The Development of Novel Photoactivatable Antagonists for the $GABA_A$ Receptor

Favaad Iqbal

Supervisors Dr. James Baker and Prof. Trevor Smart

A thesis submitted to University College London in partial fulfilment of the requirements for the degree of Doctor of Philosophy

I, Favaad Iqbal, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

……

Abstract

This thesis describes the development of novel photoactivatable antagonists for the $GABA_A$ receptor. $GABA_A$ receptors are ligand-gated Cl^- channels belonging to the Cys-loop superfamily of ionotropic receptors, which play an important role inhibiting cell excitation in the nervous system. To date, no research has probed the mobility of native, unmutated $GABA_A$ receptors. In order to do this, we planned to design a series of molecules that could bind to the $GABA_A$ receptor covalently, causing inhibition. A brief overview of the $GABA_A$ receptor and the concept of photoaffinity labelling are outlined in the first chapter, followed by a chapter detailing our results and discussion.

Firstly, our efforts focussed on the development of a lead compound. The molecule gabazine was chosen as our template, since the molecule possesses a highly specific affinity for the $GABA_A$ receptor. In our ongoing studies into the development of labelled analogues of gabazine as probes for the $GABA_A$ receptor, we discovered that a relatively minor addition to the gabazine skeleton significantly enhances its antagonist potency.

The development of several new photoaffinity-labelled reagents capable of photochemically blocking the function of the $GABA_A$ receptor was subsequently explored. We were able to demonstrate that these reagents could block up to 49% of recombinant $GABA_A$ receptors in cultured cells. Finally, our efforts centered on creating an analogue incorporating a photoaffinity label in addition to a detectable tag. It is hoped that such a compound could be useful for exploring the spatial and temporal mobility of native $GABA_A$ receptors microscopically.

The tools created are the first with the potential to knock out $GABA_A$ receptors *in vivo*, and avoid the need for any prior receptor transfection.

Contents

Ac	Acetyl
AIBN	Azobisisobutyronitrile
AKAP	A-kinase anchor protein
ALLOP	Allopregnanolone
AMPA	2-Amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid
ANQX	6-Azido-7-nitro-1,4-dihydroquinoxaline-2,3-dione
Ar	Aryl
Arg	Arginine
Asp	Aspartic acid
ATP	Adenosine triphosphate
Bn	Benzyl
Boc	*tert*-Butyl carbamate
BODIPY	Boron-dipyrromethene
Bu	Butyl
Bz	Benzoyl
cat.	Catalytic
CNS	Central nervous system
Cp	Cyclopentadienyl
Cys	Cysteine
dba	Dibenzylidene acetone
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIC	*N*,*N*'-Diisopropylcarbodiimide
DIPEA	*N*,*N*'-Diisopropylethylamine
DMAP	*N*,*N*'-Dimethylaminopyridine
DMF	*N*,*N*'-Dimethylformamide
DMSO	Dimethylsulfoxide
DNQX	6,7-Dinitroquinoxaline-2,3-dione
EC_{50}	Half maximal effective concentration
eq	Equivalents
Et	Ethyl
GABA	γ-Aminobutyric acid
GABARAP	$GABA_A$ receptor associated protein
GAD	*L*-glutamate decarboxylase
Glu	Glutamic acid
GPCR	G-protein coupled receptor
h	Planck constant
HBTU	*O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HEK	Human embryonic kidney
HIV	Human immunodeficiency virus
IC_{50}	Half maximal inhibitory concentration
^{i}Pr	Isopropyl
iso-THAZ	1,4,5,6,7,8-Hexahydro-[1,2]oxazolo[3,4-*d*]azepin-3-one
L	Ligand
LC-MS	Liquid chromatography–mass spectrometry
LGIC	Ligand-gated ion channel
Lys	Lysine
m-	*meta*-
m.p.	Melting point
Me	Methyl

MIDA	*N*-methyliminodiacetic acid
MW	Microwaves
n	Integer number
nAChR	Nicotinic acetylcholine receptor
NBS	*N*-Bromosuccinimide
NHS	*N*-Hydroxysuccinimide
NMDA	*N*-Methyl-*D*-aspartic acid
NMR	Nuclear magnetic resonance
NSF	*N*-Ethyl maleimide sensitive factor
Nu	Nucleophile
o-	*ortho*-
p-	*para*-
PEG	Poly(ethylene glycol)
Ph	Phenyl
PKA	Protein kinase A
PKC	Protein kinase C
PLIC-1	Protein linking IAP to cytoskeleton
PP1c	Protein phosphorylase 1c
ppm	Parts per million
PRIP1	Phospholipase C related catalytically inactive protein
QD	Quantum dot
RACK1	Receptor of activated protein C kinase 1
rt	Room temperature (19-22 °C)
Src	Sarcoma
t or *tert*	Tertiary
TBAF	Tetra-*n*-butylammonium fluoride
Tf	Triflyl
TFA	Trifluoroacetic acid
THDOC	Tetrahydrodeoxycorticosterone
THF	Tetrahydrofuran
THIP	4,5,6,7-Tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Ts	Tosyl
UCL	University College London
UV	Ultraviolet

First of all, I am wholly indebted to my supervisors Dr. James Baker and Prof. Trevor Smart. Jamie's infectious enthusiasm and support has been a real source of inspiration for me since day one. Without Jamie or Trevor, I fervently believe the project wouldn't exist, and I'm thankful to both for the opportunity to work on the task. I'd like to extend my gratitude to Dr. Martin Mortensen, who has helped my understanding of the project endlessly and is responsible for the biological evaluation undertaken. My appreciation is also extended to Dr. Maya Topf and Dr. Arun Pandurangan for their valuable computational studies and Ryan Ellwood for his contributions to the project.

Over the past three years at UCL, I've been honoured to work with some amazing characters. Special mentions must go to Lauren and Sam who served as exemplary mentors and friends to me in my first couple of years. I'd like to thank Chris for making me chuckle with the occasional joke and Vincent for his caustic wit. Summers would have been nothing without my footballing companions, Matt and Osman. Thanks to Andrew, Sally and Liz for coffee times and chit-chat. And thanks to Rachel and Rhian for lunchtimes. Felix, Cristina, Fil, Jarryl, Bhavesh, Niral, Elena, Mark, Ahmed, Ramiz, Eifion and João also deserve a huge thank you. We've shared some incredible moments - thank you all for making the lab an unforgettable place to work.

I'd like say thanks to Dr. Abil Aliev and Dr. Lisa Haigh for their assistance with NMR and mass spectrometry respectively.

I'd also like to extend some heartfelt thanks to ex-housemates and friends who have been ready for a Coke and a catch-up over the past three years – namely Alan, Simon, Dave, Mary, David, James, Harriet, Germander, Ed, Victoria and Alice. I've always appreciated the company, but not always been able to say it – this seemed like the most appropriate place.

Last, but by no means least, my family. Thanks Faria and Tom for hanging out with me when you've had the chance to. Thanks Mum for always being supportive, and thanks Dad for affording me the freedom to choose to do what I want with my life.

Et ignotas animum dimittit in artes naturamque nouat

– Ovid, *Metamorphoses*, VIII, 188

For my parents

1 Introduction

1.1 Introduction to the $GABA_A$ receptor

1.1.1 Neurotransmission

The word 'neurotransmission' is a compound derived of two words; from the Greek *neuro* meaning 'nerve', and the Latin *transmissio* meaning 'crossing'. In the central nervous system (CNS), nerve signals, known as action potentials are transmitted along nerve cells, known as neurons. Each action potential is a wave of current, and in order to continue transmission from one neuron to the next, the impulse must traverse the synaptic gap that separates the two. At all synapses, this transmission is achieved by inducing a postsynaptic potential – a change in electrical potential that can trigger an action potential in the postsynaptic neuron. The majority of neurons achieve this transmission by the use of chemical mediators, known as neurotransmitters.[1,2] Neurotransmission describes the process by which the neurotransmitter is secreted by a presynaptic neuron and subsequently activates a receptor on a postsynaptic neuron (**Figure 1.1**). Upon arrival at an axon terminal, an electrical impulse initiates the release of neurotransmitters from synaptic vesicles into the synaptic cleft. Receptors found on the postsynaptic neuron can then bind these molecules and respond by opening ion channels in the postsynaptic neuron or by initiating signaling cascades.

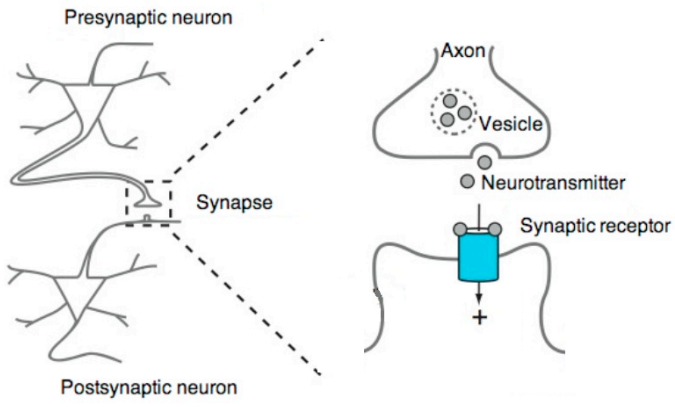


Figure 1.1 – Synaptic neurotransmission

For ionotropic receptors, the binding of a neurotransmitter directly activates an ion channel. Upon activation, ion channels selectively let ions pass through. Depending on the nature of the neurotransmitter and receptor, ions are able move in or out and alter the local transmembrane potential of the cell. These ions are characteristically Na^+, K^+, Ca^{2+} and Cl^-, and their relative concentrations ultimately influence the membrane potential in any neuron. A change in relative ion concentrations resulting in a more negative potential is known as hyperpolarisation. Conversely, a change in ion concentrations causing a more positive or less negative potential is known as depolarisation. Depolarising ion flux increases the postsynaptic potential, and if the potential increases above the threshold voltage, an action potential is triggered in the postsynaptic neuron.[3] In its resting state a neuron is polarised to approximately −70 mV relative to its surroundings. This is achieved through having a high intracellular concentration of K^+ ions and a high extracellular concentration of Na^+ ions. In a quiescent neuron, the resting voltage is around −70 mV, and the threshold voltage is around −55 mV (**Figure 1.2**).[2] When the axon hillock (the linkage between the cell body and the axon) is adequately depolarised to the threshold voltage, voltage-gated Na^+ channels open and more Na^+ ions are able to permeate into the cell. At approximately +40 mV, the Na^+ channels inactivate. K^+ channels increasingly open and hyperpolarisation occurs, where K^+ ions flow out of the cell. It is this reversal of transmembrane potential which initiates an electrical impulse.[2]

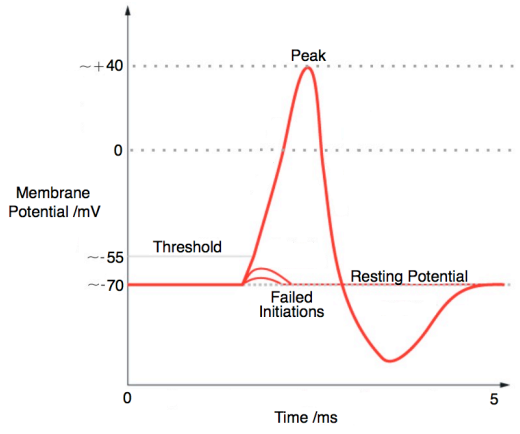


Figure 1.2 – An action poteniial

1.1.2 GABA

Two competing pathways control the overall activity of the brain. Excitation by the neurotransmitter glutamate **1** depolarises neurons,[4] whilst inhibition by the neurotransmitter γ-aminobutyric acid (GABA) **2** leads to the hyperpolarisation of neurons (**Figure 1.3**).[5]

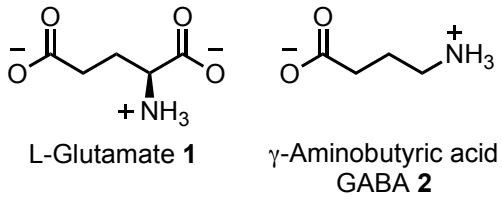


Figure 1.3

GABA is considered one of the most important inhibitory neurotransmitters in the mammalian CNS.[6] Roberts and Frankel first demonstrated the relative abundance of the amino acid within the brain, believing the biosynthetic pathway of GABA to originate from the decarboxylation of L-glutamate, a process catalysed by the enzyme L-glutamate decarboxylase (GAD).[7] GABA was finally proven as an inhibitory neurotransmitter by Krnjevic *et al.* in the 1960s.[8] Three sub-classes of receptors are activated by the release of GABA; $GABA_A$, $GABA_B$ and $GABA_C$ receptors. Both $GABA_A$ and $GABA_C$ receptors are ligand gated ion channels (LGICs), whereas $GABA_B$ receptors are G-protein coupled receptors (GPCRs).[9]

Glutamate and GABA are essential to the functionality of the CNS; they play fundamental roles in maintaining a balance between neuronal excitation and inhibition, the two major inputs that govern overall brain activity.[10, 11]

$GABA_A$ receptors are GABA-gated Cl^- ion channels, which cause the firing of the neuron to be inhibited. Upon the binding of GABA, the receptor is activated and the intrinsic Cl^- ion channel opened. This allows Cl^- ions to flow through the channel and into the cell, causing hyperpolarisation and leading to inhibition of the cell.[12]

Neurons in the CNS can display two forms of GABAergic inhibition, referred to as either phasic or tonic.[13] Phasic inhibition is a short-lived hyperpolarising event of the cell, which is brought about through the rapid, transient activation of GABA

receptors, located on the postsynaptic neuron, by GABA released in high concentration from adjacent presynaptic termini. Tonic inhibition is a result of a persistent low concentration of GABA, which has passed out of the synaptic cleft, activating receptors located at extrasynaptic positions. Instead of undergoing a short period of activity, extrasynaptic GABA receptors are tonically active. The result is a slight but prolonged hyperpolarisation of the postsynaptic neuron.[14, 15]

1.1.3 $GABA_A$ receptor structure

The $GABA_A$ receptor belongs to the Cys-loop superfamily of ionotropic receptors. Cys-loop receptors are exclusively ligand-gated ion channels (LGICs);[16] ion channels that mediate rapid synaptic transmission *via* the movement of ions through channels gated by neurotransmitters. Each Cys-loop receptor is pentameric in structure, and is found in both homo- and hetero-pentameric forms. This results in a large family of receptors, each with distinct conformational diversities (**Figure 1.4**).[17] Nineteen subunits have been identified for the $GABA_A$ and $GABA_C$ receptors. The subunits are divided into eight subfamilies, related by structure (α1-α6, β1-β3, γ1-γ3, δ, ε, θ, π, and ρ1-ρ3).[5, 18] Genes that code for $GABA_A$ subunit proteins are found in a different chromosomal locations to the genes coding for $GABA_C$ ρ-subunit proteins. As a result, subunits ρ1-ρ3 are exclusive to $GABA_C$ receptors and form homomeric LGICs. ρ-Subunits have been shown to arrange in a heteromeric fashion *in vitro*, but so far, this has not been demonstrated *in vivo*.[19]

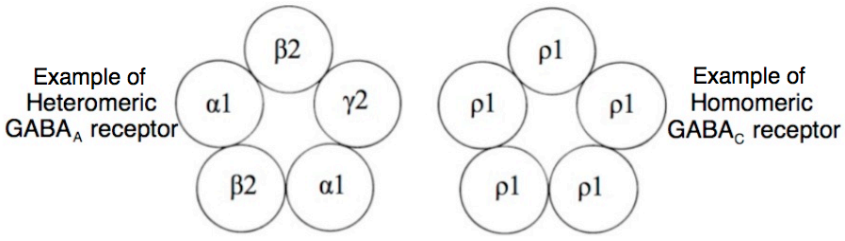


Figure 1.4 - $GABA_A$ and $GABA_C$ receptor assemblies

The distribution of receptor subunits is known to vary between regions in the brain.[20] With respect to α subunit distribution, the α1 subunit is ubiquitous throughout the brain, whereas the α6 subunit is found predominantly in the cerebellum.[21-23]

Regarding β subunit distribution, the β2 subunit is the most abundant isoform,[24] whereas the β1 subunit is the least prevalent.[24] γ1 and γ3 subunits are also fairly rare, centralised in the thalamus, whilst the γ2 subunit exists in many brain areas.[25] The δ subunit predominates extrasynaptically, and is thought to be related to the action of tonic inhibition in the brain.[26] The ε, θ and π subunits are rare subunits sparingly located in the basal ganglia, thalamus, hypothalamus and amygdala.[27] The archetypal $GABA_A$ receptor is comprised of two α, two β and one γ subunits. The α1β2γ2 receptor isoform accounts for roughly 35% of $GABA_A$ receptors encountered in the CNS.[20] A number of permutations are therefore possible for receptor subunit composition due to the number of subunits genetically encoded, and it has been suggested that the subunit composition of $GABA_A$ receptors can even vary between synapses on the same neuron.[28, 29]

Each subunit that makes up the receptor contains an extracellular N-terminal domain, which carries binding sites for agonists, antagonists and modulators.[9] GABA binding takes place at the interface between α and β subunits. Subunits also hold a sequence of 13 residues, bounded by cysteine residues that bond covalently, giving a closed loop situated between binding and channel domains giving the receptors their generic name of Cys-loop receptors.[16]

A $GABA_A$ receptor subunit also possesses four hydrophobic membrane-spanning domains (M1 to M4), an intracellular loop of variable length and a small extracellular C-terminus (**Figure 1.5**). The intracellular loop is located between the M3 and M4 domains and contains protein kinase A (PKA), protein kinase C (PKC) and tyrosine kinase phosphorylation sites. This intracellular loop is believed to be responsible for the membrane clustering of the receptor, and is required for subcellular targeting.[30] The transmembrane domain M2, from all five subunits, is arranged so that it lines the channel pore. The M2 domain is crucial for receptor gating and ionic selectivity.[9]

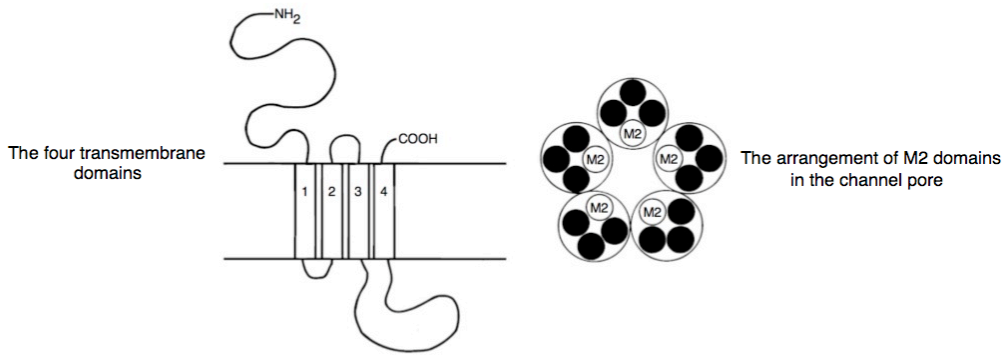


Figure 1.5 - $GABA_A$ receptor arrangement in the channel pore

1.1.4 Trafficking of $GABA_A$ receptors

The trafficking of the $GABA_A$ receptor from intracellular creation to the synapse is largely dependent on various receptor-associated proteins (**Figure 1.6**).[31]

Figure 1.6[31] - $GABA_A$ receptor life cycle

$GABA_A$ receptors are constructed in the endoplasmic reticulum and transported to the Golgi apparatus (a, **Figure 1.6**). Aided by GABARAP ($GABA_A$ receptor associated protein) and NSF (an *N*-ethyl maleimide sensitive factor), receptors are transferred *via* intracellular vesicles to the membrane surface. GABARAP assists in the clustering of $GABA_A$ receptors at a synapse as an anchoring protein (b, **Figure**

1.6).[32-34] The protein PLIC-1 (protein linking IAP to cytoskeleton) assists in membrane insertion of $GABA_A$ receptors at a synapse (c, **Figure 1.6**).

Several signalling molecules can bind to the receptor; Src (sarcoma), RACK1 (receptor of activated protein C kinase 1), PKC (protein kinase C), PKA (protein kinase A) with AKAP (A-kinase anchor protein), and gene PRIP1 (phospholipase C related catalytically inactive protein) with PP1c (protein phosphorylase 1c). It is thought that PLIC-1, PRIP1 and GABARAP are involved in an endocytotic pathway (d, **Figure 1.6**). Clathrin, a protein that assists in the formation of vesicles, is also believed to be important in the endocytotic recycling and degradation pathway.[31] Some $GABA_A$ receptors recycle back to the surface, resulting in a steady state population of the receptors at the surface.[35, 36] The cycling of receptors is significant for the efficacy of synaptic inhibition.[37, 38]

Research by the Smart group determined that receptors are dynamically mobile between synaptic and extrasynaptic domains.[39] By introducing a cysteine mutation into the channel of the common α1 subunit, they were able to demonstrate that a complete block of cell surface $GABA_A$ receptors resulted in a rapid (~10 min) replenishment of functional receptors following irreversible inhibition. The inhibition was initiated by the addition of sulfhydryl modifying agents that could permanently alter the receptor through the introduction of a disulfide bond in the channel, rendering them inactive. Partial selective inhibition of synaptic receptors resulted in a compensation mechanism, where lateral diffusion of unblocked extrasynaptic membrane receptors assisted the recovery of functional receptors at a given synapse. This mechanism superseded the insertion of $GABA_A$ receptors from the intracellular pool of receptors (**Figure 1.7**).[39]

Figure 1.7[39] – Pathways of $GABA_A$ receptor insertion

A disadvantage of this method lies in the use of a cysteine mutation to inactivate populations of receptors. While the mutation lies deep within the ion channel, it has been documented that inducing such a genetic change might lead to overexpression of transmembrane receptors.[40-43] To date, research into the trafficking of native $GABA_A$ receptors is scarce, though the mobility of other native transmembrane receptors has been examined.[44-46]

1.1.5 Compounds interacting with the $GABA_A$ receptor

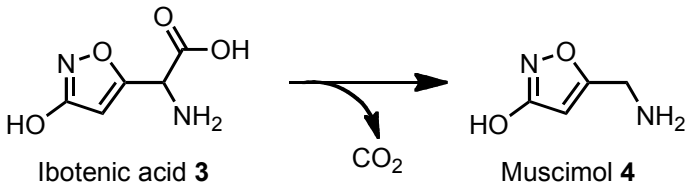
$GABA_A$ receptors are among the most complicated of the Cys-loop receptors, partly due to the large number of receptor subtypes and the variety of ligands that can interact with specific sites on the receptor. It is not known exactly how many regions are available for interaction with such structurally diverse substances; in fact many agents will act at overlapping sites on the receptor protein subunits.[18]

It has been proposed that different ligands are able to interact at different locations on the $GABA_A$ receptor.[18] These include: (i) agonist recognition sites, which are the point of interaction for agonists, partial agonists and competitive antagonists; (ii) benzodiazepine sites, which are located between the α and γ subunit; (iii) neuroactive steroid sites requiring α subunits; (iv) picrotoxinin sites that are associated with the Cl^- ion channels; (v) sedative-hypnotic barbiturate sites, which can interact with the picrotoxinin and agonist recognition regions; (vi) stereoselective sites for inhalation anaesthetics such as isoflurane; (vii) furosemide sites, which are associated with the Cl^- channels of $GABA_A$ receptors containing the α6 subunit; (viii) Zn^{2+} sites, which

are found in $GABA_A$ receptors that do not contain the γ2 subunit; (ix) divalent cation sites for ions such as Ca^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Mn^{2+}, and Mg^{2+}, which can act on the Cl^- ion channels and modulate $GABA_A$ receptor function; (x) La^{3+} regions that are different from the other cation sites. Along with these, there is also the possibility of phospholipid, phosphorylation and microtubule interacting regions.[18]

1.1.5.1 Agonists

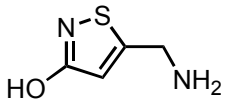
Although GABA is the principal ligand that modulates $GABA_A$ receptor function, a number of agonists are also able to bind to the same binding site and mediate the passage of Cl^- ions. One such compound is the full agonist muscimol **4** which originates from *Amanita muscaria*, more commonly known as the fly agaric, a lethal psychoactive mushroom.[47] Muscimol is the decarboxylative product of the predominant metabolite of the *Amanita* genus, ibotenic acid **3** (**Scheme 1.1**); an agonist of the ionotropic glutamate NMDA receptor.[48]



Ibotenic acid **3** Muscimol **4**

Scheme 1.1

An atomic alteration to muscimol, from an isoxazole to an isothiazole gives another agonist for the $GABA_A$ receptor, thiomuscimol **5** (**Figure 1.8**). Thiomuscimol has been reported as being equipotent to muscimol as an agonist at the $GABA_A$ receptor.[49]

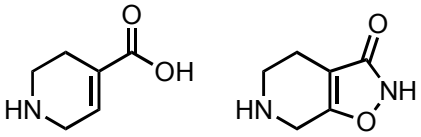


Thiomuscimol **5**

Figure 1.8

Other agonists that can modulate receptor function at the GABA binding site include the full agonist isoguvacine **6** and the partial agonist gaboxadol, also known as THIP **7** (**Figure 1.9**).[50-53] The latter has been shown to act as an analgesic in humans,

however it increases glucose metabolism in epileptic patients, limiting its clinical use.[54]



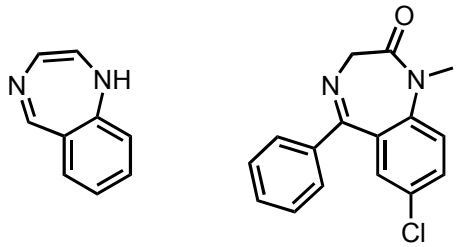
Isoguvacine **6** EC_{50} = 48 μM

THIP **7** EC_{50} = 134 μM

Figure 1.9

1.1.5.2 Benzodiazepines

Benzodiazepines are a heterocyclic class of compounds that augment the effect of GABA or other agonists. Structurally, benzodiazepines combine benzene and diazepine ring systems. In medicine, the benzodiazepine has been shown to remedy anxiety and insomnia.[55] Benzodiazepines have been used clinically since the 1960's and one of the most well-known examples is diazepam **8** (**Figure 1.10**).[56]



1,4-benzodiazepine ring system

Diazepam **8**

Figure 1.10

The location on the $GABA_A$ receptor where benzodiazepines interact is frequently referred to as the benzodiazepine site, located between the α and γ subunits of the $GABA_A$ receptor. At this location, they are able to amplify the synaptic inhibition caused by GABA.[57, 58]

1.1.5.3 Neurosteroids

Neurosteroids are the most potent endogenous modulators of $GABA_A$ receptor function in the brain. They are able to potentiate receptor activation by GABA at low concentrations (1 - 10 nM) and can directly activate the receptor in the absence of GABA at higher concentrations (200 nM - 10 μM).[59, 60] Studies by Hosie *et al.* proposed that two discrete binding sites for neurosteroid binding exist on the $GABA_A$ receptor; one for receptor activation and the other for receptor potentiation.[61, 62] In order to regulate $GABA_A$ receptor function, neurosteroids require a tetracyclic structure possessing a C3α hydroxyl moiety on the A ring and a C20 ketone group on the D ring (**Figure 1.11**).[62, 63]

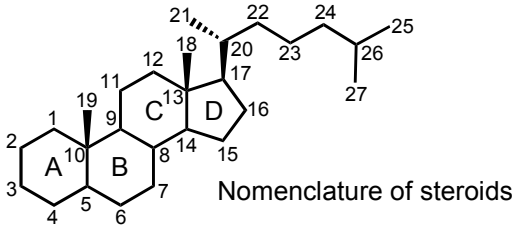
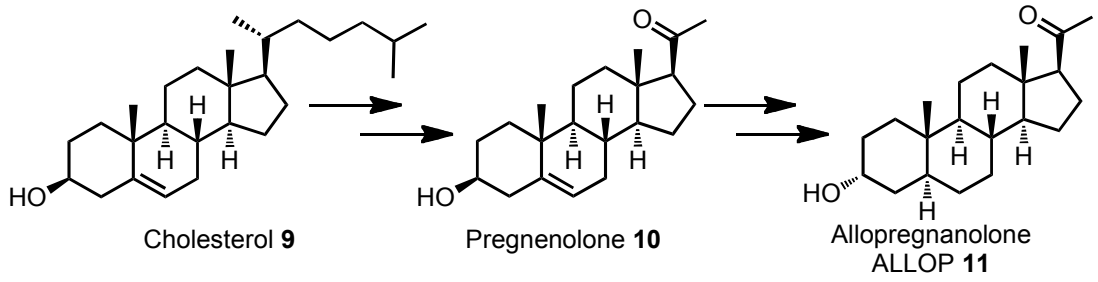


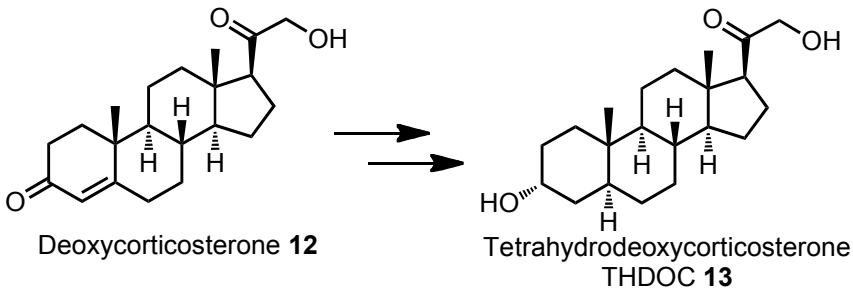
Figure 1.11

Typically, neurosteroids are synthesised in the CNS. Pregnenolone **10** and its metabolites, including allopregnanolone or ALLOP **11**, are the products from the breakdown of cholesterol **9** in the brain (**Scheme 1.2**).[64]



Scheme 1.2

A metabolite of the hormone deoxycorticosterone **12**, tetrahydrodeoxycorticosterone or THDOC **13**, is another endogenous neuroactive steroid, which is able to modulate $GABA_A$ receptor function (**Scheme 1.3**).[65]



Scheme 1.3

1.1.5.4 Antagonists

Antagonists are compounds which, when bound to a receptor, will inhibit the response caused by an activating drug. In the case of $GABA_A$ receptors, binding of an antagonist prevents inhibition of the neuron. Antagonists can work competitively or non-competitively. Competitive antagonists will bind directly to the agonist binding site and so block the access for the agonist. Non-competitive antagonists will not bind to the same location as the agonist but to another site on the receptor, potentially altering the conformational shape of the receptor and leading to the agonist-binding site being affected.

Well-known $GABA_A$ receptor antagonists include the steroid derivative R5135 **14** (**Figure 1.12**), which antagonises the $GABA_A$ receptor non-competitively by interacting with benzodiazepine binding sites.[66, 67] However, **14** is not a selective inhibitor and also blocks inhibition caused by glycine receptors. Iso-THAZ **15**, derived from the agonist THIP, inhibits the $GABA_A$ receptor competitively but also impedes neuronal inhibition by glycine.[68] Bicuculline **16**, isolated from the *Corydalis* genus, acts as a competitive and reversible antagonist and has been shown to block the action of GABA on the $GABA_A$ receptor.[69]

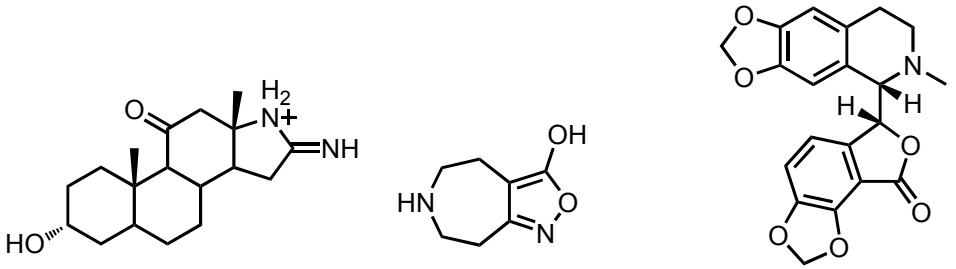


Figure 1.12 – Common $GABA_A$ receptor antagonists

1.1.5.5 Gabazine and related compounds

In 1985, Wermuth *et al.* reported the syntheses of several specific, competitive antagonists for the $GABA_A$ receptor.[66] Heaulme *et al*. showed SR 95103 **17** (**Figure 1.13**) to be a selective antagonist at the GABA binding sites on $GABA_A$ receptors.[70]

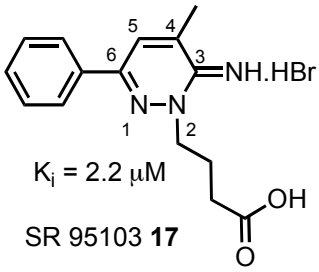


Figure 1.13

Antagonist **17** was shown to have a 20-fold greater potency for the $GABA_A$ receptor than bicuculline **16**.[70] The enhanced potency was linked to the presence of an aromatic ring at the 6-position of the pyridazine ring and it was concluded that the carboxylic acid side-chain was essential for antagonism at $GABA_A$ receptor agonist binding sites.[71] Furthermore, it was also noted that substituents attached to the peripheral aromatic ring influenced antagonist potency. Structure-activity studies showed that the removal of the pyridazine-methyl group and the inclusion of a *para*-methoxy group yielded a more potent antagonist. The ensuing compound - SR 95531 also known as gabazine **18** - has been demonstrated to be a selective, competitive and reversible antagonist for the $GABA_A$ receptor, 250 times more potent than **16** (**Figure 1.14**).[72]

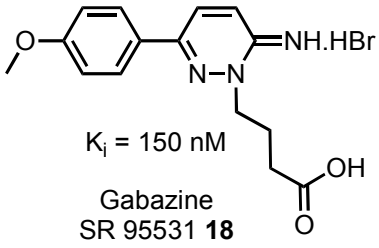


Figure 1.14

It has been suggested that the antagonistic nature of gabazine and other similar compounds originate from the *N*-substitution of GABA by a charge-delocalized guanidinic or amidinic system.[67, 72] The binding of gabazine has been probed on a microscopic level and tritiated derivatives of gabazine are commercially available.[73, 74] Ueno *et al*. demonstrated that gabazine partially inhibited direct activation of the receptor by the barbiturate pentobarbital and by the steroid alphaxolone, not by blocking their binding, but by acting as an inhibitor of $GABA_A$ receptor channel opening.[75] Since its inception, gabazine has become a widespread tool in scientific research of the $GABA_A$ receptor.[76-79]

Exploration of the core antagonist structure by the Wermuth group revealed that the potent nature of the antagonist can be negated if the pyridazine is encumbered with a sterically large group. SR 95132 **19** (**Figure 1.15**) exhibited a drastically suppressed affinity compared to **18**.[80, 81]

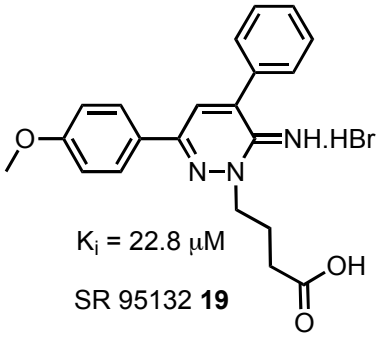


Figure 1.15

Molecular modelling of the GABA-binding site suggested that bicuculline and gabazine both inhibit the $GABA_A$ receptor competitively, but by lying in different orientations. Previous research had already indicated that the two families of

compounds possessed similar structure-activity relationships.[82] Rognan *et al*. proposed that the *para*-methoxyphenyl group on **18** and the (methylenedioxy)phenyl group on bicuculline **16** may share a common hydrogen-bonding site. SR 96073 **20** (**Figure 1.16**), a 3,4-(methylenedioxy) analogue of gabazine, was synthesised in order to test this hypothesis and was shown to be three times more potent than gabazine, supporting the theory that the compounds **16**, **18** and **20** all share a common hydrogen-bonding site.[81]

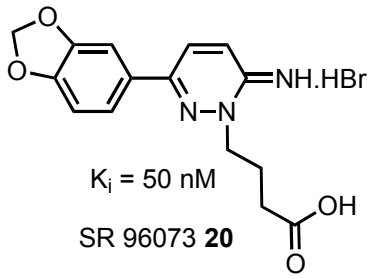


Figure 1.16

Melikian and co-workers later showed that further antagonists could be yielded from compound **20** by aminopyridazine substitution of other $GABA_A$ receptor agonists. The substitution of the GABA backbone for muscimol or thiomuscimol as bioisosteres, was envisaged to give further families of GABA antagonists. Replacement of GABA in SR 96073 by muscimol leads to analogue **21** with a similar affinity, whereas substitution by thiomuscimol lead to analogue **22** possessing enhanced affinity (**Figure 1.17**). Melikian *et al*. proposed that the enhanced affinity of **22** may originate from a greater degree of lipophilicity and lower ionization of the side chain than in analogues **18** and **20**.[83]

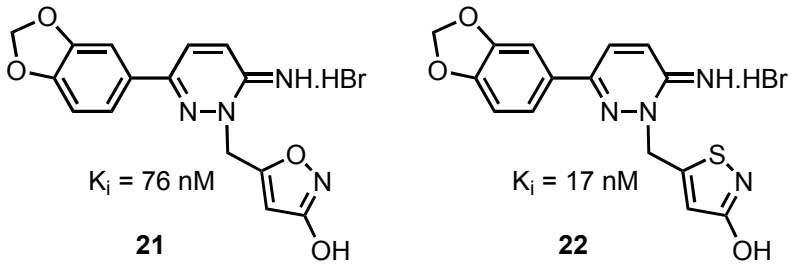


Figure 1.17

1.1.5.6 Synthesis of gabazine

As well as being essential to the antagonistic character of gabazine and its related compounds, the pyridazine core is an important building block in medicinal chemistry. Some examples of medicinally relevant pyridazine derivatives are shown in **Figure 1.18**. Compounds containing the pyridazine nucleus and the closely related pyridazinone core have been shown to display several properties, such as antidepressant,[84] antibacterial,[85] analgesic,[86] anticancer,[87] antifungal[88] and hypotensive[89, 90] activities.

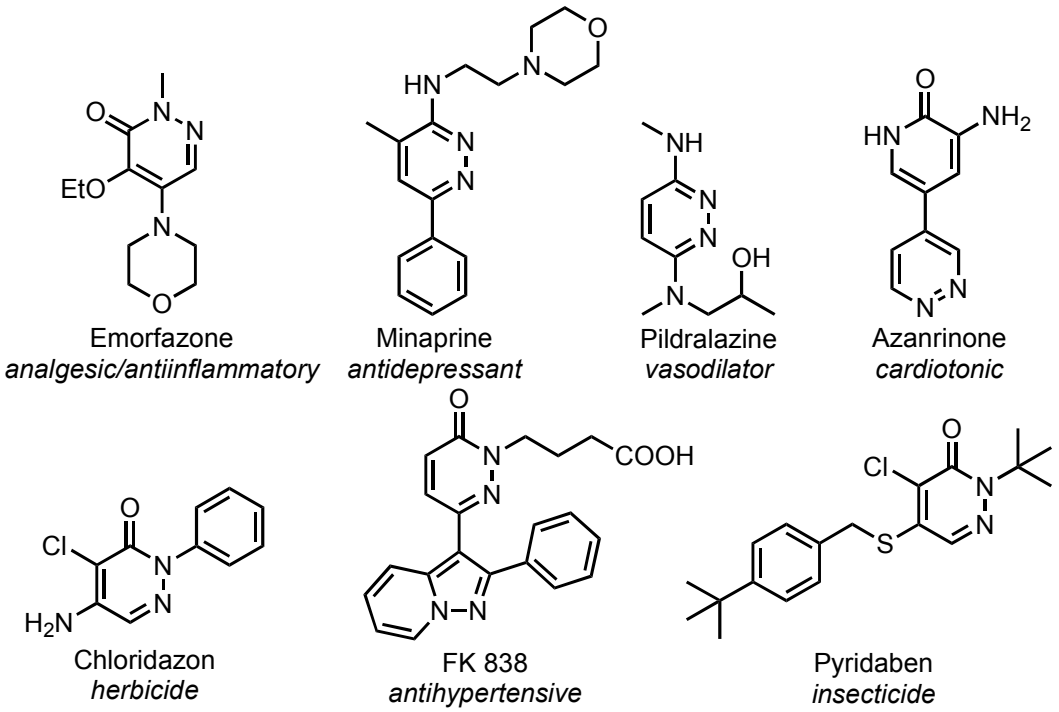
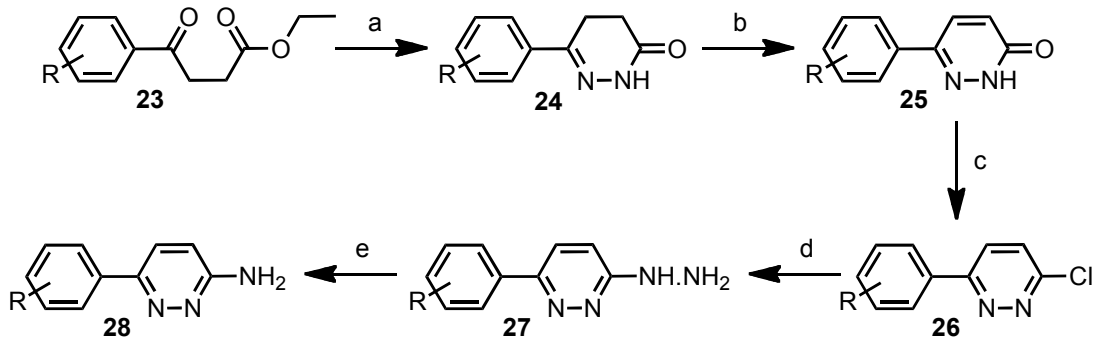


Figure 1.18 – Medicinally relevant pyridazine and pyridazinone derivatives

Gabazine contains an arylaminopyridazine functionality and historically, synthesis of this building block has been cumbersome and prolonged due to the difficult construction of this motif (**Scheme 1.4**).[91]

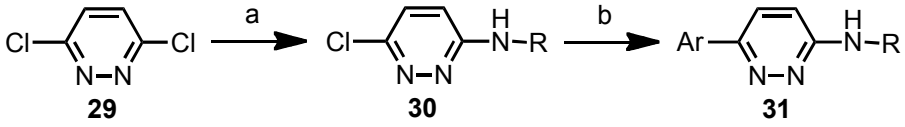


Reagents and conditions: a) Hydrazine; b) Br_2, AcOH, rt; c) $POCl_3$, $CHCl_3$, reflux; d) hydrazine, *n*-BuOH, 100 ^oC; e) H_2, Ni catalyst, MeOH, rt

Scheme 1.4

3-Benzoyl propionates **23** are reacted with hydrazine to give dihydropyridazinones **24**. Following oxidation by bromine to pyridazinones **25**, reaction with phosphoryl chloride gives the corresponding chloropyridazine **26**.[91] Reaction of **26** with hydrazine gives **27**, followed by the reduction using a Ni catalyst to furnish the desired arylaminopyridazine **28**.[92]

Following the development of Pd-catalysed carbon-carbon bond formation, several groups have put forward accelerated syntheses of arylpyridazines.[93-95] Parrot and co-workers were able to show that reaction of commercially available dichloropyridazine **29** with an appropriate amine, followed by Pd-mediated cross coupling of chloropyridazine **30** with an arylboronic acid could furnish substituted arylaminopyridazines **31** in two steps (**Scheme 1.5**).[96]

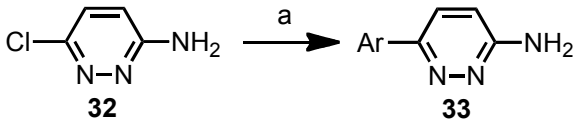


Reagents and conditions: a) R-NH_2, Acetone/H_2O, HCl (24-48 h, 80-100 ^oC); b) $Pd(PPh_3)_4$, Na_2CO_3, Ar-$B(OH)_2$, toluene/EtOH (20 h, 110 ^oC).

Scheme 1.5

3-Amino-6-arylpyridazines **33** can also be directly prepared through reaction of commercially available 3-amino-6-chloropyridazine **32** and a boronic acid, as described by Maes and researchers (**Scheme 1.6**).[94] The Wermuth group also

reported the syntheses of arylaminopyridazines **33** using analogous reaction conditions.[93]

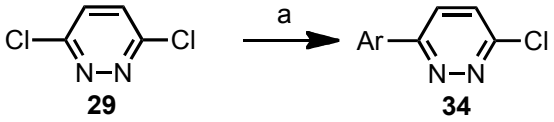


32 **33**

Reagents and conditions: a) $Pd(PPh_3)_4$, Na_2CO_3, Ar-$B(OH)_2$, toluene (5-30 h, 110 ºC)

Scheme 1.6

Perhaps the remaining drawback of the Suzuki-Miyaura conditions employed by the aforementioned groups are the long conversion times necessitated by the majority of transformations. While the approach is clearly more 'atom economical' than preceding conversions (**Scheme 1.4**), there remains a palpable desire to curtail the sluggish nature of the reaction. To this end, several research groups have tried to circumvent the issue by applying microwave irradiation.[97, 98] Numerous organic transformations, including Pd-mediated cross-couplings have been expedited by the use of microwave technology. Lin and co-workers were able to illustrate that the Pd-mediated coupling of a boronic acid with dichloropyridazine **29** under microwave conditions could swiftly lead to arylpyridazines **34** in good yields (**Scheme 1.7**).[98]



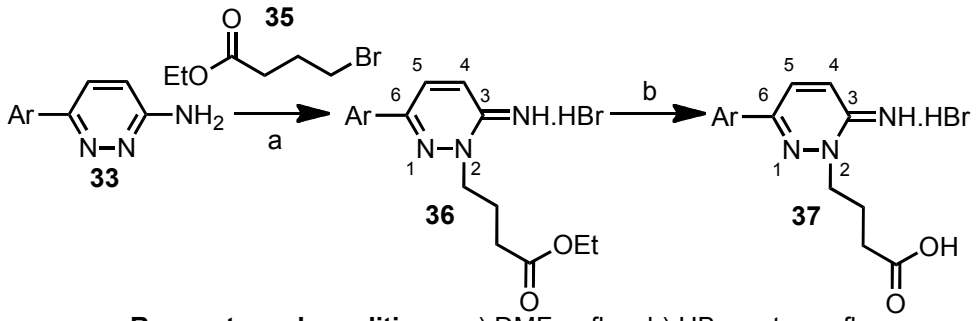
29 **34**

Reagents and conditions: a) $Pd(PPh_3)_2Cl_2$ (3 mol %), K_2CO_3, Ar-$B(OH)_2$, CH_3CN/H_2O (3:2), microwave (10 min, 120 ºC), 53-93 %

Scheme 1.7

Beyond the construction of the arylpyridazine core, in order to function as a potent $GABA_A$ antagonist, the arylpyridazine must be conjugated with a unit of GABA. It has been suggested that antagonistic nature of gabazine and other similar compounds originate from the *N*-substitution of GABA by a charge-delocalized guanidinic or amidinic system.[67, 72] Alkylation of arylpyridazines **33** by GABA is conventionally achieved through reaction with ethyl 4-bromobutyrate **35** giving protected antagonists **36**, followed by acidic deprotection, to yield $GABA_A$ antagonists **37** (**Scheme 1.8**).[71, 72, 81] The ability to selectively achieve *N*(2)-alkylation of

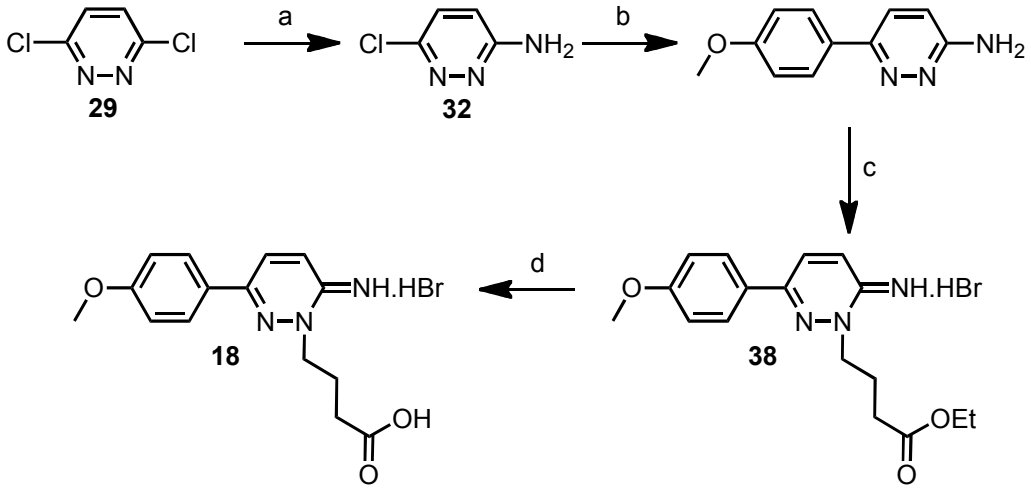
arylpyridazine **33** stems from a combination of steric effects from the adjacent aryl moiety and resonance stabilisation. If the aryl group is replaced for an alkyl group, then a mixture of *N*(1) and *N*(2)-alkylated products are often formed. Nevertheless, these are usually easily separable by recrystallisation.[72, 99]



Reagents and conditions: a) DMF, reflux; b) HBr, water, reflux

Scheme 1.8

In a recent example, Gavande *et al*. showed that microwave-accelerated synthesis can be applied to the total synthesis of gabazine **18**, achieved in just four steps from dichloropyridazine **29**, where three of the four reactions were accelerated by microwave irradiation (**Scheme 1.9**). In their synthesis, the deprotection of the ethyl ester **38** is achieved using basic conditions.[100]



Reagents and conditions: a) 30 % aq. NH_4OH, microwave (30 min, 120 ^oC), 87 %; b) $Pd(PPh_3)_4$ (5 mol %), K_2CO_3, 4-methoxyphenylboronic acid, $EtOH/H_2O$ (4:1), microwave (10 min, 120 ^oC), 94 %; c) ethyl-4-bromobutyrate, DMF, microwave (15 min, 80 ^oC), 95 %; d) NaOH, 2 h, rt, 85 %.

Scheme 1.9

1.2 Introduction to Photoaffinity Labelling

1.2.1 Photoaffinity Labelling

Photoaffinity labelling is a biochemical method that has been exploited in the analysis of many biological systems.[101]

Often, photoaffinity probes consist of two parts.[102] The first part, the photoaffinity label, forms a covalent bond between the probe and target molecule, through photoirradiation. Photoaffinity labels absorb radiation and subsequently form a highly reactive species. These reactive intermediates are then able to undergo largely non-specific reactions (*e.g.* C-H or X-H insertion reactions) with adjacent residues to form cross-linked products that are covalently bound to one another.[103] In this way, useful information can be garnered about the environment that a particular molecule may reside in (**Figure 1.19**). Photoaffinity labels are often introduced chemically, genetically or metabolically into target molecules.[104]

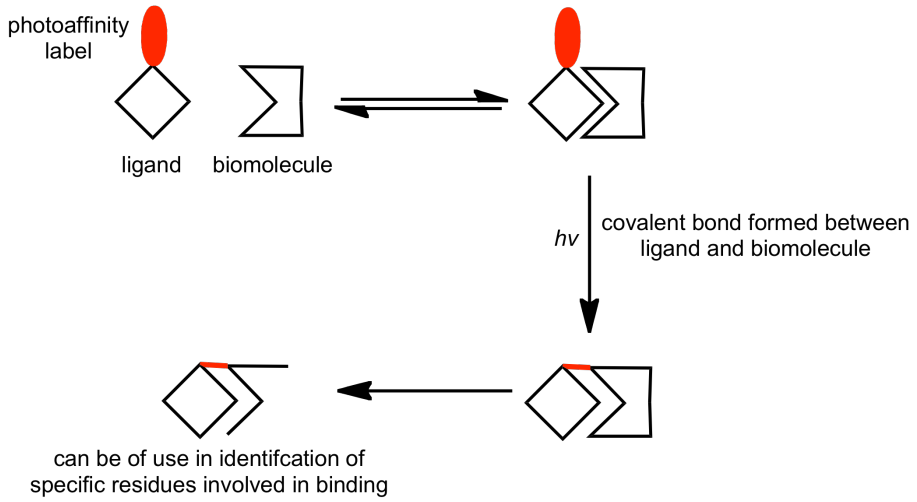
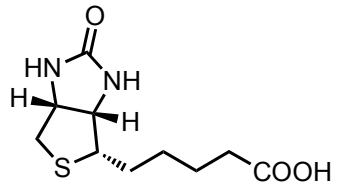


Figure 1.19 – Photoaffinity labelling in structural elucidation

The second part can be a detectable tag that is sometimes incorporated into a photoaffinity probe.[102] One such method is through the incorporation of a radiolabel. Identification of products post-photolysis can necessitate a group that can be easily traced by spectroscopic methods; in this instance, a radioisotope labelled probe becomes a valuable tool in elucidation. Isotopes commonly used include ^{3}H, ^{14}C, ^{32}P, ^{35}S and ^{125}I. Vitamin B_7, also known as biotin **39**, can also be employed as a

detectable tag, due to its high affinity to avidin, thus allowing immediate analysis or purification of labelled molecules (**Figure 1.20**).[105-107]



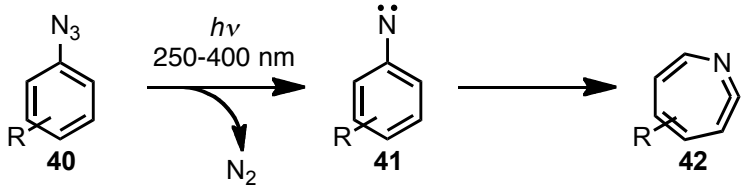
Vitamin B_7
Biotin **39**

Figure 1.20

Several photoaffinity labels have been used extensively in biology. The three discussed in detail in this chapter, the aryl azide, benzophenone and aryl diazirine, are among the most commonly used.

1.2.2 Aryl Azides

Aryl azides **40** can be photoactivated to nitrenes **41** by wavelengths ranging from 250 to 400 nm (**Scheme 1.10**).[103]



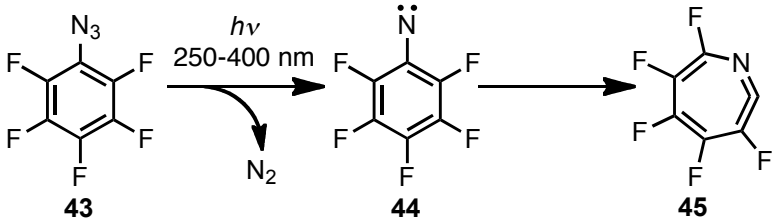
Scheme 1.10

The label is synthetically small and is in common use due, in part, to the relatively simple synthesis. Upon photoactivation, the aryl azide loses dinitrogen to become a highly reactive nitrene **41**. Aryl azides are most commonly photoactivated beneath 350 nm. The potentially high-energy radiation required to form nitrenes can hamper the environment of a photoaffinity probe, as proteins and biomolecules can be damaged by the low-wavelength irradiation.[103]

Originally, it was thought that reactions took place solely *via* the nitrene; however, it is now known that this intermediate is rather short-lived. A rapid intramolecular ring expansion of **41** is able to give the azacycloheptatetraene **42** which is highly

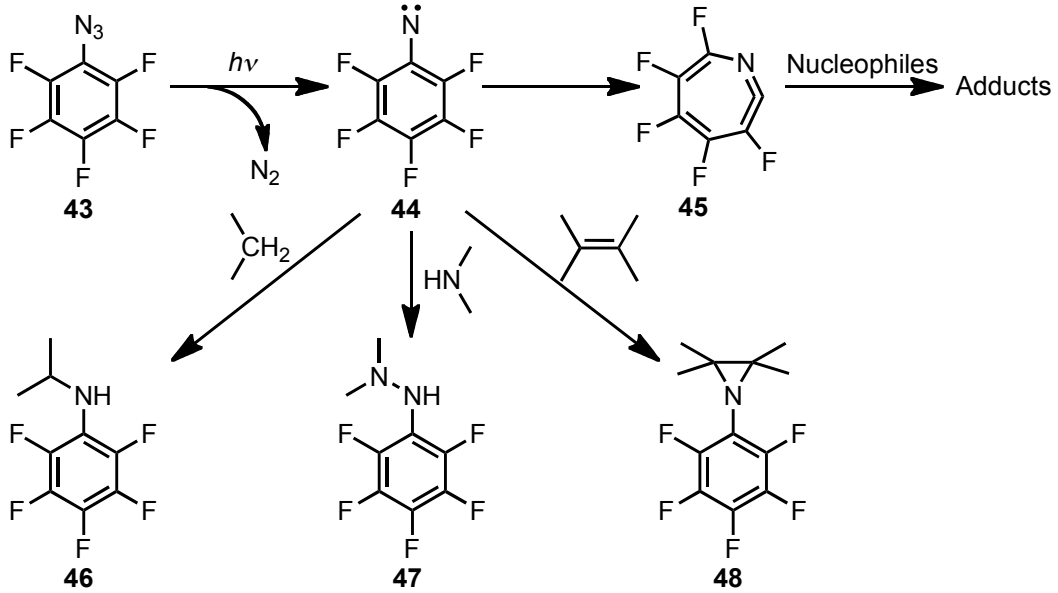
electrophilic in nature. It is thought that **42** is able to react with neighbouring nucleophilic residues. **42** is not able to insert into nonactivated C-H bonds, and as a result azacycloheptatetraenes are considered undesirable, as they are essentially less reactive than their nitrene precursors.[108-110]

One strategy to limit the unfavorable conversion to **42** is to fluorinate positions on the neighbouring aryl group. Perfluorophenylazides **43** undergo the same photochemical conversions as aryl azides **40** but fluorine substitution greatly slows down the rate of ring expansion of perfluorophenylnitrene **44** to the corresponding azacycloheptatraene **45** (**Scheme 1.11**).[111]



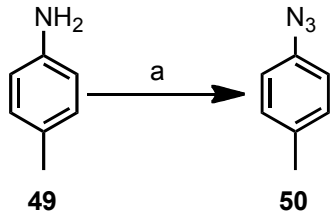
Scheme 1.11

The reactions of the photoactivated nitrene with an array of simple organic molecules are shown in **Scheme 1.12**. Reaction of nitrene **44** with secondary amines leads to hydrazines **47**, whereas reaction with alkenes leads to aziridines **48**.[103]



Scheme 1.12

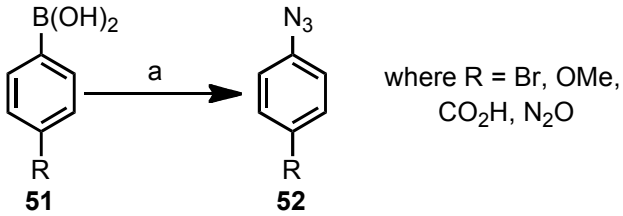
Aryl azides can be simply synthesised from the corresponding aryl amine.[112] The Sharpless group demonstrated that *para*-toluidine **49** can be converted to the corresponding azide **50** in excellent yield (**Scheme 1.13**).[113]



Reagents and conditions: a) $NaNO_2$, HCl/H_2O, -5 °C, then NaN_3, NaOAc, 0 °C, 95 %.

