UNIVERSITY OF BIRMINGHAM

The development of novel allosteric modulators of the 5-HT_3A receptor

By

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A thesis submitted to the University of Birmingham

For the degree of

DOCTOR OF PHILOSOPHY

School of Chemistry

University of Birmingham

January 2017

ACKNOWLEDGEMENTS

I would like to thank Dr. Richard S. Grainger, Dr. Sam Butterworth and Dr. Nicholas Barnes Dr. Gillian Grafton for providing me with the opportunity and support to carry out this research; Alexander Roberts for his work on the biological assay process. A special thanks to the analytical team at the School of Chemistry: Dr. Neil Spencer Dr. Cecile Le Duff, Dr. Chi Tsang, Dr. Peter Ashton, Dr. Allen Bowden, Dr. Louise Male and Mrs. Lianne Hill; without their diligent work this research would not have been possible. A big thankyou to all the Grainger group members past and present, and the 4th floor office Dr. Michael Kyle for your continual and inexhaustible sense of humour, the Tucker group members: Dr. Gemma Bullen (soon to be a Berwick), Dr. James Carr-Smith, Dr. Huy Van Nguyen, Dr. Jean-louis Duprey, Dr. Haydn Little and Dr. Matt Berwick for your good banter, working environment and all the fun we had together. A special thanks to Dr. Glenn Lees for his continual support, advice and humour. A dubious thankyou to all my MSci students that I have managed over the four years, it was a bit like herding cats at times but I am proud that I was there to help you meet your potential. I would like to thank my family for trying to understand what it is that I have been doing over the last four years and their unwavering support and kindness. Last, but not least, I must thank my wonderful wife for her dedicated support and understanding in this endeavour, I can confidently say that you have been a very stabilising influence in my life and I couldn't have achieved all that I have without you.

ABSTRACT

This thesis reports the Structure Activity Relationship study that was performed upon the 5-substituted-indole core as a means to identify Negative Allosteric Modulators of the human 5-HT_3A receptor for the development of potential drugs for the treatment of IBS-d. Herein is reported the successful identification of a PAM to NAM switch and three novel NAMs **63**, **71** and **86a** which provide the basis for further study into the treatment of IBS-d and insight into the identity of the allosteric site of the human 5-HT_3A receptor. The design, synthesis and testing of a novel fluorescent analogue of the orthosteric antagonist Quipazine is also described for the application of an improved competitive binding experiment without the need for radio-labelled ligands. Investigation into the binding mode of a reported diazirinyl-substituted indole **101** *via* its synthesis and *in vitro* testing which led to the design and synthesis of two novel photo-activated diazirinyl-indoles **110** and **123** that may be used in a photo-affinity binding study that may conclusively identify the identity of the allosteric site of the 5-HT_3A receptor. Due to the need for a fluorescent assay capable of quantifying the large range of intracellular Ca^{2+} concentrations that were observed in the testing of the PAMs generated from the SAR study, the design of a novel ratiometric tandem dye experiment led to the design and synthesis of novel BODIPY-BAPTA based fluorescent sensors **156** and **157**.

ABBREVIATIONS

Å	Angström
Ac	acetyl
Ap	apparent
aq.	aqueous
BAPTA	1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetra-acetic acid
br	broad
Bu	butyl
c.	concentrated
C	Celsius
cat.	catalytic
d	doublet
DCE	1,2-dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DMF	*N,N*-dimethylformamide
DMFDMA	*N,N*-dimethylformamidedimethyl acetal
DMSO	dimethylsulfoxide
EDG	electron-donating group
EDTA	ethylenediamine tetraacetate
EI	electron impact
eq.	equivalent
ESI	electrospray ionisation
Et	ethyl
Et_2O	diethyl ether
EWG	electron-withdrawing group
FT-IR	fourier transform infrared
g	gramme(s)
HEK-293	Human embryonic kidney cell line -293
h	hour(s)
[H]	reduction
HMBC	heteronuclear multiple bond correlation
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
Hz	hertz
i	*iso*
IR	infrared
J	coupling constant (in NMR)
L	litre
m	multiplet
M	molar
Me	methyl

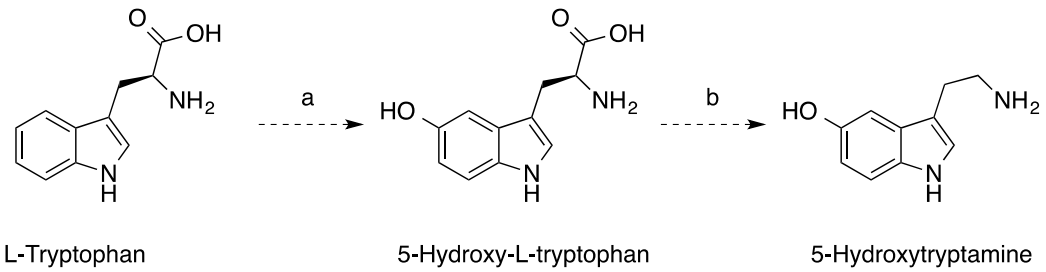
min	minute(s)
Mol	moles
MOPS	3-morpholinopropane-1-sulfonic acid
M.P.	melting point
m/z	mass/charge
N	normal
NAM	Negative allosteric modulator
NBS	*N*-bromosuccinimide
NMR	Nuclear Magnetic Resonance
[O]	oxidation
o/n	overnight
p	*para*
PAM	*Positive allosteric modulator*
petrol	60-80 °C petroleum ether
Ph	phenyl
ppm	part(s) per million
Pr	propyl
PSIG	Pounds per Square Inch Gauge
q	quartet
quant.	quantitative
r.t.	room temperature
r.d.s.	rate-determining step
RFU	Relative Fluorescence Units
s	singlet
t	*tert*
t	triplet
TAP	Tetraanisolylporphyrin
TBAF	tetrabutylammonium fluoride
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
Ts	*p*-toluenesulfonyl
u	atomic mass unit
UV	ultraviolet
vs.	versus
v	frequency

Contents

1 Introduction to the 5-HT$_3$ receptor

1.1 Biological roles of 5-HT in the human body

5-Hydroxytryptamine (**5-HT**), commonly known as serotonin, is a monoamine neurotransmitter biosynthesized from tryptophan,[1] and is involved in a wide range of signalling processes in the human body.



Scheme 1- The biosynthesis of 5-HT; Conditions: a) Tryptophan hydroxylase; b) 5-Hydroxytryptophan decarboxylase.

A small amount of the body's 5-HT serves important roles in the central nervous system (CNS) where it is synthesized by serotergic neurons, *via* the process shown above (Scheme 1), for the control and regulation of mood, appetite and sleep as well as cognitive functions such as learning and memory. Due to 5-HT's role in the regulation of mood its modulation at synapses is considered as a mode of action for several classes of antidepressant drugs, including Selective Serotonin Re-uptake Inhibitors (SSRI's). Despite the well-researched role 5-HT has in the CNS the clear majority of the human body's 5-HT, around 90%, is located in enterochromaffin cells of the epithelial lining the digestive tract, where it controls gastric motility. Over time the 5-HT diffuses into the blood where it is actively bound by blood platelets, in the event of the platelets binding a clot the 5-HT is released where it acts a vasoconstrictor and aids in the regulation of the clotting.[2]

1.2 The 5-HT_3 receptor

5-HT signals through a family of trans-membrane receptors, designated as 5-HT 1-7. Receptors 1,2 & 4-7 are G-protein-coupled receptors (GPCRs) that are found in the central and peripheral nervous systems and are responsible for the mediation of excitatory and inhibitory neurotransmission. The 5-HT_3 receptor, however, is not a GPCR but a ligand gated ion channel of the cysteine loop ligand-gated ion channel (LGIC) superfamily, a group which also includes nicotinic acetylcholine receptors (nNAChRs) and inhibitory neurotransmitter receptors for gamma-butyric amino acids (GABA).[3] As a result, the homology of the 5-HT_3 receptor is much more akin to the nACh receptors than any of the other 5-HT receptor family. The 5-HT_3 receptor is a cation-specific channel and mediates the neuronal excitation and depolarization within the central and peripheral nervous systems, where receptor activation evokes neuronal excitation and neurotransmitter release.[4]

