and dissolved in EtOAc (10 mL), then washed with sat. aq. $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (30% then 40% EtOAc/40-60 °C petroleum ether) gave **79b** as a colourless oil (14 mg, 65%); R_f 0.21 (50% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3332 (N–H, O–H) (br), 2976, 2931 (C–H) (w), 1695 (s) (C=O), 1525 (m), 1367 (m), 1285 (m), 1251 (m),1167 (s); ^1H NMR (500 MHz, $CDCl_3$), 1.43 (9H, s, C(C*H*$_3$)$_3$), 1.53 (3H, d, *J* = 6.5 Hz, CH(OH)C*H*$_3$), 2.3 (1H, br s, O*H*), 2.30 (3H, s, C(3)C*H*$_3$), 4.43 (2H, d, *J* = 6.0 Hz, C*H*$_2$NH), 4.93 (1H, q, *J* = 6.5 Hz, C*H*(OH)CH_3), 5.05 (1H, br s, N*H*), 7.17-7.22 (1H, m, C(6′)*H*), 7.33-7.44 (3H, m, C(2′)*H*, C(4′)*H*, C(5′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H_3), 25.2 (CH(OH)*C*H_3), 28.3 (C(*C*H_3)$_3$), 35.6 (*C*H_2NH), 70.0 (*C*H(OH)CH_3), 80.3 (*C*$(CH_3)_3$), 117.6 (*C*(4)), 125.1, 126.4 (*C*(2′), *C*(4′)), 127.8 (*C*(6′)), 128.9 (*C*(5′)), 129.2 (*C*(1′)), 146.8 (*C*(3′)), 155.3 (*C*=O), 158.9 (*C*(3)), 164.2 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 355.1631; $C_{18}H_{24}N_2NaO_4$ requires M^+ 355.1628; LRMS *m*/*z* (ES^+)

333 ([M+H]$^+$, 25), 355 ([M+Na]$^+$, 100). HPLC: Retention time 11.45 min, purity 95.1%.

*7.4.3 Modifications to the 5-position of **61a***

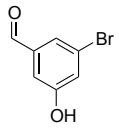
3-Bromo-5-(hydroxymethyl)phenol **134**



To a solution of 3-bromo-5-hydroxybenzoic acid (**53**, 2.5 g, 11.5 mmol, 1.0 eq) in anhydrous THF (100 mL) at 0 °C under a nitrogen atmosphere was added BH_3·THF (1 M in THF, 35 mL, 35 mmol, 3.0 eq) dropwise. The solution was warmed to rt over 16 h, after which time TLC analysis indicated complete consumption of **53**. The reaction was cooled to 0 °C and carefully quenched with MeOH (20 mL) then 5 mL conc. HCl was added dropwise. The solution was warmed to rt then concentrated *in vacuo* to remove THF and MeOH. The residues were suspended in H_2O (75 mL) and extracted with EOAc (3 × 75 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (3 × 150 mL) and brine (3 × 150 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give **134** as a pale yellow solid (2.25 g, 96%) which was used without further purification. An analytical sample was prepared by crystallisation from boiling $CHCl_3$; R_f 0.44 (75% EtOAc/40-60 °C petroleum ether); mp 88-89 °C ($CHCl_3$); ν_{max} (thin film)/cm^{-1}: 3364 (O–H) (br), 1610 (s), 1489 (m), 1277 (s), 1217 (m), 1041 (s); ^{1}H NMR (500 MHz, acetone-d_6) 4.3 (1H, br s, $CH_2O\mathit{H}$), 4.58 (2H, s, $C\mathit{H}_2OH$), 6.85-6.88 (1H, m, C(6)*H*), 6.89-6.93 (1H, m, C(2)*H*), 7.01-7.05 (1H, m, C(4)*H*), 8.7 (1H, br s, C(1)O*H*); ^{13}C NMR (126 MHz, acetone-d_6) 63.8 ($\mathit{C}H_2OH$), 113.2 (*C*(6)), 117.5 (*C*(2)), 121.2 (*C*(4)), 122.8 (*C*(3)), 147.3 (*C*(5)), 159.3 (*C*(1)); HRMS *m*/*z*

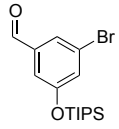
(ES^-) found $[M–H]^–$ 200.9559, 202.9539; $C_7H_6{}^{79}BrO_2$ requires $M^–$ 200.9557, $C_7H_6{}^{81}BrO_2$ requires $M^–$ 202.9536; LRMS *m*/*z* (ES^+) 201 ($[^{79}M–H]^–$, 100), 203 ($[^{81}M–H]^–$, 88). Anal. Calcd for $C_7H_7BrO_2$: C, 41.1; H, 3.5. Found: C, 41.2; H, 3.5.

3-Bromo-5-hydroxybenzaldehyde **133**[332]



134 (2.23 g, 11.0 mmol, 1.0 eq) was suspended in anhydrous CH_2Cl_2 (50 mL) and sufficient anhydrous acetone was added to dissolve the solid (c. 5 mL) with gentle heating. Activated MnO_2 (5.8 g, 67 mmol, 6 eq) was added, and the reaction stirred under a nitrogen atmosphere at rt for 2 days, after which time further MnO_2 (3.0 g, 35 mmol, 3.1 eq) was added and the reaction stirred for a further 5 days. The mixture was concentrated *in vacuo* onto Celite® and purified by manual silica gel column chromatography (50% Et_2O/petroleum ether) to give **133** as a yellow solid (1.81 g, 82%); R_f 0.30 (50% Et_2O/40-60 °C petroleum ether);[332] mp 133-135 °C (Et_2O) (lit. 134-138 °C);[332] ^1H NMR (400 MHz, acetone-d_6) 7.32-7.35 (1H, m, *Ar*), 7.35-7.38 (1H, m, *Ar*), 7.55-7.58 (1H, m, *Ar*), 9.33 (1H, s, O*H*), 9.94 (1H, s, C*H*O); LRMS *m*/*z* ($ES^–$) 199 ($[^{79}M–H]^–$, 100), 201 ($[^{81}M–H]^–$, 97). Anal. Calcd for $C_7H_5BrO_2$: C, 41.8; H, 2.5. Found: C, 41.8; H, 2.6. These data are in good agreement with literature values.[332]

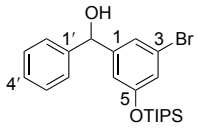
3-Bromo-5-((triisopropylsilyl)oxy)benzaldehyde **135**



To a solution of **133** (1.70 g, 8.46 mmol, 1.0 eq) and imidazole (1.44 g, 21.1 mmol, 2.5 eq) in anhydrous DMF (8 mL) at 0 °C under a nitrogen atmos-

phere was added TIPSCl (1.98 ml, 1.79 g, 9.31 mmol, 1.1 eq) dropwise. The solution was warmed to rt and stirred for 20 h, then diluted with H_2O (50 mL) and extracted with Et_2O (4 × 50 mL). The combined organic layers were washed with H_2O (2 × 200 mL), brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification though a short silica plug (eluent 3% Et_2O/petroleum ether) gave **135** as a pale yellow oil (2.90 g, 96%); R_f 0.30 (3% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 2945, 2868 (C–H) (m), 1704 (C=O) (s), 1590 (Ar C=C) (m), 1566 (Ar C=C) (m), 1445 (Ar C=C) (m), 1284 (C–O) (s), 1145 (m), 1004 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.11 (18 H, d, *J* = 7.5 Hz, Si(CH(C*H*$_3$)$_2$)), 1.24-1.33 (3H, m, Si(C*H*(CH$_3$)$_2$)$_3$), 7.27-7.30 (2H, m, C(2)*H*, C(6)*H*), 7.59 (1H, dd, *J* = 1.5, 1.5 Hz, C(4)*H*), 9.88 (1H, s, C*H*O); ^{13}C NMR (126 MHz, $CDCl_3$) 12.6 (Si(*C*H(CH$_3$)$_2$), 17.8 (Si(CH(*C*H$_3$)$_2$)$_3$), 118.6 (*C*(6)), 123.3 (*C*(3)), 125.8 (*C*(4)), 129.1 (*C*(2)), 138.7 (*C*(1)), 157.5 (*C*(5)), 190.5 (*C*HO); HRMS *m*/*z* (FI$^+$) found [M]$^+$ 356.0805, 358.0796; $C_{16}H_{25}{}^{79}BrO_2Si$ requires M^+ 356.0807, $C_{16}H_{25}{}^{81}BrO_2Si$ requires M^+ 358.0788. HPLC: Retention time 10.91 min, purity 93.4%.

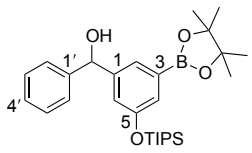
(*RS*)-(3-Bromo-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol **136**



To a solution of **135** (2.03 g, 5.68 mmol, 1.0 eq) in anhydrous THF (25 mL) at 0 °C under an argon atmosphere was added PhMgBr (0.9 M in THF, 6.9 mL, 6.2 mmol, 1.1 eq). The solution was stirred at 0 °C for 3.5 h, then warmed to rt and stirred for a further 3 h. Further PhMgBr (630 µL, 570 µmol, 0.1 eq) was added at 0 °C, and the reaction stirred at 0 °C for 1 h, after which time TLC analysis indicated complete consumption of **135**. The reaction was quenched with H_2O (50 mL), neutralised to pH7 with aq. HCl (1 M) and concentrated *in*

vacuo to remove THF. The residues were extracted with Et_2O (4 × 6 mL), and the combined organic extracts were washed with H_2O (200 mL) and brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (5% Et_2O/petroleum ether, then further purification of mixed fractions, 5% Et_2O/petroleum ether) gave **136** as a pale yellow oil (1.80 g, 73%); R_f 0.32 (20% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3348 (O–H) (br), 2945, 2867 (C–H) (m), 1595 (m), 1567 (m), 1443 (s), 1278 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.06 (18H, d, *J* = 7.3 Hz, Si(CH(C*H*$_3$)$_2$)$_3$), 1.16-1.25 (3H, m, Si(C*H*(CH$_3$)$_2$)$_3$), 2.2 (1H, br s, O*H*), 5.74 (1H, s, C*H*(OH)), 6.80 (1H, dd, *J* = 1.9, 1.2 Hz, C(6)*H*), 6.94 (1H, dd, *J* = 1.9, 1.9 Hz, C(4)*H*), 7.13 (1H, dd, *J* = 1.9, 1.2 Hz, C(2)*H*), 7.27-7.31 (1H, m, C(4´)*H*), 7.32-7.37 (4H, m, C(2´)*H*, C(3´)*H*, C(5´)*H*, C(6´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$), 12.5 (Si(*C*H(CH$_3$)$_2$)$_3$), 17.8 (Si(CH(*C*H$_3$)$_2$)$_3$), 75.5 (*C*H(OH)), 116.8 (*C*(6)), 122.1, 122.1 (*C*(2), *C*(4)), 122.5 (*C*(3)), 126.5 (*C*(2´), *C*(6´)) 127.9 (*C*(4´)), 128.6 (*C*(3´), *C*(5´)), 143.1 (*C*(1´)), 146.7 (*C*(1)), 156.9 (*C*(5)); HRMS *m*/*z* (ES$^+$) found $[M+Na]^+$ 457.1154, 459.1139; $C_{22}H_{31}{}^{79}BrNaO_2Si$ requires 457.1169, $C_{22}H_{31}{}^{81}BrNaO_2Si$ requires M^+ 459.1149; LRMS *m*/z (ES$^+$) 417 ($[^{79}M–OH]^+$, 62), 419 ($[^{81}M–OH]^+$, 100). Anal. Calcd. for $C_{22}H_{31}BrO_2Si$: C, 60.7; H, 7.2. Found: C, 60.6; H, 7.2.

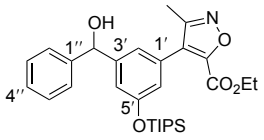
(*RS*)-Phenyl(3-(4,4,5,5-tetramethyl-1,3-dioxolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)methanol **137**



To a dry 10-20 mL microwave vial was added B_2pin_2 (975 mg, 3.84 mmol, 1.1 eq), KOAc (1.03 g, 10.5 mmol, 3.0 eq) and Pd(dppf) Cl_2·CH_2Cl_2 (143 mg,

175 μmol, 0.05 eq). The vial was sealed and purged with argon (5 × evacuate/fill). **136** (1.52 g, 3.49 mmol, 1.0 eq) in anhydrous degassed dioxane (15 mL) was added, and the suspension purged with argon for 5 min. The reaction was heated for 3 h at 80 °C, after which time TLC analysis indicated complete consumption of **136**. The reaction was cooled to rt and filtered through Celite® (eluent CH_2Cl_2) and concentrated *in vacuo*. The residues were purified by silica gel column chromatography (10%, 20%, 30% Et_2O/petroleum ether) to give **137** as an extremely viscous pale yellow gum (1.59 g, 97%); R_f 0.41 (40% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3468 (O–H) (br), 2945, 2867 (C–H) (m), 1586 (m), 1463 (m), 1425 (m), 1369 (s), 1258 (m), 1143 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.05 (18H, dd, *J* = 7.5, 3.0 Hz, Si(CH(C*H*$_3$)$_2$)$_3$), 1.15-1.24 (3H, m, Si(C*H*(CH$_3$)$_2$)$_3$), 1.34 (12H, s, OC(C*H*$_3$)$_2$C(C*H*$_3$)$_2$O), 2.24 (1H, d, *J* = 3.4 Hz, O*H*), 5.82 (1H, d, *J* = 3.4 Hz, C*H*(OH)), 6.92-6.95 (1H, m, C(2)*H*/C(6)*H*), 7.22-7.24 (1H, m, C(4)*H*), 7.24-7.28 (1H, m, C(4´)*H*), 7.30-7.35 (2H, m, C(3´)*H*, C(5´)*H*), 7.35-7.40 (2H, m, C(2´)*H*, C(6´)*H*), 7.43-7.45 (1H, m, C(6)*H*/C(2)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 12.6 (Si((*C*H(CH$_3$)$_2$)$_3$), 17.9 (Si((CH(*C*H$_3$)$_2$)$_3$), 24.8 (OC(*C*H$_3$)$_2$C(*C*H$_3$)$_2$O), 76.0 (*C*H(OH)), 83.8 (O*C*(CH$_3$)$_2$*C*(CH$_3$)$_2$O), 120.6 (*C*(2)/*C*(6)), 125.3 (*C*(4)), 125.3 (*C*(6)/*C*(2)), 126.5 (*C*(2´), *C*(6´)), 127.4 (*C*(4´)), 128.3 (*C*(3´), *C*(5´)), 143.7 (*C*(1)/*C*(1´)), 144.7 (*C*(1´)/*C*(1)), 155.8 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 505.2918, $C_{28}H_{43}BNaO_4Si$ requires M$^+$ 505.2921; LRMS *m*/*z* (ES$^+$) 465 ([M–OH]$^+$, 100), 505 ([M+Na]$^+$, 18%).

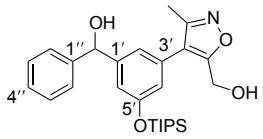
(*RS*)-Ethyl 4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazole-5-carboxylate **138**



To a 10-20 mL microwave vial was added **123** (350 mg, 1.50 mmol, 1.0 eq) and Pd(dppf)Cl_2·CH_2Cl_2 (60 mg, 73 μmol, 0.05 eq). The vial was sealed and purged with argon (5 × evacuate/fill). **137** (850 mg, 1.83 mmol, 1.2 eq) in degassed distilled DME (10 mL) was added, followed by degassed sat. aq. $NaHCO_3$ (3 mL), and the suspension was purged with argon for 5 min. The reaction mixture was heated for 2.5 h at 90 °C, then cooled to rt and filtered through Celite® (eluent CH_2Cl_2). The solution was dried ($MgSO_4$) and filtered. Purification by silica gel column chromatography (gradient elution, 2 → 15% acetone/petroleum ether) gave **138** as a pale yellow viscous gum (569 mg, 74%); R_f 0.25 (20% acetone/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3436 (O–H) (br), 2945, 2867 (C–H) (m), 1734 (C=O) (s), 1590 (s), 1454 (m), 1270 (s), 1190 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.07 (18H, d, *J* = 7.4 Hz, Si(CH(C*H*$_3$)$_2$)$_3$), 1.19 (4H, t, *J* = 7.1 Hz, OCH_2CH_3) overlapping 1.16-1.27 (3H, m, Si(C*H*$(CH_3)_2)_3$), 2.22 (3H, s, C(3)C*H*$_3$), 2.44 (1H, br s, O*H*), 4.24 (2H, q, *J* = 7.1 Hz, OCH_2CH_3), 5.81 (1H, s, C*H*(OH)), 6.71-6.74 (1H, m, C(6′)*H*), 6.91-6.94 (1H, m, C(2′)*H*), 6.94-6.97 (1H, m, C(4′)*H*), 7.25-7.30 (1H, m, C(4′′)*H*), 7.31-7.39 (4H, m, C(2′′)*H*, C(3′′)*H*, C(5′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.6 (C(3)*C*H_3), 12.6 (Si(*C*H$(CH_3)_2)_3$), 13.9 (OCH_2CH_3), 17.8 (Si(CH(*C*$H_3)_2)_3$), 61.8 (OCH_2CH_3), 75.7 (*C*H(OH)), 118.4 (*C*(4′)), 120.1 (*C*(6′)), 120.7 (*C*(2′)), 124.6 (*C*(4)), 126.6 (*C*(2′′), *C*(6′′)), 127.7 (*C*(4′′)), 128.5 (*C*(3′′), *C*(5′′)), 129.5 (*C*(1′)), 143.6 (*C*(1′′)), 145.5 (*C*(3′)), 154.7 (*C*(5)), 156.0 (*C*(5′)), 157.2 (*C*=O), 160.3 (*C*(3)); HRMS *m*/*z* (ES^+)

found $[M+Na]^+$ 532.2500; $C_{29}H_{39}NNaO_5Si$ requires 532.2490; LRMS *m*/*z* (ES^+) 510 ($[M+H]^+$, 23), 532 ($[M+Na]^+$, 100). HPLC: Retention time 16.02 min, purity 97.3%.

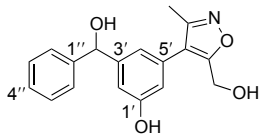
(*RS*)-(3-(5-(Hydroxymethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol **139**



To a solution of **138** (545 mg, 1.07 mmol) in anhydrous EtOH (4.5 mL) at 0 °C under an argon atmosphere was added $NaBH_4$ (101 mg, 2.67 mmol, 2.5 eq). The reaction was warmed slowly to rt over 16 h, after which time TLC analysis indicated complete consumption of **138**. The reaction was quenched with H_2O (10 mL) and aq. HCl (1 M, 5 mL), then concentrated *in vacuo* to remove EtOH. The mixture was extracted with EtOAc (4 × 10 mL), and the combined organic layers were washed with brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 25% acetone/petroleum ether) gave **139** as a colourless viscous gum (443 mg, 89%); R_f 0.34 (30% acetone/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3355 (O–H) (br), 2945, 2867 (C–H) (m), 1589 (s), 1453 (m), 1324 (s), 1227 (m), 1003 (s); ^1H NMR (500 MHz, $CDCl_3$) 1.07 (18 H, d, *J* = 7.5 Hz, Si(CH(C*H*$_3$)$_2$)$_3$), 1.17-1.26 (3H, m, Si(C*H*(CH$_3$)$_2$)$_3$), 2.25 (3H, s, C(3)C*H*$_3$), 2.58 (1H, br s, CH$_2$O*H*), 2.73 (1H, br s, CH(O*H*)), 4.62 (2H, d, *J* = 4.1 Hz, C*H*$_2$OH), 5.80 (1H, s, C*H*(OH)), 6.71-6.74 (1H, m, C(4′)*H*), 6.87-6.90 (1H, m, C(6′)*H*), 6.90-6.94 (1H, m, C(2′)*H*), 7.25-7.30 (1H, m, C(4′′)*H*), 7.32-7.38 (4H, m, C(2′′)*H*, C(3′′)*H*, C(5′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H$_3$), 12.6 (Si(*C*(H(CH$_3$)$_2$)$_3$), 17.8

(Si(CH(*C*H$_3$)$_2$)$_3$), 54.8 (*C*H$_2$OH), 75.7 (*C*H(OH)); 117.9 (*C*(6′)), 118.1 (*C*(4)), 119.4 (*C*(4′)), 120.0 (*C*(2′)), 126.5 (*C*(2′′), *C*(6′′)), 127.7 (*C*(4′′)), 128.5 (*C*(3′′), *C*(5′′)), 130.5 (*C*(3′)), 143.5 (*C*(1′′)), 146.1 (*C*(1′)), 156.5 (*C*(5′)), 158.8 (*C*(3)), 165.9 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 490.2364; $C_{27}H_{37}NNaO_4Si$ requires M$^+$ 490.2384; LRMS *m*/*z* (ES$^+$) 468 ([M+H]$^+$, 100), 492 ([M+Na]$^+$, 90). HPLC: Retention time 16.08 min, purity 96.0%.

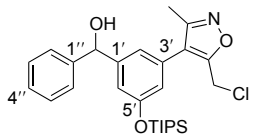
(*RS*)-3-(Hydroxy(phenyl)methyl)-5-(5-(hydroxymethyl)-3-methylisoxazol-4-yl)phenol **129a**



To a solution of **139** (56 mg, 120 μmol, 1.0 eq) in anhydrous THF (2 mL) at 0 °C under an argon atmosphere was added TBAF (1 M in THF, 120 μL, 120 μmol, 1.0 eq). The solution was stirred for 40 min at 0 °C, after which time TLC analysis indicated complete consumption of **139**. Celite® was added, and the solution concentrated *in vacuo*. Purification by repeated silica gel column chromatography (40, 60, 80, 100% acetone/petroleum ether, then 2.5% MeOH/CH_2Cl_2) gave **129a** as a colourless viscous gum (34 mg, 91%); R_f 0.17 (5% MeOH/CH_2Cl_2); ν_{max} (thin film)/cm^{-1}: 3295 (O–H) (br), 2932 (C–H) (w), 1691 (m), 1594 (s), 1323 (m), 1022 (s); ^{1}H NMR (500 MHz, acetonitrile-d_3) 2.25 (3H, s, C(3)C*H*$_3$), 3.60 (1H, br s, CH$_2$O*H*), 3.93 (1H, br s, CH(O*H*)), 4.57 (2H, s, C*H*$_2$OH), 5.78 (1H, s, C*H*(OH)), 6.72-6.76 (1H, m, C(6′)*H*), 6.87-6.91 (1H, m, C(2′)*H*), 6.92-6.96 (1H, m, C(4′)*H*), 7.25-7.30 (1H, m, C(4′′)*H*), 7.33-7.39 (2H, m, C(3′′)*H*, C(5′′)*H*), 7.41-7.46 (2H, m, C(2′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, acetonitrile-d_3) 10.0 (C(3)*C*H$_3$), 53.8 (*C*H$_2$OH), 74.8 (*C*H(OH)), 112.5 (*C*(2′)), 114.6 (*C*(6′)), 117.5

(*C*(4)) 118.6 (*C*(4′)), 126.3 (*C*(2′′), *C*(6′′)), 127.2 (*C*(4′′)), 128.3 (*C*(3′′), *C*(5′′)), 130.9 (*C*(5′)), 144.9 (*C*(1′′)), 147.6 (*C*(3′)), 157.2 (*C*(1′)), 158.6 (*C*(3)), 166.7 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 334.1058; $C_{18}H_{17}NNaO_4$ requires M^+ 334.1050; *m*/*z* (ES^-) 310 ($[M–H]^-$, 100), 621 ($[2M–H]^-$, 50). HPLC: Retention time 9.73 min, purity 98.5%.

(*RS*)-(3-(5-(Chloromethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol **143**

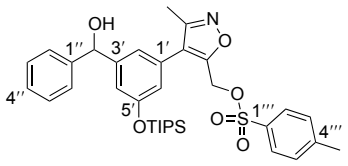


To a solution of **139** (54 mg, 115 μmol, 1.0 eq) and NEt_3 (24 μL, 17 mg, 170 μmol, 1.5 eq) in anhydrous CH_2Cl_2 (0.5 mL) at 0 °C under an argon atmosphere was added TsCl (28 mg, 140 μmol, 1.25 eq), and the solution was warmed to rt over 16 h. The reaction was then diluted with CH_2Cl_2 (10 mL) and washed with aq. HCl (1 M, 5 mL) and brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 40% EtOAc/40-60 °C petroleum ether) gave **143** as a colourless gum (35 mg, 63%) which was used immediately in the next reaction; R_f 0.62 (40% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3393 (O–H) (br), 2945, 2867 (C–H) (m), 1632 (s), 1452 (m), 1326 (s), 1220 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.09 (18H, d, *J* = 7.4 Hz, Si(CH(C*H*$_3$)$_2$)$_3$), 1.19-1.30 (3H, m, Si(C*H*(CH$_3$)$_2$)$_3$), 2.27 (3H, s, C(3)C*H*$_3$), 4.52 (2H, s, C*H*$_2$Cl), 5.83 (1H, d, *J* = 3.3 Hz, C*H*(OH)), 6.75-6.77 (1H, m, C(6′)*H*), 6.91-6.94 (1H, m, C(2′)*H*), 6.96-6.99 (1H, m, C(4′)*H*), 7.28-732 (1H, m, C(4′′)*H*), 7.34-7.41 (4H, m, C(2′′)*H*, C(3′′)*H*, C(5′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$), 10.8 (C(3)*C*H$_3$), 12.6 (Si(*C*H(CH$_3$)$_2$)$_3$), 17.8 (Si(CH(*C*H$_3$)$_2$)$_3$),

33.6 (*C*H_2Cl), 75.8 (*C*H(OH)), 118.2(*C*(6′)), 119.1 (*C*(4)), 119.3 (*C*(4′)), 119.7 (*C*(2′)), 126.4 (*C*(2′′), *C*(6′′)), 127.9 (*C*(4′′)), 128.6 (*C*(3′′), *C*(5′′)), 130.0 (*C*(3′)), 143.5 (*C*(1′′)), 146.3 (*C*(1′)), 156.7 (*C*(5′)), 158.9 (*C*(3)), 162.3 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 508.2053; $C_{27}H_{36}ClNNaO_3Si$ requires M^+ 508.2045; LRMS *m*/*z* (ES^+) 486 ($[M+H]^+$, 90), 524 ($[M+K]^+$, 100).

Further elution gave starting material **139** (2 mg, 4%).

(*RS*)-(4-(3-(Hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl 4-methylbenzenesulfonate **144**

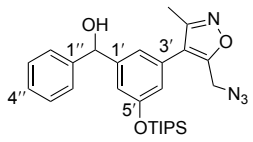


To a solution of **139** (51 mg, 110 μmol, 1.0 eq) and NEt_3 (23 μL, 17 mg, 170 μmol, 1.5 eq) in anhydrous CH_2Cl_2 (0.5 mL) at 0 °C under an argon atmosphere was added TsCl (26 mg, 140 μmol, 1.25 eq). The solution was warmed to rt and stirred for 2 h. The reaction was then diluted with CH_2Cl_2 (10 mL) and washed with aq. NH_4Cl (20% w/v, 10 mL) and brine (2 × 10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 5 → 40% EtOAc/40-60 °C petroleum ether) gave chloride **143** as a colourless gum (9 mg, 17%). Further elution gave tosylate **144** (37 mg, 55%) which was used immediately in the next reaction; R_f 012 (20% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3412 (O–H) (br), 2945, 2867 (C–H) (m), 1590 (m), 1375 (m), 1325 (m), 1190 (s), 1178 (s) (S=O); ^{1}H NMR (500 MHz, $CDCl_3$) 1.08 (18H, d, *J* = 7.3 Hz, Si(CH(C*H*$_3$)$_2$)$_3$), 1.17-1.28 (3H, m, Si(C*H*(CH$_3$)$_2$)$_3$), 2.23 (3H, s, C(3)C*H*$_3$), 2.44 (3H, s, C(4′′′)C*H*$_3$) overlapping 2.47 (1H, br s, O*H*), 5.05 (2H, s, C*H*$_2$OTs), 5.82 (1H, s, C*H*(OH)), 6.63-6.66 (1H, m,

C(6′)*H*), 6.88-6.91 (1H, m C(2′)*H*), 6.91-6.94 (1H, m, C(4′)*H*), 7.27-7.32 (3H, m, C(4′′)*H*, C(3′′′)*H*, C(5′′′)*H*), 7.34-7.42 (4H, m, C(2′′)*H*, C(3′′)*H*, C(5′′)*H*), C(6′′)*H*), 7.73-7.77 (2H, m, C(2′′′)*H*, C(6′′′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.6 (C(3)*C*H_3), 12.6 (Si(*C*H(CH_3)$_2$)$_3$), 17.8 (Si(CH(*C*H_3)$_2$)$_3$), 21.7 (C(4′′′)*C*H_3), 59.9 (*C*H_2OTs), 75.7 (*C*H(OH)), 118.4 (*C*(4′)), 119.3 (*C*(6′)), 119.9 (*C*(2′)), 121.4 (*C*(4)), 126.5 (*C*(2′′), *C*(6′′)), 127.8 (*C*(4′′)),128.0 (*C*(2′′′), *C*(6′′′)), 128.6 (*C*(3′′), *C*(5′′)), 129.4 (*C*(1′)), 129.9 (*C*(3′′′), *C*(5′′′)), 132.4 (*C*(1′′′)), 143.5 (*C*(1′′)), 145.2 (*C*(4′′′)), 146.5 (*C*(3′)), 156.6 (*C*(5′)), 158.9 (*C*(3)), 159.1 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 644.2487; $C_{34}H_{43}NNaO_6SSi$ requires M$^+$ 644.2473; LRMS *m*/*z* (ES$^+$) 604 ([M–OH]$^+$, 38), 622 ([M+H]$^+$, 100), 644 ([M+Na]$^+$, 30).

Further elution gave starting material **139** (9 mg, 18%).

(*RS*)-(3-(5-(Azidomethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol **140**

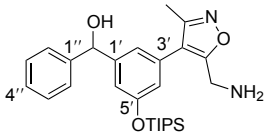


Method A: To a solution of **143** (49 mg, 100 µmol, 1.0 eq) in anhydrous DMF (0.4 mL) under an argon atmosphere was added NaN_3 (7 mg, 110 µmol, 1.1 eq). After 2 h at rt, TLC analysis indicated complete consumption of **143**. The reaction was diluted with H_2O (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (20 mL) and brine (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **140** as a pale brown oil (49 mg, 99%); R_f 0.62 (40% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3402 (O–H) (br), 2945, 2867 (C–H) (m), 2099 (N_3) (s), 1590 (s), 1453 (m), 1323 (m), 1223 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.08 (18H, d, *J* = 7.3 Hz,

Si(CH(C*H*$_3$)$_2$)$_3$), 1.18-1.28 (3H, m, Si(C*H*(CH$_3$)$_2$)$_3$), 2.27 (3H, s, C(3)C*H*$_3$), 2.32 (1H, s, O*H*), 4.33 (2H, s, C*H*$_2$N$_3$), 5.82 (1H, s, C*H*(OH)), 6.68-6.71 (1H, m, C(4´)*H*), 6.85-6.88 (1H, m, C(2´)*H*), 6.94-6.97 (1H, m, C(6´)*H*), 7.27-7.32 (1H, m, C(4´´)*H*), 7.33-7.40 (4H, m, C(2´´)*H*, C(3´´)*H*, C(5´´)*H*), C(6´´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H$_3$), 12.6 (Si(*C*H(CH$_3$)$_2$)$_3$), 17.8 (Si(CH(*C*H$_3$)$_2$)$_3$), 44.1 (*C*H$_2$N$_3$), 75.7 (*C*H(OH)), 118.1 (*C*(6´)), 119.3 (*C*(4)), 119.5 (*C*(4´)), 119.9 (*C*(2´)), 126.5 (*C*(2´´), *C*(6´´)), 127.9 (*C*(4´´)), 128.6 (*C*(3´´), *C*(5´´)), 129.9 (*C*(3´)), 143.5 (*C*(1´´)), 146.2 (*C*(1´)), 156.6 (*C*(5´)), 159.0 (*C*(3)), 161.8 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 515.2449; $C_{27}H_{36}N_4NaO_3Si$ requires 515.2449; LRMS *m*/*z* (ES$^+$) 494 ([M+H]$^+$, 100), 515 ([M+Na]$^+$, 75).

Method B: To a solution of **144** (23 mg, 37 μmol, 1.0 eq) in anhydrous DMF (0.2 mL) at 0 ˚C under an argon atmosphere was added NaN_3 (2.4 mg, 37 μmol, 1.0 eq). After 1 h at 0 ˚C, TLC analysis indicated complete consumption of **144**. The reaction was diluted with H_2O (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with H_2O (2 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **140** as a pale brown oil (15 mg, 82%). The data are identical to those reported above.

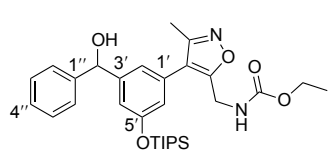
(*RS*)-(3-(5-(Aminomethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol **145**



Following the procedure of Pei and Wickham,[239] to a solution of **140** (27 mg, 55 μmol, 1.0 eq) in ^{i}PrOH (0.2 mL) was added NEt_3 (15 μL, 11 mg, 110 μmol, 2.0 eq), 1,3-propanedithiol (0.6 μL, 0.6 mg, 6 μmol, 0.1 eq) and $NaBH_4$,

(3 mg, 80 μmol, 2 eq). After 16 h, TLC analysis indicated complete consumption of **140**, and the reaction mixture was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (4 × 10 mL). The combined organic layers were washed with brine (40 ml) and dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (2% MeOH/CH_2Cl_2) gave **145** as a pale yellow oil (22 mg, 86%); R_f 0.20 (5% MeOH/CH_2Cl_2); ν_{max} (thin film)/cm^{-1}: 3373, 3308 (N–H, O–H) (br); 2945, 2867 (C–H) (s), 1589 (s), 1466 (m), 1451 (m), 1321 (s), 1008 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.08 (18H, d, J = 7.2 Hz, Si(CH(CH_3)$_2$)$_3$), 1.17-1.28 (3H, m, Si(CH(CH_3)$_2$)$_3$), 1.8 (3H, br s, OH, NH_2), 2.23 (3H, s, C(3)CH_3) 3.88 (2H, s, CH_2NH$_2$), 5.82 (1H, s, CH(OH)), 6.65-6.68 (1H, m, C(4´)H), 6.81-6.85 (1H, m, C(2´)H), 6.90-6.93 (1H, m, C(6´)H), 7.27-7.31 (1H, m, C(4´´)H), 7.33-7.40 (4H, m, C(2´´)H, C(3´´)H, C(5´´)H, C(6´´)H); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)CH$_3$), 12.6 (Si(CH(CH$_3$)$_2$)$_3$), 17.8 (Si(CH(CH_3)$_2$)$_3$), 37.1 (CH$_2$NH$_2$), 75.7 (CH(OH)), 116.4 (C(4)), 117.7 (C(6´)), 119.5 (C(4´)), 120.0 (C(2´)), 126.5 (C(2´´), C(6´´)), 127.8 (C(4´´)), 128.6 (C(3´´), C(5´´)), 130.9 (C(3´)), 143.6 (C(1´´)), 146.1 (C(1´)), 156.5 (C(5´)), 158.8 (C(3)), 168.2 (C(5)); HRMS m/z (ES$^+$) found [M+H]$^+$ 467.2725; $C_{27}H_{39}N_2O_3Si$ requires M$^+$ 467.2724; LRMS m/z (ES$^+$) 449 ([M–OH]$^+$, 100), 467 ([M+H]$^+$, 17), 489 ([M+Na]$^+$, 12).

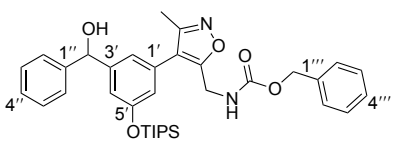
(*RS*)-Ethyl ((4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate **146**



To a solution of **145** (21 mg, 45 μmol) in anhydrous CH_2Cl_2 (1 mL) under an argon atmosphere was added NEt_3 (8 μL, 6 mg, 60 μmol, 1.3 eq) and ethyl

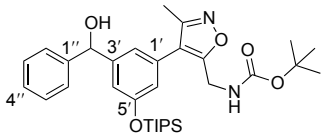
chloroformate (5 µL, 6 mg, 50 µL, 1.2 eq). After 4 h, TLC analysis indicated complete consumption of **145**. The reaction was diluted with 15 mL CH_2Cl_2, washed with aq. citric acid (10% w/v, 15 mL), sat. aq. $NaHCO_3$ (15 mL) and brine (15 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 7 → 40% EtOAc/40-60 °C petroleum ether) gave **146** as a colourless oil (18 mg, 74%); R_f 0.30 (30% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3326 (O–H, N–H) (br), 2945, 2876 (C–H) (m), 1700 (C=O) (s), 1589 (s), 1463 (m), 1323 (m), 1250 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.06 (18H, d, *J* = 7.3 Hz, Si(CH(C*H*$_3$)$_2$)$_3$), 1.16-1.28 (6H, m, Si(C*H*(CH$_3$)$_2$)$_3$, CH$_2$C*H*$_3$), 2.24 (3H, s, C(3)C*H*$_3$), 3.07 (1H, s, O*H*), 4.11 (2H, q, *J* = 7.1 Hz, C*H*$_2$CH$_3$), 4.46 (2H, d, *J* = 6.1 Hz, C*H*$_2$NH), 5.02-5.16 (1H, m, N*H*), 5.80 (1H, s, C*H*(OH)), 6.68 (1H, s, C(6´)*H*), 6.83 (C(4´)*H*), 6.95 (C(2´)*H*), 7.25-7.30 (1H, m, C(4´´)*H*), 7.31-7.41 (4H, m, C(2´´)*H*, C(3´´)*H*, C(5´´)*H*, C(6´´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*(H$_3$)), 12.6 (Si(*C*H(CH$_3$)$_2$)$_3$), 14.5 (CH$_2$*C*H$_3$), 17.8 (Si(CH(*C*H$_3$)$_2$)$_3$), 36.0 (*C*H$_2$NH), 61.4 (O*C*H$_2$CH$_3$), 75.7 (*C*H(OH)), 117.6 (*C*(4)), 118.1 (*C*(4´)), 119.2 (*C*(6´)), 120.2 (*C*(2´)), 126.5 (*C*(2´´), C(6´´)), 127.6 (*C*(4´´)), 128.4 (*C*(3´´), *C*(5´´)), 130.1 (*C*(1´)), 143.6 (*C*(1´´)), 146.4 (*C*(3´)), 156.2 (*C*=O), 156.4 (*C*(5´)), 158.9 (*C*(3)), 163.8 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 561.2742; $C_{30}H_{42}N_2NaO_5Si$ requires M$^+$ 561.2755; LRMS *m*/*z* (ES$^+$) 521 ([M–OH]$^+$, 35), 539 [M+H]$^+$, 100).

(*RS*)-Benzyl ((4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate **147**



To a solution of **145** (15 mg, 32 μmol, 1.0 eq) in anhydrous CH_2Cl_2 (0.2 mL) under an argon atmosphere was added NEt_3 (5.5 μL, 4.0 mg, 39 μmol, 1.2 eq) and benzyl chloroformate (5.5 μL, 6.0 mg, 39 μmol, 1.2 eq). After 3.5 h, TLC analysis indicated complete consumption of **145**, and the reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with aq. citric acid (10% w/v, 10 mL), H_2O (10 mL) and brine (10 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (15%, 25% EtOAc/petroleum ether) gave **147** as a colourless oil (12 mg, 62%); R_f 0.33 (30% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3413, 3226 (N–H, O–H) (br), 2945, 2867 (C–H) (m), 1702 (C=O) (s), 1589 (m), 1454 (m), 1324 (m),1257 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.07 (18H, d, *J* = 7.3 Hz, OSi(CH(C*H*$_3$)$_2$)$_3$), 1.15-1.25 (3H, m, OSi(C*H*(CH$_3$)$_2$)$_3$), 2.25 (3H, s, C(3)C*H*$_3$), 2.90 (1H, s, O*H*), 4.49 (2H, d, *J* = 6.0 Hz, C*H*$_2$NH), 5.10 (2H, s, OC*H*$_2$), 5.16 (1H, t, *J* = 6.0 Hz, CH$_2$N*H*), 5.79 (1H, s, C*H*(OH)), 6.69 (1H s, C(6´)*H*), 6.84 (1H, s, C(4´)*H*), 6.94 (1H, s, C(2´)*H*), 7.24-7.41 (10 H, m, 2 × *Ph*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H$_3$), 12.6 (Si(*C*H(CH$_3$)$_2$)$_3$), 17.8 (Si(CH(*C*H$_3$)$_2$)$_3$), 36.1 (*C*H$_2$NH), 67.2 (*C*H$_2$O), 75.7 (C*H*(OH)),117.7 (*C*(4)), 118.1 (*C*(4´)), 119.2 (*C*(6´)), 120.2 (*C*(2´)), 126.5, 127.6, 128.16, 128.23, 128.47, 128.52 (2 × Ph *C*H), 130.1 (*C*(1´)), 136.0 (*C*(1´´´)), 143.5 (*C*(1´´)), 146.4 (*C*(3´)), 156.0 (*C*=O), 156.4 (*C*(5´)), 158.9 (*C*(3)), 163.6 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 623.2913; $C_{35}H_{44}N_2NaO_5Si$ requires M$^+$ 623.2912; LRMS *m*/*z* (ES$^+$) 601 ([M+H]$^+$, 100), 623 ([M+Na]$^+$, 91).

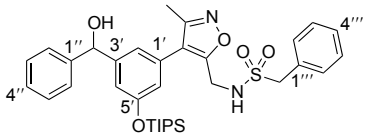
(*RS*)-*tert*-Butyl ((4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate **148**



To a solution of **145** (15 mg, 32 μmol, 1.0 eq) in anhydrous CH_2Cl_2 (0.2 mL) under an argon atmosphere was added NEt_3 (5.5 μL, 4.0 mg, 39 μmol, 1.2 eq) and Boc_2O (9 mg, 40 μmol, 1.3 eq). After 2 h, TLC analysis indicated complete consumption of **145**, and the reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with aq. citric acid (10% w/v, 10 mL), H_2O (10 mL) and brine (10 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (15%, 20% EtOAc/40-60 °C petroleum ether) gave **148** as a colourless oil (17 mg, 94%); R_f 0.29 (30% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3350 (O–H, N–H) (br), 2945, 2867 (C–H) (m), 1699 (C=O) (s), 1590 (s), 1454 (m), 1323 (m), 1249 (s), 1166 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.07 (18H, d, *J* = 7.4 Hz, OSi(CH(C*H*$_3$)$_2$)$_3$), 1.16-1.26 (3H, m, OSi(C*H*(CH$_3$)$_2$)$_3$), 1.43 (9H, s, C(C*H*$_3$)$_3$), 2.25 (3H, s, C(3)*C*H$_3$), 3.00 (1H, s, CH(O*H*)), 4.41 (2H, t, *J* = 4.7 Hz, C*H*$_2$NH), 4.95 (1H, s, N*H*), 5.80 (1H, d, *J* = 1.7 Hz, C*H*(OH)), 6.69 (1H, s, C(6′)*H*), 6.83 (1H, s, C(4′)*H*), 6.98 (1H, s, C(2′)*H*), 7.24-7.30 (1H, s, C(4′′)*H*), 7.32-7.37 (2H, m, C(3′′)*H*, C(5′′)*H*), 7.37-7.42 (2H, m, C(2′′)*H*, C(6′′)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H$_3$), 12.6 (OSi(*C*H(CH$_3$)$_2$)$_3$), 17.8 (OSi(CH(*C*H$_3$)$_2$)$_3$), 28.3 (C(*C*H$_3$)$_3$), 35.6 (*C*H$_2$NH), 75.8 (*C*H(OH)), 80.3 (O*C*(CH$_3$)$_3$), 117.4 (*C*(4)), 118.0 (*C*(4′)), 119.2 (*C*(6′)), 120.2 (*C*(2′)), 126.5 (*C*(2′′), *C*(6′′)), 127.6 (*C*(4′′)), 128.4 (*C*(3′′), *C*(5′′)), 130.3 (*C*(1′)), 143.6 (*C*(1′′)), 146.4 (*C*(3′)), 155.3 (*C*=O), 156.4 (*C*(5′)), 158.8 (*C*(3)), 164.2 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 589.3072; $C_{32}H_{46}N_2NaO_5Si$ requires M$^+$

589.3068; LRMS *m*/*z* (ES^+) 549 ($[M–OH]^+$, 100), 567 ($[M+H]^+$, 38), 589 ($[M+Na]^+$, 59).

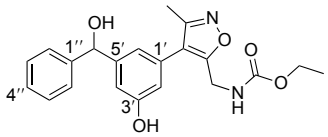
(*RS*)-*N*-((4-(3-(Hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)-1-phenylmethanesulfonamide **149**



To a solution of **145** (15 mg, 32 μmol, 1.0 eq) in anhydrous CH_2Cl_2 (0.2 mL) under an argon atmosphere was added NEt_3 (5.5 μL, 4.0 mg, 39 μmol, 1.2 eq) and phenylmethanesulfonyl chloride (7.5 mg, 39 μmol, 1.2 eq). After 5 h, further phenylmethanesulfonyl chloride (2 mg, 10 μmol, 0.3 eq) was added, and after a further 16 h, more phenylmethanesulfonyl chloride (2 mg, 10 μmol, 0.3 eq) and DMAP (0.5 mg, 4 μmol, 0.1 eq) were added. After a further 21 h, TLC analysis indicated complete consumption of **145**, and the reaction was diluted with CH_2Cl_2 (10 mL) and aq. citric acid (10% w/v, 10 mL). The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with H_2O (30 mL) and brine (30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (20%, 30% EtOAc/petroleum ether) gave **149** as a colourless oil (13 mg, 65%); R_f 0.19 (30% EtOAc/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 3278 (O–H, N–H) (br), 2965, 2867 (C–H) (m), 1589 (m), 1455 (m), 1330 (S=O) (s), 1152 (S=O) (s); ^1H NMR (500 MHz, $CDCl_3$) 1.07 (18H, d, *J* = 7.3 Hz, OSi(CO(C*H*$_3$)$_2$)$_3$), 1.16-1.25 (3H, m, OSi(C*H*(CH$_3$)$_2$)$_3$), 2.26 (3H, s, C(3)C*H*$_3$), 2.60 (1H, br s, O*H*), 4.20 (2H, s, C*H*$_2$Ph), 4.26 (2H, d, *J* = 6.0 Hz, C*H*$_2$NH), 4.63 (1H, t, *J* = 6.0 Hz, CH$_2$N*H*), 5.80 (1H, s, C*H*(OH)), 6.59-6.62 (1H, m, C(6′)*H*), 6.82-6.84

(1H, m, C(2′)*H*), 6.88-6.91 (1H, m, C(4′)*H*), 7.24-7.39 (10H, m, 2 × *Ph*); ^{13}C NMR (126 MHz, $CDCl_3$) 10.7 (C(3)*C*H_3), 12.6 (OSi(*C*H(CH$_3$)$_2$)$_3$), 17.8 (OSi(CH(*C*H$_3$)$_2$)$_3$), 37.8 (*C*H_2NH), 59.6 (*C*H_2Ph), 75.6 (*C*H(OH)), 118.2 (*C*(4′)), 118.4 (*C*(4)), 119.2 (*C*(6′)), 120.0 (*C*(2′)), 126.5, 127.8, 128.6, 128.72, 128.78, 128.80 (2 × Ph *C*H), 129.7 (*C*(1′)), 130.6 (*C*(1′′′)), 143.5 (*C*(1′′)), 146.5 (*C*(3′)), 156.6 (*C*(5′)), 159.1 (*C*(3)), 162.5 (*C*(5)); HRMS *m*/*z* (ES$^+$) found $[M+Na]^+$ 643.2637; $C_{34}H_{44}N_2NaO_5SSi$ requires M^+ 643.2632; LRMS *m*/*z* (ES$^+$) 643 ($[M+Na]^+$, 100).

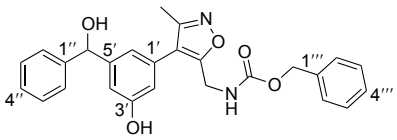
(*RS*)-Ethyl ((4-(3-hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate **129b**



To a solution of **146** (16 mg, 30 μmol, 1.0 eq) in anhydrous THF (0.2 mL) at 0 °C under an argon atmosphere was added TBAF (1 M in THF, 33 μL, 33 μmol, 1.1 eq). After 30 min, TLC analysis indicated complete consumption of **146**, and the mixture was concentrated *in vacuo*. Purification by repeated silica gel column chromatography (20, 30, 40% acetone/40-60 °C petroleum ether, then 5, 10% acetone/CH_2Cl_2) gave a colourless gum, which was precipitated from a solution in CH_2Cl_2 by addition of hexane to give **129b** as a colourless solid (6 mg, 52%); R_f 0.22 (15% acetone/CH_2Cl_2); ν_{max} (thin film)/cm^{-1}: 3309 (O–H, N–H) (br), 3028, 2986 (C–H) (w), 1696 (C=O) (s), 1595 (m), 1262 (s); ^{1}H NMR (500 MHz, acetone-d_6) 1.19 (3H, t, *J* = 6.9 Hz, CH_2CH_3), 2.21 (3H, s, C(3)C*H*$_3$), 3.92 (1H, d, *J* = 4.0 Hz, CH(O*H*)), 4.05 (2H, q, *J* = 6.9 Hz, C*H*$_2$CH$_3$), 4.38 (2H, d, *J* = 6.0 Hz, C*H*$_2$NH), 5.77 (1H, d, *J* = 4.0 Hz, C*H*(OH)), 5.97 (1H, br s, N*H*), 6.72 (1H, s, C(2′)*H*), 6.85-6.88 (1H, m, C(4′)*H*), 6.91 (1H, m, C(6′)*H*), 7.17 (1H, br s,

C(3′)O*H*), 7.25-7.30 (1H, m, C(4′′)*H*), 7.33-7.39 (2H, m, C(3′′)*H*, C(5′′)*H*), 7.41-7.46 (2H, m, C(2′′), C(6′′)*H*); ^{13}C NMR (126 MHz, acetone-d_6) 9.9 (C(3)*C*H_3), 13.9 (CH_2*C*H_3), 35.7 (*C*H_2NH), 60.8 (*C*$H_2$$CH_3$), 74.9 (*C*H(OH)), 112.5 (*C*(4′)), 114.7 (*C*(2′)), 117.0 (*C*(4)), 118.8 (*C*(6′)), 130.7 (*C*(1′)), 144.9 (*C*(1′′)), 147.5 (*C*(5′)), 156.4 (*C*=O), 157.2 (*C*(3′)), 158.8 (*C*(3)), 164.7 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 405.1402, $C_{21}H_{22}N_2NaO_5$ requires M^+ 405.1421; LRMS *m*/*z* (ES^+) 365 ($[M–OH]^+$, 48), 383 ($[M+H]^+$, 43), 405 ($[M+Na]^+$, 100). HPLC: Retention time 10.68 min, purity 97.5%.

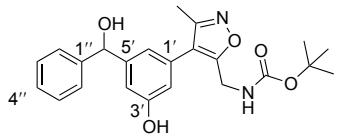
(*RS*)-Benzyl ((4-(3-hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate **129c**



To a solution of **147** (10 mg, 17 μmol, 1.0 eq) in anhydrous THF (0.2 mL) at 0 °C under an argon atmosphere was added TBAF (1 M in THF, 20 μL, 20 μmol, 1.2 eq). After 1 h, TLC analysis indicated complete consumption of **147**, and the mixture was concentrated *in vacuo*. Purification by repeated silica gel column chromatography (10%, 15% acetone/CH_2Cl_2 then 45% EtOAc/40-60°C petroleum ether) gave a colourless gum which was precipitated from a solution in CH_2Cl_2 by addition of hexane to give **129c** as a colourless solid (5 mg, 66%); R_f 0.21 (50% EtOAc/40-60 °C petroleum ether); mp 63-66 °C (CH_2Cl_2/hexane); ν_{max} (solid)/cm^{-1}: 3312 (O–H, N–H) (br), 3063, 3031 (CH) (w), 2161 (m), 2034 (m), 1977 (m, 1698 (C=O) (s), 1594 (m), 1453 (m), 1261 (C–O) (s); ^{1}H NMR (500 MHz, acetone-d_6) 2.21 (3H, s, C(3)C*H*$_3$), 4.50 (2H, d, *J* = 6.0 Hz, C*H*$_2$NH), 4.89 (1H, d, *J* = 3.9 Hz, CH(O*H*)), 5.10 (2H, s, C*H*$_2$Ph), 5.83 (1H, d, *J* = 3.9 Hz, C*H*(OH)), 6.79

(1H, s, C(2´)*H*), 6.89-7.04 (3H, m, C(4´)*H*, C(6´)*H*, CH_2N*H*), 7.20-7.52 (10H, m, 2 × *Ph*), 8.49 (1H, s, C(3´)O*H*); ^{13}C NMR (126 MHz, acetone-d_6) 10.7 (C(3)*C*H_3), 36.8 (*C*H_2NH), 66.9 (*C*H_2Ph), 75.9 (*C*H(OH)), 113.7 (*C*(4´)), 115.5 (*C*(2´)), 118.1 (*C*(4)), 119.7 (*C*(6´)), 127.3, 127.8, 128.70, 128.75, 129.0, 129.2 (2 × Ph C*H*), 131.4 (*C*(1´)), 138.1 (*C*(1´´´)), 146.3 (*C*(1´´)), 148.8 (*C*(5´)), 157.2 (*C*=O), 158.5 (*C*(3´)), 159.2 (*C*(3)), 165.3 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 467.1577; $C_{26}H_{24}N_2NaO_5$ requires M^+ 467.1577; LRMS *m*/*z* (ES^+) 427 ($[M–OH]^+$, 29), 445 ($[M+H]^+$, 73), 467 ($[M+Na]^+$, 100); HPLC: retention time 11.67 min, purity 93.3%.

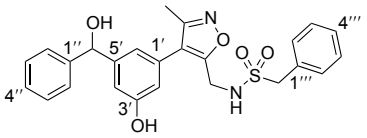
(*RS*)-*tert*-Butyl ((4-(3-hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate **129d**



To a solution of **148** (17 mg, 30 μmol, 1.0 eq) in anhydrous THF (0.2 mL) at 0 °C under an argon atmosphere was added TBAF (1 M in THF, 36 μL, 36 μmol, 1.2 eq). After 1 h, TLC analysis indicated complete consumption of **148**, and the mixture was concentrated *in vacuo*. Purification by silica gel column chromatography (10% acetone/CH_2Cl_2) gave a colourless gum which was precipitated from a solution in $CHCl_3$ by addition of hexane to give **129d** as a colourless solid (12 mg, 97%); R_f 0.16 (10% acetone/CH_2Cl_2); mp 90-92 °C ($CHCl_3$/hexane); ν_{max} (solid)/cm^{-1}: 3411, 3262 (N–H, O–H) (br), 2985, 2935 (C–H) (w), 2162 (m), 2035 (m), 1978 (m), 1678 (C=O) (s), 1594 (m), 1512 (s), 1163 (s); ^{1}H NMR (500 MHz, acetone-d_6) 1.42 (9H, s, C(C*H*$_3$)$_3$), 2.21 (3H, s, C(3)C*H*$_3$), 4.42 (2H, d, *J* = 6.0 Hz, C*H*$_2$NH), 4.89 (1H, d, *J* = 4.0 Hz, CH(O*H*)), 5.83 (1H, d, *J* = 4.0 Hz, C*H*(OH)), 6.55 (1H, br s, CH_2N*H*), 6.77 (1H, s, C(2´)*H*), 6.93-6.97 (1H,

m, C(4′)*H*), 7.01 (1H, s, C(6′)*H*), 7.20-7.26 (1H, m, C(4′′)*H*), 7.30-7.36 (2H, m, C(3′′)*H*, C(5′′)*H*), 7.45-7.53 (2H, m, C(2′′)*H*, C(6′′)*H*), 8.46 (1H, s, C(3′)O*H*); ^{13}C NMR (126 MHz, acetone-d_6) 10.8 (C(3)*C*H$_3$), 28.6 (C(*C*H$_3$)$_3$), 36.4 (*C*H$_2$NH), 75.9 (*C*H(OH)), 79.4 (*C*(CH$_3$)$_3$), 113.7 (*C*(4′)), 115.5 (*C*(2′)), 117.8 (*C*(4)), 119.7 (*C*(6′)), 127.3 (*C*(2′′), *C*(6′′)), 127.8 (*C*(4′′)), 129.0 (*C*(3′′), *C*(5′′)), 131.5 (*C*(1′)), 146.3 (*C*(1′′)), 148.8 (*C*(5′)), 156.5 (*C*=O), 158.5 (*C*(3′)), 159.1 (*C*(3)), 166.7 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 433.1727; $C_{23}H_{26}N_2NaO_5$ requires M$^+$ 433.1734; LRMS *m*/*z* (ES$^-$) 409 ([M–H]$^-$, 100); HPLC retention time 11.46 min, purity 99.6%.

(*RS*)-*N*-((4-(3-Hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)-1-phenylmethanesulfonamide **129e**



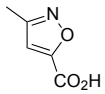
To a solution of **149** (10 mg, 17 μmol, 1.0 eq) in anhydrous THF (0.2 mL) at 0 °C under an argon atmosphere was added TBAF (1 M in THF, 20 μL, 20 μmol, 1.2 eq). After 1 h, TLC analysis indicated complete consumption of **149**, and the mixture was concentrated *in vacuo*. Purification by repeated silica gel column chromatography (15% acetone/CH_2Cl_2 then 40% EtOAc/40-60°C petroleum ether) gave a colourless gum which was precipitated from a solution in CH_2Cl_2 by addition of hexane to give **129e** as a colourless solid (5 mg, 66%); R_f 0.23 (50% EtOAc/40-60 °C petroleum ether); mp 79-82 °C (CH_2Cl_2/hexane); ν_{max} (solid)/cm^{-1}: 3266 (O–H, N–H) (br), 3030, 2971 (C–H) (w), 2161 (m), 2033 (m), 2012 (m), 1978 (m), 1739 (s), 1366 (s), 1320 (s), 1216 (s); ^{1}H NMR (500 MHz, acetone-d_6) 2.23 (3H, s, C(3)C*H*$_3$), 4.30 (2H, s, C*H*$_2$Ph), 4.39 (2H, d, *J* = 6.0 Hz, C*H*$_2$NH), 4.91 (1H, d, *J* = 4.0 Hz, CH(O*H*)), 5.84 (1H, d, *J* = 4.0 Hz, C*H*(OH)),

6.72 (1H, m, C(2´)*H*), 6.78 (1H, t, *J* = 6.0 Hz, CH_2NH), 6.95-7.01 (2H, m, C(4´)*H*, C(6´)*H*), 7.24 (1H, dddd, *J* = 7.3, 7.3, 1.2, 1.2 Hz, C(4´´)*H*), 7.29-7.39 (7H, m, C(3´´)*H*, C(5´´)*H*, C(2´´´)*H*, C(6´´´)*H*, C(3´´´)*H*, C(5´´´)*H*, C(4´´´)*H*), 7.45-7.52 (2H, m, C(2´´)*H*, C(6´´)*H*), 8.53 (1H, s, C(3´)O*H*); ^{13}C NMR (126 MHz, acetone-d_6) 9.9 (C(3)CH_3), 37.5 (CH_2NH), 58.5 (CH_2Ph), 74.9 (*C*H(OH)), 113.0 (*C*(4´)), 114.6 (*C*(2´)), 117.9 (*C*(4)), 118.7 (*C*(6´)), 126.4 (*C*(2´´), *C*(6´´)), 126.9 (*C*(4´´)), 128.1, 128.4, 130.9 (C(3´´), C(5´´), C(2´´´), C(6´´´), C(3´´´), C(5´´´), C(4´´´)), 130.1, 130.2 (*C*(1´), *C*(1´´´)), 145.4 (*C*(1´´)), 148.0 (*C*(5´)), 157.7 (*C*(3´)), 158.5 (*C*(3)), 163.4 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 487.1299; $C_{25}H_{24}N_2NaO_5S$ requires M^+ 487.1298; LRMS *m*/*z* (ES^+) 465 ($[M+H]^+$, 68), 487 ($[M+Na]^+$, 100); HPLC: retention time 11.31 min, purity 97.8%.

7.5 Chapter 5: Synthetic Procedures

*7.5.1 Fmoc-protected (S)-2-amino-3-(3-methylisoxazole-5-carboxamido)propanoic acid **155***

3-Methylisoxazole-5-carboxylic acid **162**[296]

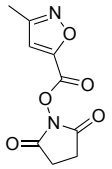


Following the procedure of Lepage *et al.*,[296] to an aq. solution of NaOH (20% w/v, 20 mL) was added hydroxylamine hydrochloride (9.04 g, 130 mmol, 1.0 eq). The solution was cooled to 0 °C and ethyl 2,4-dioxopentanoate (20.6 g, 130 mmol, 1.0 eq) was added. The solution was warmed to rt and stirred for 1 h, after which time TLC analysis indicated complete consumption of diketone. The solution was extracted with Et_2O (3 × 100 mL), and the combined organic extracts were washed with brine (200 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* at rt to give 19.4 g of yellow solid. This crude oxime was dissolved in

EtOH (150 mL), and conc. H_2SO_4 (2.4 mL) was added dropwise. The solution was heated under reflux for 4.5 h, then further conc. H_2SO_4 (0.5 mL) was added and the solution heated under reflux for a further 4 h. The reaction was cooled to rt and concentrated *in vacuo*. The residues were dissolved in Et_2O (250 mL), and washed with H_2O (250 mL) and brine (250 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by Kugelrohr distillation (90 °C, 4 mbar [lit. 122 °C, 20 mmHg][296]) gave a 87:13 mixture of desired ethyl 3-methylisoxazole-5-carboxylate (**107**) to its presumed regioisomer, ethyl 5-methylisoxazole-3-carboxylate (**110**), as a colourless solid which partially melted at rt (13.3 g, 69%).

To a solution of this mixture (2.00 g, 12.9 mmol, 1.0 eq) in THF (60 mL) and H_2O (30 mL) was added LiOH (370 mg, 15.5 mmol, 1.2 eq). The reaction was stirred at rt for 16 h, after which time TLC analysis indicated complete consumption of esters **107** and **110**. The reaction was concentrated *in vacuo* to remove THF, whereupon white needle-like crystals formed which were filtered off and washed with cold H_2O to give **162** (880 mg, 54%) as a single regioisomer; mp 138-140 ˚C (H_2O) (dec., sublimes) [lit. 204 ˚C][296]; ^{1}H NMR (400 MHz, DMSO-d_6) 2.29 (3H, s, C*H*$_3$), 7.03 (1H, s, C(4)*H*), 14.2 (1H, br s, O*H*). The ^{1}H data are in accordance with literature values.[296] Note: despite repeated recrystallisation form H_2O, the melting point was not in accordance with literature values.

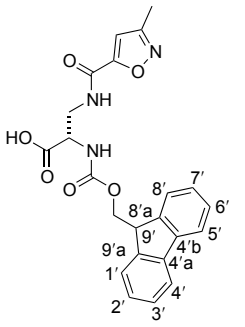
2,5-Dioxopyrrolidin-1-yl 3-methylisoxazole-5-carboxylate **163**



Following the procedure of Leonard and Brunckova,[300] to solution of **162** (318 mg, 2.5 mmol, 1.0 eq), *N*-hydroxysuccinimide (575 mg, 5.0 mmol, 2.0 eq)

and anhydrous pyridine (810 μL, 790 mg, 10.0 mmol, 4.0 eq) in anhydrous CH_2Cl_2 (12.5 mL) at 0 °C under a nitrogen atmosphere was added trifluoroacetic anhydride (700 μL, 1.05 g, 5.0 mmol, 2.0 eq) dropwise. The reaction was warmed to rt and stirred for 1 h, then diluted with CH_2Cl_2 (40 mL) and aq. HCl (1 M, 50 mL). The layers were separated, and the organic layer was washed with aq. HCl (2 × 40 mL) and sat. aq. $NaHCO_3$ (2 × 40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give **163** as a colourless solid (498 mg, 88%) which was used without further purification; R_f 0.43 (50% EtOAc/40-60 °C petroleum ether); mp 130-131 °C (EtOAc); ν_{max} (thin film)/cm^{-1}: 2962 (C–H) (w), 1738 (C=O) (s), 1205 (m), 1049 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 2.43 (3H, s, C(3)C*H*$_3$) 2.92 (4H, s, 2 × C*H*$_2$), 7.06 (1H, s, C(4)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.4 (C(3)*C*H$_3$), 25.6 (2 × *C*H$_2$), 113.1 (*C*(4)), 152.1 (C(5)*C*=O), 155.2 (*C*(5)), 160.7 (*C*(3)), 168.3 (2 × *C*=O); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 247.0331; $C_9H_8N_2NaO_5$ requires M$^+$ 247.0325; LRMS *m*/*z* (ES$^+$) 247 ([M+Na]$^+$, 100). Anal. Calcd for $C_9H_8N_2O_5$: C, 48.2; H, 3.6; N, 12.5. Found: C, 48.3; H, 3.5; N, 12.4.

(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(3-methylisoxazole-5-carboxamido)propanoic acid **156**

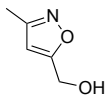


A solution of Fmoc-Dap-OH (816 mg, 2.50 mmol, 1.0 eq) and **163** (561 mg, 2.50 mmol, 1.0 eq) in anhydrous DMF (12.5 mL) was stirred under a nitrogen atmosphere for 17 h, then concentrated *in vacuo*. The residues were dissolved in

EtOAc (80 mL), washed with aq. citric acid (10% w/v, 2 × 80 mL), H_2O (4 × 80 mL), brine (80 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a pale yellow solid. Crystallisation from boiling $CHCl_3$ gave **156** as a colourless solid (915 mg, 84%); R_f 0.8 (EtOAc, 0.5% AcOH); $[\alpha]_D^{20}$ = –14.7 (*c* 1.0, MeOH); ν_{max} (thin film)/cm^{-1}: 3317 (O–H, N–H) (br), 1720, 1697 (C=O) (s), 1531 (s), 1449 (m), 1249 (s), 1052 (m); ^{1}H NMR (500 MHz, acetone-d_6) 2.32 (3H, s, C(3)C*H*$_3$), 3.83-3.99 (2H, m, NHC*H*$_2$CH), 4.22-4.28 (1H, m, OCH$_2$C*H*), 4.31-4.37 (2H, m, OC*H*$_2$CH), 4.59 (1H, ddd, *J* = 8.0, 7.8, 5.0 Hz, NHCH$_2$C*H*), 6.83 (1H, s, C(4)*H*), 6.99 (1H, d, *J* = 8.0 Hz, N*H*COO), 7.29-7.35 (1H, m, C(2´)*H*, C(7´)*H*), 7.42 (2H, dd, *J* = 7.5, 7.5 Hz, C(3´)*H*, C(6´)*H*), 7.71 (2H, d, *J* = 7.5 Hz, C(1´)*H*, C(8´)*H*), 7.87 (2H, d, *J* = 7.5 Hz, C(4´)*H*, C(5´)*H*), 8.16-8.27 (1H, m, CON*H*CH$_2$), 11.5 (1H, br s, COO*H*); ^{13}C NMR (126 MHz, acetone-d_6) 11.3 (C(3)*C*H$_3$), 41.3 (NH*C*H$_2$CH), 48.0 (OCH$_2$*C*H), 54.8 (NHCH$_2$*C*H), 67.4 (O*C*H$_2$CH), 108.1 (*C*(4)), 120.8 (*C*(4´), *C*(5´)), 126.2 (*C*(1´), *C*(8´)), 128.0 (*C*(2´), *C*(7´)), 128.5 (*C*(3´), *C*(6´)), 142.1 (*C*(4´a), *C*(4´b)), 145.0 (*C*(8´a), *C*(9´a)), 157.1 (NH*C*OO), 157.5 (C(5)*C*O), 161.6 (*C*(3)), 164.2 (*C*(5)), 171.9 (*C*OOH); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 458.1325; $C_{23}H_{21}N_3NaO_6$ requires M^+ 458.1323; LRMS *m*/*z* (ES$^+$) 436 ([M+H]$^+$, 50), 458 ([M+Na]$^+$, 100). HPLC: Retention time 11.50 min, purity 98.9%; Chiral HPLC: Retention time 15.2 min, ee 98.1%.

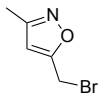
*7.5.2 Fmoc-protected (S)-2-amino-3-((3-methylisoxazol-5-yl)methoxy)propanoic acid **156***

(3-Methylisoxazol-5-yl)methanol **165**[333]



To a solution of **107** (2.00 g, 12.9 mmol, 1.0 eq) in anhydrous EtOH (25 mL) at 0 °C under an argon atmosphere was added $NaBH_4$ (585 mg, 15.5 mmol, 1.2 eq). The reaction mixture was warmed to rt and stirred for 7 h, after which time TLC analysis indicated complete consumption of **107**. The reaction was quenched with H_2O (20 mL) and aq. HCl (1 M, 10 mL), and concentrated *in vacuo* to remove EtOH. The residues were extracted with EtOAc (3 × 30 mL), and the combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (60%, 70% Et_2O/ petroleum ether) gave **165** as a pale yellow oil (989 mg, 68%); R_f 0.19 (70% Et_2O/40-60 °C petroleum ether); ^1H NMR (400 MHz, $CDCl_3$) 2.29 (3H, s, C(3)C*H*$_3$), 3.32 (1H, t, *J* = 6.3 Hz, O*H*), 4.72 (2H, d, *J* = 6.3 Hz, C*H*$_2$OH), 6.10 (1H, s, C(4)*H*); LRMS *m*/*z* (ES^+) 114 ($[M+H]^+$, 82), 136 ($[M+Na]^+$, 100). These data are in good agreement with literature values.[333]

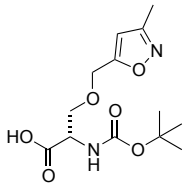
5-(Bromomethyl)-3-methylisoxazole **164**[236]



To a solution of **165** (793 mg, 7.01 mmol, 1.0 eq) in anhydrous CH_2Cl_2 (25 mL) at 0 °C under an argon atmosphere was added PPh_3Br_2 (3.55 g). The reaction was stirred in the dark for 1.5 h at 0 °C then for 1.5 h at rt, after which time TLC analysis indicated complete consumption of **165**. Most CH_2Cl_2 was removed *in vacuo*, then hexane (10 mL) was added to induce precipitation of PPh_3O. The solution was decanted off, and the residues were extracted with hexane. The combined extracts were loaded onto a short silica plug, then eluted with 20% Et_2O/petroleum ether to give **164** as a colourless oil (986 mg, 80%) which was stored in the dark at –20 °C prior to use. (The compound darkened if

stored in the light.) R_f 0.21 (20% Et_2O/40-60 °C petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) 2.33 (3H, s, C(3)C*H*$_3$), 4.46 (C*H*$_2$Br), 6.18 (C(4)*H*); LRMS *m*/*z* (ES$^+$) 176 ([^{79}M+H]$^+$, 100), 178 ([^{81}M+H]$^+$, 93). These data are in good agreement with literature values.[236]

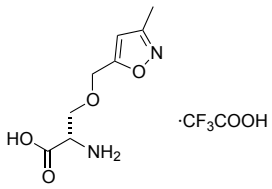
N-(*tert*-Butoxycarbonyl)-*O*-((3-methylisoxazol-5-yl)methyl)-L-serine **166**



To a solution of anhydrous *N*-(*tert*-butoxycarbonyl)-L-serine (1.03 g, 5.08 mmol, 1.0 eq) in anhydrous DMF (15 mL) at 0 °C under an argon atmosphere was added NaH (60% in mineral oil, 508 mg, 12.7 mmol, 2.5 eq) portionwise, and the mixture was stirred at 0 °C for 30 min. **164** (938 mg, 5.33 mmol, 1.05 eq) in anhydrous DMF (5 mL) was then added, and the reaction warmed to rt over 13 h. DMF was then removed *in vacuo* and the residues were diluted with H_2O (150 mL) and washed with Et_2O (3 × 100 mL). The aqueous layer was acidified to pH 2 with aq. citric acid (10% w/v) and extracted with EtOAc (3 × 150 mL). The combined EtOAc extracts were washed with H_2O (450 mL) and brine (2 × 450 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give **166** as a pale brown gum (1.30 g, 85%) which was used without further purification; $[\alpha]_D^{24}$ = +7.8 (*c* 1.0, $CHCl_3$); ν_{max} (thin film)/cm^{-1}: 3331 (O–H, N–H) (br), 2978, 2934 (C–H) (m), 1710 (C=O) (s), 1509 (m), 1368 (m), 1163 (s); ^{1}H NMR (500 MHz, $CDCl_3$) 1.47 (9H, s, C(C*H*$_3$)$_3$), 2.31 (3H, s, C(3)C*H*$_3$), 3.80 (1H, dd, *J* = 9.3, 3.1 Hz, CHC*H*$_A$H$_B$), 4.00 (1H, dd, *J* = 9.3, 2.3 Hz, CHCH$_A$*H*$_B$), 4.45-4.54 (1H, m, C*H*CH$_2$), 4.61 (2H, s, C(5)C*H*$_2$O), 5.45 (1H, d, *J* = 7.9 Hz, N*H*), 6.11 (1H, s, C(4)*H*), 7.4 (1H, br s, O*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.4 (C(3)*C*H$_3$), 28.3

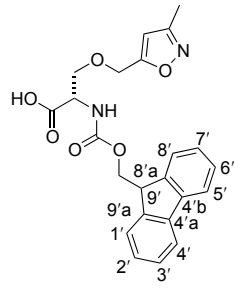
(C(CH_3)$_3$), 53.8 (*C*HCH$_2$), 64.1 (C(5)*C*H$_2$O), 70.6 (CH*C*H$_2$), 80.5 (O*C*(CH$_3$)$_3$), 104.1 (*C*(4)), 155.7 (*C*ONH), 159.9 (*C*(3)), 168.1 (*C*(5)), 174.2 (*C*OOH); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 323.1226; $C_{13}H_{20}N_2NaO_6$ requires M$^+$ 323.1214; LRMS *m*/*z* (ES$^+$) 323 ([M+Na]$^+$, 100); HPLC (220 nm): Retention time 10.26 min, purity 93.1 %.

O-((3-Methylisoxazol-5-yl)methyl)-L-serine, trifluoroacetic acid salt **153**



To a solution of **166** (1.18 g, 3.93 mmol, 1.0 eq) in CH_2Cl_2 (14 mL) was added Et_3SiH (1.88 mL, 1.37 g, 11.8 mmol, 3.0 eq) and TFA (7 mL). After 3.5 h, TLC analysis indicated complete consumption of **166**, and the mixture was concentrated *in vacuo*, dissolved in 60 mL H_2O, washed with Et_2O (4 × 60 mL) and lyophilised to give **153** as an extremely hygroscopic colourless solid (1.02 g, 83%) which was used without further purification; $[\alpha]_D^{24}$ = –1.0 (*c* 1.0, H_2O); ν_{max} (solid)/cm^{-1}: 3116 (O–N, N–H) (br), 2963, 2931 (C–H) (w), 1605 (C=O) (s), 1556 (m), 1417 (m), 1354 (m), 1138 (m), 1092 (s); ^{1}H NMR (500 MHz, methanol-d$_4$) 2.30 (3H, s, C(3)C*H*$_3$), 3.97 (2H, d, *J* = 4.7 Hz, CHC*H*$_2$O), 4.05 (1H, dd, *J* = 4.7, 4.7 Hz, C*H*CH$_2$), 4.70 (2H, s, C(5)C*H*$_2$O), 6.36 (1H, s, C(4)*H*); ^{13}C NMR (126 MHz, methanol-d$_4$) 9.7 (C(3)*C*H$_3$), 53.6 (*C*HCH$_2$), 63.2 (C(5)*C*H$_2$), 68.2 (CH*C*H$_2$O), 104.2 (*C*(4)), 116.8 (q, *J* = 293 Hz, *C*F$_3$), 160.1 (*C*(3)), 161.6 (q, *J* = 35 Hz, CF$_3$*C*OOH), 168.2 (*C*(5)), 168.8 (CH*C*OOH); ^{19}F NMR (470 MHz, methanol-d$_4$) –70.78; HRMS *m*/*z* (ES$^+$) found [M–CF$_3$COO]$^+$ 201.0877; $C_8H_{13}N_2O_4$ requires M$^+$ 201.0870; LRMS *m*/*z* (ES$^+$) 201 ([M–CF$_3$COO]$^+$, 100), 223 ([M+Na–CF$_3$COOH]$^+$, 8).

N-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*O*-((3-methylisoxazol-5-yl)methyl)-L-serine **156**

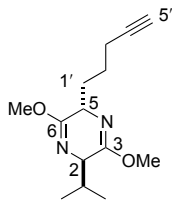


To a solution of **153** (1.00 g, 3.18 mmol, 1.0 eq) in H_2O (20 mL) and 1,4-dioxane (10 mL) was added $NaHCO_3$ (801 mg, 9.54 mmol, 3.0 eq). After 30 min, Fmoc-OSu (1.13 g, 3.34 mmol, 1.05 eq) in 1,4-dioxane (10 mL) was added dropwise over 15 min. After 3 h, TLC analysis indicated complete consumption of **153**, and the reaction was acidified to pH 2 with aq. HCl (1 M) then extracted with EtOAc (4 × 80 mL). The combined organic layers were washed with aq. citric acid (10% w/v, 250 mL), water (2 × 250 mL) and brine (2 × 250 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Repeated crystallisation from boiling 1:1 pentane/CH_2Cl_2 gave **156** as an off-white solid (1.08 g, 80%); mp 110-111 °C (1:1 pentane/CH_2Cl_2); ; $[\alpha]_D^{24}$ = +4.1 (*c* 1.0, MeOH); ν_{max} (solid)/cm^{-1}: 3312 (O–H, N–H) (br), 3067, 2950 (C–H) (w), 1720 (C=O) (s), 1512 (m), 1450 (m), 1205 (m), 1107 (m); ^{1}H NMR (400 MHz, $CDCl_3$) 2.27 (3H, s, C(3)C*H*$_3$), 3.82 (1H, dd, *J* = 9.3, 3.0 Hz, COOHCHC*H*$_A$H$_B$), 4.03 (1H, dd, *J* = 9.3, 2.8 Hz, COOHCHCH$_A$*H*$_B$), 4.23 (1H, t, *J* = 7.2 Hz, C(9′)*H*CH$_2$), 4.32-4.49 (2H, m, C(9′)HC*H*$_2$), 4.50-4.67 (3H, m, COOHC*H*CH$_2$, C(5)C*H*$_2$), 5.72 (1H, d, *J* = 8.5 Hz, N*H*), 6.08 (1H, s, C(4)*H*), 7.27-7.35 (2H, m, C(2′)*H*, C(7′)*H*), 7.35-7.45 (2H, m, C(3′)*H*, C(6′)*H*), 7.56-7.66 (2H, m, C(1′)*H*, C(8′)*H*), 7.70-7.82 (2H, m, C(4′)*H*, C(5′)*H*), 9.2 (1H, br s, O*H*); ^{13}C NMR (100 MHz, $CDCl_3$) 11.3 (C(3)*C*H$_3$), 47.1 (*C*(9′)), 54.2 (COOH*C*H), 64.0

(C(5)*C*H$_2$), 67.3 (C(9´)*C*H$_2$), 70.5 (COOHCH*C*H$_2$), 104.1 (C4)*H*), 120.0 (*C*(4´), *C*(5´)), 125.1 (*C*(1´), *C*(8´)), 127.1 (*C*(2´), *C*(7´)), 127.8 (*C*(3´), *C*(6´)), 141.3 (*C*(4´a), *C*(4´b)), 143.7, 143.8 (*C*(8´a), *C*(9´a)), 156.2 (*C*ONH), 159.9 (*C*(3)), 167.9 (*C*(5)), 173.5 (*C*OOH); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 445.1381; $C_{23}H_{22}N_2NaO_7$ requires M$^+$ 445.1370; LRMS *m*/*z* (ES$^+$) 423 ([M+H]$^+$, 57), 440 ([M+NH$_4$]$^+$, 100); HPLC: retention time 12.17 min, purity 100.0%; Chiral HPLC: Retention time 9.9 min, ee >99%.

*7.5.3 Fmoc-protected 2-amino-5-(3-methylisoxazol-5-yl)pentanoic acid **157***

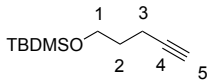
(2*R*,5*S*)-2-Isopropyl-3,6-dimethoxy-5-(pent-4-yn-1-yl)-2,5-dihydropyrazine **168**



To a stirred solution of (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine [(*R*)-**167**, 600 µL, 617 mg, 3.35 mmol] in anhydrous THF (13.4 mL) at –78 °C under a nitrogen atmosphere was added ^{n}BuLi (2.17 M in hexanes, 1.6 mL, 3.5 mmol, 1.05 eq). Stirring was continued for 15 min, then a precooled solution of 5-iodopent-1-yne (714 mg, 3.68 mmol, 1.1 eq) in anhydrous THF (1.4 mL) was added dropwise. The solution was stirred at –78 °C for 4 h, then warmed to rt and stirred for 14 h. The reaction was quenched with sat. aq. NH_4Cl (10 mL) and concentrated *in vacuo* to remove THF. The residues were diluted with H_2O (5 mL) and extracted with Et_2O (3 × 15 mL). The combined organics were washed with H_2O (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 0.5 → 8% Et_2O/petroleum ether, then further purification of mixed fractions,

0.5→6% Et_2O/petroleum ether) gave **168** as a colourless oil (643 mg, 77%); R_f 0.38 (20% Et_2O/40-60 °C petroleum ether); $[\alpha]_D^{22}$ = +3.4° (c 0.95, $CHCl_3$); ν_{max} (thin film)/cm^{-1}: 3306 (alkyne C–H) (w), 2959, 2945, 2871 (C–H) (m), 1692 (C=N) (s), 1436 (m), 1235 (s) (C–O), 1194 (m), 1007 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 0.70 (3H, d, *J* = 7.0 Hz, CH(C*H*$_3$)$_A$), 1.05 (3H, d, *J* = 7.0 Hz, CH(C*H*$_3$)$_B$), 1.46-1.60 (2H, m, C(2´)*H*$_2$), 1.74-1.84 (1H, m, C(1´)*H*$_A$), 1.90-1.98 (1H, m, C(1´)*H*$_B$) overlapping 1.95 (1H, t, *J* = 2.6 Hz, C(5´)*H*), 2.21 (2H, td, *J* = 7.3, 2.6 Hz, C(3´)*H*), 2.27 (1H, sept d, *J* = 7.0, 3.4 Hz, C*H*Me$_2$), 3.69 (3H, s, OC*H*$_3$), 3.70 (3H, s, OC*H*$_3$), 3.95 (1H, dd, *J* = 3.7, 3.4 Hz, C(2)*H*), 4.03 (1H, ddd, *J* = 6.4, 3.7, 3.7 Hz, C(5)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 16.6 (CH(*C*H$_3$)$_A$), 18.4 (*C*(3´)), 19.0 (CH(*C*H$_3$)$_B$), 23.9 (*C*(2´)), 31.7 (*C*HMe$_2$), 33.3 (*C*(1´)), 52.4 (2 × O*C*H$_3$), 55.0 (*C*(5)), 60.7 (*C*(2)), 68.2 (*C*(5´)), 84.4 (*C*(4´)), 163.6, 163.6 (*C*(3), *C*(6)); HRMS *m*/*z* (ES$^+$) found [M+H]$^+$ 251.1747; $C_{14}H_{23}N_2O_2$ requires M$^+$ 251.1754; *m*/*z* (ES$^+$) 251 ([M+H]$^+$, 100).

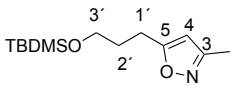
tert-Butyldimethyl(pent-4-yn-1-yloxy)silane **170**[334]



Imidazole (878 mg, 12.9 mmol, 2.4 eq) was added to a solution of 4-pentyln-1-ol (500 µL, 452 mg, 5.37 mmol, 1.0 eq) in anhydrous DMF (4.5 mL) under a nitrogen atmosphere, and stirring was continued until all solids had disappeared. TBDMSCl (971 mg, 6.44 mmol, 1.2 eq) was then added. After 26 h, TLC analysis indicated complete consumption of alcohol, and the reaction was diluted with H_2O (15 mL) and extracted with Et_2O (3 × 15 mL). The combined organic layers were washed with brine (2 × 50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by Kugelrohr distillation (90 °C, 12 mbar) gave

170 as a colourless oil (862 mg, 81%); R_f 0.60 (10% Et_2O/40-60 °C petroleum ether); ^1H NMR (400 MHz, $CDCl_3$) 0.06 (6H, s, Si(C*H*$_3$)$_2$), 0.90 (9H, s, C(C*H*$_3$)$_3$), 1.68-1.79 (2H, m, C(2)*H*$_2$), 1.93 (1H, t, *J* = 2.7 Hz, C(5)*H*), 2.28 (2H, td, *J* = 7.1, 2.7 Hz, C(3)*H*$_2$), 3.70 (2H, t, *J* = 6.0 Hz, C(1)*H*$_2$); HRMS *m*/*z* (FI^+) found 198.1436; $C_{11}H_{22}OSi$ requires M^+ 198.1440. These data are in good agreement with literature values.[334]

5-(3-((*tert*-Butyldimethylsilyl)oxy)propyl)-3-methylisoxazole **171**



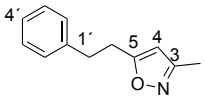
Method A: A solution of acetaldoxime (740 µL, 715 mg, 12.1 mmol, 1.0 eq) and *N*-chlorosuccinimide (1.62 g, 12.1 mmol, 1.0 eq) in DMF (35 mL) was heated to 50 °C for 90 min. The solution was cooled to rt and diluted with iced water (75 mL) and extracted with EtOAc (3 × 75 mL). The combined organic extracts were washed with H_2O (200 mL) and brine (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give presumed *N*-hydroxyacetimidoyl chloride as a yellow oil (1.07 g, 95%), which was used without further purification.

To a solution of **170** (405 mg, 2.04 mmol, 1.0 eq) and NEt_3 (420 µL, 300 mg, 3.0 mmol, 1.5 eq) in CH_2Cl_2 (10 mL) was added a solution of presumed *N*-hydroxyacetimidoyl chloride (240 mg, 2.57 mmol, 1.26 eq) in CH_2Cl_2 (1 mL). The solution was heated under reflux for 16 h, after which time further NEt_3 (420 µL, 300 mg, 3.0 mmol, 1.5 eq) and presumed *N*-hydroxyacetimidoyl chloride (240 mg, 2.57 mmol, 1.26 eq) in CH_2Cl_2 (1 mL) were added, and the reaction heated under reflux for a further 6 h. The reaction was cooled to rt and filtered through Celite® (eluent CH_2Cl_2), then washed with H_2O (30 mL) and brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica

gel column chromatography (gradient elution, 2 → 35% Et_2O/petroleum ether) gave **170** as a colourless oil (239 mg, 46%) along with unreacted starting material **170** (101 mg, 25%); R_f 0.21 (10% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 2954, 2929, 2857 (C–H) (m), 1605 (m), 1418 (m), 1254 (m), 1101 (s); ^1H NMR (500 MHz, $CDCl_3$) 0.05 (6H, s, Si(C*H*$_3$)$_2$), 0.90 (9H, s, C(C*H*$_3$)$_3$), 1.84-1.92 (2H, m, C(2´)*H*$_2$), 2.26 (3H, s, C(3)C*H*$_3$), 2.79 (t, *J* = 7.7 Hz, C(1´)*H*$_2$), 3.66 (t, *J* = 6.1 Hz, C(3´)C*H*$_3$), 5.81 (1H, s, C(4)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) –5.4 (Si(*C*H$_3$)$_2$), 11.4 (C(5)*C*H$_3$), 18.3 (*C*(CH$_3$)$_3$), 23.1 (*C*(1´)), 25.9 (C(*C*H$_3$)$_3$), 30.5 (*C*(2´)), 61.7 (*C*(3´)), 101.5 (*C*(4)). 159.7 (*C*(3)), 173.0 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 278.1540; $C_{13}H_{25}NNaO_2Si$ requires M^+ 278.1547; LRMS *m*/*z* (ES^+) 256.2 ($[M+H]^+$, 100). Anal. Calcd. for $C_{13}H_{25}NO_2Si$: C, 61.1; H, 9.9; N, 5.5. Found: C, 61.2; H, 9.9; N, 5.4.

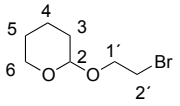
Method B: To a solution of **170** (93 mg, 480 mmol, 1.0 eq), acetaldoxime (71 µL, 69 mg, 1.2 mmol, 2.5 eq) and NEt_3 (33 µL, 24 mg, 240 µmol, 0.5 eq) in $CHCl_3$ (1.7 mL) at 0 ˚C was added sodium hypochlorite solution (>8% available chlorine, 1.7 mL). After 15 h, further oxime (71 µL, 69 mg, 1.2 mmol, 2.5 eq) and sodium hypochlorite solution (1 mL) were added. After a further 15 h, the reaction was diluted with CH_2Cl_2 (5 mL) and H_2O (5 mL). The layers were separated, and the aqueous layer extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extracts were washed with brine (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (gradient elution, 2 → 20% Et_2O/petroleum ether) gave **171** as a colourless oil (23 mg, 19%). The data are identical to those reported above.

3-Methyl-5-phenethylisoxazole **175**[335]



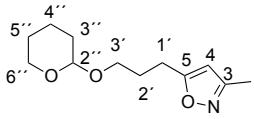
To a solution of 3,5-dimethylisoxazole (200 µL, 200 mg, 2.04 mmol, 1.0 eq) in distilled anhydrous THF (10 mL) at –78 °C under an argon atmosphere was added ^{n}BuLi (2.3 M in hexanes, 0.95 mL, 2.2 mmol, 1.1 eq) dropwise. After 1.5 h, a solution of distilled BnBr (250 µL, 360 mg, 2.14 mmol, 1.05 eq) in THF (1.5 mL) was added dropwise *via* cannula. The solution was warmed slowly to rt over 15 h, then quenched with H_2O (30 mL) and concentrated *in vacuo* to remove the THF. The residue was extracted with Et_2O (3 × 30 mL), and the combined organic layers were washed with H_2O (90 mL) and brine (90 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (petroleum ether then 15% Et_2O/petroleum ether) gave **175** as a pale yellow oil (303 mg, 79%); R_f 0.26 (20% Et_2O/40-60 °C petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) 2.26 (3H, s, C*H*$_3$), 2.95-3.08 (4H, m, C*H*$_2$C*H*$_2$), 5.77 (1H, s, C(4)*H*), 7.17-7.35 (5H, m, *Ph*); ^{13}C NMR (100 MHz, $CDCl_3$) 11.4 (*C*H_3), 28.4 (*C*H_2), 33.6 (*C*H_2), 101.9 (*C*(4)), 126.4 (*C*(4´)), 128.3, 128.5 (*C*(2´), *C*(3´), *C*(5´), *C*(6´)), 140.2 (*C*(1´)), 159.7 (*C*(3)), 172.2 (*C*(5)); LRMS *m*/*z* (ES$^+$) 188 ([M+H]$^+$, 100), 210 ([M+Na]$^+$, 45%). Anal. Calcd. for $C_{12}H_{12}NO$: C, 77.0; H, 7.0; N, 7.5. Found: C, 77.1; H, 7.1; N, 7.5. These data are in good agreement with literature values.[335]

(*RS*)-2-(2-Bromoethoxy)tetrahydro-2*H*-pyran **173**[319]



Following the procedure of Anantanaryan *et al.*,[319] to a vigorously-stirred mixture of 2-bromoethanol (7.9 mL, 13.9 g, 110 mmol, 1.0 eq) and 3,4-dihydro-2*H*-pyran (10.0 mL, 9.2 g, 110 mmol, 1.0 eq) at 0 °C was added 1 drop of conc. HCl. The reaction mixture was warmed to rt and stirred for 3 h. Solid $NaHCO_3$ (3 g) was added and the suspension was stirred for 1 h then filtered. The product was purified by vacuum distillation to give **173** as a colourless oil (19.8 g, 86%) which was stored under Ar over 3 Å molecular sieves in the dark at –20 ˚C prior to use; bp 84 °C (9 mbar) [lit. 82 °C (0.5 mmHg)][319]; R_f 0.53 (50% Et_2O/40-60 °C petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) 1.48-1.94 (6H, m, C(3)H_2, C(4)H_2, C(5)H_2), 3.48 (3H, m), 3.74-3.82 (1H, m), 3.86-3.96 (1H, m), 3.98-4.07 (1H, m, C(1´)H_2, C(2´)H_2, C(6)H_2), 4.66-4.71 (C(2)*H*); *m*/*z* (ES$^+$) 209 ([M+H]$^+$, 100). These data are in good agreement with literature values.[319]

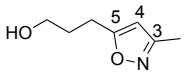
(*RS*)-3-Methyl-5-(3-((tetrahydro-2H-pyran-2-yl)oxy)propyl)isoxazole **174**[318]



To a solution of 3,5-dimethylisoxazole (5.0 mL, 5.0 g, 51 mmol, 1.0 eq) in distilled anhydrous THF (100 mL) at –78 °C under an argon atmosphere was added ^{n}BuLi (2.3 M in hexanes, 23.3 mL, 53 mmol, 1.05 eq) over 20 min. After 1.5 h, a solution of distilled **173** (7.7 mL, 10.7 g, 51 mmol, 1.0 eq) in THF (15 mL) was added over 30 min *via* cannula. The solution was warmed slowly to rt over 17 h, then quenched with H_2O (100 mL) and concentrated *in vacuo* to remove THF. The residue was extracted with Et_2O (3 × 100 mL), and the combined organic layers were washed with H_2O (300 mL) and brine (300 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (20% then 40% Et_2O/petroleum ether, 0.5% NEt_3) gave **174** as a

pale yellow oil (8.08 g, 70%); R_f 0.27 (40% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 2942, 2871 (C–H) (m), 1605 (m), 1418 (m), 1136 (m), 1120 (m), 1034 (s); ^{1}H NMR (500 MHz, acetone-d_6) 1.44-1.60 (4H, m, C(3´´)H_A, C(4´´)H_A, C(5´´)H_2), 1.61-1.71 (1H, m, C(3´´)H_B), 1.76-1.87 (1H, m, C(4´´)H_B), 1.90-2.00 (2H, m, C(2´´)H_2), 2.21 (3H, s, C(3)CH_3), 2.80-2.86 (2H, m, C(1´)H_2), 3.39-3.50 (2H, m, C(3´)H_A, C(6´´)H_A), 3.72-3.84 (2H, m, C(3´)H_B, C(6´´)H_B), 4.57-4.62 (1H, m, C(2´´)H), 6.04 (1H, s, C(4)H); ^{13}C NMR (126 MHz, acetone-d_6) 11.3 (C(3)CH$_3$), 20.1 (C(4´´)), 24.0 (C(1´)), 26.3 (C(5´´)), 28.6 (C(2´)), 31.4 (C(3´´)), 62.2 (C(6´´)), 66.6 (C(3´)), 99.2 (C(2´´)), 102.3 (C(4)), 160.1 (C(3)), 173.6 (C(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 248.1265; $C_{12}H_{19}NNaO_3$ requires M^+ 248.1257; LRMS *m*/*z* (ES^+) 226 ($[M+H]^+$, 69), 248 ($[M+Na]^+$, 100). These data are in good agreement with the literature values.[318]

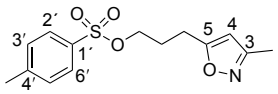
3-(3-Methylisoxazol-5-yl)propan-1-ol **172**[236,318,336]



Method A: A solution of **174** (7.46 g, 33.1 mmol, 1.0 eq) and TsOH·H_2O (315 mg, 1.66 mmol, 0.05 eq) in MeOH (150 mL) was stirred for 2 h. After TLC analysis indicated complete consumption of **174**, Celite® was added and the mixture was concentrated *in vacuo*. Purification by silica gel column chromatography (20%, 30% acetone/petroleum ether) gave **172** as a pale yellow oil (4.38 g, 94%); R_f 0.21 (30% acetone/40-60 °C petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) 1.73-1.82 (1H, m, OH), 1.90-2.01 (2H, m, $CH_2CH_2CH_2$), 2.28 (3H, s, C(3)CH_3), 2.85 (2H, t, J = 7.6 Hz, C(5)CH_2), 3.68-3.78 (2H, m, CH_2OH), 5.86 (1H, s, C(4)H); LRMS *m*/*z* (ES^+) 142 ($[M+H]^+$, 93), 164 ($[M+Na]^+$, 100). These data are in good agreement with literature values.[236,318,336]

Method B: To a solution of 3,5-dimethylisoxazole (100 µL, 99 mg, 1.02 mmol, 1.0 eq) in anhydrous THF (5 mL) at –78 °C under an argon atmosphere was added ^{n}BuLi (2.3 M in hexanes, 490 µL, 1.13 mmol, 1.1 eq) dropwise. The solution was stirred at –78 °C for 2 h, then a solution of 1,3,2-dioxathiolane-2,2-dioxide (139 mg, 1.12 mmol, 1.1 eq) in THF (1 mL) was added and the reaction was warmed slowly to rt over 16 h. The reaction was concentrated *in vacuo*, and THF (5 mL) and H_2O (100 µL) were added. A few drops of conc. H_2SO_4 were added (pH 1), and the mixture was heated under reflux for 14 h, then cooled to rt. The reaction was concentrated *in vacuo* and the residues were suspended in 10 mL sat. aq. $NaHCO_3$ then extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine (30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (20%, 30% acetone/petroleum ether) gave **172** as a pale yellow oil (62 mg, 43%). The data are identical to those reported above.