Scheme 1.13

Aryl azides **52** can also be formed from a corresponding boronic acid. Grimes *et al*. recently reported the copper(II)-catalysed formation of aryl azides by addition of sodium azide to arylboronic acids **51** (**Scheme 1.14**).[114]



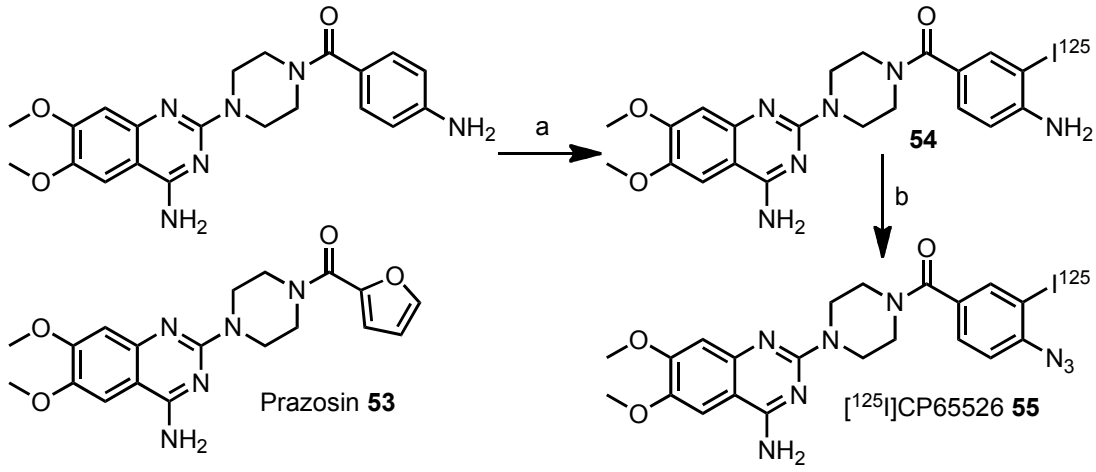
Reagents and conditions: a) NaN_3 (1.5 eq.), $Cu(OAc)_2$ (10 mol %), MeOH (1-3 h, 55 °C), 68-89 %

Scheme 1.14

1.2.2.1 Aryl azides in biological probes

One instance where aryl azides have been employed as photoaffinity labels for receptor study was by Seidman and co-workers in their synthesis of the radiolabelled prazosin analogue, [^{125}I]CP65526 **55**.[115] Prazosin **53** is a high affinity α_1-adrenoceptor blocker used to treat high blood pressure. The synthesis of the analogue relies on the formation of an aryl amine precursor **54**, which is simply converted to the desired azide **55** in the final step (**Scheme 1.15**). The resulting photoaffinity probe **55** was able to bind competitively to the α_1-adrenoceptor prior to photolysis of the azide. Following photoactivation, Seidman *et al*. were able to demonstrate that the binding site of probe **55** was identical to that of prazosin **53**. **55** was also recently

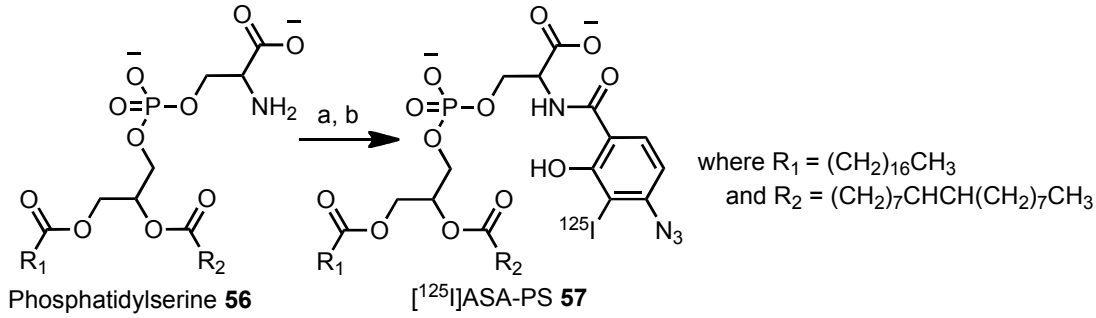
used as a research tool in competition assays with other drug molecules towards the eradication of HIV reservoirs in the brain.[116]



Reagents and conditions: a) NaI^{125}, Chloramine, NaOAc buffer (pH 5.6, 10 min, rt), 37 %; b) $NaNO_2$, HCl/H_2O, then NaN_3, rt, 33 %.

Scheme 1.15

Blanton and co-workers were able to exploit the aryl azide photoaffinity label for the specific labelling of the nicotinic acetylcholine receptor, in the synthesis of the photoaffinity labelled ligand [^{125}I]ASA-PS **57**. This was achieved by incorporating an aryl azide into the structure of a known phospholipid, phosphatidylserine **56** (Scheme 1.16). Subunits of the receptor that covalently incorporated the ligand could subsequently be mapped *via* a *Staphylococcus aureus* protease digestion. [117]

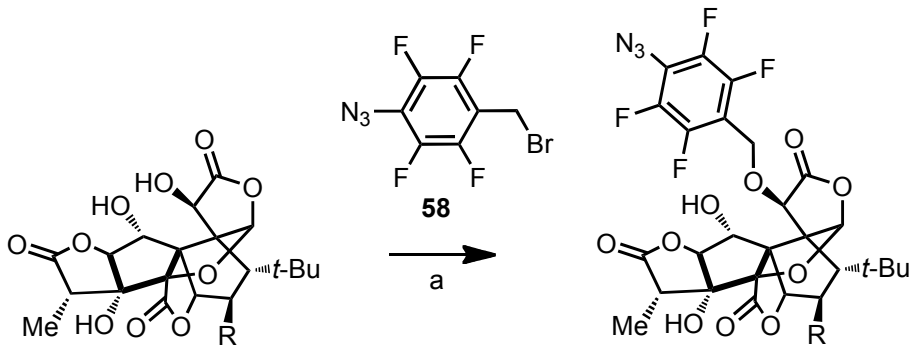


Reagents and conditions: b) NaI^{125}, Chloramine-T, Na-Phosphate buffer (pH 7.4, 2 min, rt); a) *N*-hydroxysuccinimidyl-4-azidosalicylic acid, NEt_3, $CHCl_3$ (48 h, rt).

Scheme 1.16

Photoaffinity labelling has been used to probe the specific binding sites of numerous natural products.[118-121] Strømgaard *et al*. were able to create photoaffinity labelled

analogues of terpenes from the *Ginkgo* genus, which are interesting synthetic targets because of their positive effects on a number of degenerative disorders including Alzheimer's disease.[122] The group incorporated a perfluorinated aryl azide **58** into Ginkgolide B **59** and Ginkgolide C **61** through Williamson ether syntheses, to give analogues **60** and **62** respectively (**Scheme 1.17**).[123]



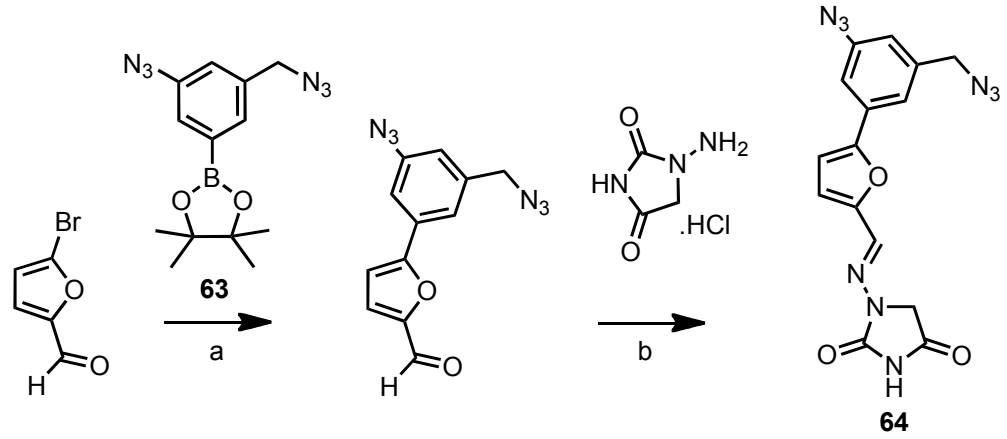
Ginkgolide B **59**, R = H
Ginkgolide C **61**, R = OH

60, R = H
62, R = OH

Reagents and conditions: a) KH, THF (4 h, rt), 50-54 %

Scheme 1.17

Hosoya *et al*. were able to incorporate the aryl azide **63** through a Pd-mediated cross-coupling reaction for the synthesis of a photoaffinity labelled dantrolene analogue **64**, a selective inhibitor of Ca^{2+} release from the sarcoplasmic reticulum (**Scheme 1.18**). The benzylic azide in ligand **64** also allows for the possible incorporation of a detectable tag through a Staudinger ligation or the Cu-mediated azide-alkyne Huisgen cycloaddition. [102]

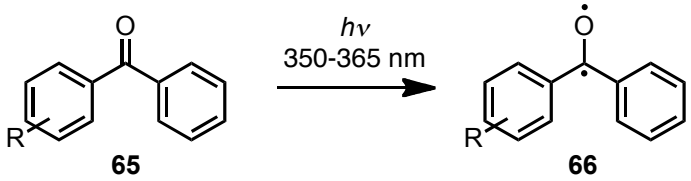


Reagents and conditions: a) $Pd(PPh_3)_4$ (5 mol %), K_3PO_4, DMF (3 h, 80 °C), 68 %; b) aq. HCl, DMF (2 h, rt), 88 %.

Scheme 1.18

1.2.3 Benzophenones

Another class of photoaffinity labels are the benzophenones **65**, which can be photoactivated by wavelengths usually ranging from 350 to 365 nm, depending on the substitution of the aryl groups (**Scheme 1.19**).



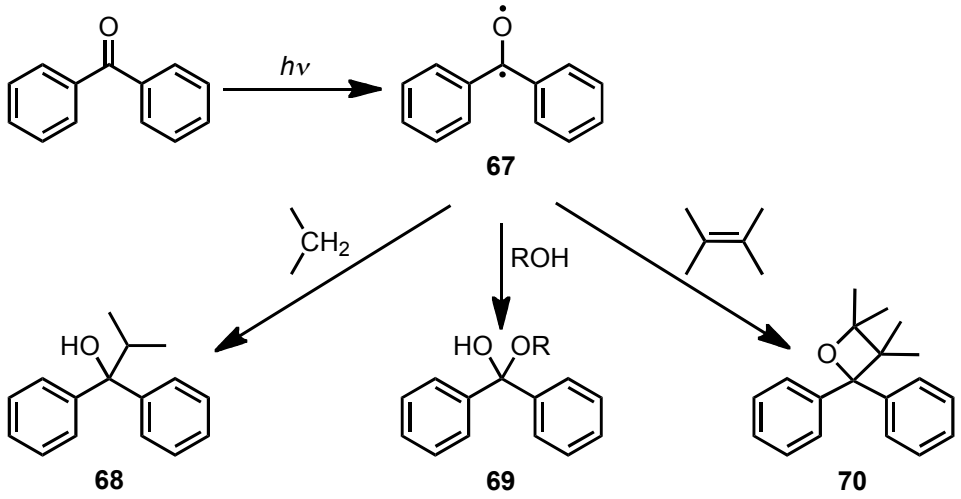
Scheme 1.19

The photolysis at these wavelengths actuates an *n* to π* transition, resulting in the formation of a triplet biradical **66**, which is known to be effective at hydrogen abstraction.[124] Benzophenones can be photoactivated in aqueous media, without the inconvenience of the label being quenched.[103] An additional facet of the photoaffinity label is that benzophenones are able to fall back to their ground state, should they not find a partner to covalently attach to. The benzophenone can therefore be irradiated again to give the reactive biradical. Hence, ligands possessing benzophenone photoaffinity labels generally have a greater degree of covalent bond formation to their intended substrates compared to their aryl azide and aryl diazirine

analogues. This is due to their capacity to be repeatedly irradiated without irreversible breakdown of the probe to an unreactive form.[125]

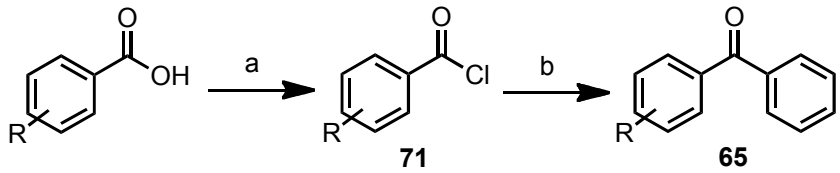
Although the benzophenone carries many advantages compared to its photolabel counterparts, the label is relatively large in size and incorporation of such a sizeable moiety can be detrimental to the binding affinity of a given ligand. In addition, the label can necessitate comparatively long photoirradiation times for tagging.[101]

The reactions of the photoactivated biradical with an array of simple organic molecules are shown in **Scheme 1.20**. Exposure of **67** to simple alkanes leads to alcohol **68**, reaction with alcohols leads to hemiketals **69**. Contact of the biradical **67** with alkenes is documented to leads to oxetane **70** in high yields.[126, 127]



Scheme 1.20

Benzophenones are usually synthesised *via* the Friedel-Crafts acylation of a relevant benzoyl chloride with benzene, catalysed by aluminium trichloride (**Scheme 1.21**).[128]



Reagents and conditions: a) $SOCl_2$, reflux; b) $AlCl_3$ catalyst, benzene, reflux

Scheme 1.21

1.2.3.1 Benzophenones in biological probes

Benzophenone substituted ligands have been used in the labelling of several targets[129] including the angiotensin II receptor,[130, 131] human luteinizing receptor subunits[132] and the insulin receptor.[133] Nakamoto and co-workers were able to label the parathyroid hormone receptor-binding site in 1995 by substituting amino acid residues on the hormone for photoaffinity labelled amino acid residues **72** and **73** (**Figure 1.21**).[134, 135] Suva *et al*. subsequently incorporated **72** in analogues of the hormone calcitonin in order to label the calcitonin receptor.[136]

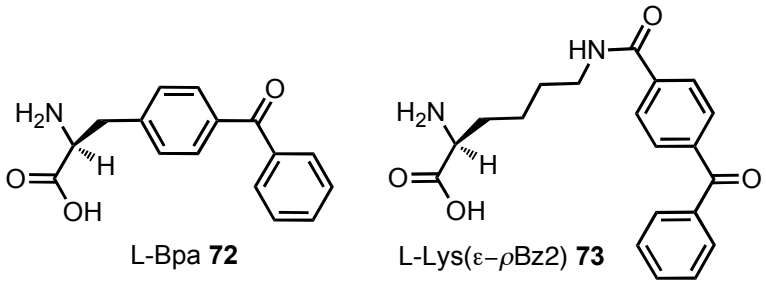
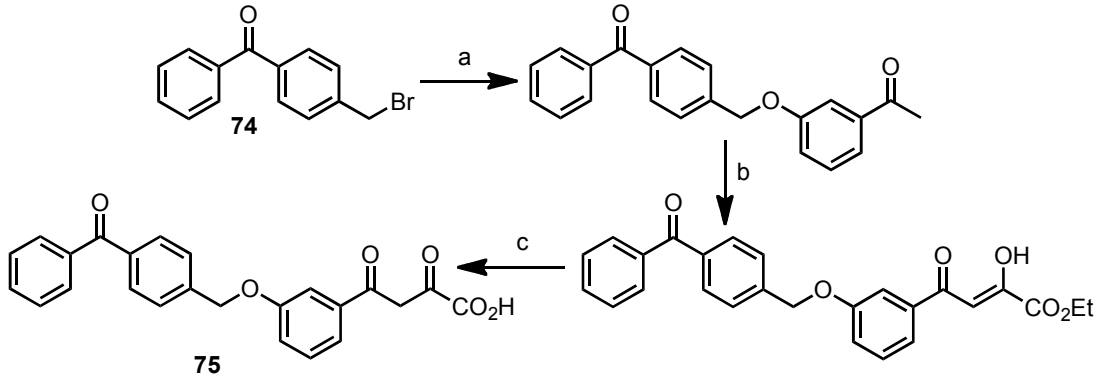


Figure 1.21

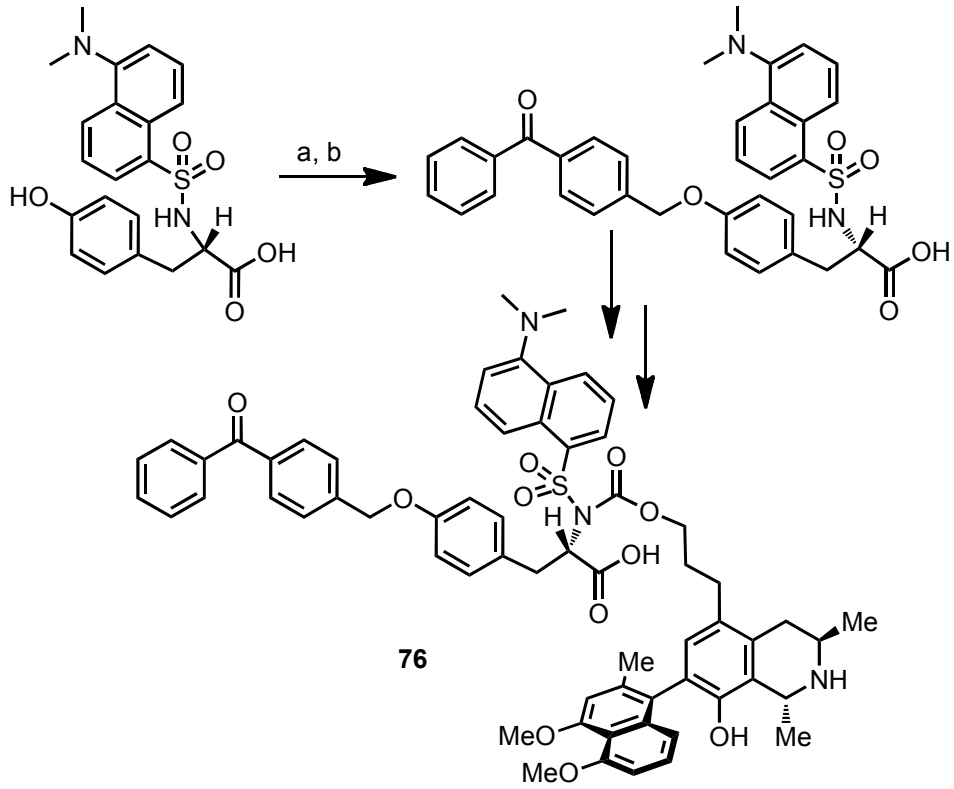
In 2003, Zhang *et al*. synthesised HIV-1 integrase inhibitors containing benzophenones for studying enzyme interactions. In their synthesis of inhibitor **75**, benzophenone **74** is introduced to the core structure through a Williamson ether synthesis (**Scheme 1.22**). While the photoaffinity labelled nature of the ligand was not explored, **75** was found to inhibit integrase activities in low micromolar ranges.[137]



Reagents and conditions: a) K_2CO_3, 1-(3-hydroxyphenyl)-ethane-1-one, DMF (70 ^oC), 56 %; b) diethyl oxalate, NaH, toluene (60 ^oC), quant.; c) NaOH, dioxane/water, 66 %.

Scheme 1.22

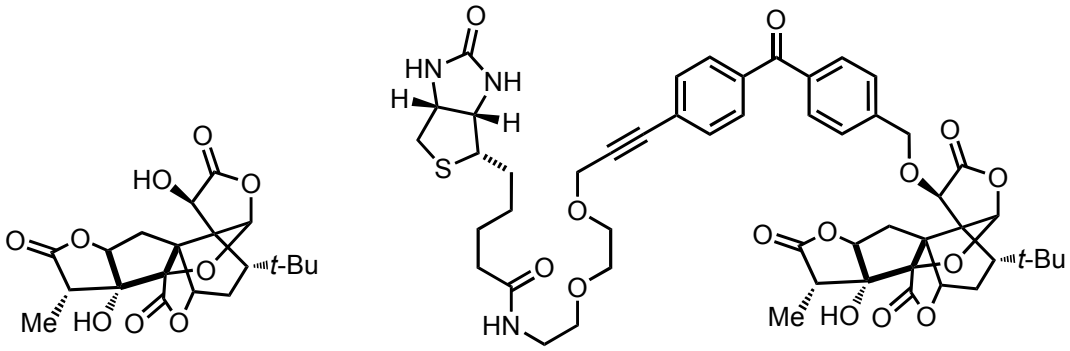
Bringmann and co-workers introduced benzophenone **74** in a similar fashion, for the synthesis of a photoactivatable, fluorescently labelled naphthylisoquinoline alkaloids **76**, which exhibit strong antiplasmodial activities (**Scheme 1.23**). Pharmacological and microscopic investigations were carried out by the group in order to examine the distribution of **76** within *Plasmodium*-infected red blood cells.[138]



Reagents and conditions: a) NaH, $BF_3.Et_2O$, THF (2 h, 65 °C);
b) **74**, Cs_2CO_3, acetone (9 h, 55 °C), then water (30 min, 100 °C), 63 % over two steps.

Scheme 1.23

More recently, the benzophenone photoaffinity label has also been used to tag Ginkgolide A **77** (**Figure 1.22**). Advancing the previous work mentioned by Strømgaard, Kato *et al*. were interested in probing the mode of action of the Ginkgolide family with biological receptors. They were able to create probe **78**, which incorporated a biotin moiety *via* the photoaffinity label to the core natural product. Probe **78** could also be used for the discovery of novel Ginkgolide binding sites.[139]

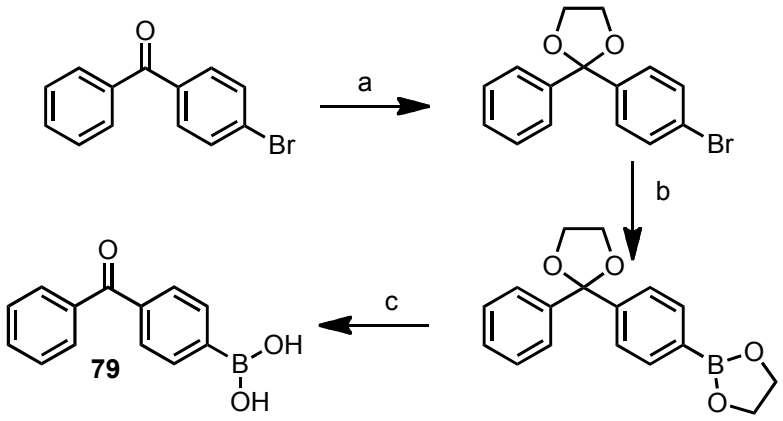


Ginkgolide A **77** **78**

Figure 1.22

In the aforementioned example, probe **78** possesses a photoaffinity label which is substituted on both phenyl groups, yet this substitution does not alter the efficiency of covalent bond formation. The alkyne is introduced *via* a Pd-mediated Sonogashira coupling.

A boronic acid derivative **79** of the benzophenone has been synthesised by the Jones group (**Scheme 1.24**).[140]

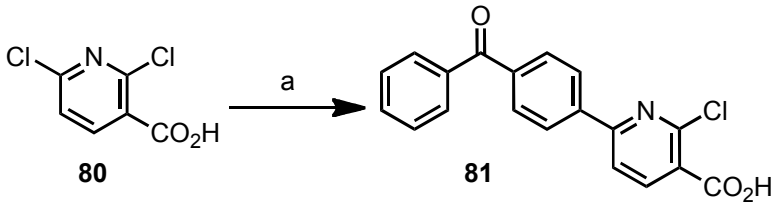


Reagents and conditions: a) Ethylene glycol, *p*-TsOH, benzene (48 h, 80 °C), quant.; b) *n*-BuLi, $B(OMe)_3$, THF (30 min, 78 °C - 0 °C), then H_2SO_4, H_2O (30 min, 0 °C), then ethylene glycol, Et_2O (1 h, rt), 88 %; c) HCl, acetone (48 h, rt), 87 %.

Scheme 1.24

However, the potential of this compound was not demonstrated in a Pd cross-coupling reaction. Instead, **79** was used to label the active site of a chemically modified enzyme. Nevertheless, the application of the benzophenone boronic acid in cross-coupling reactions has since been capitalised on by several research groups.[141-

[145] In 2010, the Houpis group demonstrated the Pd-mediated coupling of an aryl chloride **80** with boronic acid **79** to give pyridine **81** (**Scheme 1.25**).[143]

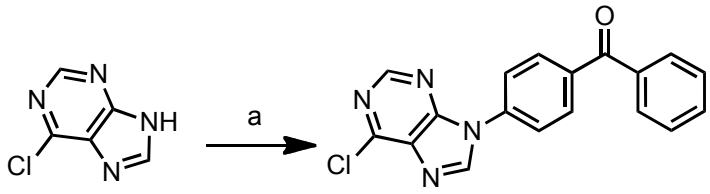


80 **81**

Reagents and conditions: a) **79**, $Pd(OAc)_2$ (3 mol %), PPh_3, Na_2CO_3, MeOH (18 h, 55 ^oC), 73 %.

Scheme 1.25

Benzophenone **79** has also been utilised in copper(II)-mediated cross-coupling reactions for the synthesis of enterovirus inhibitors. Aguado *et al*. were able to synthesise arylpurine **83** through the Cu-catalysed reaction of purine **82** and benzophenone **79** (**Scheme 1.26**). The photoaffinity labelled nature of inhibitor **83** was not further explored, but such an inhibitor could be used for mapping novel binding sites.[146]



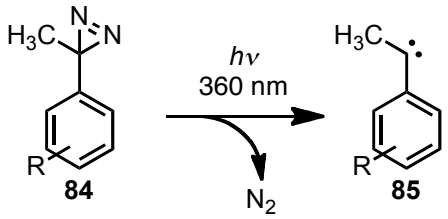
82 **83**

Reagents and conditions: a) **79**, $Cu(OAc)_2$, 1,10-phenanthroline, DMF (72 h, rt), 39 %.

Scheme 1.26

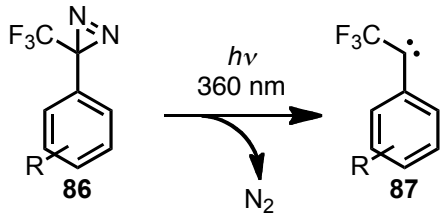
1.2.4 Aryl Diazirines

Aryl diazirines are the final class of photoaffinity labels discussed in this chapter. Aryl diazirines **84** can be photoactivated to a reactive carbene **85** by wavelengths around 360 nm and like the aryl azide photoaffinity label, upon photoactivation these labels lose dinitrogen (**Scheme 1.27**).[147]



Scheme 1.27

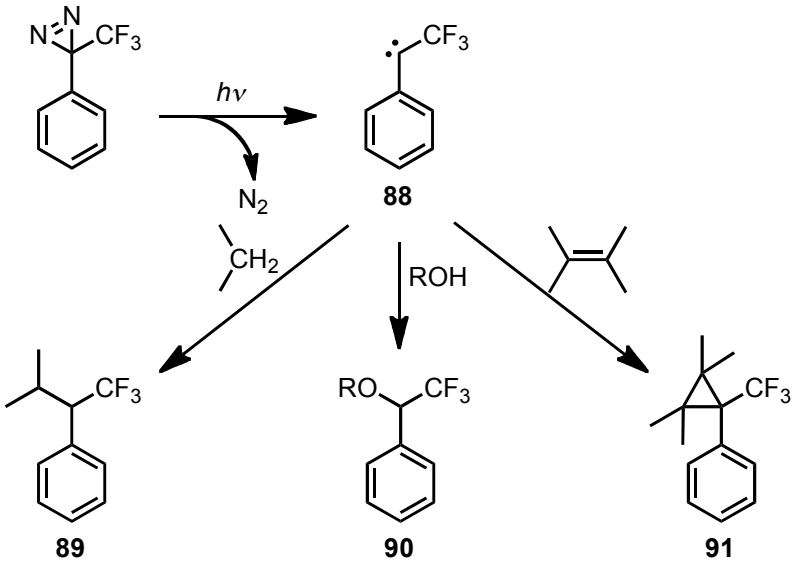
The generated carbenes **85** will insert into any C-H bond in the vicinity. The reactive intermediate is able to form cross-links to biomolecules with rapid photoirradiation times. However, the photolysis of 3-aryl diazirines can result in diazo isomerisation, presenting unwanted intermediates.[103] This unfortunate circumstance can be quelled by the attachment of a flanking trifluoromethyl group, resulting in the trifluoromethylaryl diazirine **86** (**Scheme 1.28**).[148]



Scheme 1.28

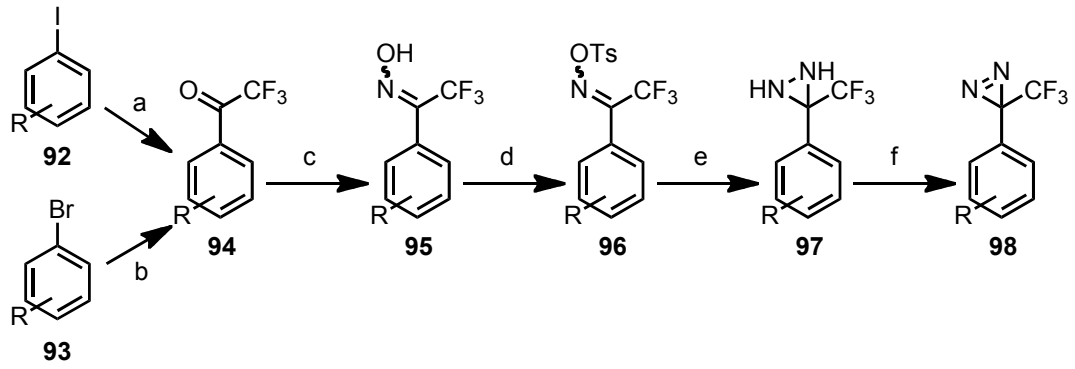
The presence of the adjacent trifluoromethyl group prevents the conversion of the active carbene **87** to undesired intermediates. The trifluoromethylaryl diazirine has been reported to have a remarkably high insertion efficiency, with insertion yields of more than 70 %.[149]

The conversions of carbene **88** with a selection of simple organic molecules are depicted in **Scheme 1.29**. Reaction of **88** with alkanes leads to compounds **89**, whilst exposure of **88** to alcohols leads to ethers **90**. Reaction with alkenes leads to cyclopropanes **91**.[103]



Scheme 1.29

Although this photoaffinity label carries many advantages over those discussed already, use of the trifluoromethylaryl diazirine is fairly limited. A plausible reason for this could be the laborious synthesis required to install the photoaffinity label.[101] Most syntheses of the three-membered diazirinyl ring entail a five-step synthesis from the corresponding aryl halide derivative (**Scheme 1.30**).



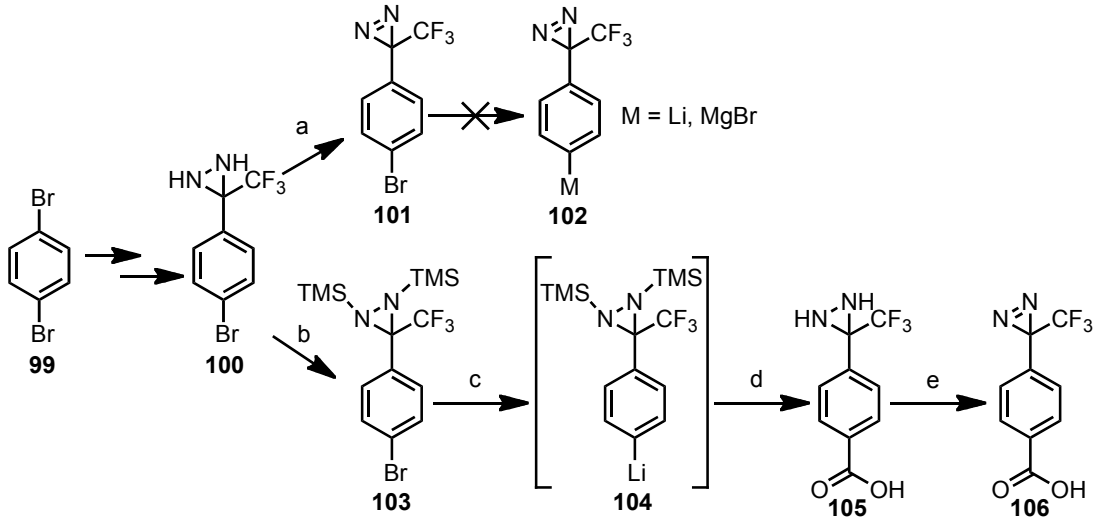
Reagents and conditions: a) Mg, CF_3CO_2Et, THF; b) *n*-BuLi, CF_3CO_2Et, THF; c) $NH_2OH.HCl$, pyridine, reflux; d) *p*-TsCl, pyridine, reflux; e) NH_3, Et_2O, -78 ^oC; f) I_2, NEt_3, DCM, 0 ^oC

Scheme 1.30

Lithium-halogen exchange of an aryl iodide **92** with ethyl trifluoroacetate or a Grignard reaction between an aryl bromide **93** and ethyl trifluoroacetate furnishes trifluoroacetophenone **94**.[150,151] Reaction with hydroxylamine hydrochloride and a base gives oxime **95**. After tosylation to give **96**, reaction with ammonia gives

diaziridine **97**, which can be oxidised using iodine and a base to the desired photoaffinity label **98**.[101]

Work by Bender *et al.* details the synthesis to diazirinylbenzoic acid **106** (**Scheme 1.31**).[152]

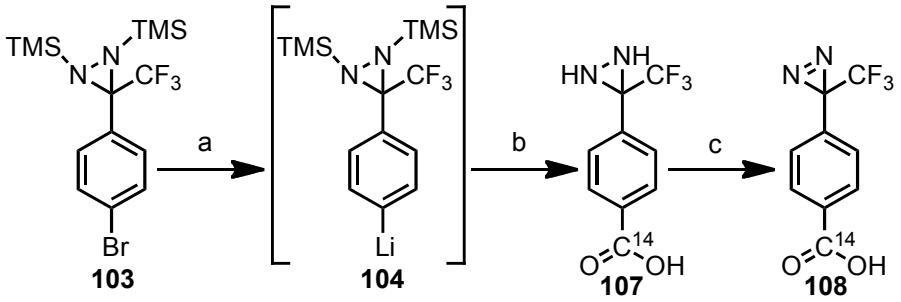


Reagents and conditions: a) *t*-BuOCl, NEt_3, EtOH (2 h, 0 °C), 85 % ; b) TMSOTf, NEt_3, CH_2Cl_2 (3 h, -78 °C), 95 %; c) *n*-BuLi, THF, 0 °C; d) CO_2, THF, (16 h -78 °C), 89 %; e) I_2, NEt_3, CH_3OH (2 h, 0 °C), 90 %.

Scheme 1.31

In this instance, 1,4-dibromobenzene **99** is the aryl halide precursor, and the synthesis of the diaziridine **100** is achieved in four steps in a manner similar to that described in Scheme 1.30. Following oxidation to diazirine **101**, the diazirine does not undergo a metal-halogen exchange to the transmetallated species **102**. *n*-BuLi results in nucleophilic attack on the diazirine ring, whilst no reaction is seen with freshly prepared magnesium. It has been suggested that this inactivity may be due to the diazirinyl nitrogens coordinating to an activated metal surface. Subsequently, it was found that treating the aryl diaziridine **100** with trimethylsilyl triflate gave the silylated diaziridine **103**. Treating **103** with *n*-BuLi provides the lithiated intermediate **104**, which can be reacted with excess carbon dioxide to give diaziridine **105**. A final oxidation step gives the diazirinylbenzoic acid **106**.[152]

Radiolabelled diazirinylbenzoic acid **108** can be created by reacting **103** with radiolabelled barium carbonate (a source of $^{14}CO_2$). Diaziridinylbenzoic acid **107** can be readily oxidised to furnish **108** (**Scheme 1.32**).[152]



Reagents and conditions: a) *n*-BuLi, THF, 0 °C;
b) $Ba^{14}CO_3$, THF (5 h, -78 °C), 78 %; c) I_2, NEt_3, CH_3OH, (2 h, 0 °C), 83 %.

Scheme 1.32

Synthetic routes have been described to a multitude of functionalised aryl diazirines but an accepted pitfall of this label is that a functional handle has to be incorporated very early in a synthesis. In 1994 however, Hatanaka *et al.* showed that the 3-trifluoromethyldiazirinyl moiety was stable to a plethora of organic conversions. By creating a *meta*-methoxy substituted aryl diazirine **109**, electrophilic aromatic substitution is greatly favoured at the 6-position, as a result of both the directing effects of the methoxy-group and the steric bulk of the trifluoromethyldiazirinyl group (**Figure 1.23**).[153] In addition, the methoxy-group could be deprotected using boron tribromide to reveal the phenol. Transformations of **109** include Friedel-Crafts acylations and subsequent alkylations. Aryl diazirines can also be radioiodinated and tritiated *via* this method.[154]

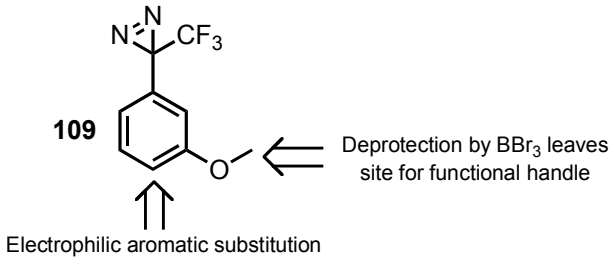
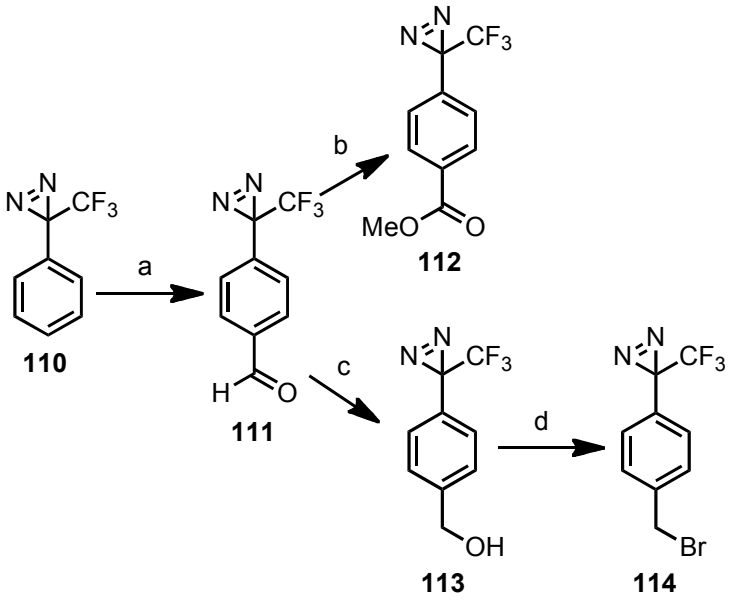


Figure 1.23

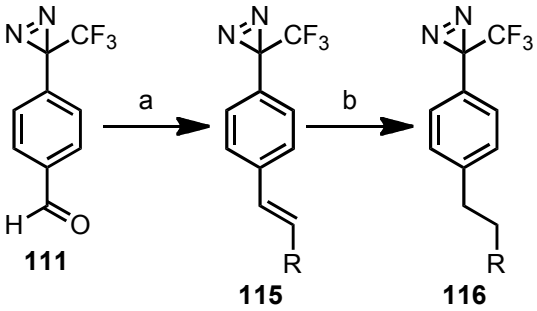
In 2006, Nakashima *et al*. reported the direct formylation of the unsubstituted aryl diazirine **110** to give the formylated aryl diazirine **111** (**Scheme 1.33**). This can be further elaborated to the corresponding ester **112** with methanol and a strong acid, or reduced to the benzylic alcohol **113** by sodium borohydride. Benzylic alcohol **113** can be easily converted to the benzyl bromide **114** *via* the Appel reaction.[155]



Reagents and conditions: a) Cl_2CHOCH_3, $TiCl_4$, TfOH, 80 %;
b) H_2SO_5 (Caro's acid), CH_3OH (3 h, rt), 97 %; c) $NaBH_4$, EtOH (4 h, rt), quant.;
d) CBr_4, PPh_3, CH_2Cl_2 (4 h, rt), quant..

Scheme 1.33

Flexibility can be introduced from the formylated aryl diazirine **111**, through a Wittig reaction to give alkene **115**. The alkene can then be reduced using Wilkinson's catalyst to give **116** (**Scheme 1.34**).[156]

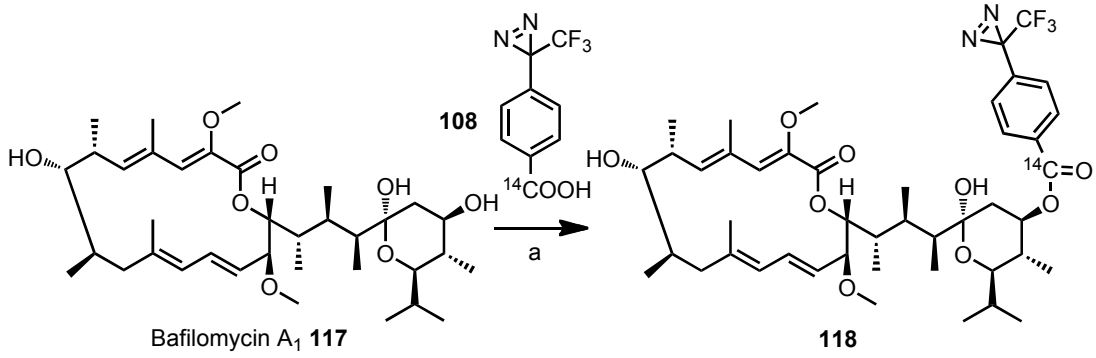


Reagents and conditions: a) Ph_3PCH_2R, CH_2Cl_2 (2 h, rt), 40-98 %;
b) $(Ph_3P)_3RhCl$, H_2, CH_3OH (10 h, rt), 20-45 %.

Scheme 1.34

1.2.4.1 Aryl diazirines in biological probes

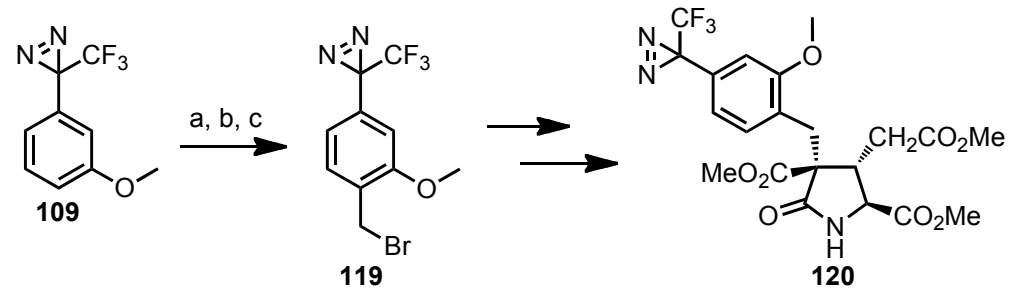
Diazirine **108** has been used in studies to locate binding sites on various ATPases. Malfunctioning ATPases are implicated in a variety of disorders including osteoporosis and male infertility. Bender *et al*. developed a simple coupling reaction between the acidic moiety on photoaffinity label **108** and an alcohol group on the V-ATPase inhibitor Bafilomycin A_1 **117** to create probe **118** (**Scheme 1.35**).[152]



Reagents and conditions: a) DMAP, EDCI, CH_2Cl_2 (16 h, rt), 41 %.

Scheme 1.35

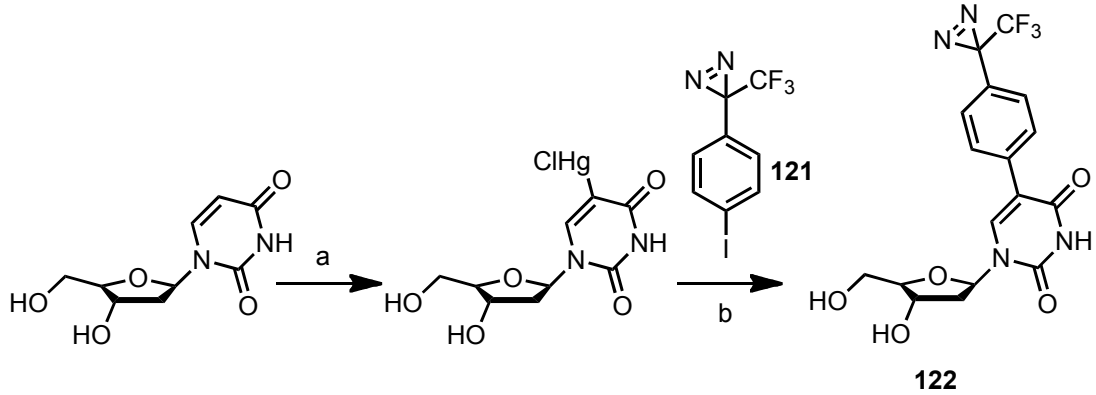
Bentz and co-workers were able to create an aryl diazirine-labelled pyroglutamate **120** for the purpose of amassing binding site information on excitatory amino acid receptors. Employing the methods hitherto described by Hatanaka *et al*., the group used a *para*-methoxy moiety on aryl diazirine **109** as a directing group to introduce the benzyl bromide functionality in aryl diazirine **119** (**Scheme 1.36**).[157]



Reagents and conditions: a) $TiCl_4$, Cl_2CHOMe, CH_2Cl_2, 54 %; b) $NaBH_4$, 91 %; c) PPh_3, CBr_4, Et_2O, 90 %

Scheme 1.36

It has been suggested in the literature that diazirines might be sensitive to palladium catalysis.[158, 159] Nonetheless, an iodide derivative **121** has been utilised in some Pd-mediated conversions. In 1998, Topin *et al*. reported the use of aryl diazirine **121** in a cross-coupling reaction to give a labelled nucleoside analogue **122** (**Scheme 1.37**). One of the drawbacks of this reaction is that a stoichiometric amount of catalyst was used.[160]

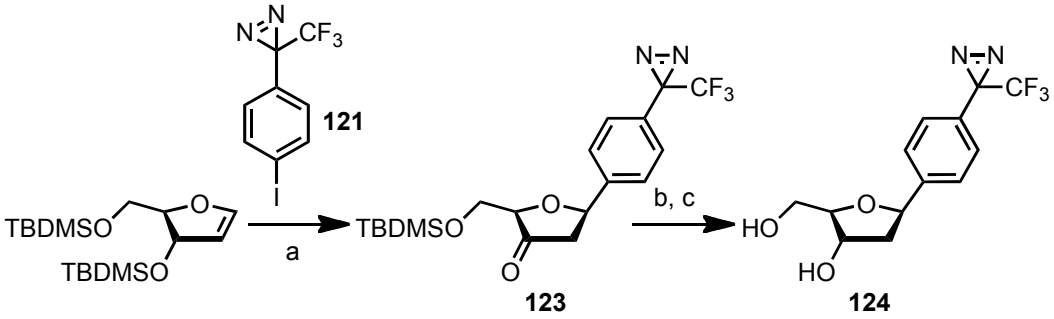


Reagents and conditions: a) $Hg(OAc)_2$, NaCl, 86 %; b) Li_2PdCl_4, CH_3OH (72 h, rt), 10 %.

Scheme 1.37

More recently, aryl diazirine **121** was employed in a Heck reaction to give another labelled nucleoside analogue **124**.[161] By incorporating the aryl diazirine directly onto the ribose, DNA was effectively labelled intrahelically (**Scheme 1.38**), whereas other research groups had only managed to label DNA extrahelically using diazirines.[162-164] The reaction conditions suggest the diazirine motif is able to withstand mild heating for prolonged periods, without undergoing degradation. Ribose **123** is

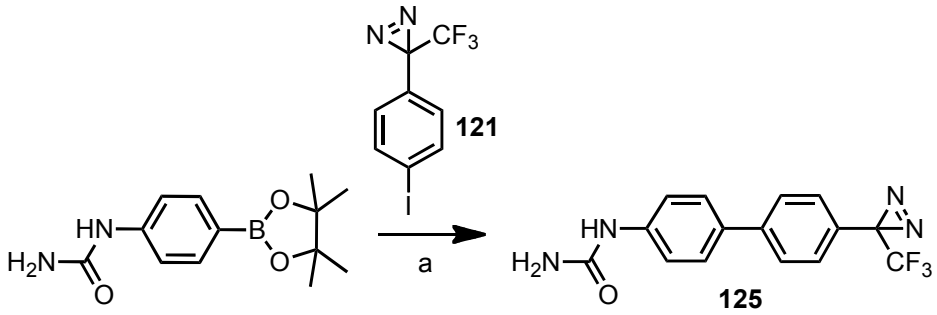
deprotected by TBAF before reduction by $Na(OAc)_3BH$, exhibiting the resistance of the diazirine to mild reducing conditions.



Reagents and conditions: a) $Pd(OAc)_2$, *n*-$Bu_4N^+Cl^-$, Cy_2NCH_3, DMF (40 h, 60 °C), 46 %; b) TBAF, THF/AcOH (1.5 h, 0 °C), 92 %; c) $Na(OAc)_3BH$, CH_3CN/AcOH, (30 min, -10 °C), 56 %.

Scheme 1.38

In 2007, Luo *et al*. used diazirine **121** in a Suzuki-Miyaura cross-coupling reaction to create a photoaffinity labelled ATP competitive inhibitor **125** possessing low nanomolar affinity (**Scheme 1.39**).[165]



Reagents and conditions: a) $Pd(PPh_3)_4$, K_2CO_3, DMF (6 d, rt), 20 %.

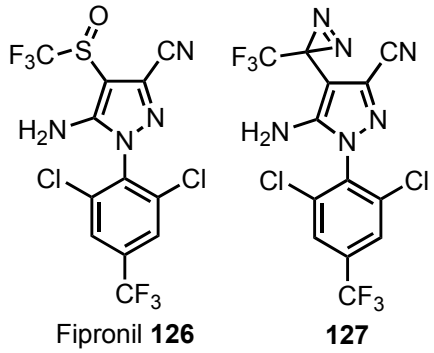
Scheme 1.39

Although no boronic acid analogue of the aryl diazirine has been reported, many of these reactions show that aryl diazirines are stable to metal-mediated catalysis.

1.2.5 Photoaffinity based probes for the $GABA_A$ receptor

Multiple photoaffinity-based probes have been synthesised in order to locate various binding sites on the $GABA_A$ receptor. In 2003, Sammelson and Casida were able to create a diazirine labelled probe **127** based on the insecticide Fipronil **126** (**Figure 1.24**). The probe non-competitively blocks the $GABA_A$ receptor by binding to an

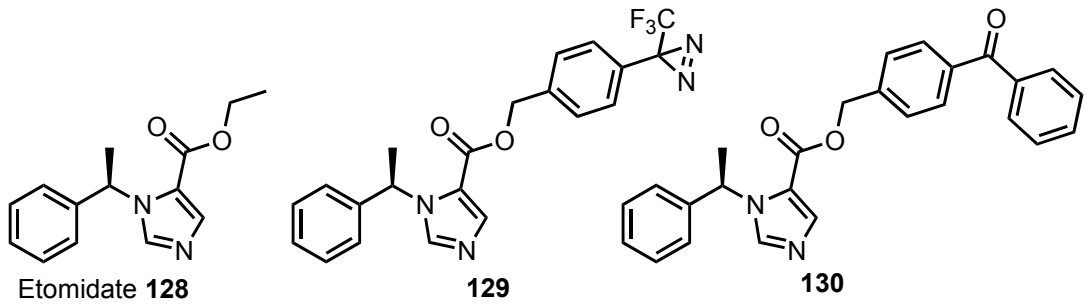
allosteric site. Neither **126** nor **127** are selective, and both also bind to glutamate-gated Cl^- channels, limiting their use as research tools with the sole purpose of blocking the $GABA_A$ receptor.[166]



Fipronil **126** **127**

Figure 1.24

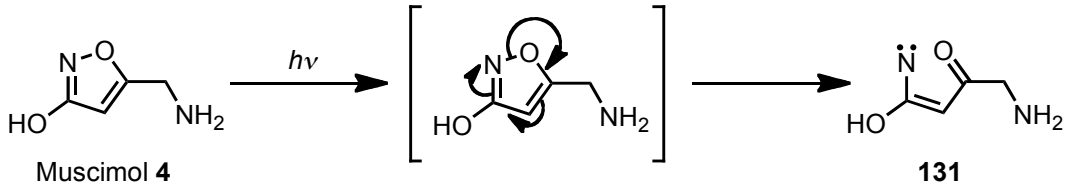
Husain *et al*. synthesised photoaffinity based probes in order to locate an anaesthetic binding site on the $GABA_A$ receptor. Etomidate **128** is a modulator of the $GABA_A$ receptor and an enhanced GABA-induced current is observed at receptors carrying either a β2 or β3 subunit.[167, 168] Despite the incorporation of a photoaffinity label in probes **129** and **130** resulting in more sizeable analogues, both analogues remain potent general anaesthetics (**Figure 1.25**).



Etomidate **128** **129** **130**

Figure 1.25

An esoteric instance of photoaffinity labelling is observed with two hitherto discussed agonists. Muscimol **4** and thiomuscimol **5** have both been exploited as a photoaffinity labelled agonists with moderate success.[169] When **4** is irradiated with ultraviolet radiation, N-O bond cleavage results in the formation of a reactive intermediate **131**, which is able to irreversibly bind to the receptor (**Scheme 1.40**).



Scheme 1.40

Thiomuscimol is regarded more useful as a photoaffinity labelled agonist, since it absorbs UV light at a higher wavelength than muscimol, desirable for *in vivo* systems where high energy irradiation damages cells and proteins.[170, 171] Tritiated analogues of thiomuscimol may also be useful for studying the localisation of receptors.[170, 172]

1.2.6 Photochemically inactivating receptors using photoactivatable antagonists

While the principal use of photoaffinity labelling has traditionally been to acquire structural information about a particular binding site, some groups have used the concept as a way of blocking certain biological processes. Chambers and co-workers for example, pioneered a novel method of tracking receptors *in vivo*. Populations of glutamate receptors can be deactivated by the addition of photochemically reactive antagonists. AMPA receptors are a major type of ionotropic glutamate receptor found in the brain, where they are responsible for fast excitatory neurotransmission. The method of deactivation involves the modification of a known antagonist, DNQX **132**, by the substitution of a nitro group for an azide moiety, forming ANQX **133** (**Figure 1.26**).

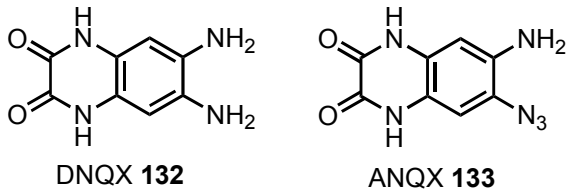


Figure 1.26

Upon photoactivation, an active nitrene is formed, which is able to insert into the amino acid side chains of the protein to which it is bound. Since **133** is a competitive antagonist at the AMPA receptor, when irradiated the compound binds to the receptor irreversibly and renders the receptor inactive.[44] Synaptic currents can be

recorded to measure the rate of AMPA receptor insertion at the cell membrane *in tandem* with the photoactivation of **133**. By electrophysiological patch-clamp recording, receptors that have been inactivated can be distinguished from those that remain active. Recording of synaptic currents requires a patch-clamp configuration with a UV light source as described by England.[45]

Furthermore, photoaffinity labelling has been used to probe the localisation and function of $GABA_B$ receptors. In this instance, an aryl diazirine is linked to a known antagonist. A fluorophore, BODIPY, is then installed by virtue of a copper-catalysed [3+2] azide-alkyne cycloaddition, to give a photoaffinity and fluorophore labelled probe **134** (**Figure 1.27**).[173]

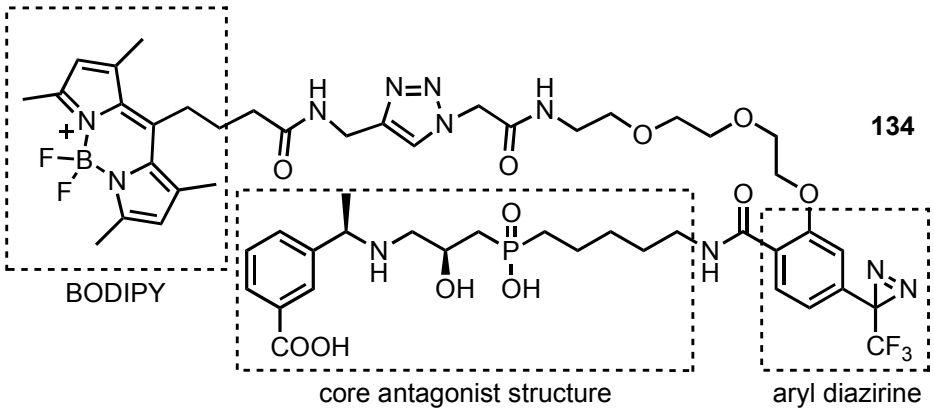


Figure 1.27 – Photoaffinity probe for the $GABA_B$ receptor

1.3 Summary

A brief overview of the $GABA_A$ receptor and photoaffinity labelling has been given in this chapter.

Firstly, the concept of neurotransmission was introduced and an overview of the neurotransmitter GABA and its role in the mammalian CNS was given. Along with this, several classes of compounds have been discussed, demonstrating the vast array of compounds able to interact with the receptor.