Despite the receptors initial discovery in 1957 from the work of Gaddum and Parcelli et al[5] at the outset of our studies there were no high resolution ×-Ray 3D-structures available for this receptor, this is due to the fragility of the extracellular domain causing degradation during purification[6,7]. Since there are no high-resolution 3D-structures available efforts have been made to relate the close structural relationship between 5-HT_3 receptors and nNAChRs, which are much better characterized. This has led to combinatory modelling of drug binding affinities with cultured 5-HT_3 receptors compared to drug-docked 3D-structures of nAChRs. The functional and structural relation of 5-HT_3 receptors and nACh receptors is indeed so similar that "chimeric receptors" which are comprised of the ECD of the α7-nACh receptor and the TMD of the 5-HT_3A receptor respond to ACh and present the channel properties of the 5-HT_3A receptor.[8,9]

In correlation to all members of the cys-loop family, 5-HT_3 receptors are assembled as a pentamer of subunits that flank a central ion channel in a pseudo-symmetric manner,[10,11] with each individual subunit comprising a large extracellular domain (ECD) as well as four transmembrane domains (TMD) formed by α-helices (M1-4) which are connected by intracellular domains (ICD) (M1-M2, M3-M4) as well as extracellular (M2-M3) loops and an extracellular C-terminus;[12,13] see Figure 2**.**

The five subunits may be homo-pentameric, that is all five are 5-HT_{3A} or hetero-pentameric where each of the subunits may be different combinations of 5-HT_{3A-E} subunits, but predominantly these hetero-pentameric 5-HT_3 receptors are made of 5-HT_{3A} and 5-HT_{3B} subunits and for the receptor to be functional there must be at least one 5-HT_{3A} subunit present.[14–16] The 5-HT_3 receptor subunits C-E were first identified from human tissue and it has since been confirmed that genes for these proteins exist in several other mammalian species, rodent are not included in this group.[17] The agonist-binding site is formed at the interface of two adjacent subunits in the extracellular N-terminal domain and consists of three loops (A-C) from one of the subunits and three β-strands from the other subunit (loops D-F), which is a feature common to all Cys-loop receptors. Only a small proportion of the residues within the loops face into the binding pocket with the rest fulfilling a structural role and possibly facilitating the conformational changes that occur as the channel opens or closes; see Figure 1 and Figure 2.[1819]

The first crystal structure of a 5-HT3 receptor, specifically the mouse 5-HT3a, was subsequently reported in 2014 by *Vogel et al*[20]. The structure was obtained through the use of stabilising peptide-nanobodies, derived from llama single-chain antibodies VHH15, which possess nanomolar affinity for the 5-HT_3 receptor;[21] (depected as the purple domains in Figure 1). The binding peptides were shown to be functional antagonists so it is likely that the structure

represents a closed/inhibited form of the channel and thus it is not certain that this would accurately represent either the native closed state or the antagonised (drug-bound) conformation of the protein. Unfortunately, the binding peptides occlude the orthosteric binding site and therefore it is unlikely this system will be suitable for obtaining ligand-bound structures even if the protein is in the relevant conformation. A 3D rendering of the ×-ray crystal data is shown below (Figure 1).

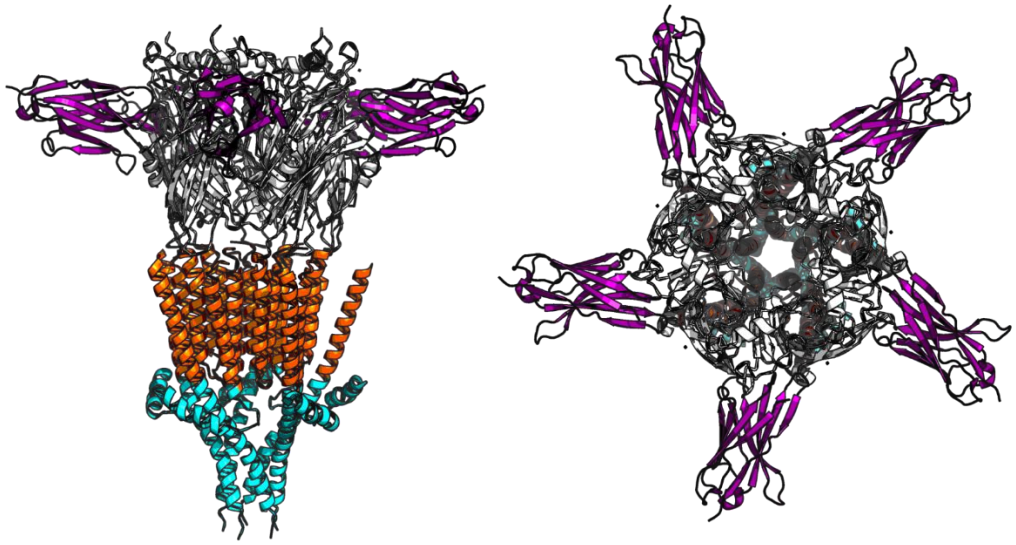


Figure 1- ×-ray crystal structure of the mouse 5-HT_3A receptor as reported by *Vogel et al;* (left) viewpoint perpendicular to the axis of the ion-pore; (right) viewpoint along the axis of the ion pore from the extracellular domain. Stabilising VHH15 proteins (purple) shown binding at the 5-HT binding sites. (PDB: 4PIR)