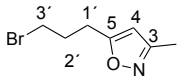
3-(3-Methylisoxazol-5-yl)propyl 4-methylbenzenesulfonate **176**



To a solution of **172** (530 mg, 3.75 mmol, 1.0 eq) and DMAP (23 mg, 190 µmol, 0.05 eq) in anhydrous CH_2Cl_2 (7.5 mL) under an argon atmosphere was added TsCl (930 mg, 4.88 µmol, 1.3 eq) and NEt_3 (680 µL, 490 mg, 4.9 mmol, 1.3 eq). After 25 h, TLC analysis indicated complete consumption of **172**. The reaction was diluted with H_2O (30 mL), the layers were separated and the aqueous layer extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (2 × 75 mL) aq. citric acid (10% w/v, 2 × 75 mL) and brine (75 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*.

Crystallisation from boiling Et_2O gave **176** as off-white crystals (754 mg, 68%); mp 52-53 °C (Et_2O); R_f 0.28 (30% acetone/40-60°C petroleum ether); ν_{max} (solid)/cm^{-1}: 3131, 3069, 2967, 2930 (C–H) (w), 1602 (m), 1348 (S=O) (s), 1167 (S=O) (s), 1096 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.99-2.08 (2H, m, $CH_2CH_2CH_2$), 2.24 (3H, s, C(3)CH_3), 2.46 (3H, s, C(4´)CH_3), 2.78 (2H, t, *J* = 7.4 Hz, C(5)CH_2), 4.06 (2H, t, *J* = 6.0 Hz, OCH_2), 5.76 (1H, s, C(4)*H*), 7.36 (2H, d, *J* = 8.1 Hz, C(3´)*H*, C(5´)*H*), 7.79 (2H, d, *J* = 8.1 Hz, C(2´)*H*, C(6´)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.4 (C(3)CH_3), 21.6 (C(4´)CH_3), 22.6 (C(5)CH_2), 26.8 ($CH_2CH_2CH_2$), 68.8 (OCH_2), 102.2 (*C*(4)), 127.9 (*C*(2´), *C*(6´)), 129.9 (*C*(3´), *C*(5´)), 132.8 (*C*(1´)), 144.9 (*C*(4´)), 159.8 (*C*(3)), 170.9 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 318.0772, $C_{14}H_{17}NNaO_4S$ requires M^+ 318.0770; LRMS *m*/*z* (ES^+) 296 ($[M+H]^+$, 100), 318 ($[M+Na]^+$, 71), 613 ($[2M+Na]^+$, 38). HPLC: retention time 12.41 min, purity 100.0%

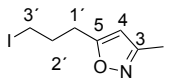
5-(3-Bromopropyl)-3-methylisoxazole **177**[315]



To a solution of **172** (500 mg, 3.54 mmol, 1.0 eq), PPh_3 (1.39 g, 5.31 mmol, 1.5 eq) and anhydrous pyridine (430 µL, 420 mg, 5.3 mmol, 1.5 eq) in anhydrous CH_2Cl_2 (50 mL) was added bromine (270 µL, 850 mg, 5.3 mmol, 1.5 eq) at 0 °C under an argon atmosphere. Further PPh_3 was added until the brown colour disappeared. The solution was stirred for 30 min at 0 °C until TLC analysis indicated complete consumption of **172**, then warmed to rt and washed with aq. HCl (1 M, 3 × 50 mL) and sat. aq. $NaHCO_3$ (50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* at rt. The resultant off-white solid was extracted with hexane (4 × 5 mL) and the hexane extracts loaded onto a short plug of silica.

Elution with 20% Et_2O/petroleum ether gave **177** as a pale yellow oil (662 mg, 92%); R_f 0.26 (25% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 2966, 2933 (C–H) (m), 1607 (s), 1449 (m), 1417 (m), 1268 (m); ^1H NMR (500 MHz, $CDCl_3$) 2.20-2.27 (2H, m, C(2′)H_2), 2.27 (3H, s, C(3)CH_3), 2.91 (2H, t, J = 7.3 Hz, C(1′)H_2), 3.43 (2H, t, J = 6.4 Hz, C(3′)H_2), 5.88 (1H, s, C(4)H); ^{13}C (126 MHz, $CDCl_3$) 11.4 (C(3)CH$_3$), 25.0 (C(1′)), 30.2 (C(2′)), 32.2 (C(3′)), 102.3 (C(4)), 159.8 (C(3)), 171.1 (C(5)); HRMS m/z (ES^+) found $[M+H]^+$ 204.0020, 205.9995; $C_7H_{11}{}^{79}BrNO$ requires M^+ 204.0019, $C_7H_{11}{}^{81}BrNO$ requires M^+ 205.9998; LRMS m/z (ES^+) 204 ($[^{79}M+H]^+$, 90), 206 ($[^{81}M+H]^+$, 100). The ^1H data are in good agreement with literature values.[315]

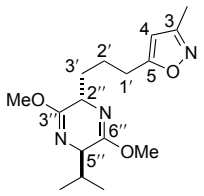
5-(3-Iodopropyl)-3-methylisoxazole **178**



To a solution of PPh_3 (2.42 g, 9.21 mmol, 1.3 eq) and imidazole (627 mg, 9.21 mmol, 1.3 eq) in anhydrous CH_2Cl_2 (30 mL) at 0 °C under an argon atmosphere was added iodine (2.34 g, 9.21 mmol, 1.3 eq) in portions. After 15 min, a solution of **172** (1.00 g, 7.08 mmol, 1.0 eq) in CH_2Cl_2 (5 mL) was added and the reaction was stirred for 2 h at 0 °C, after which time TLC analysis indicated complete consumption of **172**. The reaction was diluted with hexane (70 mL) and filtered through Celite® (eluent hexane), then concentrated *in vacuo* at rt in the dark. The residue was then extracted with hexane and loaded onto a short silica plug, then eluted with 20% Et_2O/petroleum ether to give **178** as a pale yellow oil (1.59 g, 90%) which was stored in the dark at –20 °C prior to use. (The compound darkened if stored in the light.) R_f 0.24 (20% Et_2O/40-60 °C petroleum ether); ν_{max} (thin film)/cm^{-1}: 2962, 2931 (C–H) (m), 1606 (s), 1447 (m), 1416 (s),

1259 (m), 1208 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 2.14-2.22 (2H, m, C(2′)H_2), 2.27 (3H, s, C(3)CH_3), 2.86 (2H, t, J = 7.2 Hz, C(1′)H_2), 3.21 (2H, t, J = 6.8 Hz, C(3′)H_2), 5.88 (1H, s, C(4)H); ^{13}C NMR (126 MHz, $CDCl_3$) 4.97 (*C*(3′)), 11.4 (C(3)*C*H_3), 27.3 (*C*(1′)), 30.8 (*C*(2′)), 102.3 (*C*(4)). 159.8 (*C*(4)), 171.0 (*C*(5)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 273.9695; $C_7H_{10}INNaO$ requires M^+ 273.9699; LRMS *m*/*z* (ES^+) 252 ($[M+H]^+$, 100).

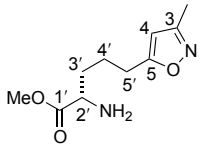
5-(3-((2*S*,5*R*)-5-Isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)propyl)-3-methylisoxazole **169**



To a solution of (*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine [(*R*)-**167**, 1.90 mL, 1.96 g, 10.6 mmol, 1.0 eq] in distilled anhydrous THF (28 mL) at –78 °C under an argon atmosphere was added ^{n}BuLi (2.3 M in hexanes, 4.8 mL, 11.1 mmol, 1.05 eq) dropwise. After 15 min, a precooled solution of **178** (2.66 g, 10.6 mmol, 1.0 eq) in THF (7.5 mL) was added dropwise *via* cannula. The reaction mixture was stirred at –78 °C for 2 h, then between –40 °C and –60 °C for 5 h, then slowly warmed to rt over 14 h. The reaction was quenched with H_2O (50 mL) and pH 7 phosphate buffer (1 M, 50 mL). The mixture was concentrated *in vacuo* to remove THF, then extracted with Et_2O (4 × 100 mL). The combined organic layers were washed with brine (400 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by repeated silica gel column chromatography (15%, 20, 35% Et_2O/petroleum ether, then repurification of mixed fractions, 7% EtOAc/petroleum ether) gave (2*S*,5*R*)-**169** as a pale yellow oil (2.28 g, 70%); R_f 0.14 (30% Et_2O/40-60 °C petroleum ether); $[\alpha]_D^{24}$ = –1.5 (*c* 1.0, $CHCl_3$); ν_{max} (thin

film)/cm^{-1}: 2945, 2871 (C–H) (m), 1692 (s), 1436 (m), 1235 (s), 1196 (m), 1007 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 0.70 (3H, d, *J* = 6.8 Hz, CH(C*H*$_3$)$_A$), 1.05 (3H, d, *J* = 6.8 Hz, CH(C*H*$_3$)$_B$), 1.58-1.73 (2H, m, C(2′)*H*), 1.73-1.82 (1H, m, C(3′)*H*$_A$), 1.85-1.94 (1H, m, C(3′)*H*$_B$), 2.26 (3H, s, C(3)C*H*$_3$) overlapping 2.22-2.30 (1H, m, C*H*(CH$_3$)$_2$), 2.72 (2H, t, *J* = 7.6 Hz, C(1′)*H*$_2$), 3.68 (3H, s, OC*H*$_3$), 3.70 (3H, s, OC*H*$_3$), 3.95 (1H, t, *J* = 3.5 Hz, C(5′′)*H*), 4.01-4.06 (1H, m, C(2′′)*H*), 5.81 (1H, s, C(4)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.4 (C(3)*C*H$_3$), 16.6 (CH(*C*H$_3$)$_A$), 19.0 (CH(*C*H$_3$)$_B$), 22.7 (*C*(2′)), 26.5 (*C*(1′)), 31.8 (*C*H(CH$_3$)$_2$), 33.4 (*C*(3′)), 52.4 (O*C*H$_3$), 52.4 (O*C*H$_3$), 55.0 (*C*(2′′)), 60.8 (*C*(5′′)), 101.4 (*C*(4)), 159.6 (*C*(3)), 163.4, 163.8 (*C*(3′′), *C*(6′′)), 173.1 (*C*(5)); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 330.1779; $C_{16}H_{25}N_3NaO_3$ requires M$^+$ 330.1788; LRMS *m*/*z* (ES$^+$) 308 ([M+H]$^+$, 100), 330 ([M+Na]$^+$, 15).

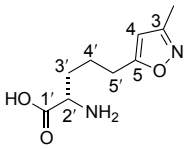
(*S*)-Methyl 2-amino-5-(3-methylisoxazol-5-yl)pentanoate **179**



To a solution of (2*S*,5*R*)-**169** (2.23 g, 7.25 mmol) in MeCN (22 mL) and H_2O (7 mL) was added TFA (3.5 mL) and the reaction mixture was stirred at rt. After 1.5 h, TLC analysis indicated complete consumption of **169**, and the mixture was concentrated *in vacuo*. The residues were dissolved in 25 mL CH_2Cl_2, and basified to pH 8 with sat. aq. $NaHCO_3$. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (3% MeOH/CH_2Cl_2) followed by removal of remaining methyl valinate *in vacuo* gave **179** as a yellow

oil (1.24 g, 81%); R_f 0.23 (5% MeOH/CH_2Cl_2); $[\alpha]_D^{24}$ = +12.3 (*c* 1.0, $CHCl_3$); ν_{max} (thin film)/cm^{-1}: 3379 (N–H) (br), 2953 (C–H) (m), 1733 (C=O) (s), 1605 (m), 1437 (m), 1418 (m), 1198 (m), 1173 (m); ^{1}H NMR (500 MHz, $CDCl_3$) 1.46-1.69 (3H, m, C(3´)H_A, NH_2), 1.71-1.86 (3H, m, C(3´)H_B, C(4´)H_2), 2.26 (3H, s, C(3)CH_3) 2.73 (3H, t, *J* = 7.1 Hz, C(5´)H_2), 3.46 (1H, dd, *J* = 7.5, 4.5 Hz, C(2´)*H*), 3.72 (3H, s, OCH_3), 5.82 (C(4)*H*); ^{13}C NMR (126 MHz, $CDCl_3$) 11.4 (C(3)*C*H_3), 23.7 (*C*(4´)), 26.3 (*C*(5´)), 34.1 (*C*(3´)), 52.0 (O*C*H_3), 54.1 (*C*(2´)), 101.6 (*C*(4)), 159.7 (*C*(3)), 172.5 (*C*(5)), 176.2 (*C*(1´)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 235.1046; $C_{10}H_{16}N_2NaO_3$ requires M^+ 235.1053; LRMS *m*/*z* (ES^+) 213 ($[M+H]^+$, 100), 235 ($[M+Na]^+$, 15); HPLC (220 nm): Retention time 7.84 min, purity 93.1%.

(*S*)-2-Amino-5-(3-methylisoxazol-5-yl)pentanoic acid **154**

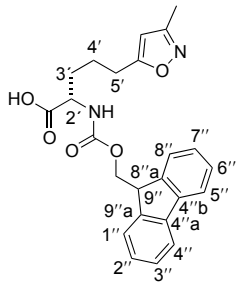


Method A: To a solution of (*S*)-**179** (1.24 g, 5.84 mmol) in THF (30 mL) was added a solution of LiOH (168 mg, 7.01 mmol, 1.2 eq) in H_2O (6 mL) and the reaction was stirred at rt. After 2 h, TLC analysis indicated complete consumption of **179**, and the mixture was acidified to pH 5 with aq. HCl (1 M). The resultant precipitate was filtered, washed with a small amount of ice-cold H_2O, ice-cold THF and Et_2O and dried in a vacuum desiccator to give (*S*)-**154** as a colourless hygroscopic solid (870 mg, 75%); $[\alpha]_D^{24}$ = +4.1 (*c* 1.0, H_2O); ν_{max} (solid)/cm^{-1}: 3118, 3035, 2940, 2596 (N–H, O–H) (br), 1604 (s), 1580 (C=O) (s), 1509 (s), 1409 (s), 1325 (m); ^{1}H NMR (500 MHz, D_2O) 1.59-188 (4H, m, C(3´)H_2, C(4´)H_2), 2.16 (3H, s, C(3)CH_3), 2.73 (2H, t, *J* = 7.2 Hz, C(5´)H_2), 3.66 (1H, app t, *J* = 6.0 Hz, C(2´)*H*), 6.03 (1H, s, C(4)*H*); ^{13}C NMR (126 MHz, D_2O) 10.3 (C(3)*C*H_3),

22.4 (*C*(4′)), 25.3 (C(5′)), 29.7 (*C*(3′)), 54.4 (C(2′)), 102.5 (*C*(4)), 161.7 (*C*(3)), 173.2 (*C*(5)), 174.5 (*C*(1′)); HRMS *m*/*z* (ES^+) found $[M+Na]^+$ 221.0898; $C_9H_{14}N_2NaO_3$ requires M^+ 221.0897; LRMS *m*/*z* (ES^+) 199 ($[M+H]^+$, 100), 221 ($[M+Na]^+$, 14), 397 ($[2M+H]^+$, 10).

Method B: To a solution of (*S*)-**179** (110 mg, 518 µmol) in THF (5 mL) and H_2O (1 mL) was added LiOH (17 mg, 710 µmol, 1.4 eq) and the reaction was stirred at rt. After 90 min, TLC analysis indicated complete consumption of **179**, and the mixture was acidified to pH 1 with conc. HCl. Purification by ion-exchange chromatography (Dowex 50WX8, 100-200 mesh), washing with water then eluting with 1 M NH_4OH gave (*S*)-**154** as a colourless powder after lyophilisation (52 mg, 51%). The data are identical to those reported above; HPLC (220 nm): retention time 7.34 min, purity 96.8%.

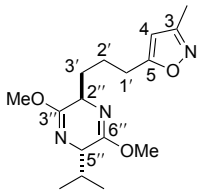
(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(3-methylisoxazol-5-yl)pentanoic acid **157**



To a suspension of (*S*)-**154** (863 mg, 4.35 mmol, 1.0 eq) in H_2O (40 mL) and 1,4-dioxane (20 mL) was added $NaHCO_3$ (731 mg, 8.70 mmol, 2.0 eq). After 30 min, Fmoc-OSu (1.54 g, 4.57 mmol, 1.05 eq) in 1,4-dioxane (12 mL) was added dropwise over 15 min. After 2.5 h, TLC analysis indicated complete consumption of **154**, and the reaction was acidified to pH 2 with aq. HCl (1 M) then extracted with EtOAc (4 × 50 mL). The combined organic layers were washed

with aq. citric acid (10% w/v, 200 mL), water (3 × 200 mL) and brine (200 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Crystallisation from boiling 2:1 hexane/CH_2Cl_2 gave (*S*)-**157** as a colourless solid (1.67 g, 91%); $[\alpha]_D^{24}$ = –7.5 (*c* 1.0, MeOH); mp 92-94 °C (2:1 hexane/CH_2Cl_2); ν_{max} (solid)/cm^{-1}: 3347 (O–H, N–H) (br), 3038, 2961 (C–H) (w), 1709, 1682 (C=O) (s), 1528 (s), 1250 (s); ^{1}H NMR (500 MHz, DMSO-d_6) 1.52-1.85 (4H, m, C(3′)C*H*$_2$, C(5′)C*H*$_2$), 2.19 (3H, s, C(3)C*H*$_3$), 2.63-2.78 (2H, m, C(4′)*H*$_2$), 3.94-4.04 (1H, m, C(2′)*H*), 4.19-4.35 (3H, m, OC*H*$_2$, OCH$_2$C(9′′)*H*), 6.10 (1H, s, C(4)*H*), 7.28-7.36 (2H, m, C(2′′)*H*, C(7′′)*H*), 7.38-7.45 (2H, m, C(3′′)*H*, C(6′′)*H*), 7.61-7.77 (3H, m, N*H*, C(1′′)*H*, C(8′′)*H*), 7.86-7.94 (2H, m, C(4′′)*H*, C(5′′)*H*), 12.5 (1H, br s, O*H*); ^{13}C NMR (126 MHz, DMSO-d_6) 11.0 (C(3)*C*H$_3$), 23.7 (*C*(5′)), 25.4 (*C*(4′)), 30.1 (*C*(3′)), 46.6 (*C*(9′′)), 53.4 (*C*(2′)), 65.6 (O*C*H$_2$), 101.9 (*C*(4)), 120.10, 120.12 (*C*(4′′), *C*(5′′)), 125.25, 125.28 (*C*(1′′), *C*(8′′)), 127.1 (*C*(2′′), *C*(7′′)), 127.6 (*C*(3′′), *C*(6′′)), 140.70, 140.72 (*C*(4′′a), *C*(4′′b)), 143.77, 143.84 (*C*(8′′a), *C*(9′′a)), 156.2 (NH*C*OO), 159.3 (*C*(3)), 172.4 (*C*(5)), 173.7 (*C*OOH); HRMS *m*/*z* (ES$^+$) found [M+Na]$^+$ 443.1580; $C_{24}H_{24}N_2NaO_5$ requires M$^+$ 443.1577; LRMS *m*/*z* (ES$^+$) 421 ([M+H]$^+$, 97), 443 ([M+Na]$^+$, 100). HPLC: Retention time 12.37 min, purity 99.2 %; Chiral HPLC: Retention time 10.0 min, ee 76.1%.

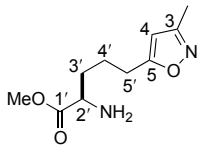
5-(3-((2*R*,5*S*)-5-Isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)propyl)-3-methylisoxazole **169**



To a solution of (*S*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine [(*S*)-**167**, 250 µL, 258 mg, 1.40 mmol, 1.0 eq] in anhydrous THF (5.6 mL) at –78 °C

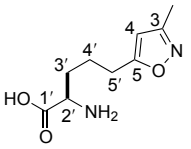
under an argon atmosphere was added ^{n}BuLi (2.3 M in hexanes, 640 µL, 1.47 mmol, 1.05 eq) dropwise. After 15 min, a precooled solution of **178** (351 mg, 1.40 mmol, 1.0 eq) in THF (1 mL) was added dropwise *via* cannula. The reaction mixture was stirred at –78 °C for 2 h, then between –40 °C and –60 °C for 6 h, then slowly warmed to rt over 15 h. The reaction was quenched with H_2O (10 mL) and pH 7 phosphate buffer (1 M, 5 mL), then extracted with Et_2O (3 × 15 mL). The combined organic layers were washed with brine (40 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (15%, 25% Et_2O/petroleum ether) gave (2*R*,5*S*)-**169** as a pale yellow oil (326 mg, 76%). Data as for (2*S*,5*R*)-**169**; $[\alpha]_D^{24}$ = +1.8 (*c* 1.0, $CHCl_3$).

(*R*)-Methyl 2-amino-5-(3-methylisoxazol-5-yl)pentanoate **179**



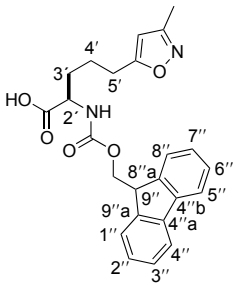
To a solution of (2*R*,5*S*)-**169** (303 mg, 986 µmol) in MeCN (3 mL) and H_2O (1 mL) was added TFA (0.5 mL) and the reaction was stirred at rt. After 3 h, TLC analysis indicated complete consumption of **169**, and the reaction was concentrated *in vacuo*. The residues were dissolved in 10 mL CH_2Cl_2, and basified to pH 8 with sat. aq. $NaHCO_3$. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (3% MeOH/CH_2Cl_2) gave (*R*)-**179** as a yellow oil (193 mg, 92%); Data as for (*S*)-**179**; $[\alpha]_D^{24}$ = –10.2 (*c* 1.0, $CHCl_3$).

(*R*)-2-Amino-5-(3-methylisoxazol-5-yl)pentanoic acid **154**



To a solution of (*R*)-**179** (170 mg, 801 µmol) in THF (5 mL) and H_2O (1 mL) was added LiOH (23 mg, 961 µmol, 1.2 eq) and the reaction was stirred at rt. After 2 h, TLC analysis indicated complete consumption of **179**, and the mixture was acidified to pH 1 with conc. HCl and concentrated *in vacuo* to remove THF, then lyophilised. Purification by ion-exchange chromatography (Dowex 50WX8, 100-200 mesh), washing with water then eluting with 1 M NH_4OH gave (*R*)-**154** as a colourless powder after lyophilisation (46 mg, 29%); Data as for (*S*)-**154**; $[\alpha]_D^{24}$ = –4.9 (*c* 1.0, H_2O); HPLC (220 nm): retention time 7.38 min, purity 97.2%.

(*R*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(3-methylisoxazol-5-yl)pentanoic acid **157**

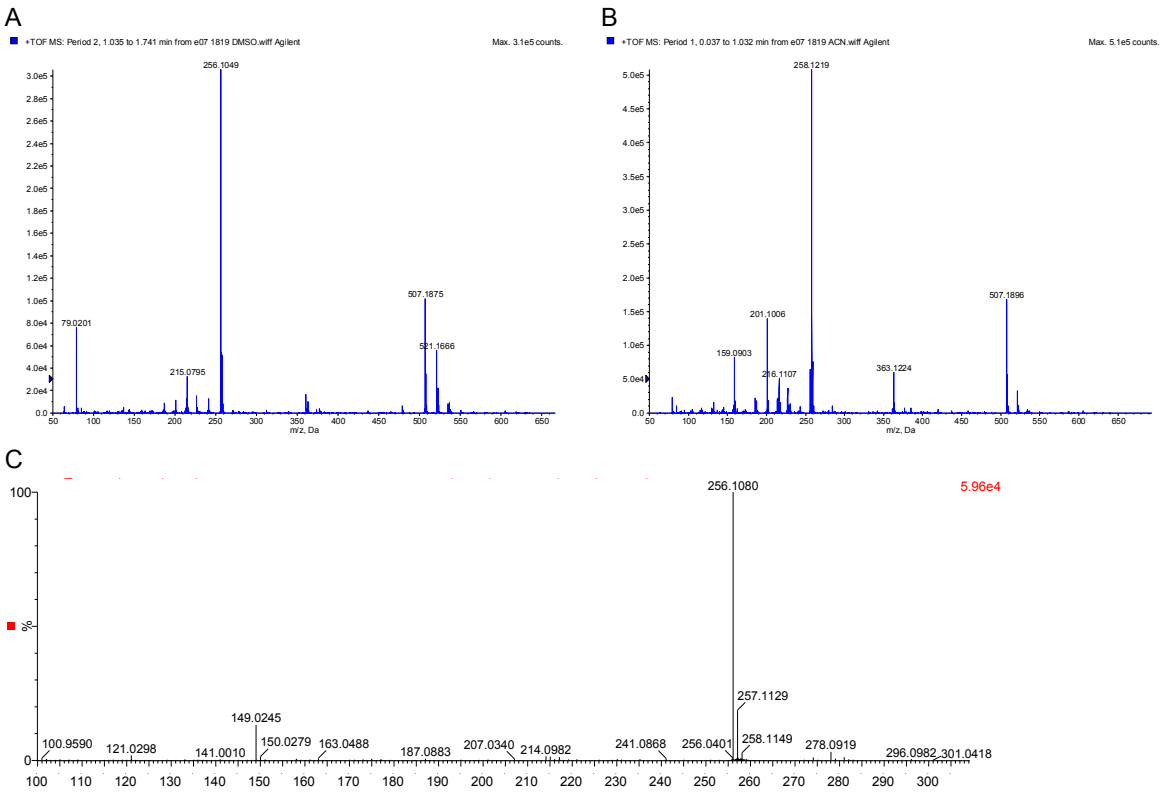


To a suspension of (*R*)-**154** (20 mg, 101 µmol, 1.0 eq) in H_2O (0.75 mL) and 1,4-dioxane (0.38 mL) was added $NaHCO_3$ (17 mg, 202 µmol, 2.0 eq). After 30 min, Fmoc-OSu (36 mg, 106 µmol, 1.05 eq) in 1,4-dioxane (0.38 mL) was added dropwise over 15 min. After 3.5 h, TLC analysis indicated complete consumption of **154**, and the reaction was acidified to pH < 3 with aq. HCl (1 M) then

extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with aq. citric acid (10% w/v, 30 mL), water (3 × 30 mL) and brine (30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Crystallisation from boiling 2:1 hexane/CH_2Cl_2 gave (*R*)-**157** as a colourless solid (24 mg, 56%); Data as for (*S*)-**157**; mp 92-94 °C; $[\alpha]_D^{24}$ = +0.9 (*c* 1.0, MeOH). HPLC: retention time 12.39 min, purity 99.1%; Chiral HPLC: Retention time 31.6 min, ee 54.2%.

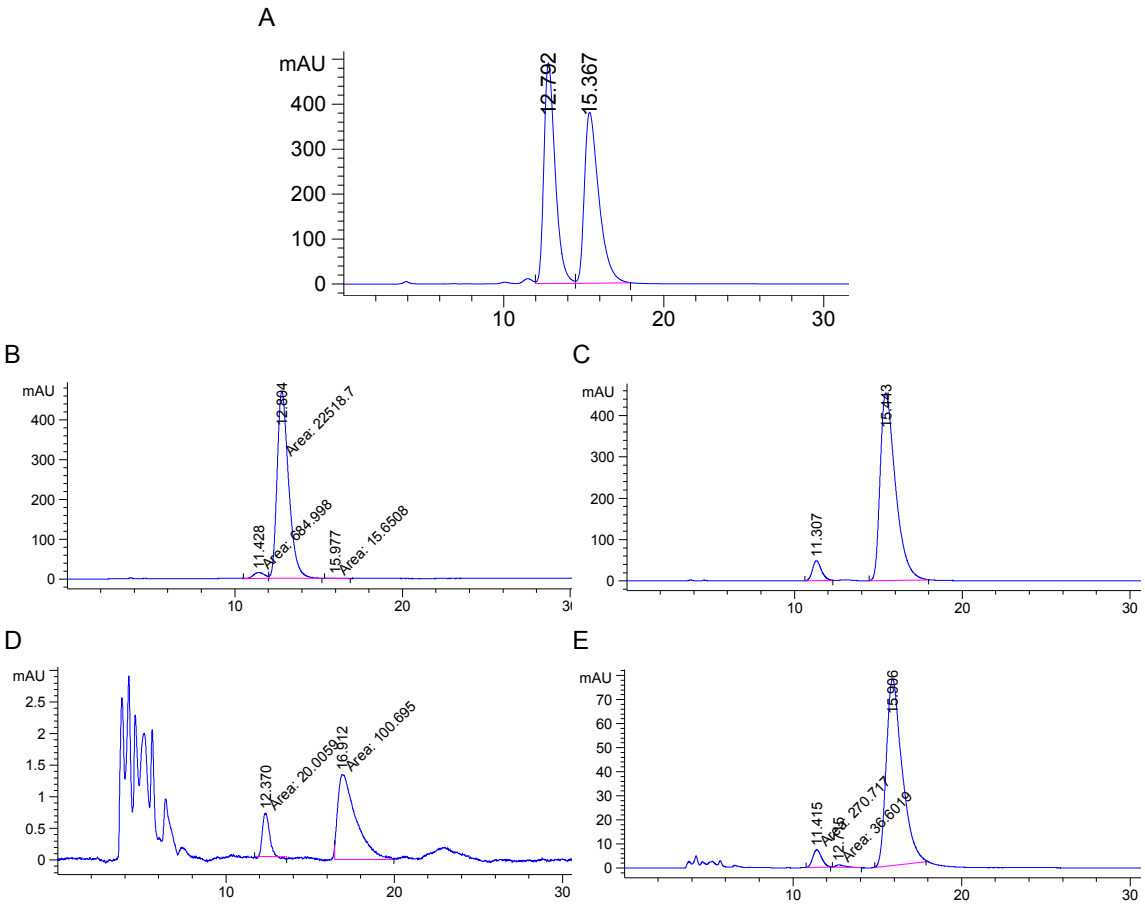
Appendices

Appendix 1: Mass spectra of 45



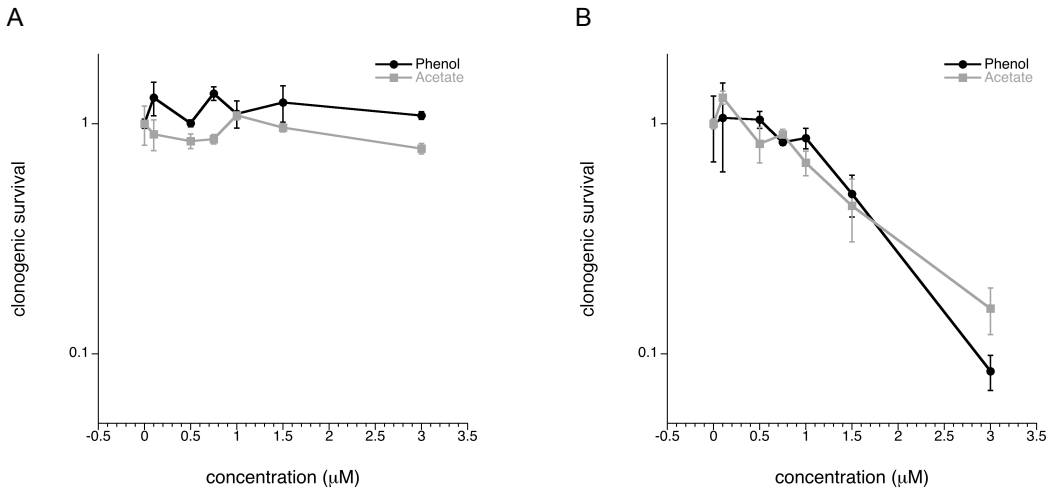
45a is liable to oxidation under laboratory conditions. A: Mass spectrum of purchased **45a** after storage in DMSO for one month; $[M-H]^+$ is likely to arise from oxidation at the benzylic position. B: Mass spectrum of freshly-prepared MeCN suspension of purchased **45a**; $[M+H]^+$ is observed, but $[M-H]^+$ is not; C: High-resolution LCMS of resynthesized **45a** after stirring in DMSO for 5 days; $[M-H]^+$ present. For **45a**, calculated $[M+H]^+$: 258.1237, $[M-H]^+$: 256.1081. Details of MS experiments are given in Section 7.1.

Appendix 2: Preparative chiral HPLC separation of (+)-61a and (–)-61a.



Chiral HPLC can separate the enantiomers of 61a, which do not racemise in buffer. A: Racemic **61a**. B: (–)-**61a**. C: (+)-**61a**; analytical chiral HPLC indicates >99% ee in both cases. D: AlphaScreen® buffer only. E: (+)-**61a** after 2 h in AlphaScreen® buffer; analytical chiral HPLC indicates >98% ee and 95% purity. UV absorbance monitored at 230 nM.

Appendix 3: Clonogenic assay for A549 and H1975 cells.



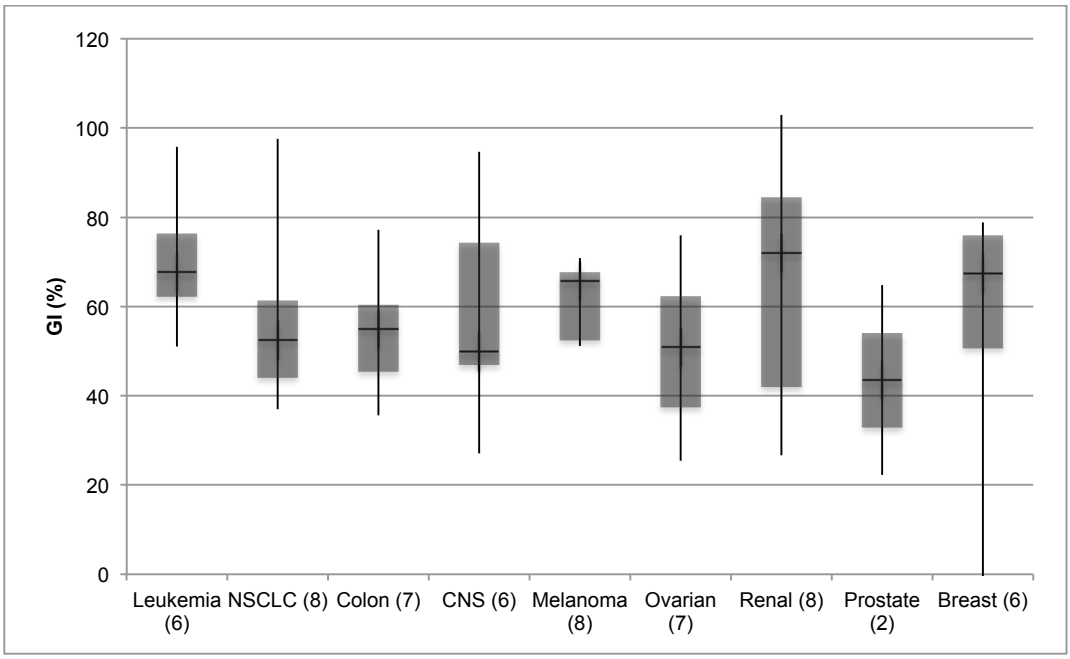
Clonogenic (colony formation) assay for lung adenocarcinoma cell lines A549 (**A**) and H1975 (**B**) after treatment with **61a** (phenol) or **61b** (acetate).

Appendix 4. Comparison of NCI-60 DTP Human Tumour Cell Line Screen for 61a, PFI-1 and XD14

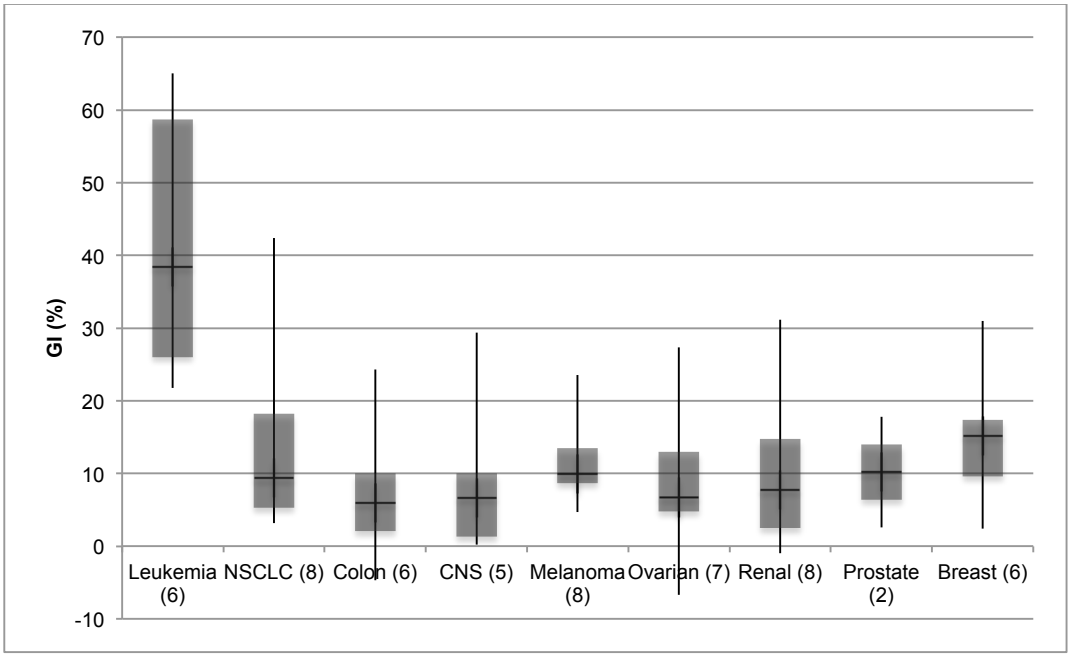
		GI (%)			GI_{50} (μM)		TGI (μM)	
	Cell line	**61a**	PFI-1	XD14	**61a**	PFI-1	**61a**	PFI-1
Leukemia	CCRF-CEM	53.3	51.1	23.0	14.8	7.8		
	HL-60(TB)	90.0	78.1	65.0	2.17	3.4	22.7	
	K-562	57.9	71.4	42.0	8.69	4.6		
	MOLT-4	41.3	61.6	34.8	17.9	10.5	80.7	
	RPMI-8226	85.1	95.7	21.8	1.94	2.2	9.7	
	SR	72.9	64.0	64.2	1.01	3.7		
NSCLC	A549/ATCC	44.6	49.5	4.1	22.4	19.5		
	HOP-62	52.8	55.7	5.7	4.89	2.5		
	HOP-92	74.4	97.6	15.8	1.02	1.2	72.9	70.8
	NCI-H226	34.5	37.0	9.7	21.3	14.1		
	NCI-H23	31.9	43.3	8.9		8.5		
	NCI-H322M	52.4	57.1	25.4	8.41	6.0		
	NCI-H460	32.8	44.2	3.2	25.1	9.3		
	NCI-H522	77.9	73.9	42.4	27	8.7		
Colon	COLO 205	62.9	61.6	-4.7	5.25	3.0		
	HCC-2998	37.8	35.6		8.55	9.1		93.3
	HCT-116	42.0	50.3	10.9	7.08	4.5		
	HCT-15	63.3	59.2	4.7	3.52	3.2		
	HT29	65.4	77.2	1.2	4.54	3.0		
	KM12	46.9	55.0	24.3	8.2	10.7		
	SW-620	27.9	40.6	7.3	24.6	12.0		
CNS	SF-268	28.5	27.1	0.2	39.3	28.8		
	SF-295	57.5	94.7	10.0	7.31	13.5		
	SF-539	37.5	49.9		4.56	4.5		
	SNB-19	47.3	45.9	1.3	5.69	4.8		
	SNB-75	113.2	82.5	29.4	0.423	0.4		
	U251		50.0	6.6	15	15.1		
Melanoma	LOX IMVI	72.7	70.9		3.6	3.5	80.5	21.4
	MALME-3M	40.0	67.8	4.7	28.9			
	M14	44.0	52.2	15.2	4.48	2.9		
	MDA-MB-435	56.1	65.8	9.6	3.48	2.6		
	SK-MEL-2	39.6	52.4	9.1	23.5	29.5		
	SK-MEL-28	38.8	51.1	7.2	9.15	6.2		
	SK-MEL-5	45.8	59.5	12.9	5.95	6.3	29.5	
	UACC-257	59.6	66.2	10.3	4.66	3.6		
	UACC-62	68.5	70.7	23.5		0.7		

		GI (%)			GI_{50} (µM)		TGI (µM)	
	Cell line	**61a**	PFI-1	XD14	**61a**	PFI-1	**61a**	PFI-1
Ovarian	IGROV1	54.0	76.0	27.3	23.2	6.3		
	OVCAR-3	53.3	67.8	11.2	6.64	4.0		87.1
	OVCAR-4	67.5	56.9	14.8	6.35	6.3		
	OVCAR-5	26.7	25.5	-6.7	31.8	10.5		
	OVCAR-8	61.4	43.8	6.7	8.33	9.1		
	NCI/ADR-RES	40.8	31.1	3.2	6.32	17.4		
	SK-OV-3	56.3	50.9	6.4	7.01	4.9		
Renal	786-0	45.2	27.3	15.6	27.1	12.0		
	A498	103.3	102.9	14.5	2.6	1.8	20.3	6.0
	ACHN	39.4	65.3	3.6	4.76	7.8		
	CAKI-1	76.2	78.8	6.1	4.58	3.6		
	RXF 393		26.7	-1.0	17.2	7.9		
	SN12C	25.5	46.8	9.4	3.99	6.8		
	TK-10	87.4	82.7	-0.7	1.08	1.6		58.9
	UO-31	83.1	89.8	31.1	4.58	1.1		
s-tate	PC-3	51.6	64.7	17.8		28.2		
	DU-145	12.5	22.2	2.6	6.95	28.8		
Breast	MCF7	79.1	78.5	15.1	1.7	1.5		
	MDA-MB-231/ATCC	37.6	45.3	2.4	3.92	3.5		
	HS 578T	66.3	66.3	18.0	1.93	1.9	90.7	63.1
	BT-549	35.3	-0.7	15.3	28.2	14.8		
	T-47D	52.9	68.6	31.0	7.7	3.7		
	MDA-MB-468	75.1	78.9	7.7	1.89	1.5	40.6	47.9

Results of NCI-60 DTP Human Tumour Cell Screen for 61a, PFI-1 (16) and XD-14 (20). Growth is determined by sulforhodamine B fluorescence after 48 h incubation with **61a**. GI = 100 – growth (%); growth is measured following treatment at 10 µM and is expressed as a reduction in protein level relative to control (DMSO). GI_{50} is the drug concentration resulting in a 50% reduction in the net protein increase in control cells during the drug incubation. TGI is the concentration resulting in total growth inhibition. The maximum concentration tested was 100 µM; if total growth inhibition was not achieved at this concentration, it can be inferred that TGI > 100 µM. Red indicates greatest activity (high GI, low GI_{50}), green indicates least activity. Data for XD14 are from Lucas *et al.*[141]



Box plot indicating median, range and quartiles of growth inhibition (GI) for PFI-1 (**16**) after 48 h treatment at 10 µM, grouped by cancer type. The number in brackets is the number of cell lines of each type in the NCI-60 panel.



Box plot indicating median, range and quartiles of growth inhibition (GI) for XD14 (**20**) after 48 h treatment at 10 µM, grouped by cancer type. The number in brackets is the number of cell lines of each type in the NCI-60 panel.

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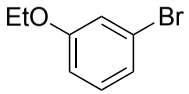
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Selected NMR Spectra

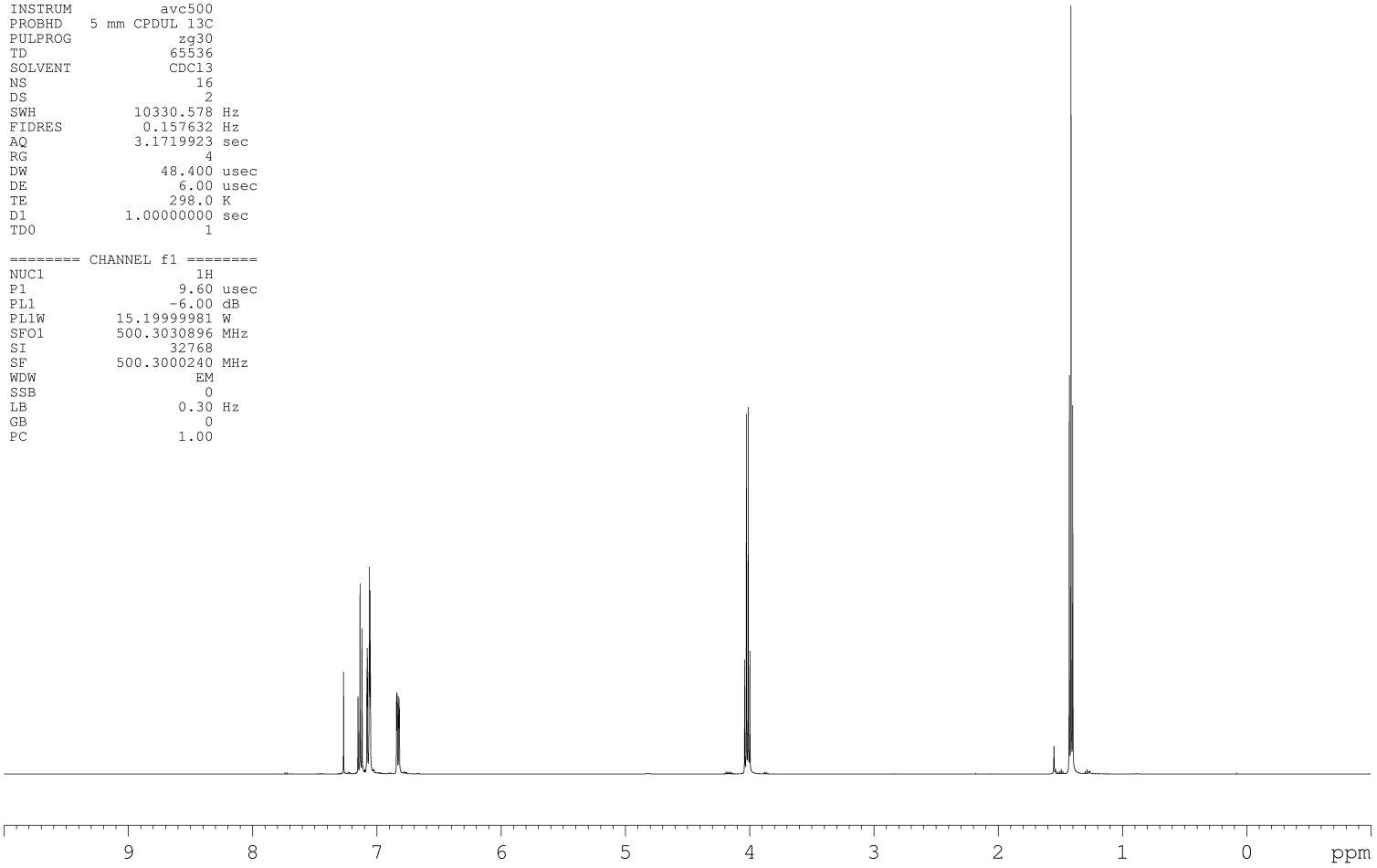
^{1}H, ^{13}C and (where appropriate) ^{19}F spectra are reported for all novel compounds, i.e. those for which this data has not previously been reported. Spectra are presented in the order in which the compounds appear in Chapter 7 (Experimental Procedures).

1-bromo-3-ethoxybenzene 52

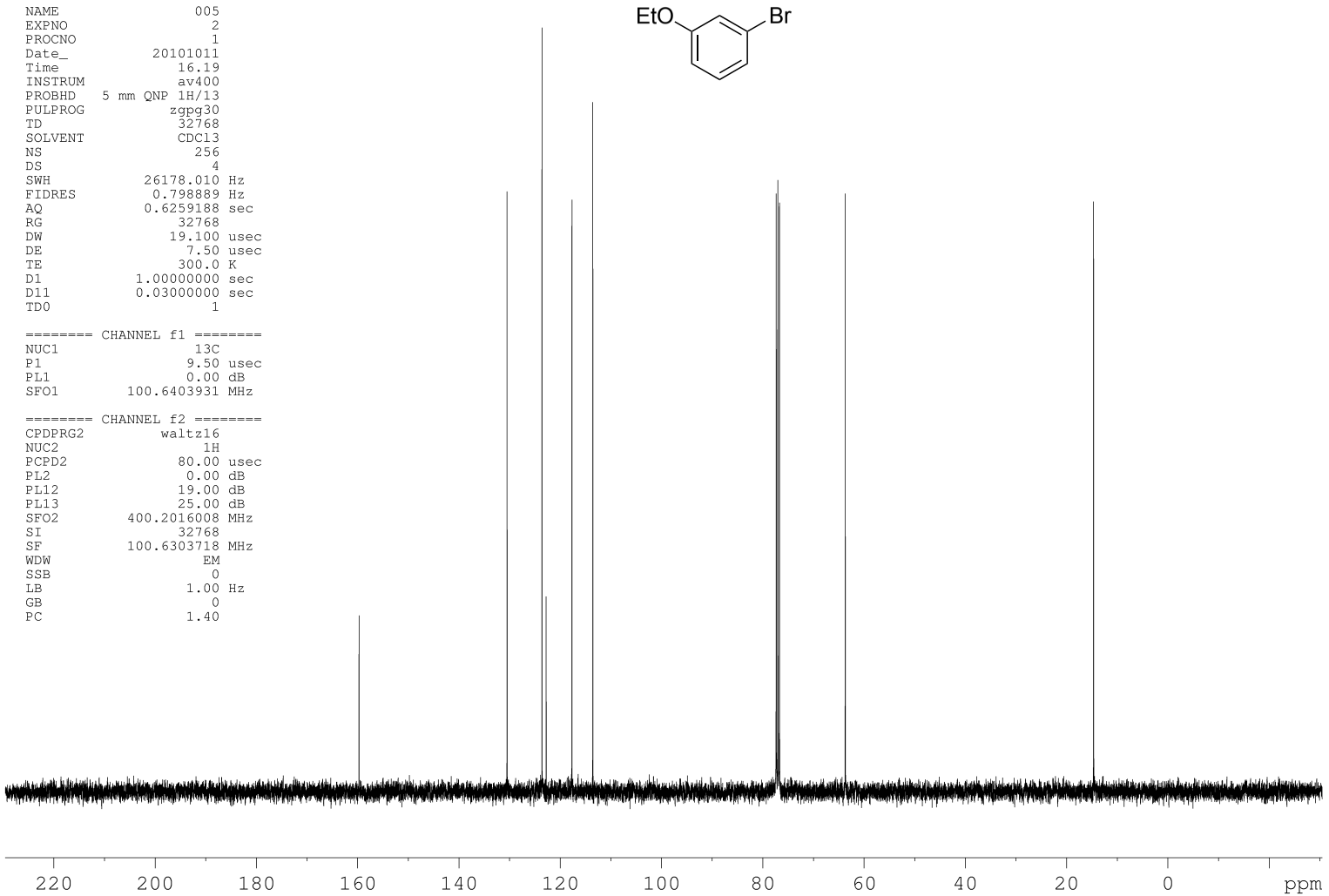



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PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ              3.1719923 sec
RG                     4
DW                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
SI                 32768
SF           500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



1-bromo-3-ethoxybenzene 52

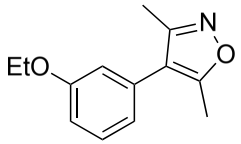



```
NAME                 005
EXPNO                  2
PROCNO                 1
Date_           20101011
Time                16.19
INSTRUM           av400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                 32768
SOLVENT            CDCl3
NS                   256
DS                     4
SWH            26178.010 Hz
FIDRES          0.798889 Hz
AQ              0.6259188 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                 0.00 dB
SFO1         100.6403931 MHz

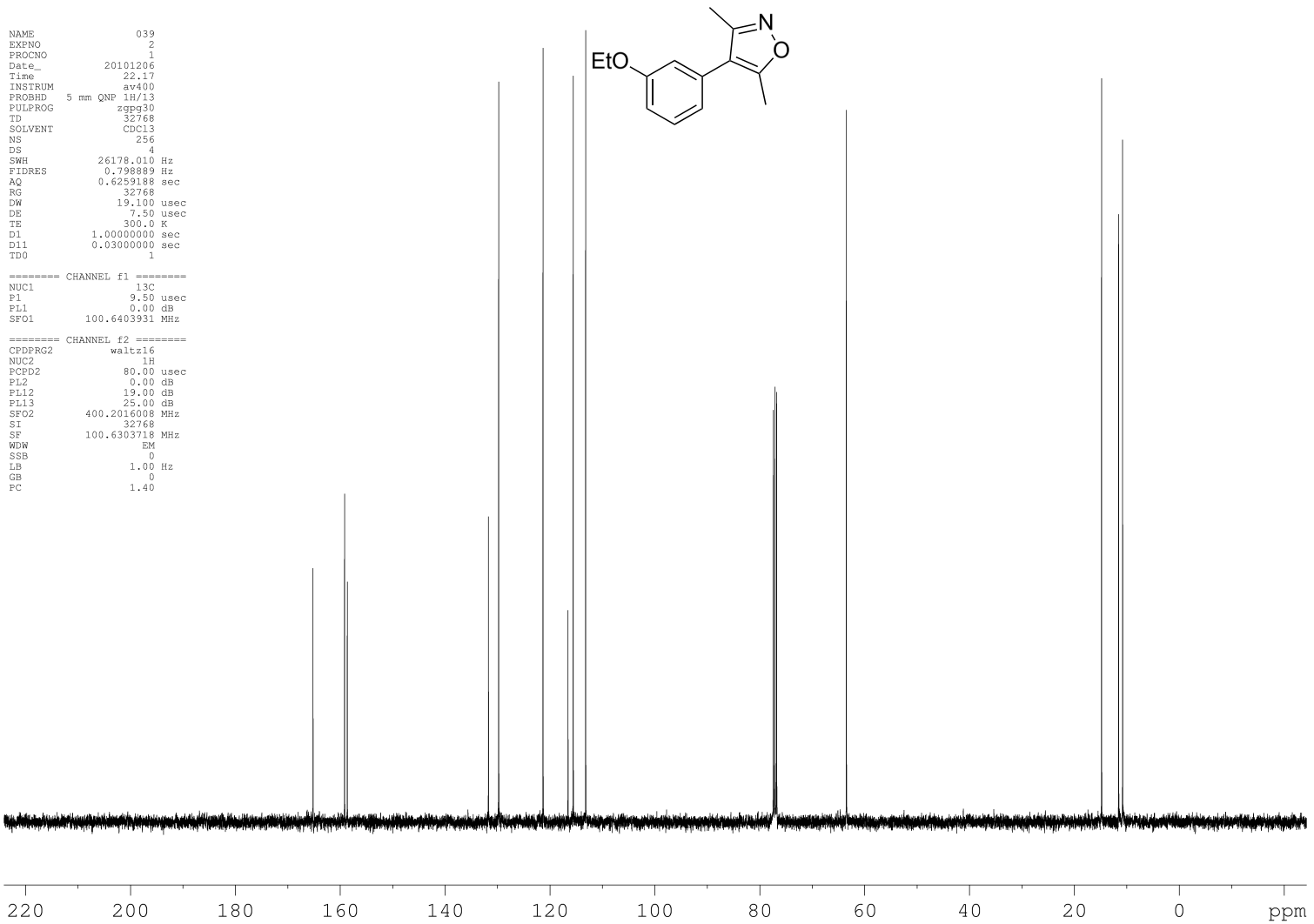
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                 0.00 dB
PL12               19.00 dB
PL13               25.00 dB
SFO2         400.2016008 MHz
SI                 32768
SF           100.6303718 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

4-(3-Ethoxyphenyl)-3,5-dimethylisoxazole 46c

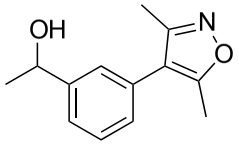


NAME	039
EXPNO	1
PROCNO	1
Date_	20101206
Time	22.09
INSTRUM	av400
PROBHD	5 mm QNP 1H/13
PULPROG	zg60
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	32
DW	60.400 usec
DE	7.50 usec
TE	300.0 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	9.00 usec
PL1	0.00 dB
SFO1	400.2024714 MHz
SI	32768
SF	400.2000028 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

4-(3-Ethoxyphenyl)-3,5-dimethylisoxazole 46c

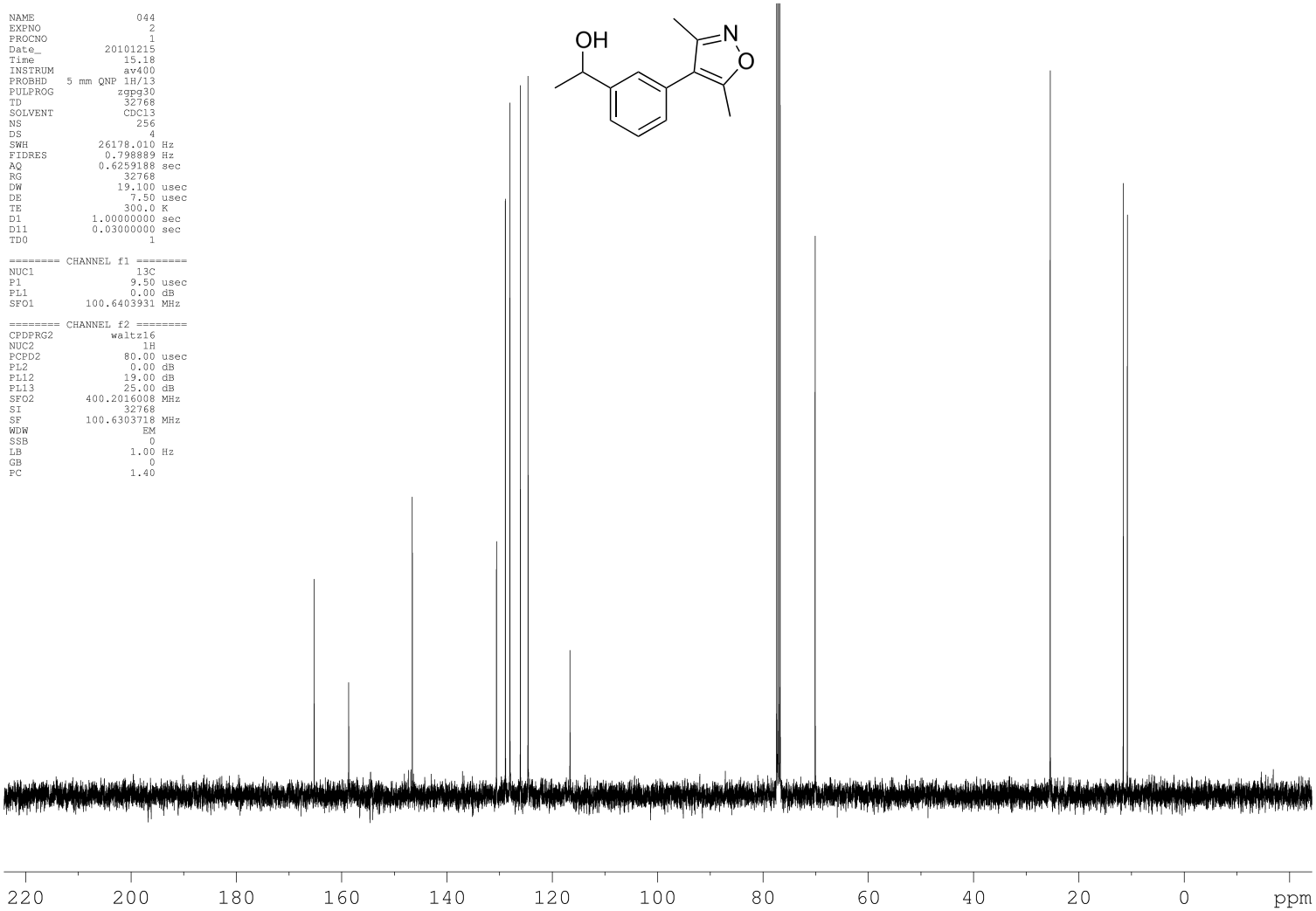


(*RS*)-1-(3-(3,5-Dimethylisoxazol-4-yl)phenyl)ethanol 46d

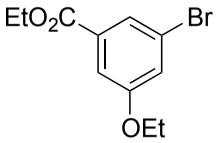


NAME	044
EXPNO	1
PROCNO	1
Date_	20101215
Time	15.10
INSTRUM	av400
PROBHD	5 mm QNP 1H/13
PULPROG	zg60
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	90.5
DW	60.400 usec
DE	7.50 usec
TE	300.0 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	9.00 usec
PL1	0.00 dB
SFO1	400.2024714 MHz
SI	32768
SF	400.2000028 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

(*RS*)-1-(3-(3,5-Dimethylisoxazol-4-yl)phenyl)ethanol 46d

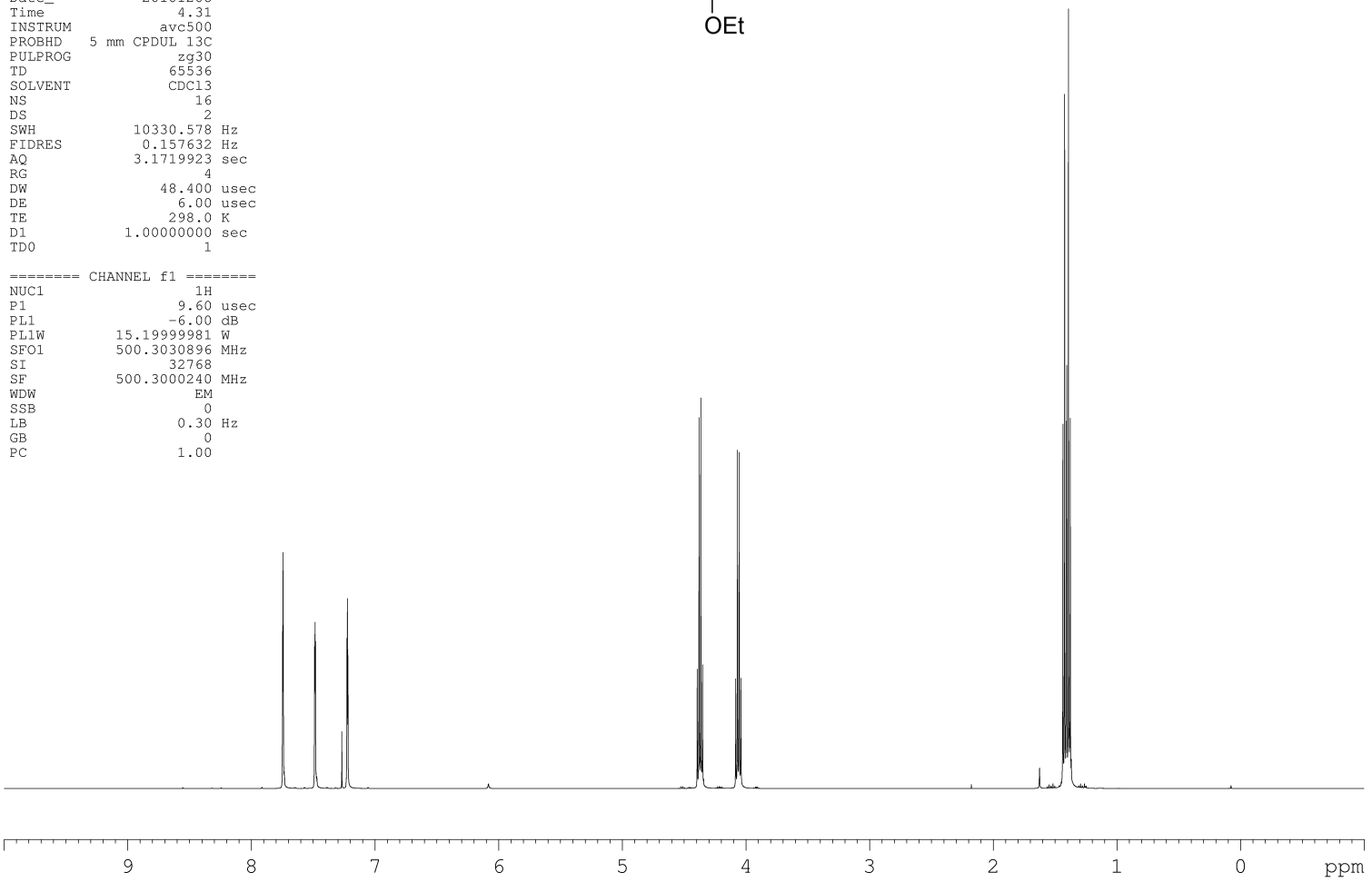


Ethyl 3-bromo-5-ethoxybenzoate 50

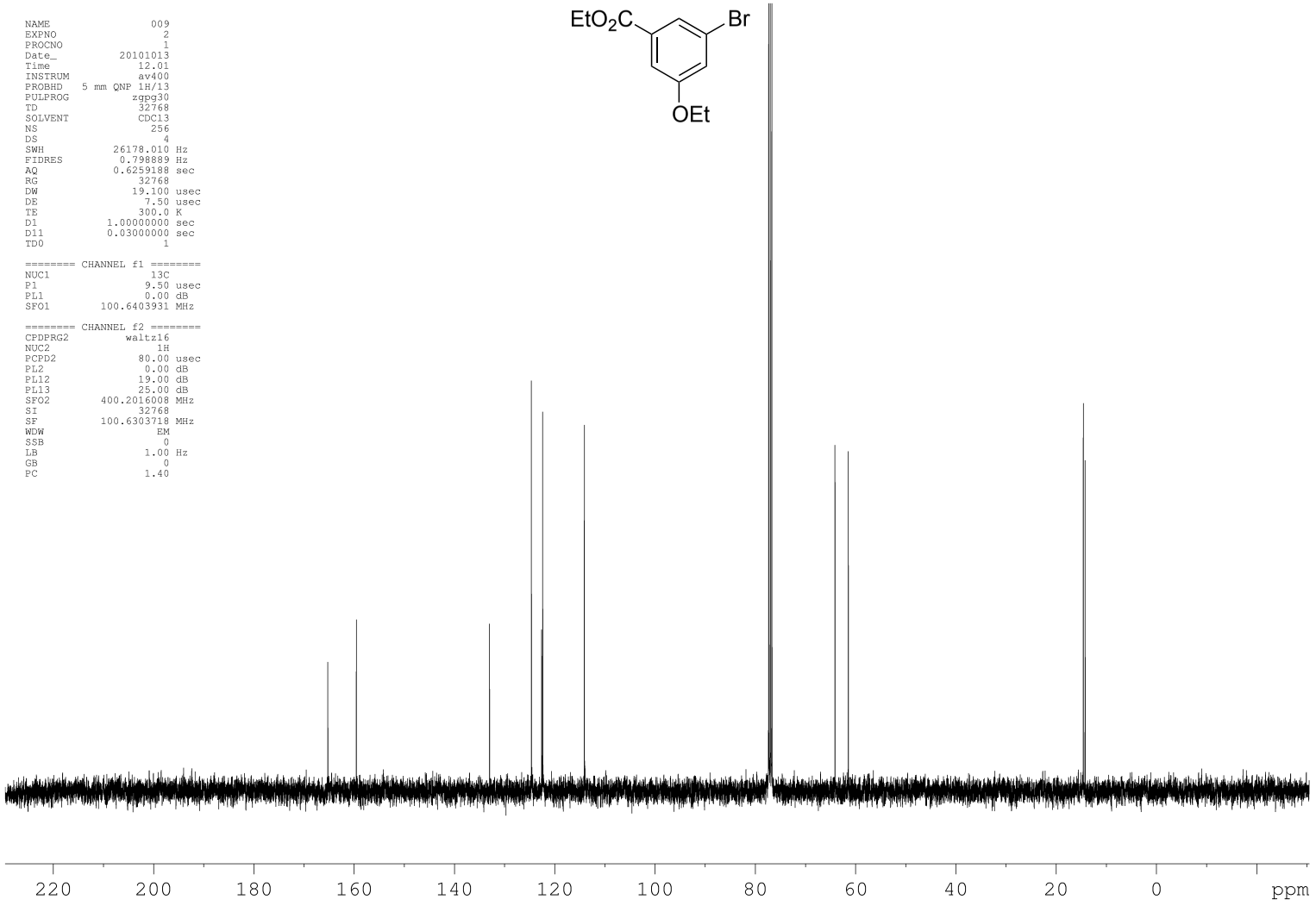



```
NAME      009 H and HMBC
EXPNO                  1
PROCNO                 1
Date_           20101208
Time                4.31
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ             3.1719923 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
SI                 32768
SF           500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



Ethyl 3-bromo-5-ethoxybenzoate 50

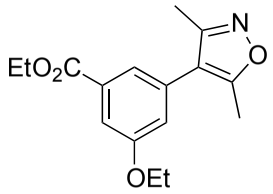



```
NAME                  009
EXPNO                   2
PROCNO                  1
Date_            20101013
Time                12.01
INSTRUM             av400
PROBHD   5 mm QNP 1H/13
PULPROG            zgpg30
TD                  32768
SOLVENT             CDCl3
NS                    256
DS                      4
SWH             26178.010 Hz
FIDRES          0.798889 Hz
AQ              0.6259188 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                  0.00 dB
SFO1         100.6403931 MHz

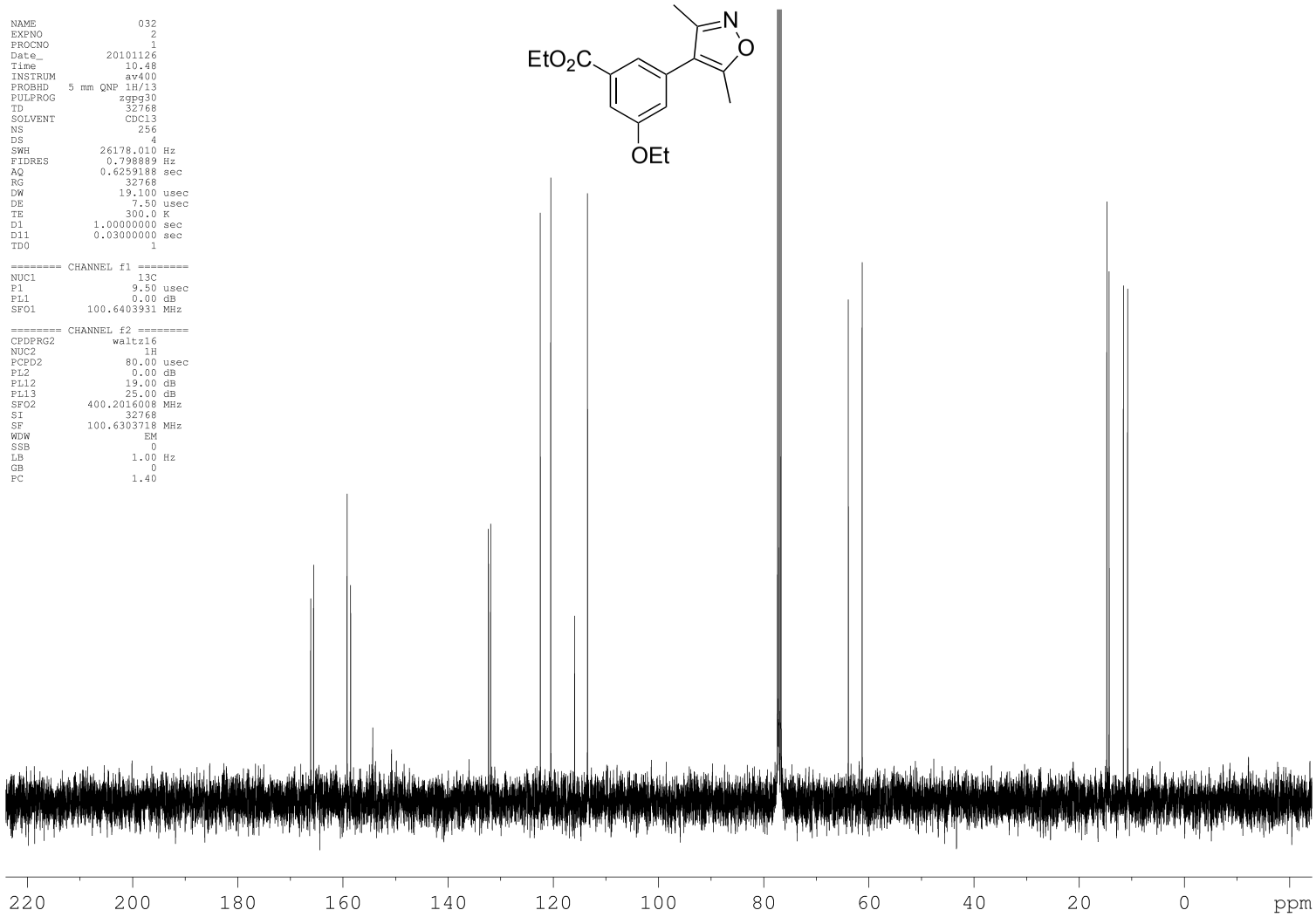
======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                  1H
PCPD2               80.00 usec
PL2                  0.00 dB
PL12                19.00 dB
PL13                25.00 dB
SFO2         400.2016008 MHz
SI                  32768
SF           100.6303718 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

Ethyl 3-(3,5-dimethylisoxazol-4-yl)-5-ethoxybenzoate 47a

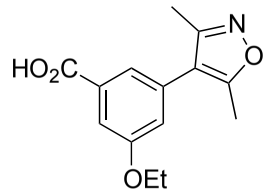


NAME	032
EXPNO	1
PROCNO	1
Date_	20101126
Time	10.40
INSTRUM	av400
PROBHD	5 mm QNP 1H/13
PULPROG	zg60
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	90.5
DW	60.400 usec
DE	7.50 usec
TE	300.0 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	9.00 usec
PL1	0.00 dB
SFO1	400.2024714 MHz
SI	32768
SF	400.2000028 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

Ethyl 3-(3,5-dimethylisoxazol-4-yl)-5-ethoxybenzoate 47a



3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxybenzoic acid 47b

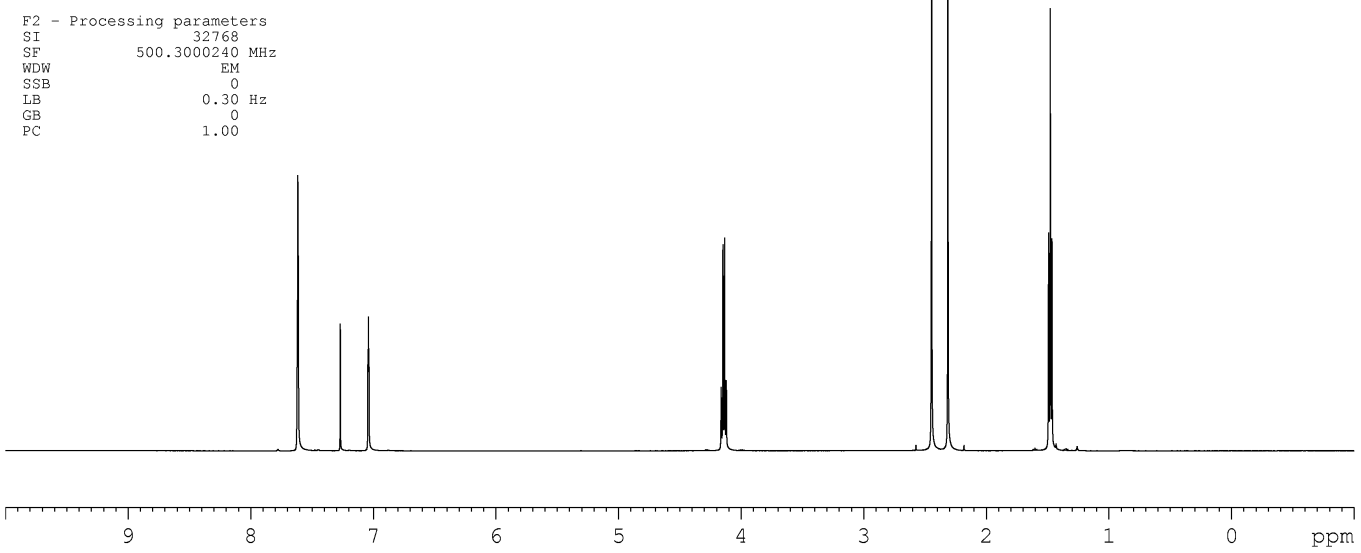



```
Current Data Parameters
NAME         103 recryst
EXPNO                  1
PROCNO                 1

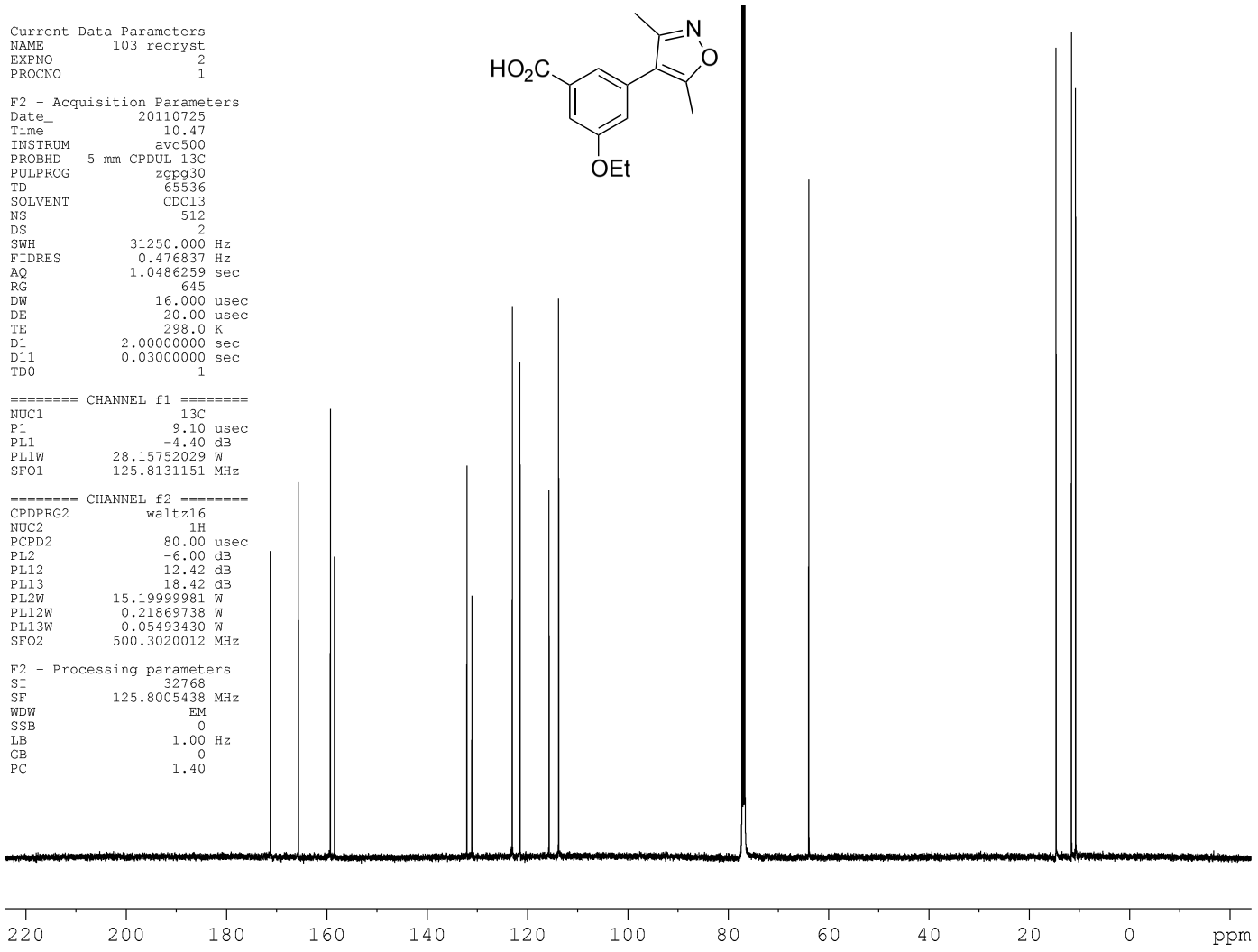
F2 - Acquisition Parameters
Date_           20110725
Time               10.20
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ             3.1719923 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

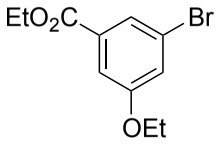
F2 - Processing parameters
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxybenzoic acid 47b



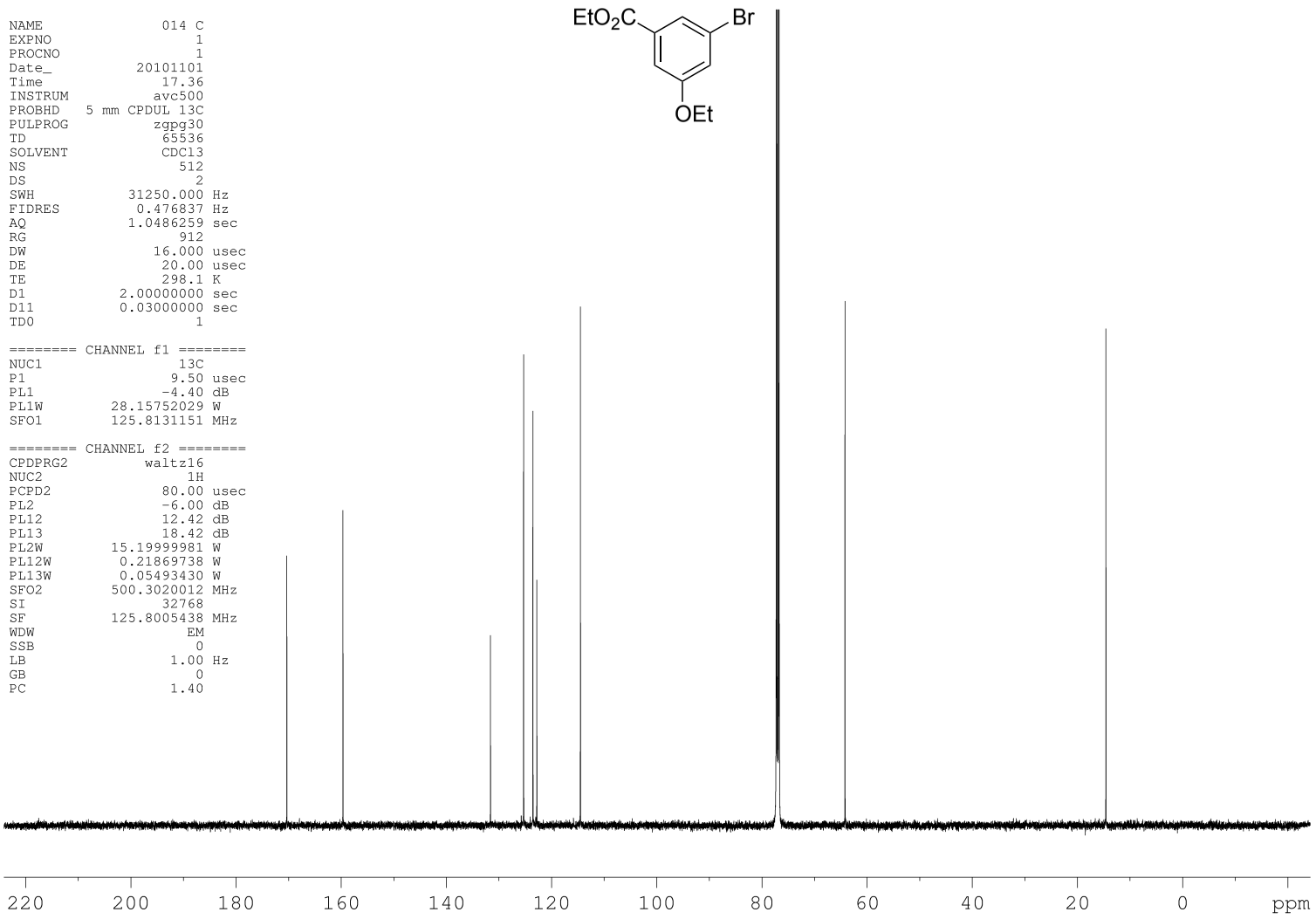
3-Bromo-5-ethoxybenzoic acid 54




```
NAME                 014
EXPNO                  1
PROCNO                 1
Date_           20101101
Time               11.16
INSTRUM            av400
PROBHD   5 mm QNP 1H/13
PULPROG             zg60
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ             3.9584243 sec
RG                   512
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                0.00 dB
SFO1        400.2024714 MHz
SI                 32768
SF          400.2000028 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                 1.00
```

3-Bromo-5-ethoxybenzoic acid 54




```
NAME               014 C
EXPNO                1
PROCNO               1
Date_           20101101
Time               17.36
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   512
DS                     2
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ             1.0486259 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

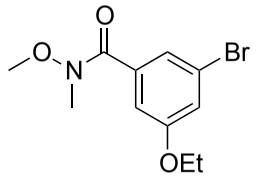
======== CHANNEL f1 ========
NUC1                 13C
P1                  9.50 usec
PL1                -4.40 dB
PL1W         28.15752029 W
SFO1         125.8131151 MHz

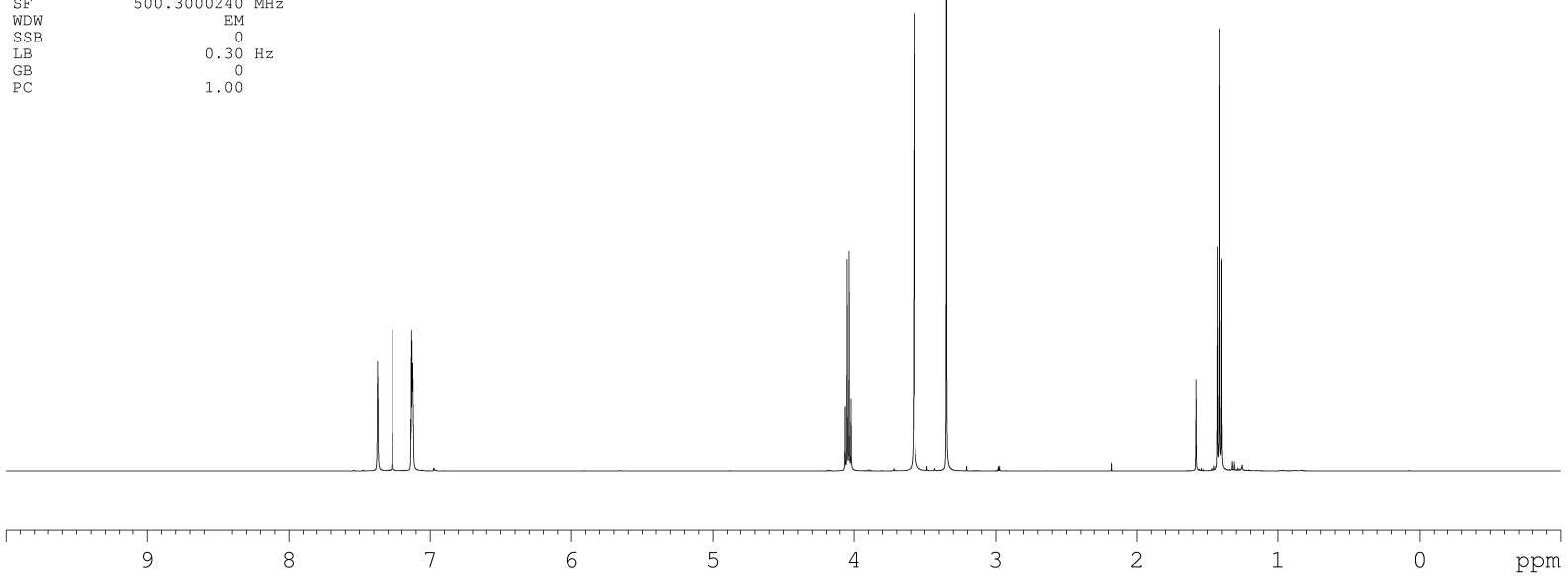
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               12.42 dB
PL13               18.42 dB
PL2W         15.19999981 W
PL12W         0.21869738 W
PL13W         0.05493430 W
SFO2         500.3020012 MHz
SI                 32768
SF           125.8005438 MHz
WDW                  EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
```

3-Bromo-5-ethoxy-*N*-methoxy-*N*-methylbenzamide 55

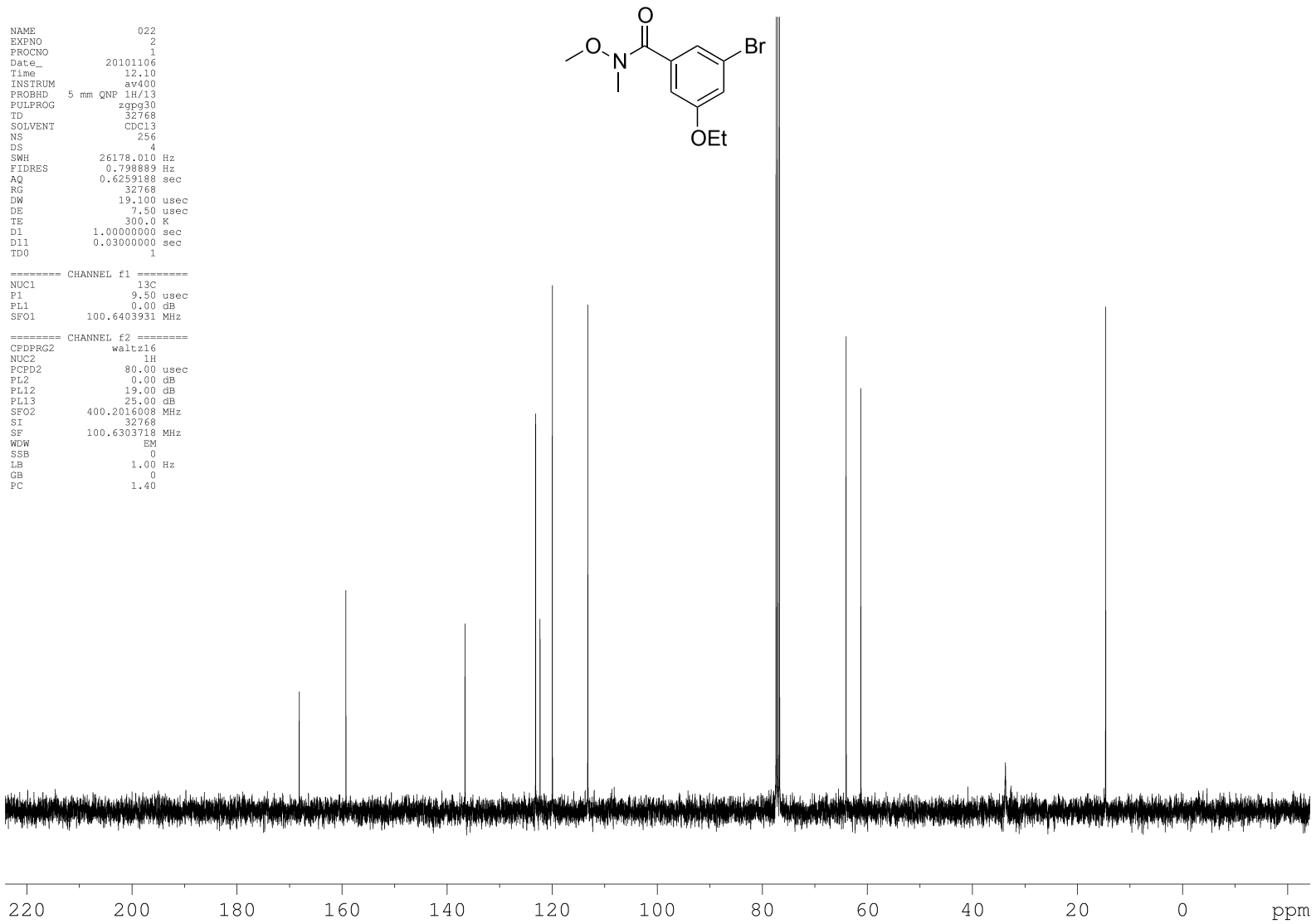
```
NAME      022 H and HMBC
EXPNO                  1
PROCNO                 1
Date_           20101208
Time                5.45
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 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
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

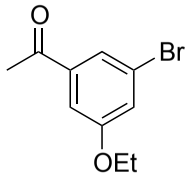




3-Bromo-5-ethoxy-*N*-methoxy-*N*-methylbenzamide 55

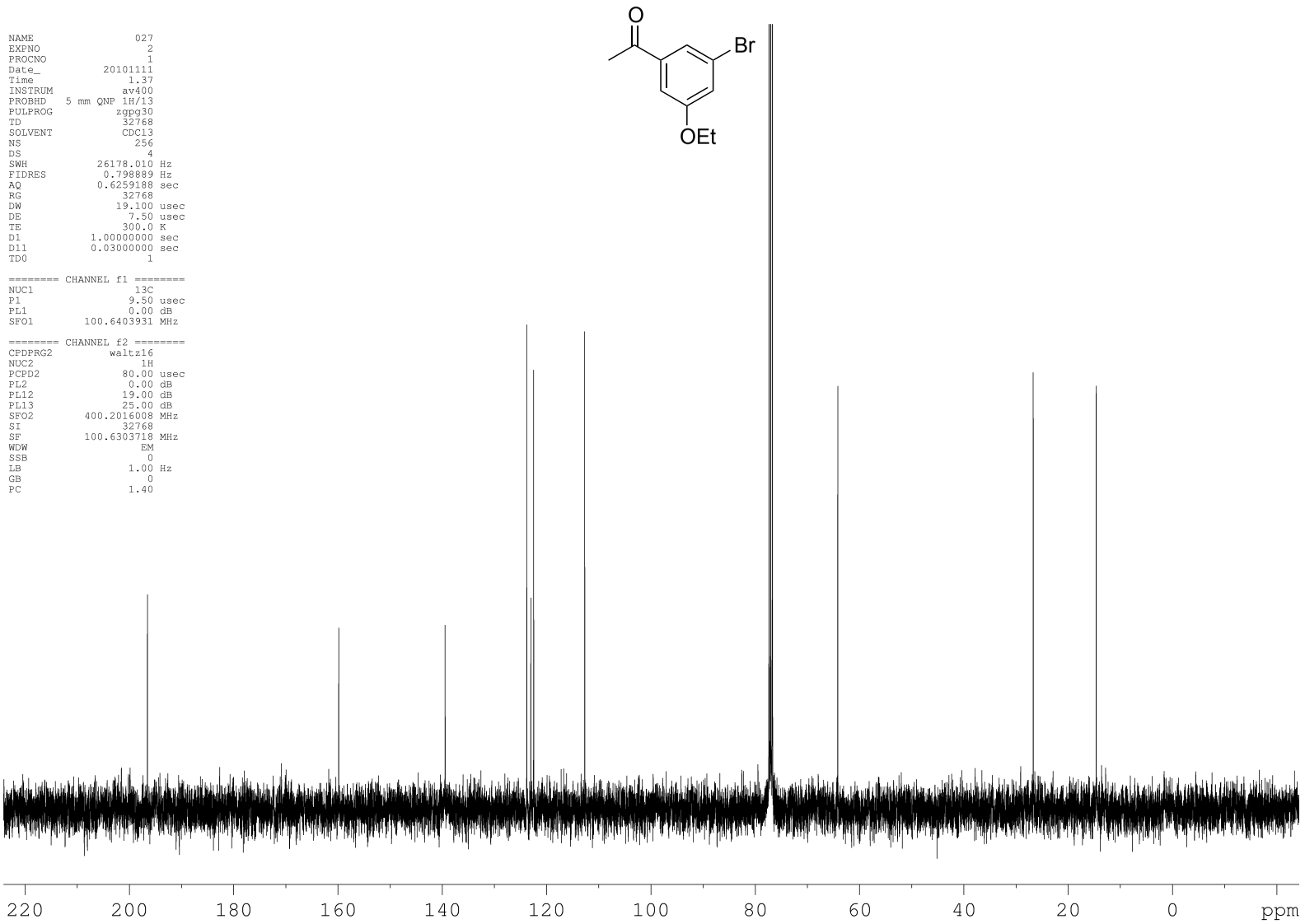


1-(3-Bromo-5-ethoxyphenyl)ethanone 56



NAME	027
EXPNO	1
PROCNO	1
Date_	20101111
Time	1.29
INSTRUM	av400
PROBHD	5 mm QNP 1H/13
PULPROG	zg60
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	228.1
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	0.00 dB
SFO1	400.2024714 MHz
SI	32768
SF	400.2000028 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

1-(3-Bromo-5-ethoxyphenyl)ethanone 56




```
NAME                 027
EXPNO                  2
PROCNO                 1
Date_           20101111
Time                1.37
INSTRUM            av400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                 32768
SOLVENT            CDCl3
NS                   256
DS                     4
SWH            26178.010 Hz
FIDRES             0.798889 Hz
AQ              0.6259188 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

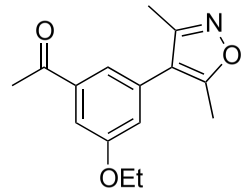
======== CHANNEL f1 ========
NUC1                  13C
P1                   9.50 usec
PL1                  0.00 dB
SFO1         100.6403931 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                  1H
PCPD2               80.00 usec
PL2                  0.00 dB
PL12                19.00 dB
PL13                25.00 dB
SFO2         400.2016008 MHz
SI                 32768
SF           100.6303718 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