Secondly, a number of commonly used photoaffinity labels have been discussed in detail, stating their respective advantages and disadvantages. Each photoaffinity label was then linked to examples in the literature, highlighting the ease of incorporation

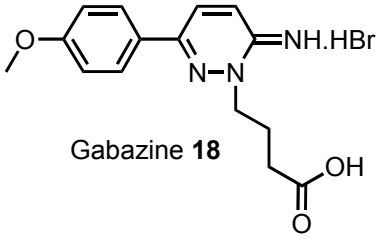
of various photoaffinity labels into a multitude of ligands, including natural products. Additionally, some examples have described a photoaffinity label incorporated directly onto a known antagonist.

Leading on from observations made in the literature, chapter two discusses our efforts to create a lead compound for the $GABA_A$ receptor, and also our efforts to incorporate a photoaffinity label into the core antagonist structure. Using the methodology developed by Chambers *et al*. we eventually hope to be able to knock out $GABA_A$ receptors *in vivo*, using a suitably labelled antagonist. As mentioned, the cycling of receptors is thought to be significant for the efficacy of synaptic inhibition. To extract further information regarding the mobility of $GABA_A$ receptor subunits and their life cycle requires the development of a traceable molecule that will affect function irreversibly. This project aims to create several gabazine analogues to probe the mobility of $GABA_A$ receptors in the mammalian CNS.

2 Results and Discussion

2.1 Aim

This project aims to create gabazine analogues to probe the mobility of $GABA_A$ receptors in the mammalian CNS. In order to track the movement of native receptors, a tag such as a photoaffinity labelled antagonist is required to covalently bind to the receptor *in situ*, thereby affecting its function. By using a set-up similar to that described by England in section **1.2.6**, we hope to be able to track the movement of $GABA_A$ receptors in neurons.[44, 45] As mentioned, gabazine **18** (**Figure 2.1**) is a selective, potent competitive antagonist for the $GABA_A$ receptor, making it an ideal candidate for the template molecule of our project.



Gabazine **18**

Figure 2.1

Gabazine binds to the GABA binding site, thus preventing GABA from binding. It has been shown that the arylpyridazine core can be functionalised without causing a loss in selectivity, discussed in section **1.1.5.5**.[70, 72] By incorporating a photoaffinity label onto the core structure, we hope to create photoactivatable antagonists that upon irradiation irreversibly bind to the receptor, thus blocking its function (**Figure 2.2**).

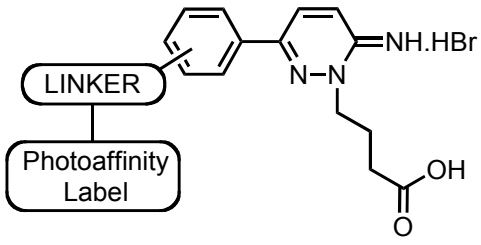


Figure 2.2

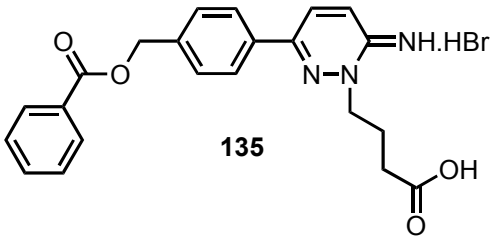
It was not known whether the addition of a photoaffinity label to the gabazine skeleton would diminish or enhance the potency and selectivity for the $GABA_A$ receptor. Our initial efforts focussed on the development of a lead compound, outlined in section **2.2**.

This lead compound replaced the photoaffinity label with a surrogate phenyl group, since the synthesis of some photolabels can be time consuming and expensive. Such an analogue would also help determine whether making a photolabelled derivative of the same compound would be worthwhile; we first wanted to check that the binding of new antagonists were retained through the incorporation of new aromatic groups. Any successful synthetic route to a lead compound would also be applicable to the syntheses of photolabelled derivatives.

2.2 Lead compound synthesis

2.2.1 Benzoyl ester antagonist

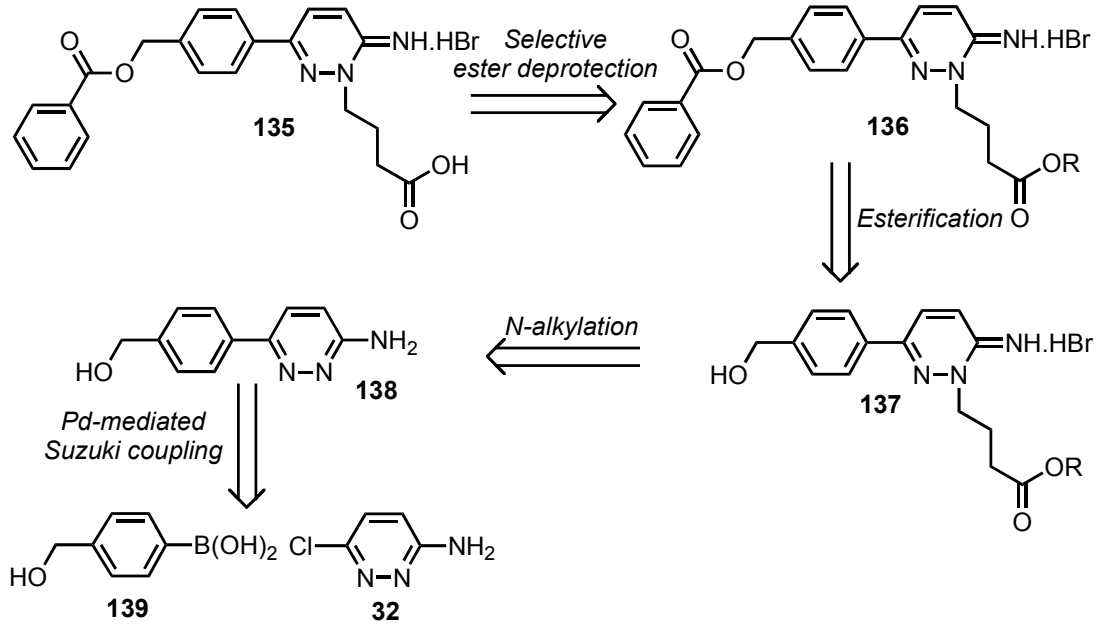
We initially attempted to create the benzoyl ester antagonist **135** (**Figure 2.3**).



135

Figure 2.3

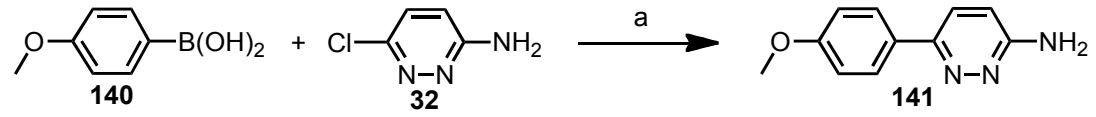
The retrosynthetic route to compound **135** is outlined in **Scheme 2.1**.



Scheme 2.1

As shown in the retrosynthesis, **135** could be formed through the deprotection of ester **136**. A potential site for a photoaffinity label could be introduced through the coupling of a benzoyl chloride and alcohol **137**. *N*-Alkylation of arylpyridazines has been documented in the literature, and we felt that it was possible to follow the methods outlined previously for conversion of arylpyridazine **138** to **137**. We planned the construction of the arylpyridazine core through the Suzuki-Miyaura coupling of chloropyridazine **32** and boronic acid **139**.

Our synthesis began by testing the feasibility of the Pd-mediated conversion of pyridazine **32** and boronic acid **140** (**Scheme 2.2**), replicating a reaction that had been documented in the literature.[174]



Reagents and conditions: a) $Pd(PPh_3)_4$, Na_2CO_3, toluene, reflux.

Scheme 2.2

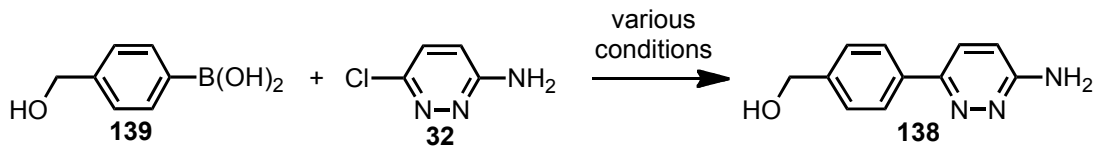
We initially chose to use conditions employed by Maes *et al*., but were disappointed

with the low yield of arylpyridazine **141** achieved from the reaction.[174] An increase in catalyst loading and slight adjustment with the delicate ratio of the reagents however, led to a vast improvement in yield (**Table 2.1**).

Entry	Ratio of reagents (**140** : **32**)	Catalyst/ mol %	Duration/ h	**Yield**
i	1.5 : 1	3	20	26 %
ii	1.1 : 1	5	8	35 %
iii	1.5 : 1	5	10	76 %

Table 2.1

With these results, we proceeded to use our best conditions in our system (**Scheme 2.3**). However, we were disappointed to find low yields, perhaps attributable to the naked alcohol of boronic acid **139**. At this juncture, we investigated the use of microwave irradiation to see if we could enhance the poor yields. No change was observed when using our previous optimised conditions, but upon adopting microwave conditions employed by the Lin group, we observed a dramatic increase in yield (**Table 2.2**).[98]



Scheme 2.3

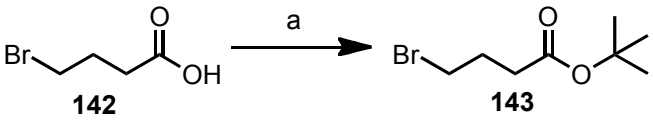
Entry	Ratio of reagents (**139** : **32**)	Catalyst (mol %)	Conditions	**Yield**
i	1.5 : 1	$Pd(PPh_3)_4$ (5)	10 h, 110 °C, toluene	33 %
ii	1.5 : 1	$Pd(PPh_3)_4$ (5)	10 min, m/w irradiation, 120 °C, toluene	33 %
iii	1.2 : 1	$Pd(PPh_3)_2Cl_2$ (3)	10 min, m/w irradiation, 120 °C, CH_3CN/water (3:2)	71 %

Table 2.2

Possible reasons for the observed increase in yield could be due to the use of an air stable catalyst or due to the increased polarity of the solvent system. A boronic acid possessing a polar substituent such as a methoxy- or hydroxy- group, such as **139**, may benefit from a reaction conducted in a more polar medium, such as this. Lin *et al*. reported that a similar reaction conducted in a toluene/DMF mix severely diminishes the yield.[98]

Since compound **136** includes two ester linkages, we thought it important to employ a mild method to facilitate the selective deprotection of just one ester. In addition, it was felt that upon eventual replacement of the phenyl group for a photoaffinity label such as an aryl diazirine, it would be unable to tolerate the strongly acidic deprotection conditions required to cleave an ethyl ester, as previously employed by Wermuth.[72] Accordingly, two bromoesters with mild deprotection conditions were synthesised for use in our syntheses. We considered the formation of a *tert*-butyl ester and an allyl ester as being the most useful for our syntheses. The *tert*-butyl group can be deprotected using a mild acid at approximately pH 4, whilst an allyl group can be cleaved using a Pd catalyst and appropriate scavenger.

The synthesis of ester **143** from acid **142** was achieved following methods described by Patel *et al*. (**Scheme 2.4**), through the synthesis of the mixed anhydride *in situ*, followed by displacement using *tert*-butanol.[175, 176]

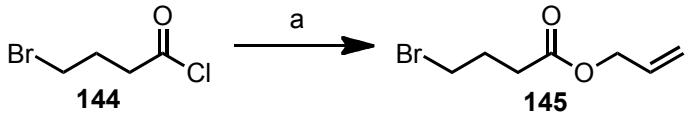


142 **143**

Reagents and conditions: a) *t*-BuOH, $(CF_3CO)_2O$, THF (16 h, -40 ºC to rt), 56 %.

Scheme 2.4

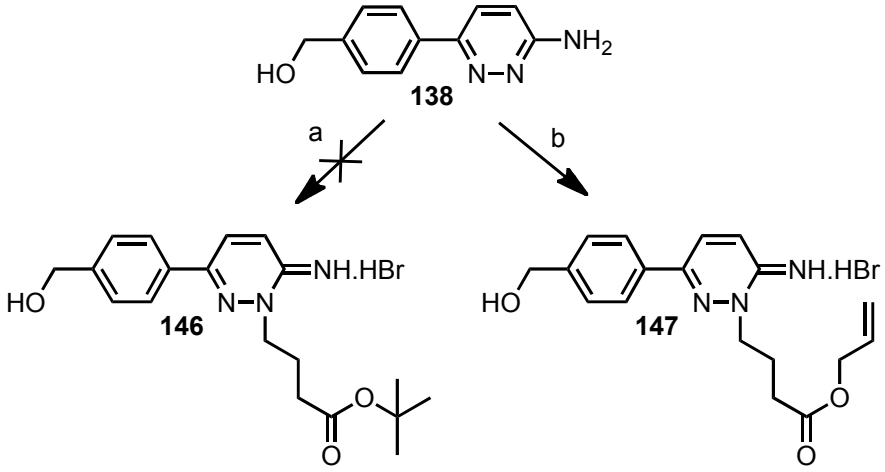
Similarly, the synthesis of allyl ester **145** was achieved following a procedure outlined by Gellerman and co-workers (**Scheme 2.5**), by reaction of the commercially available acyl chloride **144** with allyl alcohol.[177]



Reagents and conditions: a) Allyl alcohol, THF (2 h, 0 °C, then 3 h, rt), 77 %.

Scheme 2.5

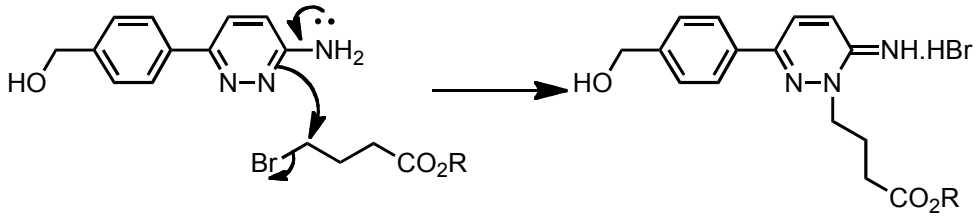
Attempted *N*-alkylation of **138** using the *tert*-butyl ester **143** was unsuccessful resulting in full recovery of both starting materials. *N*-Alkylation using the allyl ester **145** gave the product **147**, isolable by the precipitation of the reaction mixture (**Scheme 2.6**). It remains unclear as to why reaction with *tert*-butyl ester **143** does not lead to compound **146**, but we did not dwell on the result due to the successful nature of the reaction with allyl ester **145**.



Reagents and conditions: a) **143**, DMF (8 h, 80 °C); b) **145**, DMF (8 h, 80 °C), 76 %.

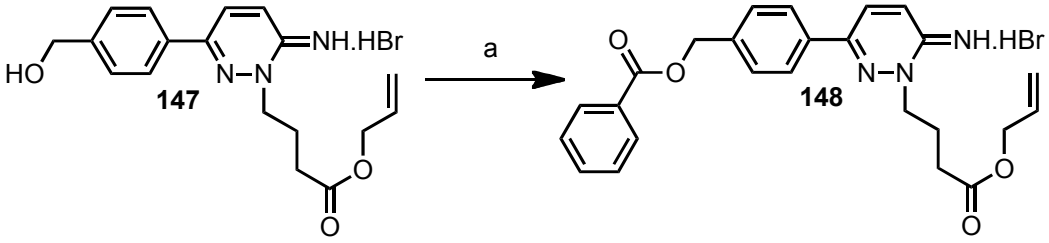
Scheme 2.6

A mechanism for the *N*(2)-alkylation of arylpyridazines with bromoesters is outlined in **Scheme 2.7**. The neighbouring amine aids substitution of the pyridazine at the *N*(2) position. In systems that lack a neighbouring aromatic group, *N*(1)-alkylation sometimes results.[72]



Scheme 2.7

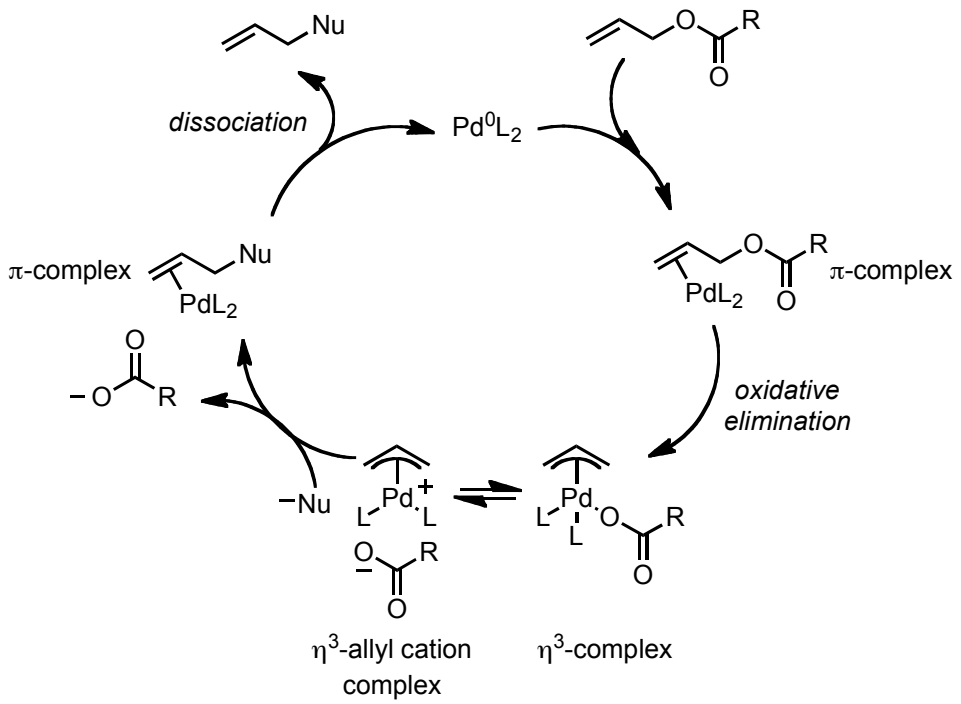
Subsequent treatment of **147** with benzoyl chloride gave the protected target molecule **148** (**Scheme 2.8**). Despite isolation of the desired product, the reaction was particularly unreliable. We observed a mixture of products potentially originating through cross-reactivity of benzoyl chloride with the pyridazine moiety. Several recrystallisations were necessary in order to separate the desired product from unwanted side products.



Reagents and conditions: a) Benzoyl chloride, pyridine, DMF (5 h, 80 ºC), 60 %.

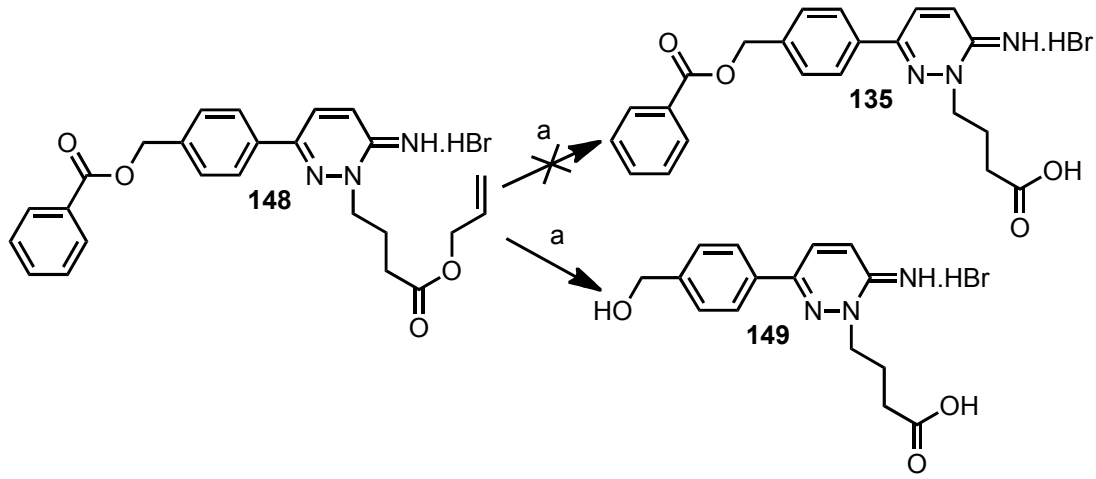
Scheme 2.8

In an attempt to selectively deprotect the allyl ester **148**, we adapted a method highlighted by Seki *et al*. using $Pd(OAc)_2$ and triethylphosphite, with dimedone as a nucleophilic scavenger.[178] A general mechanism for allyl ester deprotections mediated by palladium and a nucleophilic allyl scavenger are shown in **Scheme 2.9**.



Scheme 2.9

Unfortunately, the allyl deprotection of compound **148** did not furnish the desired acid **135**, but instead furnished compound **149** as both esters were cleaved (**Scheme 2.10**).



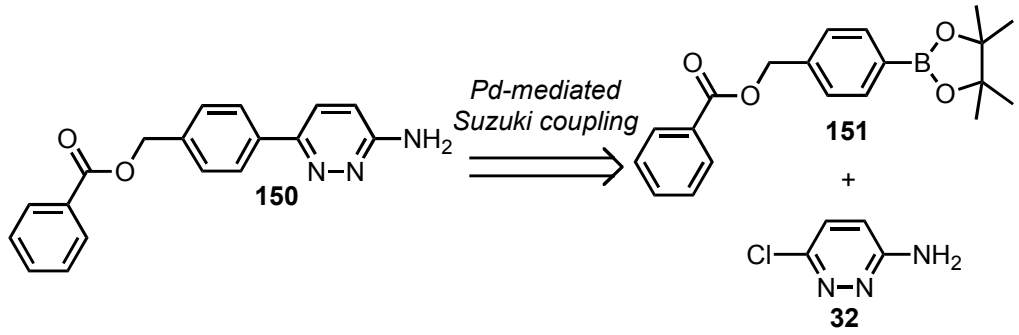
Reagents and conditions: a) $Pd(OAc)_2$, $P(OEt)_3$, dimedone, $NaHCO_3$, THF/H_2O (1:1) (15 h, 35 ^oC), 53%.

Scheme 2.10

Ultimately, our desire was to retry the reaction under anhydrous conditions as we felt that water may have acted as a nucleophile in the collapse of the benzoate fragment.

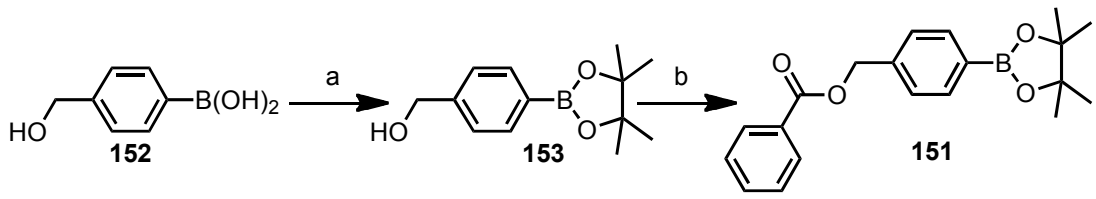
Another possible explanation for the loss of the benzoate group may be that it is also prone to deprotection by the conditions necessitated for the allyl deprotection. Alternatively, transesterification of acid **135** could also result in the formation of the acid **149**.

Attempts were made to reform allyl ester **148** using the methods outlined in **Scheme 2.8**; however, these proved to be unreliable and temperamental in nature. As a result, we considered an alternative method to create allyl ester **148**. Our subsequent efforts focussed on the synthesis of the pyridazine **150**, which could consequently be *N*-alkylated (**Scheme 2.11**).



Scheme 2.11

Boronate ester **151** was prepared by protection of boronic acid **152** as the pinacol ester **153** followed by reaction with benzoyl chloride (**Scheme 2.12**). We believe that the relatively low yields in these reactions stem from the relative insolubility of **152** and **153** in the solvent media. [179-181]

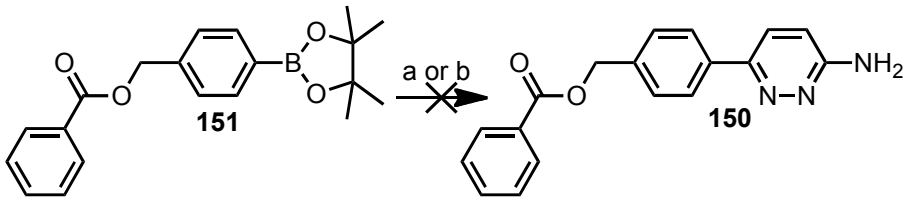


Reagents and conditions: a) Pinacol, Et_2O (6 h, rt), 39 %;
b) benzoyl chloride, pyridine, Et_2O (4 h, rt), 48 %.

Scheme 2.12

Attempts were made to make the triaryl **150** *via* a Pd-mediated method (**Scheme 2.13**). Unfortunately, preparation of the desired triaryl proved unsuccessful under

either standard Pd catalysis conditions, or our previously optimised conditions, resulting in complex reaction mixtures, possibly from transesterification of the boronate ester **151**.



Reagents and conditions: a) **32**, $Pd(PPh_3)_4$, Na_2CO_3, toluene, reflux;
b) **32**, $Pd(PPh_3)_2Cl_2$, K_2CO_3, CH_3CN/H_2O (3:2), microwave (10 min, 120 ^oC).

Scheme 2.13

Due to the capricious nature of the reactions undertaken and the challenges faced in selectively deprotecting an allylic ester in preference to a benzylic ester, an alternative target was pursued and efforts to form the benzoyl ester antagonist **135** were abandoned. It was felt that the synthesis of an antagonist containing two ester functionalities was problematic, so following careful consideration we decided to pursue a revised target compound in order that this could be avoided.

2.2.2 Benzyl ether antagonist

Our revised target was the benzyl ether antagonist **154** shown in **Figure 2.4**.

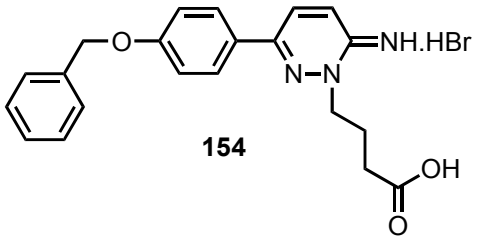
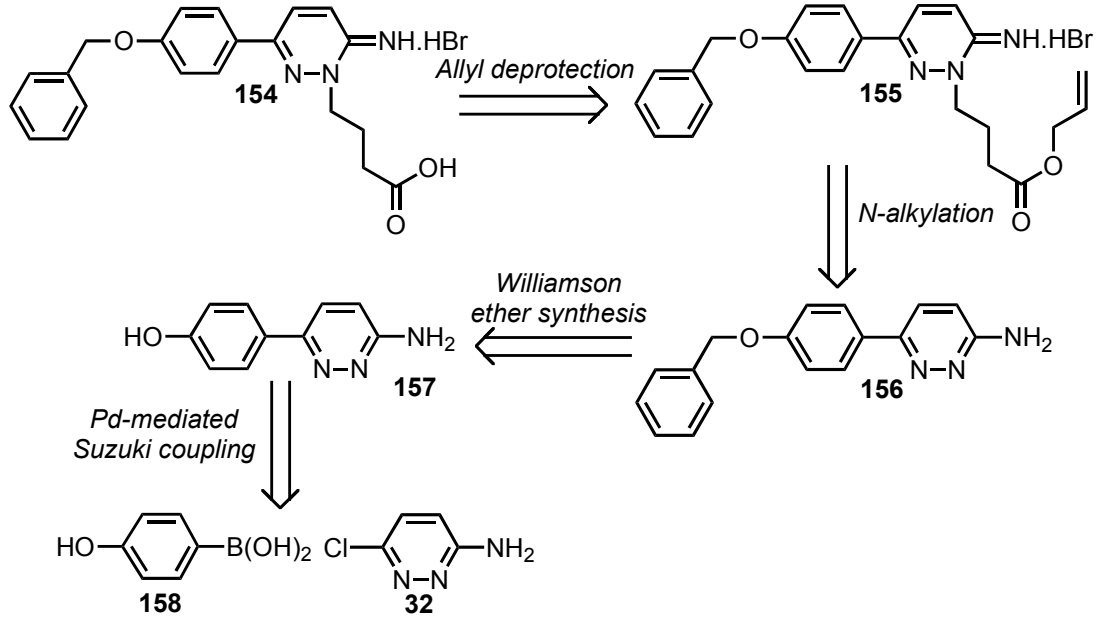


Figure 2.4

We were intrigued by adopting a phenolic system, since we felt that a phenoxide system would be easier to functionalise than a benzylic alcohol, as in arylpyridazine **138**. In order to circumvent the problems encountered with selective ester deprotection, we decided that a possible site for incorporation of a photoaffinity label could be introduced through a Williamson ether synthesis with an appropriate benzyl iodide or bromide. Syntheses of photoaffinity labels containing such functionalities

has been reported and therefore, it was felt that the benzyl ether antagonist **154** would be a suitable target.

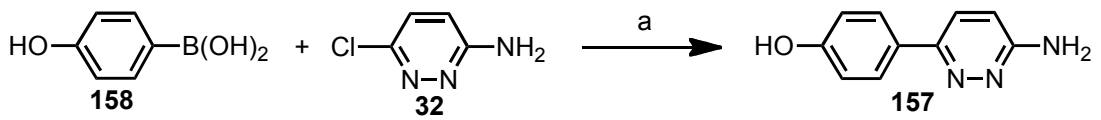
The retrosynthetic route to antagonist **154** is outlined in **Scheme 2.14**.



Scheme 2.14

In the revised retrosynthesis, antagonist **154** could be formed through the deprotection of the allyl ester **155**. The requisite site for a photoaffinity label on triaryl **156** could be introduced through the coupling of a benzyl bromide and arylpyridazine **157** *via* a Williamson ether synthesis. We planned to achieve the synthesis of arylpyridazine **157** through the optimised Suzuki-Miyaura coupling conditions obtained thus far.

Optimised conditions were applied to the microwave coupling of chloropyridazine **32** to boronic acid **158** (**Scheme 2.15**).



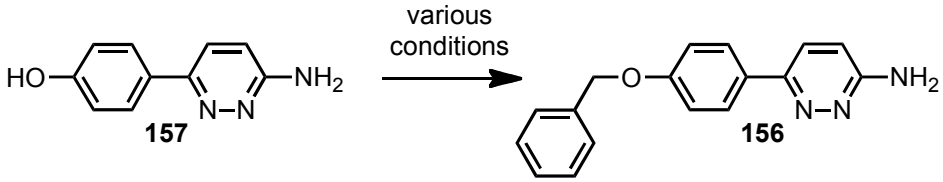
Reagents and conditions: a) $Pd(PPh_3)_2Cl_2$, K_2CO_3, CH_3CN/H_2O (3:2), microwave (10 min, 120 ^oC), 72 %.

Scheme 2.15

We were delighted with the high yield of the reaction. Interestingly, replicating the reaction thermally with analogous conditions failed to furnish any of the product.

With arylpyridazine **157** in hand, our focus turned to the Williamson ether synthesis. It was anticipated that the nucleophilicity of the pyridazine amine might lead to a mixture of regioisomers, so with this in mind, we wanted to cleave the phenolic proton of arylpyridazine **157** to favour reaction at the resulting phenoxide.

The coupling was attempted with two bases, caesium carbonate and sodium hydride (**Scheme 2.16**). Caesium carbonate can be an effective base for the reaction, but as noted, the reaction does not tolerate prolonged heating. With these promising initial results, we pursued the influence of using a stronger base in the reaction to remove the phenolic proton prior to reaction with benzyl bromide. Gratifyingly, we found that using sodium hydride enabled us to access pyridazine **156** in acceptable yield. Pyridazine **156** was isolated *via* both methods as summarised in **Table 2.3**.



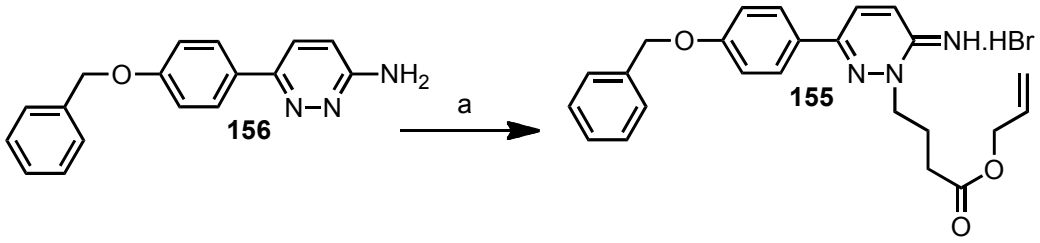
Reagents and conditions: a) Benzyl bromide, $CsCO_3$ or NaH, DMF.

Scheme 2.16

Entry	Base (eq.)	Conditions	**Yield**
i	Cs_2CO_3 (3)	DMF, 50°C, 4 h	23%
ii	Cs_2CO_3 (3)	DMF, 50°C, 8 h	-
iii	NaH (1)	DMF, 0°C, 2 h	41%

Table 2.3

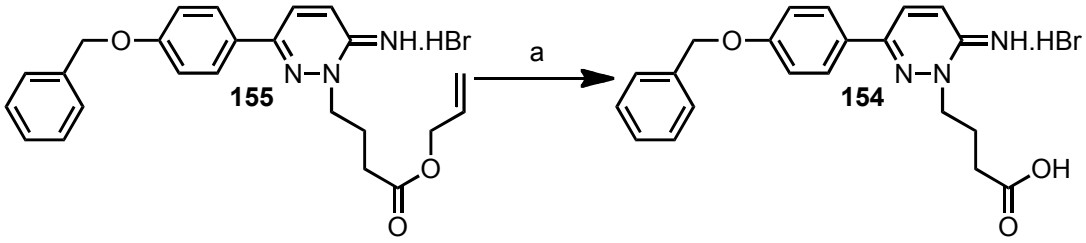
N(2)-Alkylation of pyridazine **156** was achieved in good yield using the previously synthesised allyl-4-bromobutyrate **145** (**Scheme 2.17**). No other products were observed by TLC and no *N*(1)-alkylated product was isolated.



Reagents and conditions: a) **145**, DMF (16 h, 80 ^{o}C), 59 %.

Scheme 2.17

Finally, an allyl deprotection was necessary to remove the allyl group from **155** thus furnishing the desired model antagonist **154** (**Scheme 2.18**). We adopted conditions used by Seki *et al*., as detailed previously.



Reagents and conditions: a) $Pd(OAc)_2$, $P(OEt)_3$, THF/H_2O (1:1) (17 h, 35 ^{o}C), 56 %.

Scheme 2.18

We were delighted to note that the deprotection of the allyl ester proceeded without incident, thus affording the lead compound **154** in just four steps. NMR characterisation of the product proved difficult due to the insolubility of **154** in d_6-DMSO, d_6-acetone, D_2O, CD_3OD, CD_3CN and $CDCl_3$. However, a 1:1 mix of $CDCl_3$ and CD_3OD dissolved the compound.

2.2.3 Biological Evaluation

The potency of compound **154** was examined by our collaborators in the Smart group, using whole-cell patch clamp electrophysiology with recombinant $\alpha1\beta2\gamma2S$ $GABA_A$ receptors transiently expressed in HEK293 cells.[182, 183]

Patch clamp electrophysiology is a technique which allows the direct study of ion channels in cells.[184] In our system, recombinant $\alpha1\beta2\gamma2S$ $GABA_A$ receptors are expressed as they are the most abundant isoform of the receptor, and exist throughout the brain. Receptors are expressed in Human Embryonic Kidney 293 (HEK293) cells

which are widely used in receptor studies since they are readily transfected, simple to culture and lack innate $GABA_A$ receptors.[185-187] Patch clamping can be used to record an electrophysiological output from single or multiple ion channels. For the construction of potency data of an antagonist, multiple ion channels are required and for this purpose, patch clamping is carried out on a whole cell. Typically, the potency of a compound can be expressed as the half maximal inhibitory concentration (IC_{50}), which is described as the concentration of an antagonist required to inhibit a biological process by half. In order to work out the IC_{50} of compound **154** by patch clamp electrophysiology, one must first work out the half maximal effective concentration (EC_{50}) of the endogenous ligand on a specific receptor isoform, in this case GABA.

Through constructing concentration inhibition curves for antagonism of the response to EC_{50} GABA, the lower IC_{50} for **154** (IC_{50} = 11 nM) indicated a 32-fold increase in antagonist potency compared to gabazine **18** (IC_{50} = 349 nM), which is in accordance to literature values (**Figure 2.5**).

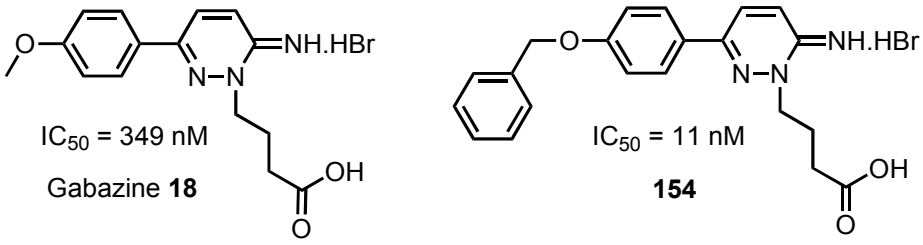


Figure 2.5

While this project aimed to retain focus on creating photoaffinity labelled antagonists, further work was conducted by a Masters student, Ryan Ellwood, who concentrated on creating analogues of the benzyl ether antagonist **154**. To this end, the *meta*-methoxy analogue **159** was synthesised. The relative potency of this compound was increased further (IC_{50} = 7 nM) compared to gabazine (**Figure 2.6**). Interestingly, installing an electron-withdrawing nitro-group in **160** even further enhanced antagonist potency (IC_{50} = 3 nM) (**Figure 2.6**).[188]

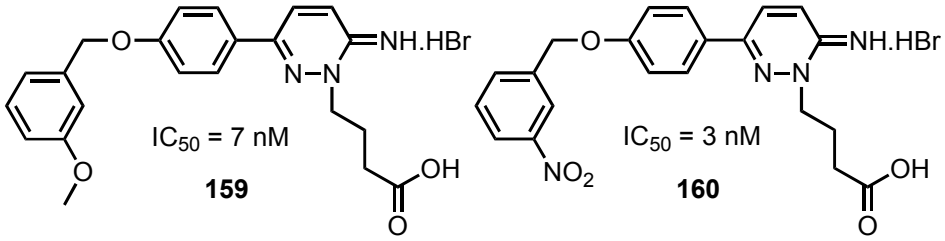


Figure 2.6

The structure-activity relationships of these compounds are outlined in **Table 2.4**.

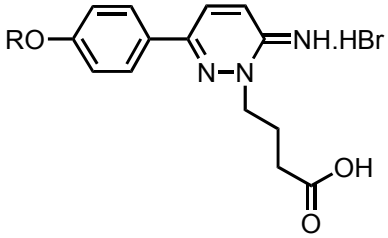


Figure 2.7

Entry	Compound	IC_{50} /nM	Relative Potency
i	Gabazine **18** (R = CH_3)	349	1
ii	**154** (R = $C_6H_5CH_2$)	11	32
iii	**159** (R = *m*-$MeOC_6H_4CH_2$)	7	50
iv	**160** (R = *m*-$NO_2C_6H_4CH_2$)	3	116

Table 2.4

Our findings indicate that there is significant scope to modify the gabazine skeleton on the alkoxy substituent and considerably enhance antagonist potency at the $GABA_A$ receptor (**Figure 2.8**). [188]

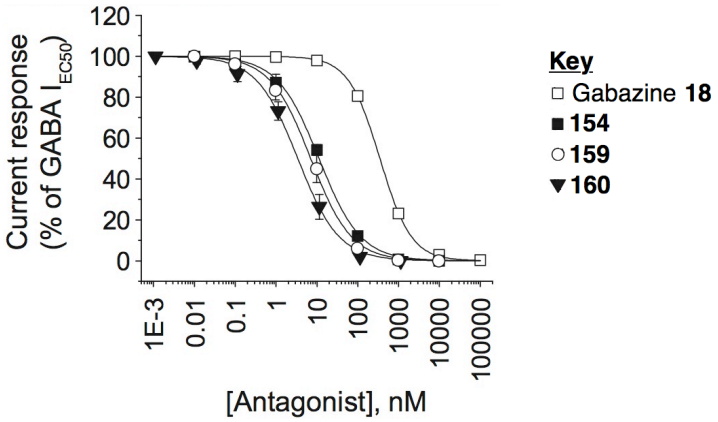
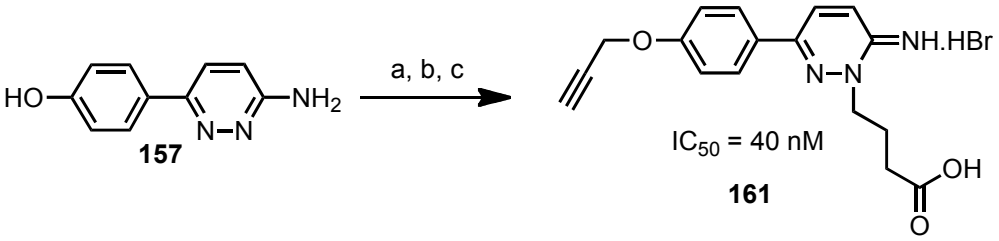


Figure 2.8 – Concentration inhibition curves for analogues 154, 159 and 160.

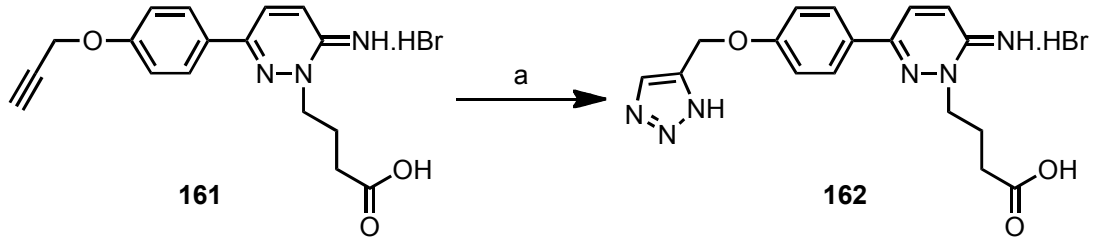
In another analogue **161** (**Scheme 2.19**), we were able to install a propargyloxy group in place of the methoxy group on **18**, thereby creating an alternative diverse point of attachment for prospective labelling groups. **161** again showed an increased potency (IC_{50} = 40 nM; n=6) compared to gabazine.[188]



Reagents and conditions: a) Propargyl bromide, sodium hydride, DMF (3 h, 0 °C), 71 %; b) allyl-4-bromobutyrate, DMF (5 h, 80 °C), 88 %; (c) $Pd(PPh_3)_4$, morpholine, 86 %.

Scheme 2.19

We were able to demonstrate that alkyne **161** can be further elaborated *via* click chemistry. **161** was further converted into the triazole-containing antagonist **162** through the Cu-mediated azide-alkyne Huisgen cycloaddition. Due to a low purity of the resultant compound, we did not obtain potency data on compound **162** (**Scheme 2.20**).



Reagents and conditions: a) CuI, TMS-N_3, DMF/CH_3OH (9:1) (7 h, 100 °C), 22 %.

Scheme 2.20

In conclusion, we have demonstrated that by the simple addition of a benzyl group, the antagonist potency of arylpyridazine analogues of GABA can be greatly increased. This is exemplified by antagonist **154**, where we observed a 32-fold increase in potency compared to gabazine.

2.3 Photoaffinity labelled antagonists

Having developed the potent benzyl ether antagonist **154**, we pursued the synthesis of benzyl ether antagonists **163**. We were interested in creating photoaffinity labelled analogues that could block the function of the $GABA_A$ receptor permanently upon irradiation. Since each photoaffinity label possesses a different reactivity profile, we chose to create several analogues incorporating the three predominately used photolabels (aryl azide, benzophenone and aryl diazirine), discussed in section **1.2**. Further to this, our desire was to establish which photoaffinity labelled analogue was able to block the function of the $GABA_A$ receptor most efficiently.

We were also interested in targeting a set of directly labelled analogues **164**. Other research groups have modified the methoxy- group of gabazine without observing a fluctuation in selectivity for the receptor.[72] We felt that directly labelled analogues **164** bore a significant structural resemblance to reported potent gabazine analogues, and thus we hoped that the synthesis of these additional photolabelled analogues would offer us the best chance of blocking the function of the $GABA_A$ receptor upon irradiation. Directly labelled analogues were also of methodological interest to us, as their syntheses would entail the synthesis of novel building blocks for photoaffinity label synthesis. Our general targets are illustrated in **Figure 2.9**.

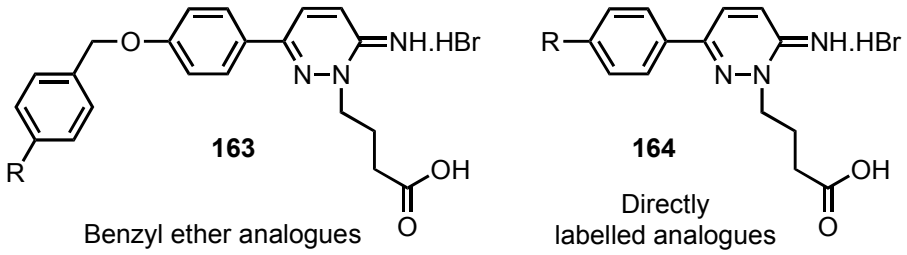


Figure 2.9

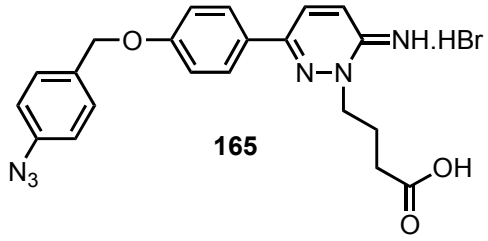
2.3.1 Benzyl ether analogues

This section outlines the strategies taken to synthesise photoaffinity labelled compounds based on the antagonist **154**. Retrosyntheses of these compounds follow the same strategy outlined previously in **Scheme 2.14**. Our plan was to try and incorporate photoaffinity labels on the *para*-position of the benzyl group, since the

synthesis of *para*-substituted photoaffinity labels are thoroughly documented in the literature.[101, 123, 138, 152]

2.3.1.1 Azide analogue

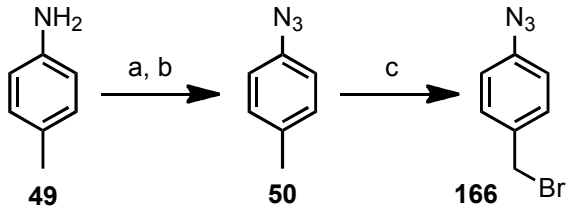
We first addressed the synthesis of azide labelled antagonist **165** (**Figure 2.10**), due to the relatively straightforward synthesis of the azide component compared with an analogous diazirine constituent, and the relative small size of the photoaffinity label compared to a benzophenone.



165

Figure 2.10

A straightforward synthesis of the appropriate benzyl bromide **166** was completed in two steps from commercially available *para*-toluidine **49**. This was achieved through formation of the diazonium salt, followed by displacement with sodium azide to give intermediate **50**, which was subjected to radical bromination by AIBN and *N*-bromosuccinimide (**Scheme 2.21**).[189]

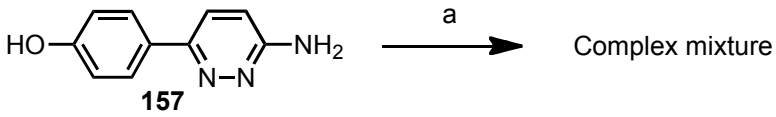


49 **50** **166**

Reagents and conditions: a) $NaNO_2$, HCl (2M, 5 min, -5 ^oC); b) NaN_3, NaOAc, H_2O (2 h, 0 ^oC), 86 % across two steps; c) AIBN, *N*-bromosuccinimide, benzene (8 h, 80 ^oC), 44 %.

Scheme 2.21

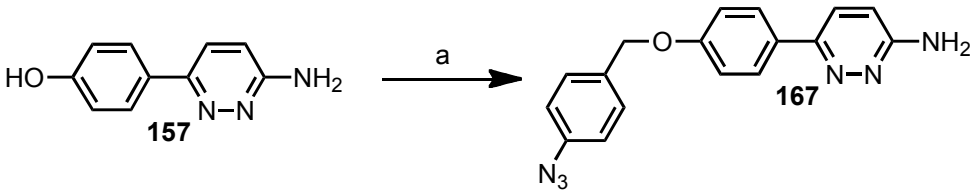
We proceeded to couple bromide **166** with arylpyridazine **157** in a Williamson ether synthesis. However, the product obtained appeared to be a multi-alkylated compound, isolation of which was not achieved, despite numerous attempts at purification by column chromatography (**Scheme 2.22**).



Reagents and conditions: a) **166**, NaH, DMF (2 h, 0 °C).

Scheme 2.22

We felt that a strong base was required in order to fully deprotonate the phenolic proton of the arylpyridazine prior to Williamson ether synthesis in order to ensure that reaction took place at the phenoxide rather than the nucleophilic amine. Due to the problems encountered with the use of sodium hydride, we investigated the use of potassium *tert*-butoxide. We were able to isolate the desired pyridazine **167**, through a Williamson ether synthesis conducted under anhydrous conditions, in acceptable yield (**Scheme 2.23**).

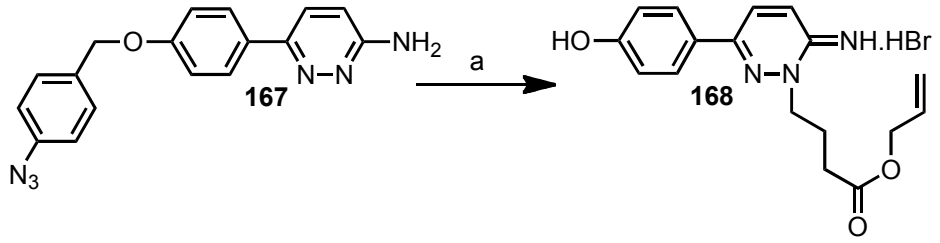


Reagents and conditions: a) **166**, potassium *tert*-butoxide, DMF (3 h, 0 °C), 38 %.

Scheme 2.23

Extraction of **167** during work-up was particularly difficult in this case, due to poor organic solubility of pyridazine **167**. It should also be noted that longer reaction times led to the formation of further products, as observed by TLC. The best results were obtained through the slow addition of one equivalent of benzyl bromide **166** to a DMF solution of arylpyridazine **157** that had been deprotonated *in situ* at 0 °C by potassium *tert*-butoxide.

Treatment of pyridazine **167** with allyl-4-bromobutyrate led to an unexpected result. We replicated reaction conditions used for the formation of ester **155**, by heating the reaction mixture for 18 h in DMF. However, the prolonged heating was not tolerated well in this instance, and unexpectedly, we isolated the debenzylated compound **168** (**Scheme 2.24**).



Reagents and conditions: a) Allyl-4-bromobutyrate, DMF (18 h, 80 ^{o}C), 28 %.

Scheme 2.24

It is thought that the debenzylated compound **168** could have been formed through the elimination of the benzyl bromide. Cleavage of benzyl ethers has been reported in the presence of strong acids, and it is possible that a small amount of nucleophilic bromide is present in the reaction mixture. Figure 2.11 also demonstrates the influence that the *para*-substituted azide may have in the observed debenzylation.

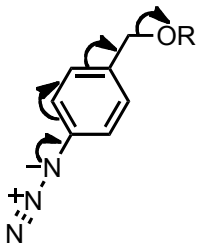
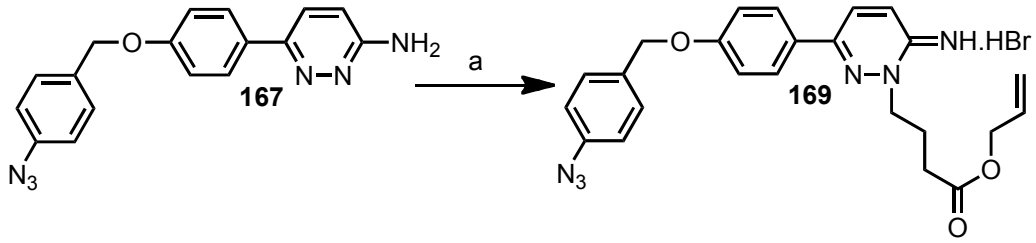


Figure 2.11

Undeterred, we repeated the reaction with a decreased reaction time, hoping to avoid the undesired formation of **168**. Pleasingly, ester **169** was isolated as the sole product from the reaction, in satisfactory yield (**Scheme 2.25**).

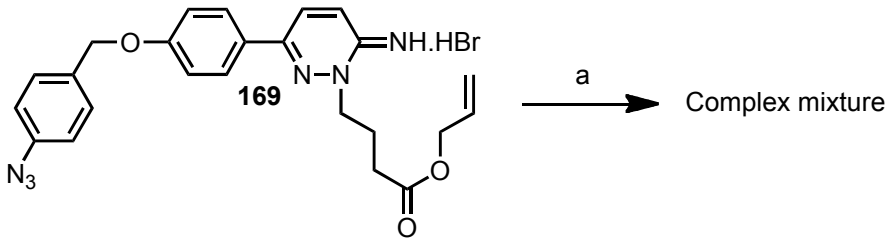


Reagents and conditions: a) Allyl-4-bromobutyrate, DMF (6 h, 80 ^{o}C), 37 %.

Scheme 2.25

Finally, given the success of the Pd-mediated deprotection of the allyl ester in the synthesis of the lead compound **154**, we chose to employ identical reaction

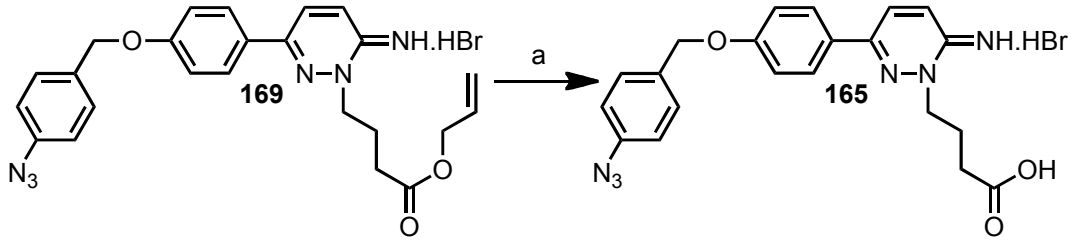
conditions for the conversion. However, we found that the parent ester **154** gave a complex mixture of inseparable products when subjected to these reaction conditions (**Scheme 2.26**).



Reagents and conditions: a) $Pd(OAc)_2$, $P(OEt)_3$, dimedone, THF/H_2O (1:1) (17 h, 35 ^oC).

Scheme 2.26

We sought to use an alternative protocol for the allyl deprotection as we felt that some of the problems may be attributable to lengthy reaction times, having observed debenzylation in the previously extended reaction. Through an alternative rapid protocol using $Pd(PPh_3)_4$ and morpholine as an allyl scavenger, the desired azide analogue **165** was cleanly afforded in excellent yield (**Scheme 2.27**).[190, 191]



Reagents and conditions: a) $Pd(PPh_3)_4$, morpholine, THF/CH_3OH (4:1) (30 min, rt), 94 %.

Scheme 2.27

In summary, the synthesis of the azide analogue **165** was concluded in six steps from commercially available *para*-toluidine **49**.

2.3.1.2 Benzophenone analogue

We next chose to tackle the synthesis of a benzophenone labelled antagonist **170** (**Figure 2.12**), due to its relatively facile preparation compared to the analogous diazirine labelled antagonist. Furthermore, the required benzophenone bromide is commercially available.

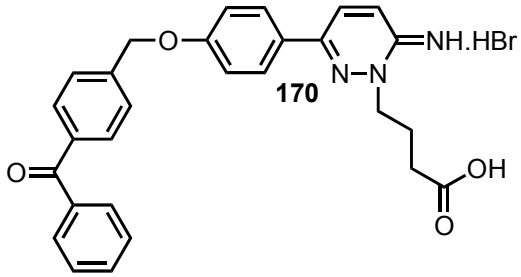
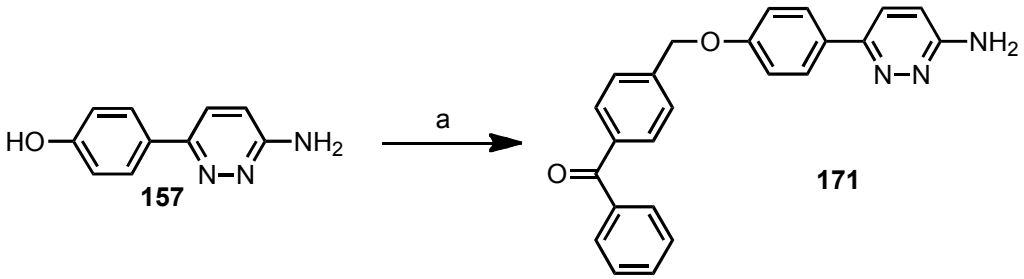


Figure 2.12

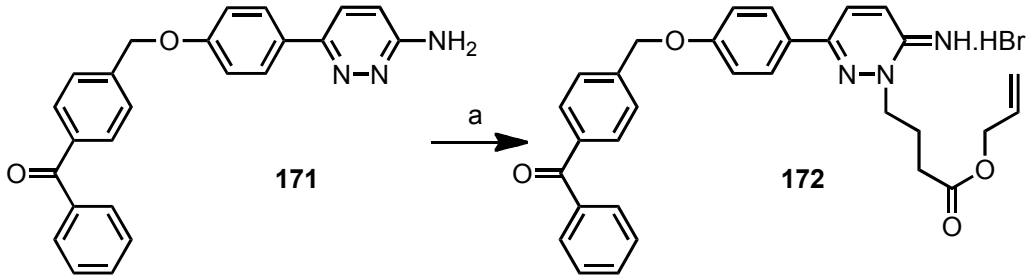
Making use of the optimised conditions from the synthesis of the azide analogue, we employed potassium *tert*-butoxide as the base for the Williamson ether synthesis. Although low yielding, pyridazine **171** was cleanly isolated by column chromatography (**Scheme 2.28**).



Reagents and conditions: a) 4-(Bromomethyl)benzophenone, potassium *tert*-butoxide, DMF (3 h, 0 ^{o}C), 31 %.