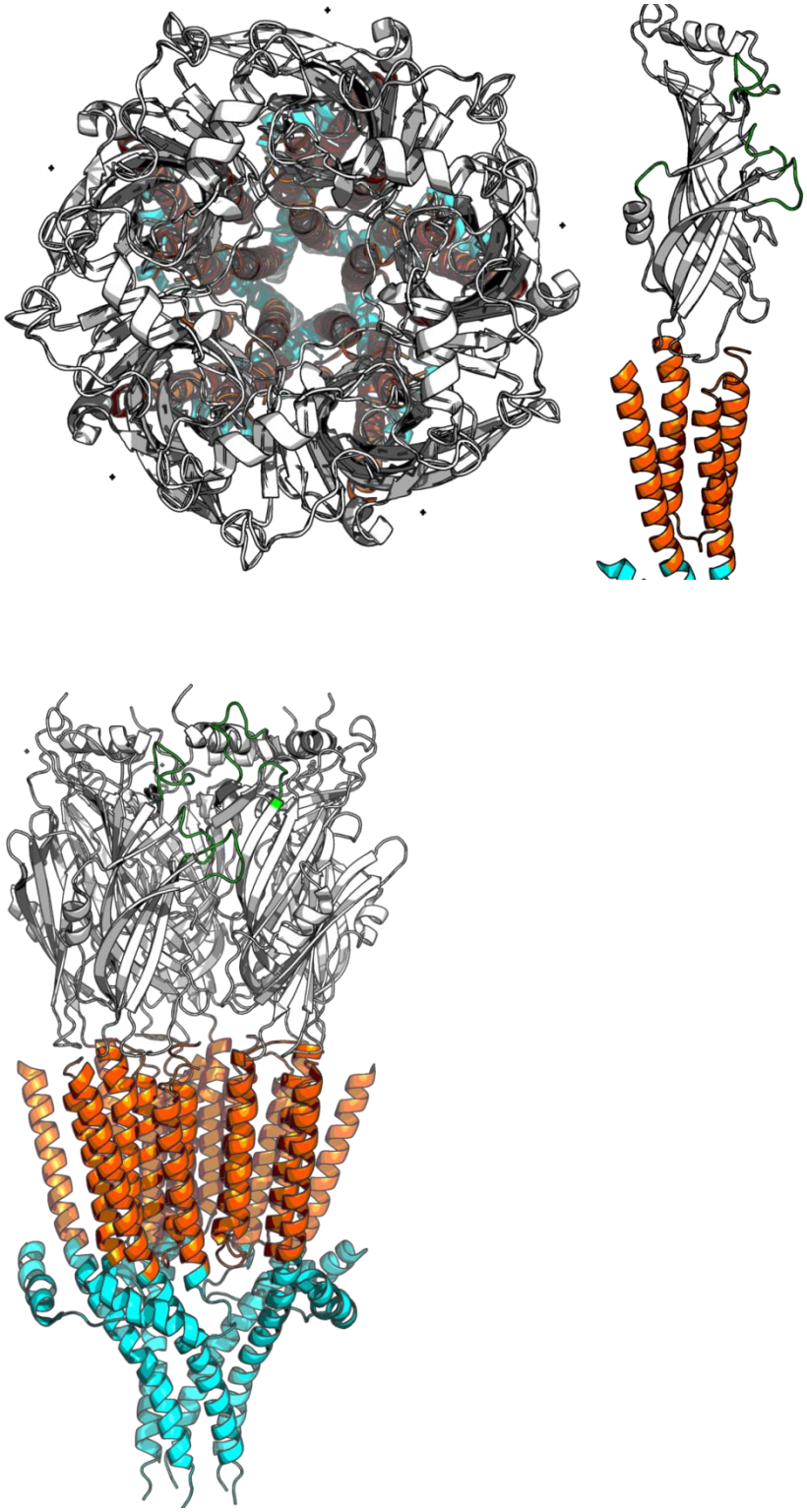


Figure 2- Mouse 5-HT_3A receptor with nanobodies removed to show 5-HT binding domain; grey- Extracellular domain, Green- 5-HT binding domain binding-loops, Orange- Trans-membrane domain and Cyan- Intracellular domain. (Top left) View along the axis of the ion-channel looking from the extracellular domain into the trans-cellular domain. (Top right) A single mouse 5-HT_3A receptor subunit. (Bottom) View of whole 5-HT_3 receptor perpendicular to the axis of the ion-channel. (PDB- 4PIR)

1.3 The role of 5-HT_3 receptor in chemotherapy-induced emesis.

5-HT_3 receptors present themselves in several key sites involved in emesis, namely the vagal afferents, the solitary tract nucleus (STN) and the area prostrema (AP), which are components in the brain and central nervous system that are responsible for vomiting and several other autonomic functions involved in the cardiovascular system as well as the feeding and metabolic systems. The introduction of chemotherapeutic agents stimulates the release of serotonin from the enterochromaffin cells in the gastro intestinal tract, which causes a systemic rise in blood serotonin concentration. This leads to stimulation of the chemoreceptor trigger zone (CTZ) in the area prostrema at the bottom of the solitary tract nucleus of the vagus nerve. Due to the lack of a blood-brain barrier the CTZ can monitor both the blood and cerebrospinal fluid constantly for toxins and in this case elevated levels of serotonin thus stimulating the 5-HT_3 receptors present the product of this stimulation is nausea which triggers the vomit reflex. With this concept in mind, competitive antagonists of 5-HT_3 receptors can be employed to suppress the nausea and vomiting by preventing the serotonin from binding to the respective 5-HT_3 receptors, the highest concentration of which are located in the solitary tract nucleus.[22] Specific 5-HT_3 receptor antagonists such as Ondansetron, Granisetron and Tropisetron (Figure 3) have been developed as antiemetics and work by competitive inhibition at central and peripheral 5-HT_3 receptor sites.[23] These drugs are able to successfully control the emetic response to chemotherapeutics and widely used in this context.

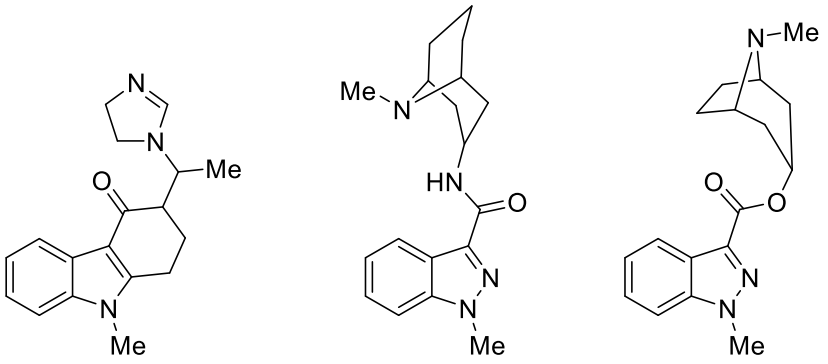


Figure 3-Structures of commercially available 5-HT_3 antagonists for the treatment of chemotherapy-induced emesis

Hibert et al reported a viable model for 5-HT_3 pharmacophore design, which they developed *via* a conformational analysis study, which consists of three key components: a basic-nitrogen, a carbonyl group participating in a H-bonding interaction in plane with the third component, an aromatic ring (Figure 4)[24,25].

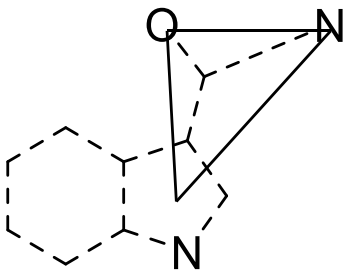


Figure 4-Schematic of Hibert *et al's* template of orthosteric antagonist pharmacophore

The schematic described in Figure 4 can be seen to closely fit each of the drugs in Figure 3 above.

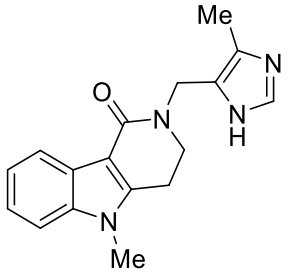
1.4 Role of the 5-HT_3 receptor in Irritable Bowel Disease

Irritable bowel syndrome (IBS) is a major functional disorder of the bowel that is often debilitating and associated with severe abdominal pain. The disease is characterized into three forms; one defined by constipation (IBS-c) and another by diarrhea (IBS-d), and a third where the condition alternates between constipation and diarrhea (IBS-a).[26] It is currently estimated that 5-15% of adults suffers from the disease, of which around ~33% of patients suffer from the diarrhoea-predominant disease (IBS-d).[27] In 2006 the estimated direct medical care costs associated with IBS treatment was $8 billion dollars in the USA alone, the indirect medical costs were estimated at $25 billion. [28] A more recent study by *Carson et al* in 2014 suggests that these early estimates may be conservative.[29] 5-HT_4 agonists are shown to potentiate peristalsis initiated by 5-HT_1 receptor stimulation and thus 5-HT_4 agonists have found clinical use in the treatment of constipation predominant form of IBS (IBS-C) and chronic constipation. 5-HT_3 antagonists, such as those shown in Figure 3, prevent activation of the 5-HT_3 receptors located on (peripheral) afferent neurons and are shown to decrease patient pain associated with IBS as well as to retard the small-intestinal and colonic transit, and consequently have shown significant clinical benefit in the treatment of IBS-d.[30]

1.5 Issues associated with competitive antagonists of the 5-HT_3 receptor

Despite the clear demand for medication, to date there is no readily available effective treatment for IBS. As discussed, drugs that target the 5-HT_3 receptor, such as those in Figure 3 have been widely studied for use in IBS-d therapy and show strong clinical efficacy. Unfortunately in all cases thus far there are unacceptable side effects such as ischemic cholitis which is essentially constriction and reduced blood flow to the gut that can lead to sepsis and, in rare cases, death.[31]

Alosetron is a 5-HT_3 receptor orthosteric antagonist that was approved by the FDA in February 2000 for the treatment of severe IBS-d in women. However in just a 9 month period the FDA removed Alosetron from practice due to it being linked with 49 cases of ischemic cholitis and 21 cases of severe constipation. In 2002 Alosetron was re-released under restrictive sale license for IBS-D patients with no other options[32] (Figure 5).