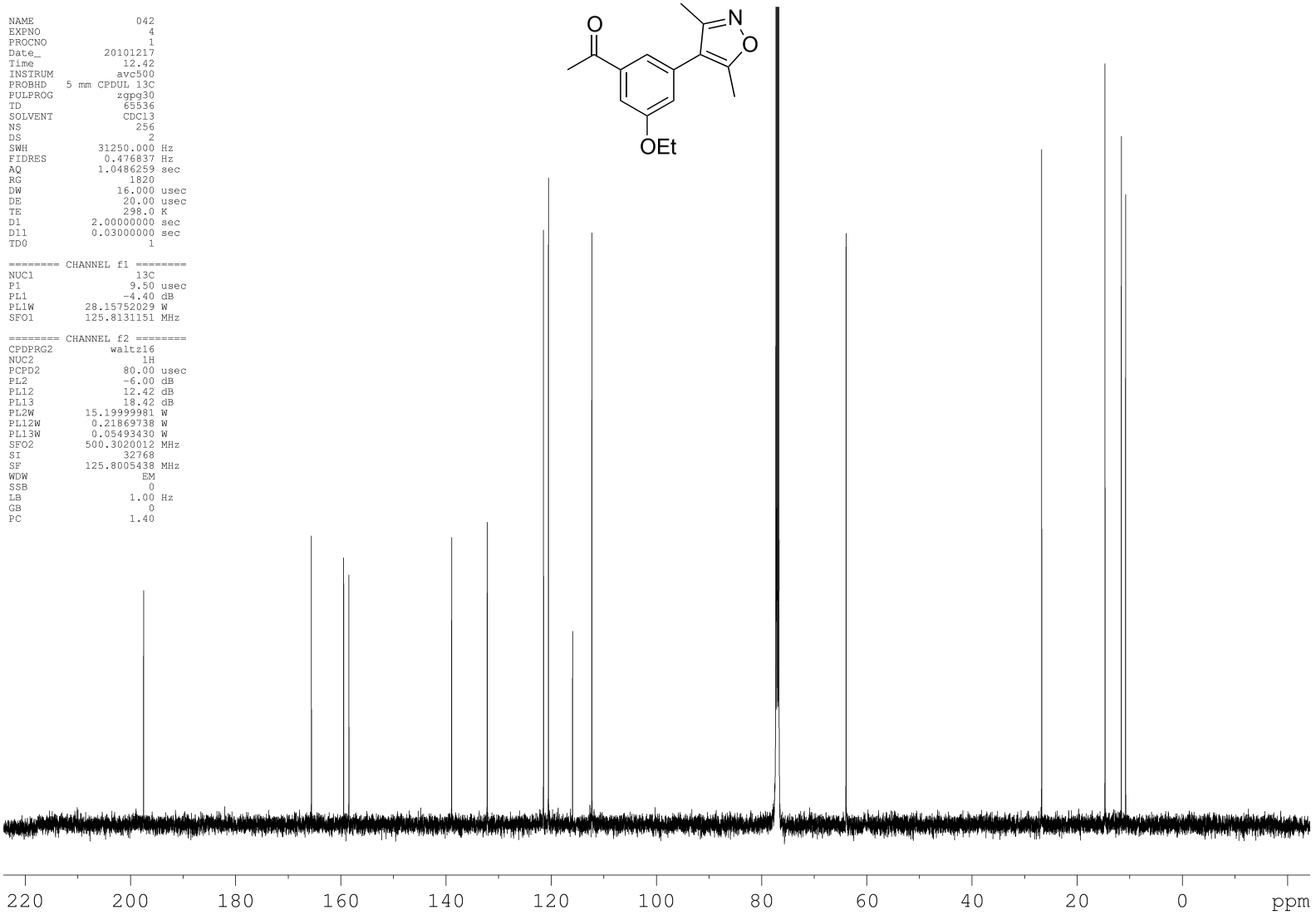
1-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)ethanone 47c

```
NAME                  042
EXPNO                   1
PROCNO                  1
Date_            20101217
Time                12.12
INSTRUM           avc500
PROBHD    5 mm CPDUL 13C
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ             3.1719923 sec
RG                      4
DW                 48.400 usec
DE                  6.00 usec
TE                 298.0 K
D1            1.00000000 sec
TD0                     1

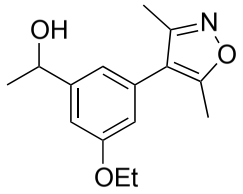
======== CHANNEL f1 ========
NUC1                   1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W         15.19999981 W
SFO1         500.3030896 MHz
SI                 32768
SF           500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



1-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)ethanone 47c

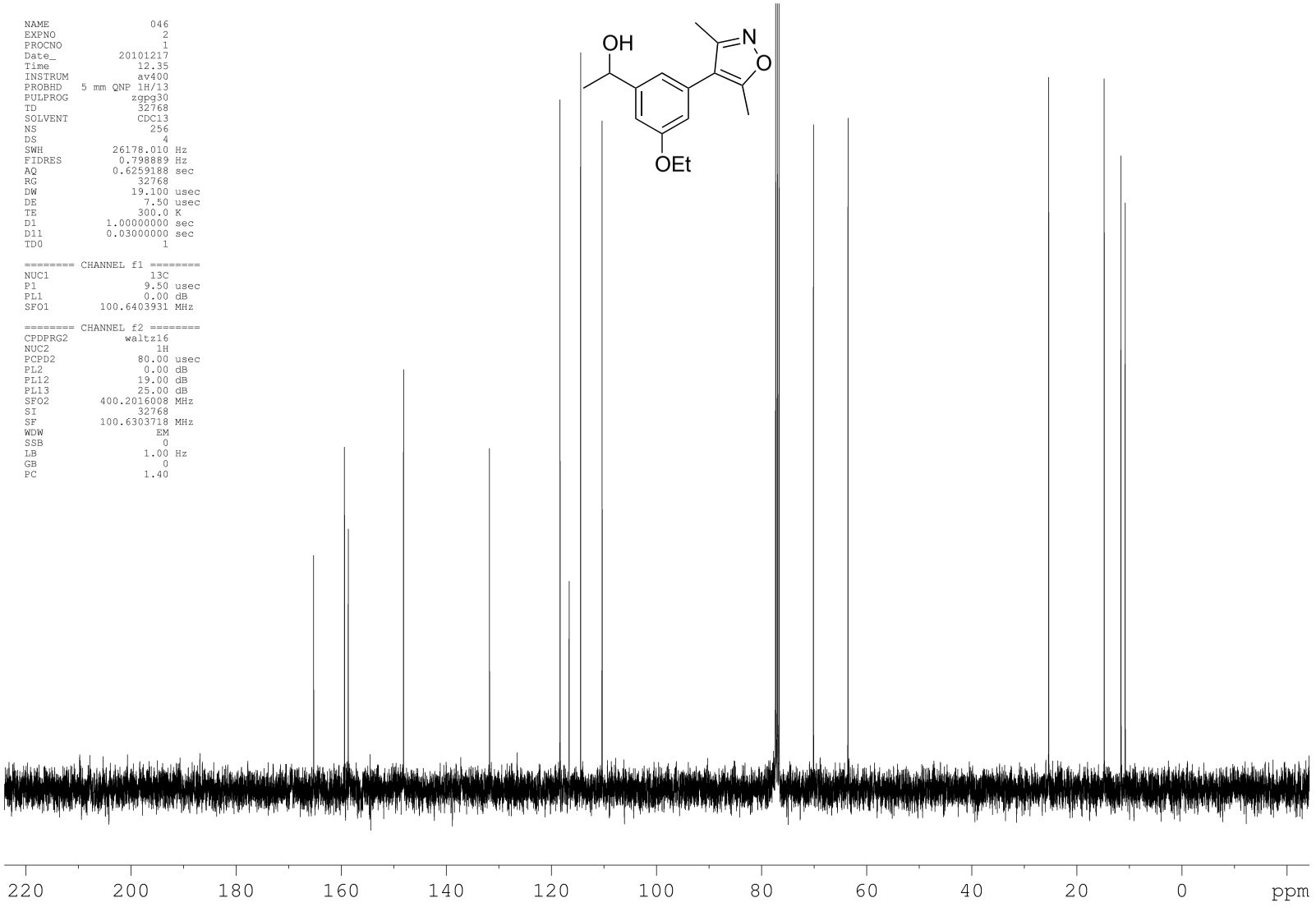


(*RS*)-1-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)ethanol 47d

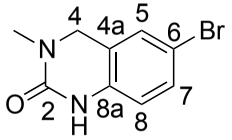


NAME	046
EXPNO	1
PROCNO	1
Date_	20101217
Time	12.27
INSTRUM	av400
PROBHD	5 mm QNP 1H/13
PULPROG	zg60
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	90.5
DW	60.400 usec
DE	7.50 usec
TE	300.0 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	9.00 usec
PL1	0.00 dB
SFO1	400.2024714 MHz
SI	32768
SF	400.2000028 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

(*RS*)-1-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)ethanol 47d



6-Bromo-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one 57

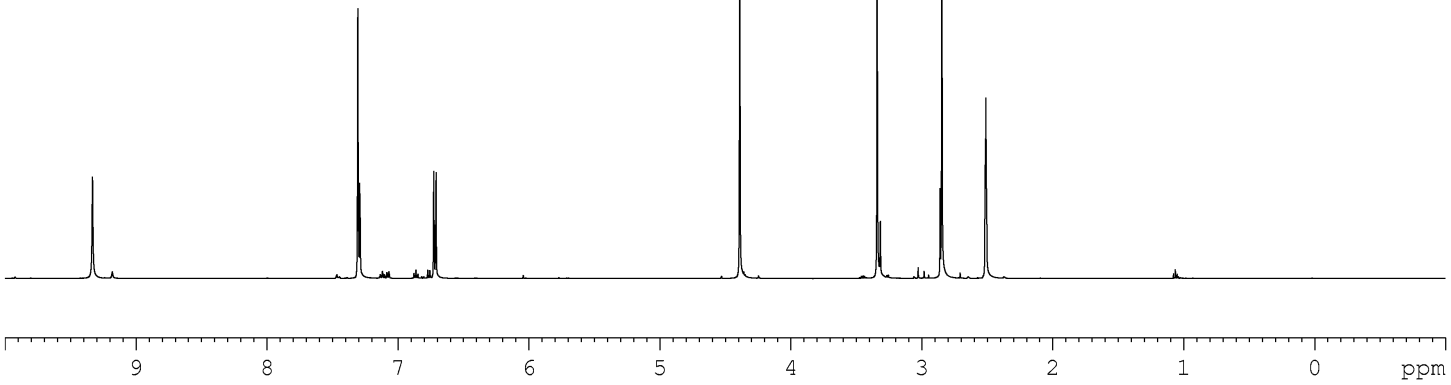



```
Current Data Parameters
NAME     bromodihydroquinazolinone
EXPNO                 1
PROCNO                1

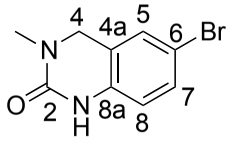
F2 - Acquisition Parameters
Date_           20110721
Time              22.04
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                65536
SOLVENT             DMSO
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 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
```



6-Bromo-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one 57




```
Current Data Parameters
NAME     bromodihydroquinazolinone
EXPNO                 2
PROCNO                1

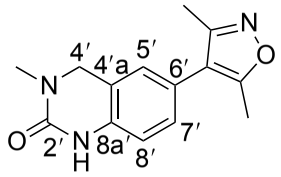
F2 - Acquisition Parameters
Date_           20110721
Time              22.31
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                65536
SOLVENT             DMSO
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                  9.10 usec
PL1                -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
```

6-(3,5-Dimethylisoxazol-4-yl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one 45a

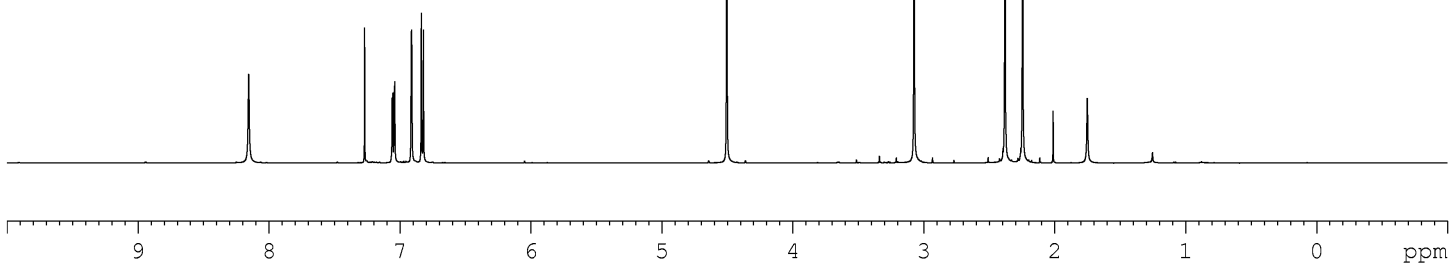



```
Current Data Parameters
NAME                  121
EXPNO                   1
PROCNO                  1

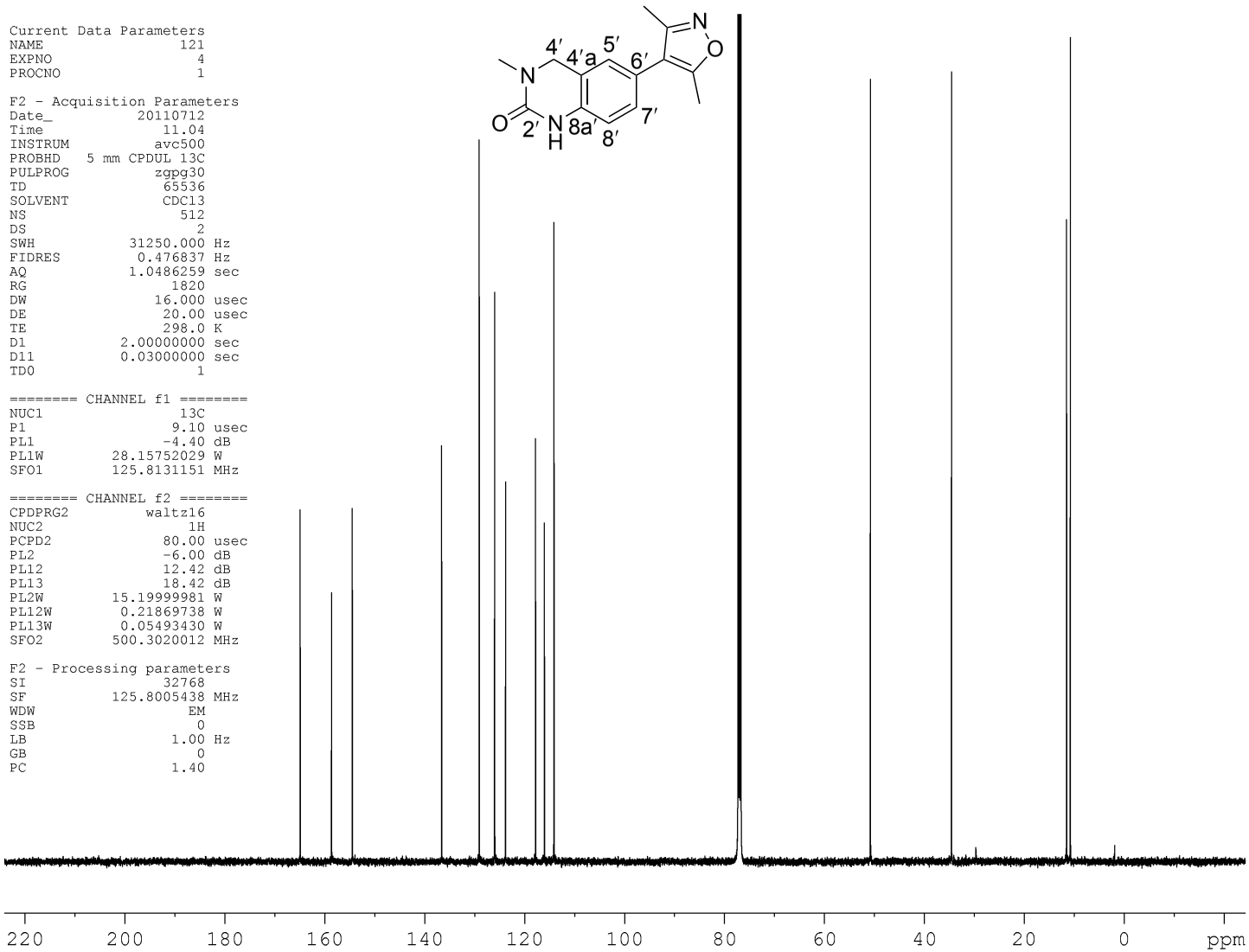
F2 - Acquisition Parameters
Date_            20110712
Time                10.21
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 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

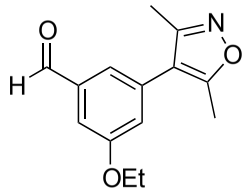
F2 - Processing parameters
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



6-(3,5-Dimethylisoxazol-4-yl)-3-methyl-3,4-dihydroquinazolin-2(1*H*)-one 45a

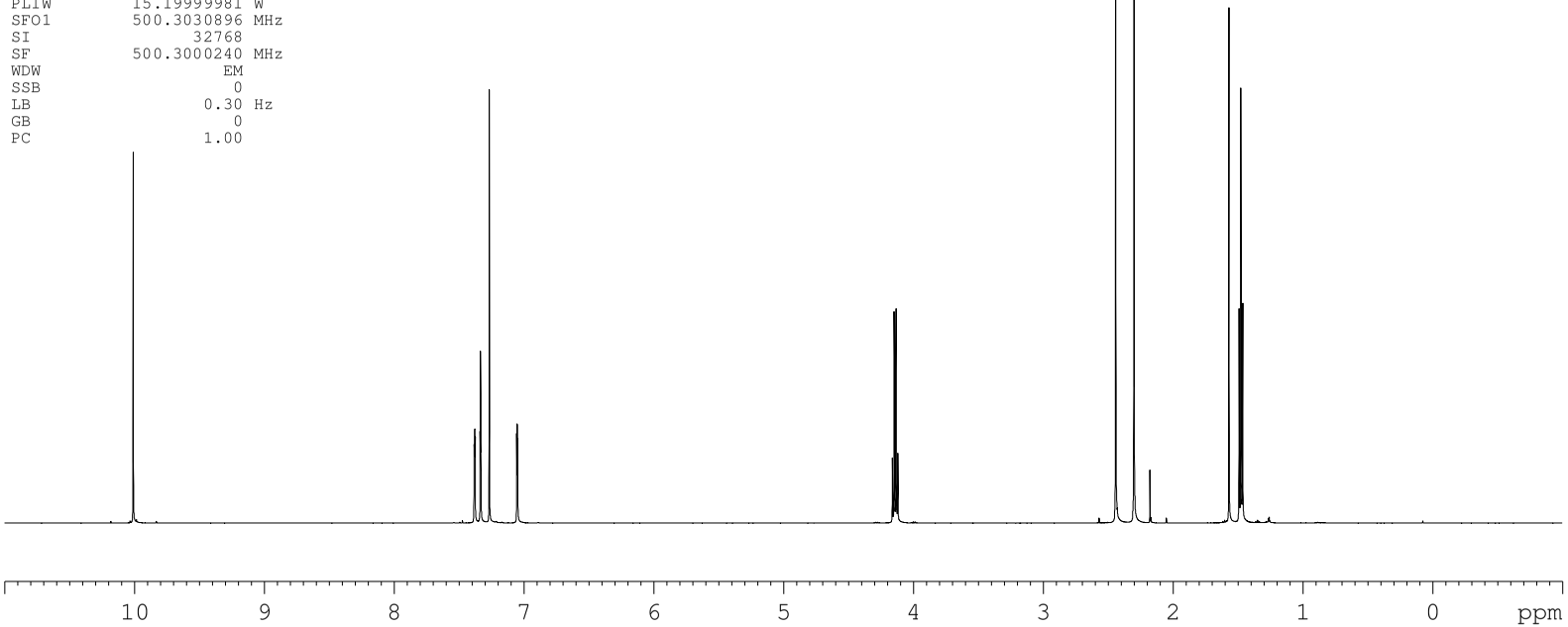


3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxybenzaldehyde 60

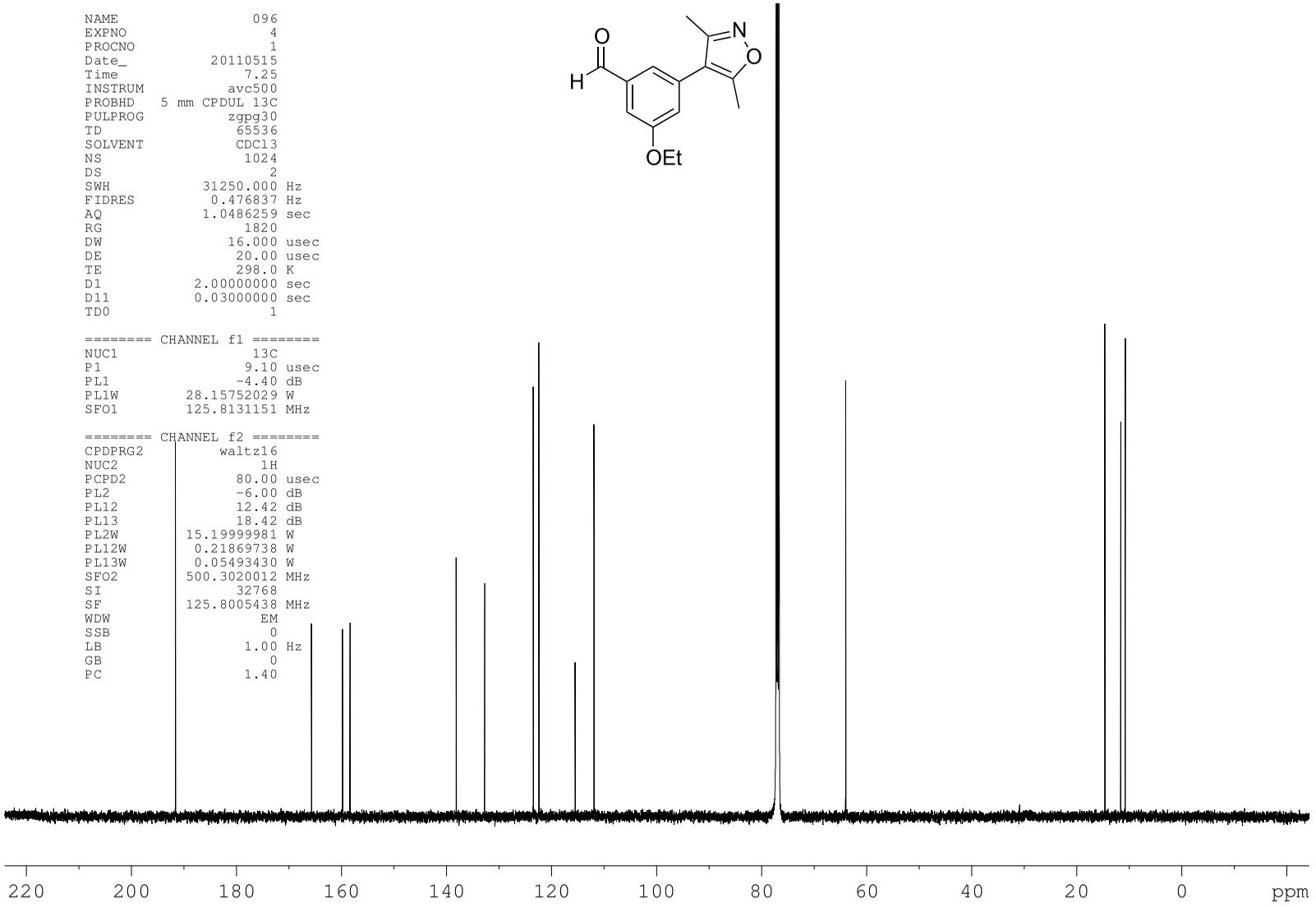


NAME	096
EXPNO	1
PROCNO	1
Date_	20110515
Time	6.16
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

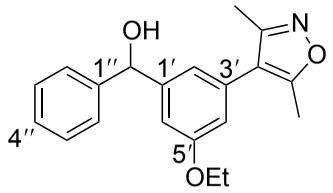
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxybenzaldehyde 60

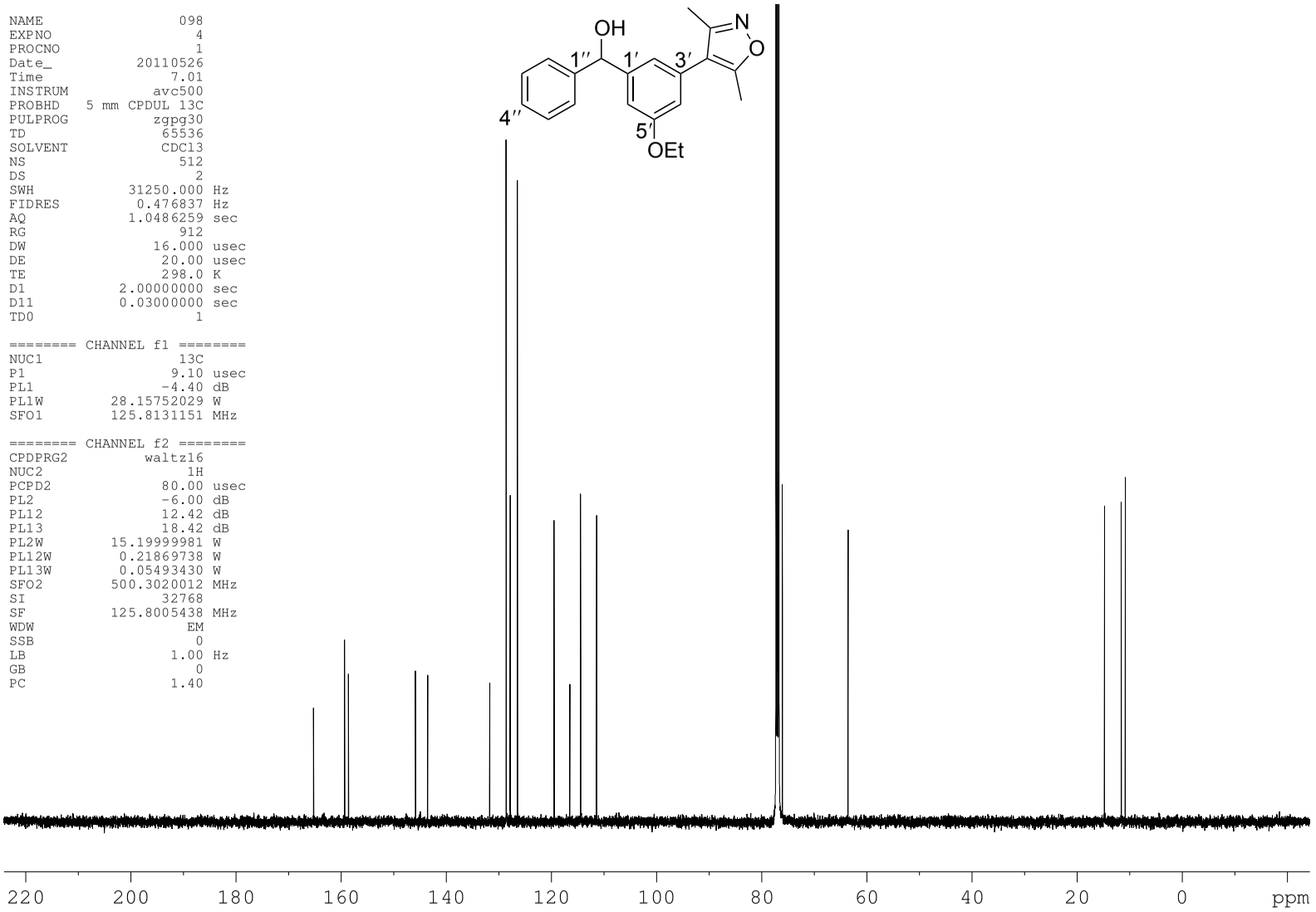


(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(phenyl)methanol 58a

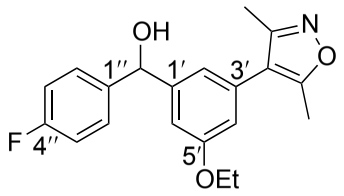


NAME	098
EXPNO	1
PROCNO	1
Date_	20110526
Time	6.18
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

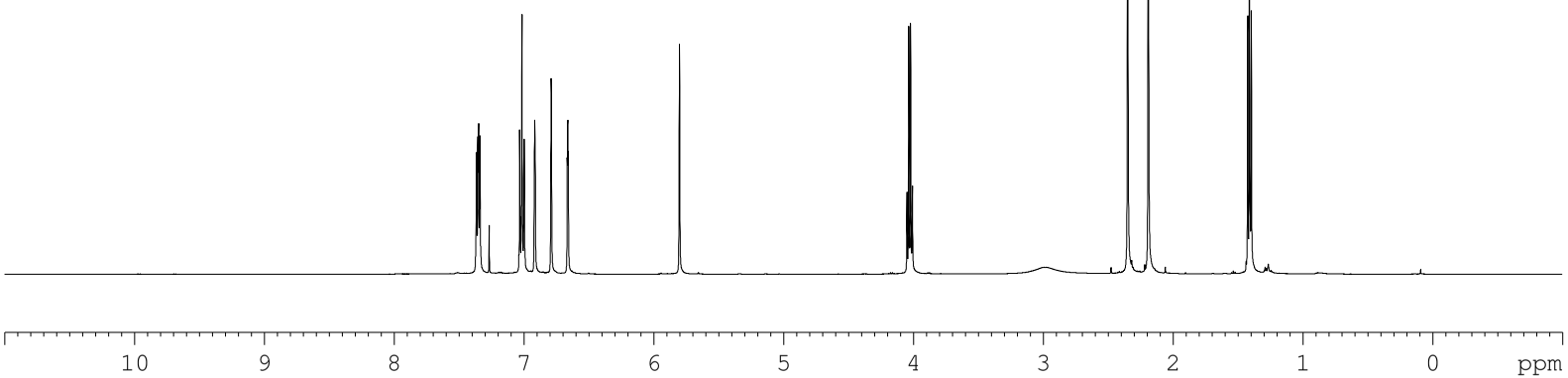
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(phenyl)methanol 58a



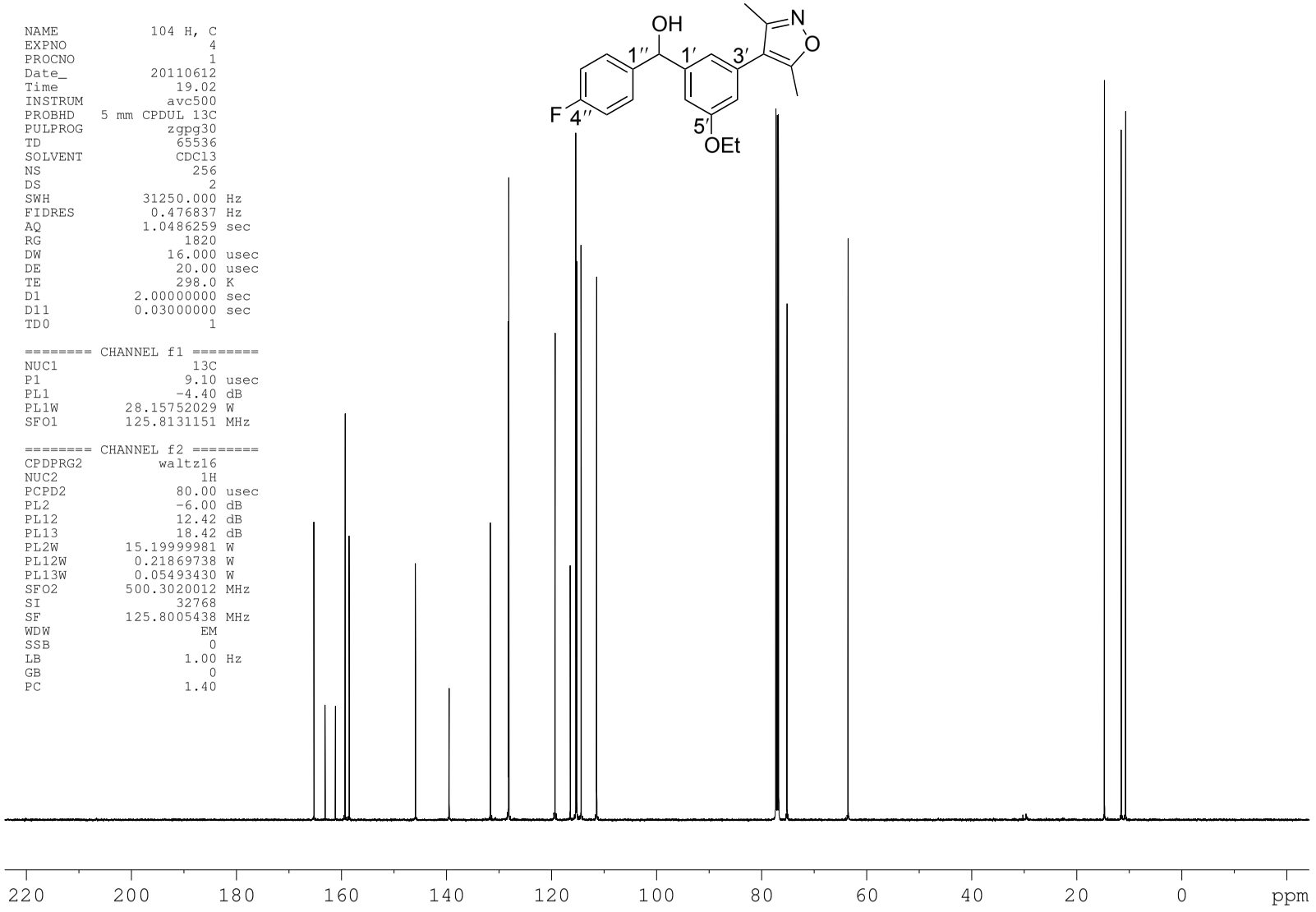
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(4-fluorophenyl)methanol 58b



NAME	104 H, C
EXPNO	1
PROCNO	1
Date_	20110612
Time	18.32
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(4-fluorophenyl)methanol 58b




```
NAME            104 H, C
EXPNO                4
PROCNO               1
Date_         20110612
Time             19.02
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             1.0486259 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                9.10 usec
PL1              -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

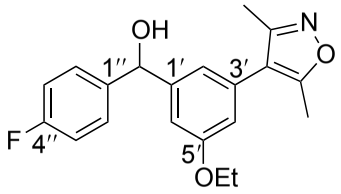
======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                1H
PCPD2            80.00 usec
PL2              -6.00 dB
PL12             12.42 dB
PL13             18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz
SI               32768
SF         125.8005438 MHz
WDW                 EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
```

(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(4-fluorophenyl)methanol 58b

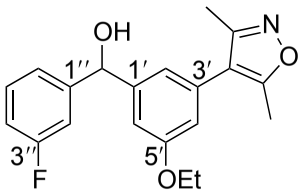
```
NAME                104 F
EXPNO                 1
PROCNO                1
Date_          20110620
Time              17.57
INSTRUM           avb500
PROBHD   5 mm PATXI 1H/
PULPROG         zgfhigqn
TD                131072
SOLVENT             CDCl3
NS                    16
DS                     4
SWH           113636.367 Hz
FIDRES          0.866977 Hz
AQ             0.5767668 sec
RG                   812
DW                 4.400 usec
DE                 6.50 usec
TE                298.0 K
D1           1.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 19F
P1                 10.00 usec
PL1                 0.90 dB
SFO1         470.4041911 MHz

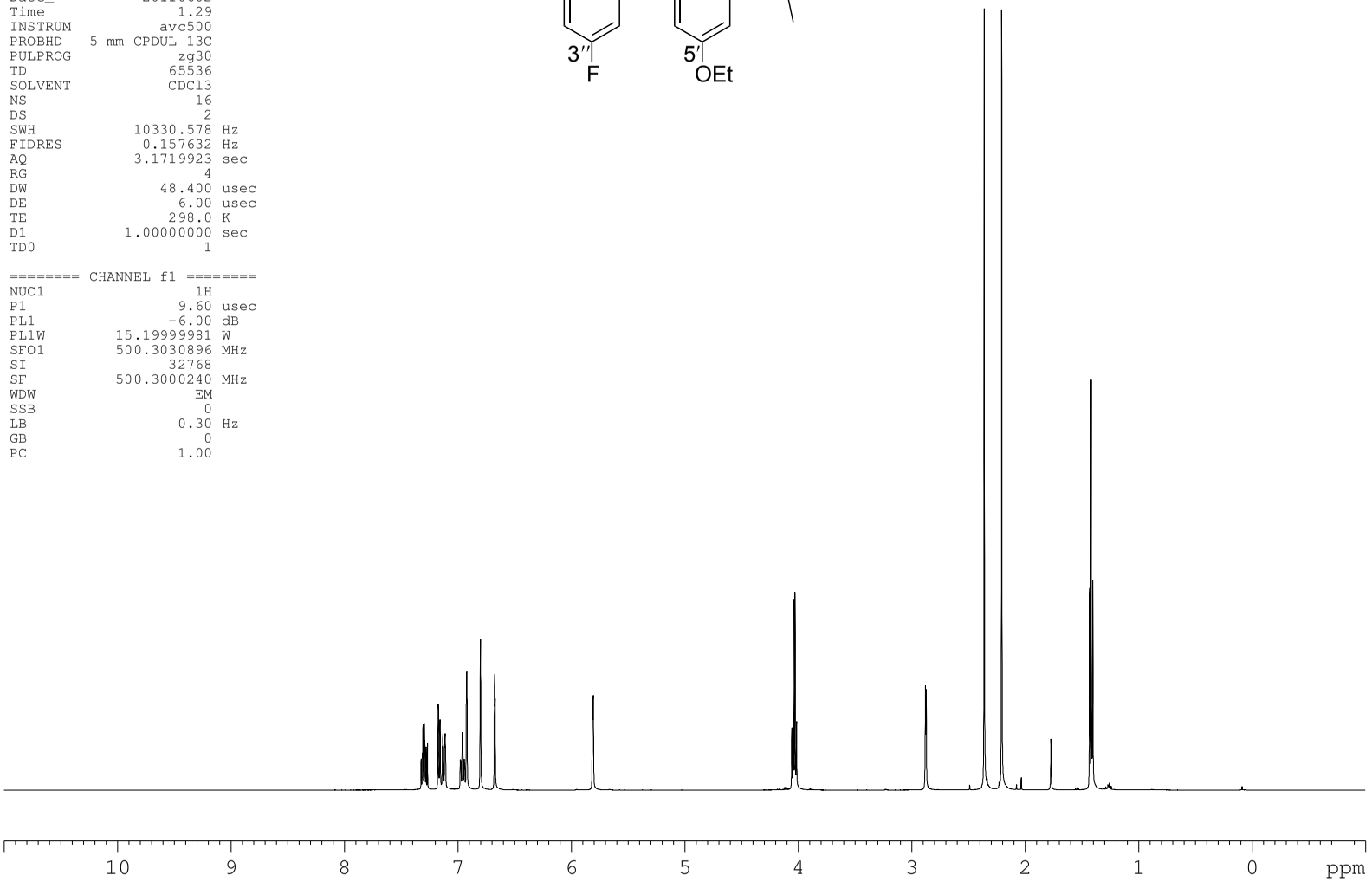
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                 1.00 dB
PL12               22.00 dB
PL2W         13.20863628 W
PL12W         0.10491993 W
SFO2         499.9819999 MHz
SI                65536
SF           470.4512360 MHz
WDW                  EM
SSB                   0
LB                 2.00 Hz
GB                    0
PC                10.00
```



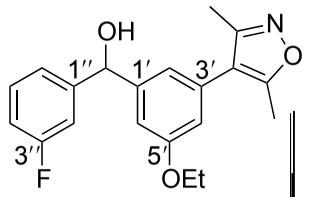
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(3-fluorophenyl)methanol 58c



NAME	102 H, C
EXPNO	1
PROCNO	1
Date_	20110602
Time	1.29
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



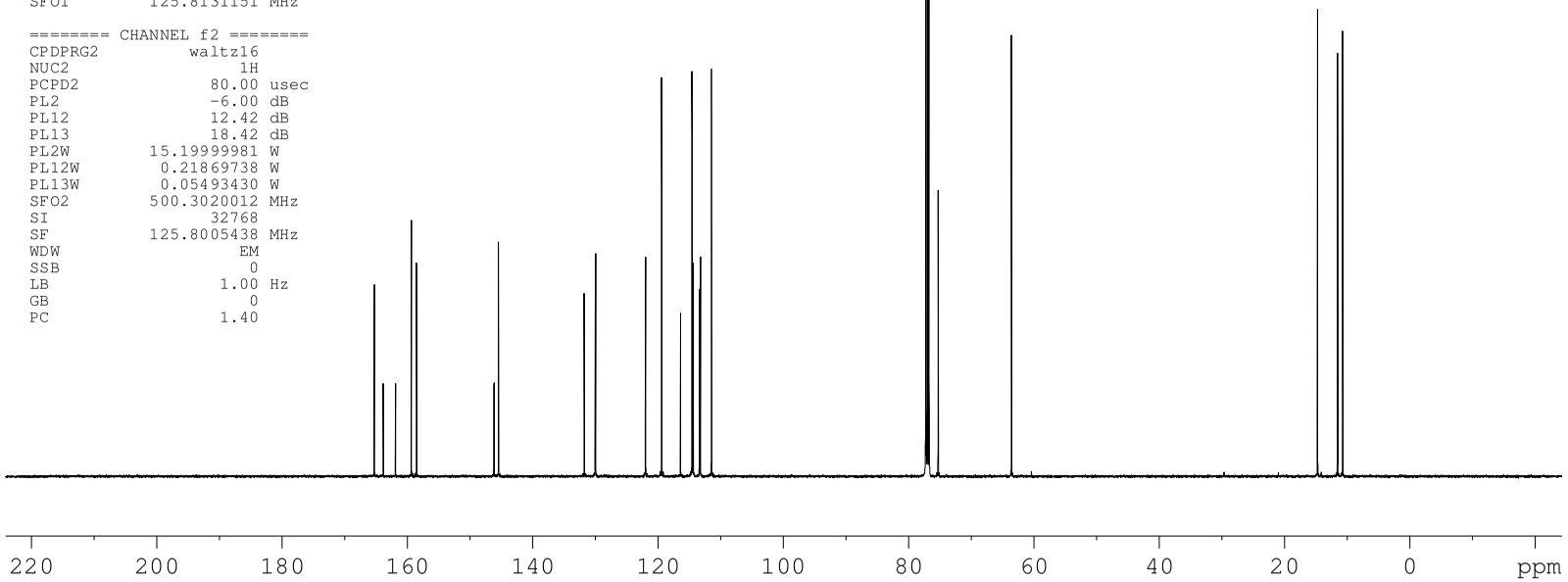
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(3-fluorophenyl)methanol 58c



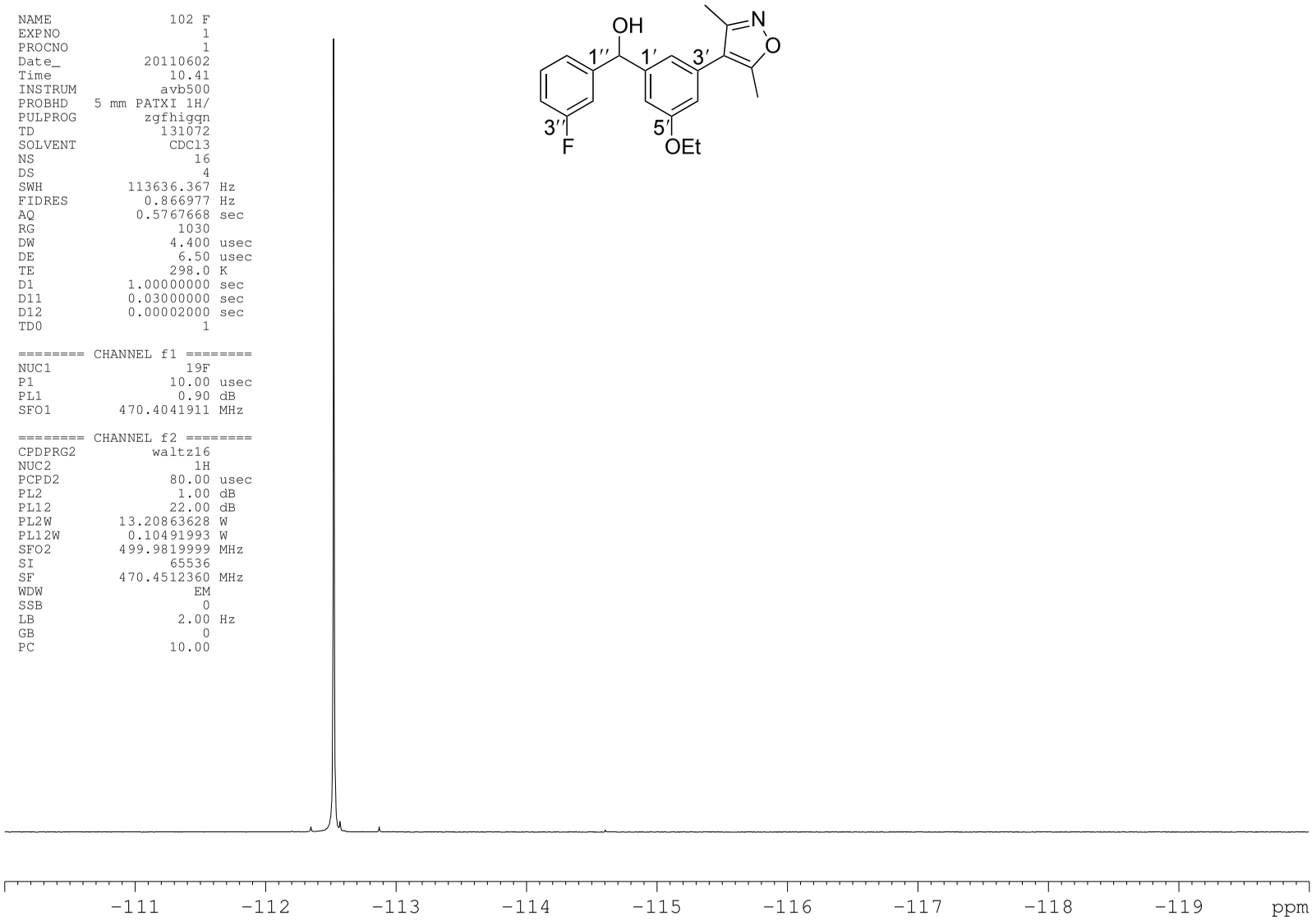

```
NAME            102 H, C
EXPNO                  4
PROCNO                 1
Date_           20110602
Time                1.59
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                  9.10 usec
PL1                -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                 32768
SF          125.8005438 MHz
WDW                 EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
```

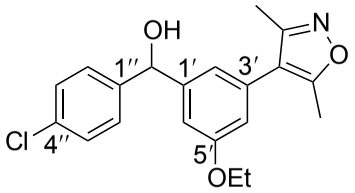


(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-ethoxyphenyl)(3-fluorophenyl)methanol 58c

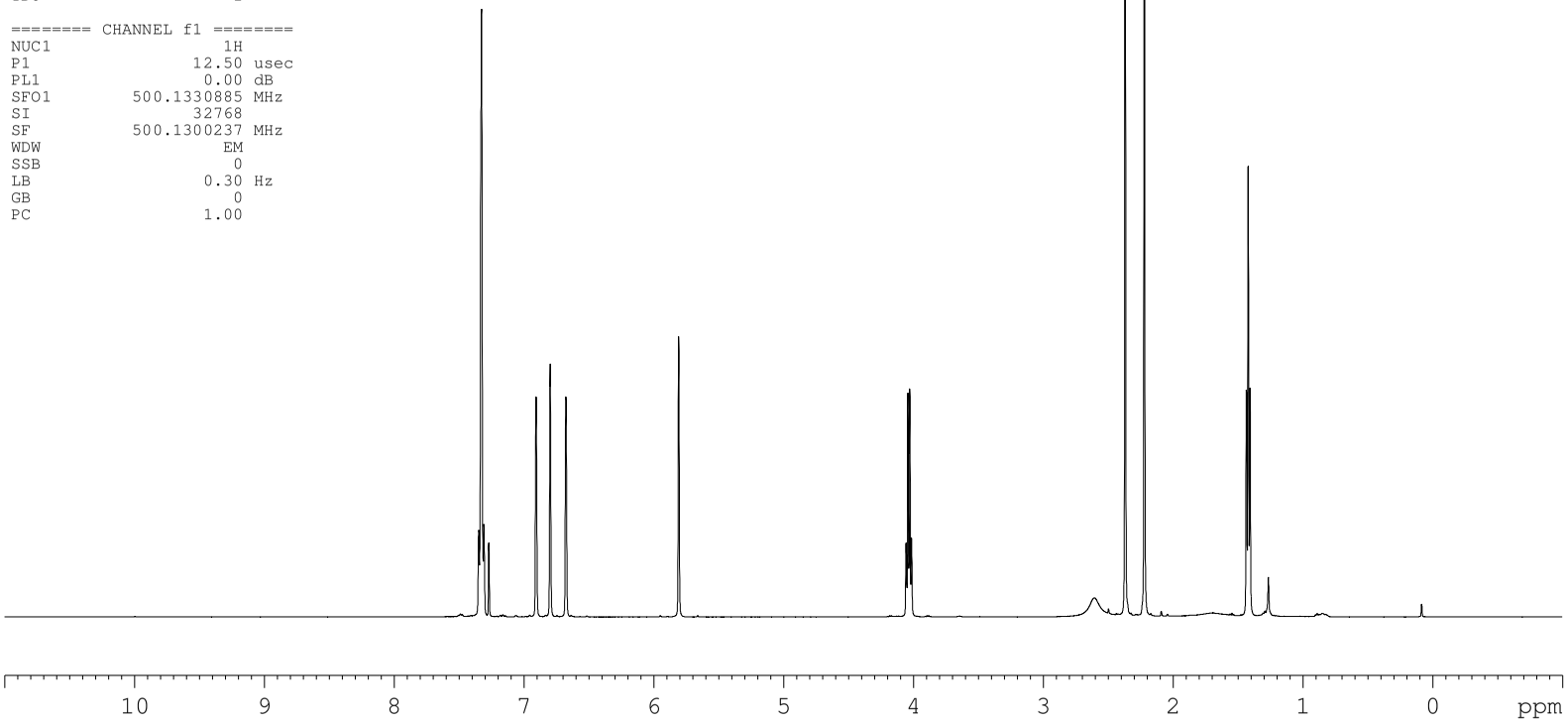


NAME	102 F	
EXPNO	1	
PROCNO	1	
Date_	20110602	
Time	10.41	
INSTRUM	avb500	
PROBHD	5 mm PATXI 1H/	
PULPROG	zgfhigqn	
TD	131072	
SOLVENT	CDCl3	
NS	16	
DS	4	
SWH	113636.367	Hz
FIDRES	0.866977	Hz
AQ	0.5767668	sec
RG	1030	
DW	4.400	usec
DE	6.50	usec
TE	298.0	K
D1	1.00000000	sec
D11	0.03000000	sec
D12	0.00002000	sec
TD0	1	
======== CHANNEL f1 ========		
NUC1	19F	
P1	10.00	usec
PL1	0.90	dB
SFO1	470.4041911	MHz
======== CHANNEL f2 ========		
CPDPRG2	waltz16	
NUC2	1H	
PCPD2	80.00	usec
PL2	1.00	dB
PL12	22.00	dB
PL2W	13.20863628	W
PL12W	0.10491993	W
SFO2	499.9819999	MHz
SI	65536	
SF	470.4512360	MHz
WDW	EM	
SSB	0	
LB	2.00	Hz
GB	0	
PC	10.00	

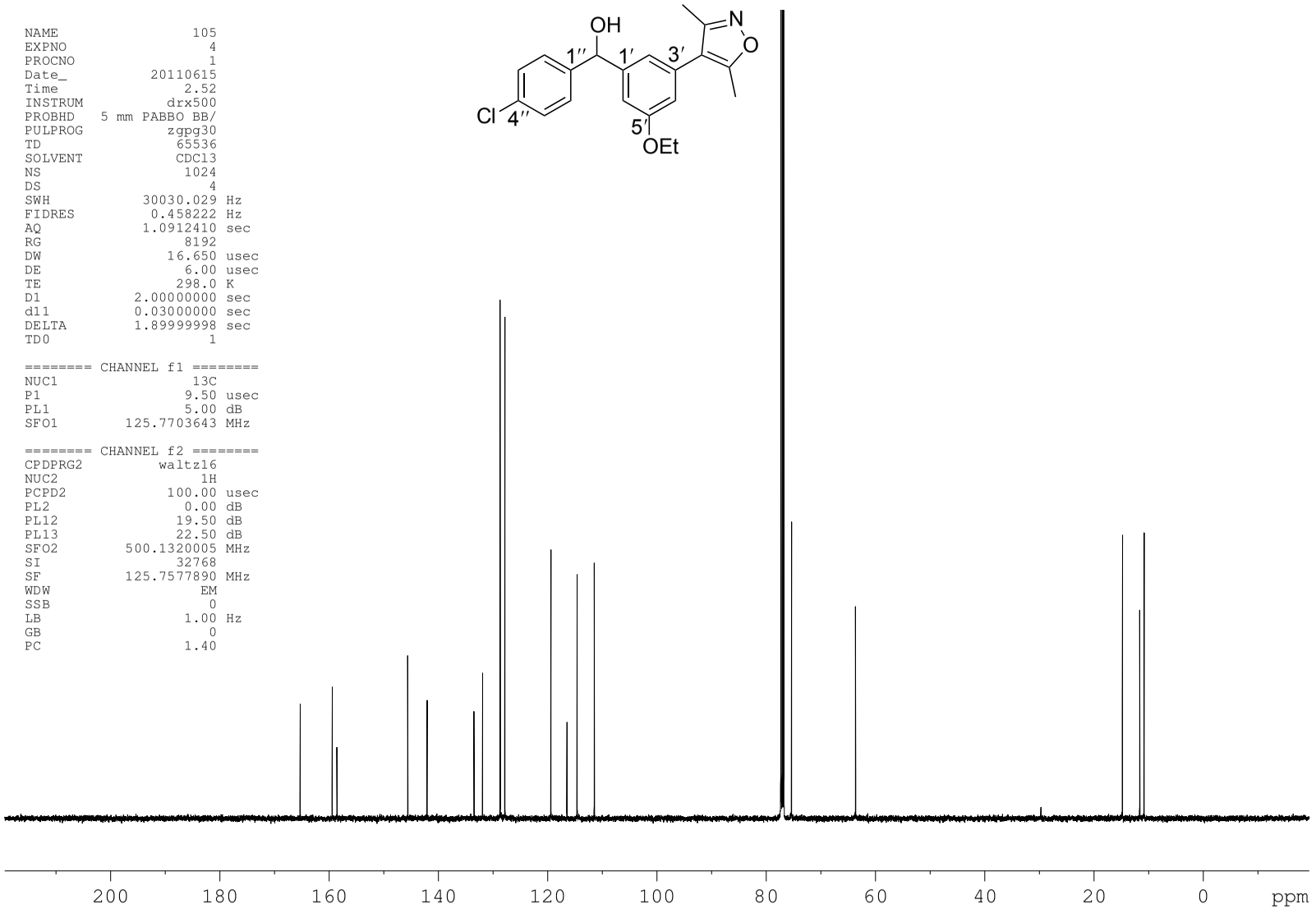
(*RS*)-(4-Chlorophenyl)(3-(3,5-dimethylisoxazol-4-yl)-5-ethoxyphenyl)methanol 58d



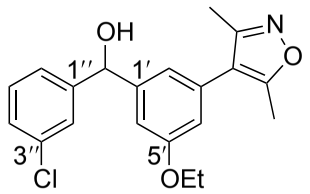
NAME	105
EXPNO	1
PROCNO	1
Date_	20110615
Time	1.36
INSTRUM	drx500
PROBHD	5 mm PABBO BB/
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	114
DW	48.400 usec
DE	6.00 usec
TE	298.1 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	12.50 usec
PL1	0.00 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300237 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-(4-Chlorophenyl)(3-(3,5-dimethylisoxazol-4-yl)-5-ethoxyphenyl)methanol 58d

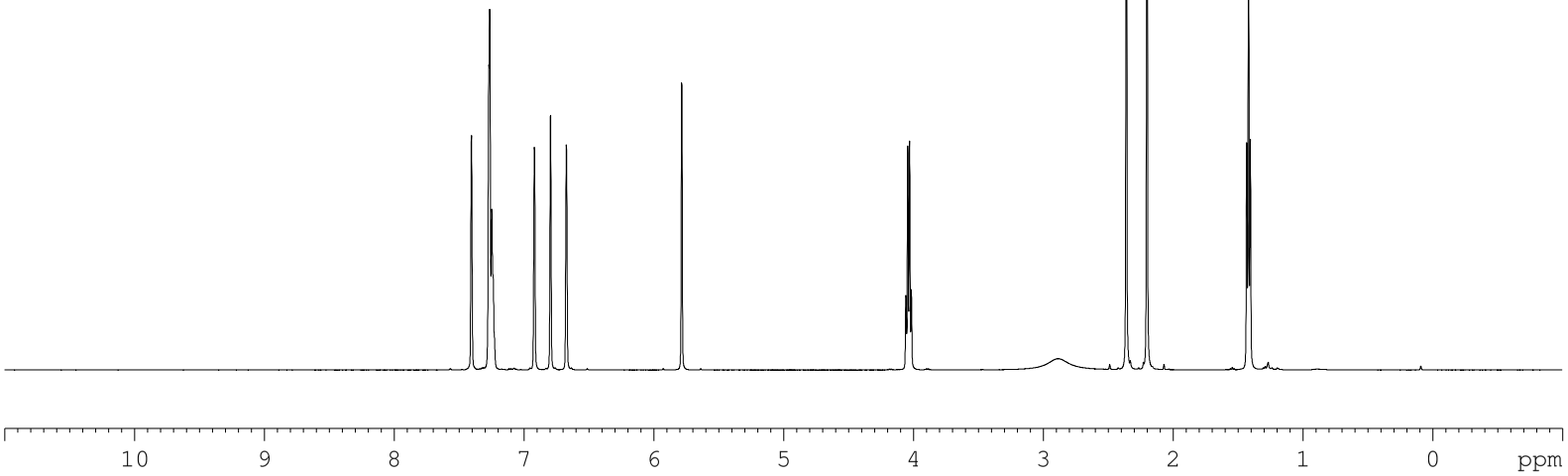


(*RS*)-(3-Chlorophenyl)(3-(3,5-dimethylisoxazol-4-yl)-5-ethoxyphenyl)methanol 58e

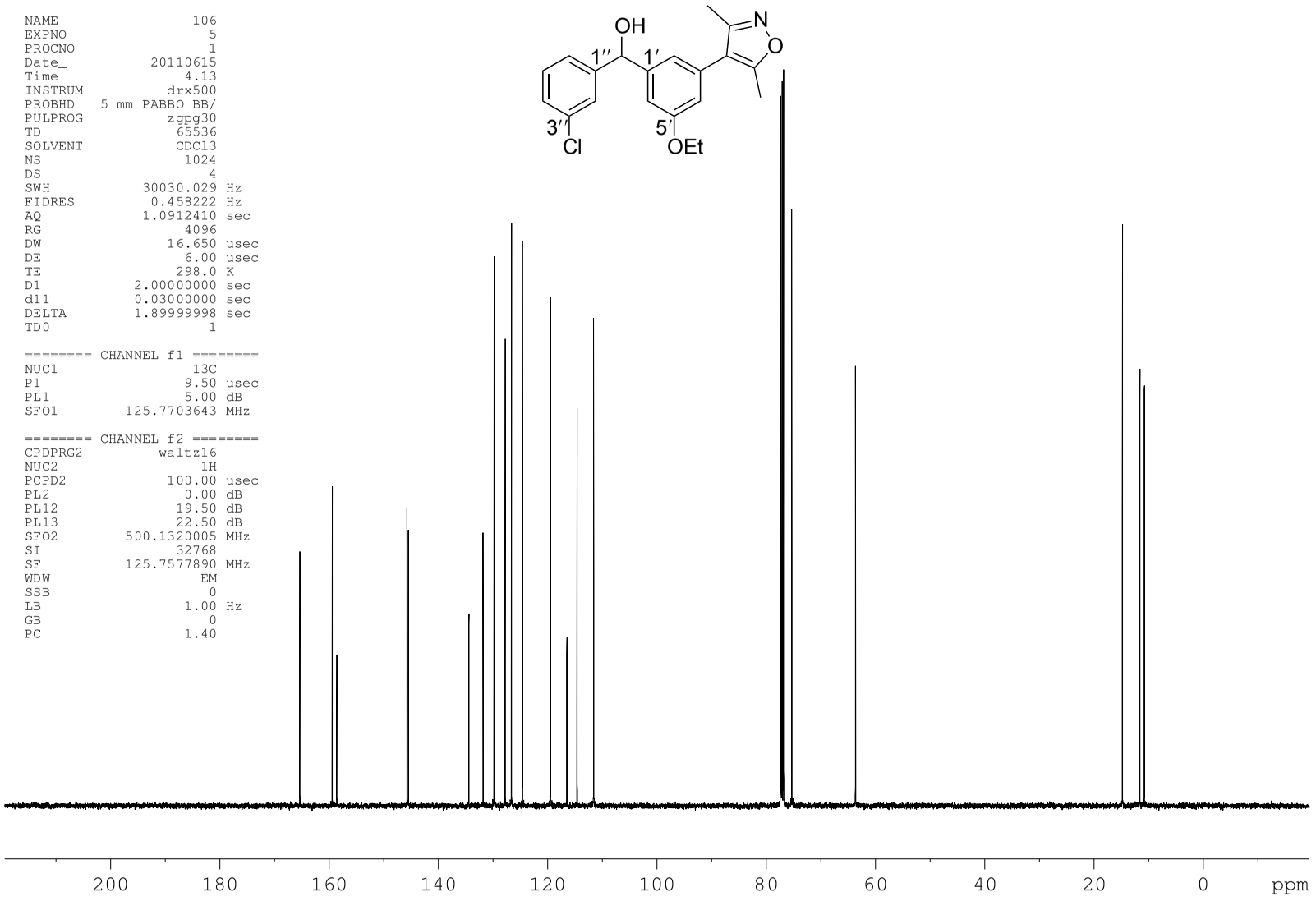



```
NAME                 106
EXPNO                  1
PROCNO                 1
Date_           20110615
Time                2.58
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1720407 sec
RG                    64
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

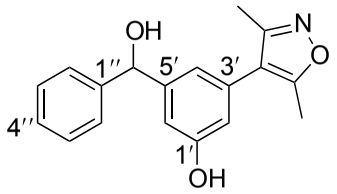
======== CHANNEL f1 ========
NUC1                  1H
P1                12.50 usec
PL1                0.00 dB
SFO1        500.1330885 MHz
SI                 32768
SF          500.1300237 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                 1.00
```



(*RS*)-(3-Chlorophenyl)(3-(3,5-dimethylisoxazol-4-yl)-5-ethoxyphenyl)methanol 58e

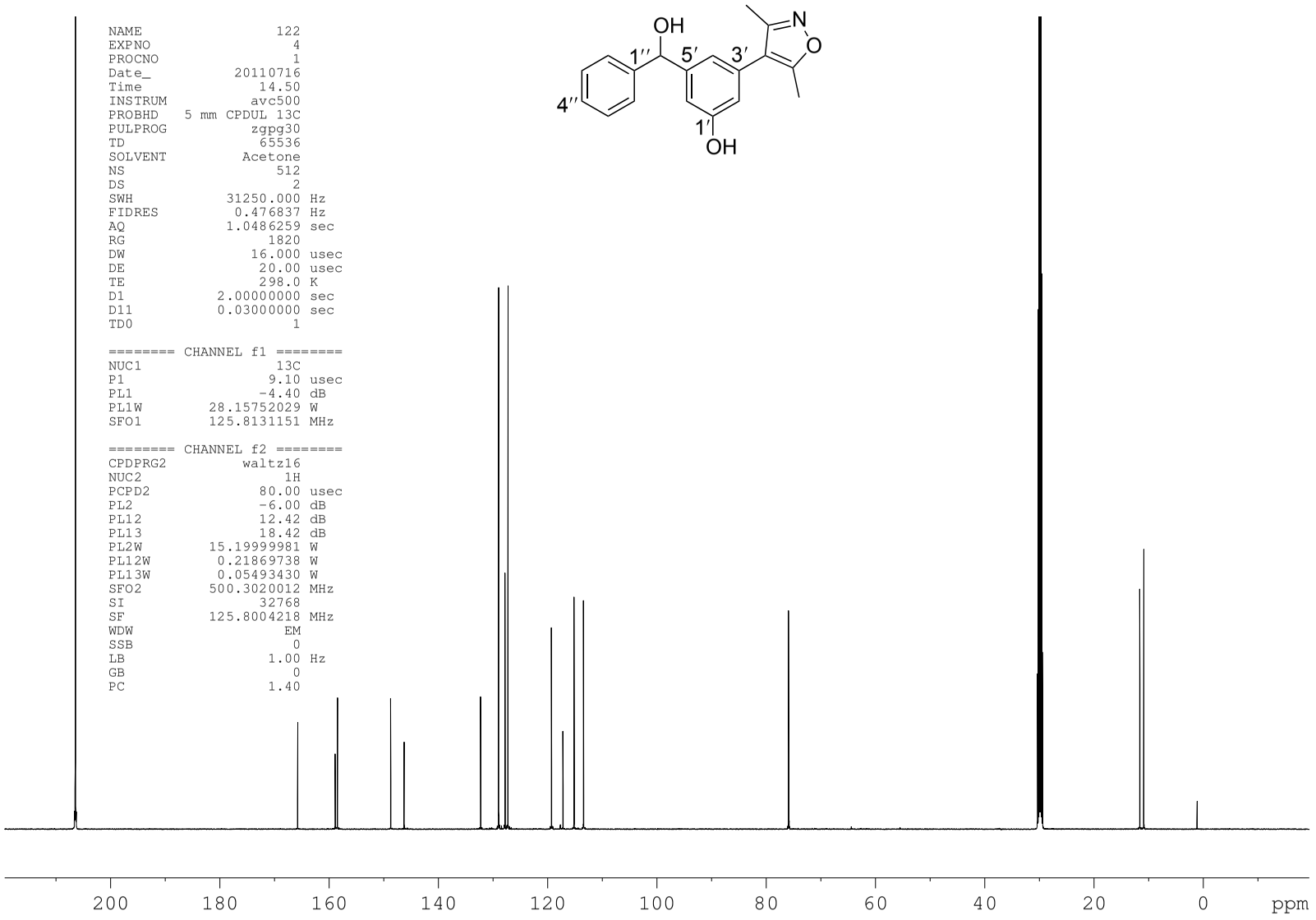


(*RS*)-3-(3,5-Dimethylisoxazol-4-yl)-5-(hydroxy(phenyl)methyl)phenol 61a



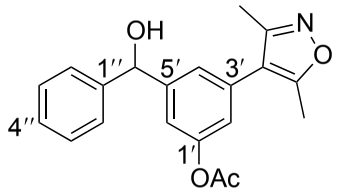
NAME	122
EXPNO	1
PROCNO	1
Date_	20110716
Time	14.07
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	Acetone
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

(*RS*)-3-(3,5-Dimethylisoxazol-4-yl)-5-(hydroxy(phenyl)methyl)phenol 61a

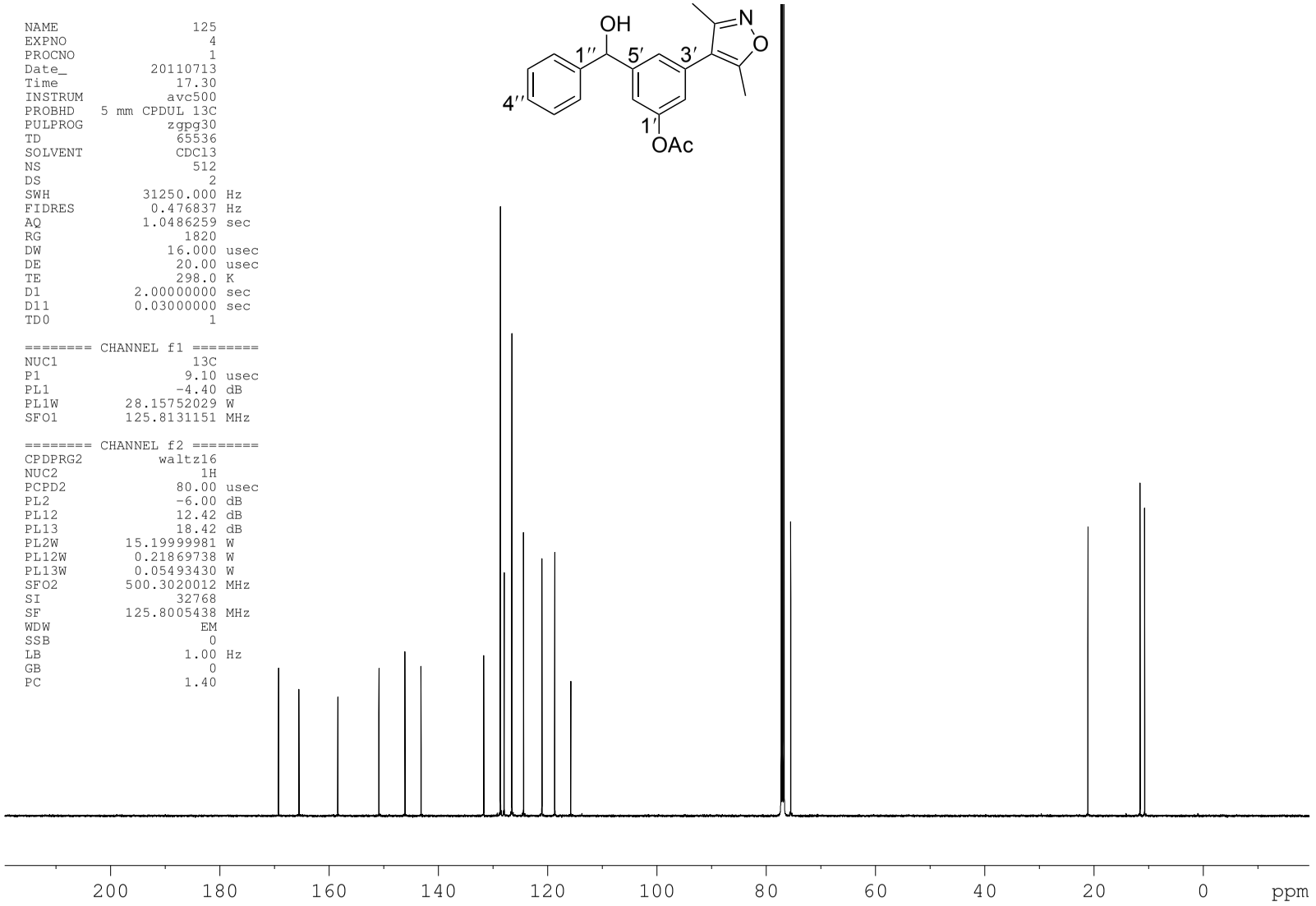


(*RS*)-3-(3,5-Dimethylisoxazol-4-yl)-5-(hydroxy(phenyl)methyl)phenyl acetate 61b

NAME	125
EXPNO	1
PROCNO	1
Date_	20110713
Time	16.47
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-3-(3,5-Dimethylisoxazol-4-yl)-5-(hydroxy(phenyl)methyl)phenyl acetate 61b

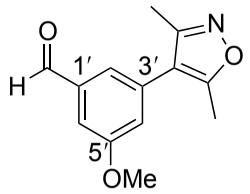



```
NAME                125
EXPNO                 4
PROCNO                1
Date_          20110713
Time              17.30
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

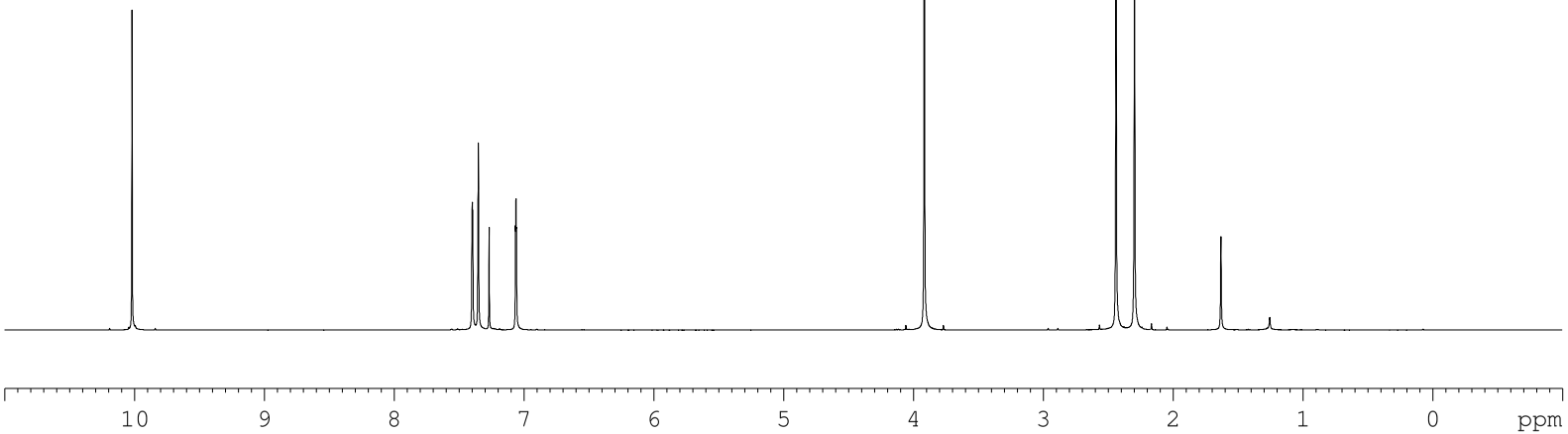
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2            80.00 usec
PL2              -6.00 dB
PL12             12.42 dB
PL13             18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

3-(3,5-Dimethylisoxazol-4-yl)-5-methoxybenzaldehyde 62

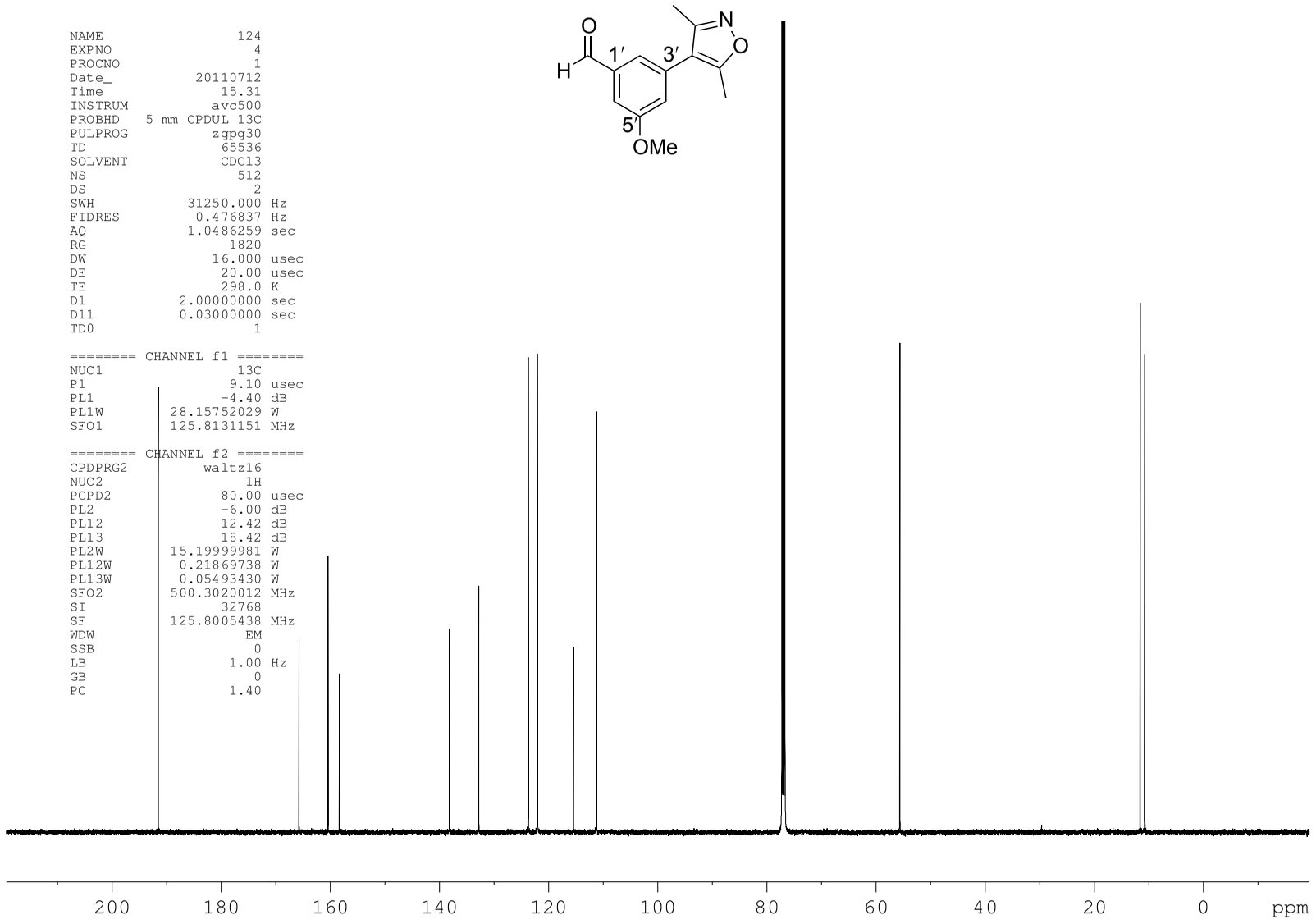


NAME	124
EXPNO	1
PROCNO	1
Date_	20110712
Time	14.48
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.1 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



3-(3,5-Dimethylisoxazol-4-yl)-5-methoxybenzaldehyde 62

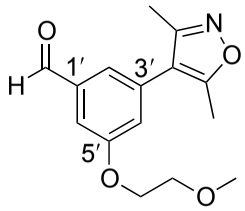



```
NAME                 124
EXPNO                  4
PROCNO                 1
Date_           20110712
Time               15.31
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   512
DS                     2
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ             1.0486259 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

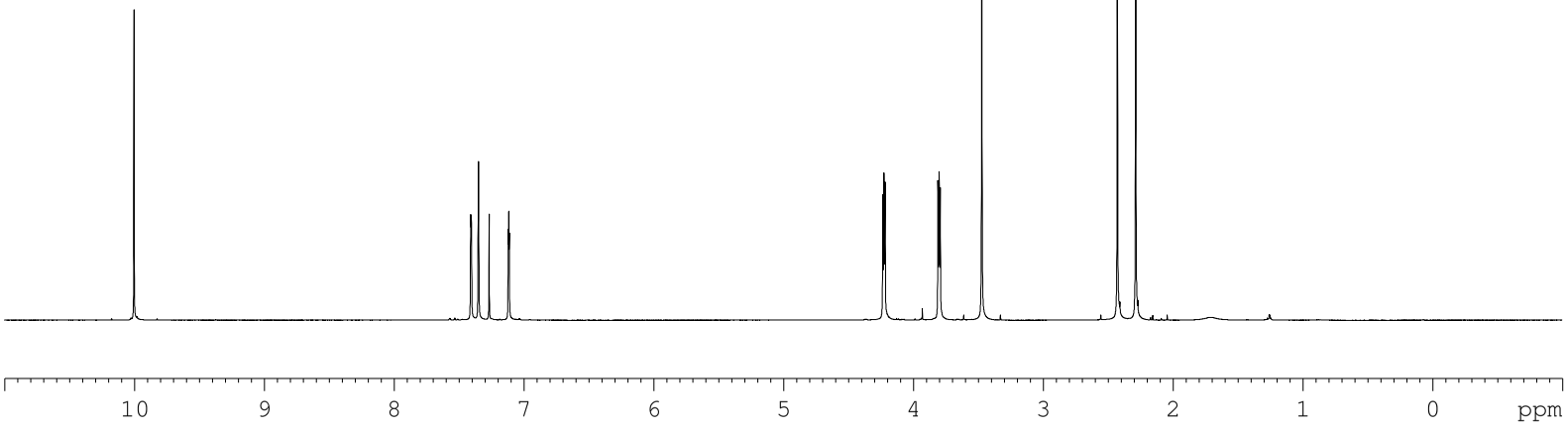
======== CHANNEL f1 ========
NUC1                13C
P1                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI               32768
SF         125.8005438 MHz
WDW                 EM
SSB                 0
LB                1.00 Hz
GB                  0
PC                1.40
```

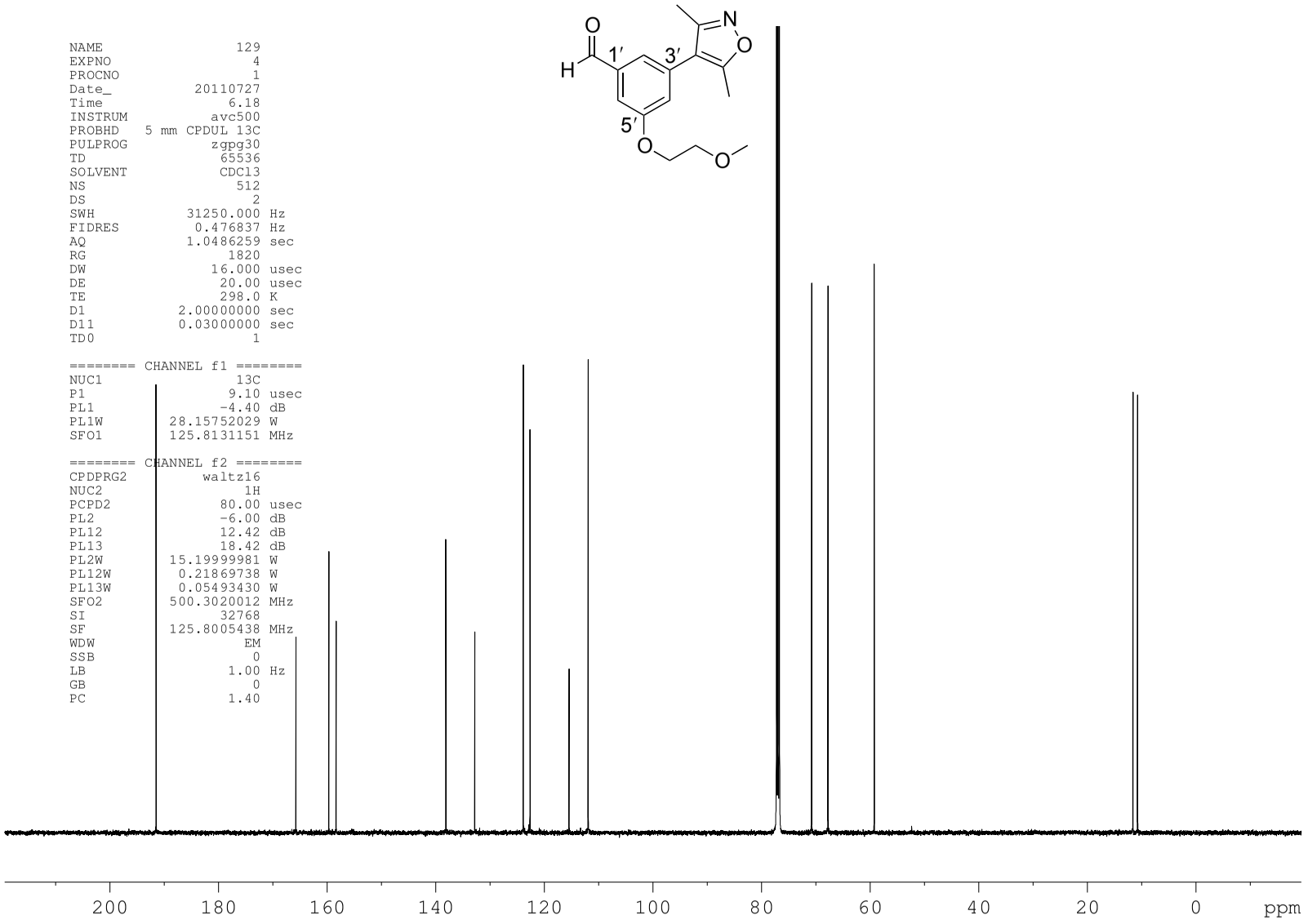
3-(3,5-Dimethylisoxazol-4-yl)-5-(2-methoxyethoxy)benzaldehyde 63



NAME	129
EXPNO	1
PROCNO	1
Date_	20110727
Time	5.35
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



3-(3,5-Dimethylisoxazol-4-yl)-5-(2-methoxyethoxy)benzaldehyde 63

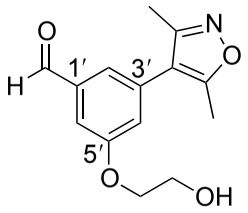



```
NAME                129
EXPNO                 4
PROCNO                1
Date_          20110727
Time               6.18
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0486259 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                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

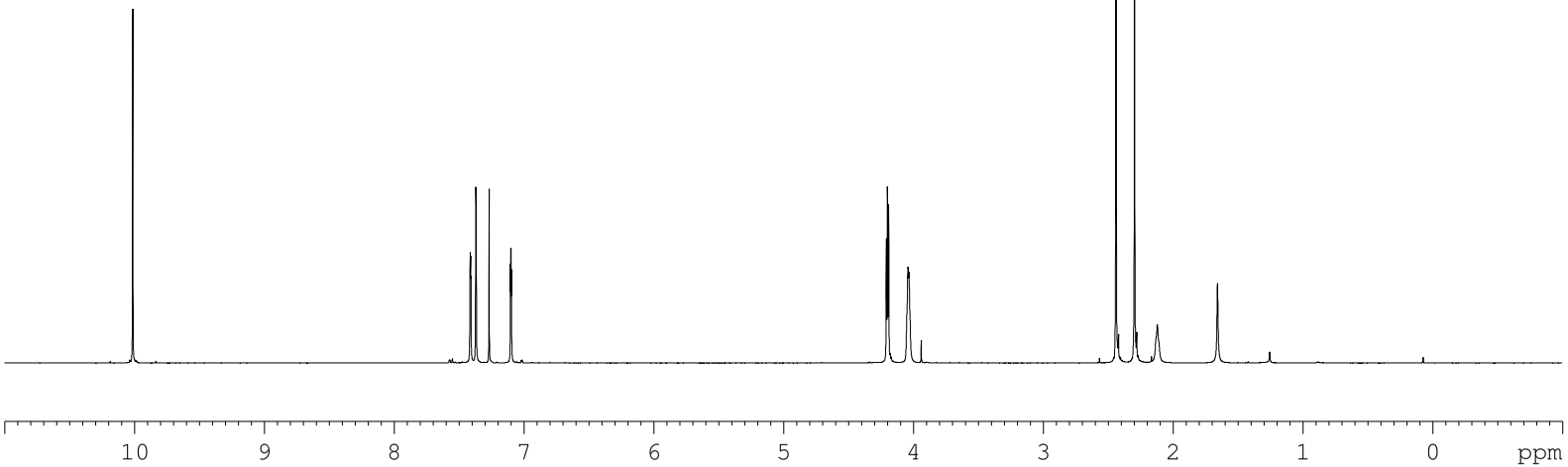
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               12.42 dB
PL13               18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

3-(3,5-Dimethylisoxazol-4-yl)-5-(2-hydroxyethoxy)benzaldehyde 64

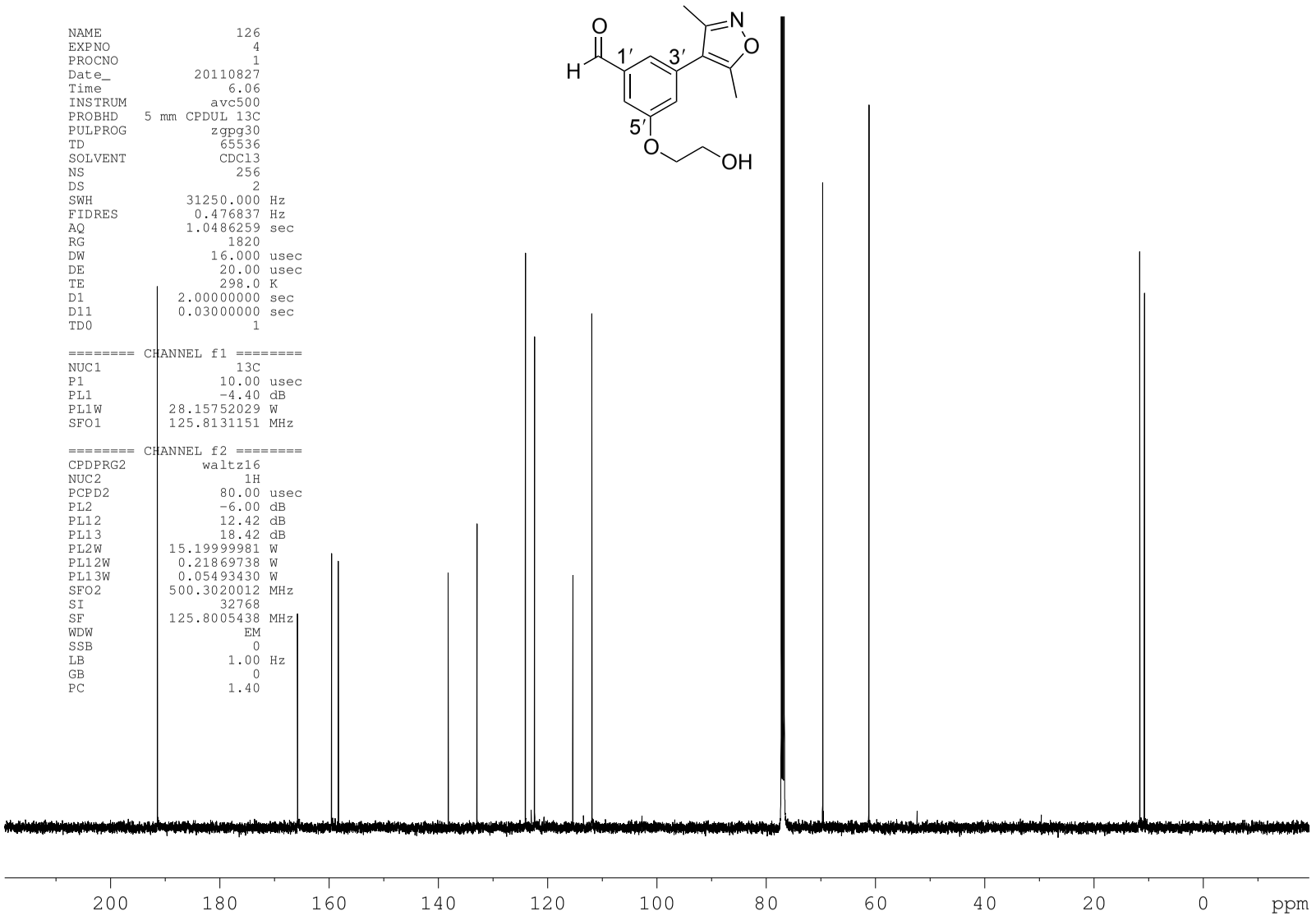


NAME	126
EXPNO	1
PROCNO	1
Date_	20110827
Time	5.36
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



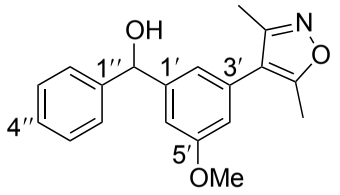
3-(3,5-Dimethylisoxazol-4-yl)-5-(2-hydroxyethoxy)benzaldehyde 64

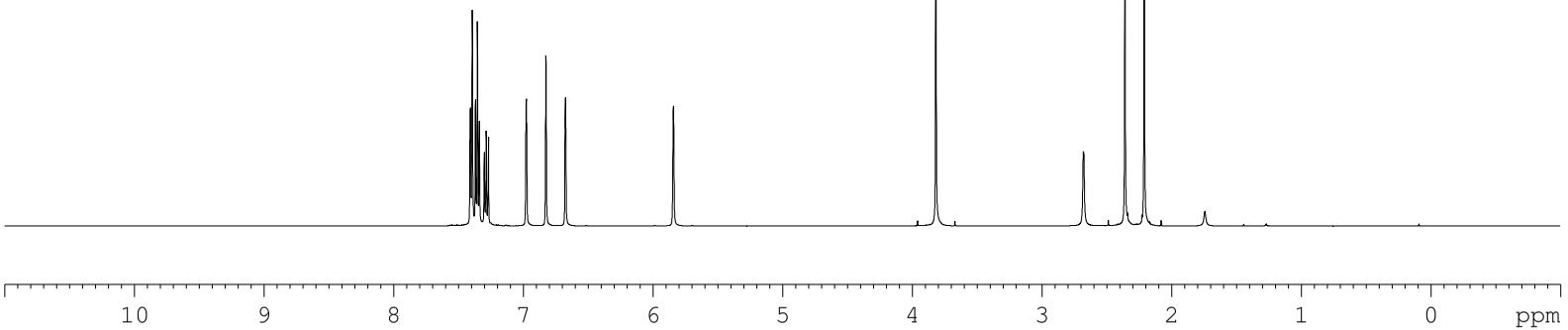


(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-methoxyphenyl)(phenyl)methanol 61c

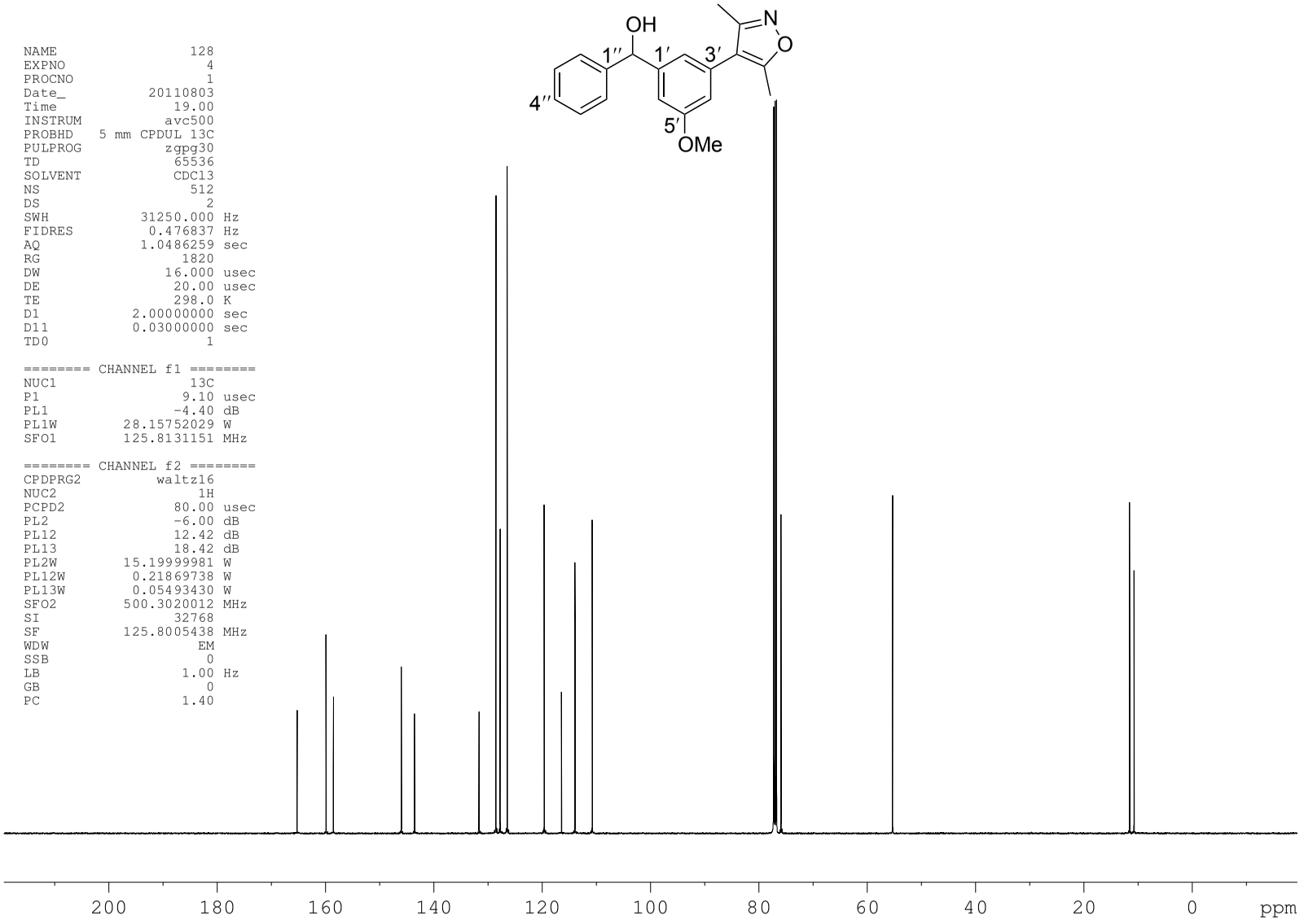
```
NAME                 128
EXPNO                  1
PROCNO                 1
Date_           20110803
Time               18.17
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 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
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```





(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-methoxyphenyl)(phenyl)methanol 61c

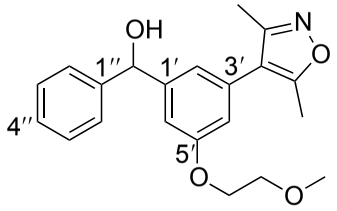



```
NAME                128
EXPNO                 4
PROCNO                1
Date_          20110803
Time              19.00
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

