The Synthesis of Heterocyclic Systems for use in Biological Imaging

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A thesis submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy

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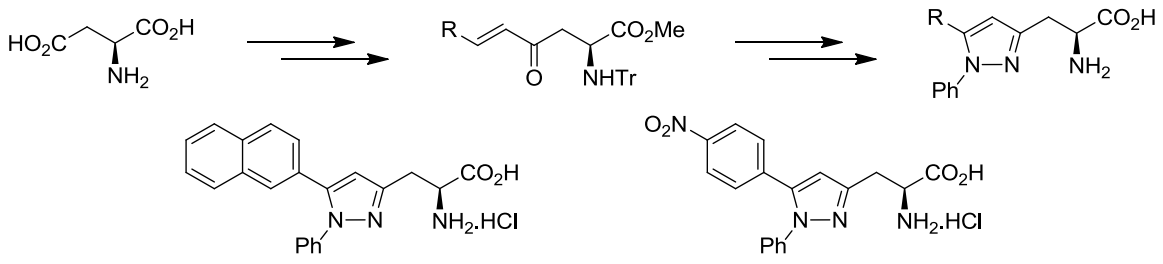
September 2013

Abstract

The first research project described in this thesis is the development of new SPECT and PET imaging agents for group II metabotropic glutamate receptors (mGluR2/3). Investigation of these receptors is of great interest as they have been implicated in many psychiatric disorders. A small library of 1,5-benzodiazepinones were synthesised with potential radiolabelling sites incorporated in the 7- and 8-positions around the benzodiazepinone core. Once synthesised the binding affinity of the compounds with mGluR2 was determined using the [^{35}S]GTPγS binding assay, which revealed them to be highly potent. Physicochemical properties were also investigated to determine whether compounds were likely to be brain penetrant. The 8-trifluoromethyl-7-methoxy and 8-iodo substituted compounds were found to have the required properties to be progressed. Work then focused on the synthesis of radiolabelling precursors of the hit compounds.

The second research project outlined in thesis involves the synthesis of novel heterocycle containing α-amino acids. Previous work in the Sutherland group achieved the synthesis of enone containing amino acids from L-aspartic acid. Building upon this, such enones were employed to form a small library of phenylpyrazole containing amino acids. The fluorescence properties of these compounds were then investigated which revealed the naphthalene and nitrophenyl substituted analogues to be fluorescent and thus have potential to be used as peptide labels for fluorescence imaging.



Contents

Acknowledgements

I would like to thank my supervisor Dr Andrew Sutherland for giving me the opportunity to carry out this research and for the help and support he has provided throughout my studies.

For advice and guidance in carrying out the binding assay I would like to thank Dr Brian Morris, Dr Sally Pimlott and Dr Judith Pratt. I would also like to thank Dr Judith Pratt for allowing me to make use of her laboratory at the University of Strathclyde.

Thank you to the technical staff at the University of Glasgow for their assistance in the collection of characterisation data for the compounds I synthesised: Dr David Adam for NMR spectroscopy, Jim Tweedy and Harry Jackson for mass spectrometry, and Kim Wilson for elemental analysis.

Financial support from the SINAPSE is gratefully acknowledged.

Thanks to the members of the Sutherland group who made my work enjoyable of a day-to-day basis: Alastair, Louise, Lindsay, Mark D, Fiona, Sajjad, Ahmed, Adele, Mark G, Ewen and Filip. I would particularly like to thank Adele for the HPLC work she carried out on my compounds. The Hartley group also played a big part in creating a great atmosphere in the Loudon Lab, so thanks to Stuart, Stephen, Andrew, Rachel, Kishore and Justyna.

Thank you to Mum, Dad, Lisa and the rest of my family and for their encouragement throughout my PhD studies, and for providing me with distractions from chemistry when I got too stressed.

I would like to finish with a huge thank you to Adam for being a massive support without which I would have found the last few years a lot more difficult, and for always providing curry and/or cake when I had a particularly bad day.

Author's Declaration

This thesis represents the original work of Lynne Gilfillan unless explicitly stated otherwise in the text. The research was carried out at the University of Glasgow in the Loudon Laboratory under the supervision of Dr Andrew Sutherland during the period of October 2009 to September 2012. Portions of the work described herein have been published elsewhere as listed below.

L. Gilfillan, A. Blair, B. J. Morris, J. A. Pratt, L. Schweiger, S. Pimlott and A. Sutherland, *Med. Chem. Commun.*, 2013, **4**, 1118−1123.

Abbreviations

Ac	acetyl
ACPD	aminocyclopentane-1,3-dicarboxylic acid
AMPA	2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid
aq	aqueous
BBB	blood brain barrier
Boc	*tert*-butyloxycarbonyl
Bn	benzyl
br	broad
Bu	butyl
Cbz	carboxybenzyl
CCK1	cholecystokinin A receptor
Chk1	checkpoint kinase 1
CHO	chinese hamster ovary
CI	chemical ionisation
CNS	central nervous system
conc.	concentrated
COSY	correlation spectroscopy
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	distortionless enhancement by polarisation transfer
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DPM	disintegrations per minute
DTT	dithiothreitol

ESI	electrospray ionisation
EI	electron ionisation
Eq.	equivalent(s)
Et	ethyl
fod	6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato
FAB	fast atom bombardment
g	gram(s)
GDP	guanosine diphosphate
GPCR	G protein coupled receptor
GTP	guanosine triphosphate
h	hour(s)
HMDS	hexamethyldisilazide
HPLC	high performance liquid chromatography
HSA	human serum albumin
HSQC	heteronuclear single quantum coherence
IAM	immobilised artificial membrane
IC_{50}	half maximal inhibitory concentration
iGluR	ionotropic glutamate receptor
K_m	membrane partition coefficient
K222	Kryptofix® 222
m	multiplet
M	molar
m-CPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
mGluR	metabotropic glutamate receptor
mL	millilitres
mmol	millimoles
MOM	methoxymethyl
Mp	melting point
MW	molecular weight
N	normal

nM	nanomolar
nm	nanometers
NMDA	*N*-methyl-D-aspartate
NMO	*N*-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
PAM	positive allosteric modulator
PCP	phencyclidine
PET	positron emission tomography
P_m	permeability
PPB	plasma protein binding
PPTS	pyridinium *p*-toluenesulfonate
Pr	propyl
q	quartet
quin	quintet
RCY	radiochemical yield
rt	room temperature
s	singlet
S.M.	starting material
SPECT	single photon emission computed tomography
t	triplet
TBS	tert-butylsalicylaldiminato
Tf	trifluoromethylsulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
Tr	trityl (triphenylmethyl)
Ts	*p*-toluenesulfonyl
UV	ultraviolet
μM	micromolar
μw	microwave

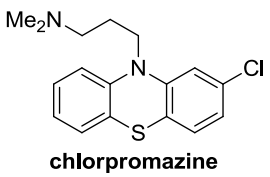
1 Introduction

1.1 Schizophrenia

Schizophrenia is a chronic and severe mental disorder that is thought to effect up to 1% of the population of developed countries.[1] Sufferers of this condition will usually experience a combination of symptoms, characterised as: positive (hallucinations, delusions), negative (lack of emotion, social and occupational dysfunction) and cognitive (disorganised thought patterns, difficulty concentrating and following instructions, memory problems).[2-4] Although schizophrenia has been recognised for over 100 years, the underlying neurochemical mechanism of the disease is not yet known.

1.1.1 Treatment of Schizophrenia

Currently schizophrenia is treated using antipsychotic drugs that target dopamine receptors (particularly D_2) to reduce dopamine transmission in the brain. The use of antidopaminergic drugs in schizophrenia was discovered quite by accident in the 1950s when it was observed that chlorpromazine (Figure 1.1), originally developed as an antihistamine, could treat psychosis.[5,6] Chlorpromazine is a potent antagonist of the D_2 dopamine receptor, but also acts as an agonist at other dopamine receptors, as well as serotonin, histamine, adrenergic, and muscarinic acetylcholine receptors.[7] The discovery of chlorpromazine launched the first generation of antipsychotic drugs, known as typical antipsychotics, which are primarily blockers of D_2. Although these drugs are effective in treating the positive symptoms of schizophrenia they have a number of undesirable side effects such as sedation, amnesic symptoms, withdrawal upon discontinuation of the drug, prolactin elevation, extrapyramidal symptoms and weight gain.[8]



chlorpromazine

Figure 1.1 Structure of chlorpromazine

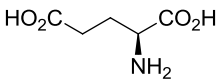
Atypical antipsychotics such as clozapine and olanzapine were then developed as the second generation of antipsychotic drugs (Figure 1.2). These compounds tend to be active at the same groups of receptors as typical antipsychotics, and are associated with the same side effects as typical antipsychotics but to a lesser extent.[9] However, a 2009 meta-analysis comparing typical and atypical antipsychotic drugs demonstrated that due the variability of the pharmacology, efficacy and side effects of typical and atypical antipsychotics, neither class represents a homogenous group and such drugs should be considered on an individual basis.[10]

Figure 1.2 Structures of clozapine and olanzapine

The ability of these drugs to treat psychosis led to the dopamine hypothesis of schizophrenia, which proposes that psychosis is related to excessive dopaminergic activity in the brain.[11] More recent research has given rise to the glutamate hypothesis of schizophrenia, which suggests that schizophrenia is caused by irregularities in glutamatergic synaptic function.[12-14]

1.2 Glutamate Receptors

Glutamate receptors are a class of excitatory amino acid receptors activated by synaptically released L-glutamate (Figure 1.3), the most abundant neurotransmitter in the central nervous system (CNS) of vertebrates. These receptors can be divided into two subtypes, ionotropic glutamate receptor and metabotropic glutamate receptors. Ionotropic glutamate receptors are ligand gated ion channels, whereas metabotropic glutamate receptors are a form of G-protein coupled receptor.[15]



L-glutamic acid

Figure 1.3 Structure of L-glutamic acid

1.2.1 Ionotropic Glutamate Receptors

Ionotropic glutamate receptors are tetrameric assemblies with each subunit containing an extracellular *N*-terminal domain connected to a transmembrane region (M1) connected to a re-entrant loop (M2), followed by two more membrane spanning regions (M3 and M4) and ending with an intracellular *C*-terminal domain (Figure 1.4).[15]

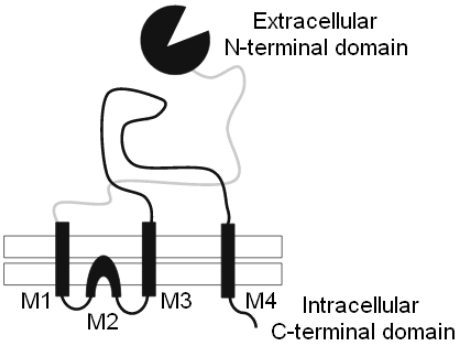


Figure 1.4 Structure of an ionotropic glutamate receptor[15]

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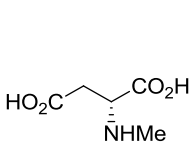
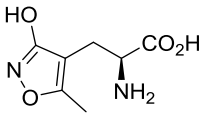
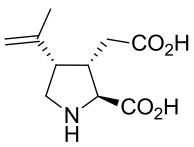
There are three distinct classes of ionotropic glutamate receptors termed NMDA, AMPA and kainate receptors, named after the synthetic agonists used to selectively activate them (Figure 1.5).[16,17]



NMDA



(S)-AMPA



kainic acid

Figure 1.5 Structures of ionotropic glutamate receptor agonists

NMDA receptor activation requires the binding of two agonists, glutamate and glycine. In addition to ligand gating NMDA receptors are also voltage dependent as the ion channel can be blocked by the binding of magnesium ions at normal resting potentials or when the cell is hyperpolarised.[18] When the cell is depolarised the channel becomes unblocked allowing sodium and calcium ions to enter and potassium ions to leave the cell. Binding to the AMPA or kainate receptors results in the opening of an ion channel, allowing the influx of sodium ions and efflux of potassium ions. Ionotropic glutamate receptors play a critical role in many cerebral functions such as synaptic plasticity, and learning and memory processes.[19]

1.2.2 Metabotropic Glutamate Receptors

Structurally, metabotropic glutamate receptors are proteins embedded within the cell membrane containing regions both outside and inside the cell. The extracellular bi-lobed *N*-terminal domain, which binds glutamate, is connected to the intracellular *C*-terminal domain by a peptide chain consisting of seven transmembrane helices (Figure 1.6).[15]

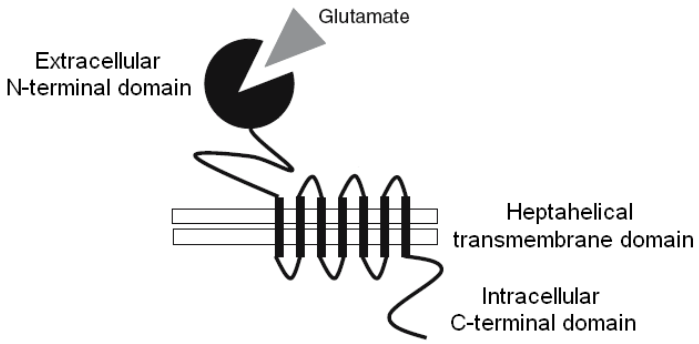


Figure 1.6 Structure of a metabotropic glutamate receptor[15]

(Reprinted from *Psychopharmacology*, 2005, **179**, 4−29 with kind permission from Springer Science and Business Media)

Unlike ionotropic glutamate receptors, metabotropic glutamate receptors do not affect ion channels or enzymes directly. Instead, binding of glutamate causes a conformational change which activates a G-protein, starting a signalling cascade. Evidence for the existence of metabotropic glutamate receptors was not discovered until the late 1980s, when glutamate was found to take part in biological processes via a receptor that did not

belong to the ionotropic glutamate receptors.[20,21] Their existence was confirmed in 1991 when the cloning and characterization of the first metabotropic glutamate receptor was reported.[22,23] To date eight members of the family of metabotropic glutamate receptors have been cloned, termed mGluR1 to mGluR8, which are separated into three groups based on their signal transduction pathway and sequence homology. Group I receptors (mGluR1 and mGluR5) are positively coupled to the activity of phospholipase C, whereas group II (mGluR2 and 3) and group III (mGluR4, 6, 7, and 8) receptors are both negatively coupled to the activity of adenyl cyclase but have different pharmacology. An important function of metabotropic glutamate receptors is their modulatory effect on both *pre* and *post*-synaptic glutamatergic neurotransmission. Group I receptors increase presynaptic glutamate release whereas group II and group III receptors limit glutamate release, particularly during conditions of spillover from the synaptic cleft.[24,25]

1.2.3 Glutamate Receptors as Pharmaceutical Targets

Compounds which bind to glutamate receptors are widely considered to be among the most important new targets in CNS drug discovery. Their widespread expression causes glutamate receptors to participate in a variety of functions of the central nervous system, and irregularities in glutamatergic neurotransmission have been observed in many psychological disorders such as schizophrenia, attention deficit hyperactivity disorder, bipolar disorder, major depressive disorder, general anxiety disorder and obsessive-compulsive disorder.[26-28]

The glutamate hypothesis of schizophrenia in particular has sparked great interest in the investigation of glutamate receptor targeting pharmaceuticals. The origins of this theory can be traced back to the late 1950s, when phencyclidine (PCP) was shown to induce the psychotic symptoms associated with schizophrenia in humans.[29,30] It has since been discovered that PCP exerts this effect by acting as an antagonist at a binding site located within the NMDA receptor ion channel, leading to NMDA receptor hypofunction.[31] Another consequence of PCP binding is glutamate efflux, and hence increased levels of extracellular glutamate, potentially caused by increased activity of non-NMDA glutamate receptors.[14,32] In 1998, work by Moghaddam and Adams showed that the mGluR2/3 agonist LY345470 (Figure 1.7, discussed further in Section 1.3.1) was able to reverse the effects of PCP on working memory, stereotypy, locomotion, and cortical glutamate efflux

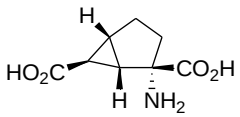
in rats.[12] This has led to much research into the use of compounds targeting metabotropic glutamate receptors to regulate glutamatergic neurotransmission.

1.3 mGluR2/3 Ligands

Since the discovery in 1998 that a mGluR2/3 agonist could potentially be used to normalise excess glutamatergic activity, many mGluR2/3 targeting compounds have been developed as potential drugs for various psychological disorders. The compounds discovered to date cover various structural and binding classes.

1.3.1 Agonists

The mGluR2/3 agonist LY345470 (developed by Eli Lily) was the compound first used to reverse the effect of PCP in rats. Although it was this compound that initially stimulated research into the link between mGluR2/3 and schizophrenia, most studies using LY354740 have investigated its anxiolytic properties, which have been demonstrated in various animal models and in human studies.[33-36] Structurally LY354740 is an amino acid containing fused three and five-membered rings (Figure 1.7).

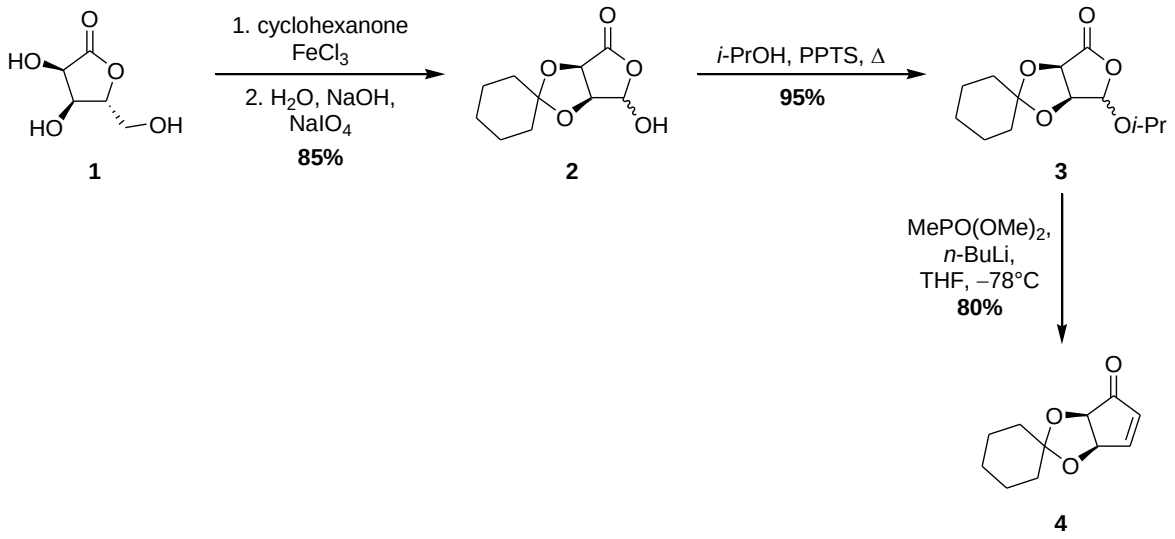


LY354740

Figure 1.7 Structure of mGluR2/3 agonist LY354740

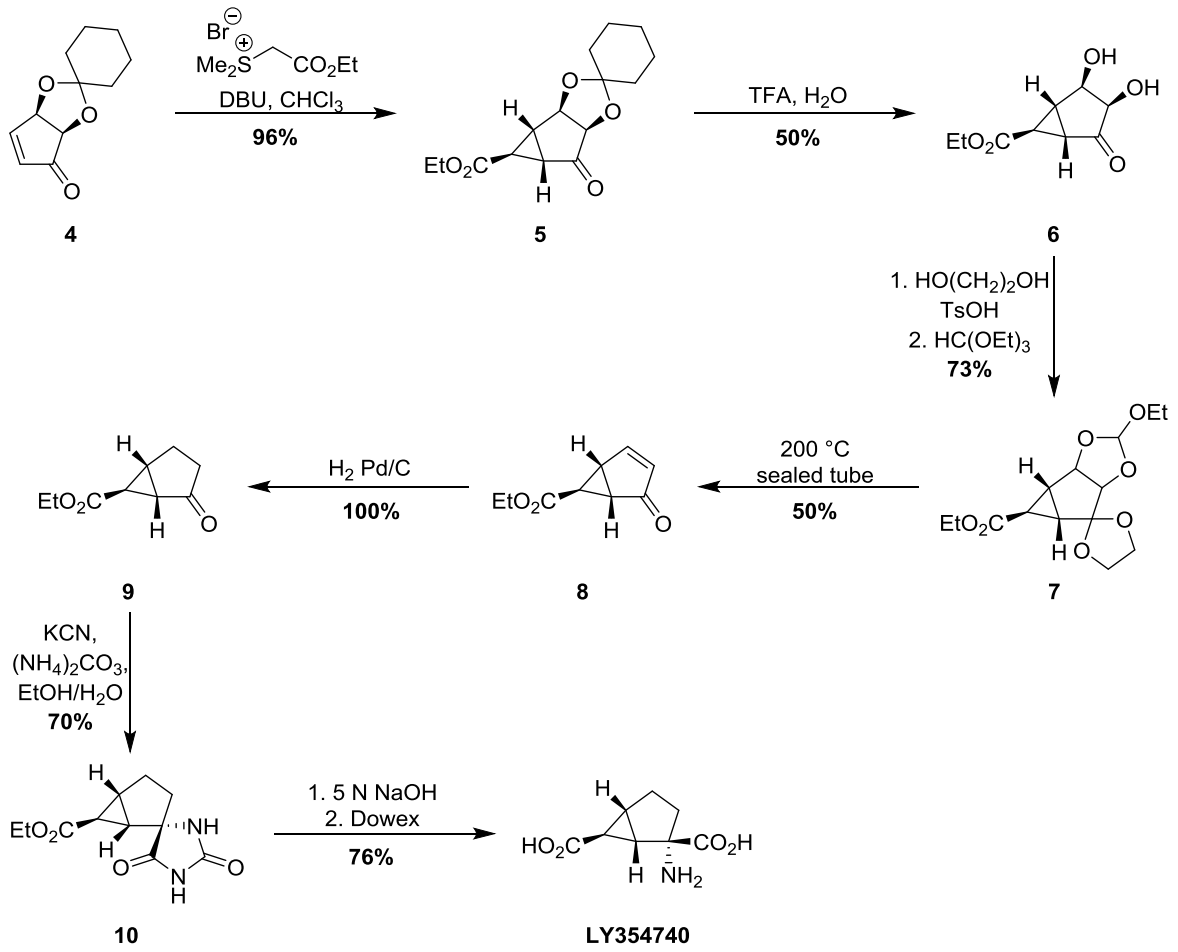
A synthesis of LY354740 is illustrated in Schemes 1.1 and 1.2. The first part of the synthetic route utilised the procedure of Borcherding and co-workers to form protected dihydroxycyclopentenone **4** in four steps from D-(+)-ribonic acid γ-lactone **1** (Scheme 1.1).[37] The 1,2-diol moiety was first protected, then base mediated lactone opening allowed periodate cleavage of the resulting 1,2-diol before reforming the lactone to give compound **2**. Transacetalisation of the non-protected alcohol with isopropylalcohol afforded compound **3**. Finally, treatment with dimethyl methylphosphonate and butyl

lithium resulted in opening of the lactone with elimation of the propoxy group to form a phosphonate ester which then underwent an intramolecular Horner-Wadsworth-Emmons reaction to produce cyclopentenone **4**.



Scheme 1.1 Synthesis of cyclopentenone **4**

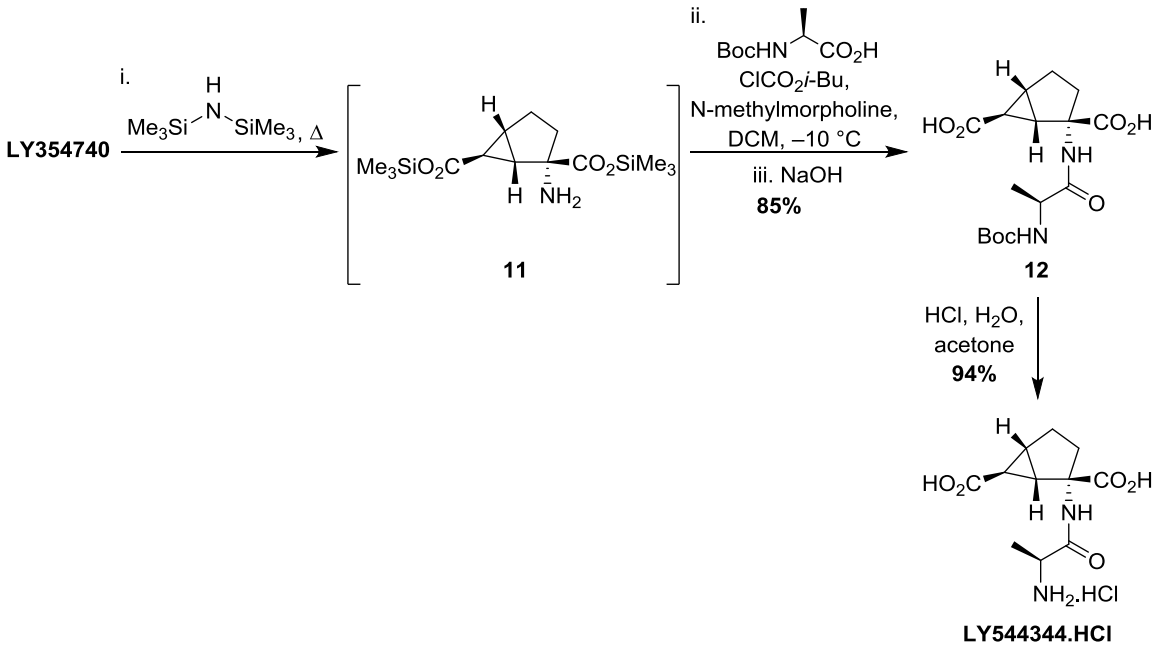
The second part of synthesis was carried out by Dominguez and co-workers (Scheme 1.2).[38] Cyclopropanation using a sulfur ylide produced fused bicyclic compound **5**, after which removal of the protected hydroxyl groups gave ketone **9**. The Bucherer-Bergs reaction using potassium cyanide and ammonium carbonate was then employed to form hydantoin **10**, the hydrolysis of which afforded the target compound, amino acid LY354740.



Scheme 1.2 Synthesis of mGluR2/3 agonist LY354740

During human trials LY354740 was found to have poor oral bioavailability of 3−5%, which led to the investigation of a prodrug approach.[39] A prodrug is a derivative of a drug compound, designed to undergo either an enzymatic or chemical transformation *in vivo* to release the active parent drug.[40] In the case of LY354740, the amine and carboxylic acid groups are highly polar, causing the compound to have difficulty in crossing the cell membrane. To overcome this LY354740 was acylated with L-alanine to form LY544344 (Scheme 1.3).

LY544344 was synthesised from LY354740 by first protecting both carboxylic acid groups as trimethylsilyl esters giving diester **11**, followed by acylation by treatment with the anhydride produced from Boc-L-alanine and isobutyl chloroformate to afford amide **12**. Hydrolysis of the trimethylsilyl groups and Boc deprotection then gave prodrug LY544344 as a hydrochloride salt (Scheme 1.3).[39]



Scheme 1.3 Synthesis of prodrug LY544344

The dipeptide moiety present in LY544344 was incorporated in order to aid crossing the cell membrane through active transport by targeting the peptide transporter PepT1. Using this strategy the oral bioavailability in rats was increased to 84% (compared to 10% for LY354740) with no prodrug circulating after thirty minutes.[41] When trialled in humans for the treatment of general anxiety disorder LY544344 produced a greater improvement in anxiety levels than placebo, however, the trial was discontinued early due to observation of convulsions in pre-clinical species (rat and dog).[42]

Another highly potent mGluR2/3 agonist that has drug potential is LY404039, in this case for the treatment of Schizophrenia. Structurally, LY404039 is very similar to previously discussed LY354740, with the only difference between them being the incorporation of a sulfone in the five membered ring (Figure 1.8).

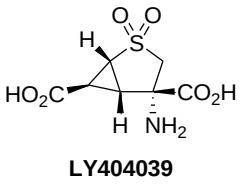
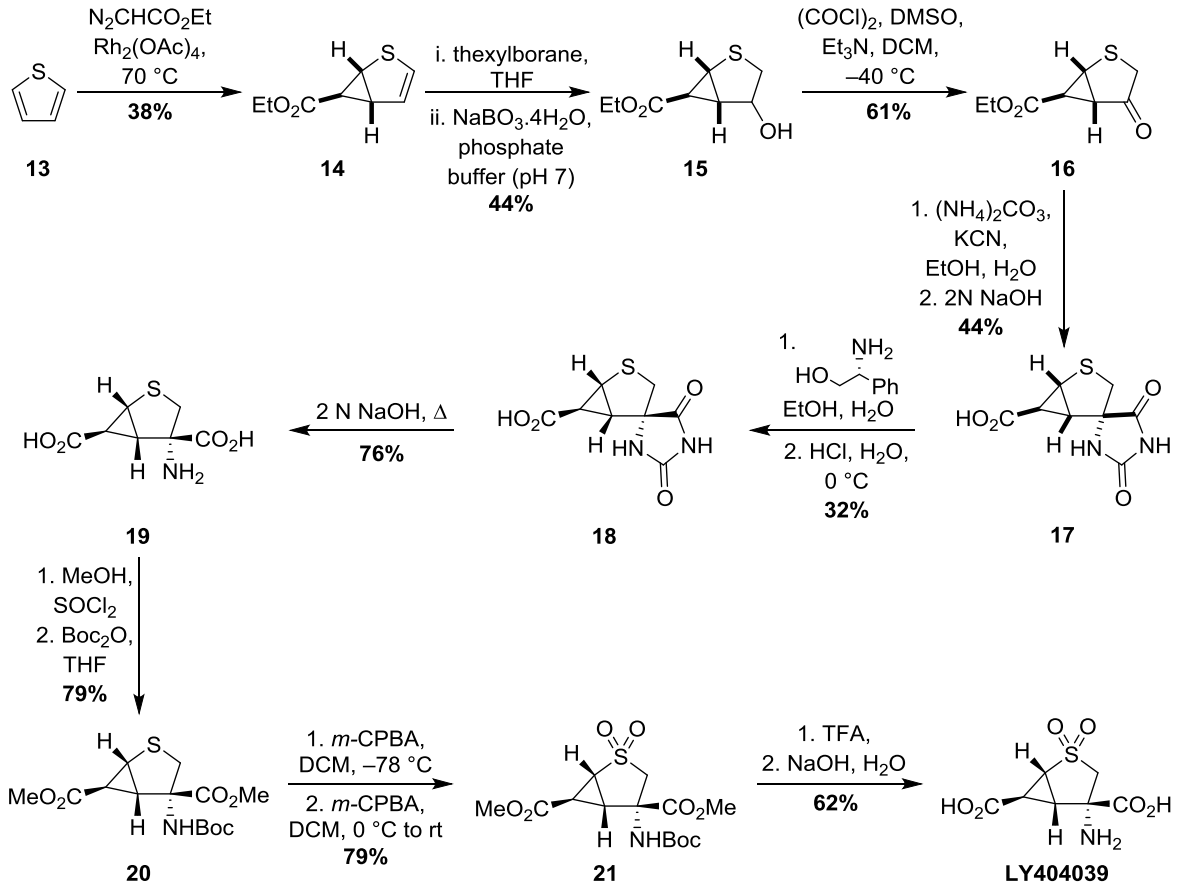


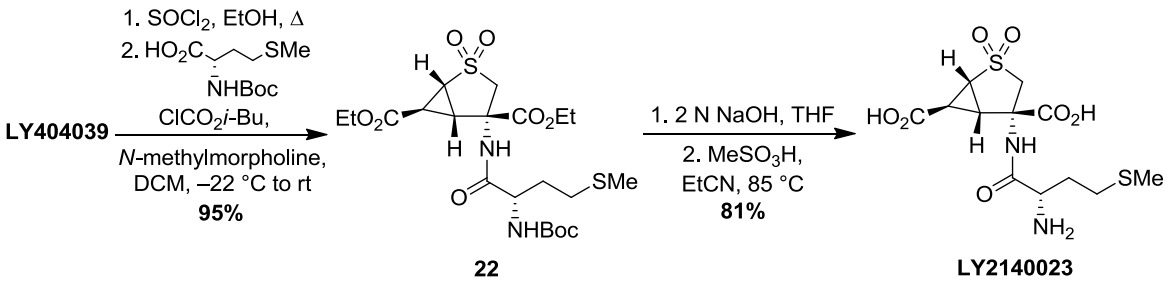
Figure 1.8 Structure of mGluR2/3 agonist LY404039

A synthesis of LY404039 is provided in Scheme 1.4.[43,44] Starting from thiophene **13** cyclopropanation was achieved via a rhodium(II)-catalysed reaction with ethyl diazoacetate to form bicyclic compound **14**. Hydroboration followed by *in situ* oxidation gave alcohol **15**, which was converted to ketone **16** by a Swern oxidation. The Bucherer-Bergs reaction and subsequent ester hydrolysis produced hydantoin **17** as a racemic mixture. The enantiomers were resolved at this stage by crystallisation of the (*R*)-phenylglycinol salt, followed by treatment with acid to form the desired single enantiomer **18**. Hydrolysis of the hydantoin moiety, followed by protection of the resulting amino acid gave compound **20**. Finally, oxidation of the sulfide to the corresponding sulfone **21** and removal of the protecting groups afforded LY404039.



Scheme 1.4 Synthesis of LY404039

Like the previously discussed mGluR2/3 agonist (LY354740), a prodrug was used for human testing. The prodrug named LY2140023 was synthesised from LY404039 by ester formation and amide coupling, followed by ester hydrolysis and Boc deprotection (Scheme 1.5).[45]



Scheme 1.5 Synthesis of prodrug LY2140023

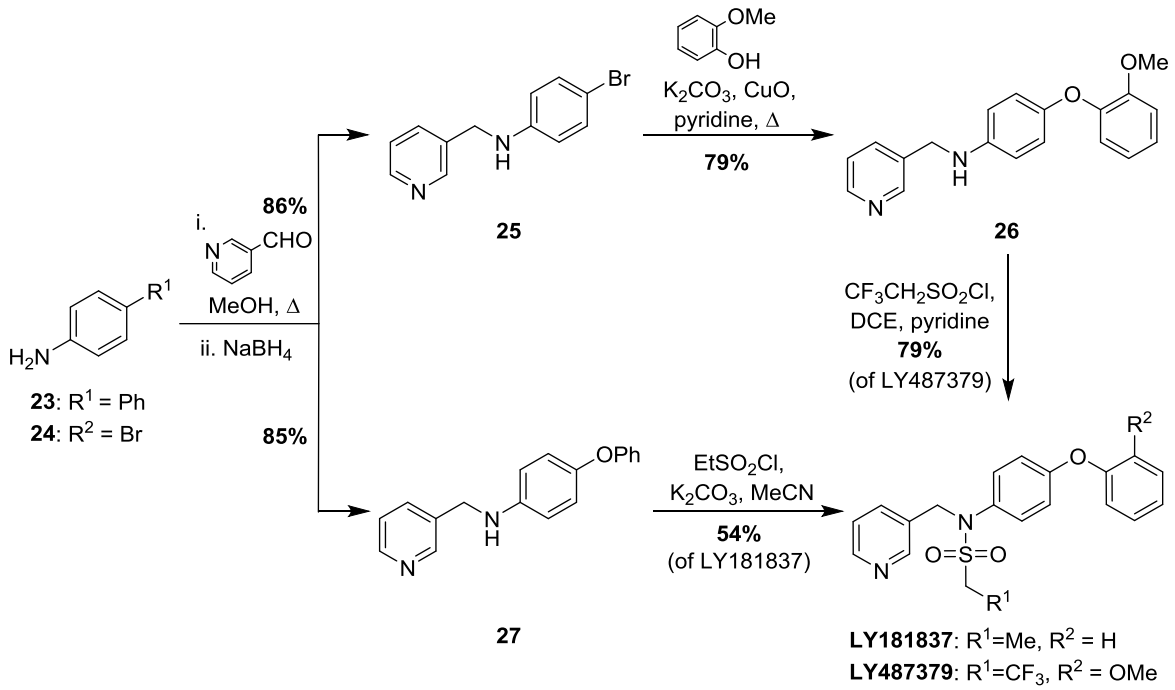
In 2007, LY2140023 underwent a phase II clinical trial with schizophrenia patients in which it was found to produce similar results to the standard olanzapine treatment without many of the previously described side-effects.[46] Unfortunately further trials produced inconclusive results and a press release in 2012 announced the termination of an on-going phase III study.[47]

1.3.2. Positive Allosteric Modulators

With mGluR2/3 agonists not providing the desired results clinically, attention was turned to the development of positive allosteric modulators (PAMs) as potential drug compounds. A positive allosteric modulator provokes a biological response upon binding to an allosteric (rather than the orthosteric) site on a receptor. The mGluR2 PAMs developed to date tend to be less complex to produce synthetically and more lipophilic than the previously described mGluR2/3 agonists, and so do not require a prodrug strategy.

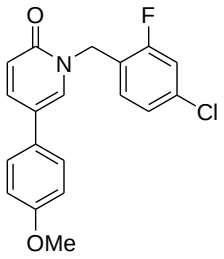
Eli Lilly developed the first mGluR2 PAMs, which were published in 2003.[48] This work initially identified LY181837 as a mGluR2 PAM, and the synthesis of further analogues in an attempt to improve potency produced LY487379 (Scheme 1.6). The synthesis of these compounds is illustrated in Scheme 1.6. LY181837 was formed in two steps by the reductive amination of phenoxy-substituted aniline **23** to give secondary amine **27**, followed by sulfonamide formation. LY487379 was formed via a similar synthetic route, employing the analogous starting material, 4-bromoaniline **24**. Reductive animation followed by Ullmann reaction formed diaryl ether **26**, and subsequent treatment with a trifluoroethyl substituted sulfonyl chloride afforded LY487379.[48]

Animal studies showed that like the previously described orthosteric agonists, LY487379 is able to reverse amphetamine or PCP induced hyperlocomotion in mice (animal model for psychosis).[49] LY487379 has also demonstrated potent activity in an animal behaviour test for anxiety.[50]



Scheme 1.6 Synthesis of mGluR2 positive allosteric modulators LY181837 and LY487379

Currently the most advanced mGluR2/3 PAM for the treatment of Schizophrenia is ADX71149, produced from a collaboration between Addex Therapeutics and Janssen Research and Development. Unfortunately the structure of ADX71149 has not been disclosed, but insight can be gained into the likely structure based upon the fact that Addex and Janssen have previously published papers and patents for mGluR2 PAMs based around two structural classes, substituted pyridones such as compound **28** and triazolopyridines such as compound **29** (Figure 1.9).[51-54]



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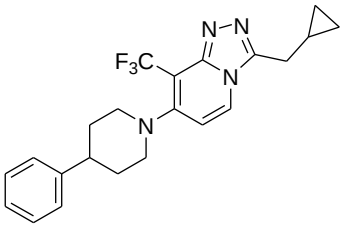

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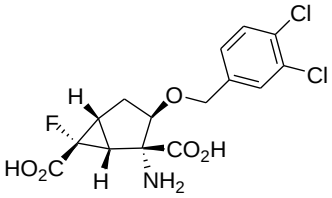
Figure 1.9 Structures of known mGluR2 PAMs

ADX71149 has been progressed into phase II trials and a press release in late 2012 revealed that ADX71149 had met safety and tolerability objectives, as well as demonstrating an effect in patients with residual negative symptoms when taken as an adjunctive therapy to their currently prescribed antipsychotics.[55] This represents a very encouraging result and in-patient trials of ADX71149 are continuing.

1.3.3 Antagonists

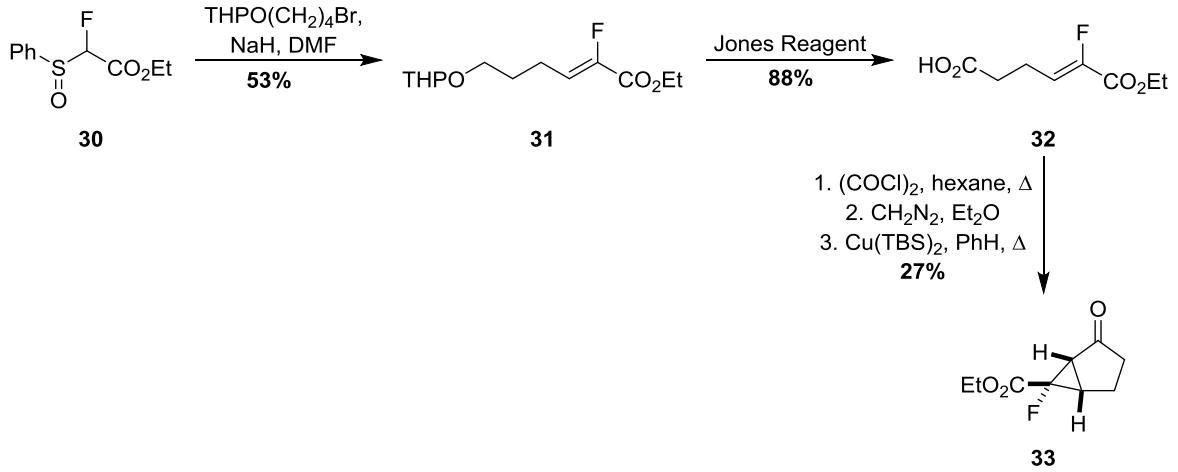
A number of potent antagonists of mGluR2/3 have also been developed, such as MGS0039 (discovered by Taisho Pharmaceutical), which has exhibited both antidepressant and anxiolytic effects in various animal behaviour models (Figure 1.10).[56-58]



MGS0039

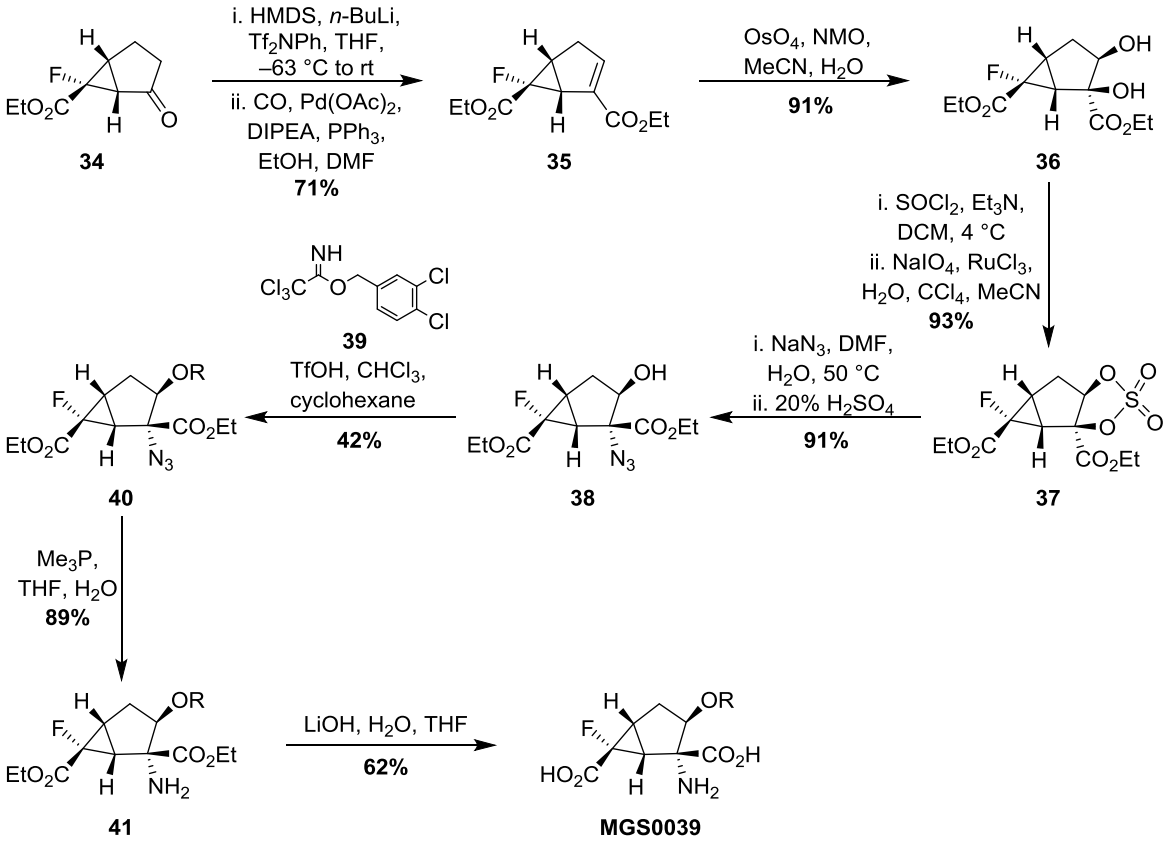
Figure 1.10 Structures mGluR2/3 antagonist MGS0039

A synthesis of MGS0039 is shown in Schemes 1.7 and 1.8. Starting from sulfoxide **30,** *Z*-alkene **31** was formed by alkylation and sulfoxide elimination. Treatment with the Jones reagent hydrolysed the THP protecting group and oxidised the resulting alcohol to carboxylic acid **32**. Diazotisation and copper-mediated cyclopropanation were performed to give bicyclic compound **33** as a racemic mixture.[59]



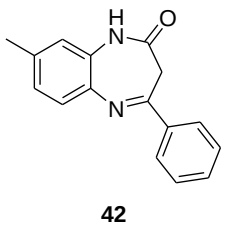
Scheme 1.7 Synthesis of bicyclic compound **33**

As this stage, the two enantiomers were separated by chiral HPLC to give enantiopure **34**. The enol triflate of ketone **34** was formed, followed by a palladium-catalysed carbonylation reaction to afford ester **35** (Scheme 1.8).[60] Dihydroxylation was then performed in which osmium(VIII) tetroxide reacted selectively at the face opposite to the fused cyclopropane ring, and the hydroxyl groups were then protected to produce cyclic sulfate **37**. Nucleophilic attack by azide followed by sulfate hydrolysis produced azide **38**. The dichlorobenzyl ether side chain was attached by reaction of trichloroacetimidate **39** with the remaining hydroxyl to form compound **40**. Finally, Staudinger reduction and ester hydrolysis gave access to MGS0039.



Scheme 1.8 Synthesis of mGluR2/3 antagonist MGS0039

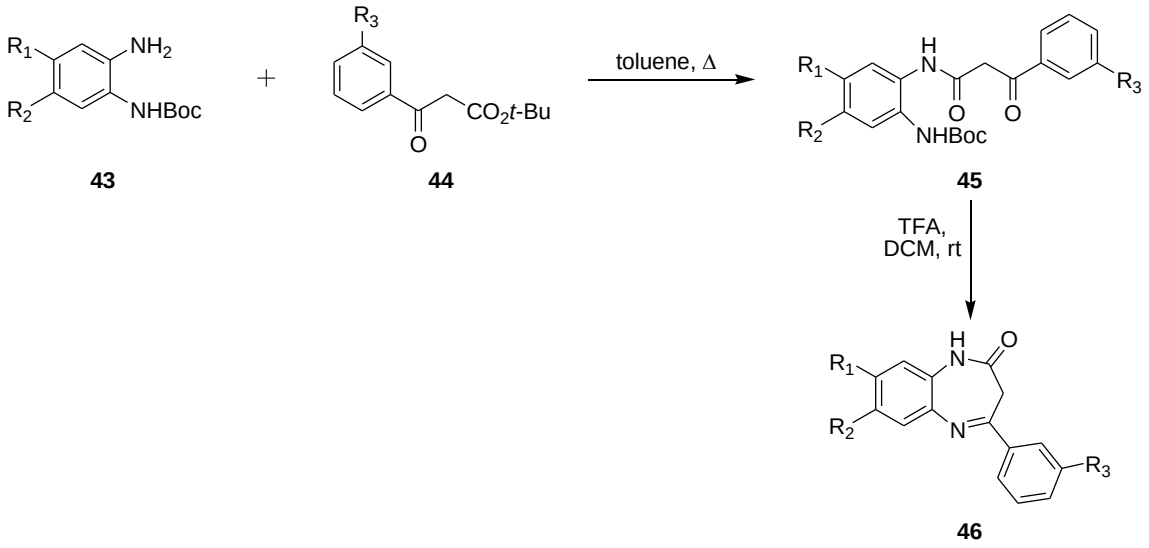
A series of papers published between 2007 and 2010 by Woltering and co-workers from Hoffmann-La Roche identified a series of compounds that are non-competitive antagonists of mGluR2/3.[61-64] The initial hit compound discovered through random screening was 1,5-benzodiazepin-2-one **42** (Figure 1.11). Its binding affinity was assessed by displacement of [^{3}H]-LY354740 (mGluR2/3 agonist) from recombinant rat mGluR2 expressed in CHO cells. As benzodiazepinone **42** is a non-competitive antagonist it was found to partially inhibit [^{3}H]-LY354740 (10 nM) binding to mGluR2 (leaving a residual of 25% specific bound) with an IC_{50} of 6.4 μM.



42

Figure 1.11 Structure of benzodiazepinone **42**

Based on this initial hit, a library of compounds were synthesised to investigate the structure activity relationship and attempt to improve potency. These compounds were prepared using the general strategy of the condensation of mono-Boc protected 1,2-diaminobenzene fragments with the general structure **43**, with β-keto esters **44**, followed by treatment with acid to form target 1,5-benzodiazepinones **46** (Scheme 1.9 – See tables 1.1 and 1.2 for R groups). This is a particularly advantageous method for forming highly substituted 1,5-benzodiazepinones as many other procedures utilise non-protected 1,2-diaminobenzenes, which limits the scope of substituents in the 7- (R^2) and 8- (R^1) positions due to regioselectivity issues (further discussed in Section 1.4).



Scheme 1.9 Synthesis of benzodiazepinones

Binding was evaluated using the assay described previously for benzodiazepinone **42**. By investigating the effect of different substituents around the aromatic rings, it was quickly established that substitution in the R^3-position resulted in increased activity, with *N*-linked imidazoles and triazoles allowing access to particularly potent compounds. Various groups were also investigated in the R^1- and R^2-positions, which revealed two particularly potent classes of compound.

The first is those with either a halide or phenyl halide in the R^1-position and hydrogen in the R^2-position. Table 1.1 details the binding data for selected compounds from this series. From these results it can be seen that the presence of a halogen substituent offers increased activity over the non-substituted phenyl ring in the R^1-position. The two *ortho* fluorinated

compounds (**48** and **50**) were evaluated in vivo which revealed them to be brain penetrant. Their antagonistic activity was also demonstrated in vivo by complete reversal of LY354740 induced hypolocomotion in mice when neither compound caused a significant increase in locomotor activity when administered alone.

Compound	R^1	R^2	R^3	Rat mGluR2 [^{3}H]-LY354740 (10 nM) binding, IC_{50} (nM)
47	Ph	H	1-imidazolyl	39
48	2-F-C_6H_4-	H	1-imidazolyl	12
49	4-F-C_6H_4-	H	1-imidazolyl	17
50	2-F-C_6H_4-	H	1,2,3-triazolyl	16
51	Br	H	1-imidazolyl	72
52	Br	H	1,2,3-triazolyl	130
53	Cl	H	1,2,3-triazolyl	60

Table 1.1 Biological screening results of a series of mGluR2/3 antagonists.

The second class of highly potent compounds identified were those with a trifluoromethyl group in the R^1-position and either an alkyl, alkoxy and amine substituent in the R^2-position. Selected binding data obtained from this series are described in Table 1.2. From these results it can be seen that alkyl, alkoxy, and amine substituents (compounds **55−59**) in the R^2-position all give increased potency over a hydrogen atom (compound **54**).

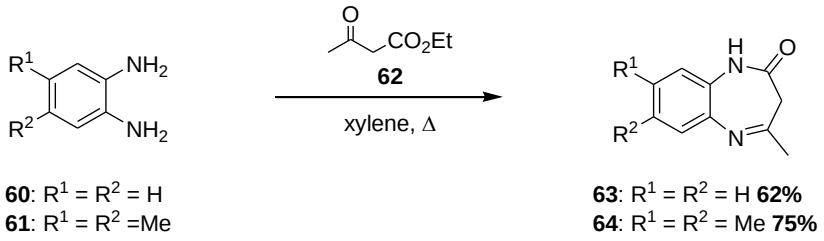
Compound	R^1	R^2	R^3	Rat mGluR2 [^{3}H]-LY354740 (10 nM) binding, IC_{50} (nM)
54	CF_3	H	1-imidazolyl	43
55	CF_3	NMe_2	1-imidazolyl	9
56	CF_3	NH*i*-Bu	1-imidazolyl	19
57	CF_3	Me	1-imidazolyl	12
58	CF_3	OMe	1,2,3-triazolyl	29
59	CF_3	OEt	1,2,3-triazolyl	7

Table 1.2 Biological screening results of a series of mGluR2/3 antagonists.

1.4 Synthesis of 1,5-Benzodiazepinones

Benzodiazepine derivatives are heterocyclic systems that are commonly found in biologically active compounds. 1,5-Benzodiazepinones in particular have shown activity as mGluR2/3 antagonists (discussed in Section 1.3.3), Chk-1 inhibitors and CCK1 receptor agonists, to name a few.[65,66] Due to the potential applications of 1,5-benzodiazepinones there has been much research into the development of synthetic methodology to produce such compounds.

Probably the simplest method of 1,5-benzodiazepinone synthesis is the treatment of a 1,2-diaminobenzene with a β-keto ester. The synthesis of 1,5-benzodiazepinones **63** and **64** by Achour and co-workers using this procedure is illustrated in Scheme 1.10.[67] When utilising this method the 1,2-aminobenzenes employed tend to be either unsubstituted or have the same substituent in the R^1- and R^2-positions to ensure that there is only one possible product regioisomer.

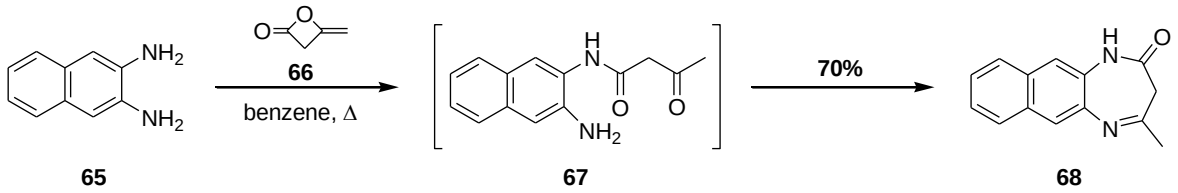


60: $R^1 = R^2 = H$
61: $R^1 = R^2 =$Me

63: $R^1 = R^2 = H$ **62%**
64: $R^1 = R^2 =$ Me **75%**

Scheme 1.10 Synthesis of 1,5-benzodiazepinones **63** and **64**

An early synthesis of a 1,5-benzodiazepinone using the treatment of diaminonaphthalene **65** with diketene **66** was published in 1959 by Ried and co-workers.[68] In this reaction, one of the amine groups of diaminonaphthalene **65** attacks the carbonyl moiety of diketene **66**, which opens to form β-keto ester **67**. This can then undergo imine condensation with the remaining free amine to produce benzodiazepinone **68** (Scheme 1.11).



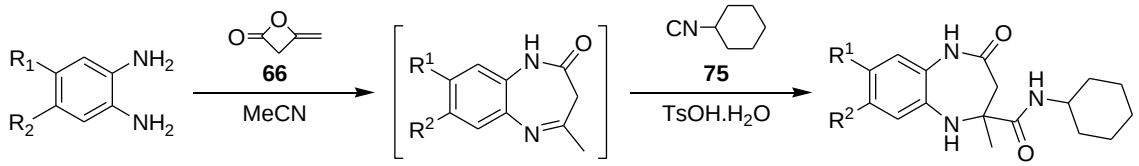
65 **67** **68**

Scheme 1.11 Synthesis of 1,5-benzodiazepionone **68**

In 2010, Shaabani and co-workers reported a procedure applying the diketene methodology to the synthesis of more complex 1,5-benzodiazepinone derivatives.[69] In this reaction, the 1,5-benzodiazepinone intermediates **63**, **64** and **72−74** were formed in the same way as the previous example, but isocyanide **75** and *p*-tosic acid were then added to incorporate an amide substituent in the 4-position of the benzodiazepinone products **76−80** (Scheme 1.12).

This reaction displays a high degree of regiocontrol. When employing benzoyl substituted **70** as the starting material, the single regioisomer product **79** was formed in a high yield of 90%. The electron-withdrawing effect of the ketone in the R^1-position of 1,2-diaminobenzene **70** results in the amine *meta* to it being the most nucleophilic, and hence carrying out the initial attack on diketene. Therefore, the benzoyl substituent appears in the 8-position of benzodiazepinone **79**. In the case of methyl substituted starting material **71**, donation of electrons from this substituent results in the amine *para* to it being the most

nucleophilic, resulting in the methyl substituent appearing in the 7-position of benzodiazepinone product **80**.



60: $R_1 = R_2 = H$
61: $R_1 = R_2 = Me$
69: $R_1 = R_2 = Cl$
70: $R_1 = COPh$, $R_2 = H$
71: $R_1 = H$, $R_2 = Me$

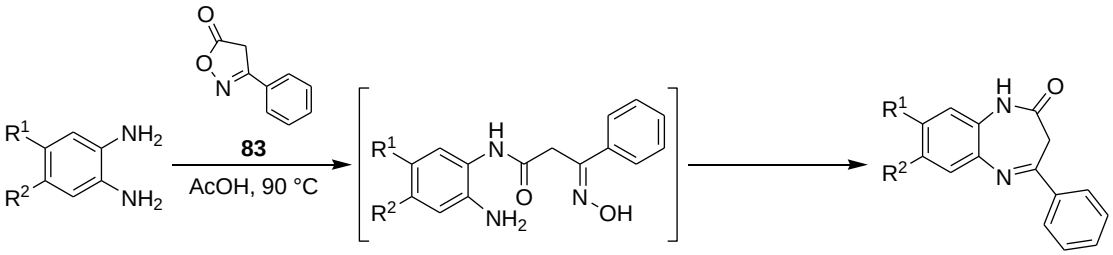
63: $R_1 = R_2 = H$
64: $R_1 = R_2 = Me$
72: $R_1 = R_2 = Cl$
73: $R_1 = COPh$, $R_2 = H$
74: $R_1 = H$, $R_2 = Me$

76: $R_1 = R_2 = H$ **90%**
77: $R_1 = R_2 = Me$ **92%**
78: $R_1 = R_2 = Cl$ **87%**
79: $R_1 = COPh$, $R_2 = H$ **90%**
80: $R_1 = H$, $R_2 = Me$ **75%**

Scheme 1.12 Synthesis of substituted benzodiazepinones **76−80**

1,5-Benzodiazepinones can also be produced from the reaction between an oxazolone and a 1,2-diaminobenzene.[70] In 1992, Rao and co-workers published the synthesis of benzodiazepinones **88−91** using this method (Scheme 1.13). This reaction proceeds by amide coupling to give oxime intermediates **84−87**, followed by imine formation with the loss of hydroxylamine.