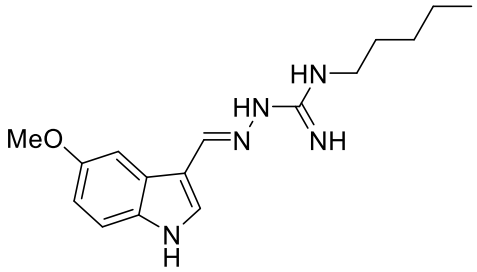


Alosetron

Figure 5-5-HT_3 antagonist Alosetron

It is believed that the incidents of ischemic cholitis stemming from the use of 5-HT_3 orthosteric antagonists such as Alosetron (Figure 5) may be due to the slow kinetics of the drug becoming un-bound from the receptor leading to long periods of 5-HT_3 de-sensitisation to the

natural ligand (5-HT). This phenomenon is apparently not unique to the 5-HT_3 receptor and has been observed in the target of the 5-HT_4 receptor with Tegaserod, a 5-HT_4 antagonist, which was FDA approved in 2002 for the treatment of IBS-C. In 2007 it was removed from clinical use due to studies showing it significantly increased (10 fold increase) patient's likelihood of myocardial infarction, stroke and angina[30] (Figure 6).



Tegaserod

Figure 6-5-HT_4 antagonist removed from clinics by FDA

1.6 Orthosteric and allosteric modulation

A traditional approach to drug development targeting ligand-gated ion channels involves development of a competitive agonist or antagonist with which to modulate the receptors function. By definition competitive modulators affect the function of receptors through the formation of a complex that cannot also bind the natural ligand; as a result the modulator and natural ligand compete for binding to the receptor. Competitive modulators generally bind at the same site of the receptor as the natural ligand and in these cases can also be termed orthosteric ligands. In the instance of the 5-HT_3 receptor this would be targeting the same binding site as described in Figure 2, and the drug molecule, such as those described in Figure 3 and Figure 5, directly compete with the natural ligand 5-HT for this site, as summarised below (Figure 7).

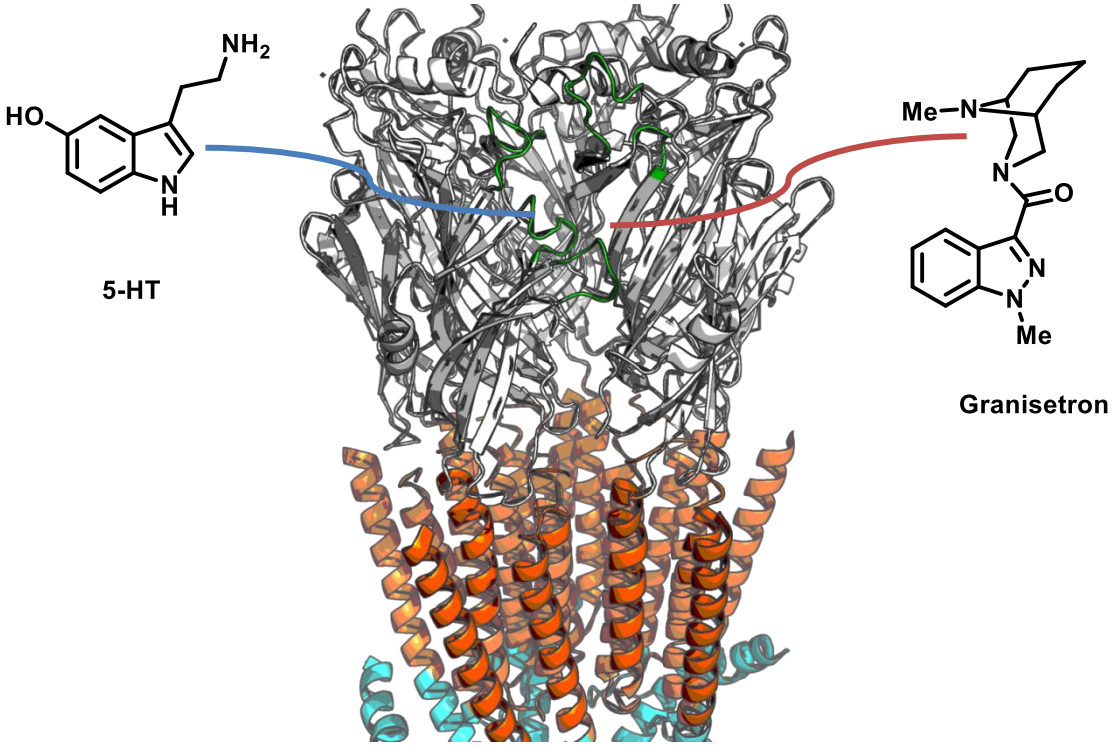


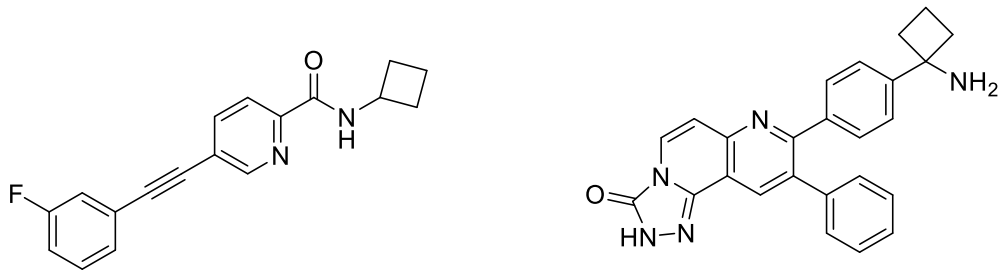
Figure 7-Diagram describing competitive modulation of the 5-HT_3 receptor. Both the natural ligand (5-HT) and the competing drug (Granisetron) are competing for the same (orthosteric) site on the receptor.

As described in section 1.5 above, there are well documented harmful side effects associated with treating conditions with competitive orthosteric agents for 5-HT_3 and 5-HT_4 receptors due to their slow off-rate of binding to the receptor leading to desensitisation to the natural ligand. [33]

An alternative approach involves the concept of non-competitive binding at an alternate binding site known as an allosteric site. This allosteric binding agent gains its effect from binding at this secondary site and changing the function of the protein or the affinity of the receptors main agonist-binding site for the natural agonist by conformational changes in the receptor, thus modulating the receptors function.[34] Depending on their specific activity allosteric modulators may achieve complete agonism or antagonism of the receptor independently of ligand binding, such as benzodiazepines binding GABA receptors[35], or may modulate the function of the protein

only in the presence of the natural ligand such as the effect of Ivermectin upon α7-nACh receptor[36]. In the latter case this allows enhanced agonism of the receptor whilst retaining some physiological control from the natural ligand (PAMs), or alternatively may allow a partial antagonism of a receptor through reducing rather than fully blocking signalling upon binding of the natural ligand (NAMs).

Allosteric modulators account for a significant proportion of clinical drugs available today, such as VU0403602 which is a pro-drug PAM of the metabotropic-glutamate receptor 5 ($mGlu_5$) and is used to treat epilepsy[37] or MK-2206 which is a selective NAM of the human Akt_3 kinase and is used in cancer therapy[38] (Figure 8).



VU0403602 MK-2206

Figure 8-Clinical allosteric modulators

Lummis *et al*[17] suggested several potential allosteric binding sites of the 5-HT_3 receptor, using the closely related nACh receptor to predict the site topology. Potential sites were identified in the receptors' ECD, inter-helical site in the TMD and one lipid trans-membrane site of membrane-receptor boundary, (Figure 9).