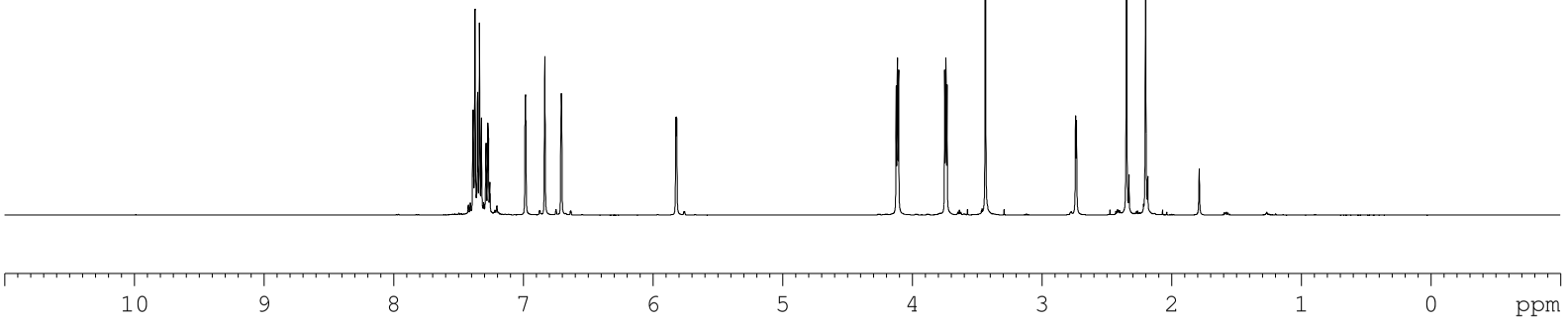
======== CHANNEL f1 ========
NUC1                13C
P1                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2         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
SI                32768
SF         125.8005438 MHz
WDW                EM
SSB                 0
LB               1.00 Hz
GB                  0
PC               1.40
```

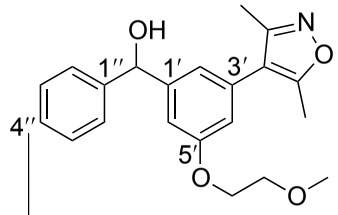
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-methoxyphenyl)(phenyl)methanol 61d



NAME	133	
EXPNO	1	
PROCNO	1	
Date_	20110803	
Time	19.38	
INSTRUM	avc500	
PROBHD	5 mm CPDUL 13C	
PULPROG	zg30	
TD	65536	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	10330.578	Hz
FIDRES	0.157632	Hz
AQ	3.1719923	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
SI	32768	
SF	500.3000240	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	



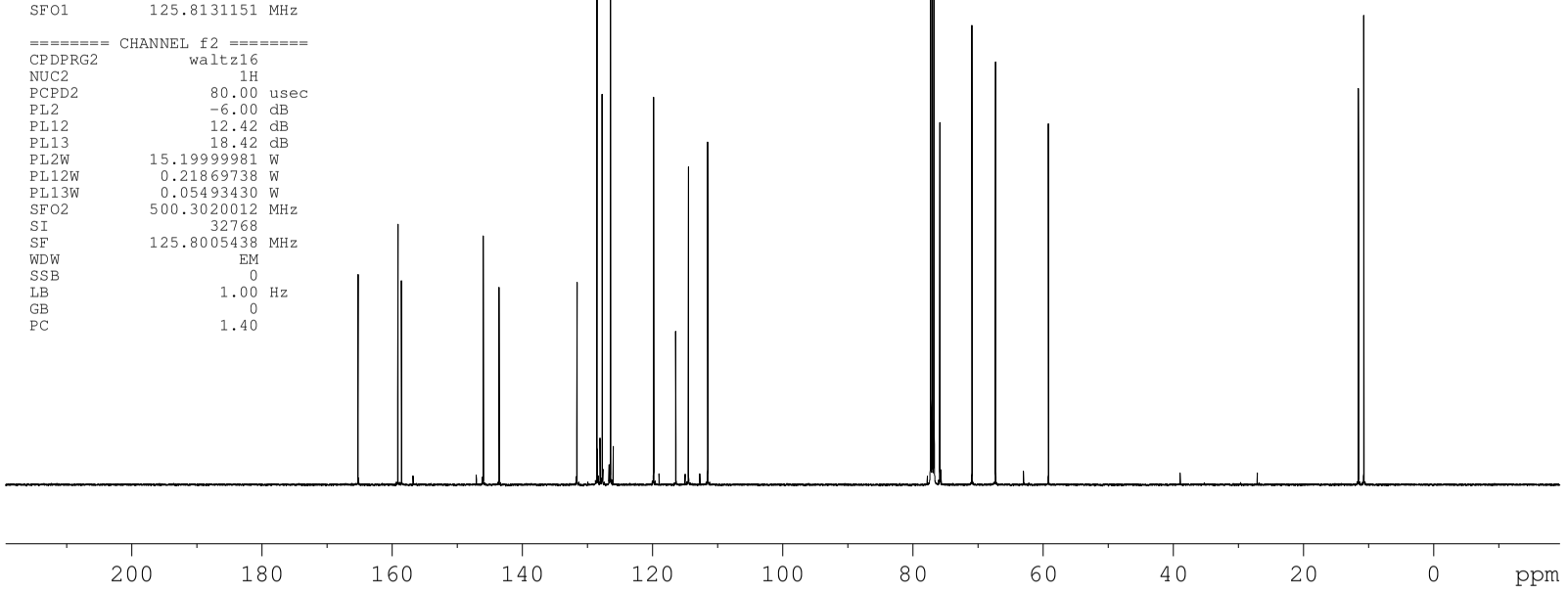
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)-5-methoxyphenyl)(phenyl)methanol 61d




```
NAME                133
EXPNO                 4
PROCNO                1
Date_          20110803
Time              20.21
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

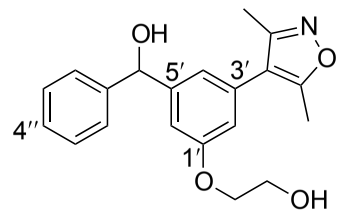
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2            80.00 usec
PL2              -6.00 dB
PL12             12.42 dB
PL13             18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz
SI                32768
SF         125.8005438 MHz
WDW                EM
SSB                 0
LB               1.00 Hz
GB                  0
PC               1.40
```



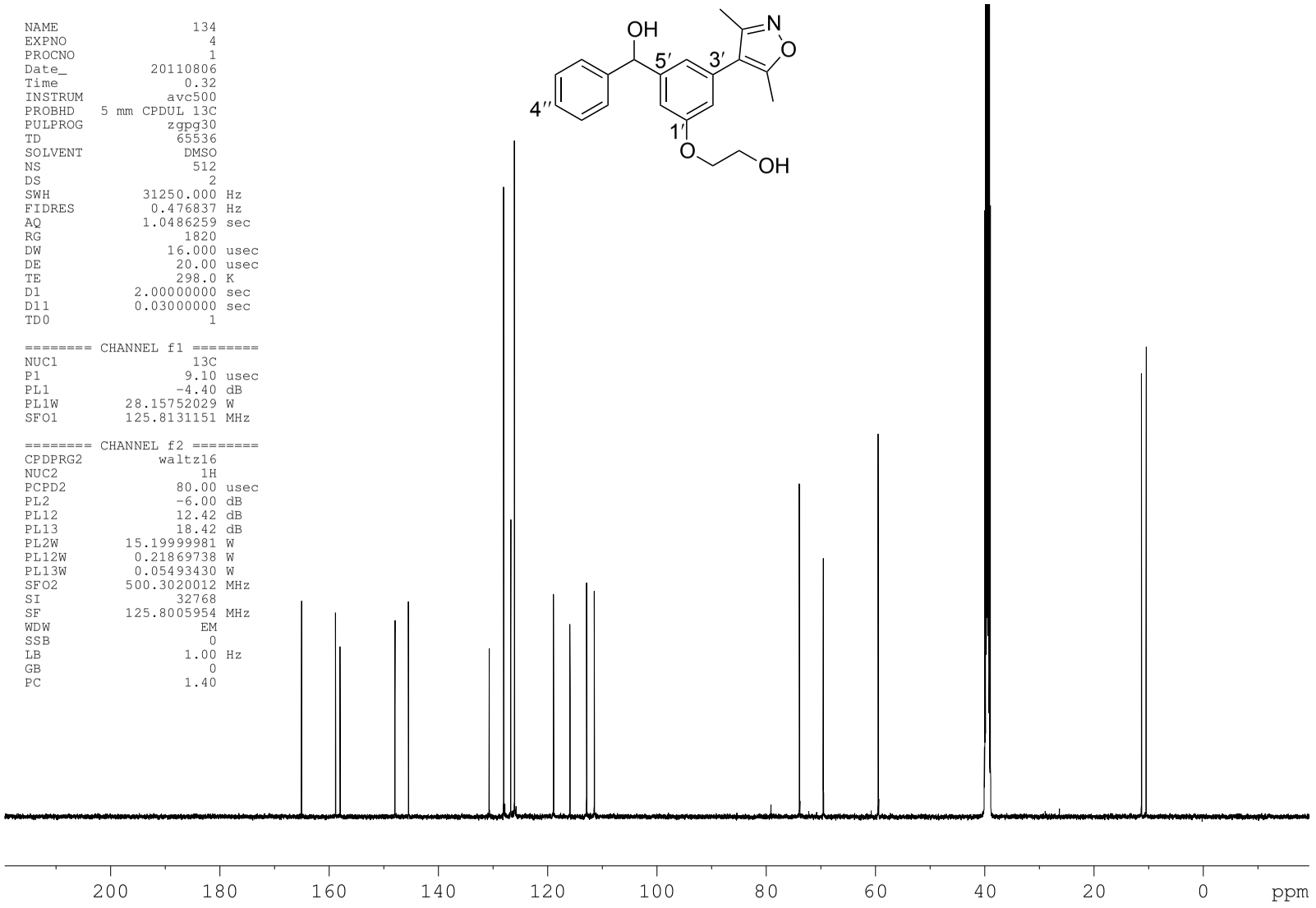
(*RS*)-2-(3-(3,5-Dimethylisoxazol-4-yl)-5-(hydroxy(phenyl)methyl)phenoxy)ethanol 61e

```
NAME                 134
EXPNO                  1
PROCNO                 1
Date_           20110805
Time               23.49
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            3.1719923 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

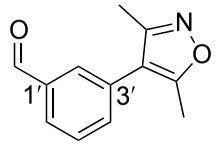
======== CHANNEL f1 ========
NUC1                  1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz
SI                 32768
SF          500.3000000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



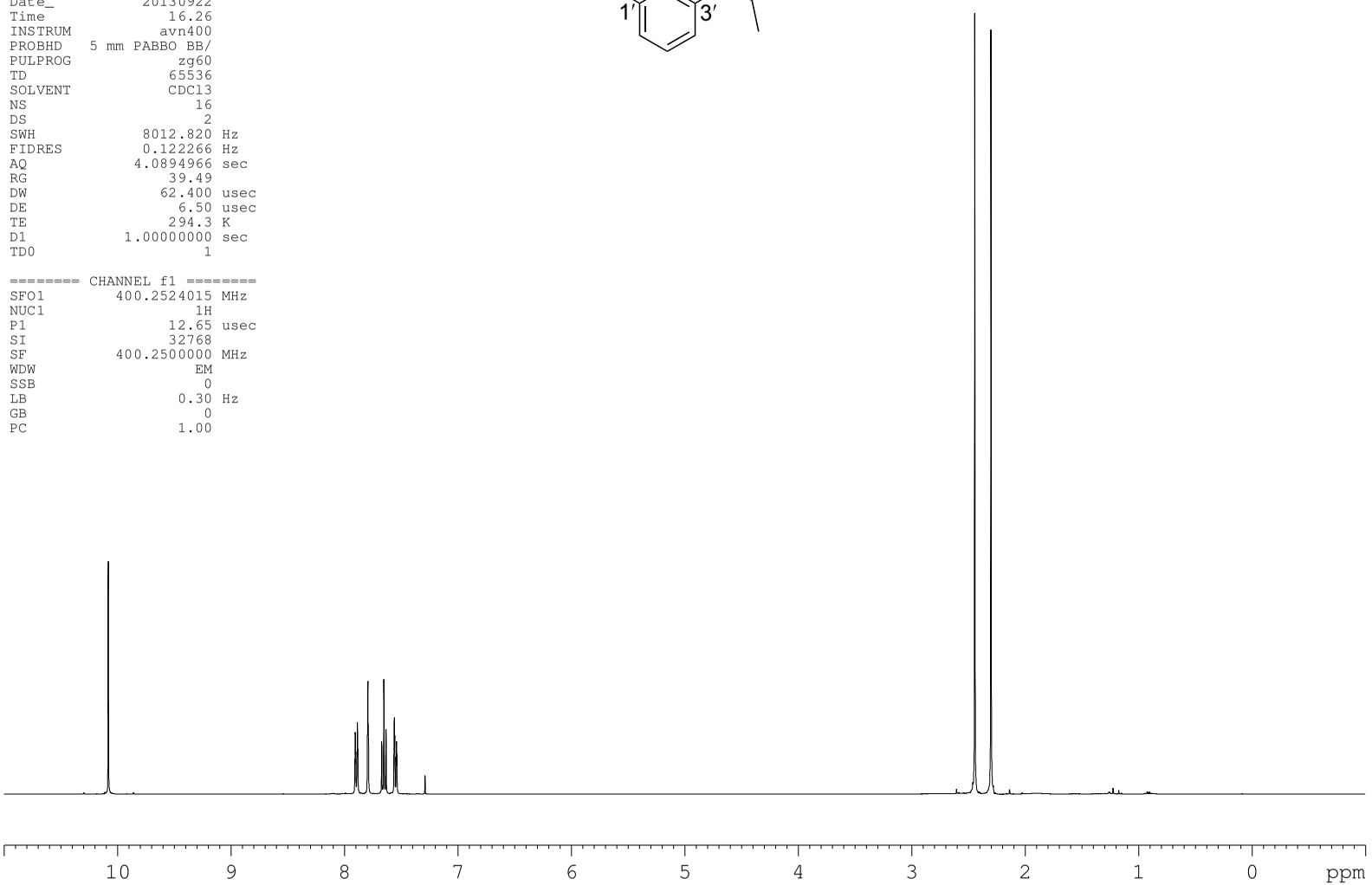
(*RS*)-2-(3-(3,5-Dimethylisoxazol-4-yl)-5-(hydroxy(phenyl)methyl)phenoxy)ethanol 61e



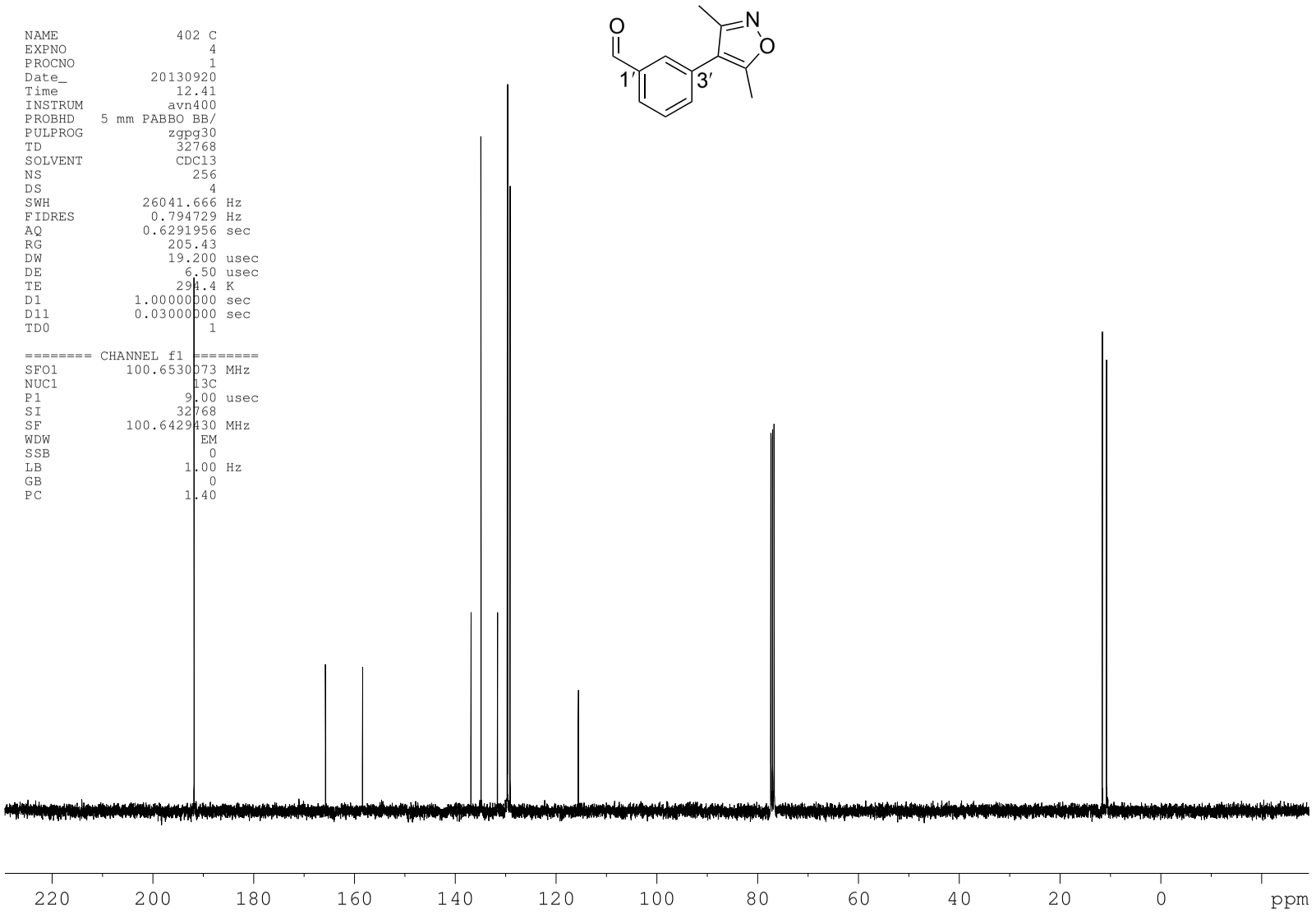
3-(3,5-Dimethylisoxazol-4-yl)benzaldehyde 67



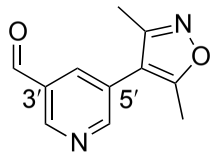
NAME	402
EXPNO	1
PROCNO	1
Date_	20130922
Time	16.26
INSTRUM	avn400
PROBHD	5 mm PABBO BB/
PULPROG	zg60
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8012.820 Hz
FIDRES	0.122266 Hz
AQ	4.0894966 sec
RG	39.49
DW	62.400 usec
DE	6.50 usec
TE	294.3 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
SFO1	400.2524015 MHz
NUC1	1H
P1	12.65 usec
SI	32768
SF	400.2500000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



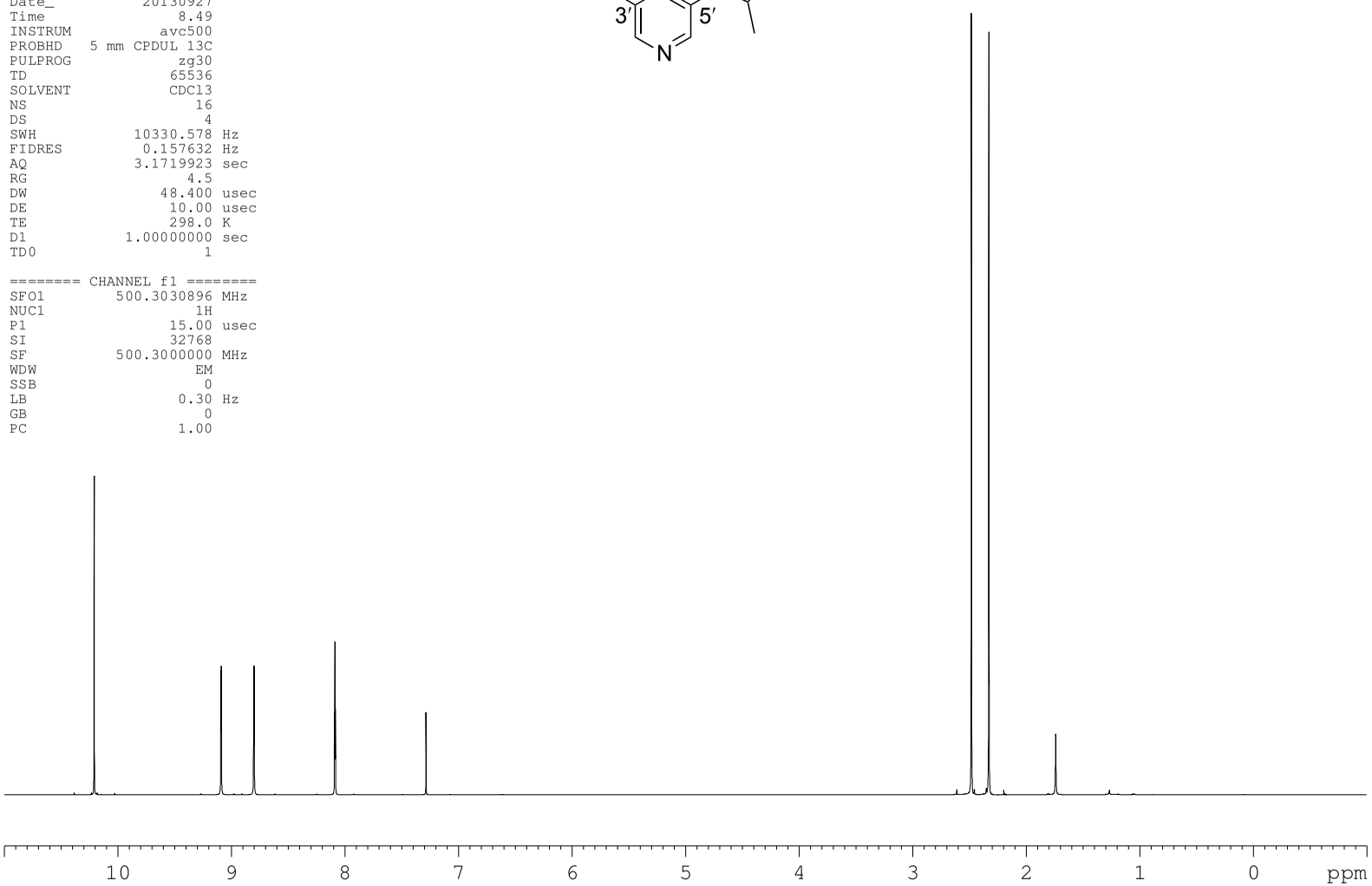
3-(3,5-Dimethylisoxazol-4-yl)benzaldehyde 67



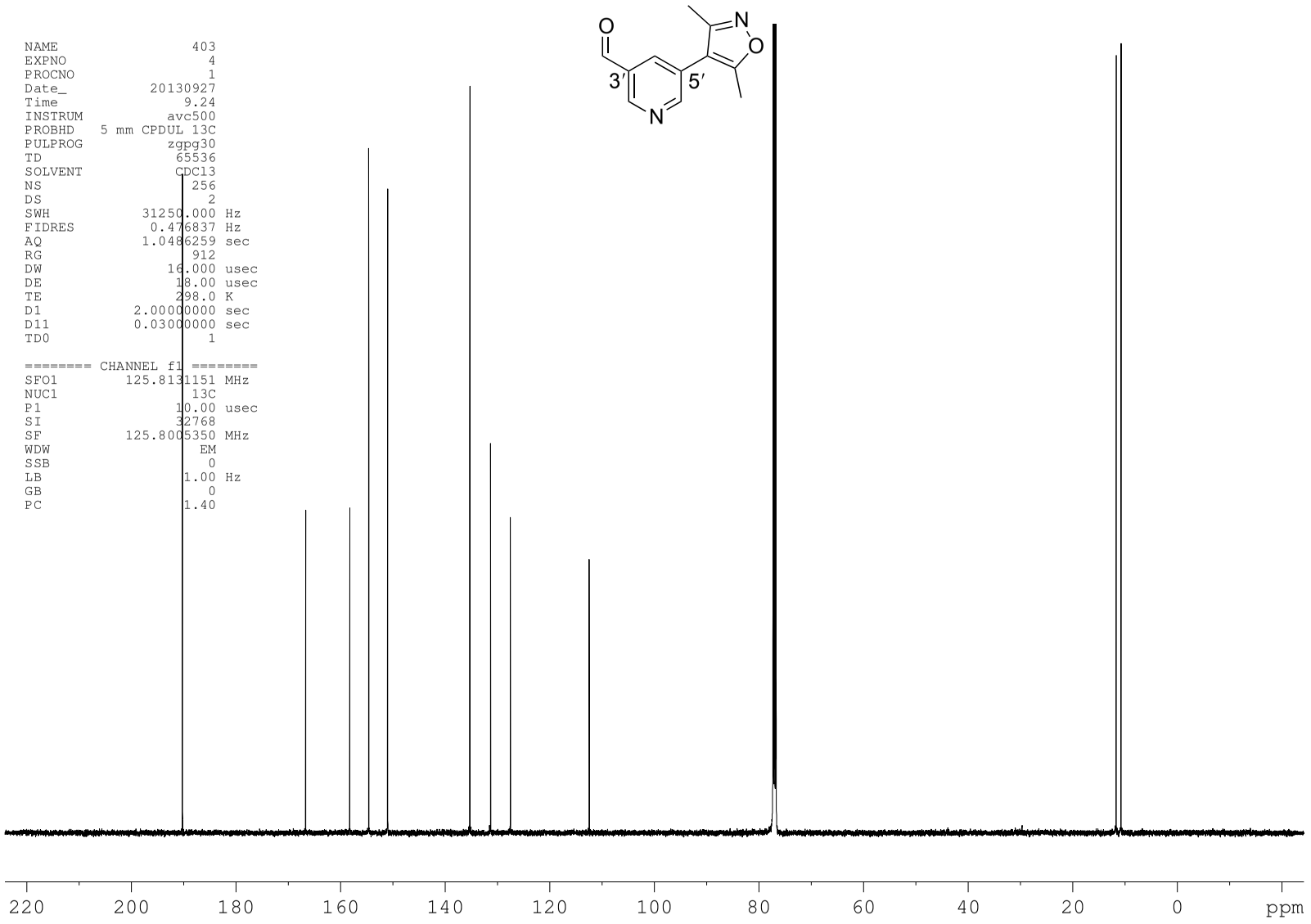
5-(3,5-Dimethylisoxazol-4-yl)-3-pyridinecarboxaldehyde 68



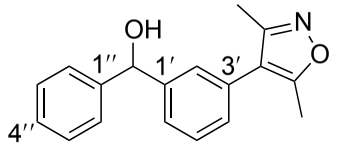
NAME	403
EXPNO	1
PROCNO	1
Date_	20130927
Time	8.49
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4.5
DW	48.400 usec
DE	10.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
SFO1	500.3030896 MHz
NUC1	1H
P1	15.00 usec
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



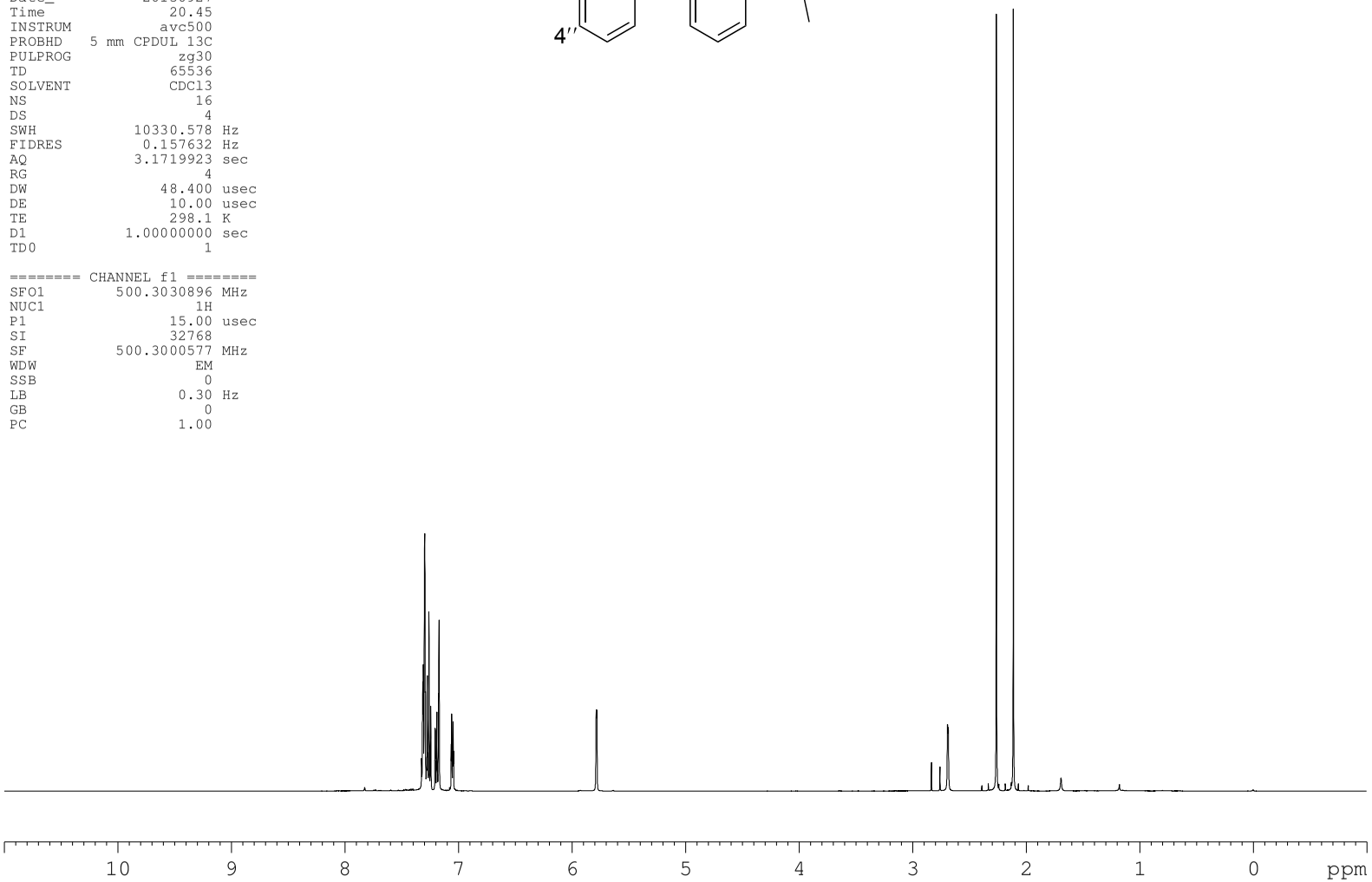
5-(3,5-Dimethylisoxazol-4-yl)-3-pyridinecarboxaldehyde 68



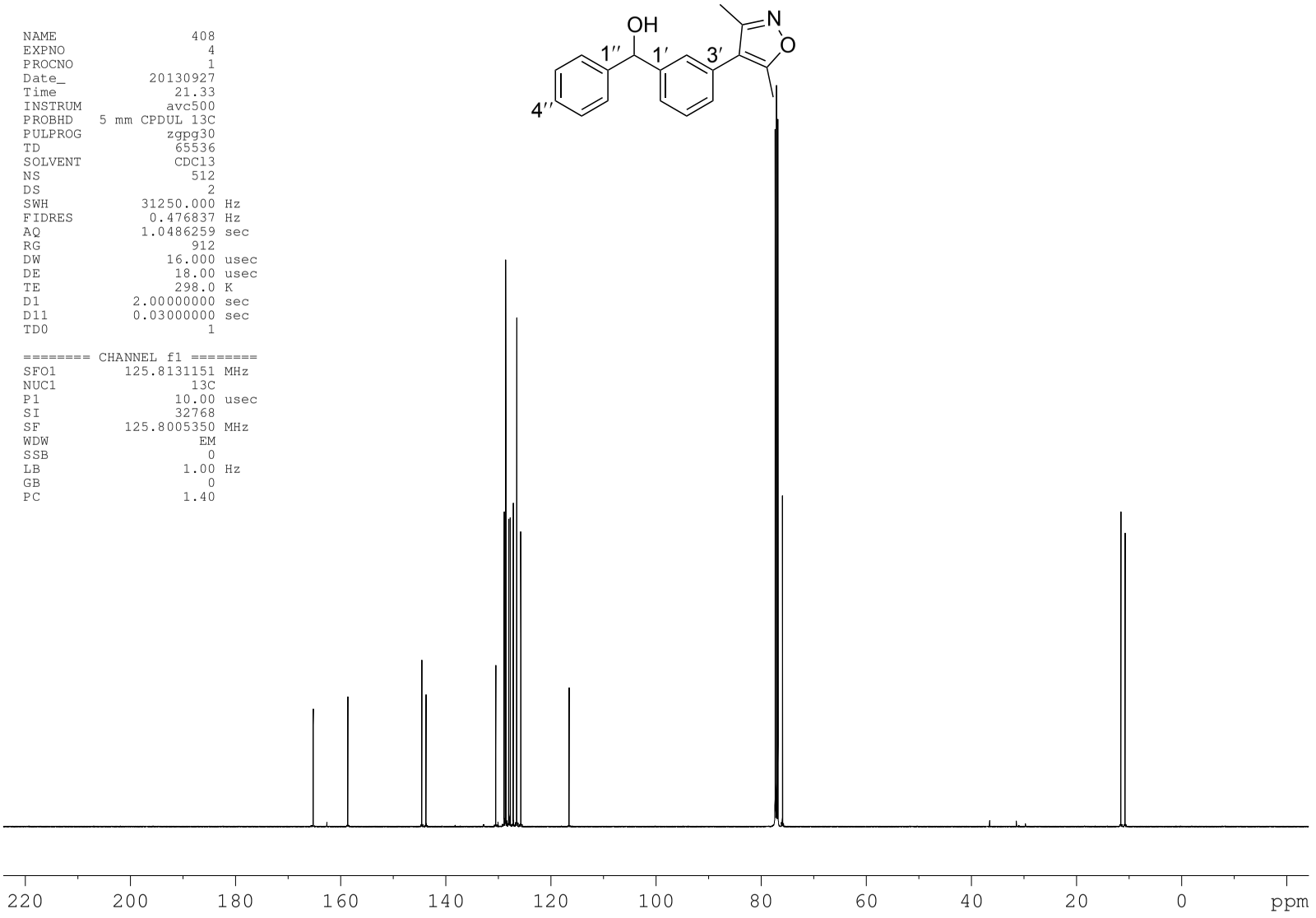
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)phenyl)(phenyl)methanol 61f



NAME	408
EXPNO	1
PROCNO	1
Date_	20130927
Time	20.45
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	10.00 usec
TE	298.1 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
SFO1	500.3030896 MHz
NUC1	1H
P1	15.00 usec
SI	32768
SF	500.3000577 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



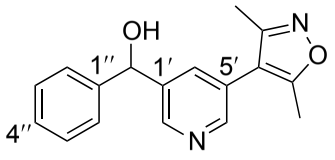
(*RS*)-(3-(3,5-Dimethylisoxazol-4-yl)phenyl)(phenyl)methanol 61f




```
NAME                408
EXPNO                 4
PROCNO                1
Date_          20130927
Time              21.33
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

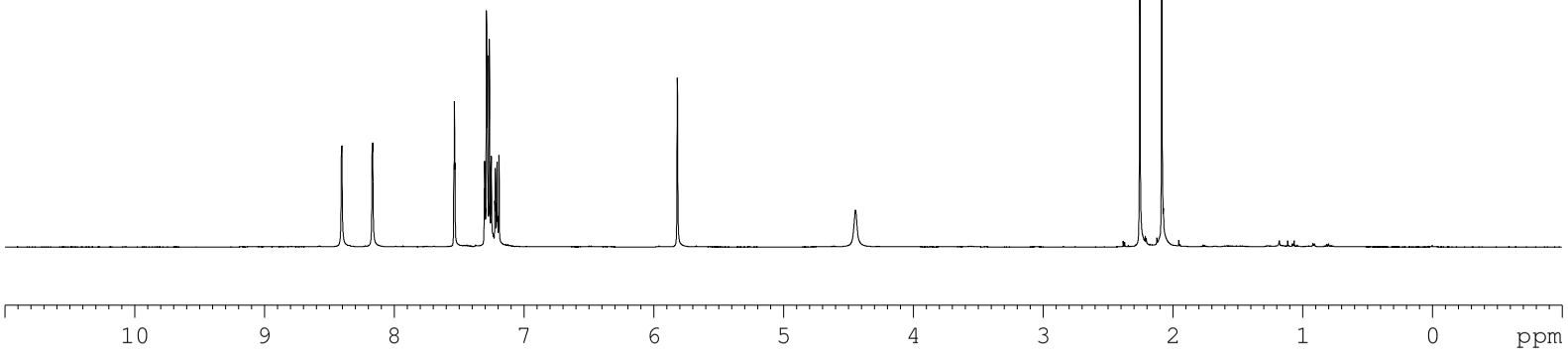
======== CHANNEL f1 ========
SFO1         125.8131151 MHz
NUC1                13C
P1                10.00 usec
SI                32768
SF          125.8005350 MHz
WDW                 EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
```

(*RS*)-(5-(3,5-Dimethylisoxazol-4-yl)pyridin-3-yl)(phenyl)methanol 61g

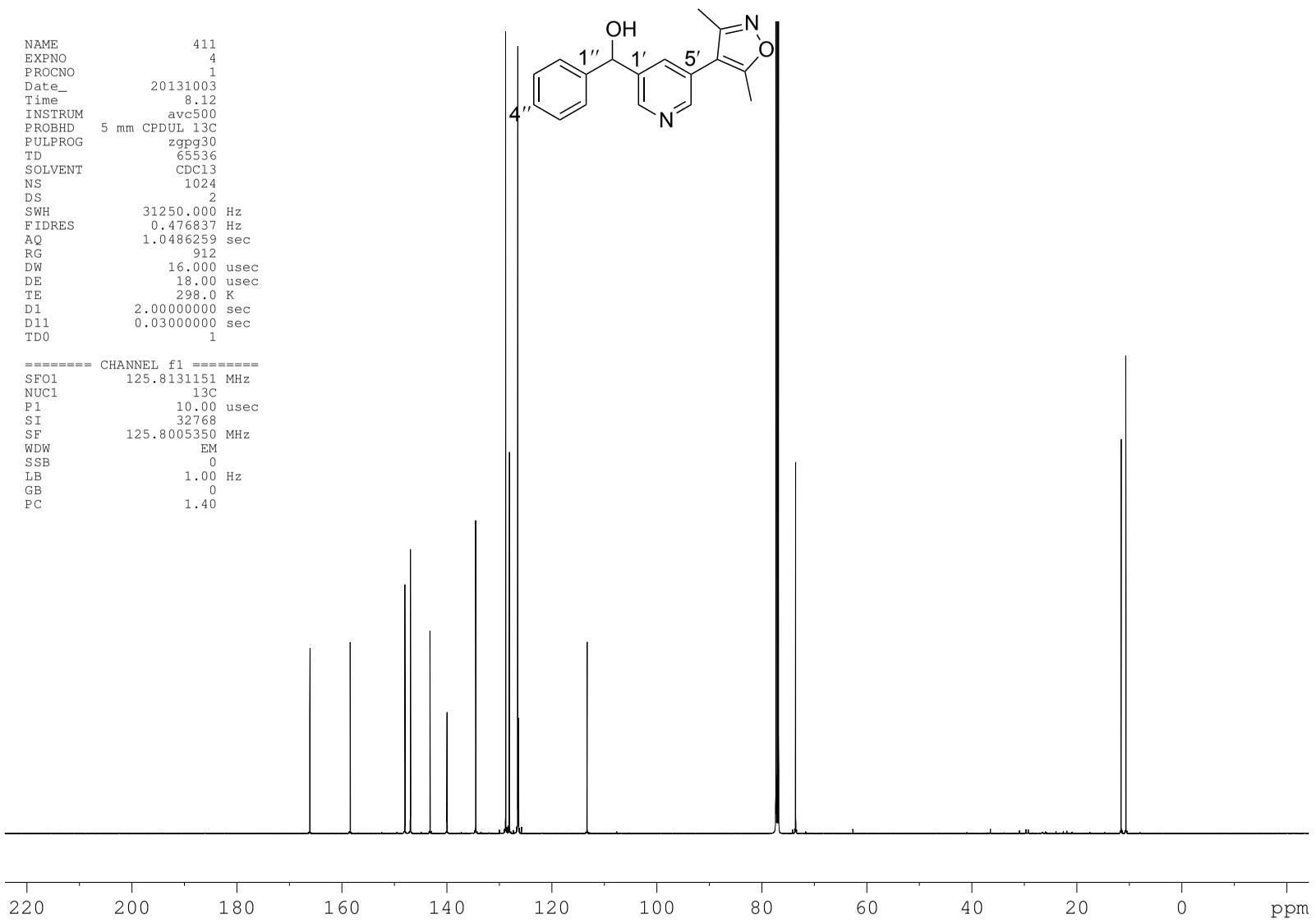



```
NAME                 411
EXPNO                  1
PROCNO                 1
Date_           20131003
Time                6.57
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 sec
RG                     4
DW               48.400 usec
DE               10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

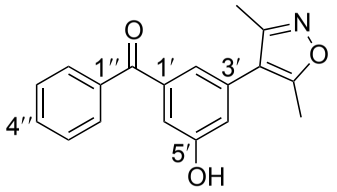
======== CHANNEL f1 ========
SFO1        500.3030896 MHz
NUC1                   1H
P1                 15.00 usec
SI                 32768
SF          500.3000470 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



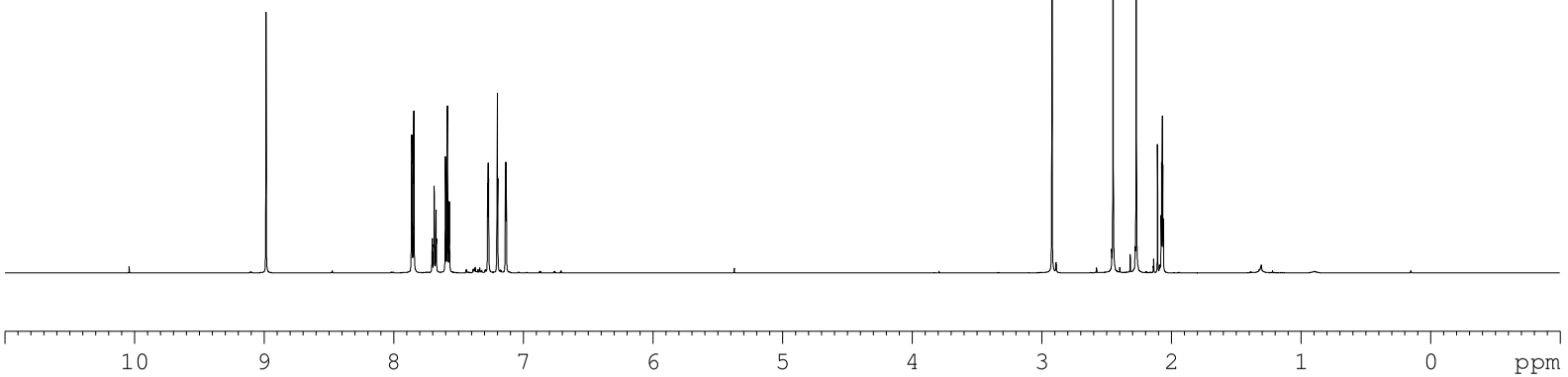
(*RS*)-(5-(3,5-Dimethylisoxazol-4-yl)pyridin-3-yl)(phenyl)methanol 61g



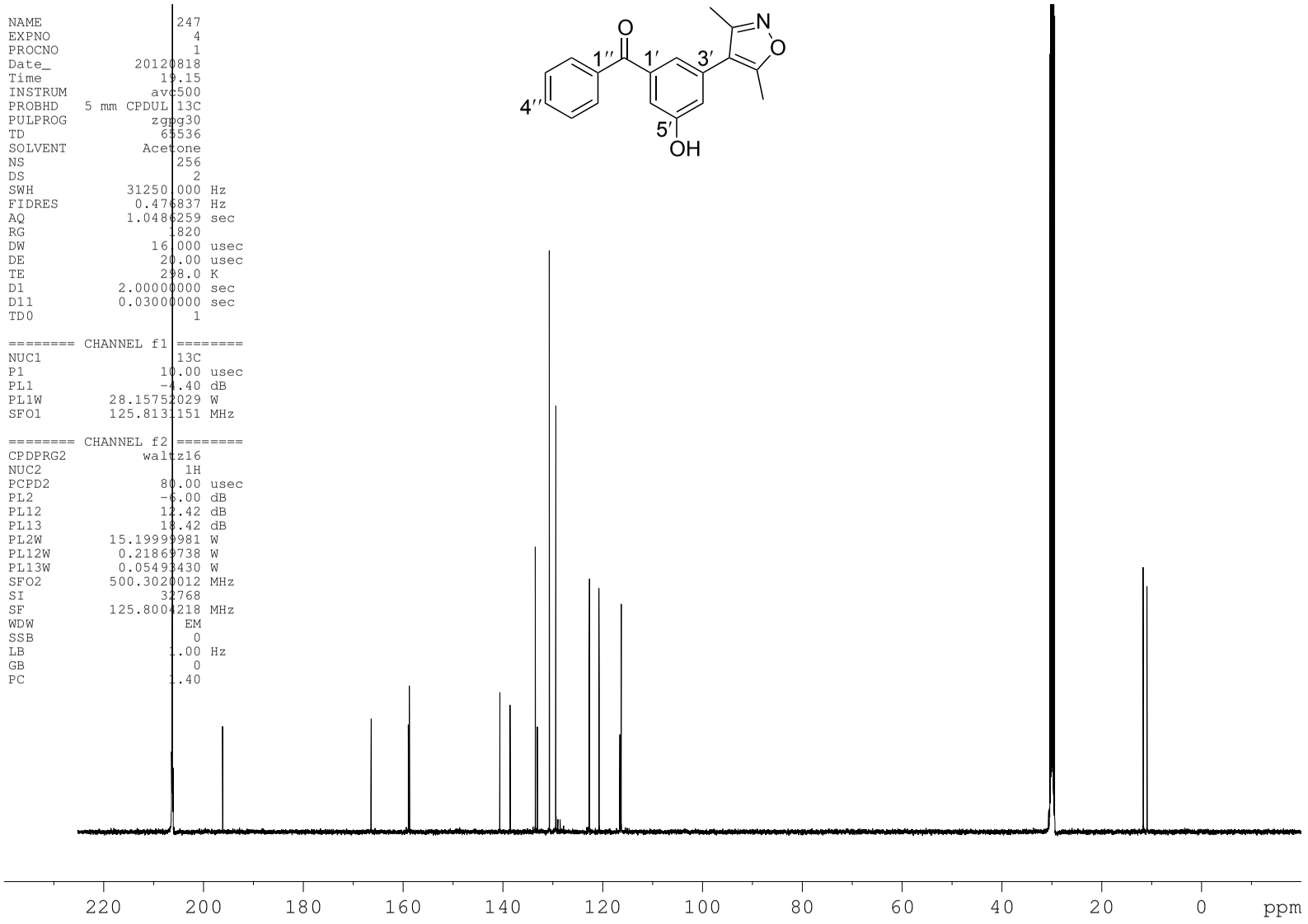
(3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxyphenyl)(phenyl)methanone 69a



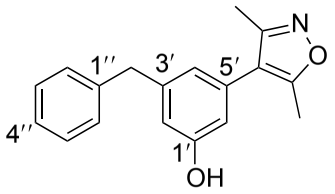
NAME	247
EXPNO	1
PROCNO	1
Date_	20120818
Time	18.40
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	Acetone
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(3-(3,5-Dimethylisoxazol-4-yl)-5-hydroxyphenyl)(phenyl)methanone 69a

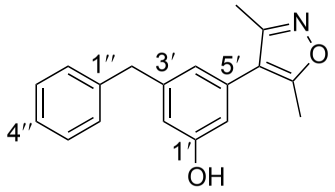


3-Benzyl-5-(3,5-dimethylisoxazol-4-yl)phenol 69b



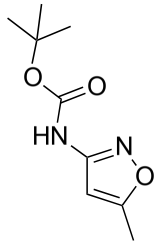
NAME	406
EXPNO	1
PROCNO	1
Date_	20130925
Time	3.17
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	Acetone
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4.5
DW	48.400 usec
DE	10.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
SFO1	500.3030896 MHz
NUC1	1H
P1	15.00 usec
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

3-Benzyl-5-(3,5-dimethylisoxazol-4-yl)phenol 69b



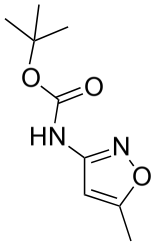
NAME	408
EXPNO	4
PROCNO	1
Date_	20130927
Time	21.33
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	512
DS	2
SWH	31250.000 Hz
FIDRES	0.476837 Hz
AQ	1.0486259 sec
RG	912
DW	16.000 usec
DE	18.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
SFO1	125.8131151 MHz
NUC1	13C
P1	10.00 usec
SI	32768
SF	125.8005350 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

tert-Butyl (5-methylisoxazol-3-yl)carbamate 84



NAME	055
EXPNO	1
PROCNO	1
Date_	20110528
Time	0.06
INSTRUM	av400
PROBHD	5 mm QNP 1H/13
PULPROG	zg60
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	90.5
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	0.00 dB
SFO1	400.2024714 MHz
SI	32768
SF	400.2000028 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

tert-Butyl (5-methylisoxazol-3-yl)carbamate 84




```
NAME                 055
EXPNO                  2
PROCNO                 1
Date_           20110528
Time                0.15
INSTRUM            av400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                 32768
SOLVENT            CDCl3
NS                   256
DS                     4
SWH            26178.010 Hz
FIDRES          0.798889 Hz
AQ             0.6259188 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

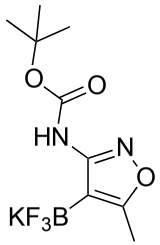
======== CHANNEL f1 ========
NUC1                13C
P1                 9.50 usec
PL1                0.00 dB
SFO1        100.6403931 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2              80.00 usec
PL2                 0.00 dB
PL12               19.00 dB
PL13               25.00 dB
SFO2        400.2016008 MHz
SI                 32768
SF          100.6303718 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

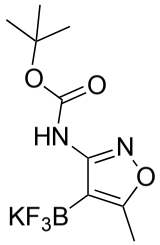
Potassium (3-((*tert*-butoxycarbonyl)amino)-5-methylisoxazol-4-yl)trifluoroborate 85

```
NAME                 066
EXPNO                  1
PROCNO                 1
Date_           20110228
Time                9.30
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            3.1719923 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
SI                 32768
SF          500.3000000 MHz
WDW                   no
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.00
```



Potassium (3-((*tert*-butoxycarbonyl)amino)-5-methylisoxazol-4-yl)trifluoroborate 85

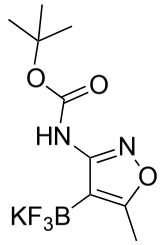



```
NAME                066
EXPNO                 3
PROCNO                1
Date_          20110227
Time               0.13
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                  1024
DS                     2
SWH            31250.000 Hz
FIDRES             0.476837 Hz
AQ              1.0486259 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                  9.50 usec
PL1                -4.40 dB
PL1W         28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF          125.8005954 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

Potassium (3-((*tert*-butoxycarbonyl)amino)-5-methylisoxazol-4-yl)trifluoroborate 85

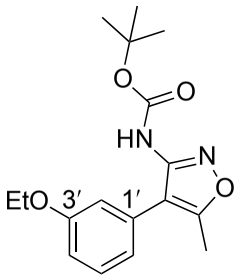



```
NAME               066 F
EXPNO                1
PROCNO               1
Date_           20110224
Time                9.59
INSTRUM          avb500
PROBHD   5 mm PATXI 1H/
PULPROG         zgfhigqn
TD                131072
SOLVENT              DMSO
NS                     16
DS                      4
SWH           113636.367 Hz
FIDRES          0.866977 Hz
AQ             0.5767668 sec
RG                  2050
DW                 4.400 usec
DE                  6.50 usec
TE                 298.0 K
D1            1.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 19F
P1                 10.00 usec
PL1                 0.90 dB
SFO1         470.4041911 MHz

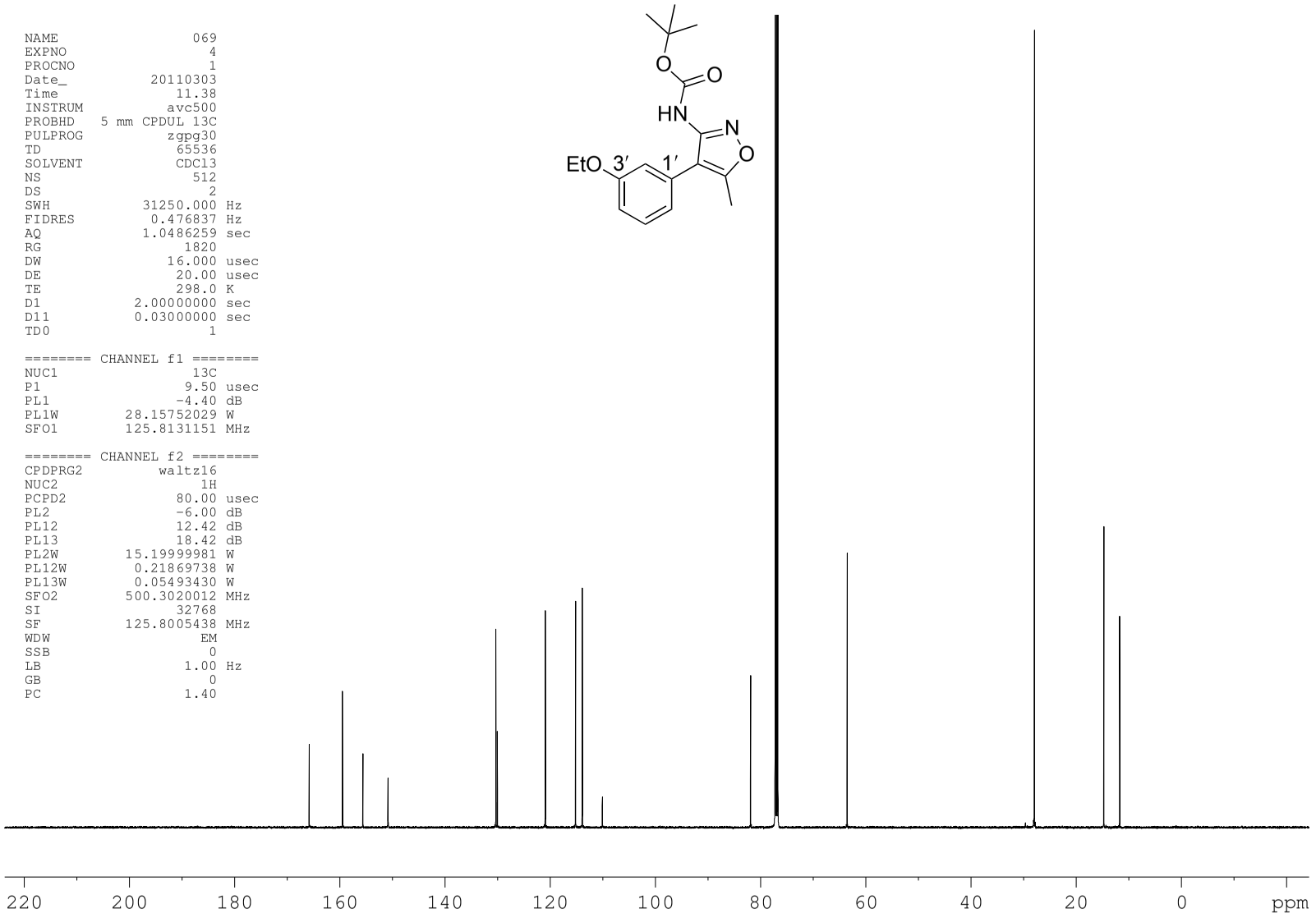
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                 1.00 dB
PL12               22.00 dB
PL2W         13.20863628 W
PL12W         0.10491993 W
SFO2         499.9819999 MHz
SI                 65536
SF           470.4512360 MHz
WDW                  EM
SSB                   0
LB                 2.00 Hz
GB                    0
PC                 1.00
```

tert-Butyl (4-(3-ethoxyphenyl)-5-methylisoxazol-3-yl)carbamate 86

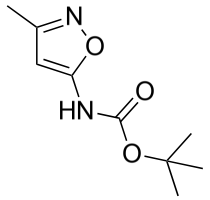


NAME	069
EXPNO	1
PROCNO	1
Date_	20110303
Time	10.54
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

tert-Butyl (4-(3-ethoxyphenyl)-5-methylisoxazol-3-yl)carbamate 86

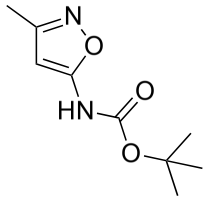


tert-Butyl (5-methylisoxazol-3-yl)carbamate 88



NAME	057
EXPNO	1
PROCNO	1
Date_	20110211
Time	21.50
INSTRUM	av400
PROBHD	5 mm QNP 1H/13
PULPROG	zg60
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	90.5
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	0.00 dB
SFO1	400.2024714 MHz
SI	32768
SF	400.2000028 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

tert-Butyl (5-methylisoxazol-3-yl)carbamate 88

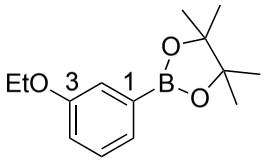



```
NAME                 057
EXPNO                  2
PROCNO                 1
Date_           20110211
Time               21.58
INSTRUM            av400
PROBHD   5 mm QNP 1H/13
PULPROG          zgpg30
TD                 32768
SOLVENT            CDCl3
NS                   256
DS                     4
SWH            26178.010 Hz
FIDRES          0.798889 Hz
AQ             0.6259188 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                0.00 dB
SFO1        100.6403931 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2              80.00 usec
PL2                 0.00 dB
PL12               19.00 dB
PL13               25.00 dB
SFO2        400.2016008 MHz
SI                 32768
SF          100.6303718 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

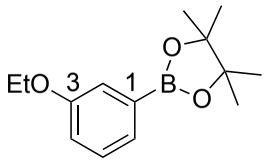
2-(3-Ethoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane 90



NAME	089
EXPNO	1
PROCNO	1
Date_	20110429
Time	22.21
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

2-(3-Ethoxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane 90

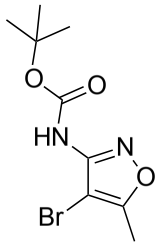



```
NAME                089
EXPNO                 4
PROCNO                1
Date_          20110429
Time              23.30
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

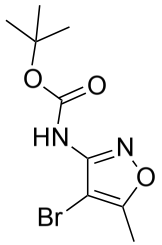
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2            80.00 usec
PL2              -6.00 dB
PL12             12.42 dB
PL13             18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

tert-Butyl (4-bromo-5-methylisoxazol-3-yl)carbamate 93



NAME	088
EXPNO	1
PROCNO	1
Date_	20110427
Time	21.20
INSTRUM	drx500
PROBHD	5 mm PABBO BB/
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	101.6
DW	48.400 usec
DE	6.00 usec
TE	297.5 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	12.50 usec
PL1	0.00 dB
SFO1	500.1330885 MHz
SI	32768
SF	500.1300237 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

tert-Butyl (4-bromo-5-methylisoxazol-3-yl)carbamate 93

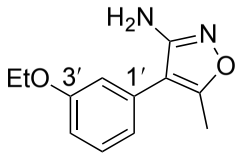



```
NAME                 088
EXPNO                  2
PROCNO                 1
Date_           20110427
Time               22.16
INSTRUM           drx500
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1024
DS                     4
SWH            30030.029 Hz
FIDRES          0.458222 Hz
AQ             1.0912410 sec
RG                4597.6
DW                16.650 usec
DE                 6.00 usec
TE                298.0 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  13C
P1                   9.50 usec
PL1                  5.00 dB
SFO1         125.7703643 MHz

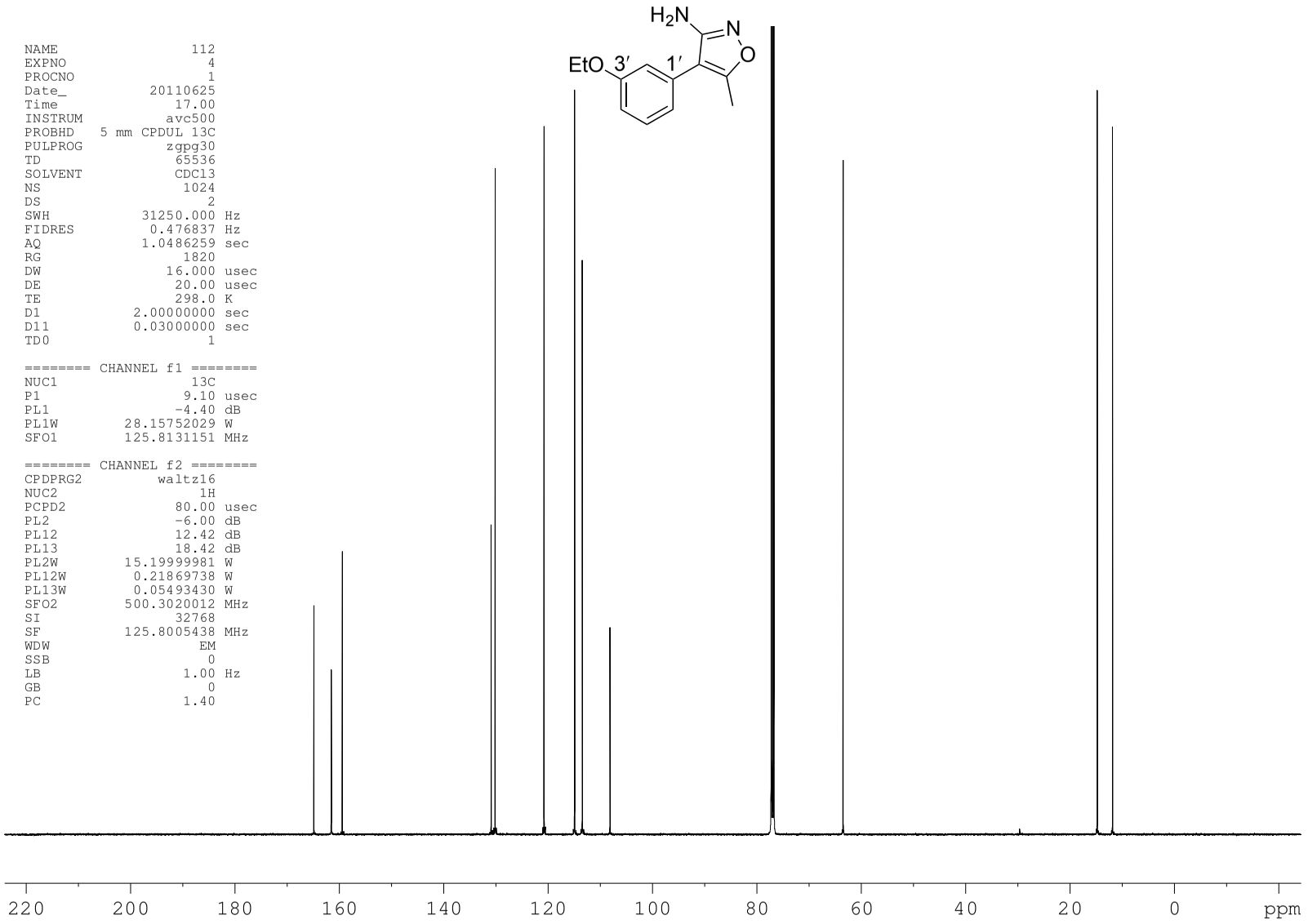
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2              100.00 usec
PL2                  0.00 dB
PL12                19.50 dB
PL13                22.50 dB
SFO2         500.1320005 MHz
SI                 32768
SF           125.7577890 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

4-(3-Ethoxyphenyl)-5-methylisoxazol-3-amine 70a



NAME	112
EXPNO	1
PROCNO	1
Date_	20110625
Time	15.51
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

4-(3-Ethoxyphenyl)-5-methylisoxazol-3-amine 70a



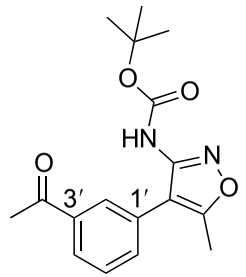

```
NAME                 112
EXPNO                  4
PROCNO                 1
Date_           20110625
Time               17.00
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  1024
DS                     2
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ             1.0486259 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                  9.10 usec
PL1                -4.40 dB
PL1W         28.15752029 W
SFO1        125.8131151 MHz

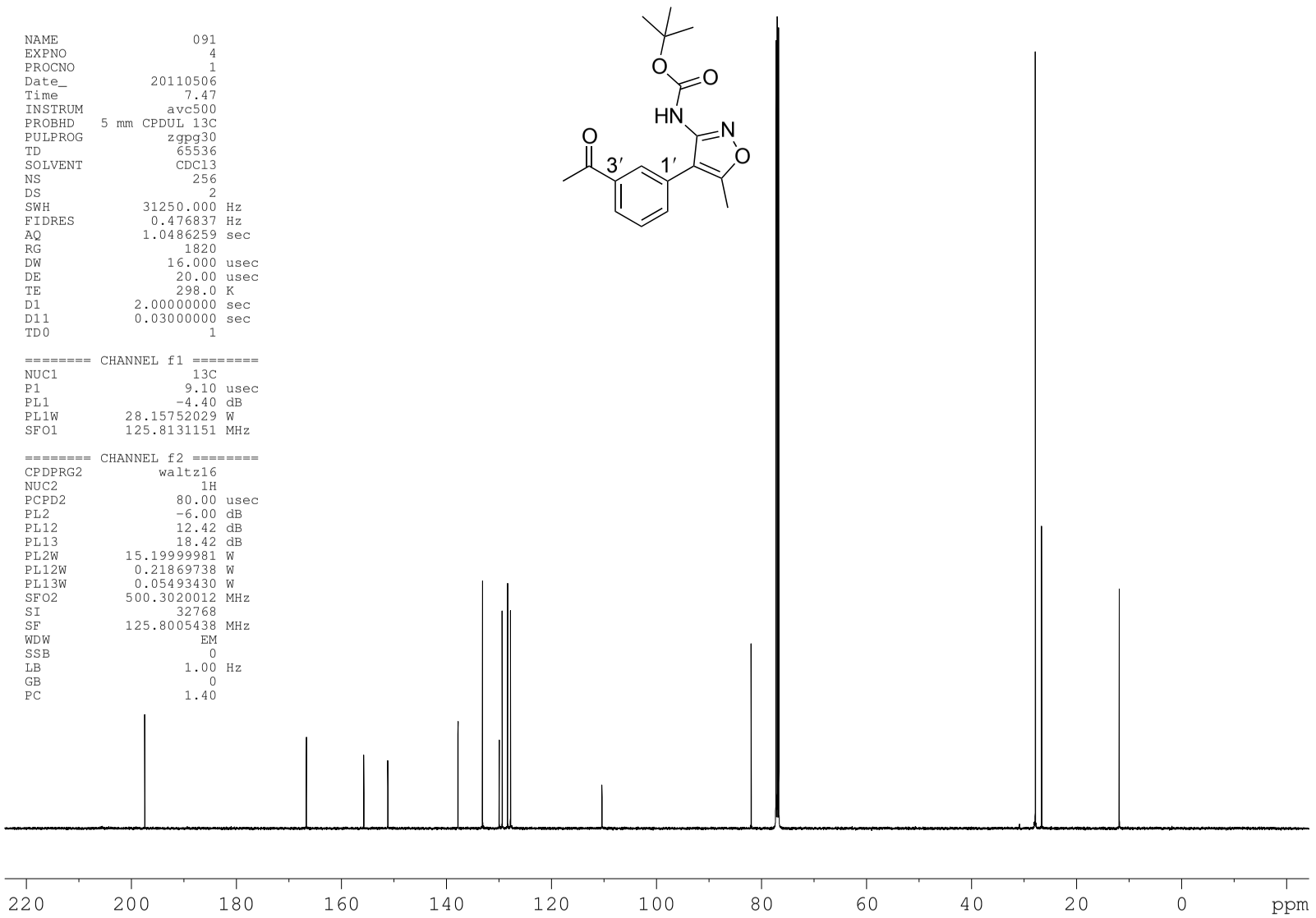
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               12.42 dB
PL13               18.42 dB
PL2W         15.19999981 W
PL12W         0.21869738 W
PL13W         0.05493430 W
SFO2        500.3020012 MHz
SI                 32768
SF          125.8005438 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

tert-Butyl (4-(3-acetylphenyl)-5-methylisoxazol-3-yl)carbamate 94

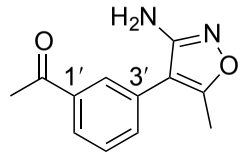
NAME	091
EXPNO	1
PROCNO	1
Date_	20110506
Time	7.17
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



tert-Butyl (4-(3-acetylphenyl)-5-methylisoxazol-3-yl)carbamate 94



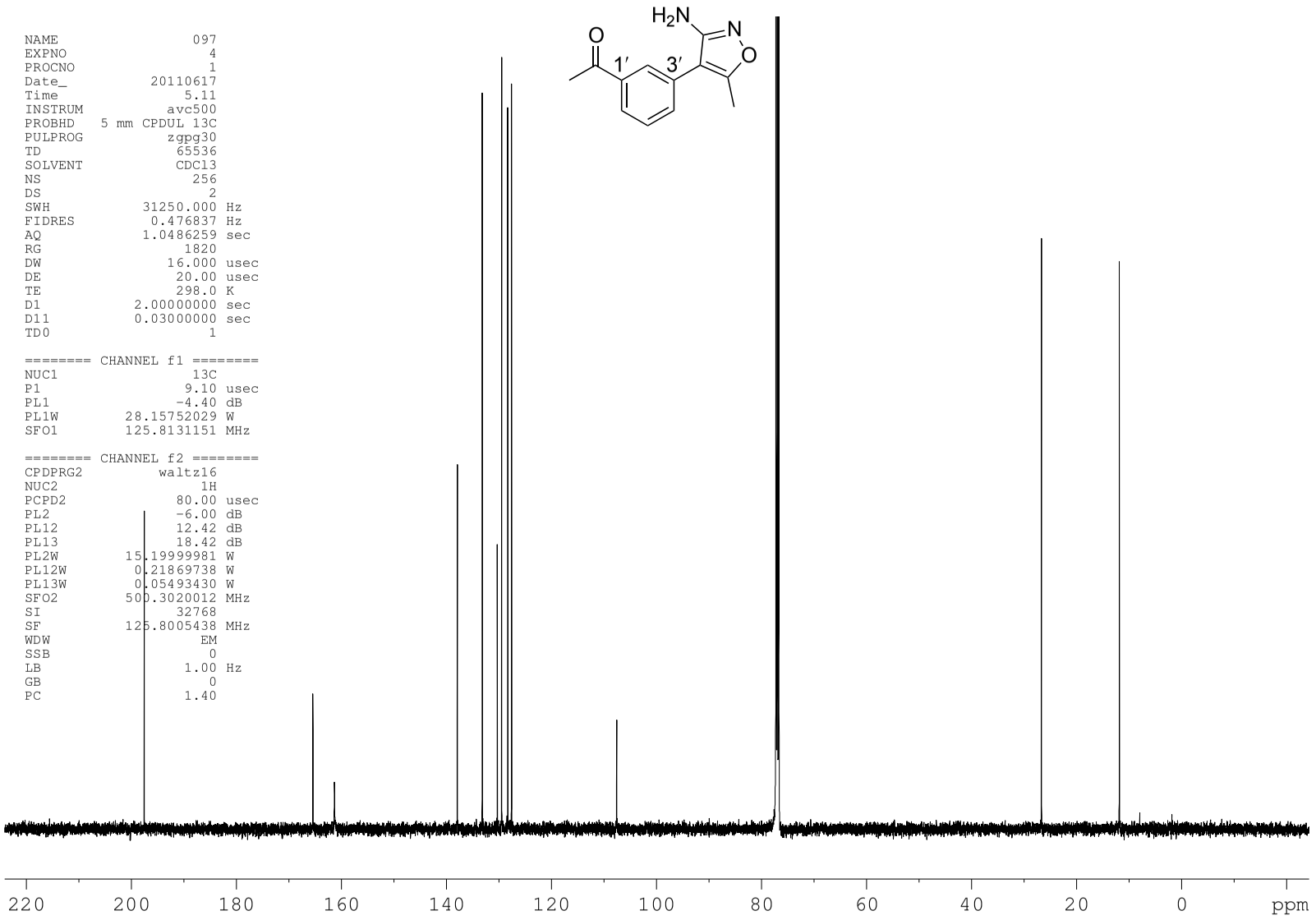
tert-Butyl (4-(3-acetylphenyl)-5-methylisoxazol-3-yl)carbamate 70b



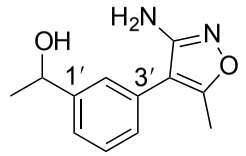

```
NAME                  097
EXPNO                   1
PROCNO                  1
Date_            20110617
Time                4.40
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 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
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

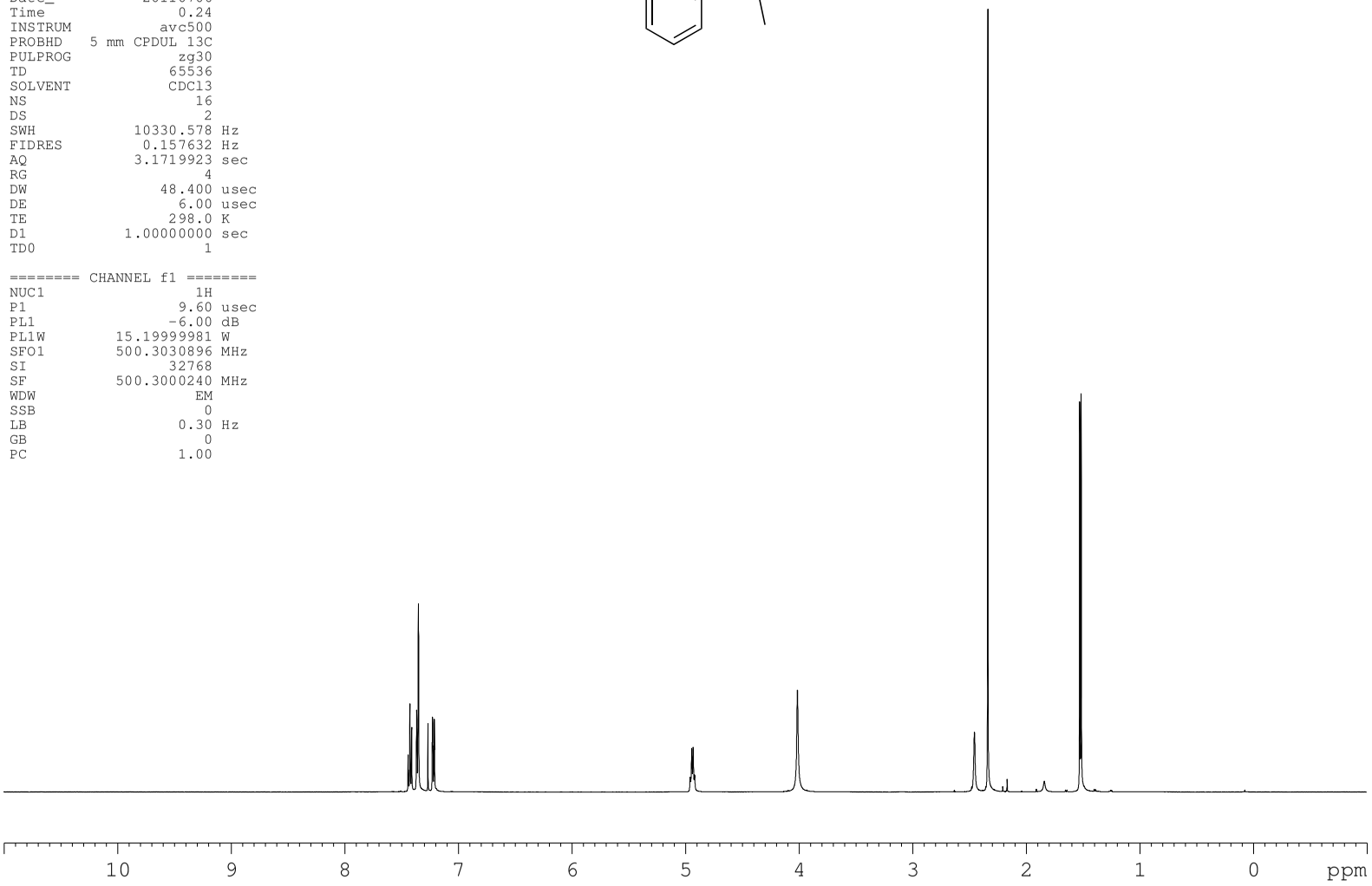
tert-Butyl (4-(3-acetylphenyl)-5-methylisoxazol-3-yl)carbamate 70b



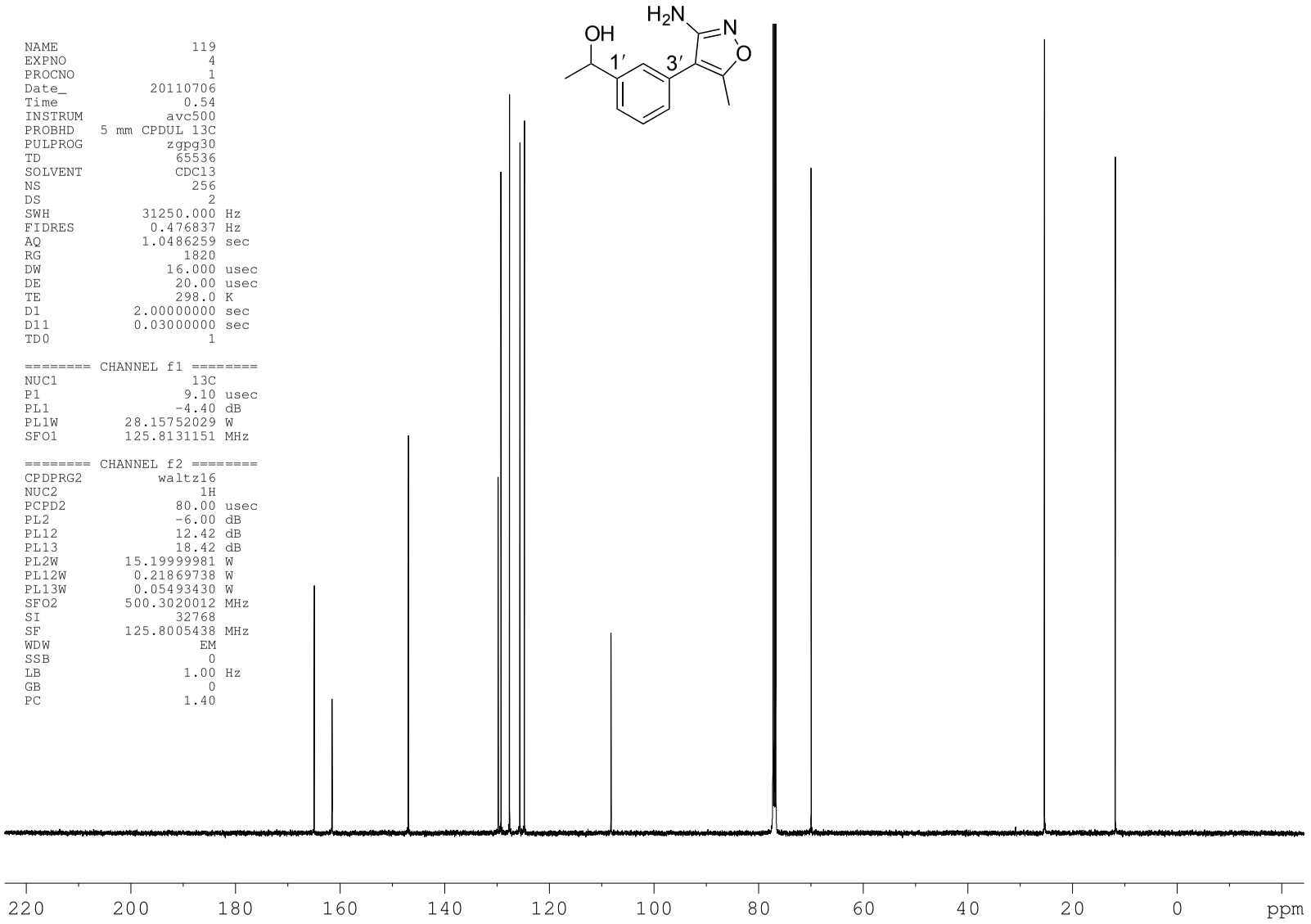
(*RS*)-1-(3-(3-Amino-5-methylisoxazol-4-yl)phenyl)ethanol 70c



NAME	119
EXPNO	1
PROCNO	1
Date_	20110706
Time	0.24
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-1-(3-(3-Amino-5-methylisoxazol-4-yl)phenyl)ethanol 70c

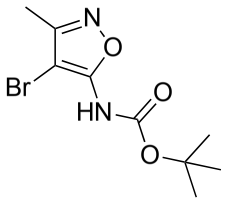



```
NAME                119
EXPNO                 4
PROCNO                1
Date_          20110706
Time               0.54
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

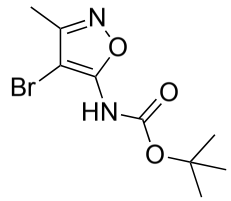
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2            80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

tert-Butyl (4-bromo-3-methylisoxazol-5-yl)carbamate 97



NAME	086
EXPNO	1
PROCNO	1
Date_	20110418
Time	11.05
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	2.9295092 sec
RG	101.6
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	0.00 dB
SFO1	400.1320007 MHz
SI	32768
SF	400.1300182 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	0.60

tert-Butyl (4-bromo-3-methylisoxazol-5-yl)carbamate 97

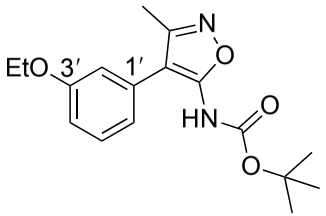



```
NAME                  086
EXPNO                   2
PROCNO                  1
Date_            20110422
Time                 0.42
INSTRUM            avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                     512
DS                       2
SWH              31250.000 Hz
FIDRES           0.476837 Hz
AQ               1.0486259 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                    9.10 usec
PL1                  -4.40 dB
PL1W          28.15752029 W
SFO1          125.8131151 MHz

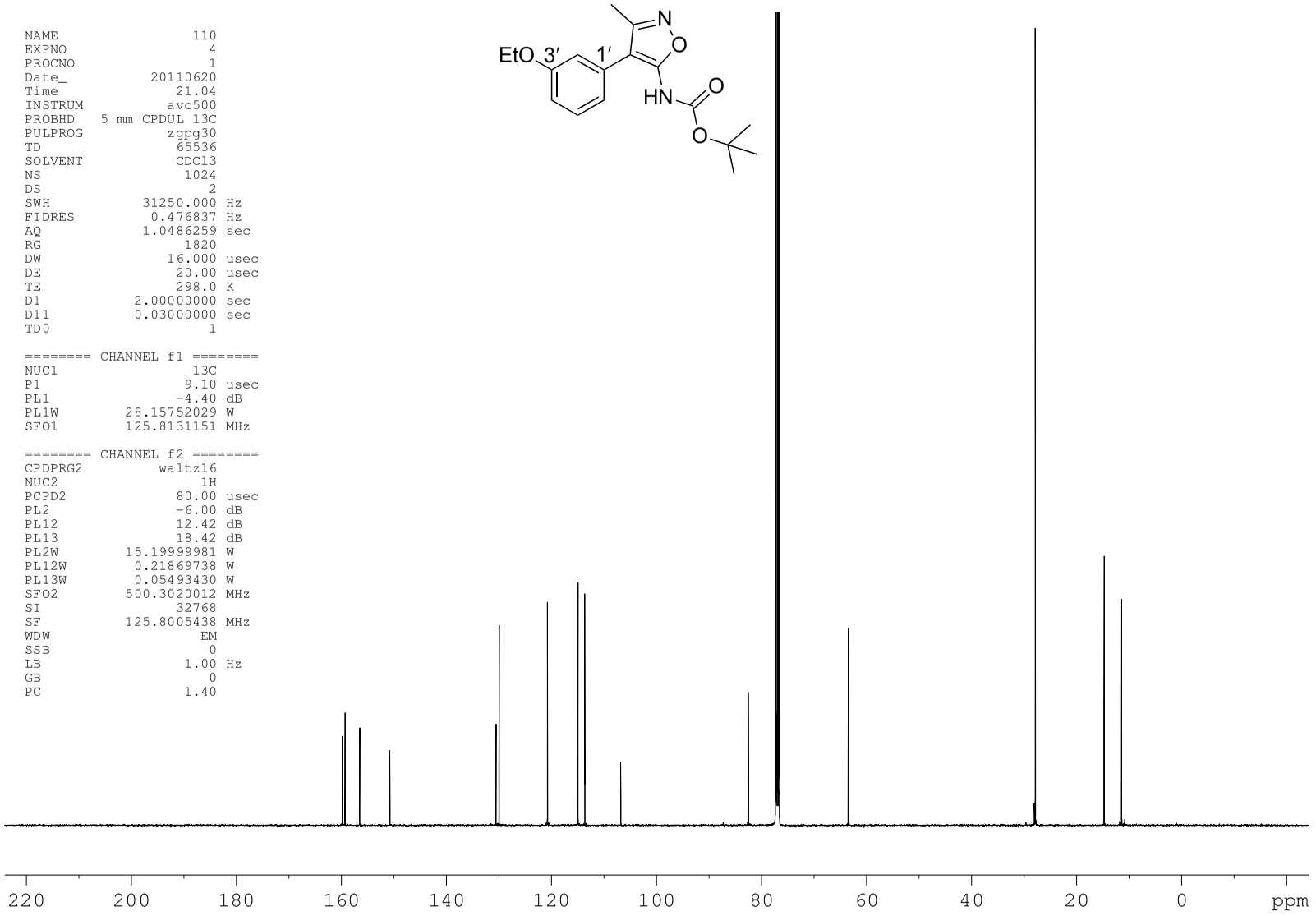
======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                    1H
PCPD2                80.00 usec
PL2                  -6.00 dB
PL12                 12.42 dB
PL13                 18.42 dB
PL2W          15.19999981 W
PL12W          0.21869738 W
PL13W          0.05493430 W
SFO2          500.3020012 MHz
SI                   32768
SF            125.8005438 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

tert-Butyl (4-(3-ethoxyphenyl)-3-methylisoxazol-5-yl)carbamate 98



NAME	110
EXPNO	1
PROCNO	1
Date_	20110620
Time	19.55
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

tert-Butyl (4-(3-ethoxyphenyl)-3-methylisoxazol-5-yl)carbamate 98

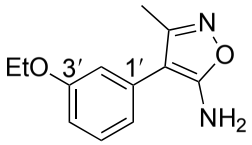



```
NAME                110
EXPNO                 4
PROCNO                1
Date_          20110620
Time              21.04
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

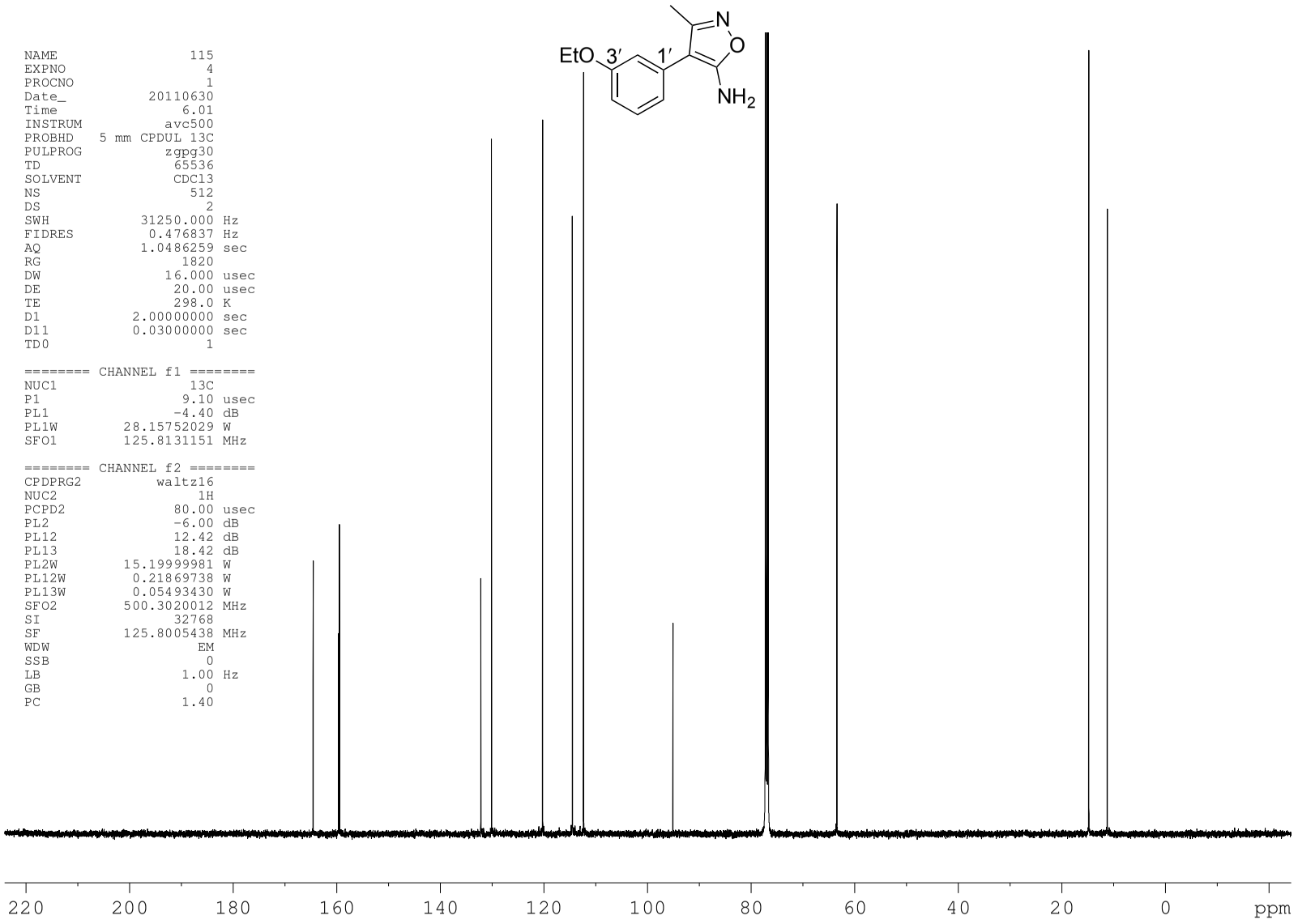
4-(3-ethoxyphenyl)-3-methylisoxazol-5-amine 71a



NAME	115
EXPNO	1
PROCNO	1
Date_	20110630
Time	5.17
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

4-(3-ethoxyphenyl)-3-methylisoxazol-5-amine 71a

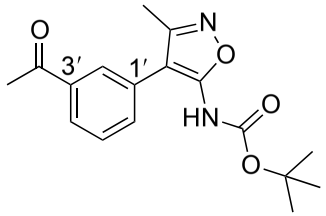



```
NAME                 115
EXPNO                  4
PROCNO                 1
Date_           20110630
Time                6.01
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   512
DS                     2
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ              1.0486259 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                  9.10 usec
PL1                -4.40 dB
PL1W         28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                 32768
SF          125.8005438 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

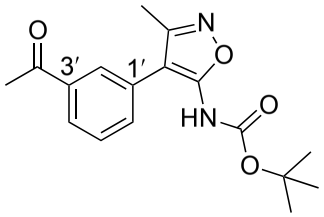
tert-Butyl (4-(3-acetylphenyl)-3-methylisoxazol-5-yl)carbamate 99




```
NAME                 108
EXPNO                  1
PROCNO                 1
Date_           20110619
Time               21.08
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 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
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

tert-Butyl (4-(3-acetylphenyl)-3-methylisoxazol-5-yl)carbamate 99

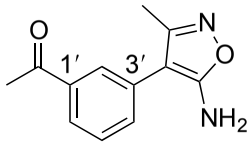



```
NAME                108
EXPNO                 4
PROCNO                1
Date_          20110619
Time              21.52
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

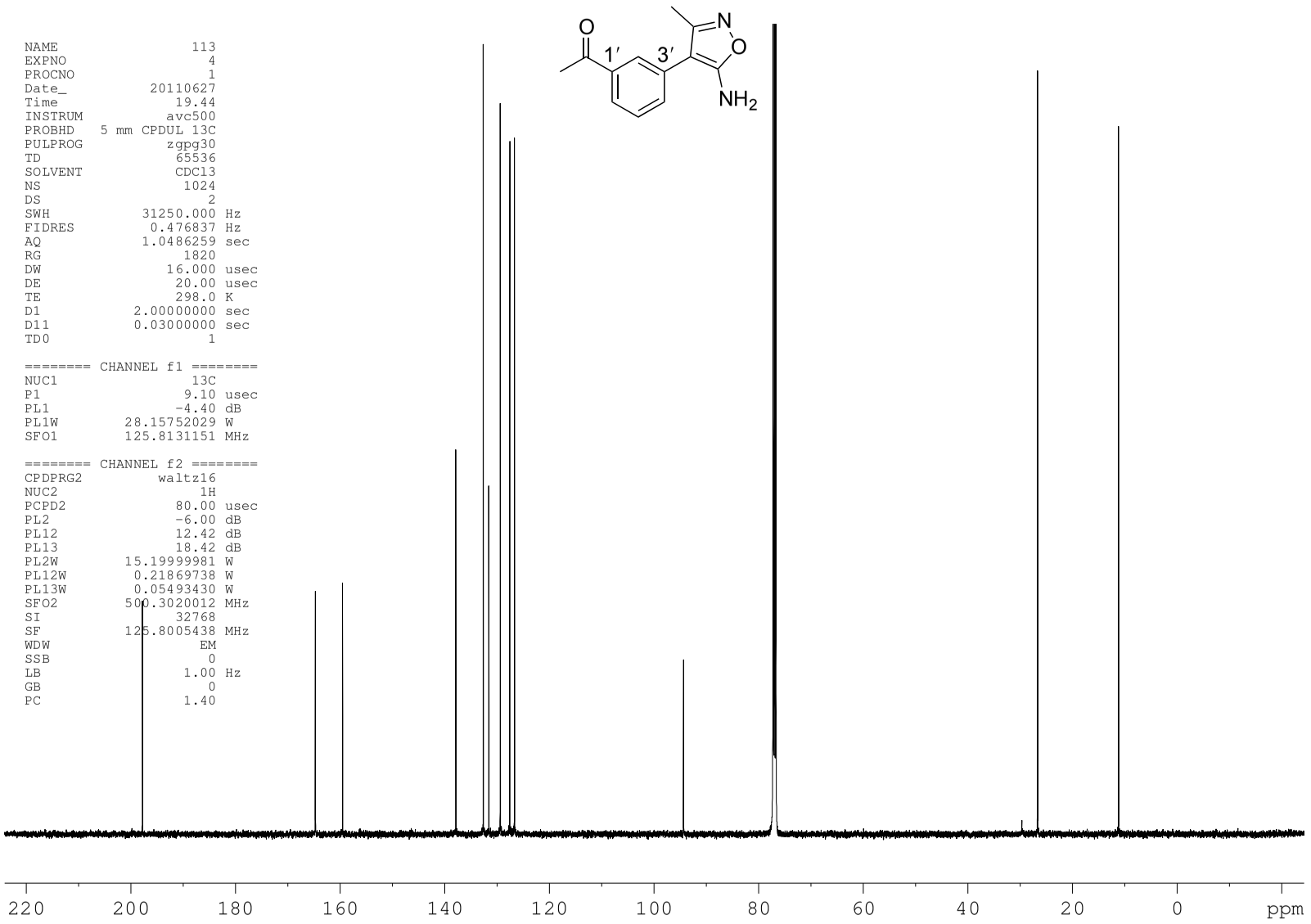
1-(3-(5-amino-3-methylisoxazol-4-yl)phenyl)ethanone 71b



NAME	113
EXPNO	1
PROCNO	1
Date_	20110627
Time	18.34
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

1-(3-(5-amino-3-methylisoxazol-4-yl)phenyl)ethanone 71b

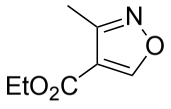



```
NAME                 113
EXPNO                  4
PROCNO                 1
Date_           20110627
Time               19.44
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

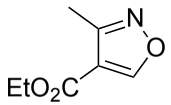
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2            80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz
SI               32768
SF         125.8005438 MHz
WDW                 EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
```

Ethyl 3-methylisoxazole-4-carboxylate 108



NAME	168-2
EXPNO	1
PROCNO	1
Date_	20111127
Time	21.16
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