The regiocontrol displayed in this process is the same as in the diketene reaction, producing single regioisomers with electron donating methyl substituent on the 7-position, and electron withdrawing nitro on the 8-position of benzodiazepinones **89** and **90**, respectively. Chloro substituted benzodiazepinone **91** was also produced by this method. Although halide substituents on a benzene ring can be considered to be electron withdrawing by induction due to the fact that halogens are more electronegative than carbon, they are also electron donating through one of the lone pairs being conjugated into the benzene ring. The resulting resonance forms make halogen substituents *ortho* and *para* directing, and so the most reactive amine of 1,2-diaminobenzene **82** will be the one *para* to the chloro substituent. Therefore the initial attack of oxazolone **83** will occur from that amine, resulting in the chloro substituent occupying the 7-position in the final product.



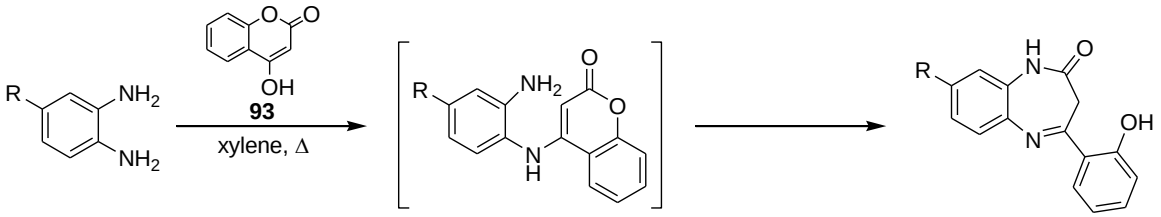
60: $R_1 = R_2 = H$
71: $R_1 = H$, $R_2 = Me$
81: $R_1 = NO_2$, $R_2 = H$
82: $R_1 = H$, $R_2 = Cl$

84: $R_1 = R_2 = H$
85: $R_1 = H$, $R_2 = Me$
86: $R_1 = NO_2$, $R_2 = H$
87: $R_1 = H$, $R_2 = Cl$

88: $R_1 = R_2 = H$ **70%**
89: $R_1 = H$, $R_2 = Me$ **80%**
90: $R_1 = NO_2$, $R_2 = H$ **65%**
91: $R_1 = H$, $R_2 = Cl$ **68%**

Scheme 1.13 Synthesis of 1,5-benzodiazepinones **88−91**

Another 1,5-benzodiazepinone forming reaction which employs a heterocyclic starting material is the reaction between a hydroxycoumarin and a 1,2-diaminobenzene, first published by Hamdi and co-workers in 1994 (Scheme 1.14).[71] This reaction proceeds with the opposite regioselectivity to the previous two examples. Starting from 1,2-diaminobenzenes **60**, **71**, **82** and **92**, treatment with hydroxycoumarin **93**, produced enamine intermediates **94−97**, rather than the β-keto amide intermediates formed in the previous examples. The enamine substituents are the result of the most reactive amine reacting with the enol moiety of hydroxycoumarin **93**. Intramolecular amide formation then produced the 1,5-benzodiazepinone products **98−101** with the substituents in the 8-position of the benzodiazepine ring.



60: R = H
71: R = Me
82: R = Cl
92: R = F

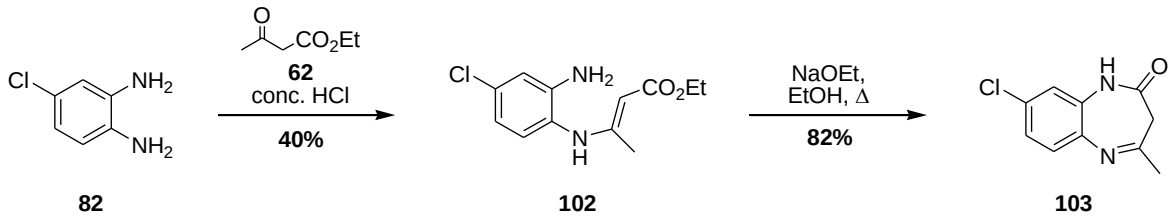
94: R = H
95: R = Me
96: R = Cl
97: R = F

98: R = H **85%**
99: R = Me **75%**
100: R = Cl **90%**
101 R = F **75%**

Scheme 1.14 Synthesis of 1,5-benzodiazepinones **98−101**

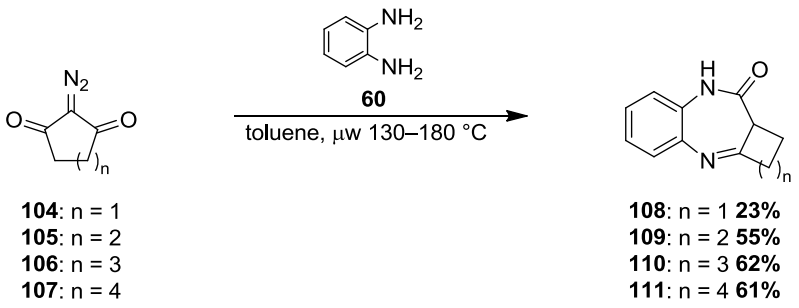
A 1,5-benzodiazepinone synthesis carried out by Capuano and co-workers as part of the synthesis of a series of compounds with affinity for dopamine and serotonin receptors is shown in Scheme 1.15.[72] Like the previous example, an enamine is first formed, followed by amide coupling. However, unlike the previous example this occurs over two steps. 1,2-

Diaminobenzene **82** was treated with β-keto ester **62** under acidic conditions to favor imine formation at the most reactive amine. The imine then isomerised to give enamine **102** which was isolated and then underwent amide formation under basic conditions to give 1,5-benzodiazepinone **103**.



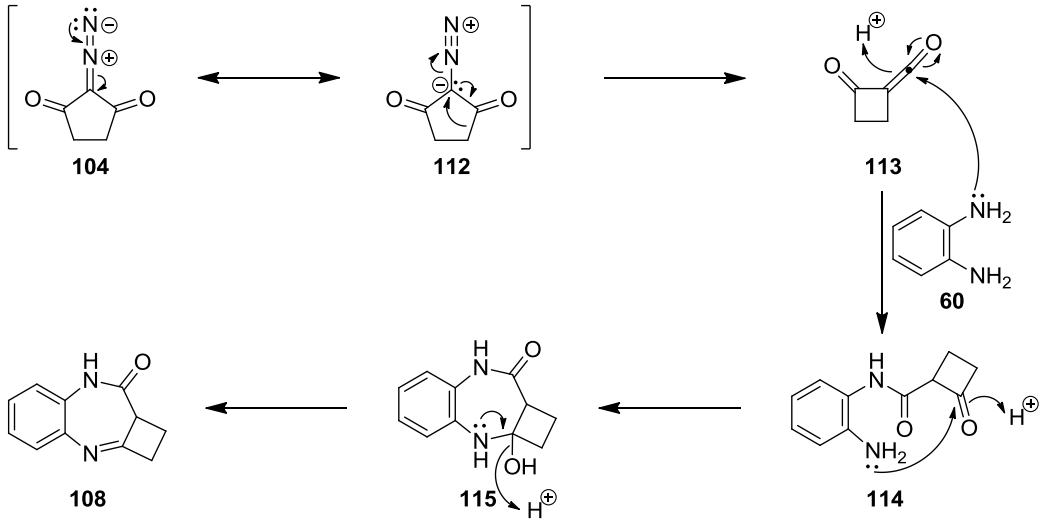
Scheme 1.15 Synthesis of 1,5-benzodiazepinone **103**

A synthesis of 1,5-benzodiazepinones by a microwave mediated reaction between a cyclic 2-diazo-1,3-diketone (**104−107**) and 1,2-diaminobenzene **60** was reported by Castilo and co-workers in 2012.[73] Using this method 1,5-benzodiazepines **108−111** were produced, containing a fused carbocycle in the 3,4-position (Scheme 1.16).



Scheme 1.16 Synthesis of 1,5-benzodiazepinones **108−111**

The mechanism for the transformation of 2-diazo-1,3-diketone **104** to 1,5-benzodiazepinone **108** is demonstrated in Scheme 1.17. Initially, a Wolff rearrangement takes place to form α-oxo ketene **113**. It has previously been shown that thermal Wolff rearrangements of 2-diazo-1,3-diketone proceed by a concerted process rather than via a carbene intermediate.[74] Nucleophilic trapping of α-oxo ketene **113** with 1,2-diamino benzene **60** then occurs to form β-keto amide **114**, followed by intramolecular imine condensation to give 1,5-benzodiazepinone **108.**



Scheme 1.17 Mechanism of the formation of 1,5-benzodiazepinone **108**

All of the methods for the synthesis of 1,5-benzodiazepinones described in this section are limited by the relative lack of substituents around the benzodiazepinone core due to the issue of regioselectivity. The procedure employed by Woltering and co-workers (discussed in Section 1.3.3) in which one of the amine groups of the 1,2-diaminobenzene starting material is protected allows complete control of the position of the substituents in the final product, and so allows for the greatest amount of versatility in terms of substitution.

The ability of the method used by Woltering and co-workers to allow many different substituents around the benzodiazepinone core makes such compounds ideal candidates for the incorporation of radiolabels for use in nuclear imaging.

1.5 Nuclear Imaging

Nuclear imaging is a widely used technique in which a biologically active compound is labelled with a radionuclide that emits radiation allowing its position to be traced. Images of tumours, and organs such as the brain, heart and lungs can be produced using this method.[75] The compounds used in this procedure are known as radiotracers and there are various properties required of a successful radiotracer. An ideal radiotracer should have both a decay half-life and a biological half-life similar to the length of the test (a few hours). Alpha and beta particles have a short range and so will only deliver a high

radiation dose to the patient and not contribute to the image, so choice of radioisotope is limited to gamma and positron emitters. The radiotracer should also ideally only localise in the area of interest. The specific techniques used in nuclear imaging are single photon emission computed tomography (SPECT) and positron emission tomography (PET).

1.5.1 Single Photon Emission Computed Tomography (SPECT)

SPECT is a medical imaging technique that makes use of radioisotopes which decay with the emission of single photons. The most commonly used are iodine-123, technetium-99m and indium-111 (Table 1.3).[76]

Radioisotope	Decay	Photon energy (keV)	Half-life
^{99m}Tc	electron capture	140	6.03 hours
^{111}In	electron capture	173, 247	2.81 days
^{123}I	electron capture	160	13.0 hours

Table 1.3 Radioisotopes commonly used in SPECT

Once the radiopharmaceutical has been injected, a gamma camera is rotated 360º around the patient, detecting photon emission at a range of angles. A lead collimator is used to reduce the number of photons that strike the detector, allowing only those travelling perpendicular to the detector to travel through (Figure 1.12). This process allows positional information to be obtained, as without it there would be no relationship between where photons are coming from and where they strike the detector. As a typical collimator only accepts 0.01% of gamma rays, this decreases the sensitivity of the technique, making collimator design a compromise between sensitivity and positional resolution.[77] Gamma rays can then strike the scintillation crystal which produces signals that can be processed to form an image.

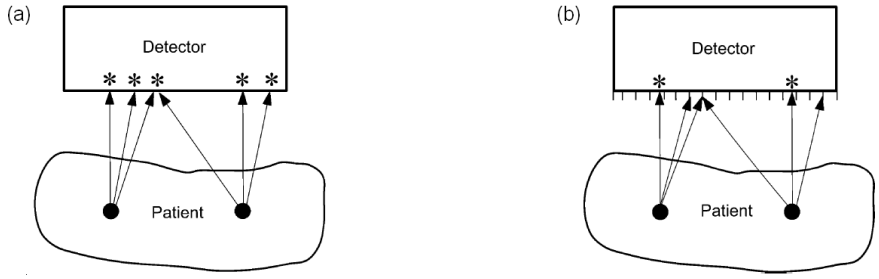


Figure 1.12 (a) emitted radiation striking detector with no collimator, (b) emitted radiation striking detector with collimator[75]

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1.5.2 Positron Emission Tomography (PET)

PET imaging employs low molecular mass radioisotopes such as carbon-11, nitrogen-13, oxygen-15 and fluorine-18 that decay by positron emission (Table 1.4).[78] These are radioisotopes of elements more commonly found in biologically active compounds than those used in SPECT.

Radioisotope	Decay	Photon Energy (keV)	Half-Life
^{11}C	positron emission	511	20.3 min
^{13}N	positron emission	511	10.0 min
^{15}O	positron emission	511	2.07 min
^{18}F	positron emission	511	110 min

Table 1.4 Radioisotopes commonly used in PET

Positron emission originates from a proton in the nucleus being converted to a neutron with the emission of a positron and a neutrino. The positron then undergoes annihilation with an electron to create two photons with an energy of 511 keV, emitted in opposite directions, at 180º to each other (Figure 1.13). Simultaneous detection of these photons allows their emission point to be determined. As this removes the need for a lead

collimator, PET is a more sensitive technique than SPECT. It also provides better spatial resolution than SPECT of around 5 mm due to the distance covered by the positron before annihilation.[75] However, there are disadvantages. The radioisotopes used in PET have a short half-life. As a consequence of this for a facility to carry out PET, they must produce these radioisotopes on site using a cyclotron. The expense of requiring a cyclotron causes PET to be significantly more costly than SPECT.

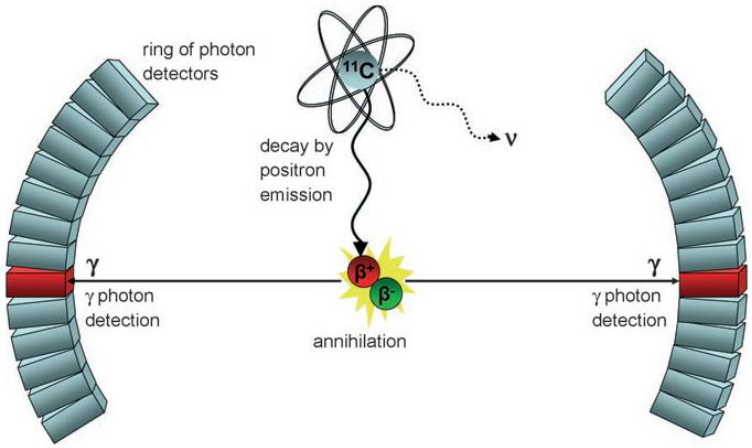


Figure 1.13 Positron annihilation in PET imaging[78]

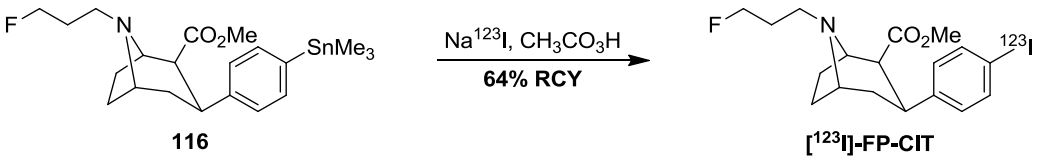
(Reprinted with permission from *Angew. Chem. Int. Ed.*, 2008, **47**, 8998−9033. Copyright 2008 John Wiley and Sons)

1.5.3 Synthesis and Use of Imaging Agents

In the synthesis of imaging agents it is essential to incorporate the radionuclide in the final stages of the synthetic route to avoid the chemist having to work with a radioactive substance in subsequent steps, and to ensure the labelled compound decays as little as possible before use. The result of radiosynthesis is measured in terms of radiochemical yield (RCY), in which the yield is expressed as the percentage of the initial radioactivity incorporated in to the reaction product. Therefore, it is the reagent containing the radionuclide that is the limiting compound, with the imaging agent precursor in excess. Radiochemical yield can be expressed as either corrected to take account of the decay that occurred during the reaction or as an uncorrected value. The corrected value only takes into account the efficiency of the incorporation of the radionuclide into the target compound, whereas the uncorrected value also incorporates the speed of the process. To

be used in radiolabelling, a reaction must be high yielding in terms of the transformation to the target compound, but also must proceed quickly.

A commonly used SPECT imaging agent is [^{123}I]-FP-CIT, which has high affinity for presynaptic dopamine transporters. This compound is produced from trimethylstannane precursor **116**, which is transformed to the iodine-123 labelled imaging agent by oxidative iododestannylation using iodine-123 labelled sodium iodide. A preparation published in 1994 by Neumeyer and co-workers achieved this transformation in a 64% radiochemical yield (Scheme 1.18).[79]



116 **[^{123}I]-FP-CIT**

Scheme 1.18 Radiosynthesis of SPECT imaging agent [^{123}I]-FP-CIT

Once labelled [^{123}I]-FP-CIT can be used in the diagnosis and monitoring of Parkinson's disease. A feature of Parkinson's disease is the reduction in dopaminergic neurones in the striatal region of the brain. This can be observed by SPECT imaging as a scan of a patient with Parkinson's disease clearly shows reduced binding of [^{123}I]-FP-CIT when compared to a patient without Parkinson's disease (Figure 1.14).

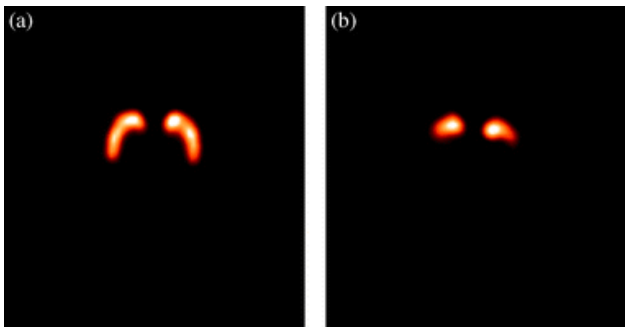
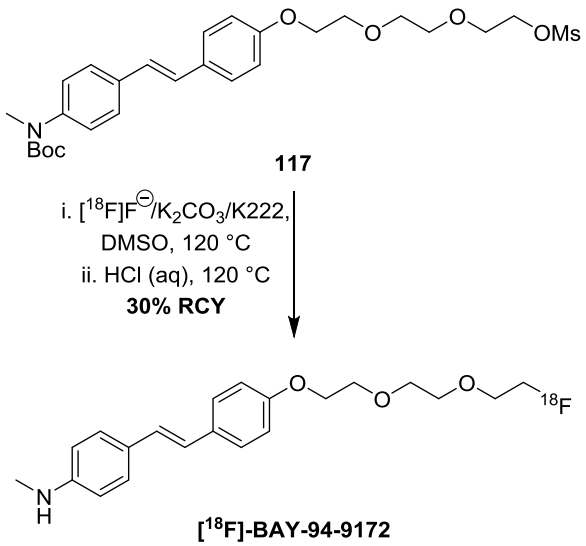


Figure 1.14 SPECT images in a (a) normal and (b) Parkinson's disease patient[76]

(Reproduced from Ref. 76 with permission from The Royal Society of Chemistry)

PET has been used to image *β*-amyloid plaques and tangles (twisted fibres mainly composed of hyperphosphorylated tau protein) which accumulate in the brain of Alzheimer's sufferers using [^{18}F]-BAY-94-9172, a fluorine-18 labelled PET radiotracer. Starting from mesylate precursor **117**, nucleophilic radiofluorination and Boc deprotection produced [^{18}F]-BAY-94-9172 in a decay corrected radiochemical yield of 30%, with a total preparation time of 90 minutes (Scheme 1.19).[80]



Scheme 1.19 Radiosynthesis of [^{18}F]-BAY-94-9172

By comparing the images obtained from a 75-year old subject with Alzheimer's disease (top picture) and a 76-year old healthy control (bottom picture) the presence of *β*-amyloid plaques and/or tangles can be clearly observed, making PET imaging an important tool in diagnosing and monitoring the progression of Alzheimer's disease (Figure 1.15).

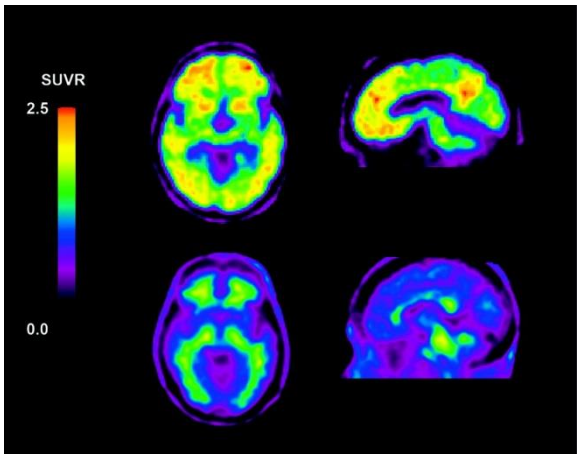


Figure 1.15 PET images showing binding of [^{18}F]-BAY-94-9172 in an Alzheimer's disease patient (top picture) and a healthy control (bottom picture)[81]

(Reprinted with permission from *Chem. Rev*., 2008, **108**, 1501−1516. Copyright 2008 American Chemical Society)

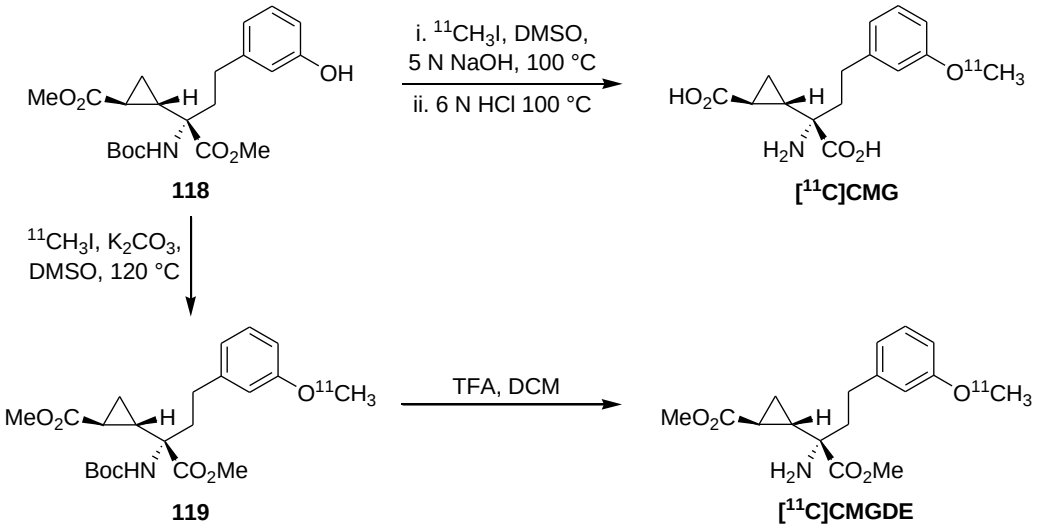
These examples illustrate the effectiveness of nuclear imaging using SPECT and PET in the diagnosis and monitoring of various conditions.

1.5.4 Imaging of mGluR2/3

Currently there are no approved radiotracers for use in imaging mGluR2/3. However a small amount of work has been published on the development of potential mGluR2/3 radiotracers for use in PET imaging. In 2012, Wang and co-workers reported the radiosynthesis and micro-PET imaging of potential mGluR2/3 imaging agents [^{11}C]CMG and [^{11}C]CMGDE.[82]

The synthesis of these compounds is provided in Scheme 1.20. Dicarboxylic acid [^{11}C]CMG was formed by alkylation of phenol precursor **118** using carbon-11 labelled iodomethane, followed by Boc deprotection and ester hydrolysis. A prodrug version of this compound ([^{11}C]CMGDE) in which the methyl esters were kept intact was also synthesised, as it was thought that this would provide a more suitable physicochemical profile for brain penetration. In order to prevent ester hydrolysis during the radiosynthesis of [^{11}C]CMGDE, alkylation was carried out using potassium carbonate rather than sodium

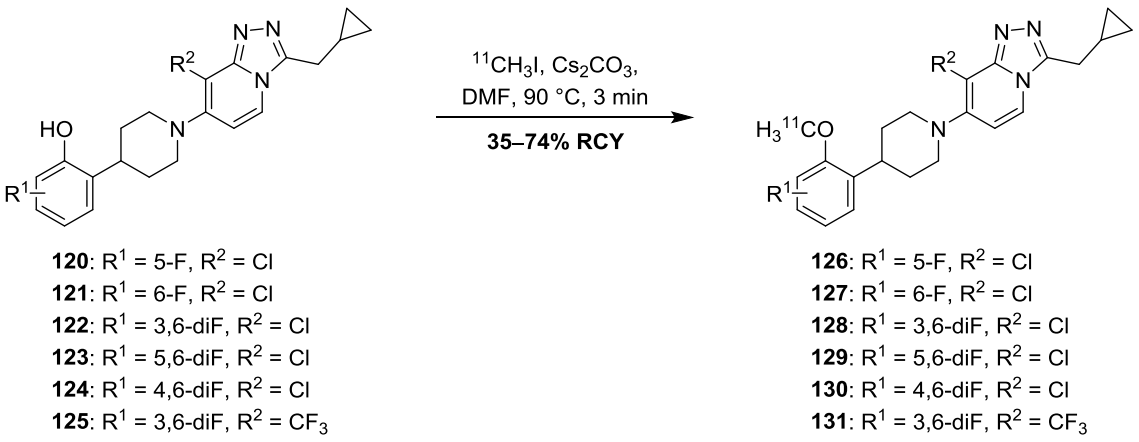
hydroxide to form compound **119**, and milder Boc deprotection was employed. No yields were included in the publication.



Scheme 1.20 Radiosynthesis of [^{11}C]CMG and [^{11}C]CMGDE

μPET imaging studies using rats showed no brain penetration upon injection with [^{11}C]CMG. However, prodrug [^{11}C]CMGDE was found to be incorporated into the brain and the resulting radiotracer (not identified, presumed to be [^{11}C]CMG or a monoester formed by *in vivo* hydrolysis) was found to bind to several brain areas known to express mGluR2/3. They were also able to demonstrate some binding specificity by observing a reduction in binding of 20−30% when LY341495 was also injected as a blocking agent.

Andrés and co-workers also published the radiosynthesis of potential mGluR2 radiotracers and μPET imaging results in 2012.[83] The compounds were derived from a series of mGluR2 positive allosteric modulators developed by Janssen Research and Development containing a triazolopyridine core. A library of compounds containing a methoxy group as potential carbon-11 labelling site were found to have low nanomolar affinity for mGluR2, and so radiolabelled versions were synthesised by alkylation of phenol precursors **120**−**125** (Scheme 1.21). This produced six carbon-11 labelled compounds in nondecay corrected radiochemical yields of from 35% to 74%.



120: R^1 = 5-F, R^2 = Cl
121: R^1 = 6-F, R^2 = Cl
122: R^1 = 3,6-diF, R^2 = Cl
123: R^1 = 5,6-diF, R^2 = Cl
124: R^1 = 4,6-diF, R^2 = Cl
125: R^1 = 3,6-diF, R^2 = CF_3

126: R^1 = 5-F, R^2 = Cl
127: R^1 = 6-F, R^2 = Cl
128: R^1 = 3,6-diF, R^2 = Cl
129: R^1 = 5,6-diF, R^2 = Cl
130: R^1 = 4,6-diF, R^2 = Cl
131: R^1 = 3,6-diF, R^2 = CF_3

Scheme 1.21 Radiosynthesis of a library of potential mGlur2 radiotracers

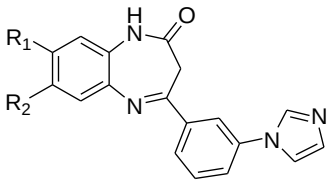
Compounds **126−131** were then subjected to studies of biodistribution and metabolism both of which identified **128** as the lead. Triazolopyridine **128** was then used in rat μPET imaging, where it showed uptake in the brain. The specificity and reversibility of this binding was demonstrated by the fact that injection of known mGluR2 PAM at 30 mins displaced radioactivity in all brain regions.

This represents a promising start in the development of imaging agents for mGluR2/3, however, the fact that these two studies represent the only work published to date shows that the imaging of mGluR2/3 is at a very early stage and further research is needed.

1.6 Proposed Research

The primary aim of this research was to develop new radiotracers to be used in SPECT and PET imaging of mGluR2/3. Non-competitive antagonists of mGluR2/3 described by Woltering and co-workers containing a 1,5-benzodiazepinone core were chosen as a starting point for the development of radiotracers.[61-64]

Compounds with the general structure of 1,5-benzodiazepinone **132** containing a phenylimidazole substituent in the 4-position were proposed as synthetic targets (Figure 1.16). It was planned to use the 7- (R^2) and 8- (R^1) positions to incorporate groups containing either iodine, carbon or fluorine which could eventually be labeled with iodine-123 for SPECT, and carbon-11 or fluorine-18 for PET imaging.



132

Figure 1.16 Structure of potential imaging agents

Following the synthesis of a small library of non-radiolabelled compounds it was intended to determine their binding affinity with mGluR2/3, and investigate their physicochemical properties to determine whether they are likely to be brain penetrant. Once the most suitable analogues have been identified they could be radiolabelled and evaluated *in vivo* as potential radiotracers to be used in SPECT and PET imaging of mGluR2/3.

2 Development of Imaging Agents for mGluR2/3

2.1 Synthesis of Potential SPECT imaging agents

The first stage of work in this project was the synthesis of compounds that could be of use for SPECT imaging. Initial targets were designed based on the compounds previously published by Woltering and co-workers containing a benzodiazepinone core and phenylimidazole group.[63] Iodine was introduced in the *ortho*, *meta* and *para* positions around a benzene ring to give compounds **133**, **134** and **135** as the first synthetic targets (Figure 2.1). These compounds could eventually be radiolabelled with iodine-123 to provide compounds for SPECT imaging.

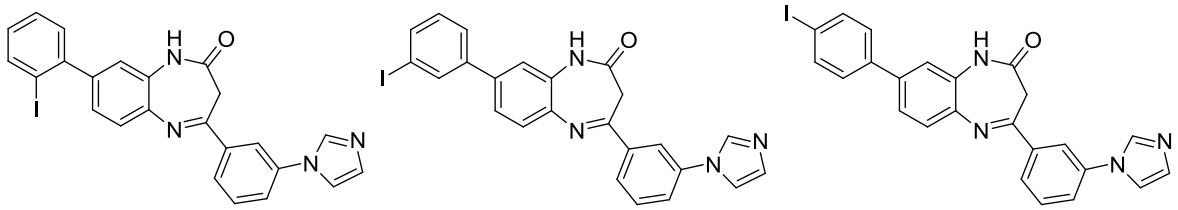


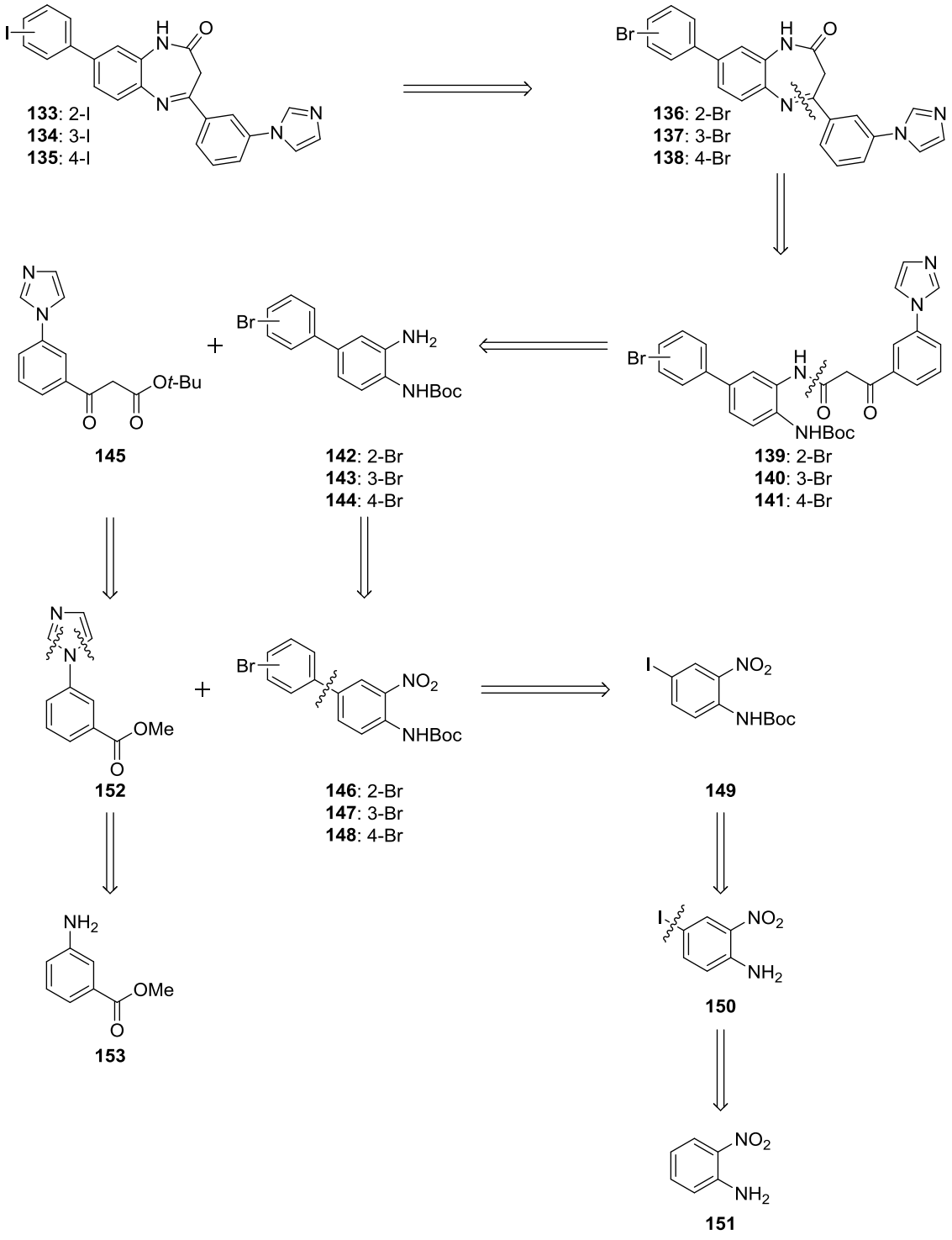
Figure 2.1 SPECT target compounds

Firstly, non-radiolabelled versions of the compounds were synthesised in order to investigate their physicochemical properties and biological activity.

2.1.1 Retrosynthetic Analysis

A retrosynthetic analysis of these initial targets is shown in Scheme 2.1. It was proposed that aryl iodides **133**, **134**, and **135** could be prepared by halogen exchange from the corresponding aryl bromides **136**, **137** and **138**. Disconnecting the imine then opens the benzodiazepinone core to reveal a β-keto amide and a protected amine. Disconnection of the amide linkage then provides amine fragments **142**, **143** and **144**, and β-keto ester fragment **145**. From the amine fragments, functional group interconversion leads to nitro benzenes **146**, **147** and **148**, which then can be disconnected between the benzene rings to give iodobenzene **149**. Removal of the protecting group on the amine followed by

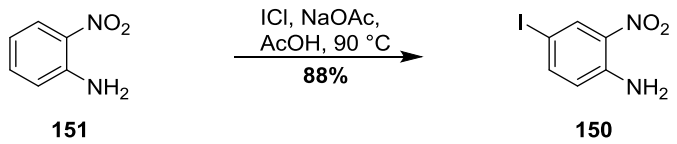
functional group interconversion and disconnection of the iodide bond, leads to commercially available 2-nitroaniline **151**. On the β-keto ester path, removal of the *tert*-butyl acetate side chain gives ester **152**, in which the imidazole heterocycle could be formed from commercially available methyl 3-aminobenzoate **153**. As this strategy involves forming the iodide in the final step it could also be used to produce radiolabelled compounds.



Scheme 2.1 Retrosynthetic analysis of SPECT targets

2.1.2 Synthesis of the Amine Fragments

The first step in the synthesis of the aminobenzene fragments was the selective iodination of 2-nitroaniline **151** to give aniline **150** by electrophilic aromatic substitution, which proceeded in an excellent yield of 88% (Scheme 2.2).[84] Iodine monochloride acts as a source of electrophilic iodine in this reaction due to the difference in electronegativity between iodine (2.66) and chlorine (3.16). The regioselectivity of this reaction can be explained by the combination of the strongly activating amino group which favours *ortho* and *para* substitution, and the strongly deactivating nitro group which favours *meta* substitution. As a result of this, the 4- and 6-positions are strongly favoured electronically for electrophilic substitution. However, the 6-position has an ortho amino group which results in it being disfavoured sterically. Therefore substitution occurs at the 4-position.



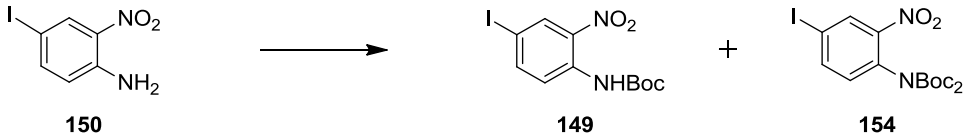
151 **150**

Scheme 2.2 Iodination of nitroaniline **151**

Protection of the amine with a Boc group was then required. Various reaction conditions were investigated as outlined in Scheme 2.3 and Table 2.1. The first method was the treatment of aniline **150** with di-*tert*-butyl dicarbonate, DMAP and triethylamine in dichloromethane. After stirring overnight there was still amine present so a further equivalent of di-*tert*-butyl dicarbonate was added. Once complete consumption of the amine was observed the reaction was purified to give the desired compound **149** in 35% yield, which could not be separated from an unidentified close running impurity, along with the bis-protected compound **154** as the major product in 65% yield (entry 1). In an attempt to reduce the amount of bis-Boc compound being formed, the reaction was repeated with a total of one equivalent of di-*tert*-butyl dicarbonate, which was added in batches. This did lead to an increased yield of the desired compound **149** of 59%, but compound **154** was still formed and the close running impurity was still present (entry 2).

At this point it became clear that an alternate approach would be necessary for this transformation. Mono-Boc protection was attempted by treating amine **150** with di-*tert*-

butyl dicarbonate under reflux in THF.[85] However, after stirring for two days only starting material was observed (entry 3). The most successful method for forming compound **149** used the conditions of Kelly and co-workers.[86] Treating amine **150** with lithium hexamethyldisilazide at 0 ºC followed by the dropwise addition of a solution of di-*tert*-butyl dicarbonate produced mono-protected amine **149** in a moderate yield of 60% and the bis-protected compound **154** in 15% yield. Although compound **154** was still produced it was a cleaner reaction than that described previously, with only starting material, desired product and bis-protected product being observed.



Scheme 2.3 Boc protection of aniline **150**

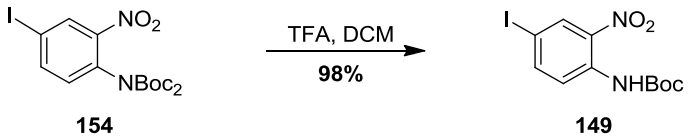
Entry	Reagent(s)	Conditions	149 (Yield)	154 (Yield)
1	Boc_2O, DMAP, Et_3N	DCM	35%*	65%
2	Boc_2O (1 eq. added in batches), DMAP, Et_3N	DCM	59%*	not recorded
3	Boc_2O	THF, Δ	0%	0%
4	**Boc_2O, LiHMDS**	**0 °C to rt**	**60%**	15%

*with unknown impurity

Table 2.1 Conditions attempted for Boc protection of aniline **150**

The NH protons of aniline **150** are more acidic than those of an unsubstituted aniline, due to the presence of the strongly electron withdrawing *ortho* nitro group. Once aniline **150** has been mono-Boc protected to form compound **149** the remaining NH proton will have further increased acidity. This allows the formation of bis-protected aniline **154** by deprotonation of mono-protected compound **149** and reaction with di-*tert*-butyl dicarbonate.

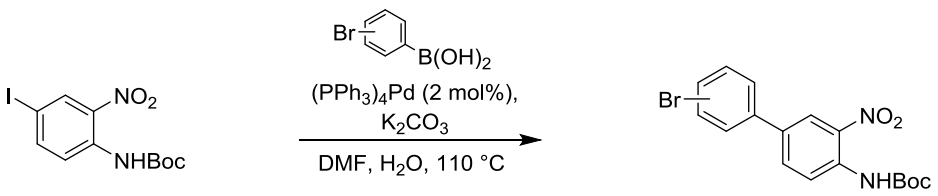
As bis-protected compound **154** was being produced in significant amounts, the mono-deprotection of this compound was attempted to investigate whether this material could be used to form mono-Boc protected amine **149**. It was found that bis-protected amine **154** underwent conversion to mono-protected amine **149** in high yield upon treatment with two equivalents of trifluoroacetic acid in dilute dichloromethane (Scheme 2.4).[87] The various attempts at forming compound **149** gave enough material to carry forward to subsequent steps. However, if this reaction were to be repeated alternative conditions would be employed using sufficient equivalents of di-*tert*-butyl dicarbonate to achieve complete conversion of aniline **150** to bis-protected **154**, followed by mono-deprotection. This would remove the need for separation of the Boc and bis-Boc protected compounds and should result in a significantly higher yield.



154 **149**

Scheme 2.4 Mono-deprotection of bis-Boc protected amine **154**

At this point, the route diverged with iodobenzene **149** being employed in a palladium(0)-catalysed Suzuki cross coupling reaction with either 2-, 3- or 4-bromophenylboronic acid (Scheme 2.5). Using these conditions, compounds **146**, **147** and **148** were produced in moderate to good yields of 62%, 72% and 78% respectively.



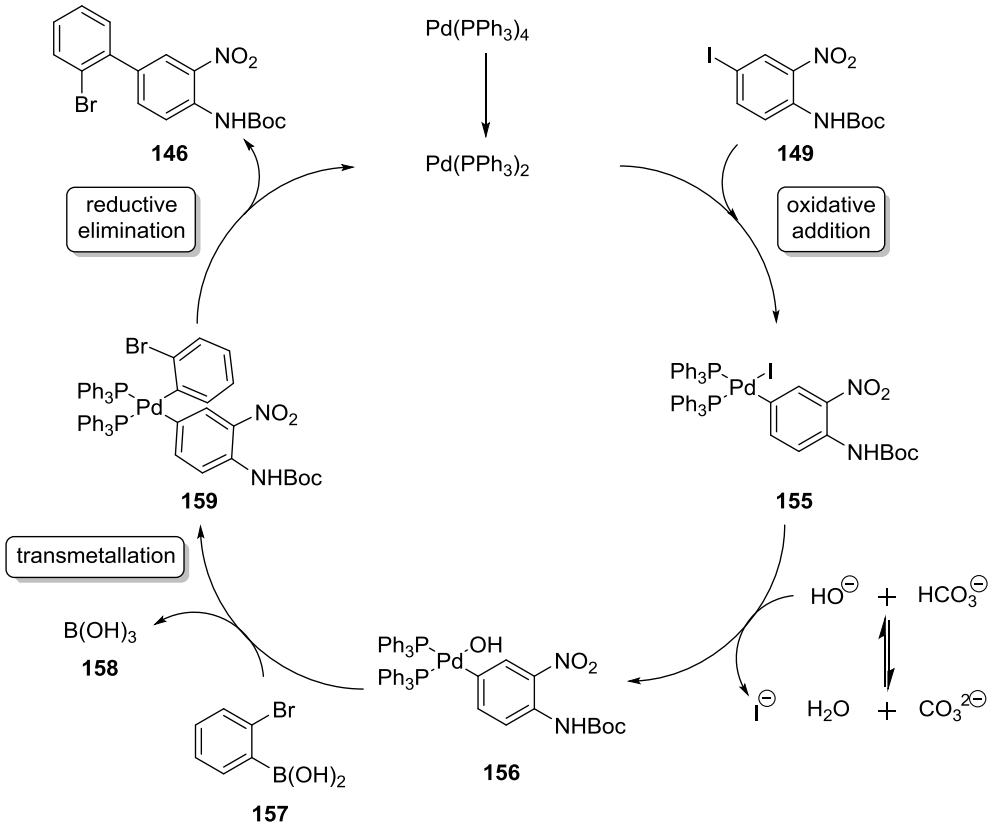
149

146: 2-Br **62%**
147: 3-Br **72%**
148: 4-Br **78%**

Scheme 2.5 Formation of biaryl compounds **146**, **147** and **148** by the Suzuki reaction

The Suzuki cross-coupling reaction proceeds via a palladium(0) to palladium(II)-catalytic cycle (Scheme 2.6). Oxidative addition of palladium(0) into the aryl halide bond of **149** first occurs to give palladium(II) species **155**, followed by exchange of the halide attached to palladium with hydroxide (formed from reaction between carbonate and water) to give

palladium(II) species **156**. Transmetalation then occurs with boronic acid **157** to form intermediate **159**. Finally, a carbon-carbon bond is formed by the reductive elimination of biaryl compound **146** and the palladium(0) species is regenerated, allowing the cycle to begin over again.[88]



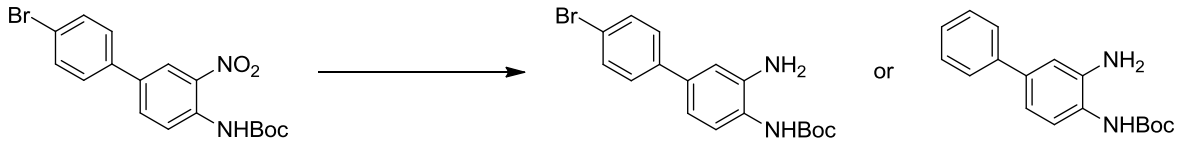
Scheme 2.6 Suzuki reaction mechanism

The Suzuki reaction was followed by nitro reduction to complete the synthesis of the amine fragments. *Para*-substituted compound **148** was used to optimise this step as detailed in Scheme 2.7 and Table 2.2. Reduction of nitrobenzene **148** to amine **144** was first attempted using palladium-catalysed hydrogenation with ammonium formate as a hydrogen transfer agent,[89] as literature precedent had been found for performing a selective nitro reduction in the presence of an aryl bromide under these conditions (entries 1 and 2).[90] The reaction was first performed at room temperature but only starting material was observed. As no reaction occurred at room temperature, the reaction was repeated at 55 ^{o}C, which resulted in the formation of debrominated compound **160** in 77% yield. To determine whether it was possible to reduce the nitro group in the presence of the aryl bromide using ammonium formate the reaction was repeated, starting at room temperature

and slowly increasing the temperature whilst monitoring the reaction by TLC. No reaction was observed until the temperature reached 40 ºC, which produced a mixture of starting material **148** and de-brominated product **160**.

An alternate method was then investigated using tin(II) chloride dihydrate following the conditions reported by Bellamy and Ou, in which nitro compounds are reduced to anilines in a non-acidic medium (entry 3).[91] Under these conditions the reaction is thought to proceed by a sequence of electron and proton transfers with tin acting as the electron source.[92] Using this procedure, the desired amine **144** was isolated but in a low yield of 11%, most likely due to loss of the Boc group.

Returning to the literature revealed a number of examples where pyridine was present in the reaction when the desired compound contained a Boc group.[93,94] Replacing methanol used in the previous reaction with ethyl acetate and pyridine, and reducing the temperature to room temperature resulted in the reaction proceeding in a yield of 63% (entry 4).

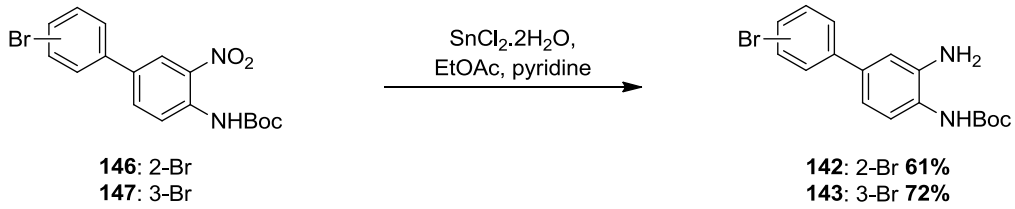


Scheme 2.7 Nitro Reduction of nitrobenzene **148**

Entry	Reagent(s)	Solvent(s)	Temperature	144 (Yield)	160 (Yield)
1	NH_4HCO_2, 10% Pd/C	MeOH,	rt	-	-
2	NH_4HCO_2, 10% Pd/C	MeOH,	55 °C	-	77%
3	$SnCl_2.H_2O$	MeOH,	70 °C	11%	-
4	**$SnCl_2.H_2O$**	**EtOAc, pyridine**	**rt**	**63%**	-

Table 2.2 Optimisation of nitro reduction

With the reaction optimised, nitrobenzenes **146** and **147** were exposed to the same conditions to give amines **142** and **143** (Scheme 2.8).



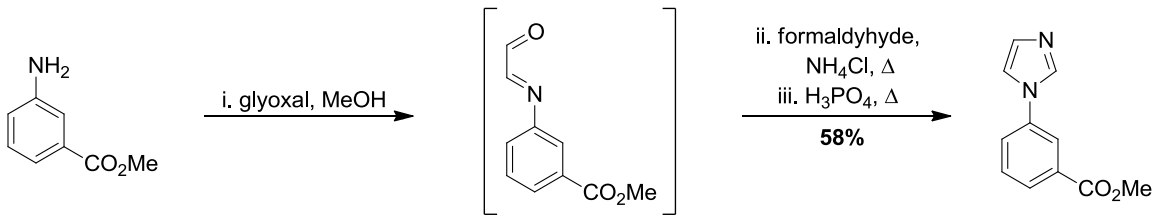
146: 2-Br
147: 3-Br

142: 2-Br **61%**
143: 3-Br **72%**

Scheme 2.8 Nitro reduction of nitrobenzenes **146** and **147**

2.1.3 Synthesis of the β-Keto Ester Fragment

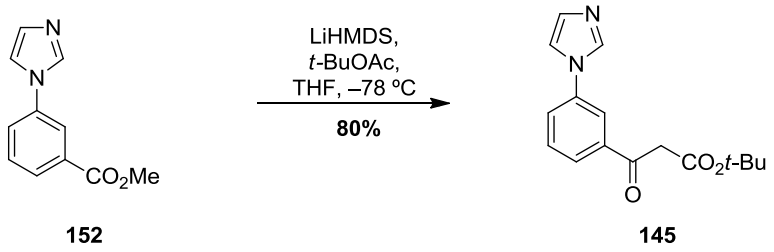
To synthesise β-keto ester fragment **145**, commercially available methyl 3-aminobenzoate **153** first underwent an imidazole forming reaction using the conditions described by Zhang and co-workers,[95] which proceeded in a yield of 58%. In this reaction phenylimidazole **152** is formed by a two stage process in which glyoxal and methyl 3-aminobenzoate condense to form imine intermediate **161**, followed by the addition of formaldehyde, ammonium chloride and phosphoric acid to complete the reaction (Scheme 2.9).



153 **161** **152**

Scheme 2.9 Synthesis of imidazole **152**

A Claisen condensation was then employed to complete the synthesis of the β-keto ester fragment (Scheme 2.10).[96] In this reaction, *tert*-butyl acetate was first treated with lithium hexamethyldisilazide at –78 ºC to form a lithium enolate, which then reacted with imidazolylbenzoic ester **152** to give β-keto ester **145** in a high yield of 80%.

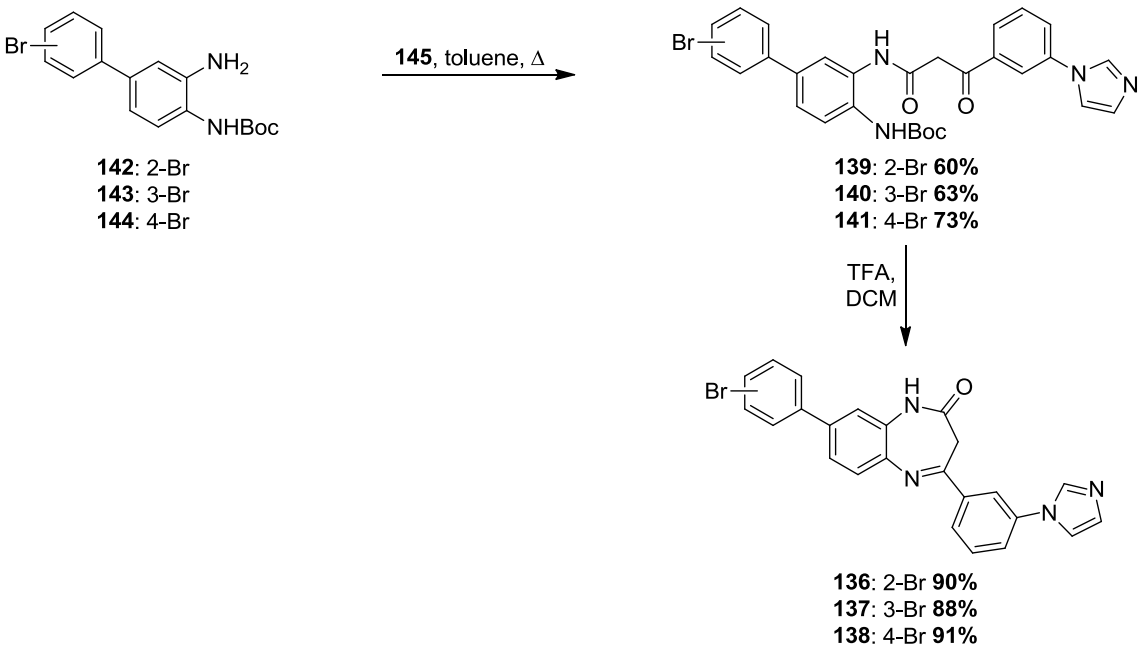


152 **145**

Scheme 2.10 Claisen condensation to form β-keto ester **145**

2.1.4 Completion of Synthetic Route

The two fragments were then brought together in a condensation reaction performed by heating amines **142**, **143** and **144** with β-keto ester **145** under reflux in toluene, forming β-keto amides **139**, **140** and **141** in good yields. Although the β-keto amides were isolated, full analysis was not performed on these compounds as their NMR spectra appeared as a mixture of keto-enol tautomers. The benzodiazepinone core was accessed through the reaction of the β-keto amides with trifluoroacetic acid giving benzodiazepinones **136**, **137**, and **138** in excellent yields of 90%, 88% and 91% respectively (Scheme 2.11). Under the acidic conditions the Boc group is removed to form an amine which immediately performs an intramolecular amine condensation reaction with the ketone to form the benzodiazepinone core.



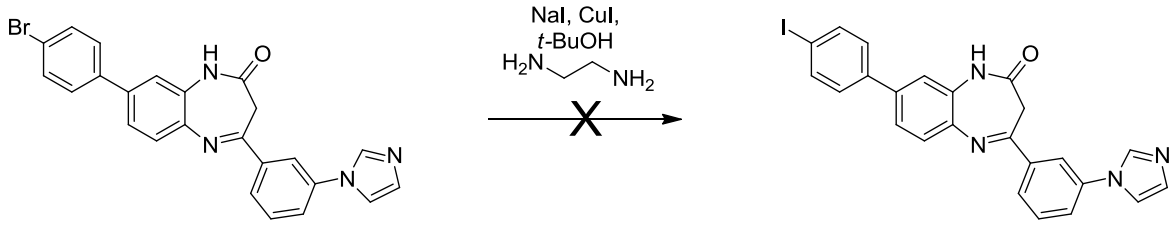
142: 2-Br
143: 3-Br
144: 4-Br

139: 2-Br **60%**
140: 3-Br **63%**
141: 4-Br **73%**

136: 2-Br **90%**
137: 3-Br **88%**
138: 4-Br **91%**

Scheme 2.11 β-Keto amide formation and cyclisation to form benzodiazepinone core

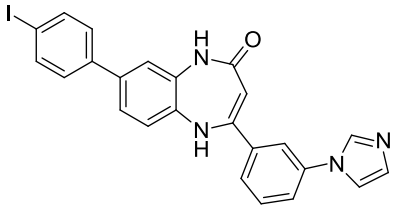
The final step in the proposed route was a copper catalysed halogen exchange reaction with a diamine chelating ligand using conditions described by Klapars and Buchwald.[97] This reaction was first attempted with benzodiazepinone **138** by heating to 140 ºC for six days. Upon inspection of the ^{1}H NMR spectrum, the product recovered from this reaction did appear to contain iodine but the original benzodiazepinone core did not appear to be intact. The reaction was repeated using milder conditions of heating to 120 ºC for 20 hours but again no desired product was isolated (Scheme 2.12).



138 **135**

Scheme 2.12 Attempted halogen exchange reaction.

The major product of the reaction showed the correct mass by mass spectrometry but the ^{1}H NMR spectra did not include the expected benzodiazepinone methylene signal. A possible explanation for this is that the iodination could have occurred but the benzodiazepinone core was isomerised to give compound **162** (Figure 2.2). Unfortunately the product could not be isolated sufficiently cleanly to perform full analysis to confirm this.

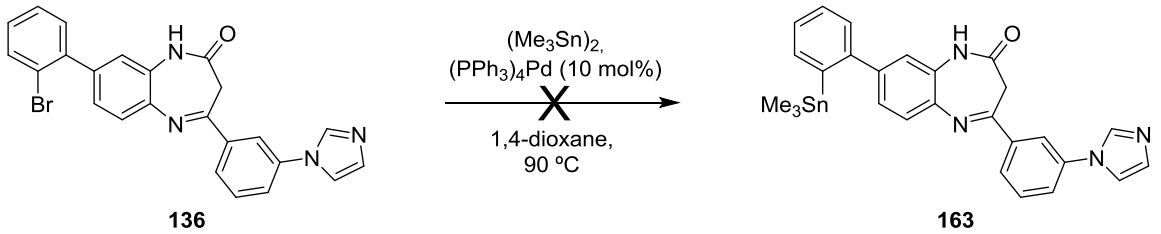


162

Figure 2.2 Structure of proposed isomerised compound **162**

As the direct halogen exchange reaction had proved unsuccessful an alternate approach was devised using stannylation of the bromide followed by iododestannylation. Palladium(0)-catalysed stannane formation was attempted on *ortho*-bromo substituted compound **136** but no reaction occurred (Scheme 2.13). Consulting the literature revealed

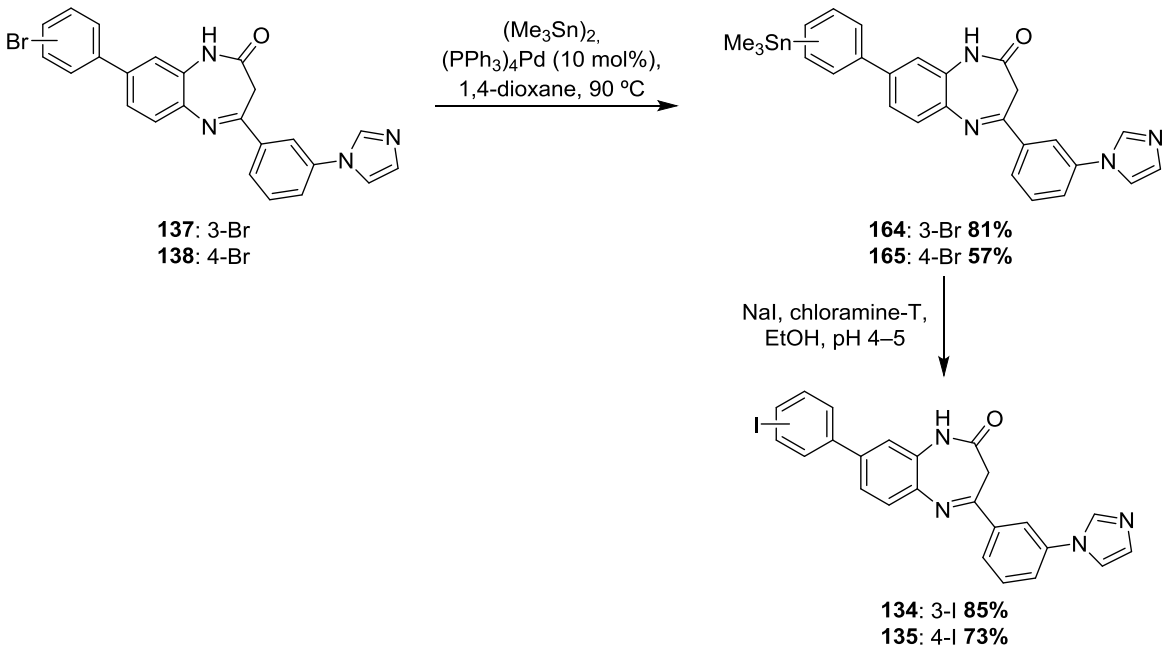
that examples of stannylation in the *ortho* position of a biaryl system used the conditions of *n*-butyllithium or *t*-butyllithium and a trimethyltin halogen species.[98,99] The harsh conditions required to overcome the steric hindrance in the *ortho*-bromo analogue would not be compatible with the more sensitive functionality found elsewhere in the compound. Therefore the synthesis of the *ortho*-substituted compound was not pursued any further using this synthetic route.