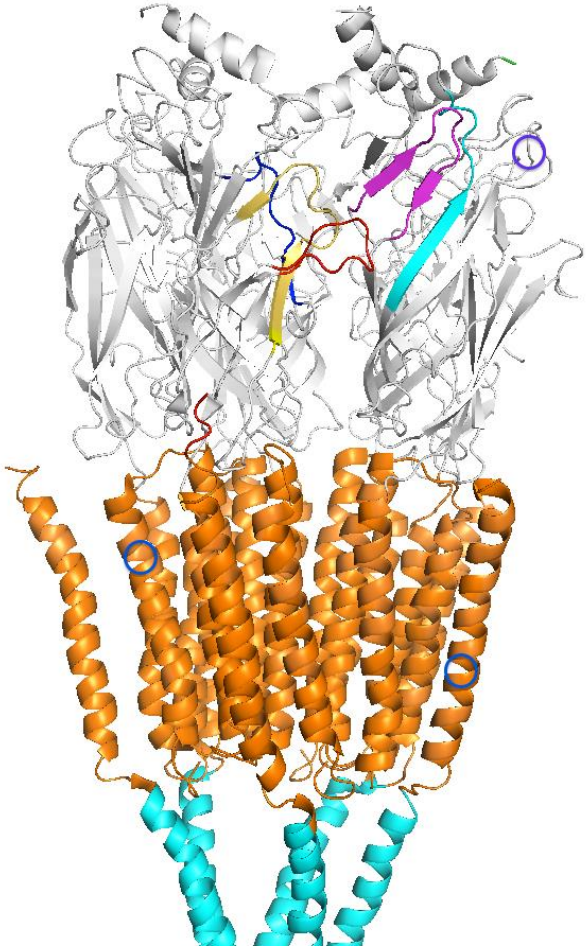


Figure 9-nACh receptor- Proposed allosteric binding domains highlighted with the blue rings. Main agonist binding domain loops included for clarity with the same colour scheme as figure 1

1.7 The hit compound

In a recent publication from our collaborators, *Barnes et al*[39] have identified that 5-chloroindole is a highly ligand efficient PAM of the 5-HT_3 receptor. They have shown that 5-chloroindole effectively potentiates the 5-HT_3 signal when co-dosed with 5-HT but when 5-chloroindole is added alone there is no observed stimulation of the receptor. With doses between 3-100 µM of 5-chloroindole, *Barnes et al* have shown that 5-chloroindole significantly slowed the decay of the Ca^{2+} signal in the presence of 3 µM of 5-HT as shown in Figure 10 below.

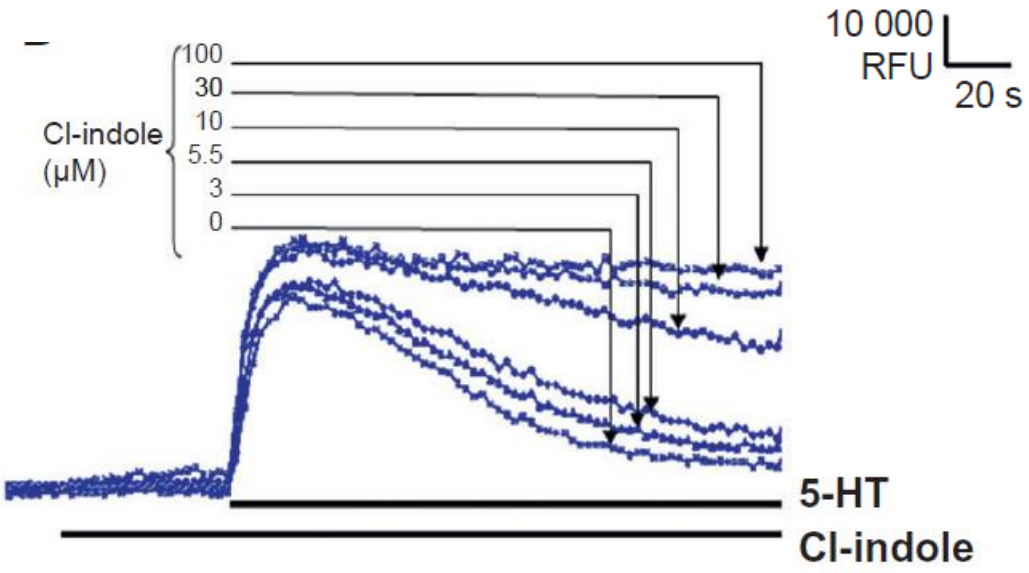


Figure 10-*Barnes et al* dose response 5 uM 5-HT

This prolongation of the Ca^{2+} signal may provide a useful feature for an allosteric modulator drug with which to treat IBS-d as it offers a potentially long lasting effect that can be returned to base line activity of the receptor with an increased dose of 5-HT, which the body should be able to self-regulate.

To gain a clinically useful compound for the treatment of IBS-d a negative allosteric modulator (NAM) is required and the compound reported by *Barnes et al* is a PAM. There are examples of SAR studies whereby relatively conservative changes in molecular structure yield a PAM/NAM switch in signalling such as the switch observed by *Lindsley et al* [40] (Figure 11) in their SAR study of $mGlu_5$ receptor allosteric modulators.

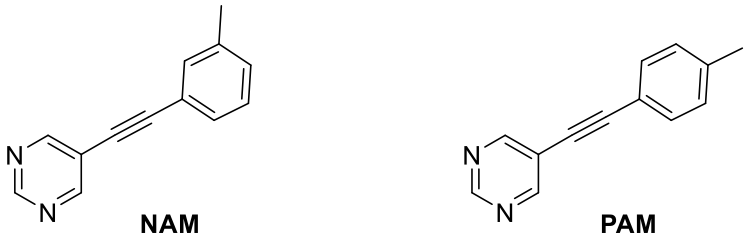


Figure 11-PAM/NAM switch reported by *Lindsley et al*

Encouraged by the very conservative structural change observed by *Lindsley et al* yielding a complete switch from PAM to NAM, the SAR study commenced around the indole core with with the aim of developing NAMs of the 5-HT_3 receptor for drug discovery.

1.8 Project Aims

The hypothesis that allosteric modulators may provide safer drugs with which to medicate IBS-d compared to orthosteric agents such as Alosetron (Figure 5) is based on the uncompetitive binding mode of allosteric modulators. It is believed that due to the nature of the uncompetitive allosteric binding, the receptor is always able to bind the natural ligand (5-HT) and indeed requires the natural ligand for function, the receptor is fundamentally always under the control of the natural ligand. Because of this the receptor 'blockade' effect, that is hypothesised to be the cause of the side effects of the otherwise efficacious orthosteric antagonists such as Alosetron, could be avoided providing drugs that have the desired efficacy without the associated negative side effects.

Following their published findings, a collaborative project with *Barnes et al* began; initially focused upon a Structure Activity Relationship (SAR) study with the PAM they identified (5-chloroindole) as the basis molecule. Given the lack of available structural data for the receptor at the outset of the project as well as an on-going uncertainty as to the binding site of allosteric modulators, our strategy was to systematically modify each position of the indole core to empirically establish the SAR for both the affinity of the compounds with the 5-HT_3A receptor and the pharmacological effects following binding.

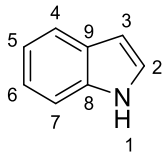


Figure 12-The numbering convention of indole

The initial aim was to explore the effects of varying the identity of the group at the 5-position to determine the significance of the chlorine substituent present in 5-chloroindole**.** Following this initial SAR, a systematic increase to the steric bulk at each position of the indole core *via* the addition of a methyl group was to be explored to assess the steric constraint of the allosteric site in a conservative manner. Furthermore, the effects of replacing the aromatic carbon framework with heteroatoms such as oxygen and nitrogen was to be explored to assess the effect of reducing the lipophilicity and altering the hydrogen bonding potential. In doing this it was anticipated that making discrete systematic changes to both the electronic and steric nature of the molecules could potentially provide both insight about the allosteric binding site's tolerance to such changes and lead candidates for drug discovery.