Scheme 2.28

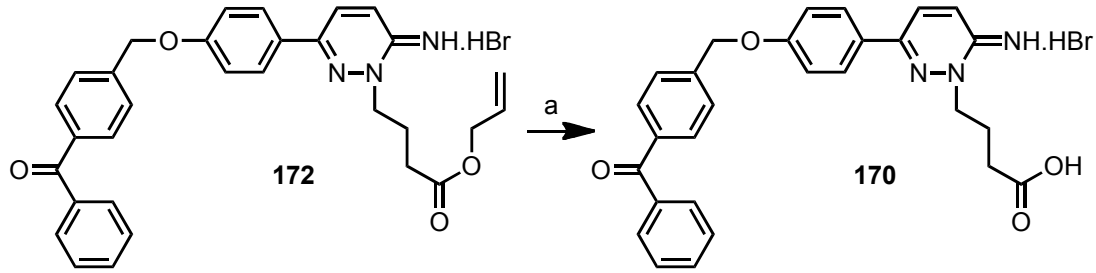
We attempted to alkylate pyridazine **171** with allyl-4-bromobutyrate. Pleasingly, the desired ester **172** was isolated in a good yield as a single product from the reaction (**Scheme 2.29**). In this instance we observed no debenzylation, despite prolonged heating.



Reagents and conditions: a) Allyl-4-bromobutyrate, DMF (18 h, 80 ^{o}C), 60 %.

Scheme 2.29

Finally, the allyl group on ester **172** was successfully deprotected using $Pd(PPh_3)_4$ and morpholine as described previously, to leave the desired benzophenone derivative **170** (**Scheme 2.30**).



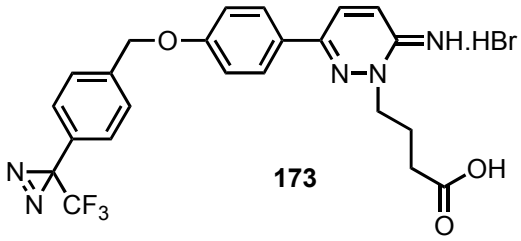
Reagents and conditions: a) $Pd(PPh_3)_4$, morpholine, THF/CH_3OH (4:1) (30 min, rt), 65 %.

Scheme 2.30

In summary, the synthesis of the benzophenone analogue **170** was conveniently completed in three steps from the commercially available 4-(bromomethyl)benzophenone.

2.3.1.3 Diazirine analogue

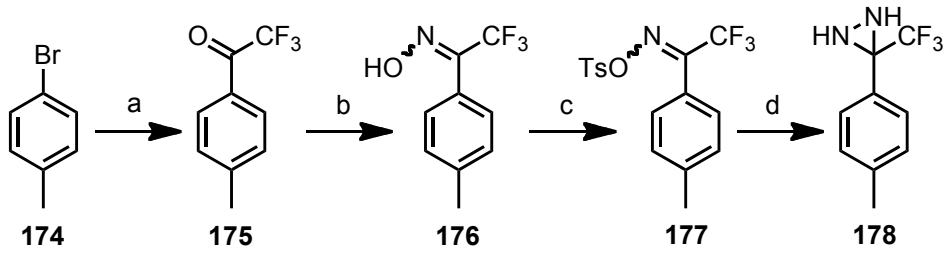
In our final benzyl ether analogue, we hoped to attach a diazirine photolabel to form analogue **173** (**Figure 2.13**). However, the diazirine benzyl bromide component **114** is not commercially available and demands a lengthy synthesis.



173

Figure 2.13

Synthesis of the appropriate benzyl bromide required six steps from commercially available 4-bromotoluene **174**. We embarked on the first four steps (**Scheme 2.31)**, culminating in the synthesis of diaziridine **178**.



174 **175** **176** **177** **178**

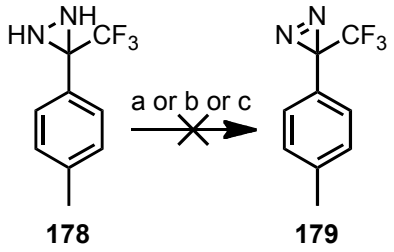
Reagents and conditions: a) *n*-BuLi, ethyl trifluoroacetate, Et_2O (5 h, -78 ^oC), 38 %; b) hydroxylamine hydrochloride, pyridine (3 h, 70 ^oC), 99 %; c) *p*-TsCl, pyridine (18 h, 110 ^oC), 75 %; d) NH_3, Et_2O (8 h, -78 ^oC), 88 %.

Scheme 2.31

4–Bromotoluene undergoes a straightforward lithium-halogen exchange before conversion to the ketone **175**. Accounts in the literature often purify this compound *via* distillation, however it was found that the reaction could be suitably purified by column chromatography.[150] A mixture of oximes **176** resulted, as expected from literature precedent, when the parent ketone **175** was reacted with hydroxylamine hydrochloride. We continued to the next step with a 1:1 mixture of isomers where **176** was reacted with *para*-toluenesulfonyl chloride to furnish the corresponding tosylate **177** in good yield. Finally, diaziridine **178** is formed through a low temperature reaction with ammonia, condensed into a sealed vessel at -78 °C, furnishing diaziridine **178** in excellent yield.

Our attention then turned to forming the diazirine. Frustratingly, several attempts to oxidise diaziridine **178** to diazirine **179**, following literature methods in methanol or

ethanol were unsuccessful. Diaziridine **178** was recovered in each case, despite the use of several freshly prepared oxidizing agents (**Scheme 2.32**).[101, 152, 160]

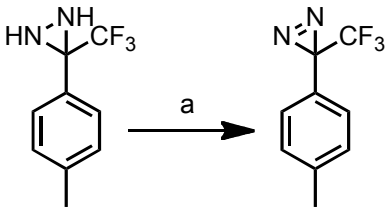


178 **179**

Reagents and conditions: a) *tert*-Butyl hypochlorite, NEt_3, EtOH (2 h, 0 °C); b) I_2, NEt_3, MeOH (2 h, 0 °C); c) Ag_2O, NEt_3, MeOH (2 h, 0 °C)

Scheme 2.32

We then investigated a method used by Strømgaard and co-workers, which employed iodine in an aprotic chlorinated solvent to mediate the conversion.[123] Using their procedure, diazirine **179** was isolated in good yield (**Scheme 2.33**).

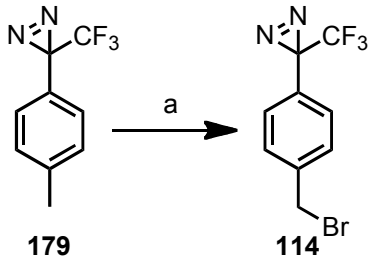


178 **179**

Reagents and conditions: a) I_2, NEt_3, CH_2Cl_2 (5 min, 0 °C), 61 %.

Scheme 2.33

We next attempted to brominate the tolyl position of diazirine **179** *via* radical bromination using AIBN and *N*-bromosuccinimide in CCl_4.[123] We successfully isolated bromide **114** in good yield (**Scheme 2.34**).



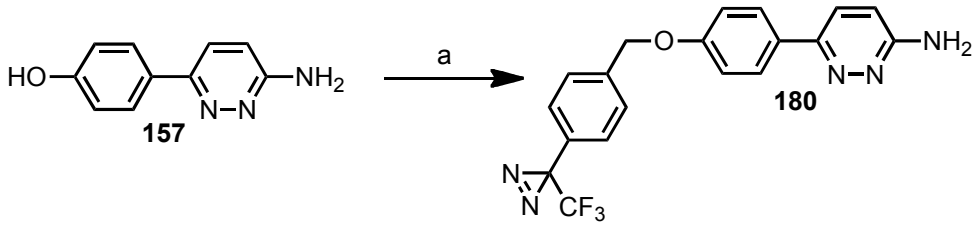
179 **114**

Reagents and conditions: a) AIBN, *N*-bromosuccinimide, CCl_4 (4 h, 70 °C), 65 %.

Scheme 2.34

One interesting point, which is rarely commented upon in the literature, is the volatility of both diazirine **179** and bromide **114** in this reaction. The volatility of both products necessitated that any concentration *in vacuo* must be conducted at low temperature, otherwise loss of bromide **114** under reduced pressure was observed. This may have led to the suppressed yields in reactions involving these reagents.

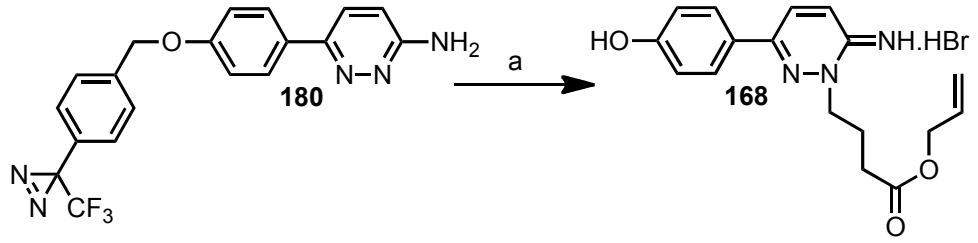
Owing to the precious nature of the bromide we chose to react the compound with an excess of arylpyridazine **157**. Additionally, we felt that it might be beneficial to add a crown ether to the reaction mixture to facilitate clean removal of the acidic proton prior to reaction. Crown ethers are often used in conjunction with bases in order to coordinate metal cations to the central cavity of a crown ether. For potassium bases, 18-crown-6 is commonly used.[192-194] To our delight, pyridazine **180** was afforded in good yield through using this adapted procedure (**Scheme 2.35**).



Reagents and conditions: a) **114**, potassium *tert*-butoxide, 18-crown-6, DMF (3 h, 0 ^{o}C), 62 %.

Scheme 2.35

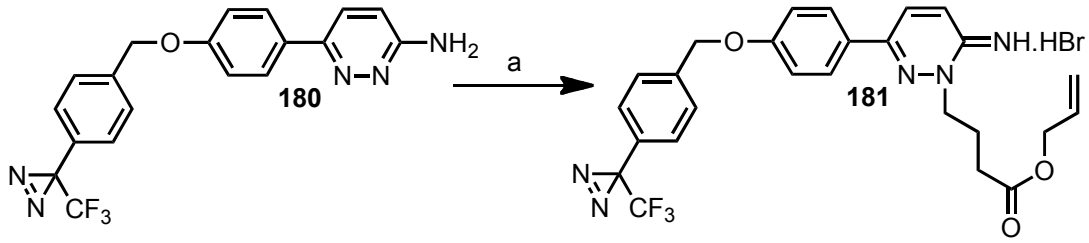
Following on from this we attempted to *N*-alkylate the compound as previously described. However, under the strain of extended heating, we again isolated the debenzylated compound **168** (**Scheme 2.36**).



Reagents and conditions: a) Allyl-4-bromobutyrate, DMF (19 h, 80 ^{o}C), 21 %.

Scheme 2.36

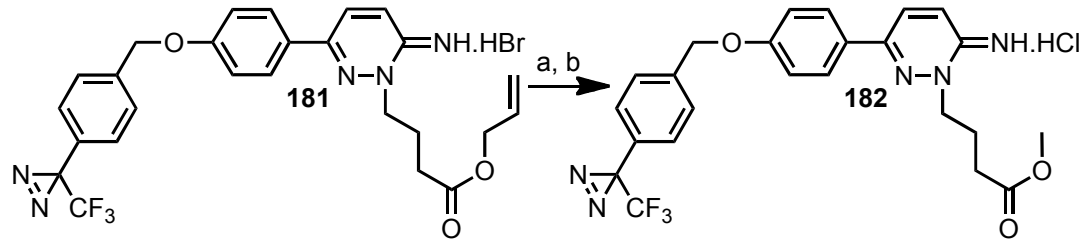
We predicted the conversion to ester **168** could again be alleviated through decreasing the exposure of reaction mixture to heating for long periods. As a compromise, we sacrificed the yield of the reaction for the purity of ester **181**, which was isolated in moderate yield (**Scheme 2.37**).



Reagents and conditions: a) Allyl-4-bromobutyrate, DMF (5 h, 80 ^{o}C), 41 %.

Scheme 2.37

Our preferred method of deprotection using $Pd(PPh_3)_4$ and morpholine gave unfortunate results. After conducting the reaction, the desired compound could not be seen on TLC. This was a result of the excess morpholine used as the scavenger ligand. We postulated that the compound was left as the carboxylate species, which could not pass through the column without elution with acetic acid. An attempt to re-acidify the compound with HCl in methanol, followed by a trituration with water to remove the morpholide salts was promising; however, instead of the desired antagonist we isolated the methyl ester **182** (**Scheme 2.38**).

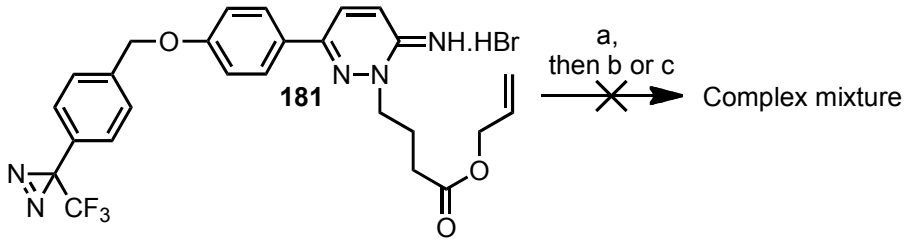


Reagents and conditions: a) $Pd(PPh_3)_4$, morpholine, THF/CH_3OH (4:1) (30 min, rt); b) HCl in MeOH (2M) (2 h, rt), 45 % over two steps.

Scheme 2.38

Given this result, it was felt that the desired acid **173** could be isolated with work-up with either aqueous HCl or HCl in diethyl ether after Pd deprotection in order to avoid the esterification observed with methanol. However, we found that the

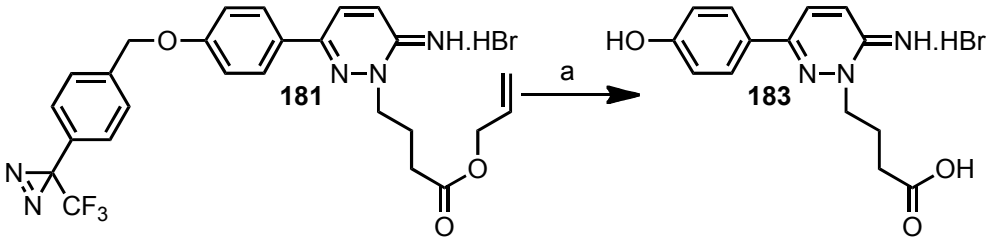
compound could not be isolated with either protocol and instead led to a complex mixture by NMR. (**Scheme 2.39**).



Reagents and conditions: a) $Pd(PPh_3)_4$, morpholine, THF/CH_3OH (4:1) (30 min, rt); b) HCl in H_2O (2M) (2 h, rt); c) HCl in Et_2O (2M) (2 h, rt).

Scheme 2.39

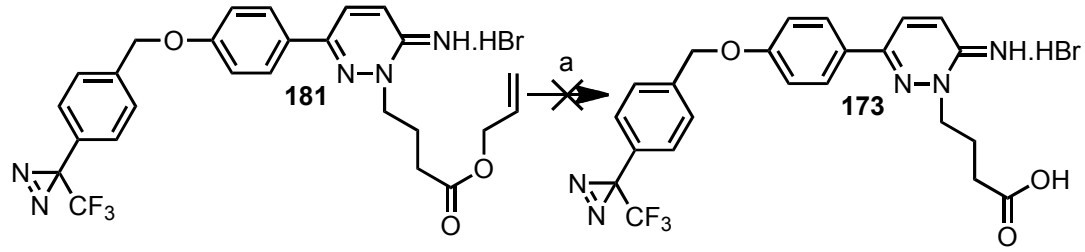
Deprotection of the allyl ester was also attempted using a classical acidic hydrolysis method outlined by the Wermuth group.[72] While this led to removal of the allyl group, debenzylation of **181** also occurred to give acid **183** (**Scheme 2.40**).



Reagents and conditions: a) HBr, AcOH (18 h, 100 ^oC), 74 %.

Scheme 2.40

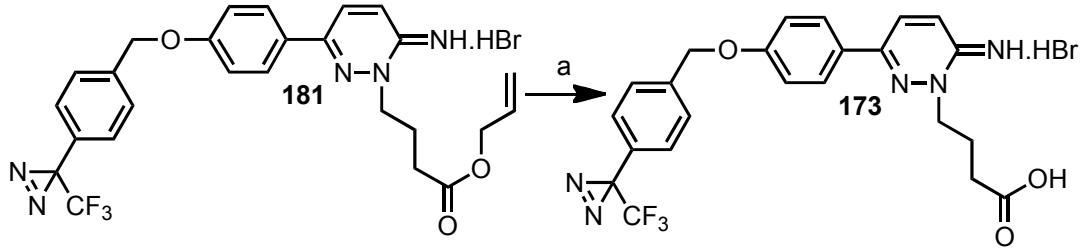
Since many of the problems with the allyl deprotection originated from the basic nature of the morpholine scavenger, we examined the literature for examples where acidic allyl scavengers had been used in allyl deprotections. In one report, acetic acid was used but when this protocol was adapted, only starting material was observed (**Scheme 2.41**).[195]



Reagents and conditions: a) $Pd(PPh_3)_4$, AcOH (1 h, 80 ^oC)

Scheme 2.41

Finally, we tried 1,3-dimethylbarbituric acid, an alternative acidic scavenger that is commonly employed in allyl deprotections.[196-198] To our delight, the synthesis of the diazirine analogue **173** was concluded in this way, with the final step achieved in good yield (**Scheme 2.42**).



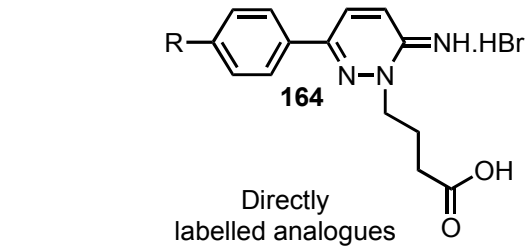
Reagents and conditions: a) $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, THF/CH_3OH, (3 h, rt), 59 %.

Scheme 2.42

No degradation or reduction to an inactive diaziridine form was observed, hence we were able to demonstrate the stability of the diazirine moiety to palladium. The diazirine labelled antagonist **173** was therefore synthesised in nine steps from commercially available 4-bromotoluene **174**.

2.3.2 Directly labelled analogues

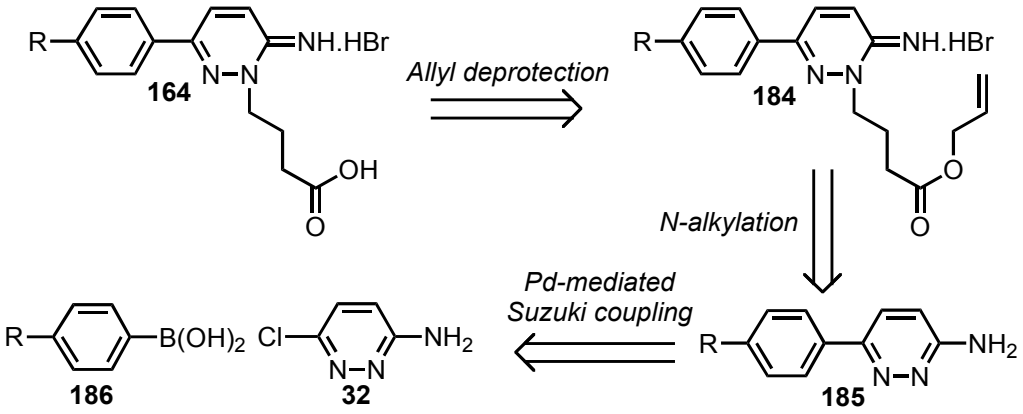
Our efforts to create directly labelled analogues are discussed in this section. Directly labelled analogues **164** are depicted in **Figure 2.14**. We chose to target such compounds for two main reasons; firstly because other research groups have modified the methoxy- group of gabazine without observing a drop in selectivity[72]; and secondly, these targets are methodologically interesting, as they could entail the synthesis of novel building blocks for photoaffinity labelling.



(where R = COPh (benzophenone), $C(N)_2CF_3$ (diazirine))

Figure 2.14

Our general retrosynthetic route to compounds **164** is outlined in **Scheme 2.43**.



Scheme 2.43

Acid **164** could be formed through the deprotection of ester **184**. Ester **184** is formed through the reaction of arylpyridazine **185** and allyl-4-bromobutyrate. Synthesis of arylpyridazine **185** could be achieved through a Pd-mediated Suzuki-Miyaura coupling of chloropyridazine **32** and boronic acid **186**. We believed that it would be possible to incorporate a photoaffinity label *via* a boronic acid. The benzophenone boronic acid already exists[140], but an analogous diazirine analogue would demand formation of a novel diazirinyl boronic acid. We decided to abandon efforts to create an analogous azide analogue, due to our concerns over the high-energy irradiation required to activate such an analogue. Conditions such as these have been reported as being deleterious to cells.[199]

2.3.2.1 Benzophenone analogue

For our first analogue **187**, we hoped to attach a benzophenone photolabel (**Figure 2.15**).

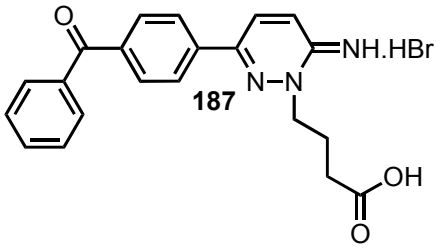
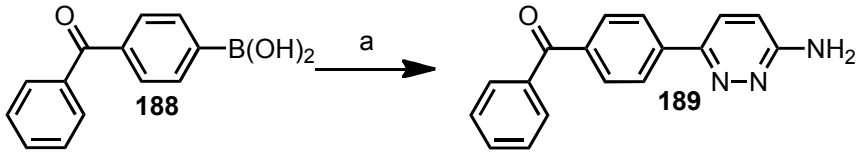


Figure 2.15

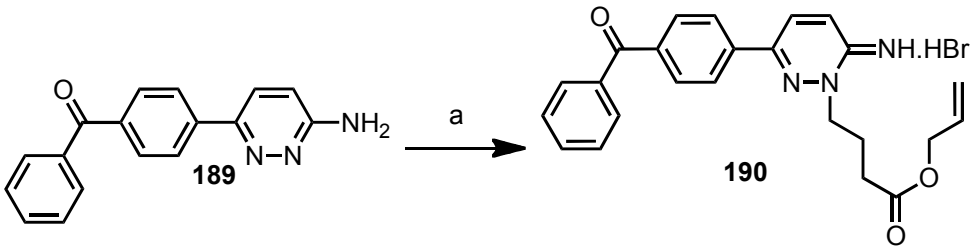
We intended to introduce the photoaffinity label *via* a Suzuki-Miyaura coupling between commercially available benzophenone boronic acid **188** and chloropyridazine **32**. Our previously optimised conditions were applied to coupling (**Scheme 2.44**). We isolated the desired pyridazine **189** in good yield.



Reagents and conditions: a) **32**, $Pd(PPh_3)_2Cl_2$, K_2CO_3, CH_3CN/H_2O (3:2), microwave (10 min, 120 ^oC), 61 %.

Scheme 2.44

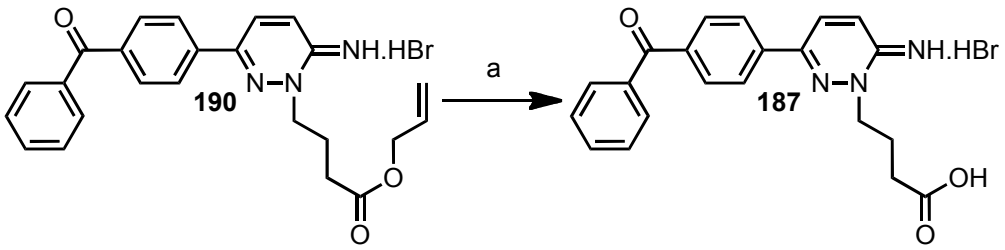
N-Alkylation was then carried out on pyridazine **189**, following similar protocols to those described previously. We were able to isolate ester **190** as a single product from the reaction in excellent yield (**Scheme 2.45**).



Reagents and conditions: a) Allyl-4-bromobutyrate, DMF (18 h, 80 ^oC), 76 %.

Scheme 2.45

Finally, deprotection of the ester was carried out using similar conditions to those previously employed, culminating in excellent yield (**Scheme 2.46**).



Reagents and conditions: a) $Pd(PPh_3)_4$, morpholine, THF/CH_3OH (4:1) (30 min, rt), 92 %.

Scheme 2.46

Gratifyingly, we were able to isolate the desired photoaffinity labelled antagonist **187** in three steps.

2.3.2.2 Diazirine analogue

Our focus then shifted towards creating diazirine analogue **191** (**Figure 2.16**).

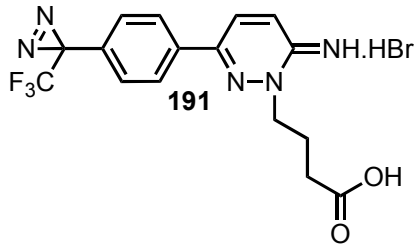
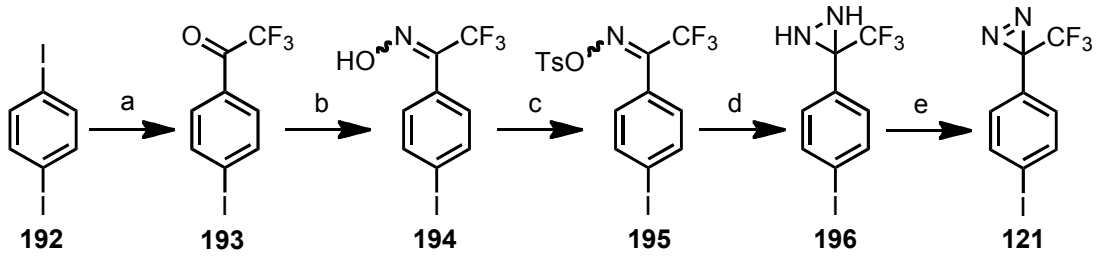


Figure 2.16

It was envisaged that the synthesis of **191** would require the creation of a novel diazirinyl boronic acid. We felt that such a chemical segment is missing from the chemist's armoury, so in an effort to develop a new and unique way to incorporate the photoaffinity label, we sought to explore the synthesis of the boronic acid. Boronic acids possess many qualities that render them useful reagents in organic chemistry; in this case they could aid facile insertion of a complicated photoaffinity label *via* a relatively simple C-C bond forming reaction through a Pd-mediated procedure.

Our planned synthesis of the boronic acid would necessitate the formation of diazirinyl aryl iodide **121**, which could itself be synthesised from commercially available 1,4-diiodobenzene **192**. We carried out the synthesis of iodide **192** following a procedure outlined by Topin *et al*. (**Scheme 2.47**), which follows similar

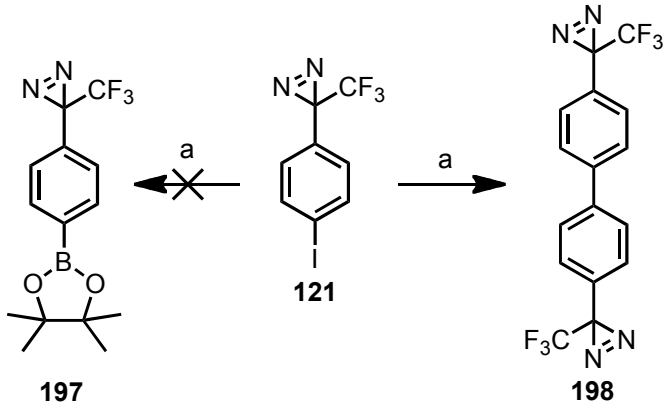
methods to those described previously for the synthesis of the diazirine **179** (**Scheme 2.31** and **Scheme 2.33**).[160]



Reagents and conditions: a) *n*-BuLi, ethyl trifluoroacetate, Et_2O (18 h, -78 °C), 41 %; b) hydroxylamine hydrochloride, pyridine, EtOH (4 h, 60 °C), 82 %; c) *p*-TsCl, pyridine (18 h, 110 °C), 59 %; d) NH_3, Et_2O (8 h, -78 °C), 48 %; e) I_2, NEt_3, CH_2Cl_2 (5 min, 0 °C), 64 %.

Scheme 2.47

With iodide **121** in hand, we attempted to form the boronic acid **197**. Our initial attempts focussed on the Pd-mediated substitution of a boronate ester prior to cleavage using acid. However all our initial attempts to create the boronate ester lead to complex mixtures by NMR and we were unable to retrieve any of the desired boronate ester. Nevertheless, we did observe small amounts of the homocoupled product **198**, leading us to believe that we were forming the boronate ester (**Scheme 2.48**).



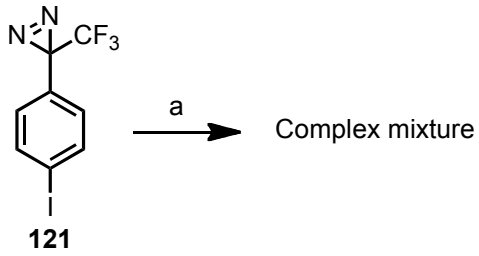
Reagents and conditions: a) $Pd(dppf)Cl_2$, B_2pin_2, KOAc, DMSO (4 h, 80 °C), 12 %.

Scheme 2.48

One explanation for the observation of the homocoupled product **198** is that as soon as the boronate ester **197** is formed, it couples to the aryl iodide **121** and undergoes a

Pd-mediated Suzuki-Miyaura coupling. It is possible that the labile nature of the iodide atom makes aryl iodide **121** an attractive partner for coupling to proceed with, in the presence of a Pd catalyst and base. Homocoupling of aryl bromides and aryl iodides has been reported in the literature upon when attempting to mediate the formation of the boronate ester with palladium.[200]

Further attempts were made to create the boronic acid through a direct borylation of iodide **121**. We had concerns about using this method, due to reports in the literature of the high sensitivity of the diazirinyl nitrogens to *n*-BuLi.[152] Unfortunately, we observed a complex NMR profile suggesting that degradation of the parent iodide had indeed occurred (**Scheme 2.49**).



121

Reagents and conditions: a) *n*-BuLi, $B(OMe)_3$, THF (3 h, -78 ^oC), then HCl (2M) (1 h, rt).

Scheme 2.49

At this point, due to promising results from our other analogues and the long route to creating the aryl bromide and aryl chloride diazirine derivatives, we halted our work on diazirine analogue **191**. Literature reports do indicate that aryl chloride substrates may be superior precursors for boronic acids, in some cases circumventing the formation of homocoupled compounds.[201, 202] We feel that the creation of a boronic acid is viable and our pertinent results thus far indicate a platform upon which to base future work in this area.

2.3.3 Biological Evaluation

The potency of compounds **165**, **170**, **173** and **187** (**Figure 2.17**) were examined by our collaborators using patch clamp electrophysiology with recombinant $\alpha1\beta2\gamma2S$ $GABA_A$ receptors transiently expressed in HEK293 cells.[182, 183] The potencies from inhibition curves and affinities from Schild analysis of these compounds are shown

in **Table 2.5**.

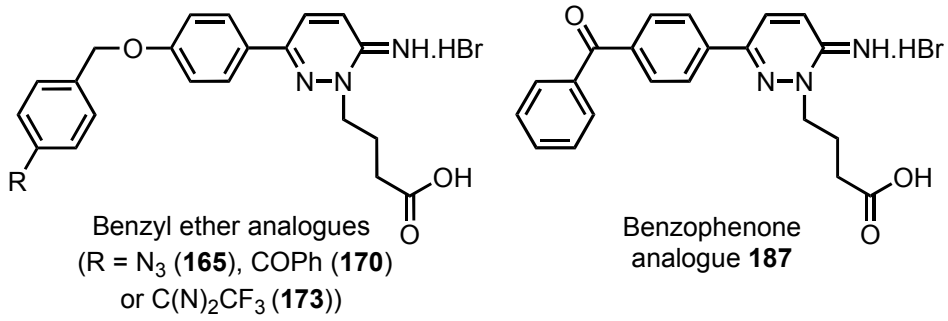


Figure 2.17

Entry	**$GABA_A$ antagonist**	**IC_{50} /nM**	**K_i /nM**
i	**165** (R = N_3)	28 ± 7	44 [34;57]
ii	**170** (R = COPh)	171 ± 34	153 [93;230]
iii	**173** (R = $C(N)_2CF_3$)	59 ± 5	132 [69;254]
iv	**187**	233 ± 33	318 [190;475]

Table 2.5

As can be seen, the effect of a small photoaffinity label such as an azide in **165** (Entry i, **Table 2.5**) or a diazirine in **173** (Entry iii, **Table 2.5**) has a slight effect on the potency of the respective compound, which could be attributed to the subtle changes in electronics of the compound as well as the additional steric bulk. Benzophenone analogue **170** (Entry ii, **Table 2.5**) was found to be less potent that any of the other analogues, but this could once again be attributed to a more significant steric bulk, or to the electron withdrawing nature of the arylketone. A significant drop in potency is observed with benzophenone analogue **187** (Entry iv, **Table 2.5**), which could arise from the loss of the ether functionality and introduction of an electron withdrawing ketone moiety. Reports in the literature have highlighted the importance of the electron donating oxygen atom in the structures of gabazine and another competitive antagonist bicuculline, and by removing this atom, it is possible that a reduction in affinity is induced.[81] Nevertheless, it should be noted that benzophenone analogue **173** still possesses a greater affinity than the parent compound gabazine, adding gravitas to the notion that the presence of the ether

functionality is not the only way of creating a potent analogue. It is possible that the presence of an additional phenyl group may facilitate an increase in potency, as exemplified by the elevated potency of our compounds when compared to gabazine.

Our collaborators then further investigated whether our photolabelled antagonists would effectively block the function of $GABA_A$ receptors *in vitro*. By irradiating each photoactivatable analogue in the presence of $GABA_A$ receptors, the synaptic block of receptors could be measured using patch-clamp electrophysiology. Irradiation was achieved using a Rapp Optoelectronic JML-C2 Xenon flashlamp system, with a 240-395 nm band-pass filter. The findings from these experiments are summarised in **Table 2.6**.

Entry	**$GABA_A$ antagonist**	**λ_{max} /nm**	**Photolytic Block**
i	**165** (R = N_3)	313	29 ± 7%
ii	**170** (R = COPh)	303	49 ± 2%
iii	**173** (R = $C(N)_2CF_3$)	335	21 ± 5%
iv	**187**	306	2 ± 4%

Table 2.6

In these experiments, patch clamp electrophysiology is used to record the membrane current from multiple ion channels on a whole cell. An EC_{50} concentration of GABA (10 μM) is applied to the cell and the postsynaptic current is measured, before the agonist is washed away. A high concentration of antagonist (10 μM) is then applied to the cell. UV radiation is then exposed to the cell *via* an optic fibre as 10 brief flashes (0.6 ms) every 20 seconds. Subsequent to this, any antagonist that has not covalently bound to the receptor is washed away for 5 minutes. The original concentration of GABA (10 μM) is then applied to the cell and the GABA current is recorded. The difference in GABA current prior to and after irradiation is referred to as the photolytic block, and this denotes the total percentage of $GABA_A$ receptors that have been inactivated on a whole cell following irradiation in the presence of a photoaffinity labelled antagonist.

As can be seen, no block of $GABA_A$ receptors is observed with benzophenone analogue **187** (Entry iv, **Table 2.6**) and a moderate block is observed from both the azide analogue **165** (Entry i, **Table 2.6**) and diazirine analogue **173** (Entry iii, **Table 2.6**) analogues, whereas benzophenone analogue **170** (Entry ii, **Table 2.6**) blocks the receptor significantly, accounting for a 49% block of $GABA_A$ receptors (**Figure 2.18**). No irreversible block is observed after UV exposure with gabazine **18**, which lacks a photoaffinity label, shown in **Figure 2.18**.

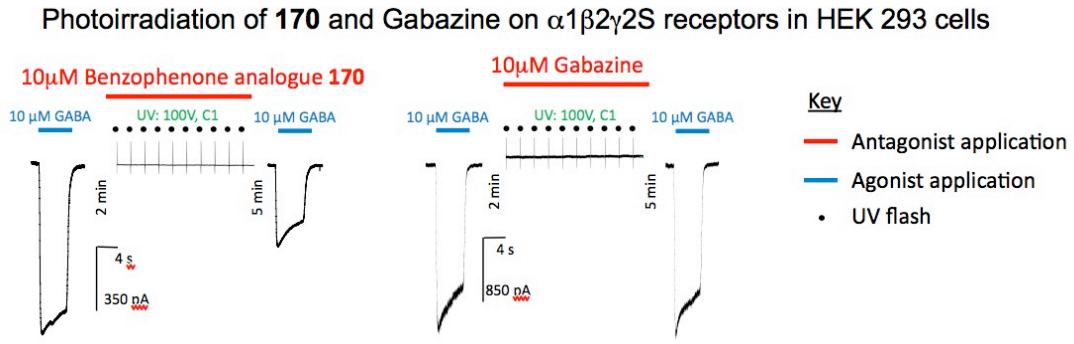


Figure 2.18 – Photoirradiation of 170 and Gabazine on $GABA_A$ receptors

In this recombinant *in vitro* system, there is no mechanism by which the receptors can be replenished from an intracellular pool of $GABA_A$ receptors.[39] If the GABA current is recorded over time, we observe no recovery to the pre-irradiation GABA current (**Figure 2.19**).

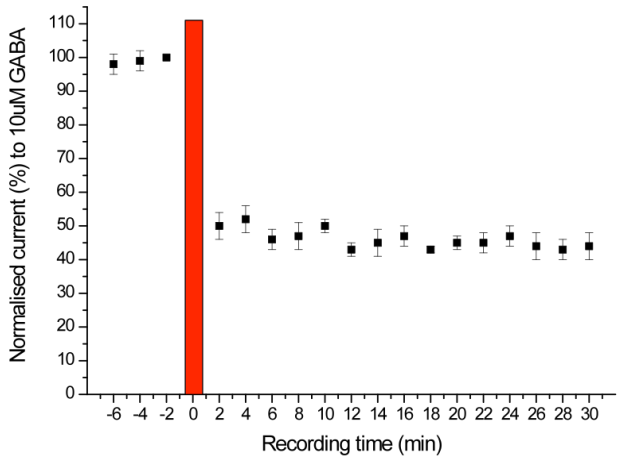


Figure 2.19 – GABA activated current pre- and post-photoirradiation

Work is currently underway to test for photoinhibition of GABA currents in hippocampal neurons, where replenishment of receptors is believed to occur.[39] In such a system, we could expect to see recovery of the GABA current following $GABA_A$ receptor block.

In collaboration with the Topf group, computational studies have been used to give insights into how benzophenone analogue **170** binds to the $GABA_A$ receptor. Unfortunately, the crystal structure of the $GABA_A$ receptor has not been solved, so molecular modelling and docking studies rely heavily on sequence homology with the structurally similar nicotinic acetylcholine receptor (nAChR). With this in mind, we feel that key interactions indicated by docking experiments would need to be scrutinised further by mutagenesis. To this end, mutagenesis studies have commenced to investigate the pertinence of several key residues in binding as presented by the docking studies.

Molecular modelling revealed that the benzophenone label of benzophenone analogue **170** could lie in a hydrophobic pocket between α and β subunits in α1β2γ2 WT receptors, where the label can be excited (**Figure 2.20**).

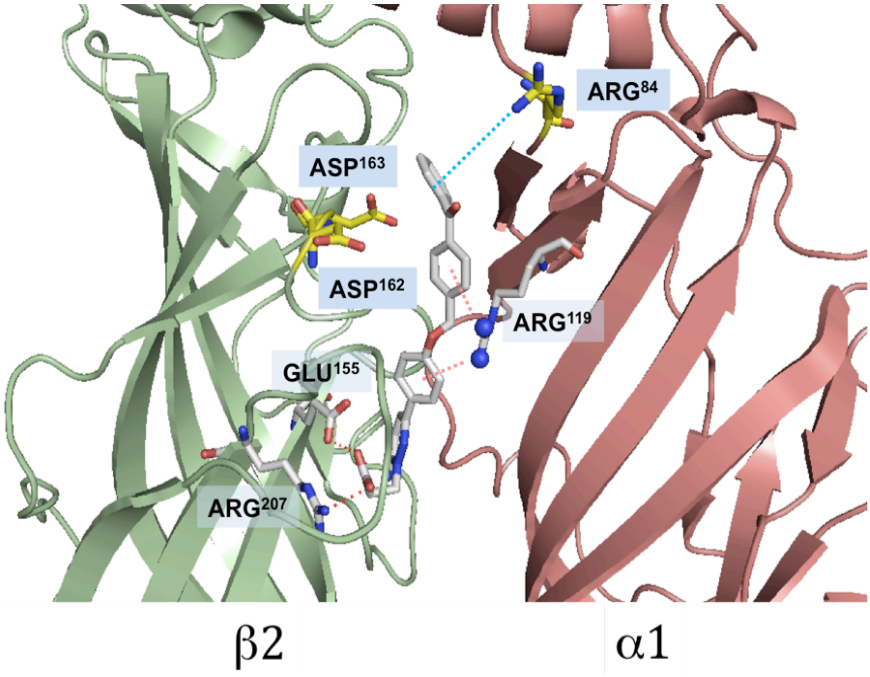


Figure 2.20 – Homology model of 170 within α1 and β2 subunits

We propose that the α1R84 and α1R119 residues may be involved in cation-π stacking interaction between the benzophenone and receptor. Both residues could also be involved in allowing radical recombination to occur after photoirradiation. Substitution of a less polar residue by mutagenesis may lead to a loss of this interaction, which in turn could lead the benzophenone analogue not being held as tightly in the binding site of the receptor. These considerations are currently under exploration by our collaborators in the Smart group.

With this result in hand, the next step in our research was to track native receptors microscopically by labelling them with a fluorescent marker such as a quantum dot. In the following section, we describe our efforts towards creating an analogue for this goal.

2.4 Photoaffinity labelled probes for following receptor mobility

The denouement of our research concerned efforts to create a gabazine analogue incorporating the most effective blocking photoaffinity label, *i.e*. a benzophenone, in addition to a fluorescent marker, enabling the tracing of receptor mobility microscopically. Quantum dots (QDs) are intensely fluorescent nanoparticles usually made from CdSe, encased in zinc sulfide. They hold many advantages over traditional dye molecules; principally that they do not suffer photobleaching, whilst possessing wide absorption bands yet narrow and distinct emission spectra.[203] The attachment of a QD to a biomolecule is however problematic.[204] To our knowledge, there are no instances where a QD has been used *in tandem* with photoaffinity labelling. Nevertheless, we felt that various approaches could be attempted in order to label the $GABA_A$ receptor. Three of the most relevant methods are outlined below.

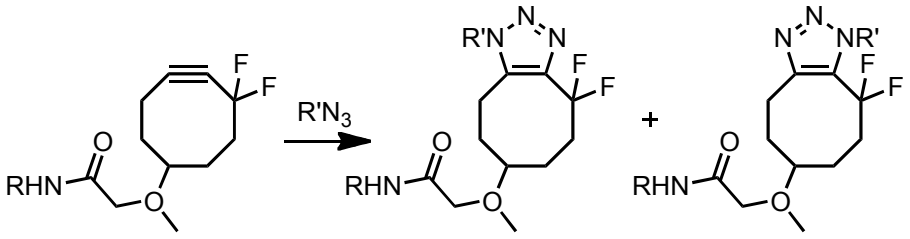
2.4.1 Methods of QD conjugation

2.4.1.1 Direct attachment

A direct link could be made between a given biomolecule and a QD. This approach has been applied to the tracking of $GABA_C$ receptors, where muscimol was directly tethered to a quantum dot *via* a polyethylene glycol (PEG) linker.[205, 206] Through this method however, there is little control over the number of muscimol units per quantum dot, with some estimations suggesting that as many as 150 muscimol units may be bound to each QD. Therefore, a substantial concern of this method is that receptors are labelled non-specifically, and many receptors may be tethered to a single QD.

2.4.1.2 Indirect attachment - Strain-promoted azide-alkyne coupling

The Cu-mediated azide-alkyne Huisgen cycloaddition is widely used in bioconjugation but QD fluorescence is quenched in the presence of Cu.[207] This outcome can be circumvented by reacting a strained alkyne with an azide (**Scheme 2.50**).[208-211]



Scheme 2.50

This method is essentially a two-step approach. One element could contain the reactive alkyne (usually the biomolecule), while a QD could be functionalised with azides. Azide-coated QD's are not commercially available, however these could be synthesised from an amine-coated QD precursor. A significant drawback of this approach is that the reaction between azide and alkyne takes place over several hours, and for the tracking of $GABA_A$ receptors this would therefore be unsuitable.

2.4.1.3 Indirect attachment - Streptavidin-biotin coupling

The streptavidin-biotin non-covalent interaction is one of the most powerful non-covalent interactions in nature. Streptavidin coated QD's have been used by other groups for receptor mobility and are commercially available.[212, 213] With this method, a biotinylated photoaffinity labelled ligand could be synthesised, and subsequently conjugated to a streptavidin coated QD. Compared to the strain-promoted azide-alkyne coupling, the streptavidin-biotin coupling would be more suitable for the tracking of receptors, due to the accelerated rate of coupling between the two components and the rate of receptor mobility.

2.4.2 Photoaffinity probe design

We felt that the streptavidin-biotin coupling was the strongest method to achieve specific labelling of $GABA_A$ receptors, due to the strong and expedient nature of the coupling between streptavidin and biotin. Moreover, the commercial availability of streptavidin-coated QDs meant that we could concentrate on creating a benzophenone analogue that would incorporate a biotin moiety. However, the attachment of biotin to the core antagonist structure necessitated a thorough investigation of the factors that could affect the compound's labelling efficiency (**Figure 2.21**).

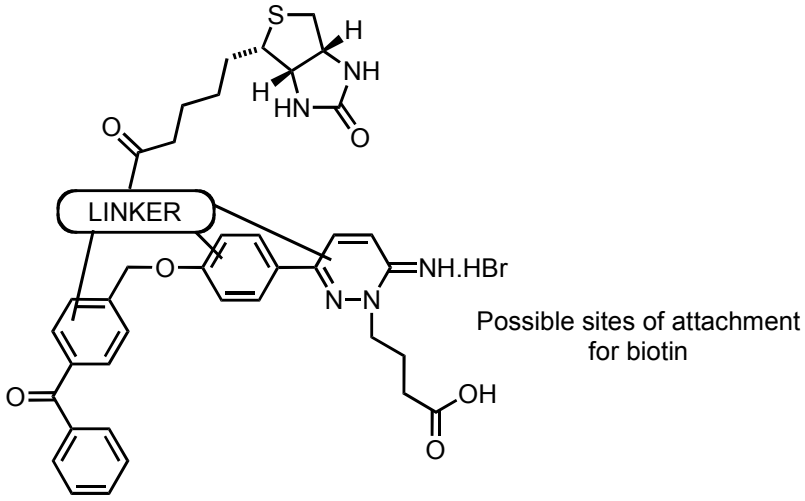


Figure 2.21

Previous reports indicate that functionalisation on the pyridazine is poorly tolerated and significantly affects the high potency of gabazine related compounds.[72, 81, 83] We were thus keen to avoid disturbing the two available positions on the pyridazine.[72] The ether functionality also plays a dual role; for the attachment of the benzophenone photoaffinity label, and as a hydrogen bond donor.[81] Furthermore, from collaborative computational work, we were able to theorise that the bulk of the benzophenone photoaffinity label in analogue **170** lies in a hydrophobic pocket between α and β subunits on the $GABA_A$ receptor (**Figure 2.22**).

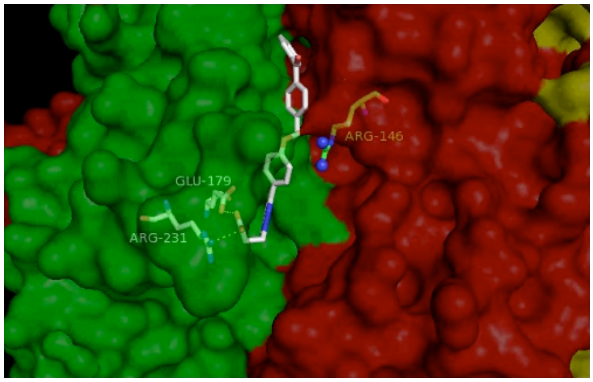


Figure 2.22 – Proposed orientation of 170 within $GABA_A$ receptor binding site

We were keen not to perturb the orientation of the photoaffinity label within this location, though a potential point of attachment for a biotin moiety was a position on the internal phenyl of the benzophenone.

Given these considerations, we considered synthesising the photoaffinity probe **199** shown in **Figure 2.23**.

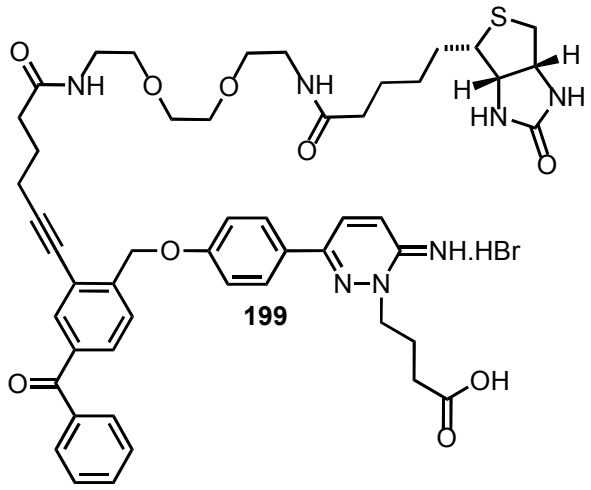
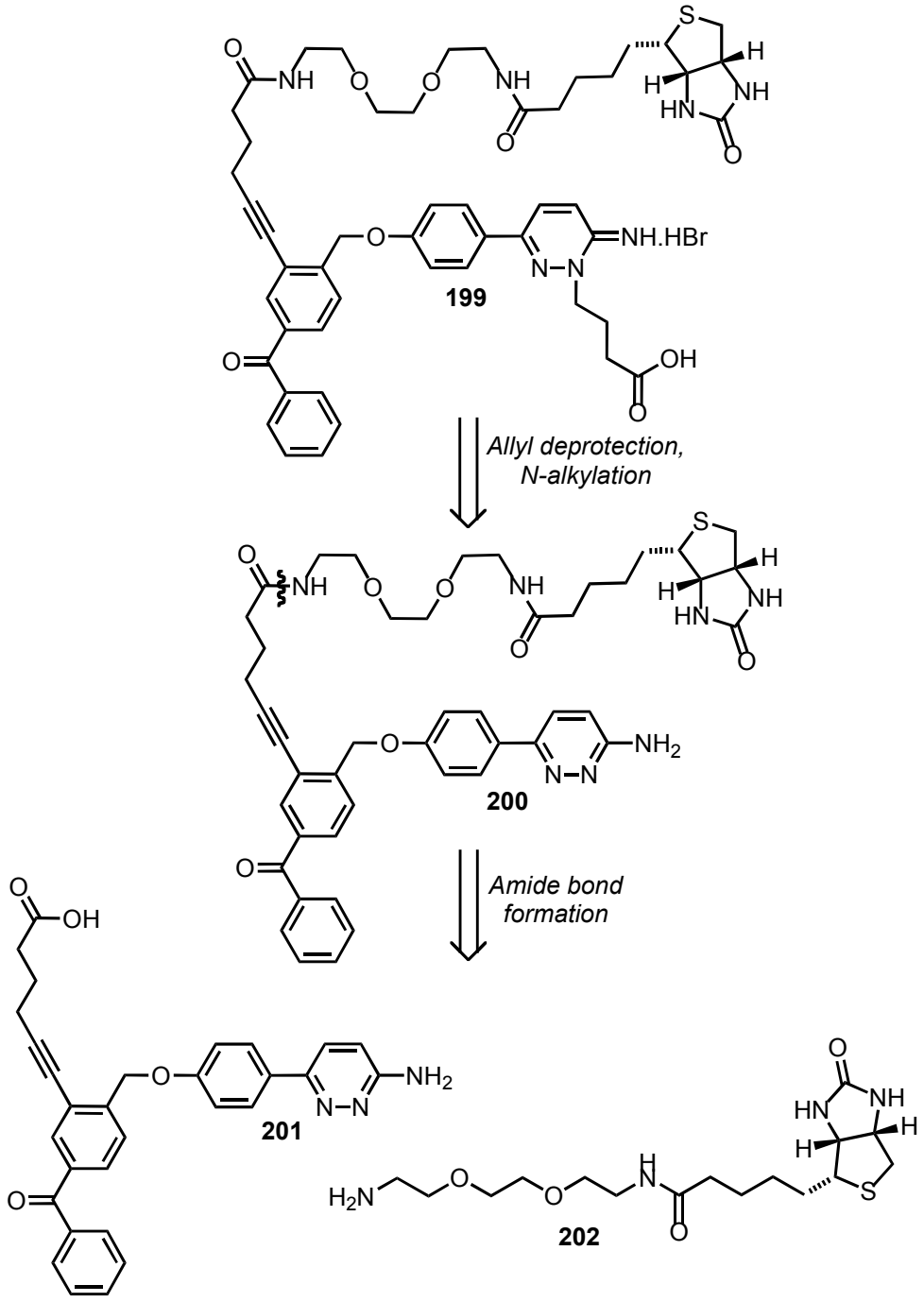


Figure 2.23

The biotin moiety would need to be placed sufficiently away from the receptor to facilitate coupling to streptavidin and we therefore envisioned that a spacer from the core antagonist structure to biotin would be required. It was envisaged that an ethylene glycol linker could be used for this purpose. An alkyne was chosen as the most appropriate way of attaching a linker for several reasons. Firstly, our computational modelling studies had shown that space exists for a linear linker at this location on the benzophenone (Figure 2.22). Secondly, alkyne-substituted

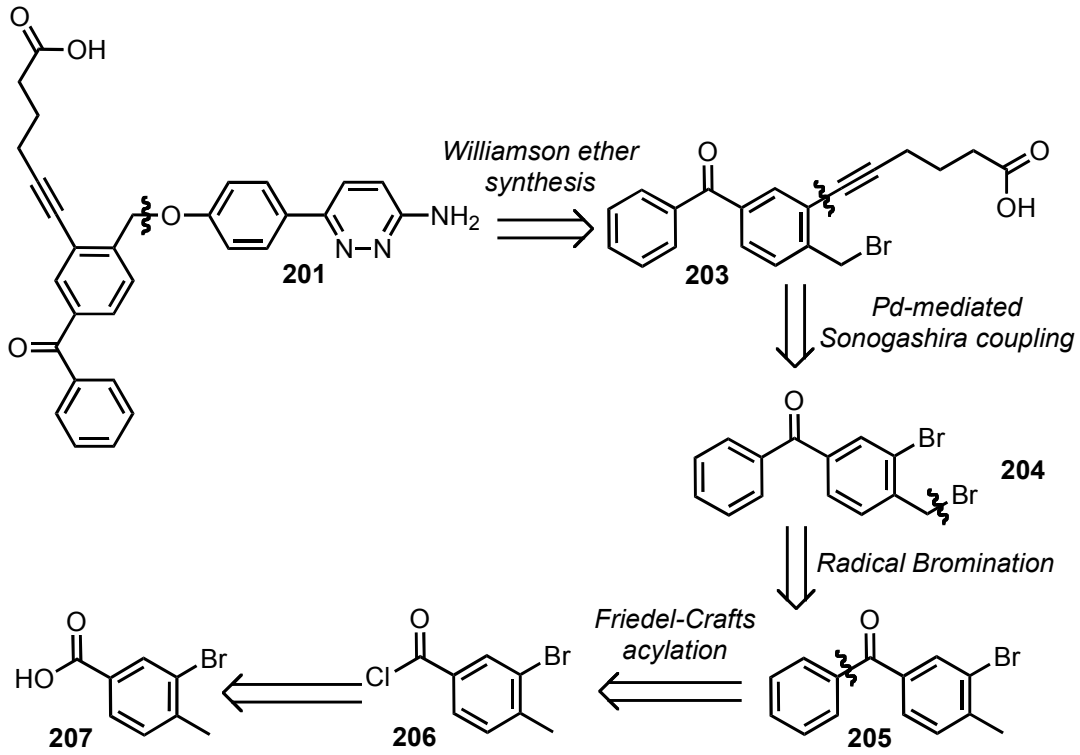
benzophenones have previously been used as effectively as photoaffinity labels without any detrimental effect on the cross-linking ability originating from the presence of a conjugated alkyne.[139]

The preliminary retrosynthetic route to **199** is outlined in **Scheme 2.51**.



Scheme 2.51

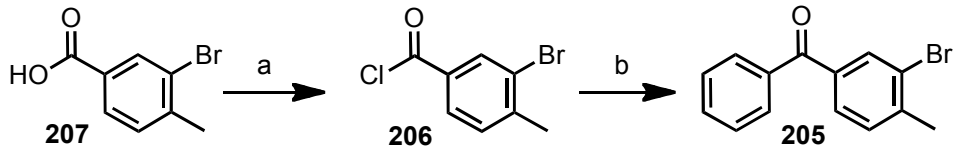
We believed that acid **199** could be synthesised through the alkylation and subsequent deprotection of pyridazine **200**, using identical procedures to those used for analogues previously described. Pyridazine **200** could be made by a coupling reaction between acid **201** and amine **202**. Synthesis of the amine fragment **202** has been thoroughly documented in the literature[214-216], whereas acid fragment **201** required a novel synthetic route. Outlined below in **Scheme 2.52** was our proposed path for creating acid **201**.



Scheme 2.52

We proposed that acid **201** could be formed through a Williamson ether synthesis between bromide **203** and arylpyridazine **157**, as routinely conducted in the syntheses of other analogues. The acid functionality can be introduced through a selective Sonogashira coupling between bromide **204** and 5-hexynoic acid. Bromide **204** could be created through the radical bromination of the tolyl position on benzophenone **205**. Benzophenone **205** is not commercially available, but its synthesis has been documented.[217] Thus, we anticipated that benzophenone **205** could be created through a Friedel-Crafts acylation of the corresponding acid chloride **206**, itself easily synthesised from the acid **207**.