136 **163**

Scheme 2.13 Attempted iodination of compound **136**

However, the milder stannylation method was suitable for use on the *meta* and *para*-bromo substituted analogues. Palladium(0)-catalysed stannylation using hexamethylditin was employed, followed by iododestannylation under oxidative conditions to access target compounds **134** and **135** (Scheme 2.14).[100] As the iodide was inserted in the final step this synthetic route could be used to form radiolabelled compounds.



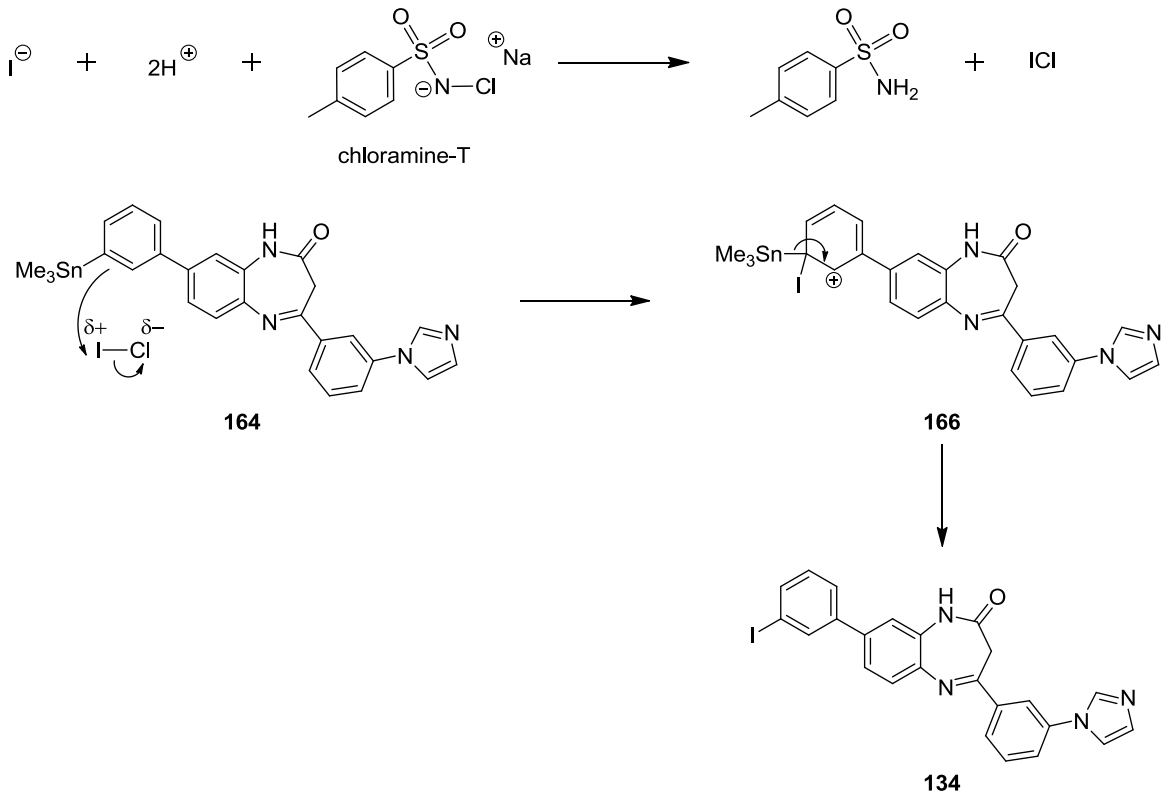
137: 3-Br
138: 4-Br

164: 3-Br **81%**
165: 4-Br **57%**

134: 3-I **85%**
135: 4-I **73%**

Scheme 2.14 Pd(0)-catalysed stannylation followed by oxidative iododestannylation

The mechanism of the iododestannylation reaction is provided in Scheme 2.15. Under acidic conditions sodium iodide and chloramine-T can form iodine monochloride *in situ*, which acts as a source of electrophilic iodine.[101] Demetallation then occurs to form the desired aryl iodide (Scheme 2.15).

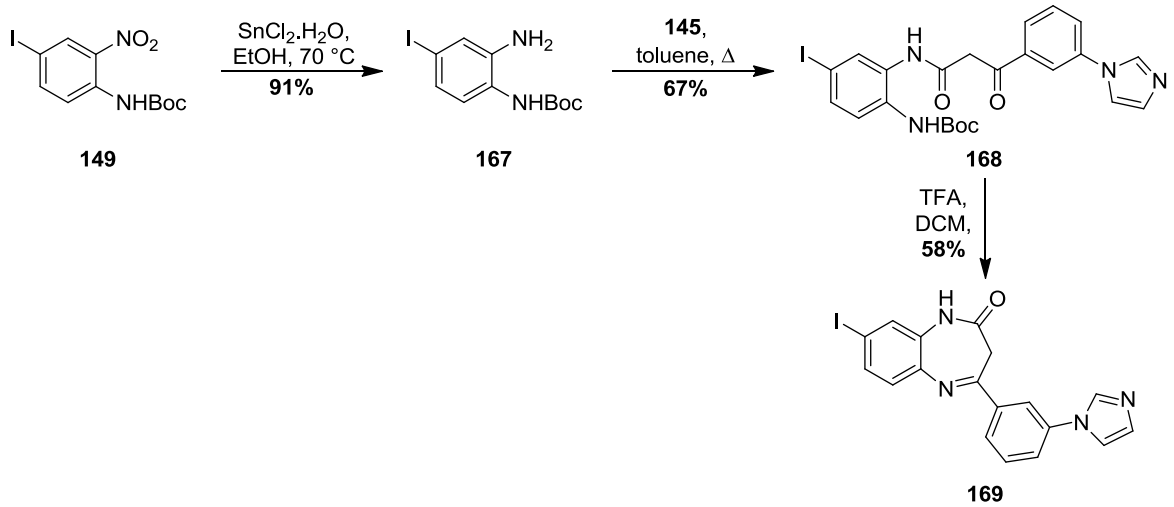


Scheme 2.15 Iododestannylation mechanism

This sequence of reactions could raise some safety concerns due to the high toxicity of organotin compounds. However, it is very commonly used in radioiodination to form SPECT imaging agents, followed by purification by HPLC to ensure any organotin impurities are removed.[102] It is so popular because the iododestannylation reaction is extremely fast and can be performed under very mild conditions compared to analogous iododesilylation and iododeborylation procedures.[103,104] The increased reactivity arises from the fact the carbon-tin bond has a bond dissociation energy (DBE) of 217 kJ/mol, compared to 311 kJ/mol for carbon-silicon and 365 kJ/mol for carbon-boron.[105] Therefore, the more labile carbon-tin bond more readily undergoes electrophilic aromatic substitution.

2.1.5 Synthesis of an Additional SPECT Target

An additional SPECT target was synthesised to investigate what effect the removal of the benzene ring at position-8 of the benzodiazepinone would have on the physicochemical properties of these compounds. Benzodiazepinone **169** was synthesised in three steps from nitrobenzene **149** by nitro reduction, β-keto amide formation and cyclisation (Scheme 2.16).



Scheme 2.16 Synthesis of benzodiazepinone **169**

2.2 Synthesis of Potential PET Imaging Agents

Another aim of this project was to design and synthesise compounds for PET imaging. Two target compounds were initially proposed, **170** containing a methoxy group and **171** incorporating a 3-fluoropropoxy chain (Figure 2.3). These compounds could be radiolabelled with carbon-11 and fluorine-18 respectively to provide compounds for PET imaging.

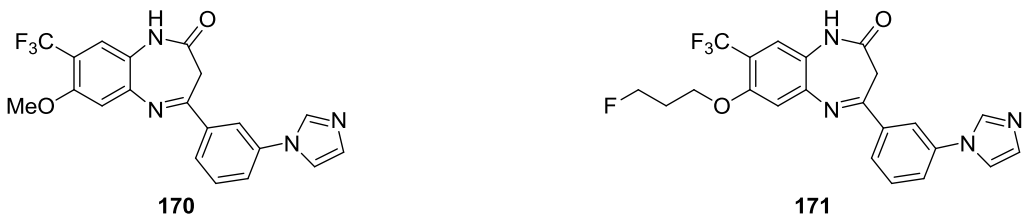
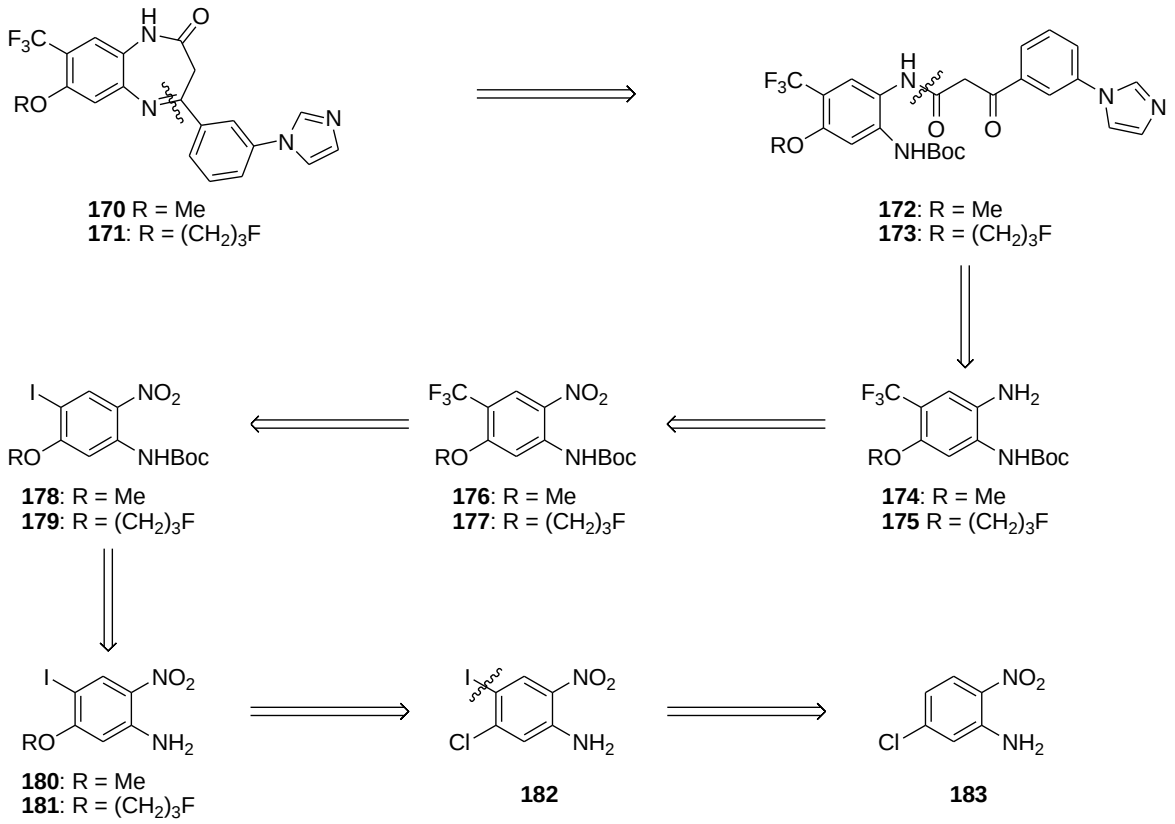


Figure 2.3 Target compounds for PET imaging

2.2.1 Retrosynthetic Analysis

The proposed route to synthesise the non-labelled compounds employed the same strategy as for the SPECT compounds. It was planned to form the benzodiazepinone core by cyclisation of β-keto amides **172** and **173**, which would be formed from amines **174** and **175**, and β-keto ester **145**. Commercially available 5-chloro-2-nitroaniline **183** was proposed as the starting material for the synthesis of amine fragments **174** and **175**, with the alkoxy and trifluoromethyl groups being inserted by nucleophilic aromatic substitution and copper catalysed trifluoromethylation, respectively (Scheme 2.17).

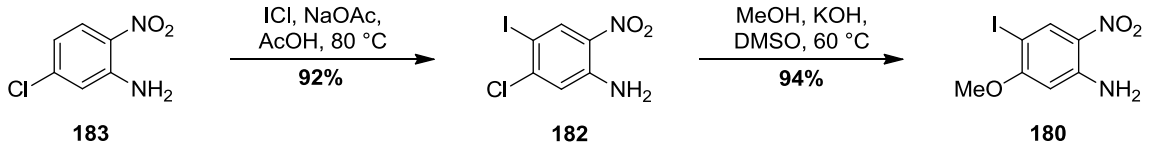


Scheme 2.17 Retrosynthetic analysis of target compounds **170** and **171**

2.2.2 Synthesis of Target Compounds

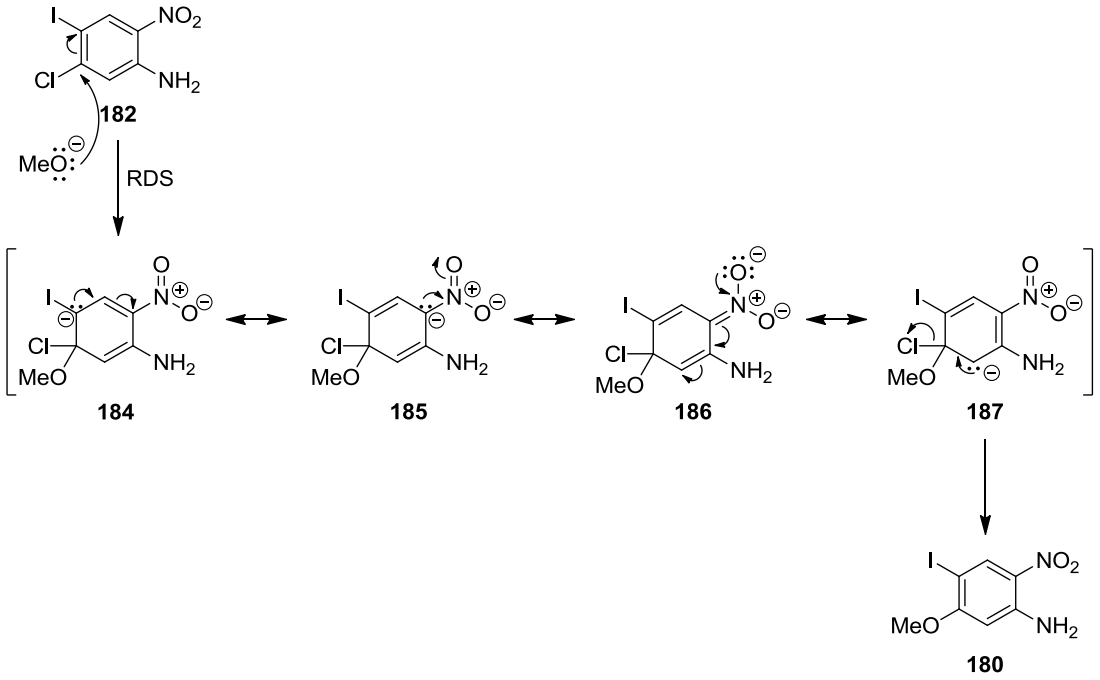
Commercially available 5-chloro-2-nitroaniline **183** was treated with iodine monochloride to give aniline **182** in 92% yield. To optimise the synthetic route, the planned sequence of reactions was first performed on the methoxy substituted analogue. Nucleophilic aromatic

substitution was employed to insert the methoxy group and gave compound **180** in an excellent 94% yield (Scheme 2.18).[61]



Scheme 2.18 Iodination of aniline **183** followed by S_NAr to insert a methoxy group

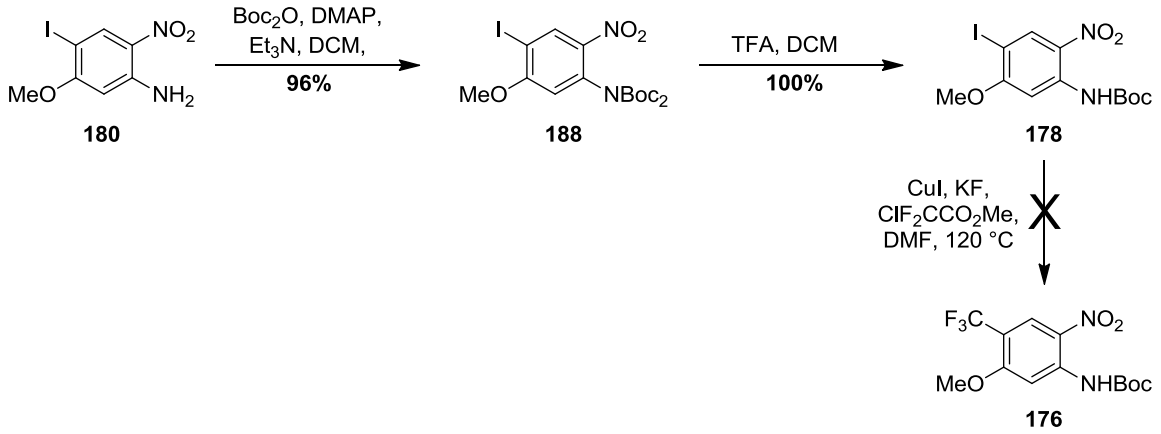
Although there are two halides present in compound **182**, the aromatic nucleophilic substitution reaction is completely selective for the chloride position. In nucleophilic aromatic substitution the rate determining step is the initial attack by the nucleophile on a carbon attached to a halide because this step breaks the aromaticity of the ring. Removal of the halide leaving group is fast as this step restores aromaticity. Attack at the chloride position is favoured as it has a strongly electron withdrawing nitro group *para* to it, which activates the ring towards nucleophilic attack and stabilises the Meisenheimer complex through resonance (Scheme 2.19).[106] Aryl chlorides are in fact more reactive than aryl iodides in nucleophilic aromatic substitution, as the increased electronegativity of chlorine over iodine results in greater polarisation of the carbon halide bond, making the carbon a better electrophile.



Scheme 2.19 Nucleophilic aromatic substitution mechanism

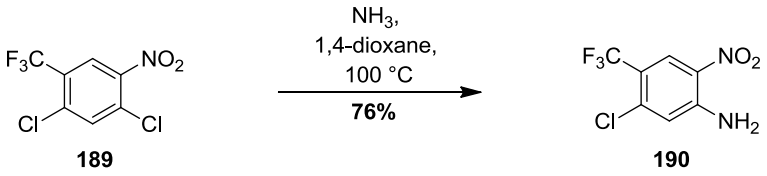
At this point, mono-Boc protection of amine **180** was attempted. However, significant amounts of the bis-protected compound were observed when monitoring the reaction. A further equivalent of di-*tert*-butyl dicarbonate was added to give complete conversion to bis-protected aniline **188** in a yield of 96%. Treatment of this material with trifluoroacetic acid allowed quantitative mono-deprotection to give protected amine **178** (Scheme 2.20).

The next step in the planned route was a copper-catalysed trifluoromethylation reaction using methyl chlorodifluoroacetate to give trifluoromethylbenzene **176** (Scheme 2.20). In this reaction, a trifluoromethyl copper complex is produced which reacts with the aryl iodide.[107,108] Unfortunately, the reaction was unsuccessful with only deprotected amine **180** being observed. When the reaction was carried out using amine **180**, again, trifluoromethylation did not occur. However, compound **178** was still of use as it was employed in the synthesis of compounds with multiple labelling sites which are discussed in Section 2.3.



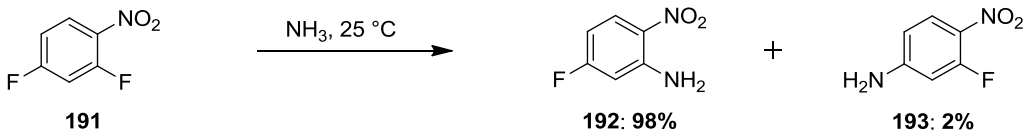
Scheme 2.20 Boc protection of amine **180** and attempted trifluoromethylation

As the trifluoromethylation reaction was unsuccessful, an alternate synthetic route was devised using a starting material already containing the trifluoromethyl group. Starting from commercially available dichlorobenzene **189**, a selective amination was performed to give amine **190** (Scheme 2.21).[109]



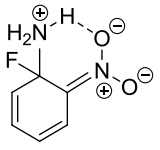
Scheme 2.21 Amination of dichlorobenzene **189**

This reaction is completely selective for the *ortho*-nitro position even though both halides have strongly electron withdrawing groups in the *ortho* and *para* positions. When searching the literature it was found that when an aromatic system contains a nitro group with a halide in both the *ortho* and *para* positions there is a bias towards a nucleophilic aromatic substitution with primary amines occurring at the *ortho* position.[110,111] This effect does not appear with other strongly electron withdrawing groups such as trifluoromethyl.[112,113] One example which illustrates this well is the amination of 2,4-difluoronitrobenzene **191**, performed by Ji and co-workers.[114] Performing this reaction in ammonia at 25 °C produced the *ortho*-aminated product **192** in a 98% yield, and *para*-aminated product **193** in a yield of only 2% (Scheme 2.22).



Scheme 2.22 Amination of 2,4-difluoronitrobenzene **191**

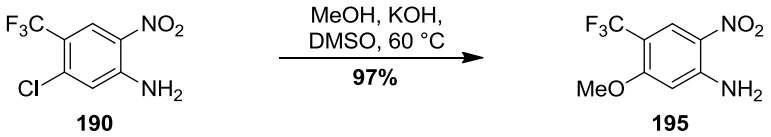
Kinetic studies of the amination of 2-fluoronitrobenzene and 4-fluoronitrobenzene separately found that the reaction with 2-fluoronitrobenzene was twenty seven times faster. To explain this, intermediate **194** was proposed, in which hydrogen bonding between oxygen of the nitro group and hydrogen of the amine stabilises the Meisenheimer complex (Figure 2.4). The same Meisenheimer complex stabilisation explains the selectivity observed in the amination of dichlorobenzene **189**.



194

Figure 2.4 Stabilised Meisenheimer Complex **194**

With aniline **190** in hand, the synthetic route became divergent, with aromatic nucleophilic substitution reactions used to insert the alkoxy substituents. Reaction at the remaining chloride position afforded methoxy substituted aniline **195** in a 97% yield (Scheme 2.23).

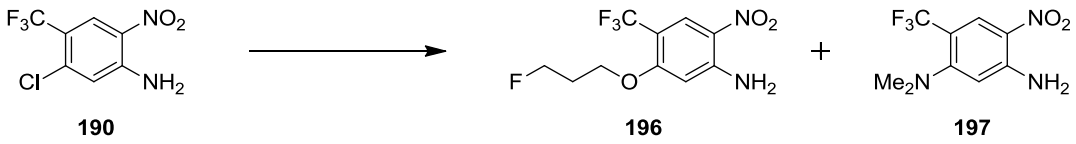


190 **195**

Scheme 2.23 S_NAr to insert methoxy group

To synthesise the 3-fluoropropoxy substituted compound **196**, chlorobenzene **190** underwent a nucleophilic aromatic substitution reaction with 3-fluoropropanol (Scheme 2.24 and Table 2.3). This was first attempted following a literature procedure for a similar compound, employing potassium carbonate as the base and performing the reaction in DMF.[115] However, a disappointing yield of 34% was obtained (entry 1). This low yield was due to material being lost during purification, as a result of a close running impurity which was particularly difficult to remove. A small amount of this impurity was isolated and ^{1}H NMR spectroscopy revealed it to be dimethylamine **197**. Referring to the ^{1}H NMR spectrum of the crude material from this reaction showed that the ratio of desired compound **196** to dimethylamine **197** to be 2:1. Under the basic reaction conditions, some of the DMF solvent was being hydrolysed to produce dimethylamine, which could then undergo an aromatic nucleophilic substitution with chlorobenzene **190**.[116]

To overcome this problem, the reaction was repeated without any dimethylformamide, performing the reaction neat in 3-fluoropropanol (entry 2). However, after two days only starting material was observed. DMSO was then used as the solvent and under these conditions, the desired reaction proceeded in a 71% yield (entry 3).

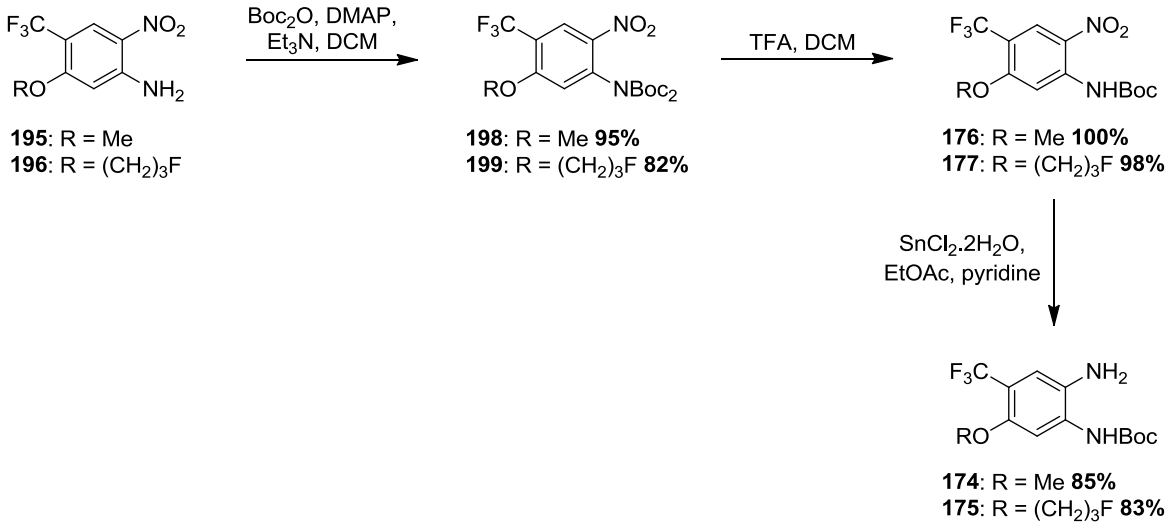


Scheme 2.24 S_NAr to insert 3-fluoropropoxy group

Entry	Reagent(s)	Solvent(s)	Temperature	196 (Yield)
1	3-fluoropropanol, K_2CO_3	DMF	120 °C	34%
2	3-fluoropropanol, K_2CO_3	-	120 °C	-
3	**3-fluoropropanol, K_2CO_3**	**DMSO**	**120 °C**	**71%**

Table 2.3 S_NAr conditions

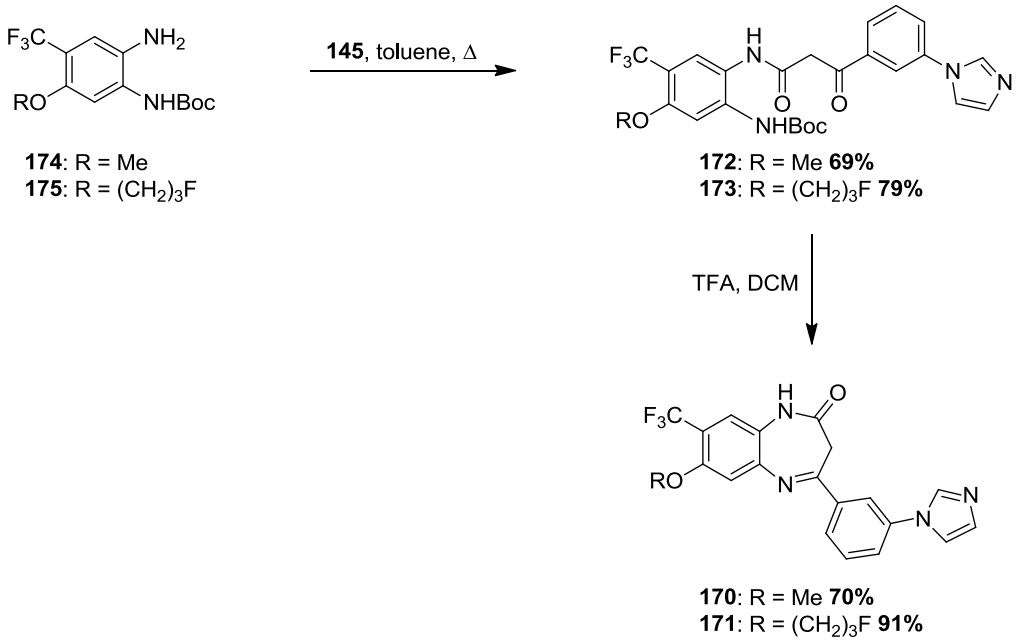
Bis-Boc protection followed by mono-deprotection allowed the amine to be protected to successfully form Boc-protected anilines **176** and **177**. Nitro reduction with tin(II) chloride dihydrate completed the synthesis of amine fragments **174** and **175** (Scheme 2.25).



Scheme 2.25 Synthesis of amine fragments **174** and **175**

Treatment of the amine fragments with β-keto ester **145** gave β-keto amides **172** and **173** in good yields of 69% and 79%. Finally, treatment with trifluoroacetic acid deprotected the

amine and allowed cyclisation to occur to give benzodiazepinones **170** and **171** (Scheme 2.26). This completed the synthesis of the target compounds for PET imaging.



Scheme 2.26 Synthesis of final target compounds **170** and **171**

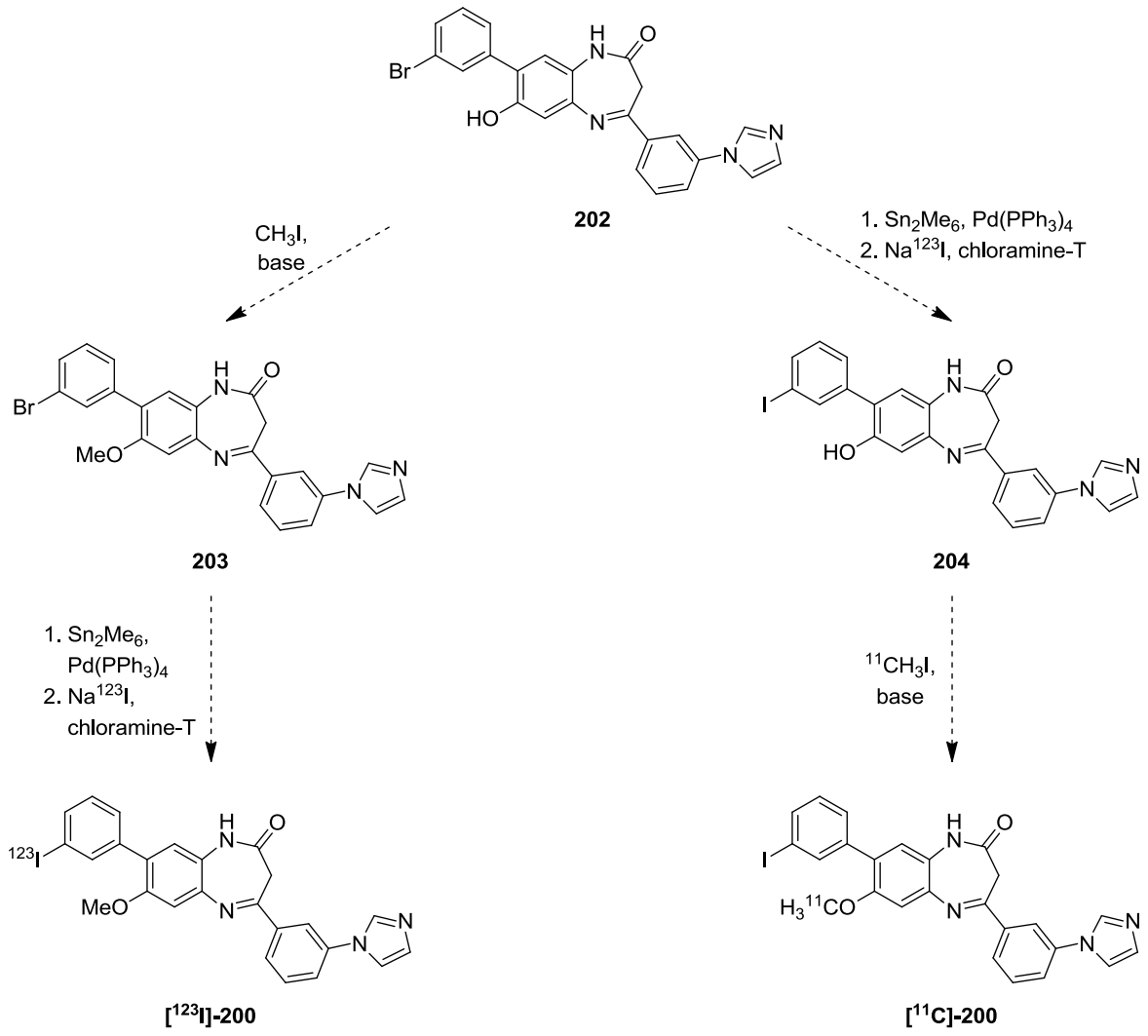
2.3 Synthesis of Potential SPECT or PET Imaging Agents

The final class of compound this project aims to develop are compounds with multiple labelling sites, which could be used for either SPECT or PET imaging. The first stage in the development of this series was the synthesis of non-labelled compounds for initial biological testing. Compounds **200** and **201** were proposed, containing both the aryl iodide part of the potential SPECT imaging agents **134** and **135**, and the methoxy group found in potential PET imaging agent **170** (Figure 2.5).

Figure 2.5 Target compounds with multiple labelling sites

2.3.1 Multiple Labelling Sites Concept

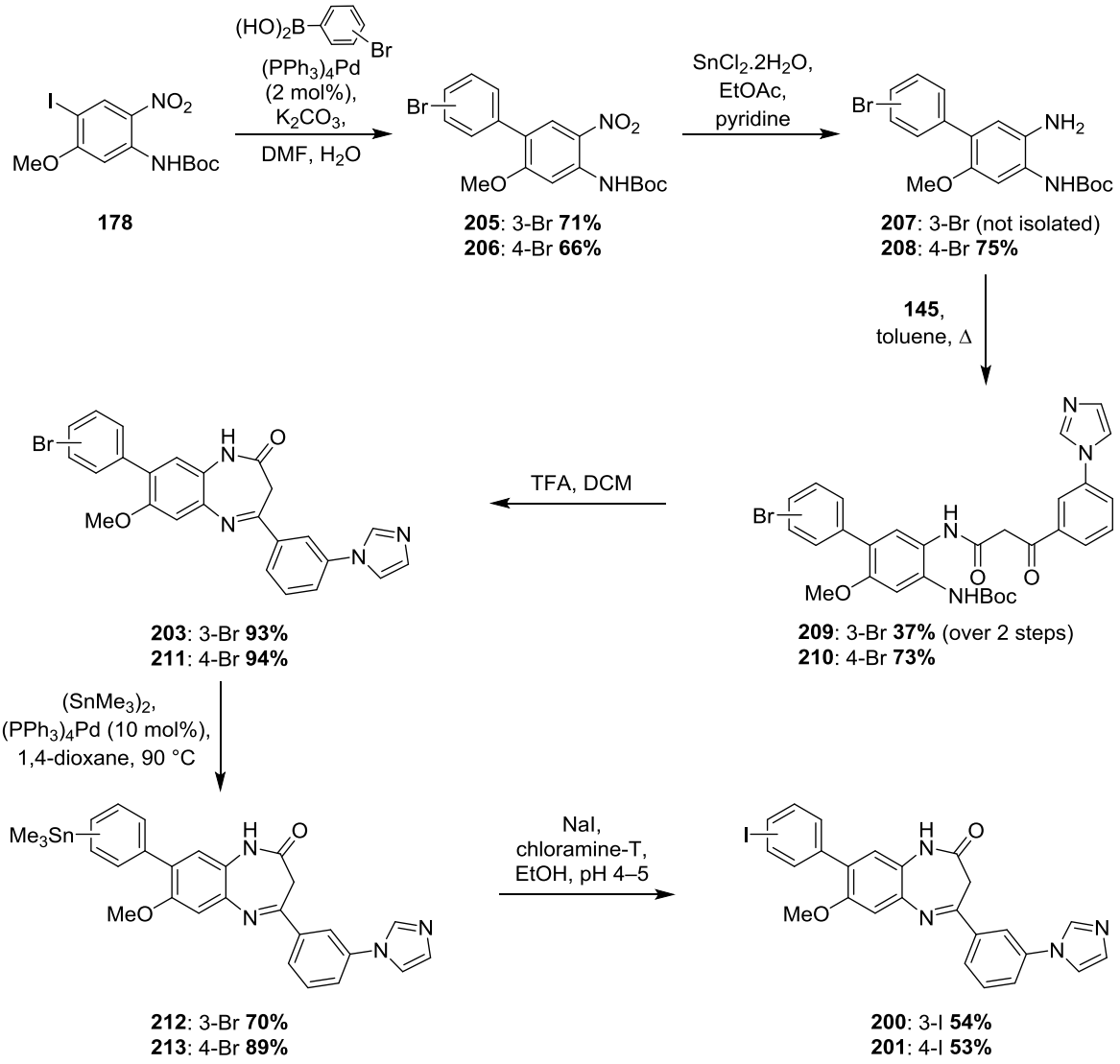
From one common precursor the order in which the final steps are performed can be altered to produce either a SPECT or PET imaging agent as shown in Scheme 2.27. From compound **202** containing an aryl bromide and phenol, performing alkylation of the phenol followed by iodination using radiolabelled sodium iodide could form SPECT imaging agent **^{123}I-200**. However, if iodination was performed first, then alkylation using radiolabelled iodomethane, PET imaging agent **^{11}C-200** would be formed.



Scheme 2.27 Concept of compounds with multiple labelling sites

2.3.2 Synthesis of Target Compounds

Methoxy substituted aryl iodide **178** was employed as the starting point in the synthesis of compounds **200** and **201**. Aryl iodide **178** had originally been synthesised when attempting to form amine fragment **174**, but the trifluoromethylation reaction on this substrate was unsuccessful. From this point, the same series of reactions that had been used in the synthesis of potential SPECT imaging agents **134** and **135** were employed to form benzodiazepinones **200** and **201** (Scheme 2.28). The yield of the formation of β-keto amide **209** was taken over two steps due to the instability of the amine intermediate **207**.



Scheme 2.28 Synthesis of target compounds **200** and **201**

With a library of seven benzodiazepinones completed (Figure 2.6), attention was then turned to investigating the properties of these compounds.

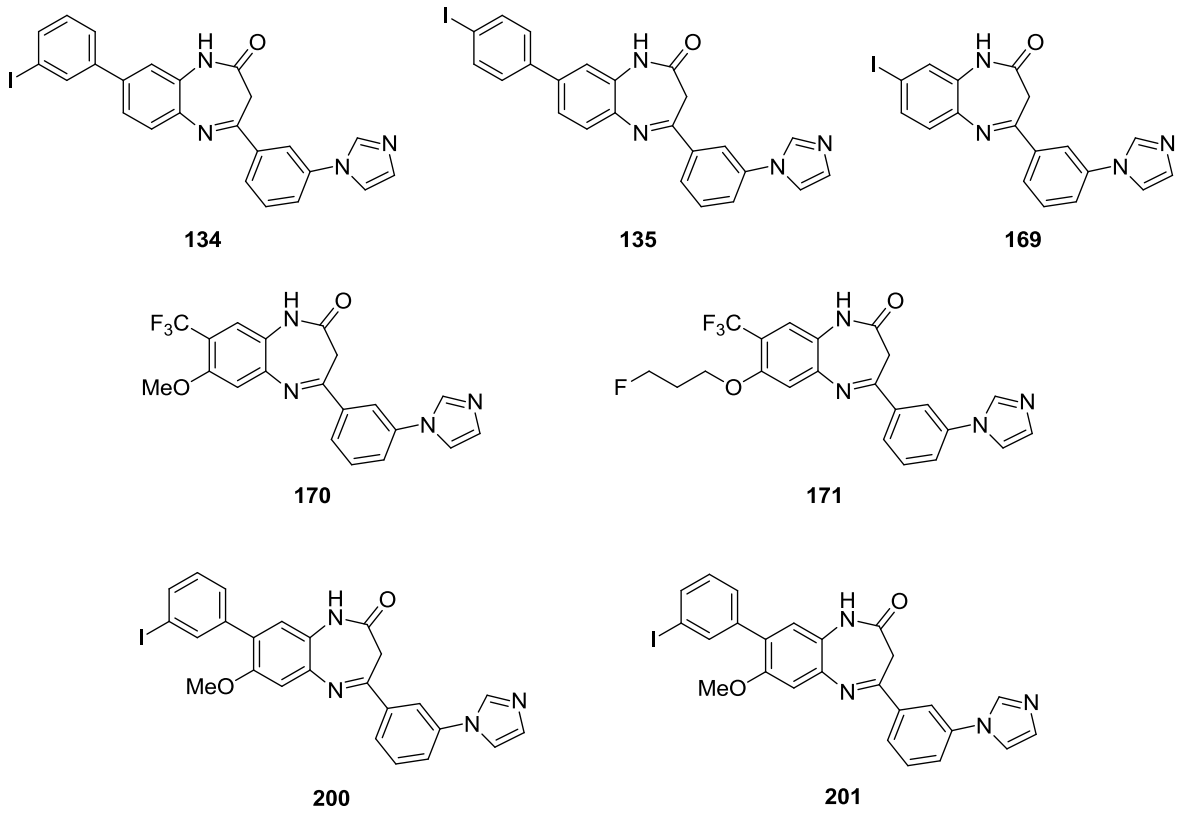


Figure 2.6 Library of seven benzodiazepinones

2.4 Investigation of Physicochemical Properties

It was important to investigate the physicochemical properties of the compound library as it is not just binding to the biological target that determines whether a compound has potential as a radiotracer. When a compound enters the body it has to have the correct physicochemical properties in order to reach the biological target. As the compounds have been proposed for use in brain imaging it is vital that the compounds are able to cross the blood brain barrier.

2.4.1 Blood Brain Barrier

Evidence for the existence of the blood brain barrier (BBB) was first found in 1885 when Ehrlich observed that soluble dyes injected into the bloodstream of animals caused staining by the dye in all organs except the brain and spinal cord. In 1913 Goodman then observed that when dye was injected into the spinal cord staining was found in the brain and spinal cord but not in other organs. It is now known that this is because the central nervous system (CNS) is separated from the blood by a permeability barrier. Blood capillaries in the brain are lined by endothelial cells which are connected by cell adhesion molecules that form tight junctions, rather than containing pores found in blood capillaries outside the CNS. Although the BBB is formed by the endothelial cells, pericytes and astrocytes which surround the blood vessel also contribute to the barrier (Figure 2.7).[117]

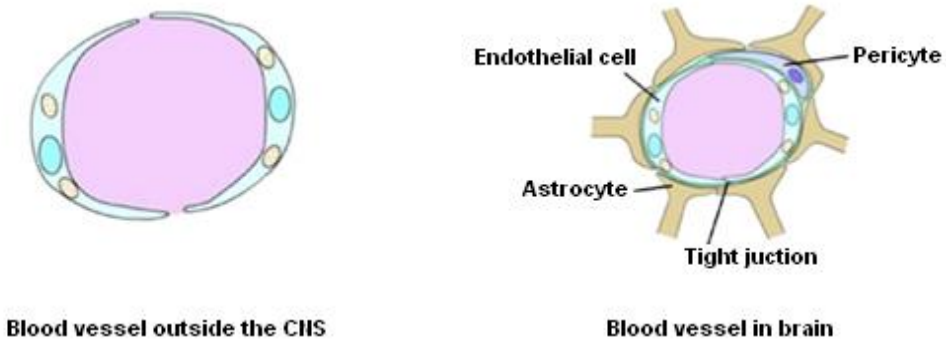


Figure 2.7 Structure of the BBB[117]

(Reprinted from A. M. Palmer, *Neurobiol. Dis.*, 2010, **37**, 3−12. Copyright 2010, with permission from Elsevier)

The BBB has many important functions such as ion regulation, separation of central and peripheral nervous systems (which use many of the same neurotransmitters), and the prevention of neurotoxins and macromolecules from entering the brain.[118]

For a drug to be brain penetrant, it must cross the blood brain barrier. There are various mechanisms by which a molecule can pass from the blood to the brain. Small lipophilic molecules can cross the BBB by passive diffusion across the cell membrane, whereas hydrophilic molecules are more likely to cross the BBB by methods such as saturable transporters, endocytosis and transcytosis, or paracellular transport.[119]

2.4.2 Partition Coefficient

The partition coefficient is defined as the ratio of concentrations of an unionised compound partitioned between two immiscible phases at equilibrium. The logarithmic form of the octanol-water partition coefficient (expressed as Log P) is often used as an indicator of the likelihood of a compound to cross the cell membrane (Figure 2.8).

$$\log P = \log \left(\frac{[\text{compound}]_{\text{octanol}}}{[\text{compound}]_{\text{water}}} \right)$$

Figure 2.8 Equation for the calculation of Log P

In order for a compound to cross the BBB by diffusion, it must be sufficiently lipophilic to pass through the cell membrane. However, if a compound is too lipophilic, the aqueous solubility can decrease to the point where problems arise from poor aqueous solubility, metabolic instability or high plasma protein binding.[120] For CNS targets, it is generally observed that Log P should be below 4 and ideally between 1 and 3.5.[121]

The traditional octanol-water method of determining Log P is time consuming and not suitable for obtaining data for large numbers of compounds. To overcome this, many algorisms have been developed to provide Log P values *in silico*. Log P can also be determined using reverse phase high performance liquid chromatography (HPLC) with a C18 column.[122,123] Log P values for the library of benzodiazepinones were determined using four different *in silico* methods and by HPLC, which was performed by Adele Blair, a PhD student from the Sutherland group.

It can be seen that compounds **169**, **170** and **171** generally have a lower Log P than compounds **134**, **135**, **200** and **201** (Table 2.4). However, depending on the method used there is a difference of 1.85 to 2.36 between the highest and lowest values for one compound. Recent work by Tavares and co-workers has shown that there can be a large variation in Log P depending on the method used.[124] Therefore, Log P alone should not be used as an indicator of whether a compound is likely to cross the blood brain barrier. Log

P is a very simplistic way of modelling BBB penetration and there are other methods that can provide more information.

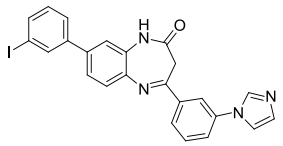
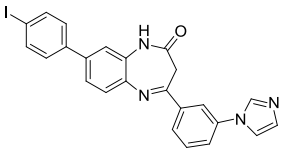
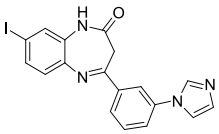
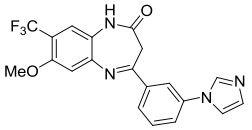
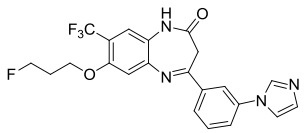
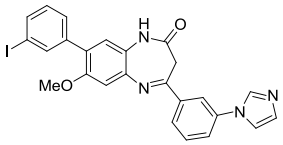
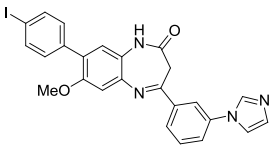
Compound	Log P (Chem Sketch)	Log P (Marvin Sketch)	Log P (Chem Draw)	cLog P (Chem Draw)	cLog P (HPLC)
 **134**	3.88	5.12	4.64	5.60	5.73
 **135**	3.88	5.12	4.64	5.60	5.77
 **169**	2.68	3.44	2.96	3.92	4.60
 **170**	2.71	3.08	2.40	3.92	4.67
 **171**	3.23	3.29	2.70	4.40	5.06
 **200**	3.64	4.87	4.51	5.09	5.67
 **201**	3.64	4.87	4.51	5.09	5.72

Table 2.4 Log P values obtained using HPLC and *in silico* methods

2.4.3 Membrane Partition Coefficient and Permeability

For compounds expected to cross the BBB by passive diffusion through the cell membrane it is important to consider compound-membrane interactions. For each compound-membrane lipid mixture there is a characteristic equilibrium constant for compound partitioning into fluid membranes, defined as the membrane partition coefficient (K_m). Values for this parameter can be obtained by HPLC using an immobilised artificial membrane (IAM) column. An IAM column has phospholipid analogues immobilised on chromatographic material to mimic fluid cell membranes (Figure 2.9). By observing the retention time of a compound on an IAM column, a value for K_m can be calculated.[125,126]

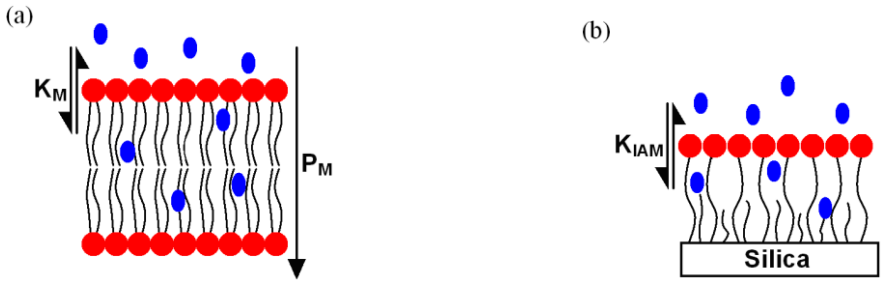


Figure 2.9 Solute interacting with (a) membrane bilayers (b) IAM column surface

When considering molecule transport, K_m does not directly predict membrane transport. To permeate the cytosol a molecule must enter and exit the membrane, and for molecules travelling though the cell, a second membrane entry and exit must occur. Therefore, when a molecule passes through a cell membrane, partitioning occurs four times. To account for this dynamic process, permeability (P_m) must be considered. P_m is directly proportional to K_m but also accounts for molecular size and membrane thickness. P_m can be defined by the equation provided in Figure 2.10 (a), where D_m is the membrane diffusion coefficient and L is the membrane thickness. D_m is inversely proportional to molecular size (V). Assuming molecular size is proportional to molecular weight (MW) and the membrane thickness is constant, P_m can be calculated by the equation in Figure 2.10 (c).[126]

(a) $$P_m = \frac{D_m \times K_m}{L}$$

(b) $$D_m \propto \frac{1}{V} \propto \frac{1}{MW}$$

(c) $$P_m = \frac{K_m}{MW}$$

Figure 2.10 Equations relating K_m to P_m

2.4.4 Plasma Protein Binding

Once a compound reaches the blood it can become bound to the proteins present in blood plasma. As this binding is usually reversible, an equilibrium exists between the bound and unbound species. Only the unbound fraction can undergo processes such as binding to the target receptor and metabolism. Although there are many proteins in the blood that are capable of binding drugs, the most significant are human serum albumin (HSA, the most abundant protein in human blood plasma) and α_1-acid glycoprotein (AGP).[127]

A column with HSA immobilised on a chromatographic support is used to measure plasma protein binding by HPLC. Compounds with high plasma protein binding will have a greater affinity for HSA and will therefore elute later than compounds with lower binding. Valko and co-workers have illustrated a good correlation between PPB measured *in vivo* and values obtained by HPLC.[128]

2.4.5 HPLC results for Compound Library

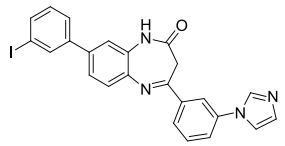
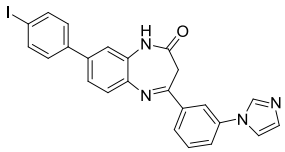
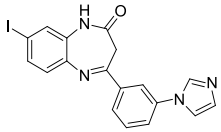
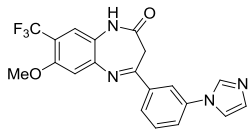
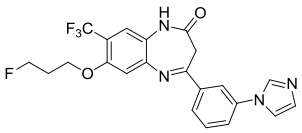
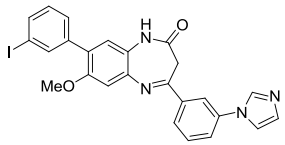
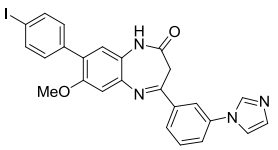
Tavares and co-workers have shown that relationships exist between properties determined by HPLC and *in vivo* data.[124] This gave rise to a set of parameters that a compound should adhere to in order to be progressed as a potential radiotracer (Table 2.5).

Parameter	HPLC Value
Permeability (P_m)	< 0.5
Membrane partition coefficient (K_m)	< 250
Plasma Protein Binding (PPB)	< 95%

Table 2.5 Progression limits for compounds

The library of benzodiazepinones were subjected to these HPLC techniques by Adele Blair (Table 2.6). From this data it can be seen that the compounds split into two groups with compounds **134**, **135**, **200** and **201** being outwith every parameter. It is clear that the

additional benzene results in compounds with high lipophilicity and is unfavourable for physicochemical properties. Compound **171** is very close to the required value for PPB but K_m and P_m, although much lower than that of the biaryl compounds, are too high. Methoxy compound 1**70** is the best candidate, with iodide **169** also suitable for progression.

Structure	P_m (IAM column)	K_m (IAM column)	%PPB (HSA column)
 **134**	> 2.27	> 1144.81	98.46
 **135**	> 2.27	> 1144.81	98.67
 **169**	0.51	218.40	94.92
 **170**	0.44	176.15	92.41
 **171**	0.77	343.73	95.12
 **200**	> 2.15	> 1148.85	98.08
 **201**	> 2.13	> 1138.17	98.32

Key: Within limits, marginally above limits, above limits

Table 2.6 Physicochemical properties determined by HPLC

2.5 Biological Evaluation

In order to be of use as a radiotracer, a compound must have high affinity with the receptor it is targeting. Therefore, the affinity of the library of benzodiazepinones with group II metabotropic glutamate receptors had to be investigated.

2.5.1 Non-Competitive Antagonism

Previously described benzodiazepinones have been shown to function as non-competitive antagonists of mGluR2/3.[129,130] Agonist binding activates a receptor, whereas antagonist binding inhibits the agonist mediated response. Competitive antagonism occurs when the antagonist binds at the same site as an agonist (Figure 2.11). When an antagonist binds to an allosteric site on the receptor it is defined as a non-competitive antagonist. Binding to the allosteric site prevents receptor activation by either causing a conformational change to agonist site to reduce agonist binding, or by blocking the effect of agonist binding at some point in the signalling cascade.

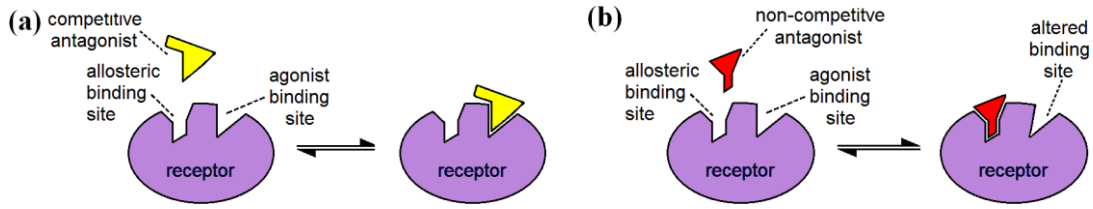


Figure 2.11 (a) Competitive binding, (b) non-competitive binding

Functional assays which measure a biological response of binding to the allosteric site are used to determine the inhibitory potency of non-competitive antagonists.

2.5.2 Principals of the [^{35}S]GTPγS Assay

The [^{35}S]GTPγS assay is a functional assay commonly used to determine binding to G protein coupled receptors (GPCRs) *in vitro*. The assay makes use of the fact that upon activation G proteins exchange bound guanosine diphosphate (GDP) for guanosine triphosphate (GTP) (Figure 2.12).

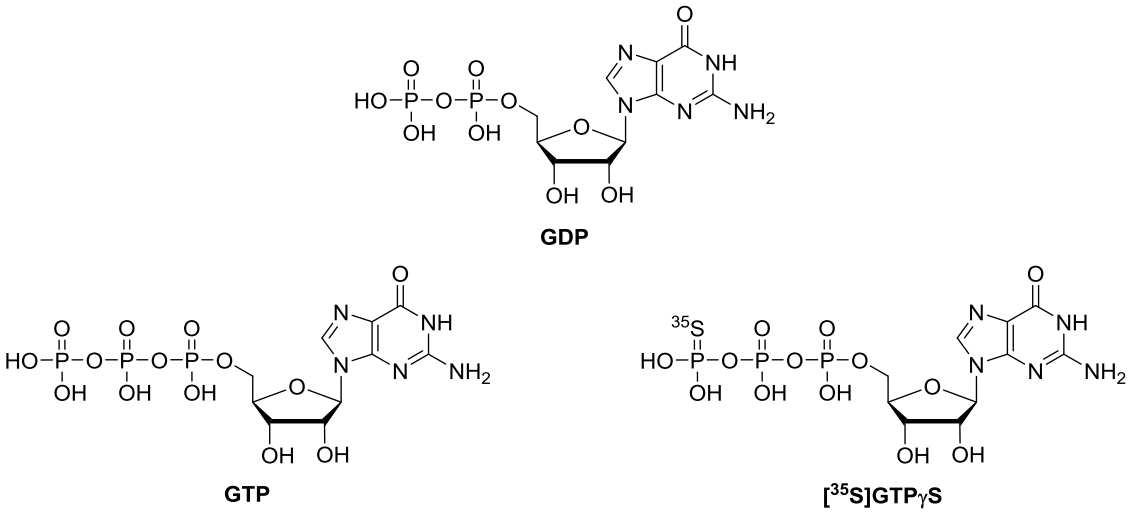


Figure 2.12 Structures of GPD, GTP and [^{35}S]GTPγS

The G protein exists as a Gαβγ heterotrimer with GDP bound to the Gα subunit. Upon binding of an agonist to a GPCR, GDP is exchanged for GTP on the Gα subunit of the G-protein, which becomes dissociated from the Gβγ subunits. GTP is then hydrolysed by GTPase activity to GDP, and the Gα subunit recombines with the Gβγ subunits to complete the cycle (Figure 2.13).