2 Results and discussion-Summary of biological data and SAR

2.1 Drug profiling intracellular Ca^{2+} assay

The compounds synthesised during the SAR study were tested in an intracellular Ca^{2+} assay using HEK293 cells that stably express the human 5-HT_3A homopentameric receptors (HEKh5-HT_3A cells[41]). Agonism of the 5-HT_3 receptors expressed by these cells results in channel opening and influx of calcium ions. In order to assess intracellular calcium concentration, the HEKh5-HT_3A cells are treated with a calcium specific fluorescent PET quenching sensor (typically Fluo-4AM). The AM-protected dyes are processed by cellular esterases which liberates the membrane-impermeable and sensory active tetra-acid that is quenched in the unbound state, but is highly fluorescent once this quenching has been relieved through the binding of calcium ions. Changes in intracellular Ca^{2+} were measured using a FlexStation with fluorescence levels assessed every 3 seconds. Either buffer or compound of interest is added after 20 seconds followed by 5-HT at 80 seconds and finally a positive control is added at 320 seconds (initially carbachol). Four different vehicle controls were run per plate and the responses were normalised to the appropriate vehicle. Each compound of interest was assessed at 7 different concentrations in triplicate studies and across 4 separate plates (Figure 13).

This assay allows for the identification of a range of compound activities: 1) Inactive compounds will give an identical trace to 5-HT treatment alone, 2) 5-HT_3 agonists will stimulate a calcium response in the cells ahead of 5-HT addition, 3) 5-HT_3 antagonists will supress the 5-HT response and 4) Positive Allosteric Modulators will enhance the magnitude of the 5-HT response (but not cause a signal ahead of 5-HT addition).

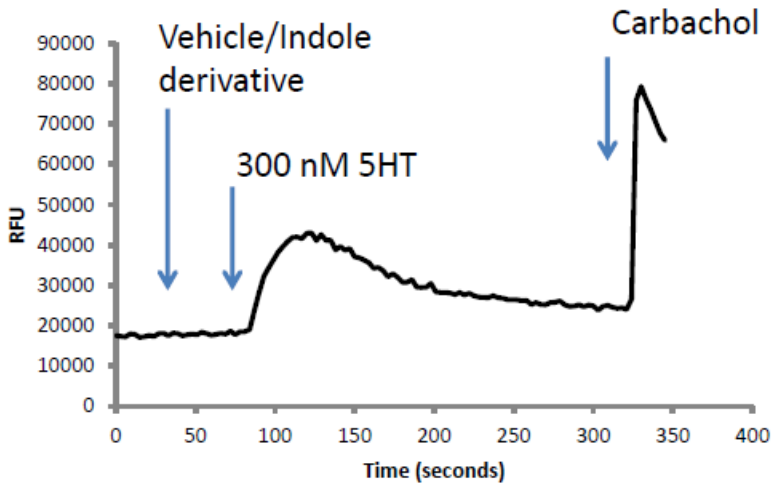


Figure 13-Time resolved fluorescence of HEKh5-HT$_3$A cells; arrows denote the addition of captioned compounds; RFU= Relative Fluorescent Units

As Figure 13 above shows an increase in RFU is observed upon addition of 5-HT, which is exactly as would be expected due to the 5-HT$_3$ receptors expressed by the HEK cells becoming activated and allowing the efflux of Ca^{2+} into the cells loaded with the Ca^{2+} fluorescent dyes. An issue that was encountered at the onset of this research is the 5-HT signal observed is already at the upper quartile of the linear dynamic range that the fluorescent dye within the cells can reach. This led to some variability in the observation of the effect of potentiation of the PAMs developed from this SAR research which are listed in the below sections. The differing percentage of potentiation expressed in the observation columns of the below tables 1-8 may be attributed to a variance in response to the control agent (carbachol) rather than a significant compound variability. This is reflected in the larger variability generally seen within replicates for single compounds once activity reaches >150-200% of the 5-HT response.

The Biological data reported in this thesis was collected, processed and compiled by Mr. Alexander Roberts (PhD candidate) and Dr. Gillian Grafton (School of Clinical and Experimental Medicine), both of whom are members of the Barnes research group.

2.2 SAR at the 5-position

As the publication that this research stemmed from identified 5-chloroindole[42] as a potent PAM of the 5-HT_3 receptor, the effects of different substituents at the 5-position of the indole core was explored (Table 1).

Entry	Compound	EC_{50}	Observation
1a	5-(Trifluoromethyl)indole, **6**	2.4 μM	PAM- 200% potentiation at 100 μM
1b	5-Iodoindole*	12 μM	PAM- 600% potentiation at 100 μM
1c	5-Bromoindole*	15 μM	PAM- 700% potentiation at 100 μM
1d	5-Methylindole*	15 μM	PAM- 500% potentiation at 100 μM
1e	5-Chloroindole, **7**	10 μM	PAM- 500% potentiation at 100 μM
1f	5-Fluoroindole*	149 μM	PAM- 400% potentiation at 100 μM
1g	5-Phenylindole*	499 μM	PAM- 150% potentiation at 100 μM
1h	5-Hydroxyindole*	2 mM	Orthosteric Inhibition- Residual activity 30% at 100 μM

Table 1-Summary of SAR at 5-position of indole core by drug profiling intracellular Ca^{2+} assay; * purchased compound

As can be seen from Table 1 all the compounds, with the exception of **1h**, are observed to retain PAM functionality which is remarkable when one considers the variance in steric volume from a fluorine to an iodine or even a phenyl substituent. This suggests that the allosteric site at which the PAMs are binding allows the molecules to dock in a way that does incur significant steric interactions at the 5-position of the indole motif with groups smaller than phenyl, and may be the result of the 5-position of the molecule being partially or fully exposed from the binding site or perhaps interacting with a lipophilic pocket. The lowest EC_{50} values reported in the table

feature electron-withdrawing functionality which is suggested to be due to inductive weakening of the N-H bond thus making the hydrogen atom a more available H-bonding donor. Another observation is that the 5-halogen series, from chlorine to bromine and up to iodine, all have very similar EC_{50} values. This is tentatively assigned to being due to the increase in lipophilicity of the molecules (Cl→Br→I) with cLogP estimated (*via* chemdraw) to be 3.0, 3.2 and 3.4 respectively. More lipophilic molecules are less well solvated in water, and, if they are sufficiently soluble in water to be able to interact with the 5-HT receptor, this will increase the thermodynamic driving force for the molecule to fill the predominantly lypophilic binding site within the protein. 5-Fluoroindole might be expected on electronic grounds to have a superior EC_{50} than that observed for Cl/Br/I and the fact that it is observed to have a relatively poor (high) EC_{50} can be explained again by cLogP at 2.4, which implies the compound will be comparatively much better solvated in water than the other halogen-functionalised compounds. 5-Bromoindole was found to have the greatest potentiation in the series at 1.4 times greater than 5-chloroindole (Figure 55 *vs.* Figure 56) however as was discussed previously, this may result from assay viability rather than a genuine change in activity. 5-(Trifluoromethyl)indole **1a** exhibits the highest affinity of all the compounds in table 1 which can be rationalised in the same way as above regarding the acidity of the N-H (Figure 57). As described above, an indole appended with a strongly electron withdrawing group would be expected to afford a stronger H-bonding donor, which may be a crucial interaction at the allosteric site which may explain the increase in binding affinity. The trifluoromethyl functionality increases the cLogP to 3.3 *vs.* 3.0 for chlorine which, as discussed above, favours the molecule docking with the lipophilic protein environments over solvation. However, a decrease in potentiation *i.e.* the percentage of maximum response is observed from the assay, apparently creating a trade-off between magnitudes of activity (potentiation percent) and binding affinity.

2.3 1-position of indole

To explore the SAR at the 1-position of the indole core (Figure 12) compounds **6** and **7** were *N*-methylated to afford compounds **8** and **9** which were used to probe the importance of the N-H hydrogen bond donor and to provide insight into the steric capacity at the allosteric site. A benzofuran derivative **14** was synthesised as a classical isostere of **8** as the N-H bond in this instance is essentially replaced for a lone-pair of electrons, thus no longer possessing a hydrogen bond donor, an alternative perspective of the importance of the indole N-H bond was provided.