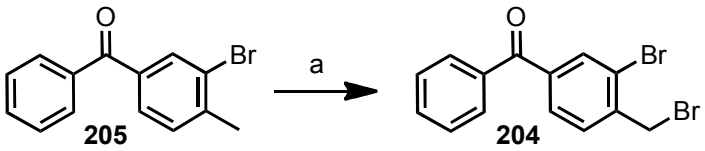
We embarked upon the synthesis of benzophenone **205**. Following conditions described by Ding *et al*. we were delighted to obtain benzophenone **205** in excellent yield. This was achieved through chlorination of acid **207** in refluxing $SOCl_2$, followed immediately by Friedel-Crafts acylation of the resulting acid chloride **206** in refluxing benzene, mediated by a catalytic amount of $AlCl_3$ (**Scheme 2.53**).[217]



Reagents and conditions: a) $SOCl_2$ (2 h, 80 ^oC), quant.;
b) $AlCl_3$, benzene (2 h, 50 ^oC), 98 %.

Scheme 2.53

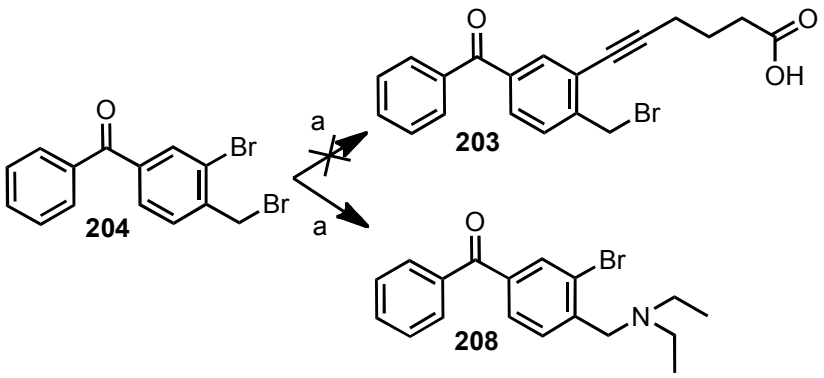
The bromide **204** is formed through radical bromination of benzophenone **205** with *N*-bromosuccinimide and AIBN as a radical initiator. The low yield is in part a result of multiple bromination of the tolyl position, as well as difficulties encountered with purification by column chromatography due to benzophenones **204** and **205** possessing similar retention factors (**Scheme 2.54**).[217]



Reagents and conditions: a) AIBN, *N*-Bromosuccinimide, CCl_4 (17 h, 70 ^oC), 27 %.

Scheme 2.54

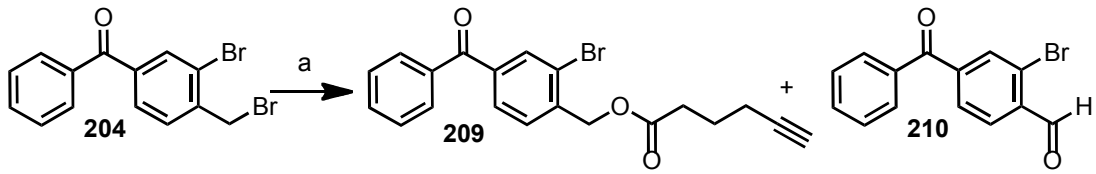
Following this, we attempted to proceed with a Sonogashira coupling of bromide **204** with 5-hexynoic acid. Unfortunately we were unable to isolate any of the desired acid **203** and instead obtained the amine **208**. This was formed through the S_N2 displacement of the bromide on benzophenone **204**, by diethylamine (**Scheme 2.55**).



Reagents and conditions: a) 5-Hexynoic acid, $Pd(PPh_3)_2Cl_2$, CuI, $HNEt_2$ (12 h, rt), 51 %.

Scheme 2.55

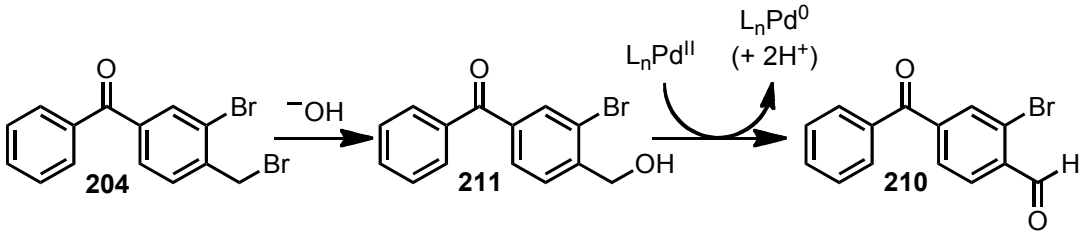
We considered that the reaction would proceed cleanly in triethylamine, so attempted the Sonogashira coupling using this approach. Synthesis of the desired compound however was not achieved, instead isolating the alkyne **209** and the aldehyde **210** (**Scheme 2.56**).



Reagents and conditions: a) 5-Hexynoic acid, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3 (48 h, rt), **209**, 31 %; **210**, 9.5 %.

Scheme 2.56

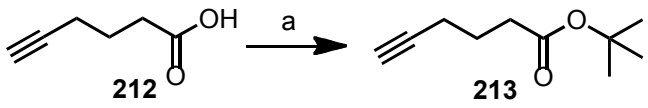
Alkyne **209** is formed through the *in situ* deprotonation of 5-hexynoic acid, followed by the S_N2 displacement of the bromide on benzophenone **204**. Nevertheless, the isolation of aldehyde **210** is unusual. We propose that the triethylamine used may have been wet, in which case it is possible that the benzyl bromide could have been converted to the benzyl alcohol **211**. Several reports have detailed the Pd^{II}-catalysed formation of benzaldehydes from benzyl alcohols, and we believe that aldehyde **210** may have been formed in this way (**Scheme 2.57**).[218, 219]



Scheme 2.57

We hoped that the formation of alkyne **209** could be abated through the Sonogashira coupling of bromide **204** with an ester, which could subsequently be deprotected. Hence, we synthesised a *tert*-butyl ester, which can be deprotected using a mild acid at approximately pH 4.

The synthesis of ester **213** was achieved *via* the synthesis of the mixed anhydride of 5-hexynoic acid **212** *in situ*, followed by displacement using *tert*-butanol. However, the synthesis culminated in a very low yield, which we thought was due to the high volatility of the ester **213** (**Scheme 2.58**).

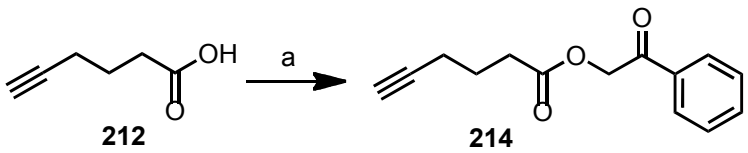


Reagents and conditions: a) *t*-BuOH, $(CF_3CO)_2O$, THF (16 h, -40 °C to rt), 6 %.

Scheme 2.58

We considered that the volatility of ester **213** might be problematic in our planned Sonogashira coupling and envisaged that a bulkier ester might be beneficial for our syntheses. Accordingly, we synthesised the phenacyl ester, which is deprotected using copper in the presence of acetic acid.

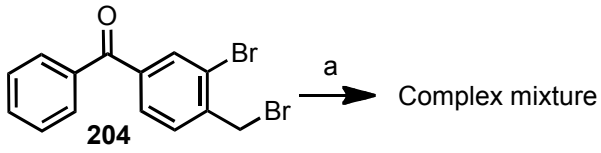
The synthesis of ester **214** was achieved *via* the *in situ* formation of phenacyl iodide from phenacyl chloride with tetrabutylammonium iodide, followed by S_N2 displacement of the iodide by 5-hexynoic acid. Ester **214** was isolated in acceptable yield (**Scheme 2.59**).



Reagents and conditions: a) Phenacyl chloride, tetrabutylammonium iodide, NEt_3, EtOAc (3 h, rt), 47 %.

Scheme 2.59

The Sonogashira coupling of ester **214** to bromide **204** was attempted. Unfortunately however, we obtained a complex mixture by NMR, containing none of the desired adduct (**Scheme 2.60**).

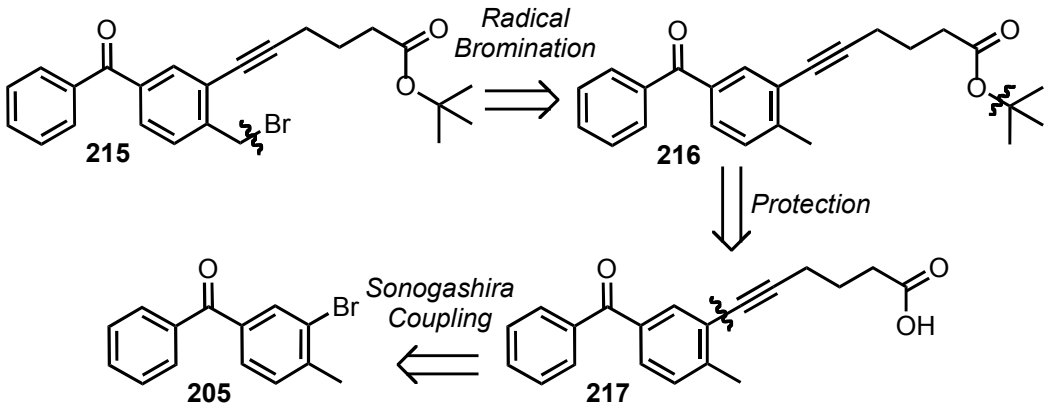


Reagents and conditions: a) **214**, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3 (48 h, rt).

Scheme 2.60

We believe that the phenacyl ester may have been partially deprotected by the copper present in the reaction mixture, which is necessary for the Sonogashira coupling.

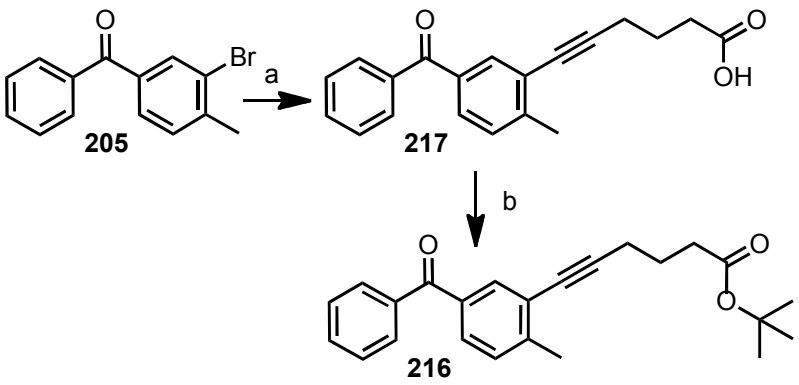
Due to the difficulties encountered towards the synthesis of bromide **204**, we altered our retrosynthesis so that a Sonogashira coupling could be attempted prior to bromide formation, with the aim of creating the ester **216**. Outlined in **Scheme 2.61** is our revised retrosynthesis for the formation of bromide **215**.



Scheme 2.61

We postulated that bromide **215** could be synthesised *via* the radical bromination of ester **216** with AIBN and *N*-bromosuccinimide. We predicted that potential cross-reactivity of the alkyne with *N*-bromosuccinimide could be a problem, particularly as the formed tolyl radical would be in conjugation with the alkyne. Nevertheless, we hoped that we would be able to create bromide **215** in this way. Acid **217** could be synthesised *via* a Sonogashira coupling between 5-hexynoic acid and benzophenone **205**.

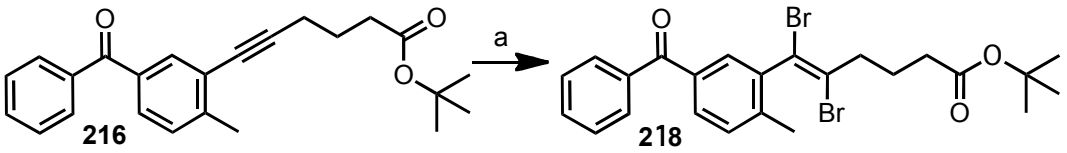
Consequently, Sonogashira coupling of benzophenone **205** to 5-hexynoic acid was attempted using our previously favoured conditions. Acid **217** was isolated in acceptable yield. We then protected acid **217** with a *tert*-butyl group, using conditions adopted previously, affording ester **216** in reasonable yield (**Scheme 2.62**).



Reagents and conditions: a) 5-Hexynoic acid, $Pd(PPh_3)_2Cl_2$, CuI, $HNEt_2$ (48 h, rt), 45 %; b) *t*-BuOH, $(CF_3CO)_2O$, THF (3 h, -40 ^oC to rt), 47 %.

Scheme 2.62

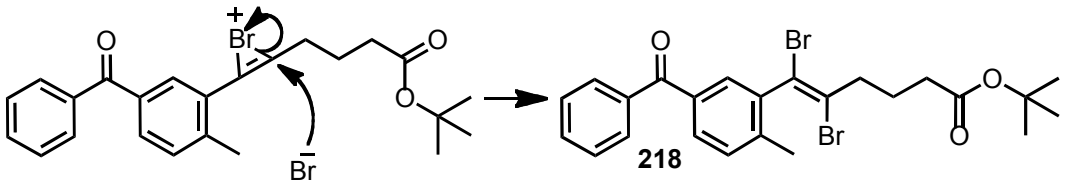
Following from this, we attempted a radical bromination of ester **216**, hoping to brominate on the tolyl position. However, the only product isolated from the reaction was the dibromoalkene **218** (**Scheme 2.63**).



Reagents and conditions: a) AIBN, *N*-bromosuccinimide, benzene (6 h, 60 ºC), 39 %.

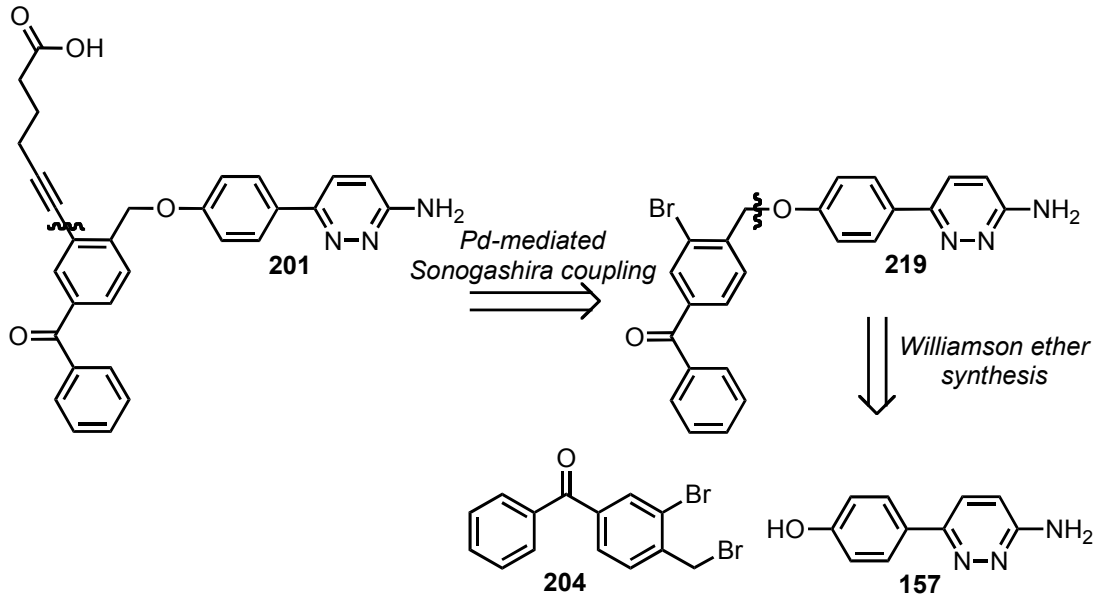
Scheme 2.63

The undesired product is likely to be the result of the displacement of a bromonium ion by nucleophilic bromide in the reaction mixture. (**Scheme 2.64**)



Scheme 2.64

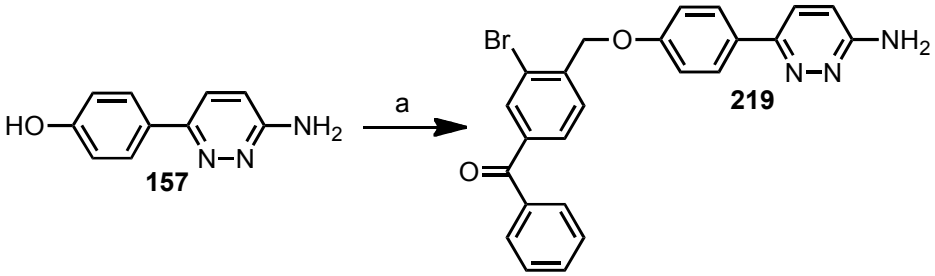
Unfortunately, we were not able to create the desired bromide **215** in this way. An alternative plan of action was then devised due to the complexity of trying to create bromide **203**. We then considered arriving at acid **201** *via* the retrosynthetic pathway outlined in **Scheme 2.65**.



Scheme 2.65

We proposed that acid **201** could be created through Sonogashira coupling of pyridazine **219** with 5-hexynoic acid. Pyridazine **219** could be synthesised *via* Williamson ether synthesis of bromide **204** with the previously synthesised arylpyridazine **157**.

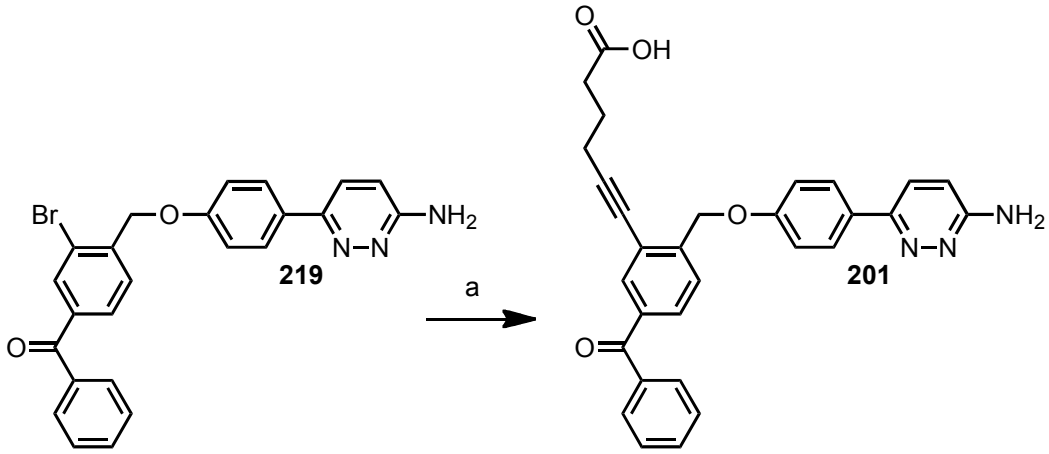
Using bromide **204**, we attempted a Williamson ether synthesis with arylpyridazine **157**, employing our favoured conditions from previous ether syntheses. We were delighted to obtain the desired pyridazine **219** in excellent yield (**Scheme 2.66**).



Reagents and conditions: a) **204**, potassium *tert*-butoxide, 18-crown-6, DMF (3 h, 0 ^{o}C), 81 %.

Scheme 2.66

Sonogashira coupling of pyridazine **219** was attempted using our preferred conditions and the desired acid **201** was isolated in good yield (**Scheme 2.67**).

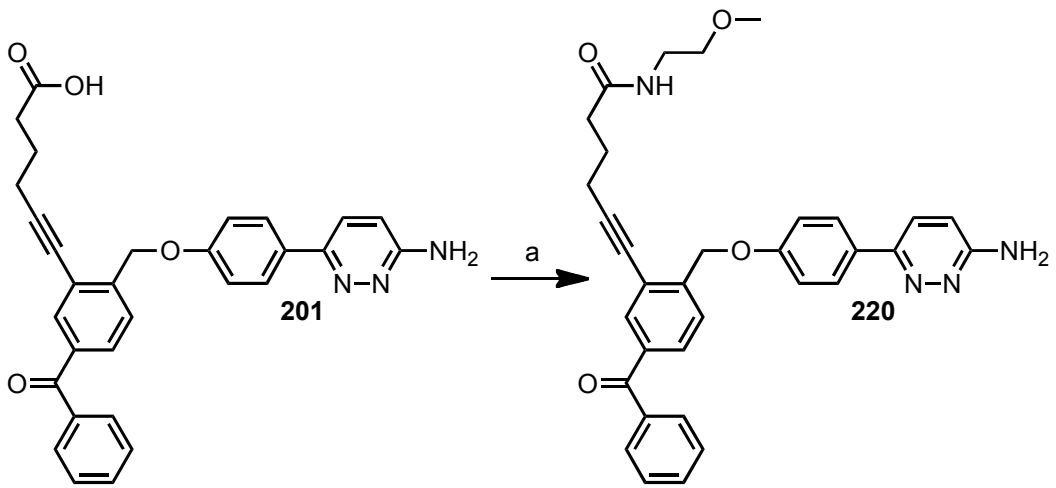


Reagents and conditions: a) 5-Hexynoic acid, $Pd(PPh_3)_2Cl_2$, CuI, $HNEt_2$ (48 h, rt), 77 %.

Scheme 2.67

Reproduction of the Sonogashira coupling was ineffectual under our optimised conditions, and we were not able to repeat this reaction successfully. Due to time constraints, this is an issue that remains to be addressed. Nevertheless, we continued our synthesis towards probe **199** with the isolated amount of acid **201**. We discovered that acid **201** was very insoluble, possibly due to the compound preferring to exist as a zwitterion. Consequently, we were unable to obtain either ^{1}H NMR or ^{13}C NMR spectra for this compound, due to its insolubility in common deuterated solvents. The correct mass was obtained by mass spectrometry, and in order to definitively prove the isolation of acid **201**, we decided to derivatise the compound with a simple amine, hoping to lower its polarity such that conclusive NMR spectra could be obtained.

Accordingly, an amide coupling was attempted *via* the *in situ* generation of the NHS ester of acid **201** and 2-methoxyethylamine, mediated by diisopropylcarbodiimide hydrochloride. Pyridazine **220** was afforded in good yield (**Scheme 2.68**).

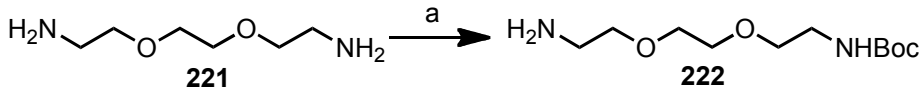


Reagents and conditions: a) 2-Methoxyethylamine, *N*-hydroxysuccinimide, diisopropylcarbodiimide hydrochloride, DMF (3 h, rt), 60 %.

Scheme 2.68

With pyridazine **220** conclusively proven to be the product of the previous reaction, we sought to create the amine fragment **202** for coupling to acid **201**. Amine **202** has been synthesised previously in the literature, so we followed those procedures outlined in our synthesis.[214-216]

We began with mono-Boc protection of diamine **221** to furnish the amine **222** in moderate yield (**Scheme 2.69**).



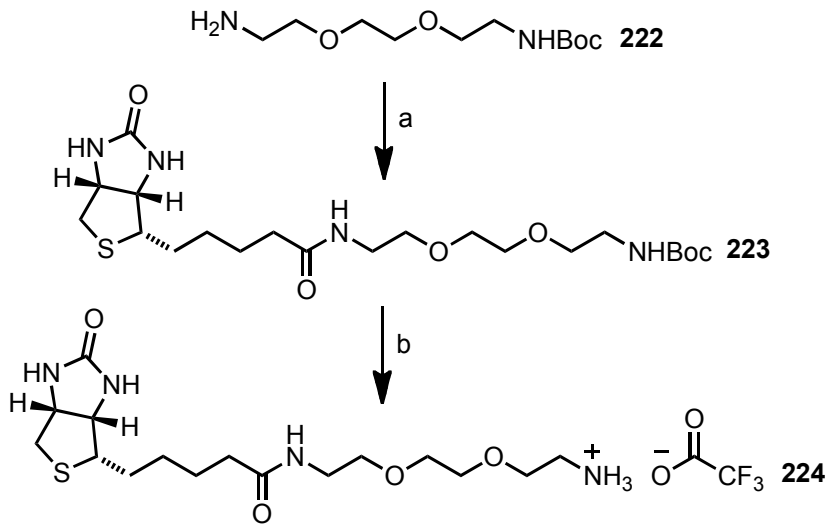
221 **222**

Reagents and conditions: a) Di-*tert*-butyl-dicarbonate, CH_2Cl_2 (24 h, rt), 56 %.

Scheme 2.69

We believe that the moderate yield can be accounted for by diamine **221** reacting twice with di-*tert*-butyl dicarbonate, hence leading to some doubly protected amine.

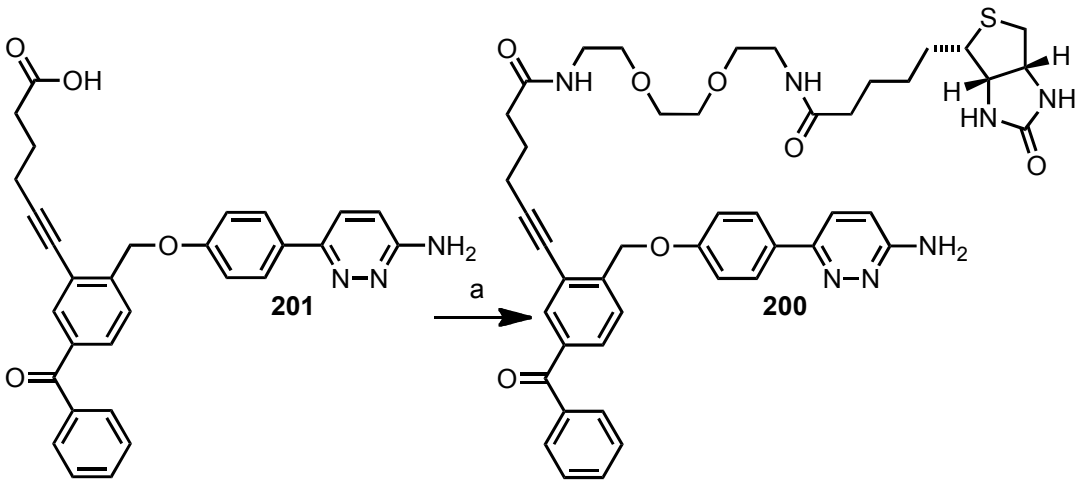
Amide coupling of amine **222** to biotin, mediated by HBTU and *N*,*N*-diisopropylethylamine (DIPEA) led to amine **223** in excellent yield. Subsequent treatment of amine **223** with trifluoroacetic acid led to the removal of the Boc group to reveal the desired amine **224**, as the trifluoroacetate salt in quantitative yield (**Scheme 2.70**).[214-216]



Reagents and conditions: a) Biotin, HBTU, DIPEA, DMF (2 h, rt), 83 %;
b) TFA/CH_2Cl_2 (1:1) (3 h, rt), quant..

Scheme 2.70

With acid fragment **201** and amine fragment **224** in hand, we attempted the coupling of the two reagents using standard amide coupling conditions, as previously employed. We isolated the desired pyridazine **200**, but in a poor yield of 12% (**Scheme 2.71**).

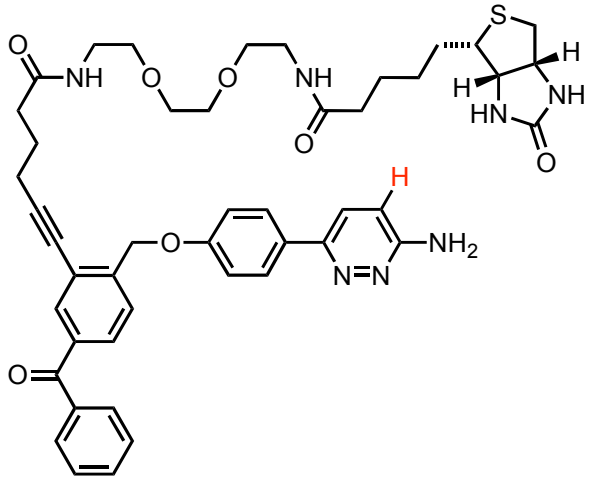


Reagents and conditions: a) **224**, HBTU, DIPEA, DMF (3 h, rt), 12 %.

Scheme 2.71

It is believed that the poor yield was attributable to the compound being sensitive to column chromatography, as we were only able to isolate a small amount of the desired compound pure.

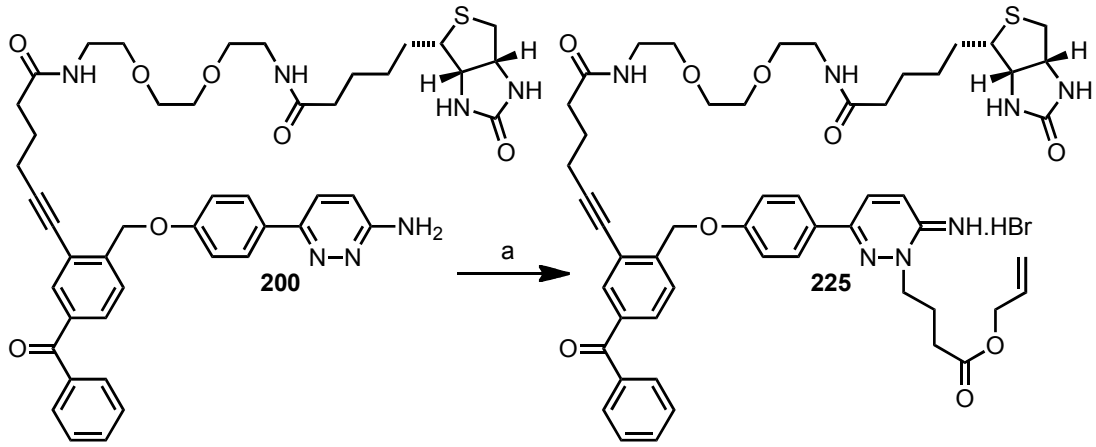
Intriguingly, by ^{1}H NMR, one aromatic signal was missing in protic deuterated solvents. It was found that one pyridazine hydrogen, highlighted in **Scheme 2.72**, is readily exchangeable in this environment. The deuteration is not observed in DMSO.



Scheme 2.72

A tentative explanation for the apparent deuteration of the proton could be that the delivery of a deuterium is passed to the enamine by the neighbouring pendant chain.

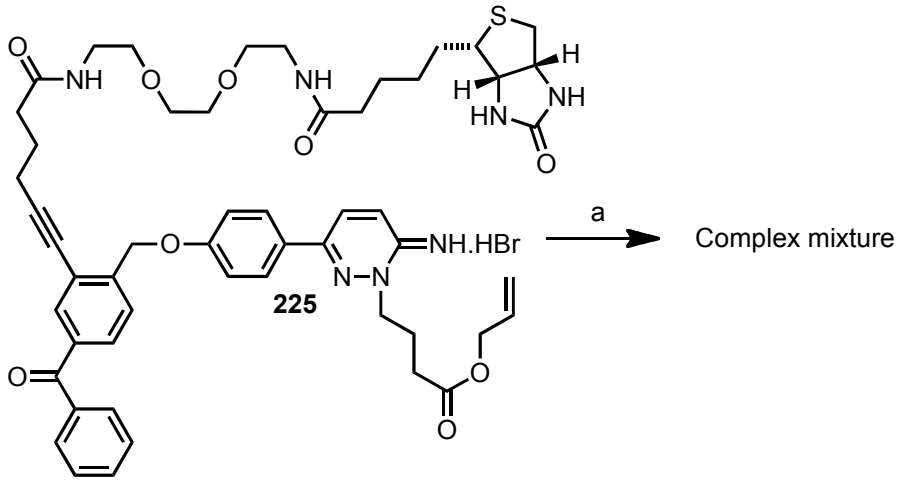
Whilst the poor yield of the previous reaction remains to be addressed, we continued to the penultimate step of our synthesis. Treatment of pyridazine **200** with allyl-4-bromobutyrate in DMF furnished the desired ester **225** through selective *N*(2)-alkylation of the pyridazine (**Scheme 2.73**).



Reagents and conditions: a) Allyl-4-bromobutyrate, DMF (5 h, 80 ºC), 43 %.

Scheme 2.73

Deprotection of ester **225** was attempted with our favoured conditions for allyl deprotection, using $Pd(PPh_3)_4$ and 1,3-dimethylbarbituric acid as the allyl scavenger. However, treatment of ester **225** with this system led to complete degradation, resulting in a complex mixture by NMR. Analyses by LC-MS also failed to reveal the desired compound (**Scheme 2.74**).

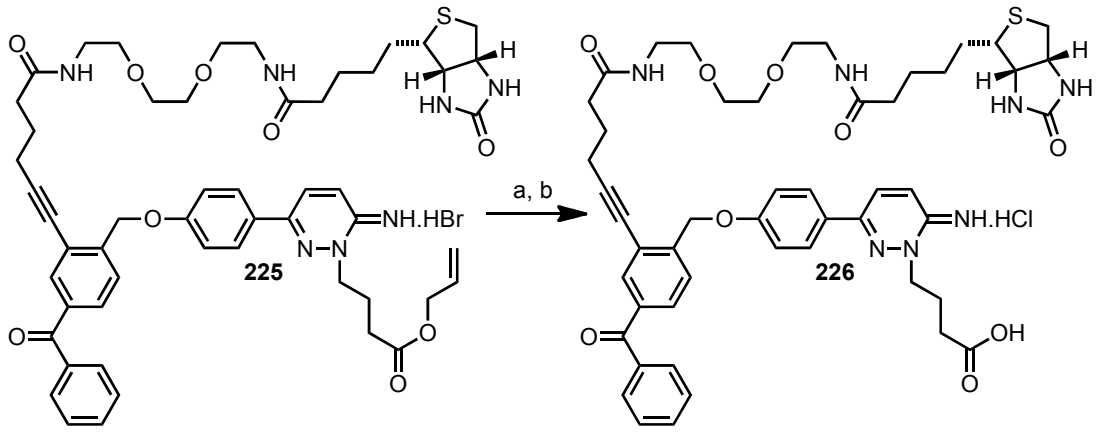


Reagents and conditions: a) $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, THF/CH_3OH, (3 h, rt).

Scheme 2.74

A plausible reason for the failure of the Pd-mediated deprotection method could be due to poisoning of the catalyst through the sulfur atom of biotin. Additionally, coordination of the ethylene glycol unit to the catalyst may contribute to its inactivity.

Although other metal-mediated allyl deprotections have been reported[220], we were concerned about the potential poisoning of catalysts. As a result, we chose to investigate a simple saponification method described by Gavande and co-workers.[100] To our delight, we were able to isolate compound **226** as the hydrochloride salt in acceptable yield (**Scheme 2.75**).



Reagents and conditions: a) NaOH, THF/H_2O (1:1) (3 h, 50 ºC);
b) HCl (0.1 M in H_2O), 52 % over both steps

Scheme 2.75

As a result, we were able to isolate a small amount (2.6 mg) of the desired photoaffinity probe **226** across eight steps from the commercially available 3-bromo-4-methylbenzoic acid **207**.

2.4.3 Biological Evaluation

Our collaborators in the Smart group investigated the potency of compound **226** using patch clamp electrophysiology with recombinant α1β2γ2S $GABA_A$ receptors transiently expressed in HEK293 cells by our collaborators in the Smart group.[182, 183] Through construction of concentration inhibition curves for the response to EC_{50} GABA, the IC_{50} for probe **226** was calculated as 1.49 μM (**Figure 2.24**), a nine-fold decrease in potency compared to the benzophenone analogue **170**.

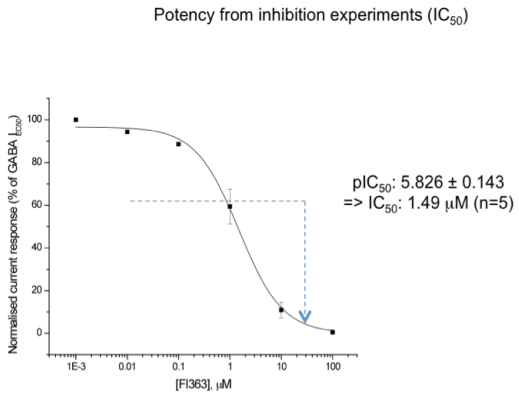


Figure 2.24 – Concentration inhibition curve for probe 226

Our collaborators further investigated whether probe **226** would effectively block the function of $GABA_A$ receptors *in vitro*. The findings from these initial experiments are summarised in **Table 2.7**.

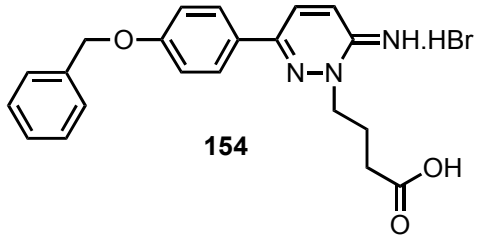
Entry	**$GABA_A$ antagonist**	**Photolytic Block**
i	**226**	32 ± 14%

Table 2.7

Due to the relatively small quantity of photoaffinity probe **226** synthesised, research examining the mobility of the $GABA_A$ receptor relies upon further synthesis. We have not been able to attempt the QD labelling of receptors due to the small amount of compound synthesised, but we have so far demonstrated that probe **226** blocks receptors in HEK cells.

2.5 Conclusions and Future Work

A number of $GABA_A$ antagonists based on the gabazine structure have been made for use as research tools for the receptor. We have demonstrated that by the simple addition of a benzyl group, the antagonist potency of arylpyridazine analogues of GABA can be greatly increased. This is exemplified by antagonist **154**, where we observed a 32-fold increase in potency compared to gabazine (**Figure 2.25**).



154

Figure 2.25

Our preliminary findings also indicated that the inclusion of an electron-donating methoxy- group or an electron-withdrawing nitro- group on the *meta*-position of the benzyl ring (**159** and **160** respectively) were not only tolerated but also enabled further increases in potency. Furthermore, we demonstrated that the presence of an additional benzyl group is not the only means of eliciting an increase in antagonist potency. In the case of **161**, a propargyloxy group will suffice.[188]

In all, four photoaffinity labelled antagonists were created (**Figure 2.26**). Of these, the benzophenone analogue **170** was shown to block 49% of $GABA_A$ receptors in cultured HEK cells. Future work in this area will concentrate on the optimization of blocking $GABA_A$ receptors *in vivo*.

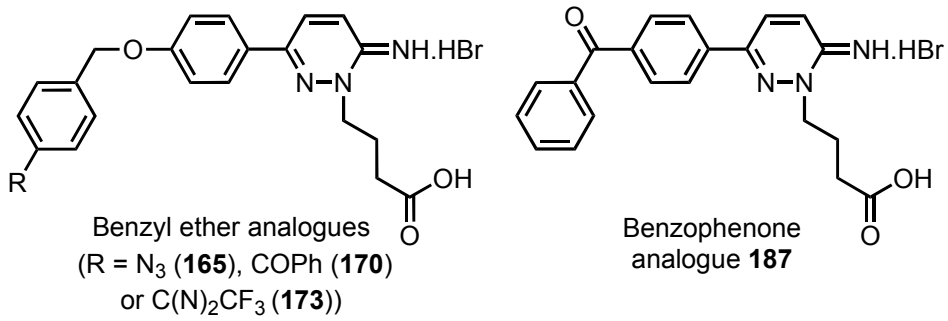


Figure 2.26

The tools created are the first with the potential to irreversibly inactivate $GABA_A$ receptors *in vivo*, and avoid the need to use mutant receptors expressed in neurons. Receptor trafficking could potentially be probed in specific surface areas of a neuron with the use of spot illumination methods, whereby photolysis can be limited to defined areas of neurons.[221, 222] It is also possible that some of the antagonists created thus far may be subtype specific. If this is the case, then the mobility of particular receptor subtypes could also be explored.

We were able to create a benzophenone labelled photoaffinity probe **226** for the $GABA_A$ receptor (**Figure 2.27**).

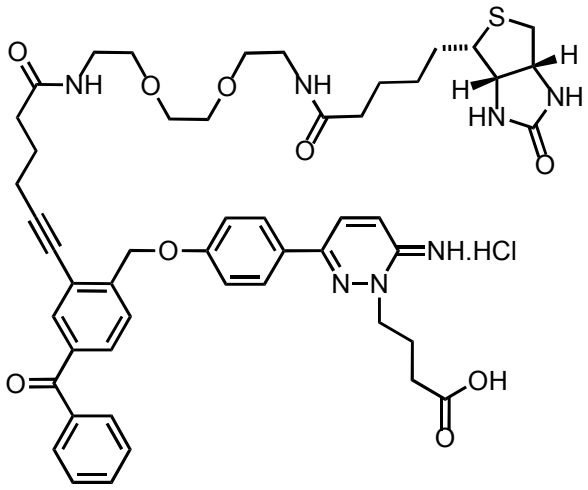


Figure 2.27

It is hoped that such a compound could be useful for exploring the spatial and temporal mobility of native $GABA_A$ receptors microscopically. Optimisation of receptor labelling studies may necessitate the synthesis of further photoaffinity probes, with variable tether lengths. Future work will therefore focus on the synthesis of analogues with variable PEG lengths.

Unfortunately, we abandoned plans to create diazirine analogue **191** due to time constraints (**Figure 2.28**).

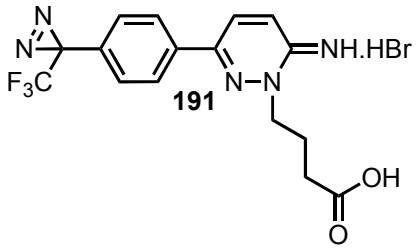
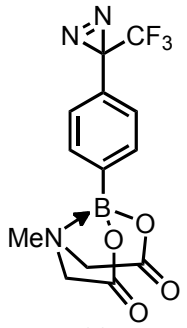


Figure 2.28

Attempts to create a diazirinyl boronic acid were fruitless, but we remain hopeful that a boronic acid could still be synthesised. If a diazirinyl boronic acid is unstable, we may have to consider a method by which to stabilise the compound. One such way, is through the creation of an air stable *N*-methyliminodiacetic acid (MIDA)

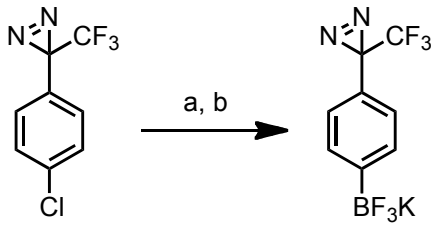
boronate of the diazirine. MIDA boronates promote the slow release of an unstable boronic acid, with impressive isolated cross-coupling yields from a series of unstable boronic acid precursors. Hence, the synthesis of a diazirinyl MIDA boronate **227** could circumvent those problems encountered with the synthesis of the diazirinyl boronic acid (**Figure 2.29**).



227

Figure 2.29

Similarly, trifluoroborate salts have become fashionable as partners in Pd-mediated cross-couplings.[223-226] This is in part due to their easy-to-handle nature and stability compared to their analogous boronic acids. Molander *et al*. recently established a one-pot procedure to generate trifluoroborates from their respective aryl chlorides.[201] This route could be compatible with a diazirinyl aryl chloride **228**, where one could create potentially create trifluoroborate salt **229** (**Scheme 2.76**).



228 **229**

Reagents and conditions: a) Pd cat., XPhos, sodium *tert*-butoxide, diboronic acid, KOAc, EtOH (18 h, 80 ^{o}C); b) KHF_2.

Scheme 2.76

Finally, the synthesis of new photoaffinity labelled antagonists that could be used to probe the mobility of $GABA_A$ receptors may entail the synthesis of various analogues containing a muscimol or thiomuscimol backbone in place of the GABA backbone. Muscimol **4** and thiomuscimol **5** have been exploited as photoaffinity

labelled agonists, as discussed in section **1.2.5**. Antagonists **21** and **22** were created by the Wermuth group, yet their use as photoaffinity labelled antagonists remain unexplored (**Figure 2.30**).[83]

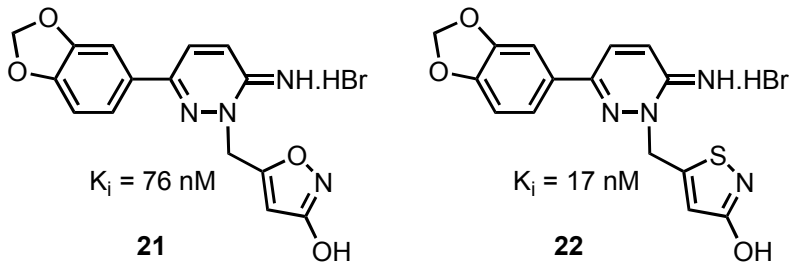


Figure 2.30

Future work in this area will concentrate on the synthesis of lead compound analogues such as analogues **230** and **231** depicted in **Figure 2.31**.

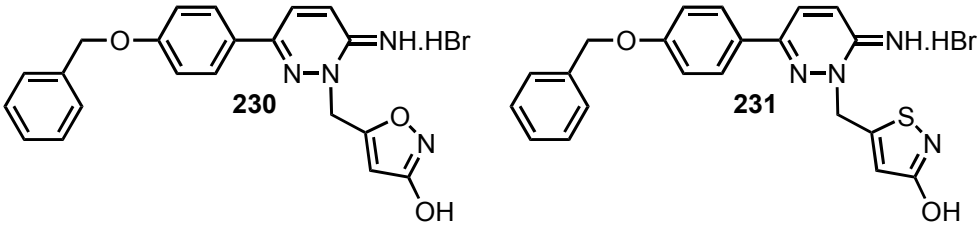


Figure 2.31

If successful, the resulting analogues could be derivatised to furnish other photoaffinity probes for the $GABA_A$ receptor.

3 Experimental Section

3.1 General Methods

All reactions were carried out at atmospheric pressure with stirring unless otherwise stated. All reagents and solvents were purchased from suppliers and used without further purification unless otherwise stated. Anhydrous toluene and THF were obtained from an Anhydrous Engineering (USA) solvent system after drying over alumina granules or pellets. NBS was recrystallised from water prior to use.

Reactions were monitored by TLC or ^{1}H NMR as stated. TLC plates pre-coated with silica gel 60 F_{254} on aluminium (Merck KGaA) were used, being visualized by UV (254 or 365 nm) or chemical stain ($KMnO_4$ or vanillin). Normal phase silica gel (BDH) was used for flash chromatography.

^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz respectively on a Bruker AMX500 at ambient temperature, unless otherwise stated; all chemical shifts were referenced to the residual proton impurity of the deuterated solvent. All spectra were recorded at 298 K. The multiplicity of the signal is indicated as s (singlet), d (doublet), t (triplet), dd (doublet of doublets), dt (doublet of triplets), quint (quintet) and m (multiplet); defined as all multipeak signals where overlap or complex coupling of signals makes definitive descriptions of peaks difficult. All peaks should be taken as sharp unless otherwise described. Coupling constants are defined as *J* given in Hz. Coupling constants are quoted in Hz to one decimal place. For NMR experiments, $CDCl_3$ denotes deuterated (d^3) chloroform, DMSO denotes deuterated (d^6) dimethylsulfoxide, and CD_3OD denotes deuterated (d^4) methanol. Deuterated solvents were chosen according to the position of solvent peak in spectra and solubility of substrate.

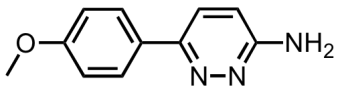
High and low resolution mass spectrometry was performed using a VG70 SE operating in modes CI, EI, ES and FAB.

Infrared spectra were obtained on a Perkin Elmer Spectrum 100 FTIR Spectrometer operating in ATR mode. Melting points were measured with a Gallenkamp apparatus

and are uncorrected. Room temperature (rt) is defined as between 19-22 ºC. *In vacuo* is used to describe solvent removal by Büchi rotary evaporation between 20 ºC and 60 ºC, at approximately 10 mmHg unless otherwise stated. The term 'degassed' refers to the process of removing O_2 from a solution by bubbling Argon through a sealed vessel containing the solution prior to use. Microwave irradiation was carried out in a CEM 150W microwave reactor.

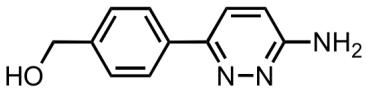
3.2 Experimental Procedures

6-(4-Methoxyphenyl)-pyridazin-3-ylamine[94] (141)



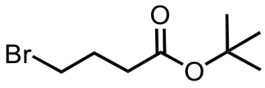
To a solution of 3-amino-6-chloropyridazine (50 mg, 0.386 mmol) in toluene (5 mL) was added 4-methoxybenzeneboronic acid (88 mg, 0.579 mmol), tetrakis(triphenylphosphine)palladium(0) (23 mg, 0.019 mmol) and Na_2CO_3 (3 mL, 2 M in H_2O) then degassed at rt for 10 min. The reaction mixture was then heated to 110 °C for 10 h. After cooling to rt, the solvent was removed *in vacuo*. EtOAc (20 mL) was added and the mixture placed in an ultrasound bath for 10 min. The mixture was filtered, and the residue was washed with EtOAc (2 × 40 mL). The solvent was then removed *in vacuo* and the residue was purified by column chromatography (EtOAc) to give the *pyridazine* **141** (59 mg, 0.293 mmol, 76%) as a white solid. m.p. 166-168 °C; lit. m.p.[94] 171 °C; R_f = 0.15 (EtOAc); IR υ_{max} (film)/cm^{-1} 3460, 3104, 1651, 1610, 1456; ^{1}H NMR (DMSO, 500 MHz) δ 3.79 (3 H, s, OCH_3), 6.45 (2 H, s, NH_2), 6.83 (1 H, d, *J* 9.3, NH_2CCH), 7.01 (2 H, d, *J* 7.3, CH_3OCCH), 7.78 (1 H, d, *J* 9.3, NH_2CCHCH), 7.87 (2 H, d, *J* 7.3, $CH_3OCCHCH$); ^{13}C NMR (DMSO, 125 MHz) δ 55.2 (CH_3), 114.1 (CH), 114.7 (CH), 125.2 (CH), 126.7 (CH), 129.4 (C), 149.6 (C), 159.2 (C), 159.6 (C); *m/z* (CI+) 201.0905 (M^+, $C_{11}H_{11}N_3O$ requires 201.0902).

[4-(6-Aminopyridazin-3-yl)-phenyl]-methanol (138)



To a solution of 3-amino-6-chloropyridazine (50 mg, 0.386 mmol) in CH_3CN (1.2 mL) and water (0.8 mL) in a microwave vial was added 4-(hydroxymethyl)-benzeneboronic acid (70 mg, 0.463 mmol), bis(triphenylphosphine)palladium(II) dichloride (8.0 mg, 0.0115 mmol) and K_2CO_3 (80 mg, 0.579 mmol) consecutively. The resulting solution was degassed at rt for 5 min then subjected to microwave irradiation for 10 min at 120 °C. The mixture was diluted with water (50 mL) and extracted with EtOAc (3 × 100 mL), washed with brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was subjected to column chromatography (EtOAc/CH_3OH 19:1) to give the *pyridazine* **138** (55 mg, 0.273 mmol, 71%) as a white solid. m.p. 190-194 °C; R_f = 0.15 (EtOAc); IR υ_{max} (film)/cm^{-1} 3423, 3133, 1647, 1611, 1459; ^{1}H NMR (CD_3OD, 500 MHz) δ 4.65 (2 H, s, HOC*H*$_2$), 6.45 (2 H, s, NH_2), 7.00 (1 H, d, *J* 9.3, NH_2CC*H*), 7.45 (1 H, d, *J* 7.3, $HOCH_2$CCHC*H*), 7.78 (1 H, d, *J* 9.3, NH_2CCHC*H*), 7.86 (2 H, d, *J* 7.3, $HOCH_2$CC*H*); ^{13}C NMR (CD_3OD, 125 MHz) δ 64.8 (CH_2), 117.4 (CH), 127.1 (CH), 128.3 (CH), 128.4 (CH), 137.0 (C), 143.6 (C), 152.7 (C), 161.0 (C); *m*/*z* (CI+) 201.0911 (M^+, $C_{11}H_{11}N_3O$ requires 201.0902), 184 (50).

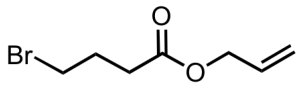
***tert*-Butyl-4-bromobutyrate[175, 176] (143)**



Trifluoroacetic anhydride (2.00 g, 9.52 mmol) was slowly added to a solution of 4-bromobutyric acid (1.71 g, 10.2 mmol) in anhydrous THF (15 mL) at –40 °C and the resulting solution was stirred at –40 °C for 30 min. *tert*-Butyl alcohol (12.5 mL) was added to the solution, which was then warmed to rt and stirred for 16 h. The reaction mixture was poured onto a mixture of crushed ice and saturated $NaHCO_3$ (25 mL). The product was extracted using EtOAc (50 mL) and the organic phase washed with water (50 mL), brine (50 mL), and dried (Na_2SO_4). The filtrate was concentrated *in*

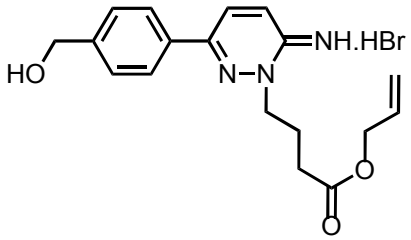
vacuo to obtain an oil, dissolved in methyl *tert*-butyl ether (50 mL). The resulting solution was passed through a short pad of silica gel. The filtrate was concentrated *in vacuo* and dried under high vacuum to yield the *ester* **143** (1.19 g, 5.34 mmol, 56%) as a colourless oil. IR υ_{max} (film)/cm^{-1} 2995, 1732; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.42 (9H, s, $CO_2C(CH_3)_3$), 2.10 (2 H, tt, *J* 7.2 and 6.5, $BrCH_2CH_2$), 2.37 (2 H, t, *J* 7.2, $BrCH_2CH_2CH_2$), 3.41 (2 H, t, *J* 6.5, $BrCH_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.2 (CH_2), 28.3 (CH_3), 32.9 (CH_2), 33.8 (CH_2), 63.7 (C), 172.3 (C=O); *m*/z (CI+) 223.0339 (M+H, $C_8H_{16}O_2{}^{79}Br$ requires 223.0334), 225 (100), 151 (100), 149 (100).

Allyl-4-bromobutyrate[177] (145)



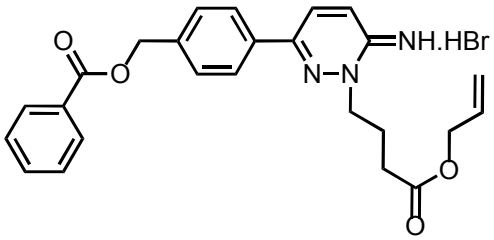
4-Bromobutyrylchloride (2.31 g, 12.5 mmol) was added dropwise to allyl alcohol (5.85 g, 101 mmol) at 0 °C over 2 h. After stirring at rt for a further 3 h, the solvent was removed *in vacuo* and the residue dissolved in CH_2Cl_2 (25 mL), washed with $NaHCO_3$ (25 mL), brine (25 mL) and dried (Na_2SO_4). The filtrate was concentrated *in vacuo* and dried under high vacuum to yield the *ester* **145** (1.99 g, 9.61 mmol, 77%) as a colourless oil. R_f = 0.75 (petrol/EtOAc 1:1); IR υ_{max} (film)/cm^{-1} 2983, 1774, 1737; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.17 (2 H, tt, *J* 6.9 and 6.5, $BrCH_2CH_2$), 2.51 (2 H, t, *J* 6.9, $BrCH_2CH_2CH_2$), 3.44 (2 H, t, *J* 6.5, $BrCH_2$), 4.57 (2 H, d, *J* 12.5, $CO_2CH_2CHCH_2$), 5.21 (1 H, dd, *J* 10.4 and 1.3, CO_2CH_2CHCHH), 5.29 (1 H, dd, *J* 17.3 and 1.3, CO_2CH_2CHCHH), 5.88 (1 H, m, CO_2CH_2CH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.9 (CH_2), 32.4 (CH_2), 32.7 (CH_2), 65.3 (CH_2), 118.4 (CH_2), 132.1 (CH), 172.3 (C=O); *m*/z (CI+) 207.0024 (M+H, $C_7H_{12}O_2{}^{79}Br$ requires 207.0021), 209 (100), 151 (60), 149 (60), 127 (75).

1-(3-Allyloxycarbonylpropyl)-6-amino-3-(4-hydroxymethylphenyl)-pyridazinium bromide (147)



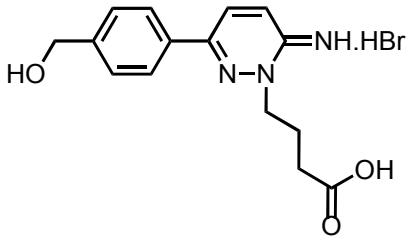
To a solution of 3-amino-6-(4-hydroxymethylphenyl)pyridazine (100 mg, 0.497 mmol) in DMF (0.3 mL) was added allyl-4-bromobutyrate (154 mg, 0.743 mmol). The solution was heated to 80 °C for 8 h. The hot solution was poured into EtOAc (6 mL) to yield a solid, which was then isolated by filtration. The product was dried under high vacuum to give the *ester* **147** (155 mg, 0.379 mmol, 76%) as a white solid. m.p. degraded > 200 °C; R_f = 0.05 (EtOAc/CH_3OH 9:1); IR υ_{max} (film)/cm^{-1} 3034, 1726, 1655, 1579, 1534; ^1H NMR (CD_3OD, 500 MHz) δ 2.27 (2 H, m, NCH_2CH_2), 2.62 (2 H, t, *J* 6.8, $NCH_2CH_2CH_2$), 4.46-4.48 (4 H, m, NCH_2 and $CO_2CH_2CHCH_2$), 4.69 (2 H, s, $HOCH_2$), 5.15 (1 H, dd, *J* 10.4 and 1.1, CO_2CH_2CHCHH), 5.23 (1 H, dd, *J* 17.1 and 1.1, CO_2CH_2CHCHH), 5.82 (1 H, m, CO_2CH_2CH), 7.53 (2 H, d, *J* 8.2, $HOCH_2CCHCH$), 7.64 (1 H, d, *J* 9.3, $NHCCH$), 7.97 (2 H, d, *J* 8.2, $HOCH_2CCH$), 8.33 (1 H, d, *J* 9.3, $NHCCHCH$); ^{13}C NMR (CD_3OD, 125 MHz) δ 22.5 (CH_2), 31.3 (CH_2), 57.0 (CH_2), 64.9 (CH_2), 66.5 (CH_2), 118.6 (CH_2), 126.9 (CH), 127.8 (CH), 128.5 (CH), 132.8 (CH), 133.0 (C), 133.4 (CH), 146.4 (C), 152.1 (C), 154.2 (C), 174.0 (C=O); *m*/*z* (ES+) 327.1611 (M+H, $C_{18}H_{21}N_3O_3$ requires 327.1582), 200 (35).