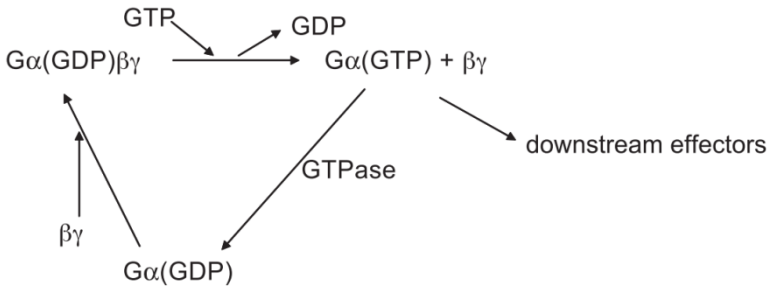


Figure 2.13 GDP/GTP exchange[131]

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When this process occurs in the presence of sulphur-35 labelled GTP, known as [^{35}S]GTPγS (Figure 2.12), the GDP is exchanged with [^{35}S]GTPγS upon agonist activation. Unlike GTP, [^{35}S]GTPγS is non-hydrolysable by GTPase activity. Therefore rather than being hydrolysed back to GDP and reforming the heterotrimer, Gα bound [^{35}S]GTPγS accumulates (Figure 2.14).

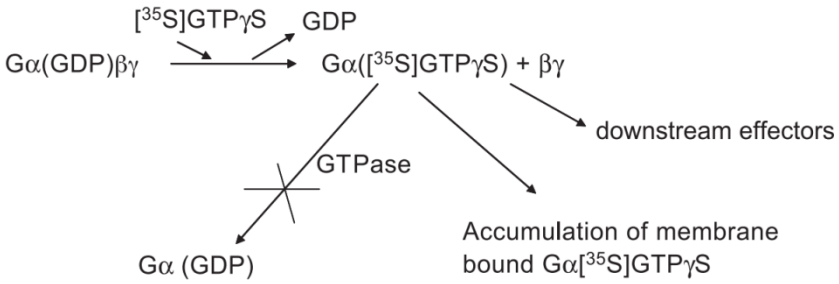

Figure 2.14 GDP/GTP exchange in the presence of [^{35}S]GTPγS[131]

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The assay measures the level of G-protein activation following binding of an agonist. The amount of sulphur-35 and hence the level of activation can be quantified by passing the assay mixture through a glass-fibre membrane filter and performing liquid scintillation analysis to count the level of radioactivity retained on the filter. In the presence of a non-competitive antagonist, agonist binding will be disrupted and so the level of activation lowered. By performing measurements at a range of non-competitive antagonist concentrations, IC_{50} values can be determined.

2.5.3 Control Experiments

Before testing the library of benzodiazepinones, a number of control experiments had to be performed to confirm the assay was functioning correctly. Firstly, to determine the occurrence of agonist stimulation and sulfur-35 accumulation on the protein, the basal and stimulated binding had to be determined. Running the experiment without any agonist present defines the basal binding and with agonist, the stimulated binding. To ensure that the increased binding reflects actions at mGluR2/3, an experiment using an agonist and competitive antagonist was proposed. Finally, carrying out an experiment with a large excess of cold GTPγS was planned to define the non-specific binding of [^{35}S]GTPγS. The required results to proceed with the assay are detailed in Table 2.7.

Conditions	Agonist	Antagonist	Cold GTPγS	Expected Result
1*	✗	✗	✗	basal binding
2*	✓	✗	✗	higher than basal binding
3*	✓	✓	✗	close to basal binding
4*	✓	✗	✓	lower than basal binding

*All conditions contain protein, GDP, [^{35}S]GTPγS and assay buffer.

✓ = present in experiment, ✗ = not present in experiment

Table 2.7 Conditions for control experiments

2.5.4 Initial Attempts Using Rat Brain Homogenate

Control experiments were initially performed using rat brain cortex homogenate. The Bradford protein assay was carried out to quantify the protein so that known amounts could be used in the assay. As rat brain contains various glutamate receptors a specific mGluR2/3 agonist had to be used to ensure that any observed stimulation was from actions at mGluR2/3. Specific agonist LY354740[33] (which has been used to stimulate [^{35}S]GTPγS binding autoradiographically[132]) and the competitive antagonist LY341495[133] were used, both of which have low nanomolar affinity for mGluR2/3 (Figure 2.15).

LY354740 **LY341495**

Figure 2.15 Compounds used in control experiments

The first conditions tested were based on work by Richards and co-workers who performed mGluR2/3 stimulation autoradiographically.[132] [^{35}S]GTPγS was present at a concentration of 0.1 nM and 1 μM LY354740 was used to stimulate. A lower DPM (disintegrations per minute) value was observed for the experiment containing 10 μM cold GTPγS, but no significant stimulation was observed. Initial efforts to increase stimulation focused on

altering the level of GDP present in the assay. Changing from 100 μM to 2 mM and then 5 mM GDP decreased the DPM values (Figure 2.16). It was hoped that by decreasing the basal binding, stimulated binding would become observable. The experiments were performed incubating at 37 °C for 30 minutes and 22 °C for 2 hours but no stimulation was observed in either case.

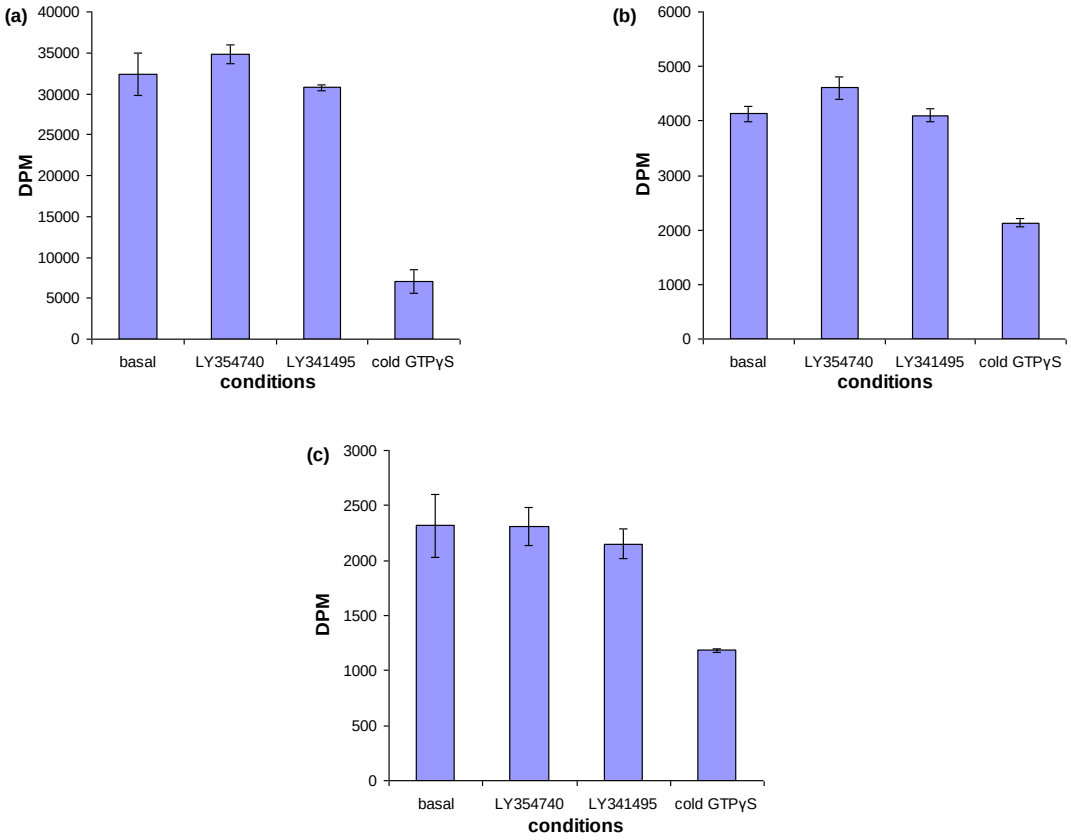


Figure 2.16 Control experiments with (a) 100 μM GPD, (b) 2 mM GDP, (c) 5 mM GDP

Whilst this was being carried out a paper was published by Odagaki and co-workers in which they thoroughly investigated [^{35}S]GTPγS assay conditions using rat cerebral cortex homogenate.[134] They were able to achieve a 50% increase in specific binding when stimulating with L-glutamate. Ionotropic glutamate agonists such as NMDA, AMPA and kainic acid showed no stimulatory effects. Therefore, the observed stimulation was due to activation of G-proteins coupled with metabotropic glutamate receptors.

In their conditions, reducing agent dithiothreitol (DTT) was used as an additive. [^{35}S]GTPγS autoradiography performed by Happe and co-workers showed a reduction in basal and non-specific binding in the presence of DTT, thus improving ratio of stimulated

to basal binding.[135] The control experiments were performed with the addition of 0.2 mM DTT, 20 μM GDP, 30 μg protein and varied concentrations of agonist LY354740, incubating at 22 °C for 2 hours (Figure 2.17). At both 1 μM and 3 μM agonist, a small increase in binding over basal was observed.

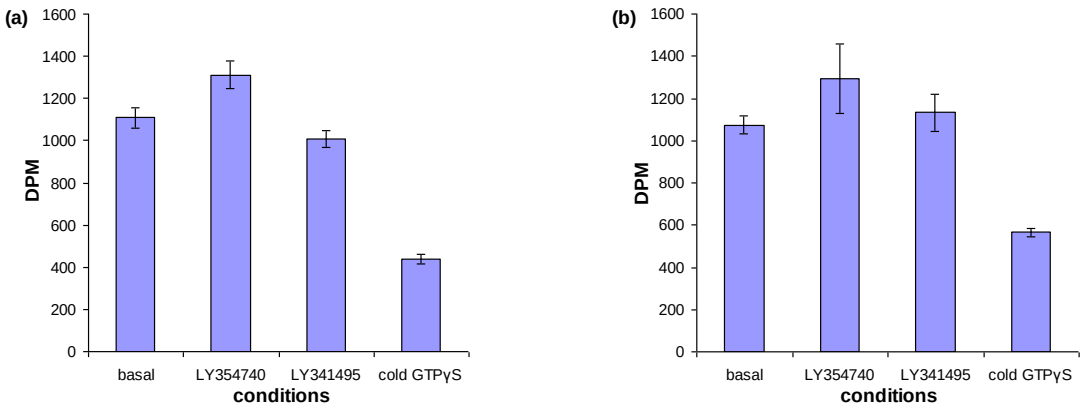


Figure 2.17 Control experiments with (a) 1μM agonist, (b) 3 μM agonist.

The best conditions from the Odagaki paper were then performed using LY354740 instead of L-glutamate. It was decided not to move to L-glutamate as it would introduce stimulation of other metabotropic glutamate receptors. Incubation conditions of 30 °C for 1 hour, and a [^{35}S]GTPγS concentration of 0.2 nM were used. Similar results to those produced previously were obtained (Figure 2.18). Further variations to the concentration of [^{35}S]GTPγS and amount of protein were also investigated but no stimulation was observed.

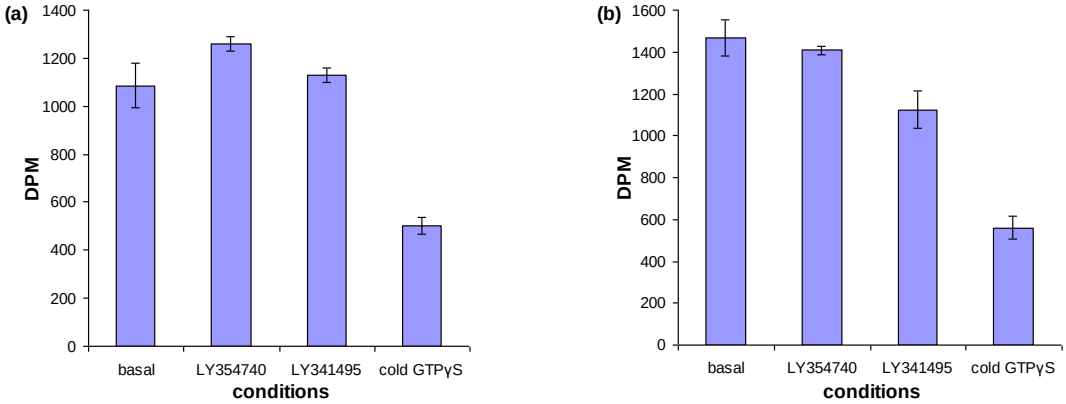


Figure 2.18 Odagaki conditions with (a) 1 μM and (b) 3 μM.

Saponin is a permeabilizing agent and has been shown in [^{35}S]GTPγS studies with other GPCRs to facilitate the interaction of guanine nucleotides with GPCRs.[136] To investigate whether saponin could offer any improvement it was added to the assay mixture at a concentration of 100 μg/mL. However, this again offered no increase over basal binding (Figure 2.19).

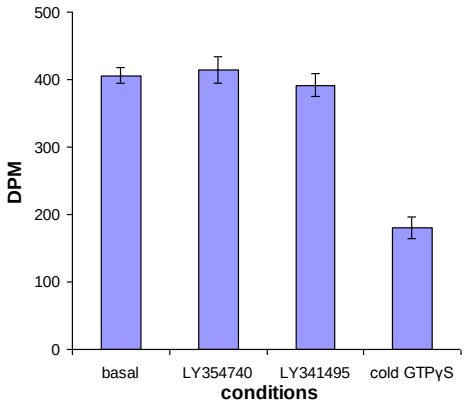


Figure 2.19 Controls with 100 μg/mL saponin

By this point some small stimulation had been observed but could not be consistently repeated. The work by Odagaki and co-workers was the only example in the literature performing the [^{35}S]GTPγS assay using metabotrobic glutamate receptor stimulation with rat brain homogenate. As the best conditions from the paper and close alterations did not provide a significant increase over basal binding, work using homogenates was terminated at this stage.

The lack of stimulation could be attributed to many factors. It may be the case that the increased binding due to agonist binding to mGluR2/3 did take place but was not high enough to be detected above the noise of basal and non-specific binding. Also the presence of other biological material in the homogenates could have caused an interaction that prevented stimulation from occurring.

2.5.5 Using Membrane Expressing Human mGluR2

Failure of the control experiments using rat brain homogenate led to the decision to use membranes from cells expressing human recombinant mGluR2, which were obtained from Millipore (ChemiSCREEN™ mGLU2 metabotropic glutamate receptor membrane

preparation).[137] As the membranes were obtained from cells expressing mGluR2 a specific agonist was not needed and L-glutamate was used. The control experiments were carried out and provided very positive results. All of the expected effects described in Table 2.7 were observed (Figure 2.20). Stimulating with 100 μM and 10 μM L-glutamate gave a 117% and 95% increase over basal binding receptively. Reducing the amount of glutamate by a factor of ten gave only a small reduction in the level of stimulation, therefore, a glutamate concentration of 10 μM was used when performing the assay to determine IC_{50} values. The addition of 10 μM LY341495 reduced stimulated binding back down to basal level, and 10 μM cold GTPγS gave a very low level of non-specific binding.

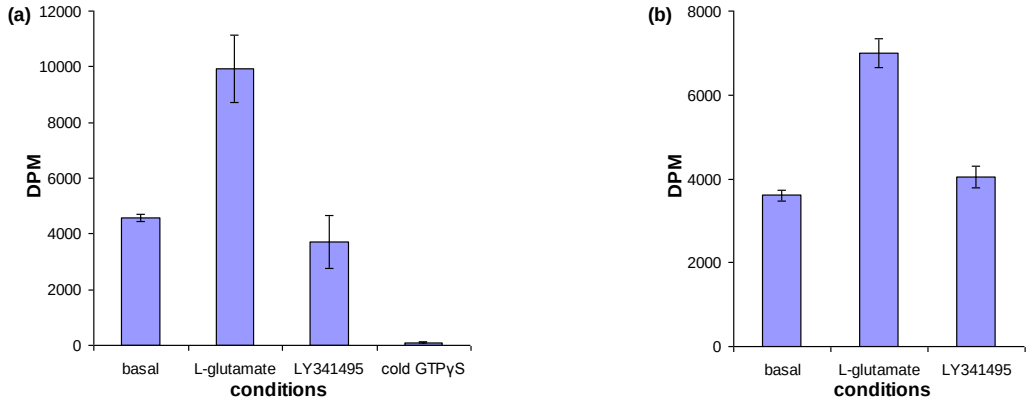


Figure 2.20 Graphs of control experiments; (a) 100 μM glutamate, (b) 10 μM glutamate.

Work then moved to testing the affinity of non-competitive antagonists. A compound of known affinity was first tested to ensure that the expected result could be obtained. Commercially available Ro 64-5229 (Figure 2.21) was chosen for this purpose, which has a published IC_{50} of 0.11 μM in the [^{35}S]GTPγS assay.[138] This previously published IC_{50} value for Ro 64-5229 was obtained using rat mGluR2 transfected cell membrane rather than human mGluR2. Also the agonist used was 1*S*,3*R*-ACPD (Figure 2.21) at a concentration of 10 μM rather than L-glutamate. These differences should not lead to a large variation in binding affinity as testing in rat and human mGluR2 has previously been shown to give very similar IC_{50} values.[139] Using L-glutamate instead of 1*S*,3*R*-ACPD should also not be significant as they have very similar IC_{50} values for binding to mGluR2 of 1 μM and 1.3 μM respectively.[140]

Ro 64-5229 **(1*S*,3*R*)-ACPD**

Figure 2.21 Structure of test compound

The assay was performed using Ro 64-5229 in three separate experiments performed in triplicate. Data obtained was plotted to give a sigmoidal curve, from which an IC_{50} value of 0.53 ± 0.03 μM (n = 3) was obtained (Figure 2.22). This is of the same order of magnitude as the previous result which was determined using slightly different experimental conditions.

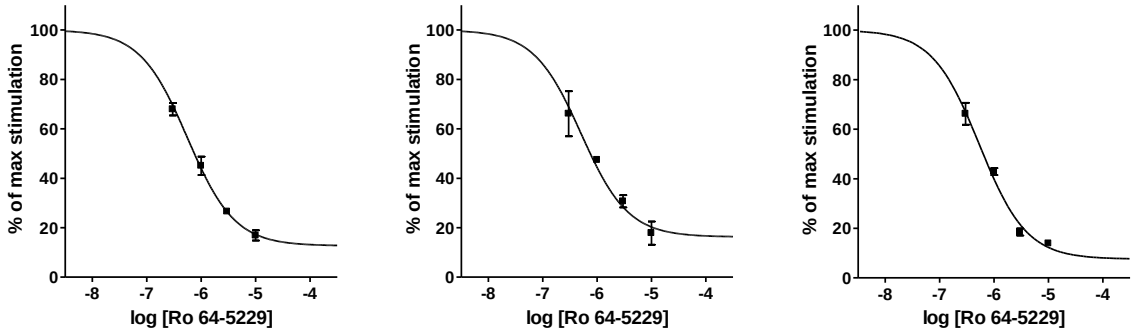


Figure 2.22 Graph of Ro 64-5229 binding

With this excellent result, the binding affinity of the library of benzodiazepinones could be tested. Due to the high cost of performing the assay, not all of the compounds were tested three times. Instead, all compounds that performed well in the HPLC screening were tested three times, and all data possible with the remaining resources was obtained for the other compounds.

The compounds were all found to have similar binding to mGluR2, providing IC_{50} values between 89 and 133 nM (Table 2.8). It is difficult to compare binding affinity of these compounds to previously published benzodiazepinones by Woltering and co-workers, due to differences in the binding assays used.[61-64] What can be observed is that the

benzodiazepinones have greater affinity for mGluR2 than Ro 64-5229 and comparable affinity to benzodiazepinones published by Hemstapat and co-workers, which were determined using similar conditions.[130] The sigmodial curves produced by binding of the benzodiazepinones all have a hill slope of greater than 1. This is precedented as Cartmell and co-workers tested a range of compounds that bind to mGluR2 and found great variation in the hill slopes of from 0.6 to 2.[140]

Compounds **169**, **170** and **201** produced the most potent inhibition of agonist stimulated [^{35}S]GTPγS binding, however, compound **201** gave a large error due to the fact that it was only tested twice. Fortunately, compounds **169** and **170** also produced the best HPLC results and consequently were selected for further research.

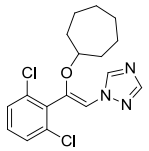
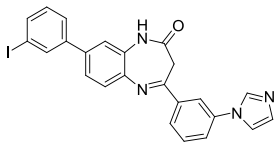
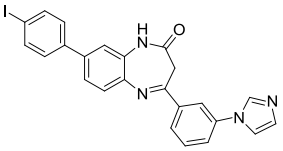
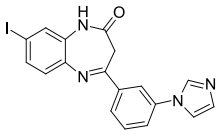
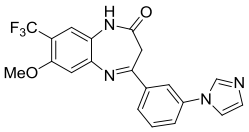
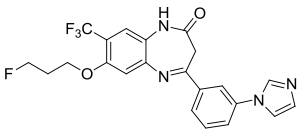
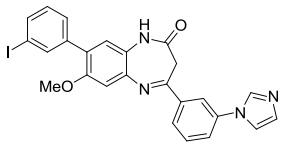
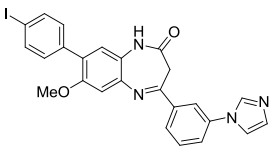
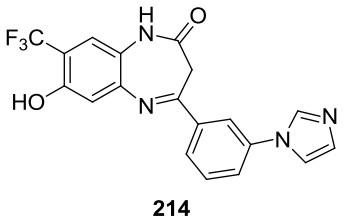
Compound	IC_{50}	Hill Slope
 **Ro 64-5229**	533 ± 27 nM (n=3)	1.00
 **134**	132 ± 7 nM (n=3)	1.65 ± 0.13
 **135**	133 ± 24 nM (n=2)	1.75 ± 0.38
 **169**	89 ± 5 nM (n=3)	1.80 ± 0.14
 **170**	91 ± 30 nM (n=3)	1.49 ± 0.36
 **171**	100 ± 14 nM (n=3)	2.05 ± 0.15
 **200**	not tested	not tested
 **201**	89 ± 46 (n=2)	2.52 ± 1.10

Table 2.8 Binding affinity of benzodiazepinones

2.6 Synthesis of a Labelling Precursor

After examining the physicochemical properties and biological affinity results, methoxy substituted compound **170** emerged as a lead compound for further work, such as radiolabelling. In the synthesis of potential PET imaging agent **170**, an aromatic nucleophilic substitution was used to attach the methoxy group early in the synthetic route. To access a radiolabelled analogue, an alternate strategy was required in which the alkyl group could be added in the final step. If phenol **214** (Figure 2.23) could be accessed, a radiolabelled compound could be formed by alkylation using carbon-11 labelled iodomethane. Further non-labelled analogues in this series could also be quickly accessed by alkylation of phenol **214**.

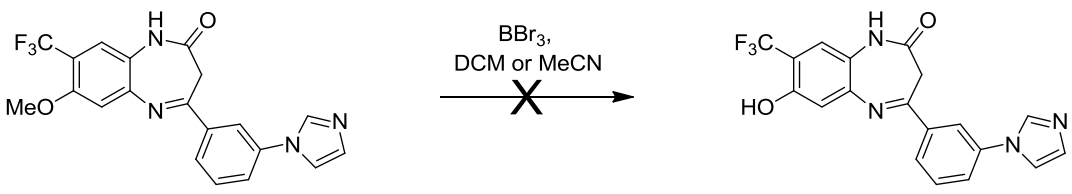


214

Figure 2.23 Structure of radiolabelling precursor **214**

2.6.1 Attempted Synthesis by Demethylation

Initial attempts to synthesise phenol **214** by Lewis acid mediated demethylation of methoxy substituted compound **170** with boron tribromide proved to be unsuccessful with only decomposition of the starting material being observed at both room temperature and under reflux (Scheme 2.29).

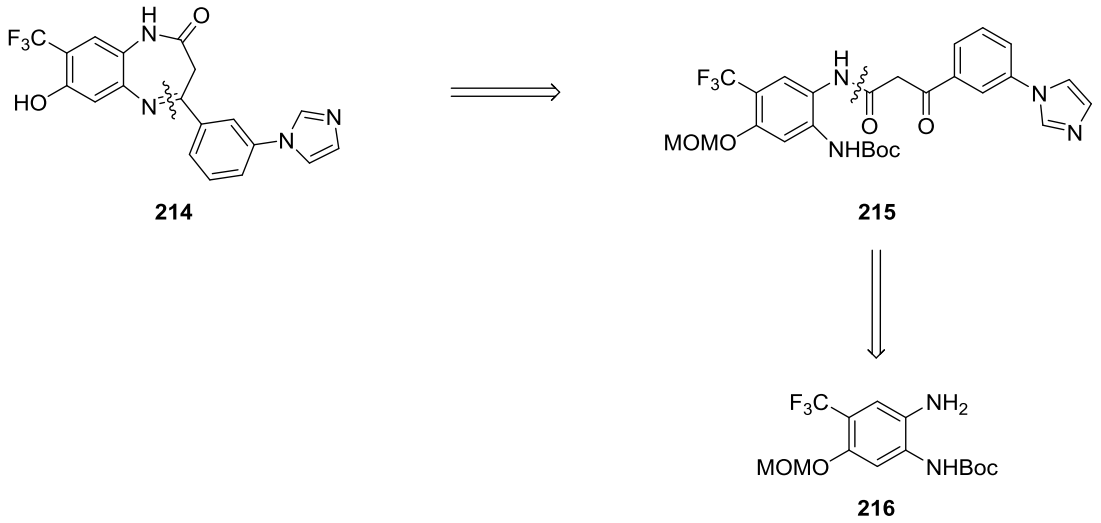


170 **214**

Scheme 2.29 Attempted demethylation of compound **170**

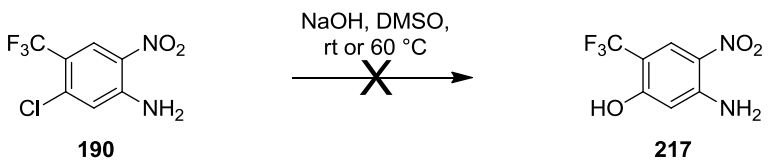
2.6.2 Attempted Synthesis Using a Methoxymethyl Ether Protecting Group

A new method for the synthesis of phenol **214** was then proposed in which the benzodiazepinone would be formed from β-keto amide **215** containing a methoxymethyl (MOM) ether protecting group on the phenol, which would be removed under the acidic cyclisation conditions. To achieve this, amine fragment **216** containing a MOM-protected phenol had to be synthesised (Scheme 2.30).



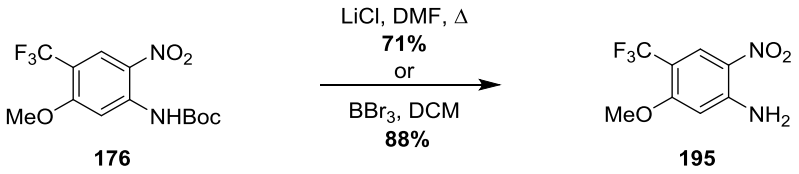
Scheme 2.30 Retrosynthetic analysis of phenol **214**

Preliminary efforts to synthesise amine fragment **216** focused on first producing phenol **217** and then protecting with a MOM group. Formation of phenol **217** via a nucleophilic aromatic substitution of chlorobenzene **190** using sodium hydroxide was attempted following the procedure of Jacobs, which had been successful on a similar compound.[141] Unfortunately only starting material was observed under these conditions (Scheme 2.31).



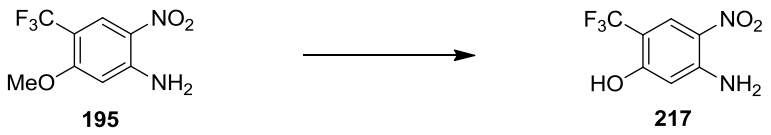
Scheme 2.31 Attempted nucleophilic aromatic substitution

As the phenol could not be prepared directly from chlorobenzene **190**, demethylation of methoxybenzene **176** was attempted. Reactions using lithium chloride or boron tribromide were carried out but neither gave the desired phenol **217**. Instead, only removal of the Boc protecting group occurred to give aniline **195** (Scheme 2.32).



Scheme 2.32 Attempted demethylation of methoxybenzene **176**

Attention was then turned to demethylation reactions using aniline **195**. Various conditions were investigated as outlined in Scheme 2.33 and Table 2.9. Lewis acid mediated methods using boron tribromide and aluminium trichloride produced either starting material or decomposition (entries 1 and 2). Lithium chloride gave some success with desired phenol **217** being isolated in a yield of 26% (entries 3 and 4). The best results were obtained using pyridine hydrochloride (entries 5 to 8).[142] Optimisation was required as no reaction occurred at 140 °C, but using the high temperature of 210 °C resulted in decomposition. A best yield of 61% was obtained using a lower reaction temperature of 150 °C. Increasing the reaction time at this temperature resulted in a poorer yield as product **217** began to decompose before starting material **195** was completely consumed.

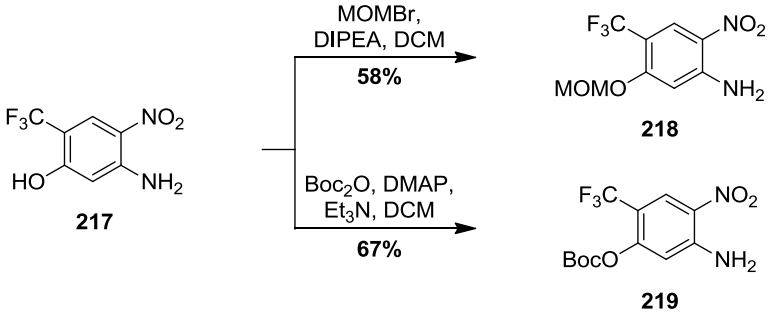


Scheme 2.33 Demethylation of methoxybenzene **195**

Entry	Reagent	Conditions	Outcome
1	BBr_3	DCM, rt or Δ	S.M.
2	$AlCl_3$	DCM, rt	Decomposition
3	LiCl	DMF, Δ, 18 h	Product observed but not isolated
4	LiCl	DMSO, 150 °C, 18 h	26%
5	Pyridine.HCl	140 °C, 3 h	S.M.
6	Pyridine.HCl	210°C, 3 h	Decomposition
7	**Pyridine.HCl**	**150 °C, 12 h**	**61%**
8	Pyridine.HCl	150 °C, 24 h	52%

Table 2.9 Optimisation of demethylation of methoxybenzene **195**

With phenol **217** in hand, the next step was to protect the phenol and aniline groups. Treating compound **217** with either bromomethyl methyl ether or di-*tert*-butyl dicarbonate resulted in protection of the phenol functional group in modest yields of 58% and 67%, respectively (Scheme 2.34).

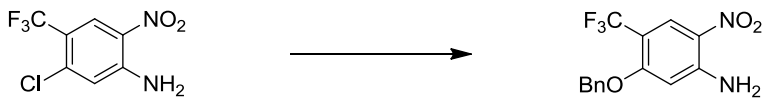


Scheme 2.34 Protection of phenol **217**

Due to the fact that the synthetic route was producing moderate yields, it was felt that the desired compound could be reached more efficiently using an alternate synthetic route. As such, the sequence was terminated at this stage.

2.6.3 Synthesis Using a Benzyl Protecting Group

A new synthetic route was devised in which a benzyl protected phenol was introduced by nucleophilc aromatic substitution using benzyl alcohol, rather than forming a phenol then protecting it as had been carried out previously. Various conditions were trialled (Scheme 2.35 and Table 2.10) with the most successful being those of Boltze and co-workers, in which the reaction was performed neat in benzyl alcohol with phase transfer catalyst tetrabutylammonium bromide present to encourage solvation of the hydroxide.[143]

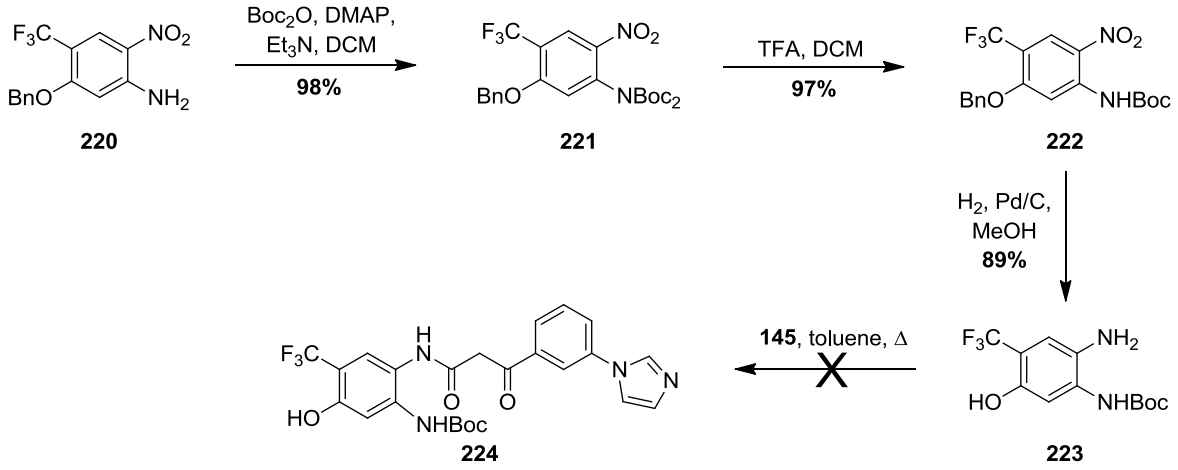


Scheme 2.35 Synthesis of benzyl ether **220**

Entry	Reagent(s)	Solvent	Temperature	Outcome
1	BnOH, NaH	DMF	50 °C	inseparable mixture
2	BnOH, KOH	DMSO	60 °C	starting material
3	**BnOH, NBu_4Br, KOH**	**-**	**60 °C**	**60%**

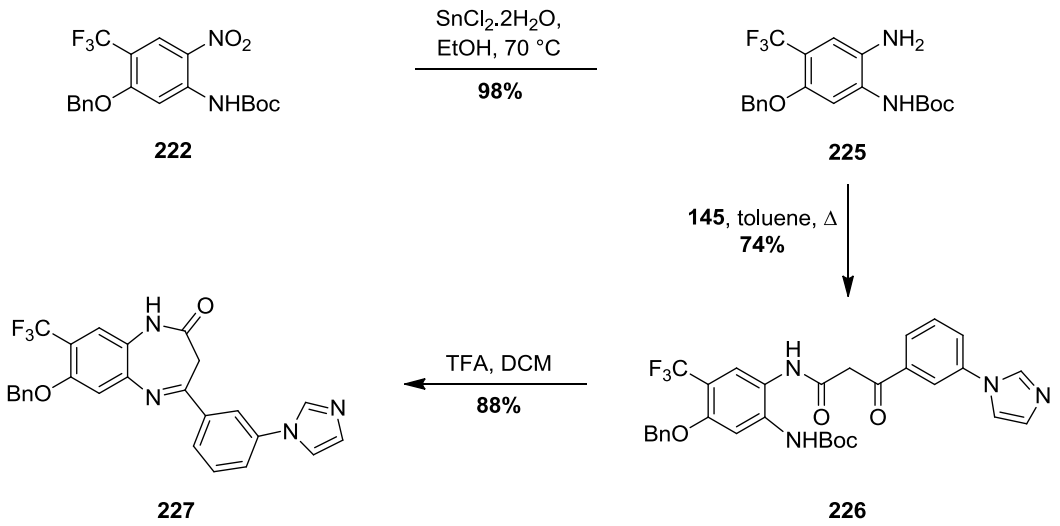
Table 2.10 SnAr reaction with benzyl alcohol

Protection of the amine moiety of compound **220** was achieved by bis-Boc protection followed by mono-deprotection to give protected amine **222**. Hydrogenation of compound **222** achieved both nitro reduction and benzyl ether cleavage to give phenol **223**. β-keto amide formation was attempted on phenol **223** but a mixture of products were observed due to reaction at the phenol (Scheme 2.36). It became clear that the phenol would need to remain protected during this step.



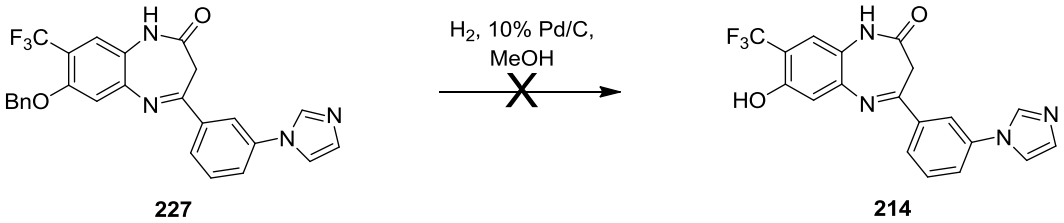
Scheme 2.36 Attempted synthesis of β-keto amide **224**

Tin(II) chloride was employed to selectively reduce the nitro group of compound **222** in the presence of the benzyl ether. This reaction gave amine **225** in an excellent yield of 98%. Treatment with β-keto ester **145** produced β-keto amide **226**, and intramolecular imine formation then furnished benzodiazepinone **227** (Scheme 2.37).



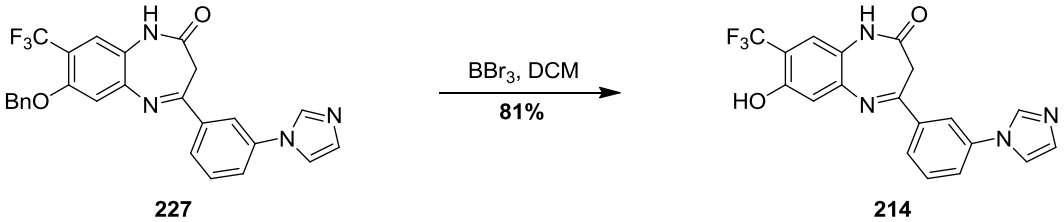
Scheme 2.37 Synthesis of benzodiazepinone **227**

Deprotection of the phenol was then required to complete the synthetic route. Ether cleavage by hydrogenation was trialled as work by Janciene and co-workers revealed the benzodiazepinone core to be stable to palladium-catalysed hydrogenation.[144] However, this reaction produced a mixture of compounds from which the desired phenol **214** could not be isolated (Scheme 2.38).



Scheme 2.38 Attempted debenzylation of benzodiazepinone **227**

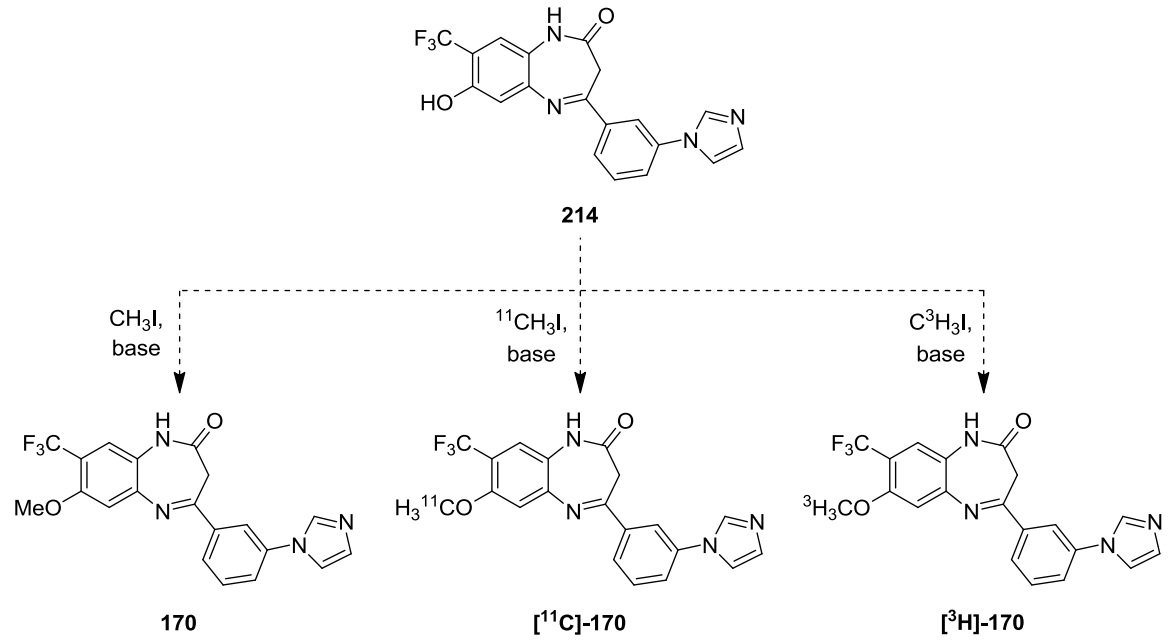
Lewis acid mediated debenzylation using boron tribromide was then performed which afforded phenol **214** in a high 81% yield (Scheme 2.39). It is worth noting that this reaction follows the commonly observed order of reactivity of Lewis acid mediated ether cleavage, with successful debenzylation of benzyl ether **227** under these conditions, whereas attempted demethylation of the analogous methyl ether **170** was unsuccessful.[145]



Scheme 2.39 Debenzylation of benzodiazepinone **227**

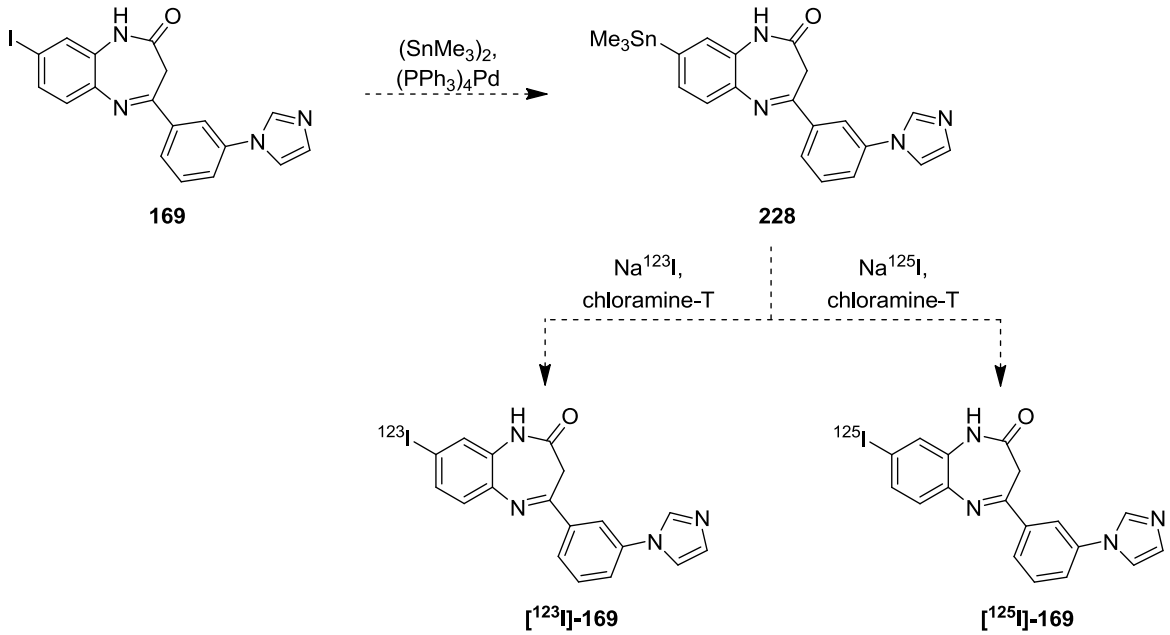
2.7 Future Work

With phenol **214** in hand, the next stage of work on this project will be to optimise the alkylation of this compound. Once this compound has been successfully alkylated, a carbon-11 labelled version could be produced for animal PET imaging, or a tritiated (hydrogen-3 labelled) version for autoradiography studies (Scheme 2.40).



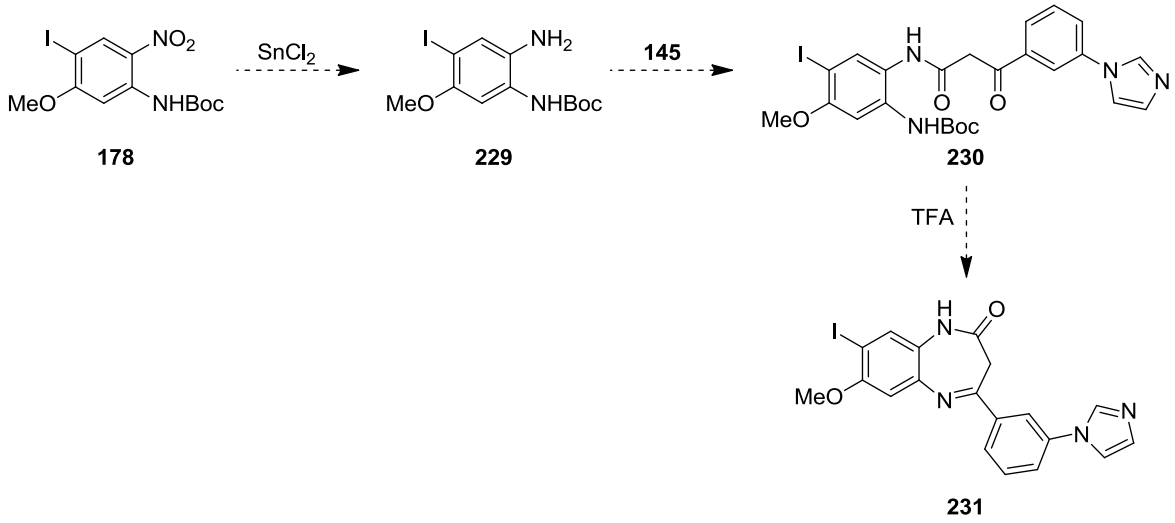
Scheme 2.40 Alkylation of phenol **214**

Potential SPECT imaging agent **169** was also selected for labelling studies. A suitable labelling precursor **228** could be synthesised from aryl iodide **169** by treatment with hexamethyditin and palladium(0). Radiolabelling could then be performed with iodine-123 for animal SPECT studies or iodine-125 for autoradiography to further investigate the suitability of benzodiazepinone **169** as a SPECT imaging agent (Scheme 2.41).



Scheme 2.41 Stannylation of iodide **169** followed by iododestannylatiom

The only series that did not provide a compound suitable for progression were the compounds with multiple labelling sites that could be used for SPECT or PET imaging. In this series the two compounds (benzodiazepinones **200** and **201**) had poor physicochemical properties. To overcome this, compound **231** is proposed which removes one of the benzene rings present in compounds **200** and **201**, which caused the lipophilicity to be too high. Benzodiazepinone **231** could be easily synthesised in three steps from previously used nitrobenzene **178** (Scheme 2.42).



Scheme 2.42 Proposed synthesis of benzodiazepinone **231**

2.8 Conclusions

A library of seven benzodiazepinones were synthesised following the general strategy of forming separate amine and β-keto ester fragments, coupling together and cyclising to form the benzodiazepinone core. This synthetic route allowed access to a variety of analogues around the benzodiazepinone core. A particular success of this chemistry was the synthesis of the highly substituted amine fragments which were synthesised in good to very high yields from inexpensive starting materials.

Investigating the physicochemical properties of these compounds revealed the addition of a benzene ring at the 8-position of the benzodiazepinone caused compounds **134**, **135**, **200** and **201** to become too highly lipophilic. Compounds **169** and **170** were found to have the necessary partition coefficient, permeability and plasma protein binding to be considered

for further research. Using the [^{35}S]GTPγS binding assay showed all the compounds to have similar mGluR2 potency, comparable to existing benzodiazepinones. Therefore, the binding was considered to be sufficient to progress the compounds to labelling studies.

Iodide **169** and methoxy substituted compound **170** were selected for radiolabelling and a synthetic route to labelling precursor **214** was successfully developed. Future work on this project will focus on the synthesis of labelling precursor **228**, and the radiolabelling experiments that will allow further biological investigation to take place both *in vitro* and *in vivo*.

Therefore this project achieved its aim to identify compounds with good binding to mGluR2 and favourable physicochemical properties, which with further research will have the potential to be used as SPECT or PET imaging agents.

3 Novel Heterocycle Containing α-Amino Acids

3.1 Introduction

The importance of α-amino acids in chemistry, biology and medicine is well established. Proteinogenic amino acids provide the building blocks for proteins and enzymes, and are involved in enzyme catalysed reactions. Amino acids are important in signal transduction with compounds such as L-glutamate, L-aspartate and glycine functioning as neurotransmitters,[146] whilst other proteinogenic amino acids act as the precursors in the biosynthesis of neurotransmitters.[147,148] In organic chemistry, proteinogenic amino acids are regularly employed as starting materials in the synthesis of natural products and biologically active compounds, using a chiral pool approach.[149-151]

Non-proteinogenic amino acids include those that are produced naturally but are not found in proteins, and amino acids which do not occur naturally. These compounds are also of great interest due to their medicinal applications[152,153] and use as probes of protein structure and function.[154]

3.1.1 Heterocycle Containing α-Amino Acids

An interesting subset of α-amino acids are those which contain heterocyclic rings. Among the proteinogenic α-amino acids, L-tryptophan, L-histidine and L-proline fall into this category (Figure 3.1).

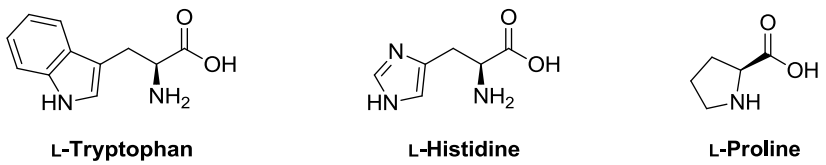


Figure 3.1 Naturally occurring heterocycle containing amino acids

L-Tryptophan contains an indole side chain and is of particular biological significance as it is the biochemical precursor of the neurotransmitter serotonin,[148] the hormone melatonin[155] and the vitamin niacin (Figure 3.2).[156]

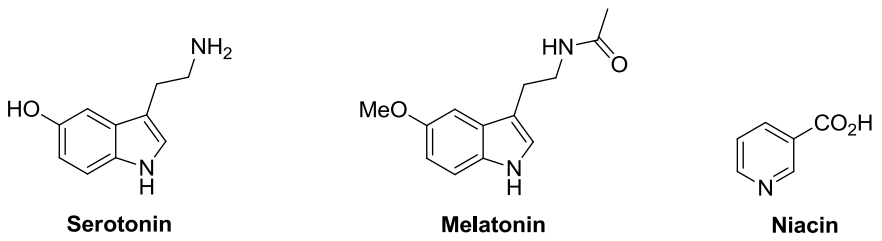


Figure 3.2 Structures of serotonin, melatonin and niacin

L-Histidine is found at many enzyme active sites as the imidazole ring present in L-histidine allows it to act as either a proton donor or acceptor and also permits for the coordination of metal ions.[157] The pyrrolidine ring of L-proline makes it unique among the proteinogenic amino acids, being the only one containing a secondary amine. L-Proline has important applications in organic chemistry in which it is commonly used as an organocatalyst in asymmetric synthesis.[158,159]

Non-proteinogenic heterocycle containing α-amino acids are also of great interest. Many of these compounds occur naturally. One example of this is (−)-dysiherbaine, which was isolated from the marine sponge dysidea herbacea by Sakai and co-workers in 1997 (Figure 3.3).[160] This compound showed binding activity for non-NMDA ionotropic glutamate receptors. The biological activity coupled with the synthetically challenging structure of contiguous stereogenic centers made this compound an ideal total synthesis target. Snider and co-workers published the first total synthesis of this compound in 2000[161] and there have been many more total syntheses since.[162]

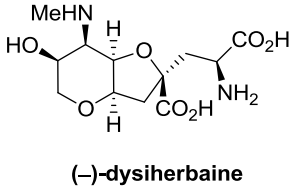


Figure 3.3 Structure of (–)-disiherbaine

A number of unnatural heterocycle containing α-amino acids also show activity at ionotropic glutamate receptors. The AMPA receptor is named due to the fact that it can be stimulated by the α-amino acid (*S*)-AMPA (Figure 3.4).[17] Heterocycle containing α-amino acid (*S*)-5-fluorowillardiine is also an agonist of this receptor (Figure 3.4).[163]

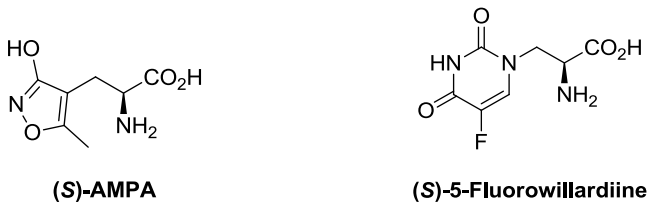


Figure 3.4 AMPA receptor agonists

3.1.2 Fluorescent Heterocycle Containing α-Amino Acids

Many heterocycle-containing α-amino acids have fluorescent properties. Fluorescence is a process which involves the emission of light from a compound that has absorbed light of a different, usually shorter, wavelength. Upon excitation, an electron is promoted to an excited state (S_1 or S_2). A singlet to singlet transition then occurs (S_1 to S_0) with the release of energy as a photon to return the electron to its ground state (Figure 3.5). As this is an allowed transition it occurs on a fast nanosecond scale.[164]

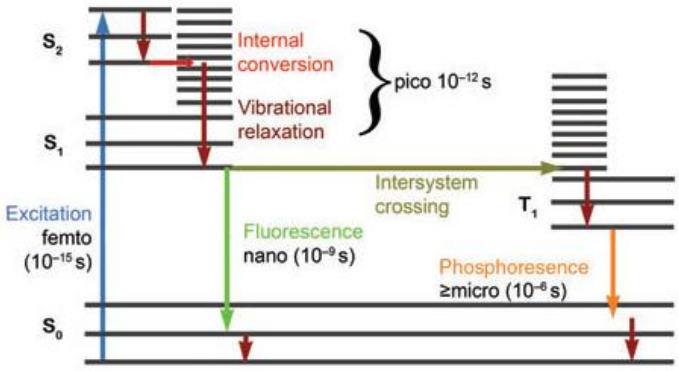


Figure 3.5 Molecular relaxation pathways[164]

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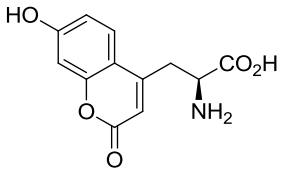
Structures with a high degree of aromaticity and conjugation are more likely to be fluorescent as the large π-system results in a small difference in energy between the ground and excited states, which allows low energy photons to promote electrons into excited states.

A number of naturally occurring amino acids such as phenylalanine, tyrosine and tryptophan are fluorescent and emit at 282 nM, 303 nM and 348 nM, respectively.[165] Amino acids containing a fluorescent side chain can be used in biology to label a protein of interest. Fluorescence microscopy can then be employed to monitor the location of the protein in cells. Brun and co-workers synthesised coumarin containing α-amino acid **232** which was found to be fluorescence with emission at 464 nM (Figure 3.6).[166] As this wavelength is significantly higher than the emission of the naturally occurring fluorescent amino acids, compound **232** could be used as a fluorescent label for peptides. This was demonstrated by synthesising an amino acid **232** labelled version of the peptide penetratin and imaging its internalisation into HeLa cells.



232

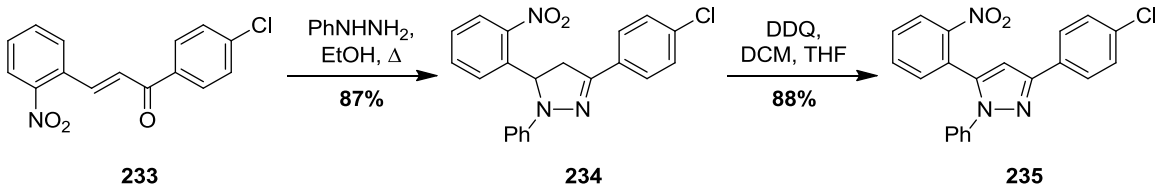
Figure 3.6 Structure of coumarin containing amino acid **232**

3.1.3 Formation of Heterocycles from Enones

Many heterocycles can be formed by exploiting the reactivity of the enone moiety to form a variety of nitrogen, oxygen and sulfur containing five, six or seven membered heterocycles.

Five membered heterocycles such as pyrazole can be formed from an enone starting material. The synthesis of pyrazole **235** was carried out by Duggineni and co-workers as part of the synthesis of a substrate for a Pictet-Spengler reaction.[167] Treatment of enone

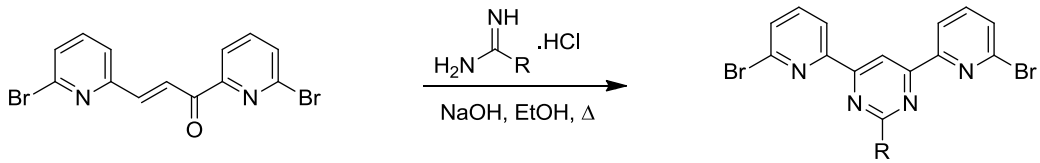
233 with phenylhydrazine produced pyrazoline **234** which subsequently underwent oxidation to give the corresponding pyrazole **233**, both in excellent yields (Scheme 3.1).