Entry	Compound	EC_{50}	Observation
2a	5-Chloro-1-methylindole, **8**	60 μM	PAM- 350% potentiation at 100 μM
2b	5-(Trifluoromethyl)-1-methylindole, **9**	211 μM	PAM- 300% potentiation at 100 μM
2c	5-Chlorobenzofuran, **14**	N/A	No effect.
2d	5-(Trifluoromethyl)-1-benzylindole, **11**	30.6 μM	Competitive inhibition. Residual activity 55% at 100 μM
2e	5-Chloro-1-benzylindole, **10**	29.2 μM	Competitive inhibition. Residual activity 40% at 100 μM
2f	PU-02, **15** [43]	2.1 μM	NAM-Residual activity 28% at 100 μM

Table 2-Summary of SAR data for the 1-position of the indole core by drug profiling intracellular Ca^{2+} assay

compounds **8** and **9** were both observed to behave as PAMs in the drug profiling intracellular Ca^{2+} assay as was observed for the parent molecules (table entries **6** and **7** respectively) which provides evidence that the indole N-H bond is not strictly required for the PAM effect to be observed, this in turn rules out the presence of a crucial H-bonding interaction with the indole N-H being the H-bonding donor (Figure 58 and Figure 59). Although both **8** and **9** were clearly active PAMs it was observed that there was a decrease in compound binding affinity (EC_{50}) for both compounds. It is also clear that the effect of *N*-methylation of **6** has a lesser effect upon binding affinity, within the same order of magnitude, whereas *N*-methylation of **7** showed a nearly 100-fold decrease in binding affinity.

The isosteric substitution of the indole N-H for oxygen in the benzofuran **2c** appears to render the compound devoid of any activity within the drug profiling intracellular Ca^{2+} assay. This observation is consistent with the hypothesis that the indole N-H is forming a stabilising hydrogen bond with the receptor, if correct the benzofuran **2c** oxygen lone pair of electrons in the sp^2-orbital would therefore have a strong repulsion with a lone-pair within the receptor for example an amide carbonyl (Figure 14).

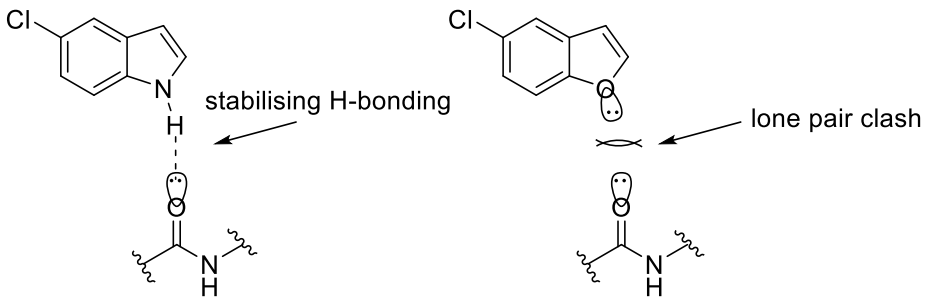
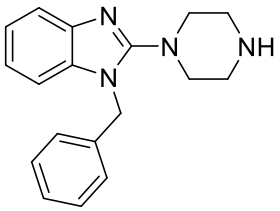
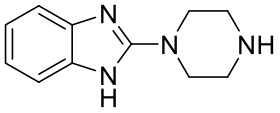


Figure 14-Benzofuran sp^2 lone-pair of electrons repelling with amide C=O sp^2 lone-pair of electrons.

Following the encouraging results of the N-methylated derivatives **2a** and **2b** synthesis and testing of the *N*-benzylated derivatives **2d** and **2e** was performed. *Jensen et al*[43] report a variety of benzylated and naphthylated allosteric modulators of the 5-HT_3A receptor, such as **2f** (Figure 62) which was synthesised for comparison the NAMs described in this thesis. It was found that *N*-benzylation creates a change in binding mode from that of a PAM to instead that of a competitive orthosteric antagonist. interestingly both compounds retain reasonable affinity for the receptor that is comparable to the parent compounds **1a** and **1e** (Figure 60 and Figure 61). The observation of the shift in binding mode to change from PAM to orthosteric antagonist upon *N*-benzylation which could be best explained in correlation with the findings of *Kirschbaum et al*[24] whereby they report identifying a key stabilising interaction at the orthosteric site in the development of partial agonist-analogues of Lerisetron (Figure 15).

Lerisetron

IC_{50}= 0.36 nM

De-benzyl-Lerisetron

IC_{50}= 19.2 nM

Figure 15-Lerisetron and debenzylated-Leristron

In their report *Kirschbaum et al* suggest that the benzyl substituent at the 1-position helps encourage the molecule to fit the receptor in a way that increases the antagonistic properties.

2.4 2-position of indole

Entry	Compound	EC_{50}	Observation
3a	5-Chloro-2-methyl-1H-indole, **20**	14 μM	PAM- 800% potentiation at 100 μM
3b	5-(Trifluoromethyl)-2-methyl-1-H-indole, **21**	39 μM	PAM- 600% potentiation at 100 μM
3c	2-(5-Chloro-1H-indol-2-yl)ethan-1-ol, **25**	9.8 μM	PAM- 460% potentiation at 100 μM
3d	2-(5-(Trifluoromethyl)-1H-indol-2-yl)ethan-1-ol,**26**	16.8 μM	PAM- 430% potentiation at 100 μM
3e	2-(5-Bromo-1H-indol-2-yl)ethan-1-ol, **29**	14.9 μM	PAM- 670% potentiation at 100 μM
3f	5-Chloro-2-phenyl-1H-indole, **33**	N/A	No effect
3g	2-Cyclopropyl-5-(trifluoromethyl)-1H-indole, **40**	19 μM	Orthosteric antagonist- 55% residual response at max dose. at 100 μM
3h	5-Bromo-2-phenyl-1H-indole, **35**	N/A	No effect
3i	5-Bromo-2-oxindole, **36**	N/A	No effect
3j	5-Bromoindazole*	296 μM	PAM- 600% potentiation at 100 μM

Table 3-Summary of 2-position SAR by drug profiling intracellular Ca^{2+} assay;* purchased sample

Methylation at the 2-position of the indole core afforded **20** and **21** which both exhibit the PAM mode of interaction with the receptor. Compound **20** shows very little decrease in binding affinity compared to its parent compound **7** whereas compound **21** is observed to decrease in affinity by an order of magnitude, the reasons for this are currently unknown.

With a view to increasing the steric bulk at the 2-position slightly more than the methylated compounds (entries **20** and **21**) **40** was synthesised and was observed to behave as an antagonist in the drug-profiling intracellular Ca^{2+} assay; with only a slight decrease in binding affinity for the receptor when compared to the parent indole **7** .Further radio-ligand competitive binding studies are underway to identify whether **40** interacts as a competitive-orthosteric inhibitor or as a negative allosteric modulator. Following the results of successful substitution with a 2-methyl and cyclopropyl-groups, the 2-hydroxyethyl moiety was appended at the 2-position yielding table entries **25, 26** and **29.** (Figure 66, Figure 67 and Figure 68).

Introduction of the 2-hydroxyethyl moiety increases the steric bulk at the 2-position quite markedly as there is unrestricted rotation around the alkyl C-C and hydroxyl O-H bonds. furthermore, due to the hydroxyl functional group, the molecule has gained another H-bond donor and acceptor at that end of the molecule that may cause interactions with the amino acid residues within the allosteric binding site as well as with solvating water and salts. Pleasingly it was observed that table entries **25, 26** and **29** all retain PAM activity as well as maintaining very similar EC_{50} values to that of their parent compounds, thus providing more evidence that the allosteric biding site can tolerate a variance in chemical shape and size. To increase the steric bulk further compounds **33** and **35** were synthesised and found not to have any measurable affinity for the 5-HT_3 receptor. 5-Bromoindazole was explored as a classical isostere of 5-bromoindole; due to its ready availability this compound was purchased and found to behave as a PAM although with a reduced binding affinity compared to the parent molecule. The reduction in binding affinity may ber attributed to the decrease in cLogP associated with substituting the C-H for an aromatic nitrogen atom (Figure 69).