1-(3-Allyloxycarbonylpropyl)-6-amino-3-(4-benzoyloxymethylphenyl)-pyridazinium bromide (148)



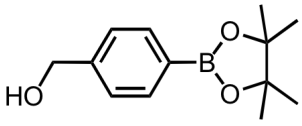
To a solution of **147** (53 mg, 0.130 mmol) and pyridine (10 mg, 0.130 mmol) in DMF (0.8 mL) at 80 °C, was added benzoyl chloride (18 mg, 0.130 mmol) and the reaction mixture was heated at 80 °C for 3 h. EtOAc (16 mL) was added to the hot solution to give a white solid. The solid was recrystallised from ethanol. The product was dried under high vacuum to give the *ester* **148** (40 mg, 0.078 mmol, 60%) as a white solid. m.p. degraded > 200 °C; R_f = 0.05 (EtOAc/CH_3OH 9:1); IR υ_{max} (film)/cm^{-1} 3030, 1726, 1720, 1647, 1560, 1531; ^1H NMR (CD_3OD, 500 MHz) δ 2.25-2.28 (2 H, m, NCH_2CH_2), 2.61-2.63 (2 H, m, $NCH_2CH_2CH_2$), 4.45-4.48 (4 H, m, NCH_2 and $CO_2CH_2CHCH_2$), 4.68 (2 H, s, OCH_2), 5.15 (1 H, dd, *J* 10.2 and 1.3, CO_2CH_2CHCHH), 5.23 (1 H, dd, *J* 16.7 and 1.3, CO_2CH_2CHCHH), 5.82 (1 H, m, CO_2CH_2CH), 7.52 (2 H, d, *J* 8.2, OCH_2CCHCH), 7.67 (1 H, d, *J* 9.3, NHCCH), 7.96 (2 H, d, *J* 8.2, OCH_2CCH), 8.13-8.15 (2 H, m, $CO_2CCHCHCH$), 8.33 (1 H, d, *J* 9.3, NHCCHCH), 8.69 (1 H, t, *J* 7.5, $CO_2CCHCHCH$), 8.90 (2 H, d, *J* 7.7, $CO_2CCHCHCH$); ^{13}C NMR (CD_3OD, 125 MHz) δ 22.6 (CH_2), 31.4 (CH_2), 57.1 (CH_2), 64.5 (CH_2), 66.5 (CH_2), 118.8 (CH_2), 127.0 (CH), 127.8 (CH), 128.6 (CH), 128.9 (CH), 132.9 (CH), 133.0 (C), 133.5 (CH), 143.0 (CH), 146.4 (C), 148.1 (C), 148.5 (CH), 152.0 (C), 154.2 (C), 174.0 (C=O), 174.9 (C=O); *m*/*z* (ES+) 431.1795 (M+H, $C_{25}H_{25}N_3O_4$ requires 431.1845), 326 (30), 304 (40).

1-(3-Carboxypropyl)-6-amino-3-(4-hydroxymethylphenyl)-pyridazinium bromide (149)



To a solution of $NaHCO_3$ (11 mg, 0.130 mmol), and dimedone (11 mg, 0.078 mmol) in water (1 mL) was successively added THF (1 mL), triethyl phosphite (7.0 mg, 0.043 mmol) and palladium(II) acetate (1.5 mg, 0.0089 mmol) under argon. After stirring for 3 min, **148** (40 mg, 0.078 mmol) was added and the mixture was stirred at 35 °C for 15 h. The mixture was diluted with water (5 mL) and washed thoroughly with CH_2Cl_2 (3 × 15 mL). The aqueous phase was separated and evaporated *in vacuo*. The residue was purified by column chromatography ($CHCl_3$/CH_3OH 9:1) to give the *acid* **149** (15 mg, 0.042 mmol, 54%) as a white solid. m.p. degraded > 200 °C; R_f = 0.05 (EtOAc/CH_3OH 8:2); IR υ_{max} (film)/cm^{-1} 3030, 1721, 1660, 1544; ^1H NMR (CD_3OD, 500 MHz) δ 2.14 (2 H, m, NCH_2CH_2), 2.34 (2 H, t, *J* 6.8, $NCH_2CH_2CH_2$), 4.43 (4 H, t, *J* 6.8, NCH_2), 4.67 (2 H, s, $HOCH_2$), 7.54 (2 H, d, *J* 8.1, $HOCH_2CCHCH$), 7.63 (1 H, d, *J* 9.1, NHCCH), 7.97 (2 H, d, *J* 8.1, $HOCH_2CCH$), 8.28 (1 H, d, *J* 9.1, NHCCHCH); ^{13}C NMR (CD_3OD, 125 MHz) δ 22.3 (CH_2), 31.1 (CH_2), 56.4 (CH_2), 64.9 (CH_2), 128.0 (CH), 128.5 (CH), 132.6 (CH), 132.9 (C), 133.2 (CH), 146.7 (C), 152.0 (C), 153.7 (C), 177.3 (C=O); *m*/*z* (ES+) 287.1268 (M+H, $C_{15}H_{17}N_3O_3$ requires 287.1270), 200 (25).

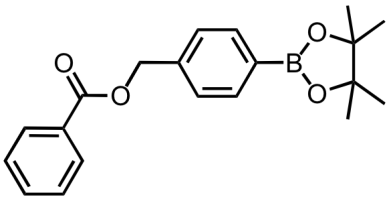
[4-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenyl] methanol (153)



To a solution of 4-(hydroxymethyl)benzeneboronic acid (640 mg, 4.23 mmol) in Et_2O (25 mL) was added pinacol (499 mg, 4.23 mmol) and stirred at rt for 6 h. The

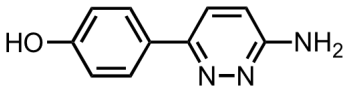
solvent was removed *in vacuo* and the residue subjected to column chromatography (petrol/EtOAc 4:1) to give the *boronate ester* **153** (388 mg, 1.66 mmol, 39%) as a white solid. m.p. 90-93 °C; R_f = 0.80 (EtOAc); IR υ_{max} (film)/cm^{-1} 3023, 1610; ^{1}H NMR (DMSO, 500 MHz) δ 1.23 (12 H, s, 4 × C*H*$_3$), 4.47 (2 H, d, *J* 5.7, HOC*H*$_2$), 5.26 (1 H, t, *J* 5.7, O*H*), 7.35 (2 H, d, *J* 8.0, BCCHC*H*), 7.65 (2 H, d, *J* 8.0, BCC*H*); ^{13}C NMR (DMSO, 125 MHz) δ 24.6 (CH_3), 62.7 (CH_2), 83.5 (C), 125.7 (CH), 126.7 (C), 134.3 (CH), 146.0 (C); *m*/*z* (EI) 234.1419 (M^+, $C_{13}H_{19}O_3B$ requires 234.1422), 135 (50).

Benzoic acid 4-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzyl ester (151)



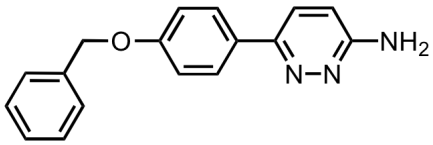
To a solution of **153** (100 mg, 0.427 mmol) and pyridine (33 mg, 0.427 mmol) in Et_2O (1 mL) was added benzoyl chloride (60 mg, 0.427 mmol). The reaction mixture was stirred at rt for 4 h. EtOAc (50 mL) was then added and the mixture was washed with water (50 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was subjected to column chromatography (petrol/EtOAc 4:1) to give the *boronate ester* **151** (70 mg, 0.207 mmol, 48%) as a white solid. m.p. 60-63 °C; R_f = 0.60 (petrol/EtOAc 1:1); IR υ_{max} (film)/cm^{-1} 3031, 1727, 1603; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.23 (12 H, s, 4 × C*H*$_3$), 5.28 (2 H, s, CO_2CH_2), 7.52-7.54 (4 H, m, $CO_2CCHCHCH$ and $CO_2CCHCHCH$), 7.56 (1 H, t, *J* 7.2, $CO_2CCHCHCH$), 7.83 (2 H, d, *J* 7.2, BCCHC*H*), 8.07 (2 H, d, *J* 7.2, BCC*H*); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 24.9 (CH_3), 66.7 (CH_2), 83.9 (C), 127.4 (CH), 128.5 (CH), 129.3 (C), 129.8 (CH), 130.2 (C), 133.1 (CH), 135.2 (CH), 139.1 (C), 166.5 (C=O); *m*/*z* (EI) 338.1699 (M^+, $C_{13}H_{19}O_3B$ requires 338.1684), 239 (50), 105 (25).

4-(6-Amino-pyridazin-3-yl)-phenol (157)



To a microwave vial containing 3-amino-6-chloropyridazine (102 mg, 0.740 mmol), 4-hydroxyphenylboronic acid (163 mg, 1.26 mmol), bis(triphenylphosphine)palladium(II) dichloride (27 mg, 0.040 mmol) and K_2CO_3 (202 mg, 1.46 mmol) were added CH_3CN (2.0 mL) and H_2O (1.3 mL). The resulting solution was degassed for 5 min and subjected to microwave irradiation for 10 min at 120 °C. The mixture was diluted with water (50 mL) and extracted with EtOAc (3 × 100 mL), washed with brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc/CH_3OH 19:1) to give the *pyridazine* **157** (87 mg, 0.459 mmol, 62%) as an orange solid. m.p. 250-252 °C; R_f = 0.10 (EtOAc); IR υ_{max} (film)/cm^{-1} 3414, 3121, 1646, 1617, 1447; ^{1}H NMR ($CD_3OD/CDCl_3$ 1:1, 600 MHz) δ 6.88 (2 H, d, *J* 8.9, HOCC*H*), 6.97 (1 H, d, *J* 9.3, NH_2CCH), 7.64 (1 H, d, *J* 9.3, NH_2CCHCH), 7.68 (2 H, d, *J* 8.9, HOCCHC*H*); ^{13}C NMR (CD_3OD, 125 MHz) δ 114.4 (CH), 115.5 (CH), 124.7 (CH), 126.7 (CH), 128.0 (C), 150.0 (C), 157.8 (C), 159.3 (C); *m*/*z* (EI) 187.0732 (M^+, $C_9H_9N_3O$ requires 187.0740).

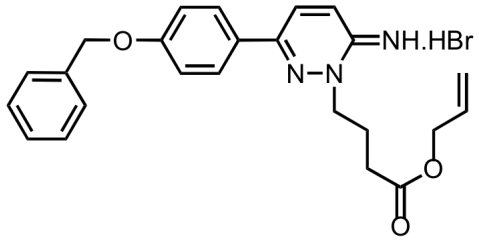
6-(4-Benzyloxyphenyl)-pyridazin-3-yl amine (156)



157 (250 mg, 1.34 mmol) and sodium hydride (54 mg, 1.34 mmol) in DMF (2 mL) was cooled to 0 °C. Benzyl bromide (239 mg, 1.40 mmol) was added dropwise and the reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was extracted with Et_2O (100 mL) and washed with water (100 mL). The aqueous layer was further extracted with Et_2O (4 × 50 mL). The organic extracts were combined and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue subjected to column chromatography (EtOAc) to give the *pyridazine* **156** (151 mg, 0.544 mmol, 41%) as

a white solid. m.p. 190-192 °C; R_f = 0.30 (EtOAc/CH_3OH 20:1); IR υ_{max} (film)/cm^{-1} 3431, 3282, 3113, 1647, 1609; ^1H NMR (CD_3OD, 500 MHz) δ 5.13 (2 H, s, OC*H*$_2$), 6.98 (1 H, d, *J* 9.3, NH_2CCH), 7.08 (2 H, d, *J* 6.8, OCC*H*), 7.30 (1 H, t, *J* 7.2, $OCH_2CCHCHCH$), 7.36 (2 H, dd, *J* 7.3 and 7.2, $OCH_2CCHCHCH$), 7.44 (2 H, d, *J* 7.3, $OCH_2CCHCHCH$), 7.72 (1 H, d, *J* 9.3, NH_2CCHCH), 7.81 (2 H, d, *J* 6.8, OCCHC*H*); ^{13}C NMR (CD_3OD, 125 MHz) δ 71.0 (CH_2), 116.3 (CH), 117.7 (CH), 127.9 (C), 128.2 (CH), 128.5 (CH), 128.6 (CH), 129.0 (CH), 129.6 (CH), 130.7 (C), 136.2 (C), 138.5 (C), 161.0 (C); *m*/*z* (ES+) 278.1293 (M+H, $C_{17}H_{15}N_3O$ requires 278.1281), 243 (45), 187 (50).

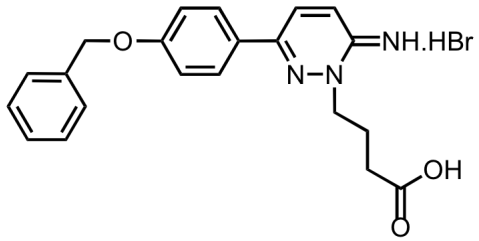
1-(3-Allyloxycarbonylpropyl)-6-amino-3-(4-benzyloxyphenyl)-pyridazinium bromide (155)



To a solution of **156** (50 mg, 0.180 mmol) in DMF (0.2 mL) was added allyl-4-bromobutyrate (56 mg, 0.271 mmol). The solution was heated to 80 °C for 16 h. The hot solution was poured into EtOAc (5 mL) to yield a solid, which was then isolated by filtration. The product was dried under high vacuum to give the *ester* **155** (51 mg, 0.105 mmol, 59%) as a grey solid. m.p. 174-177 °C; R_f = 0.60 ($CHCl_3$/CH_3OH 8:2); IR υ_{max} (film)/cm^{-1} 2930, 1724, 1681, 1649, 1612, 1554, 1541, 1509; ^1H NMR (CD_3OD, 500 MHz) δ 2.14-2.19 (2 H, m, NCH_2CH_2); 2.60 (2 H, t, *J* 6.8, $NCH_2CH_2CH_2$), 4.44-4.46 (4 H, m, NCH_2 and $CO_2CH_2CHCH_2$), 5.13 (1 H, dd, *J* 8.0 and 1.5, CO_2CH_2CHCHH), 5.15 (2 H, s, OC*H*$_2$), 5.22 (1 H, dd, *J* 15.7 and 1.5, CO_2CH_2CHCHH), 5.81 (1 H, m, CO_2CH_2CH), 7.13 (2 H, d, *J* 5.6, OCC*H*), 7.32 (1 H, t, *J* 7.1, $OCH_2CCHCHCH$), 7.37 (2 H, dd, *J* 7.4 and 7.1, $OCH_2CCHCHCH$), 7.44 (2 H, d, *J* 7.4, $OCH_2CCHCHCH$), 7.59 (1 H, d, *J* 9.6, NHCC*H*), 7.93 (2 H, d, *J* 5.6, OCCHC*H*), 8.27 (1 H, d, *J* 9.6, NHCCHC*H*); ^{13}C NMR (CD_3OD, 125 MHz) δ 22.5 (CH_2), 31.3 (CH_2), 56.9 (CH_2), 66.5 (CH_2), 71.1 (CH_2), 116.7 (CH), 118.6 (CH_2),

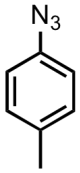
126.6 (CH), 126.7 (C), 128.6 (CH), 129.1 (CH), 129.3 (CH), 129.6 (CH), 132.6 (CH), 133.4 (CH), 138.2 (C), 151.9 (C), 153.9 (C), 162.7 (C), 174.0 (C=O); *m*/*z* (ES+) 403.1864 (M+H, $C_{24}H_{25}N_3O_3$ requires 403.1896).

1-(3-Carboxypropyl)-6-amino-3-(4-benzyloxyphenyl)-pyridazinium bromide (154)



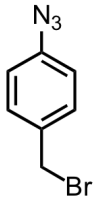
To a solution of $NaHCO_3$ (20 mg, 0.240 mmol), and dimedone (18 mg, 0.128 mmol) in water (0.70 mL) was successively added THF (4.5 mL), triethyl phosphite (11 mg, 0.066 mmol) and palladium(II) acetate (2.0 mg, 0.0089 mmol) under argon. After stirring for 3 min, **155** (51 mg, 0.105 mmol) was added and the mixture was stirred at 35 °C for 17 h. The mixture was diluted with water (5 mL) and washed thoroughly with CH_2Cl_2 (3 × 15 mL). The aqueous phase was separated and evaporated *in vacuo*. The residue was purified by column chromatography ($CHCl_3$/CH_3OH 9:1) to give the *acid* **154** (26 mg, 0.059 mmol, 56%) as a white solid. m.p. 180-183 °C; R_f = 0.30 ($CHCl_3$/CH_3OH 8:2); IR υ_{max} (film)/cm^{-1} 2924, 2214, 1712, 1671, 1647, 1609, 1566, 1535, 1512; ^{1}H NMR ($CDCl_3$/CD_3OD 1:1, 600 MHz) δ 2.14-2.19 (2 H, m, NCH_2CH_2), 2.45-2.50 (2 H, m, $NCH_2CH_2CH_2$), 4.41-4.44 (2 H, m, NCH_2), 5.15 (2 H, s, OCH_2), 7.11 (2 H, d, *J* 8.8, OCC*H*), 7.33 (1 H, t, *J* 7.3, $OCH_2CCHCHCH$), 7.39 (2 H, dd, *J* 7.6 and 7.3, $OCH_2CCHCHCH$), 7.44 (2 H, d, *J* 7.6, $OCH_2CCHCHCH$), 7.66 (1 H, d, *J* 12.7, NHCC*H*), 7.88 (2 H, d, *J* 8.8, OCCHC*H*), 8.14 (1 H, d, *J* 12.7, NHCCHC*H*); ^{13}C NMR ($CDCl_3$/CD_3OD 1:1), 150 MHz) δ 22.2 (CH_2), 30.9 (CH_2), 56.2 (CH_2), 70.1 (CH_2), 115.5 (CH), 125.0 (C), 125.4 (CH), 127.4 (CH), 128.0 (CH), 128.1 (CH), 128.5 (CH), 131.1 (CH), 136.3 (C), 150.5 (C), 152.2 (C), 161.3 (C), 177.3 (C=O); *m*/*z* (ES+) 364.1661 (M+H, $C_{21}H_{22}N_3O_3$ requires 364.1671), 278 (25), 273 (20).

4-Azidotoluene[113] (50)



To a solution of 4-toluidine (3.75 g, 35.0 mmol) in HCl (50 mL, 2 M) at −5 °C was added sodium nitrite (2.90 g, 42.0 mmol) in water (10 mL). The temperature was maintained at −5 °C for 5 min. Urea (250 mg, 4.16 mmol) was then added to the reaction mixture. The resulting solution was added over 5 min to a solution of sodium azide (4.55 g, 70.0 mmol) and sodium acetate (8.4 g, 105 mmol) in water (50 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h, then extracted with Et_2O (2 × 100 mL), washed with water (2 × 100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was dried under high vacuum to give the *azide* **50** (4.00 g, 30.0 mmol, 86%) as a yellow oil. R_f = 0.25 (petrol); IR υ_{max} (film)/cm^{-1} 2933, 2111; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.33 (3 H, s, C*H*$_3$), 6.93 (2 H, d, *J* 9.0, N$_3$CCHC*H*), 7.15 (2 H, d, *J* 9.0, N$_3$CC*H*); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.9 (CH_3), 118.9 (CH), 130.4 (CH), 134.7 (C), 137.2 (C); *m*/*z* (ES+) 133.0641 (M^+, $C_7H_7N_3$ requires 133.0640), 105 (50).

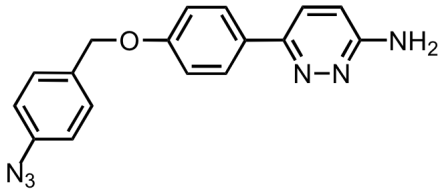
1-Azido-4-bromomethyl-benzene[113] (166)



To a solution of **50** (2.27 g, 17.0 mmol) in anhydrous benzene (20 mL) was added NBS (3.78 g, 21.3 mmol) and AIBN (1.40 g, 8.52 mmol). The reaction mixture was heated to 80 °C for 4 h. After cooling to rt, the solvent was concentrated *in vacuo*. The residue was purified by column chromatography (petrol) to give the *azide* **166** (1.60 g, 7.58 mmol, 44%) as a colourless oil. R_f = 0.30 (petrol); IR υ_{max} (film)/cm^{-1} 2954, 2125; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.48 (2 H, s, C*H*$_2$Br), 7.01 (2 H, d, *J* 8.5,

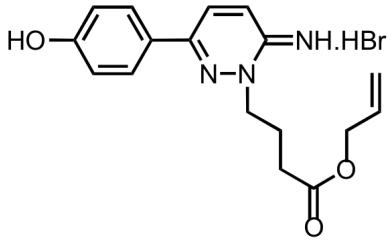
N_3CCHCH), 7.37 (2 H, d, *J* 8.5, N_3CCH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 33.0 (CH_2), 119.4 (CH), 130.9 (CH), 134.6 (C), 140.3 (C); *m/z* (EI) 210.9745 (M^+, $C_7H_6N_3{}^{79}Br$ requires 210.9740), 212 (100), 132 (90).

6-[4-(4-Azidobenzyloxy)-phenyl]-pyridazin-3-yl amine (167)



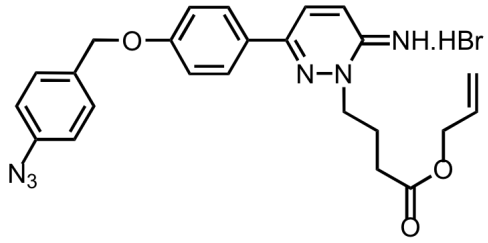
157 (300 mg, 1.60 mmol) and potassium *tert*-butoxide (186 mg, 1.66 mmol) in DMF (2 mL) were cooled to 0 °C. **166** (348 mg, 1.65 mmol) was dissolved in DMF (1 mL) and added dropwise. The reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was extracted with EtOAc (100 mL) and washed with water (100 mL). The aqueous layer was further extracted with EtOAc (4 × 50 mL). The organic extracts were combined and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue subjected to column chromatography ($CHCl_3/CH_3OH$ 19:1) to give the *pyridazine* **167** (193 mg, 0.606 mmol, 38%) as a white solid. m.p. 204-206 °C; R_f = 0.20 ($CHCl_3/CH_3OH$ 19:1); IR υ_{max} (film)/cm^{-1} 3431, 3282, 3113, 2954, 2125, 1647; ^1H NMR (DMSO, 500 MHz) δ 5.13 (2 H, s, OCH_2), 6.37 (2 H, s, NH_2), 6.82 (1 H, d, *J* 9.6, NH_2CCH), 7.07 (2 H, d, *J* 8.6, OCC*H*), 7.15 (2 H, d, *J* 8.7, N_3CCHCH), 7.49 (2 H, d, *J* 8.7, N_3CCH), 7.74 (1 H, d, *J* 9.6, NH_2CCHCH), 7.88 (2 H, d, *J* 8.6, OCCHC*H*); ^{13}C NMR (DMSO, 125 MHz) δ 66.7 (CH_2), 114.4 (CH), 115.0 (CH), 119.2 (CH), 125.0 (CH), 126.6 (CH), 129.5 (CH), 129.8 (C), 133.9 (C), 138.9 (C), 149.6 (C), 158.5 (C), 159.4 (C); *m/z* (EI) 319.1312 (M+H, $C_{17}H_{14}N_6O$ requires 319.1307), 242 (80).

1-(3-Allyloxycarbonyl-propyl)-6-amino-3-(4-hydroxy-phenyl)-pyridazinium bromide (168)



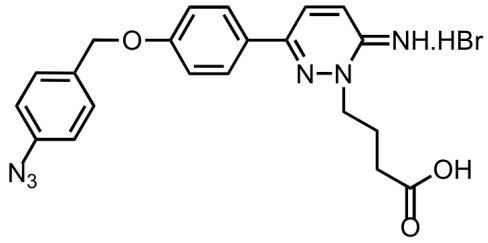
To a solution of **167** (194 mg, 0.609 mmol) in DMF (1 mL) was added allyl-4-bromobutyrate (194 mg, 0.937 mmol). The solution was heated to 80 °C for 18 h. The hot solution was then poured into EtOAc (20 mL) to yield a solid, which was then isolated by filtration. The residue was purified by column chromatography ($CHCl_3/CH_3OH$ 9:1) to give the *ester* **168** (64 mg, 0.162 mmol, 27%) as a white solid. m.p. >250 °C; R_f = 0.10 (EtOAc/CH_3OH 9:1); IR υ_{max} (film)/cm^{-1} 3113, 1731, 1614, 1553; ^{1}H NMR (DMSO, 600 MHz) δ 2.10 (2 H, m, NCH_2CH_2), 2.55 (2 H, m, $NCH_2CH_2CH_2$), 4.37 (2 H, m, NCH_2), 4.48 (2 H, m, $CO_2CH_2CHCH_2$), 5.17 (1 H, dd, *J* 11.0 and 1.2, CO_2CH_2CHCHH), 5.27 (1 H, dd, *J* 17.5 and 1.2, CO_2CH_2CHCHH), 5.82 (1 H, m, CO_2CH_2CH), 6.92 (2 H, d, *J* 8.6, OCCH), 7.61 (1 H, d, *J* 9.3, NHCCHCH), 7.82 (2 H, d, *J* 8.6, OCCHCH), 8.35 (1 H, d, *J* 9.3, NHCCH); ^{13}C NMR (DMSO, 150 MHz) δ 21.6 (CH_2), 30.0 (CH_2), 55.0 (CH_2), 64.6 (CH_2), 116.0 (CH), 118.0 (CH_2), 125.7 (C), 128.1 (CH), 131.0 (CH), 132.5 (CH), 149.5 (C), 151.9 (C), 160.1 (C), 171.9 (C=O); *m/z* (ES+) 314.1507 (M+H, $C_{17}H_{20}N_3O_3$ requires 314.1505), 188 (20).

1-(3-Allyloxycarbonyl-propyl)-6-amino-3-[4-(4-azido-benzyloxy)-phenyl]-pyridazinium bromide (169)



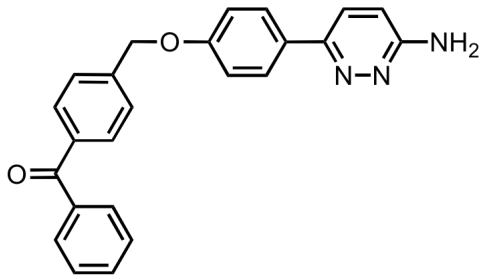
To a solution of **167** (194 mg, 0.609 mmol) in DMF (1 mL) was added allyl-4-bromobutyrate (194 mg, 0.937 mmol). The solution was heated to 80 °C for 6 h. The hot solution was then poured into EtOAc (20 mL) to yield a solid, which was then isolated by filtration. The product was dried under high vacuum to give the *ester* **169** (120 mg, 0.228 mmol, 37%) as a white solid. m.p. degraded >200 °C; R_f = 0.20 (EtOAc/CH_3OH 9:1); IR υ_{max} (film)/cm^{-1} 3184, 2112, 1727, 1646, 1607, 1543; ^1H NMR (CD_3OD, 500 MHz) δ 2.27 (2 H, m, $NCH_2CH_2CH_2$), 2.61 (2 H, t, *J* 8.5, $NCH_2CH_2CH_2$), 4.46-4.48 (4 H, m, NCH_2 and $CO_2CH_2CHCH_2$), 5.11 (2 H, s, OCH_2), 5.17 (1 H, dd, *J* 10.5 and 1.1, CO_2CH_2CHCHH), 5.25 (1 H, dd, *J* 17.3 and 1.1, CO_2CH_2CHCHH), 5.82 (1 H, m, CO_2CH_2CH), 7.03 (2 H, d, *J* 8.7, N_3CCHCH), 7.07 (2 H, d, *J* 8.6, OCCH), 7.45 (2 H, d, *J* 8.7, N_3CCH), 7.74 (1 H, d, *J* 9.6, NHCCHCH), 7.88 (2 H, d, *J* 8.6, OCCHCH), 8.20 (1 H, d, *J* 9.6, NHCCH); ^{13}C NMR (CD_3OD, 125 MHz) δ 22.4 (CH_2), 31.3 (CH_2), 56.8 (CH_2), 66.5 (CH_2), 70.6 (CH_2), 117.0 (CH), 118.6 (CH_2), 120.1 (CH), 125.0 (C), 126.5 (CH), 129.4 (CH), 130.5 (C), 130.5 (CH), 131.4 (C), 132.5 (CH), 133.4 (CH), 152.2 (C), 153.8 (C), 161.9 (C), 174.0 (C=O); *m*/*z* (FAB+) 445.1994 (M+H, $C_{24}H_{25}N_6O_3$ requires 445.1988), 307 (40).

6-Amino-3-[4-(4-azido-benzyloxy)-phenyl]-1-(3-carboxy-propyl)-pyridazinium bromide (165)



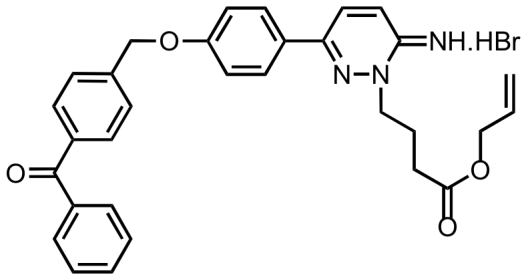
To a solution of **169** (120 mg, 0.228 mmol) in THF (4 mL) and CH_3OH (1 mL) was added morpholine (198 mg, 2.28 mmol) and tetrakis(triphenylphosphine)palladium(0) (26 mg, 0.023 mmol) under argon. The reaction mixture was stirred at rt for 30 min, then concentrated *in vacuo*. The residue was purified by column chromatography ($CHCl_3/CH_3OH$/AcOH 17:2.9:0.1) to give the *acid* **165** (104 mg, 0.214 mmol, 94%) as a white solid. m.p. degraded >200 °C; R_f = 0.20 ($CHCl_3/CH_3OH$ 17:3); IR υ_{max} (film)/cm^{-1} 2930, 2111, 1710, 1656, 1538, 1509; ^1H NMR ($CDCl_3/CD_3OD$ 1:1, 600 MHz) δ 2.05-2.11 (2 H, m, NCH_2CH_2), 2.33-2.37 (2 H, m, $NCH_2CH_2CH_2$), 4.41-4.47 (2 H, m, NCH_2), 5.14 (2 H, s, OCH_2), 7.06 (2 H, d, *J* 8.4, N_3CCHCH), 7.10 (2 H, d, *J* 8.6, $OCCH$), 7.45 (2 H, d, *J* 8.4, N_3CCH), 7.71 (1 H, d, *J* 9.5, $NHCCHCH$), 7.87 (2 H, d, *J* 8.6, $OCCHCH$), 8.12 (1 H, d, *J* 9.5, $NHCCH$); ^{13}C NMR ($CDCl_3/CD_3OD$ 1:1, 150 MHz) δ 22.7 (CH_2), 31.9 (CH_2), 56.7 (CH_2), 69.4 (CH_2), 115.5 (CH), 119.1 (CH), 125.3 (C), 125.4 (CH), 128.0 (CH), 128.7 (CH), 129.1 (CH), 131.0 (CH), 133.1 (C), 139.9 (C), 150.3 (C), 152.2 (C), 161.0 (C), 177.9 (C=O); *m/z* (ES-) 403.1529 (M^+, $C_{21}H_{20}N_3O_3$ requires 403.1519); UV ($CHCl_3/CH_3OH$ 1:1) ε_{283} = 11900 and ε_{313} = 2165 cm^{-1} M^{-1} d^3.

{4-[4-(6-Amino-pyridazin-3-yl)-phenoxymethyl]-phenyl}-phenyl-methanone (171)



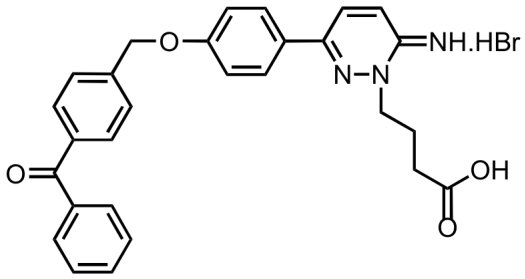
A solution of **157** (300 mg, 1.60 mmol) and potassium *tert*-butoxide (181 mg, 1.60 mmol) in DMF (2 mL) were cooled to 0 °C. 4-Bromomethyl benzophenone (486 mg, 1.77 mmol) was dissolved in DMF (1 mL) and added dropwise. The reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was extracted with EtOAc (100 mL) and washed with water (100 mL). The aqueous layer was further extracted with EtOAc (4 × 50 mL). The organic extracts were combined and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue subjected to column chromatography ($CHCl_3$/CH_3OH 19:1) to give the *pyridazine* **171** (190 mg, 0.498 mmol, 31%) as a white solid. m.p. 167-169 °C; R_f = 0.30 ($CHCl_3$/CH_3OH 9:1); IR υ_{max} (film)/cm^{-1} 2923, 2491, 1727, 1644, 1596; ^{1}H NMR (DMSO, 500 MHz) δ 5.29 (2 H, s, OC*H*$_2$), 6.38 (2 H, s, NH_2), 6.82 (1 H, d, *J* 9.6, NH_2CC*H*), 7.12 (2 H, d, *J* 8.6, OCC*H*), 7.56 (2 H, dd, *J* 7.6 and 7.4, O=CCCHC*H*CH), 7.68 (3 H, m, OCH_2CC*H* and O=CCCHCHC*H*), 7.76 (5 H, m, NH_2CCHC*H*, OCH_2CCHC*H* and O=CCC*H*CHCH), 7.90 (2 H, d, *J* 8.6, OCCHC*H*); ^{13}C NMR (DMSO, 125 MHz) δ 69.0 (CH_2), 114.8 (CH), 115.5 (CH), 125.4 (CH), 127.1 (CH), 127.8 (CH), 129.1 (CH), 130.0 (CH), 130.3 (CH), 130.6 (C), 133.2 (CH), 136.8 (C), 137.5 (C), 142.5 (C), 144.9 (C), 158.8 (C), 159.9 (C), 195.9 (C=O); *m*/*z* (EI) 381.1485 (M^+, $C_{24}H_{19}N_3O_2$ requires 381.1472), 195 (10).

1-(3-Allyloxycarbonyl-propyl)-6-amino-3-[4-(4-benzoyl-benzyloxy)-phenyl]-pyridazinium bromide (172)



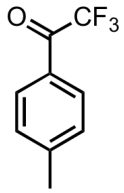
To a solution of **171** (147 mg, 0.385 mmol) in DMF (1 mL) was added allyl-4-bromobutyrate (120 mg, 0.578 mmol). The solution was heated to 80 °C for 18 h. The hot solution was then poured into EtOAc (20 mL) to yield a solid, which was then isolated by filtration. The product was dried under high vacuum to give the *ester* **172** (135 mg, 0.229 mmol, 60%) as a white solid. m.p. 160-162 °C; R_f = 0.20 (EtOAc/CH_3OH 9:1); IR υ_{max} (film)/cm^{-1} 3042, 1732, 1644, 1606, 1541; ^1H NMR (CD_3OD, 600 MHz) δ 2.28 (2 H, quint, $NCH_2CH_2CH_2$), 2.64 (2 H, t, *J* 6.8, $NCH_2CH_2CH_2$), 4.46-4.48 (4 H, m, NCH_2 and $CO_2CH_2CHCH_2$), 5.17 (1 H, dd, *J* 10.4 and 1.1, CO_2CH_2CHCHH), 5.25 (1 H, dd, *J* 17.4 and 1.1, CO_2CH_2CHCHH), 5.32 (2 H, s, OCH_2), 5.84 (1 H, m, CO_2CH_2CH), 7.20 (2 H, d, *J* 8.8, OCCH), 7.55 (2 H, dd, *J* 7.6 and 7.4, O=CCCHCHCH), 7.63 (1 H, d, *J* 9.6, NHCCHCH), 7.66-7.68 (3 H, m, OCH_2CCH and O=CCCHCHCH), 7.78 (4 H, m, OCH_2CCHCH and O=CCCHCHCH), 7.99 (2 H, d, *J* 8.8, OCCHCH), 8.31 (1 H, d, *J* 9.6, NHCCH); ^{13}C NMR (CD_3OD, 150 MHz) δ 21.1 (CH_2), 29.9 (CH_2), 55.5 (CH_2), 65.1 (CH_2), 69.0 (CH_2), 115.3 (CH), 117.3 (CH_2), 125.3 (CH), 125.5 (C), 126.9 (CH), 128.0 (CH), 128.2 (CH), 129.6 (CH), 130.0 (CH), 131.2 (CH), 132.0 (CH), 132.5 (CH), 136.9 (C), 137.4 (C), 141.9 (C), 150.4 (C), 152.5 (C), 161.0 (C), 172.6 (C=O), 196.7 (C=O); *m*/*z* (FAB+) 508.2243 (M+H, $C_{31}H_{30}N_3O_4$ requires 508.2236), 313 (5).

6-Amino-3-[4-(4-benzoyl-benzyloxy)-phenyl]-1-(3-carboxy-propyl)-pyridazinium bromide (170)



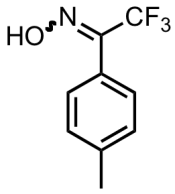
To a solution of **172** (150 mg, 0.254 mmol) in THF (4 mL) and CH_3OH (1 mL) was added 1,4-dimethyl barbituric acid (398 mg, 2.54 mmol) and tetrakis(triphenylphosphine)palladium(0) (29 mg, 0.025 mmol) under argon. The reaction mixture was stirred at rt for 3 h, then concentrated *in vacuo*. The residue was purified by column chromatography ($CHCl_3/CH_3OH$ 17:3), then triturated with water to give the *acid* **170** (90 mg, 0.164 mmol, 65%) as a white solid. m.p. 152-154 °C; R_f = 0.25 ($CHCl_3/CH_3OH$ 8:2); IR υ_{max} (film)/cm^{-1} 3418, 1734, 1720, 1645, 1578; ^1H NMR ($CDCl_3/CD_3OD$ 1:1, 600 MHz) δ 2.10-2.17 (2 H, m, NCH_2CH_2), 2.39-2.42 (2 H, m, $NCH_2CH_2CH_2$), 4.41-4.45 (2 H, m, NCH_2), 5.28 (2 H, s, OCH_2), 7.14 (2 H, d, *J* 8.2, OCCH), 7.49 (2 H, dd, *J* 7.6 and 7.4, O=CCCHCHCH), 7.59-7.62 (4 H, m, NHCCHCH, OCH_2CCH and O=CCCHCHCH), 7.79-7.83 (4 H, m, OCH_2CCHCH and O=CCCHCHCH), 7.90 (2 H, d, *J* 8.2, OCCHCH), 8.13 (1 H, d, *J* 9.6, NHCCH); ^{13}C NMR ($CDCl_3/CD_3OD$ 1:1, 150 MHz) δ 23.8 (CH_2), 32.8 (CH_2), 57.9 (CH_2), 70.7 (CH_2), 116.9 (CH), 126.7 (CH) 126.8 (C), 128.4 (CH), 129.5 (CH), 129.7 (CH), 131.3 (CH), 131.7 (CH), 132.5 (CH), 134.2 (CH), 138.4 (C), 138.6 (C), 143.0 (C), 151.7 (C), 153.6 (C), 162.3 (C), 179.5 (C=O), 198.6 (C=O); *m*/*z* (FAB+) 468.1910 (M+H, $C_{28}H_{26}N_3O_4$ requires 468.1923), 391 (60); UV ($CHCl_3/CH_3OH$ 1:1) ε_{303} = 10300 cm^{-1} M^{-1} d^3.

2,2,2-Trifluoro-1-(4-methylphenyl)-1-ethanone[150] (175)



4-Bromotoluene (10.0 g, 58.5 mmol) was dissolved in Et_2O (280 mL) and cooled to −40 °C. *n*-BuLi (40.5 mL, 60.7 mmol, 1.1 M in hexanes) was added dropwise, and the solution was warmed to 0 °C over 2 h. The solution was then cooled to −78 °C and a solution of ethyl trifluoroacetate (9.55 g, 67.2 mmol) in Et_2O (60 mL) was added. The reaction mixture was stirred at −78 °C for 3 h, before being warmed to rt. The solution was hydrolysed with saturated ammonium chloride solution (50 mL), then washed with water (3 × 50 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue purified by column chromatography (petrol) to give the *ketone* **175** (4.24 g, 22.5 mmol, 38%) as a colourless oil. R_f = 0.50 (petrol); IR υ_{max} (film)/cm^{-1} 3433, 1719; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.15 (3 H, s, C*H*$_3$), 7.30 (2 H, d, *J* 8.2, CH_3CCH), 7.97 (2 H, d, *J* 8.2, CH_3CCHCH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 22.8 (CH_3), 118.1 (q, $^1J_{CF}$ 288.5, CF_3), 127.3 (C), 130.0 (CH), 130.7 (CH), 147.4 (C), 174.9 (q, $^2J_{CF}$ 37.6, C=O); *m*/*z* (EI) 188.1512 (M^+, $C_9H_7F_3O$ requires 188.1465).

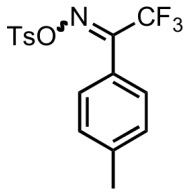
2,2,2-Trifluoro-1-(4-methylphenyl)-1-ethanone oxime[123] (176)



To a solution of **175** (13.5 g, 71.7 mmol) dissolved in pyridine (155 mL), was added hydroxylamine hydrochloride (14.9 g, 215 mmol). The reaction mixture was then heated at 70 °C for 3 h. After cooling to rt, the solvent was removed *in vacuo*. The remaining residue was dissolved in Et_2O (300 mL) and washed with aqueous HCl (300 mL, 0.01 M), water (3 × 50 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give the *oxime* **176** (14.5 g, 71.4 mmol, 99%) as a pale yellow solid used

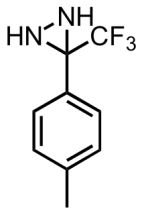
without further purification as a 1:1 mixture of isomers. R_f = 0.60 (petrol/EtOAc 3:1); IR υ_{max} (film)/cm^{-1} 3290, 1888, 1631; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.43 (6 H, s, 2 × C*H*$_3$), 7.25-7.35 (8 H, m, 2 × CH_3CCH and 2 × CH_3CCHCH), 8.30 (1 H, s, OH), 8.42 (1 H, s, OH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 21.8 (CH_3), 118.2 (q, $^1J_{CF}$ 277.5, CF_3), 123.3 (C), 129.0 (CH), 130.0 (CH), 141.7 (C), 148.8 (q, $^2J_{CF}$ 37.6, CNO); *m*/*z* (EI) 203.0551 (M^+, $C_9H_8F_3NO$ requires 203.0558).

2,2,2-Trifluoro-1-(4-methylphenyl)-1-ethanone *O*-(*p*-toluenesulfonyl) oxime[123] (177)



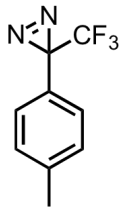
To a solution of **176** (14.5 g, 71.4 mmol) dissolved in pyridine (250 mL) was added *p*-toluenesulfonyl chloride (20.5 g, 107 mmol). The reaction mixture was refluxed at 110 °C for 18 h. After cooling to rt, the solvent was removed *in vacuo*, and the residue was purified by column chromatography (CH_2Cl_2) to give the *tosylate* **177** (19.2 g, 53.5 mmol, 75%) as a white solid used without further purification as a 1:1 mixture of isomers. R_f = 0.80 ($CHCl_3$); IR υ_{max} (film)/cm^{-1} 2994, 1917, 1637, 1588, 1489; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.40 (3 H, s, C*H*$_3$), 2.49 (3 H, s, C*H*$_3$) 7.29-7.34 (4 H, m, Ar-*H*), 7.38 (2 H, d, *J* 7.1, SO_2CCHCH), 7.88 (2 H, d, *J* 7.1, SO_2CCH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 21.8 (CH_3), 22.0 (CH_3), 119.3 (q, $^1J_{CF}$ 264, CF_3), 123.6 (C), 126.8 (C), 129.4 (CH), 130.2 (CH), 130.3 (CH), 131.0 (C), 132.2 (CH), 147.5 (C), 155.0 (q, $^2J_{CF}$ 36.5, CNO); *m*/*z* (EI) 357.3481 (M^+, $C_{16}H_{14}F_3NO_3S$ requires 357.3475).

3-*p*-Tolyl-3-trifluoromethyl-diaziridine[123] (178)



177 (19.2g, 53.6 mmol) was added to a sealed vessel containing Et_2O (130 mL) at −78 °C. Ammonia (25 mL) was condensed in dropwise and the solution was stirred at −78 °C for 8 h. The vessel was then unsealed and allowed to warm to rt. The solution was then extracted with Et_2O (300 mL) and washed with water (300 mL). The organic layer was dried ($MgSO_4$), and the solvent removed *in vacuo*. The product was purified by flash column chromatography ($CHCl_3$) to give the *diaziridine* **178** (9.46 g, 47.3 mmol, 88%) as a white solid. m.p. 59-61 °C; R_f = 0.40 ($CHCl_3$); IR υ_{max} (film)/cm^{-1} 3005, 1650, 1598, 1489; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.18 (1 H, s, NH), 2.40 (3 H, s, C*H*$_3$), 2.80 (1 H, s, NH), 7.36 (2 H, d, *J* 8.3, CH_3CCH), 7.81 (2 H, d, *J* 8.3, CH_3CCHCH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 21.8 (CH_3), 57.7 (q, $^2J_{CF}$ 30.2, $C(NH)_2$), 123.0 (q, $^1J_{CF}$ 276.6, CF_3), 129.9 (CH), 131.4 (C), 138.4 (CH), 140.0 (C); *m*/*z* (EI) 202.1753 (M^+, $C_9H_9F_3N_2$ requires 202.1764).

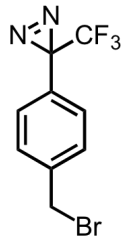
3-*p*-Tolyl-3-trifluoromethyl-3*H*-diazirine[123] (179)



To a solution of **178** (1.00 g, 4.95 mmol) dissolved in CH_2Cl_2 (20 mL) was added triethylamine (2.06 mL, 14.8 mmol) at 0 °C. Iodine (1.38 g, 5.45 mmol) was added gradually, until the solution became brown in colour. The reaction mixture was washed with aqueous NaOH (20 mL, 1 M), water (20 mL), brine (20 mL) and dried ($MgSO_4$). The solvent was carefully removed *in vacuo* at 20 °C owing to the volatility of the product. The residue was purified by column chromatography

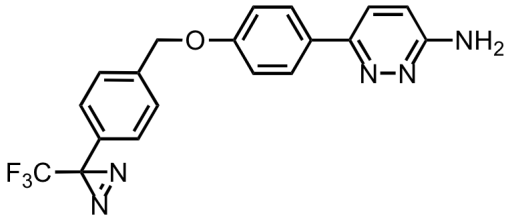
(petrol/CH_2Cl_2 20:1) to give the *diazirine* **179** (601 mg, 3.00 mmol, 61%) as a colourless oil. R_f = 0.90 ($CHCl_3$); IR υ_{max} (film)/cm^{-1} 3196, 1650; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.39 (3 H, s, C*H*$_3$), 7.10 (2 H, d, *J* 8.0, CH_3CCH), 7.21 (2 H, d, *J* 8.0, CH_3CCHCH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 21.3 (CH_3), 28.4 (q, $^2J_{CF}$ 40.5, $C(N)_2$), 122.7 (q, $^1J_{CF}$ 273.0, CF_3), 126.2 (C), 126.5 (CH), 129.6 (CH), 140.0 (C); *m*/*z* (EI) 200.1110 (M^+, $C_9H_7F_3N_2$ requires 200.1065).

3-(4-Bromomethylphenyl)-3-trifluoromethyl-3*H*-diazirine[123] (114)



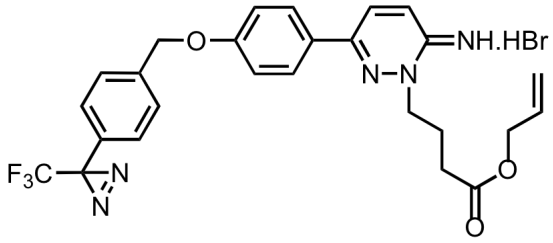
To a solution of **179** (1.80 g, 8.97 mmol) in CCl_4 (40 mL) was added NBS (2.39 g, 13.5 mmol) and AIBN (20 mg, 0.128 mmol). The reaction mixture was refluxed at 70 °C for 4 h. After cooling to rt, the precipitate was filtered and the solvent was removed *in vacuo* at 20 °C owing to the volatility of the product. The residue was purified by column chromatography (petrol/CH_2Cl_2 20:1) to give the *bromide* **114** (1.63 g, 5.83 mmol, 65%) as a colourless oil. R_f = 0.45 (petrol/CH_2Cl_2 19:1); IR υ_{max} (film)/cm^{-1} 3277, 1644; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.49 (2 H, s, C*H*$_2$Br), 7.19 (2 H, d, *J* 8.0, Ar-*H*), 7.46 (2 H, d, *J* 8.0, Ar-*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 28.4 (q, $^2J_{CF}$ 40.5, $C(N)_2$), 32.1 (CH_2), 122.1 (q, $^1J_{CF}$ 273.0, CF_3), 127.0 (CH), 129.3 (C), 129.6 (CH), 139.5 (C); *m*/*z* (EI) 277.9732 (M^+, $C_9H_6{}^{79}BrF_3N_2$ requires 277.9666), 279 (100).

6-{4-[4-(3-Trifluoromethyl-3*H*-diazirin-3-yl)-benzyloxy]-phenyl}-pyridazin-3-ylamine (180)



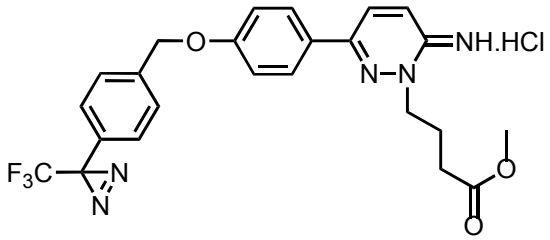
A solution of **157** (160 mg, 0.854 mmol), 18-crown-6 (226 mg, 0.854 mmol) and potassium *tert*-butoxide (96 mg, 0.854 mmol) in DMF (3 mL) were cooled to 0 °C. **114** (140 mg, 0.501 mmol) was dissolved in DMF (1 mL) and added dropwise, then stirred at 0 °C for 3 h. The reaction mixture was extracted with EtOAc (100 mL) and washed with water (100 mL). The aqueous layer was further extracted with EtOAc (4 × 50 mL). The organic extracts were combined and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue subjected to column chromatography ($CHCl_3$/CH_3OH 19:1) to give the *pyridazine* **180** (120 mg, 0.311 mmol, 62%) as a white solid. m.p. degraded >150 °C; R_f = 0.25 ($CHCl_3$/CH_3OH 9:1); IR υ_{max} (film)/cm^{-1} 2930, 1654, 1562; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.18 (2 H, s, OC*H*$_2$), 6.99 (1 H, d, *J* 9.3, NH_2CCH), 7.07 (2 H, d, *J* 7.7, OCC*H*), 7.25 (2 H, d, *J* 8.5, OCH_2CCHCH), 7.55 (2 H, d, *J* 8.5, OCH_2CCH), 7.70 (1 H, d, *J* 9.3, NH_2CCHCH), 7.79 (2 H, d, *J* 7.7, OCCHC*H*); ^{13}C NMR (CD_3OD, 150 MHz) δ 29.6 (q, $^2J_{CF}$ 39.2 $C(N)_2$), 70.2 (CH_2), 116.3 (CH), 117.8 (CH), 123.7 (q, $^1J_{CF}$ 274.7, CF_3), 127.6 (CH), 127.8 (CH), 128.7 (CH), 129.0 (CH), 129.7 (C), 131.0 (C), 133.1 (C), 140.6 (C), 152.8 (C), 160.6 (C); *m*/*z* (FAB+) 386.1222 (M+H, $C_{19}H_{14}N_5OF_3$ requires 386.1229), 279 (90).

1-(3-Allyloxycarbonyl-propyl)-6-amino-3-{4-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)-benzyloxy]-phenyl}-pyridazinium bromide (181)



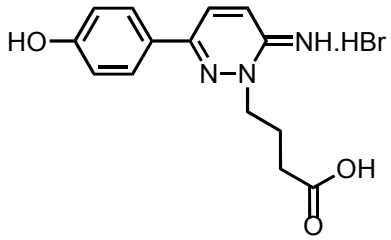
To a solution of **180** (120 mg, 0.311 mmol) in DMF (1 mL) was added allyl-4-bromobutyrate (96 mg, 0.467 mmol). The solution was heated to 80 °C for 5 h. The hot solution was then poured into EtOAc (20 mL) to yield a solid, which was then isolated by filtration. The product was dried under high vacuum to give the *ester* **181** (80 mg, 0.135 mmol, 44%) as a white solid. m.p. degraded >200 °C; R_f = 0.20 (EtOAc/CH_3OH 8:2); IR υ_{max} (film)/cm^{-1} 3033, 1732, 1647, 1541; ^1H NMR (CD_3OD, 600 MHz) δ 2.27 (2 H, quint, $NCH_2CH_2CH_2CH_2$), 2.62 (2 H, t, *J* 6.7, $NCH_2CH_2CH_2CH_2$), 4.43-4.47 (4 H, m, NCH_2 and CO_2CH_2CH), 5.15 (1 H, dd, *J* 9.9 and 1.6, CO_2CH_2CHCHH), 5.22-5.23 (3 H, m, CO_2CH_2CHCH and OCH_2), 5.82 (1 H, m, CO_2CH_2CH), 7.15 (2 H, d, *J* 8.9, OCCH), 7.28 (2 H, d, *J* 8.4, OCH_2CCHCH), 7.58 (2 H, d, *J* 8.4, OCH_2CCH), 7.61 (1 H, d, *J* 9.5, NHCCHCH), 7.94 (2 H, d, *J* 8.9, OCCHCH), 8.29 (1 H, d, *J* 9.5, NHCCH); ^{13}C NMR (CD_3OD, 150 MHz) δ 22.5 (CH_2), 29.4 (q, $^2J_{CF}$ 40.1, $C(N)_2$), 31.3 (CH_2), 56.9 (CH_2), 66.5 (CH_2), 70.2 (CH_2), 116.7 (CH), 118.6 (CH_2), 123.6 (q, $^1J_{CF}$ 273.9, CF_3), 126.7 (CH), 126.9 (C), 127.9 (CH), 128.6 (CH), 129.1 (CH), 129.5 (C), 132.6 (CH), 133.4 (CH), 140.6 (C), 151.8 (C), 153.9 (C), 162.4 (C), 174.0 (C=O); *m*/*z* (FAB+) 512.1900 (M+H, $C_{26}H_{25}N_5O_3F_3$ requires 512.1900), 484 (100), 458 (10).

1-(3-Methoxycarbonyl-propyl)-6-amino-3-{4-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)-benzyloxy]-phenyl}-pyridazinium chloride (182)



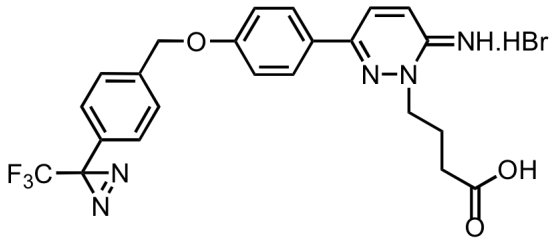
To a solution of **181** (2.0 mg, 0.0034 mmol) in THF (0.4 mL) and CH_3OH (0.1 mL) was added morpholine (2.9 mg, 0.034 mmol) and tetrakis(triphenylphosphine)palladium(0) (1.0 mg, 0.00087 mmol) under argon. The reaction mixture was stirred at rt for 30 min, then concentrated *in vacuo*. Methanolic HCl (0.5 mL) was added to the residue and the mixture was stirred at rt for 2 h, then concentrated *in vacuo*. The residue was purified by column chromatography ($CHCl_3$/CH_3OH 17:3) to give the *ester* **182** (0.8 mg, 0.0015 mmol, 45%) as a white solid. m.p. degraded >200 °C; R_f = 0.25 (EtOAc/CH_3OH 8:2); IR υ_{max} (film)/cm^{-1} 3010, 1727, 1651, 1559; ^{1}H NMR (CD_3OD, 600 MHz) δ 2.25 (2 H, quint, NCH_2CH_2), 2.59 (2 H, t, *J* 6.7, $NCH_2CH_2CH_2$), 3.57 (3 H, s, CO_2CH_3), 4.42 (2 H, t, *J* 6.7, NCH_2), 5.22 (2 H, s, OCH_2), 7.15 (2 H, d, *J* 8.8, OCC*H*), 7.28 (2 H, d, *J* 8.4, OCH_2CCHCH), 7.58-7.61 (4 H, m, OCH_2CCH and NHCCHC*H*), 7.95 (2 H, d, *J* 8.8, OCCHC*H*), 8.28 (1 H, d, *J* 9.5, NHCC*H*); ^{13}C NMR (CD_3OD, 150 MHz) δ 22.5 (CH_2), 29.5 (q, $^2J_{CF}$ 40.1, $C(N)_2$), 31.1 (CH_2), 52.3 (CH_3), 56.8 (CH_2), 70.2 (CH_2), 116.7 (CH), 123.5 (q, $^1J_{CF}$ 273.9, CF_3), 126.7 (CH), 126.9 (C), 127.8 (CH), 128.6 (CH), 129.1 (CH), 129.4 (CH), 129.5 (C), 132.6 (CH), 140.6 (C), 151.8 (C), 153.9 (C), 162.4 (C), 174.9 (C=O); *m*/*z* (FAB+) 486.1753 (M+H, $C_{24}H_{23}N_5O_3F_3$ requires 486.1759), 458 (10).