Ethyl 3-methylisoxazole-4-carboxylate 108

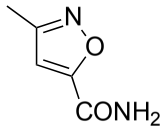



```
NAME                168-2
EXPNO                   4
PROCNO                  1
Date_            20111127
Time                22.04
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                 65536
SOLVENT             CDCl3
NS                    512
DS                      2
SWH             31250.000 Hz
FIDRES           0.476837 Hz
AQ              1.0486259 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

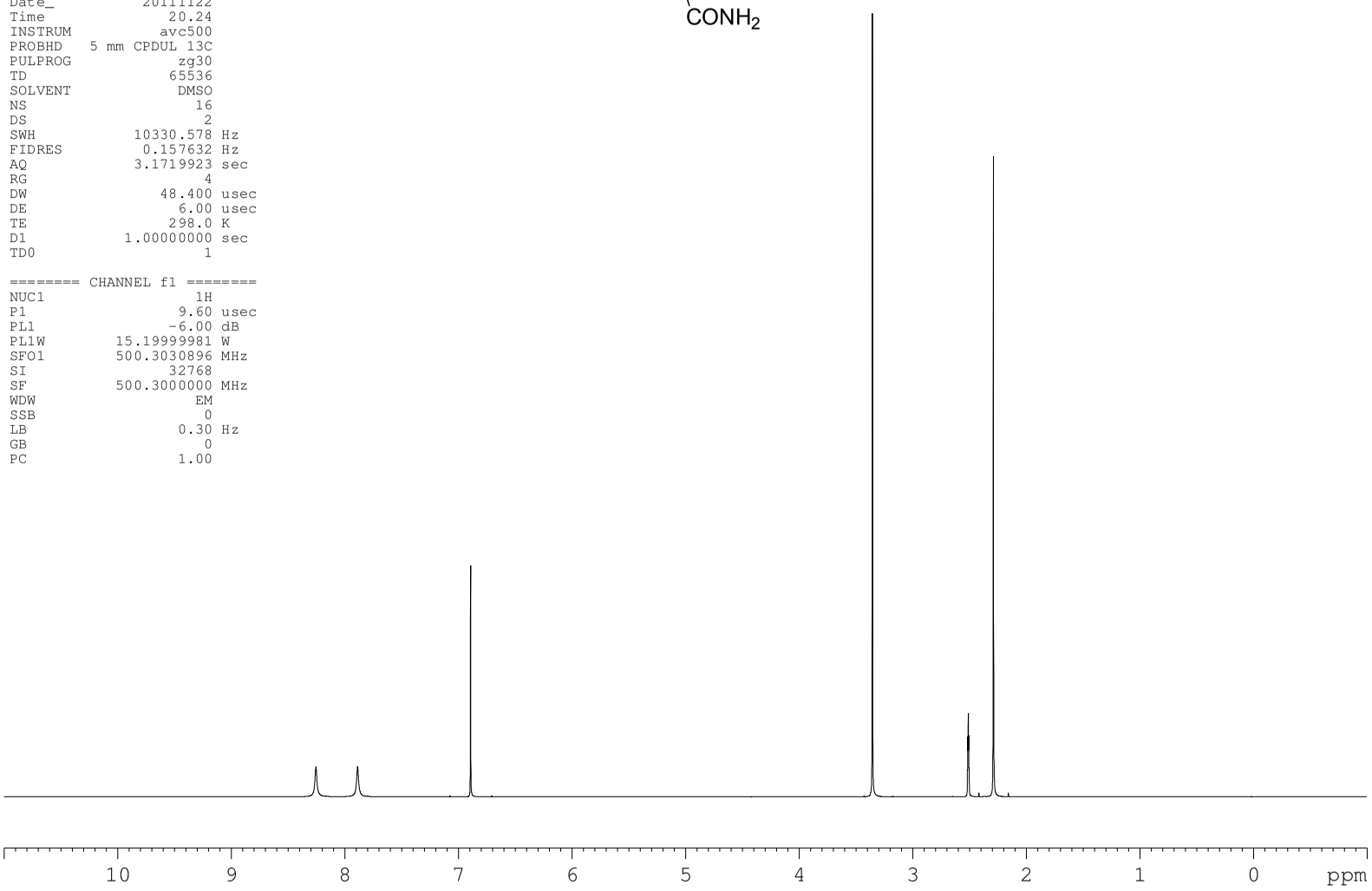
======== CHANNEL f1 ========
NUC1                  13C
P1                 10.00 usec
PL1                -4.40 dB
PL1W         28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2           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
SI                 32768
SF          125.8005438 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

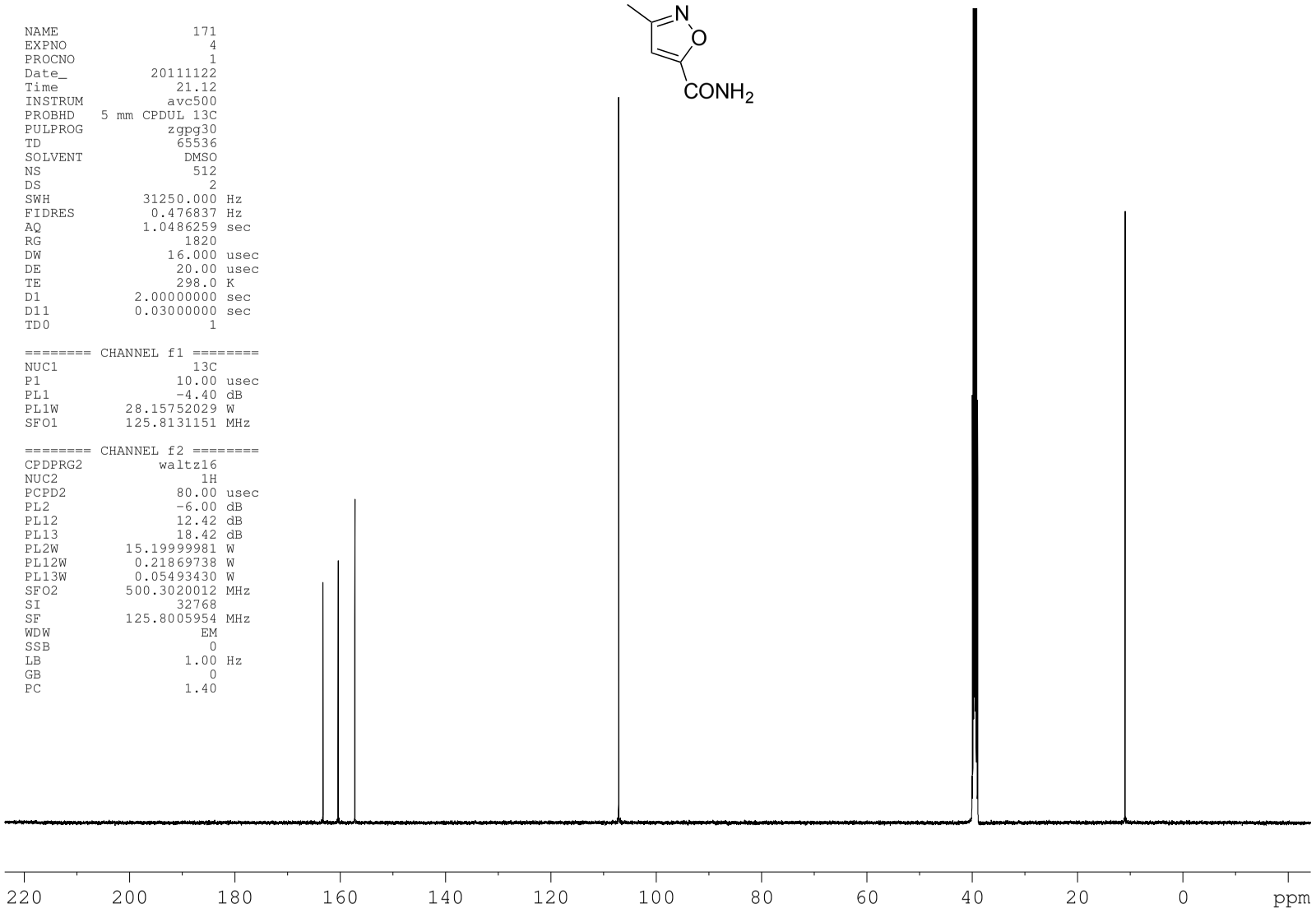
3-Methylisoxazole-5-carboxamide 106



NAME	171
EXPNO	1
PROCNO	1
Date_	20111122
Time	20.24
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	3.1719923 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
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



3-Methylisoxazole-5-carboxamide 106

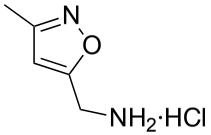



```
NAME                171
EXPNO                 4
PROCNO                1
Date_          20111122
Time              21.12
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

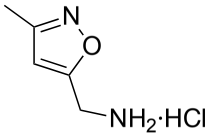
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz
SI                32768
SF         125.8005954 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

(3-Methylisoxazol-5-yl)methanamine hydrochloride 109



NAME	172
EXPNO	1
PROCNO	1
Date_	20111124
Time	14.00
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	3.1719923 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
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

(3-Methylisoxazol-5-yl)methanamine hydrochloride 109

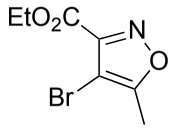



```
NAME                172
EXPNO                 4
PROCNO                1
Date_          20111124
Time              14.35
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                   256
DS                     2
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ             1.0486259 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

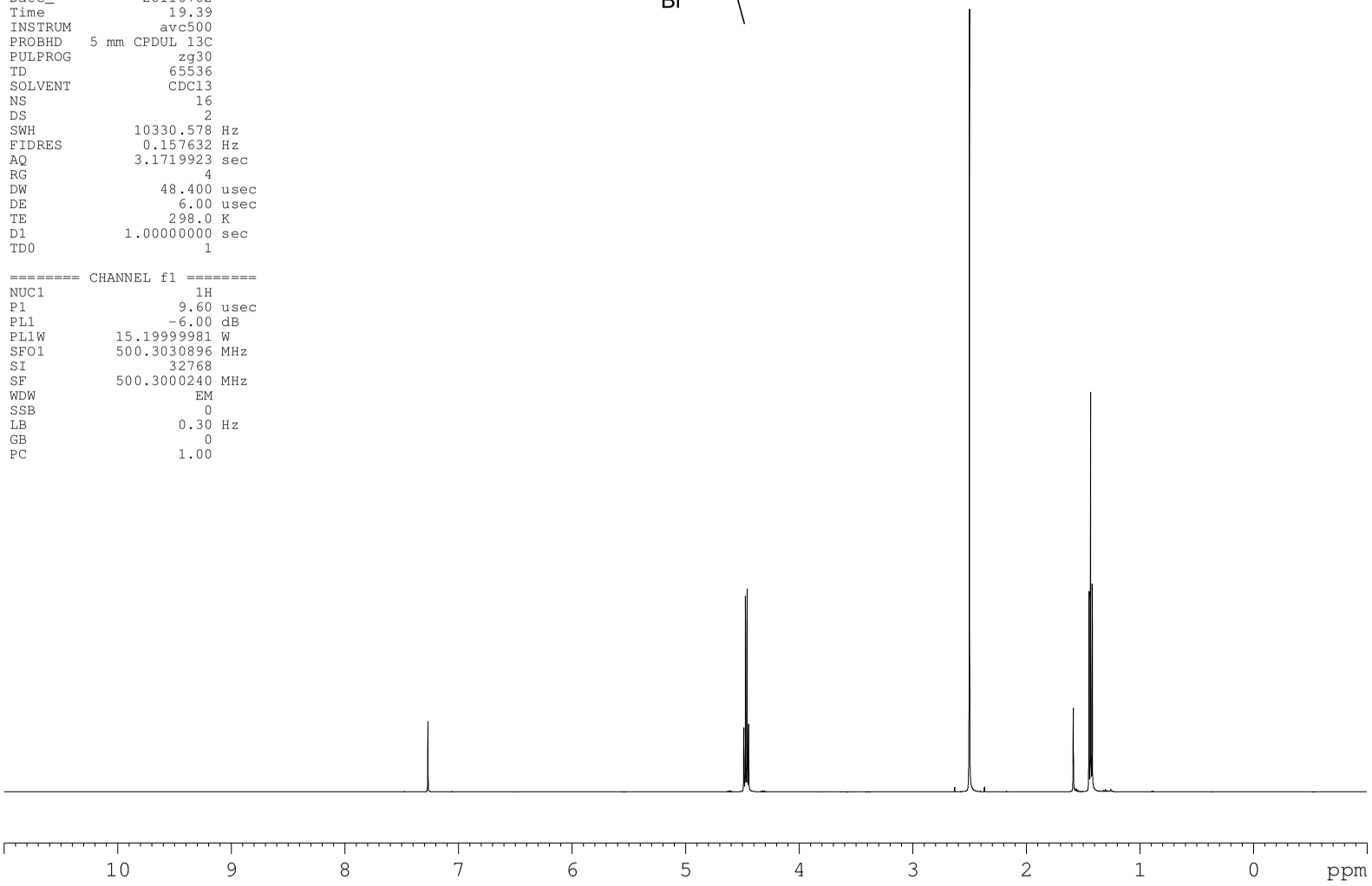
======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF         125.8005954 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

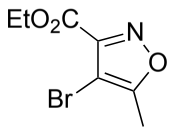
Ethyl 4-bromo-5-methylisoxazole-3-carboxylate 111



NAME	118
EXPNO	1
PROCNO	1
Date_	20110702
Time	19.39
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



Ethyl 4-bromo-5-methylisoxazole-3-carboxylate 111

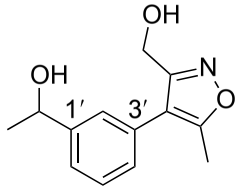



```
NAME                118
EXPNO                 4
PROCNO                1
Date_          20110702
Time              20.49
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

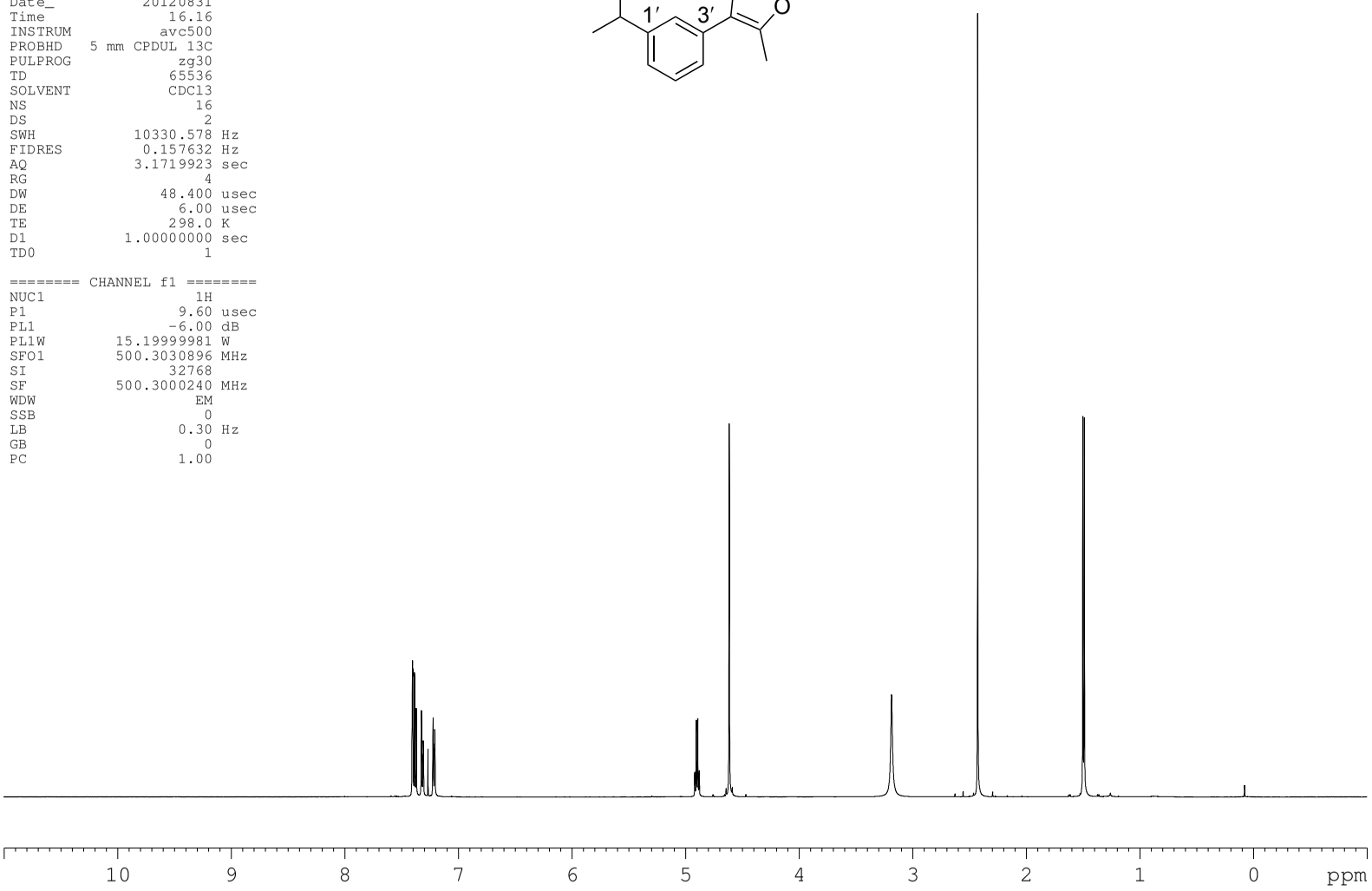
======== CHANNEL f1 ========
NUC1                13C
P1                 9.10 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

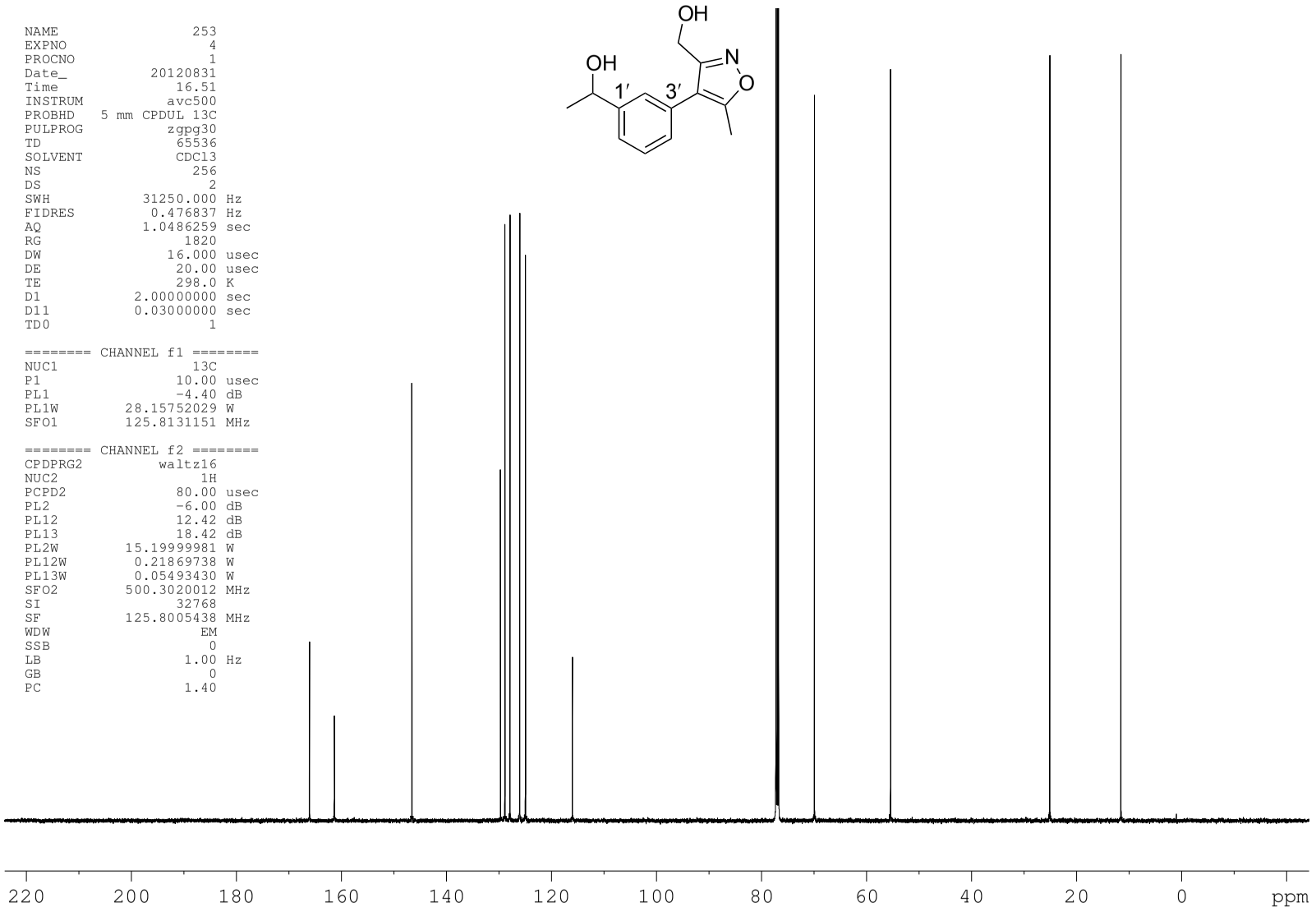
(*RS*)-1-(3-(3-(Hydroxymethyl)-5-methylisoxazol-4-yl)phenyl)ethanol 72c



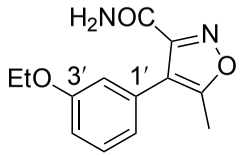
NAME	253
EXPNO	1
PROCNO	1
Date_	20120831
Time	16.16
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



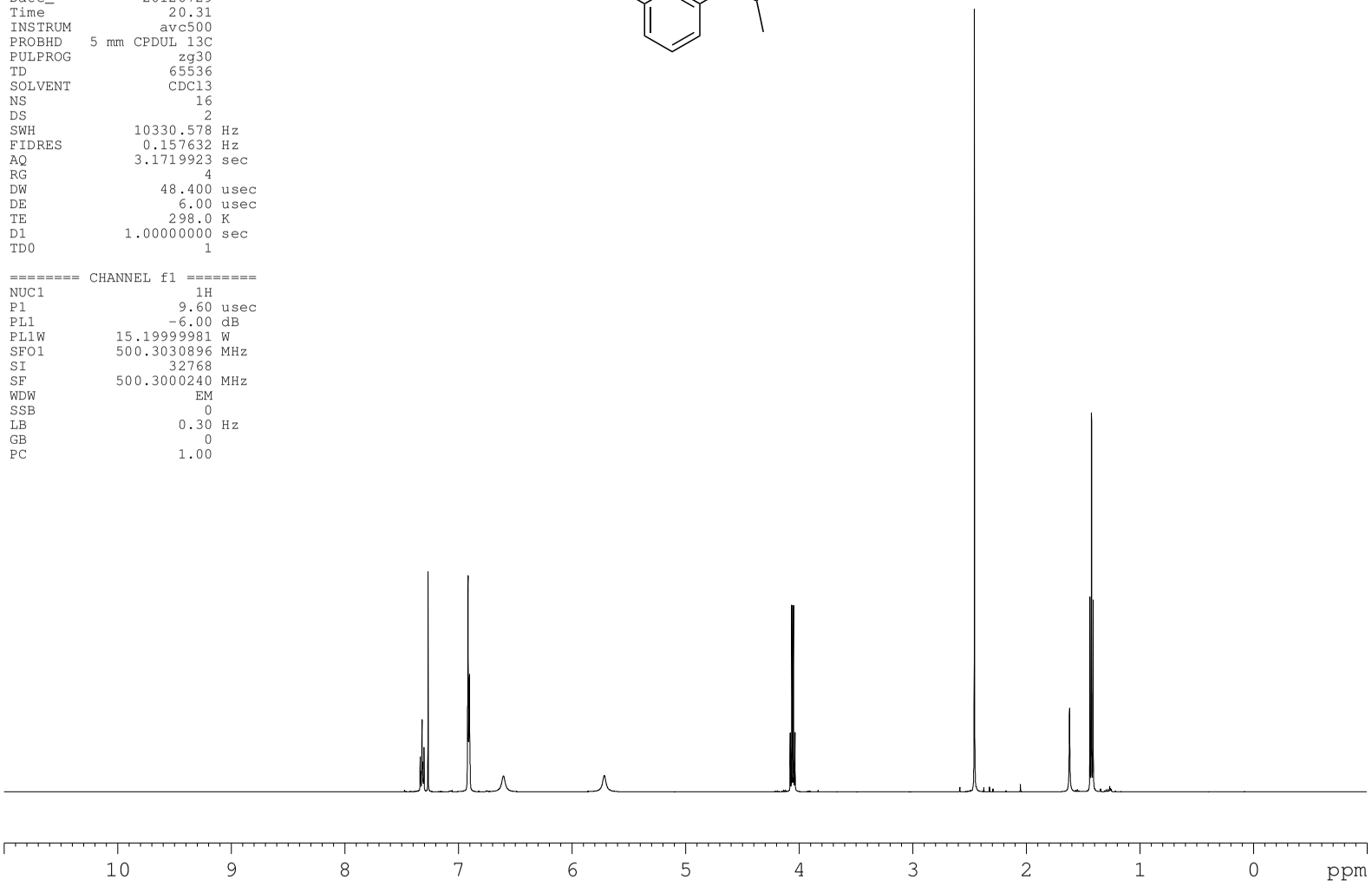
(*RS*)-1-(3-(3-(Hydroxymethyl)-5-methylisoxazol-4-yl)phenyl)ethanol 72c



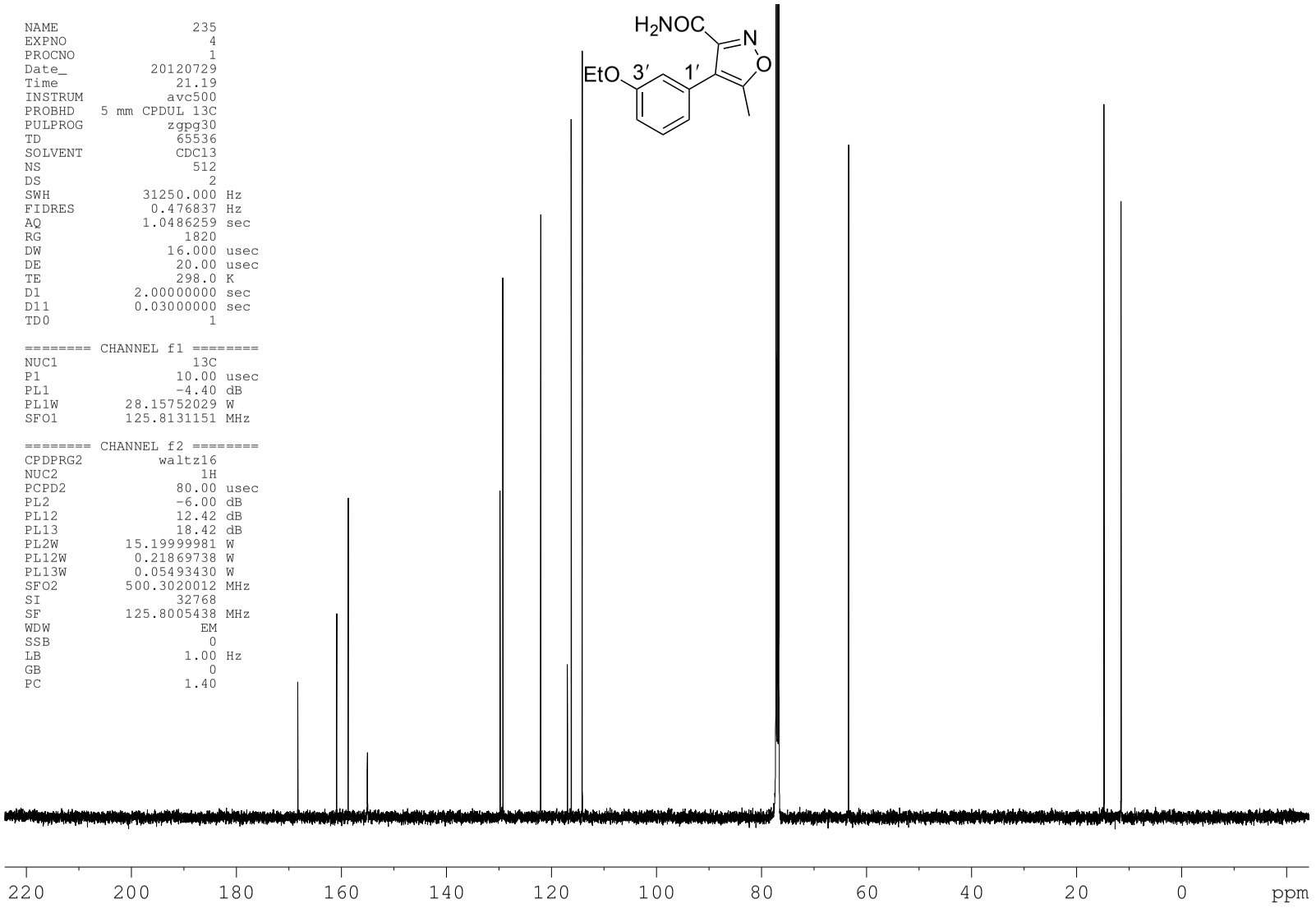
4-(3-Ethoxyphenyl)-5-methylisoxazole-3-carboxamide 73



NAME	235
EXPNO	1
PROCNO	1
Date_	20120729
Time	20.31
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



4-(3-Ethoxyphenyl)-5-methylisoxazole-3-carboxamide 73

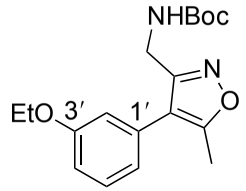



```
NAME                235
EXPNO                 4
PROCNO                1
Date_          20120729
Time              21.19
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

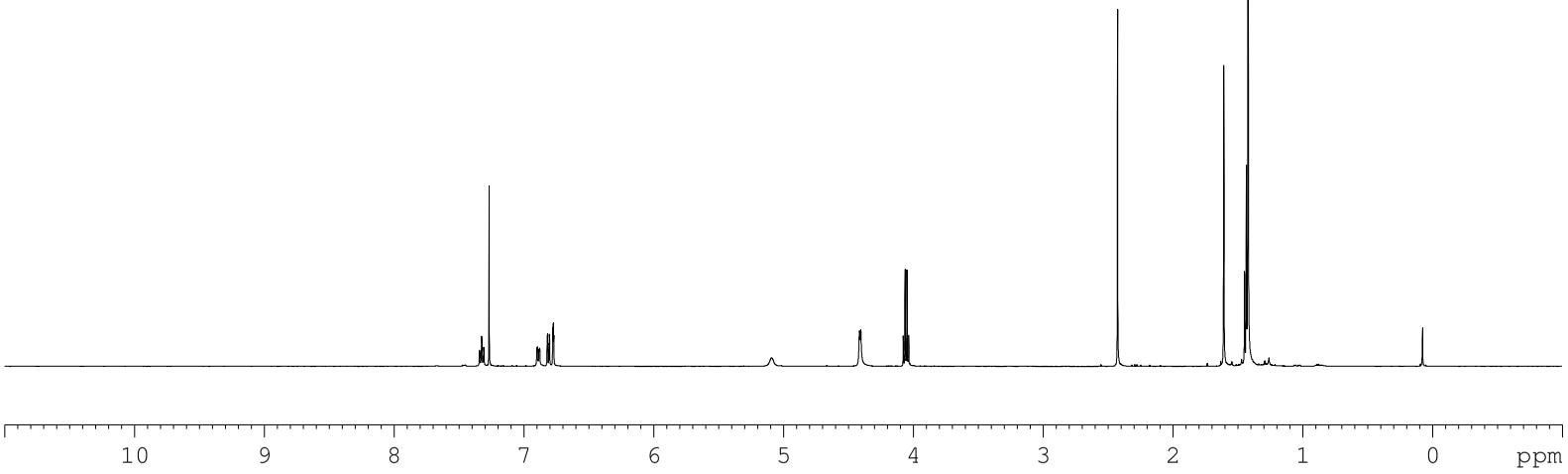
======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

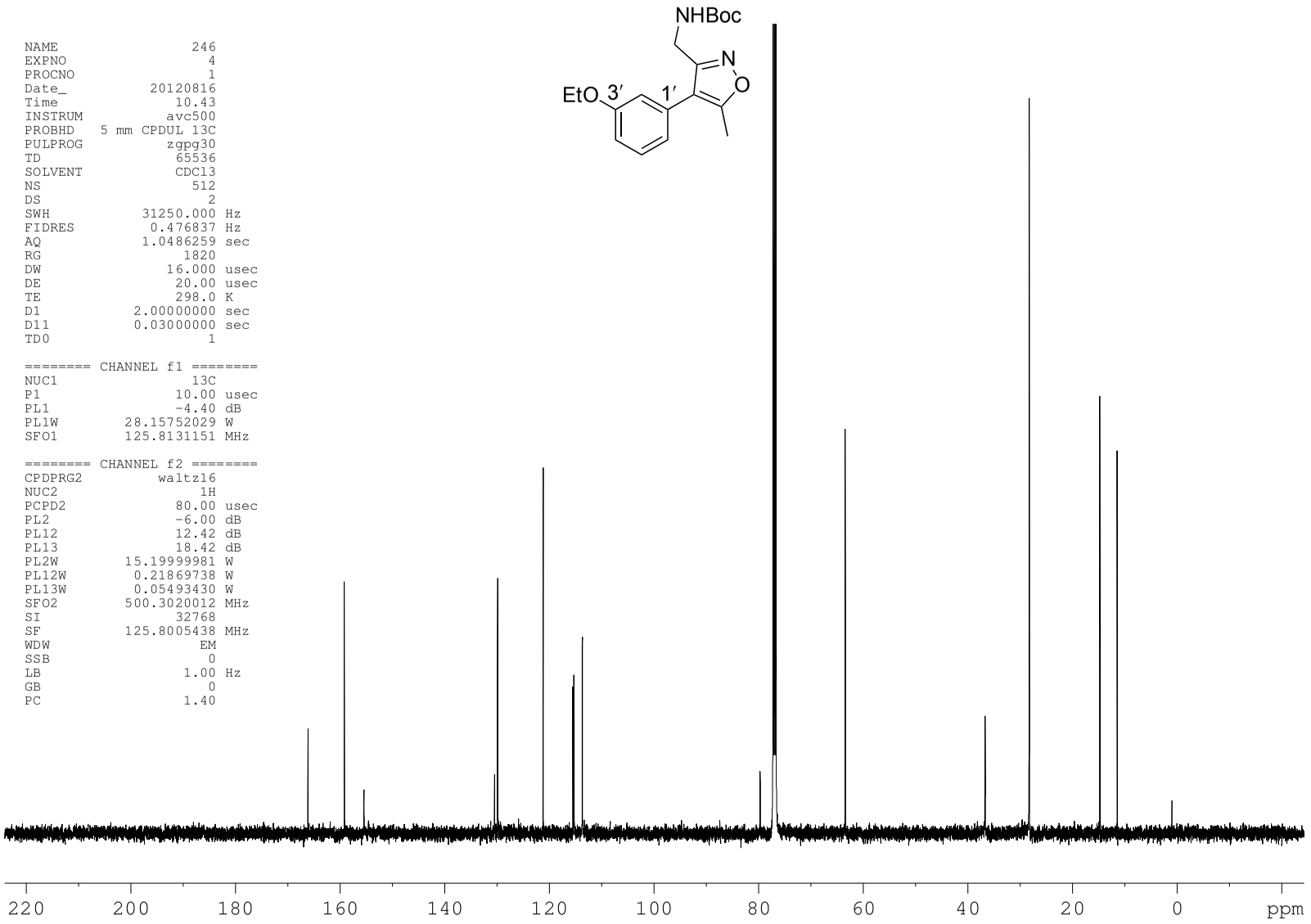
tert-Butyl ((4-(3-ethoxyphenyl)-5-methylisoxazol-3-yl)methyl)carbamate 114



NAME	246
EXPNO	1
PROCNO	1
Date_	20120816
Time	7.59
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



tert-Butyl ((4-(3-ethoxyphenyl)-5-methylisoxazol-3-yl)methyl)carbamate 114

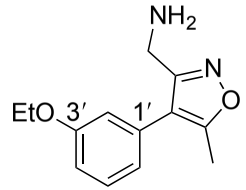



```
NAME                 246
EXPNO                  4
PROCNO                 1
Date_           20120816
Time               10.43
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   512
DS                     2
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ             1.0486259 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

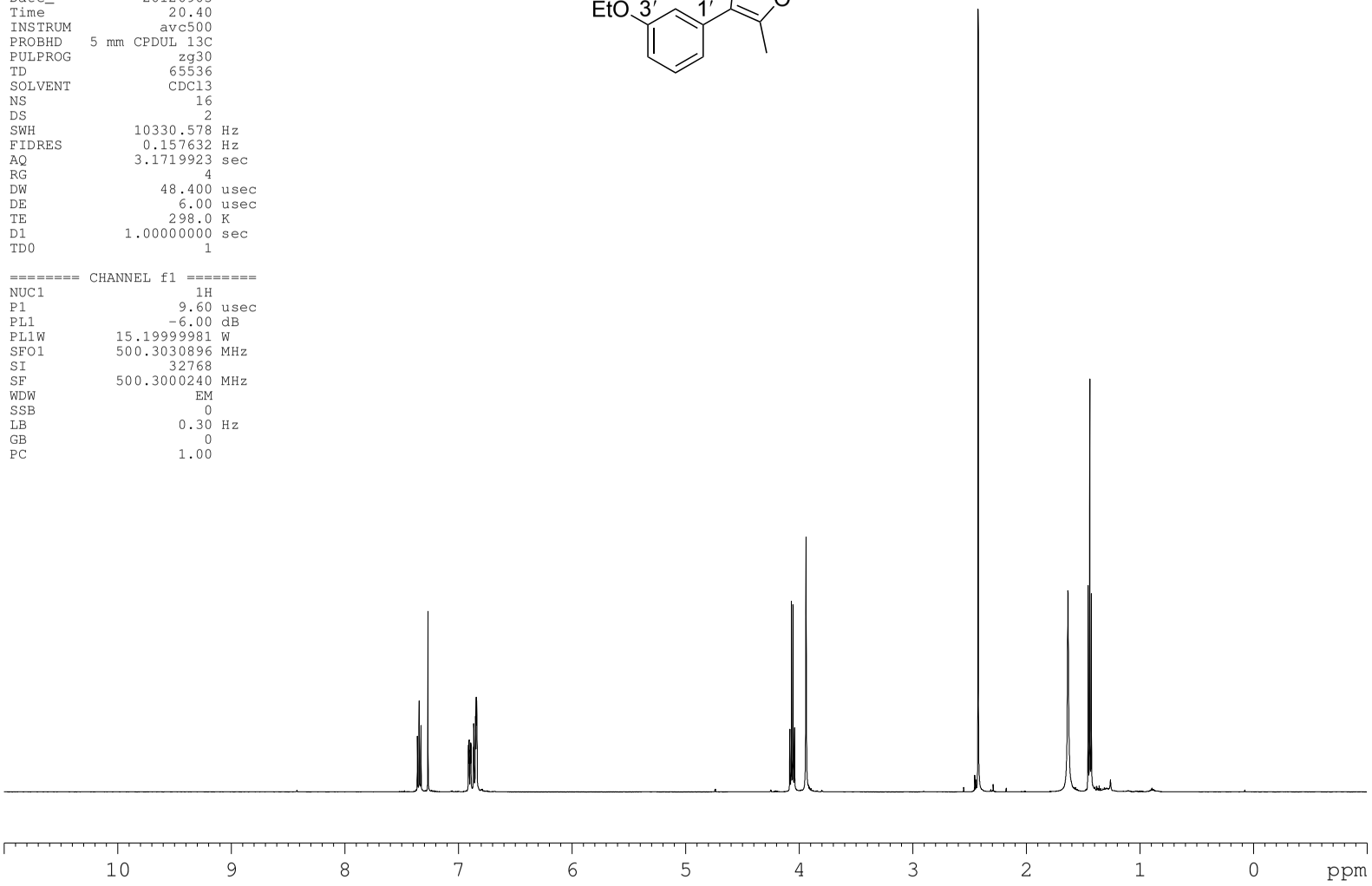
======== CHANNEL f1 ========
NUC1                 13C
P1                 10.00 usec
PL1                -4.40 dB
PL1W         28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                 32768
SF          125.8005438 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

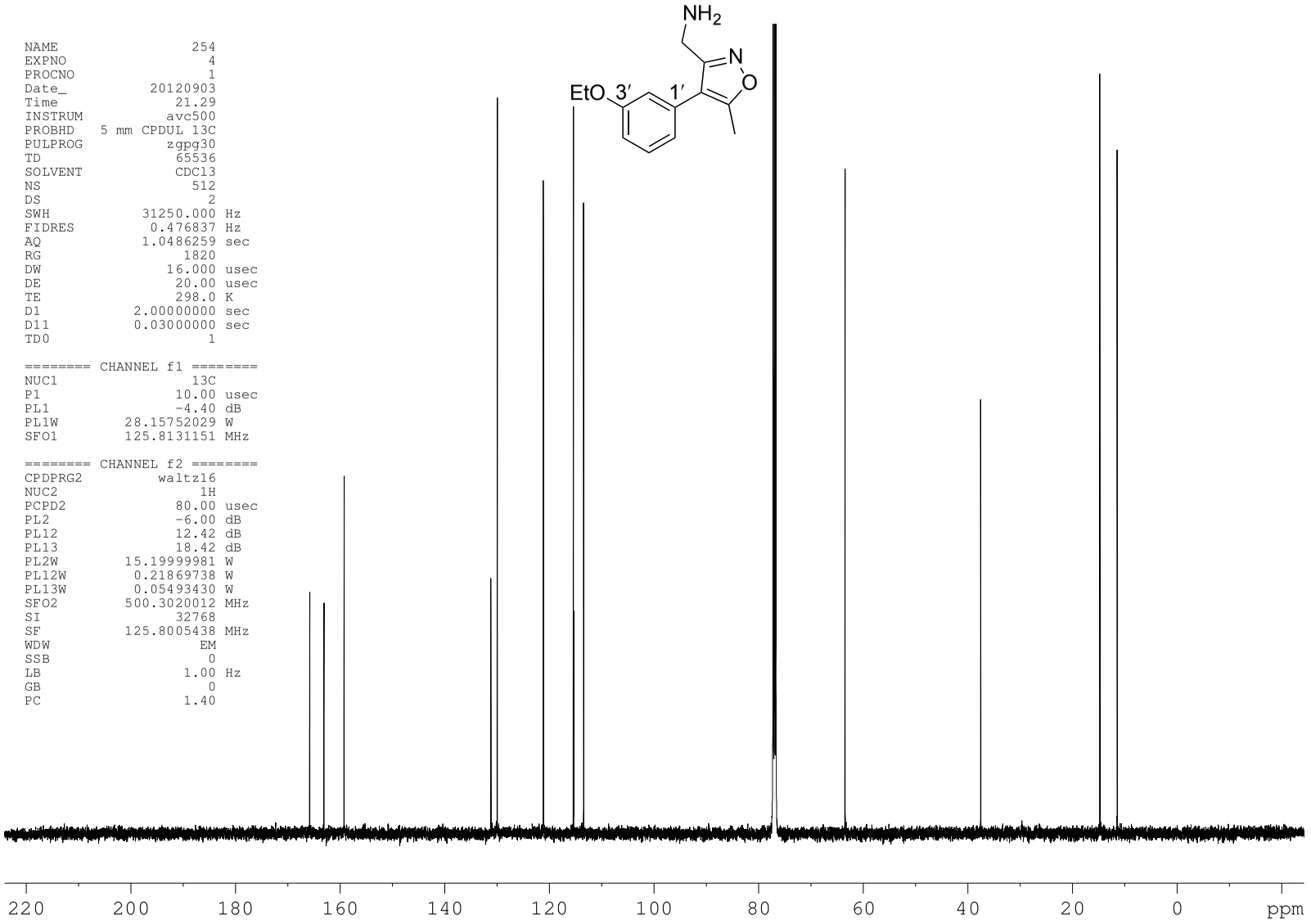
(4-(3-Ethoxyphenyl)-5-methylisoxazol-3-yl)methanamine 74a



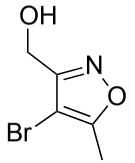
NAME	254
EXPNO	1
PROCNO	1
Date_	20120903
Time	20.40
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



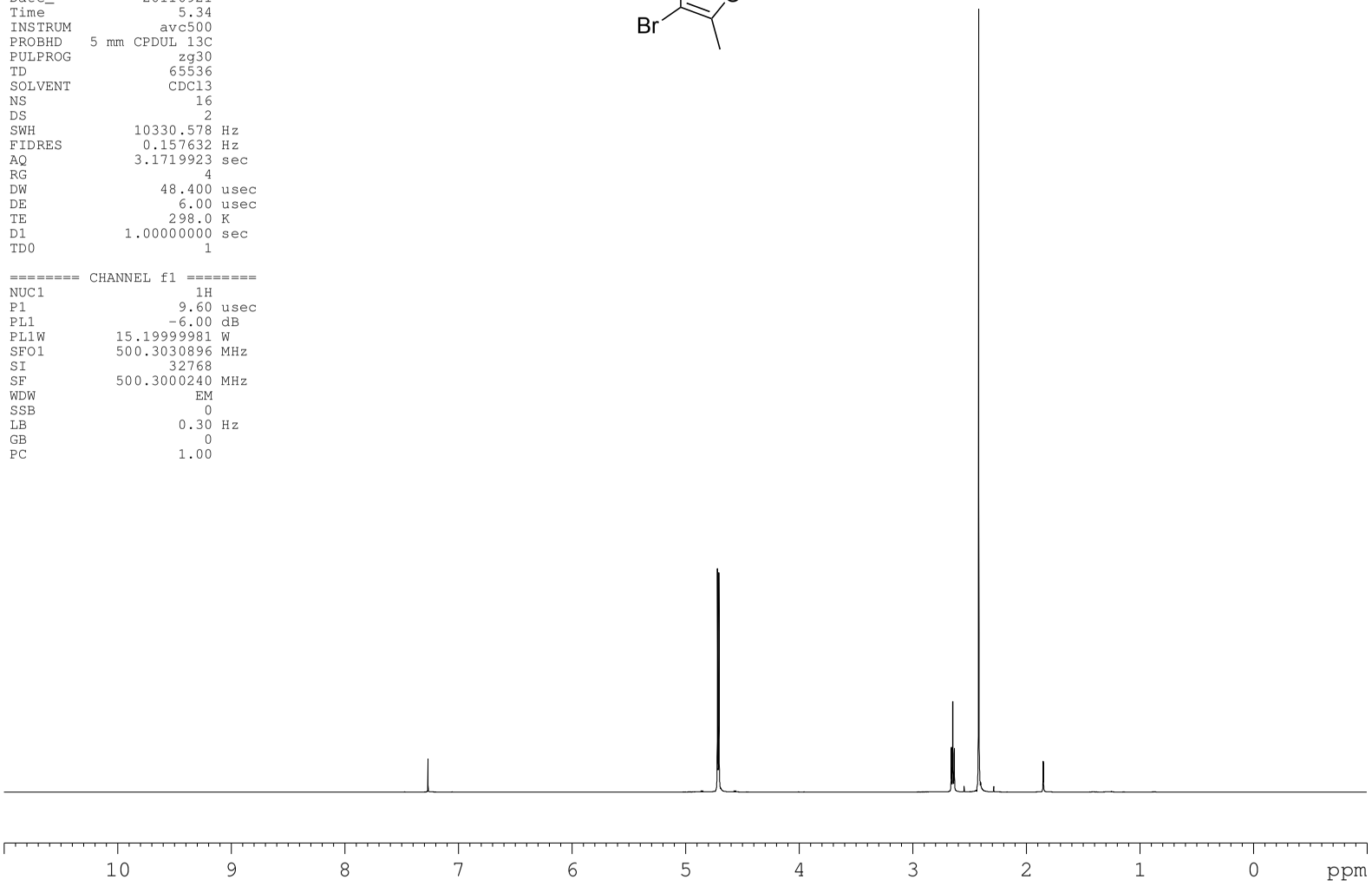
(4-(3-Ethoxyphenyl)-5-methylisoxazol-3-yl)methanamine 74a



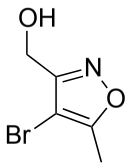
(4-Bromo-5-methylisoxazol-3-yl)methanol 115



NAME	138
EXPNO	1
PROCNO	1
Date_	20110921
Time	5.34
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(4-Bromo-5-methylisoxazol-3-yl)methanol 115

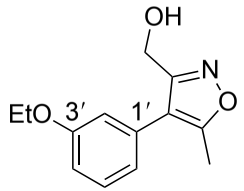



```
NAME                138
EXPNO                 4
PROCNO                1
Date_          20110921
Time               6.44
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

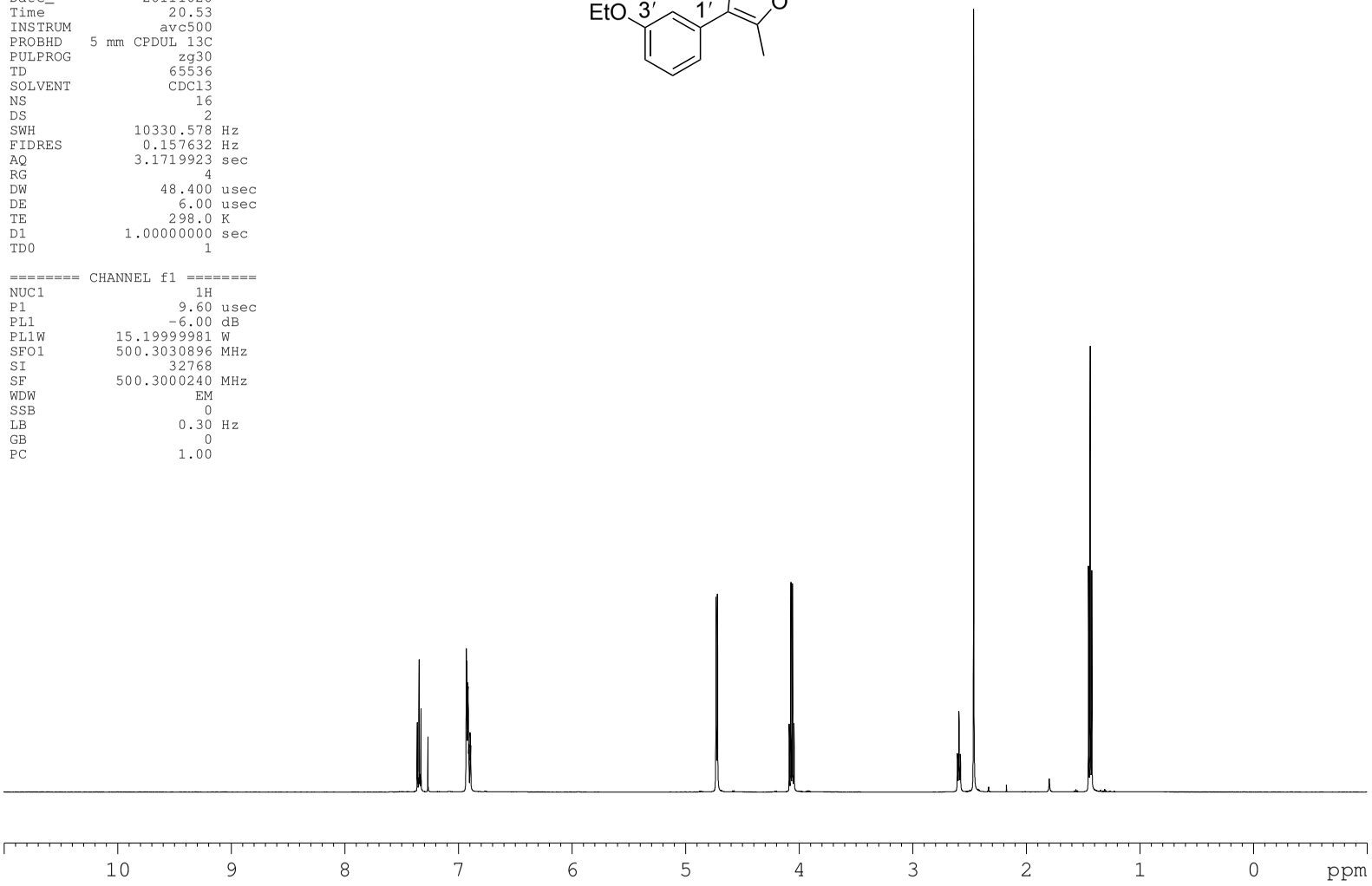
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

(4-(3-Ethoxyphenyl)-5-methylisoxazol-3-yl)methanol 72a

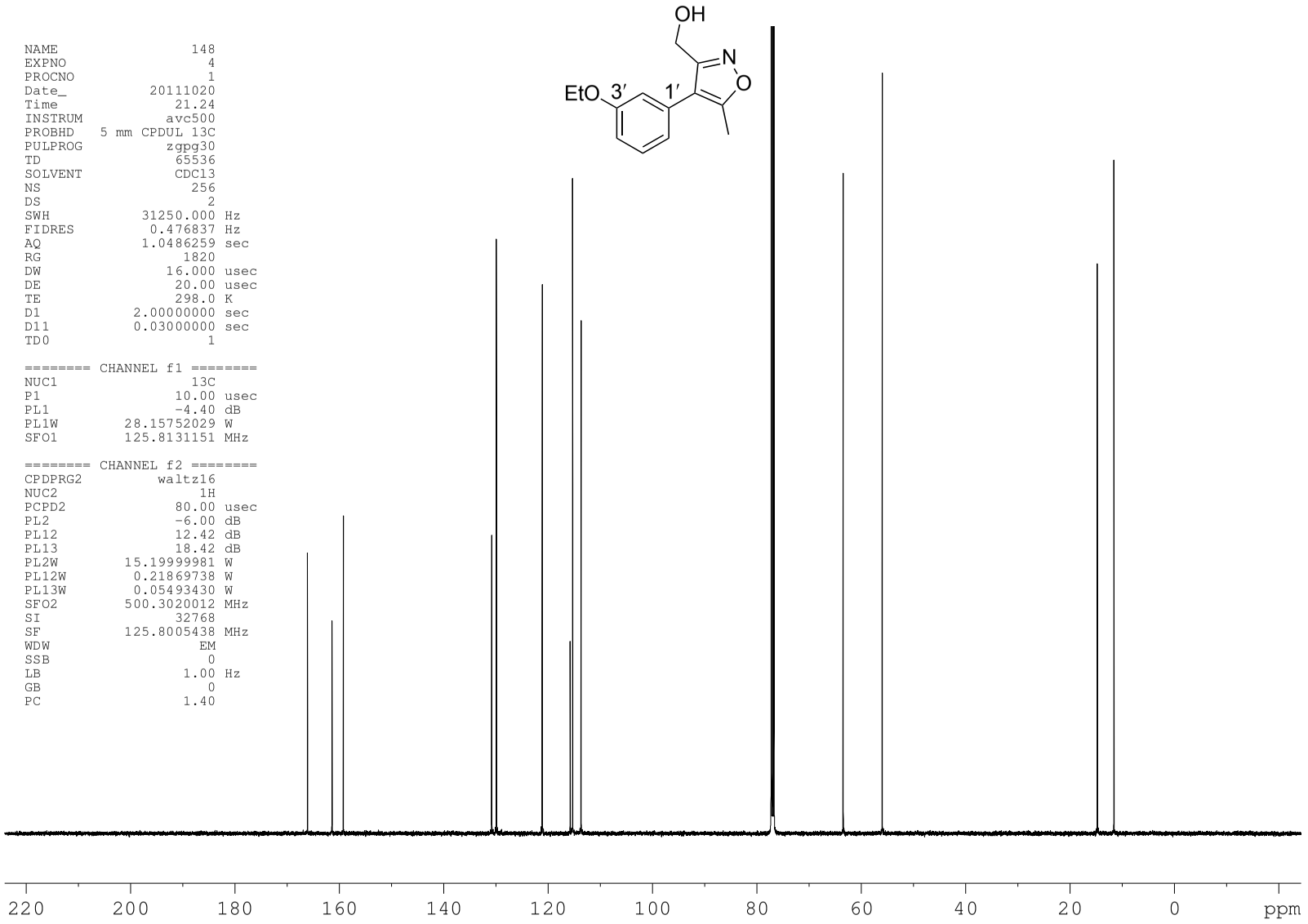


NAME	148
EXPNO	1
PROCNO	1
Date_	20111020
Time	20.53
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(4-(3-Ethoxyphenyl)-5-methylisoxazol-3-yl)methanol 72a

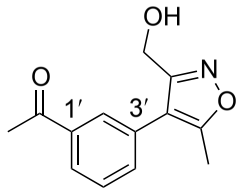



```
NAME                148
EXPNO                 4
PROCNO                1
Date_          20111020
Time              21.24
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

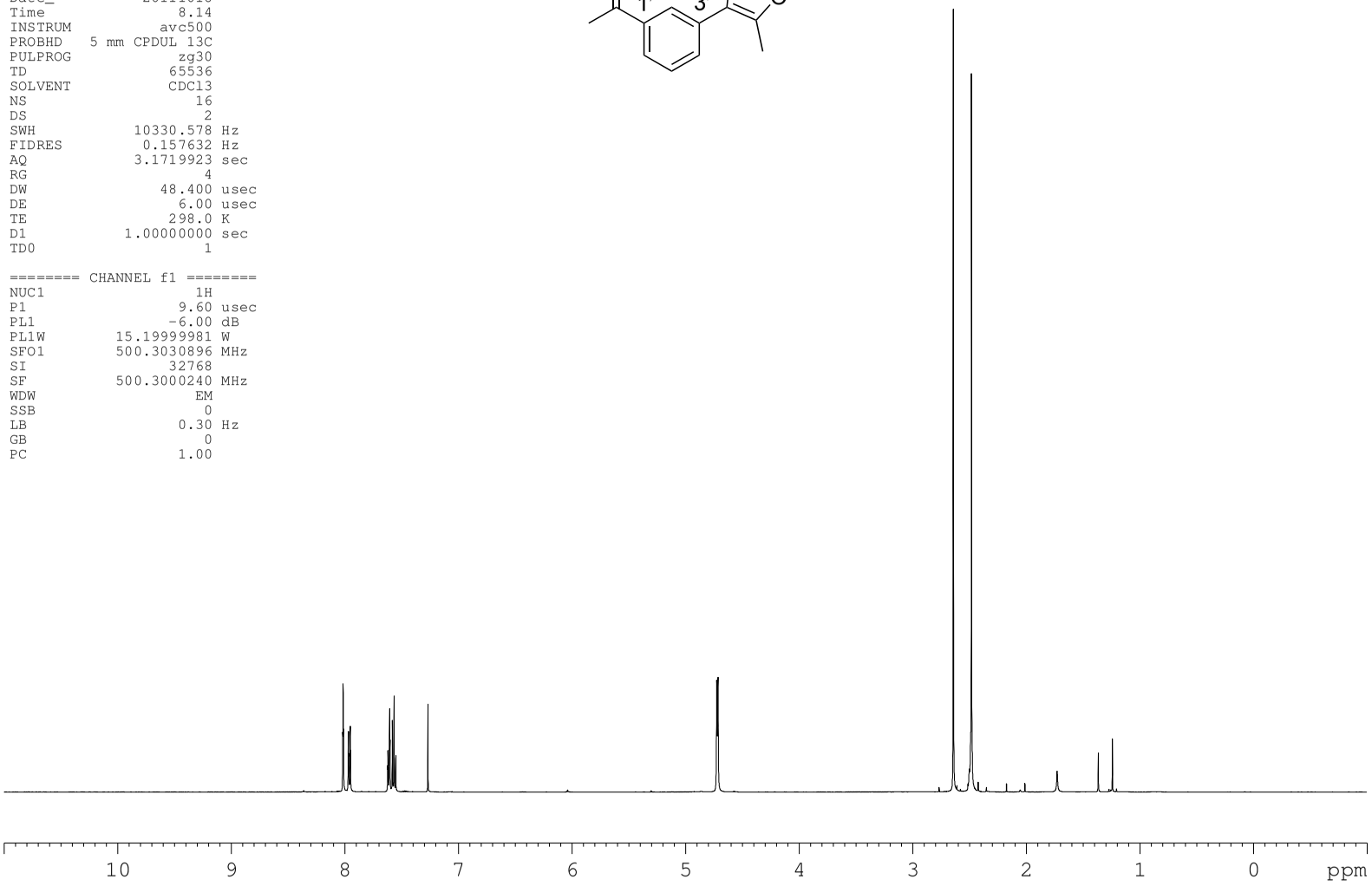
======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

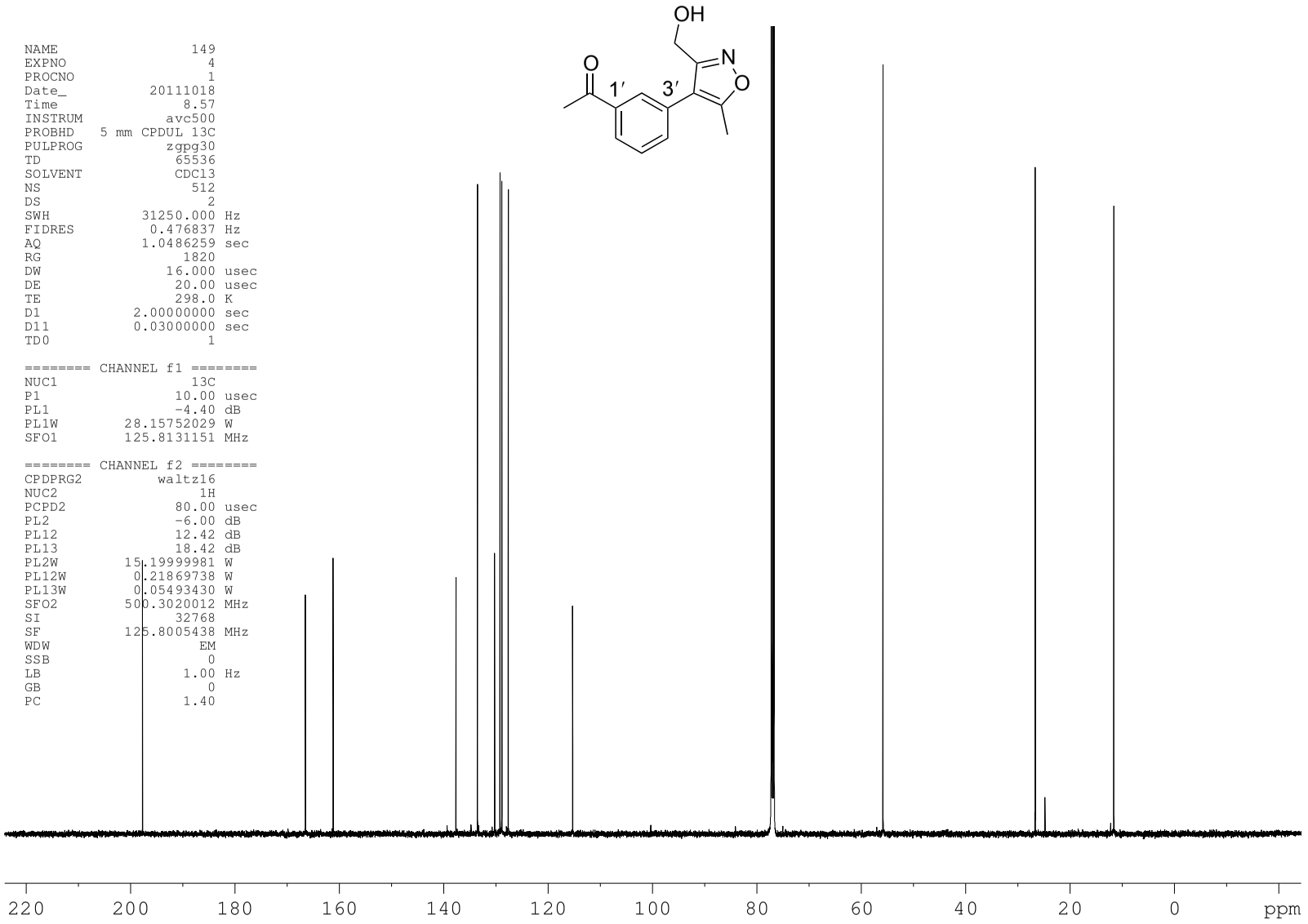
1-(3-(3-(Hydroxymethyl)-5-methylisoxazol-4-yl)phenyl)ethanone 72b



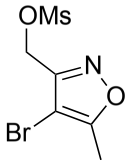
NAME	149
EXPNO	1
PROCNO	1
Date_	20111018
Time	8.14
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



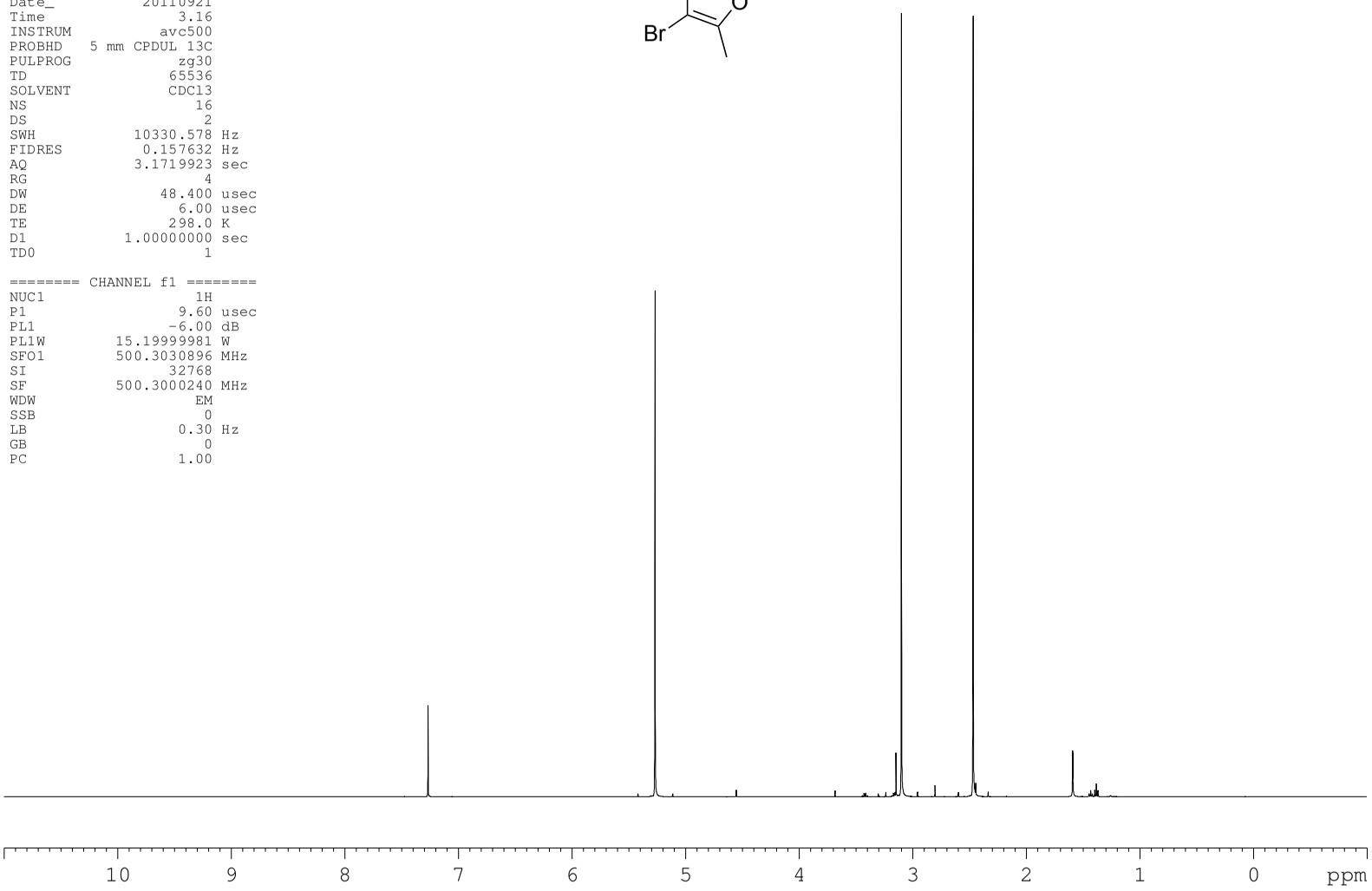
1-(3-(3-(Hydroxymethyl)-5-methylisoxazol-4-yl)phenyl)ethanone 72b



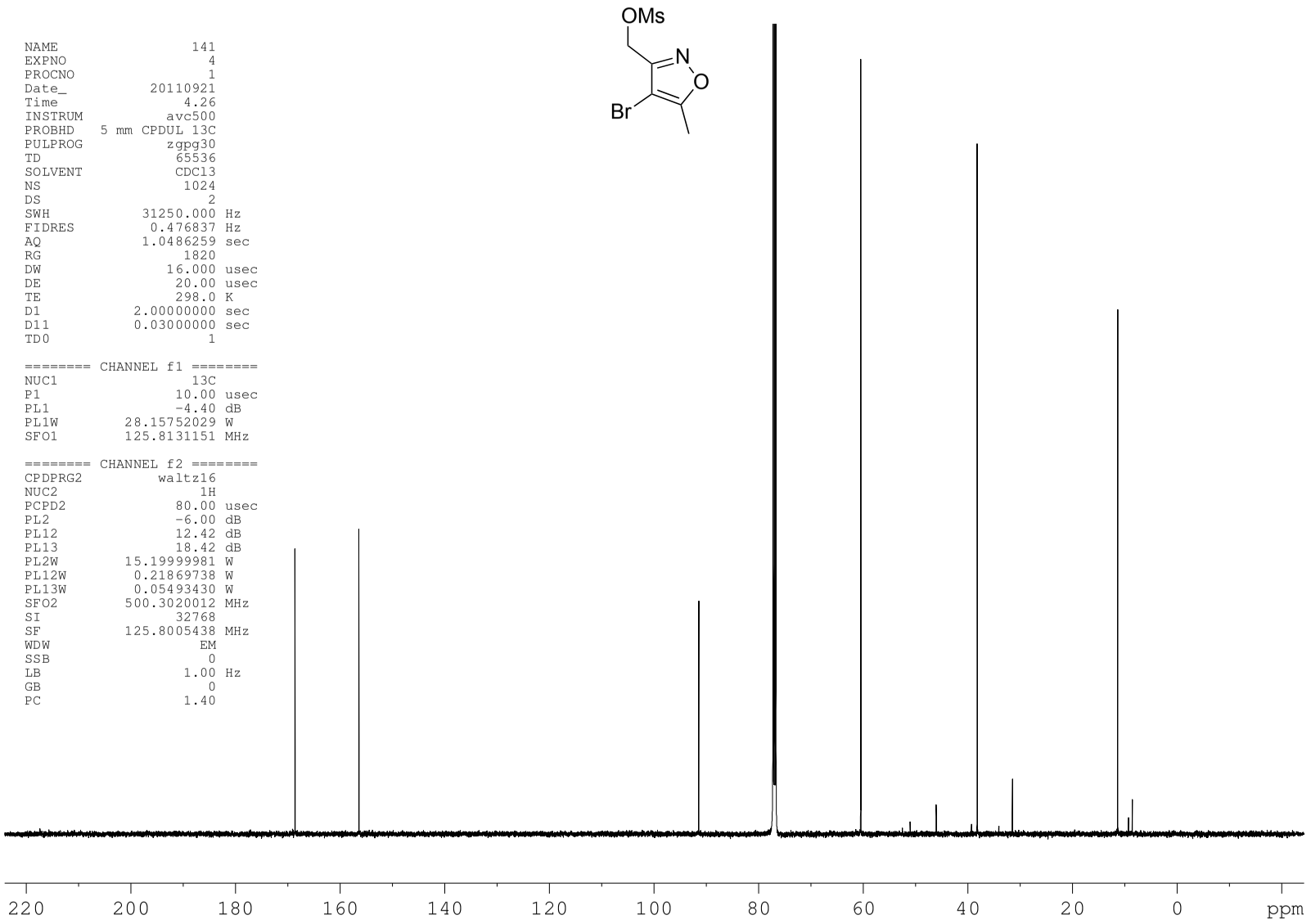
(4-Bromo-5-methylisoxazol-3-yl)methyl methanesulfonate 116



NAME	141
EXPNO	1
PROCNO	1
Date_	20110921
Time	3.16
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(4-Bromo-5-methylisoxazol-3-yl)methyl methanesulfonate 116

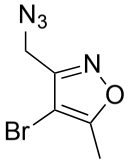



```
NAME                141
EXPNO                 4
PROCNO                1
Date_          20110921
Time               4.26
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

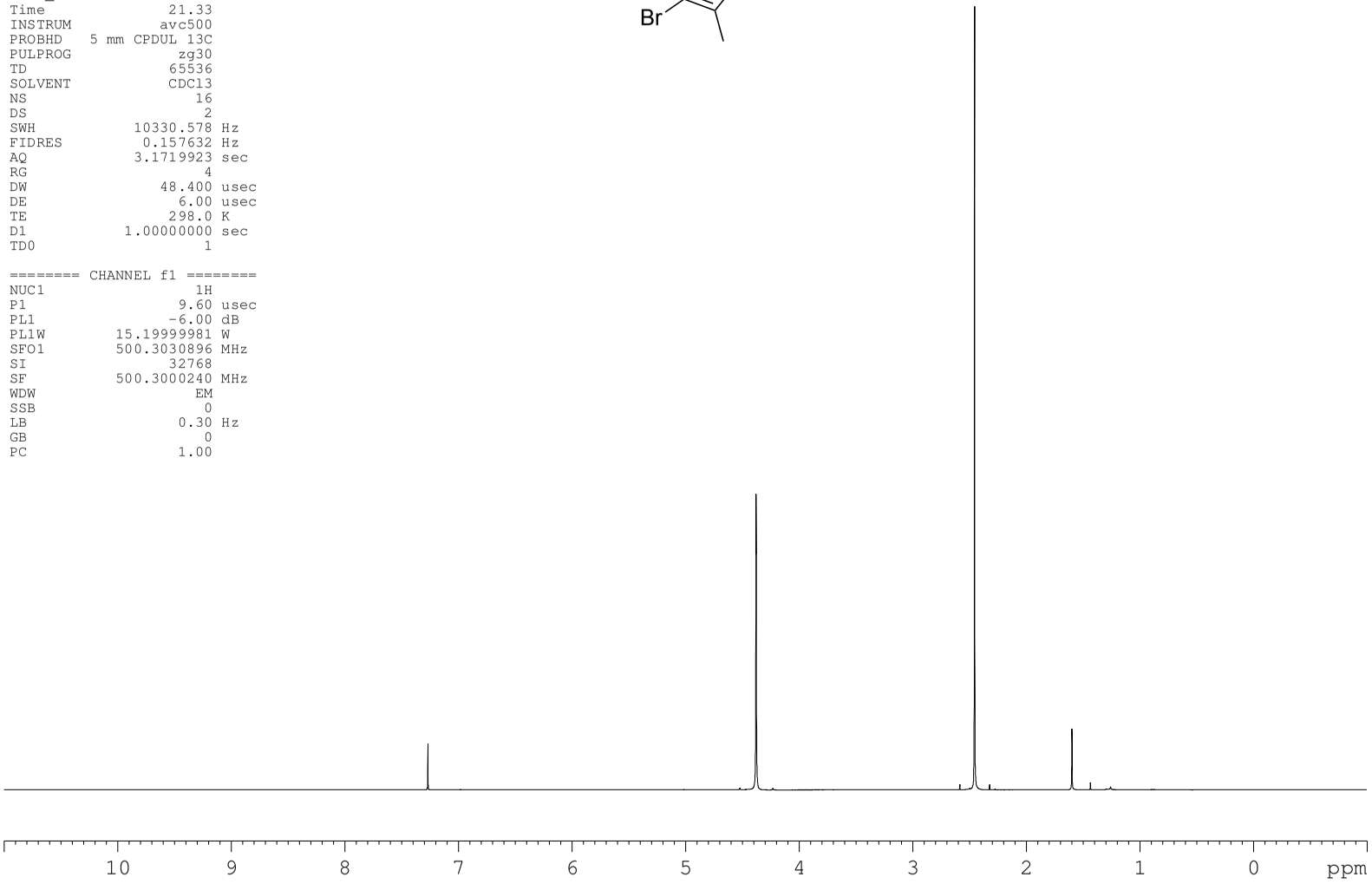
======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

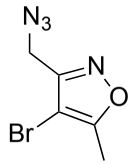
3-(Azidomethyl)-4-bromo-5-methylisoxazole 117



NAME	142
EXPNO	1
PROCNO	1
Date_	20110922
Time	21.33
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



3-(Azidomethyl)-4-bromo-5-methylisoxazole 117

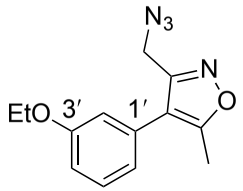



```
NAME                142
EXPNO                 4
PROCNO                1
Date_          20110922
Time              22.17
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

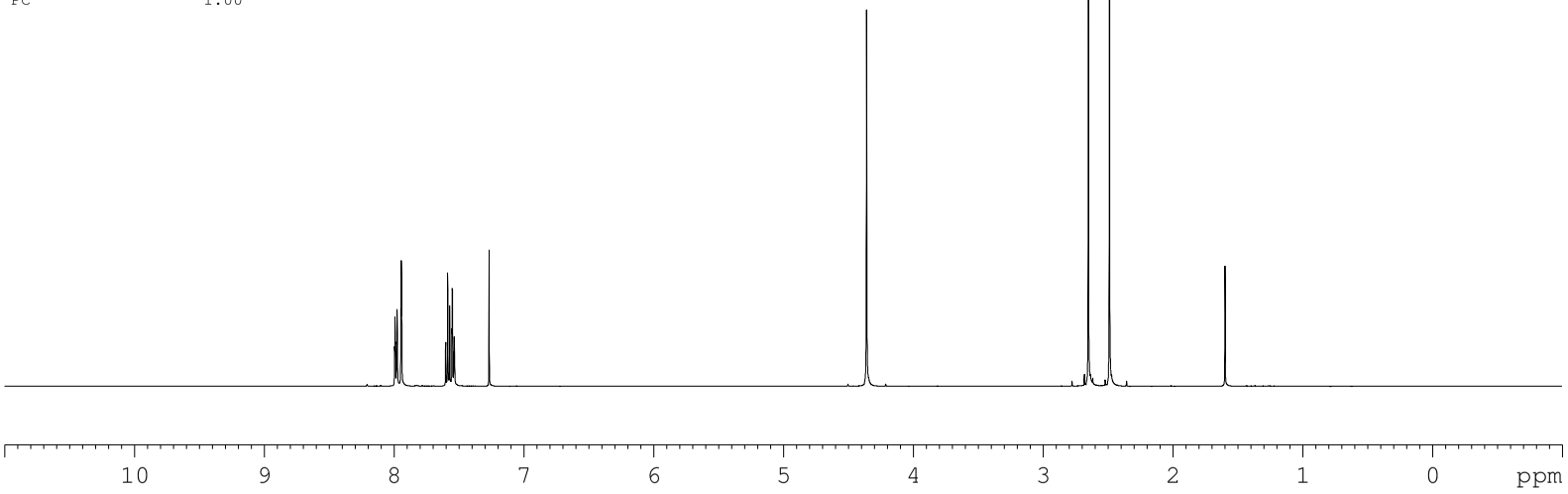
======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

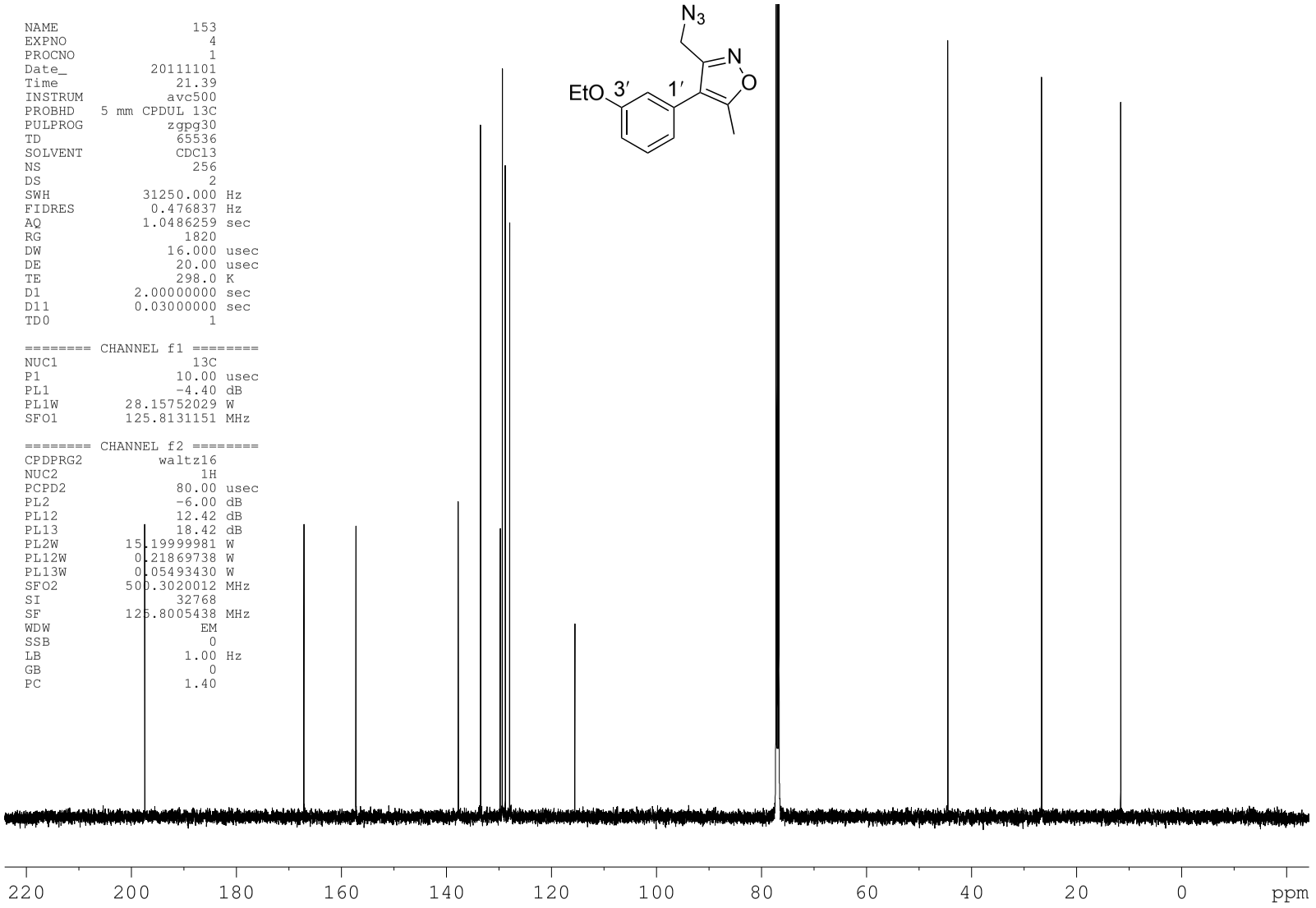
3-(Azidomethyl)-4-(3-ethoxyphenyl)-5-methylisoxazole 118



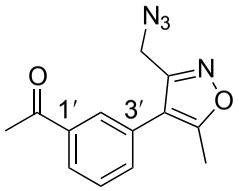
NAME	153
EXPNO	1
PROCNO	1
Date_	20111101
Time	21.04
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



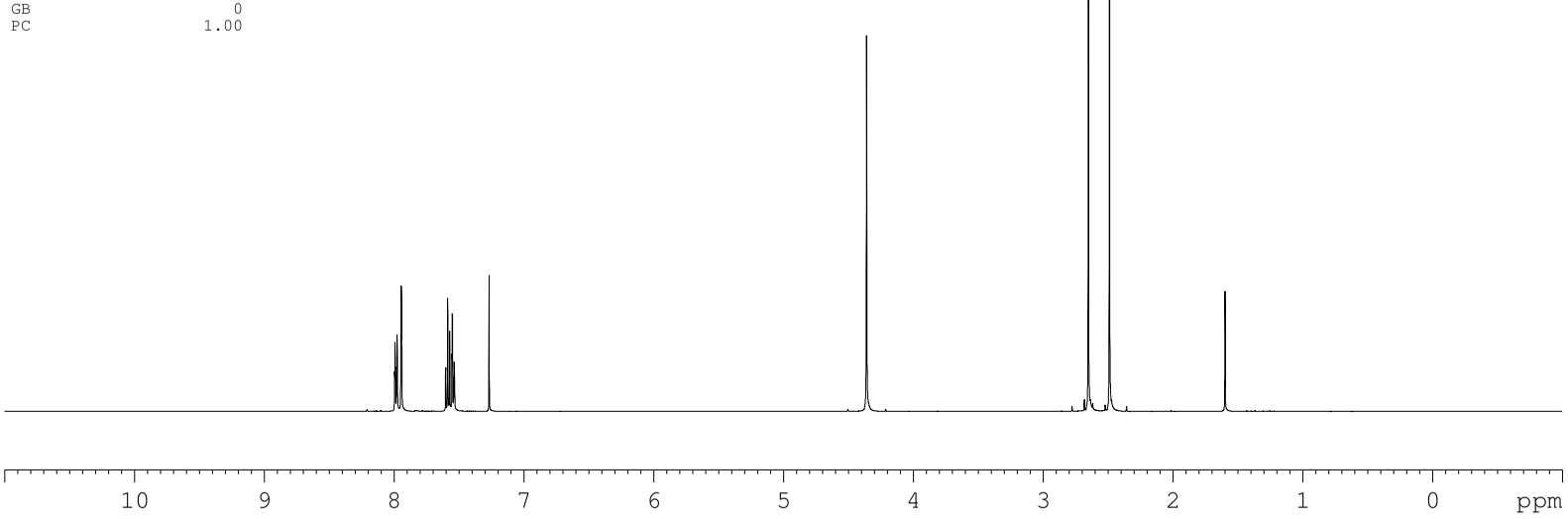
3-(Azidomethyl)-4-(3-ethoxyphenyl)-5-methylisoxazole 118



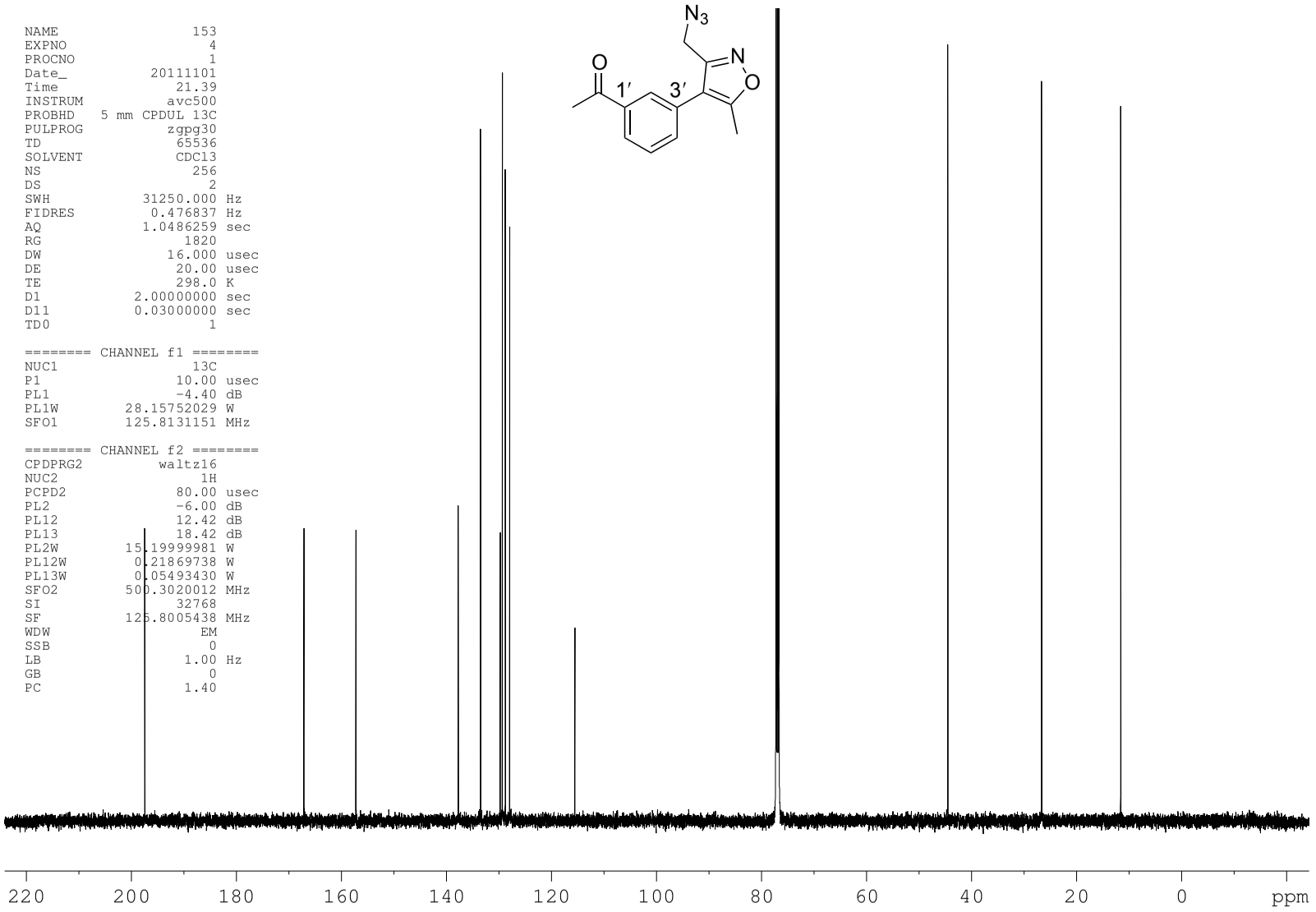
1-(3-(3-(Azidomethyl)-5-methylisoxazol-4-yl)phenyl)ethanone 119



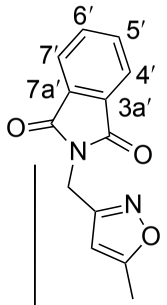
NAME	153
EXPNO	1
PROCNO	1
Date_	20111101
Time	21.04
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



1-(3-(3-(Azidomethyl)-5-methylisoxazol-4-yl)phenyl)ethanone 119

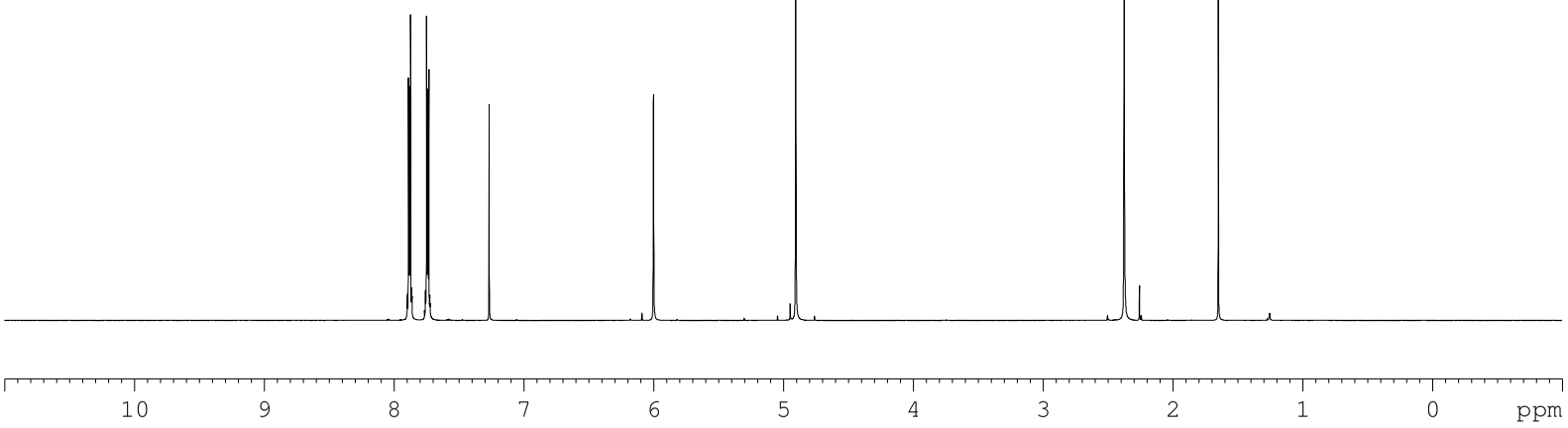


1-(3-(3-(Azidomethyl)-5-methylisoxazol-4-yl)phenyl)ethanone 121

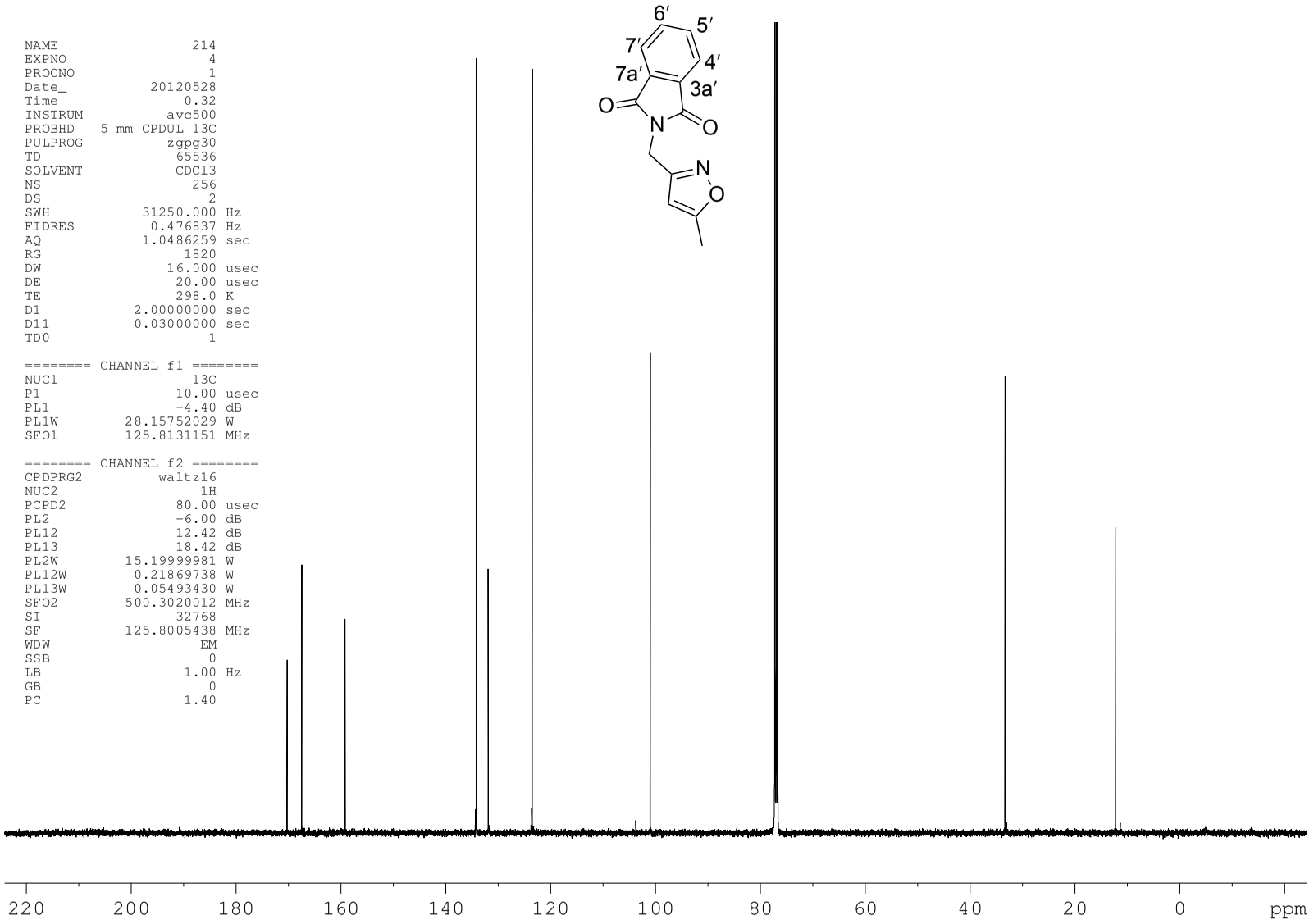


NAME	214
EXPNO	1
PROCNO	1
Date_	20120527
Time	23.57
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