233 **234** **235**

Scheme 3.1 Formation of pyrazole **235**

Substituted pyrimidines are an example of a six membered ring that can be formed from an enone starting material. In the synthesis of pyrimidine based ligands by Jones and co-workers, enone **236** was treated with range of amidine hydrochlorides to form a library of 2-substituted pyrimidines (**237−240)** in moderate to high yield (Scheme 3.2).[168]

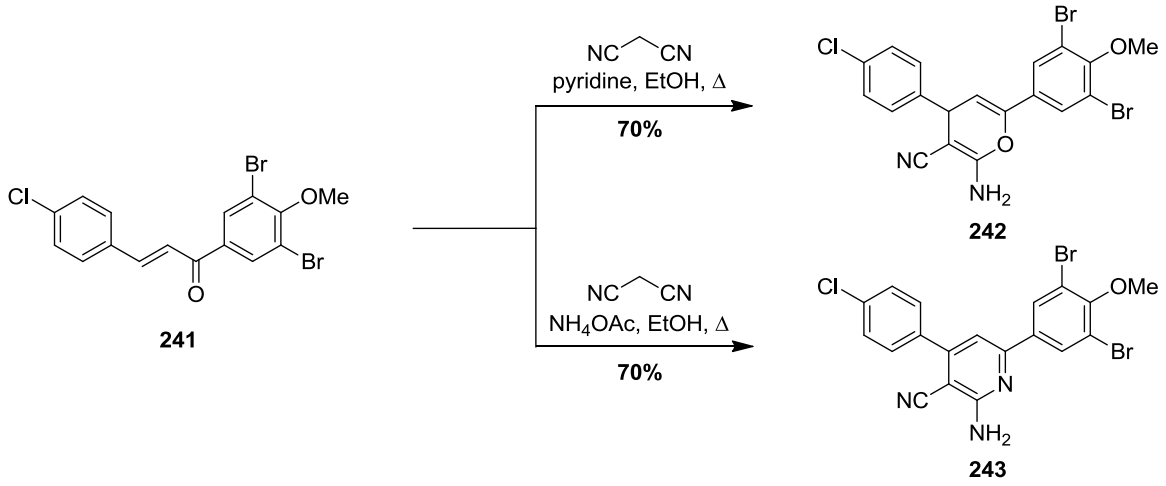


236

237: R = $CH(OEt)_2$ **47%**
238: R = Me **52%**
239: R = Ph **53%**
240: R = NH_2 **85%**

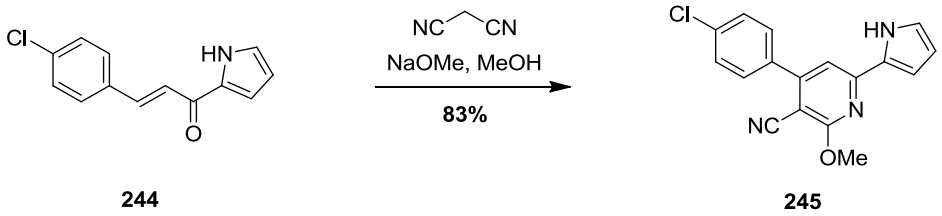
Scheme 3.2 Formation of pyrimidines **237−240**

Malononitrile is an ideal Michael donor for reaction with an enone due to the presence of the electron withdrawing cyano groups. Michael addition of malononitrile to enone **241**, followed by cyclisation and air mediated oxidative aromatisation produced 2-amino-3-cyanopyran **242** (Scheme 3.3). However, in the presence of ammonium acetate, imine formation occurs prior to cyclisation and oxidation to form 2-amino-3-cyanopyridine **243**.[169]



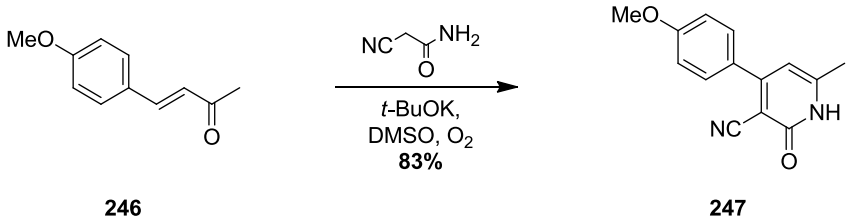
Scheme 3.3 Synthesis of pyran **242** and pyridine **243**

Using an alkoxide base in the reaction instead of ammonium acetate provides an alkoxy-substituent in place of the amino group. Reaction of enone **244** with malononitrile and sodium methoxide produced 3-cyano-2-methoxypyridine **245** (Scheme 3.4).[170]



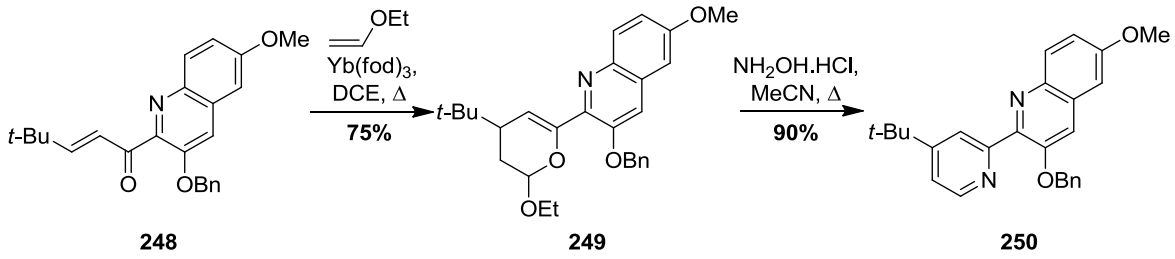
Scheme 3.4 Formation of pyridine **245**

Jain and co-workers reported the synthesis of 3-cyano-2-pyridones from the reaction between an enone and cyanoacetamide under basic conditions in an oxygen atmosphere (Scheme 3.5).[171] This reaction is believed to occur by Michael addition of cyanoacetamide to enone **246** followed by imine formation and oxygen mediated oxidation to form cyano-substituted pyridone **247**.



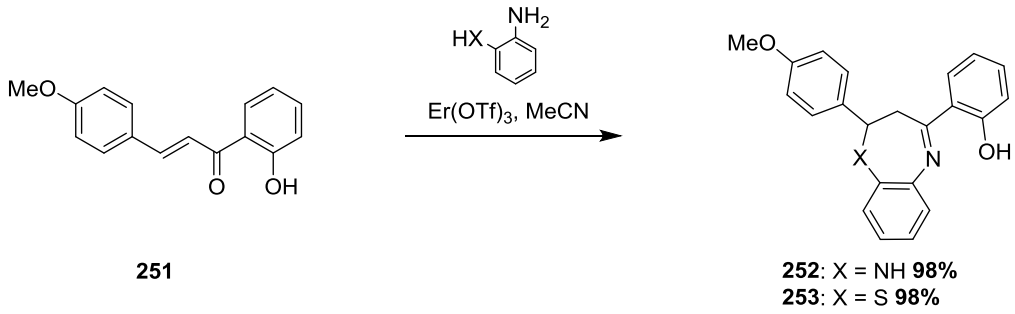
Scheme 3.5 Formation of 3-cyano-2-pyridone **247**

Enones can also function as the diene component in hetero Diels-Alder reactions. Ciufolini and co-workers used a ytterbium(III)-catalysed Diels-Alder reaction between enone **248** and ethyl vinyl ether to form dihydropyran **249** as part of the synthesis of a model substrate in their total synthesis of (+)-camptothecin.[172] Treatment with hydroxylamine then transformed the dihydropyran to pyridine **250** (Scheme 3.6).



Scheme 3.6 Synthesis of pyridine **250**

Finally, enones can also be used to form seven membered heterocycles. An erbium(III)-catalysed reaction with either 1,2-diaminobenzene or 2-aminothiophenol has been shown to produce 1,5-benzodiazepines and 1,5-benzothiazepines, respectively (Scheme 3.7).[173] The analogous 1,5-benzoxazepine can be formed by reaction of an enone with 2-aminophenol.[174]

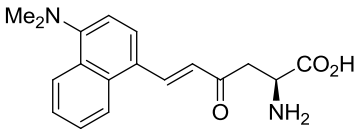


Scheme 3.7 Synthesis of 1,5-benzodiazepine **252** and 1,5-benzothiazepine **253**

3.1.4 Proposed Research

Firstly, a library of enone containing α-amino acids were to be synthesised utilising the synthetic route previously developed in the Sutherland group by Lindsay Fowler, in which such enones were produced in four steps from L-aspartic acid **255** (Scheme 3.8).[175] During

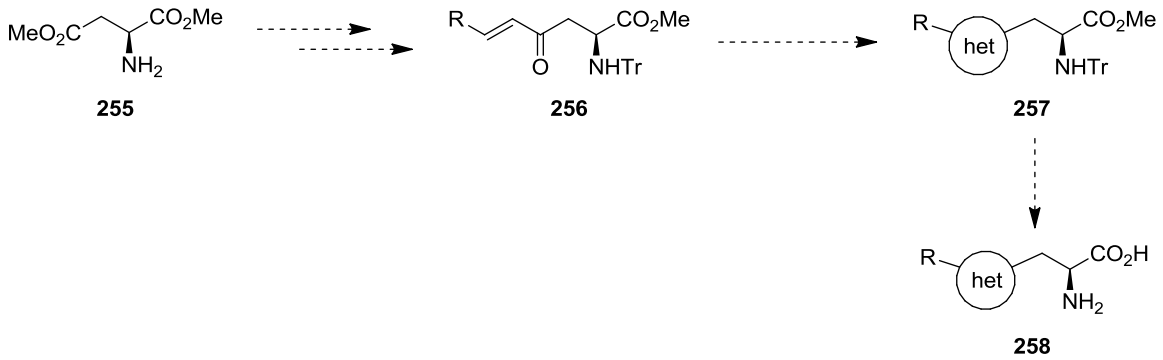
this previous work, a fluorescent enone containing α-amino acid was prepared (Figure 3.7), but was found to be unstable after several days at room temperature.



254

Figure 3.7 Structure of fluorescent amino acid **254**

It was therefore proposed to explore the reactivity of the enone moiety to form heterocycles, using the reactions discussed in Section 3.1.3 (Scheme 3.8). It was then planned to remove the protecting groups to produce a library of novel heterocycle containing α-amino acids, the fluorescent properties of which could then be investigated.



255 **256** **257** **258**

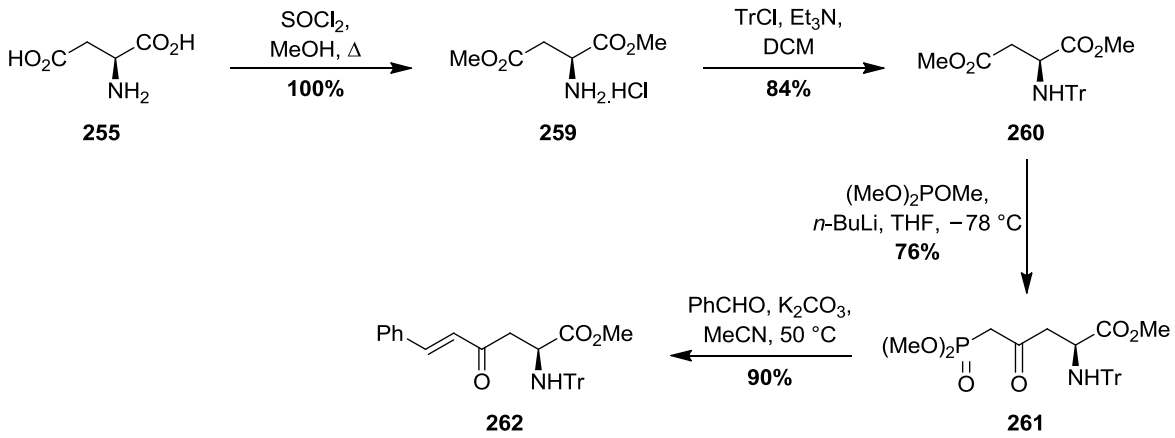
Scheme 3.8 Proposed synthesis of novel heterocycle containing α-amino acids

3.2 Synthesis of Novel Heterocycle-Containing Amino Acids

3.2.1 Synthesis of Enones

A library of enones was synthesised according to the route previously developed in the Sutherland group by Lindsay Fowler.[175] Starting from L-aspartic acid **255**, treatment with thionyl chloride in methanol allowed quantitative methyl ester formation. Amine **259** was then protected with the bulky trityl group, which directed the subsequent reaction with the anion of dimethyl methylphosphonate onto the β-methyl ester, to form phosphonate ester

261. A Horner-Wadsworth-Emmons reaction using benzaldehyde was then employed to form enone **262** in an excellent yield (Scheme 3.9).



Scheme 3.9 Synthesis of enone **262**

Phosphonate ester **261** was then reacted with various aldehydes to form six further enones (**263−268**) in moderate to high yields (Figure 3.8). Using Horner-Wadsworth-Emmons conditions led exclusively to formation of the *E*-enones, with no *Z*-enone being observed. This was confirmed by ^{1}H NMR spectroscopy in which the coupling constant between the alkene protons varied from 15.7 to 16.2 Hz, all within the expected range for an *E*-alkene.

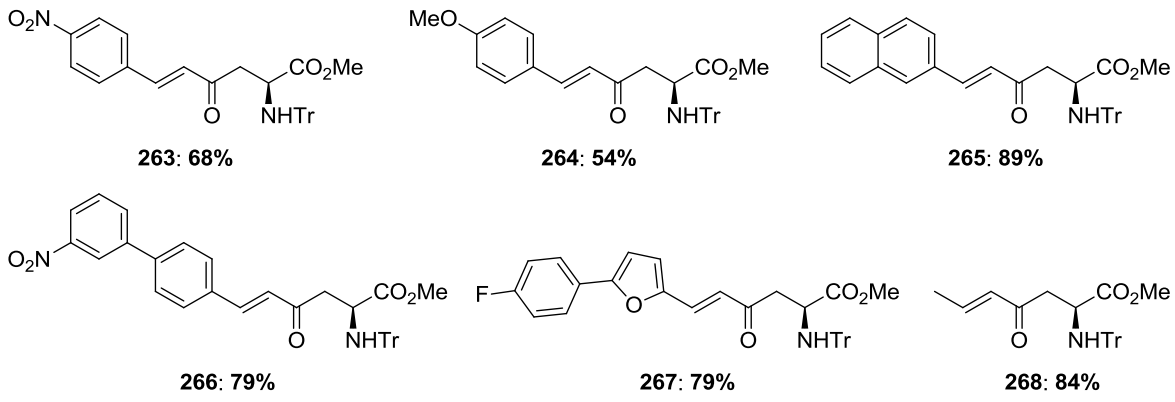
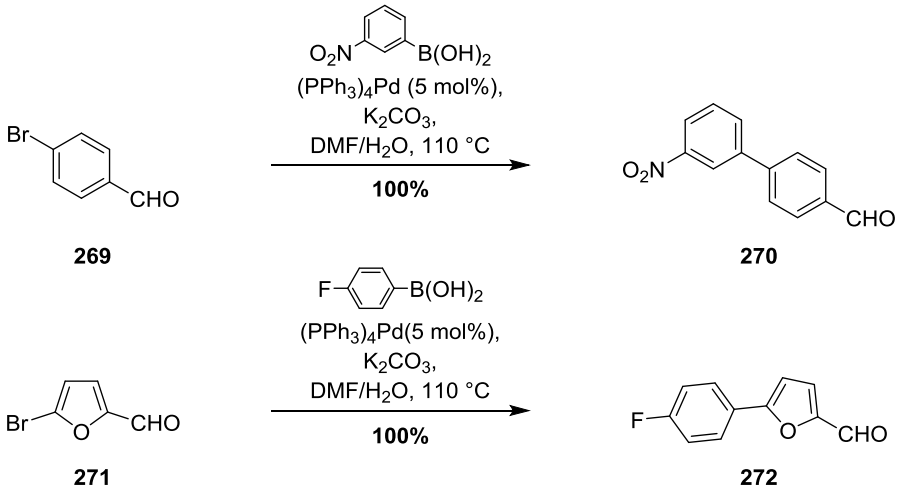


Figure 3.8 Structures of enones **263−268**

Most of the aldehydes used were commercially available, however, biaryl aldehydes **270** and **272** had to be synthesised. Suzuki coupling reactions were employed to form these

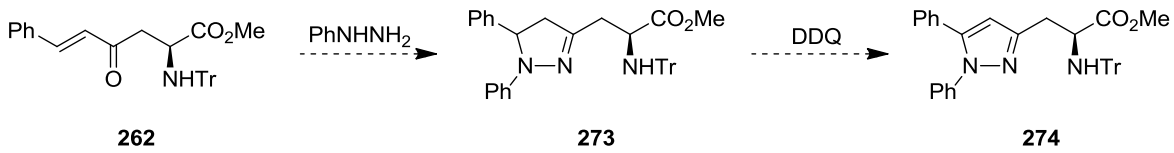
compounds quantitatively from readily available starting materials **269** and **271** (Scheme 3.10).



Scheme 3.10 Suzuki reactions to form biaryl aldehydes **270** and **272**

3.2.2 Synthesis of Pyrazoles

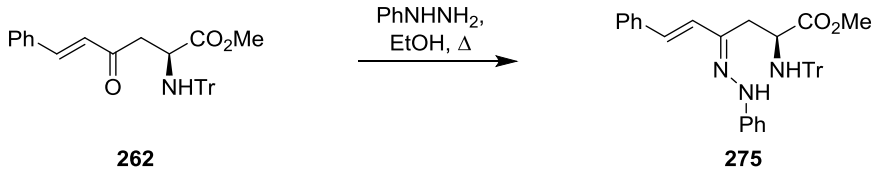
With a small library of enones in hand, attention was then turned to exploiting the reactivity of the enone moiety to form a heterocycle. It was planned to treat phenyl substituted enone **262** with phenylhydrazine to form pyrazoline **273**, which could then be oxidised to form pyrazole **274** (Scheme 3.11).



Scheme 3.11 Planned synthesis of pyrazole **274**

However, when the reaction was carried out under both neutral[176] and basic[177] conditions, pyrazoline **273** was not observed. Analysis by ^{1}H NMR spectroscopy revealed that although the starting material had been consumed, the alkene protons were still intact and a new peak was present at 9.26 ppm. This is consistent with the formation of hydrazone **275** by imine condensation with the new signal being the hydrazone NH moiety (Scheme 3.12). Although it was encouraging that the first part of the reaction had taken place, the Michael

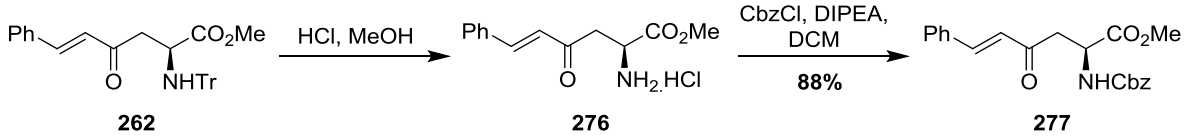
addition required to generate pyrazoline **273** had not occurred. A potential explaination for the lack of Michael addition is that the hydazone may have been formed as the *E*-isomer to avoid steric clash with the alkene substituents, which cannot rotate to avoid this, unlike the group on the other side of the hydrazone. With this geometry the Michael addition to form a pyrazoline would not be able to occur.



262 **275**

Scheme 3.12 Formation of hydrazone **275**

Reviewing the literature revealed that this reaction had often been performed under acidic conditions.[178,179] In order to conduct the reaction using acid mediated conditions, it was necessary to replace the acid labile trityl group with a protecting group that would be stable to acid. A carboxybenzyl (Cbz) group was chosen for this purpose. Treatment of the trityl protected enone **262** with aqueous hydrochloric acid in methanol formed the amine **276** as a hydrochloride salt. The crude product of this reaction was then treated with benzyl chloroformate to form Cbz protected enone **277** (Scheme 3.13).



262 **276** **277**

Scheme 3.13 Synthesis of Cbz protected **277**

This sequence was successful for all the enones with aromatic substituents (**278−282**), although the furan analogue **282** was obtained in a low yield of 31% (Figure 3.9). Methyl substituted enone **283** could not be isolated using this method. It was observed that the crude product of the trityl deprotection reaction on these two substrates contained many more impurities than the other compounds. The removal of excess aqueous hydrochloric acid under vacuum during work-up required temperatures greater than 50 °C. It was proposed that decomposition of the furan and methyl substrates could occur under these

conditions due to the reduced stability of these enones compared to the phenyl substituted compounds.

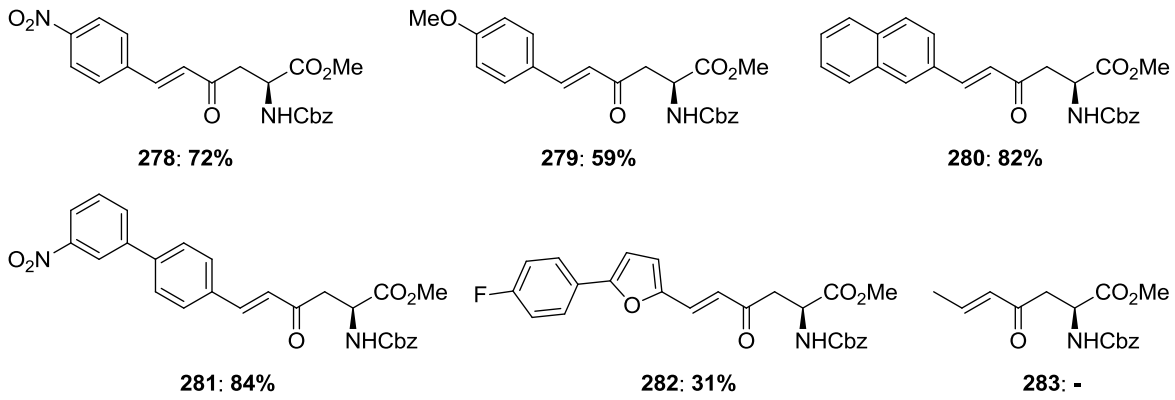
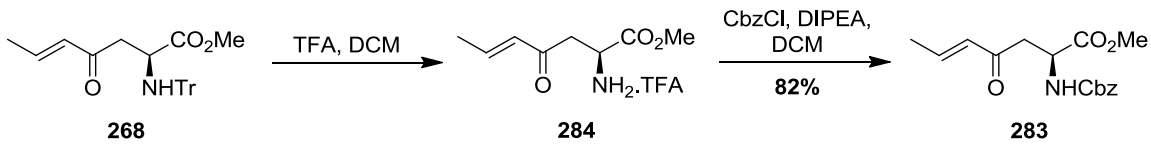


Figure 3.9 Structure of Cbz protected compounds **278−283**

To Cbz protect methyl substituted enone **283**, an alternative trityl deprotection was carried out by treatment of enone **268** with two equivalents of trifluoroacetic acid in dichloromethane. This milder procedure provided a much cleaner amine and hence much cleaner Cbz protected product, which was able to be isolated in a high yield of 82% (Scheme 3.14).



Scheme 3.14 Synthesis of Cbz protected **283**

The method was simpler to perform practically and so was used when bringing through additional material for some of the aromatic analogues. For the phenyl, nitrophenyl and methoxyphenyl compounds (**277−279**) a similar or improved yield was obtained (Figure 3.10).

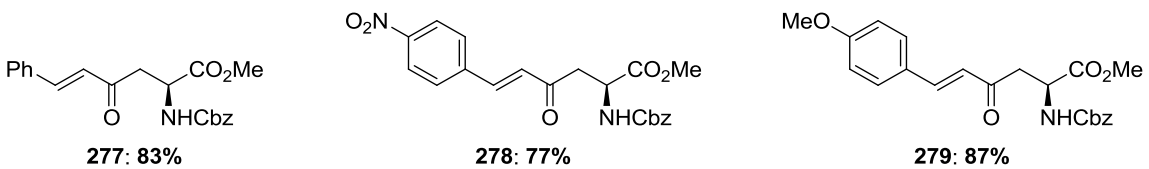
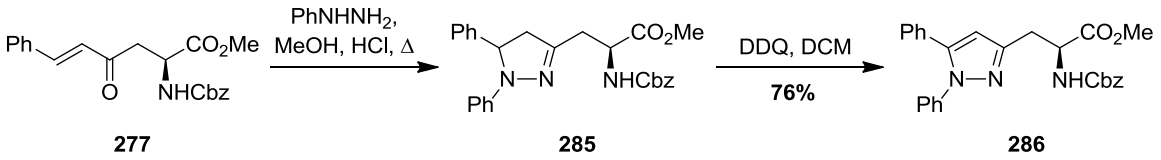


Figure 3.10 Cbz protected compounds synthesised under milder conditions

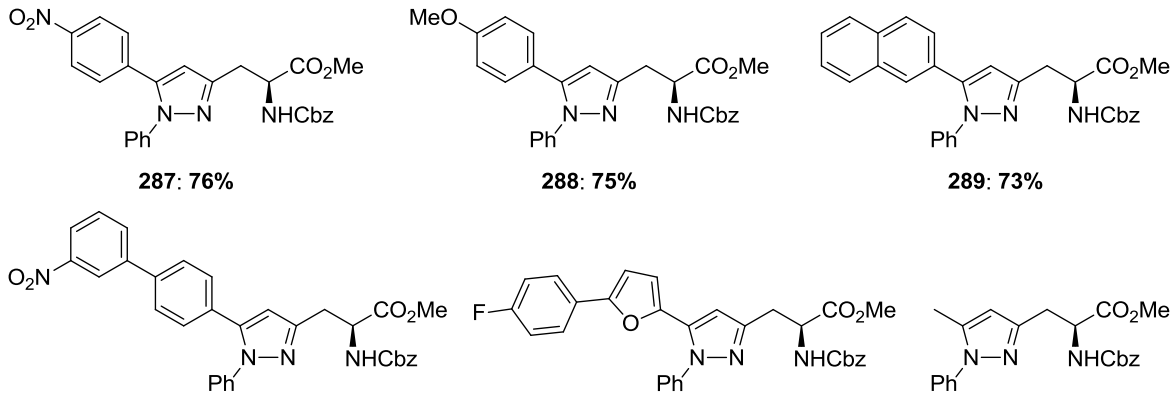
Phenyl substituted enone **277** was then subjected to the pyrazoline forming reaction under acid mediated conditions (Scheme 3.15). Pyrazoline **285** was obtained as a mixture of diastereomers. As one of the stereocenters would be lost during the subsequent oxidation, the ratios and assignment of the diastereomers was not investigated. The crude product was then oxidised to the corresponding pyrazole **286** using DDQ in 76% yield over the two steps.



277 **285** **286**

Scheme 3.15 Synthesis of pyrazole **286**

This method was used to produce pyrazoles **287−290** in high yields of from 73% to 84% (Figure 3.11). Fluorophenyl analogue **291** could not be formed under these conditions as the reaction produced a complex mixture of compounds. A possible explanation for this is decomposition of the furan under acidic conditions. Formation of methyl analogue **292** was observed but was unable to be isolated from a complex mixture, most likely due to the reduced stability of the methyl substituted enone compared to the aromatic analogues.



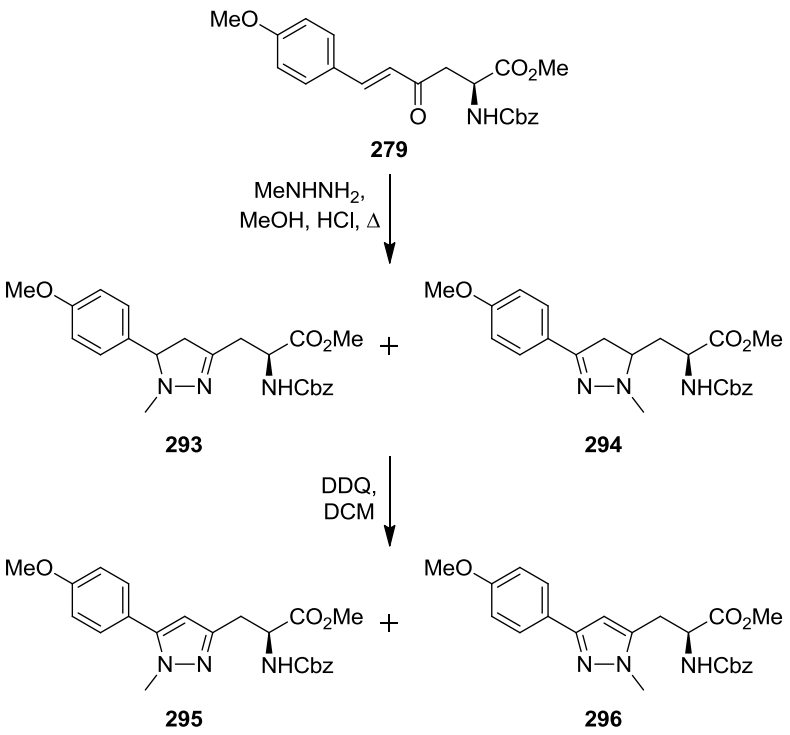
287: **76%** **288**: **75%** **289**: **73%**

290: **84%** **291**: **-** **292**: **-**

Figure 3.11 Structures of pyrazoles **287−292**

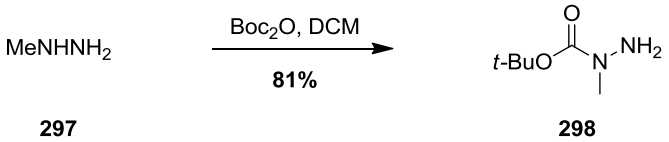
In an attempt to investigate alternate substitution around the pyrazole, the pyrazoline forming reaction was attempted using methylhydrazine rather than phenylhydrazine. Various analogues were attempted, however, when using methylhydrazine clean products

could not be isolated. When methoxy substituted compound **279** was used, a mixture of regioisomers **295** and **296** appeared to be formed. The ratio of the major to minor regioisomer was 3:1 but the compounds could not be isolated sufficiently cleanly to perform full analysis to assign which was the major regioisomer (Scheme 3.16). It was thought that the formation of regioisomers could be the reason that reactions with methylhydrazine were failing to produce clean products.



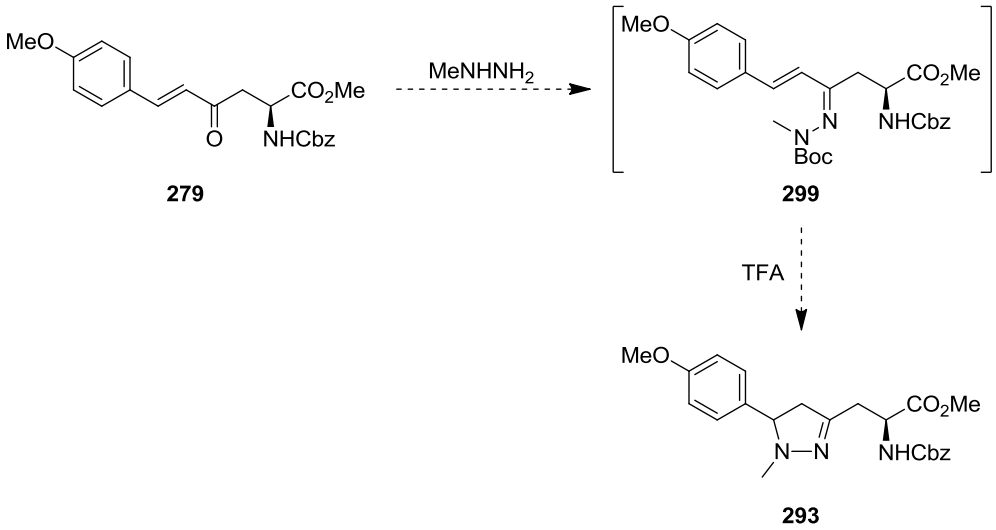
Scheme 3.16 Attempted synthesis of a methyl substituted pyrazole

Unlike phenylhydrazine, the nitrogen atoms in methylhydrazine are not sufficiently different in terms of their nucleophilicity to lead to one product. In an attempt to differentiate the reactivity of the nitrogen atoms, methylhydrazine **297** was Boc protected following a literature procedure to form compound **298** (Scheme 3.17).[180]



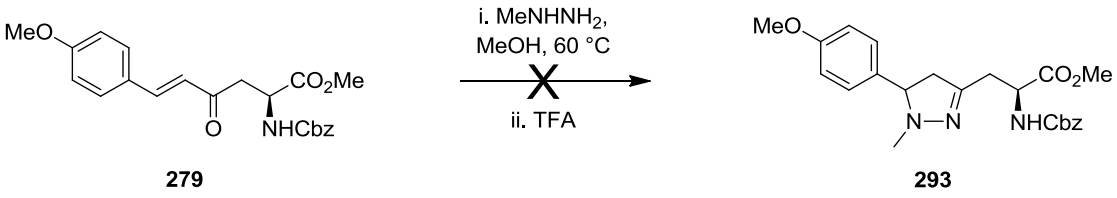
Scheme 3.17 Boc protection of methylhydrazine

It was hoped that the Boc protected methylhydrazine would undergo imine formation, followed by treatment with acid which would remove the Boc group and allow the Michael addition to occur (Scheme 3.18).



Scheme 3.18 Proposed synthesis of methyl substituted pyrazoline **293**

There was some precedent in the literature for the use of Boc protected methylhydrazine in the synthesis of methyl substituted pyrazoles.[181] Unfortunately, when this was attempted neither the intermediate hydrazone **299** or target pyrazoline **293** were observed (Scheme 3.19). As there was not sufficient time to investigate this approach any further work on the synthesis of methyl substituted compounds was terminated at this point.

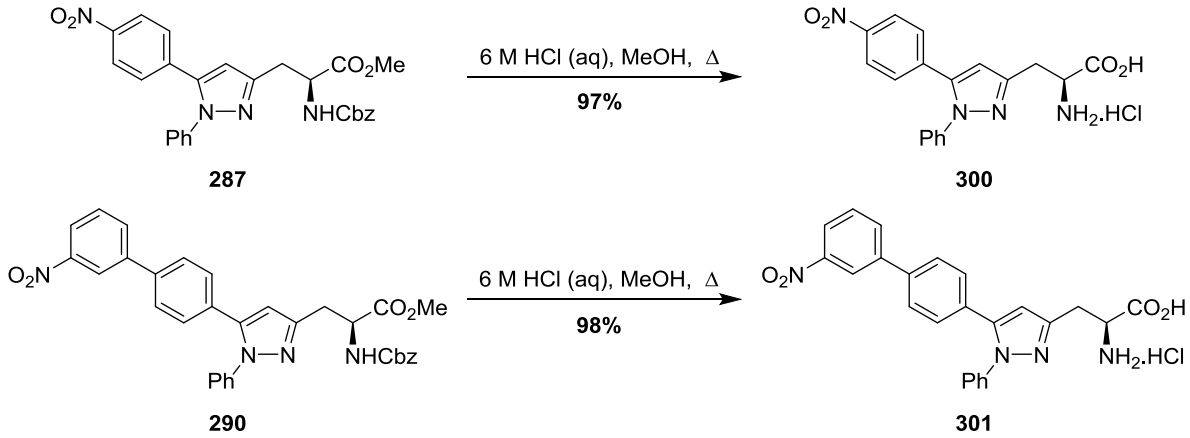


Scheme 3.19 Attempted synthesis of pyrolazine **293**

3.2.3 Deprotection to Parent α-Amino Acids

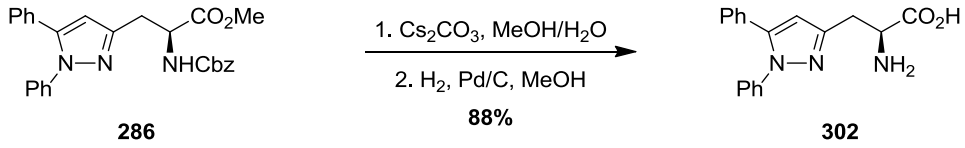
To complete the synthetic route, deprotection to the parent amino acids was required. Ester hydrolysis, followed by removal of the Cbz group by hydrogenation was considered

to be the ideal sequence for deprotection as this would potentially allow for the free carboxylic acid to be coupled to a peptide before amine deprotection. For the nitro containing compounds **287** and **290** removal of the Cbz group by hydrogenation was not suitable as this would result in reduction of the nitro group to the corresponding aniline. Treatment of these compounds with 6 M aqueous hydrochloric acid under reflux achieved both removal of the Cbz group and methyl ester hydrolysis to give parent amino acids **300** and **301** in excellent yields of 97% and 98%, respectively (Scheme 3.20).



Scheme 3.20 Deprotection to form amino acids **300** and **301**

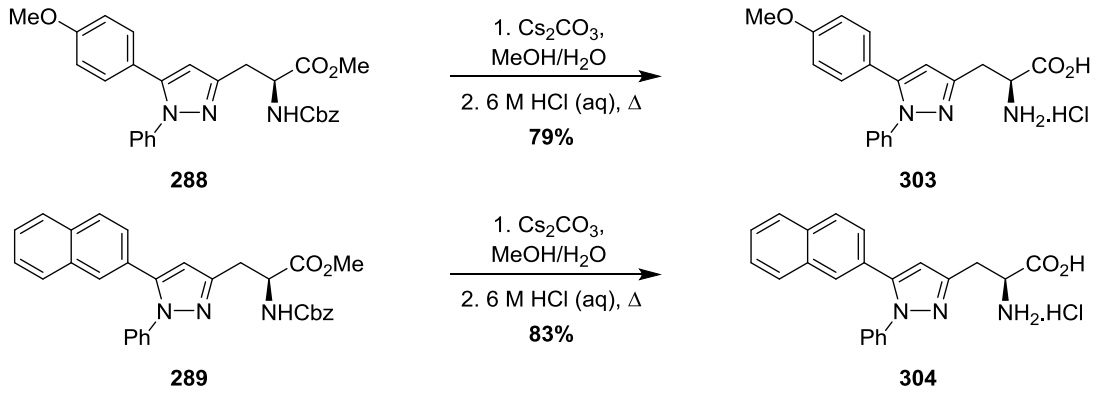
For the compounds that do not contain a nitro group, a two-step deprotection strategy was employed. Basic ester hydrolysis followed by palladium-catalysed hydrogenation was used to remove the protecting groups on phenyl substituted pyrazole **286** to give amino acid **302** in 88% yield (Scheme 3.21).



Scheme 3.21 Deprotection to form amino acid **302**

The same sequence of reactions was attempted on methoxyphenyl and naphthalene substituted compounds **288** and **289** but the products of these reactions could not be isolated sufficiently cleanly to perform full analysis of the products. Analysis of the ^{1}H NMR spectra obtained during these reactions showed the ester hydrolysis had proceeded

cleanly, but small impurities were present after hydrogenation to remove the Cbz group. To overcome this problem, acid mediated Cbz removal was instead used. Under these conditions, methoxy and naphthalene substituted pyrazoles **303** and **304** were formed in high yields of 79% and 83%, respectively (Scheme 3.22).



Scheme 3.22 Deprotection to form amino acids **303** and **304**

The specific rotation was measured for all compounds at each stage of this synthetic sequence, and all were found to be optically active. In the timeframe of this project it was not possible to determine whether the chiral centre present in the starting material had been fully retained throughout the synthetic sequence or if some degree of racemisation had occurred. Work on this project is continuing within the Sutherland group and this determination will be performed in the future using chiral HPLC.

3.3 Fluorescence of Amino Acids

Once the synthesis of the library of amino acids was completed, the physical properties of these compounds were investigated. The absorption maxima for each compound were determined and these values were then used as the excitation wavelength for obtaining emission spectra. Nitrophenyl substituted amino acid **300** and naphthalene substituted amino acid **304** were found to be fluorescent with emission at 415 nm and 356 nm, respectively (Figure 3.12).

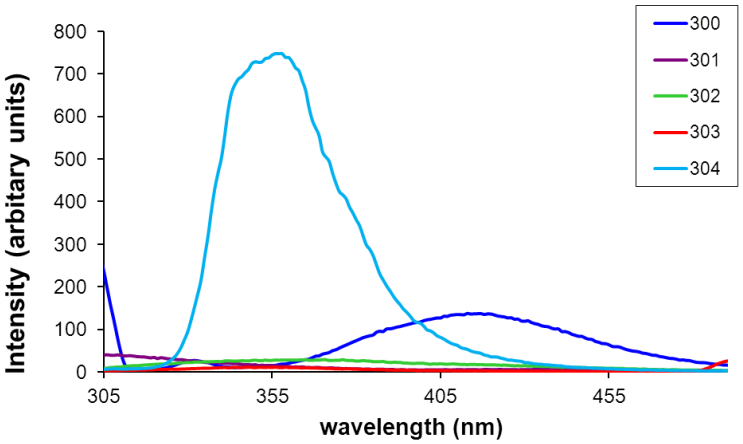
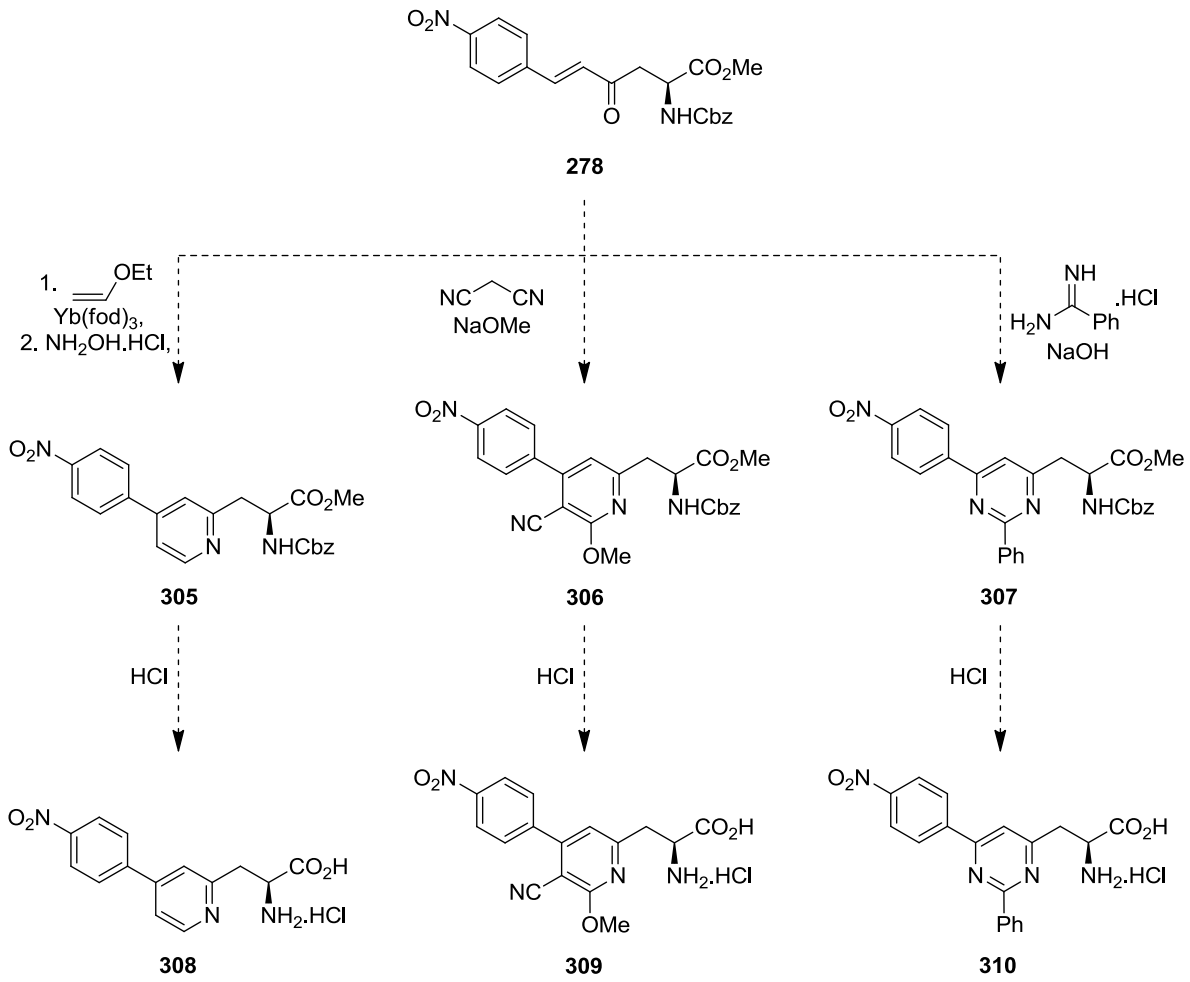


Figure 3.12 Emission spectra of amino acids (all performed in MeOH)

To be of use as a fluorescent label for peptides emission should occur at a higher wavelength than that of the fluorescent proteinogenic amino acids to ensure that their emission does not overlap. The emission of naphthalene substituted amino acid **304** occurs at a very similar wavelength to that of tryptophan and so does not meet this criteria. However, the emission of nitrophenyl substituted amino acid **300** occurs at over 400 nm and therefore should be easily distinguishable from fluorescent proteinogenic amino acids, and so has the potential to be used a fluorescent label.

3.4 Future Work

Future work on this project will focus on using the nitrophenyl substituted analogue to form different heterocycles from enone intermediate **278**. Using the reactions discussed in Section 3.1.3, α-amino acids such as pyridine **308**, 3-cyano-2-methoxypyridine **309** and pyrimidine **310** could potentially be synthesised.



Scheme 3.23 Structure of amino acids **308−310** that could potentially be synthesised

Another avenue for future work on this project could focus on the incorporation of a known fluorophore via the enone substituent. *N*,*N*-Dimethylaminonaphthyl substituted enone **254** (Figure 3.7), has previously been shown to be fluorescent with emission at 540 nM.[175] Heterocycle containing derivatives of this structure could also be produced to give compounds such as α-amino acids **311−314** (Figure 3.13). If such amino acids were found to be highly fluorescent the use of these compounds as fluorescent labels for peptides could then be investigated.

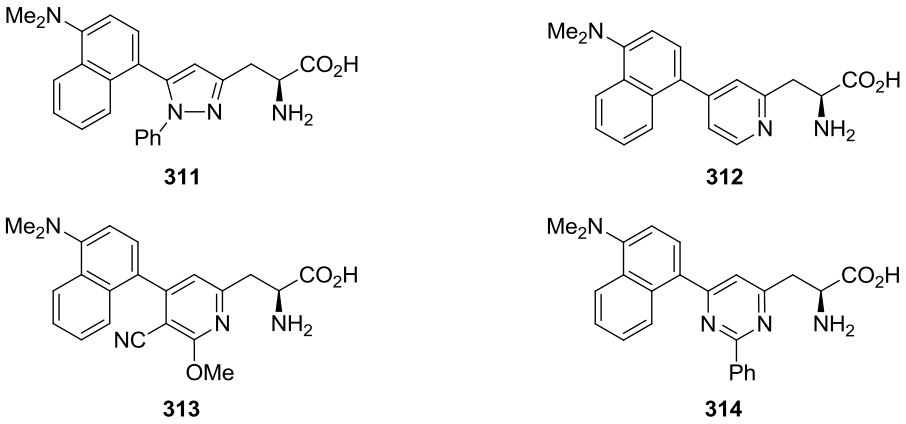


Figure 3.13 Structures of amino acids **311−314**

3.5 Conclusions

A library of five novel heterocycle containing α-amino acids was synthesised starting from inexpensive L-aspartic acid. Enone substituted α-amino acids were prepared and reaction with phenylhydrazine followed by oxidation formed phenylpyrazole substituted compounds in high yield. The parent α-amino acids were the accessed by removal of the protecting groups on the amine and carboxylic acid. Investigation of their physical properties revealed amino acids **300** and **304** to be fluorescent, with nitrophenyl substituted compound emitting at 415 nm and hence having potential use as in fluorescence imaging.

This project achieved its aim to synthesise novel heterocycle containing α-amino acids some of which are fluorescent. Further research will hopefully allow further compounds to be produced to provide a library of novel fluorescent α-amino acids. Such compounds could then be investigated as peptide labels for fluorescence imaging.

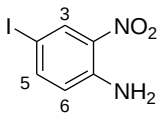
4 Experimental

4.1 General Experimental

Reagents and starting materials were obtained from commercial sources and used as received. Dry solvents were purified using a PureSolv 500 MD solvent purification system. Brine refers to a saturated aqueous solution of sodium chloride in deionised water. Flash column chromatography was carried out using Fisher matrix silica 60. Macherey-Nagel aluminium-backed plates pre-coated with silica gel 60 (UV_{254}) were used for thin layer chromatography and were visualised by staining with potassium permanganate. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 400 or 500 spectrometer. ^{1}H shift values are reported in ppm relative to tetramethylsilane (δ_H 0.00), residual chloroform (δ_H 7.26), dimethylsulfoxide (δ_H 2.50), or methanol (δ_H 3.31) as standard. ^{13}C chemical shift values are reported in ppm relative to tetramethylsilane (δ_C 0.00), $CDCl_3$ (δ_C 77.16), $(CD_3)_2SO$ (δ_C 39.52) or CD_3OD (δ_C 49.00) as standard. Proton and carbon assignments are based on two-dimensional COSY and DEPT experiments, respectively. Infrared spectra were obtained neat using a SHIMADZU spectrometer. Mass spectra were obtained using a JEOL JMS-700 or Bruker Microtof-q spectrometer. Melting points were determined on a Gallenkamp melting point apparatus. Optical rotations were determined as solutions irradiating with the sodium D line (λ = 589 nm) using an Autopol V polarimeter. $[\alpha]_D$ values are given in units 10^{-1} deg cm^2 g^{-1}.

4.2 Experimental Procedures and Spectroscopic Data for Compounds

4-Iodo-2-nitroaniline (150)[182]

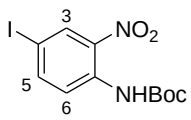


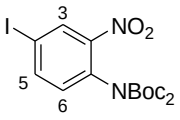
Iodine monochloride (1.0 M in dichloromethane) (19.0 mL, 19.0 mmol) was added to a solution of 2-nitroaniline (**151**) (2.50 g, 18.1 mmol) and sodium acetate (1.56 g, 19.0

mmol) in acetic acid (20 mL). The reaction mixture was stirred at 90 °C for 0.5 h before being allowed to cool to room temperature and poured on to ice water. A precipitate formed that was collected by filtration to give 4-iodo-2-nitroaniline (**150**) as an orange solid (4.20 g, 88%). Mp 117−119 °C (lit.,[182] 118−119 °C); δ_H (400 MHz, $CDCl_3$) 6.11 (1H, br s, NH_2), 6.61 (1H, d, *J* 8.8 Hz, 6-H), 7.57 (1H, dd, *J* 8.8, 1.9 Hz, 5-H), 8.44 (1H, d, *J* 1.9 Hz, 3-H); δ_C (101 MHz, $CDCl_3$) 75.9 (C), 120.6 (CH), 133.2 (C), 134.4 (CH), 143.8 (CH), 144.0 (C); *m/z* (CI) 265 (MH^+, 56%), 235 (24), 186 (10), 158 (24), 139 (32), 79 (98).

1-(*tert*-Butoxycarbonylamino)-4-iodo-2-nitrobenzene (149)[183] and

1-[Bis(*tert*-butoxycarbonyl)amino]-4-iodo-2-nitrobenzene (154)



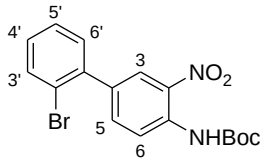


Method A: Lithium hexamethyldisilazide (1.0 M in tetrahydrofuran) (26.8 mL, 26.8 mmol) was added dropwise to a solution of 4-iodo-2-nitroaniline (**150**) (3.54 g, 13.4 mmol) in tetrahydrofuran (50 mL) at 0 °C. The solution was then allowed to warm to room temperature and a solution of di-*tert*-butyl dicarbonate (3.51 g, 16.1 mmol) in tetrahydrofuran (20 mL) was added dropwise. The reaction mixture was stirred at room temperature for 18 h then quenched with a saturated aqueous solution of ammonium chloride (150 mL), extracted with ethyl acetate (3 × 100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 10% ethyl acetate in petroleum ether (40−60) to give 1-[bis(*tert*-butoxycarbonyl)amino]-4-iodo-2-nitrobenzene (**154**) as a white solid (0.93 g, 15%), then 1-(*tert*-butoxycarbonylamino)-4-iodo-2-nitrobenzene (**149**) as a yellow solid (2.94 g, 60%). Data for 1-[bis(*tert*-butoxycarbonyl)amino]-4-iodo-2-nitrobenzene (**154**); Mp 121−123 °C; ν_{max}/cm^{-1} (neat) 2984 (CH), 1763 (CO), 1742 (CO), 1525, 1346, 1273, 1150, 1109, 1001, 827; δ_H (400 MHz, $CDCl_3$) 1.41 (18H, s, 6 × CH_3), 7.06 (1H, d, *J* 8.3 Hz, 6-H), 7.95 (1H, dd, *J* 8.3, 2.0 Hz, 5-H), 8.37 (1H, d, *J* 2.0 Hz, 3-H); δ_C (101 MHz, $CDCl_3$) 27.9 (6 × CH_3), 84.2 (C), 92.3 (C), 132.8 (CH), 133.2 (C), 133.8 (CH), 142.8 (CH), 146.0 (C), 150.0 (C); *m/z* (EI) 464.0443 (M^+. $C_{16}H_{21}IN_2O_6$ requires 464.0444), 364 (30%), 308 (100), 264 (74), 216 (37). Data for 1-(*tert*-butoxycarbonylamino)-4-iodo-2-nitrobenzene (**149**); Mp 96−98 °C (lit.,[183] 92−94 °C); ν_{max}/cm^{-1} (neat) 3364 (NH), 2970

(CH), 1728 (CO), 1566, 1497, 1335, 1242, 1142, 895; δ_H (400 MHz, $CDCl_3$) 1.54 (9H, s, 3 × CH_3), 7.85 (1H, dd, *J* 9.0, 1.9 Hz, 5-H), 8.37 (1H, d, *J* 9.0 Hz, 6-H), 8.50 (1H, d, *J* 1.9 Hz, 3-H), 9.61 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 82.5 (C), 83.1 (C), 122.5 (CH), 134.2 (CH), 135.9 (C), 136.4 (C), 144.3 (CH), 152.0 (C); *m/z* (EI) 363.9921 (M^+. $C_{11}H_{13}IN_2O_4$ requires 363.9920), 308 (16%), 264 (23), 216 (6), 84 (81), 57 (100).

Method B: To a solution of 1-[bis(*tert*-butoxycarbonyl)amino]-4-iodo-2-nitrobenzene (**154**) (1.17 g, 2.52 mmol) in dichloromethane (30.0 mL) at 0 ºC was added trifluoroacetic acid (0.39 mL, 5.0 mmol). The mixture then warmed to room temperature and was stirred for 3 h. The reaction mixture was diluted with a saturated aqueous solution of sodium hydrogen carbonate (20 mL), extracted with dichloromethane (3 × 20 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 1-(*tert*-butoxycarbonylamino)-4-iodo-2-nitrobenzene (**149**) as a yellow solid (0.900 g, 98%). Spectroscopic data as reported above.

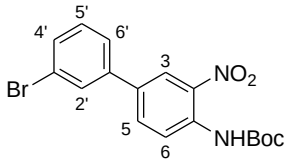
4-(2'-Bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (146)



To a solution of 1-(*tert*-butoxycarbonylamino)-2-nitro-4-iodobenzene (**149**) (1.50 g, 3.81 mmol) in *N*,*N*-dimethylformamide and water (100 mL, 9:1) was added 2-bromophenylboronic acid (0.820 g, 4.12 mmol), potassium carbonate (1.42 g, 10.3 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.092 g, 0.08 mmol). The reaction mixture was heated to 110 ºC and stirred for 1.5 h. After cooling to room temperature the solution was concentrated *in vacuo*, redissolved in chloroform (100 mL) and filtered through Celite® and concentrated *in vacuo*. The resulting solid was dissolved in diethyl ether (100 mL), washed with water (6 × 50 mL) and brine (2 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography eluting with 10% ethyl acetate in petroleum ether (40–60) to give 4-(2'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (**146**) as a yellow oil (1.01 g, 62%). ν_{max}/cm^{-1} (neat) 3370 (NH), 2980 (CH), 1734 (CO), 1517, 1470, 1338, 1238, 1139, 1022, 753; δ_H (500 MHz, $CDCl_3$) 1.53 (9H, s, 3 × CH_3), 7.25 (1H, ddd, *J* 8.0, 7.3, 1.8 Hz, 4'-H), 7.33 (1H, dd, *J* 7.7, 1.8 Hz, 6'-H), 7.39–7.41 (1H, m, 5'-H), 7.67–7.72 (2H, m, 5-H and 3'-H), 8.27 (1H, d, *J* 2.1 Hz, 3-H), 8.64 (1H, d, *J* 8.8 Hz, 6-H), 9.74 (1H, br s, NH); δ_C (126 MHz,

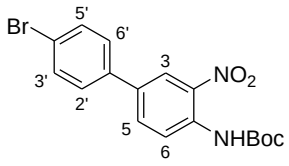
$CDCl_3$) 28.4 (3 × CH_3), 82.2 (C), 120.4 (CH), 122.7 (C), 126.6 (CH), 127.9 (CH), 129.7 (CH), 131.2 (CH), 133.5 (CH), 134.9 (C), 135.5 (C), 135.6 (C), 137.0 (CH), 139.8 (C), 152.4 (C); *m/z* (EI) 392.0385 ($M^{+\cdot}$. $C_{17}H_{17}{}^{79}BrN_2O_4$ requires 392.0372), 336 (49%), 292 (95), 167 (58), 139 (37).

4-(3'-Bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (147)



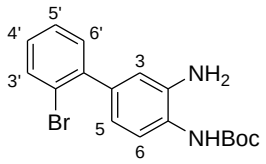
The reaction was carried out according to the above procedure using 1-(*tert*-butoxycarbonylamino)-4-iodo-2-nitrobenzene (**149**) (0.100 g, 0.27 mmol), 3-bromophenylboronic acid (0.082 g, 0.41 mmol), potassium carbonate (0.094 g, 0.68 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.006 g, 0.005 mmol) in *N*,*N*-dimethylformamide and water (8 mL, 9:1) to give 4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (**147**) as a yellow solid (0.076 g, 72%). Mp 107−109 °C; ν_{max}/cm^{-1} 3345 (NH), 2986, (CH), 1732 (CO), 1577 (C=C), 1522, 1340, 1248, 1152, 892; δ_H (400 MHz, $CDCl_3$) 1.56 (9H, s, 3 × CH_3), 7.34 (1H, t, *J* 7.9 Hz, 5'-H), 7.50−7.54 (2H, m, 4'-H and 6'-H), 7.73 (1H, t, *J* 1.8 Hz, 2'-H), 7.81 (1H, dd, *J* 8.9, 2.3 Hz, 5-H), 8.39 (1H, d, *J* 2.3 Hz, 3-H), 8.66 (1H, d, *J* 8.9 Hz, 6-H), 9.69 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 82.3 (C), 121.4 (CH), 123.4 (C), 124.0 (CH), 125.5 (CH), 129.9 (CH), 130.8 (CH), 131.2 (CH), 133.6 (C), 134.2 (CH), 135.6 (C), 136.2 (C), 140.4 (C), 152.3 (C); *m/z* (EI) 392.0363 (M^+. $C_{17}H_{17}{}^{79}BrN_2O_4$ requires 392.0372), 336 (32%), 292 (78), 246 (19), 167 (43), 57 (99).

4-(4'-Bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (148)



The reaction was carried out according to the above procedure using 1-(*tert*-butoxycarbonylamino)-2-nitro-4-iodobenzene (**149**) (3.00 g, 8.24 mmol), 4-bromophenylboronic acid (1.74 g, 8.65 mmol), potassium carbonate (2.85 g, 20.6 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.18 g, 0.16 mmol) in *N*,*N*-dimethylformamide and water (125 mL, 9:1) to give 4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (**148**) as a yellow solid (2.51 g, 78%). Mp 134−136 °C; ν_{max}/cm^{-1} (neat) 3366 (NH), 2970 (CH), 1730 (CO), 1489, 1341, 1244, 1143, 812; δ_H (400 MHz, $CDCl_3$) 1.56 (9H, s, 3 × CH_3), 7.46 (2H, d, *J* 8.4 Hz, 2'-H and 6'-H), 7.60 (2H, d, *J* 8.4 Hz, 3'-H and 5'-H), 7.81 (1H, dd, *J* 8.9, 2.1 Hz, 5-H), 8.39 (1H, d, *J* 2.1 Hz, 3-H), 8.65 (1H, d, *J* 8.9 Hz, 6-H), 9.69 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.2 (3 × CH_3), 82.1 (C), 121.3 (CH), 122.4 (C), 123.6 (CH), 128.2 (2 × CH), 132.2 (2 × CH), 133.8 (C), 133.9 (CH), 135.2 (C), 136.1 (C), 137.0 (C), 152.1 (C); *m/z* (EI) 392.0367 (M^+. $C_{17}H_{17}{}^{79}BrN_2O_4$ requires 392.0372), 336 (40%), 292 (59), 246 (22), 167 (21), 139 (24), 57 (100).