2-(3-Carboxypropyl)-6-amino-3-(4-hydroxy-phenyl)-pyridazinium bromide[72] (183)



To a solution of **181** (2.0 mg, 0.0034 mmol) in AcOH (0.4 mL) was added aqueous HBr (0.5 mL, 5 M) at rt. The reaction mixture was stirred at 80 °C for 18 h, then concentrated *in vacuo* to give the *acid* **183** (0.9 mg, 0.0025 mmol, 74%) as a white solid. m.p. degraded >200 °C; R_f = 0.25 ($CHCl_3$/CH_3OH 8:2); IR υ_{max} (film)/cm^{-1} 3032, 1728, 1641, 1529; ^{1}H NMR (DMSO, 600 MHz) δ 2.11-2.14 (2 H, m, NCH_2CH_2), 2.52-2.54 (2 H, m, $NCH_2CH_2CH_2$), 4.34-4.36 (2 H, m, NCH_2), 6.93 (2 H, d, *J* 8.8, OCC*H*), 7.60 (1 H, d, *J* 9.3, NHCCHC*H*), 7.83 (2 H, d, *J* 8.8, OCCHC*H*), 8.29 (1 H, d, *J* 9.3, NHCC*H*); ^{13}C NMR (DMSO, 150 MHz) δ 21.6 (CH_2), 30.0 (CH_2), 55.0 (CH_2), 116.7 (CH), 126.7 (C), 128.0 (CH), 131.0 (CH), 132.5 (CH), 151.5 (C), 153.9 (C), 162.1 (C), 179.5 (C=O); *m*/*z* (ES+) 274.1197 (M+H, $C_{14}H_{16}N_3O_3$ requires 274.1192), 188 (30).

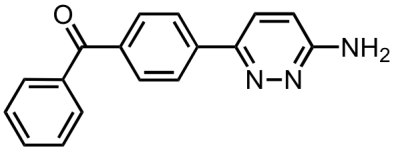
6-Amino-1-(3-carboxy-propyl)-3-{4-[4-(3-trifluoromethyl-3*H*-diazirin-3-yl)-benzyloxy]-phenyl}-pyridazinium bromide (173)



To a solution of **181** (10.0 mg, 0.0168 mmol) in THF (0.2 mL) and CH_3OH (0.05 mL) was added 1,4-dimethyl barbituric acid (26 mg, 0.168 mmol) and tetrakis(triphenylphosphine)palladium(0) (2.0 mg, 0.0017 mmol) under argon. The reaction mixture was stirred at rt for 3 h, then concentrated *in vacuo*. The residue was

purified by column chromatography ($CHCl_3/CH_3OH$ 17:3), then triturated with water to give the *acid* **173** (5.5 mg, 0.010 mmol, 59%) as a white solid. m.p. degraded >200 °C; R_f = 0.25 ($CHCl_3/CH_3OH$ 8:2); IR υ_{max} (film)/cm^{-1} 3420, 1719, 1650, 1577; ^{1}H NMR ($CDCl_3/CD_3OD$ 1:1, 400 MHz) δ 2.10-2.17 (2 H, m, NCH_2CH_2), 2.39-2.45 (2 H, m, $NCH_2CH_2CH_2$), 4.41-4.43 (2 H, m, NCH_2), 5.20 (2 H, s, OCH_2), 7.11 (2 H, d, *J* 8.8, OCCH), 7.24 (2 H, d, *J* 8.0, OCH_2CCHCH), 7.53 (2 H, d, *J* 8.0, OCH_2CH), 7.58 (1 H, d, *J* 9.6, NHCCHCH), 7.89 (2 H, d, *J* 8.8, OCCHCH), 8.16 (1 H, d, *J* 9.6, NHCCCH); ^{13}C NMR ($CDCl_3/CD_3OD$ 1:1, 150 MHz) δ 23.7 (CH_2), 29.4 (q, $^2J_{CF}$ 40.4, $C(N)_2$), 32.7 (CH_2), 57.8 (CH_2), 70.4 (CH_2), 116.8 (CH), 123.5 (q, $^1J_{CF}$ 272.6, CF_3), 126.7 (CH) 126.8 (C), 128.0 (CH), 129.1 (CH), 129.4 (CH), 130.0 (C), 132.5 (CH), 140.0 (C), 151.8 (C), 153.7 (C), 162.3 (C), 179.5 (C=O); *m/z* (ES+) 472.1574 (M+H, $C_{23}H_{21}N_5O_3F_3$ requires 472.1596), 444 (75); UV ($CHCl_3/CH_3OH$ 1:1) ε_{280} = 6100 and ε_{335} = 2100 cm^{-1} M^{-1} d^3.

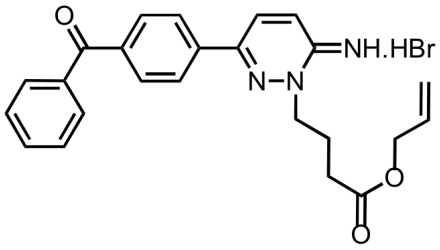
[4-(6-Amino-pyridazin-3-yl)-phenyl]-phenyl-methanone (189)



To a microwave vial containing 3-amino-6-chloropyridazine (101 mg, 0.779 mmol), 4-benzoylphenylboronic acid (262 mg, 1.16 mmol), bis(triphenylphosphine)palladium(II) dichloride (27 mg, 0.040 mmol) and K_2CO_3 (201 mg, 1.45 mmol) were added CH_3CN (2.0 mL) and H_2O (1.3 mL). The resulting solution was degassed for 5 min and subjected to microwave irradiation for 10 min at 120 °C. The mixture was diluted with water (50 mL), extracted with EtOAc (3 × 100 mL), washed with brine (100 mL) and dried ($MgSO_4$). The solvent was concentrated *in vacuo*. The residue was purified by column chromatography ($EtOAc/CH_3OH$ 19:1) to give the *pyridazine* **189** (130 mg, 0.472 mmol, 61%) as a white solid. m.p. 147-149 °C; R_f = 0.15 (EtOAc); IR υ_{max} (film)/cm^{-1} 3052, 2923, 1727, 1644, 1596; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.02 (1 H, d, *J* 9.3, NH_2CCH), 7.49 (2 H, dd, *J* 7.9 and 7.5, O=CCCHCHCH), 7.61 (1 H, t, *J* 7.5, O=CCCHCHCH), 7.77 (3 H, m, NH_2CCHCH and $NH_2CCHCHCCCH$), 7.85 (2 H, d, *J* 7.9, O=CCCHCHCH), 8.00 (2

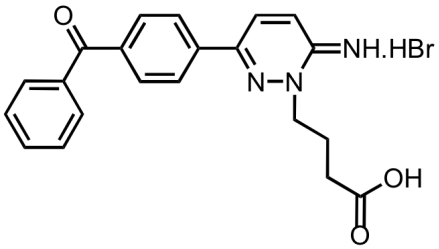
H, s, *J* 7.5, $NH_2CCHCHCCCHCH$); ^{13}C NMR (CD_3OD, 125 MHz) δ 117.4 (CH), 126.8 (CH), 128.3 (CH), 129.3 (CH), 130.9 (CH), 131.6 (CH), 133.7 (CH), 138.3 (C), 138.4 (C), 141.5 (C), 151.4 (C), 160.4 (C), 198.1 (C=O); *m*/*z* (FAB+) 276.1141 (M+H, $C_{17}H_{14}N_3O$ requires 276.1137), 238 (20), 169 (35).

1-(3-Allyloxycarbonyl-propyl)-6-amino-3-(4-benzoyl-phenyl)-pyridazinium bromide (190)



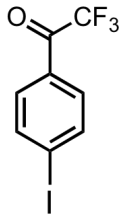
To a solution of **189** (100 mg, 0.363 mmol) in DMF (1 mL) was added allyl-4-bromobutyrate (103 mg, 0.497 mmol). The solution was heated to 80 °C for 18 h. The hot solution was then poured into EtOAc (20 mL) to yield a solid, which was then isolated by filtration. The product was dried under high vacuum to give the *ester* **190** (133 mg, 0.276 mmol, 76%) as a white solid. m.p. 142-144 °C; R_f = 0.20 (EtOAc/CH_3OH 8:2); IR υ_{max} (film)/cm^{-1} 3165, 2999, 1725, 1648, 1533; ^1H NMR ($CDCl_3/CD_3OD$ 1:1, 600 MHz) δ 2.32 (2 H, quint, NCH_2CH_2), 2.67 (2 H, t, *J* 6.7, $NCH_2CH_2CH_2$), 4.51-4.54 (4 H, m, NCH_2 and $CO_2CH_2CHCH_2$), 5.17 (1 H, dd, *J* 10.4 and 1.2, CO_2CH_2CHCHH), 5.27 (1 H, dd, *J* 17.2 and 1.2, CO_2CH_2CHCHH), 5.86 (1 H, m, CO_2CH_2CH), 7.57 (2 H, dd, *J* 7.8 and 7.6, O=CCCHCHCH), 7.71 (1 H, t, *J* 7.6, O=CCCHCHCH), 7.74 (1 H, d, *J* 9.5, NHCCHCH), 7.82 (2 H, d, *J* 8.2, NHCCHCHCCCH), 7.95 (2 H, d, *J* 7.8, O=CCCHCHCH), 8.18 (2 H, d, *J* 8.2, NHCCHCHCCCHCH), 8.45 (1 H, d, *J* 9.5, NHCCH); ^{13}C NMR (CD_3OD, 150 MHz) δ 21.1 (CH_2), 29.9 (CH_2), 55.7 (CH_2), 65.1 (CH_2), 117.2 (CH_2), 125.7 (CH), 126.4 (CH), 128.3 (CH), 129.7 (CH), 130.3 (CH), 131.5 (CH), 132.0 (CH), 132.9 (CH), 136.4 (C), 136.9 (C), 139.3 (C), 149.7 (C), 153.1 (C), 172.7 (C=O), 196.1 (C=O); *m*/*z* (FAB+) 402.1822 (M+H, $C_{24}H_{24}N_3O_3$ requires 402.1818), 238 (35).

6-Amino-3-(4-benzoyl-phenyl)-1-(3-carboxy-propyl)-pyridazinium bromide (187)



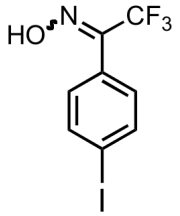
To a solution of **190** (64 mg, 0.133 mmol) in THF (4 mL) and CH_3OH (1 mL) was added morpholine (116 mg, 1.33 mmol) and tetrakis(triphenylphosphine)palladium(0) (15 mg, 0.0133 mmol) under argon. The reaction mixture was stirred at rt for 30 min, then concentrated *in vacuo*. The residue was purified by column chromatography ($CHCl_3/CH_3OH$/AcOH 17:2.9:0.1) to give the *acid* **187** (54 mg, 0.122 mmol, 92%) as a white solid. m.p. 140-142 °C; R_f = 0.25 ($CHCl_3/CH_3OH$ 8:2); IR υ_{max} (film)/cm^{-1} 2933, 1740, 1651, 1570, 1533, 1511; ^1H NMR ($CDCl_3/CD_3OD$ 1:1, 600 MHz) δ 2.10-2.15 (2 H, m, NCH_2CH_2), 2.37-2.43 (2 H, m, $NCH_2CH_2CH_2$), 4.47-4.51 (2 H, m, NCH_2), 7.54 (2 H, dd, *J* 7.7 and 7.5, O=CCCHCHCH), 7.67 (1 H, t, *J* 7.5, O=CCCHCHCH), 7.78 (1 H, d, *J* 9.5, NHCCHCH), 7.80 (2 H, d, *J* 8.2, NHCCHCHCCCH), 7.93 (2 H, d, *J* 7.7, O=CCCHCHCH), 8.09 (2 H, d, *J* 8.2, NHCCHCHCCCHCH), 8.25 (1 H, d, *J* 9.5, NHCCH); ^{13}C NMR ($CDCl_3/CD_3OD$ 1:1, 150 MHz) δ 22.7 (CH_2), 31.9 (CH_2), 56.9 (CH_2), 126.1 (CH), 126.4 (CH), 128.5 (CH), 130.0 (CH), 130.7 (CH), 131.1 (CH), 133.1 (CH), 136.3 (C), 136.8 (C), 139.3 (C), 149.3 (C), 152.9 (C), 179.3 (C=O), 196.6 (C=O); *m/z* (FAB+) 362.1510 (M+H, $C_{21}H_{20}N_3O_3$ requires 362.1505), 329 (80); UV ($CHCl_3/CH_3OH$ 1:1) ε_{306} = 10600 cm^{-1} M^{-1} d^3.

1-(4-Iodophenyl)-2,2,2-trifluoroacetophenone[160] (193)



1,4-Diiodobenzene (15.0 g, 45.5 mmol) was dissolved in THF (60 mL) and cooled to −78 °C. *n*-BuLi (30 mL, 48.0 mmol, 1.6 M in hexanes) was added dropwise. Ethyl trifluoroacetate (9.05 g, 63.7 mmol) was then added in one portion. The reaction mixture was stirred at −78 °C for 18 h, before being warmed to rt. The solution was hydrolysed with saturated ammonium chloride solution (40 mL) then washed further with aqueous HCl (50 mL, 6 M), water (2 × 100 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue purified by vacuum distillation to give the *ketone* **193** (5.65 g, 18.8 mmol, 41%) as a brown oil. R_f = 0.10 (petrol); IR υ_{max} (film)/cm^{-1} 3530, 1722, 1594, 1493, 1410; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (2 H, d, *J* 10.8, ICC*H*), 7.93 (2 H, d, *J* 10.8, ICCHC*H*); ^{13}H NMR (500 MHz, $CDCl_3$) δ 104.7 (C), 117.1 (q, $^1J_{CF}$ 290.5, CF_3), 129.2 (C), 131.1 (CH), 138.7 (CH), 180.1 (q, $^2J_{CF}$ 35.5, C=O); *m*/*z* (EI) 299.9241 (M^+, $C_8H_4IF_3O$ requires 299.9254).

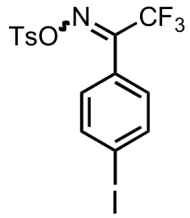
1-(4-Iodophenyl)-2,2,2-trifluoroethanone oxime[160] (194)



To a solution of **193** (5.11 g, 17.0 mmol) in pyridine (5 mL), was added a solution of hydroxylamine hydrochloride (14.9 g, 215 mmol) in pyridine (5 mL) and ethanol (16 mL). The reaction mixture was then heated at 60 °C for 4 h. After cooling to rt, the solvent was removed *in vacuo*. The residue was dissolved in Et_2O (100 mL) and washed with aqueous HCl (2 × 100 mL, 0.1 M), water (100 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo*. The residue was subjected to column

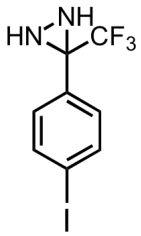
chromatography (petrol/EtOAc 3:1) to give the *oxime* **194** (4.40 g, 14.0 mmol, 82%) used without further purification as a 1:1 mixture of isomers. R_f = 0.60 (petrol/EtOAC 3:1); IR υ_{max} (film)/cm^{-1} 3284, 1896, 1641, 1586, 1493, 1459; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.25-7.35 (4 H, m, 2 × ICCHC*H*), 7.74-7.82 (4 H, m, 2 × ICC*H*), 8.60 (1 H, s, OH), 8.63 (1 H, s, OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 96.4 (C), 119.7 (q, $^1J_{CF}$ 161.3, CF_3), 130.4 (CH), 137.6 (CH), 138.7 (C), 146.3 (C), 148.8 (q, $^2J_{CF}$ 37.5, CNO); *m*/*z* (EI) 314.9370 (M^+, $C_8H_5F_3INO$ requires 314.9363).

1-(4-Iodophenyl)-2,2,2-trifluoroethanone *O*-(*p*-tolylsulfonyl) oxime[160] (195)



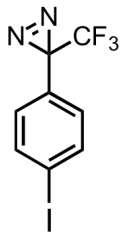
To a solution of **194** (4.39 g, 13.94 mmol) in pyridine (55 mL) was added *p*-toluenesulfonyl chloride (3.32 g, 17.43 mmol). The reaction mixture was refluxed at 110 °C for 18 h. After cooling to rt, the solvent was removed *in vacuo*, and the residue was purified by column chromatography ($CHCl_3$) to give the *tosylate* **195** (3.89 g, 8.29 mmol, 59%) as a white solid. R_f = 0.50 (petrol/EtOAc 2:1); IR υ_{max} (film)/cm^{-1} 2920, 1917, 1647, 1589, 1486; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.48 (3 H, s, C*H*$_3$), 7.12 (2 H, d, *J* 8.5, Ar-*H*), 7.38 (2 H, d, *J* 8.4, SO_2CCHCH), 7.84 (2 H, d, *J* 8.5, Ar-*H*), 7.89 (2 H, d, *J* 8.4, SO_2CCH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.9 (CH_3), 123.9 (q, $^1J_{CF}$ 276.3, CF_3), 127.1 (C), 129.4 (CH), 129.9 (CH), 130.0 (CH), 130.3 (C), 138.3 (C), 138.3 (CH), 146.3 (C), 153.0 (q, $^2J_{CF}$ 33.7, CNO); *m*/*z* (EI) 468.9477 (M^+, $C_{15}H_{11}IF_3NO_3S$ requires 468.9451).

3-*p*-Iodophenyl-3-trifluoromethyl-diaziridine[160] (196)



195 (1.94g, 4.15 mmol) was added to a sealed vessel containing CH_2Cl_2 (9 mL) at −78 °C. Ammonia (2.5 mL) was condensed in dropwise and the solution was stirred at −78 °C for 8 h. The vessel was then unsealed and allowed to warm to rt. The solution extracted with CH_2Cl_2 (25 mL) and washed with water (25 mL). The organic layer was dried (Na_2SO_4) and then concentrated *in vacuo*. The product was purified by flash column chromatography (petrol/EtOAc 4:1) to give the *diaziridine* **196** (632 mg, 1.98 mmol, 48%) as a white solid. m.p. 57-59 °C R_f = 0.30 (petrol/EtOAc 2:1); IR υ_{max} (film)/cm^{-1} 3196, 1666, 1591, 1489, 1393; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.19 (1 H, s, NH), 2.77 (1 H, s, NH), 7.36 (2 H, d, *J* 7.5, ICC*H*), 7.76 (2 H, d, *J* 7.5, ICCHC*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 57.7 (q, $^2J_{CF}$ 36.2, $C(NH)_2$), 96.7 (C), 123.0 (q, $^1J_{CF}$ 276.6, CF_3), 129.8 (CH), 131.8 (C), 138.1 (CH); *m*/*z* (EI) 312.9452 (M-H, $C_8H_6IF_3N_2$ requires 312.9450).

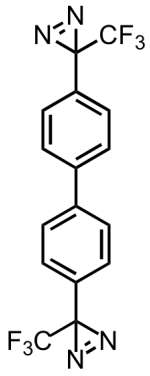
3-*p*-Iodophenyl-3-trifluoromethyl-3*H*-diazirine[123, 160] (121)



To a solution of **196** (150 mg, 0.479 mmol) dissolved in CH_2Cl_2 (3 mL) was added triethylamine (0.301 mL, 2.21 mmol) at 0 °C. Iodine (203 mg, 0.799 mmol) was added gradually, until the solution became brown in colour. The reaction mixture was then washed with aqueous NaOH (3 mL, 1 M), water (10 mL), brine (10 mL) and dried ($MgSO_4$). The solvent was carefully removed *in vacuo* at 20 °C owing to

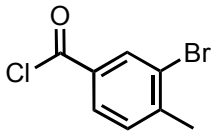
the volatility of the product. The residue was purified by column chromatography (petrol/CH_2Cl_2 20:1) to give the *diazirine* **121** (95 mg, 0.304 mmol, 64%) as a colourless oil. R_f = 0.90 ($CHCl_3$); IR υ_{max} (film)/cm^{-1} 3132, 1659; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.92 (2 H, d, *J* 8.3, ICC*H*), 7.21 (2 H, d, *J* 8.3, ICCHC*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 28.4 (q, $^2J_{CF}$ 40.7, $C(N)_2$), 96.1 (C), 122.0 (q, $^1J_{CF}$ 274.6, CF_3), 128.2 (CH), 128.9 (C), 138.0 (CH); *m*/*z* (EI) 311.9371 (M^+, $C_8H_4F_3IN_2$ requires 311.9372).

4,4'-Bis-(3-trifluoromethyl-3*H*-diazirine)-biphenyl (198)



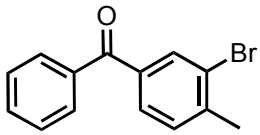
To a solution of **121** (166 mg, 0.532 mmol) in DMSO (5 mL) was added successively [1,1-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (11 mg, 0.015 mmol), bis(pinacolato)diboron (202 mg, 1.06 mmol) and potassium acetate (157 mg, 1.06 mmol). The reaction mixture was heated to 80 °C for 8 h. After cooling to rt, the mixture was diluted with EtOAc (200 mL) and washed with aqueous HCl (100 mL, 0.1 M), water (2 × 100 mL), brine (100 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2) to give the *diazirine* **198** (24 mg, 0.063 mmol, 12%) as a white solid. R_f = 0.70 ($CHCl_3$); IR υ_{max} (film)/cm^{-1} 3122, 1642; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.30 (4 H, d, *J* 7.5, Ar-*H*), 7.59 (4 H, d, *J* 7.5, Ar-*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 28.5 (q, $^2J_{CF}$ 40.7, $C(N)_2$), 122.2 (q, $^1J_{CF}$ 274.6, CF_3), 127.2 (CH), 127.6 (CH), 128.9 (C), 141.2 (C); *m*/*z* (EI) 370.0649 (M^+, $C_8H_4F_3IN_2$ requires 370.0653).

3-Bromo-4-methyl-benzoyl chloride[217] (206)



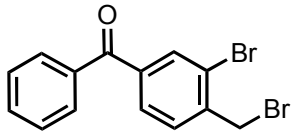
To 3-bromo-4-methylbenzoic acid (1.00 g, 3.95 mmol) was added to $SOCl_2$ (12 mL) and the reaction mixture was stirred at rt for 2 min, then under reflux at 80 °C for 2 h. The solvent was removed *in vacuo* and further evaporated using CH_2Cl_2 (2 × 25 mL) to give the *acid chloride* **206** (1.08 g, 3.95 mmol, quant.) as a brown solid which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.49 (3 H, s, C*H*$_3$), 7.38 (1 H, d, *J* 7.8, BrCCC*H*), 7.94 (1 H, dd, *J* 7.8 and 1.8, BrCCCHC*H*), 8.27 (1 H, d, *J* 1.8, BrCC*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 23.6 (CH_3), 125.4 (C), 130.2 (CH), 131.3 (CH), 132.5 (C), 135.1 (CH), 146.4 (C), 167.1 (C=O).

(3-Bromo-4-methyl-phenyl)-phenyl-methanone[217] (205)



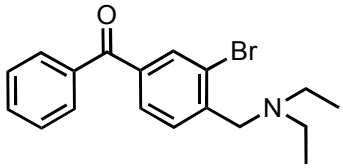
To a solution of **206** (1.08 g, 3.95 mmol) in benzene (12 mL) was added $AlCl_3$ (930 mg, 6.97 mmol) at rt. The reaction mixture was then heated to 50 °C for 2 h. The solvent was removed *in vacuo*. The residue was purified by column chromatography (petrol) to give the *ketone* **205** (1.06 g, 3.85 mmol, 98%) as a white solid. m.p. 80-82 °C; R_f = 0.30 (petrol); IR υ_{max} (film)/cm^{-1} 2933, 1740, 1651; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.48 (3 H, s, C*H*$_3$), 7.34 (1 H, d, *J* 7.8, BrCCC*H*), 7.48 (2 H, dd, *J* 7.6 and 7.5, O=CCCHC*H*CH), 7.60 (1 H, t, *J* 7.5, O=CCCHCHC*H*), 7.63 (1 H, dd, *J* 7.8 and 1.7, BrCCCHC*H*), 7.77 (2 H, d, *J* 7.6, O=CCC*H*CHCH), 7.99 (1 H, d, *J* 1.7, BrCC*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 23.4 (CH_3), 125.0 (C), 128.5 (CH), 129.1 (CH), 130.1 (CH), 130.7 (CH), 132.7 (CH), 134.0 (CH), 137.0 (C), 137.4 (C), 143.0 (C), 195.2 (C=O); *m*/*z* (EI) 273.9996 (M+H, $C_{14}H_{11}O^{79}Br$ requires 273.9988), 276 (100).

(3-Bromo-4-bromomethyl-phenyl)-phenyl-methanone[217] (204)



To a solution of **205** (1.04 g, 3.78 mmol) in CCl_4 (20 mL) was added AIBN (82 mg, 0.50 mmol) and NBS (672 mg, 3.78 mmol) at rt. The reaction mixture was then heated under reflux at 70 °C for 17 h. The solvent was removed *in vacuo*. The residue was purified by column chromatography (petrol) to give the *ketone* **204** (368 mg, 1.04 mmol, 27%) as a white solid. m.p. 53-55 °C; R_f = 0.35 (petrol); IR υ_{max} (film)/cm^{-1} 2994, 1737; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.64 (2 H, s, BrCH_2), 7.51 (2 H, dd, *J* 7.6 and 7.5, O=CCCHC*H*CH), 7.57 (1 H, d, *J* 7.8, BrCCC*H*), 7.63 (1 H, t, *J* 7.5, O=CCCHCHC*H*), 7.71 (1 H, dd, *J* 7.8 and 1.9, BrCCCHC*H*), 7.79 (2 H, d, *J* 7.6, O=CCC*H*CHCH), 8.02 (1 H, d, *J* 1.9, BrCC*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 32.5 (CH_2), 124.6 (C), 128.5 (CH), 129.5 (CH), 130.1 (CH), 131.2 (CH), 133.1 (CH), 134.7 (CH), 136.8 (C), 139.2 (C), 141.2 (C), 194.6 (C=O); *m*/*z* (EI) 351.9099 (M+H, $C_{14}H_{10}O^{79}Br_2$ requires 351.9093), 356 (50), 354 (100).

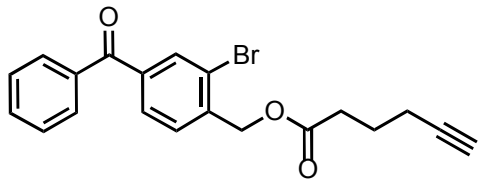
(3-Bromo-4-diethylaminomethyl-phenyl)-phenyl-methanone (208)



To a solution of **204** (80 mg, 0.225 mmol) in diethylamine (4 mL) was added bis(triphenylphosphine)palladium(II) dichloride (24 mg, 0.034 mmol) and copper(I) iodide (3.0 mg, 0.015 mmol) at rt. The reaction mixture was stirred for 30 min. 5-Hexynoic acid (65 mg, 0.282 mmol) in diethylamine (2 mL) was added dropwise over a period of 3 h. The reaction mixture was then stirred at rt for 12 h. The solvent was removed *in vacuo*. The residue was dissolved in Et_2O (100 mL) and washed with aqueous HCl (2 × 100 mL, 0.1 M), water (100 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo*. The residue was subjected to column chromatography (petrol/EtOAc 19:1) to give the *amine* **208** (40 mg, 0.116 mmol, 51%) as a colourless

oil. R_f = 0.30 (petrol/EtOAc 19:1); IR υ_{max} (film)/cm^{-1} 2968, 1747, 1658, 1597, 1548; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.09 (6 H, t, *J* 7.1, C*H*$_3$CH$_2$N), 2.59 (4 H, q, *J* 7.1, CH$_3$C*H*$_2$N), 3.72 (2 H, s, CH$_3$CH$_2$NC*H*$_2$), 7.51 (2 H, dd, *J* 7.8 and 7.7, O=CCCHC*H*CH), 7.62 (1 H, t, *J* 7.7, O=CCCHCHC*H*), 7.74 (2 H, m, CH$_3$CH$_2$NCH$_2$CC*H* and CH$_3$CH$_2$NCH$_2$CCHC*H*), 7.81 (2 H, d, *J* 7.8, O=CCC*H*CHCH), 7.99 (1 H, s, BrCC*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 12.1 (CH_3), 47.5 (CH_2), 57.4 (CH_2), 123.9 (C), 128.5 (CH), 129.1 (CH), 130.1 (CH), 130.2 (CH), 132.8 (CH), 134.0 (CH), 137.3 (C), 137.4 (C), 144.8 (C), 195.3 (C=O); *m*/*z* (CI) 345.0728 (M^+, $C_{18}H_{20}ON^{79}Br$ requires 345.0734), 347 (100), 247 (95), 245 (95).

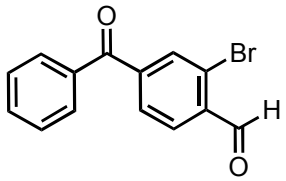
Hex-5-ynoic acid 4-benzoyl-2-bromo-benzyl ester (209)



To a solution of **204** (257 mg, 0.727 mmol) in triethylamine (6 mL) was added bis(triphenylphosphine)palladium(II) dichloride (68 mg, 0.097 mmol) and copper(I) iodide (14 mg, 0.073 mmol). The reaction mixture was stirred at rt for 30 min. 5-Hexynoic acid (112 mg, 1.00 mmol) in triethylamine (2 mL) was added dropwise over a period of 3 h. The reaction mixture was then stirred at rt for 48 h. The solvent was removed *in vacuo*. The remaining residue was dissolved in Et_2O (100 mL) and washed with aqueous HCl (2 × 100 mL, 0.1 M), water (100 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo*. The residue was subjected to column chromatography (petrol/EtOAc 19:1) to give the *alkyne* **209** (20 mg, 0.069 mmol, 9.5%) as a colourless oil. R_f = 0.45 (petrol/EtOAc 4:1); IR υ_{max} (film)/cm^{-1} 3301, 2939, 2127, 1736, 1659; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.93 (2 H, quint, *J* 7.1, O=CCH$_2$C*H*$_2$), 2.01 (1 H, t, *J* 2.6, *H*CCCH$_2$), 2.34 (2 H, td, *J* 7.1 and 2.6, O=CCH$_2$CH$_2$C*H*$_2$), 2.62 (2 H, t, *J* 7.1, O=CC*H*$_2$), 5.29 (2 H, s, OC*H*$_2$), 7.52 (3 H, m, O=CCCHC*H*CH and BrCCC*H*), 7.63 (1 H, t, *J* 7.4, O=CCCHCHC*H*), 7.74 (2 H, dd, *J* 7.7 and 1.7, BrCCCHC*H*), 7.77 (2 H, d, *J* 7.7, O=CC*H*CHCH), 8.02 (1 H, d, *J* 1.7, BrCC*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 18.0 (CH_2), 23.6 (CH_2), 32.9 (CH_2), 65.6

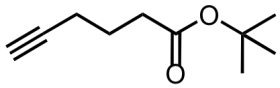
(CH_2), 69.5 (C), 83.2 (CH), 123.1 (C), 128.6 (CH), 129.1 (CH), 129.1 (CH), 130.1 (CH), 133.1 (CH), 134.2 (CH), 136.9 (C), 138.9 (C), 139.7 (C), 172.7 (C=O), 194.9 (C=O); *m*/*z* (EI) 384.0366 (M^+, $C_{20}H_{17}O_3{}^{79}Br$ requires 384.0361).

4-Benzoyl-2-bromo-benzaldehyde (210)



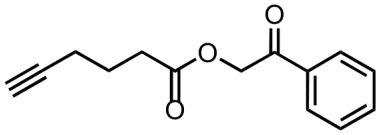
To a solution of **204** (257 mg, 0.727 mmol) in triethylamine (6 mL) was added bis(triphenylphosphine)palladium(II) dichloride (68 mg, 0.097 mmol) and copper(I) iodide (14 mg, 0.073 mmol) at rt. The reaction mixture was stirred for 30 min. 5-Hexynoic acid (112 mg, 1.00 mmol) in triethylamine (2 mL) was added dropwise over a period of 3 h. The reaction mixture was then stirred at rt for 48 h. The solvent was removed *in vacuo*. The remaining residue was dissolved in Et_2O (100 mL) and washed with aqueous HCl (2 × 100 mL, 0.1 M), water (100 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo*. The residue was subjected to column chromatography (petrol/EtOAc 19:1) to give the *aldehyde* **210** (86 mg, 0.223 mmol, 31%) as a colourless oil. R_f = 0.80 (petrol/EtOAc 4:1); IR υ_{max} (film)/cm^{-1} 2970, 1724, 1698, 1662; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.55 (2 H, dd, *J* 7.7 and 7.4, O=CCCHC*H*CH), 7.67 (1 H, t, *J* 7.4, O=CCCHCHC*H*), 7.81 (3 H, m, O=CC*H*CHCH and BrCCC*H*), 8.01 (2 H, d, *J* 7.4, BrCCCHC*H*), 8.04 (1 H, s, BrCC*H*), 10.48 (1 H, s, C*H*O); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 126.9 (C), 128.8 (CH), 129.0 (CH), 129.8 (CH), 130.2 (CH), 133.6 (CH), 135.0 (CH), 135.6 (C), 136.3 (C), 143.6 (C), 191.4 (CHO), 194.4 (C=O); *m*/*z* (EI) 287.9786 (M^+, $C_{14}H_9O_2{}^{79}Br$ requires 287.9785), 290 (100), 213 (40), 211 (40).

Hex-5-ynoic acid *tert*-butyl ester (213)



To a solution of 5-hexynoic acid (1.00 g, 8.91 mmol) in THF (15 mL) at –40 °C, was added trifluoroacetic anhydride (1.68 g, 8.00 mmol) dropwise across 30 min. *tert*-Butyl alcohol (10 mL) was added in one portion. The reaction mixture was stirred at –40 °C for 3 h. The solvent was removed *in vacuo* and the residue was purified by column chromatography (petrol/EtOAc 9:1) to give the *ester* **213** (80 mg, 0.475 mmol, 6%). R_f = 0.80 (petrol/EtOAc 4:1); IR υ_{max} (film)/cm^{-1} 3304, 2970, 2152, 1727, 1661; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.44 (9 H, s, OC(CH_3)$_3$), 1.80 (2 H, quint, *J* 7.1, O=CCH$_2$CH_2), 1.95 (1 H, t, *J* 2.6, *H*CCCH$_2$), 2.24 (2 H, td, *J* 7.1 and 2.6, O=CCH$_2$CH$_2$CH_2), 2.32 (2 H, t, *J* 7.1, O=CCH_2); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 18.0 (CH_2), 23.9 (CH_2), 28.2 (CH_3), 34.8 (CH_2), 69.1 (C), 80.4 (C), 83.6 (CH), 172.4 (C=O); *m*/*z* (EI) 168.1148 (M^+, $C_{10}H_{16}O_2$ requires 168.1150), 105 (65).

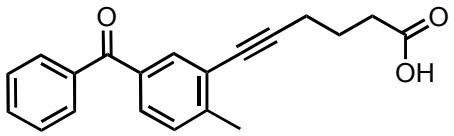
Hex-5-ynoic acid 2-oxo-2-phenyl-ethyl ester (214)



To a solution of phenacyl chloride (1.52 g, 9.83 mmol), triethylamine (1.13 g, 11.1 mmol) and tetrabutylammonium iodide (3.62 g, 9.83 mmol) in EtOAc (20 mL) was added 5-hexynoic acid (1.00 g, 8.91 mmol) in one portion. The reaction mixture was stirred at rt for 3 h. The solvent was removed *in vacuo* and the residue was purified by column chromatography (petrol/EtOAc 4:1) to give the *ester* **214** (974 mg, 4.23 mmol, 47%). R_f = 0.55 (petrol/EtOAc 4:1); IR υ_{max} (film)/cm^{-1} 3288, 2938, 2157, 1740; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.92 (2 H, quint, *J* 7.4, O=CCH$_2$CH_2CH$_2$), 1.99 (1 H, t, *J* 2.6, *H*CCCH$_2$), 2.33 (2 H, td, *J* 7.4 and 2.6, O=CCH$_2$CH$_2$CH_2), 2.65 (2 H, t, *J* 7.3, O=CCH_2CH$_2$CH$_2$), 5.35 (2 H, s, CO$_2$CH_2), 7.49 (2 H, dd, *J* 7.8 and 7.7, O=CCCHC*H*), 7.61 (1 H, t, *J* 7.8, O=CCCHCHC*H*), 7.91 (2 H, d, *J* 7.8, O=CCC*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 17.9 (CH_2), 23.8 (CH_2), 32.7 (CH_2), 66.1 (CH_2), 69.3

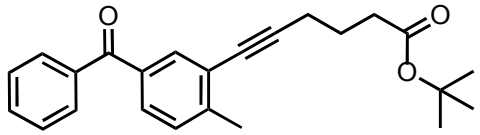
(C), 83.6 (CH), 127.9 (CH), 129.0 (CH), 134.1 (CH), 134.3 (C), 172.7 (C=O), 192.2 (C=O); *m*/*z* (CI) 231.1024 (M+H, $C_{14}H_{15}O_3$ requires 231.1021).

6-(5-Benzoyl-2-methyl-phenyl)-hex-5-ynoic acid (217)



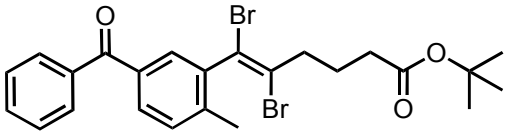
To a solution of **205** (200 mg, 0.727 mmol) in diethylamine (6 mL) was added bis(triphenylphosphine)palladium(II) dichloride (68 mg, 0.097 mmol) and copper(I) iodide (14 mg, 0.073 mmol) at rt. The reaction mixture was stirred for 30 min. 5-Hexynoic acid (112 mg, 1.00 mmol) in diethylamine (2 mL) was added dropwise over a period of 3 h. The reaction mixture was then stirred at rt for 48 h. The solvent was removed *in vacuo*. The remaining residue was dissolved in Et_2O (100 mL) and washed with aqueous HCl (100 mL, 0.1 M), water (2 × 100 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo*. The residue was subjected to column chromatography (EtOAc) to give the *acid* **217** (100 mg, 0.327 mmol, 45%) as a white solid. m.p. 100-102 °C; R_f = 0.20 (EtOAc); IR υ_{max} (film)/cm^{-1} 3332, 2213, 1741, 1659; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.98 (2 H, quint, *J* 7.2, O=CCH$_2$C*H*$_2$), 2.51 (3 H, s, C*H*$_3$) 2.56-2.61 (4 H, m, O=CCH$_2$CH$_2$C*H*$_2$ and O=CC*H*$_2$), 7.31 (1 H, d, *J* 7.9, CH$_3$CC*H*), 7.52 (2 H, dd, *J* 7.6 and 7.4, O=CCCHC*H*CH), 7.59 (1 H, t, *J* 7.4, O=CCCHCHC*H*), 7.65 (1 H, dd, *J* 7.9 and 2.0, CH$_3$CCHC*H*), 7.79 (1 H, d, *J* 2.0, CH$_3$CCC*H*), 7.81 (2 H, d, *J* 7.6, O=CCC*H*CHCH); ^{13}C NMR (DMSO, 150 MHz) δ 18.3 (CH$_3$), 20.6 (CH$_2$), 48.6 (CH$_2$), 65.6 (CH$_2$), 69.5 (C), 83.2 (C), 123.1 (C), 128.6 (CH), 129.1 (CH), 130.1 (CH), 131.6 (CH), 133.1 (CH), 134.2 (CH), 136.9 (C), 138.9 (C), 139.7 (C), 172.7 (C=O), 194.9 (C=O); *m*/*z* (EI) 306.1254 (M^+, $C_{20}H_{18}O_3$ requires 306.1250), 247 (30).

6-(5-Benzoyl-2-methyl-phenyl)-hex-5-ynoic acid *tert*-butyl ester (216)



To a solution of **217** (290 mg, 0.946 mmol) in THF (4 mL) at –40 °C, was added trifluoroacetic anhydride (189 mg, 0.900 mmol) dropwise across 30 min. *tert*-Butyl alcohol (1 mL) was added in one portion. The reaction mixture was stirred at –40 °C for 3 h. The solvent was removed *in vacuo* and the residue was purified by column chromatography (petrol/EtOAc 9:1) to give the *ester* **216** (153 mg, 0.422 mmol, 47%) as a colourless oil. R_f = 0.80 (petrol/EtOAc 4:1); IR υ_{max} (film)/cm^{-1} 3221, 2209, 1741, 1726; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.44 (9 H, s, OC(C*H*$_3$)$_3$), 1.89 (2 H, quint, *J* 7.4, O=CCH$_2$C*H*$_2$), 2.41 (2 H, t, *J* 7.4, O=CC*H*$_2$), 2.49 (3 H, s, Ar-C*H*$_3$), 2.51 (2 H, t, *J* 7.4, O=CCH$_2$CH$_2$C*H*$_2$), 7.30 (1 H, d, *J* 7.9, CH$_3$CC*H*), 7.48 (2 H, dd, *J* 7.6 and 7.4, O=CCCHC*H*CH), 7.59 (1 H, t, *J* 7.4, O=CCCHCHC*H*), 7.63 (1 H, dd, *J* 7.9 and 1.8, CH$_3$CCHC*H*), 7.77 (2 H, d, *J* 7.6, O=CCC*H*CHCH), 7.79 (1 H, d, *J* 1.8, CH$_3$CCC*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 19.1 (CH_2), 21.2 (CH_3), 24.3 (CH_2), 28.2 (CH_3), 34.5 (CH_2), 79.4 (C), 80.5 (C), 94.4 (C), 124.0 (C), 128.4 (CH), 129.3 (CH), 129.5 (CH), 130.1 (CH), 132.5 (CH), 133.7 (CH), 135.2 (C), 137.7 (C), 145.1 (C), 172.6 (C=O), 196.1 (C=O); *m*/*z* (EI) 362.1881 (M^+, $C_{24}H_{26}O_3$ requires 362.1882), 306 (50).

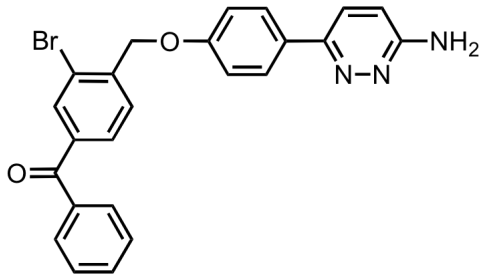
6-(5-Benzoyl-2-methyl-phenyl)-5,6-dibromo-hex-5-enoic acid *tert*-butyl ester (218)



To a solution of **216** (150 mg, 0.413 mmol) in benzene (1 mL) was added NBS (91 mg, 0.516 mmol) and AIBN (33 mg, 0.206 mmol). The reaction mixture was heated to 60 °C for 6 h. After cooling to rt, the precipitate was filtered and the solvent was removed *in vacuo*. The residue was purified by column chromatography

(petrol/EtOAc 9:1) to give the *ester* **218** (84 mg, 0.160 mmol, 39%) as a colourless oil. R_f = 0.30 (petrol/EtOAc 9:1); IR υ_{max} (film)/cm^{-1} 3317, 1742, 1731; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.47 (9 H, s, OC(CH_3)$_3$), 2.00 (2 H, m, O=CCH_2CH_2), 2.35 (2 H, t, *J* 7.4, O=CCH_2), 2.37 (3 H, s, Ar-CH_3), 2.81 (1 H, dt, *J* 13.4 and 6.4, O=CCH$_2$CH$_2$C*H*H), 2.94 (1 H, dt, *J* 13.4 and 6.4, O=CCH$_2$CH$_2$CH*H*), 7.34 (1 H, d, *J* 7.9, CH$_3$CC*H*), 7.48 (2 H, dd, *J* 7.6 and 7.4, O=CCCHC*H*CH), 7.59 (1 H, t, *J* 7.4, O=CCCHCHC*H*), 7.60 (1 H, d, *J* 1.7, CH$_3$CCC*H*), 7.72 (1 H, dd, *J* 7.9 and 1.7, CH$_3$CCHC*H*), 7.79 (2 H, d, *J* 7.6, O=CCC*H*CHCH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 19.6 (CH_3), 23.0 (CH_2), 28.2 (CH_3), 34.2 (CH_2), 39.5 (CH_2), 80.6 (C), 115.6 (C), 124.8 (C), 128.5 (C), 130.1 (CH), 130.6 (CH), 130.7 (CH), 130.9 (CH), 132.5 (CH), 135.7 (C), 137.7 (C), 140.4 (C), 140.9 (C), 172.4 (C=O), 195.9 (C=O); *m*/*z* (EI) 520.0245 (M^+, $C_{24}H_{26}{}^{79}Br_2O_3$ requires 520.0249), 522 (100), 524 (50).

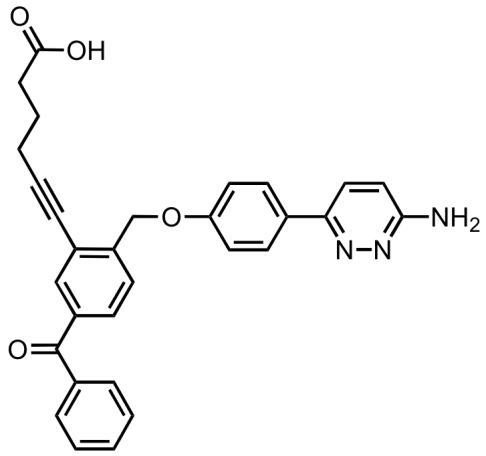
{4-[4-(6-Amino-pyridazin-3-yl)-phenoxymethyl]-3-bromo-phenyl}-phenyl-methanone (219)



A solution of **157** (260 mg, 1.39 mmol), 18-crown-6 (157 mg, 1.39 mmol) and potassium *tert*-butoxide (157 mg, 1.39 mmol) in DMF (6 mL) were cooled to 0 °C. A solution of **205** (368 mg, 1.04 mmol) in DMF (2 mL) was added dropwise and the reaction mixture stirred at 0 °C for 3 h. The reaction mixture was extracted with EtOAc (100 mL) and washed with water (100 mL). The aqueous layer was further extracted with EtOAc (4 × 50 mL). The organic extracts were combined and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue subjected to column chromatography ($CHCl_3$/CH_3OH 19:1) to give the *pyridazine* **219** (389 mg, 0.845 mmol, 81%) as a white solid. m.p. 164-166 °C; R_f = 0.30 ($CHCl_3$/CH_3OH 19:1); IR υ_{max} (film)/cm^{-1} 3013, 1735, 1646; ^{1}H NMR ($CDCl_3$/CD_3OD 1:1, 600 MHz) δ 5.25 (2 H, s, OCH_2), 7.04 (1 H, d, *J* 9.0, NH$_2$CC*H*), 7.10 (2 H, d, *J* 8.3, OCC*H*) 7.51 (2 H,

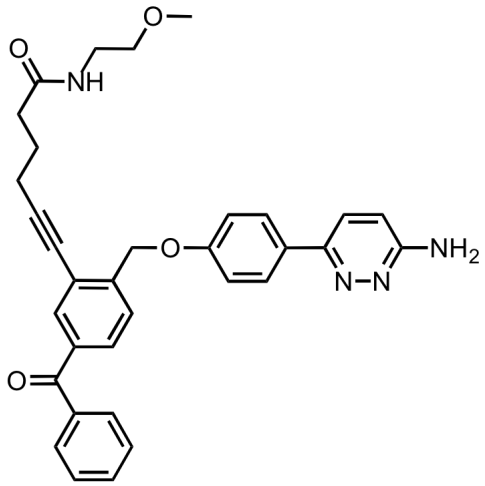
dd, *J* 7.6 and 7.5, O=CCCHC*H*CH), 7.63 (1 H, t, *J* 7.5, O=CCCHCHC*H*), 7.69-7.75 (3 H, m, BrCCC*H*, BrCCCHC*H* and NH_2CCHC*H*), 7.76 (2 H, d, *J* 7.6, O=CCC*H*CHCH), 7.76 (2 H, d, *J* 8.3, OCCHC*H*), 8.00 (1 H, d, *J* 1.8, BrCC*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 70.4 (CH_2), 116.5 (CH), 118.7 (CH), 123.4 (C), 129.0 (CH), 129.0 (CH), 129.8 (CH), 129.9 (CH), 130.6 (CH), 130.9 (C), 131.2 (C), 131.3 (CH), 134.5 (CH), 135.2 (CH), 138.1 (C), 139.7 (C), 142.2 (C), 160.5 (C), 197.0 (C=O) * 1 (C) absent; *m*/*z* (ES+) 460.0641 (M+H, $C_{24}H_{19}N_3O_2{}^{79}Br$ requires 460.0661), 462 (100).

6-{2-[4-(6-Amino-pyridazin-3-yl)-phenoxymethyl]-5-benzoyl-phenyl}-hex-5-ynoic acid (201)



To a solution of **219** (200 mg, 0.434 mmol) in diethylamine (15 mL) and THF (15 mL) was added bis(triphenylphosphine)palladium(II) dichloride (40 mg, 0.057 mmol) and copper(I) iodide (8.0 mg, 0.043 mmol) at rt. The reaction mixture was stirred for 30 min. 5-Hexynoic acid (67 mg, 2.17 mmol) in diethylamine (10 mL) was added dropwise over a period of 4 h. The reaction mixture was then stirred at rt for 48 h. The solvent was removed *in vacuo*. The residue was dissolved in THF (100 mL), washed with aqueous HCl (2 × 100 mL, 0.1 M), water (100 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo*. The residue was subjected to column chromatography ($CHCl_3$/CH_3OH 9:1) to give the *acid* **201** (164 mg, 0.333 mmol, 77%) as a white solid. m.p. 186-188 °C; R_f = 0.30 ($CHCl_3$/CH_3OH 9:1); IR υ_{max} (film)/cm^{-1} 3328, 2178, 1740, 1638; *m*/*z* (ES+) 492.1901 (M+H, $C_{30}H_{26}N_3O$ requires 492.1923).

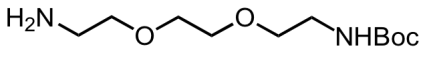
6-{2-[4-(6-Amino-pyridazin-3-yl)-phenoxymethyl]-5-benzoyl-phenyl}-hex-5-ynoic acid (2-methoxy-ethyl)-amide (220)



To a solution of **201** (5.0 mg, 0.0102 mmol) in DMF (0.3 mL) at rt, was added *N*-hydroxysuccinimide (1.3 mg, 0.011 mmol) and stirred at rt for 30 min. The solution was added dropwise to a solution of diisopropylcarbodiimide hydrochloride (2.0 mg, 0.012 mmol) and 2-methoxyethamine (2.0 mg, 0.027 mmol) in DMF (0.3 mL) and the reaction mixture was stirred at rt for 3 h. The solvent was removed *in vacuo* and the residue was purified by column chromatography (EtOAc) to give the *pyridazine* **220** (3.3 mg, 0.0060 mmol, 60%) as a white solid. R_f = 0.45 ($CHCl_3$/CH_3OH 9:1); IR υ_{max} (film)/cm^{-1} 3277, 2137, 1734, 1644; ^1H NMR ($CDCl_3$, 600 MHz) δ 1.87 (2 H, quint, *J* 7.3, O=CCH_2CH_2), 2.34 (2 H, t, *J* 7.4, O=CCH_2), 2.51 (2 H, t, *J* 7.4, O=CCH_2CH_2CH_2), 3.38 (2 H, t, *J* 7.0, NHCH_2), 5.37 (2 H, s, COCH_2C), 7.00 (1 H, d, *J* 9.3, NH_2CC*H*), 7.13 (2 H, d, *J* 8.8, OCC*H*) 7.55 (2 H, dd, *J* 7.6 and 7.4, O=CCCHC*H*CH), 7.59 (1 H, t, *J* 7.4, O=CCCHCHC*H*), 7.70-7.75 (2 H, m, OCH_2CC*H* and NH_2CCHC*H*), 7.76-7.79 (3 H, m, OCH_2CCHC*H* and OCCHC*H*), 7.81 (1 H, d, *J* 1.8, OCH_2CCC*H*), 7.87 (2 H, d, *J* 7.6, O=CCC*H*CHCH); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 19.7 (CH_2), 25.9 (CH_2), 35.9 (CH_2), 40.2 (CH_2), 58.9 (CH_3), 69.2 (CH_2), 71.9 (CH_2), 79.6 (C), 97.3 (C), 116.2 (CH), 117.5 (CH), 124.5 (C), 128.0 (CH), 128.6 (CH), 128.6 (CH), 129.6 (CH), 130.4 (CH), 131.0 (CH), 131.2 (C), 134.0 (CH), 134.4 (CH), 138.3 (C), 138.5 (C), 144.4 (C), 152.1 (C), 160.9 (C), 175.4

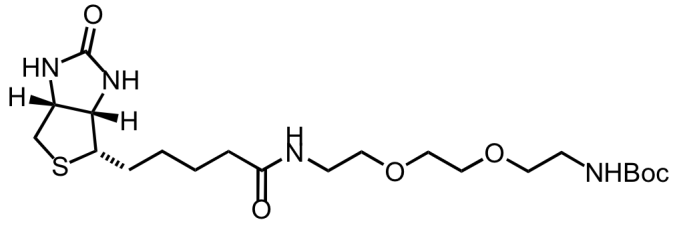
(C=O), 197.4 (C=O), *1 (C) absent; *m*/*z* (ES+) 571.2321 ($[MNa]^+$, $C_{33}H_{32}N_4O_4Na$ requires 571.2321), 549 (100).

tert-Butyl _N_-(2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (222)



To a solution of di-*tert*-butyl-dicarbonate (1.10 g, 5.00 mmol) in CH_2Cl_2 (5 mL) was added a solution of 2-[2-(2-aminoethoxy)ethoxy]ethanamine (7.32 g, 50.0 mmol) in CH_2Cl_2 (15 mL). The resulting reaction mixture was stirred at rt for 24 h. The solvent was removed *in vacuo*. EtOAc (125 mL) was added to the residue and the resulting white precipitate was washed with Na_2CO_3 (3 × 50 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue was purified by column chromatography (CH_2Cl_2/CH_3OH 8:2) to give the *amine* **222** (690 mg, 2.80 mmol, 56%) as a colourless oil. R_f = 0.30 (CH_2Cl_2/CH_3OH 8:2); IR υ_{max} (film)/cm^{-1} 3344, 2869, 1692; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.35 (9 H, s, CH_3), 2.05 (2 H, s, NH_2), 2.80 (2 H, t, *J* 5.0, NCH_2), 3.22-3.23 (2 H, m, NCH_2), 3.42-3.47 (4 H, m, OCH_2), 3.52-3.54 (4 H, m, OCH_2), 5.27 (1 H, s, NH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.4 (CH_3), 40.3 (CH_2), 41.6 (CH_2), 70.2 (CH_2), 70.2 (CH_2), 73.2 (CH_2), 79.1 (C), 156.1 (C=O), * 1 CH_2 absent; *m*/*z* (CI) 249.1825 (M+H, $C_{11}H_{25}N_2O_4$ requires 249.1814).

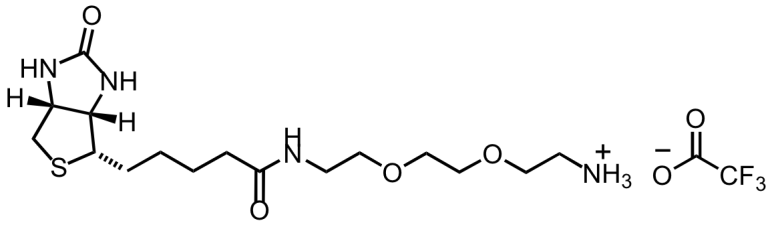
tert-Butyl-_N_-(2-(2-(2-(5-(2-oxo-1,3,3a,4,6,6a-hexahydrothieno(3,4-_d_)imidazol-6-yl)pentanoylamino)ethoxy)ethoxy)ethyl)carbamate (223)



A solution of biotin (590 mg, 2.42 mmol), HBTU (790 mg, 2.10 mmol) and DIPEA (606 mg, 2.60 mmol) in DMF (15 mL) was stirred for 20 min at rt before being added dropwise to a solution of **222** (400 mg, 1.61 mmol) in DMF (10 mL). The reaction mixture was stirred for 2 h at rt. The solvent was removed *in vacuo* and the residue was purified by column chromatography (CH_2Cl_2/CH_3OH 19:1) to give the

amine **223** (640 mg, 1.34 mmol, 83%) as a white solid. m.p. 106-108 °C; R_f = 0.30 (CH_2Cl_2/CH_3OH 19:1); $[\alpha]_D^{20.0}$ +23.0 (*c* 0.6, CH_2Cl_2); IR υ_{max} (film)/cm^{-1} 3317, 2945, 1697; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.47 (11 H, m, $C(CH_3)_3$ and $NHC{=}OCH_2CH_2CH_2CH_2$), 1.71 (4 H, m, $NHC{=}OCH_2CH_2CH_2CH_2$), 2.27 (2 H, t, *J* 7.0, $NHC{=}OCH_2CH_2CH_2$), 2.79 (1 H, d, *J* 13.0, SC*H*H), 2.92 (1 H, dd, *J* 13.0 and 5.0, SCH*H*), 3.17 (1 H, dt, *J* 5.0 and 3.0, SC*H*), 3.31 (2 H, m, NCH_2), 3.46 (2 H, m, NCH_2), 3.55-3.59 (2 H, m, OCH_2), 3.62 (6 H, m, OCH_2), 4.36 (1 H, dd, *J* 7.5 and 5.0, CHNHC=ONHC*H*), 4.55 (1 H, dd, *J* 7.5 and 5.0, C*H*NHC=ONHCH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.2 (CH_2), 27.7 (CH_2), 27.8 (CH_2), 28.1 (CH_3), 35.4 (CH_2), 38.9 (CH_2), 40.0 (CH_2), 40.2 (CH_2), 55.2 (CH), 60.1 (CH), 61.6 (CH), 69.7 (CH_2), 70.0 (CH_2), 79.1 (C), 156.0 (C=O), 163.9 (C=O), 173.7 (C=O), * 2 CH_2 absent; *m*/*z* (ES) 497.2423 ($[MNa]^+$, $C_{21}H_{38}N_4O_6SNa$ requires 497.2410).