1-(3-(3-(Azidomethyl)-5-methylisoxazol-4-yl)phenyl)ethanone 121

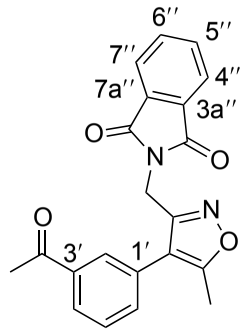



```
NAME                214
EXPNO                 4
PROCNO                1
Date_          20120528
Time               0.32
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

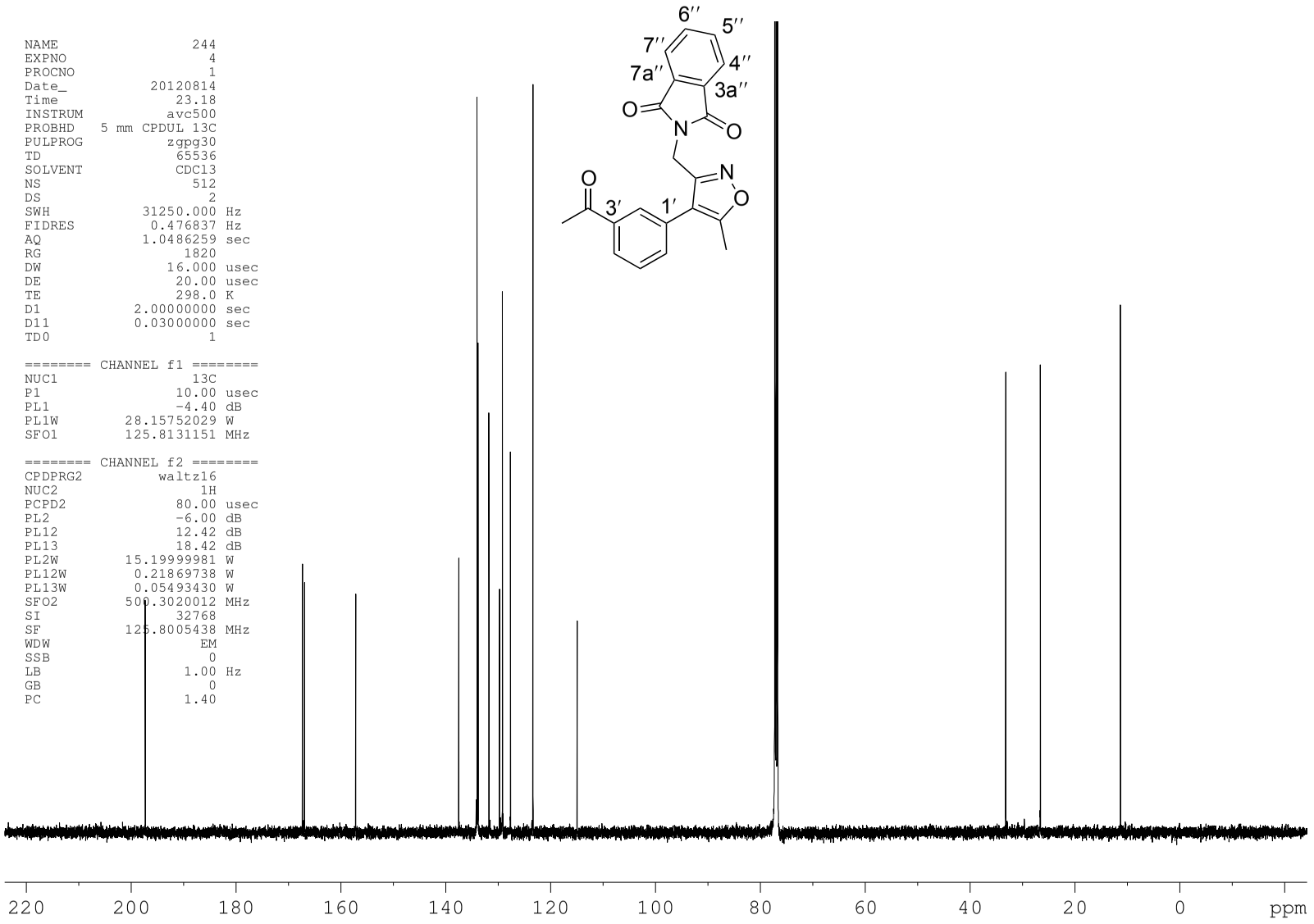
2-((4-(3-Acetylphenyl)-5-methylisoxazol-3-yl)methyl)isoindoline-1,3-dione 122



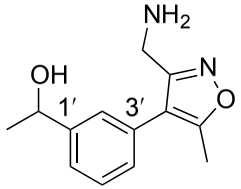
NAME	244
EXPNO	1
PROCNO	1
Date_	20120814
Time	22.30
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

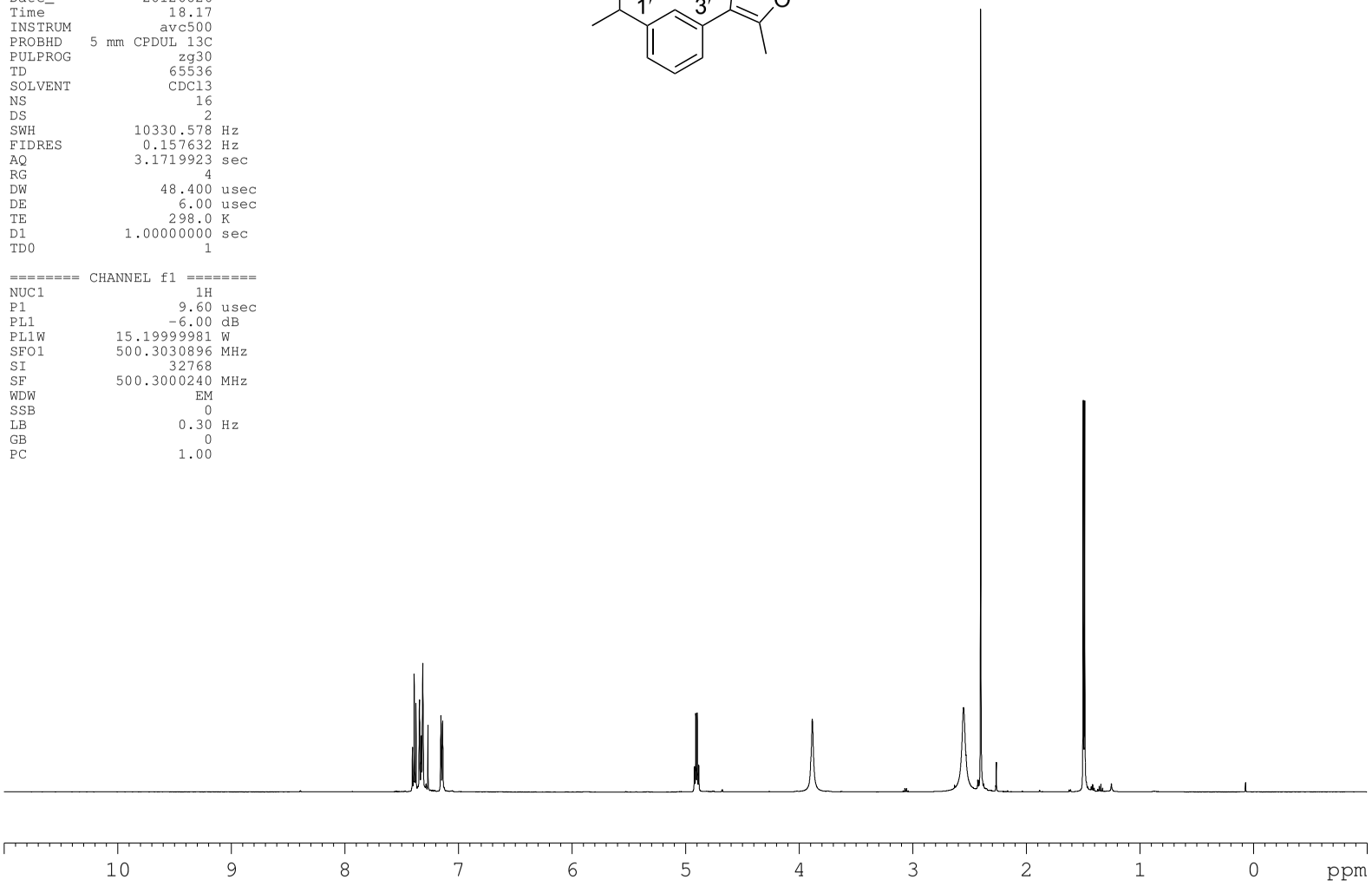
2-((4-(3-Acetylphenyl)-5-methylisoxazol-3-yl)methyl)isoindoline-1,3-dione 122



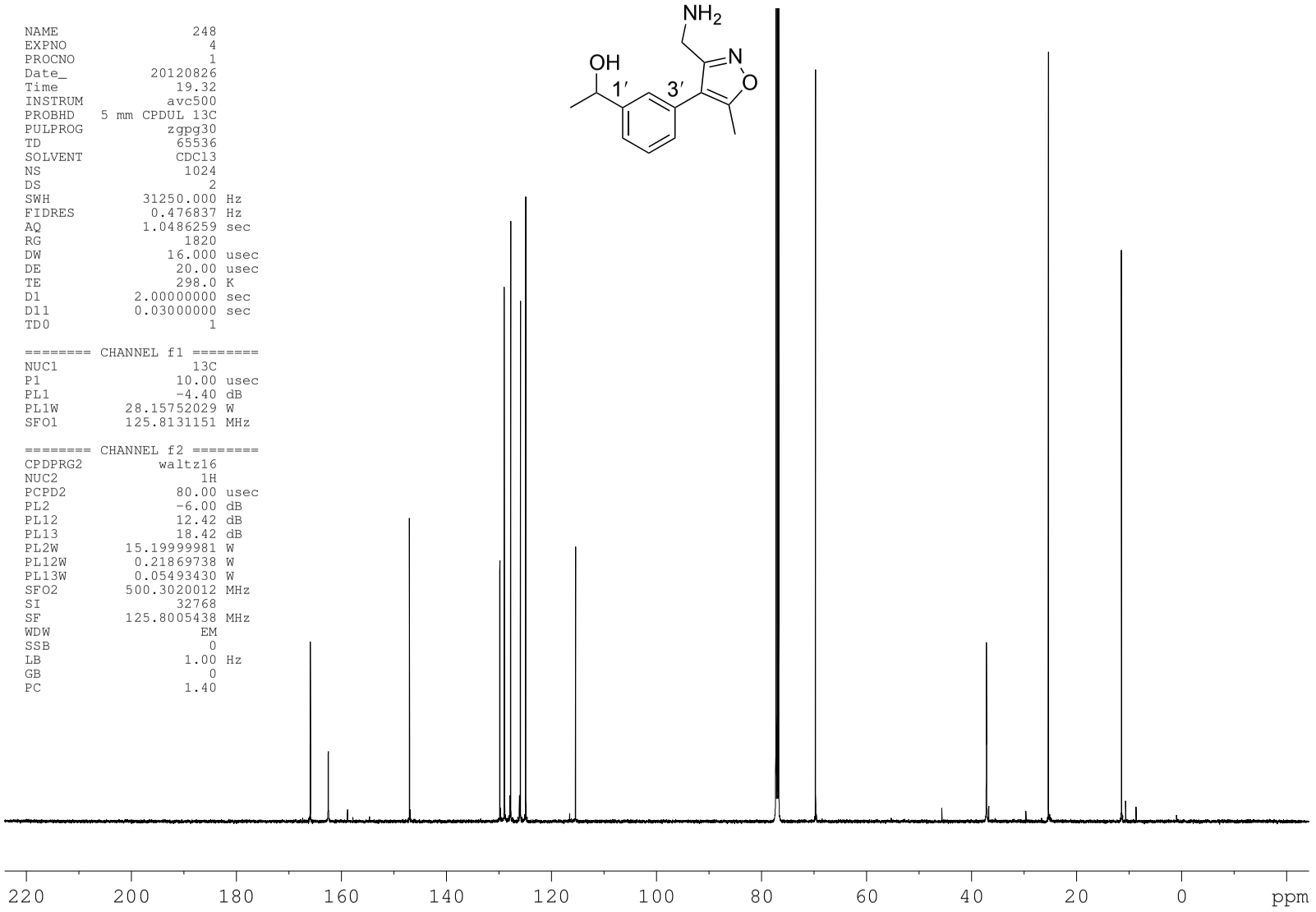
1-(3-(3-(Aminomethyl)-5-methylisoxazol-4-yl)phenyl)ethanol 74b



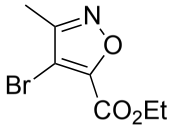
NAME	248
EXPNO	1
PROCNO	1
Date_	20120826
Time	18.17
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



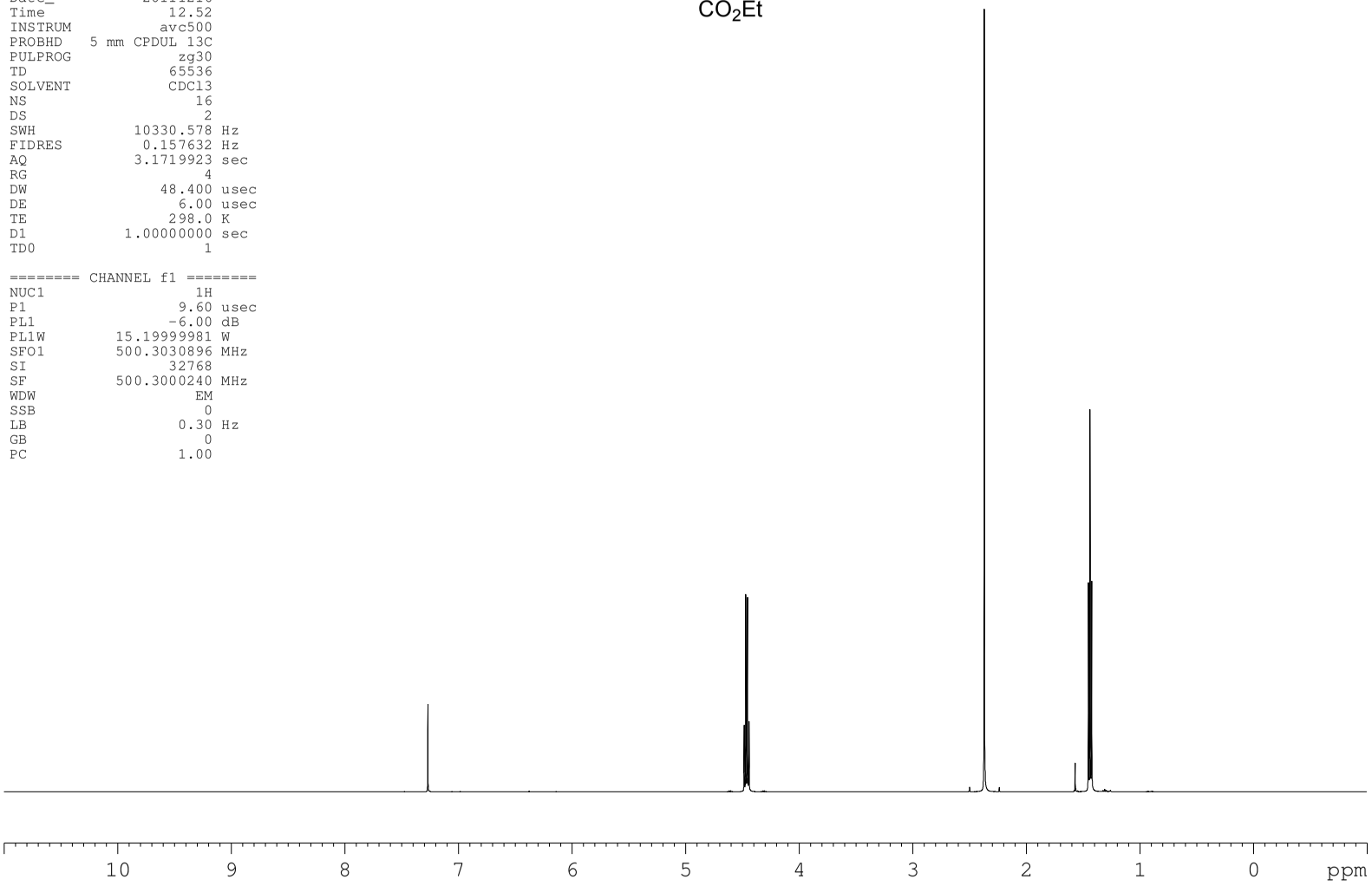
1-(3-(3-(Aminomethyl)-5-methylisoxazol-4-yl)phenyl)ethanol 74b



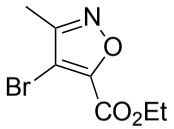
Ethyl 4-bromo-3-methylisoxazole-5-carboxylate 123



NAME	174
EXPNO	1
PROCNO	1
Date_	20111216
Time	12.52
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



Ethyl 4-bromo-3-methylisoxazole-5-carboxylate 123

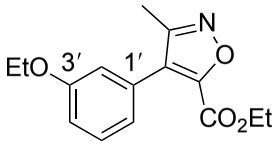



```
NAME                174
EXPNO                 3
PROCNO                1
Date_          20111216
Time              13.16
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

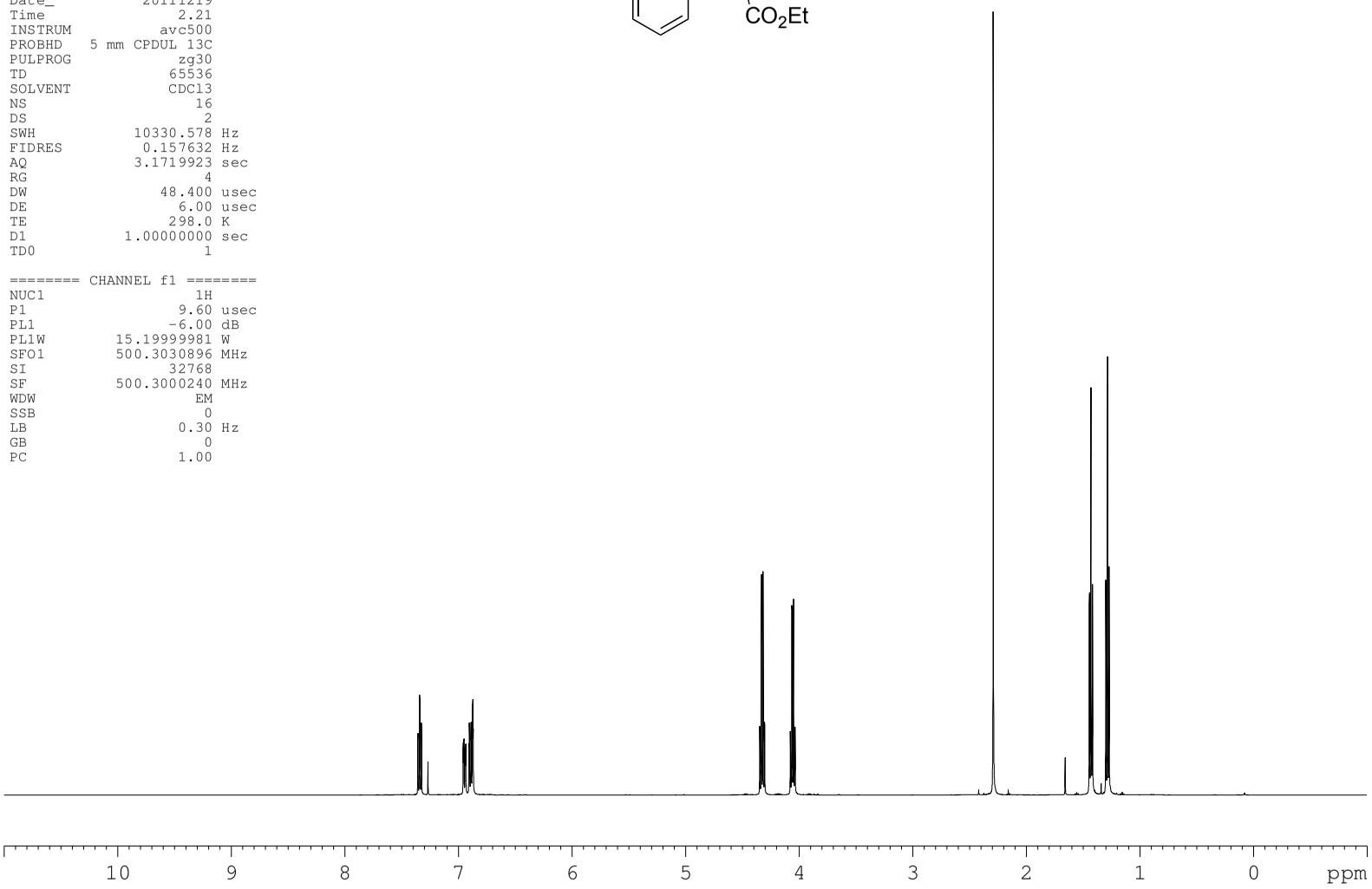
======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

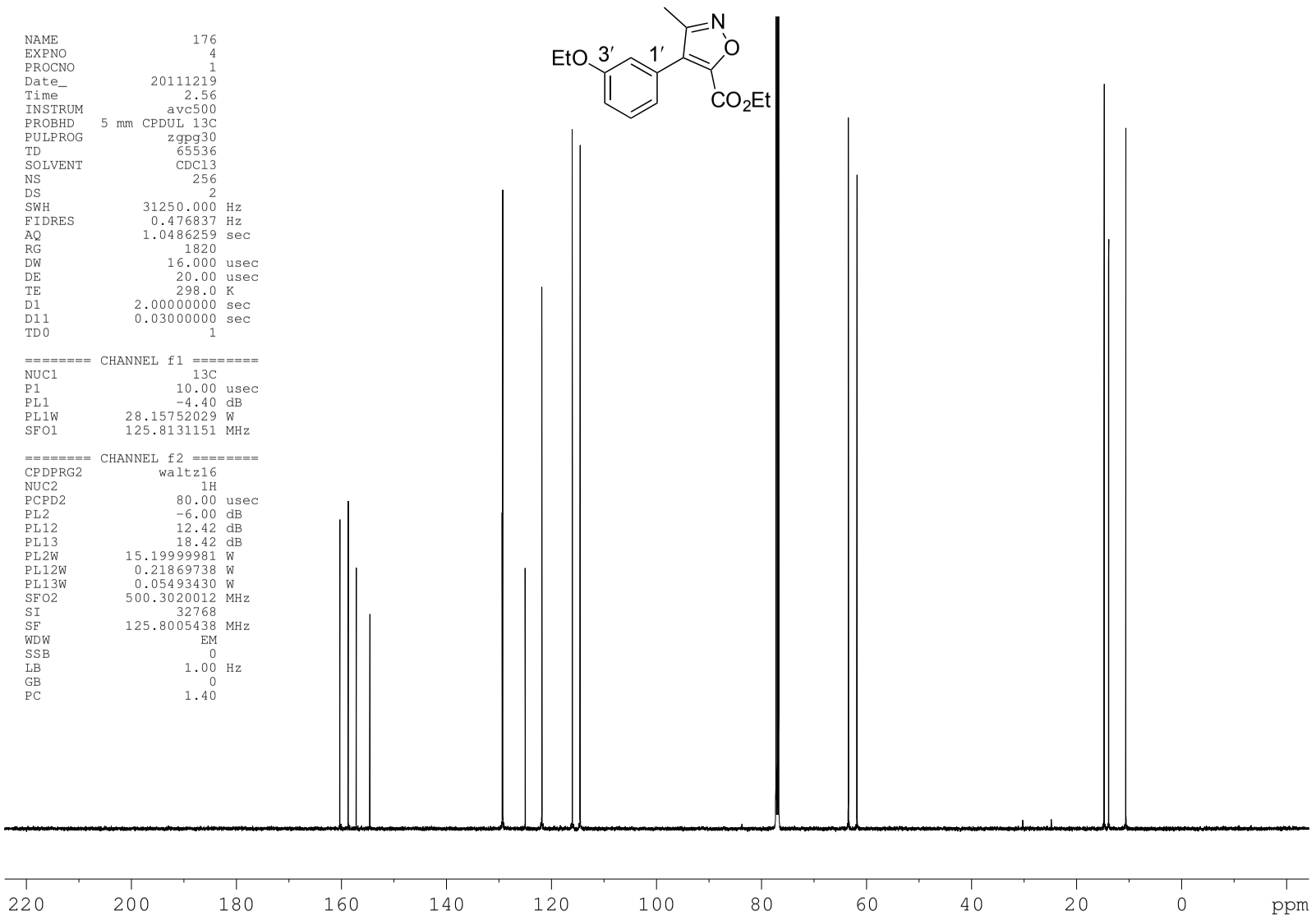
Ethyl 4-(3-ethoxyphenyl)-3-methylisoxazole-5-carboxylate 75a



NAME	176
EXPNO	1
PROCNO	1
Date_	20111219
Time	2.21
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



Ethyl 4-(3-ethoxyphenyl)-3-methylisoxazole-5-carboxylate 75a

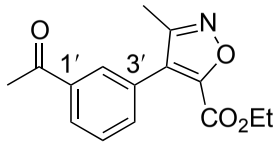



```
NAME                176
EXPNO                 4
PROCNO                1
Date_          20111219
Time               2.56
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

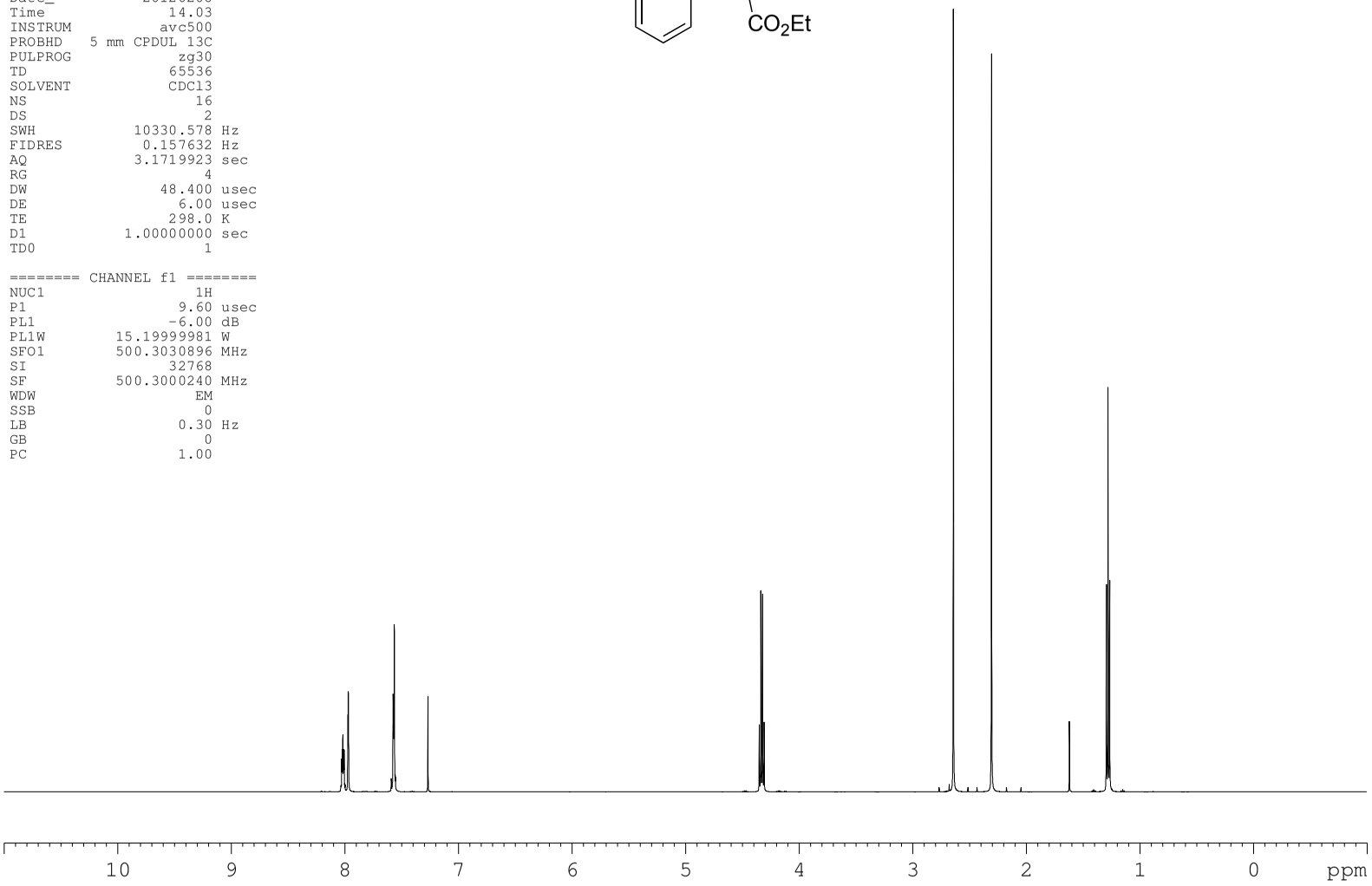
======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1       125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

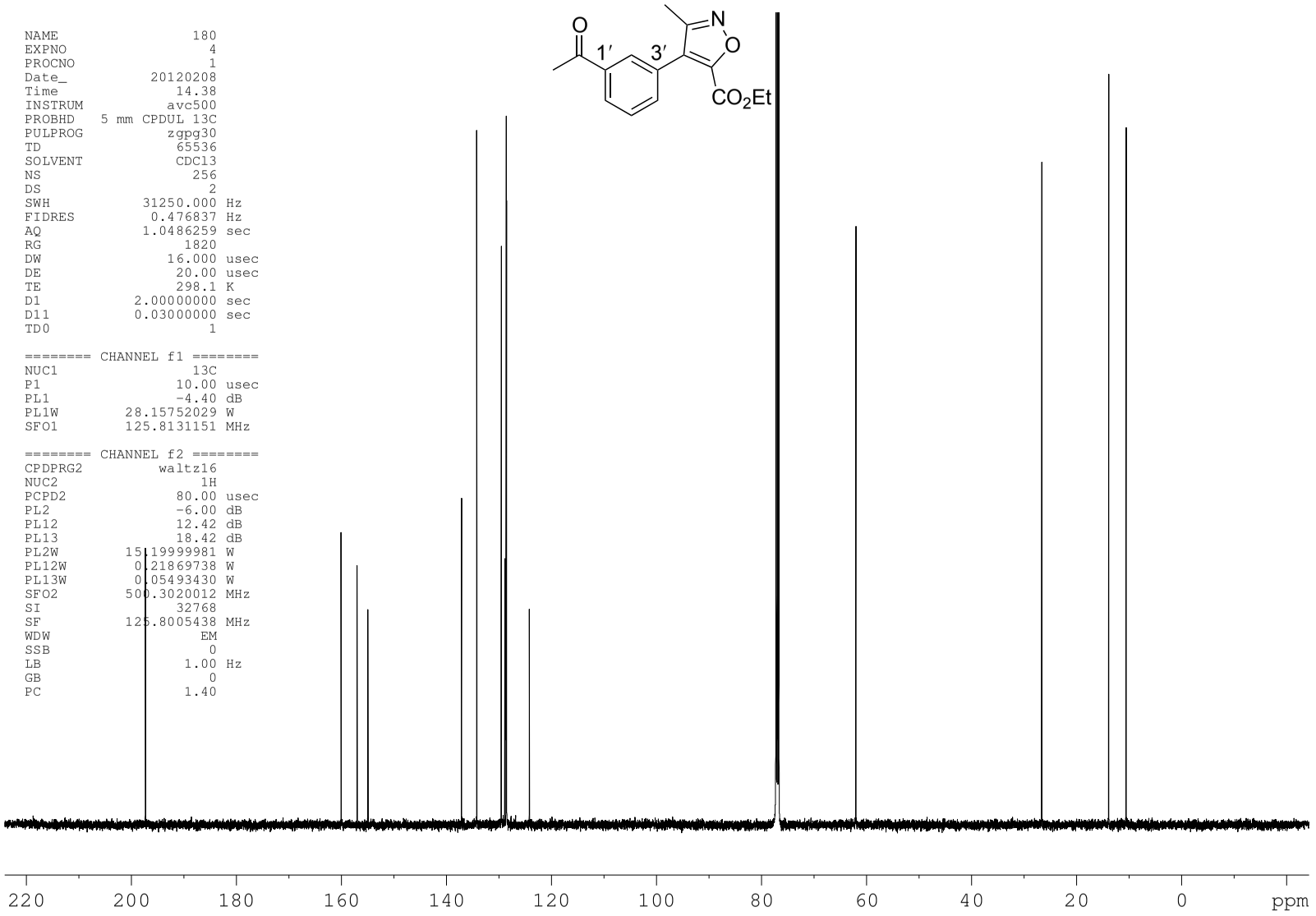
Ethyl 4-(3-acetylphenyl)-3-methylisoxazole-5-carboxylate 75b



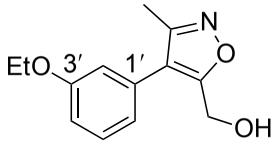
NAME	180
EXPNO	1
PROCNO	1
Date_	20120208
Time	14.03
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



Ethyl 4-(3-acetylphenyl)-3-methylisoxazole-5-carboxylate 75b

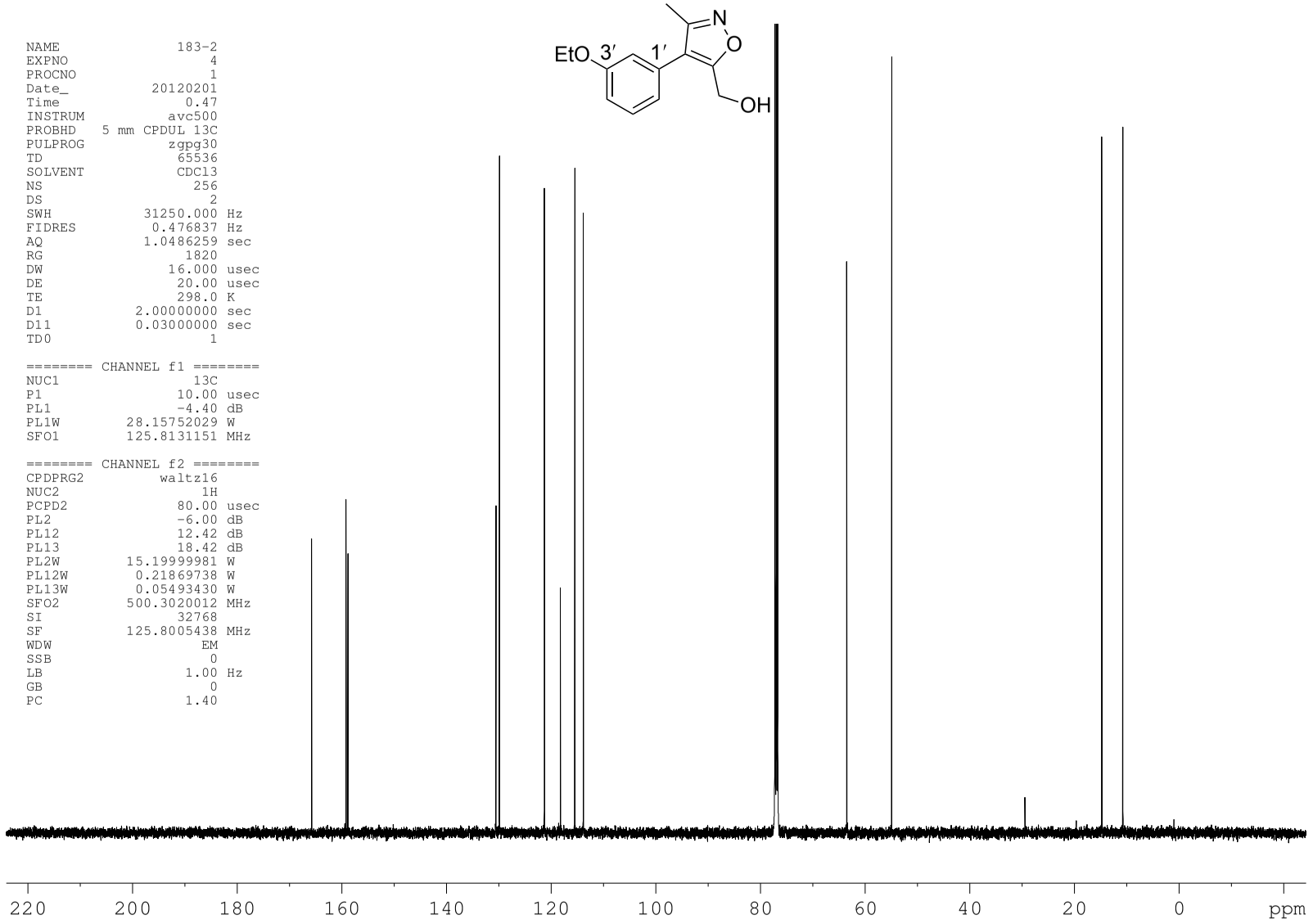


Ethyl 4-(3-acetylphenyl)-3-methylisoxazole-5-carboxylate 76a

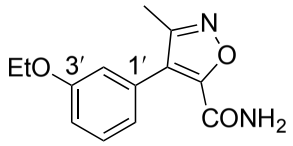


NAME	294
EXPNO	1
PROCNO	1
Date_	20130107
Time	22.15
INSTRUM	av400
PROBHD	5 mm QNP 1H/13
PULPROG	zg60
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	8278.146 Hz
FIDRES	0.126314 Hz
AQ	3.9584243 sec
RG	90.5
DW	60.400 usec
DE	7.50 usec
TE	300.0 K
D1	1.00000000 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	10.10 usec
PL1	0.00 dB
SFO1	400.2024714 MHz
SI	32768
SF	400.2000028 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

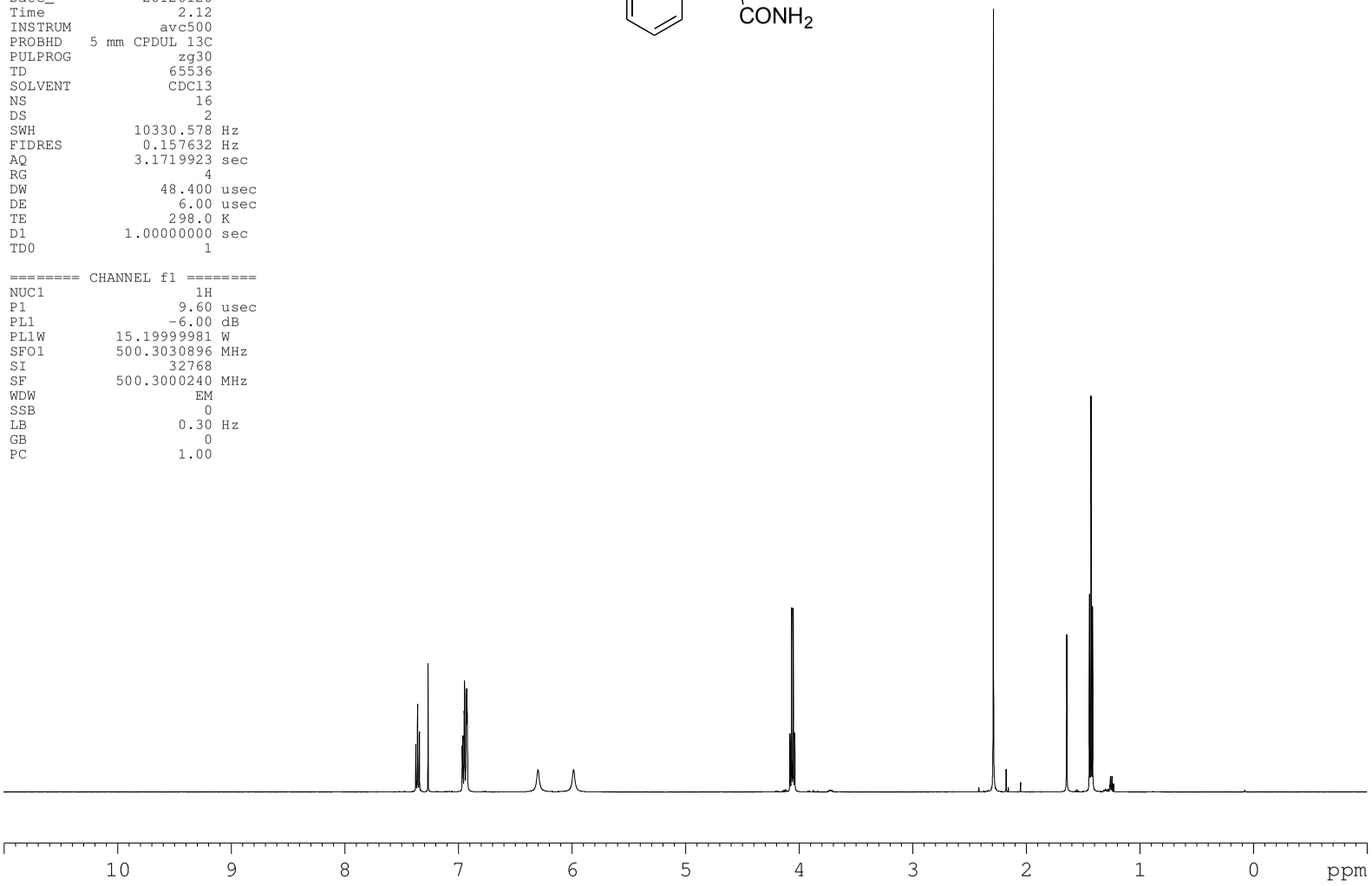
Ethyl 4-(3-acetylphenyl)-3-methylisoxazole-5-carboxylate 76a



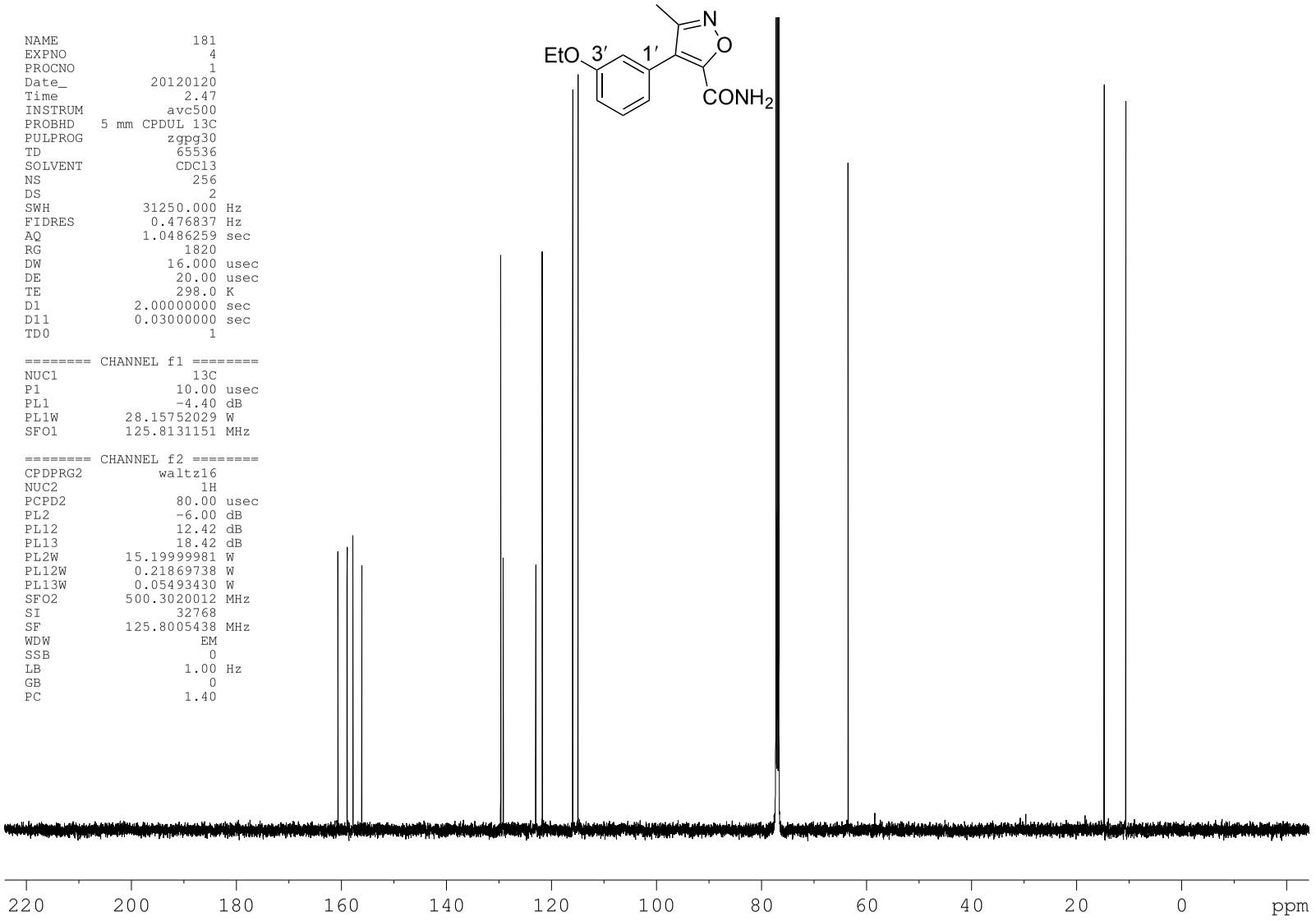
4-(3-Ethoxyphenyl)-3-methylisoxazole-5-carboxamide 77



NAME	181
EXPNO	1
PROCNO	1
Date_	20120120
Time	2.12
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



4-(3-Ethoxyphenyl)-3-methylisoxazole-5-carboxamide 77

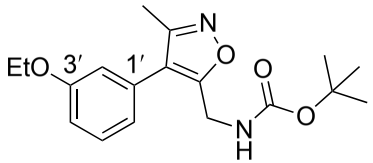



```
NAME                181
EXPNO                 4
PROCNO                1
Date_          20120120
Time               2.47
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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 ========
CPDPRG2          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
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

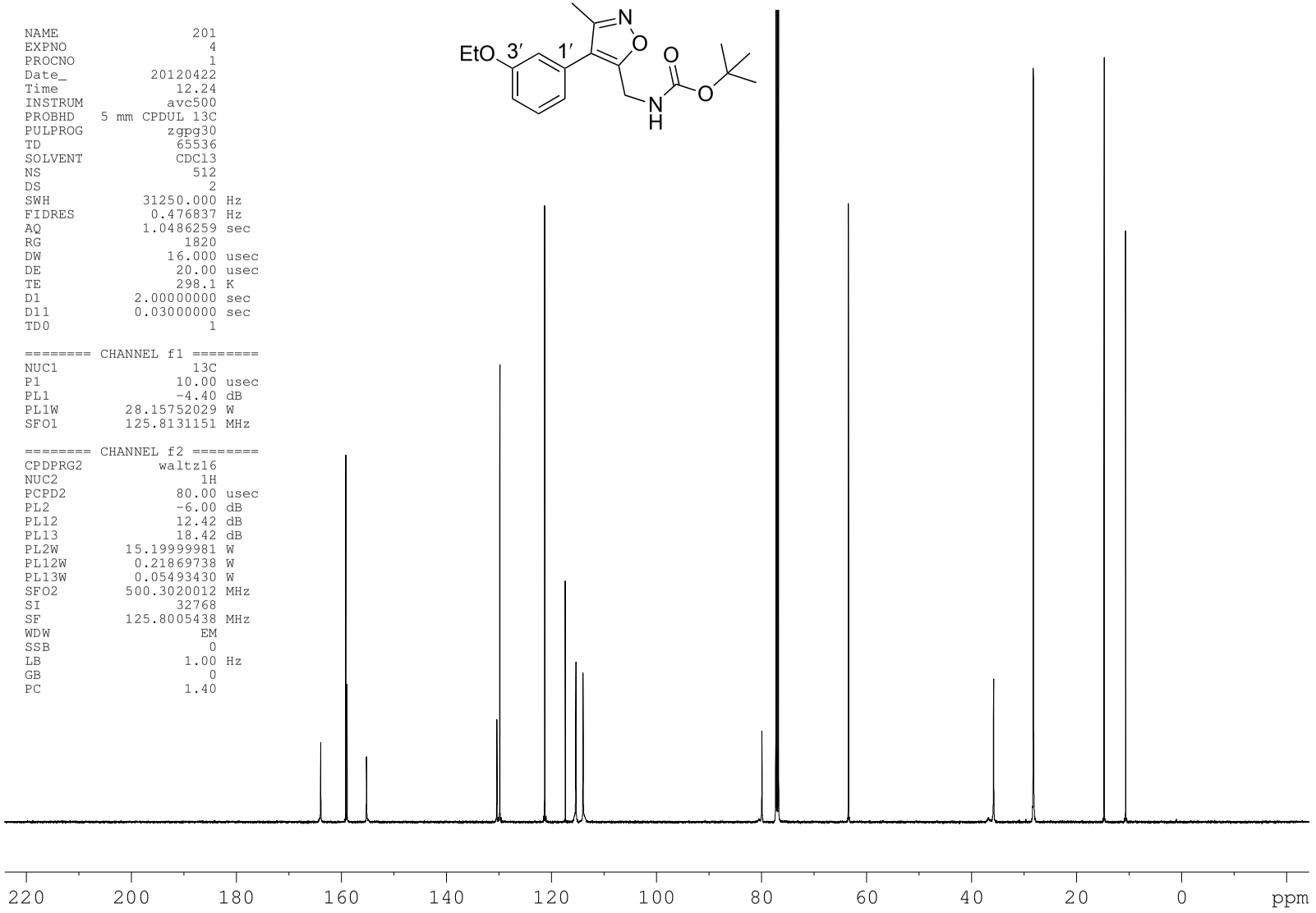
tert-Butyl ((4-(3-ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)carbamate 79a



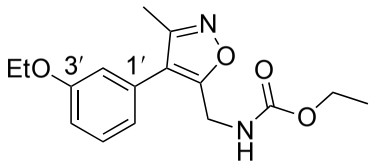

```
NAME                 201
EXPNO                  1
PROCNO                 1
Date_           20120422
Time               11.36
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 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
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

tert-Butyl ((4-(3-ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)carbamate 79a

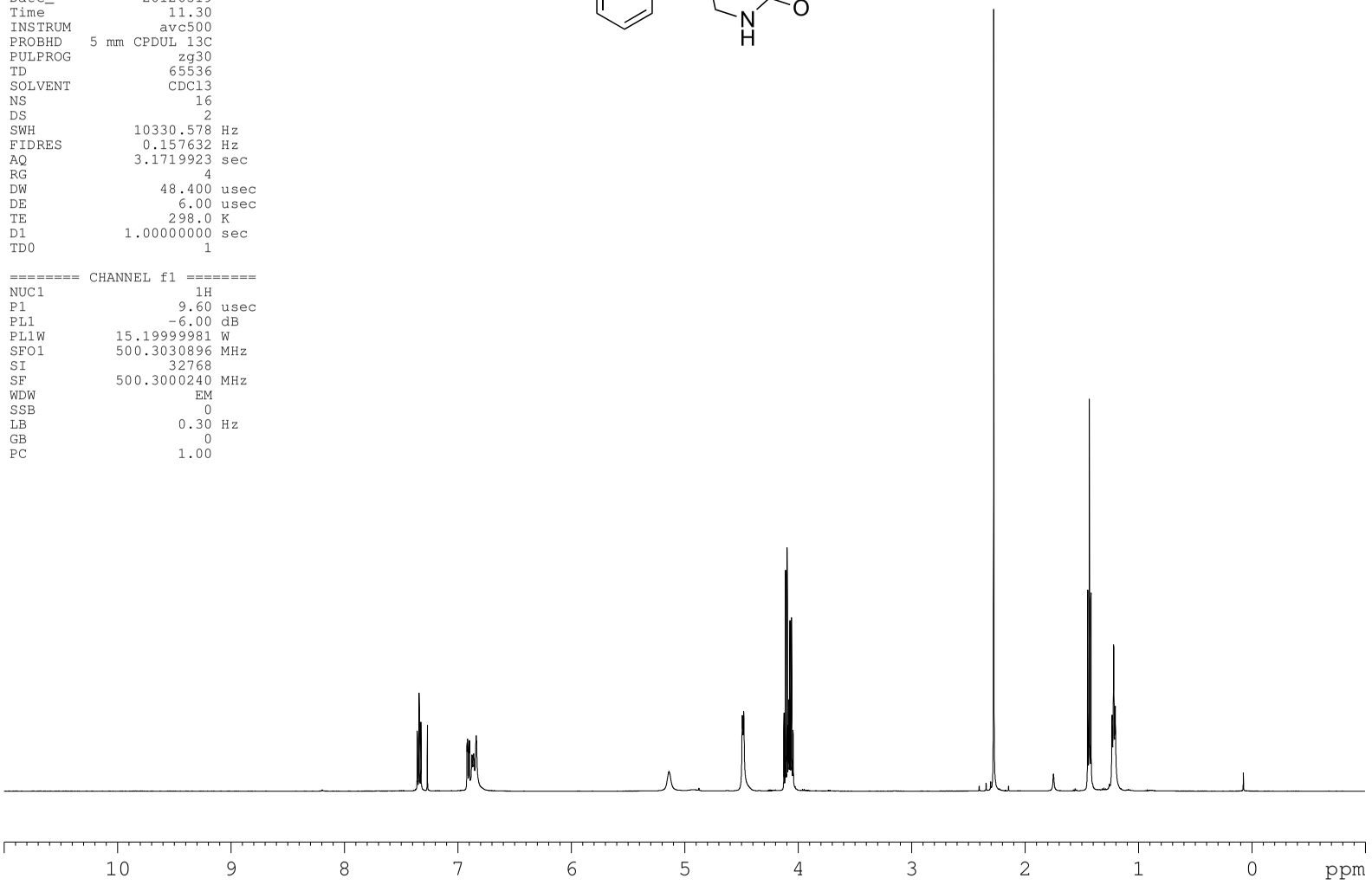


Ethyl ((4-(3-ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)carbamate 80

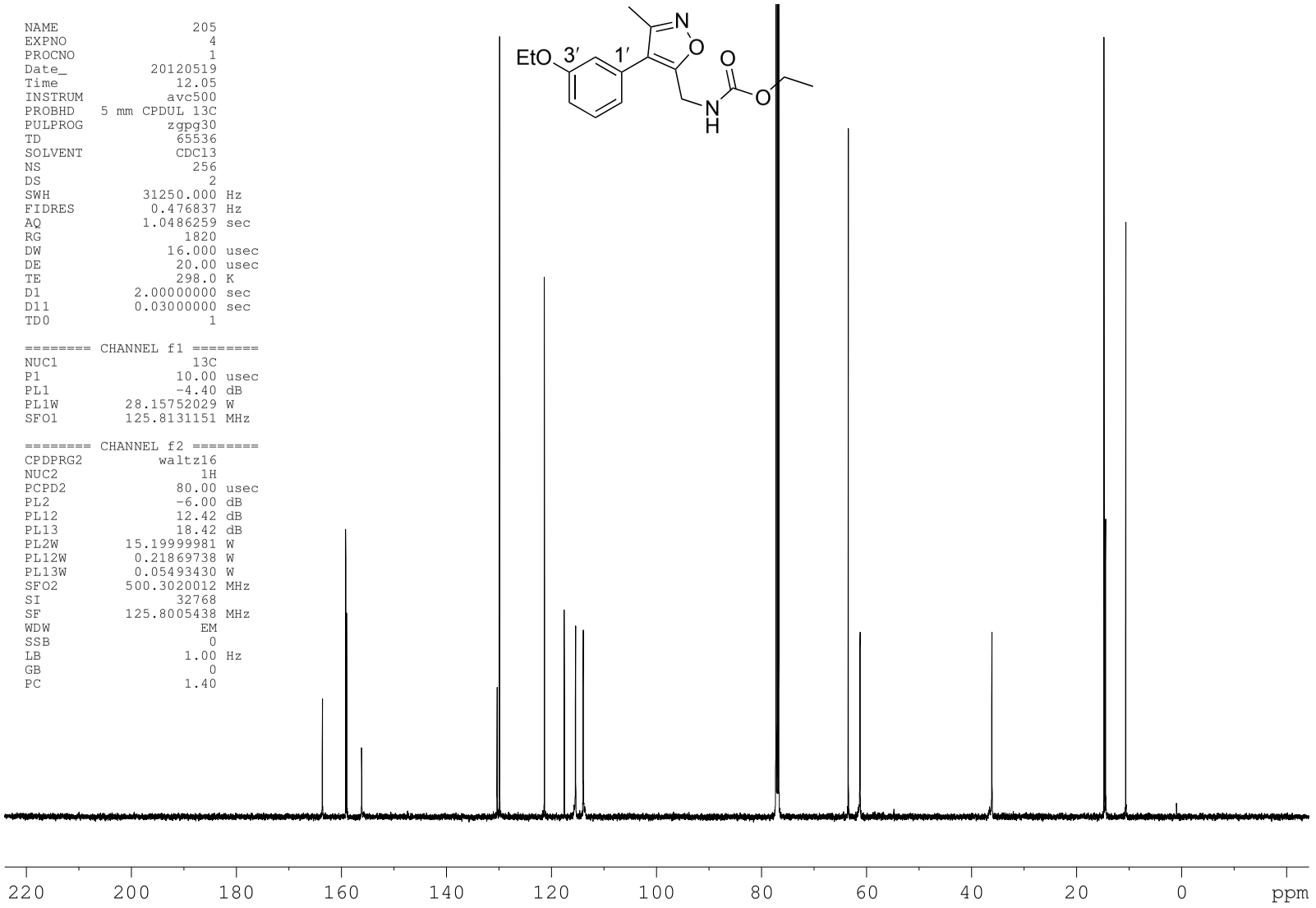


NAME	205
EXPNO	1
PROCNO	1
Date_	20120519
Time	11.30
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

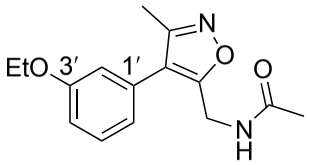
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



Ethyl ((4-(3-ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)carbamate 80

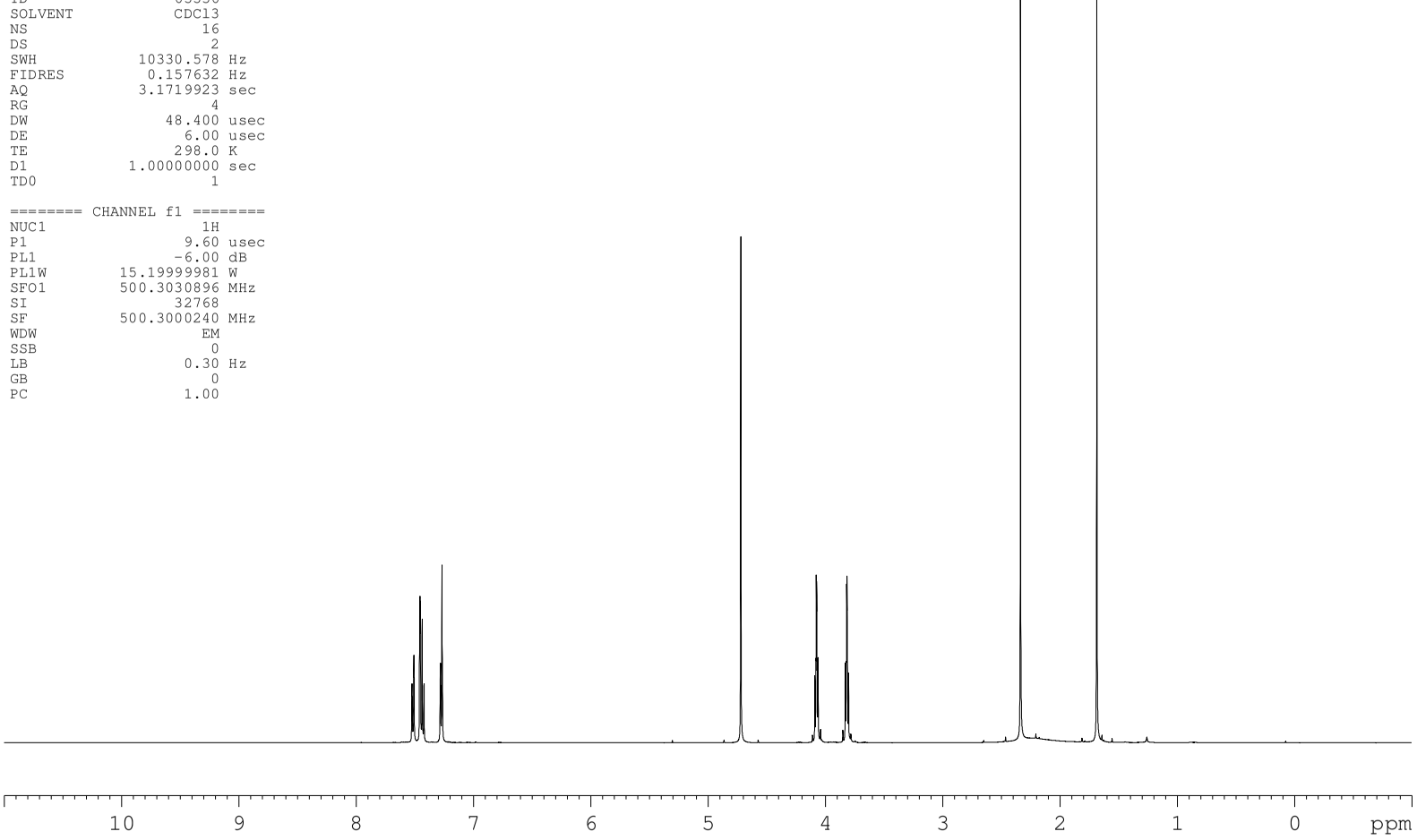


N-((4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)acetamide 81

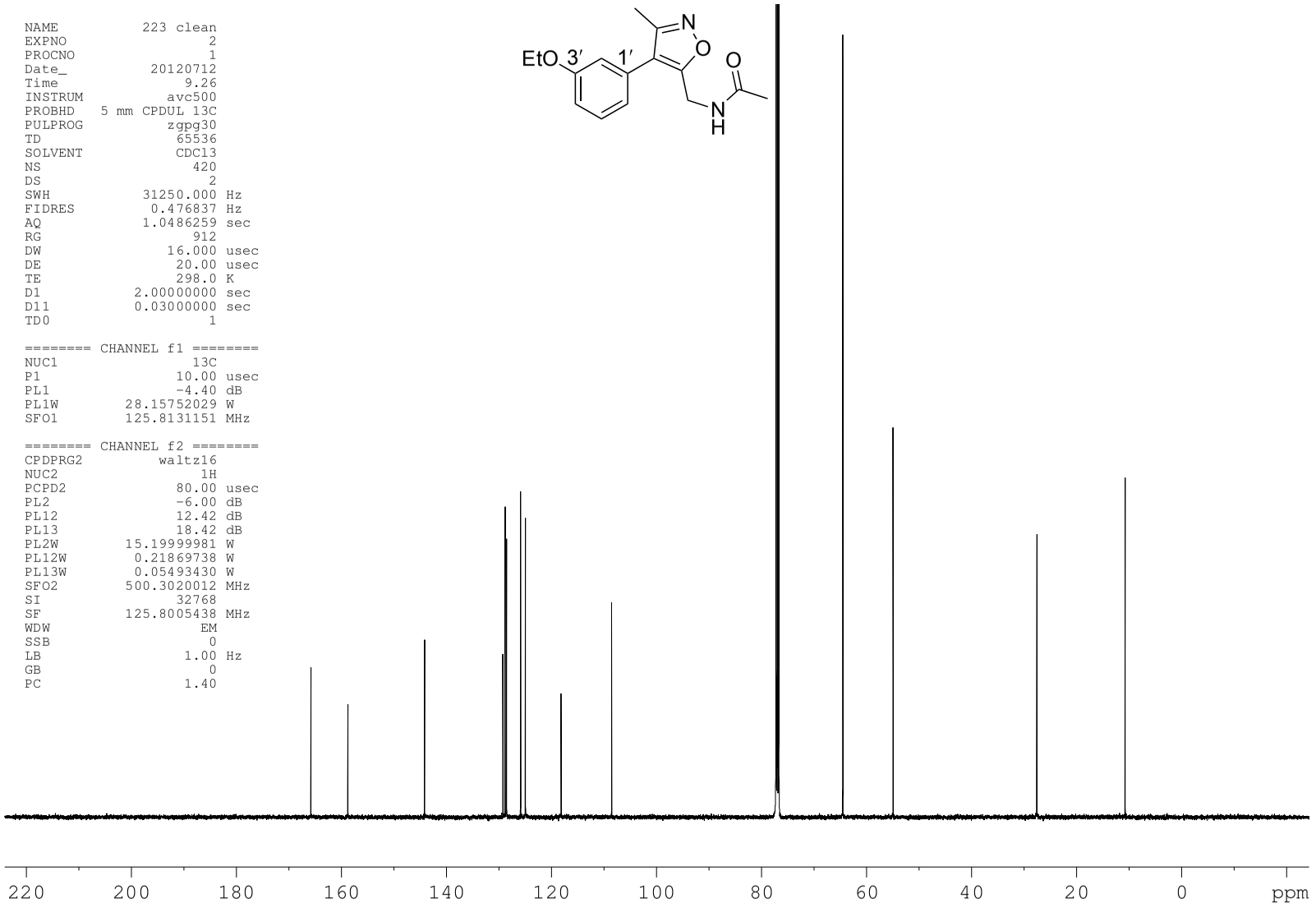



```
NAME           223 clean
EXPNO                  1
PROCNO                 1
Date_           20120712
Time               9.23
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

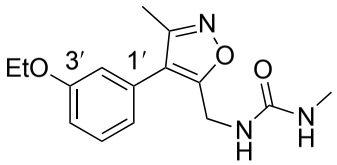
======== CHANNEL f1 ========
NUC1                  1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



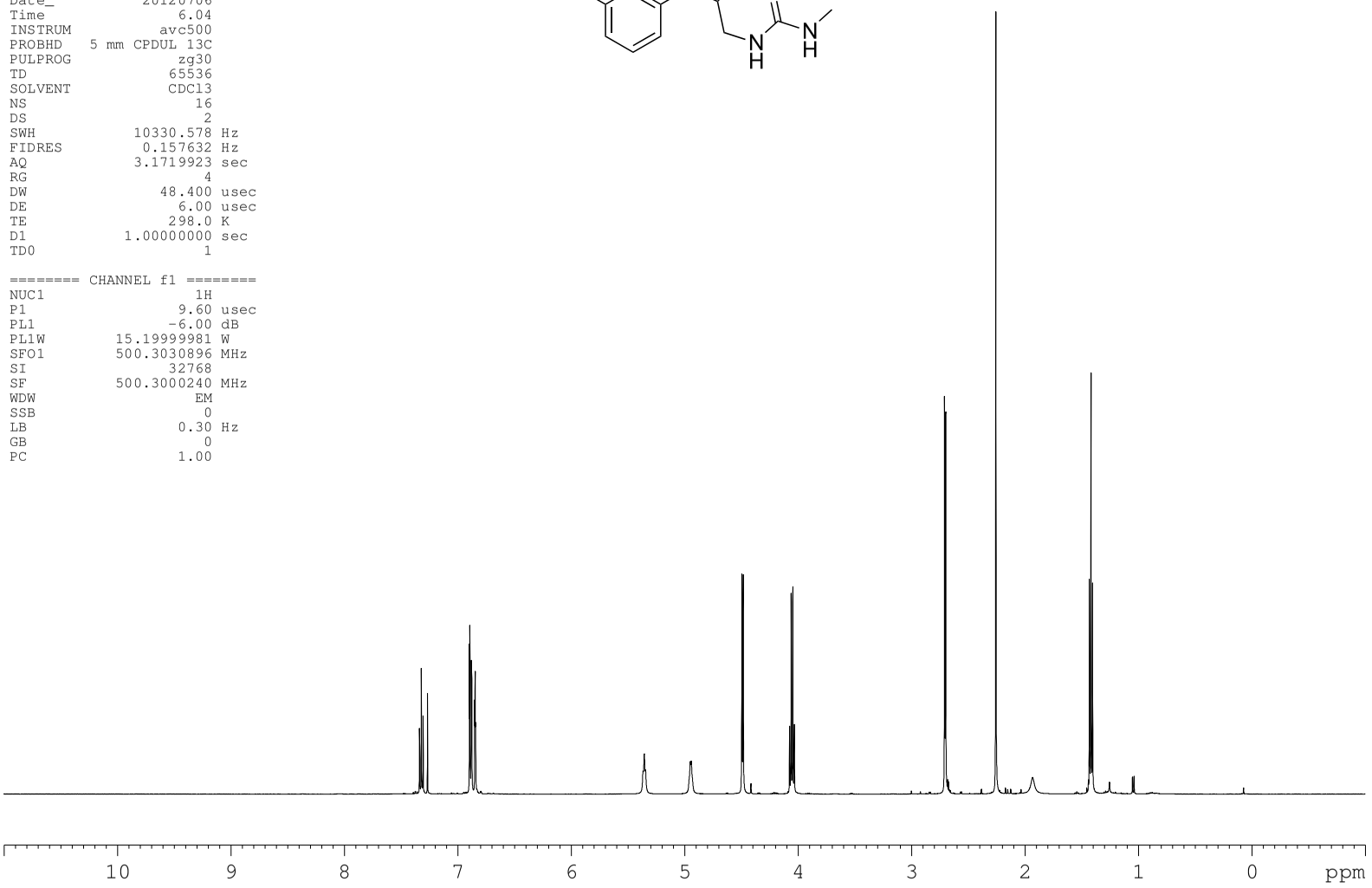
N-((4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)acetamide 81



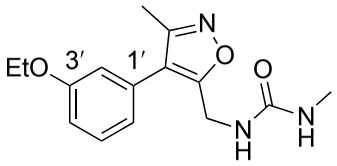
1-((4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)-3-methylurea 82



NAME	227
EXPNO	1
PROCNO	1
Date_	20120706
Time	6.04
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



1-((4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methyl)-3-methylurea 82

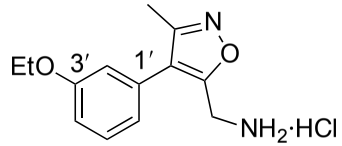



```
NAME                 227
EXPNO                  4
PROCNO                 1
Date_           20120706
Time                6.39
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ             1.0486259 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

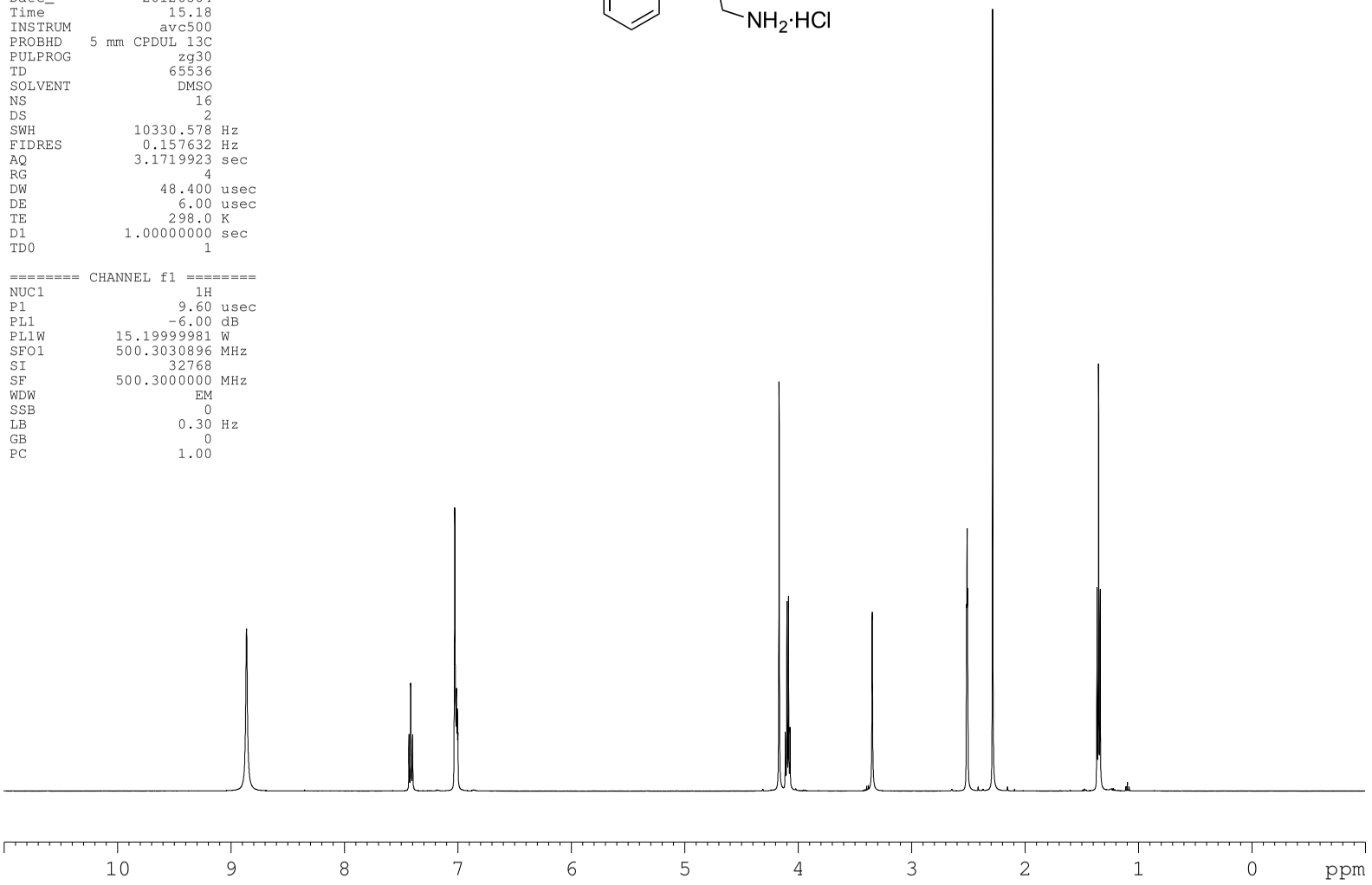
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

(4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methanamine hydrochloride 78a

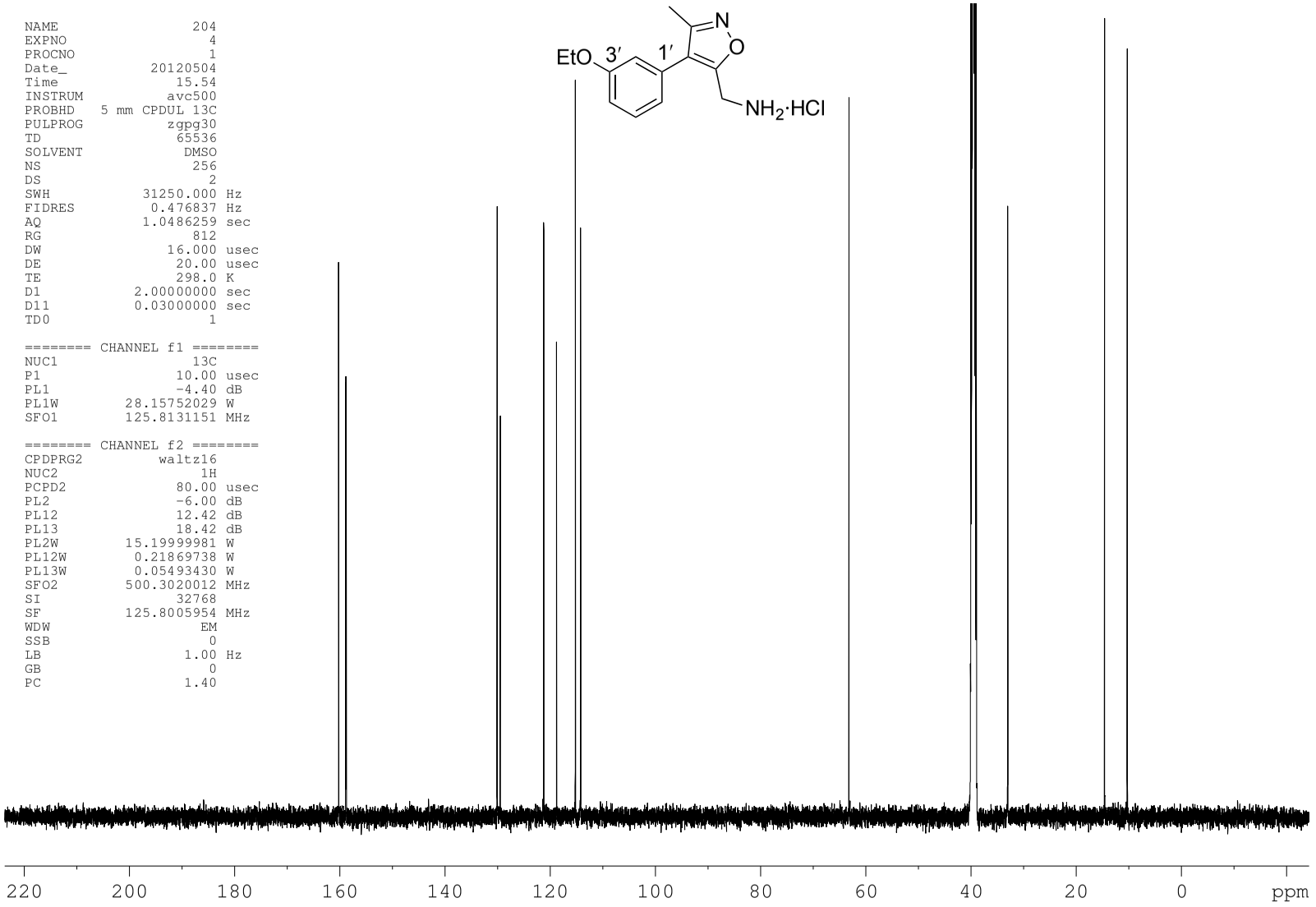



```
NAME                 204
EXPNO                  1
PROCNO                 1
Date_           20120504
Time               15.18
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719923 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

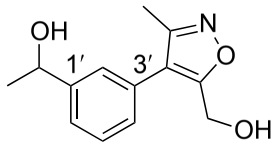
======== CHANNEL f1 ========
NUC1                  1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz
SI                 32768
SF          500.3000000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



(4-(3-Ethoxyphenyl)-3-methylisoxazol-5-yl)methanamine hydrochloride 78a

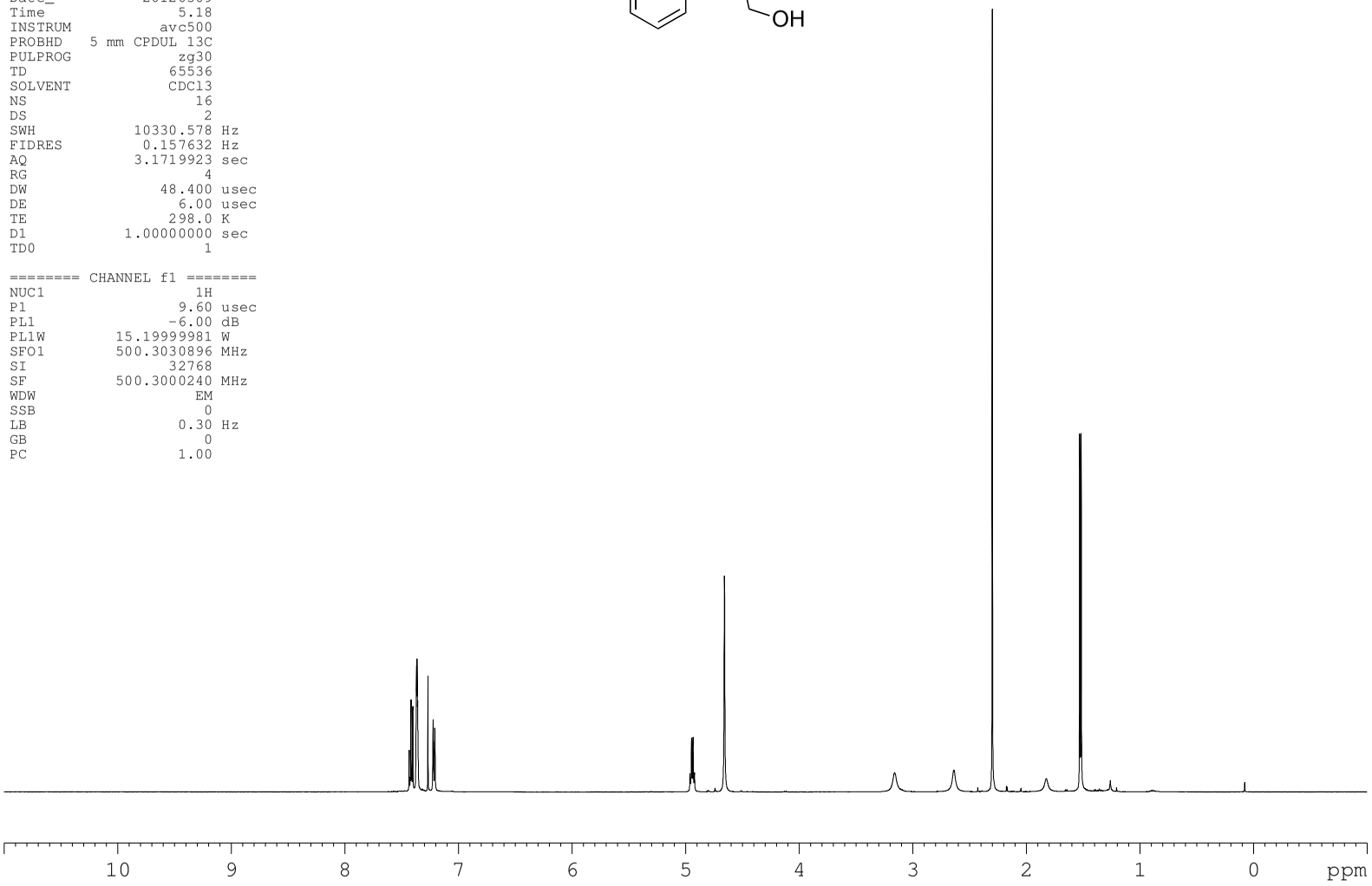


(*RS*)-1-(3-(5-(Hydroxymethyl)-3-methylisoxazol-4-yl)phenyl)ethanol 76c

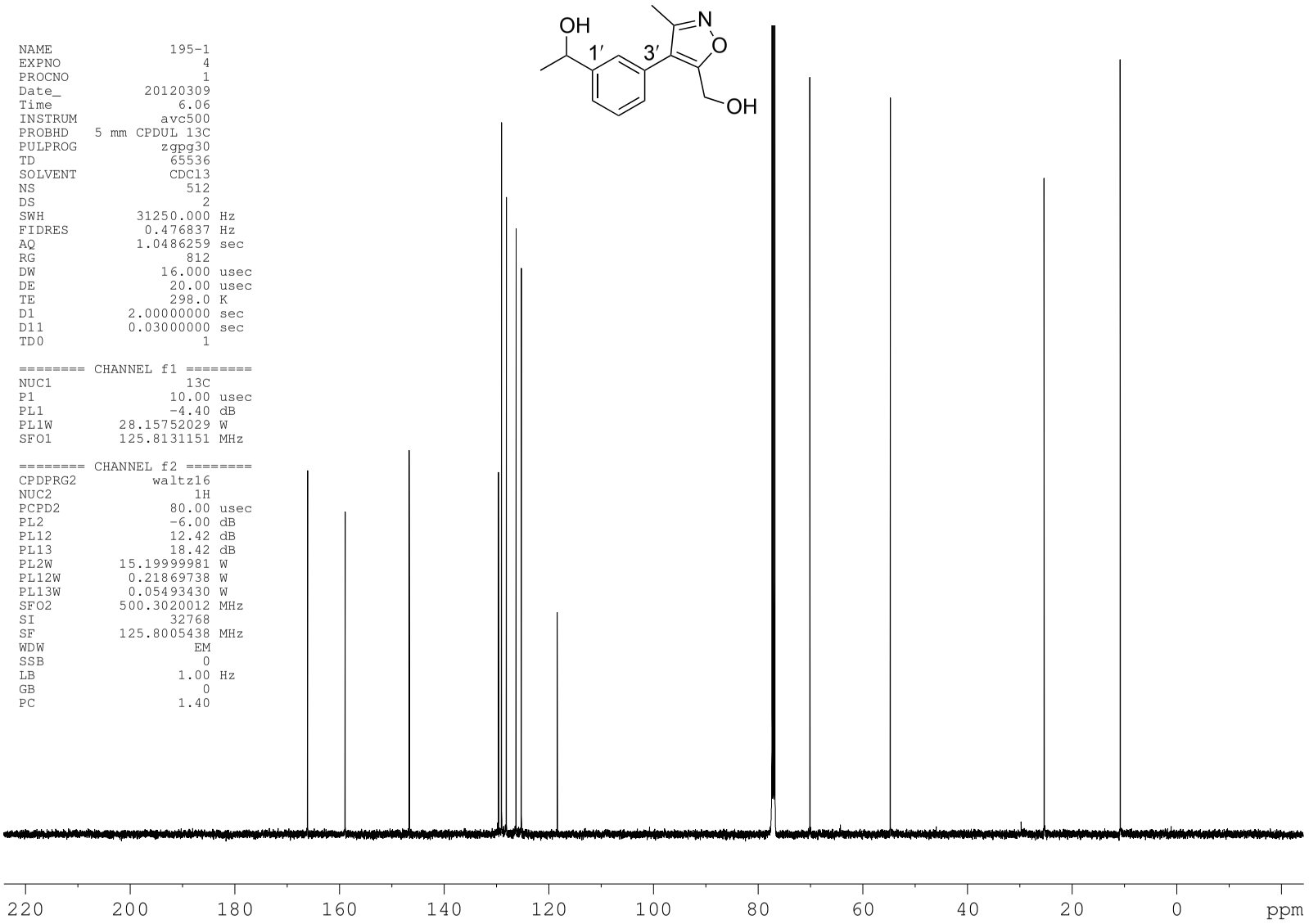



```
NAME                195-1
EXPNO                   1
PROCNO                  1
Date_            20120309
Time                5.18
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ             3.1719923 sec
RG                      4
DW                 48.400 usec
DE                  6.00 usec
TE                 298.0 K
D1           1.00000000 sec
TD0                     1

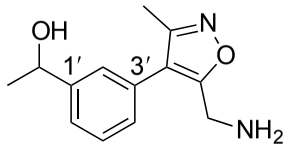
======== CHANNEL f1 ========
NUC1                   1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W         15.19999981 W
SFO1         500.3030896 MHz
SI                  32768
SF           500.3000240 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```



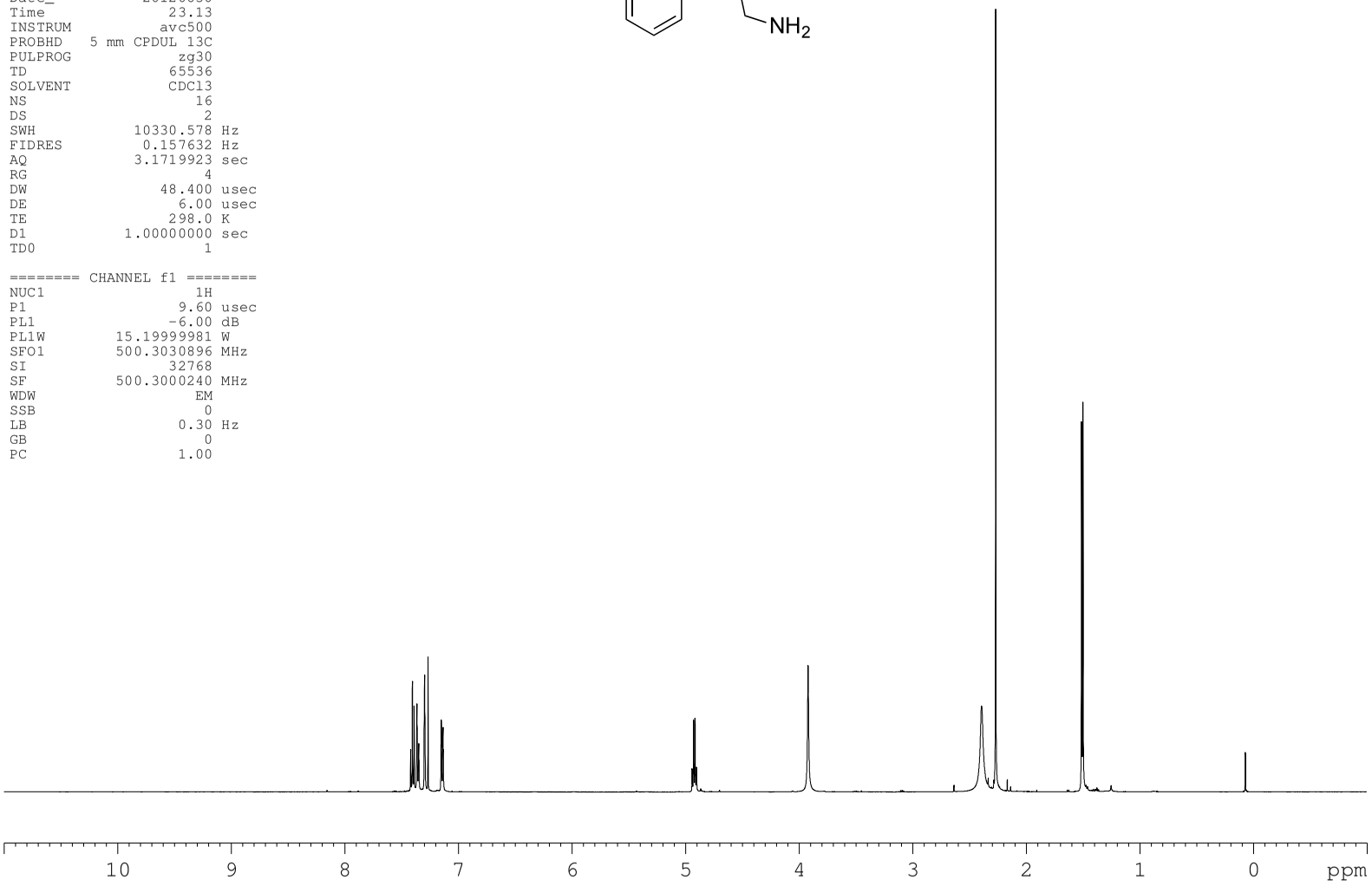
(*RS*)-1-(3-(5-(Hydroxymethyl)-3-methylisoxazol-4-yl)phenyl)ethanol 76c



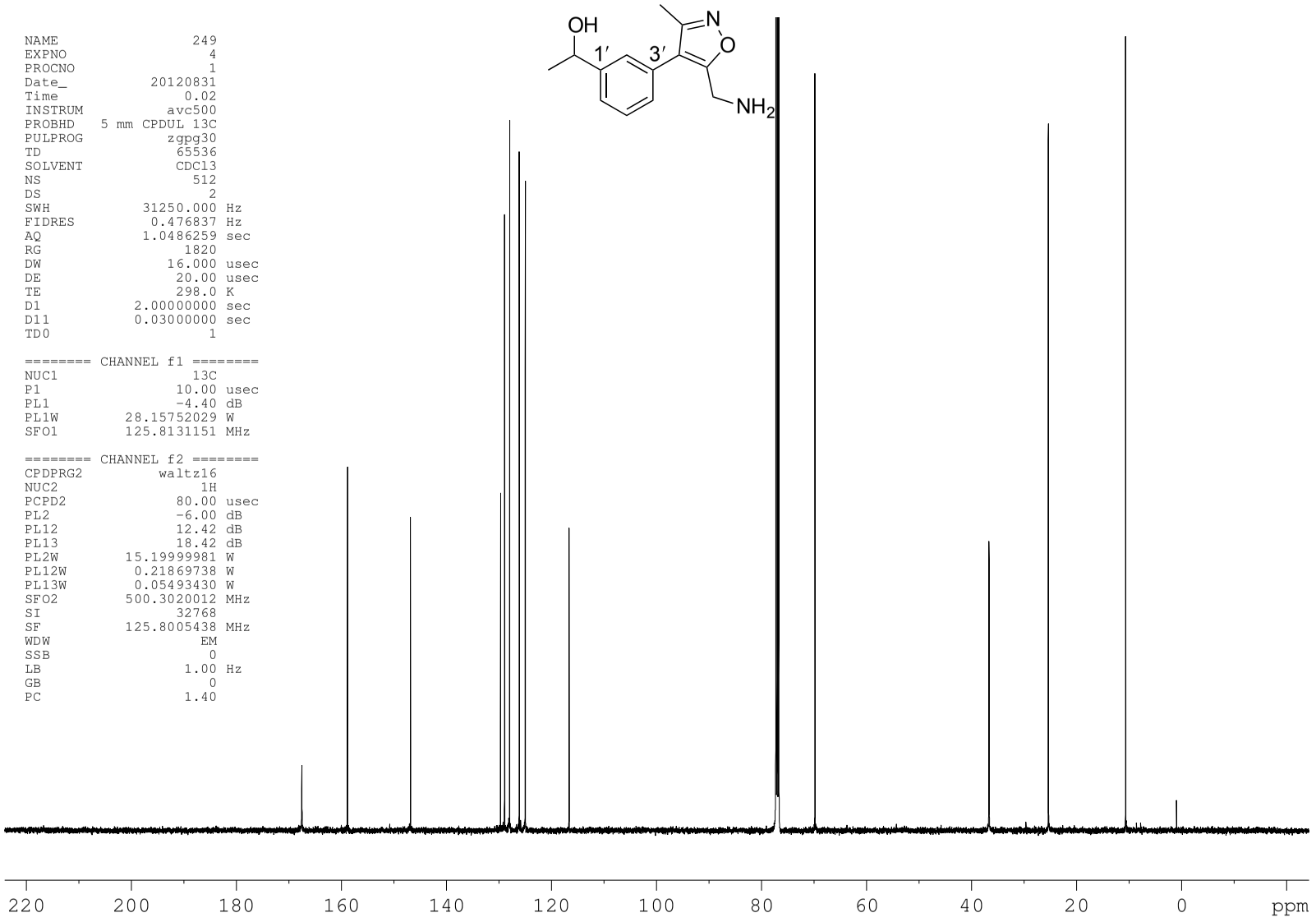
(*RS*)-1-(3-(5-(Aminomethyl)-3-methylisoxazol-4-yl)phenyl)ethanol 78b



NAME	249
EXPNO	1
PROCNO	1
Date_	20120830
Time	23.13
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-1-(3-(5-(Aminomethyl)-3-methylisoxazol-4-yl)phenyl)ethanol 78b

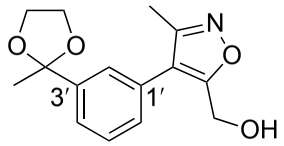



```
NAME                 249
EXPNO                  4
PROCNO                 1
Date_           20120831
Time                0.02
INSTRUM           avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   512
DS                     2
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ             1.0486259 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

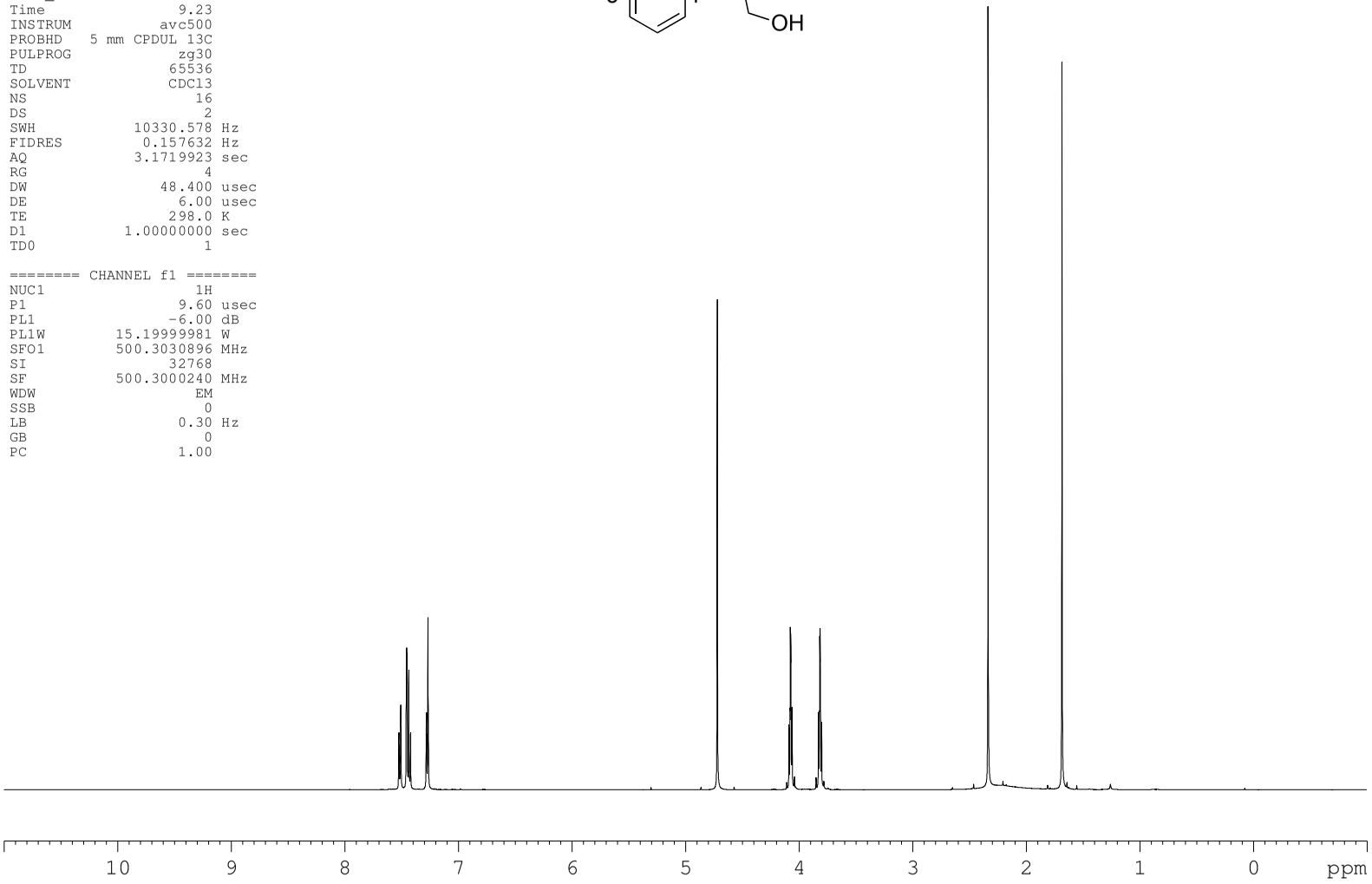
======== CHANNEL f1 ========
NUC1                 13C
P1                 10.00 usec
PL1                -4.40 dB
PL1W         28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                 32768
SF          125.8005438 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

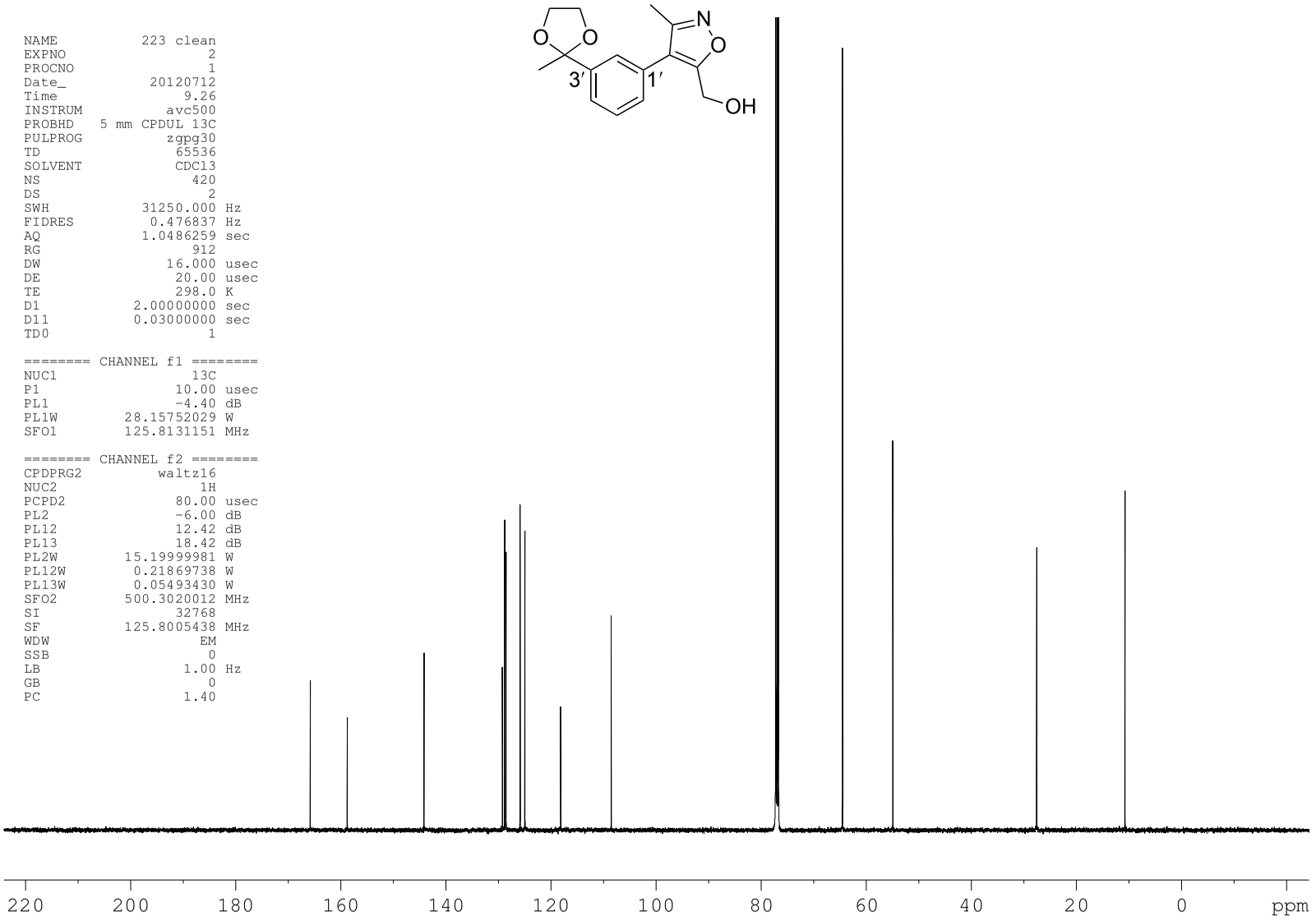
(3-Methyl-4-(3-(2-methyl-1,3-dioxolan-2-yl)phenyl)isoxazol-5-yl)methanol 126