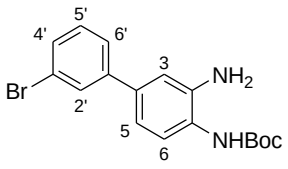
2-Amino-4-(2'-bromophefnyl)-1-(*tert*-butoxycarbonylamino)benzene (142)



To a solution of 4-(2'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (**146**) (0.680 g, 1.73 mmol) in ethyl acetate and pyridine (70 mL, 6:1) was added tin(II) chloride dihydrate (1.95 g, 8.65 mmol), and the mixture stirred at room temperature for 6 h. The reaction mixture was then filtered through Celite® and concentrated *in vacuo*. The resulting solid was redissolved in ethyl acetate (100 mL), washed with water (4 × 50 mL) and brine (2 × 20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material

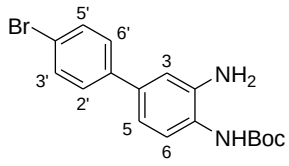
was purified by flash column chromatography eluting with 0–50% ethyl acetate in petroleum ether (40−60) to give 2-amino-4-(2'-bromophenyl)-1-(*tert*-butoxycarbonyl amino)benzene (**142**) as a white solid (0.380 g, 61%). Mp 152–154 °C; ν_{max}/cm^{-1} (neat) 3375 (NH), 2970 (CH), 1705 (CO), 1532, 1360, 1243, 1164, 1048, 886; δ_H (500 MHz, $CDCl_3$) 1.53 (9H, s, 3 × CH_3), 3.79 (2H, br s, NH_2), 6.27 (1H, br s, NH), 6.82 (1H, d, *J* 1.8 Hz, 3-H), 6.84 (1H, dd, *J* 8.0, 1.8 Hz, 5-H), 7.17 (1H, ddd, *J* 8.0, 7.1, 2.0 Hz, 4'-H), 7.29 (1H, dd, *J* 7.6, 2.0 Hz, 6'-H), 7.30–7.34 (1H, m, 5'-H), 7.36 (1H, d, *J* 8.0 Hz, 6-H), 7.64 (1H, dd, *J* 8.0, 1.0 Hz, 3'-H); δ_C (126 MHz, $CDCl_3$) 28.5 (3 × CH_3), 80.9 (C), 118.9 (CH), 121.1 (CH), 122.7 (C), 124.1 (CH), 124.5 (C), 127.4 (CH), 128.7 (CH), 131.4 (CH), 133.2 (CH), 139.0 (C), 139.3 (C), 142.4 (C), 152.9 (C); *m/z* (CI) 363.0708 (MH^+. $C_{17}H_{20}{}^{79}BrN_2O_2$ requires 363.0708), 307 (53%), 285 (32), 263 (10), 229 (35), 172 (100).

2-Amino-4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (143)



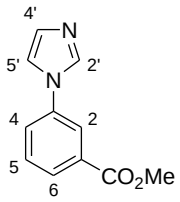
The reaction was carried out according to the above procedure using 4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (**147**) (0.266 g, 0.676 mmol) and tin(II) chloride dihydrate (0.767 g, 3.40 mmol) in ethyl acetate and pyridine (30 mL, 6:1) to give 2-amino-4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (**143**) as a white solid (0.177 g, 72%). Mp 146–149 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2925 (CH), 1685 (CO), 1503, 1247, 1158, 1058, 862; δ_H (400 MHz, $CDCl_3$) 1.53 (9H, s, 3 × CH_3), 3.87 (2H br s, NH_2), 6.25 (1H, br s, NH), 6.96 (1H, d, *J* 1.8 Hz, 3-H), 6.99 (1H, dd, *J* 8.1, 1.8 Hz, 5-H), 7.27 (1H, t, *J* 7.9 Hz, 5'-H), 7.37 (1H, d, *J* 8.1 Hz, 6-H), 7.42–7.47 (2H, m, 4'-H and 6'-H), 7.67 (1H, t, *J* 1.7 Hz, 2'-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 80.9 (C), 116.3 (CH), 118.7 (CH), 122.9 (C), 124.9 (CH), 124.9 (C), 125.7 (CH), 130.1 (2 × CH), 130.3 (CH), 137.6 (C), 140.1 (C), 143.1 (C), 153.9 (C); *m/z* (EI) 362.0630 (M^+. $C_{17}H_{19}{}^{79}BrN_2O_2$ requires 362.0630), 306 (14%), 289 (20), 262 (99), 234 (13), 181 (15), 154 (19), 139 (16), 127 (11), 91 (10), 57 (99).

2-Amino-4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (144)



The reaction was carried out according to the above procedure using 4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-nitrobenzene (**148**) (1.82 g, 4.63 mmol) and tin(II) chloride dihydrate (5.22 g, 23.1 mmol) in ethyl acetate and pyridine (175 mL, 6:1) to give 2-amino-4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (**144**) as a white solid (1.06 g, 63%). Mp 194–196 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2978 (CH), 1686 (CO), 1502, 1248, 1163, 1057, 805; δ_H (400 MHz, $CDCl_3$) 1.53 (9H, s, 3 × CH_3), 3.84 (2H, br s, NH_2), 6.25 (1H, br s, NH), 6.95 (1H, d, *J* 2.0 Hz, 3-H), 6.98 (1H, dd, *J* 8.1, 2.0 Hz, 5-H), 7.36 (1H, d, *J* 8.1 Hz, 6-H), 7.39 (2H, d, *J* 8.5 Hz, 2'-H and 6'-H), 7.52 (2H, d, *J* 8.5 Hz, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 80.9 (C), 116.2 (CH), 118.5 (CH), 121.4 (C), 124.7 (C), 125.0 (CH), 128.7 (2 × CH), 131.9 (2 × CH), 138.0 (C), 139.9 (C), 140.1 (C), 153.9 (C); *m/z* (EI) 362.0626 (M^+. $C_{17}H_{19}{}^{79}BrN_2O_2$ requires 362.0630), 306 (15%), 262 (95), 234 (16), 181 (12), 154 (19), 83 (58).

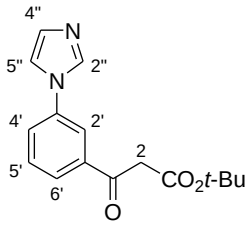
Methyl 3-(1'*H*-imidazol-1'-yl)benzoate (152)[95]



Methyl 3-aminobenzoate (**153**) (10.0 g, 66.2 mmol) was dissolved in methanol (45 mL) and 40% aqueous glyoxal solution (7.61 mL, 66.2 mmol) was added. The mixture was stirred at room temperature for 18 h before the addition of ammonium chloride (7.08 g, 132 mmol), 37% aqueous formaldehyde (10.7 mL, 132 mmol), and methanol (240 mL). The reaction mixture was heated under reflux and stirred for 1 h before adding 85% aqueous phosphoric acid (9.26 mL). The mixture was heated under reflux for a further 5 h before concentrating *in vacuo*. The resulting residue was dissolved in water (300 mL),

basified to pH 9 using 30% aqueous potassium hydroxide, and extracted with diethyl ether (4 × 300 mL). The organic extracts were combined, dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 50−100% ethyl acetate in petroleum ether (40−60) to give methyl 3-(1'*H*-imidazol-1'-yl)benzoate (**152**) as a brown oil (7.48 g, 58%). Spectroscopic data in accordance with literature. δ_H (400 MHz, $CDCl_3$) 3.96 (3H, s, CH_3), 7.23 (1H, br s, 4'-H), 7.35 (1H, t, *J* 1.3 Hz, 5'-H), 7.54−7.62 (2H, m, 4-H and 5-H), 7.93 (1H, br s, 2'-H), 8.03 (1H, dt, *J* 7.3, 1.6 Hz, 6-H), 8.05−8.07 (1H, m, 2-H); δ_C (101 MHz, $CDCl_3$) 52.6 (CH_3), 118.2 (CH), 122.3 (CH), 125.5 (CH), 128.5 (CH), 130.1 (CH), 130.5 (CH), 132.1 (C), 135.5 (CH), 137.5 (C), 165.9 (C); *m/z* (EI) 202 (M^+, 100%), 171 (93), 143 (47), 116 (50), 84 (31).

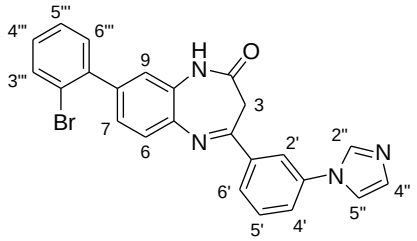
tert-Butyl 3-[3'-(1"*H*-imidazol-1"-yl)phenyl]-3-oxopropanoate (145)



A solution of lithium hexamethyldisilazide (1.0 M in tetrahydrofuran) (41.5 mL, 41.5 mmol) in dry tetrahydrofuran (100 mL) was cooled to –78 ºC under argon and *tert*-butanol (5.57 mL, 41.5 mmol) was added. After stirring for 1 h, methyl 3-(1'*H*-imidazol-1'-yl)benzoate (**152**) (3.50 g, 17.3 mmol) was added and stirring was continued at –78 °C for a further 2 h. The reaction mixture was allowed to warm to room temperature before the addition of a saturated aqueous solution of ammonium chloride (200 mL). The mixture was extracted with ethyl acetate (4 × 100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting oil was purified by flash column chromatography eluting with 0−5% methanol in dichloromethane. Further purification was carried out by flash column chromatography eluting with 100% ethyl acetate to give *tert*-butyl 3-[3'-(1"*H*-imidazol-1"-yl)phenyl]-3-oxopropanoate (**145**) as a brown oil (3.94 g, 80%). ν_{max}/cm^{-1} (neat) 3117, 2978 (CH), 1728 (CO), 1690 (CO), 1589 (C=C), 1505, 1312, 1250, 1142, 1057, 795; NMR spectra are a mixture of keto-enol tautomers (1:0.13) Signals are given for the major tautomer. δ_H (400 MHz, DMSO-d_6) 1.39 (9H, s, 3 × CH_3), 4.18 (2H, s, 2-H_2), 7.15 (1H, br s, 4"-H), 7.69 (1H, t, *J* 7.9 Hz, 5'-H), 7.84−8.01 (3H, m, 4'-H, 6'H and 5"-H), 8.15 (1H, br s, 2'-H), 8.38 (1H, br s, 2"-H); δ_C (101 MHz, DMSO-d_6) 27.6 (3 × CH_3), 47.0 (CH_2), 80.9

(C), 118.1 (CH), 120.0 (CH), 125.1 (CH), 126.4 (CH), 130.1 (CH), 130.4 (CH), 135.7 (CH), 137.2 (C), 137.4 (C), 166.7 (C), 193.2 (C); *m/z* (FAB) 287.1395 (MH^+. $C_{16}H_{19}N_2O_3$ requires 287.1396), 231 (17%), 213 (22), 187 (30), 147 (12), 73 (30).

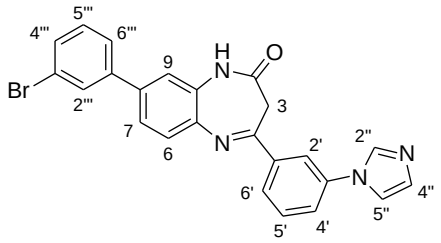
8-(2”’-Bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (136)



To a solution of 2-amino-4-(2’-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (**142**) (0.283 g, 0.779 mmol) in toluene (2 mL) was added *tert*-butyl 3-[3’-(1”*H*-imidazol-1”-yl)phenyl]-3-oxopropanoate (**145**) (0.223 g, 0.779 mmol). The solution was heated under reflux for 6 h, cooled to room temperature and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 0–2.5% ethanol in dichloromethane to give 4-(2””-bromophenyl)-1-(*tert*-butylcarbonylamino)-2-{3’-[3”(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}benzene (**139**) as a white solid (0.269 g, 60%). To a solution of 4-(2””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’-*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}benzene (**139**) (0.214, 0.372 mmol) in dichloromethane (4 mL) at 0 °C was added trifluoroacetic acid (0.5 mL). The reaction mixture was warmed to room temperature and stirred for 1 h. A saturated aqueous solution of sodium hydrogen carbonate (10 mL) was added, the mixture extracted with dichloromethane (3 × 5 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was then triturated with toluene to give 8-(2”’-bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**136**) as a white solid (0.151 g, 90%). Mp 179–181 °C (decomposition); ν_{max}/cm^{-1} (neat) 3086 (NH), 2924 (CH), 1682 (CO), 1505, 1462, 1308, 1254, 1049, 891; δ_H (500 MHz, DMSO-d_6) 3.69 (2H, s, 3-H_2), 7.16 (1H, br s, 4”-H), 7.23 (1H, d, *J* 1.9 Hz, 9-H), 7.30 (1H, dd, *J* 8.3, 1.9 Hz, 7-H), 7.33–7.38 (1H, m, 4”’-H), 7.44 (1H, dd, *J* 7.6, 1.8 Hz, 6”’-H), 7.48–7.54 (1H, m, 6-H and 5”’-H), 7.70 (1H, t, *J* 7.9 Hz, 5’-H), 7.77 (1H, dd, *J* 8.0, 1.0 Hz, 3”’-H), 7.83–7.89 (2H, m, 4’-H and 5”-H), 8.07 (1H, br d *J* 7.9 Hz, 6’-H), 8.25 (1H, t, *J* 1.8 Hz, 2’-H), 8.37 (1H, br s,

2”-H), 10.74 (1H, br s, NH); δ_H (126 MHz, DMSO-d_6) 39.9 (CH_2), 118.3 (CH), 119.6 (CH), 121.7 (C), 122.4 (CH), 123.2 (CH), 125.2 (CH), 126.2 (CH), 127.8 (CH), 128.2 (CH), 129.7 (CH), 129.9 (C), 130.1 (CH), 130.5 (CH), 131.4 (CH), 133.2 (CH), 135.9 (CH), 137.4 (C), 138.5 (2 × C), 138.8 (C), 140.9 (C), 157.8 (C), 166.1 (C); *m/z* (FAB) 457.0660 (MH^+. $C_{24}H_{17}{}^{79}BrN_4O$ requires 457.0664), 238 (62%), 220 (15), 169 (100), 85 (100).

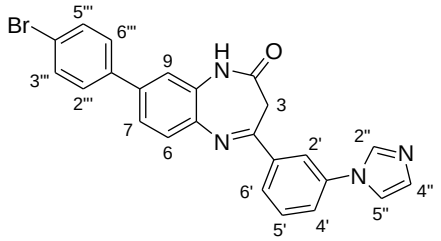
8-(3”’-Bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (137)



The reaction was carried out according to the above procedure, using 2-amino-4-(3’-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (**143**) (0.347 g, 0.955 mmol) and *tert*-butyl 3-[3’-(1”*H*-imidazol-1”-yl)phenyl]-3-oxopropanoate (**145**) (0.275 g, 0.960 mmol) in toluene (10 mL) to give 4-(3””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}benzene (**140**) as a white foam (0.344 g, 63%). Cyclisation was performed as described above, using 4-(3””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}benzene (**140**) (0.080 g, 0.139 mmol) and trifluoroacetic acid (5 mL) in dichloromethane (0.5 mL) to give 8-(3”’-bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**137**) as a white solid (0.056 g, 88%). Mp 204–205 °C (decomposition); ν_{max}/cm^{-1} (neat) 3055 (NH), 2854 (CH), 1683 (CO), 1503, 1256, 1112, 1061, 913, 863, 776; δ_H (500 MHz, DMSO-d_6) 3.68 (2H, s, 3-H_2), 7.16 (1H, br s, 4”-H), 7.47 (1H, t, *J* 7.9 Hz, 5”’-H), 7.53 (1H, d, *J* 1.9 Hz, 9-H), 7.55 (1H, d, *J* 8.4 Hz, 6-H), 7.58–7.62 (2H, m, 7-H and 6”’-H), 7.67–7.72 (2H, m, 5’-H and 4”’-H), 7.79–7.88 (3H, m, 4’-H, 5”-H and 2”’-H), 8.06 (1H, br d, *J* 7.9 Hz, 6’-H), 8.24 (1H, br s, 2’-H), 8.32 (1H, br s, 2”-H), 10.53 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 39.9 (CH_2), 118.3 (CH), 119.6 (CH), 120.1 (CH), 122.5 (C), 122.8 (CH), 123.2 (CH), 125.7 (CH), 126.2 (CH), 128.8 (CH), 129.2 (CH), 130.1 (CH), 130.4 (CH), 130.6 (CH), 130.7 (C),

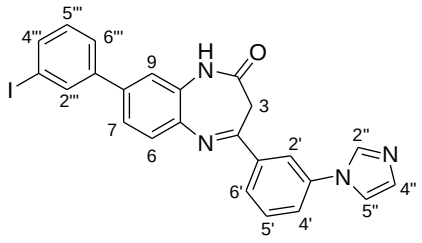
131.3 (CH), 135.9 (CH), 136.5 (C), 137.4 (C), 138.8 (C), 139.0 (C), 141.5 (C), 157.9 (C), 166.1 (C); *m/z* (ESI) 457.0644 (MH^+.$C_{24}H_{18}{}^{79}BrN_4O$ requires 457.0659).

8-(4”’-Bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (138)



The reaction was carried out according to the procedure described above, using 2-amino-4-(4’-bromophenyl)-1-(*tert*-butoxycarbonylamino)benzene (**144**) (0.178 g, 0.490 mmol) and *tert*-butyl 3-[3’-(1”*H*-imidazol-1”-yl)phenyl]-3-oxopropanoate (**145**) (0.109 g, 0.381 mmol) in toluene (10 mL) to give 4-(4””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}benzene (**141**) as a white foam (0.160 g, 73%). Cyclisation was performed as described above, using 4-(4””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}benzene (**141**) (0.230 g, 0.400 mmol) and trifluoroacetic acid (1 mL) in dichloromethane (8 mL) to give 8-(4”’-bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**138**) as a white solid (0.166 g, 91%). Mp 200–202 °C (decomposition); ν_{max}/cm^{-1} (neat) 3079 (NH), 2923 (CH), 1682 (CO), 1579, 1504, 1474, 1315, 1216, 1059, 812; δ_H (400 MHz, DMSO-d_6) 3.68 (2H, s, 3-H_2), 7.16 (1H, br s, 4”-H), 7.49 (1H, d, *J* 1.9 Hz, 9-H), 7.54 (1H, d, *J* 8.4 Hz, 6-H), 7.59 (1H, dd, *J* 1.9, 8.4 Hz, 7-H), 7.62–7.73 (5H, m, 5’-H, 2”’-H, 3”’-H, 5”’-H and 6”’-H), 7.83–7.89 (2H, m, 4’-H and 5”-H), 8.06 (1H, br d, *J* 7.9 Hz, 6’-H), 8.25 (1H, br s, 2’-H), 8.37 (1H, br s, 2”-H), 10.69 (1H, br s, NH); δ_C (101 MHz, DMSO) 39.9 (CH_2), 118.3 (CH), 119.5 (CH), 119.7 (CH), 121.2 (C), 122.4 (CH), 123.1 (CH), 126.1 (CH), 128.6 (2 × CH), 128.7 (CH), 130.0 (CH), 130.3 (CH), 130.6 (C), 132.0 (2 × CH), 135.8 (CH), 136.8 (C), 137.4 (C), 138.2 (C), 138.8 (2 × C), 157.7 (C), 166.0 (C); *m/z* (FAB) 457.0668 (MH^+. $C_{24}H_{18}{}^{79}BrN_4O$ requires 457.0664), 441 (34%), 219 (60), 203 (22), 169 (81), 147 (34), 84 (100).

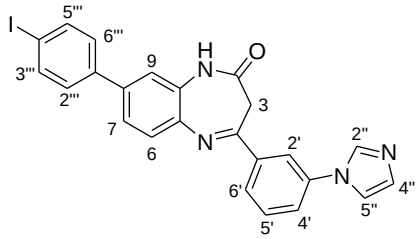
4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-(3"'-iodophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (134)



A solution of 8-(3"'-bromophenyl)-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-(3"'-bromophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**137**) (0.030 g, 0.066 mmol) in 1,4-dioxane (1 mL) was degassed for 0.25 h before the addition of hexamethylditin (54 μL, 0.26 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.008 g, 0.007 mmol). The reaction mixture was heated to 90 ºC and stirred under Ar for 48 h. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography on silica eluting with 0–4% ethanol in dichloromethane to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-[3"'-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**164**) (0.290, 81%). To a solution of 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-[3"'-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**164**) (0.029 g, 0.054 mmol) in ethanol (50 mL) was added sodium iodide (0.011 g, 0.073 mmol) in 0.01 M aqueous sodium hydroxide (11 mL). The solution was then acidified to pH 4–5 using 0.05 M aqueous hydrochloric acid. A solution of chloramine-T (0.032 g, 0.14 mmol) in water (30 mL) was added and the mixture stirred at room temperature for 0.5 h. The reaction was then quenched by the addition of sodium metabisulfite (0.400 g) in water (20 mL). The solution was then diluted with a saturated aqueous solution of sodium hydrogen carbonate (40 mL), extracted with dichloromethane (4 × 80 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting solid was then triturated with diethyl ether to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-(3"'-iodophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**134**) as a white solid (0.023 g, 85%). Mp 208–209 °C (decomposition); ν_{max}/cm^{-1} (neat) 3190 (NH), 3090 (CH), 1676 (CO), 1551, 1505, 1312, 1269, 1055, 874, 777; δ_H (400 MHz, DMSO-d_6) 3.68 (2H, s, 3-H_2), 7.16 (1H, br s, 4"-H), 7.31 (1H, t, *J* 7.7 Hz, 5"'-H), 7.49 (1H, br s, 9-H), 7.53 (1H, d, *J* 8.3 Hz, 6-H), 7.59 (1H, br d, *J* 8.3 Hz, 7-H), 7.65–7.80 (3H, m, 5'-H, 4"'-H and 6"'-H), 7.82–7.90 (2H, m, 4'-H and 5"-H), 8.00–8.10 (2H, m, 6'-H and 2"'-H), 8.25 (1H, br s, 2'-H), 8.38 (1H, br s, 2"-H), 10.66 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 39.9 (CH_2), 95.7 (C), 118.3 (CH), 119.5 (CH), 120.0 (CH), 122.7 (CH), 123.2 (CH), 126.0 (CH), 126.1

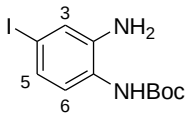
(CH), 128.7 (CH), 130.1 (CH), 130.4 (CH), 130.6 (C), 131.2 (CH), 135.0 (CH), 135.8 (CH), 136.4 (CH), 136.5 (C), 137.4 (C), 138.8 (C), 138.9 (C), 141.4 (C), 157.8 (C), 166.0 (C); *m/z* (ESI) 505.0508 (MH^+. $C_{24}H_{18}IN_4O$ requires 505.0520).

4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-(4"'-iodophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (135)



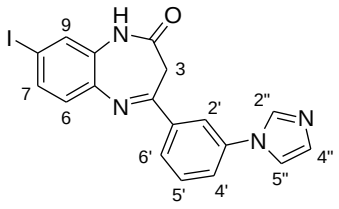
The reaction was carried out as described above, using 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-(4"'-bromophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**138**) (0.200 g, 0.437 mmol), hexamethylditin (0.36 mL, 1.8 mmol) and tetrakis(triphenylphosphine) palladium(0) (0.051 g, 0.044 mmol) in 1,4-dioxane (4.8 mL) to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-[4"'-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**165**) (0.136 g, 57%). Iododestannylation was performed as described above, using 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-[4"'-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**165**) (0.035 g, 0.065 mmol), sodium iodide (0.010 g, 0.065 mmol) and chloramine-T (0.030 g, 0.13 mmol) in ethanol (25 mL) to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-(4"'-iodophenyl)-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**135**) as a white solid (0.024 g, 73%). Mp 217–218 °C (decomposition); ν_{max}/cm^{-1} (neat) 2919 (CH), 1672 (CO), 1500, 1314, 1242, 1102, 1057, 802; δ_H (500 MHz, DMSO-d_6) 3.67 (2H, s, 3-H_2), 7.17 (1H, br s, 4"-H), 7.44–7.60 (5H, m, 6-H, 7-H, 9-H, 2"'H and 6"'-H), 7.69 (1H, t, *J* 7.6 Hz, 5'-H), 7.77–7.91 (4H, m, 4'-H, 5"-H, 3"'-H and 5"'-H), 8.06 (1H, br d, *J* 7.6 Hz, 6'-H), 8.25 (1H, br s, 2'-H), 8.33 (1H, br s, 2"-H), 10.58 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 39.9 (CH_2), 94.2 (C), 118.3 (CH), 119.5 (CH), 119.6 (CH), 122.4 (CH), 123.2 (CH), 126.1 (CH), 128.7 (3 × CH), 130.1 (CH), 130.4 (CH), 130.7 (C), 135.8 (CH), 137.0 (C), 137.4 (C), 137.9 (2 × CH), 138.5 (C), 138.8 (2 × C), 157.7 (C), 166.0 (C); *m/z* (ESI) 505.0507 (MH^+. $C_{24}H_{18}IN_4O$ requires 505.0520).

2-Amino-1-(*tert*-butoxycarbonylamino)-4-iodobenzene (167)[183]



To a solution of 1-(*tert*-butoxycarbonylamino)-4-iodo-2-nitrobenzene (**149**) (0.200 g, 0.550 mmol) in ethanol (17 mL) was added tin(II) chloride dihydrate (0.620 g, 2.75 mmol). The reaction mixture was heated to 70 °C and stirred for 3 h. After cooling to room temperature the mixture was concentrated *in vacuo*. The resulting solid was dissolved in ethyl acetate (20 mL) and a saturated aqueous solution of sodium hydrogen carbonate (10 mL) was added. The mixture was then extracted with ethyl acetate (3 × 20 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 2-amino-1-(*tert*-butoxycarbonylamino)-4-iodobenzene (**167**) as an off-white solid (0.170 g, 91%). Mp 140−141 °C (lit.,[183] 127−130 °C); ν_{max}/cm^{-1} (neat) 3349 (NH), 2922 (CH), 1678 (CO), 1587 (C=C), 1506, 1489, 1410, 1248, 1157, 1051, 853; δ_H (400 MHz, $CDCl_3$) 1.50 (9H, s, 3 × CH_3), 3.74 (2H, br s, NH_2), 6.18 (1H, br s, NH), 7.01 (1H, d, *J* 8.2 Hz, 6-H), 7.05–7.11 (2H, m, 3-H and 5-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 80.9 (C), 89.8 (C), 124.7 (C), 126.0 (CH), 126.1 (CH), 128.5 (CH), 141.3 (C), 153.6 (C); *m/z* (CI) 335.0265 (MH^+. $C_{11}H_{16}IN_2O_2$ requires 335.0257), 311 (10%), 279 (100), 209 (22), 153 (43), 113 (17), 69 (64).

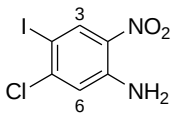
4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-iodo-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (169)



To a solution of 2-amino-1-(*tert*-butoxycarbonylamino)-4-iodobenzene (**167**) (0.145 g, 0.434 mmol) in toluene (1 mL) was added *tert*-butyl 3-[3'-(1"*H*-imidazol-1"-yl)phenyl]-3-oxopropanoate (**145**) (0.149 g, 0.520 mmol). The solution was heated under reflux for 4 h before cooling to room temperature and concentrating *in vacuo*. The resulting material was purified by flash column chromatography eluting with 0–4% ethanol in

dichloromethane to give 1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-4-iodobenzene (**168**) as a white foam (0.158 g, 67%). To a solution of 1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-4-iodobenzene (**168**) (0.124 g, 0.227 mmol) in dichloromethane (1 mL) at 0 °C was added trifluoroacetic acid (0.3 mL). The mixture was warmed to room temperature and stirred for 0.5 h. A saturated aqueous solution of sodium hydrogen carbonate (10 mL) was added, the solution extracted with dichloromethane (2 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was then triturated with toluene to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-iodo-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**169**) as a white solid (0.056 g, 58%). Mp 238−240 °C (decomposition); ν_{max}/cm^{-1} (neat) 3074 (NH), 2082 (CH), 1679 (CO), 1587, 1502, 1311, 1229, 1054, 909; δ_H (400 MHz, DMSO-d_6) 3.65 (2H, s, 3-CH_2), 7.16 (1H, br s, 4"-H), 7.23 (1H, d, *J* 8.8 Hz, 6-H), 7.53–7.59 (2H, m, 7-H and 9-H), 7.68 (1H, t, *J* 7.9 Hz, 5'-H), 7.82–7.89 (2H, m, 4'-H and 5-"H), 8.03 (1H, br d, *J* 7.9 Hz, 6'-H), 8.22 (1H, br s, 2'-H), 8.37 (1H, br s, 2"-H), 10.64 (1H, br s NH); δ_C (101 MHz, DMSO-d_6) 39.9 (CH_2), 91.0 (C), 118.3 (CH), 119.5 (CH), 123.2 (CH), 126.1 (CH), 129.8 (CH), 130.0 (2 × CH), 130.4 (CH), 131.6 (C), 132.6 (CH), 135.8 (CH), 137.4 (C), 138.7 (C), 138.8 (C), 158.2 (C), 166.0 (C); *m/z* (EI) 428.0128 (M^+. $C_{18}H_{13}IN_4O$ requires 428.0134), 386 (84%), 359 (13), 260 (18), 193 (10), 170 (10), 78 (11).

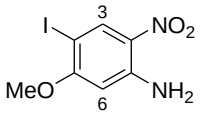
5-Chloro-4-iodo-2-nitroaniline (182)[184]



Iodine monochloride (1.0 M in dichloromethane) (45.7 mL, 45.7 mmol) was added to a solution of 5-chloro-2-nitroaniline (**183**) (7.00 g, 40.6 mmol) and sodium acetate (3.75 g, 45.7 mmol) in acetic acid (35 mL). The reaction mixture was heated to 80 °C and stirred for 3 h. The mixture was then allowed to cool to room temperature and the remaining dichloromethane removed *in vacuo*. The resulting residue was poured onto ice water with stirring and the precipitate formed was collected by filtration to give 5-chloro-4-iodo-2-nitroaniline (**182**) as an orange solid (11.1 g, 92%). Mp 200–201 °C (lit.,[184] 202−203 °C); ν_{max}/cm^{-1} (neat) 3350 (NH), 1609 (C=C), 1543, 1478, 1309, 1234, 1124, 892; δ_H (400 MHz, DMSO-d_6) 7.26 (1H, s, 6-H), 7.59 (2H, br s, NH_2), 8.36 (1H, s, 3-H); δ_C (101 MHz,

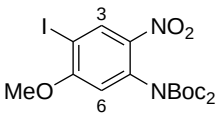
DMSO-d_6) 79.0 (C), 118.5 (CH), 130.3 (C), 136.0 (CH), 143.8 (C) 146.0 (C); *m/z* (EI) 297.8999 (M^+. $C_6H_4{}^{35}ClIN_2O_2$ requires 297.9006), 268 (10%), 252 (32), 225 (9), 172 (4), 125 (37).

4-Iodo-5-methoxy-2-nitroaniline (180)[184]



5-Chloro-4-iodo-2-nitroaniline (**182**) (5.00 g, 16.8 mmol) was added to a solution of potassium hydroxide (2.08 g, 37.0 mmol) in dimethylsulfoxide (17 mL) and methanol (17 mL). The mixture was heated to 60 °C and stirred for 6 h. After allowing to cool to room temperature the solution was poured onto 1 M aqueous hydrochloric acid (100 mL) and extracted with ethyl acetate (3 × 70 mL). The organic extracts were combined, washed with 1 M aqueous hydrochloric acid (6 × 50 mL) and brine (2 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 4-iodo-5-methoxy-2-nitroaniline (**180**) as an orange solid (4.66 g, 94%). Mp 182–183 °C (lit.,[184] 189 °C); ν_{max}/cm^{-1} (neat) 3340 (NH), 1639 (C=C), 1571, 1332, 1233, 1107, 921, 840; δ_H (400 MHz, $CDCl_3$) 3.90 (3H, s, OCH_3), 6.11 (1H, s, 6-H), 6.25 (2H, br s, NH_2), 8.55 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 56.8 (CH_3), 71.2, (C), 97.7 (CH), 127.9 (C), 137.1 (CH), 146.8 (C), 163.1 (C); *m/z* (EI) 293.9504 (M^+. $C_7H_7IN_2O_3$ requires 293.9501), 264 (47%), 248 (53), 121 (48), 106 (50).

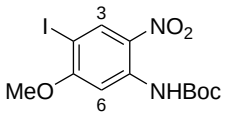
1-[Bis(*tert*-butoxycarbonyl)amino]-4-iodo-5-methoxy-2-nitrobenzene (188)



To a solution 4-iodo-5-methoxy-2-nitroaniline (**180**) (2.95 g, 10.0 mmol) in dichloromethane (135 mL) was added di-*tert*-butyl dicarbonate (4.80 g, 22.0 mmol), 4-dimethylaminopyridine (0.240 g, 2.00 mmol) and triethylamine (3.07 mL, 22.0 mmol) and the solution stirred at room temperature for 18 h. The reaction mixture was then diluted with water (100 mL), extracted with dichloromethane (3 × 70 mL), dried ($MgSO_4$) and

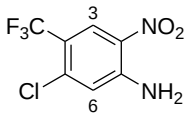
concentrated *in vacuo*. The resulting material was then purified by flash chromatography eluting with 0–50% ethyl acetate in petroleum ether (40–60) to give 1-[bis(*tert*-butoxycarbonyl)amino]-4-iodo-5-methoxy-2-nitrobenzene (**188**) as a white solid (4.76 g, 96%). Mp 186–188 °C; ν_{max}/cm^{-1} (neat) 2977 (CH), 1793 (CO), 1570 (C=C), 1515, 1339, 1282, 1229, 1153, 1098, 1009, 847; δ_H (400 MHz, $CDCl_3$) 1.43 (18H, s, 6 × CH_3), 3.97 (3H, s, OCH_3), 6.66 (1H, s, 6-H), 8.60 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 27.9 (6 × CH_3), 57.4 (CH_3), 84.2 (C), 84.3 (C), 112.4 (CH), 135.8 (C), 136.3 (CH), 139.1 (C), 150.2 (C), 162.4 (C); *m/z* (EI) 494.0552 (M^+. $C_{17}H_{23}IN_2O_7$ requires 494.0550), 394 (12%), 338 (93), 321 (10), 294 (58), 246 (10), 61 (28), 43 (100).

1-(*tert*-Butoxycarbonylamino)-4-iodo-5-methoxy-2-nitrobenzene (178)



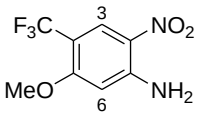
To a solution of 1-[bis(*tert*-butoxycarbonyl)amino]-4-iodo-5-methoxy-2-nitrobenzene (**188**) (1.14 g, 2.31 mmol) in dichloromethane (30 mL) at 0 ºC was added trifluoroacetic acid (0.35 mL, 4.6 mmol). The reaction mixture then warmed to room temperature and was stirred for 2 h. The reaction mixture was diluted with a saturated aqueous solution of sodium hydrogen carbonate (30 mL) and extracted with dichloromethane (3 × 40 mL). The organic extracts were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give 1-(*tert*-butoxycarbonylamino)-4-iodo-5-methoxy-2-nitrobenzene (**178**) as a yellow solid (0.910 g, 100%). Mp 193–194 °C; ν_{max}/cm^{-1} (neat) 3347 (NH), 2989 (CH), 1719 (CO), 1572 (C=C), 1430, 1330, 1229, 1042, 845; δ_H (400 MHz, $CDCl_3$) 1.55 (9H, s, 3 × CH_3), 4.00 (3H, s, OCH_3), 8.20 (1H, s, 6-H), 8.65 (1H, s, 3-H), 10.06 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 57.4 (CH_3), 76.1 (C), 82.4 (C), 100.6 (CH), 130.0 (C), 136.9 (CH), 139.1 (C), 152.3 (C), 163.9 (C); *m/z* (EI) 394.0029 (M^+. $C_{12}H_{15}IN_2O_5$ requires 394.0026), 338 (45%), 294 (50), 248 (10), 168 (9), 83 (58), 44 (100).

5-Chloro-2-nitro-4-(trifluoromethyl)aniline (190)[109]



A solution of 1,5-dichloro-2-nitro-4-(trifluoromethyl)benzene (**189**) (4.80 g, 18.5 mmol) in 1,4-dioxane (10 mL) was saturated with ammonia gas. The reaction vessel was sealed and heated to 100 °C for 4 days. The solution was again saturated with ammonia gas, the reaction vessel sealed and heated to 100 °C for a further 2 days. The reaction mixture was allowed to cool to room temperature, then poured on to water (100 mL) and stirred until a yellow precipitate formed. The precipitate was collected by filtration to give 5-chloro-2-nitro-4-(trifluoromethyl)aniline (**190**) as a yellow solid (3.34 g, 76%). Mp 111–112 °C (lit.,[109] 113–114 °C); δ_H (400 MHz, $CDCl_3$) 6.39 (2H, br s, NH_2), 6.97 (1H, s, 6-H), 8.50 (1H, s, 3-H); δ_C (126 MHz, $CDCl_3$) 117.3 (q, $J_{C\text{-}C\text{-}F}$ 33.4 Hz, C), 120.6 (CH), 122.3 (q, $J_{C\text{-}F}$ 271.8 Hz, C), 127.0 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.7 Hz, CH), 129.6 (C), 139.1 (C), 146.5 (C); *m/z* (EI) 240.0 (M^+, 62%), 210 (12), 194 (35), 182 (16), 132 (13), 83 (100).

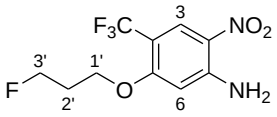
5-Methoxy-2-nitro-4-(trifluoromethyl)aniline (195)



5-Chloro-2-nitro-4-(trifluoromethyl)aniline (**190**) (2.30 g, 9.56 mmol) was added to a solution of potassium hydroxide (1.18 g, 21.0 mmol) in dimethylsulfoxide (10 mL) and methanol (10 mL). The reaction mixture was heated to 60 °C and stirred for 5 h. After cooling to room temperature the reaction was poured onto 1 M aqueous hydrochloric acid (40 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were then washed with water (3 × 30 mL) and brine (2 × 30 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 5-methoxy-2-nitro-4-(trifluoromethyl)aniline (**195**) as a yellow solid (2.19 g, 97%). Mp 136–138 °C; ν_{max}/cm^{-1} (neat) 3341 (NH), 1636 (C=C), 1566, 1420, 1327, 1234, 1111, 918, 841; δ_H (400 MHz, $CDCl_3$) 3.92 (3H, s, OCH_3), 6.21 (1H, s, 6-H), 6.46 (2H, br s, NH_2), 8.41 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 56.5 (CH_3),

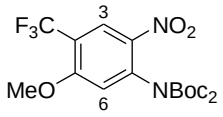
99.0 (CH), 109.8 (q, $J_{C\text{-}C\text{-}F}$ 32.9 Hz, C), 125.2 (C), 122.8 (q, $J_{C\text{-}F}$ 271.2 Hz, C), 127.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$, 5.7 Hz, CH), 149.0 (C), 162.4 (C); *m/z* (EI) 236.0406 (M^+. $C_8H_7F_3N_2O_3$ requires 236.0409), 217 (22%), 206 (82), 190 (38), 178 (24), 147 (30), 83 (68).

5-(3'-Fluoropropoxy)-2-nitro-4-(trifluoromethyl)aniline (196)



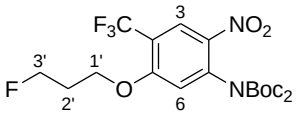
To a solution of 5-chloro-2-nitro-4-(trifluoromethyl)aniline (**190**) (1.00 g, 4.16 mmol) in dimethylsulfoxide (2.00 mL) was added 3-fluoropropanol (3.13 mL, 41.6 mmol) and potassium carbonate (1.15 g, 8.32 mmol) was added. The reaction mixture was stirred at 90 °C for 24 h. Potassium carbonate (1.15 g, 8.32 mmol) was added and the mixture stirred for a further 48 h at 90 °C. The reaction mixture was then diluted with 1 M aqueous hydrochloric acid (80 mL) and extracted with dichloromethane (3 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified using flash chromatography on silica eluting with 30% ethyl acetate in petroleum ether (40–60) to give 5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)aniline (**196**) as a yellow solid (0.880 g, 71%). Mp 148–149 °C; (Found: C, 42.49; H, 3.47; N, 9.81. $C_{10}H_{10}F_4N_2O_3$ requires C, 42.56; H, 3.57; N, 9.93%); ν_{max}/cm^{-1} (neat) 3480 (NH), 3354 (NH), 1641 (C=C), 1571, 1306, 1245, 1115, 1034, 914, 851; δ_H (400 MHz, $CDCl_3$) 2.22 (2H, dquin, *J* 26.4, 5.7 Hz, 2'-H_2), 4.18 (2H, t, *J* 5.7 Hz, 1'-H_2), 4.65 (2H, dt, *J* 46.9, 5.7 Hz, 3'-H_2), 6.22 (1H, s, 6-H), 6.45 (2H, br s, NH_2), 8.42 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 30.1 (d, $J_{C\text{-}C\text{-}F}$ 20.2 Hz, CH_2), 64.9 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 4.9 Hz, CH_2), 80.1 (d, $J_{C\text{-}F}$ 164.9 Hz, CH_2), 99.6 (CH), 109.9 (q, $J_{C\text{-}C\text{-}F}$ 33.2 Hz, C), 122.9 (q, $J_{C\text{-}F}$ 269.5 Hz, C), 125.4 (C), 127.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.6 Hz, CH), 148.8 (C), 161.5 (C); *m/z* (CI) 283 (MH^+, 100%), 253 (18), 233 (8), 133 (16), 85 (34).

1-[Bis(*tert*-butoxycarbonyl)amino]-5-methoxy-2-nitro-4-(trifluoromethyl)benzene (198)



To a solution of 5-methoxy-2-nitro-4-(trifluoromethyl)aniline (**195**) (3.27 g, 13.8 mmol) in dichloromethane (150 mL) was added di-*tert*-butyl dicarbonate (6.66 g, 30.5 mmol), 4-dimethylaminopyridine (0.340 g, 2.76 mmol) and triethylamine (4.25 mL, 30.5 mmol) and the solution stirred at room temperature for 18 h. The reaction mixture was then diluted with water (100 mL) and extracted with dichloromethane (3 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was then purified by flash chromatography eluting with 30% ethyl acetate in petroleum ether (40–60) to give 1-[bis(*tert*-butoxycarbonyl)amino]-5-methoxy-2-nitro-4-(trifluoromethyl)benzene (**198**) as a white solid (5.70 g, 95%). Mp 123–125 °C; ν_{max}/cm^{-1} (neat) 2986 (CH), 1805 (CO), 1620 (C=C), 1589, 1528, 1319, 1242, 1150, 1096, 849; δ_H (400 MHz, $CDCl_3$) 1.44 (18H, s, 6 × CH_3), 4.01 (3H, s, OCH_3), 6.91 (1H, s, 6-H), 8.43 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 28.0 (6 × CH_3), 57.2 (CH_3), 84.6 (C), 114.8 (CH), 118.9 (q, $J_{C\text{-}C\text{-}F}$ 33.1 Hz, C), 122.1 (q, $J_{C\text{-}F}$ 273.1 Hz, C), 125.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.4 Hz, CH), 138.0 (C), 139.0 (C), 150.2 (C), 161.1 (C); *m/z* (EI) 336.0925 ($M^+-C_5H_8O_2$. $C_{13}H_{15}F_3N_2O_5$ requires 336.0933), 280 (8%), 236 (12), 83 (90), 57 (100).

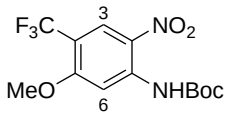
1-[Bis(*tert*-butoxycarbonyl)amino]-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene (199)



The reaction was performed according to the above procedure using 5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)aniline (**196**) (1.33 g, 4.71 mmol), di-*tert*-butyl dicarbonate (2.26 g, 10.4 mmol), 4-dimethylaminopyridine (0.120 g, 0.940 mmol) and triethylamine (1.44 mL, 10.4 mmol) in dichloromethane (65 mL). The material was then purified by flash chromatography eluting with 20% ethyl acetate in petroleum ether (40–60) to give 1-

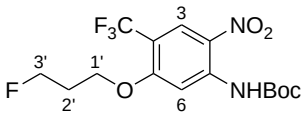
[bis(*tert*-butoxycarbonyl)amino]-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene (**199**) as a white solid (1.86 g, 82%). Mp 84–86 °C; (Found: C, 49.84; H, 5.46; N, 5.87. $C_{20}H_{26}F_4N_2O_7$ requires C, 49.79; H, 5.43; N, 5.81%); ν_{max}/cm^{-1} (neat) 2982 (CH), 1794 (CO), 1620 (C=C), 1586, 1523, 1327, 1254, 1138, 1099, 1038, 918, 849; δ_H (400 MHz, $CDCl_3$) 1.44 (18H, s, 6 × CH_3), 2.26 (2H, dquin, *J* 26.3, 5.8 Hz, 2'-H_2), 4.27 (2H, t, *J* 5.8 Hz, 1'-H_2), 4.66 (2H, dt, *J* 46.9, 5.8 Hz, 3'-H_2), 6.91 (1H, s, 6-H), 8.43 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 28.0 (6 × CH_3), 30.1 (d, $J_{C\text{-}C\text{-}F}$ 20.1 Hz, CH_2), 65.6 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 4.7 Hz, CH_2), 79.9 (d, $J_{C\text{-}F}$ 165.2 Hz, CH_2), 84.7 (C), 115.4 (CH), 119.0 (q, $J_{C\text{-}C\text{-}F}$ 33.0 Hz, C), 122.2 (q, $J_{C\text{-}F}$ 272.9 Hz, C), 125.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.4 Hz, CH), 138.2 (C), 139.0 (C), 150.2 (C), 160.2 (C); *m/z* (CI) 383 ($MH^+ - C_5H_8O_2$, 37%), 353 (24), 327 (98), 283 (61), 253 (30).

1-(*tert*-Butoxycarbonylamino)-5-methoxy-2-nitro-4-(trifluoromethyl)benzene (176)



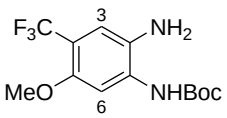
To a solution of 1-[bis(*tert*-butoxycarbonyl)amino]-5-methoxy-2-nitro-4-(trifluoromethyl) benzene (**198**) (1.30 g, 2.98 mmol) in dichloromethane (40 mL) at 0 ºC was added trifluoroacetic acid (0.46 mL, 6.0 mmol). The reaction mixture warmed to room temperature and was stirred for 2 h. The reaction mixture was diluted with a saturated aqueous solution of sodium hydrogen carbonate (30 mL) and extracted with dichloromethane (3 × 20 mL). The organic extracts were combined, dried ($MgSO_4$), and concentrated *in vacuo* to give 1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitro-4-(trifluoromethyl)benzene (**176**) as a yellow solid (1.00 g, 100%). Mp 108–109 °C; ν_{max}/cm^{-1} (neat) 3395 (NH), 1636 (CO), 1481, 1335, 1258, 1134, 964, 856; δ_H (400 MHz, $CDCl_3$) 1.56 (9H, 3 × CH_3), 4.03 (3H, s, CH_3), 8.38 (1H, s, 6-H), 8.50 (1H, s, 3-H), 10.20 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.0 (3 × CH_3), 56.7 (CH_3), 82.7 (C), 101.7 (CH), 112.5 (q, $J_{C\text{-}C\text{-}F}$ 33.1 Hz, C), 122.3 (q, $J_{C\text{-}F}$ 271.9 Hz, C), 126.2 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.5 Hz, CH), 127.4 (C), 141.5 (C), 151.8 (C), 162.6 (C); *m/z* (EI) 336.0929 (M^+. $C_{13}H_{15}F_3N_2O_5$ requires 336.0933), 280 (8%), 236 (24), 206 (9), 83 (100).

1-(*tert*-Butoxycarbonylamino)-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl) benzene (177)



The reaction was carried out according to the above procedure using 1-[bis(*tert*-butoxycarbonyl)amino]-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene (**199**) (1.55 g, 3.21 mmol) and trifluoroacetic acid (0.49 mL, 6.4 mmol) in dichloromethane (40 mL) to give 1-(*tert*-butoxycarbonylamino)-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene (**177**) as a yellow solid (1.20 g, 98%). Mp 75–76 °C; (Found: C, 47.13; H, 4.71; N, 7.21. $C_{15}H_{18}F_4N_2O_5$ requires C, 47.12; H, 4.75; N, 7.33%); ν_{max}/cm^{-1} (neat) 3356 (NH), 2988 (CH), 1743 (CO), 1631 (C=C), 1580, 1440, 1343, 1233, 1133, 1049, 924, 849; δ_H (400 MHz, $CDCl_3$) 1.55 (9H, s, 3 × CH_3), 2.24 (2H, dquin, *J* 25.8, 5.8 Hz, 2'-H_2), 4.33 (2H, t, *J* 5.8 Hz, 1'-H_2), 4.66 (2H, dt, *J* 46.9, 5.8 Hz, 3'-H_2), 8.39 (1H, s, 6-H), 8.50 (1H, s, 3-H), 10.19 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 30.0 (d, $J_{C\text{-}C\text{-}F}$ 20.4 Hz, CH_2), 65.4 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.0 Hz, CH_2), 80.0 (d, $J_{C\text{-}F}$ 165.2 Hz, CH_2), 82.9 (C), 102.5 (CH), 112.9 (q, $J_{C\text{-}C\text{-}F}$ 33.0 Hz, C), 122.5 (q, $J_{C\text{-}F}$ 271.8 Hz, C), 126.6 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.4 Hz, CH), 127.8 (C), 141.6 (C), 152.0 (C), 162.0 (C); *m/z* (EI) 382 (M^+, 3%), 282 (23), 222 (17), 131 (6), 57 (100).

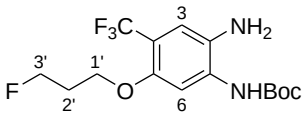
2-Amino-1-(*tert*-butoxycarbonylamino)-5-methoxy-4-(trifluoromethyl)benzene (174)



To a solution of 1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitro-4-(trifluoromethyl) benzene (**176**) (0.880 g, 2.62 mmol) in ethyl acetate and pyridine (6:1, 70 mL) was added tin(II) chloride dihydrate (2.96 g, 13.1 mmol) and the reaction mixture stirred at room temperature for 18 h. The precipitate formed was removed by filtration through Celite®. The filtrate was diluted with water (150 mL), extracted with ethyl acetate (3 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by triturating with petroleum ether (40–60) to give 2-amino-1-(*tert*-butoxycarbonylamino)-5-

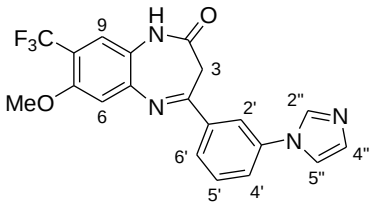
methoxy-4-(trifluoromethyl)benzene (**174**) as a white solid (0.680 g, 85%). Mp 151–152 °C; ν_{max}/cm^{-1} (neat) 3372 (NH), 2986 (CH), 1690 (CO), 1528, 1497, 1427, 1296, 1111, 1057, 887; δ_H (400 MHz, $CDCl_3$) 1.53 (9H, s, 3 × CH_3), 3.16 (2H, br s, NH_2), 3.87 (3H, s, OCH_3), 6.92 (1H, br s, NH), 7.04 (1H, s, 3-H), 7.58 (1H, s, 6-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 56.6 (CH_3), 81.5 (C), 105.4 (CH), 113.5 (q, $J_{C\text{-}C\text{-}F}$ 31.0 Hz, C), 119.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.2 Hz, CH), 123.7 (q, $J_{C\text{-}F}$ 271.7 Hz, C), 126.0 (C), 133.9 (C), 153.0 (C), 153.5 (C); *m/z* (EI) 306.1192 (M^+. $C_{13}H_{17}F_3N_2O_3$ requires 306.1191), 250 (52%), 232 (14), 206 (70), 191 (42), 163 (15), 134 (13), 83 (100), 57 (100).

2-Amino-1-(*tert*-butoxycarbonylamino)-5-(3'-fluoropropoxy)-4-(trifluoromethyl) benzene (175)



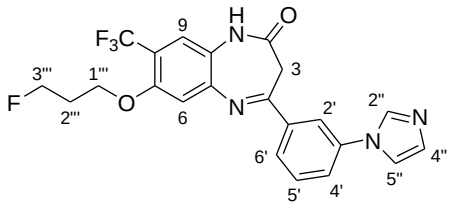
The reaction was carried out according to the above procedure using 1-(*tert*-butoxycarbonylamino)-5-(3'-fluoropropoxy)-2-nitro-4-(trifluoromethyl)benzene (**177**) (1.13 g, 2.96 mmol) and tin(II) chloride dihydrate (3.34 g, 14.8 mmol) in ethyl acetate and pyridine (12 mL, 6:1). The crude material was purified using flash column chromatography eluting with 0–30% ethyl acetate in petroleum ether (40–60) to give 2-amino-1-(*tert*-butoxycarbonylamino)-5-(3'-fluoropropoxy)-4-(trifluoromethyl)aniline (**175**) as a white solid (0.890 g, 83%). Mp 121–122 °C; (Found: C, 51.02; H, 5.69; N, 7.81. $C_{15}H_{20}F_4N_2O_3$ requires C, 51.13; H, 5.72; N, 7.95%); ν_{max}/cm^{-1} (neat) 3358 (NH), 2994 (CH), 1690 (CO), 1526, 1499, 1441, 1296, 1219, 1111, 1059, 957, 880; δ_H (400 MHz, $CDCl_3$) 1.52 (9H, s, 3 × CH_3), 2.16 (2H, dquin, *J* 25.5, 5.8 Hz, 2'-H_2), 3.18 (2H, br s, NH_2), 4.13 (2H, t, *J* 5.8 Hz, 1'-H_2), 4.64 (2H, dt, *J* 47.0, 5.8 Hz, 3'-H_2), 6.92 (1H, br s, NH), 7.03 (1H, s, 3-H), 7.57 (1H, s, 6-H); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 30.4 (d, $J_{C\text{-}C\text{-}F}$ 20.0 Hz, CH_2), 64.7 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.6 Hz, CH_2), 80.7 (d, $J_{C\text{-}F}$ 163.9 Hz, CH_2), 81.3 (C), 105.9 (CH), 113.5 (q, $J_{C\text{-}C\text{-}F}$ 31.2 Hz, C), 118.7 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 4.8 Hz, CH), 123.7 (q, $J_{C\text{-}F}$ 271.7 Hz, C), 127.5 (C), 133.3 (C), 152.0 (C), 152.9 (C); *m/z* (CI) 353 (MH^+, 24%), 297 (98), 279 (10), 253 (38), 81 (42).

4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-7-methoxy-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (170)



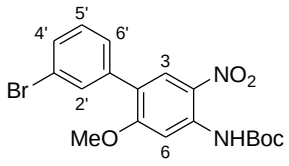
To a solution of 2-amino-1-(*tert*-butoxycarbonylamino)-5-methoxy-4-(trifluoromethyl) benzene (**174**) (0.654 g, 2.14 mmol) in toluene (4 mL) was added *tert*-butyl 3-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-3-oxopropanoate (**145**) (0.733 g, 2.56 mmol). The solution was heated under reflux for 3.5 h before cooling to room temperature and concentrating *in vacuo*. The resulting material was purified by flash column chromatography eluting with 0–5% ethanol in dichloromethane to give 1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-5-methoxy-4-(trifluoromethyl)benzene (**172**) as a white solid (0.723 g, 69%). To a solution of 1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-5-methoxy-4(trifluoromethyl)benzene (**172**) (0.490, 0.945 mmol) in dichloromethane (5 mL) at 0 °C was added trifluoroacetic acid (1 mL). The reaction mixture was warmed to room temperature and stirred for 2 h. A saturated aqueous solution of sodium hydrogen carbonate (10 mL) was added, the solution extracted with dichloromethane (3 × 5 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude material was then triturated with toluene to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**170**) as a white solid (0.266 g, 70%). Mp 222–223 °C (decomposition); ν_{max}/cm^{-1} (neat) 2842 (CH), 1685 (CO), 1497, 1403, 1300, 1223, 1101, 1044, 919, 827; δ_H (400 MHz, DMSO-d_6) 3.69 (2H, s, 3-H_2), 3.93 (3H, s, OCH_3), 7.16 (1H, br s, 4"-H), 7.24 (1H, s, 6-H), 7.48 (1H, s, 9-H), 7.70 (1H, t, *J* 7.9 Hz, 5'-H), 7.83–7.90 (2H, m, 4'-H and 5"-H), 8.06 (1H, d, *J* 7.9 Hz, 6'-H), 8.27 (1H, br s, 2'-H), 8.36 (1H, br s, 2"-H), 10.56 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 40.0 (CH_2), 56.3 (CH_3), 110.6 (CH), 114.9 (q, $J_{C\text{-}C\text{-}F}$ 30.8 Hz, C), 118.1 (CH), 119.6 (CH), 120.7 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.4 Hz, CH), 123.0 (C), 123.1 (q, $J_{C\text{-}F}$ 271.9, C), 123.4 (CH), 126.1 (CH), 129.9 (CH), 130.2 (CH), 135.6 (CH), 137.3 (C), 138.4 (C), 143.1 (C), 152.8 (C), 159.8 (C), 165.7 (C); *m/z* (FAB) 401.1224 (MH^+. $C_{20}H_{16}F_3N_4O_2$ requires 401.1225), 238 (15%), 169 (26), 136, (13), 107 (16), 86 (100).