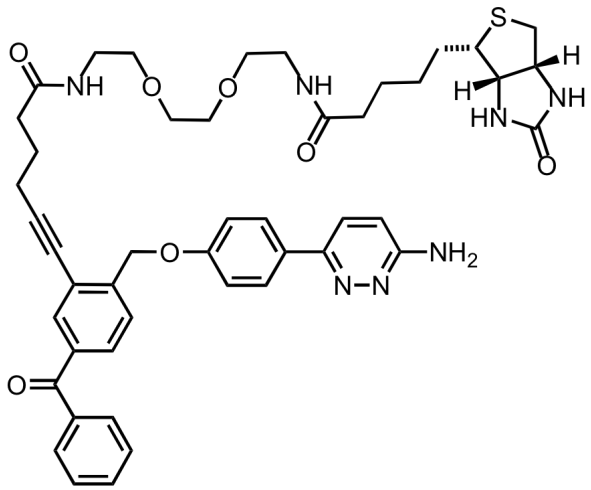
2-(2-(2-(5-(2-oxo-1,3,3a,4,6,6a-hexahydrothieno(3,4-*d*)imidazol-6-yl)pentanoylamino)ethoxy)ethoxy)ethylammonium 2,2,2-trifluoroacetate salt (224)



A solution of **223** (610 mg, 1.29 mmol) in CH_2Cl_2 (5 mL) and TFA (5 mL) was stirred at rt for 3 h. The solvent was removed *in vacuo* to give the *amine* **224** (630 mg, 1.29 mmol, quant.) as a colourless oil. R_f = 0.05 (CH_2Cl_2/CH_3OH 19:1); $[\alpha]_D^{20.0}$ +40.0 (*c* 0.5, CH_3OH); IR υ_{max} (film)/cm^{-1} 3312, 2936, 1689; ^1H NMR (CD_3OD, 400 MHz) δ 1.43-1.76 (6 H, m, $NHC{=}OCH_2CH_2CH_2CH_2$), 2.24 (2 H, t, *J* 7.5, $NHC{=}OCH_2CH_2CH_2$), 2.74 (1 H, d, *J* 13.0, SC*H*H), 2.94 (1 H, dd, *J* 13.0 and 5.0, SCH*H*), 3.21 (1 H, dt, *J* 8.5 and 5.0, SC*H*), 3.38 (2 H, t, *J* 5.0, OCH_2), 3.57 (2 H, t, *J* 5.0, OCH_2), 3.65 (4 H, m, OCH_2), 3.71 (2 H, t, *J* 5.0, $OCH_2CH_2NH_3$), 4.33 (1 H, dd, *J* 7.5 and 5.0, CHNHC=ONHC*H*), 4.53 (1 H, dd, *J* 7.5 and 5.0, C*H*NHC=ONHCH); ^{13}C NMR (CD_3OD, 125 MHz) δ 25.4 (CH_2), 28.1 (CH_2), 28.3 (CH_2), 35.3 (CH_2), 38.8 (CH_2), 39.3 (CH_2), 39.7 (CH_2), 55.6 (CH), 60.4 (CH), 62.1 (CH), 66.5 (CH_2),

69.2 (CH_2), 69.8 (CH_2), 69.9 (CH_2), 164.8 (C=O), 175.0 (C=O); *m*/*z* (ES) 375.2060 (M+H, $C_{16}H_{31}N_4O_4S$ requires 375.2066).

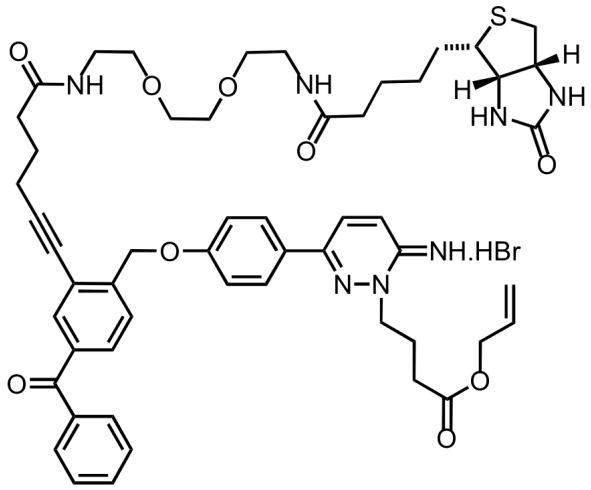
6-{2-[4-(6-Amino-pyridazin-3-yl)-phenoxymethyl]-5-benzoyl-phenyl}-hex-5-ynoic acid [2-(2-{2-[5-(2-oxo-1,3,3a,4,6,6a-hexahydro-thieno[3,4-*d*]imidazol-6-yl)-pentanoylamino]-ethoxy}-ethoxy)-ethyl]-amide (200)



To a solution of **224** (87 mg, 0.177 mmol) in DMF (5 mL) at rt, was added HBTU (67 mg, 0.177 mmol) and DIPEA (68 mg, 0.531 mmol). The reaction mixture was stirred at rt for 20 min. The resulting solution was then added dropwise to a solution of **201** (83 mg, 0.177 mmol) in DMF (5 mL). The reaction mixture was stirred at rt for 3 h. The solvent was removed *in vacuo*. The residue was purified by column chromatography ($CHCl_3$/CH_3OH 9:1) to give the *pyridazine* **200** (18 mg, 0.021 mmol, 12%). m.p. degraded >150 °C; R_f = 0.20 ($CHCl_3$/CH_3OH 9:1); $[\alpha]_D^{20.0}$ +27.0 (*c* 0.2, CH_3OH); IR υ_{max} (film)/cm^{-1} 3295, 2930, 2102, 1737, 1660, 1597, 1446; ^1H NMR ($CDCl_3$/CD_3OD 1:1, 600 MHz) δ 1.43-1.76 (6 H, m, NHC=OCH$_2$C*H*$_2$C*H*$_2$C*H*$_2$), 1.90 (2 H, quint, *J* 7.1, NHC=OCH$_2$C*H*$_2$CH$_2$CC), 2.16 (2 H, t, *J* 7.0, NHC=OC*H*$_2$CH$_2$CH$_2$CH$_2$), 2.34 (2 H, t, *J* 7.1, NHC=OC*H*$_2$CH$_2$CH$_2$CC), 2.51 (2 H, t, *J* 7.1, NHC=OCH$_2$CH$_2$C*H*$_2$CC), 2.69 (1 H, d, *J* 12.8, SC*H*H), 2.94 (1 H, dd, *J* 12.8 and 4.7, SCH*H*), 3.21 (1 H, m, SC*H*), 3.35 (4 H, m, 2 × NHC*H*$_2$CH$_2$O), 3.57-3.63 (8 H, m, 4 × OC*H*$_2$), 4.26 (1 H, m, CHNHC=ONHC*H*), 4.53 (1 H, m, C*H*NHC=ONHCH), 5.35 (2 H, s, OC*H*$_2$C), 7.08 (2 H, d, *J* 8.3, OCC*H*), 7.50 (2 H, dd, *J* 7.6 and 7.5, O=CCCHC*H*CH), 7.60-7.70 (4

H, m, O=CCCHCHC*H,* OCH_2CC*H*, OCH_2CCHC*H* and NH_2CCHC*H*), 7.73-7.76 (3 H, m, OCCHC*H* and OCH_2CCC*H*), 7.83 (2 H, m, O=CCC*H*CHCH) * 1 H absent – NH_2CC*H* not observed due to deuteration in $CDCl_3/CD_3OD$ but signal is observed at 6.82 ppm in DMSO; ^{13}C NMR ($CDCl_3/CD_3OD$ 1:1, 150 MHz) δ 20.3 (CH_2), 26.0 (CH_2), 26.9 (CH_2), 29.5 (CH_2), 29.8 (CH_2), 36.2 (CH_2), 37.0 (CH_2), 40.3 (CH_2), 40.4 (CH_2), 41.5 (CH_2), 57.0 (CH), 61.6 (CH), 63.4 (CH), 69.4 (CH_2), 70.9 (CH_2), 70.9 (CH_2), 71.4 (CH_2), 79.1 (C), 97.6 (C), 116.5 (CH), 123.9 (C), 128.4 (CH), 129.0 (CH), 129.0 (CH), 129.8 (CH), 130.6 (C), 130.7 (CH), 131.3 (CH), 134.3 (CH), 134.9 (CH), 138.2 (C), 138.4 (C), 144.3 (C), 160.9 (C), 165.9 (C=O), 175.2 (C=O), 176.0 (C=O), 197.9 (C=O) * 1 CH_2, 1 CH and 2 (C) absent; *m/z* (ES+) 870.3621 ($[MNa]^+$, $C_{46}H_{53}N_7O_7SNa$ requires 870.3625), 848 (25).

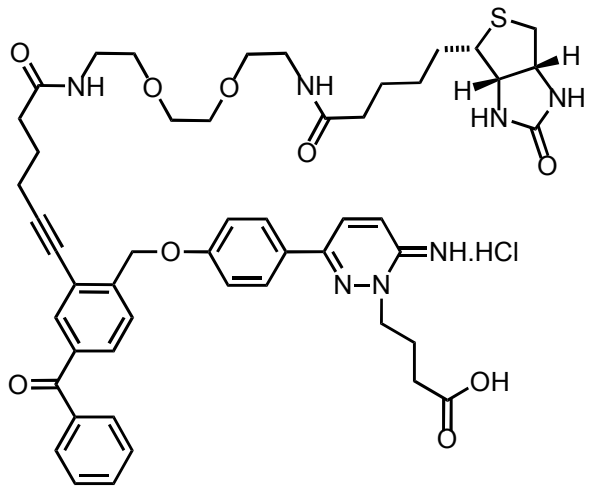
1-(3-Allyloxycarbonyl-propyl)-6-amino-3-[4-(4-benzoyl-2-{5-[2-(2-{2-[5-(2-oxo-1,3,3a,4,6,6a-hexahydro-thieno[3,4-*d*]imidazol-6-yl)-pentanoylamino]-ethoxy}-ethoxy)-ethylcarbamoyl]-pent-1-ynyl}-benzyloxy)-phenyl]-pyridazinium bromide (225)



To a solution of **200** (17 mg, 0.020 mmol) in DMF (0.5 mL) was added allyl-4-bromobutyrate (6.0 mg, 0.029 mmol). The solution was heated to 80 °C for 5 h. The hot solution was then poured into EtOAc (2 mL) to yield a solid, which was then isolated by filtration. The product was dried under high vacuum to give the *ester* **225** (9.2 mg, 0.0085 mmol, 43%) as a white solid. m.p. degraded >150 °C; R_f = 0.10 ($EtOAc/CH_3OH$ 9:1); $[\alpha]_D^{20.0}$ +29.0 (*c* 0.1, CH_3OH); IR υ_{max} (film)/cm^{-1} 3318, 2943,

2831, 2120, 1725, 1666, 1449; ^{1}H NMR ($CDCl_3/CD_3OD$ 1:1, 600 MHz) δ 1.45-1.71 (6 H, m, NHC=OCH$_2$C*H*$_2$C*H*$_2$C*H*$_2$), 1.92 (2 H, quint, *J* 7.4, NHC=OCH$_2$C*H*$_2$CH$_2$CC), 2.17 (2 H, t, *J* 7.0, NHC=OC*H*$_2$CH$_2$CH$_2$CH$_2$), 2.29-2.36 (4 H, m, NHC=OC*H*$_2$CH$_2$CH$_2$CC and NNCH$_2$C*H*$_2$CH$_2$), 2.50-2.55 (4 H, m, NHC=OCH$_2$CH$_2$C*H*$_2$CC and NNCH$_2$CH$_2$C*H*$_2$), 2.69 (1 H, d, *J* 12.6, SC*H*H), 2.89 (1 H, dd, *J* 12.6 and 4.7, SCH*H*), 3.13 (1 H, m, SC*H*), 3.35 (4 H, m, 2 × NHC*H*$_2$CH$_2$O), 3.53-3.58 (8 H, m, 4 × OC*H*$_2$), 4.28 (1 H, m, CHNHC=ONHC*H*), 4.49-4.57 (5 H, m, C*H*NHC=ONHCH, CO$_2$C*H*$_2$CH and NNC*H*$_2$CH$_2$CH$_2$), 5.18 (1 H, dd, *J* 10.2 and 1.6, CO$_2$CH$_2$CHC*H*H), 5.23 (1 H, dd, *J* 17.4 and 1.6, CO$_2$CH$_2$CHCH*H*), 5.35 (2 H, s, OC*H*$_2$C), 5.82 (1 H, m, CO$_2$CH$_2$C*H*), 7.14 (2 H, d, *J* 8.3, OCC*H*), 7.50 (2 H, dd, *J* 7.6 and 7.5, O=CCCHC*H*CH), 7.60-7.66 (3 H, m, O=CCCHCHC*H,* OCH$_2$CC*H*, OCH$_2$CCHC*H*), 7.69 (1 H, d, *J* 9.3, NHCCHC*H*), 7.76 (2 H, d, *J* 7.6, O=CCC*H*CHCH), 7.84 (1 H, d, *J* 1.4, OCH$_2$CCC*H*), 7.96 (2 H, d, *J* 8.3, OCCHC*H*), 8.37 (1 H, d, *J* 9.3, NHCC*H*); ^{13}C NMR ($CDCl_3/CD_3OD$ 1:1, 150 MHz) δ 20.4 (CH_2), 26.1 (CH_2), 26.9 (CH_2), 29.5 (CH_2), 29.8 (CH_2), 32.1 (CH_2), 36.4 (CH_2), 37.0 (CH_2), 40.4 (CH_2), 40.4 (CH_2), 41.6 (CH_2), 43.8 (CH_2), 57.0 (CH), 58.0 (CH_2), 61.6 (CH), 63.3 (CH), 66.8 (CH_2), 69.4 (CH_2), 70.9 (CH_2), 71.4 (CH_2), 71.4 (CH_2), 79.1 (C), 97.8 (C), 117.0 (CH), 119.7 (CH_2), 123.8 (C), 126.7 (C), 127.1 (CH), 127.2 (CH), 128.3 (CH), 129.9 (CH), 130.8 (CH), 131.3 (CH), 133.1 (CH), 133.3 (CH), 134.4 (CH), 135.0 (CH), 138.2 (C), 143.8 (C), 153.8 (C), 153.9 (C), 158.9 (C), 162.3 (C), 165.8 (C=O), 173.8 (C=O), 175.1 (C=O), 175.9 (C=O), 197.9 (C=O) * 1 CH_2 absent; *m*/*z* (ES+) 974.4557 (M+H, $C_{53}H_{64}N_7O_9S$ requires 974.4486), 788 (50).

6-[4-(4-Benzoyl-2-{5-[2-(2-{2-[5-(2-oxo-1,3,3a,4,6,6a-hexahydro-thieno[3,4-*d*]imidazol-6-yl)-pentanoylamino]-ethoxy}-ethoxy)-ethylcarbamoyl]-pent-1-ynyl}-benzyloxy)-phenyl]-2-(3-carboxy-propyl)-pyridazinium chloride (226)



To a solution of **225** (7.0 mg, 0.0065 mmol) in THF (0.5 mL) and water (0.5 mL) was added NaOH (1.0 mg, 0.025 mmol) at rt. The resulting mixture was stirred at 50 °C for 3 h. After cooling to 10 °C, the reaction mixture was washed with ethyl acetate (2 mL). The aqueous layer was separated and treated with aqueous HCl (0.1M) to adjust the pH to 1.0 at rt. The resulting mixture was then stirred at 0°C for 1 h, and the solvent was removed *in vacuo*. The reaction vessel was then triturated with water (3 × 2 mL), and the residue was dried under high vacuum to give the *ester* **226** (3.3 mg, 0.0034 mmol, 53%) as a white solid. m.p. degraded >150 °C; R_f = 0.10 (CH_3Cl/CH_3OH 8:2); $[\alpha]_D^{20.0}$ +20.0 (*c* 0.05, CH_3OH); IR υ_{max} (film)/cm^{-1} 3359, 2143, 1730, 1644, 1457; ^1H NMR ($CDCl_3/CD_3OD$ 1:1, 600 MHz) δ 1.45-1.66 (6 H, m, NHC=OCH$_2$C*H*$_2$C*H*$_2$C*H*$_2$), 1.91 (2 H, quint, *J* 7.4, NHC=OCH$_2$C*H*$_2$CH$_2$CC), 2.16 (4 H, m, NHC=OC*H*$_2$CH$_2$CH$_2$CH$_2$ and NHC=OC*H*$_2$CH$_2$CH$_2$CC), 2.36 (2 H, t, *J* 6.8, NNCH$_2$C*H*$_2$CH$_2$), 2.50-2.57 (4 H, m, NHC=OCH$_2$CH$_2$C*H*$_2$CC and NNCH$_2$CH$_2$C*H*$_2$), 2.69 (1 H, d, *J* 12.6, SC*H*H), 2.89 (1 H, dd, *J* 12.6 and 4.7, SCH*H*), 3.13 (1 H, m, SC*H*), 3.35 (4 H, m, 2 × NHC*H*$_2$CH$_2$O), 3.51-3.59 (8 H, m, 4 × OC*H*$_2$), 4.28 (1 H, m, CHNHC=ONHC*H*), 4.42-4.48 (3 H, m, C*H*NHC=ONHCH and NNC*H*$_2$CH$_2$CH$_2$), 5.35 (2 H, s, OC*H*$_2$C), 7.14 (2 H, d, *J* 8.3, OCC*H*), 7.52 (2 H, dd, *J* 7.6 and 7.5, O=CCCHC*H*CH), 7.65-7.69 (3 H, m, O=CCCHCHC*H,*

OCH_2CCH, OCH_2CCHCH), 7.71 (1 H, d, *J* 9.3, NHCCHC*H*), 7.76 (2 H, d, *J* 7.6, O=CCC*H*CHCH), 7.81 (1 H, s, OCH_2CCCH), 7.92 (2 H, d, *J* 8.3, OCCHC*H*), 8.22 (1 H, d, *J* 9.3, NHCC*H*); ^{13}C NMR ($CDCl_3/CD_3OD$ 1:1, 150 MHz) δ 20.2 (CH_2), 26.0 (CH_2), 26.9 (CH_2), 29.5 (CH_2), 30.9 (CH_2), 31.7 (CH_2), 36.3 (CH_2), 37.0 (CH_2), 40.1 (CH_2), 40.3 (CH_2), 40.4 (CH_2), 57.0 (CH_2), 59.2 (CH), 61.6 (CH), 63.3 (CH), 69.4 (CH_2), 69.5 (CH_2), 70.8 (CH_2), 71.4 (CH_2), 71.4 (CH_2), 79.2 (C), 97.6 (C), 116.9 (CH), 123.7 (C), 126.6 (C), 126.8 (CH), 128.4 (CH), 129.6 (CH), 129.8 (CH), 130.7 (CH), 131.3 (CH), 132.8 (CH), 134.4 (CH), 134.8 (CH), 138.2 (C), 144.0 (C), 152.1 (C), 153.8 (C), 158.9 (C), 162.7 (C), 169.8 (C=O), 175.9 (C=O), 176.5 (C=O), 177.1 (C=O), 198.0 (C=O) * 1 CH_2 absent; *m*/*z* (ES+) 956.3971 ($[MNa]^+$, $C_{50}H_{59}N_7O_9SNa$ requires 956.3993), 933 (15).

4 References

1. Purves, D.; Augustive, G. J.; Fitzpatrick, D., *Synaptic Transmission*. 4th ed.; Sinauer Associates: Sunderland, MA, **2001**.

2. Rang, H. P.; Dale, M. M.; Ritter, J. M.; Flower, R. J., *Pharmacology*. 6th ed.; **2007**.

3. Zubay, G. L., *Biochemistry*. 4th ed.; Wm. C. Brown Publishers: **1996**.

4. Krogsgaard-Larsen, P.; Frølund, B.; Jorgensen, F. S.; Schousboe, A., *J. Med. Chem.,* **1994**, *37* (16), 2489-2505.

5. Mehta, A. K.; Ticku, M. K., *Brain Res. Rev.,* **1999**, *29* (2-3), 196-217.

6. Bowery, N. G.; Smart, T. G., *Br. J. Pharmacol.,* **2006**, *147* (1), S109-S119.

7. Roberts, E.; Frankel, S., *J. Biol. Chem.,* **1950**, *187* (1), 55-63.

8. Krnjevic, K.; Schwartz, S., *Exp. Brain Res.,* **1967**, *3* (4), 320-336.

9. Chebib, M.; Johnston, G. A. R., *J. Med. Chem.,* **2000**, *43* (8), 1427-1447.

10. Foster, A. C.; Kemp, J. A., *Curr. Opin. Pharmacol.,* **2006**, *6* (1), 5-6.

11. Foster, A. C.; Kemp, J. A., *Curr. Opin. Pharmacol.,* **2006**, *6* (1), 7-17.

12. Whiting, P. J., *Curr. Opin. Pharmacol.,* **2006**, *6* (1), 24-29.

13. Vogt, K. E.; Prenosil, G. A.; Gasser, E. M. S.; Rudolph, U.; Keist, R.; Fritschy, J. M., *J. Neurophysiol.,* **2006**, *96* (2), 846-857.

14. Fritschy, J. M.; Brünig, I., *Pharmacol. Ther.,* **2003**, *98* (3), 299-323.

15. Stell, B. M.; Mody, I., *J. Neurosci.,* **2002**, *22* (10), RC223.

16. Sine, S. M.; Engel, A. G., *Nature,* **2006**, *440* (7083), 448-455.

17. Bowery, N. G.; Smart, T. G., *Br. J. Pharmacol.,* **2006**, *147*, S109-S119.

18. Johnston, G. A. R., *Pharmacol. Ther.,* **1996**, *69* (3), 173-198.

19. Qian, H. H.; Ripps, H., *Proceedings of the Royal Society B,* **1999**, *266* (1436), 2419-2425.

20. McKernan, R. M.; Whiting, P. J., *Trends Neurosci.,* **1996**, *19* (4), 139-143.

21. Baude, A.; Sequier, J. M.; McKernan, R. M.; Olivier, K. R.; Somogyi, P., *Neuroscience,* **1992**, *51* (4), 739-748.

22. Marowsky, A.; Fritschy, J. M.; Vogt, K. E., *Eur. J. Neurosci.,* **2004**, *20* (5), 1281-1289.

23. Rissman, R. A.; Mishizen-Eberz, A. J.; Carter, T. L.; Wolfe, B. B.; De Blas, A. L.; Miralles, C. P.; Ikonomovic, M. D.; Armstrong, D. M., *Neuroscience,* **2003**, *120* (3), 695-704.

24. Machu, T. K.; Olsen, R. W.; Browning, M. D., *J. Neurochem.,* **1993**, *61* (6), 2034-2040.

25. Maddox, F. N.; Valeyev, A. Y.; Poth, K.; Holohean, A. M.; Wood, P. M.; Davidoff, R. A.; Hackman, J. C.; Luetje, C. W., *Dev. Brain Res.,* **2004**, *149* (2), 143-151.

26. Feng, H. J.; Botzolakis, E. J.; Macdonald, R. L., *Neuropharmacology,* **2009**, *56* (1), 161-173.

27. Takehara, A.; Hosokawa, M.; Eguchi, H.; Ohigashi, H.; Ishikawa, O.; Nakamura, Y.; Nakagawa, H., *Cancer Res.,* **2007**, *67* (20), 9704-9712.

28. Fritschy, J. M.; Benke, D.; Mertens, S.; Oertel, W. H.; Bachi, T.; Mohler, H., *Proc. Natl. Acad. Sci. U. S. A.,* **1992**, *89* (15), 6726-6730.

29. Mortensen, M.; Patel, B.; Smart, T. G., *Front. Cell. Neurosci.,* **2012**, *6* (1), 1-10.

30. Cherubini, E.; Conti, F., *Trends Neurosci.,* **2001**, *24* (3), 155-162.

31. Kanematsu, T.; Terunuma, M.; Goto, H.; Kuratani, A.; Hirata, M., *Folia Pharmacol. Jpn.,* **2004**, *123* (2), 105-112.

32. Bavro, V. N.; Sola, M.; Bracher, A.; Kneussel, M.; Betz, H.; Weissenhorn, W., *EMBO Rep.,* **2002**, *3* (2), 183-189.

33. Chen, L.; Wang, H. B.; Vicini, S.; Olsen, R. W., *Proc. Natl. Acad. Sci. U. S. A.,* **2000**, *97* (21), 11557-11562.

34. Kittler, J. T.; Rostaing, P.; Schiavo, G.; Fritschy, J. M.; Olsen, R.; Triller, A.; Moss, S. J., *Mol. Cell. Neurosci.,* **2001**, *18* (1), 13-25.

35. Luscher, B.; Keller, C. A., *Pharmacol. Ther.,* **2004**, *102* (3), 195-221.

36. Moss, S. J.; Smart, T. G., *Nat. Rev. Neurosci.,* **2001**, *2* (4), 240-250.

37. Bedford, F. K.; Kittler, J. T.; Muller, E.; Thomas, P.; Uren, J. M.; Merlo, D.; Wisden, W.; Triller, A.; Smart, T. G.; Moss, S. J., *Nat. Neurosci.,* **2001**, *4* (9), 908-916.

38. Kittler, J. T.; Chen, G. J.; Honing, S.; Bogdanov, Y.; McAinsh, K.; Arancibia-Carcamo, I. L.; Jovanovic, J. N.; Pangalos, M. N.; Haucke, V.; Yan, Z.; Moss, S. J., *Proc. Natl. Acad. Sci. U. S. A.,* **2005**, *102* (41), 14871-14876.

39. Thomas, P.; Mortensen, M.; Hosie, A. M.; Smart, T. G., *Nat. Neurosci.,* **2005**, *8* (7), 889-897.

40. Bredt, D. S.; Nicoll, R. A., *Neuron,* **2003**, *40* (2), 361-379.

41. Cull-Candy, S.; Kelly, L.; Farrant, M., *Curr. Opin. Neurobiol.,* **2006**, *16* (3), 288-297.

42. Lu, W.; Shi, Y.; Jackson, A. C.; Bjorgan, K.; During, M. J.; Sprengel, R.; Seeburg, P. H.; Nicoll, R. A., *Neuron,* **2009**, *62* (2), 254-268.

43. Petrini, E. M.; Lu, J. Y.; Cognet, L.; Lounis, B.; Ehlers, M. D.; Choquet, D., *Neuron,* **2009**, *63* (1), 92-105.

44. Chambers, J. J.; Gouda, H.; Young, D. M.; Kuntz, I. D.; England, P. M., *J. Am. Chem. Soc.,* **2004**, *126* (43), 13886-13887.

45. England, P. M., *Sci. STKE,* **2006**, *2006* (331), pl1.

46. Vytla, D.; Combs-Bachmann, R.; Hussey, A.; Hafez, I.; Chambers, J. J., *Org. Biomol. Chem.,* **2011**, *9* (20), 7151-7161.

47. Michelot, D.; Melendez-Howell, L. M., *Mycol. Res.,* **2003**, *107* (2), 131-146.

48. Brehm, L.; Frydenvang, K.; Krogsgaard-Larsen, P.; Liljefors, T., *Struct. Chem.,* **1998**, *9* (2), 149-155.

49. Arnt, J.; Scheel-Kruger, J.; Magelund, G.; Krogsgaard-Larsen, P., *J. Pharm. Pharmacol.,* **1979**, *31* (5), 306-313.

50. Storustovu, S. I.; Ebert, B., *Eur. J. Pharmacol.,* **2003**, *467* (1-3), 49-56.

51. Collins, J. F.; McDonald, J. A.; Newton, R. F., *Brain Res. Bull.,* **1980**, *5* (2), 141-144.

52. Lodge, D.; Curtis, D. R.; Johnston, G. A. R., *J. Neurochem.,* **1978**, *31* (6), 1525-1528.

53. Mortensen, M.; Kristiansen, U.; Ebert, B.; Frølund, B.; Krogsgaard-Larsen, P.; Smart, T. G., *J. Physiol.,* **2004**, *557* (2), 389-413.

54. Kelly, P. A. T.; McCulloch, J., *J. Neurochem.,* **1982**, *39* (3), 613-624.

55. Lader, M. H.; Psych, F. R. C., *Eur. Neuropsychopharmacol.,* **1999**, *9* (6), S399-S405.

56. Mandelli, M.; Tognoni, G.; Garattini, S., *Clin. Pharmacokinet.,* **1978**, *3* (1), 72-91.

57. Da, S. F.; Taliani, S.; Trincavelli, M. L.; Montali, M.; Martini, C., *Curr. Med. Chem.,* **2007**, *14* (25), 2680-2701.

58. Sigel, E., *Med. Chem. Rev.,* **2005**, *2* (3), 251-256.

59. Lambert, J. J.; Belelli, D.; Hill-Venning, C.; Peters, J. A., *Trends Pharmacol. Sci.,* **1995**, *16* (9), 295-303.

60. Rupprecht, R.; Hauser, C. A. E.; Trapp, T.; Holsboer, F., *J. Steroid Biochem. Mol. Biol.,* **1996**, *56* (1-6), 163-168.

61. Smart, T. G.; Hosie, A. M.; Wilkins, M. E., *Pharmacol. Ther.,* **2007**, *116* (1), 7-19.

62. Hosie, A. M.; Wilkins, M. E.; da, S. H. M. A.; Smart, T. G., *Nature,* **2006**, *444* (7118), 486-489.

63. Harrison, N. L.; Majewska, M. D.; Harrington, J. W.; Barker, J. L., *J. Pharmacol. Exp. Ther.,* **1987**, *241* (1), 346-353.

64. Biagini, G.; Panuccio, G.; Avoli, M., *Curr. Opin. Neurol.,* **2010**, *23* (2), 170-176.

65. Reddy, D. S., *Neuroscience,* **2006**, *138* (3), 911-920.

66. Chambon, J. P.; Feltz, P.; Heaulme, M.; Restle, S.; Schlichter, R.; Biziere, K.; Wermuth, C. G., *Proc. Natl. Acad. Sci. U. S. A.,* **1985**, *82* (6), 1832-1836.

67. Hunt, P.; Clements-Jewery, S., *Neuropharmacology,* **1981**, *20* (4), 357-361.

68. Tunnicliff, G.; Ngo, T. T., *J. Neurochem.,* **1982**, *39* (4), 998-1000.

69. Zhang, J.; Xu, R., *J. Nat. Prod.,* **1988**, *51* (6), 1241-1242.

70. Heaulme, M.; Chambon, J. P.; Leyris, R.; Molimard, J. C.; Wermuth, C. G.; Biziere, K., *Brain Res.,* **1986**, *384* (2), 224-231.

71. Wermuth, C. G.; Biziere, K., *Trends Pharmacol. Sci.,* **1986**, *7* (1), 421-424.

72. Wermuth, C. G.; Bourguignon, J. J.; Schlewer, G.; Gies, J. P.; Schoenfelder, A.; Melikian, A.; Bouchet, M. J.; Chantreux, D.; Molimard, J. C.; Heaulme, M.; Chambon, J. P.; Biziere, K., *J. Med. Chem.,* **1987**, *30* (2), 239-249.

73. Heaulme, M.; Chambon, J. P.; Leyris, R.; Wermuth, C. G.; Biziere, K., *J. Neurochem.,* **1987**, *48* (6), 1677-1686.

74. Melikian, A.; Schlewer, G.; Hurt, S.; Chantreux, D.; Wermuth, C. G., *J. Labelled Compd. Radiopharm.,* **1987**, *24* (3), 267-274.

75. Ueno, S.; Bracamontes, J.; Zorumski, C.; Weiss, D. S.; Steinbach, J. H., *J. Neurosci.,* **1997**, *17* (2), 625-634.

76. Brickley, S. G.; Revilla, V.; Cull-Candy, S. G.; Wisden, W.; Farrant, M., *Nature,* **2001**, *409* (6816), 88-92.

77. Cope, D. W.; Hughes, S. W.; Crunelli, V., *J. Neurosci.,* **2005**, *25* (50), 11553-11563.

78. Tamas, G.; Lorincz, A.; Simon, A.; Szabadics, J., *Science,* **2003**, *299* (5614), 1902-1905.

79. Wei, W.; Hamby, A. M.; Zhou, K.; Feller, M. B., *Nature,* **2011**, *469* (7330), 402-406.

80. Martin, R. J.; Sitamze, J.-M.; Duittoz, A. H.; Wermuth, C. G., *Eur. J. Pharmacol.,* **1995**, *276* (1-2), 9-19.

81. Rognan, D.; Boulanger, T.; Hoffmann, R.; Vercauteren, D. P.; Andre, J. M.; Durant, F.; Wermuth, C. G., *J. Med. Chem.,* **1992**, *35* (11), 1969-1977.

82. Kardos, J.; Blasko, G.; Kerekes, P.; Kovacs, I.; Simonyi, M., *Biochem. Pharmacol.,* **1984**, *33* (22), 3537-3545.

83. Melikian, A.; Schlewer, G.; Chambon, J. P.; Wermuth, C. G., *J. Med. Chem.,* **1992**, *35* (22), 4092-7.

84. Pitarch, L.; Coronas, R.; Mallol, J., *Eur. J. Med. Chem.,* **1974**, *9* (6), 644-650.

85. Ishida, A.; Homma, K.; Kono, H.; Tamura, K.; Sasaki, Y. EP579059A1, **1994**.

86. Schacht, E.; Kurmeier, H.-A.; Gante, J.; Lissner, R.; Melzer, G.; Orth, D. EP10156A1, **1980**.

87. Friesz, A.; Csermely, G. HU6801, **1973**.

88. Takeshiba, H.; Kinoto, T.; Jojima, T. EP89650A1, **1983**.

89. Nomoto, Y.; Takai, H.; Ohno, T.; Kubo, K. EP326307A2, **1989**.

90. Slater, R. A.; Howson, W.; Swayne, G. T. G.; Taylor, E. M.; Reavill, D. R., *J. Med. Chem.,* **1988**, *31* (2), 345-351.

91. Albright, J. D.; Moran, D. B.; Wright, W. B.; Collins, J. B.; Beer, B.; Lippa, A. S.; Greenblatt, E. N., *J. Med. Chem.,* **1981**, *24* (5), 592-600.

92. Sitamze, J. M.; Schmitt, M.; Wermuth, C. G., *J. Org. Chem.,* **1992**, *57* (11), 3257-3258.

93. Guery, S.; Parrot, I.; Rival, Y.; Wermuth, C. G., *Tetrahedron Lett.,* **2001**, *42* (11), 2115-2117.

94. Maes, B. U. W.; Lemiere, G. L. F.; Dommisse, R.; Augustyns, K.; Haemers, A., *Tetrahedron,* **2000**, *56* (12), 1777-1781.

95. Parrot, I.; Ritter, G.; Wermuth, C. G.; Hibert, M., *Synlett,* **2002**, *7*, 1123-1127.

96. Parrot, I.; Rival, Y.; Wermuth, C. G., *Synthesis,* **1999**, *7*, 1163-1168.

97. Helm, M. D.; Moore, J. E.; Plant, A.; Harrity, J. P. A., *Angew. Chem. Int. Ed.,* **2005**, *44* (25), 3889-3892.

98. Lin, S.; Liu, Z.; Hu, Y., *J. Comb. Chem.,* **2007**, *9* (5), 742-744.

99. Barlin, G. B., *J. Chem. Soc., Perkin Trans. 1,* **1976**, *13*, 1424-1429.

100. Gavande, N.; Johnston, G. A. R.; Hanrahan, J. R.; Chebib, M., *Org. Biomol. Chem.,* **2011**, *8* (18), 4131-4136.

101. Hashimoto, M.; Hatanaka, Y., *Eur. J. Org. Chem.,* **2008**, *15*, 2513-2523.

102. Hosoya, T.; Inoue, A.; Hiramatsu, T.; Aoyama, H.; Ikemoto, T.; Suzuki, M., *Bioorg. Med. Chem.,* **2009**, *17* (6), 2490-2496.

103. Brunner, J., *Annu. Rev. Biochem.,* **1993**, *62* (1), 483-514.

104. Tanaka, Y.; Bond, M. R.; Kohler, J. J., *Mol. BioSyst.,* **2008**, *4* (6), 473-480.

105. Fuwa, H.; Takahashi, Y.; Konno, Y.; Watanabe, N.; Miyashita, H.; Sasaki, M.; Natsugari, H.; Kan, T.; Fukuyama, T.; Tomita, T.; Iwatsubo, T., *ACS Chem. Biol.,* **2007**, *2* (6), 408-418.

106. Kinoshita, T.; Cano-Delgado, A.; Seto, H.; Hiranuma, S.; Fujioka, S.; Yoshida, S.; Chory, J., *Nature,* **2005**, *433* (7022), 167-171.

107. Kotake, Y.; Sagane, K.; Owa, T.; Mimori-Kiyosue, Y.; Shimizu, H.; Uesugi, M.; Ishihama, Y.; Iwata, M.; Mizui, Y., *Nat. Chem. Biol.,* **2007**, *3* (9), 570-575.

108. Liang, T. Y.; Schuster, G. B., *J. Am. Chem. Soc.,* **1987**, *109* (25), 7803-7810.

109. Schrock, A. K.; Schuster, G. B., *J. Am. Chem. Soc.,* **1984**, *106* (18), 5228-5234.

110. Sigman, M. E.; Autrey, T.; Schuster, G. B., *J. Am. Chem. Soc.,* **1988**, *110* (13), 4297-4305.

111. Poe, R.; Schnapp, K.; Young, M. J. T.; Grayzar, J.; Platz, M. S., *J. Am. Chem. Soc.,* **1992**, *114* (13), 5054-5067.

112. Nielsen, P. E.; Buchardt, O., *Photochem. Photobiol.,* **1982**, *35* (3), 317-323.

113. Demko, Z. P.; Sharpless, K. B., *Angew. Chem. Int. Ed.,* **2002**, *41* (12), 2110-2113.

114. Grimes, K. D.; Gupte, A.; Aldrich, C. C., *Synthesis,* **2010**, *9*, 1441-1448.

115. Seidman, C. E.; Hess, H. J.; Homcy, C. J.; Graham, R. M., *Biochemistry,* **1984**, *23* (16), 3765-3770.

116. Namanja, H. A.; Emmert, D.; Davis, D. A.; Campos, C.; Miller, D. S.; Hrycyna, C. A.; Chmielewski, J., *J. Am. Chem. Soc.,* **2012**, *134* (6), 2976-2980.

117. Blanton, M. P.; Wang, H. H., *Biochemistry,* **1990**, *29* (5), 1186-1194.

118. Andrus, M. B.; Turner, T. M.; Sauna, Z. E.; Ambudkar, S. V., *Bioorg. Med. Chem. Lett.,* **2000**, *10* (20), 2275-2278.

119. Radeke, H. S.; Snapper, M. L., *Bioorg. Med. Chem.,* **1998**, *6* (8), 1227-1232.

120. Shen, R. C.; Inoue, T.; Forgac, M.; Porco, J. A., *J. Org. Chem.,* **2005**, *70* (9), 3686-3692.

121. Vera, M. D.; Pfizenmayer, A. J.; Ding, X. B.; Ahuja, D.; Toogood, P. L.; Joullie, M. M., *Bioorg. Med. Chem. Lett.,* **2001**, *11* (14), 1871-1874.

122. Kanowski, S.; Herrmann, W. M.; Stephan, K.; Wierich, W.; Horr, R., *Pharmacopsychiatry,* **1996**, *29* (2), 47-56.

123. Strømgaard, K.; Saito, D. R.; Shindou, H.; Ishii, S.; Shimizu, T.; Nakanishi, K., *J. Med. Chem.,* **2002**, *45* (18), 4038-4046.

124. Galardy, R. E.; Craig, L. C.; Printz, M. P., *Nat. New. Biol.,* **1973**, *242* (117), 127-128.

125. Hermanson, G. T., *Bioconjugate Techniques*. 2nd ed.; Academic Press: **2008**.

126. Hisamoto, K.; Hiraga, Y.; Abe, M., *Photochem. Photobiol. Sci., 10* (9), 1469-1473.

127. Yabuno, Y.; Hiraga, Y.; Abe, M., *Chem. Lett.,* **2008**, *37* (8), 822-823.

128. Rubidge, C. R.; Qua, N. C., *J. Am. Chem. Soc.,* **1914**, *36* (4), 732-737.

129. Dorman, G.; Prestwich, G. D., *Biochemistry,* **1994**, *33* (19), 5661-5673.

130. Bosse, R.; Servant, G.; Zhou, L. M.; Boulay, G.; Guillemette, G.; Escher, E., *Regul. Pept.,* **1993**, *44* (2), 215-223.

131. Servant, G.; Boulay, G.; Bosse, R.; Escher, E.; Guillemette, G., *Mol. Pharmacol.,* **1993**, *43* (5), 677-683.

132. Keutmann, H. T.; Rubin, D. A., *Endocrinology,* **1993**, *132* (3), 1305-1312.

133. Shoelson, S. E.; Lee, J. S.; Lynch, C. S.; Backer, J. M.; Pilch, P. F., *J. Biol. Chem.,* **1993**, *268* (6), 4085-4091.

134. Adams, A. E.; Pines, M.; Nakamoto, C.; Behar, V.; Yang, Q. M.; Bessalle, R.; Chorev, M.; Rosenblatt, M.; Levine, M. A.; Suva, L. J., *Biochemistry,* **1995**, *34* (33), 10553-10559.

135. Nakamoto, C.; Behar, V.; Chin, K. R.; Adams, A. E.; Suva, L. J.; Rosenblatt, M.; Chorev, M., *Biochemistry,* **1995**, *34* (33), 10546-10552.

136. Suva, L. J.; Flannery, M. S.; Caulfield, M. P.; Findlay, D. M.; Juppner, H.; Goldring, S. R.; Rosenblatt, M.; Chorev, M., *J. Pharmacol. Exp. Ther.,* **1997**, *283* (2), 876-884.

137. Zhang, X.; Marchand, C.; Pommier, Y.; Burke Jr, T. R., *Bioorg. Med. Chem. Lett.,* **2004**, *14* (5), 1205-1207.

138. Bringmann, G.; Gampe, C. M.; Reichert, Y.; Bruhn, T.; Faber, J. H.; Mikyna, M.; Reichert, M.; Leippe, M.; Brun, R.; Gelhaus, C., *J. Med. Chem.,* **2007**, *50* (24), 6104-6115.

139. Kato, E.; Howitt, R.; Dzyuba, S. V.; Nakanishi, K., *Org. Biomol. Chem.,* **2007**, *5* (23), 3758-3761.

140. DeSantis, G.; Paech, C.; Jones, J. B., *Bioorg. Med. Chem.,* **2000**, *8* (3), 563-570.

141. Bretonnet, A.-S.; Jochum, A.; Walker, O.; Krimm, I.; Goekjian, P.; Marcillat, O.; Lancelin, J.-M., *J. Med. Chem.,* **2007**, *50* (8), 1865-1875.

142. Gomaa, M. S.; Armstrong, J. L.; Bobillon, B.; Veal, G. J.; Brancale, A.; Redfern, C. P. F.; Simons, C., *Bioorg. Med. Chem.,* **2008**, *16* (17), 8301-8313.

143. Houpis, I. N.; Liu, R.; Wu, Y.; Yuan, Y.; Wang, Y.; Nettekoven, U., *J. Org. Chem.,* **2010**, *75* (20), 6965-6968.

144. Kalai, T.; Jeko, J.; Berente, Z.; Hideg, K., *Synthesis,* **2006**, *3*, 439-446.

145. Le, Q. S. T.; Nielsen, T. E.; Meldal, M., *J. Comb. Chem.,* **2008**, *10* (3), 447-455.

146. Aguado, L.; Thibaut, H. J.; Priego, E.-M.; Jimeno, M.-L.; Camarasa, M.-J.; Neyts, J.; Perez-Perez, M.-J., *J. Med. Chem.,* **2010**, *53* (1), 316-324.

147. Smith, R. A. G.; Knowles, J. R., *J. Am. Chem. Soc.,* **1973**, *95* (15), 5072-5073.

148. Brunner, J.; Senn, H.; Richards, F. M., *J. Biol. Chem.,* **1980**, *255* (8), 3313-3318.

149. Nassal, M., *J. Am. Chem. Soc.,* **1984**, *106* (24), 7540-7545.

150. Creary, X., *J. Org. Chem.,* **1987**, *52* (22), 5026-5030.

151. Ansong, O.; Antoine, M. D.; Nwokogu, G. C.; Hergenrother, P. M., *J. Org. Chem.,* **1994**, *59* (9), 2506-2510.

152. Bender, T.; Huss, M.; Wieezorek, H.; Grond, S.; von Zezschwitz, P., *Eur. J. Org. Chem.,* **2007**, 3870-3878.

153. Hatanaka, Y.; Hashimoto, M.; Kurihara, H.; Nakayama, H.; Kanaoka, Y., *J. Org. Chem.,* **1994**, *59* (2), 383-387.

154. Ambroise, Y.; Pillon, F.; Mioskowski, C.; Valleix, A.; Rousseau, B., *Eur. J. Org. Chem.,* **2001**, *20*, 3961-3964.

155. Nakashima, H.; Hashimoto, M.; Sadakane, Y.; Tomohiro, T.; Hatanaka, Y., *J. Am. Chem. Soc.,* **2006**, *128* (47), 15092-15093.

156. Hashimoto, M.; Kato, Y. H.; Hatanaka, Y., *Chem. Pharm. Bull.,* **2007**, *55* (10), 1540-1543.

157. Bentz, E. L.; Gibson, H.; Hudson, C.; Moloney, M. G.; Seldon, D. A.; Wearmouth, E. S., *Synlett,* **2006**, *2*, 247-250.

158. Bayley, H., *Chemistry Of Diazirines*. CRC Press: **1987**; Vol. 2.

159. Bonk, I.; Ruhmann, A., *Eur. J. Biochem.,* **2000**, *267* (10), 3017-3024.

160. Topin, A. N.; Gritsenko, O. M.; Brevnov, M. G.; Gromova, E. S.; Korshunova, G. A., *Nucleosides Nucleotides,* **1998**, *17* (7), 1163-1175.

161. Qiu, Z. H.; Lu, L. H.; Jian, X.; He, C., *J. Am. Chem. Soc.,* **2008**, *130* (44), 14398-14399.

162. Sergiev, P.; Dokudovskaya, S.; Romanova, E.; Topin, A.; Bogdanov, A.; Brimacombe, R.; Dontsova, O., *Nucleic Acids Res.,* **1998**, *26* (11), 2519-2525.

163. Yamaguchi, T.; Saneyoshi, M., *Nucleic Acids Res.,* **1996**, *24* (17), 3364-3369.

164. Zofall, M.; Bartholomew, B., *Nucleic Acids Res.,* **2000**, *28* (21), 4382-4390.

165. Luo, L.; Parrish, C. A.; Nevins, N.; McNulty, D. E.; Chaudhari, A. M.; Carson, J. D.; Sudakin, V.; Shaw, A. N.; Lehr, R.; Zhao, H.; Sweitzer, S.; Lad, L.; Wood, K. W.; Sakowicz, R.; Annan, R. S.; Huang, P. S.; Jackson, J. R.; Dhanak, D.; Copeland, R. A.; Auger, K. R., *Nat. Chem. Biol.,* **2007**, *3* (11), 722-726.

166. Sammelson, R. E.; Casida, J. E., *J. Org. Chem.,* **2003**, *68* (21), 8075-8079.

167. Husain, S. S.; Nirthanan, S.; Ruesch, D.; Solt, K.; Cheng, Q.; Li, G.-D.; Arevalo, E.; Olsen, R. W.; Raines, D. E.; Forman, S. A.; Cohen, J. B.; Miller, K. W., *J. Med. Chem.,* **2006**, *49* (16), 4818-4825.

168. Husain, S. S.; Stewart, D.; Desai, R.; Hamouda, A. K.; Li, S. G.-D.; Kelly, E.; Dostalova, Z.; Zhou, X.; Cotten, J. F.; Raines, D. E.; Olsen, R. W.; Cohen, J. B.; Forman, S. A.; Miller, K. W., *J. Med. Chem.,* **2010**, *53* (17), 6432-6444.

169. Cavalla, D.; Neff, N. H., *J. Neurochem.,* **1985**, *44* (3), 916-921.

170. Frølund, B.; Ebert, B.; Lawrence, L. W.; Hurt, S. D.; Krogsgaard-Larsen, P., *J. Labelled Compd. Radiopharm.,* **1995**, *36* (9), 877-889.

171. Nielsen, M.; Witt, M. R.; Ebert, B.; Krogsgaard-Larsen, P., *Eur. J. Pharmacol.,* **1995**, *289* (1), 109-112.

172. Krogsgaard-Larsen, P.; Frølund, B.; Kristiansen, U.; Frydenvang, K.; Ebert, B., *Eur. J. Pharm. Sci.,* **1997**, *5* (6), 355-384.

173. Li, X.; Cao, J.-H.; Li, Y.; Rondard, P.; Zhang, Y.; Yi, P.; Liu, J.-F.; Nan, F.-J., *J. Med. Chem.,* **2008**, *51* (11), 3057-3060.

174. Maes, B. U. W.; Kosmrlj, J.; Lemiere, G. L. F., *J. Heterocycl. Chem.,* **2002**, *39* (3), 535-543.

175. Chaleix, V.; Sol, V.; Huang, Y. M.; Guilloton, M.; Granet, R.; Blais, J. C.; Krausz, P., *Eur. J. Org. Chem.,* **2003**, *8*, 1486-1493.

176. Patel, R. N.; Goswami, A.; Chu, L.; Donovan, M. J.; Nanduri, V.; Goldberg, S.; Johnston, R.; Siva, P. J.; Nielsen, B.; Fan, J. Y.; He, W. X.; Shi, Z. P.; Wang, K. W.; Eiring, R.; Cazzulino, D.; Singh, A.; Mueller, R., *Tetrahedron: Asymmetry,* **2004**, *15* (8), 1247-1258.

177. Gellerman, G.; Elgavi, A.; Salitra, Y.; Kramer, I., *J. Pept. Res.,* **2001**, *57* (4), 277-291.

178. Seki, M.; Kondo, K.; Kuroda, T.; Yamanaka, T.; Iwasaki, T., *Synlett,* **1995**, *6*, 609-611.

179. Kaupp, G.; Naimi-Jamal, M. R.; Stepanenko, V., *Chem. Eur. J,* **2003**, *9* (17), 4156-4160.

180. Morandi, S.; Caselli, E.; Forni, A.; Bucciarelli, M.; Torre, G.; Prati, F., *Tetrahedron: Asymmetry,* **2005**, *16* (17), 2918-2926.

181. Jin, R. Z.; Bian, Z.; Kang, C. Q.; Guo, H. Q.; Gao, L. X., *Synth. Commun.,* **2005**, *35* (14), 1897-1902.

182. Mortensen, M.; Ebert, B.; Wafford, K.; Smart, T. G., *J. Physiol.,* **2010**, *588* (8), 1251-1268.

183. Mortensen, M.; Smart, T. G., *Nat. Protoc.,* **2007**, *2* (11), 2826-2841.

184. Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J., *Pflug. Arch. Eur. J. Phy.,* **1981**, *391* (2), 85-100.

185. Klausberger, T.; Ehya, N.; Fuchs, K.; Fuchs, T.; Ebert, V.; Sarto, I.; Sieghart, W., *J. Biol. Chem.,* **2001**, *276* (19), 16024-16032.

186. Ueno, S.; Zorumski, C.; Bracamontes, J.; Steinbach, J. H., *Mol. Pharmacol.,* **1996**, *50* (4), 931-938.

187. Zorumski, C. F.; Wittmer, L. L.; Isenberg, K. E.; Hu, Y. F.; Covey, D. F., *Neuropharmacology,* **1996**, *35* (9-10), 1161-1168.

188. Iqbal, F.; Ellwood, R.; Mortensen, M.; Smart, T. G.; Baker, J. R., *Bioorg. Med. Chem. Lett.,* **2011**, *21* (14), 4252-4254.

189. Aoki, S.; Matsuo, N.; Hanaya, K.; Yamada, Y.; Kageyama, Y., *Bioorg. Med. Chem.,* **2009**, *17* (9), 3405-3413.

190. Blart, E.; Genet, J. P.; Safi, M.; Savignac, M.; Sinou, D., *Tetrahedron,* **1994**, *50* (2), 505-514.

191. Hoffmann, R. W.; Ditrich, K., *Liebigs Ann. Chem.,* **1990**, *1990* (1), 23-29.

192. Jutzi, P.; Heidemann, T.; Neumann, B.; Stammler, H. G., *Synthesis,* **1992**, *11*, 1096-1098.

193. Takano, S.; Sugihara, Y.; Ogasawara, K., *Heterocycles,* **1994**, *39* (1), 59-66.

194. Yu, H. W.; Zhang, L. R.; Zhou, J. C.; Ma, L. T.; Zhang, L. H., *Bioorg. Med. Chem.,* **1996**, *4* (4), 609-614.

195. Lee, J. H.; Griffin, J. H.; Nicas, T. I., *J. Org. Chem.,* **1996**, *61* (12), 3983-3986.

196. Schmittberger, T.; Waldmann, H., *Bioorg. Med. Chem.,* **1999**, *7* (5), 749-762.

197. Taylor, S. D.; Harris, J., *Steroids,* **2011**, *76* (10-11), 1098-1102.

198. Tsukamoto, H.; Kondo, Y., *Synlett,* **2003**, *7*, 1061-1063.

199. McMillan, T. J.; Leatherman, E.; Ridley, A.; Shorrocks, J.; Tobi, S. E.; Whiteside, J. R., *J. Pharm. Pharmacol.,* **2008**, *60* (8), 969-976.

200. Adamo, C.; Amatore, C.; Ciofini, I.; Jutand, A.; Lakmini, H., *J. Am. Chem. Soc.,* **2006**, *128* (21), 6829-6836.

201. Molander, G. A.; Trice, S. L. J.; Dreher, S. D., *J. Am. Chem. Soc.,* **2010**, *132* (50), 17701-17703.

202. Pilarski, L. T.; Szabo, K. J., *Angew. Chem. Int. Ed.,* **2011**, *50* (36), 8230-8232.

203. Dabbousi, B. O.; RodriguezViejo, J.; Mikulec, F. V.; Heine, J. R.; Mattoussi, H.; Ober, R.; Jensen, K. F.; Bawendi, M. G., *J. Phys. Chem. B,* **1997**, *101* (46), 9463-9475.

204. Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H., *Nat. Mater.,* **2005**, *4* (6), 435-446.

205. Gussin, H. A.; Tomlinson, I. D.; Little, D. M.; Warnement, M. R.; Qian, H. H.; Rosenthal, S. J.; Pepperberg, D. R., *J. Am. Chem. Soc.,* **2006**, *128* (49), 15701-15713.

206. Gussin, H. A.; Tomlinson, I. D.; Muni, N. J.; Little, D. M.; Qian, H. H.; Rosenthal, S. J.; Pepperberg, D. R., *Bioconjugate Chem.,* **2010**, *21* (8), 1455-1464.

207. Bernardin, A.; Cazet, A.; Guyon, L.; Delannoy, P.; Vinet, F.; Bonnaffe, D.; Texier, I., *Bioconjugate Chem.,* **2010**, *21* (4), 583-588.

208. Agard, N. J.; Prescher, J. A.; Bertozzi, C. R., *J. Am. Chem. Soc.,* **2004**, *126* (46), 15046-15047.

209. Agard, N. J.; Prescher, J. A.; Bertozzi, C. R., *Glycobiology,* **2004**, *14* (11), 1197-1197.

210. Baskin, J. M.; Bertozzi, C. R., *QSAR Comb. Sci.,* **2007**, *26* (11-12), 1211-1219.

211. Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R., *Proc. Natl. Acad. Sci. U. S. A.,* **2007**, *104* (43), 16793-16797.

212. Bouzigues, C.; Morel, M.; Triller, A.; Dahan, M., *Proc. Natl. Acad. Sci. U. S. A.,* **2007**, *104* (27), 11251-11256.

213. Muir, J.; Arancibia-Carcamo, I. L.; MacAskill, A. F.; Smith, K. R.; Griffin, L. D.; Kittler, J. T., *Proc. Natl. Acad. Sci. U. S. A.,* **2010**, *107* (38), 16679-16684.

214. Guy, J.; Caron, K.; Dufresne, S.; Michnick, S. W.; Skene, W. G.; Keillor, J. W., *J. Am. Chem. Soc.,* **2007**, *129* (39), 11969-11977.

215. Trester-Zedlitz, M.; Kamada, K.; Burley, S. K.; Fenyö, D.; Chait, B. T.; Muir, T. W., *J. Am. Chem. Soc.,* **2003**, *125* (9), 2416-2425.

216. Smith, M. E. B.; Schumacher, F. F.; Ryan, C. P.; Tedaldi, L. M.; Papaioannou, D.; Waksman, G.; Caddick, S.; Baker, J. R., *J. Am. Chem. Soc.,* **2010**, *132* (6), 1960-1965.

217. Ding, D.; Zhao, Y.; Meng, Q.; Xie, D.; Nare, B.; Chen, D.; Bacchi, C. J.; Yarlett, N.; Zhang, Y.-K.; Hernandez, V.; Xia, Y.; Freund, Y.; Abdulla, M.; Ang, K.-H.; Ratnam, J.; McKerrow, J. H.; Jacobs, R. T.; Zhou, H.; Plattner, J. J., *ACS Med. Chem. Lett.,* **2010**, *1* (4), 165-169.

218. Peterson, K. P.; Larock, R. C., *J. Org. Chem.,* **1998**, *63* (10), 3185-3189.

219. Steinhoff, B. A.; Fix, S. R.; Stahl, S. S., *J. Am. Chem. Soc.,* **2002**, *124* (5), 766-767.

220. Kitamura, M.; Tanaka, S.; Yoshimura, M., *J. Org. Chem.,* **2002**, *67* (14), 4975-4977.

221. Donnert, G.; Keller, J.; Medda, R.; Andrei, M. A.; Rizzoli, S. O.; Luehrmann, R.; Jahn, R.; Eggeling, C.; Hell, S. W., *Proc. Natl. Acad. Sci. U. S. A.,* **2006**, *103* (31), 11440-11445.

222. Wang, H.; Peca, J.; Matsuzaki, M.; Matsuzaki, K.; Noguchi, J.; Qiu, L.; Wang, D.; Zhangn, F.; Boyden, E.; Deisserothl, K.; Kasai, H.; Hall, W. C.; Feng, G.; Augustine, G. J., *Proc. Natl. Acad. Sci. U. S. A.,* **2007**, *104* (19), 8143-8148.

223. Cho, Y. A.; Kim, D. S.; Ahn, H. R.; Canturk, B.; Molander, G. A.; Ham, J., *Org. Lett.,* **2009**, *11* (19), 4330-4333.

224. Molander, G. A.; Bernardi, C. R., *J. Org. Chem.,* **2002**, *67* (24), 8424-8429.

225. Molander, G. A.; Biolatto, B., *J. Org. Chem.,* **2003**, *68* (11), 4302-4314.

226. Molander, G. A.; Canturk, B., *Org. Lett.,* **2008**, *10* (11), 2135-2138.