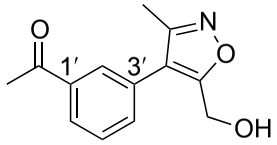
NAME	223 clean
EXPNO	1
PROCNO	1
Date_	20120712
Time	9.23
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



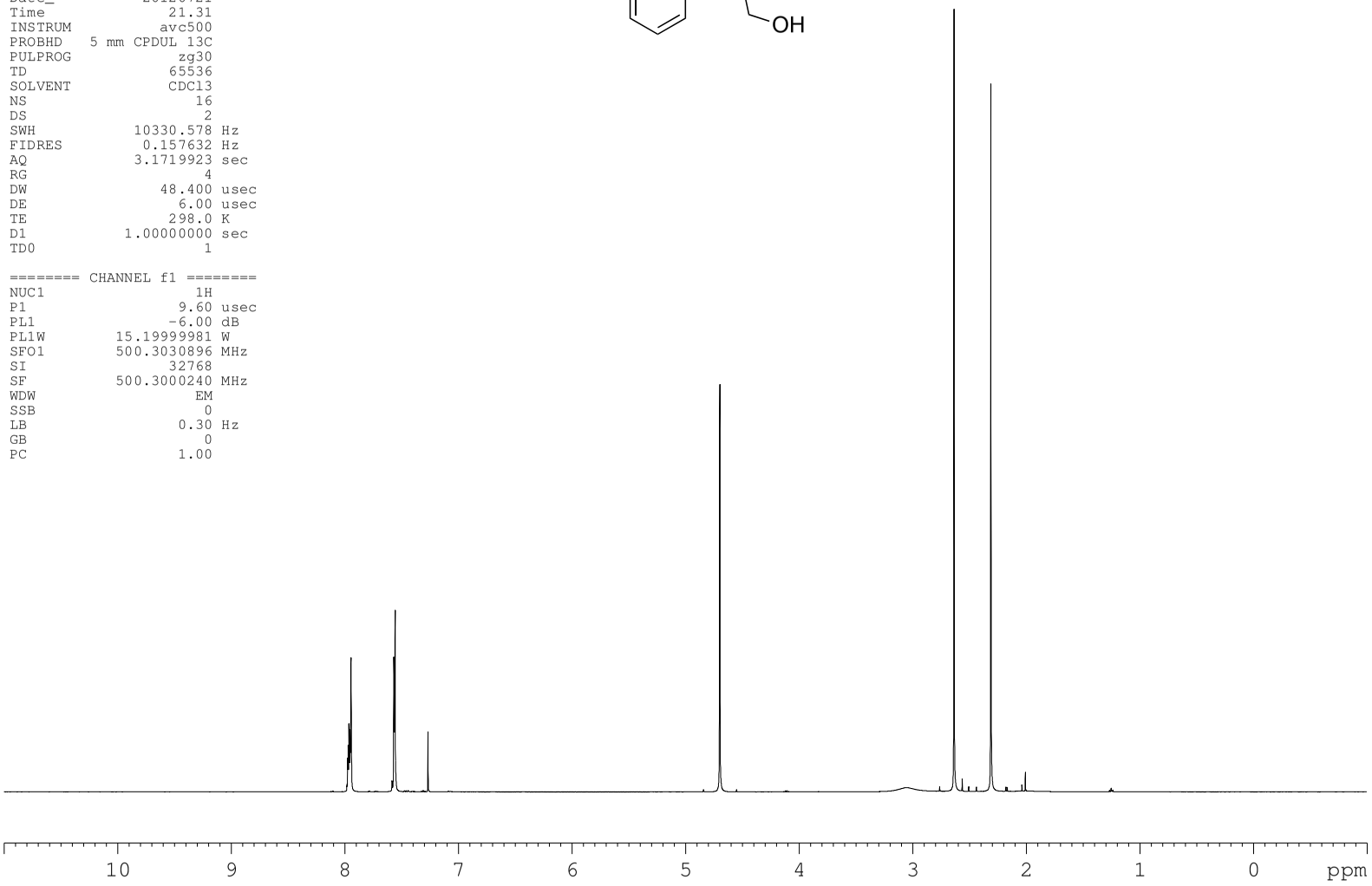
(3-Methyl-4-(3-(2-methyl-1,3-dioxolan-2-yl)phenyl)isoxazol-5-yl)methanol 126



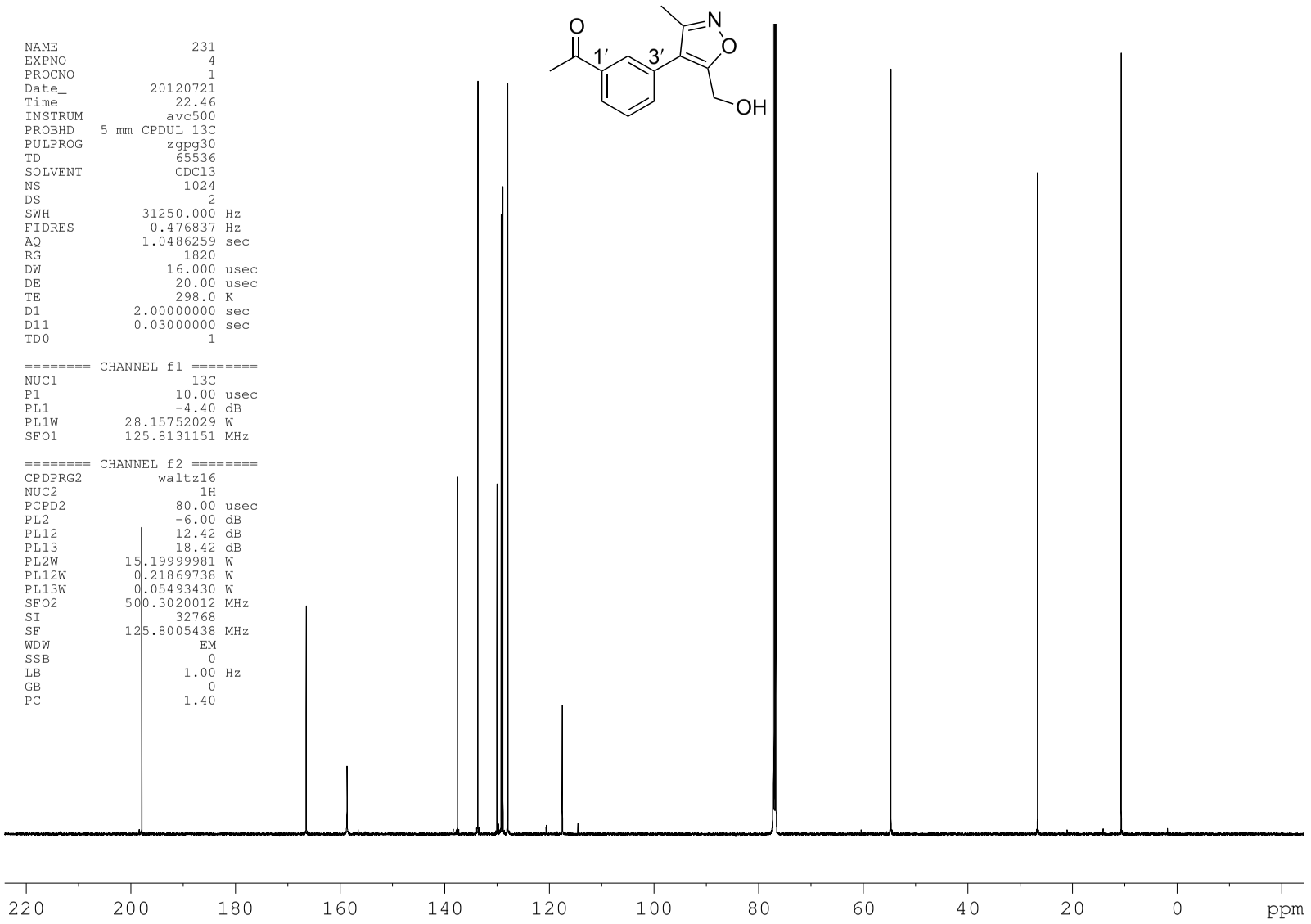
1-(3-(5-(Hydroxymethyl)-3-methylisoxazol-4-yl)phenyl)ethanone 76b



NAME	231
EXPNO	1
PROCNO	1
Date_	20120721
Time	21.31
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



1-(3-(5-(Hydroxymethyl)-3-methylisoxazol-4-yl)phenyl)ethanone 76b

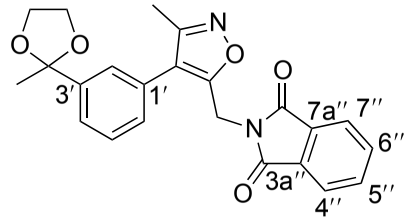



```
NAME                231
EXPNO                 4
PROCNO                1
Date_          20120721
Time              22.46
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

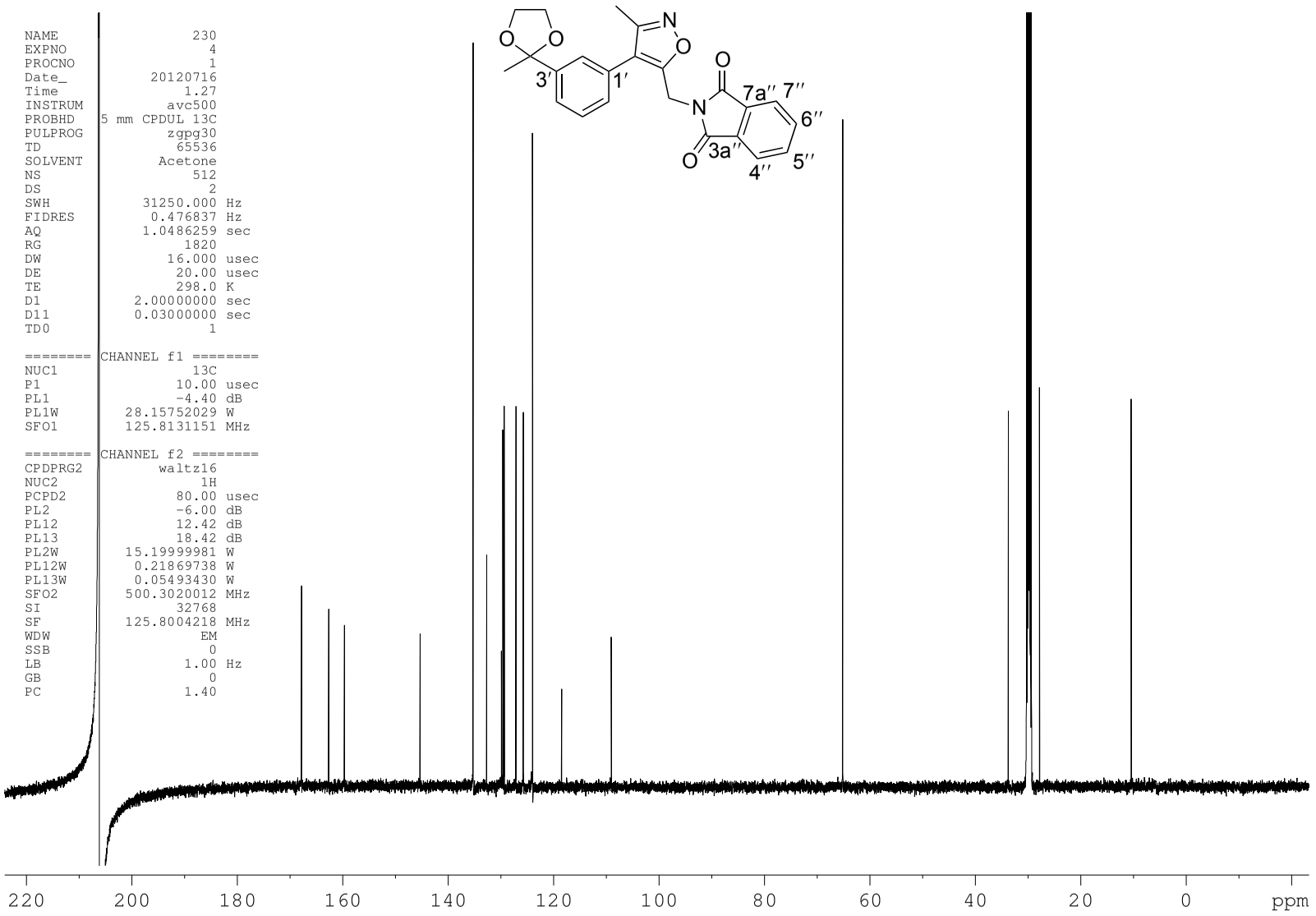
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2       500.3020012 MHz
SI                32768
SF         125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

2-((3-Methyl-4-(3-(2-methyl-1,3-dioxolan-2-yl)phenyl)isoxazol-5-yl)methyl)isoindoline-1,3-dione 127

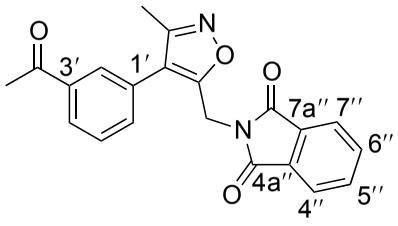


NAME	230
EXPNO	1
PROCNO	1
Date_	20120716
Time	0.39
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	Acetone
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.60 usec
PL1	-6.00 dB
PL1W	15.19999981 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

2-((3-Methyl-4-(3-(2-methyl-1,3-dioxolan-2-yl)phenyl)isoxazol-5-yl)methyl)isoindoline-1,3-dione 127

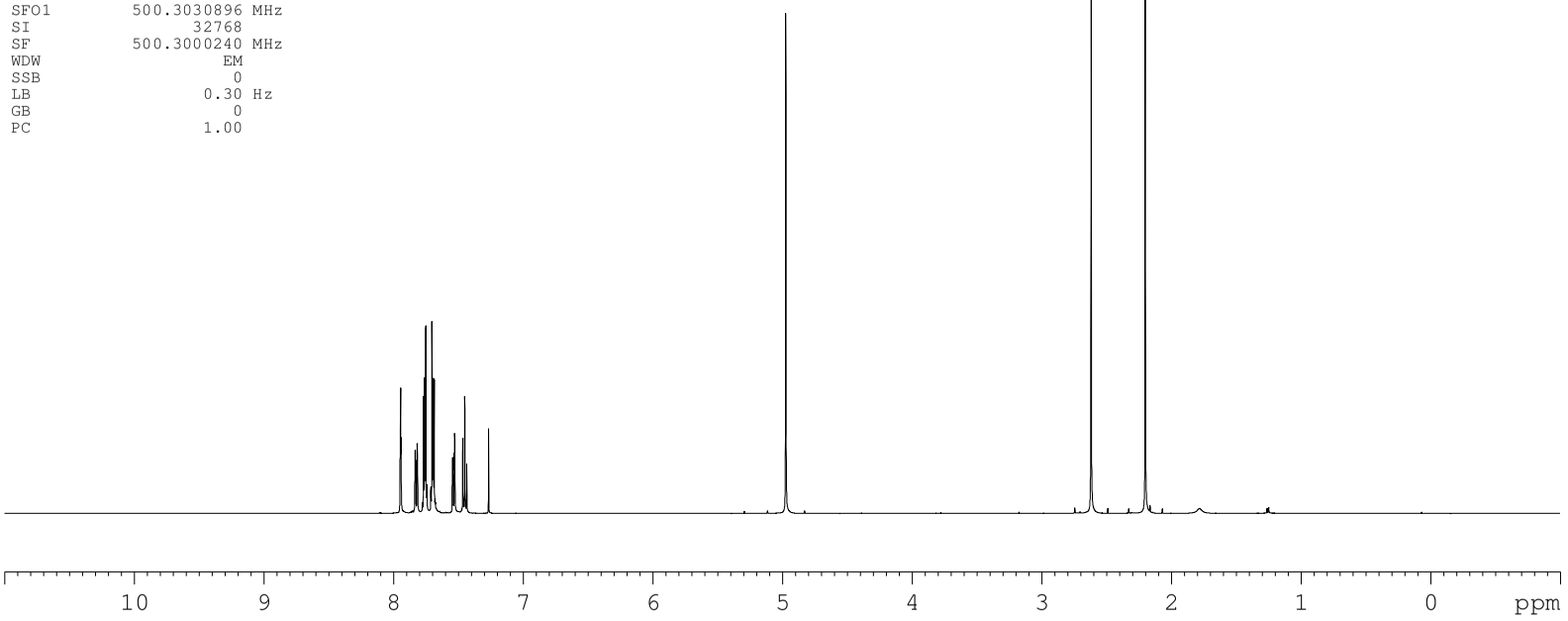


2-((4-(3-Acetylphenyl)-3-methylisoxazol-5-yl)methyl)isoindoline-1,3-dione 128

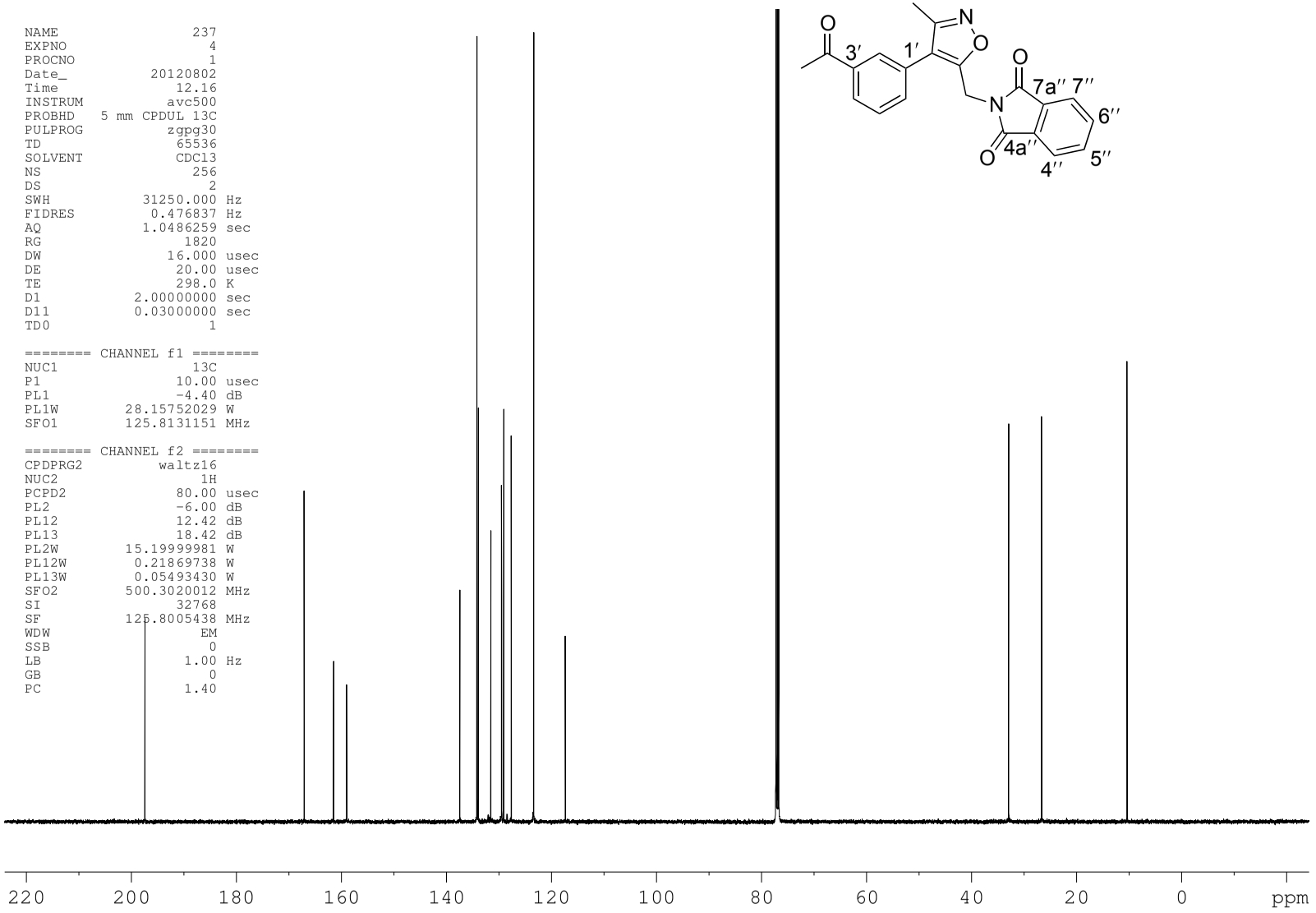



```
NAME                 237
EXPNO                  1
PROCNO                 1
Date_           20120802
Time               11.41
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

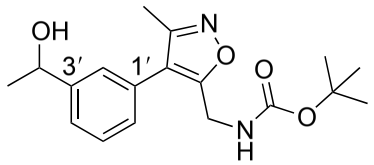
======== CHANNEL f1 ========
NUC1                  1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



2-((4-(3-Acetylphenyl)-3-methylisoxazol-5-yl)methyl)isoindoline-1,3-dione 128



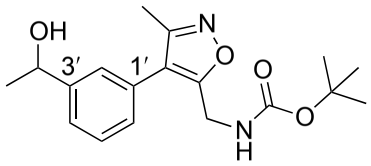
(*RS*)-*tert*-Butyl ((4-(3-(1-hydroxyethyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 79b



NAME	256
EXPNO	1
PROCNO	1
Date_	20120904
Time	14.57
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

(*RS*)-*tert*-Butyl ((4-(3-(1-hydroxyethyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 79b

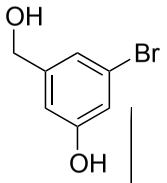



```
NAME                256
EXPNO                 4
PROCNO                1
Date_          20120905
Time               1.34
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

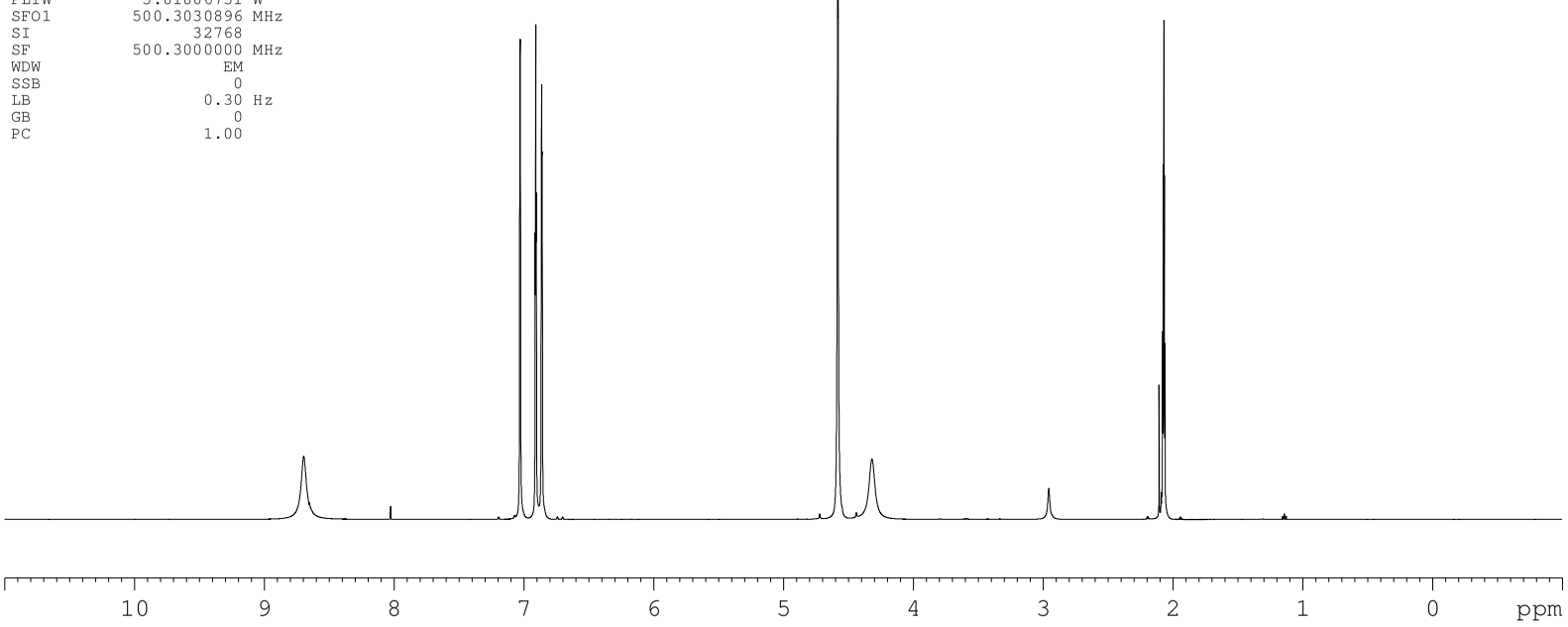
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

3-Bromo-5-(hydroxymethyl)phenol 134

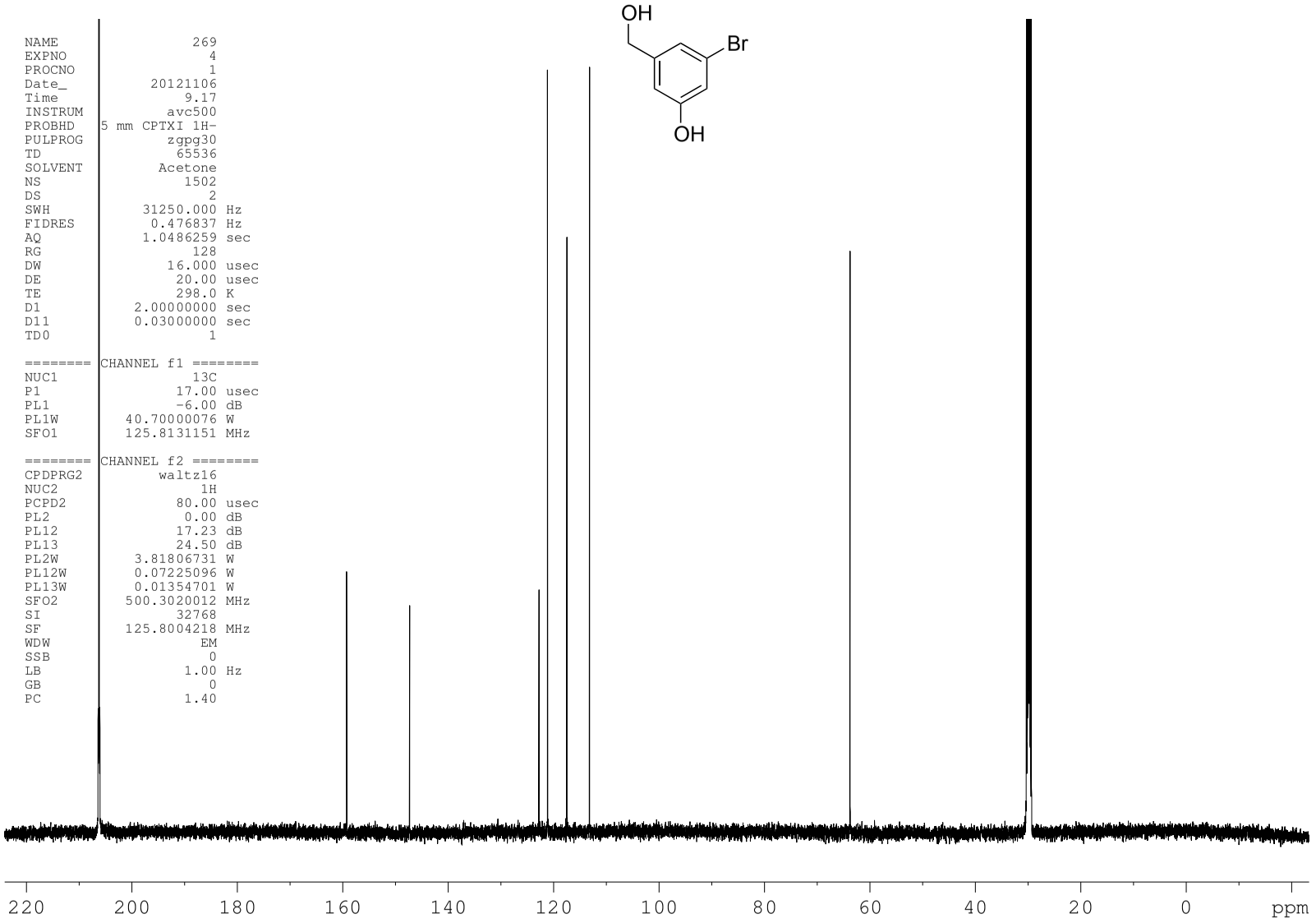


NAME	269
EXPNO	1
PROCNO	1
Date_	20121106
Time	6.49
INSTRUM	avc500
PROBHD	5 mm CPTXI 1H-
PULPROG	zg30
TD	65536
SOLVENT	Acetone
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	22.6
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========	
NUC1	1H
P1	11.00 usec
PL1	0.00 dB
PL1W	3.81806731 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

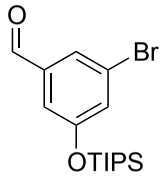


3-Bromo-5-(hydroxymethyl)phenol 134

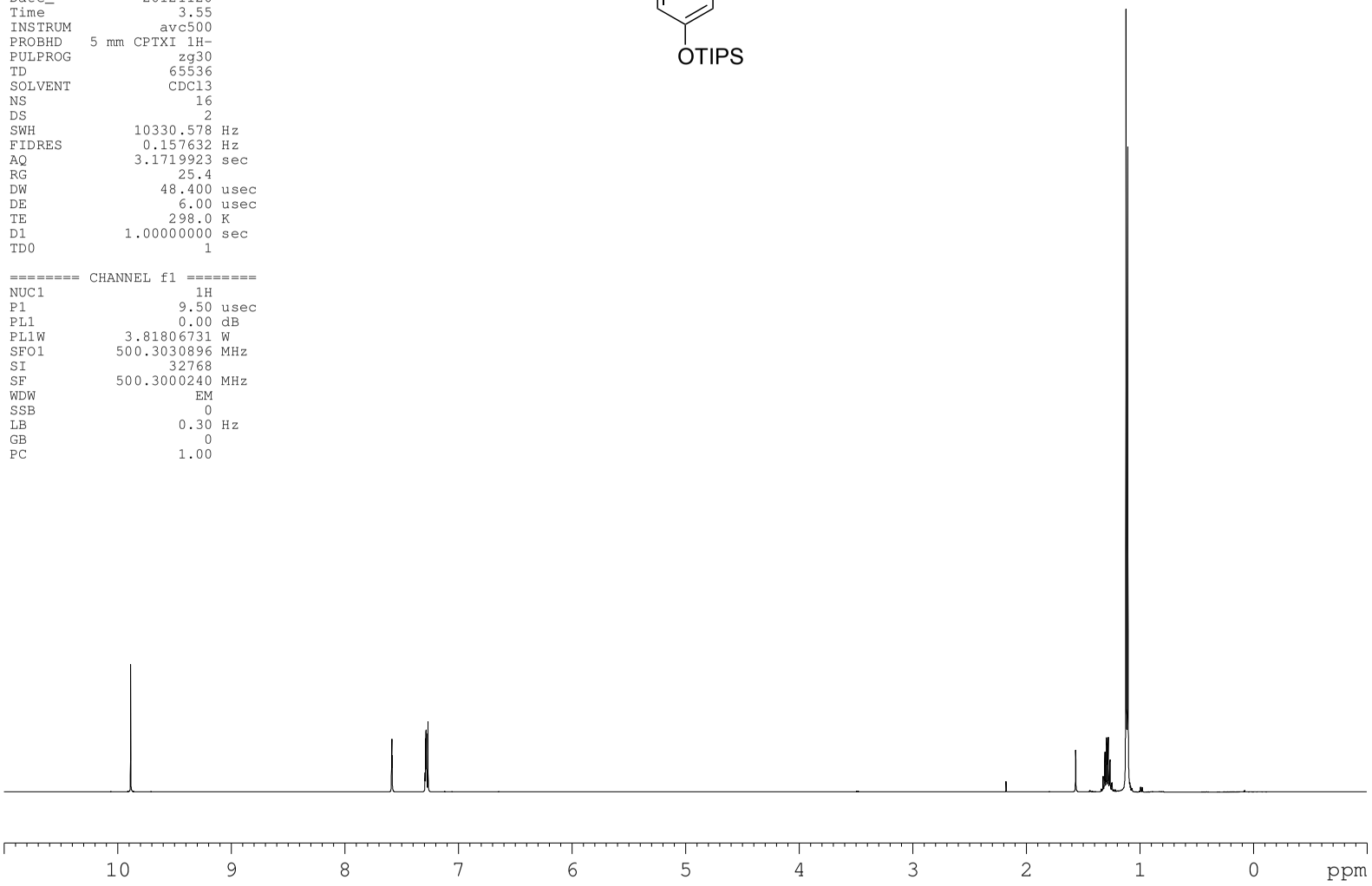


NAME	269
EXPNO	4
PROCNO	1
Date_	20121106
Time	9.17
INSTRUM	avc500
PROBHD	5 mm CPTXI 1H-
PULPROG	zgpg30
TD	65536
SOLVENT	Acetone
NS	1502
DS	2
SWH	31250.000 Hz
FIDRES	0.476837 Hz
AQ	1.0486259 sec
RG	128
DW	16.000 usec
DE	20.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
========	CHANNEL f1 ========
NUC1	13C
P1	17.00 usec
PL1	-6.00 dB
PL1W	40.70000076 W
SFO1	125.8131151 MHz
========	CHANNEL f2 ========
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	0.00 dB
PL12	17.23 dB
PL13	24.50 dB
PL2W	3.81806731 W
PL12W	0.07225096 W
PL13W	0.01354701 W
SFO2	500.3020012 MHz
SI	32768
SF	125.8004218 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

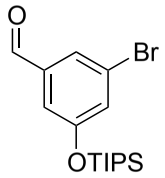
3-Bromo-5-((triisopropylsilyl)oxy)benzaldehyde 135



NAME	278
EXPNO	1
PROCNO	1
Date_	20121120
Time	3.55
INSTRUM	avc500
PROBHD	5 mm CPTXI 1H-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	25.4
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	9.50 usec
PL1	0.00 dB
PL1W	3.81806731 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



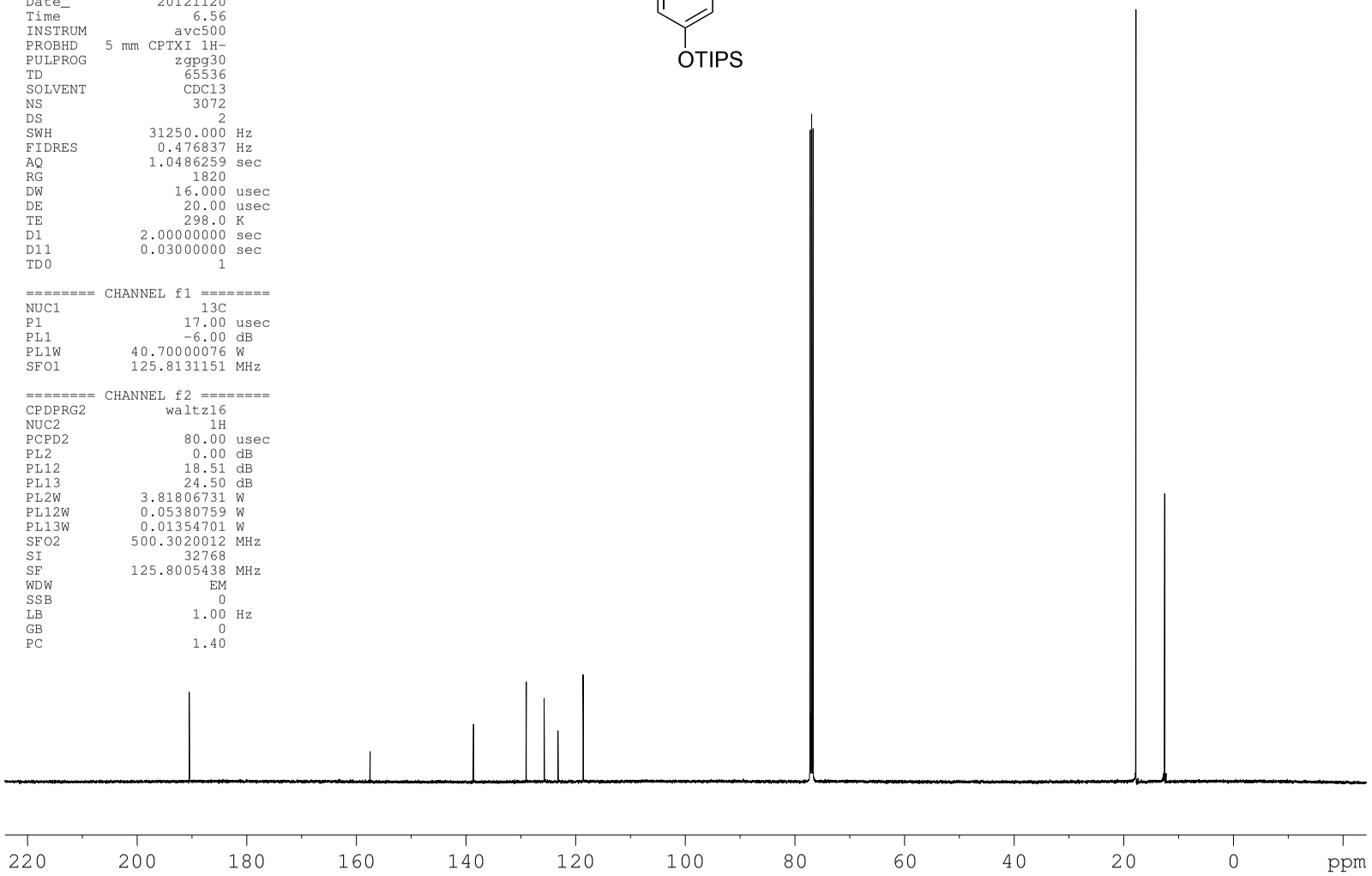
3-Bromo-5-((triisopropylsilyl)oxy)benzaldehyde 135



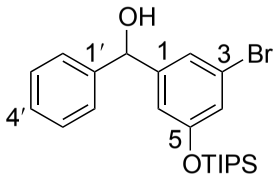

```
NAME                 278
EXPNO                  4
PROCNO                 1
Date_           20121120
Time                6.56
INSTRUM           avc500
PROBHD   5 mm CPTXI 1H-
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  3072
DS                     2
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ             1.0486259 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                 17.00 usec
PL1                -6.00 dB
PL1W         40.70000076 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                 0.00 dB
PL12               18.51 dB
PL13               24.50 dB
PL2W          3.81806731 W
PL12W         0.05380759 W
PL13W         0.01354701 W
SFO2        500.3020012 MHz
SI                 32768
SF          125.8005438 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

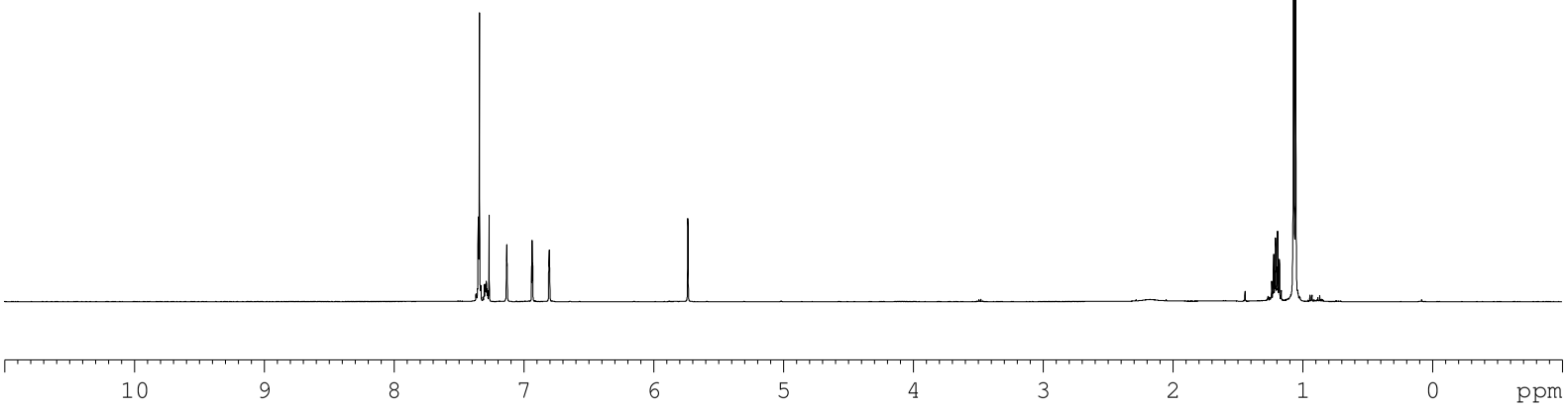


3-Bromo-5-((triisopropylsilyl)oxy)benzaldehyde 136

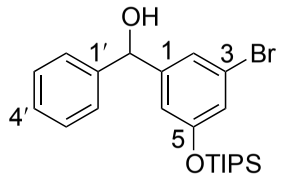


NAME	284
EXPNO	6
PROCNO	1
Date_	20121201
Time	6.46
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.8
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========	
NUC1	1H
P1	10.60 usec
PL1	6.00 dB
PL1W	0.95905519 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



3-Bromo-5-((triisopropylsilyl)oxy)benzaldehyde 136

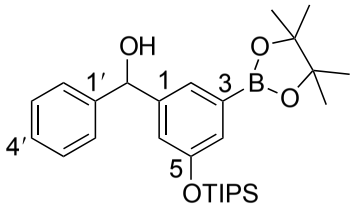



```
NAME                 284
EXPNO                  9
PROCNO                 1
Date_           20121201
Time                8.05
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

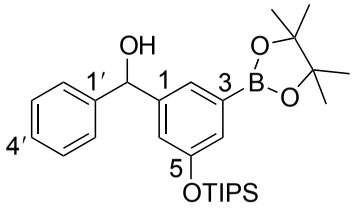
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

(*RS*)-Phenyl(3-(4,4,5,5-tetramethyl-1,3-dioxolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)methanol 137



NAME	317
EXPNO	1
PROCNO	1
Date_	20130311
Time	17.13
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.56
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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

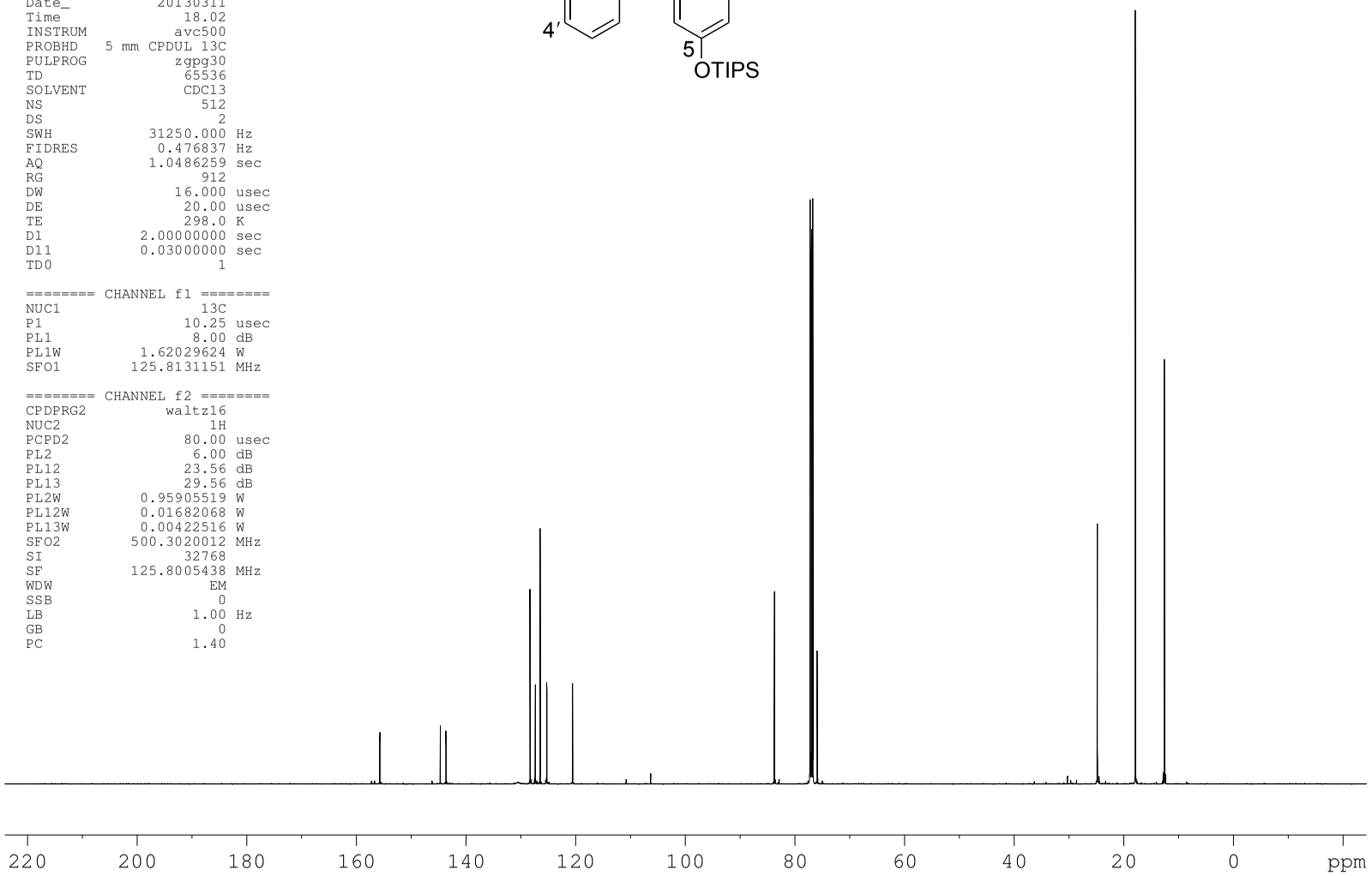
(*RS*)-Phenyl(3-(4,4,5,5-tetramethyl-1,3-dioxolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)methanol 137



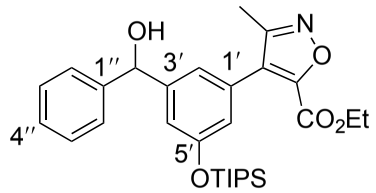

```
NAME                317
EXPNO                 4
PROCNO                1
Date_          20130311
Time              18.02
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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 ========
CPDPRG2          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
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

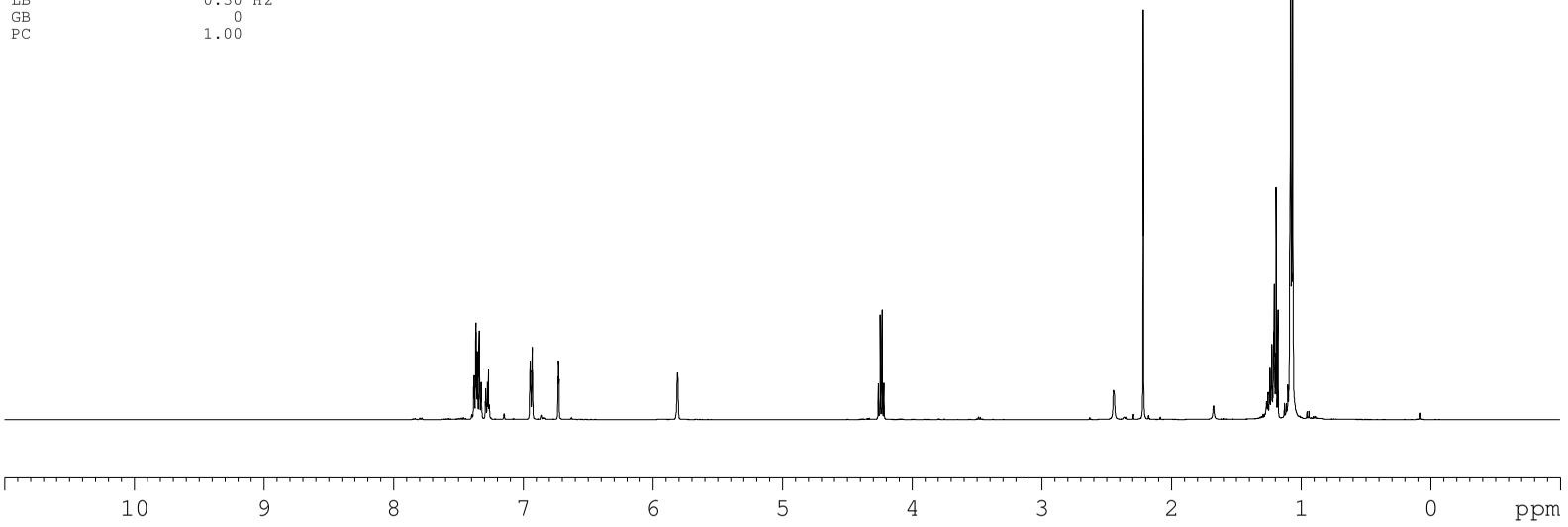


(*RS*)-Ethyl 4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazole-5-carboxylate 138

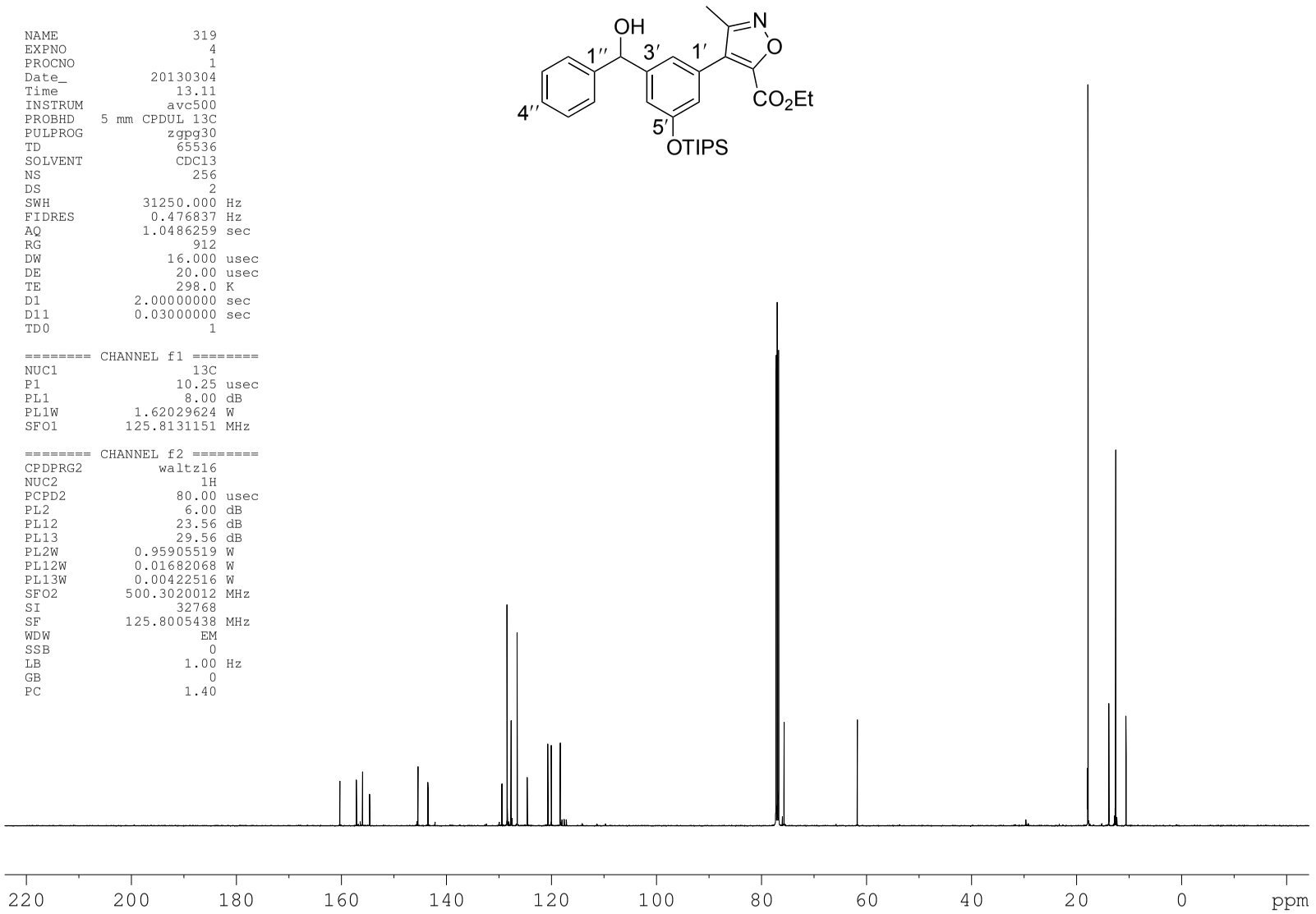



```
NAME                 319
EXPNO                  1
PROCNO                 1
Date_           20130304
Time               12.36
INSTRUM          avc500
PROBHD    5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719923 sec
RG                   2.8
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
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



(*RS*)-Ethyl 4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazole-5-carboxylate 138



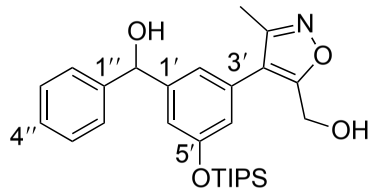

```
NAME                319
EXPNO                 4
PROCNO                1
Date_          20130304
Time              13.11
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

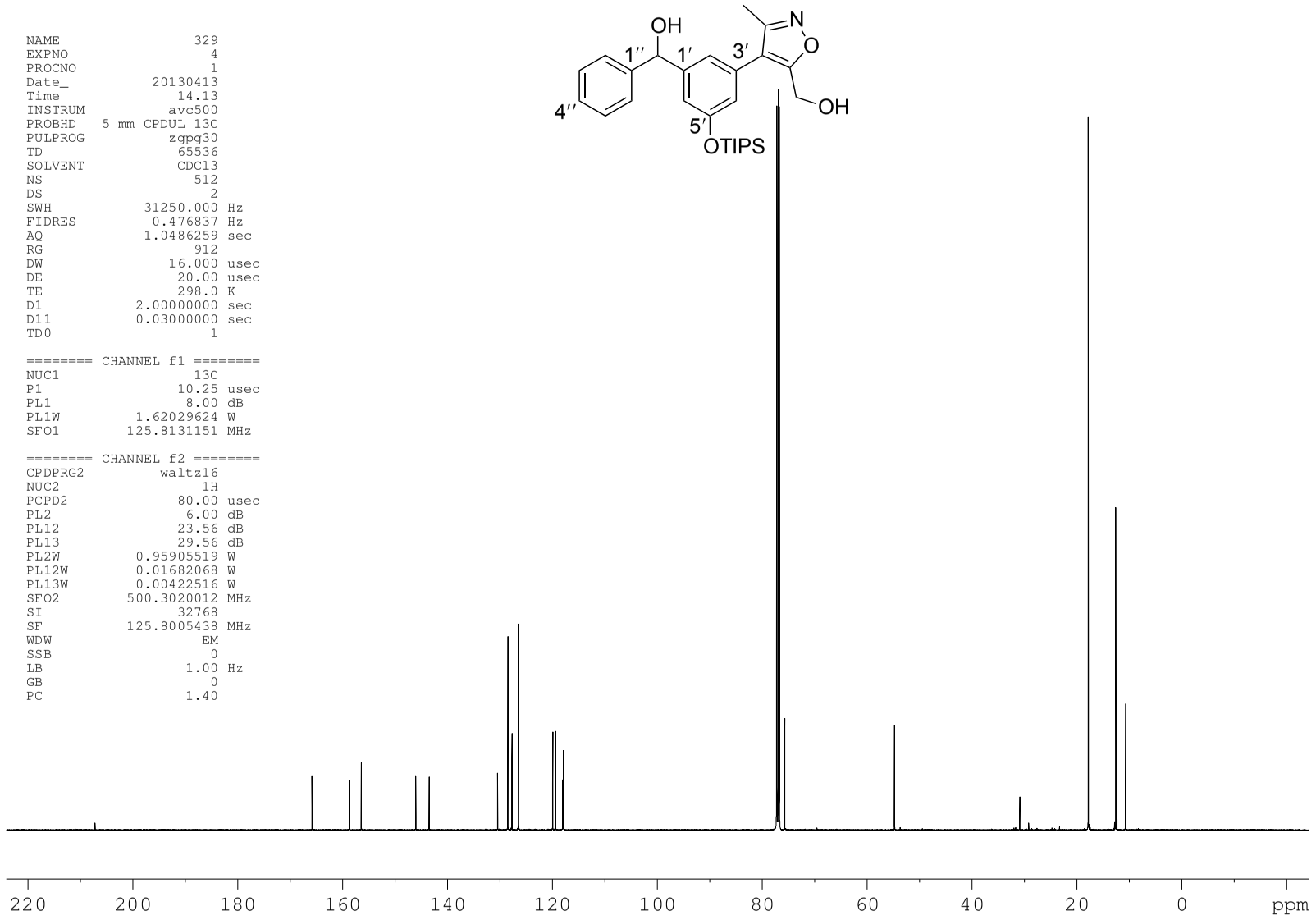
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

(*RS*)-(3-(5-(Hydroxymethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol 139

NAME	329
EXPNO	1
PROCNO	1
Date_	20130413
Time	13.24
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.8
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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-(3-(5-(Hydroxymethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol 139

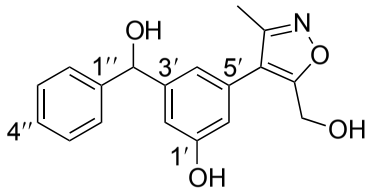



```
NAME                329
EXPNO                 4
PROCNO                1
Date_          20130413
Time              14.13
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

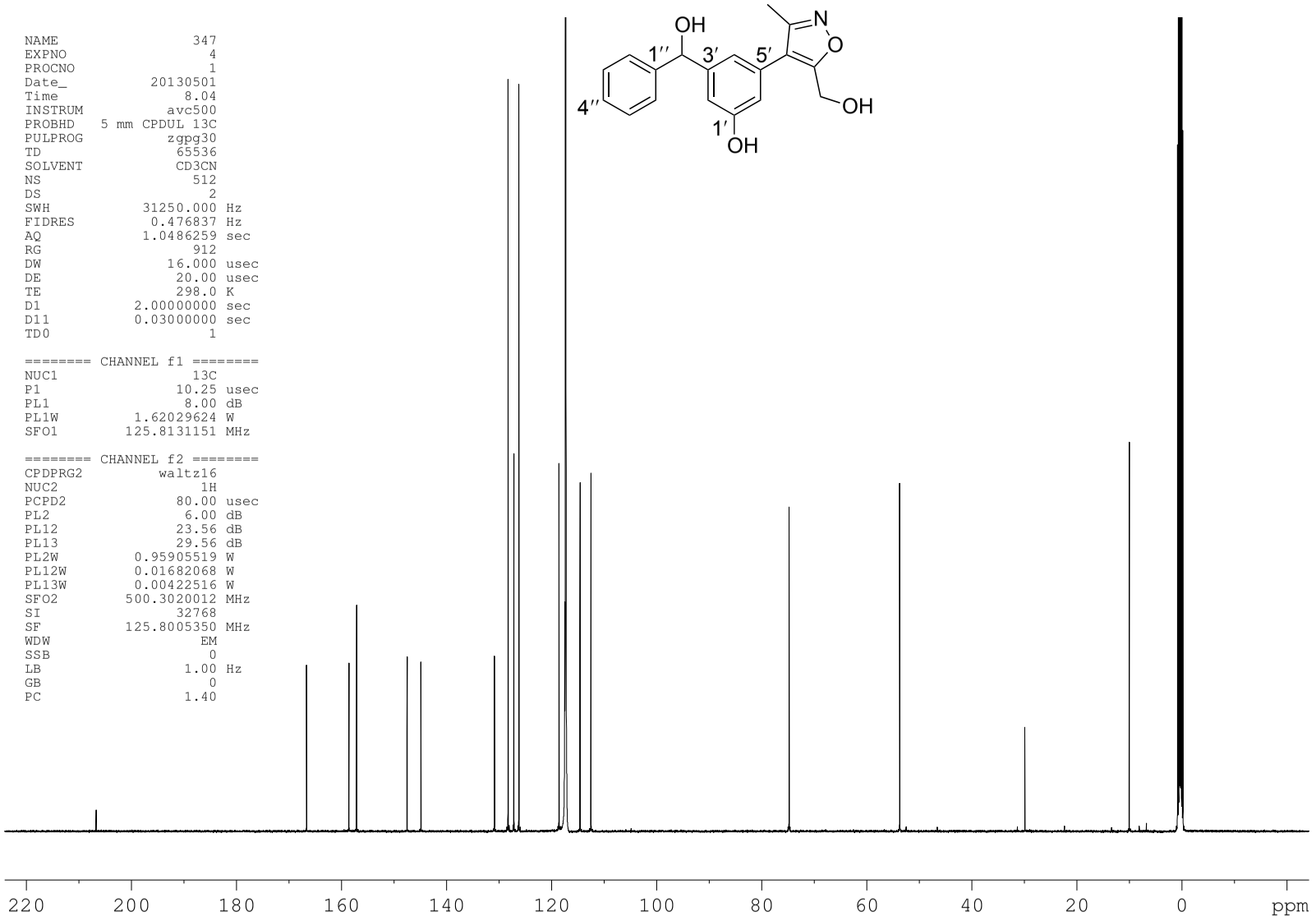
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

(*RS*)-3-(Hydroxy(phenyl)methyl)-5-(5-(hydroxymethyl)-3-methylisoxazol-4-yl)phenol 129a

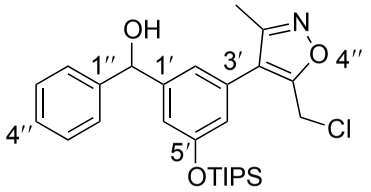


NAME	347
EXPNO	1
PROCNO	1
Date_	20130501
Time	7.15
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CD3CN
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.56
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.60 usec
PL1	6.00 dB
PL1W	0.95905519 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

(*RS*)-3-(Hydroxy(phenyl)methyl)-5-(5-(hydroxymethyl)-3-methylisoxazol-4-yl)phenol 129a

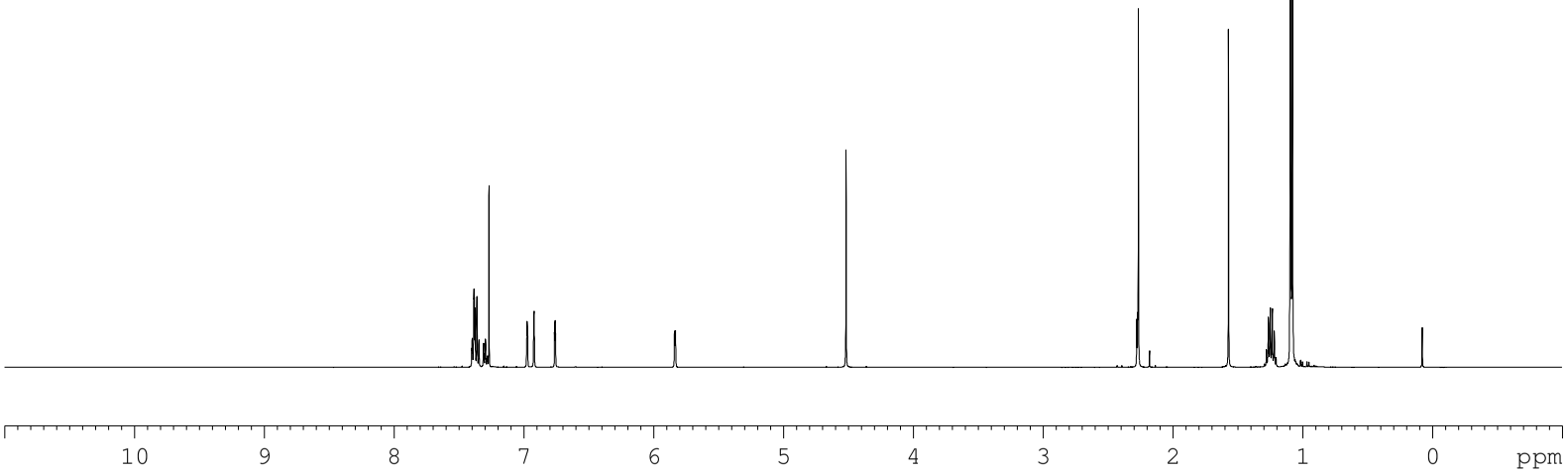


(*RS*)-(3-(5-(Chloromethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol 143

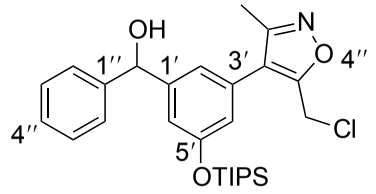


NAME	366
EXPNO	1
PROCNO	1
Date_	20130622
Time	5.17
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	3.2
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========	
NUC1	1H
P1	10.60 usec
PL1	6.00 dB
PL1W	0.95905519 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-(3-(5-(Chloromethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol 143




```
NAME                366
EXPNO                 4
PROCNO                1
Date_          20130622
Time               6.34
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

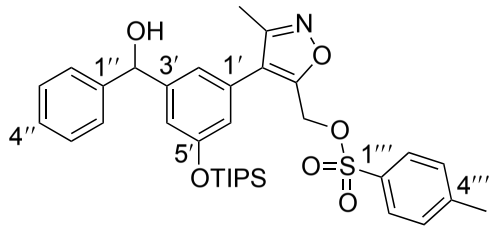
======== CHANNEL f1 ========
NUC1                13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

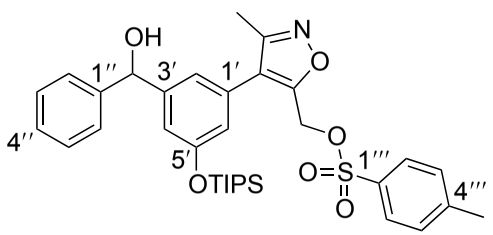
(*RS*)-(4-(3-(Hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl 4-methylbenzenesulfonate 144

```
NAME                 368
EXPNO                  1
PROCNO                 1
Date_           20130623
Time                0.37
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 sec
RG                   2.8
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
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



(*RS*)-(4-(3-(Hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl 4-methylbenzenesulfonate 144

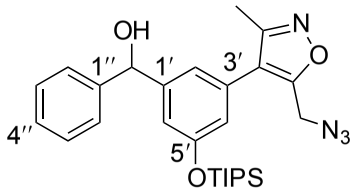



```
NAME                368
EXPNO                 4
PROCNO                1
Date_          20130623
Time               1.12
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

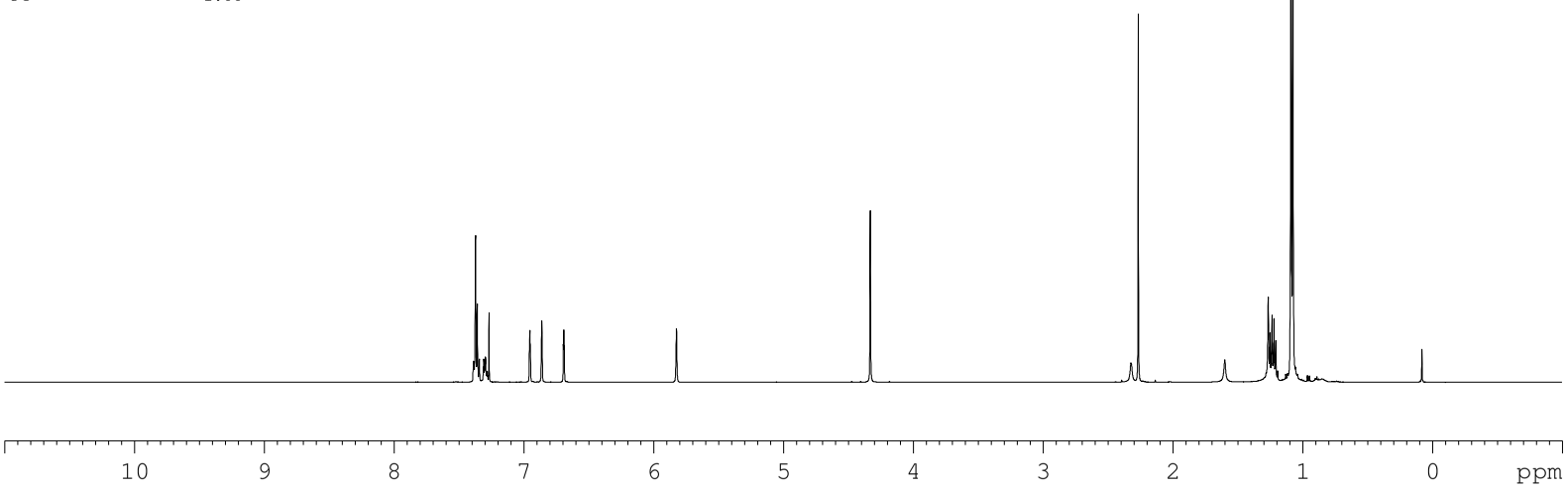
======== CHANNEL f1 ========
NUC1                13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

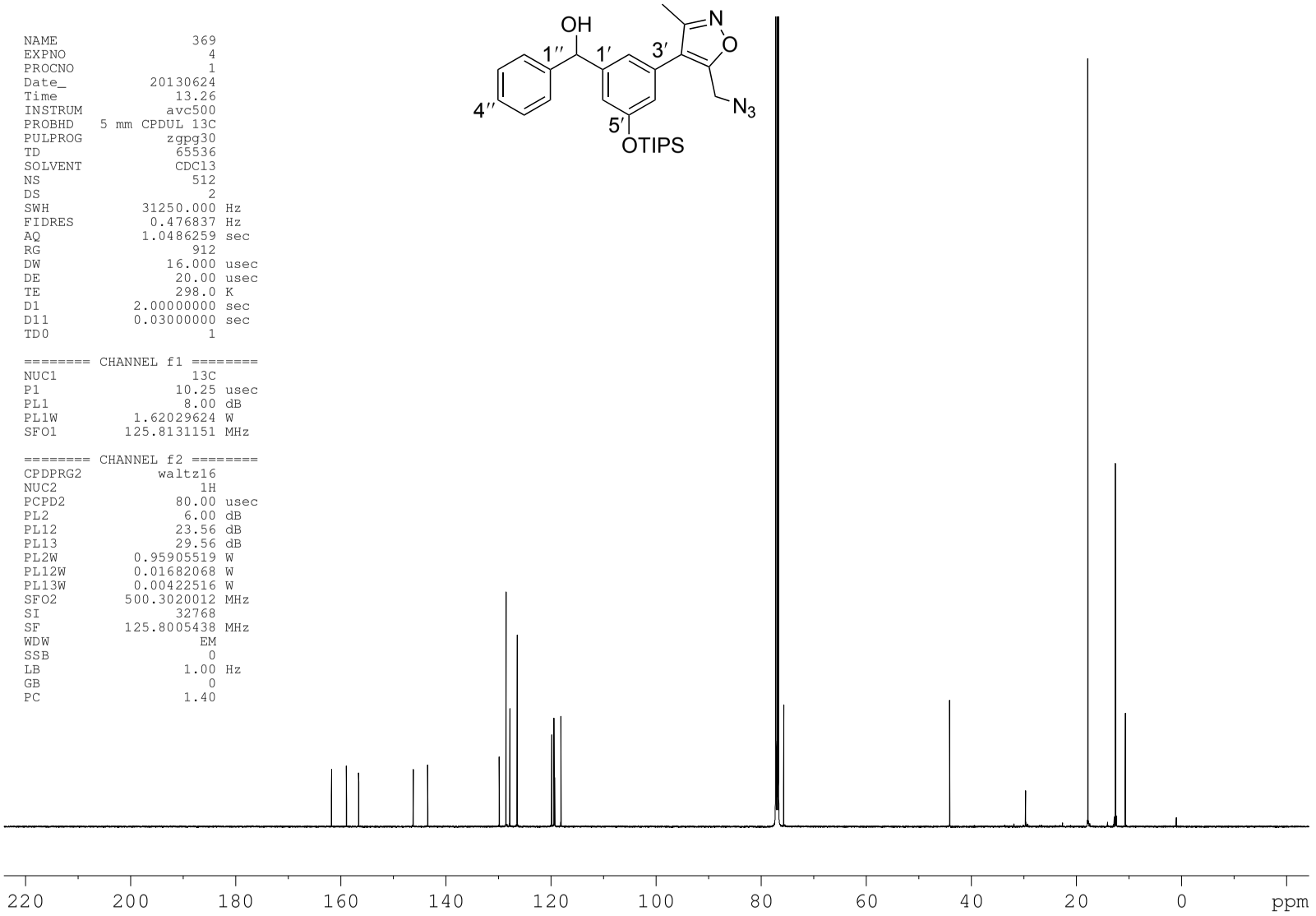
(*RS*)-(3-(5-(Azidomethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol 140



NAME	369
EXPNO	1
PROCNO	1
Date_	20130624
Time	12.37
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-(3-(5-(Azidomethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol 140

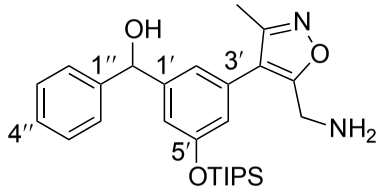



```
NAME                369
EXPNO                 4
PROCNO                1
Date_          20130624
Time              13.26
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

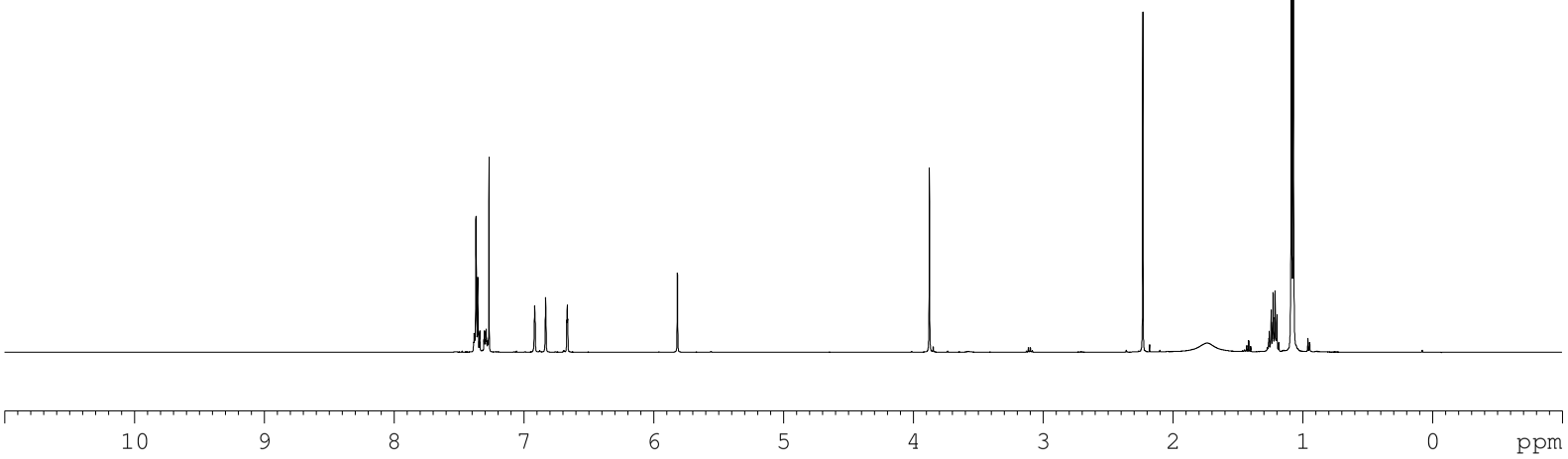
======== CHANNEL f1 ========
NUC1                13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

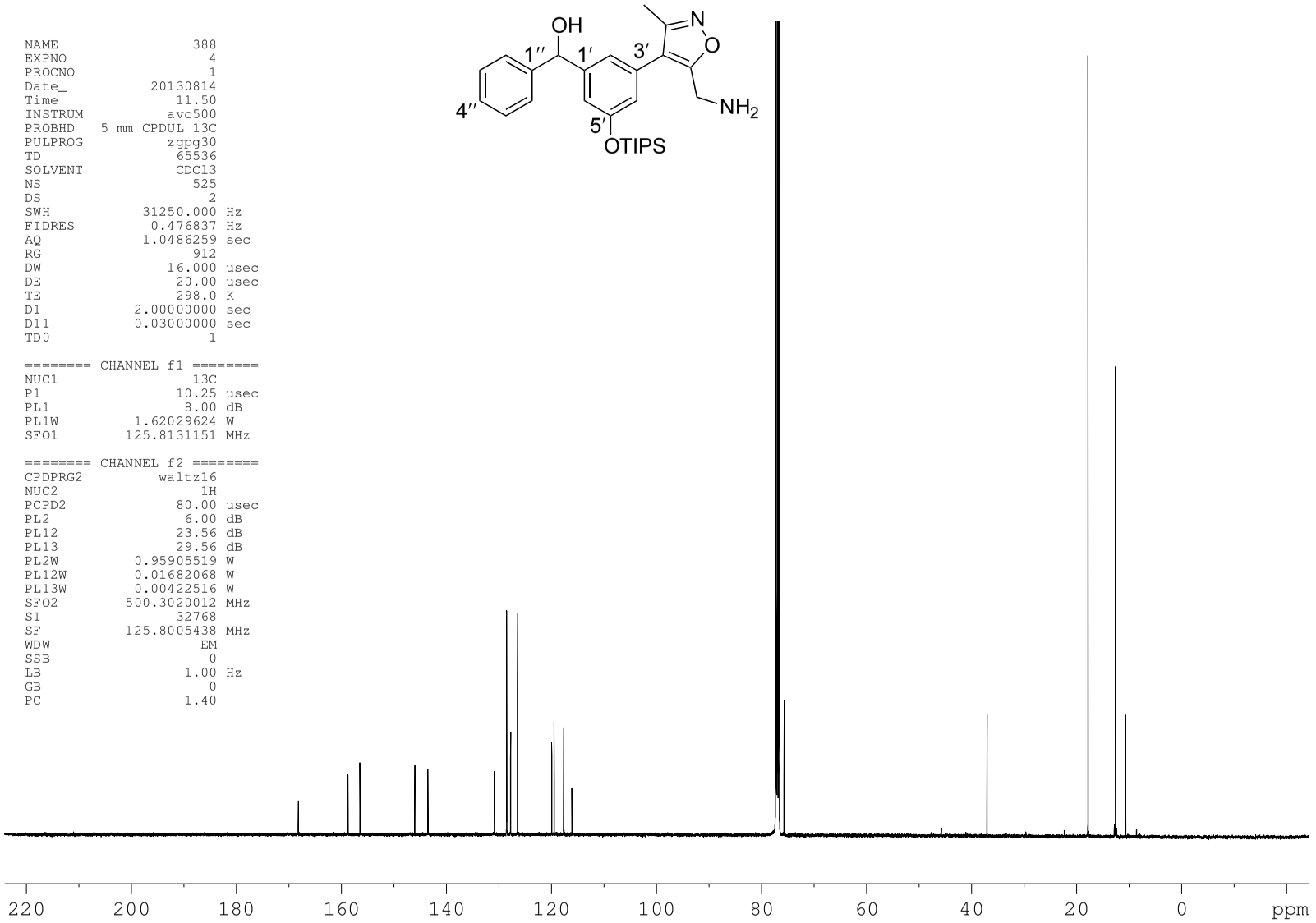
(*RS*)-(3-(5-(Aminomethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol 145



NAME	388
EXPNO	1
PROCNO	1
Date_	20130814
Time	11.02
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.8
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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-(3-(5-(Aminomethyl)-3-methylisoxazol-4-yl)-5-((triisopropylsilyl)oxy)phenyl)(phenyl)methanol 145




```
NAME                388
EXPNO                 4
PROCNO                1
Date_          20130814
Time              11.50
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  525
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

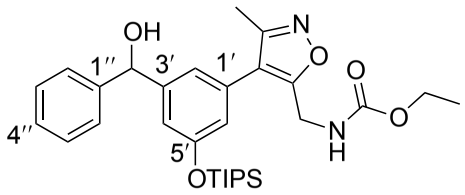
======== CHANNEL f1 ========
NUC1                13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

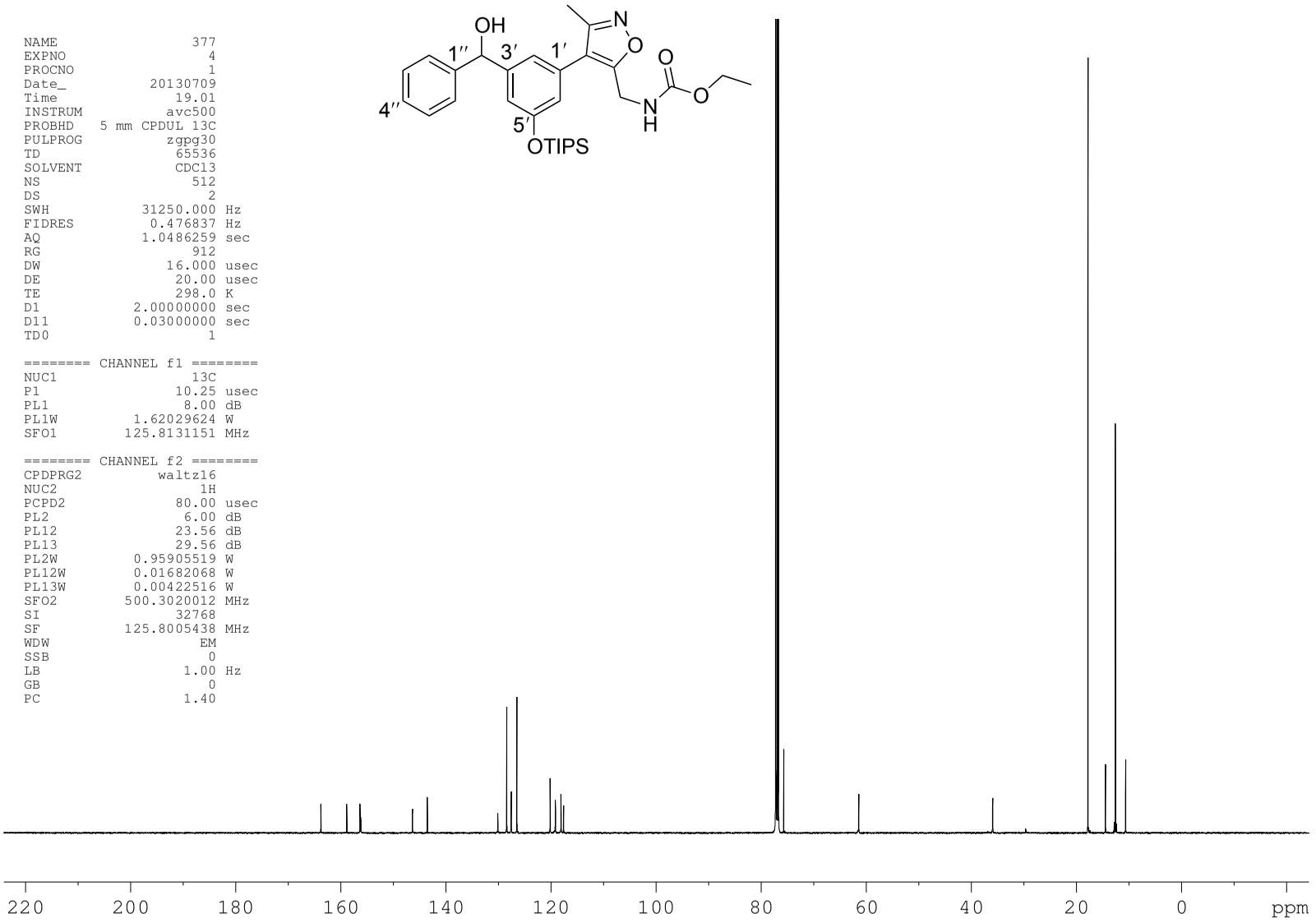
(*RS*)-Ethyl ((4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 146

```
NAME                 377
EXPNO                  1
PROCNO                 1
Date_           20130709
Time               18.12
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 sec
RG                   2.8
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
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



(*RS*)-Ethyl ((4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 146



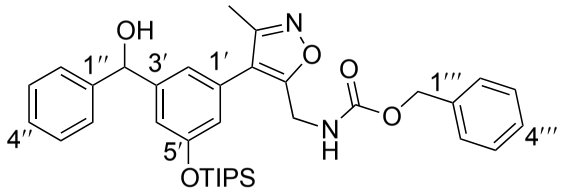

```
NAME                377
EXPNO                 4
PROCNO                1
Date_          20130709
Time              19.01
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

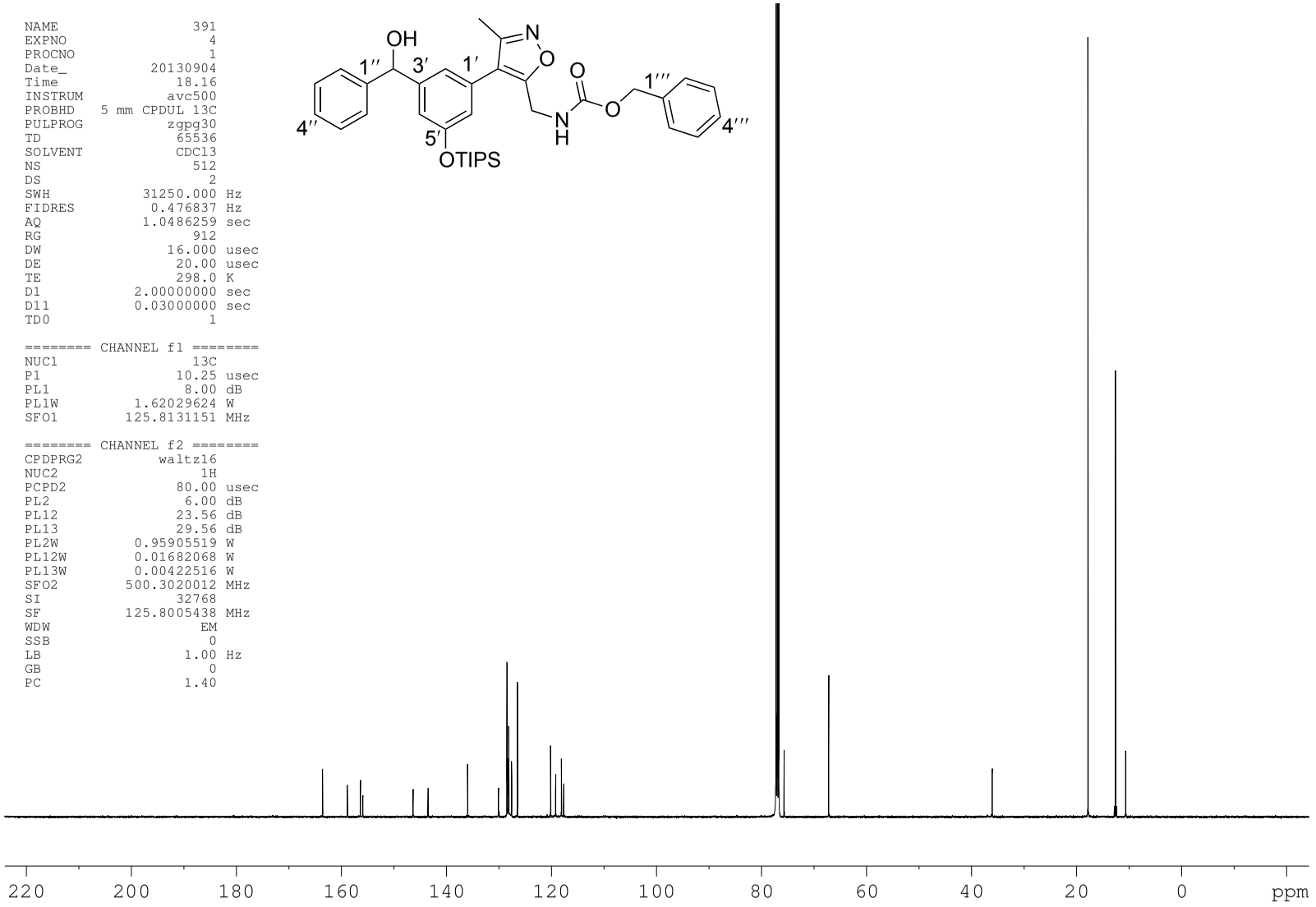
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

(*RS*)-Benzyl ((4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 147

NAME	391
EXPNO	1
PROCNO	1
Date_	20130904
Time	13.56
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-Benzyl ((4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 147

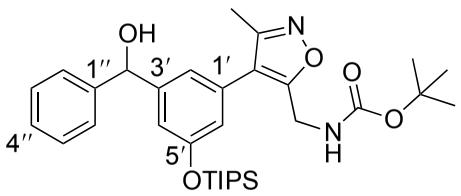



```
NAME                391
EXPNO                 4
PROCNO                1
Date_          20130904
Time              18.16
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

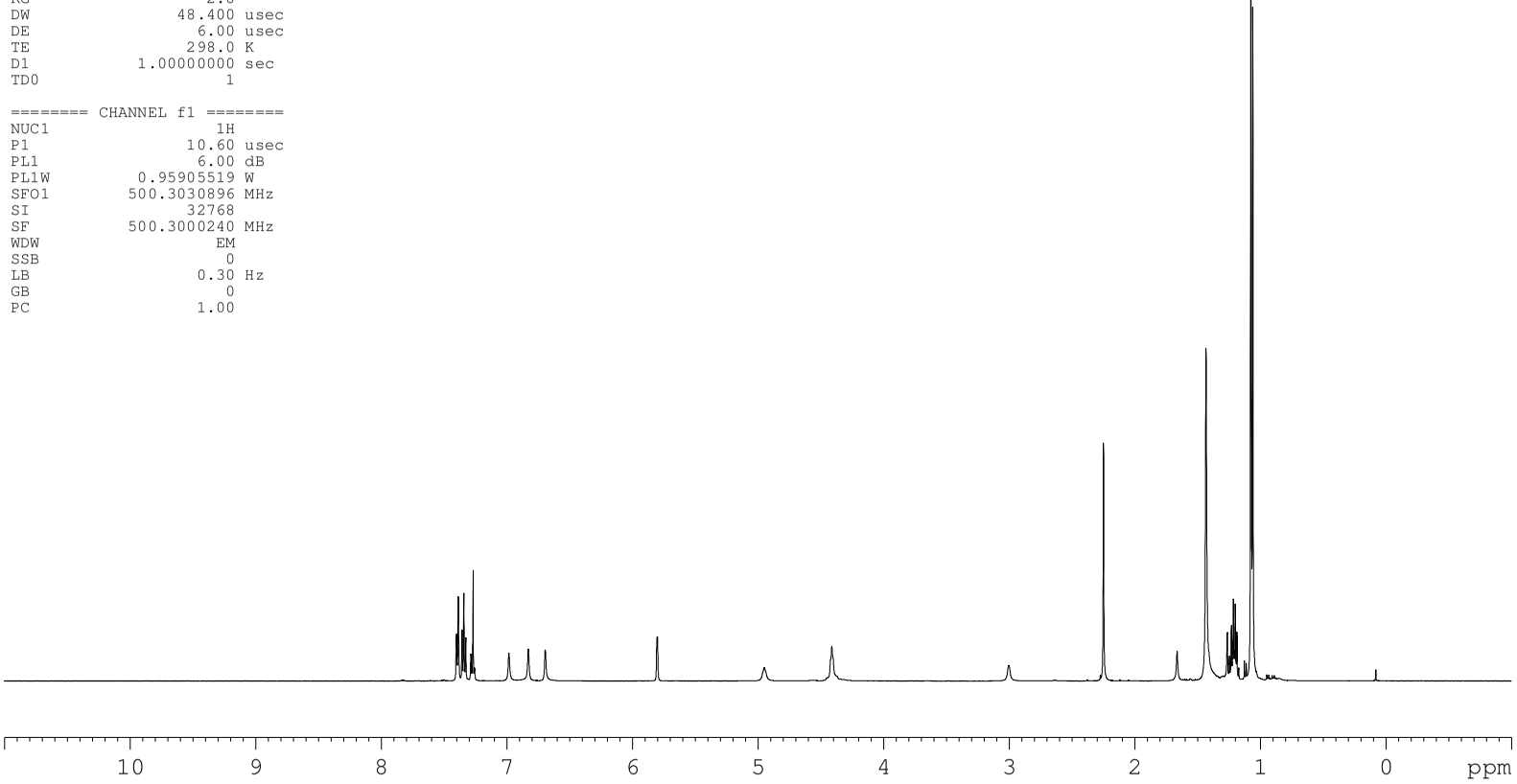
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

(*RS*)-*tert*-Butyl ((4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 148

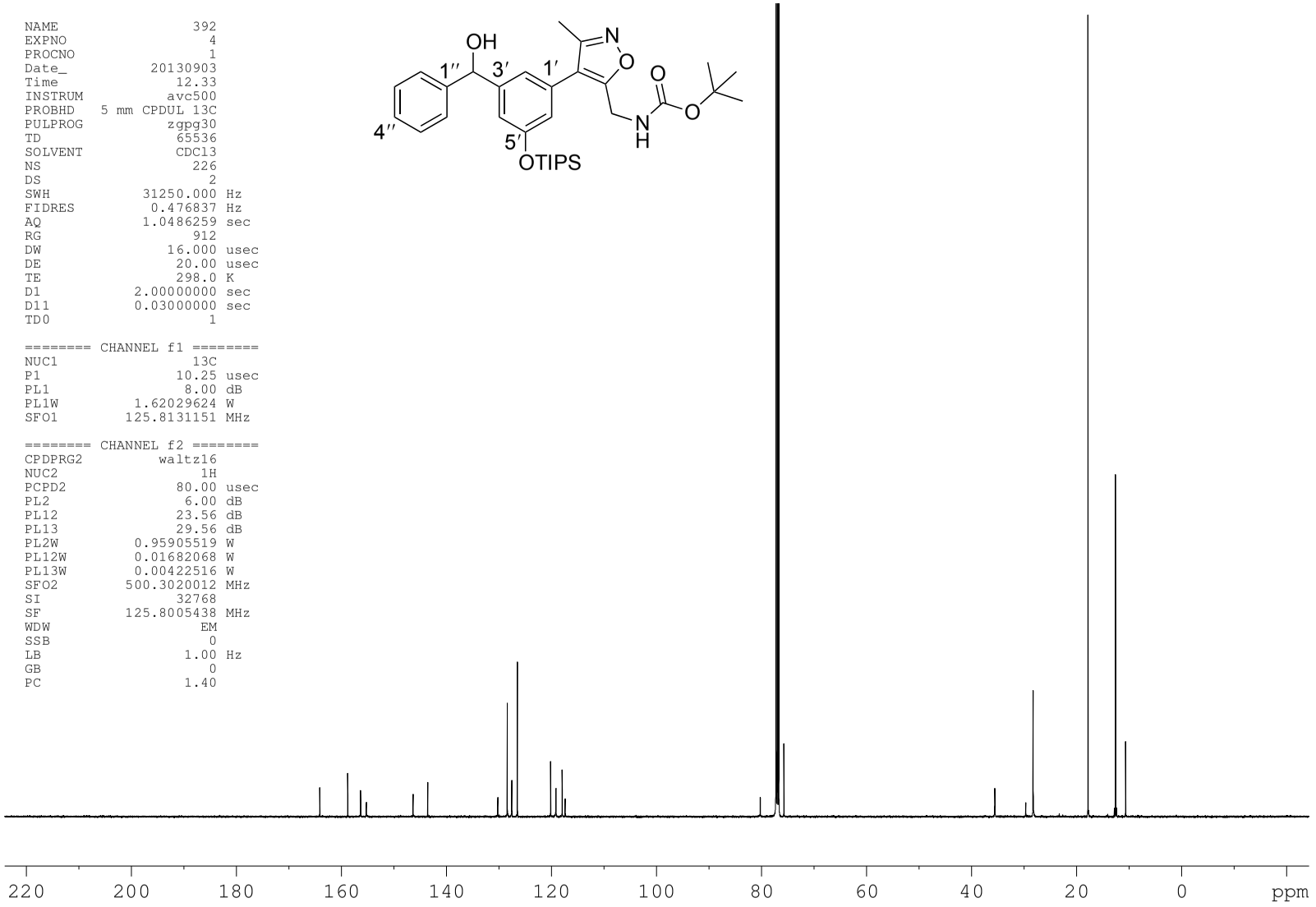