7-(3”’-Fluoropropoxy)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (171)



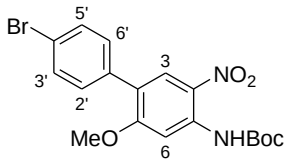
The reaction was carried out as described above, using 2-amino-1-(*tert*-butoxycarbonylamino)-5-(3’-fluoropropoxy)-4-(trifluoromethyl)benzene (**175**) (0.400 g, 1.14 mmol) and *tert*-butyl 3-[3’-(1”*H*-imidazol-1”-yl)phenyl]-3-oxopropanoate (**145**) (0.389 g, 1.36 mmol) in toluene (3 mL) to give 1-(*tert*-butoxycarbonylamino)-5-(3””-fluoropropoxy)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}-4-(trifluoromethyl)benzene (**173**) as a white solid (0.507 g, 79%). Cyclisation was performed as described above using 1-(*tert*-butoxycarbonylamino)-5-(3””fluoropropoxy)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}-4-(trifluoromethyl)benzene (**173**) (0.070 g, 0.12 mmol) and trifluoroacetic acid (0.35 mL) in dichloromethane (0.7 mL) to give 7-(3”’-fluoropropoxy)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**171**) as a white solid (0.049 g, 91%). Mp 225–227 °C (decomposition); ν_{max}/cm^{-1} (neat) 2854 (CH), 1683 (CO), 1584, 1495, 1396, 1316, 1218, 1180, 1104, 1048, 954; δ_H (500 MHz, DMSO-d_6) 2.14 (1H, dquin, *J* 25.2, 5.9 Hz, 2”’-H_2), 3.69 (2H, s, 3-H_2), 4.25 (2H, t, *J* 5.9 Hz, 1”’-H_2), 4.62 (2H, dt, *J* 47.2, 5.9 Hz, 3”’-H_2), 7.16 (1H, br s, 4”-H), 7.27 (1H, s, 6-H), 7.48 (1H, s, 9-H), 7.70 (1H, t, *J* 7.9 Hz, 5’-H), 7.84–7.90 (2H, m, 4’-H and 5”’-H), 8.06 (1H, br d, *J* 7.9 Hz, 6’-H), 8.27 (1H, br s, 2’-H), 8.37 (1H, br s, 2”-H), 10.60 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 29.6 (d, $J_{C\text{-}C\text{-}F}$ 19.9 Hz, CH_2), 40.0 (CH_2), 64.6 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.8 Hz, CH_2), 80.5 (d, $J_{C\text{-}F}$, 161.9 Hz, CH_2), 111.4 (CH), 115.2 (q, $J_{C\text{-}C\text{-}F}$ 30.9 Hz, C), 118.3 (CH), 119.7 (CH), 120.9 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.6 Hz, CH), 123.3 (q, $J_{C\text{-}F}$ 272.0 Hz, C), 123.3 (C), 123.6 (CH), 126.3 (CH), 130.1 (CH), 130.5 (CH), 135.8 (CH), 137.4 (C), 138.4 (C), 143.3 (C), 152.0 (C), 160.0 (C), 166.0 (C); *m/z* (FAB) 447.1448 (MH^+. $C_{22}H_{19}F_4N_4O_2$ requires 447.1444), 307 (21%), 289 (15), 155 (11), 89 (12).

4-(3'-Bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitrobenzene (205)



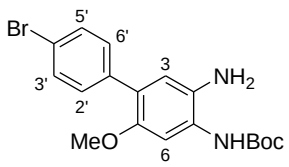
To a solution of 1-(*tert*-butoxycarbonylamino)-4-iodo-5-methoxy-2-nitrobenzene (**178**) (1.75 g, 4.44 mmol) in *N*,*N*-dimethylformamide and water (90 mL, 9:1) was added 3-bromophenylboronic acid (0.892 g, 4.44 mmol), potassium carbonate (1.23 g, 8.88 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.104 g, 0.09 mmol). The reaction mixture was heated to 110 ºC and stirred for 1.5 h. After cooling to room temperature the solution was concentrated *in vacuo*. The resulting residue was redissolved in chloroform (90 mL) and filtered through Celite® and concentrated *in vacuo*. The resulting solid was dissolved in diethyl ether (200 mL), washed with water (4 × 100 mL) and brine (2 × 100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography eluting with 0–10% ethyl acetate in petroleum ether (40–60) to give 4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitrobenzene (**205**) as a yellow solid (1.34 g, 71%). Mp 140–141 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2986 (CH), 1736 (CO), 1620 (C=C), 1574, 1273, 1141, 1018, 849; δ_H (400 MHz, $CDCl_3$) 1.57 (9H, s, 3 × CH_3), 3.97 (3H, s, OCH_3), 7.29 (1H, t, *J* 7.9 Hz, 5'-H), 7.40–7.44 (1H, m, 6'-H), 7.49 (1H, ddd, *J* 7.9, 1.8, 1.0 Hz, 4'-H), 7.65 (1H, t, *J* 1.8 Hz, 2'-H), 8.21 (1H, s, 3-H), 8.31 (1H, s, 6-H), 10.15 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 56.6 (CH_3), 82.2 (C), 101.4 (CH), 122.4 (C), 123.7 (C), 128.1 (CH), 128.4 (CH), 129.0 (C), 129.9 (CH), 130.9 (CH), 132.4 (CH), 138.0 (C), 138.7 (C), 152.4 (C), 162.6 (C); *m/z* (EI) 422.0465 (M^+. $C_{18}H_{19}{}^{79}BrN_2O_5$ requires 422.0477), 366 (17%), 322 (20), 294 (5), 197 (8), 105 (22).

4-(4'-Bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitrobenzene (206)



The reaction was carried out according to the above procedure using 1-(*tert*-butoxycarbonylamino)-4-iodo-5-methoxy-2-nitrobenzene (**178**) (2.00 g, 5.07 mmol), 4-bromophenylboronic acid (1.02 g, 5.07 mmol), potassium carbonate (1.40 g, 10.1 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.116 g, 0.101 mmol) in *N*,*N*-dimethylformamide and water (100 mL, 9:1) to give 4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitrobenzene (**206**) as a yellow solid (1.41 g, 66%). Mp 159–160 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2986 (CH), 1735 (CO), 1620 (C=C), 1574, 1443, 1273, 1142, 1018, 849; δ_H (400 MHz, $CDCl_3$) 1.56 (9H, s, 3 × CH_3), 3.96 (3H, s, OCH_3), 7.37 (1H, d, *J* 8.5 Hz, 2'-H and 6'-H), 7.54 (1H, d, *J* 8.5 Hz, 3'-H and 5'-H), 8.20 (1H, s, 3-H), 8.30 (1H, s, 6-H), 10.15 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.4 (3 × CH_3), 56.6 (CH_3), 82.2 (C), 101.4 (CH), 122.1 (C), 124.0 (C), 128.1 (CH), 129.0 (C), 131.1 (2 × CH), 131.6 (2 × CH), 134.8 (C), 138.5 (C), 152.4 (C), 162.5 (C); *m/z* (EI) 422.0482 (M^+. $C_{18}H_{19}{}^{79}BrN_2O_5$ requires 422.0477), 366 (33%), 322 (36), 292 (6), 197 (13), 154 (8), 126 (8), 105 (8), 57 (100).

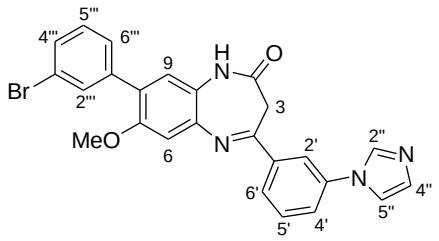
2-Amino-4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxybenzene (208)



To a solution of 4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitrobenzene (**206**) (1.00 g, 2.36 mmol) in ethyl acetate and pyridine (70 mL, 6:1) was added tin(II) chloride dihydrate (2.66 g, 11.8 mmol), and the reaction mixture stirred at room temperature for 17 h. The mixture was then filtered through Celite® and concentrated *in vacuo*. The resulting solid was redissolved in ethyl acetate (100 mL), washed with water (2 × 50 mL) and brine (2 × 50 mL), dried ($MgSO_4$) and concentrated *in*

vacuo. The resulting solid was purified by trituarating with petroleum ether (40−60) and diethyl ether to give 2-amino-4-(4'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxybenzene (**208**) as a white solid (0.697 g, 75%). Mp 188–189 °C; ν_{max}/cm^{-1} (neat) 3364 (NH), 2978 (CH), 1682 (CO), 1597 (C=C), 1505, 1420, 1250, 1165, 1057, 833; δ_H (400 MHz, $CDCl_3$) 1.54 (9H, s, 3 × CH_3), 3.32 (2H, br s, NH_2), 3.75 (3H, s, OCH_3), 6.69 (1H, br s, NH), 6.80 (1H, s, 3-H), 7.30 (1H, s, 6-H), 7.35 (2H, d, *J* 8.4 Hz, 2'-H and 6'-H), 7.49 (2H, *J* 8.4 Hz, 3'-H and 5-H); δ_C (101 MHz, $CDCl_3$) 28.5 (3 × CH_3), 56.3 (CH_3), 81.1 (C), 106.7 (CH), 121.1 (C), 122.1 (CH), 125.9 (C), 128.4 (C), 128.6 (C), 131.1 (2 × CH), 131.2 (2 × CH), 136.9 (C), 151.8 (C), 153.6 (C); *m/z* (CI) 393.0819 (MH^+. $C_{18}H_{22}{}^{79}BrN_2O_3$ requires 393.0814), 337 (51%), 315 (100), 293 (28), 259 (65), 215 (27), 113 (70), 73 (80).

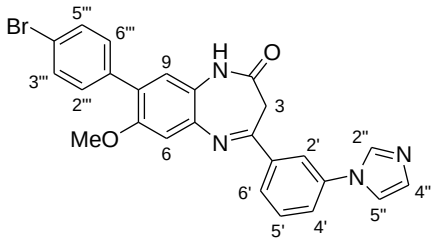
8-(3"'-Bromophenyl)-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (203)



To a solution of 4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxy-2-nitrobenzene (**205**) (0.100 g, 0.236 mmol) in ethyl acetate and pyridine (7 mL, 6:1) was added tin(II) chloride dihydrate (0.379 g, 1.68 mmol), and the reaction mixture stirred at room temperature for 17 h. The mixture was then filtered through Celite® and concentrated *in vacuo*. The resulting solid was redissolved in ethyl acetate (10 mL), washed with water (2 × 5 mL) and brine (2 × 5 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting solid was purified by flash column chromatography eluting with 10–50% ethyl acetate in petroleum ether (40−60) to give 2-amino-4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxybenzene (**207**) as a purple/black solid (0.073 g) which was used without further purification. To a solution of 2-amino-4-(3'-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxybenzene (**207**) (0.073 g) in toluene (0.5 mL) was added *tert*-butyl 3-[3'-(1"*H*-imidazol-1"-yl)pheny]-3-oxopropanoate (**145**) (0.066 g, 0.23 mmol). The solution was heated to 75 °C and stirred for 7 h before cooling to room temperature and concentrating *in vacuo*. The resulting material was purified by flash

column chromatography eluting with 0–4% ethanol in dichloromethane to give 4-(3””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}-5-methoxybenzene (**209**) as a white solid (0.054 g, 37%) (over two steps). To a solution of 4-(3””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}-5-methoxybenzene (**209**) (0.070 g, 0.12 mmol) in dichloromethane (5 mL) at 0 °C was added trifluoroacetic acid (0.15 mL). The reaction mixture was warmed to room temperature and stirred for 1.5 h. A saturated aqueous solution of sodium hydrogen carbonate (10 mL) was added, the solution extracted with dichloromethane (3 × 5 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 8-(3”’-bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**203**) as a white solid (0.052 g, 93%). Mp 299–300 °C (decomposition); ν_{max}/cm^{-1} (neat) 3395 (NH), 3189 (CH), 1668 (CO), 1502, 1235, 1166, 1058, 1014; δ_H (500 MHz, DMSO-d_6) 3.66 (2H, s, 3-H_2), 3.84 (3H, s, OCH_3), 7.14 (1H, s, 6-H), 7.17 (2H, br s, 9-H, 4”-H), 7.42 (1H, t, *J* 7.9 Hz, 5”’-H), 7.50–7.59 (2H, m, 4”’-H and 6”’-H), 7.67–7.73 (2H, m, 5’-H and 2”’-H), 7.83–7.88 (2H, m, 4’-H and 5”-H), 8.06 (1H, br d, *J* 8.0 Hz, 6’-H), 8.26 (1H, br s, 2’-H), 8.37 (1H, br s, 2”-H), 10.48 (1H, br s, NH); δ_C (126 MHz, $CDCl_3$) 39.9 (CH_2), 60.0 (CH_3), 109.7 (CH), 118.3 (CH), 119.6 (CH), 121.4 (C), 123.2 (CH), 123.6 (CH), 123.9 (C), 126.1 (CH), 126.9 (C), 128.3 (CH), 130.1 (2 × CH), 130.4 (2 × CH), 131.7 (CH), 135.8 (CH), 137.4 (C), 138.8 (C), 139.5 (C), 139.9 (C), 152.5 (C), 157.8 (C), 165.6 (C); *m/z* (ESI) 487.0751 (MH^+. $C_{25}H_{20}{}^{79}BrN_4O_2$ requires 487.0764).

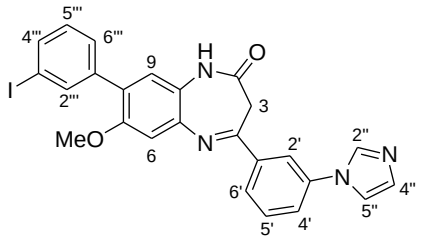
8-(4”’-Bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (211)



To a solution of 2-amino-4-(4’-bromophenyl)-1-(*tert*-butoxycarbonylamino)-5-methoxybenzene (**208**) (0.200 g, 0.509 mmol) in toluene (1 mL) was added *tert*-butyl 3-[3’-(1”*H*-imidazol-1”-yl)pheny]-3-oxopropanoate (**145**) (0.175 g, 0.611 mmol). The

solution was stirred under reflux for 4 h before cooling to room temperature and concentrating *in vacuo*. The resulting material was purified by flash column chromatography eluting with 0–3% ethanol in dichloromethane to give 4-(4””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}-5-methoxybenzene (**210**) as a white solid (0.226, 73%). To a solution of 4-(4””-bromophenyl)-1-(*tert*-butoxycarbonylamino)-2-{3’-[3”-(1”’*H*-imidazol-1”’-yl)phenyl]-3’-oxopropanamido}-5-methoxybenzene (**210**) (0.420 g, 0.694 mmol) in dichloromethane (4 mL) at 0 °C was added trifluoroacetic acid (0.5 mL). The reaction mixture was warmed to room temperature and stirred for 1.5 h. A saturated aqueous solution of sodium hydrogen carbonate (10 mL) was added, the solution extracted with dichloromethane (3 × 5 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 8-(4”’-bromophenyl)-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**211**) as a white solid (0.317 g, 94%). Mp 187–188 °C (decomposition); ν_{max}/cm^{-1} (neat) 3121 (NH), 2839 (CH), 1688 (CO), 1480, 1375, 1236, 1046, 817; δ_H (400 MHz, DMSO-d_6) 3.66 (2H, s, 3-H_2), 3.83 (3H, s, OCH_3), 7.11–7.19 (3H, m, 6-H, 9-H, 4”-H), 7.47 (2H, d, *J* 8.4 Hz, 2”’-H and 6”’-H), 7.65 (2H, d, *J* 8.4 Hz, 3”’-H and 5”’-H), 7.70 (1H, t, *J* 7.9 Hz, 5’-H), 7.83–7.89 (2H, m, 4’-H and 5”-H), 8.06 (1H, br d, *J* 7.9 Hz, 6’-H), 8.26 (1H, br s, 2’-H), 8.37 (1H, s, 2”-H), 10.52 (1H, br s, NH); δ_C (101 MHz, DMSO-d_6) 39.9 (CH_2), 55.9 (CH_3), 109.7 (CH), 118.3 (CH), 119.6 (CH), 120.7 (C), 123.2 (CH), 123.4 (CH), 123.9 (C), 126.1 (CH), 127.4 (C), 130.1 (CH), 130.4 (CH), 131.2 (2 × CH), 131.3 (2 × CH), 135.9 (CH), 136.3 (C), 137.4 (C), 138.9 (C), 139.7 (C), 152.4 (C), 157.7 (C), 165.7 (C); *m/z* (ESI) 487.0746 (MH^+. $C_{25}H_{20}{}^{79}BrN_4O_2$ requires 487.0764).

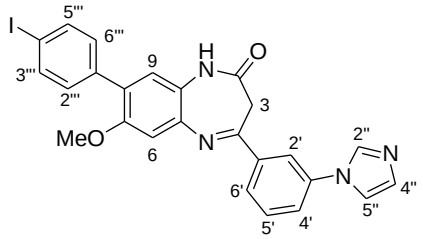
4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-(3"'-iodophenyl)-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (200)



A solution of 8-(3"'-bromophenyl)-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**203**) (0.055 g, 0.113 mmol) in 1,4-dioxane (1.4 mL) was degassed for 0.25 h before the addition of hexamethylditin (91 μL, 0.44 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.013 g, 0.011 mmol). The reaction mixture was heated to 90 °C and stirred under Ar for 6 h. The mixture was concentrated *in vacuo* and purified by flash chromatography on silica eluting with 0–5% ethanol in dichloromethane to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-8-[3"'-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**212**) (0.044 g, 70%). To a solution of 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-8-[3"'-(trimethylstannyl) phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**212**) (0.044 g, 0.077 mmol) in ethanol (50 mL) was added sodium iodide (0.012 g, 0.080 mmol) in 0.01 M aqueous sodium hydroxide (12 mL). The solution was then acidified to pH 4–5 using 0.05 M aqueous hydrochloric acid. A solution of chloramine-T (0.036 g, 0.16 mmol) in water (40 mL) was added and the mixture stirred at room temperature for 0.5 h. The reaction was then quenched by the addition of sodium metabisulfite (0.900 g) in water (90 mL). The solution was then diluted with a saturated aqueous solution of sodium hydrogen carbonate (100 mL), extracted with dichloromethane (4 × 80 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting solid was then purified by flash column chromatography eluting with 0–3% ethanol in dichloromethane to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-(3"'-iodophenyl)-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**200**) as a yellow solid (0.022 g, 54%). Mp 197–198 °C (decomposition); ν_{max}/cm^{-1} (neat) 3078 (NH), 2955 (CH), 1674 (CO), 1582, 1497, 1366, 1234, 1041, 779; δ_H (500 MHz, $CDCl_3$) 3.64 (2H, s, 3-H_2), 3.89 (3H, s, OCH_3), 7.01 (1H, s, 9-H), 7.07 (1H, s, 6-H), 7.17 (1H, t, *J* 7.8 Hz, 5"'-H), 7.27 (1H, br s, 4"-H), 7.42 (1H, br s, 5"-H), 7.50–7.56 (2H, m, 4'-H and 6"'-H), 7.62 (1H, t, *J* 7.9 Hz, 5'-H), 7.69–7.72 (1H, m, 4"'-H), 7.89 (1H, t, *J* 1.6 Hz, 2"'-H), 7.92 (1H, br s, NH), 7.99 (1H, br s, 2"-H), 8.06 (1H, br d, *J* 7.9 Hz, 6'-H), 8.23 (1H, t, *J* 1.8 Hz, 2'-H); δ_C (126

MHz, $CDCl_3$) 40.0 (CH_2), 56.0 (CH_3), 94.1 (C), 109.7 (CH), 118.4 (CH), 120.7 (CH), 122.6 (C), 123.7 (CH), 123.9 (CH), 126.8 (CH), 128.7 (C), 128.7 (CH), 129.8 (CH), 130.4 (CH), 130.5 (CH), 135.7 (CH), 136.5 (CH), 137.9 (C), 138.2 (CH), 139.0 (C) 139.5 (C), 140.1 (C), 153.7 (C), 157.6 (C), 166.3 (C); *m/z* (ESI) 535.0608 (MH^+. $C_{25}H_{20}IN_4O_2$ requires 535.0625).

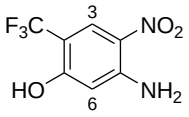
4-[3'-(1"*H*-Imidazol-1"-yl)phenyl]-8-(4"'-iodophenyl)-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (201)



The reaction was carried out as described above, using 8-(4"'-bromophenyl)-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**211**) (0.080 g, 0.16 mmol), hexamethylditin (136 μL, 0.660 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.019 g, 0.016 mmol) in 1,4-dioxane (2 mL) to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-8-[4"'-(trimethylstannyl)phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**213**) (0.084 g, 89%). Iododestannylation was then performed as described above, using 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-7-methoxy-8-[4"'-(trimethylstannyl) phenyl]-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**213**) (0.084 g, 0.15 mmol), sodium iodide (0.023 g, 0.153 mmol) and chloramine-T (0.068 g, 0.30 mmol) in ethanol (100 mL) to give 4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-(4"'-iodophenyl)-7-methoxy-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**201**) as a yellow solid (0.042 g, 53%). Mp 203–204 °C (decomposition); ν_{max}/cm^{-1} (neat) 3109 (NH), 2932 (CH), 1678 (CO), 1582 (C=C), 1500, 1477, 1312, 1230, 1038, 1003, 817; δ_H (400 MHz, DMSO-d_6) 3.65 (2H, s, 3-H_2), 3.82 (3H, s, OCH_3), 7.08–7.23 (3H, m, 6-H, 9-H and 4"-H), 7.32 (2H, d, *J* 8.2 Hz, 2"'-H and 6"'-H), 7.70 (1H, t, *J* 7.8 Hz, 5'-H), 7.81 (2H, d, *J* 8.2 Hz, 3"'-H and 5"'-H), 7.83–7.91 (2H, m, 4'-H and 5"-H), 8.06 (1H, br d, *J* 7.8 Hz, 6'-H), 8.26 (1H, br s, 2'-H), 8.38 (1H, br s, 2"-H), 10.52 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 39.9 (CH_2), 55.9 (CH_3), 93.6 (C), 109.7 (CH), 118.4 (CH), 119.6 (CH), 123.2 (CH), 123.3 (CH), 123.9 (C), 126.1 (CH), 127.5 (C), 130.1 (CH), 130.4 (CH), 131.4 (2 × CH), 136.0 (CH), 136.6 (C),

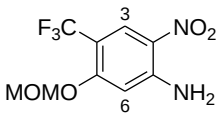
137.0 (2 × CH), 137.4 (C), 138.9 (C), 139.7 (C), 152.4 (C), 157.7 (C), 165.7 (C); *m/z* (FAB) 535.0638 (MH^+. $C_{25}H_{20}IN_4O_2$ requires 535.0631), 409 (9), 304 (7), 282 (29), 119 (21), 96 (41), 85 (50), 56 (100).

5-Hydroxy-2-nitro-4-(trifluoromethyl)aniline (217)



A mixture of pyridine (0.69 mL, 8.4 mmol) and 37% aqueous hydrochloric acid (0.75 mL, 9.1 mmol), was distilled under Ar at 250 °C to remove water. The mixture was cooled to 140 °C before adding 5-methoxy-2-nitro-4-(trifluoromethyl)aniline (**195**) (0.100 g, 0.423 mmol). After stirring at 150 °C for 8 h the reaction mixture was allowed to cool to room temperature. The mixture was then diluted with water (10 mL) and brine (5 mL), extracted with ethyl acetate (9 × 15 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 15−30% ethyl acetate in petroleum ether (40−60) to give 5-hydroxy-2-nitro-4-(trifluoromethyl)aniline (**217**) as a yellow solid (0.057 g, 61%). Mp 196−198 °C (decomposition); ν_{max}/cm^{-1} (neat) 3505 (NH), 3387 (NH), 3187 (OH), 1634 (C=C), 1568, 1416, 1310, 1201, 1103, 922, 841; δ_H (400 MHz, $CDCl_3$) 6.47 (1H, s, 6-H), 7.76 (2H, br s, NH_2), 8.15 (1H, s, 3-H), 11.54 (1H, br s, OH); δ_C (101 MHz, $CDCl_3$) 102.0 (CH), 106.3 (q, $J_{C\text{-}C\text{-}F}$ 31.6 Hz, C), 123.3 (q, $J_{C\text{-}F}$ 270.5 Hz, C), 123.4 (C), 126.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.7 Hz, CH), 150.0 (C), 160.6 (C); *m/z* (EI) 222.0248 (M^+. $C_7H_5F_3N_2O_3$ requires 222.0252), 203 (6%), 176 (8), 172 (7), 128 (6), 84 (100).

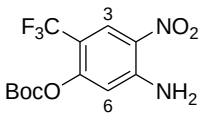
5-Methoxymethyloxy-2-nitro-4-(trifluoromethyl)aniline (218)



To a solution of 5-hydroxy-2-nitro-4-(trifluoromethyl)aniline (**217**) (0.020 g, 0.090 mmol) in dichloromethane (0.5 mL) at 0 °C was added *N*,*N*-diisopropylethylamine (0.024 mL, 0.135 mmol) and bromomethyl methyl ether (0.008 mL, 0.099 mmol). The solution was

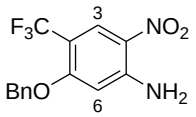
warmed to room temperature and stirred for 3 h. The reaction mixture was then diluted with water (10 mL), extracted with dichloromethane (3 × 10 mL) and ethyl acetate (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography eluting with 10−20% ethyl acetate in petroleum ether (40−60) to give 5-methoxymethyloxy-2-nitro-4-(trifluoromethyl)aniline (**218**) (0.014, 58%). Mp 127−128 °C; ν_{max}/cm^{-1} (neat) 3472 (NH), 3337 (NH), 2916 (CH), 1645 (C=C), 1570, 1306, 1225, 1084, 921, 853; δ_H (400 MHz, $CDCl_3$) 3.50 (3H, s, CH_3), 5.28 (2H, s, CH_2), 6.43 (2H, br s, NH_2), 6.53 (1H, s, 6-H), 8.43 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 56.8 (CH_3), 94.5 (CH_2), 102.1 (CH), 110.2 (q, $J_{C\text{-}C\text{-}F}$ 32.8, C), 122.8 (q, $J_{C\text{-}F}$ 271.2 Hz, C), 125.9 (C), 127.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.6 Hz, CH), 148.6 (C), 159.8 (C); *m/z* (EI) 266.0510 (M^+. $C_9H_9F_3N_2O_4$ requires 266.0514), 247 (10%), 235 (5), 159 (5), 78 (23).

5-(*tert*-Butoxycarbonyloxy)-2-nitro-4-(trifluoromethyl)aniline (219)



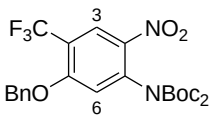
To a solution of 5-hydroxy-2-nitro-4-(trifluoromethyl)aniline (**217**) (0.040 g, 0.18 mmol) in dichloromethane (2 mL) was added di-*tert*-butyl dicarbonate (0.039 g, 0.18 mmol), 4-dimethylaminopyridine (0.002 g, 0.018 mmol) and triethylamine (0.025 mL, 0.18 mmol) and the solution stirred at room temperature for 4 h. The reaction mixture was then diluted with water (5 mL) and extracted with dichloromethane (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 20% ethyl acetate in petroleum ether (40−60) to give 5-(*tert*-butoxycarbonyloxy)-2-nitro-4-(trifluoromethyl)aniline (**219**) as a yellow solid (0.039 g, 67%). Mp 109−110 °C; ν_{max}/cm^{-1} (neat) 3491 (NH), 3383 (NH), 3003 (CH), 1763 (CO), 1653 (C=C), 1568, 1240, 1121, 1055, 916; δ_H (500 MHz, $CDCl_3$) 1.49 (9H, s, 3 × CH_3), 7.02 (1H, s, 6-H), 8.02 (2H, br s, NH_2), 8.27 (1H, s, 3-H); δ_C (126 MHz, $CDCl_3$) 27.0 (3 × CH_3), 85.0 (C), 108.4 (q, $J_{C\text{-}C\text{-}F}$ 32.8, C), 113.4 (CH), 122.5 (q, $J_{C\text{-}F}$ 270.6, C), 126.2 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.2 Hz, CH), 127.3 (C), 149.2 (C), 149.6 (C), 151.4 (C); *m/z* (EI) 322.0771 (M^+. $C_{12}H_{13}F_3N_2O_5$ requires 322.0777), 307 (12%), 279 (9), 222 (100), 203 (37), 176 (17), 149 (25).

5-Benzyloxy-2-nitro-4-(trifluoromethyl)aniline (220)



To a solution of 5-chloro-2-nitro-4-(trifluoromethyl)aniline (**190**) (1.30 g, 5.40 mmol) in benzyl alcohol (17 mL) was added tetra-*n*-butylammonium bromide (0.087 g, 0.027 mmol) and potassium hydroxide (0.727 g, 13.0 mmol). The reaction mixture was heated to 60 °C and stirred for 48 h. After cooling to room temperature the reaction was quenched with 1 M aqueous hydrochloric acid (100 mL), extracted with ethyl acetate (3 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The addition of diethyl ether (50 mL) and petroleum ether (40–60) (50 mL) resulted in the formation of a precipitate that was collected by filtration to give 5-benzyloxy-2-nitro-4-(trifluoromethyl)aniline (**220**) as a yellow solid (1.01, 60%). Mp 146–147 °C; (Found: C, 53.84; H, 3.48; N, 8.86. $C_{14}H_{11}F_3N_2O_3$ requires C, 53.85; H, 3.55; N, 8.97%); ν_{max}/cm^{-1} (neat) 3350 (NH), 1640 (C=C), 1576, 1451, 1335, 1236, 1103, 907, 833; δ_H (400 MHz, $CDCl_3$) 5.19 (2H, s, CH_2), 6.25 (1H, s, 6-H), 6.43 (2H, br s, NH_2), 7.32–7.44 (5H, m, Ph), 8.45 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 70.8 (CH_2), 100.1 (CH), 110.1 (q, $J_{C\text{-}C\text{-}F}$ 33.0 Hz, C), 122.7 (q, $J_{C\text{-}F}$ 271.2 Hz, C), 125.3 (C), 126.7 (2 × CH), 127.4 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.7 Hz, CH), 128.4 (CH), 128.8 (2 × CH), 134.9 (C), 148.6 (C), 161.1 (C); *m/z* (CI) 313 (MH^+, 100%), 283 (80), 223 (100), 147 (54), 119 (63), 89 (90).

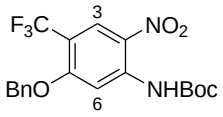
5-Benzyloxy-1-bis[*tert*-butoxycarbonyl)amino]-2-nitro-4-(trifluoromethyl)benzene (221)



To a solution of 5-benzyloxy-2-nitro-4-(trifluoromethyl)aniline (**220**) (1.70 g, 5.44 mmol) in dichloromethane (60 mL) was added di-*tert*-butyl dicarbonate (2.61 g, 12.0 mmol), 4-dimethylaminopyridine (0.130 g, 1.09 mmol) and triethylamine (1.67 mL, 12.0 mmol) and the solution stirred at room temperature for 18 h. The reaction mixture was then diluted with water (60 mL), extracted with dichloromethane (2 × 50 mL), dried ($MgSO_4$) and

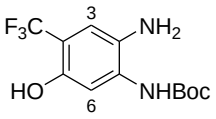
concentrated *in vacuo*. The resulting material was then purified by flash chromatography eluting with 20% ethyl acetate in petroleum ether (40–60) to give 5-benzyloxy-1-bis[*tert*-butoxycarbonyl)amino]-2-nitro-4-(trifluoromethyl)benzene (**221**) as a white solid (2.73 g, 98%). Mp 166–168 °C; (Found: C, 56.17; H, 5.29; N, 5.48. $C_{24}H_{27}F_3N_2O_7$ requires C, 56.25; H, 5.31; N, 5.47%); ν_{max}/cm^{-1} (neat) 2992 (CH), 1790 (CO), 1624 (C=C), 1530, 1343, 1233, 1150, 1098, 912, 847; δ_H (500 Hz, $CDCl_3$) 1.37 (18H, s, 6 × CH_3), 5.30 (2H, s, CH_2), 6.90 (1H, s, 6-H), 7.33–7.41 (5H, m, Ph), 8.45 (1H, s, 3-H); δ_C (126 MHz, $CDCl_3$) 27.7 (6 × CH_3), 71.3 (CH_2), 84.4 (2 × C), 115.8 (CH), 119.2 (q, $J_{C\text{-}C\text{-}F}$ 33.0 Hz, C), 122.0 (q, $J_{C\text{-}F}$ 272.9 Hz, C), 125.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.4 Hz, CH), 126.8 (2 × CH), 128.6 (CH), 129.0 (2 × CH), 134.4 (C), 137.9 (C), 138.7 (C), 149.8 (C), 159.8 (C); *m/z* (CI) 413 (MH^+−CO_2t-Bu, 59%), 401 (26), 375 (39), 357 (100), 327 (33), 313 (15), 267 (23), 237 (14), 113 (13).

5-Benzyloxy-1-(*tert*-butoxycarbonylamino)-2-nitro-4-(trifluoromethyl)benzene (222)



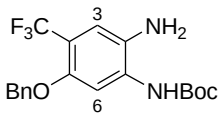
To a solution of 5-benzyloxy-1-[bis(*tert*-butoxycarbonyl)amino]-2-nitro-4-(trifluoromethyl)benzene (**221**) (2.71 g, 5.29 mmol) in dichloromethane (73 mL) at 0 ºC was added trifluoroacetic acid (0.81 mL, 11 mmol). The reaction mixture was then warmed to room temperature and stirred for 2 h. The reaction mixture was diluted with a saturated aqueous solution of sodium hydrogen carbonate (50 mL), extracted with dichloromethane (2 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 5-benzyloxy-1-(*tert*-butoxycarbonylamino)-2-nitro-4-(trifluoromethyl)benzene (**222**) as a yellow solid (2.12 g, 97%). Mp 159–160 °C; (Found: C, 55.14; H, 4.59; N, 6.74. $C_{19}H_{19}F_3N_2O_5$ requires C, 55.34; H, 4.65; N, 6.79%); ν_{max}/cm^{-1} (neat) 3343 (NH), 2992 (CH), 1732 (CO), 1632 (C=C), 1580, 1439, 1341, 1236, 1140, 978, 843; δ_H (400 MHz, $CDCl_3$) 1.57 (9H, s, 3 × CH_3), 5.30 (2H, s, CH_2), 7.32–7.50 (5H, m, Ph), 8.50 (1H, s, 6-H), 8.52 (1H, s, 3-H), 10.18 (1H, br s, NH); δ_C (101 MHz, $CDCl_3$) 28.1 (3 × CH_3), 71.2 (CH_2), 82.7 (C), 102.8 (CH), 113.1 (q, $J_{C\text{-}C\text{-}F}$ 33.0 Hz, C), 122.4 (q, $J_{C\text{-}F}$ 272.0 Hz, C), 126.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.5 Hz, CH), 127.4 (2 × CH), 127.7 (C), 128.5 (CH), 128.7 (2 × CH), 134.7 (C), 141.4 (C), 151.9 (C), 161.6 (C); *m/z* (CI) 413 (MH^+, 37%), 383 (11), 357 (100), 327 (13), 313 (13), 267 (23), 223 (12).

2-Amino-1-(*tert*-butoxycarbonylamino)-5-hydroxy-4-(trifluoromethyl)benzene (223)



To a solution of 5-benzyloxy-1-(*tert*-butoxycarbonylamino)-2-nitro-4-(trifluoromethyl)benzene (**222**) (1.16 g, 2.81 mmol) in methanol (40 mL) was added 10% palladium on carbon (0.116 g). The reaction mixture was stirred under a hydrogen atmosphere for 17 h. The mixture was then filtered through Celite® and concentrated *in vacuo*. The resulting material was then recrystallised from dichloromethane/petroleum ether (40–60) to give 2-amino-1-(*tert*-butoxycarbonylamino)-5-hydroxy-4-(trifluoromethyl)benzene (**223**) as a white solid (0.729 g, 89%). Mp 220–222 °C (decomposition); (Found: C, 48.94; H, 5.14; N, 9.29. $C_{12}H_{15}F_3N_2O_3$ requires C, 49.32; H, 5.17; N, 9.59%); ν_{max}/cm^{-1} (neat) 3331 (NH), 2993 (CH), 2739, 1678 (CO), 1518, 1433, 1277, 1155, 1119, 1074, 905, 831; δ_H (400 MHz, DMSO-d_6) 1.48 (9H, s, 3 × CH_3), 4.63 (2H, br s, NH_2), 6.86 (1H, s, 3-H), 7.30 (1H, s, 6-H), 8.40 (1H, br s, NH), 9.42 (1H, br s, OH); δ_C (101 MHz, DMSO-d_6) 28.1 (3 × CH_3), 79.4 (C), 110.1 (q, $J_{C\text{-}C\text{-}F}$ 29.8 Hz, C), 110.2 (CH), 113.2 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.1 Hz, CH), 124.3 (q, $J_{C\text{-}F}$ 271.4 Hz, C), 129.4 (C), 131.0 (C), 146.8 (C), 152.9 (C); *m/z* (CI) 293 (MH^+, 39%), 237 (100), 193 (20), 113 (31).

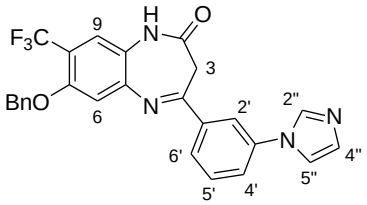
2-Amino-5-benzyloxy-1-(*tert*-butoxycarbonylamino)-4-(trifluoromethyl)benzene (225)



To a solution of 5-benzyloxy-1-(*tert*-butoxycarbonylamino)-2-nitro-4-(trifluoromethyl)benzene (**222**) (2.10 g, 5.09 mmol) in ethanol (145 mL) was added tin(II) chloride dihydrate (5.75 g, 25.5 mmol). The reaction mixture was heated to 70 °C and stirred for 6 h. After cooling to room temperature, the mixture was concentrated *in vacuo*. The resulting solid was dissolved in ethyl acetate (100 mL) and a saturated aqueous solution of sodium hydrogen carbonate (100 mL) was added. The mixture was then extracted with ethyl acetate (3 × 70 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 20% ethyl

acetate in petroleum ether (40–60) to give 2-amino-5-benzyloxy-1-(*tert*-butoxycarbonylamino)-4-(trifluoromethyl)benzene (**225**) as an off-white solid (1.91 g, 98%). Mp 129–130 °C; ν_{max}/cm^{-1} (neat) 3360 (NH), 2992 (CH), 1688 (CO), 1597 (C=C), 1497, 1437, 1296, 1223, 1123, 1063, 883; δ_H (400 MHz, $CDCl_3$) 1.54 (9H, s, 3 × CH_3), 3.12 (2H, br s, NH_2), 5.13 (2H, s, CH_2), 6.87 (1H, br s, NH), 7.07 (1H, s, 3-H), 7.28−7.49 (5H, m, Ph), 7.66 (1H, s, 6-H); δ_C (101 MHz, $CDCl_3$) 28.3 (3 × CH_3), 70.9 (CH_2), 81.2 (C), 106.4 (CH), 113.9 (q, $J_{C\text{-}C\text{-}F}$ 31.9 Hz, C), 118.9 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.2 Hz, CH), 123.6 (q, $J_{C\text{-}F}$ 271.7 Hz, C), 127.1 (2 × CH), 127.1 (C), 127.8 (CH), 128.5 (2 × CH), 133.3 (C), 136.6 (C), 152.0 (C), 152.8 (C); *m/z* (CI) 383.1590 (MH^+. $C_{19}H_{22}F_3N_2O_3$ requires 383.1583), 327 (33%), 283 (18), 113 (26), 71 (100).

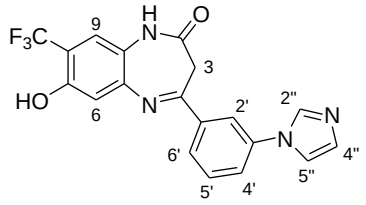
7-Benzyloxy-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (227)



To a solution of 2-amino-5-benzyloxy-1-(*tert*-butoxycarbonylamino)-4-(trifluoromethyl)benzene (**225**) (0.250 g, 0.654 mmol) in toluene (1.5 mL) was added *tert*-butyl 3-[3'-(1"*H*-imidazol-1"-yl]phenyl)-3-oxopropanoate (**145**) (0.223 g, 0.779 mmol). The solution was heated under reflux for 4 h before cooling to room temperature and concentrating *in vacuo*. The resulting material was purified by flash column chromatography eluting with 0–5% ethanol in dichloromethane to give 5-benzyloxy-1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-4-(trifluoromethyl)benzene (**226**) as a white solid (0.286 g, 74%). To a solution of 5-benzyloxy-1-(*tert*-butoxycarbonylamino)-2-{3'-[3"-(1"'*H*-imidazol-1"'-yl)phenyl]-3'-oxopropanamido}-4-(trifluoromethyl)benzene (**226**) (0.085 g, 0.14 mmol) in dichloromethane (0.75 mL) at 0 °C was added trifluoroacetic acid (0.25 mL). The reaction mixture was warmed to room temperature and stirred for 2 h. The solution was diluted with dichloromethane (5 mL). A saturated aqueous solution of sodium hydrogen carbonate (5 mL) was added, the mixture extracted with dichloromethane (3 × 5 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 7-benzyloxy-4-[3'-(1"*H*-imidazol-1"-yl)phenyl]-8-

trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**227**) as a white solid (0.059 g, 88%). Mp 209−211 °C (decomposition); ν_{max}/cm^{-1} (neat) 3100 (NH), 2870 (CH), 1674 (CO), 1493, 1404, 1306, 1223, 1121, 1055, 914, 895; δ_H (400 MHz, DMSO-d_6) 3.70 (2H, s, 3-H_2), 5.31 (2H, s, OCH_2Ph), 7.16 (1H, br s, 4”-H), 7.32−7.52 (7H, m, 6-H, 9-H and Ph), 7.71 (1H, t, *J* 7.9 Hz, 5’-H), 7.84−7.90 (2H, m, 4’-H and 5”-H), 8.06 (1H, br d, *J* 7.9 Hz, 6’-H), 8.26 (1H, t, *J* 1.8 Hz, 2’-H), 8.36 (1H, br s, 2”-H), 10.60 (1H, br s, NH); δ_C (126 MHz, DMSO-d_6) 40.1 (CH_2), 70.1 (CH_2), 111.8 (CH), 115.5 (q, $J_{C\text{-}C\text{-}F}$ 30.9 Hz, C), 118.3 (CH), 119.8 (CH), 120.9 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.2 Hz, CH), 123.4 (q, $J_{C\text{-}F}$ 271.9 Hz, C), 123.4 (C), 123.6 (CH), 126.3 (CH), 127.1 (2 × CH), 128.0 (CH), 128.6 (2 × CH), 130.1 (CH), 130.5 (CH), 135.9 (CH), 136.4 (C), 137.4 (C), 138.4 (C), 143.2 (C), 151.8 (C), 160.1 (C), 166.0 (C); *m/z* (ESI) 477.1517 (MH^+. $C_{26}H_{20}F_3N_4O_2$ requires 477.1533).

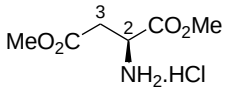
7-Hydroxy-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (214)



To a suspension of 7-benzyloxy-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**227**) (0.150 g, 0.315 mmol) in dichloromethane (4 mL) was added boron tribromide (1.0 M in dichloromethane) (1.55 mL, 1.55 mmol). After stirring for 8 h, the solution was diluted with a saturated aqueous solution of sodium hydrogen carbonate (5 mL), extracted with ethyl acetate (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting solid was then triturated with dichloromethane to give 7-hydroxy-4-[3’-(1”*H*-imidazol-1”-yl)phenyl]-8-trifluoromethyl-2,3-dihydro-1*H*-1,5-benzodiazepin-2-one (**214**) as a pale yellow solid (0.099 g, 81%). Mp 232−234 °C (decomposition); ν_{max}/cm^{-1} (neat) 3071 (NH), 2922 (CH), 2743, 1651 (CO), 1582, 1491, 1412, 1317, 1221, 1105, 1059, 889; δ_H (500 MHz, DMSO-d_6) 3.65 (2H, s, 3-H_2), 7.03 (1H, s, 6-H), 7.15, (1H, s, 4”-H), 7.40 (1H, s, 9-H), 7.69, (1H, t, *J* 7.9 Hz, 5’-H). 7.79−7.87 (2H, m, 4’-H and 5”-H), 8.04 (1H, br d, *J* 7.9 Hz, 6’-H), 8.21 (1H, br s, 2’-H), 8.32 (1H, s, 2”-H), 10.37 (1H, s, NH), 10.51 (1H, br s, OH); δ_C (126 MHz, DMSO-d_6) 39.9 (CH_2), 113.8 (CH), 114.0 (q, $J_{C\text{-}C\text{-}F}$ 30.4 Hz, C), 118.1 (CH), 119.6 (CH), 120.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ 5.3 Hz, CH),

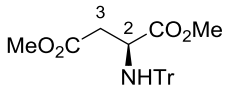
122.1 (C), 123.4 (CH), 123.4 (q, $J_{C\text{-}F}$ 272.0 Hz, C), 126.1 (CH), 129.9 (CH), 130.2 (CH), 135.7 (CH), 137.3 (C), 138.5 (C), 143.0 (C), 151.4 (C), 159.8 (C), 165.8 (C); *m/z* (EI) 386.0984 (M^+. $C_{19}H_{13}F_3N_4O_2$ requires 386.0991), 344 (50%), 324 (46), 296 (12), 169 (8), 84 (14).

Dimethyl (2*S*)-2-aminobutandioate hydrochloride (259)[149]



To a suspension of L-aspartic acid (**255**) (5.00 g, 37.6 mmol) in dry methanol (100 mL) at 0 °C under argon was added thionyl chloride (3.80 mL, 52.6 mmol). The mixture was allowed to warm to room temperature and stirred under reflux for 3 h. The solution was allowed to cool to room temperature and concentrated *in vacuo*, azeotroping with toluene to give dimethyl (2*S*)-2-aminobutandioate hydrochloride (**259**) as a white solid (7.41 g, 100%). Mp 115–116 °C (lit.,[149] 114–115 °C); $[\alpha]_D^{24}$ +22.0 (*c* 1.0, MeOH), lit.,[185] $[\alpha]_D^{25}$ +16.4 (*c* 4.4, MeOH); δ_H (400 MHz, $CDCl_3$) 3.26 (1H, dd, *J* 18.0, 4.4 Hz, 3-*H*H), 3.35 (1H, dd, *J* 18.0, 3.2 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.63 (1H, br s, 2-H), 8.73 (3H, br s, NH_3^+); δ_C (101 MHz, $CDCl_3$) 33.9 (CH_2), 49.7 (CH), 52.7 (CH_3), 53.7 (CH_3), 168.7 (C), 170.6 (C); *m/z* (CI) 162 (MH^+, 100%), 148 (5), 102 (20).

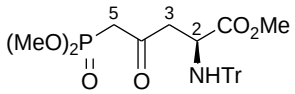
Dimethyl (2*S*)-2-(tritylamino)butandioate (260)[175]



To a suspension of dimethyl (2*S*)-2-aminobutandioate hydrochloride (**259**) (7.38 g, 37.3 mmol) in dichloromethane (300 mL), at 0 °C under argon was added triethylamine (10.4 mL, 74.6 mmol) dropwise, followed by triphenylmethyl chloride (12.5 g, 44.8 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 6 h. The mixture was diluted with dichloromethane (50 mL), washed with 2.0 M citric acid (300 mL), water (150 mL), brine (150 mL), dried ($MgSO_4$) and concentrated *in vacuo.* The resulting material was purified by flash column chromatography eluting with 20% diethyl

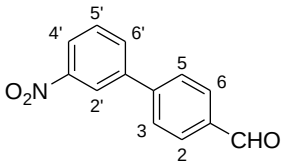
ether in petroleum ether (40−60) to give dimethyl (2*S*)-2-(tritylamino)butandioate (**260**) as a white solid (12.6 g, 84%). Mp 70–71 °C (lit.,[175] 71−72 °C); $[\alpha]_D^{25}$ +43.7 (*c* 1.0, $CHCl_3$), lit.,[175] $[\alpha]_D^{23}$ +36.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.50 (1H, dd, *J* 14.7, 7.0 Hz, 3-*H*H), 2.65 (1H, dd, *J* 14.7, 5.4 Hz, 3-H*H*), 2.93 (1H, *J* 9.8 Hz, NH), 3.26 (3H, s, OCH_3), 3.65–3.73 (4H, m, 2-H and OCH_3), 7.15–7.20 (3H, m, 3 × Ar-H), 7.23–7.28 (6H, m, 6 × Ar-H), 7.46–7.51 (6H, m, 6 × Ar-H); δ_C (101 MHz, $CDCl_3$) 40.2 (CH_2), 51.8 (CH_3), 52.0 (CH_3), 53.7 (CH), 71.2 (C), 126.5 (3 × CH), 127.9 (6 × CH), 128.8 (6 × CH), 145.7 (3 × C), 171.0 (C), 173.9 (C); *m/z* (CI) 404 (MH^+, 2%), 326 (9), 285 (11), 243 (100), 162 (95).

Methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (261)[175]



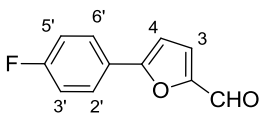
To a solution of dimethyl methylphosphonate (4.44 mL, 39.7 mmol) in dry tetrahydrofuran (125 mL) at –78 °C under argon was added *n*-butyllithium (1.6 M in hexanes) (23.3 mL, 37.2 mmol) dropwise. After stirring at –78 °C for 1 h, the solution was added via cannula to a stirring solution of dimethyl (2*S*)-2-(tritylamino)butandioate (**260**) (5.00 g, 12.4 mmol) in dry tetrahydrofuran (125 mL) at –78 °C. After stirring at –78 °C under argon for 3 h, the reaction was quenched by the addition of a saturated aqueous solution of ammonium chloride (80 mL). The solution was then concentrated *in vacuo*, redissolved in ethyl acetate (200 mL), washed with water (100 mL) and brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 80% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**261**) as a white solid (4.65 g, 76%). Mp 116−117 °C (lit.,[175] 117−119 °C); $[\alpha]_D^{25}$ +26.5 (*c* 0.5, $CHCl_3$), lit.,[175] $[\alpha]_D^{24}$ +31.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.78 (1H, dd, *J* 16.7, 6.9 Hz, 3-*H*H), 2.84−2.96 (2H, m, 3-H*H* and NH), 3.05 (1H, d, *J* 22.7, 5-*H*H), 3.06 (1H, d, *J* 22.7, 5-H*H*), 3.29 (3H, s, OCH_3), 3.70 (1H, ddd, *J* 9.6, 6.9, 4.7 Hz, 2-H), 3.76 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 7.16–7.21 (3H, m, 3 × Ar-H), 7.23–7.29 (6H, m, 6 × Ar-H), 7.45–7.49 (6H, m, 6 × Ar-H); δ_C (101 MHz, $CDCl_3$) 41.9 (d, $J_{C\text{-}P}$ 127.8 Hz, CH_2), 48.8 (CH_2), 52.0 (CH_3), 52.9 (CH), 53.1 (d, $J_{C\text{-}O\text{-}P}$ 6.0 Hz, 2 × CH_3), 71.3 (C), 126.6 (3 × CH), 127.9 (6 × CH), 128.8 (6 × CH), 145.7 (3 × C), 174.0 (C), 199.3 (d, $J_{C\text{-}C\text{-}P}$ 6.6 Hz, C); *m/z* (CI) 496 (MH^+, 18%), 473 (27), 254 (56), 243 (100).

4-(3'-Nitrophenyl)benzaldehyde (270)[186]



To a solution of 4-bromobenzaldehyde (**269**) (0.100 g, 0.540 mmol) in *N*,*N*-dimethylformamide and water (3 mL, 19:1) was added 3-nitrophenylboronic acid (0.135 g, 0.809 mmol), potassium carbonate (0.187 g, 1.35 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.031 g, 0.027 mmol). The reaction mixture was heated to 110 ºC and stirred for 4 h. After cooling to room temperature, the solution was concentrated *in vacuo*, redissolved in chloroform (10 mL), filtered through Celite® and concentrated *in vacuo*. The resulting solid was dissolved in diethyl ether (20 mL), washed with water (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography eluting with 10% ethyl acetate in petroleum ether (40–60) to give 4-(3'-nitrophenyl)benzaldehyde (**270**) as an off-white solid (0.123 g, 100%). Mp 113−114 °C (lit.,[186] 114−116 °C); δ_H (400 MHz, $CDCl_3$) 7.68 (1H, t, *J* 8.0 Hz, 5'-H), 7.81 (2H, d, *J* 8.3 Hz, 3-H and 5-H), 7.95−8.00 (1H, m, 6'-H), 8.03 (2H, d, *J* 8.3 Hz, 2-H and 6-H), 8.26−8.31 (1H, m, 4'-H), 8.51 (1H, t, *J* 2.0 Hz, 2'-H), 10.10 (1H, s, CHO); δ_C (101 MHz, $CDCl_3$) 122.4 (CH), 123.3 (CH), 128.0 (2 × CH), 130.2 (CH), 130.7 (2 × CH), 133.4 (CH), 136.2 (C), 141.6 (C), 144.5 (C), 149.0 (C), 191.8 (CH); *m/z* (CI) 228 (MH^+, 100%), 198 (10).

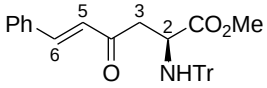
5-(4'-Fluorophenyl)-2-furaldehyde (272)[187]



The reaction was carried out according to the above procedure using 5-bromo-2-furaldehyde (**271**) (0.100 g, 0.571 mmol), 4-fluorophenylboronic acid (0.120 g, 0.858 mmol), potassium carbonate (0.198 g, 1.43 mmol) and tetrakis (triphenylphosphine)palladium(0) (0.035 g, 0.030 mmol) in *N*,*N*-dimethylformamide and water (8 mL, 9:1), to give 5-(4'-fluorophenyl)-2-furaldehyde (**272**) as an off-white solid

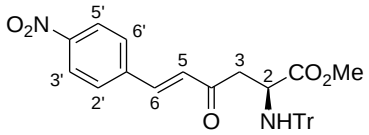
(0.108 g, 100%). Mp 76−77 °C (lit.,[187] 79−80 °C); δ_H (400 MHz, $CDCl_3$) 6.79 (1H, d, *J* 3.7 Hz, 4-H), 7.11−7.18 (2H, m, 3'-H and 5'-H), 7.32 (1H, d, *J* 3.7 Hz, 3-H), 7.79−7.85 (2H, m, 2'-H and 6'-H), 9.64 (1H, s, CHO); δ_C (101 MHz, $CDCl_3$) 107.5 (CH), 116.3 (d, $J_{C\text{-}C\text{-}F}$, 22.3 Hz, 2 × CH), 123.9 (CH), 125.5, (d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ 3.4 Hz, C), 127.5 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 8.4 Hz, 2 × CH), 152.2 (C), 158.7 (C), 163.6 (d, $J_{C\text{-}F}$ 250.9 Hz, C), 177.3 (CH); *m/z* (CI) 191 (MH^+, 100%).

Methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (262)[175]



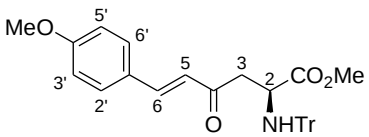
To a solution of methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**261**) (1.50 g, 3.03 mmol) in dry acetonitrile (30 mL) was added potassium carbonate (0.440 g, 3.18 mmol) and benzaldehyde (0.620 mL, 6.06 mmol). The reaction mixture was heated to 50 °C and stirred for 48 h. The mixture was then allowed to cool to room temperature before concentrating *in vacuo*. The resulting residue was then dissolved in ethyl acetate (80 mL), diluted with water (40 mL), extracted with ethyl acetate (3 × 40 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was purified by flash column chromatography eluting with 20% diethyl ether in petroleum ether (40−60) to give methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**262**) as a colourless oil (1.29 g, 90%). $[\alpha]_D^{25}$ +61.2 (*c* 0.5, $CHCl_3$), lit.,[175] $[\alpha]_D^{25}$ +111 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.79 (1H, dd, *J* 15.2, 7.0 Hz, 3-*H*H), 2.88−2.95 (1H, m, 3-H*H* and NH), 3.28 (3H, s, OCH_3), 3.79 (1H, ddd, *J* 9.9, 7.0, 5.3 Hz, 2-H), 6.69 (1H, d, *J* 16.2 Hz, 5-H), 7.15−7.20 (9H, m, 9 × Ar-H), 7.38−7.55 (12H, m, 6-H and 11 × Ar-H); δ_C (101 MHz, $CDCl_3$) 45.7 (CH_2), 52.0 (CH_3), 53.8 (CH), 71.3 (C), 126.4 (CH), 126.5 (3 × CH), 127.9 (6 × CH), 128.4 (2 × CH), 128.8 (6 × CH), 129.0 (2 × CH), 130.6 (CH), 134.4 (C), 143.3 (CH), 145.8 (3 × C), 174.5 (C), 197.5 (C); *m/z* (FAB) 476 (MH^+, 6%), 398 (10), 307 (10), 243 (100), 232 (18).

Methyl (2*S*,5*E*)-6-(4'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (263)[188]



The reaction was carried out according to the above procedure using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**261**) (2.00 g, 4.04 mmol), potassium carbonate (0.613 g, 4.44 mmol) and 4-nitrobenzaldehyde (1.22 g, 8.08 mmol) in dry acetonitrile (40 mL). The crude material was purified by flash column chromatography eluting with 30% diethyl ether in petroleum ether (40−60) to give methyl (2*S*,5*E*)-6-(4'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**263**) as a pale yellow oil (1.42 g, 68%). $[\alpha]_D^{23}$ +57.0 (*c* 1.0, $CHCl_3$), lit.,[188] $[\alpha]_D^{25}$ +43.3 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.79 (1H, dd, *J* 15.4, 6.9 Hz, 3-*H*H), 2.90 (1H, dd, *J* 15.4, 5.1 Hz, 3-H*H*), 2.94 (1H, d, *J* 10.1 Hz, NH), 3.31 (3H, s, OCH_3), 3.76–3.84 (1H, m, 2-H), 6.76 (1H, d, *J* 16.2 Hz, 5-H), 7.16–7.21 (3H, m, 3 × Ar-H), 7.22–7.28 (6H, m, 6 × Ar-H), 7.45–7.52 (7H, m, 6-H and 6 × Ar-H), 7.67 (2H, d, *J* 8.8 Hz, 2'-H and 6'-H), 8.26 (2H, d, *J* 8.8 Hz, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 46.2 (CH_2), 52.1 (CH_3), 53.7 (CH), 71.3 (C), 124.2 (2 × CH), 126.6 (3 × CH), 128.0 (6 × CH), 128.8 (6 × CH), 128.9 (2 × CH), 129.6 (CH), 139.9 (CH), 140.6 (C), 145.7 (3 × C), 148.7 (C), 174.3 (C), 196.9 (C); *m/z* (FAB) 521 (MH^+, 2%), 460 (6), 443 (5), 307 (46), 289 (26), 243 (42), 154 (100).

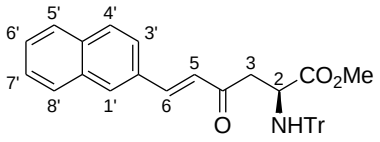
Methyl (2*S*,5*E*)-6-(4'-methoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (264)[189]



The reaction was carried out according to the above procedure using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**261**) (0.600 g, 1.21 mmol), potassium carbonate (0.176 g, 1.27 mmol) and 4-methoxybenzaldehyde (0.291 mL, 2.42 mmol) in dry acetonitrile (12 mL). The crude material was purified by flash column chromatography eluting with 30% ethyl acetate in petroleum ether (40−60) to give methyl

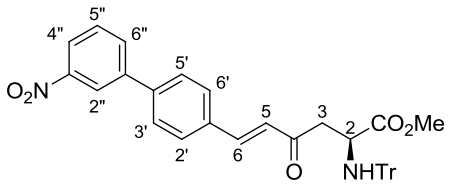
(2*S*,5*E*)-6-(4'-methoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**264**) as a colourless oil (0.328 g, 54%). $[\alpha]_D^{26}$ +44.6 (*c* 0.5, $CHCl_3$), lit.,[189] $[\alpha]_D^{23}$ +54.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.78 (1H, dd, *J* 15.0, 7.0 Hz, 3-*H*H), 2.85−2.95 (2H, m, 3-H*H* and NH), 3.26 (3H, s, OCH_3), 3.75−3.85 (4H, m, 2-H and OCH_3), 6.58 (1H, *J* 16.2 Hz, 5-H), 6.91 (2H, d, *J* 8.8 Hz, 3'-H and 5'-H), 7.13−7.19 (3H, m, 3 × Ar-H), 7.20−7.27 (6H, m, 6 × Ar-H), 7.42−7.53 (9H, 6-H, 2'-H, 6'-H and 6 × Ar-H); δ_C (101 MHz, $CDCl_3$) 45.7 (CH_2), 52.0 (CH_3), 53.9 (CH), 55.4 (CH_3), 71.3 (C), 114.5 (2 × CH), 124.3 (CH), 126.5 (3 × CH), 127.1 (C), 127.9 (6 × CH), 128.9 (6 × CH), 130.1 (2 × CH), 143.2 (CH), 145.8 (3 × C), 161.8 (C), 174.5 (C), 197.5 (C); *m/z* (FAB) 506 (MH^+, 7%), 428 (6), 262 (10), 243 (100), 161 (20).

Methyl (2*S*,5*E*)-6-(naphthalen-2'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (265)[175]



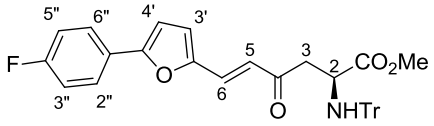
The reaction was carried out according to the above procedure using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**261**) (0.600 g, 1.21 mmol), potassium carbonate (0.176 g, 1.27 mmol) and 2-naphthaldehyde (0.378 g, 2.42 mmol) in dry acetonitrile (12 mL). The crude material was purified by flash column chromatography eluting with 10−30% diethyl ether in petroleum ether (40−60) to give methyl (2*S*,5*E*)-6-(naphthalen-2'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**265**) as a white solid (0.563 g, 89%). Mp 63−64 °C (lit.,[175] 62−63 °C); $[\alpha]_D^{26}$ +52.7 (*c* 1.0, $CHCl_3$), lit.,[175] $[\alpha]_D^{24}$ +64.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.84 (1H, dd, *J* 15.0, 7.0 Hz, 3-*H*H), 2.94 (1H, d, *J* 10.0 Hz, NH), 2.97 (1H, dd, *J* 15.0, 5.3 Hz, 3-H*H*), 3.28 (3H, s, OCH_3), 3.83 (1H, ddd, *J* 10.0, 7.0, 5.3 Hz, 2-H), 6.80 (1H, d, *J* 16.2 Hz, 5-H), 7.15−7.20 (3H, m, 3 × Ar-H), 7.22−7.29 (6H, m, 6 × Ar-H), 7.46−7.56 (8H, m, 6'-H, 7'-H and 6 × Ar-H), 7.65 (1H, d, *J* 16.2 Hz, 6-H), 7.66 (1H, dd, *J* 8.6, 1.7 Hz, 3'-H), 7.81−7.89 (3H, m, 4'-H, 5'-H and 8'-H), 7.94 (1H, br s, 1'-H); δ_C (101 MHz, $CDCl_3$) 45.8 (CH_2), 52.0 (CH_3), 53.9 (CH), 71.3 (C), 123.5 (CH), 126.5 (CH), 126.6 (3 × CH), 126.8 (CH), 127.4 (CH), 127.8 (CH), 127.9 (6 × CH), 128.1 (CH), 128.6 (CH), 128.8 (6 × CH), 130.6 (CH), 131.9 (C), 133.3 (C), 134.4 (C), 143.4 (CH), 145.8 (3 × C), 174.5 (C), 197.5 (C); *m/z* (FAB) 526 (MH^+, 5%), 448 (7), 418 (6), 382 (7), 282 (8), 243 (100), 181 (7).

Methyl (2*S*,5*E*)-6-(3”-nitrobiphen-4’-yl)-4-oxo-2-(tritylamino)hex-5-enoate (266)[175]



The reaction was carried out according to the above procedure using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**261**) (0.700 g, 1.41 mmol), potassium carbonate (0.205 g, 1.48 mmol) and 4-(3’-nitrophenyl)benzaldehyde (**270**) (0.645 g, 2.84 mmol) in dry acetonitrile (12 mL). The crude material was purified by flash column chromatography eluting with 40−60% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-6-(3”-nitrobiphen-4’-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**266**) as a yellow oil (0.664 g, 79%). $[\alpha]_D^{23}$ +55.0 (*c* 1.0, $CHCl_3$), lit.,[175] $[\alpha]_D^{23}$ +67.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.82 (1H, dd, *J* 15.2, 6.9 Hz, 3-*H*H), 2.93 (1H, d, *J* 10.0 Hz, NH), 2.94 (1H, dd, *J* 15.2, 5.2 Hz, 3-H*H*), 3.30 (3H, s, OCH_3), 3.81 (1H, ddd, *J* 10.0, 6.9, 5.2 Hz, 2-H), 6.75 (1H, d, *J* 16.2 Hz, 5-H), 7.16−7.21 (3H, m, 3 × Ar-H), 7.23−7.29 (6H, m, 6 × Ar-H), 7.49−7.55 (7H, m, 6-H and 6 × Ar-H), 7.63−7.71 (5H, m, 2’-H, 3’-H, 5’-H, 6’-H and 5”-H), 7.93−7.97 (1H, m, 6”-H), 8.23−8.27 (1H, m, 4”-H), 8.49 (1H, t, *J* 2.0 Hz, 2”-H); δ_C (101 MHz, $CDCl_3$) 45.8 (CH_2), 52.0 (CH_3), 53.8 (CH), 71.3 (C), 121.9 (CH), 122.6 (CH), 126.5 (3 × CH), 126.9 (CH), 127.7 (2 × CH), 127.9 (6 × CH), 128.8 (6 × CH), 129.1 (2 × CH), 129.9 (CH), 132.9 (CH), 134.6 (C), 140.6 (C), 141.7 (C), 142.1 (CH), 145.7 (3 × C), 148.8 (C), 174.4 (C), 197.4 (C); *m/z* (FAB) 597 (MH^+, 3%), 530 (23), 353 (9), 243 (100), 219 (18), 165 (16).

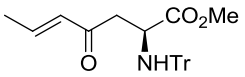
Methyl (2*S*,5*E*)-6-[5’-(4”-fluorophenyl)furan-2’-yl]-4-oxo-2-(tritylamino)hex-5-enoate (267)[175]



The reaction was carried out according to the above procedure using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**261**) (0.700 g, 1.41 mmol),

potassium carbonate (0.205 g, 1.48 mmol) and 5-(4'-fluorophenyl)-2-furaldehyde (**272**) (0.540 g, 2.84 mmol) in dry acetonitrile (14 mL). The crude material was purified by flash column chromatography eluting with 20−40% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-6-[5'-(4"-fluorophenyl)furan-2'-yl]-4-oxo-2-(tritylamino)hex-5-enoate (**267**) as a yellow oil (0.631 g, 79%). $[\alpha]_D^{23}$ +59.8 (*c* 1.0, $CHCl_3$), lit.,[175] $[\alpha]_D^{23}$ +63.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 2.76 (1H, dd, *J* 14.9, 7.0 Hz, 3-*H*H), 2.90 (1H, dd, *J* 14.9, 5.4 Hz, 3-H*H*), 2.91 (1H, d, *J* 9.5 Hz, NH), 3.27 (3H, s, OCH_3), 3.76−3.84 (1H, m, 2-H), 6.67 (1H, d, *J* 15.8 Hz, 5-H), 6.70 (1H, d, *J* 3.6 Hz, 4'-H), 6.75 (1H, d, *J* 3.6 Hz, 3'-H), 7.09−7.20 (5H, m, 3"-H, 5"-H and 3 × Ar-H), 7.22−7.29 (7H, m, 6-H and 6 × Ar-H), 7.48−7.52 (6H, m, 6 × Ar-H), 7.68−7.74 (2H, m, 2"-H and 6"-H); δ_C (101 MHz, $CDCl_3$) 46.3 (CH_2), 52.0 (CH_3), 54.0 (CH), 71.3 (C), 107.8 (CH), 116.1 (d, $J_{C\text{-}C\text{-}F}$ 22.1 Hz, 2 × CH), 118.7 (CH), 123.0 (CH), 126.1 (d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ 3.4 Hz, C), 126.3 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 8.1 Hz, 2 × CH), 126.5 (3 × CH), 127.9 (6 × CH), 128.8 (6 × CH), 128.9 (CH), 145.8 (3 × C), 150.4 (C), 155.6 (C), 162.9 (d, $J_{C\text{-}F}$ 249.4 Hz, C), 174.5 (C), 196.9 (C); *m/z* (FAB) 560 (MH^+, 25%), 482 (24), 316 (98), 243 (100), 216 (96), 166 (71), 124 (35).

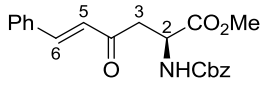
Methyl (2*S*,5*E*)-4-oxo-2-(tritylamino)hept-5-enoate (268)[189]



The reaction was carried out according to the above procedure using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**261**) (2.00 g, 4.04 mmol), potassium carbonate (0.586 g, 4.24 mmol) and acetaldehyde (0.680 mL, 12.1 mmol) in dry acetonitrile (30 mL). The crude material was purified by flash column chromatography eluting with 30% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-4-oxo-2-(tritylamino)hept-5-enoate (**268**) as a colourless oil (1.41 g, 84%). $[\alpha]_D^{25}$ +17.6 (*c* 1.0, $CHCl_3$), lit.,[189] $[\alpha]_D^{25}$ +17.1 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.89 (3H, dd, *J* 6.8, 1.6 Hz, 7-H_3), 2.66 (1H, dd, *J* 15.3, 7.0 Hz, 3-*H*H), 2.79 (1H, dd, *J* 15.3, 5.2 Hz, 3-H*H*), 2.85 (1H, d, *J* 10.0 Hz, NH), 3.26 (3H, s, OCH_3), 3.72 (1H, ddd, *J* 10.0, 7.0, 5.2 Hz, 2-H), 6.07 (1H, dq, *J* 15.7, 1.6 Hz, 5-H), 6.77 (1H, dq, *J* 15.7, 6.8 Hz, 6-H), 7.15−7.20 (3H, m, 3 × Ar-H), 7.22−7.28 (6H, m, 6 × Ar-H), 7.45−7.50 (6H, m, 6 × Ar-H); δ_C (101 MHz, $CDCl_3$) 18.3 (CH_3), 44.9 (CH_2), 51.9 (CH_3), 53.6 (CH), 71.2 (C), 126.5 (3 × CH), 127.9 (6 × CH),

128.8 (6 × CH), 132.1 (CH), 143.6 (CH), 145.8 (3 × C), 174.5 (C), 197.4 (C); *m/z* (CI) 414 (MH^+, 4%), 336 (14), 285 (36), 243 (100), 172 (47).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (277)

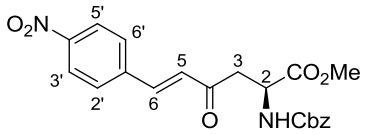


Method A: To a solution of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**262**) (0.745 g, 1.57 mmol) in methanol (33 mL) was added 37% aqueous hydrochloric acid (5.2 mL). The reaction mixture was stirred at room temperature for 1 h before diluting with water (33 mL) and concentrating *in vacuo* to remove the methanol. The remaining solution was then washed with ethyl acetate (20 mL) and the aqueous layer concentrated *in vacuo*. The resulting material was dissolved in dichloromethane (22 mL) and *N*,*N*-diisopropylethylamine (0.680 mL, 3.93 mmol) was added followed by benzyl chloroformate (0.336 mL, 2.36 mmol). The reaction mixture was stirred at room temperature for 1 h before diluting with water (50 mL). The mixture was then extracted with dichloromethane (4 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography eluting with 50% diethyl ether in petroleum ether (40−60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**277**) as a white solid (0.505 g, 88%). Mp 77−78 °C; ν_{max}/cm^{-1} (neat) 3333 (NH), 3059 (CH), 2951 (CH), 1734 (CO), 1688 (CO), 1533 (C=C), 1435, 1343, 1254, 1090, 980, 748; $[\alpha]_D^{26}$ +26.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.26 (1H, dd, *J* 17.9, 4.2 Hz, 3-*H*H), 3.48 (1H, dd, *J* 17.9, 4.2 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 4.68 (1H, dt, *J* 8.4, 4.2 Hz, 2-H), 5.12 (2H, s, O*C*H_2Ph), 5.85 (1H, d, *J* 8.4 Hz, NH), 6.69 (1H, d, *J* 16.2 Hz, 5-H), 7.26−7.43 (8H, m, 8 × Ar-H), 7.51−7.59 (3H, m, 6-H and 2 × Ar-H); δ_C (101 MHz, $CDCl_3$) 43.4 (CH_2), 50.2 (CH), 52.8 (CH_3) 67.2 (CH_2), 125.6 (CH), 128.2 (2 × CH), 128.3 (CH), 128.6 (4 × CH), 129.2 (2 × CH), 131.0 (CH), 134.2 (C), 136.4 (C), 144.2 (CH), 156.2 (C), 171.7 (C), 197.6 (C); *m/z* (CI) 368.1506 (MH^+. $C_{21}H_{22}NO_5$ requires 368.1498), 326 (8%), 260 (23), 234 (21), 219 (18), 181 (6), 147 (17), 107 (16), 85 (100).

Method B: To a solution of methyl (2*S*,5*E*)-4-oxo-6-phenyl-2-(tritylamino)hex-5-enoate (**262**) (1.06 g, 2.23 mmol) in dichloromethane (45 mL) was added trifluoroacetic acid (0.33 mL, 4.5 mmol). The reaction mixture was stirred at room temperature for 2 h before concentrating *in vacuo*. The resulting residue was dissolved in chloroform (5 mL) and

petroleum ether (40−60) was added until an orange oil formed which stuck to the flask. The solvent was then decanted off, and the remaining oil was dissolved in dichloromethane (30 mL) followed by the addition of *N*,*N*-diisopropylethylamine (0.971 mL, 5.58 mmol) and benzyl chloroformate (0.478 mL, 3.35 mmol). The reaction mixture was stirred at room temperature for 1 h before diluting with water (50 mL). The mixture was then extracted with dichloromethane (4 × 50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography eluting with 50% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**277**) as a white solid (0.682 g, 83%). Spectroscopic data as reported above.

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-nitrophenyl)-4-oxohex-5-enoate (278)

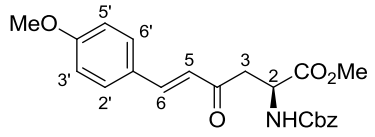


Method A: The reaction was carried out according to the previously described procedure for compound **277** (method A) using methyl (2*S*,5*E*)-6-(4'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**263**) (0.820 g, 1.58 mmol) and 37% aqueous hydrochloric acid (5.2 mL) in methanol (33 mL), followed by treatment with *N*,*N*-diisopropylethylamine (0.662 mL, 3.80 mmol) and benzyl chloroformate (0.325 mL, 2.28 mmol) in dichloromethane (25 mL). The crude material was purified by flash column chromatography eluting with 30−40% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-nitrophenyl)-4-oxohex-5-enoate (**278**) as a yellow solid (0.470 g, 72%). Mp 73−74 °C; ν_{max}/cm^{-1} (neat) 3331 (NH), 2953 (CH), 1730 (CO), 1686 (CO), 1512 (C=C), 1343, 1202, 1059, 978, 860; $[\alpha]_D^{28}$ +20.6 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.30 (1H, dd, *J* 18.1, 4.2 Hz, 3-*H*H), 3.48 (1H, dd, *J* 18.1, 4.2 Hz, 3-H*H*), 3.76 (3H, s, OCH_3), 4.70 (1H, dt, *J* 8.4, 4.2 Hz, 2-H), 5.12 (2H, s, O*C*H_2Ph), 5.82 (1H, d, *J* 8.4 Hz, NH), 6.80 (1H, d, *J* 16.2 Hz, 5-H), 7.29−7.37 (5H, m, Ph), 7.58 (1H, d, *J* 16.2 Hz, 6-H), 7.69 (2H, d, *J* 8.8 Hz, 2'-H and 6'-H), 8.26 (2H, d, *J* 8.8 Hz, 3'-H and 5'-H); δ_C (101 MHz, $CDCl_3$) 42.9 (CH_2), 50.1 (CH), 52.9 (CH_3), 67.2 (CH_2), 124.3 (2 × CH), 128.1 (2 × CH), 128.3 (CH), 128.6 (2 × CH), 128.9 (CH), 129.1 (2 × CH),

136.2 (C), 140.3 (C), 140.8 (CH), 148.9 (C), 156.1 (C), 171.5 (C), 197.0 (C); *m/z* (CI) 413.1352 (MH^+. $C_{21}H_{21}N_2O_7$ requires 413.1349), 383 (42%), 348 (38), 305 (30), 275 (30), 257 (23), 137 (68), 91 (68), 69 (100).

Method B: The reaction was carried out according to the previously described procedure for compound **277** (method B) using methyl (2*S*,5*E*)-6-(4'-nitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**263**) (0.391 g, 0.751 mmol) and trifluoroacetic acid (0.11 mL, 1.5 mmol) in dichloromethane (15 mL), followed by treatment with *N*,*N*-diisopropylethylamine (0.327 mL, 1.88 mmol) and benzyl chloroformate (0.161 mL, 1.13 mmol) in dichloromethane (10 mL). The crude material was purified by flash column chromatography eluting with 30−40% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-nitrophenyl)-4-oxohex-5-enoate (**278**) as a yellow solid (0.238 g, 77%). Spectroscopic data as reported above.

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-methoxyphenyl)-4-oxohex-5-enoate (279)

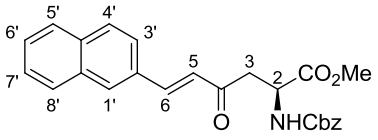


Method A: The reaction was carried out according to the previously described procedure for compound **277** (method A) using methyl (2*S*,5*E*)-6-(4'-methoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**264**) (0.318 g, 0.629 mmol) and 37% aqueous hydrochloric acid (2.3 mL) in methanol (15 mL), followed by treatment with *N*,*N*-diisopropylethylamine (0.270 mL, 1.55 mmol) and benzyl chloroformate (0.133 mL, 0.932 mmol) in dichloromethane (10 mL). The crude material was purified by flash column chromatography eluting with 30% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-methoxyphenyl)-4-oxohex-5-enoate (**279**) as a colourless oil (0.148 g, 59%). ν_{max}/cm^{-1} (neat) 3347 (NH), 2953 (CH), 1717 (CO), 1655 (CO), 1597 (C=C), 1510 (C=C), 1248, 1208, 1169, 1026, 980, 816; $[\alpha]_D^{29}$ +30.3 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.23 (1H, dd, *J* 17.9, 4.2 Hz, 3-*H*H), 3.47 (1H, dd, *J* 17.9, 4.2 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.67 (1H, dt, *J* 8.5, 4.2 Hz, 2-H), 5.12 (2H, s, OCH_2Ph), 5.88 (1H, d, *J* 8.5 Hz, NH), 6.58 (1H, d, *J* 16.2 Hz, 5-H), 6.92 (2H, d, *J* 8.8 Hz, 3'-H and 5'-H), 7.27−7.38 (5H, m, Ph), 7.46−7.56 (3H, m, 6-H, 2'-H and 6'-

H); δ_C (101 MHz, $CDCl_3$) 42.1 (CH_2), 50.1 (CH), 52.7 (CH_3), 55.4 (CH_3), 67.0 (CH_2), 114.5 (2 × CH), 123.2 (CH), 126.7 (C), 128.0 (2 × CH), 128.1 (CH), 128.5 (2 × CH), 130.2 (2 × CH), 136.2 (C), 143.9 (CH), 156.1 (C), 161.9 (C), 171.7 (C), 197.4 (C); *m/z* (EI) 397.1526 (M^+. $C_{22}H_{23}NO_6$ requires 397.1525), 336 (10%), 306 (10), 289 (19), 262 (19), 243 (45), 182 (34), 161 (100).

Method B: The reaction was carried out according to the previously described procedure for compound **277** (method B) using methyl (2*S*,5*E*)-6-(4'-methoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**264**) (0.600 g, 1.19 mmol) and trifluoroacetic acid (0.18 mL, 2.4 mmol) in dichloromethane (25 mL), followed by treatment with *N*,*N*-diisopropylethylamine (0.519 mL, 2.98 mmol) and benzyl chloroformate (0.255 mL, 1.79 mmol) in dichloromethane (16 mL). The crude material was purified by flash column chromatography eluting with 30−40% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-methoxyphenyl)-4-oxohex-5-enoate (**279**) as a colourless oil (0.412 g, 87%). Spectroscopic data as reported above.

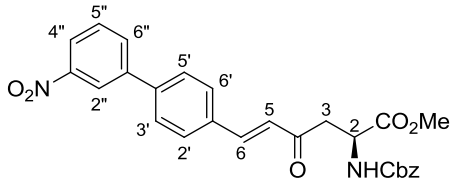
Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(naphthalen-2'-yl)-4-oxohex-5-enoate (280)



The reaction was carried out according to the previously described procedure for compound **277** (method A) using methyl (2*S*,5*E*)-6-(naphthalen-2'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**265**) (0.563 g, 1.07 mmol) and 37% aqueous hydrochloric acid (4.0 mL) in methanol (25 mL), followed by treatment with *N*,*N*-diisopropylethylamine (0.444 mL, 2.55 mmol) and benzyl chloroformate (0.218 mL, 1.53 mmol) in dichloromethane (17 mL). The crude material was purified by flash column chromatography eluting with 20−30% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(naphthalen-2'-yl)-4-oxohex-5-enoate (**280**) as a colourless oil (0.368 g, 82%). ν_{max}/cm^{-1} (neat) 3339 (NH), 3059 (CH), 2951 (CH), 1717 (CO), 1659 (CO), 1505 (C=C), 1207, 1057, 976, 812; $[\alpha]_D^{28}$ +27.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.31 (1H, dd, *J* 18.0, 4.2 Hz, 3-*H*H), 3.54 (1H, dd, *J* 18.0, 4.2 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 4.71 (1H, dt, *J* 8.6, 4.2 Hz, 2-H), 5.13 (2H, s,

OC*H*$_2$Ph), 5.89 (1H, d, *J* 8.6 Hz, NH), 6.82 (1H, d, *J* 16.2 Hz, 5-H), 7.28−7.39 (5H, m, Ph), 7.51−7.58 (2H, m, 6'-H and 7'-H), 7.67 (1H, dd, *J* 8.6, 1.5 Hz, 3'-H), 7.73 (1H, d, *J* 16.2 Hz, 6-H), 7.82−7.90 (3H, m, 4'-H, 5'-H and 8'-H), 7.96 (1H, br s, 1'-H); δ_C (101 MHz, $CDCl_3$) 42.4 (CH_2), 50.1 (CH), 52.8 (CH_3), 67.0 (CH_2), 123.4 (CH), 125.5 (CH), 126.9 (CH), 127.6 (CH), 127.8 (CH), 128.0 (2 × CH), 128.2 (CH), 128.5 (2 × CH), 128.7 (CH), 128.9 (CH), 130.9 (CH), 131.5 (C), 133.2 (C), 134.5 (C), 136.2 (C), 144.2 (CH), 156.1 (C), 171.6 (C), 197.4 (C); *m/z* (CI) 418.1653 (MH^+. $C_{25}H_{24}NO_5$ requires 418.1654), 383 (32%), 310 (48), 275 (20), 147 (30), 107 (29).

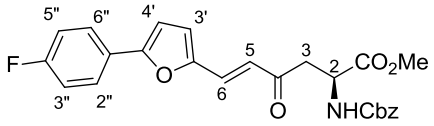
Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(3"-nitrobiphen-4'-yl)-4-oxo-hex-5-enoate (281)



The reaction was carried out according to the previously described procedure for compound **277** (method A) using methyl (2*S*,5*E*)-6-(3"-nitrobiphen-4'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**266)** (0.550 g, 0.922 mmol) and 37% aqueous hydrochloric acid (3.5 mL) in methanol (22 mL), followed by treatment with *N*,*N*-diisopropylethylamine (0.401 mL, 2.30 mmol) and benzyl chloroformate (0.197 mL, 1.38 mmol) in dichloromethane (16 mL). The crude material was purified by flash column chromatography eluting with 30−50% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(3"-nitrobiphen-4'-yl)-4-oxo-hex-5-enoate (**281**) as a pale yellow solid (0.377 g, 84%). Mp 102−104 °C; ν_{max}/cm^{-1} (neat) 3325 (NH), 3034 (CH), 1742 (CO), 1683 (CO), 1664 (CO), 1529 (C=C), 1342, 1179, 1057, 970, 802; $[\alpha]_D^{27}$ +17.2 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.29 (1H, dd, *J* 18.0, 4.2 Hz, 3-*H*H), 3.51 (1H, dd, *J* 18.0, 4.2 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 4.71 (1H, dt, *J* 8.5, 4.2 Hz, 2-H), 5.13 (2H, s, OC*H*$_2$Ph), 5.87 (1H, d, *J* 8.5 Hz, NH), 6.77 (1H, d, *J* 16.2 Hz, 5-H), 7.28−7.39 (5H, m, Ph), 7.57−7.70 (6H, m, 6-H, 2'-H, 3'-H, 5'-H, 6'-H, and 5"-H), 7.94 (1H, ddd, *J* 7.8, 1.7, 1.0 Hz, 6"-H), 8.24 (1H, ddd, *J* 8.2, 2.2, 1.0 Hz, 4"-H), 8.46−8.50 (1H, m, 2"-H); δ_C (101 MHz, $CDCl_3$) 42.6 (CH_2), 50.2 (CH), 52.9 (CH_3), 67.2 (CH_2), 122.0 (CH), 122.8 (CH), 126.2 (CH), 127.9 (2 × CH), 128.2 (2 × CH), 128.3 (CH), 128.7

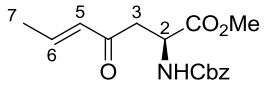
(2 × CH), 129.4 (2 × CH), 130.1 (CH), 133.0 (CH), 134.4 (C), 136.3 (C), 141.0 (C), 141.8 (C), 143.1 (CH), 148.9 (C), 156.2 (C), 171.7 (C), 197.5 (C); *m/z* (CI) 489.1664 (MH^+. $C_{27}H_{25}N_2O_7$ requires 489.1662), 459 (8%), 418 (10), 381 (42), 351 (23), 338 (32), 310 (19), 275 (10), 181 (15), 147 (26), 91 (100).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-[5'-(4"-fluorophenyl)furan-2'-yl]-4-oxohex-5-enoate (282)



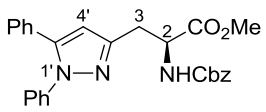
The reaction was carried out according to the previously described procedure for compound **277** (method A) using methyl (2*S*,5*E*)-6-[5'-(4"-fluorophenyl)furan-2'-yl]-4-oxo-2-(tritylamino)hex-5-enoate (**267**) (0.626 g, 1.12 mmol) and 37% aqueous hydrochloric acid (4.1 mL) in methanol (28 mL), followed by treatment with *N*,*N*-diisopropylethylamine (0.488 mL, 2.80 mmol) and benzyl chloroformate (0.240 mL, 1.68 mmol) in dichloromethane (19 mL). The crude material was purified by flash column chromatography eluting with 30% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-[5'-(4"-fluorophenyl)furan-2'-yl]-4-oxohex-5-enoate (**282**) as a yellow oil (0.157 g, 31%). ν_{max}/cm^{-1} (neat) 3344 (NH), 2919 (CH), 1717 (CO), 1600 (CO), 1506 (C=C), 1484, 1211, 1156, 1025, 967, 838; $[\alpha]_D^{26}$ +11.3 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.20 (1H, dd, *J* 17.9, 4.2 Hz, 3-*H*H), 3.44 (1H, dd, *J* 17.9, 4.2 Hz, 3-H*H*), 3.75 (3H, s, OCH_3), 4.68 (1H, dt, *J* 8.5, 4.2 Hz, 2-H), 5.12 (2H, s, O*C*H_2Ph), 5.87 (1H, d, *J* 8.5 Hz, NH), 6.66 (1H, d, *J* 15.8 Hz, 5-H), 6.70 (1H, d, *J* 3.6 Hz, 4'-H), 6.78 (1H, d, *J* 3.6 Hz, 3'-H), 7.09−7.16 (2H, m, 3"-H and 5"-H), 7.28−7.39 (6H, m, 6-H and Ph), 7.68−7.74 (2H, m, 2"-H and 6"-H); δ_C (101 MHz, $CDCl_3$) 42.7 (CH_2), 50.3 (CH), 52.9 (CH_3), 67.2 (CH_2), 108.0 (CH), 116.2 (d, $J_{C\text{-}C\text{-}F}$ 22.1 Hz, 2 × CH), 119.4 (CH), 122.0 (CH), 126.1 (d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ 3.0 Hz, C), 126.5 (d, $J_{C\text{-}C\text{-}C\text{-}F}$ 8.3 Hz, 2 × CH), 128.2 (2 × CH), 128.3 (CH), 128.7 (2 × CH), 129.6 (CH), 136.3 (C), 150.2 (C), 156.0 (C), 156.2 (C), 163.1 (d, $J_{C\text{-}F}$ 249.5 Hz, C), 171.8 (C), 196.9 (C); *m/z* (EI) 451.1434 (M^+. $C_{25}H_{22}FNO_6$ requires 451.1431), 343 (15%), 316 (22), 300 (100), 229 (20), 215 (66), 159 (26), 133 (20), 123 (23), 91 (62).

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxohept-5-enoate (283)



The reaction was carried out according to the previously described procedure for compound **277** (method B) using methyl (2*S*,5*E*)-4-oxo-2-(tritylamino)hept-5-enoate (**268**) (1.20 g, 2.90 mmol) and trifluoroacetic acid (0.43 mL, 5.8 mmol) in dichloromethane (60 mL), followed by treatment with *N*,*N*-diisopropylethylamine (1.26 mL, 7.25 mmol) and benzyl chloroformate (0.621 mL, 4.35 mmol) in dichloromethane (50 mL). The crude material was purified by flash column chromatography eluting with 30% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxohept-5-enoate (**283**) as a colourless oil (0.726 g, 82%). ν_{max}/cm^{-1} (neat) 3331 (NH), 2951 (CH), 1717 (CO), 1667 (CO), 1506 (C=C), 1437, 1207, 1028, 968, 739; $[\alpha]_D^{25}$ +26.5 (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 1.91 (3H, dd, *J* 6.9, 1.6 Hz, 7-H_3), 3.09 (1H, dd, *J* 18.0, 4.2 Hz, 3-*H*H), 3.33 (1H, dd, *J* 18.0, 4.2 Hz, 3-H*H*), 3.73 (3H, s, OCH_3), 4.62 (1H, dt, *J* 8.5, 4.2 Hz, 2-H), 5.11 (2H, s, O*C*H_2Ph), 5.80 (1H, d, *J* 8.5 Hz, NH), 6.09 (1H, dq, *J* 15.9, 1.6 Hz, 5-H), 6.88 (1H, dq, *J* 15.9, 6.9 Hz, 6-H), 7.29−7.39 (5H, m, Ph); δ_C (101 MHz, $CDCl_3$) 18.5 (CH_3), 41.6 (CH_2), 50.1 (CH), 52.7 (CH_3), 67.1 (CH_2), 128.1 (2 × CH), 128.2 (CH), 128.6 (2 × CH), 131.5 (CH), 136.4 (C), 144.6 (CH), 156.2 (C), 171.7 (C), 197.5 (C); *m/z* (CI) 306.1343 (MH^+. $C_{16}H_{20}NO_5$ requires 306.1341), 262 (33%), 198 (15), 172 (13), 155 (17), 91 (16).

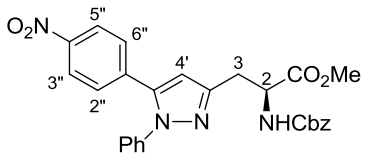
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(1',5'-diphenyl-1'*H*-pyrazol-3'-yl)propanoate (286)



To a solution of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**277**) (0.169 g, 0.460 mmol) in methanol (4 mL) was added phenylhydrazine (0.045 mL, 0.46 mmol) and 37% aqueous hydrochloric acid (0.015 mL). The reaction mixture was stirred under reflux for 17 h, cooled to room temperature and concentrated *in vacuo*.

The resulting material was diluted with ethyl acetate (10 mL) and a saturated aqueous solution of sodium hydrogen carbonate (10 mL), extracted with ethyl acetate (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting material was then dissolved in dichloromethane (30 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.104 g, 0.460 mmol) was added. After stirring at room temperature for 2 h, the reaction mixture was concentrated *in vacuo* and the resulting solid purified by flash column chromatography eluting with 20−30% ethyl acetate in petroleum ether (40−60), followed by triturating with chloroform to give methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(1',5'-diphenyl-1'*H*-pyrazol-3'-yl)propanoate (**286**) as a colourless oil (0.160 g, 76%). ν_{max}/cm^{-1} (neat) 3379 (NH), 3061 (CH), 2951 (CH), 1717 (CO), 1503 (C=C), 1375, 1207, 1051, 912, 761; $[\alpha]_D^{23}$ +29.2 (*c* 0.25, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.23 (1H, dd, *J* 14.9, 4.9 Hz, 3-*H*H), 3.30 (1H, dd, *J* 14.9, 5.8 Hz, 3-H*H*), 3.77 (3H, s, OCH_3), 4.71–4.79 (1H, m, 2-H), 5.10 (2H, d, *J* 12.2 Hz, OC*H*HPh), 5.14 (2H, d, *J* 12.2 Hz, OCH*H*Ph), 5.77 (1H, d, *J* 8.4 Hz, NH), 6.27 (1H, s, 4'-H), 7.15–7.36 (15H, m, 3 × Ph); δ_C (101 MHz, $CDCl_3$) 30.7 (CH_2), 52.5 (CH_3), 53.6 (CH), 67.0 (CH_2), 107.6 (CH), 125.1 (2 × CH), 127.4 (CH), 128.2 (3 × CH), 128.4 (CH), 128.5 (2 ×CH), 128.6 (2 ×CH), 128.8 (2 ×CH), 128.9 (2 ×CH), 130.5 (C), 136.5 (C), 140.0 (C), 144.0 (C), 148.3 (C), 156.1 (C), 172.1 (C); *m/z* (CI) 456.1925 (MH^+. $C_{27}H_{26}N_3O_4$ requires 456.1923), 368 (9%), 348 (100), 305 (11), 257 (23) 137 (59).

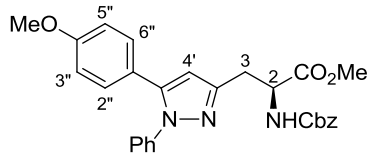
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(4"-nitrophenyl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (287)



The reaction was carried out according to the above procedure using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-nitrophenyl)-4-oxohex-5-enoate (**278**) (150 mg, 0.364 mmol), phenylhydrazine (0.035 mL, 0.364 mmol) and 37% aqueous hydrochloric acid (0.012 mL) in methanol (3 mL), followed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.082 g, 0.364 mmol) in dichloromethane (22 mL). The crude material was purified by flash column chromatography eluting with 20–40% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(4"-nitrophenyl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (**287**) as a yellow oil (0.138 g, 76%). ν_{max}/cm^{-1}

(neat) 3350 (NH), 2951 (CH), 1717 (CO), 1597, 1502 (C=C), 1346, 1207, 1051, 972, 853; $[\alpha]_D^{23}$ +30.0 (*c* 0.3, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.21−3.34 (2H, m, 3-H_2), 3.77 (3H, s, OCH_3), 4.73−4.81 (1H, m, 2-H), 5.09 (1H, d, *J* 12.2 Hz, OC*H*HPh), 5.14 (1H, d, *J* 12.2 Hz, OCH*H*Ph), 5.72 (1H, d, *J* 8.4 Hz, NH), 6.41 (1H, s, 4'-H), 7.18–7.23 (2H, m, 2 × Ar-H), 7.29–7.38 (10H, m, 2"-H, 6"-H and 8 × Ar-H), 8.14 (2H, d, *J* 8.7 Hz, 3"-H and 5"-H); δ_C (101 MHz, $CDCl_3$) 30.7 (CH_2), 52.5 (CH_3), 53.4 (CH), 67.0 (CH_2), 108.7 (CH), 123.8 (2 × CH), 125.2 (2 × CH), 128.1 (CH), 128.1 (CH), 128.2 (2 × CH), 128.5 (2 × CH), 129.3 (4 × CH), 136.3 (C), 136.5 (C), 139.3 (C), 141.5 (C), 147.3 (C), 148.8 (C), 155.9 (C), 171.9 (C); *m/z* (EI) 500.1695 (M^+. $C_{27}H_{24}N_4O_6$ requires 500.1696), 441 (10%), 392 (15), 349 (100), 278 (68), 232 (22), 91 (45).

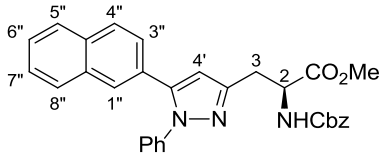
Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(4"-methoxyphenyl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (288)



The reaction was carried out according to the above procedure using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-methoxyphenyl)-4-oxohex-5-enoate (**279**) (0.080 g, 0.20 mmol), phenylhydrazine (0.20 mL, 0.20 mmol) and 37% aqueous hydrochloric acid (0.050 mL) in methanol (1.6 mL), followed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.046 g, 0.201 mmol) in dichloromethane (12 mL). The crude material was purified by flash column chromatography eluting with 20–30% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(4"-methoxyphenyl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (**288**) as a colourless oil (0.073 g, 75%). ν_{max}/cm^{-1} (neat) 3339 (NH), 2951 (CH), 1717 (CO), 1506 (C=C), 1436, 1248, 1176, 1027, 835; $[\alpha]_D^{26}$ +10.6 (*c* 0.5, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.23 (1H, dd, *J* 14.8, 4.9 Hz, 3-*H*H), 3.30 (1H, dd, *J* 14.8, 5.7 Hz, 3-H*H*), 3.78 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.72−4.78 (1H, m, 2-H), 5.11 (1H, d, *J* 12.3 Hz, OC*H*HPh), 5.15 (1H, d, *J* 12.3 Hz, OCH*H*Ph), 5.80 (1H, d, *J* 8.3 Hz, NH), 6.22 (1H, s, 4'-H), 6.82 (2H, d, *J* 8.8 Hz, 3"-H and 5"-H), 7.11 (2H, d, *J* 8.8 Hz, 2"-H and 6"-H), 7.22−7.38 (10H, m, 2 × Ph); δ_C (101 MHz, $CDCl_3$) 30.8 (CH_2), 52.5 (CH_3), 53.6 (CH), 55.4 (CH_3), 67.0 (CH_2), 107.1 (CH), 114.0 (2 × CH), 122.9 (C), 125.2 (2 × CH), 127.3 (CH), 128.2 (3 × CH), 128.6 (2 × CH),

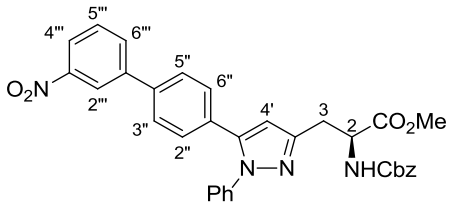
128.9 (2 × CH), 130.1 (2 × CH), 136.5 (C), 140.1 (C), 143.9 (C), 148.2 (C), 156.1 (C), 159.7 (C), 172.2 (C); *m/z* (EI) 485.1961 (M^+. $C_{28}H_{27}N_3O_5$ requires 485.1951), 426 (31), 377 (27), 334 (100), 318 (17), 263 (61), 199 (18), 91 (33).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(naphthalen-2"-yl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (289)



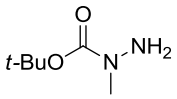
The reaction was carried out according to the above procedure using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(naphthalen-2'-yl)-4-oxohex-5-enoate (**280**) (0.150 g, 0.359 mmol), phenylhydrazine (0.036 mL, 0.36 mmol) and 37% aqueous hydrochloric acid (0.013 mL) in methanol (3 mL), followed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.082 g, 0.36 mmol) in dichloromethane (22 mL). The crude material was purified by flash column chromatography eluting with 20% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(naphthalen-2"-yl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (**289**) as a white solid (0.132 g, 73%). Mp 66−67 °C; ν_{max}/cm^{-1} (neat) 3341 (NH), 2924 (CH), 1719 (CO), 1597, 1499 (C=C), 1341, 1207, 1047, 818; $[\alpha]_D^{25}$ +14.0 (*c* 0.2, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.27 (1H, dd, *J* 15.0, 4.7 Hz, 3-*H*H), 3.33 (1H, dd, *J* 15.0, 5.7 Hz, 3-H*H*), 3.79 (3H, s, OCH_3), 4.73−4.82 (1H, m, 2-H), 5.10 (1H, d, *J* 12.3 Hz, OC*H*HPh), 5.15 (1H, d, *J* 12.3 Hz, OCH*H*Ph), 5.81 (1H, d, *J* 8.1 Hz, NH), 6.38 (1H, s, 4'-H), 7.19 (1H, dd, *J* 8.5, 1.6 Hz, 3"-H), 7.24−7.38 (10H, m, 2 × Ph), 7.46−7.53 (2H, m, 6"-H and 7"-H), 7.69−7.84 (4H, m, 1"-H, 4"-H, 5"-H and 8"-H); δ_C (101 MHz, $CDCl_3$) 30.7 (CH_2), 52.5 (CH_3), 53.5 (CH), 67.0 (CH_2), 107.9 (CH), 125.0 (2 × CH), 126.3 (CH), 126.6 (CH), 126.7 (CH), 127.4 (CH), 127.7 (CH), 127.8 (C), 128.0 (CH), 128.1 (CH), 128.1 (3 × CH), 128.2 (CH), 128.5 (2 × CH), 128.9 (2 × CH), 132.8 (C), 133.0 (C), 136.4 (C), 139.9 (C), 143.9 (C), 148.3 (C), 156.0 (C), 172.1 (C); *m/z* (EI) 505.2012 (M^+. $C_{31}H_{27}N_3O_4$ requires 505.2002), 446 (10%), 397 (55), 354 (45), 338 (20), 283 (100), 215 (10), 108 (13), 91 (27).

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(3"'-nitrobiphen-4"-yl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (290)



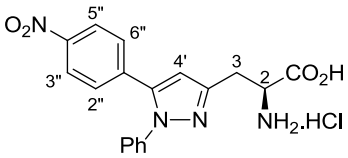
The reaction was carried out according to the above procedure using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(3"-nitrobiphen-4'-yl)-4-oxo-hex-5-enoate (**281**) (0.200 g, 0.409 mmol), phenylhydrazine (0.040 mL, 0.41 mmol) and 37% aqueous hydrochloric acid (0.012 mL) in methanol (3 mL), followed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.093 g, 0.41 mmol) in dichloromethane (25 mL). The crude material was purified by flash column chromatography eluting with 20% ethyl acetate in petroleum ether (40−60) to give methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(3"'-nitrobiphen-4"-yl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (**290**) as a yellow oil (0.199 g, 84%). ν_{max}/cm^{-1} (neat) 3359 (NH), 1716 (CO), 1508 (C=C), 1348, 1207, 1052, 803; $[\alpha]_D^{23}$ +20.5 (*c* 0.4, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.26 (1H, dd, *J* 15.0, 4.9 Hz, 3-*H*H), 3.32 (1H, dd, *J* 15.0, 5.6 Hz, 3-H*H*), 3.79 (3H, s, OCH_3), 4.73−4.81 (1H, m, 2-H), 5.11 (1H, d, *J* 12.3 Hz, OC*H*HPh), 5.15 (1H, d, *J* 12.3 Hz, OCH*H*Ph), 5.78 (1H, d, *J* 8.3 Hz, NH), 6.36 (1H, s, 4'-H), 7.26−7.39 (12H, m, 2"-H, 6"-H and 2 × Ph), 7.56 (2H, d, *J* 8.3 Hz, 3"-H and 5"-H), 7.62 (1H, t, *J* 8.0 Hz, 5"'-H), 7.88−7.93 (1H, m, 6"'-H), 8.19−8.24 (1H, m, 4"'-H), 8.44 (1H, t, *J* 1.9 Hz, 2"'-H); δ_C (101 MHz, $CDCl_3$) 30.6 (CH_2), 52.5 (CH_3), 53.4 (CH), 67.0 (CH_2), 107.7 (CH), 121.8 (CH), 122.4 (CH), 125.2 (2 × CH), 127.2 (2 × CH), 127.6 (CH), 128.1 (3 × CH), 128.5 (2 × CH), 129.0 (2 × CH), 129.4 (2 × CH), 129.9 (CH), 130.5 (C), 132.8 (CH), 136.3 (C), 138.4 (C), 139.8 (C), 141.9 (C), 143.1 (C), 148.4 (C), 148.8 (C), 156.0 (C), 172.0 (C); *m/z* (EI) 576.2007 (M^+. $C_{33}H_{28}N_4O_6$ requires 576.2009), 476 (45%), 425 (15), 409 (26), 381 (17), 354 (100), 308 (17).

1-*tert*-Butoxycarbonyl-1-methylhydrazine (298)[180]



A solution of di-*tert*-butyl dicarbonate (2.18 g, 9.99 mmol) in dichloromethane (8 mL) was added dropwise to a solution of methylhydrazine (**297**) (0.53 mL, 10 mmol) in dichloromethane (7 mL). The reaction mixture was stirred at room temperature for 1.5 h before concentrating *in vacuo*. The resulting material was purified by flash column chromatography eluting with 50% ethyl acetate in petroleum ether (40−60) to give 1-*tert*-butoxycarbonyl-1-methylhydrazine (**298**) as a colourless liquid (1.46 g, 81%). Spectroscopic data in accordance with literature.[180] δ_H (500 MHz, CD_3Cl) 1.47 (9H, s, 3 × CH_3), 3.05 (3H, s, CH_3), 4.07 (2H, br s, NH_2); δ_C (126 MHz, CD_3Cl) 28.1 (3 × CH_3), 37.9 (CH_3), 79.8 (C), 156.7 (C); *m/z* (CI) 147 (MH^+, 51%), 132 (12), 91 (100).

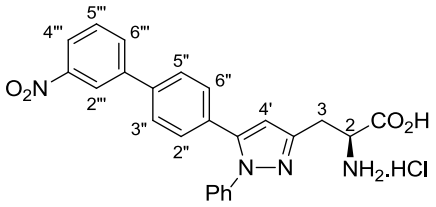
(2*S*)-2-Amino-3-[5'-(4"-nitrophenyl)-1'*H*-pyrazol-3'-yl]propanoic acid hydrochloride (300)



To a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(4"-nitrophenyl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (**287**) (0.080 g, 0.16 mmol) in methanol (0.5 mL) was added 6.0 M aqueous hydrochloric acid (4.5 mL). The reaction mixture was then stirred under reflux for 48 h. After cooling to room temperature, the mixture was concentrated *in vacuo* and triturated with diethyl ether to give (2*S*)-2-amino-3-[5'-(4"-nitrophenyl)-1'*H*-pyrazol-3'-yl]propanoic acid hydrochloride (**300**) as a pale yellow foam (0.060 g, 97%). ν_{max}/cm^{-1} (neat) 3340 (NH), 2918 (CH), 2850, 1744 (CO), 1596 (C=C), 1515, 1344, 1255, 1080, 854; $[\alpha]_D^{27}$ −6.0 (*c* 0.3, MeOH); δ_H (400 MHz, CD_3OD) 3.33−3.51 (2H, m, 3-H_2), 4.45 (1H, br s, 2-H), 6.73 (1H, s, 4'-H), 7.29−7.46 (5H, m, Ph), 7.48 (2H, d, *J* 7.7 Hz, 2"-H and 6"-H), 8.19 (2H, d, *J* 7.7 Hz, 3"-H and 5"-H); δ_C (101 MHz, CD_3OD) 29.6 (CH_2), 53.5 (CH), 109.9 (CH), 124.7 (2 × CH), 126.8 (2 × CH), 129.6

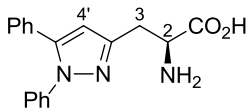
(CH), 130.4 (2 × CH), 130.8 (2 × CH), 137.5 (C), 140.6 (C), 143.8 (C), 148.7 (C), 148.9 (C), 170.9 (C); *m/z* (ESI) 351.1082 ($M–H^-$. $C_{18}H_{15}N_4O_4$ requires 351.1099).

(2*S*)-2-Amino-3-[5'-(3"'-nitrobiphen-4"-yl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoic acid hydrochloride (301)



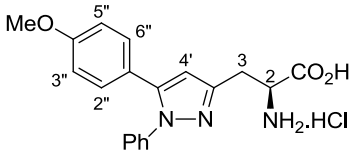
The reaction was carried out according to the above procedure using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(3"'-nitrobiphen-4"-yl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (**290**) (0.150 g, 0.260 mmol) in methanol (0.7 mL) and 6.0 M aqueous hydrochloric acid (7.0 ml) to give (2*S*)-2-amino-3-[5'-(3"'-nitrobiphen-4"-yl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoic acid hydrochloride (**301**) as an off white foam (0.118 g, 98%). ν_{max}/cm^{-1} (neat) 3026 (NH), 2861 (CH), 1731 (CO), 1525 (C=C), 1347, 1197, 973, 803; $[\alpha]_D^{28}$ −8.3 (*c* 0.3, MeOH); δ_H (400 MHz, CD_3OD) 3.37 (1H, dd, *J* 15.8, 7.5 Hz, 3-*H*H), 3.46 (1H, dd, *J* 15.8, 4.6 Hz, 3-H*H*), 4.45 (1H, dd, *J* 7.5, 4.6 Hz, 2-H), 6.64 (1H, s, 4'-H), 7.33−7.45 (7H, m, 2"-H, 6"-H and Ph), 7.66−7.73 (3H, m, 3"-H, 5"-H and 5"'-H), 8.04 (1H, ddd, *J* 7.8, 1.7, 0.9 Hz, 6"'-H), 8.23 (1H, ddd, *J* 8.2, 2.2, 0.9 Hz, 4"'-H), 8.45−8.47 (1H, m, 2"'-H); δ_C (101 MHz, CD_3OD) 29.6 (CH_2), 53.6 (CH), 108.8 (CH), 122.5 (CH), 123.4 (CH), 126.7 (2 × CH), 128.4 (2 × CH), 129.3 (CH), 130.3 (2 × CH), 130.6 (2 × CH), 131.3 (CH), 131.5 (C), 134.1 (CH), 140.2 (C), 141.1 (C), 143.0 (C), 145.5 (C), 148.5 (C), 150.3 (C), 171.0 (C); *m/z* (ESI) 429.1545 (MH^+. $C_{24}H_{21}N_4O_4$ requires 429.1557).

(2*S*)-2-Amino-3-(1',5'-diphenyl-1'*H*-pyrazol-3'-yl)propanoic acid (302)



To a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(1',5'-diphenyl-1'*H*-pyrazol-3'-yl)propanoate (**286**) (0.086 g, 0.19 mmol) in methanol and water (5 mL, 7:3) was added cesium carbonate (0.080 g, 0.25 mmol). The reaction mixture was stirred at room temperature for 17 h before concentrating *in vacuo*. The resulting residue was dissolved in water (10 mL), acidified to pH 1 with 1.0 M aqueous hydrochloric acid, extracted with dichloromethane (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. To a solution of the resulting residue in methanol (5 mL) was added 10% palladium on carbon (0.050 g), and the reaction mixture stirred under a hydrogen atmosphere for 17 h. The mixture was then filtered through Celite® and concentrated *in vacuo* to give (2*S*)-2-amino-3-(1',5'-diphenyl-1'*H*-pyrazol-3'-yl)propanoic acid (**302**) as a white foam (0.051 g, 88%). ν_{max}/cm^{-1} (neat) 3380 (NH), 3058 (CH), 1630 (CO), 1502 (C=C), 1423, 1375, 1112, 760; $[\alpha]_D^{28}$ −25.8 (*c* 0.4, MeOH); δ_H (400 MHz, CD_3OD) 3.20−3.45 (2H, m, 3-H_2), 4.02 (1H, br s, 2-H), 6.53 (1H, s, 4'-H), 7.17−7.40 (10H, m, 2 × Ph); δ_H (126 MHz, CD_3OD) 30.3 (CH_2), 55.7 (CH), 108.7 (CH), 126.7 (2 × CH), 129.0 (CH), 129.6 (3 × CH), 129.8 (2 × CH), 130.1 (2 × CH), 131.5 (C), 141.3 (C), 145.9 (C), 150.0 (C), 173.8 (C); *m/z* (ESI) 308.1382 (MH^+. $C_{18}H_{18}N_3O_2$ requires 308.1394).

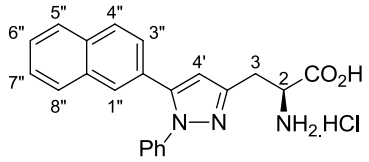
(2*S*)-2-Amino-3-[5'-(4"-methoxyphenyl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoic acid hydrochloride (303)



To a solution of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(4"-methoxyphenyl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (**288**) (0.069 g, 0.14 mmol) in methanol and water (3 mL, 7:3) was added cesium carbonate (0.059 g, 0.18 mmol). The reaction mixture was stirred at room temperature for 17 h before concentrating *in vacuo*. The resulting residue

was dissolved in water (5 mL), acidified to pH 1 with 1.0 M aqueous hydrochloric acid, extracted with dichloromethane (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting residue was suspended in 6.0 M aqueous hydrochloric acid (2 mL) and the mixture stirred under reflux for 24 h. The reaction mixture was concentrated *in vacuo* and triturated with diethyl ether to give (2*S*)-2-amino-3-[5'-(4"-methoxyphenyl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoic acid hydrochloride (**303**) as a white foam (0.041 g, 79%). ν_{max}/cm^{-1} (neat) 3359 (NH), 3211 (OH), 2921 (CH), 1729 (CO), 1611, 1506 (C=C), 1437, 1151, 834; $[\alpha]_D^{28}$ −5.0 (*c* 0.2, $CHCl_3$); δ_H (500 MHz, CD_3OD) 3.28−3.46 (2H, m, 3-H_2), 3.78 (3H, s, OCH_3), 4.38−4.43 (1H, m, 2-H), 6.46 (1H, s, 4'-H), 6.86 (2H, d, *J* 8.7 Hz, 3"-H and 5"-H), 7.14 (2H, d, *J* 8.7 Hz, 2"-H and 6"-H), 7.27−7.42 (5H, m, Ph); δ_C (126 MHz, CD_3OD) 29.6 (CH_2), 53.7 (CH), 55.9 (CH_3), 108.0 (CH), 115.1 (2 × CH), 123.6 (C), 126.6 (2 × CH), 129.0 (CH), 130.1 (2 × CH), 131.2 (2 × CH), 141.3 (C), 146.2 (C), 148.3 (C), 161.6 (C), 171.0 (C); *m/z* (ESI) 338.1489 (MH^+. $C_{19}H_{20}N_3O_3$ requires 338.1499).

(2*S*)-2-Amino-3-[5'-(naphthalen-2"-yl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoic acid hydrochloride (304)



The reaction was carried out according to the above procedure using (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[5'-(naphthalen-2"-yl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoate (**289**) (0.030 g, 0.059 mmol) and cesium carbonate (0.026 g, 0.080 mmol) in methanol and water (1 mL, 7:3), followed by treating with 6.0 M aqueous hydrochloric acid (1 mL) to give (2*S*)-2-amino-3-[5'-(naphthalen-2"-yl)-1'-phenyl-1'*H*-pyrazol-3'-yl]propanoic acid hydrochloride (**304**) as an off white foam (0.019 g, 83%). ν_{max}/cm^{-1} (neat) 3366 (NH), 2922 (CH), 1739 (CO), 1596 (C=C), 1497, 1205, 1081, 819; $[\alpha]_D^{28}$ −3.7 (*c* 0.3, MeOH); δ_H (400 MHz, CD_3OD) 3.40 (1H, dd, *J* 15.8, 7.3 Hz, 3-*H*H), 3.49 (1H, br d, *J* 15.8 Hz, 3-H*H*), 4.47 (1H, br s, 2-H), 6.70 (1H, s, 4'-H), 7.25 (1H, br d, *J* 8.2 Hz, 3"-H), 7.32−7.42 (5H, m, Ph), 7.46−7.54 (2H, m, 6"-H and 7"-H), 7.72−7.87 (4H, m, 1"-H, 4"-H, 5"-H and 8"-H); δ_C (101 MHz, CD_3OD) 29.5 (CH_2), 53.5 (CH), 109.0 (CH), 126.7 (2 × CH), 127.0 (CH), 127.8 (CH), 128.0 (CH), 128.5 (C), 128.7 (CH), 129.2 (CH), 129.3

(2 × CH), 129.3 (CH) 130.2 (2 × CH), 134.4 (2 × C), 140.9 (C), 146.3 (C), 148.4 (C), 171.0 (C); *m/z* (ESI) 358.1541 (MH^+. $C_{22}H_{20}N_3O_2$ requires 358.1550).

4.3 Procedure for [^{35}S]GTPγS Binding Assay[137]

ChemiSCREEN™ membrane preparation (recombinant human mGluR2 metabotropic glutamate receptor) was obtained from Millipore. Membranes were permeabilised by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]GTPγS (0.1 nM), glutamate (10 μM) and various concentrations of test compound, in HEPES (20 mM), sodium chloride (100 mM), magnesium chloride (10 mM), GDP (0.5 μM), pH 7.4 (final volume 100 μL). Incubation was carried out for 30 min at 30°C. Basal binding was determined without L-glutamate or test compound present, and stimulated binding without test compound present. Reactions were terminated by rapid filtration through Whatman GF/B glass fibre filters pre-soaked with water using a 24-well Brandel cell harvester. The filters were washed 3 times (1 mL per well per wash) with ice cold sodium phosphate (10 mM), pH 7.4. Disintegrations per minute were determined by liquid scintillation analysis and IC_{50} values derived from nonlinear regression analysis using GraphPad Prism Version 4 (GraphPad Software Inc).

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