```
NAME                 392
EXPNO                  1
PROCNO                 1
Date_           20130903
Time               11.59
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 sec
RG                   2.8
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
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



(*RS*)-*tert*-Butyl ((4-(3-(hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 148

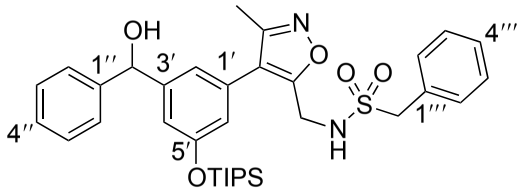



```
NAME                392
EXPNO                 4
PROCNO                1
Date_          20130903
Time              12.33
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  226
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

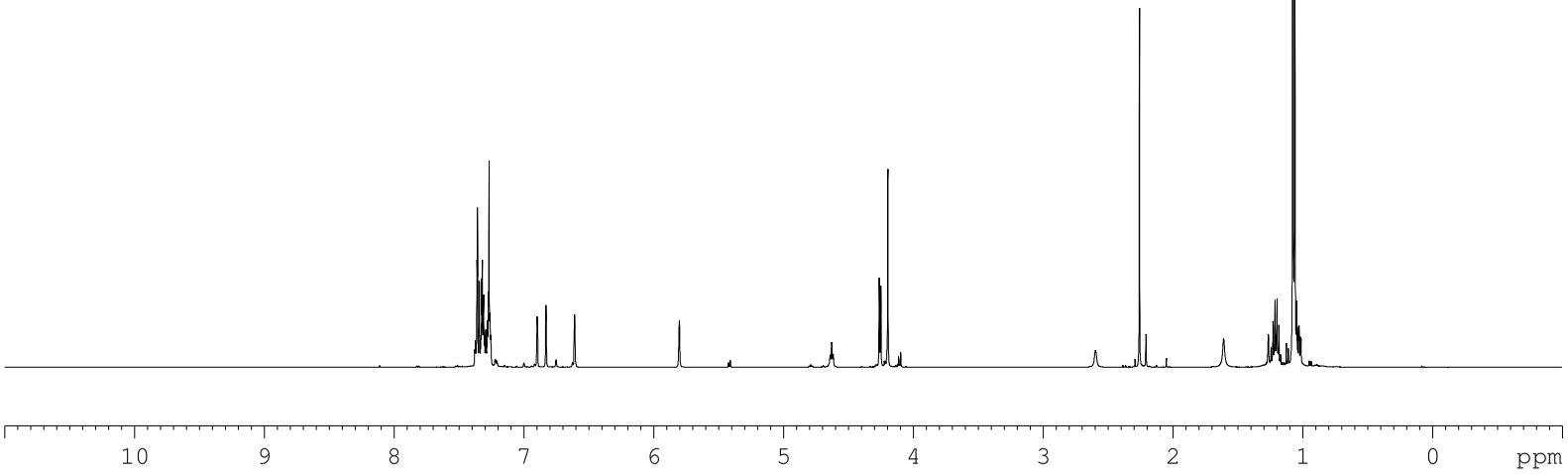
======== CHANNEL f1 ========
NUC1                13C
P1                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          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
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

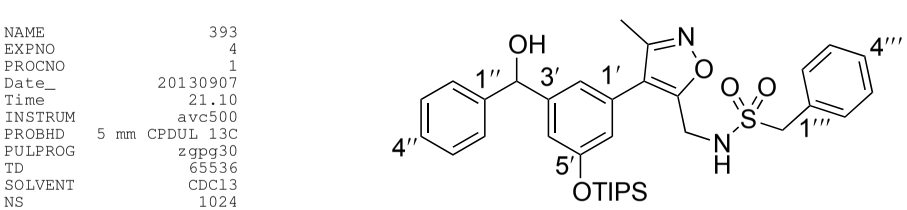
(*RS*)-*N*-((4-(3-(Hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)-1-phenylmethanesulfonamide 149



NAME	393
EXPNO	1
PROCNO	1
Date_	20130907
Time	19.55
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	3.2
DW	48.400 usec
DE	6.00 usec
TE	298.1 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-*N*-((4-(3-(Hydroxy(phenyl)methyl)-5-((triisopropylsilyl)oxy)phenyl)-3-methylisoxazol-5-yl)methyl)-1-phenylmethanesulfonamide 149

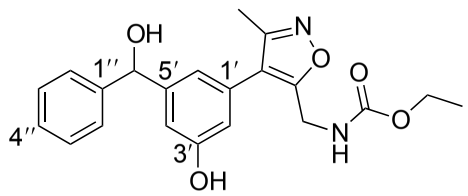



```
NAME                393
EXPNO                 4
PROCNO                1
Date_          20130907
Time              21.10
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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 ========
CPDPRG2          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
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

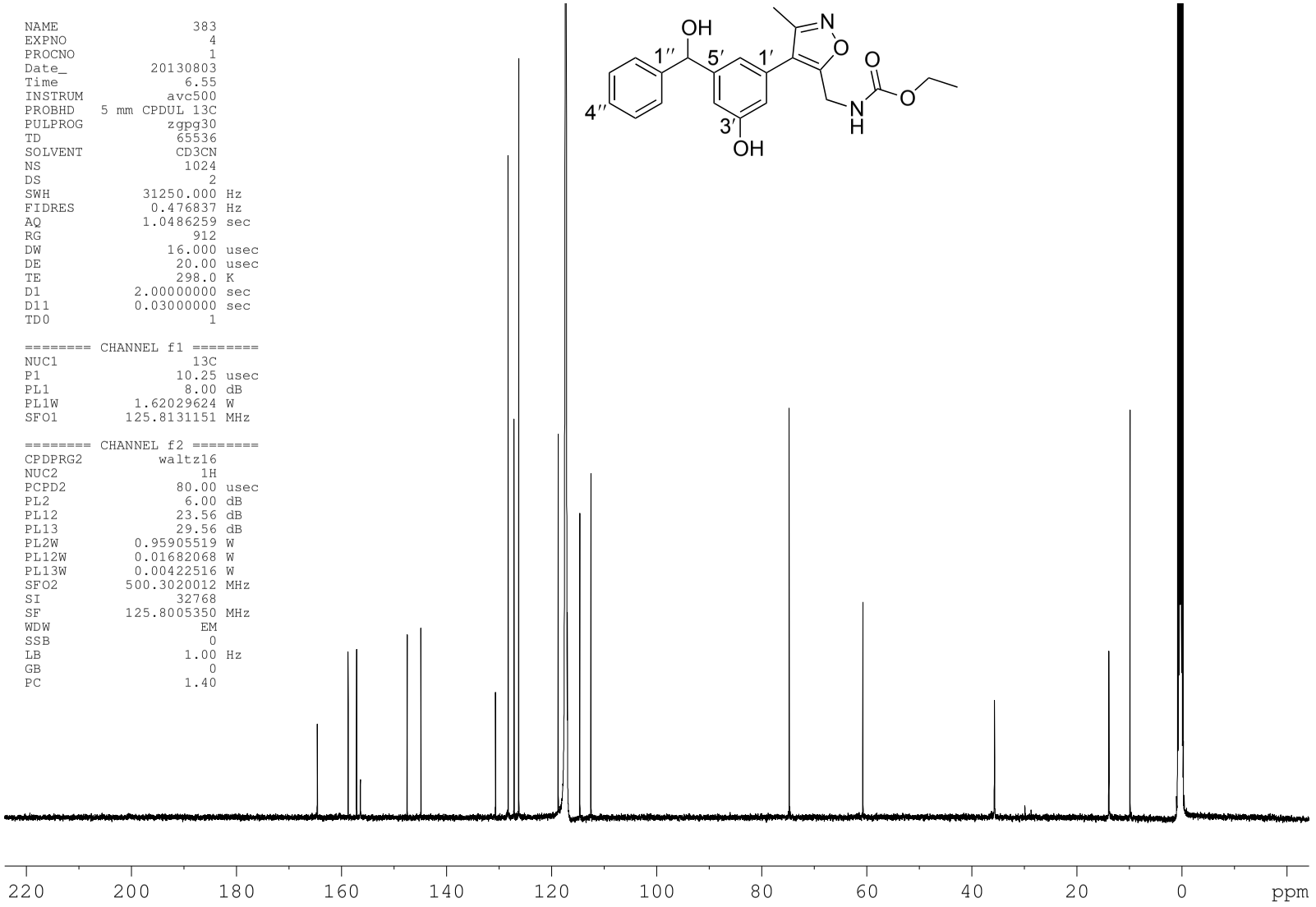
(*RS*)-Ethyl ((4-(3-hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 129b




```
NAME                 383
EXPNO                  1
PROCNO                 1
Date_           20130803
Time                5.39
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CD3CN
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719923 sec
RG                   2.8
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
SI                 32768
SF          500.3000000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

(*RS*)-Ethyl ((4-(3-hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 129b

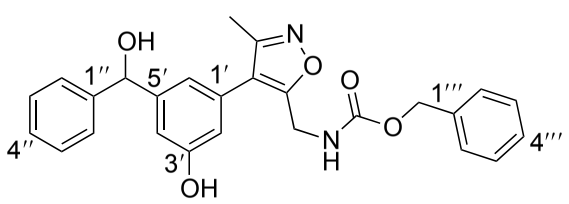



```
NAME                383
EXPNO                 4
PROCNO                1
Date_          20130803
Time               6.55
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CD3CN
NS                 1024
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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

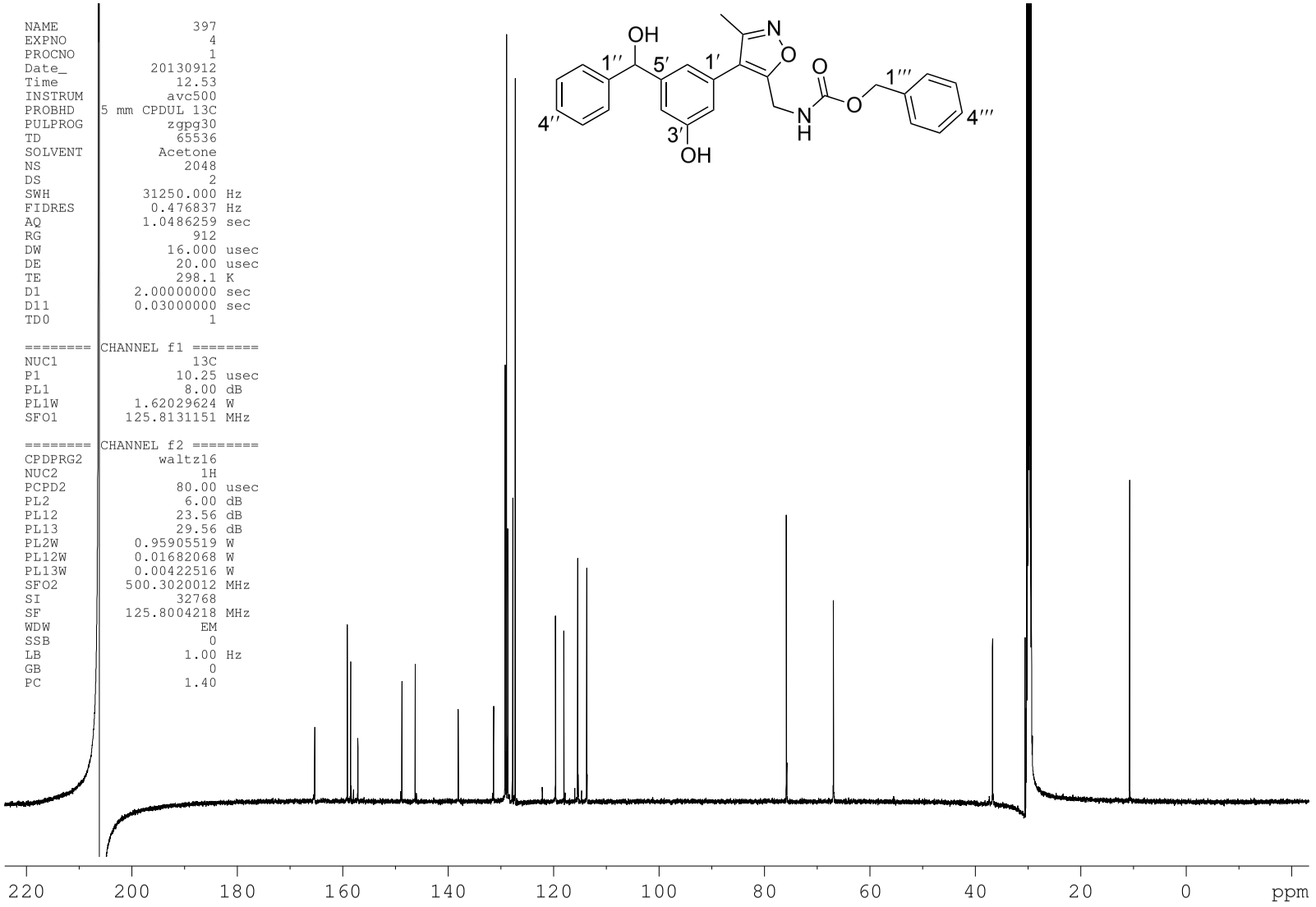
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W         0.95905519 W
PL12W        0.01682068 W
PL13W        0.00422516 W
SFO2        500.3020012 MHz
SI                32768
SF          125.8005350 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

(*RS*)-Benzyl ((4-(3-hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 129c



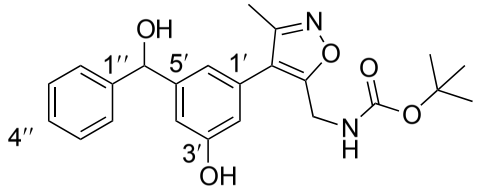
NAME	397
EXPNO	1
PROCNO	1
Date_	20130912
Time	10.44
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	Acetone
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.8
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.60 usec
PL1	6.00 dB
PL1W	0.95905519 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

(*RS*)-Benzyl ((4-(3-hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 129c

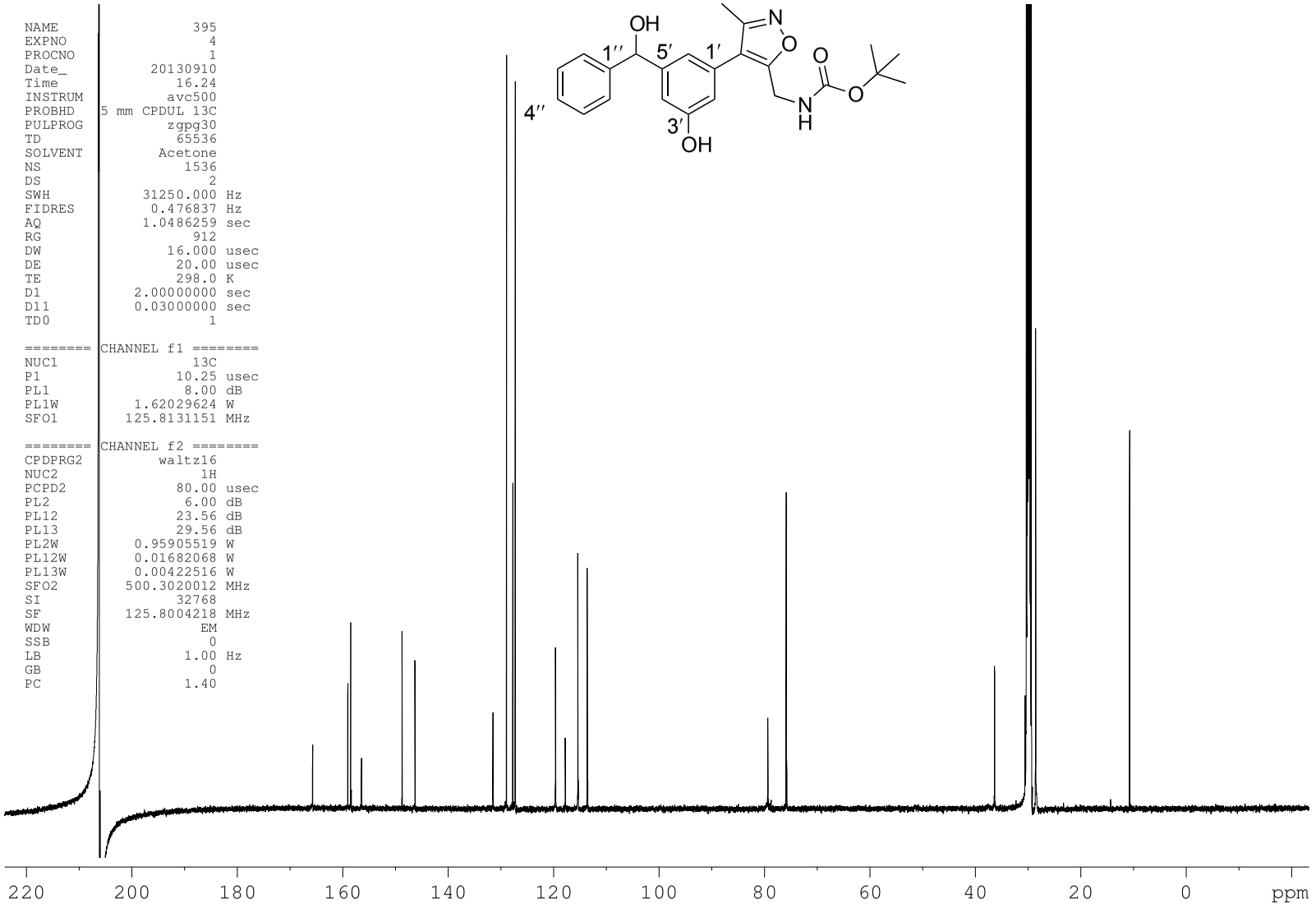


(*RS*)-*tert*-Butyl ((4-(3-hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 129d

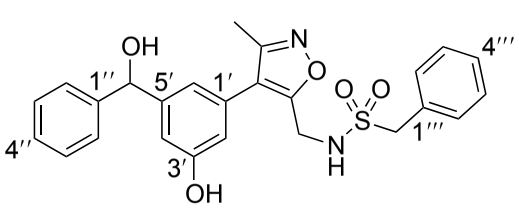
NAME	395
EXPNO	1
PROCNO	1
Date_	20130910
Time	14.41
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	Acetone
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	3.2
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.60 usec
PL1	6.00 dB
PL1W	0.95905519 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*RS*)-*tert*-Butyl ((4-(3-hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)carbamate 129d



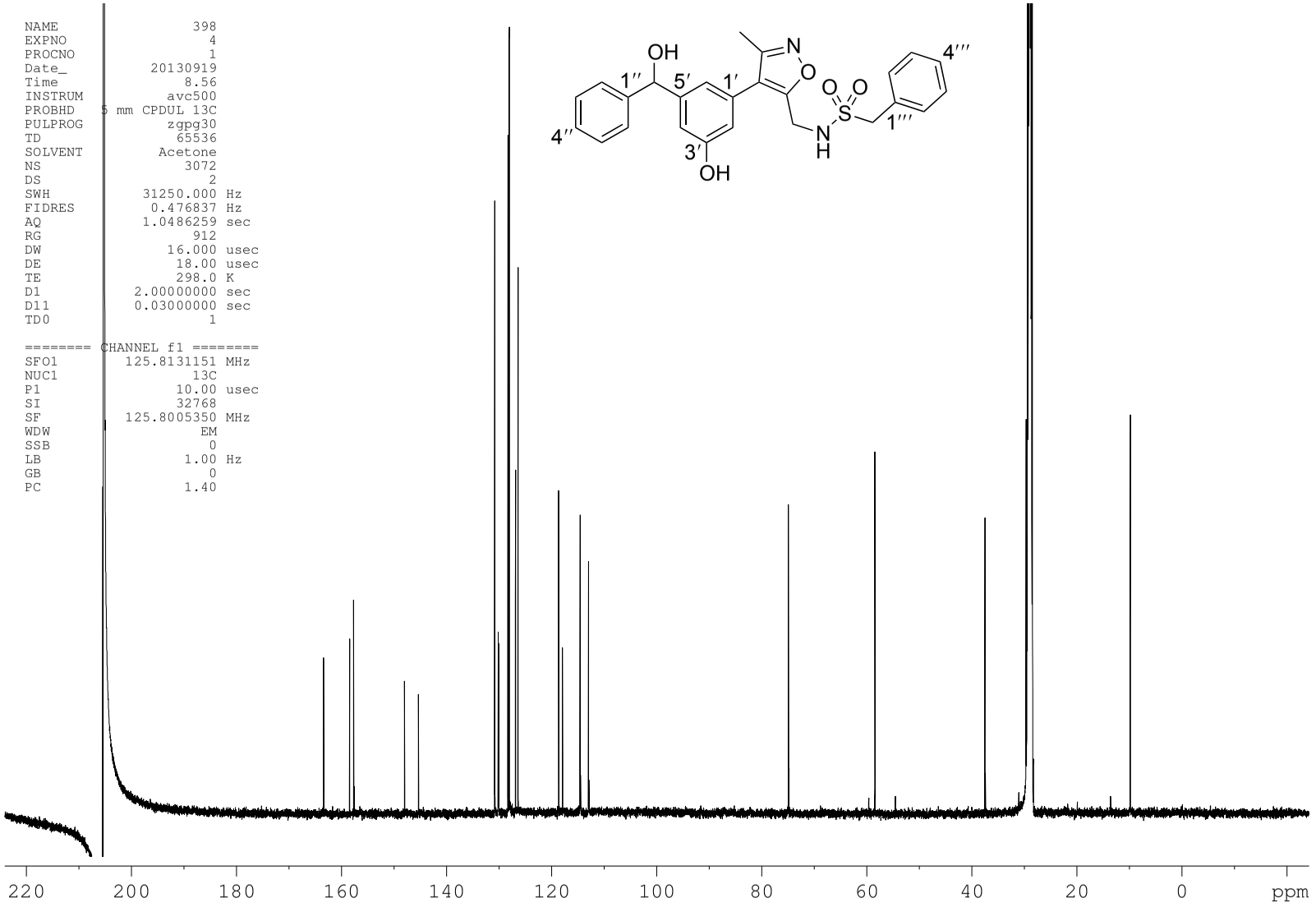
(*RS*)-*N*-((4-(3-Hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)-1-phenylmethanesulfonamide 129e




```
NAME                  398
EXPNO                   1
PROCNO                  1
Date_            20130919
Time                5.56
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              3.1719923 sec
RG                      4
DW                 48.400 usec
DE                 10.00 usec
TE                 298.1 K
D1              1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          500.3030896 MHz
NUC1                   1H
P1                  15.00 usec
SI                  32768
SF            500.3000000 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

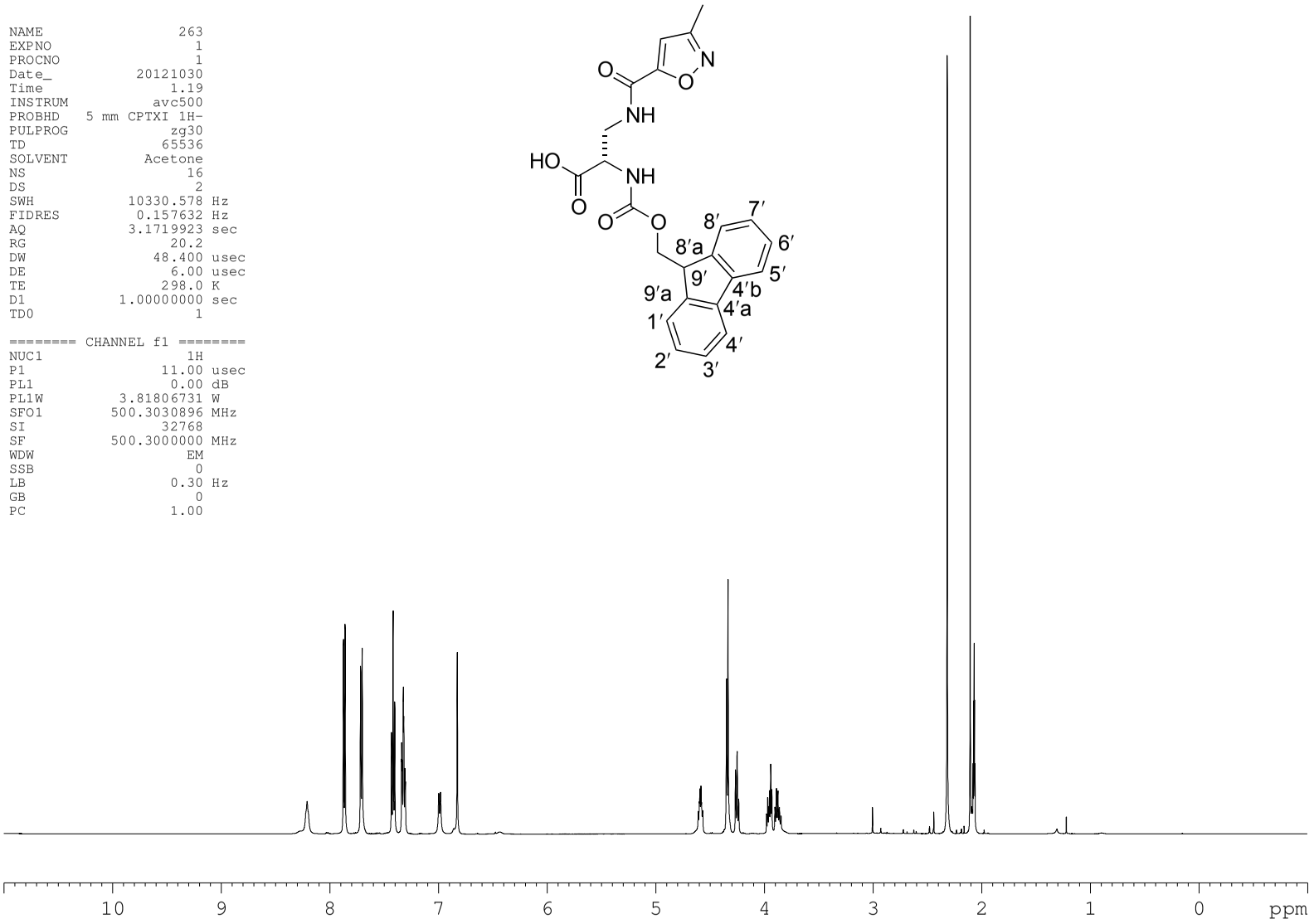
(*RS*)-*N*-((4-(3-Hydroxy-5-(hydroxy(phenyl)methyl)phenyl)-3-methylisoxazol-5-yl)methyl)-1-phenylmethanesulfonamide 129e



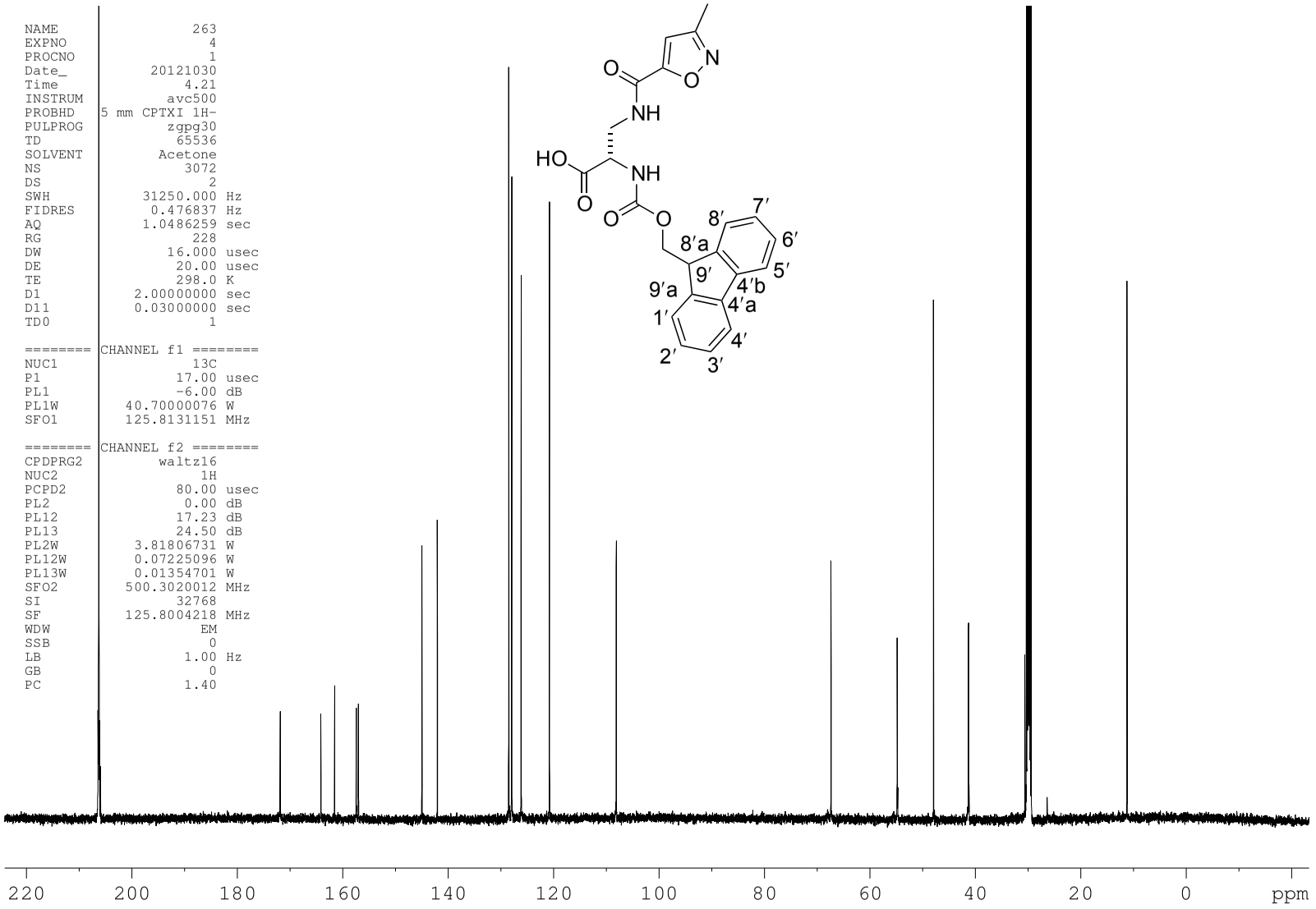
(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(3-methylisoxazole-5-carboxamido)propanoic acid 155

```
NAME                 263
EXPNO                  1
PROCNO                 1
Date_           20121030
Time                1.19
INSTRUM          avc500
PROBHD   5 mm CPTXI 1H-
PULPROG             zg30
TD                 65536
SOLVENT          Acetone
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 sec
RG                  20.2
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

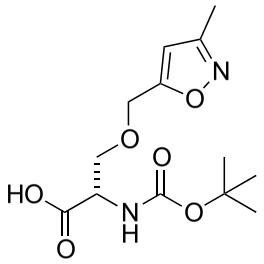
======== CHANNEL f1 ========
NUC1                  1H
P1                 11.00 usec
PL1                 0.00 dB
PL1W          3.81806731 W
SFO1        500.3030896 MHz
SI                 32768
SF          500.3000000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



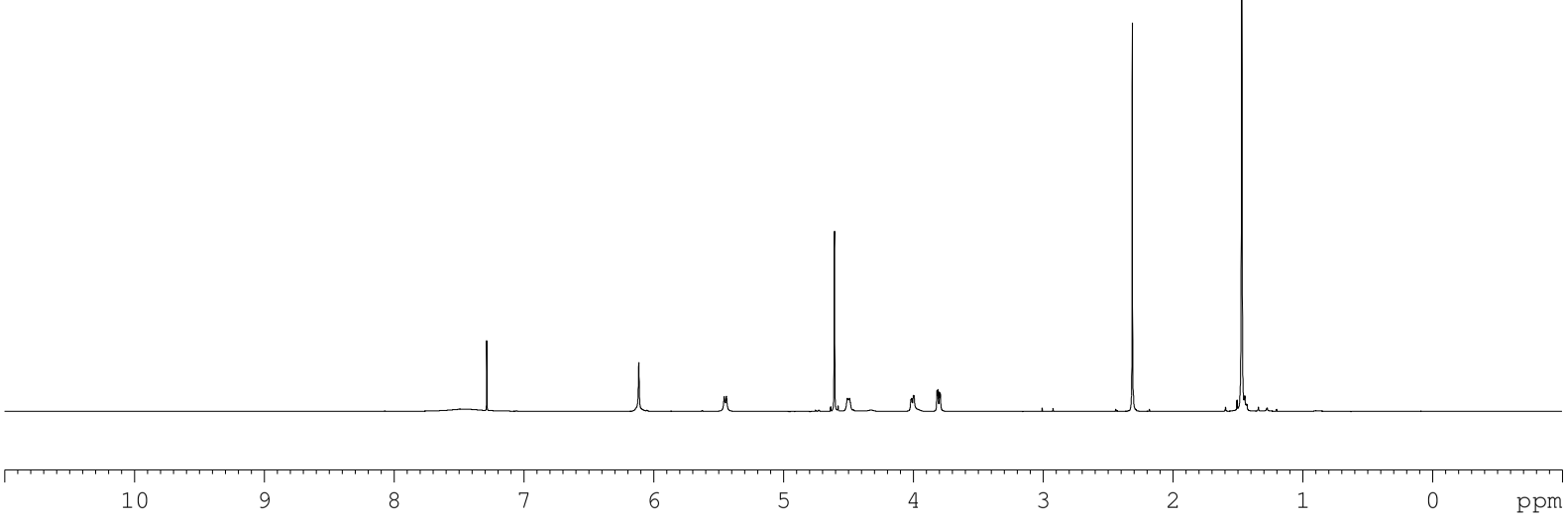
(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(3-methylisoxazole-5-carboxamido)propanoic acid 155



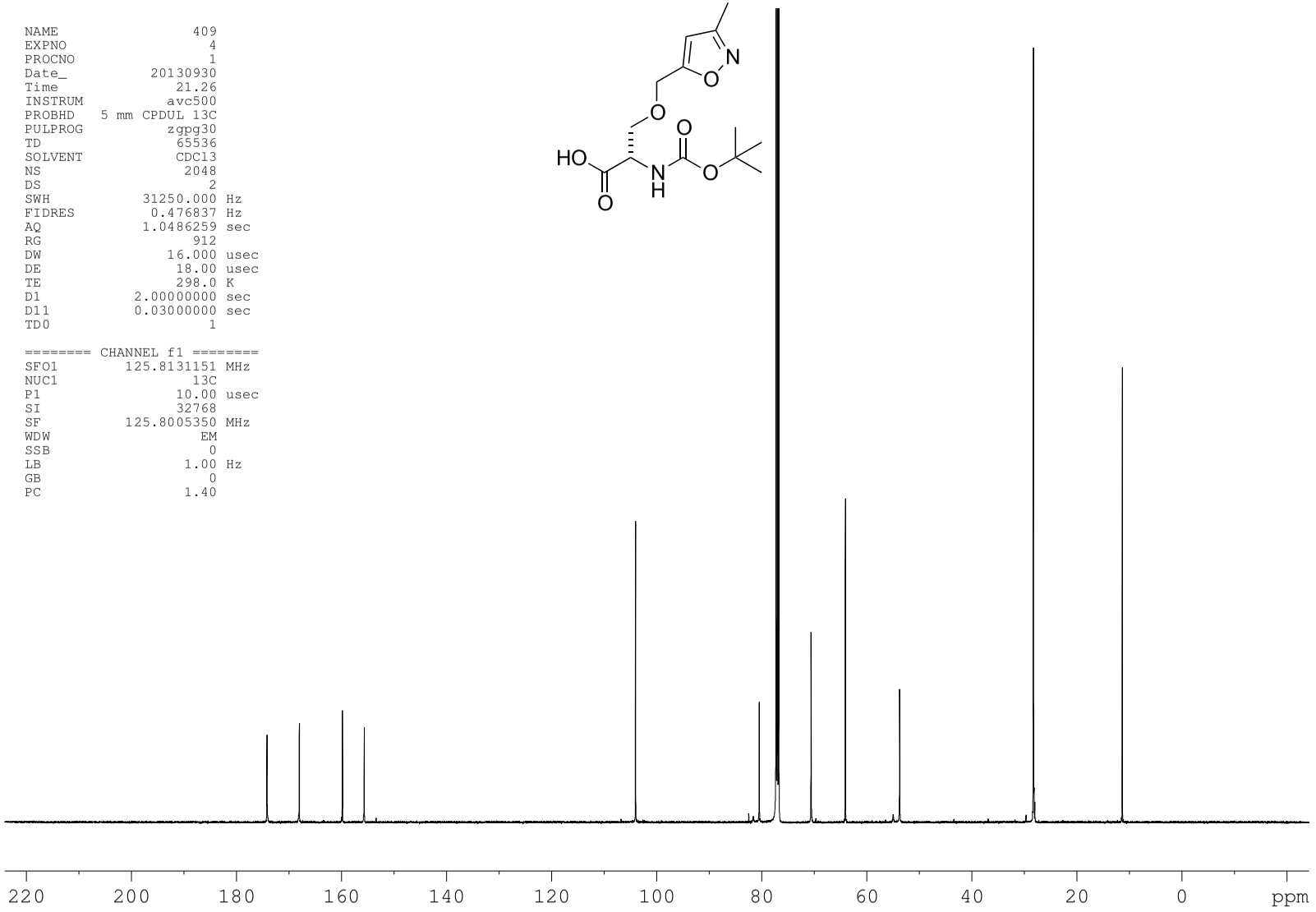
N-(*tert*-Butoxycarbonyl)-*O*-((3-methylisoxazol-5-yl)methyl)-L-serine 166



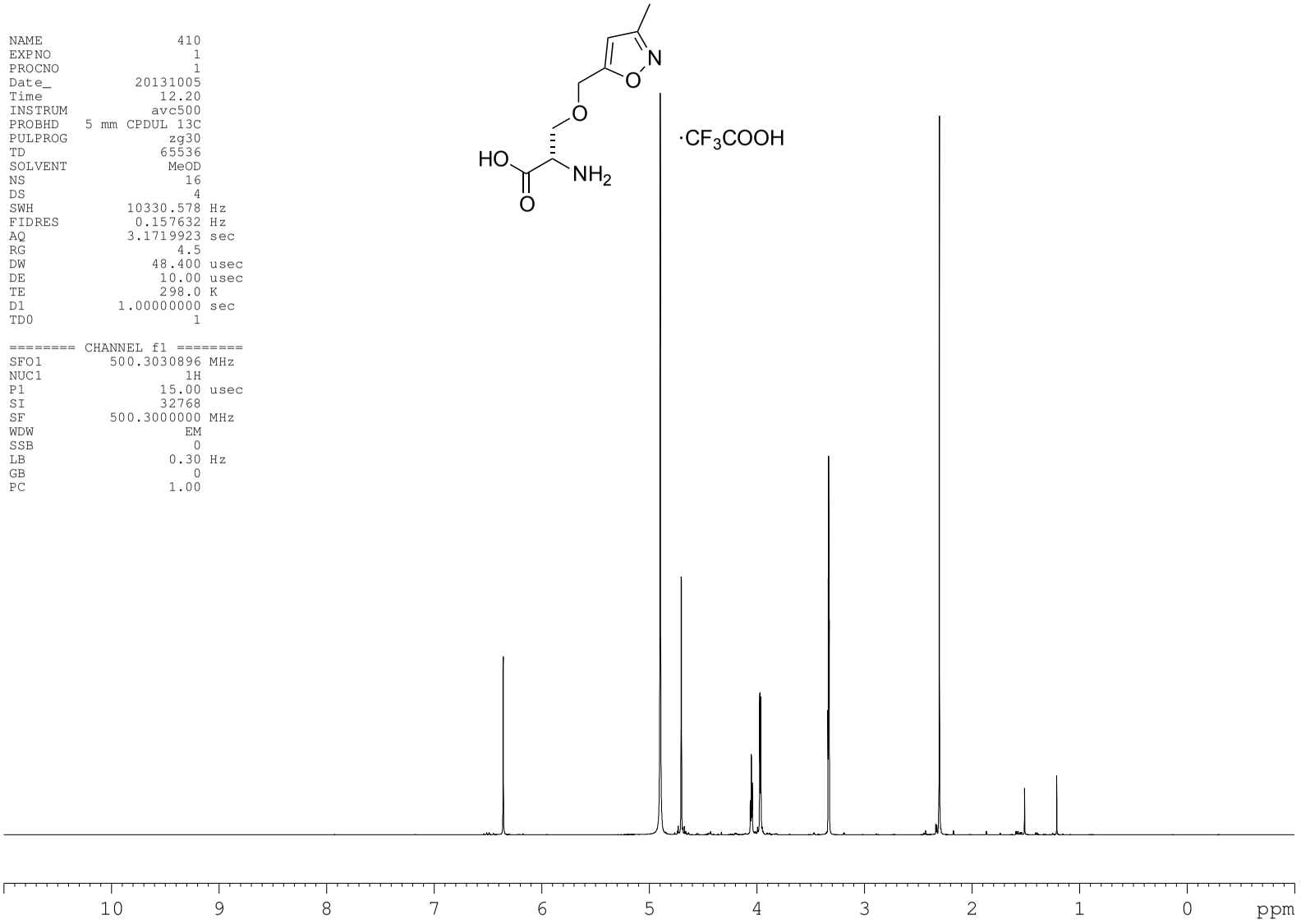
NAME	409
EXPNO	1
PROCNO	1
Date_	20130930
Time	19.19
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	4
DW	48.400 usec
DE	10.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
SFO1	500.3030896 MHz
NUC1	1H
P1	15.00 usec
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



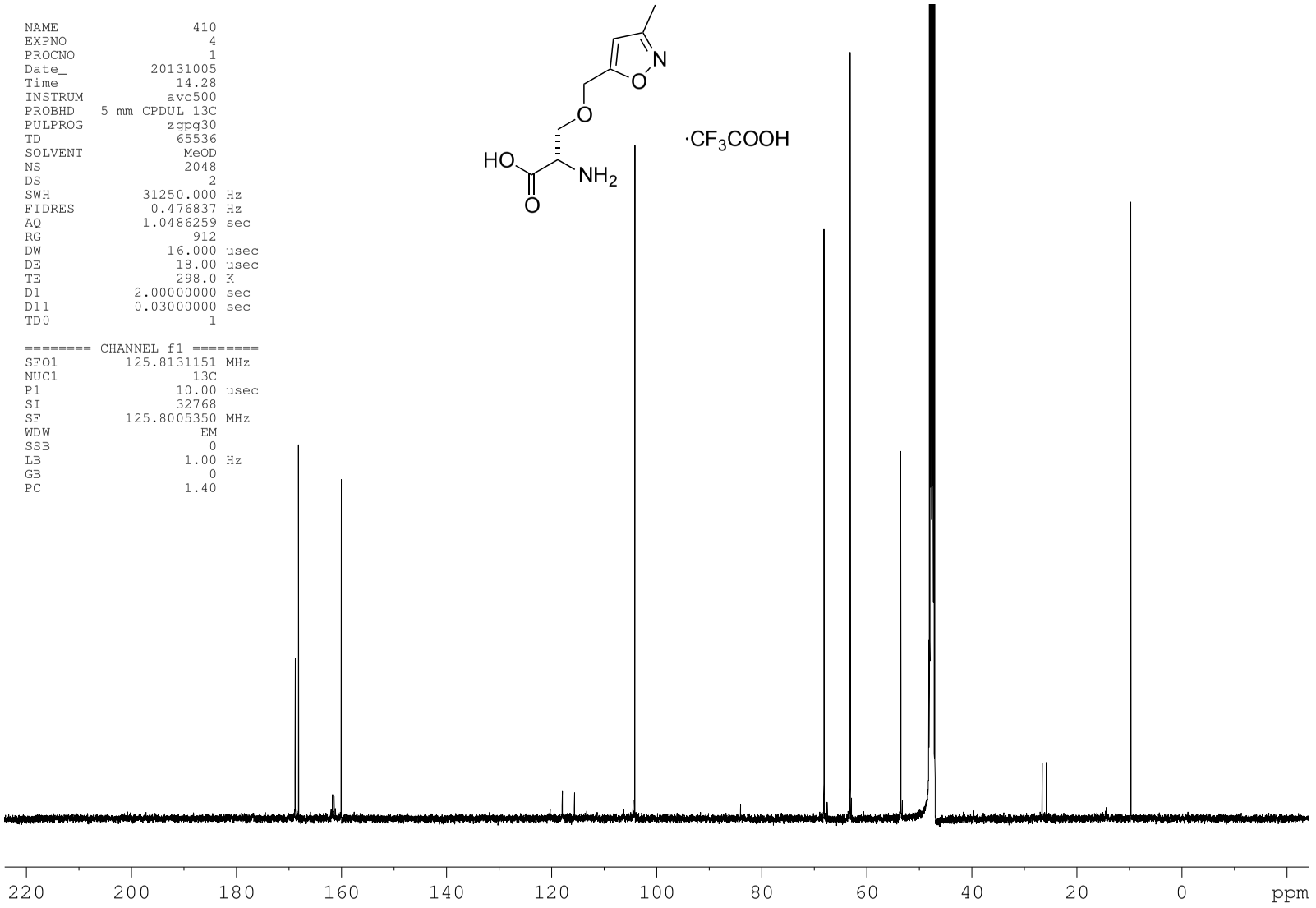
N-(*tert*-Butoxycarbonyl)-*O*-((3-methylisoxazol-5-yl)methyl)-L-serine 166



O-((3-Methylisoxazol-5-yl)methyl)-L-serine, trifluoroacetic acid salt 153



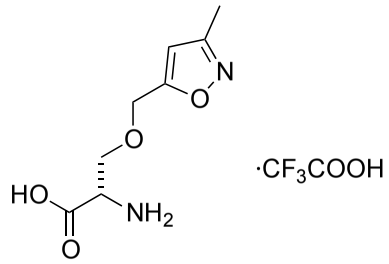
O-((3-Methylisoxazol-5-yl)methyl)-L-serine, trifluoroacetic acid salt 153



O-((3-Methylisoxazol-5-yl)methyl)-L-serine, trifluoroacetic acid salt 153

```
NAME             410 19F
EXPNO                1
PROCNO               1
Date_         20131007
Time             10.03
INSTRUM          avb500
PROBHD   5 mm PATXI 1H/
PULPROG        zgfhigqn
TD               131072
SOLVENT            CDCl3
NS                   16
DS                    4
SWH          113636.367 Hz
FIDRES         0.866977 Hz
AQ            0.5767668 sec
RG                 2050
DW                4.400 usec
DE                 6.50 usec
TE                298.0 K
D1           1.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
TD0                  1

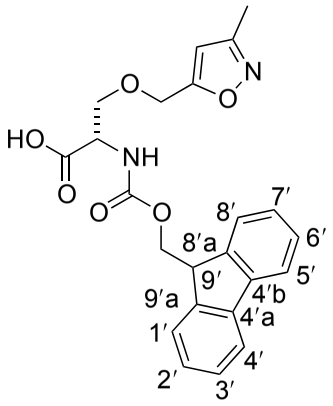
======== CHANNEL f1 ========
SFO1        470.4041911 MHz
NUC1               19F
P1                10.00 usec
SI                65536
SF          470.4512360 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```



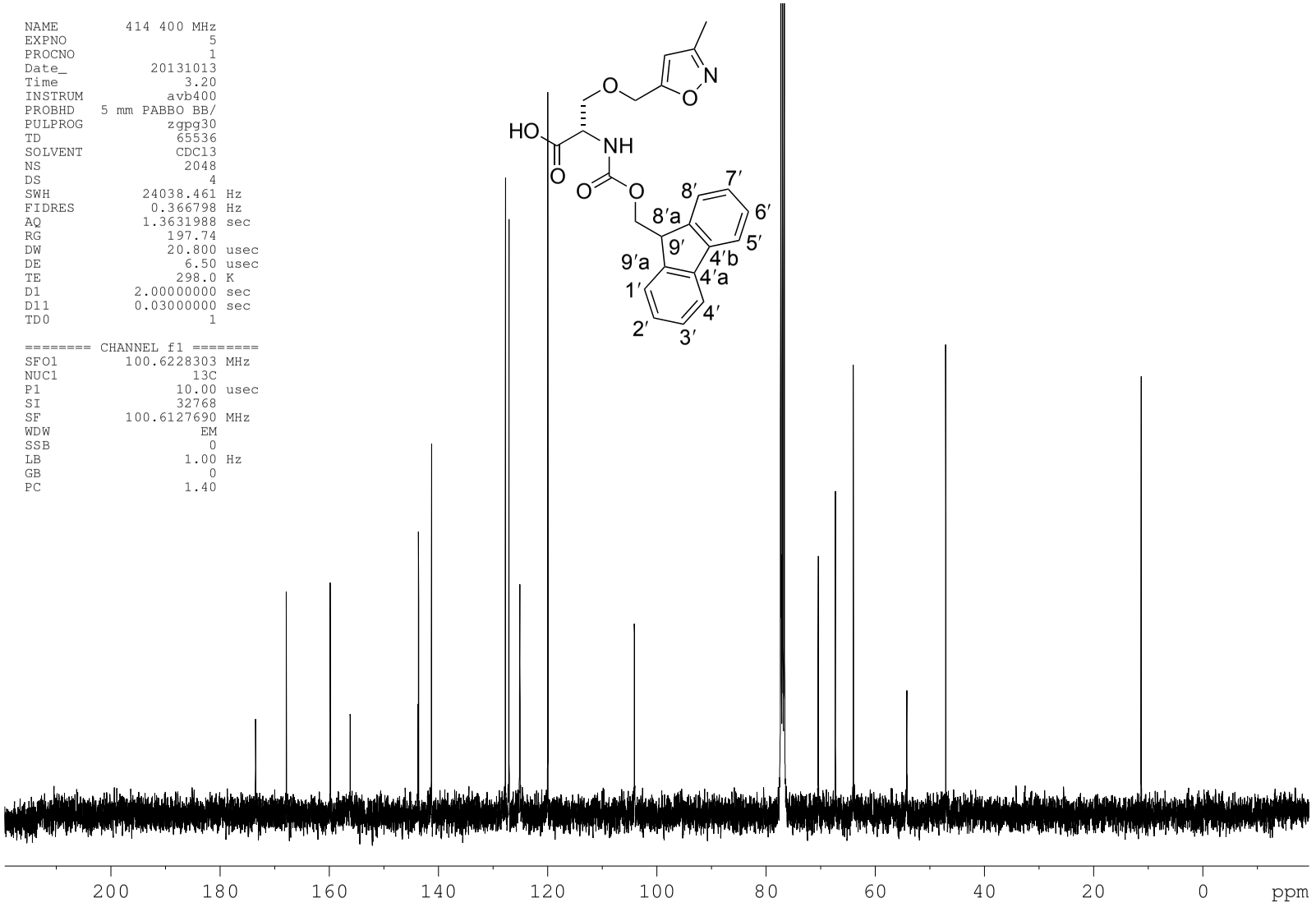
N-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*O*-((3-methylisoxazol-5-yl)methyl)-L-serine 156

```
NAME         414 400 MHz
EXPNO                  1
PROCNO                 1
Date_           20131013
Time                0.28
INSTRUM          avb400
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8012.820 Hz
FIDRES          0.122266 Hz
AQ              4.0894966 sec
RG                 126.79
DW                 62.400 usec
DE                   6.50 usec
TE                  298.0 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         400.1320007 MHz
NUC1                   1H
P1                  10.00 usec
SI                 65536
SF           400.1300000 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



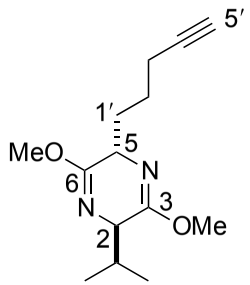
N-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-*O*-((3-methylisoxazol-5-yl)methyl)-L-serine 156



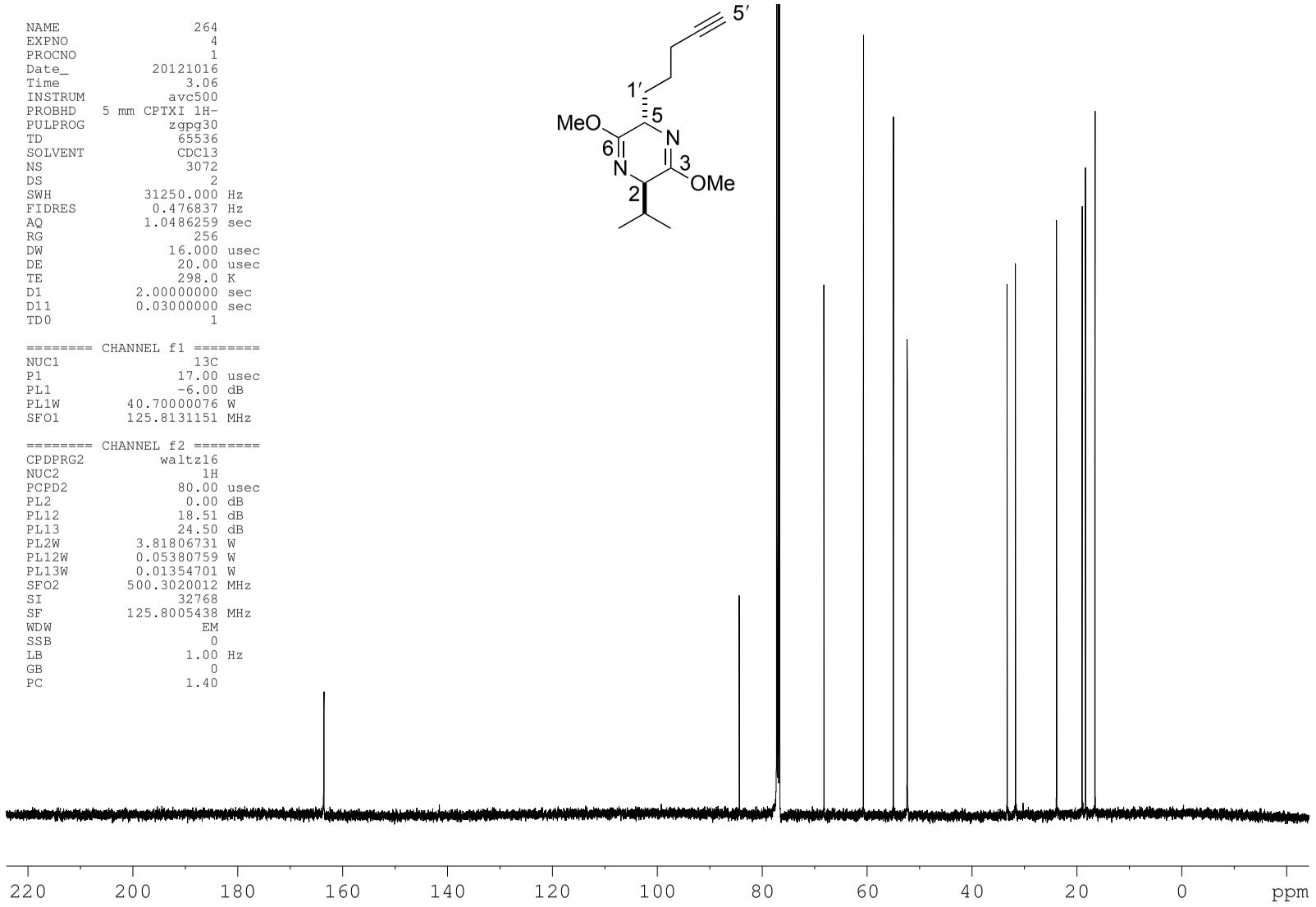
(2*R*,5*S*)-2-Isopropyl-3,6-dimethoxy-5-(pent-4-yn-1-yl)-2,5-dihydropyrazine 168

```
NAME                 264
EXPNO                  1
PROCNO                 1
Date_           20121016
Time                0.05
INSTRUM          avc500
PROBHD   5 mm CPTXI 1H-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719923 sec
RG                  25.4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                  9.50 usec
PL1                 0.00 dB
PL1W          3.81806731 W
SFO1        500.3030896 MHz
SI                 32768
SF          500.3000240 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



(2*R*,5*S*)-2-Isopropyl-3,6-dimethoxy-5-(pent-4-yn-1-yl)-2,5-dihydropyrazine 168

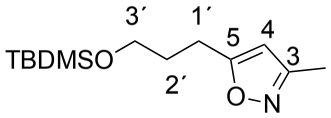



```
NAME                 264
EXPNO                  4
PROCNO                 1
Date_           20121016
Time                3.06
INSTRUM           avc500
PROBHD   5 mm CPTXI 1H-
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                  3072
DS                     2
SWH            31250.000 Hz
FIDRES             0.476837 Hz
AQ              1.0486259 sec
RG                   256
DW                16.000 usec
DE                20.00 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1

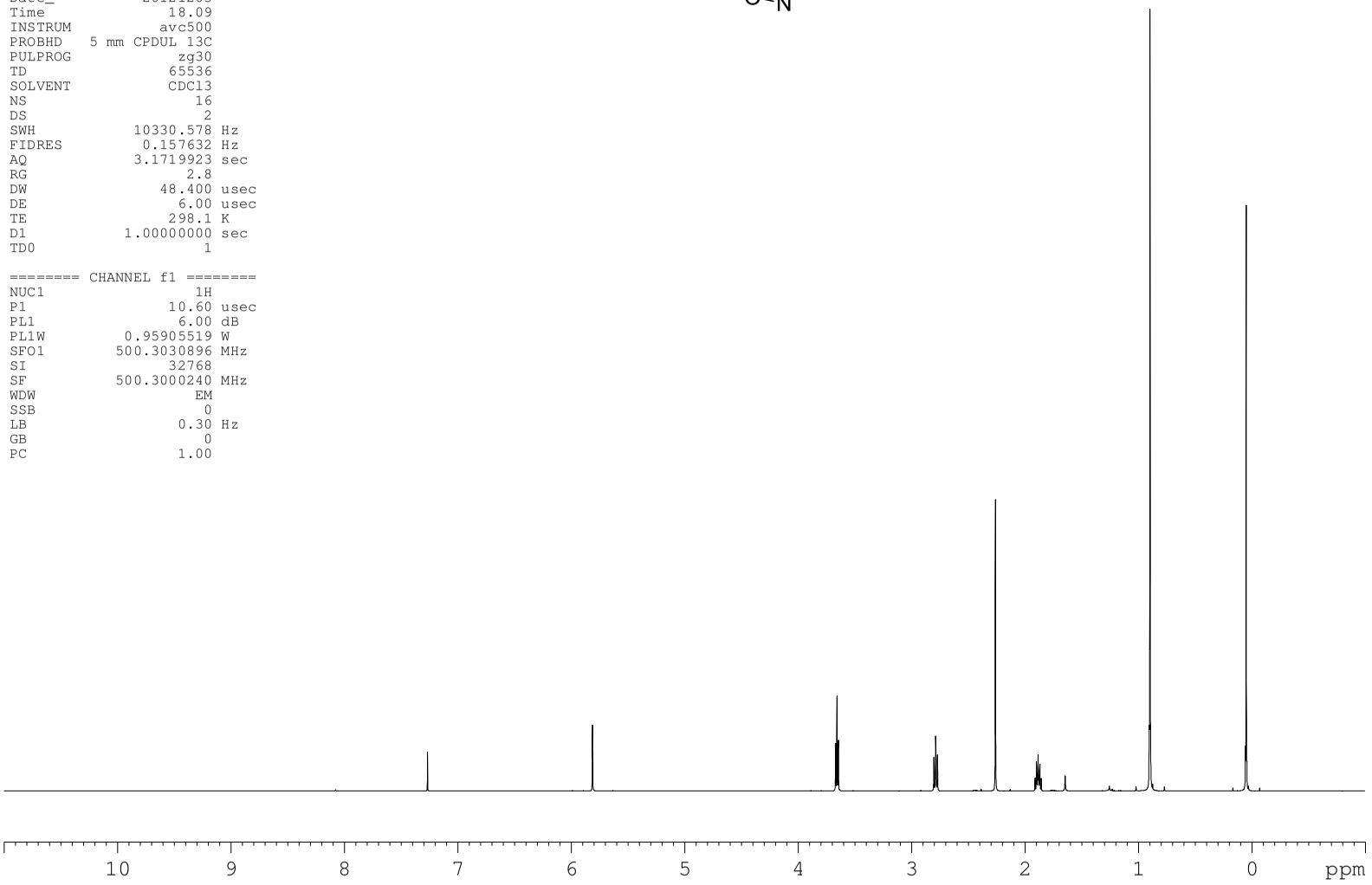
======== CHANNEL f1 ========
NUC1                 13C
P1                 17.00 usec
PL1                -6.00 dB
PL1W         40.70000076 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                 0.00 dB
PL12               18.51 dB
PL13               24.50 dB
PL2W          3.81806731 W
PL12W         0.05380759 W
PL13W         0.01354701 W
SFO2        500.3020012 MHz
SI                 32768
SF          125.8005438 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

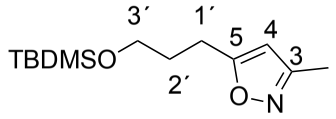
5-(3-((*tert*-Butyldimethylsilyl)oxy)propyl)-3-methylisoxazole 171



NAME	282
EXPNO	1
PROCNO	1
Date_	20121203
Time	18.09
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	2
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.8
DW	48.400 usec
DE	6.00 usec
TE	298.1 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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



5-(3-((*tert*-Butyldimethylsilyl)oxy)propyl)-3-methylisoxazole 171

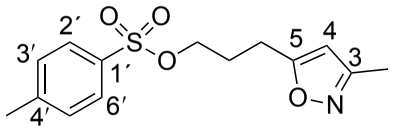



```
NAME                282
EXPNO                 4
PROCNO                1
Date_          20121203
Time              19.01
INSTRUM         avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0486259 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                10.25 usec
PL1                8.00 dB
PL1W         1.62029624 W
SFO1        125.8131151 MHz

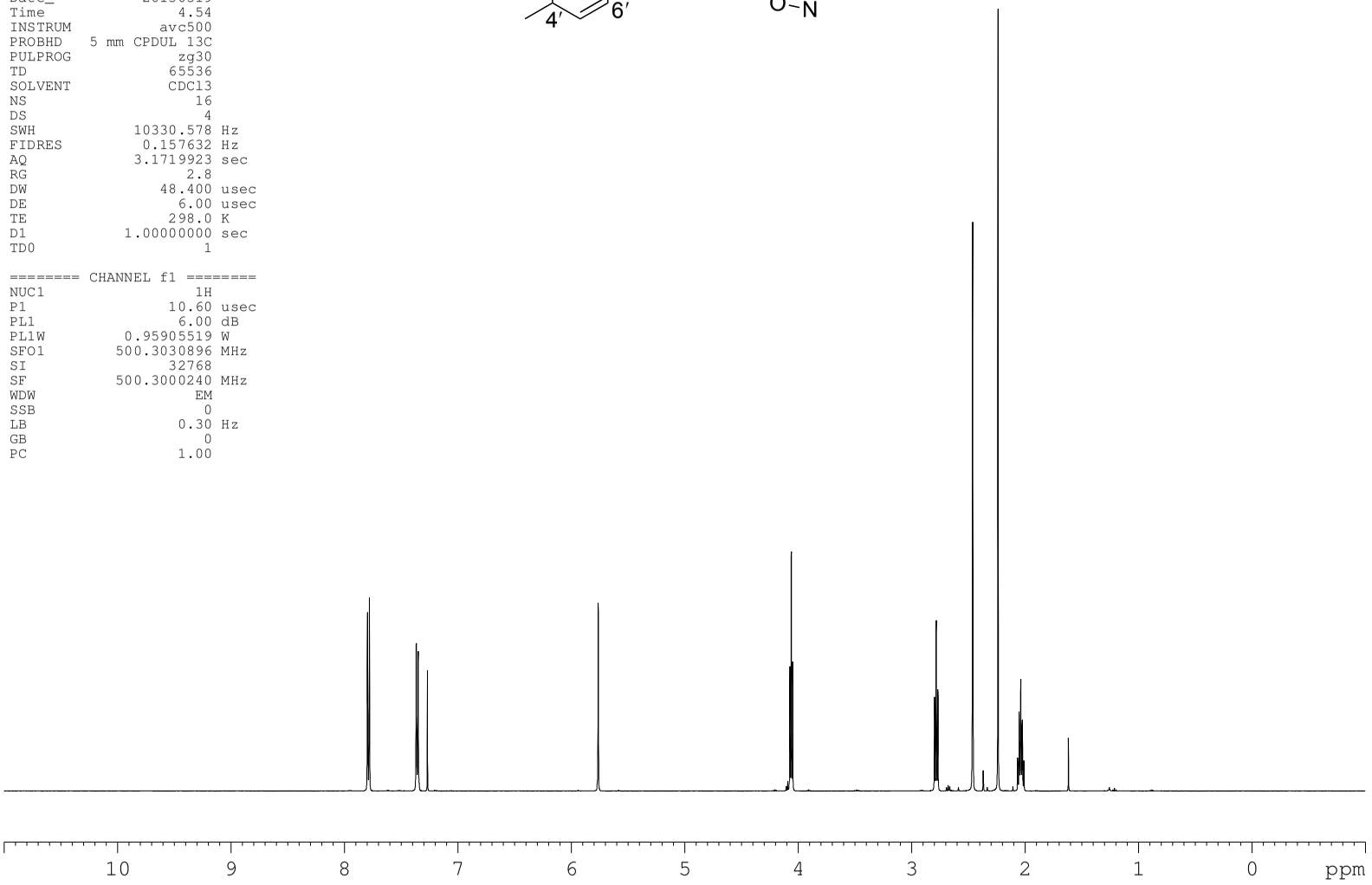
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2                6.00 dB
PL12              23.56 dB
PL13              29.56 dB
PL2W          0.95905519 W
PL12W         0.01682068 W
PL13W         0.00422516 W
SFO2        500.3020012 MHz
SI                32768
SF          125.8005438 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

3-(3-Methylisoxazol-5-yl)propyl 4-methylbenzenesulfonate 176

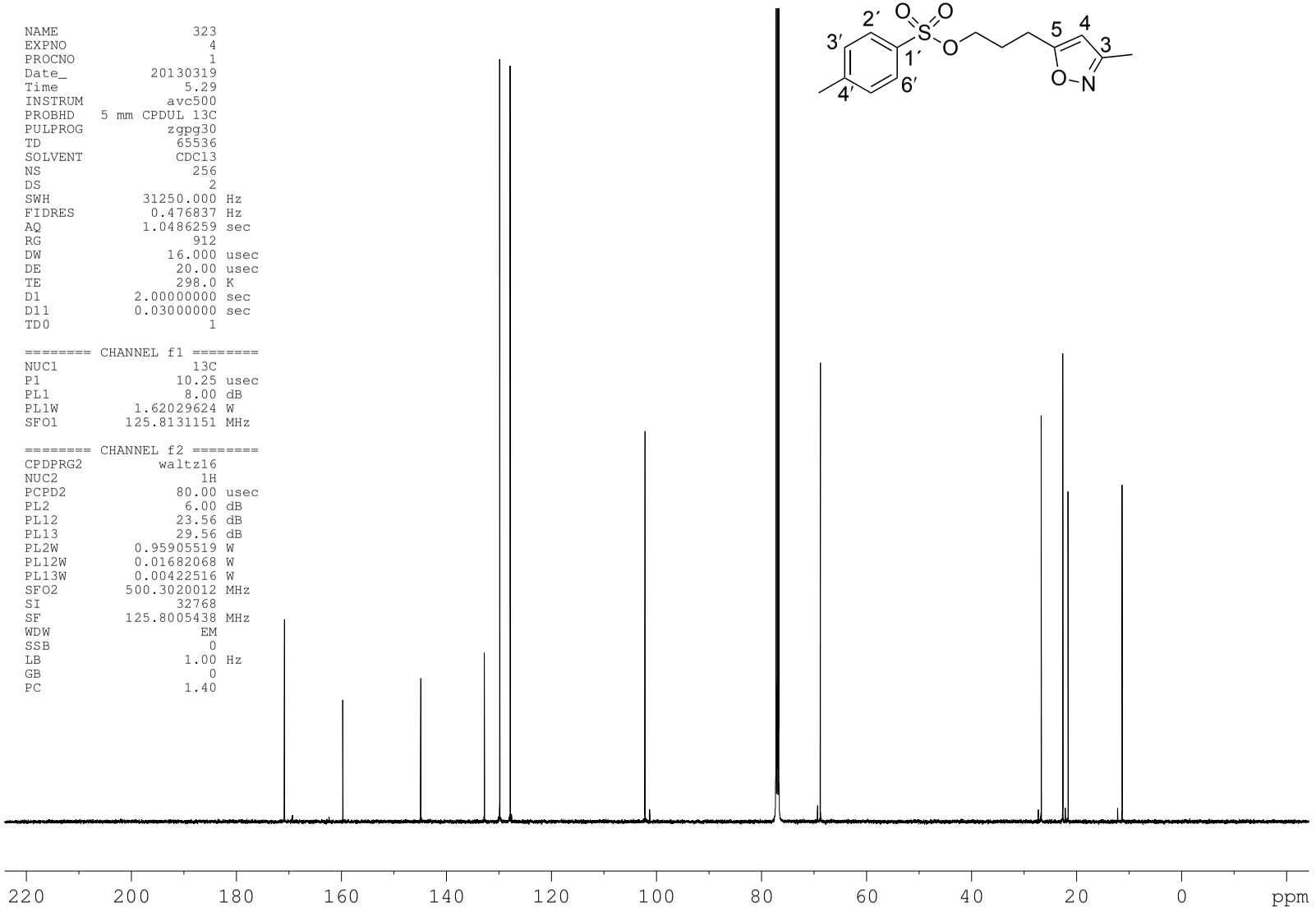


NAME	323
EXPNO	1
PROCNO	1
Date_	20130319
Time	4.54
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.8
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

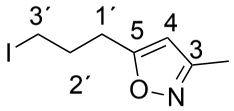
======== CHANNEL f1 ========	
NUC1	1H
P1	10.60 usec
PL1	6.00 dB
PL1W	0.95905519 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



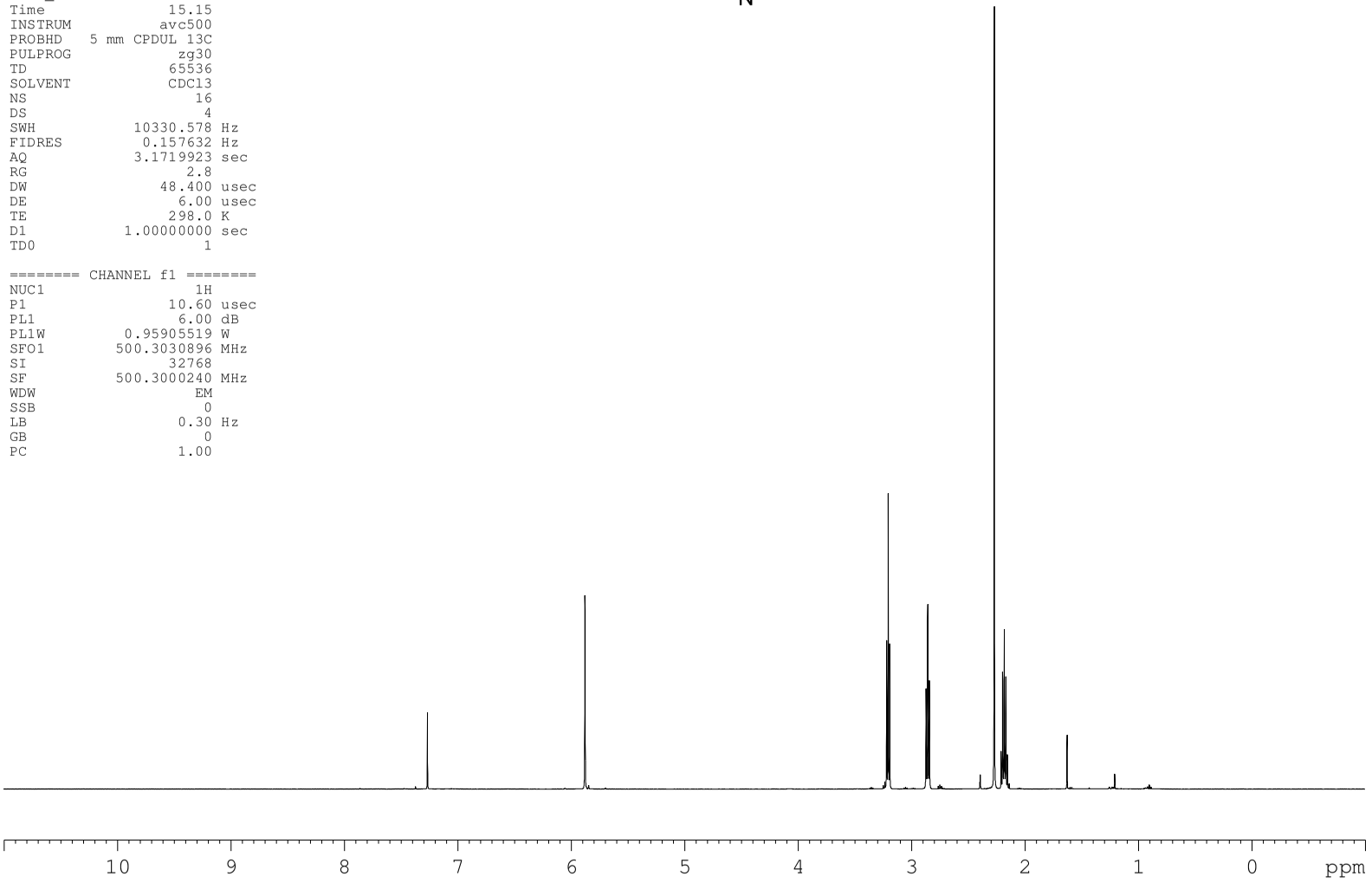
3-(3-Methylisoxazol-5-yl)propyl 4-methylbenzenesulfonate 176



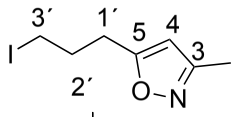
5-(3-Iodopropyl)-3-methylisoxazole 178



NAME	340
EXPNO	1
PROCNO	1
Date_	20130417
Time	15.15
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.8
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
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

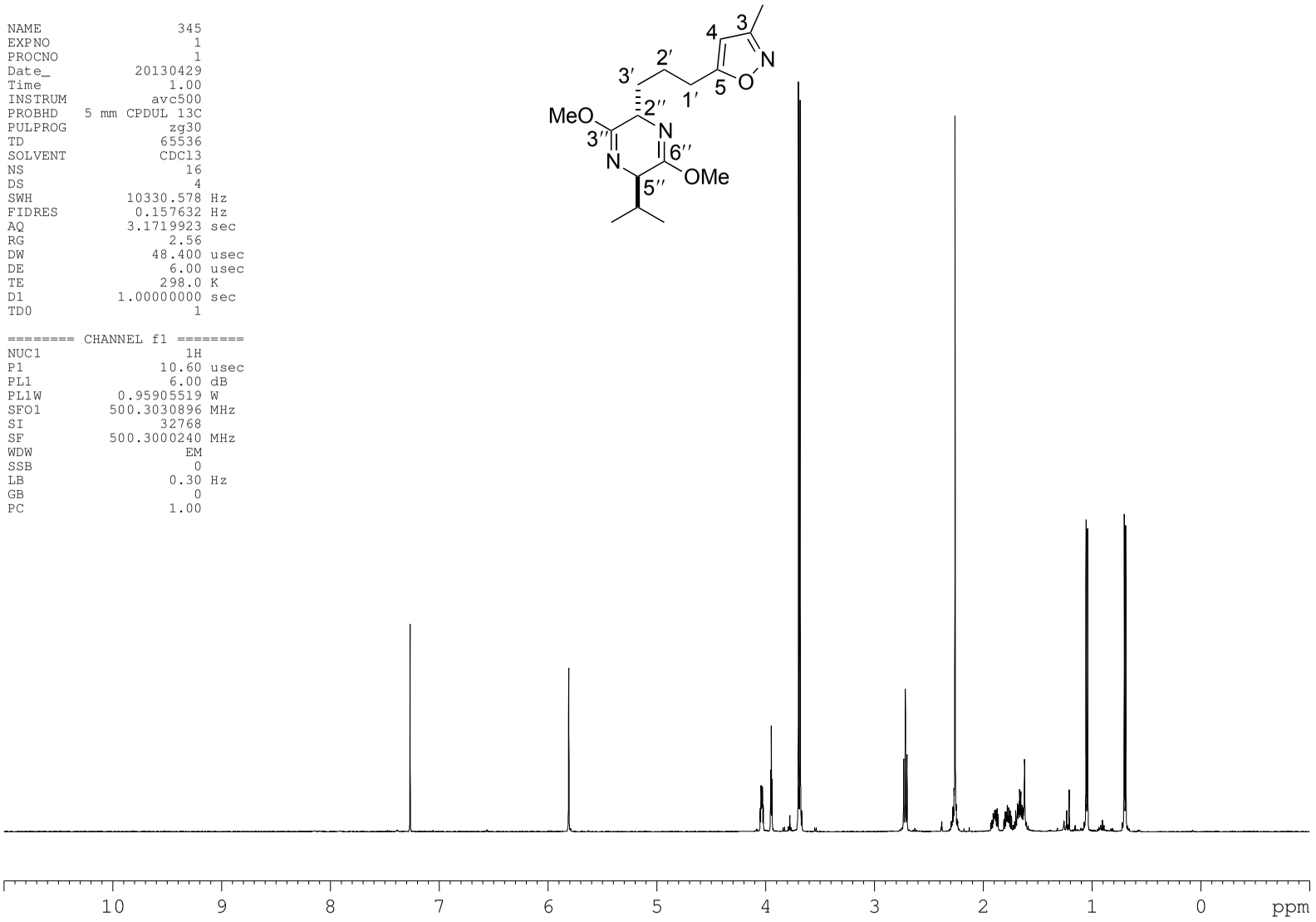


5-(3-Iodopropyl)-3-methylisoxazole 178

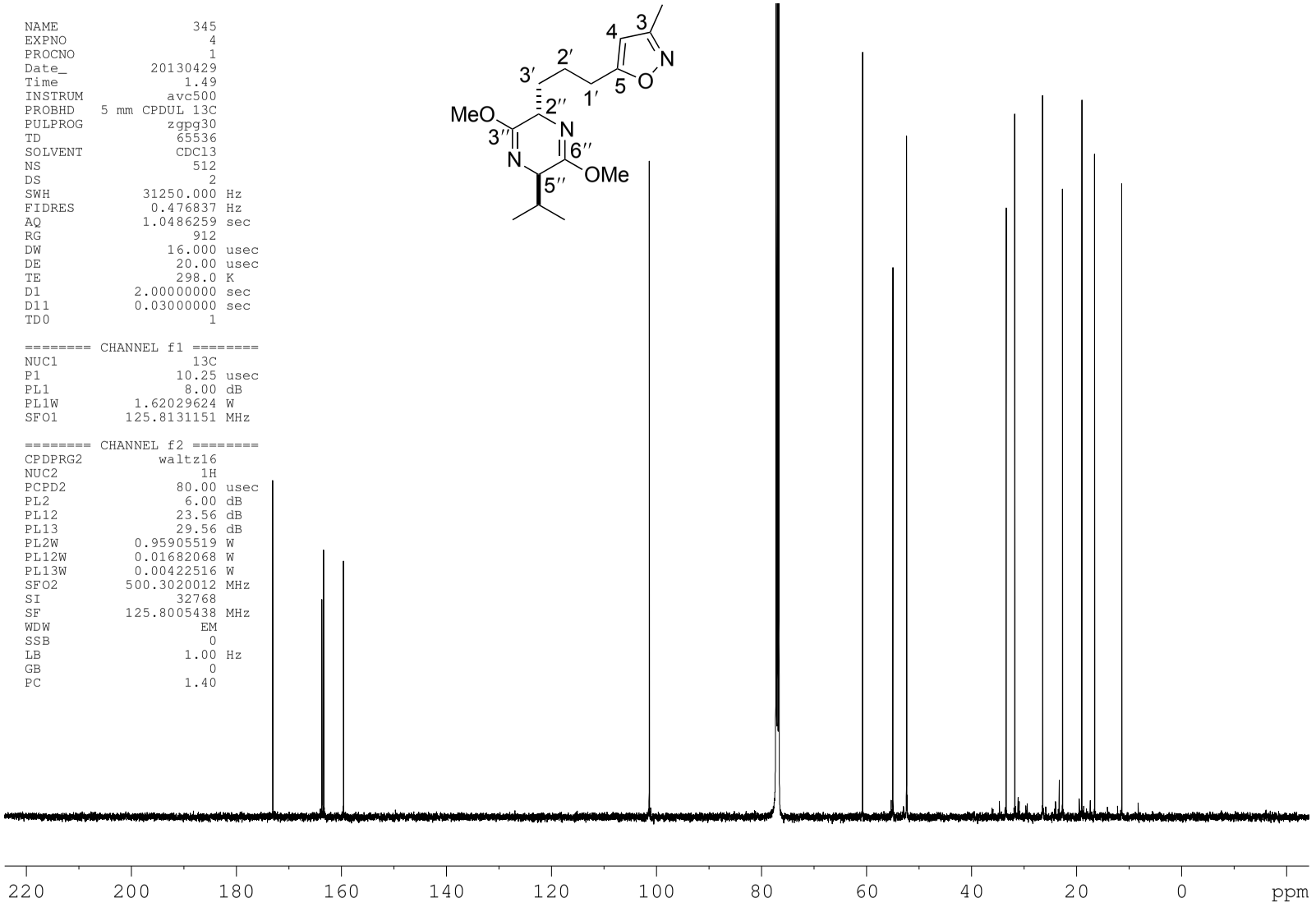


NAME	340
EXPNO	4
PROCNO	1
Date_	20130417
Time	15.52
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	256
DS	2
SWH	31250.000 Hz
FIDRES	0.476837 Hz
AQ	1.0486259 sec
RG	912
DW	16.000 usec
DE	20.00 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.25 usec
PL1	8.00 dB
PL1W	1.62029624 W
SFO1	125.8131151 MHz
======== CHANNEL f2 ========	
CPDPRG2	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
SI	32768
SF	125.8005438 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

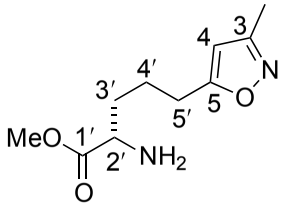
5-(3-((2*S*,5*R*)-5-Isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)propyl)-3-methylisoxazole 169



5-(3-((2*S*,5*R*)-5-Isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl)propyl)-3-methylisoxazole 169

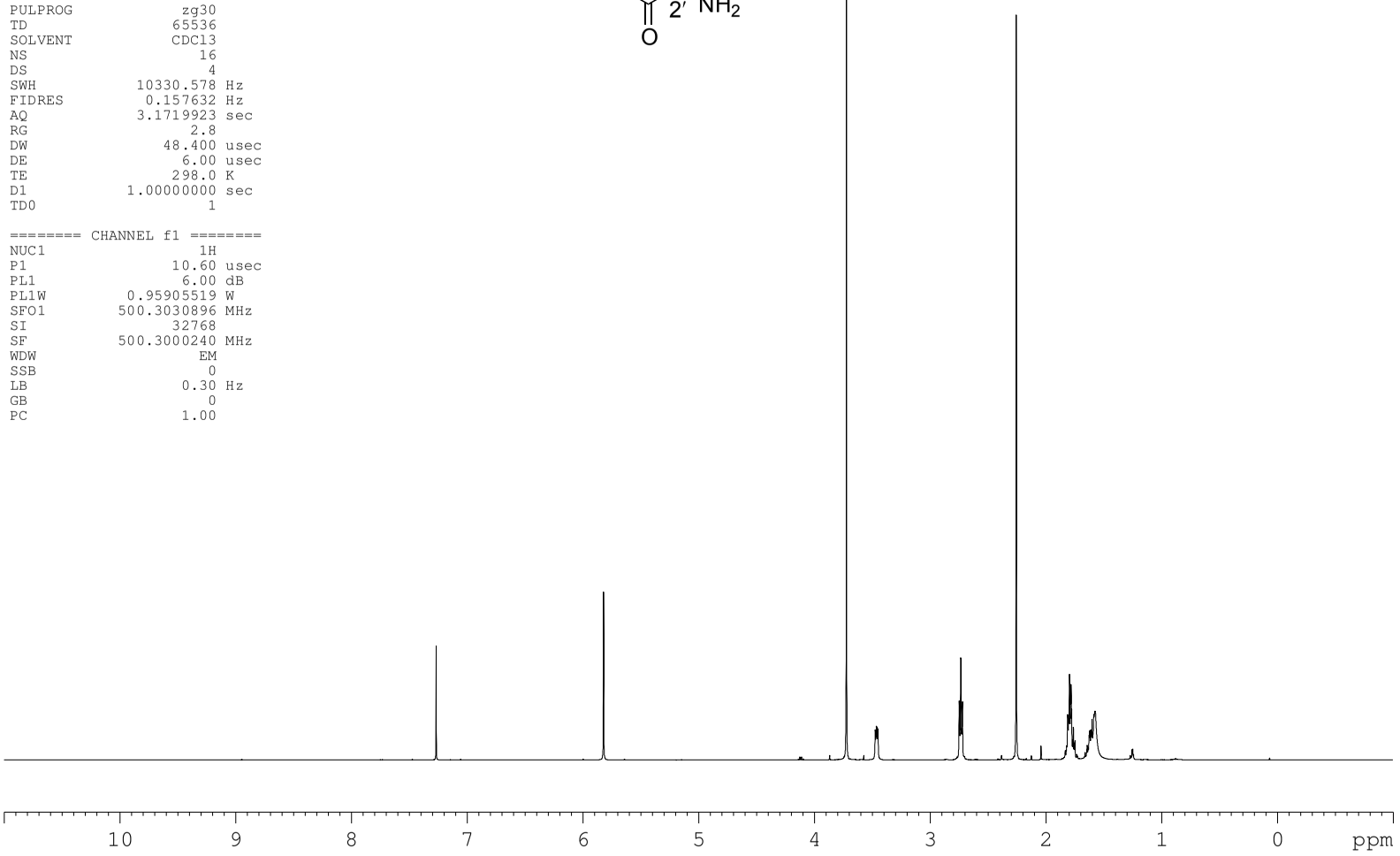


(*S*)-Methyl 2-amino-5-(3-methylisoxazol-5-yl)pentanoate 179

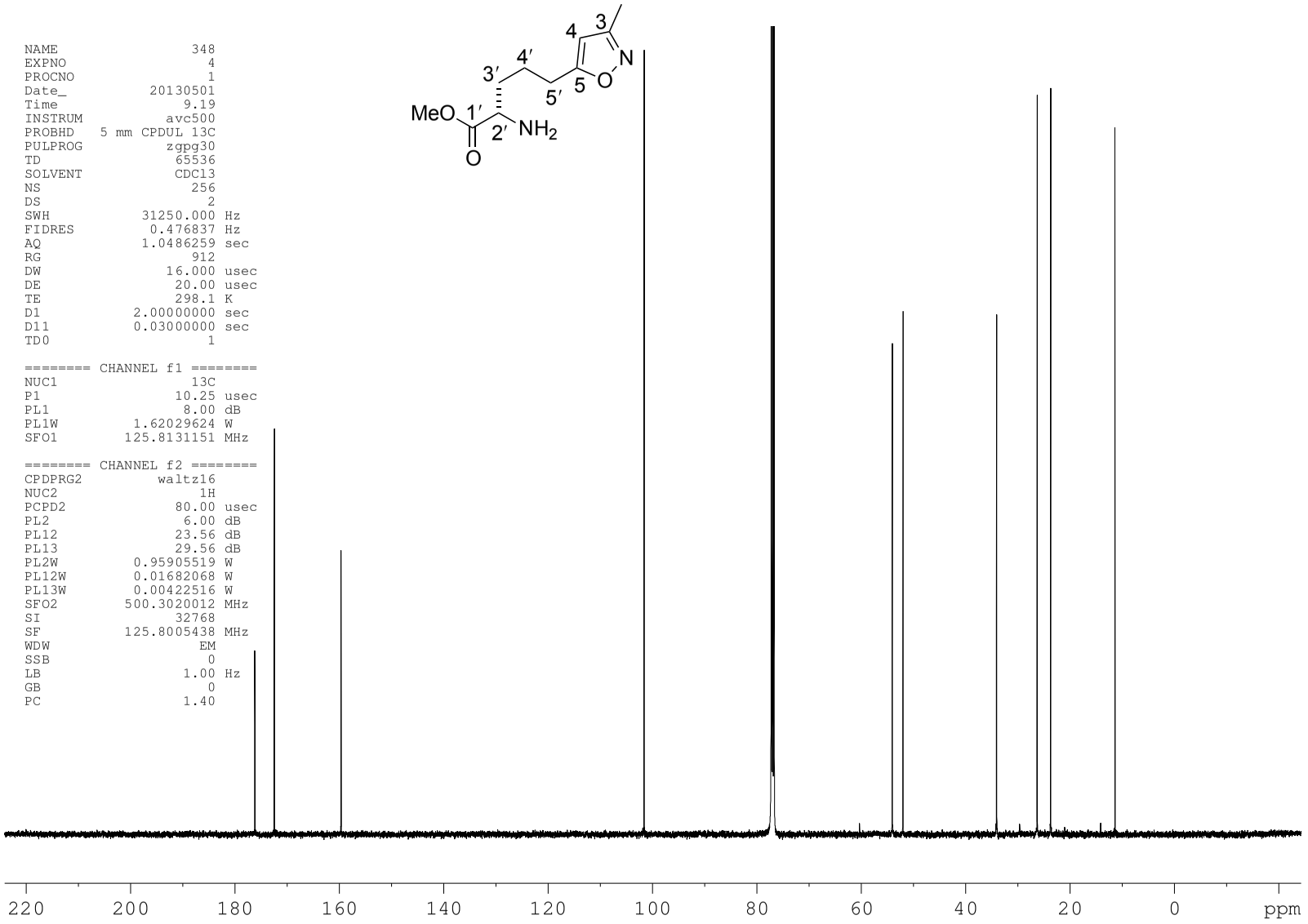


NAME	348
EXPNO	1
PROCNO	1
Date_	20130501
Time	8.43
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.8
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1

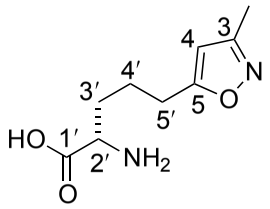
======== CHANNEL f1 ========	
NUC1	1H
P1	10.60 usec
PL1	6.00 dB
PL1W	0.95905519 W
SFO1	500.3030896 MHz
SI	32768
SF	500.3000240 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



(*S*)-Methyl 2-amino-5-(3-methylisoxazol-5-yl)pentanoate 179



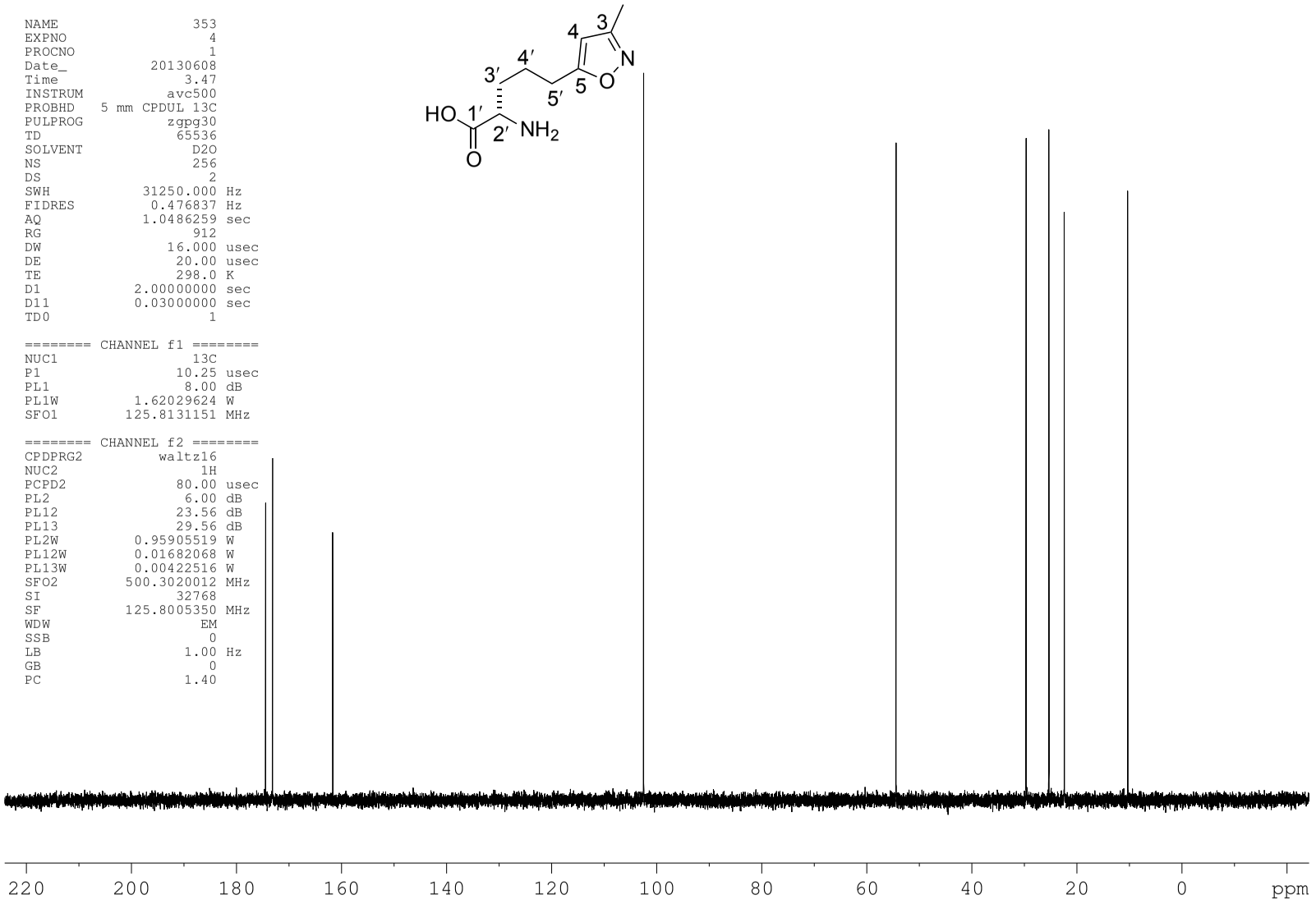
(*S*)-2-Amino-5-(3-methylisoxazol-5-yl)pentanoic acid 154



NAME	353
EXPNO	1
PROCNO	1
Date_	20130608
Time	3.11
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	D2O
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 sec
RG	2.8
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
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

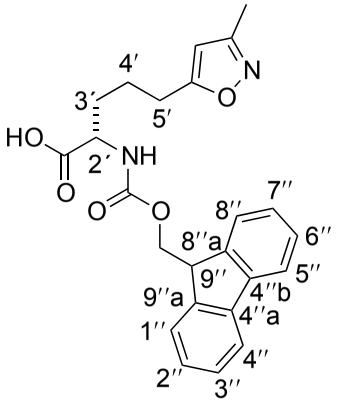
(*S*)-2-Amino-5-(3-methylisoxazol-5-yl)pentanoic acid 154

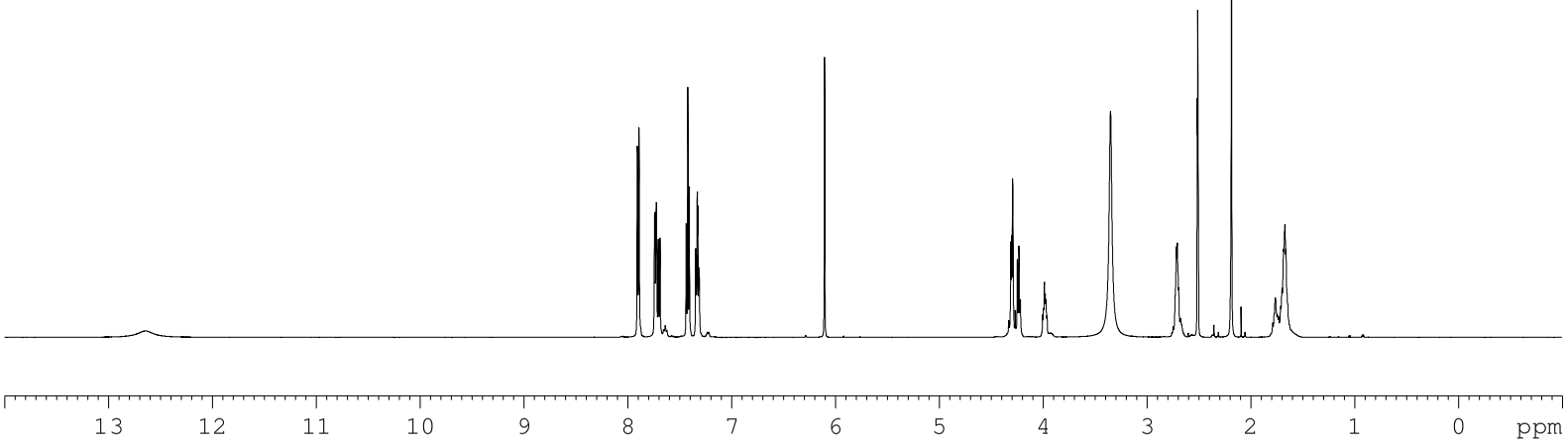


(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(3-methylisoxazol-5-yl)pentanoic acid 157

NAME	386
EXPNO	1
PROCNO	1
Date_	20130817
Time	10.20
INSTRUM	avc500
PROBHD	5 mm CPDUL 13C
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	16
DS	4
SWH	10330.578 Hz
FIDRES	0.157632 Hz
AQ	3.1719923 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
SI	32768
SF	500.3000000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00





(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-(3-methylisoxazol-5-yl)pentanoic acid 157