2.5 3-position of indole

The SAR at the 3-position of the indole core was influenced by the documented observations of *Hibert et al,* described in Figure 4 above; where the inclusion of a carbonyl H-bonding acceptor in the plane of the aromatic ring around 3 Å from the indole core with a basic amine around 5 Å from the carbonyl in this region of the chemical space is likely to yield an orthosteric antagonist. Therefore structures relating to these were deliberately avoided.

Entry	Compound	EC_{50}	Observation
4a	5-Bromobenzimidazole, **45**	17 μM	Orthosteric inhibition- Residual activity 40% at 100 μM
4b	5-(Trifluoromethyl)benzimidazole, **46**	39 μM	Orthosteric inhibition- Residual activity 50% at 100 μM
4c	5,3-Dichloro-1H-indole, **41**	51 μM	PAM- 350% potentiation at 100 μM
4d	5-Chloro-3-methyl-1H-indole, **44**	73 μM	PAM- 500% potentiation at 100 μM
4e	5-(Trifluoromethyl)-3-chloro-1H-indole, **42**	97 μM	PAM- 150% potentiation at 100 μM
4f	5-Chloro-3-bromo-1H-indole, **43**	N/A	No effect

Table 4-Summary of 3-position SAR by drug profiling intracellular Ca^{2+} assay

Methylation at the 3-position of the indole core afforded **44** which was observed to maintain the PAM binding mode of the parent compound **6** and, as was observed with compound **20,** there was a noticeable decrease in binding affinity but the overall extent of the potentiation tracks linearly with that of compound **6** (Figure 70). As the 3-position of indoles readily undergoes electrophilic aromatic substitution this reactivity was exploited to afford 3-halo-substituted indoles **41, 42** and **44**. It was observed that a chlorine substituent is tolerated by the allosteric site with a slight decrease in the binding affinity although with a significant reduction of the potentiation (**6** *vs* **41**). Compound **41** was found to potentiate at 70% the maxima of the parent

compound **6** and entry **42** was found to potentiate at 75% the maxima of the parent compound **7** (Figure 71 and Figure 72).

The introduction of a bromine substituent at the 3-position yielded compound **43** which was observed to render the compound devoid of any measurable interaction during the drug profiling intracellular Ca^{2+} assay.This is possibly identifying a steric bottle-neck that prevents **43** from fitting the allosteric site.To assess the effect of substituting the C-H at the 3-position of the indole aromatic framework with a sp^2-nitrogen atom benzimidazoles **45** and **46** were synthesised and found to both be competitive orthosteric antagonists. The reason for this change in binding mode is unclear but may be due to the tautomerization of benzimidazoles (Figure 16).

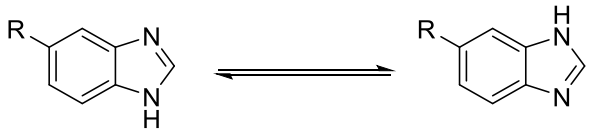


Figure 16- Benzimidazole tautomerization

The ability of compounds **45** and **46** to tautomerize essentially renders them as a mixture of 5 and 6-substitued benzimidazoles.

2.6 4-position of indole

Entry	Compound	EC_{50}	Observation
5a	5-Bromo-1H-pyrrolo[3,2-b]pyridine, **59**	154 μM	Orthosteric inhibition- Residual activity 20% at 100 μM
5b	5-Chloro-1H-pyrrolo[3,2-b]pyridine, **60**	N/A.	No effect.
5c	5-(Trifluoromethyl)-1H-pyrrolo[3,2-b]pyridine, **54**	N/A	No effect.

Table 5-Summary of 4-position SAR by drug profiling intracellular Ca^{2+} assay

With a view to assess the effect of substituting the C-H in the 4-position of the indole core compounds **59, 60** and **54** were synthesised and tested. Intriguingly only **59** appeared to have any affinity for the 5-HT_3 receptor whilst **60** and **54** presented no observable effects to the assay. Compound **59** was observed to exhibit inhibition of the 5-HT_3 receptor during the drug profiling intracellular Ca^{2+} assay (Figure 73). To conclude whether **59** is a NAM radio-ligand binding studies are currently ongoing.

Continuation of the methyl-screen to the 4-position of the indole core was unfortunately unsuccessful. An attempt was made, starting from 4-chloro-3-methylaniline, to synthesise both 5-chloro-4-methylindole and 5-chloro-6-methyl-indole. unfortunately, despite efforts to optimise the reactions leading to 5-chloro-4-methyl-indole, only the latter was successfully synthesised by this route (see Scheme 19 in synthetic chapter).

2.7 6-position of indole

Entry	Compound	EC_{50}	Observation
6a	6-(Trifluoromethyl)-1-benzylbenzimidazole, **46a**	16 μM	Competitive inhibition. Residual activity 20% at 100 μM
6b	5-Chloro-6-methyl-1H-indole, **63**	284 μM	NAM-Residual activity 42% at 100 μM
6c	5-Chloro-1H-pyrrolo[2,3-c]pyridine, **65**	N/A	No effect
6d	6-Bromoindole*	16 μM	PAM- 300% potentiation at 100 μM
6e	6-Chloroindole*	51 μM	PAM- 350% potentiation at 100 μM

Table 6-Summary of 6-position SAR by drug profiling intracellular Ca^{2+} assay; * purchased compound

Continuation of the methyl-screen **63** was synthesised and observed to act as an antagonist in the drug profiling intracellular Ca^{2+} assay (Figure 74). A radio-ligand binding study with ^{3}H-Granisetron

was performed and shows that **63** does not compete with the radio-labelled orthosteric agent and therefore identifies **63** as an allosteric modulator.

Table entries **6d** and **6e** were purchased from Sigma Aldrich and, from the initial results of the drug-profiling assay, found to act as PAMs with comparable binding affinity to **6** although with a much-decreased potentiation 60% that of the parent for **6e** and 38% that of the parent for **6d** (Figure 75 and Figure 76)

Interestingly independent indole substitution with chlorine at the 5 and 6-postions of the indole core elicits a PAM effect but dual substitution in the case of **63** shifts the binding mode to that of a negative allosteric modulator with a binding affinity around 5.5 fold lower than entry **6e.** To explore C-H/nitrogen substitution at the 6-position pyrrolopyridine **65** was synthesised. The results of the drug-profiling intracellular Ca^{2+} assay show no measurable activity for **65**. In a consistent manor to the observations of compound **11** compound **46a** was also observed to behave as an orthosteric antagonist of the 5-HT_3 receptor with comparable binding affinity to compound **11**.

2.8 7-position SAR

Entry	Compound	EC_{50}	Observation
7a	5-Chloro-7-methylindole, **71**	46 μM	NAM- 40% residual activity at 100 μM
7b	7-Methyl-5-(trifluoromethyl)indole, **72**	68 μM	PAM- 660% potentiation at 100 μM
7c	5-Chloro-7-ethynylindole, **75**	N/A	No effect
7d	5-(Trifluoromethyl)pyrrolo[2,3-b]pyridine, **84**	N/A	No effect
7e	5-Chloropyrrolo[2,3-b]pyridine, **82**	257 μM	NAM- 30% residual activity at 100 μM
7f	5-Bromopyrrolo[2,3-b]pyridine, **83**	N/A	No effect

Table 7-Summary of 7-position SAR by drug profiling intracellular Ca^{2+} assay

As the systematic methyl-screen of activity progressed to the 7-position of the indole core compound **71** was synthesised and found to behave as an antagonist in the drug-profiling intracellular Ca^{2+} assay (Figure 77). The mode of binding was determined *via* a radio-ligand competitive binding experiment with ^{3}H-Granisetron and the results reveal that **71** does not compete with the radio-labelled agent thus identifying **71** as a NAM. There was a 60% decrease in signalling of the receptor at the maximal dose of **71** with a very similar binding affinity for the allosteric site compared with the parent compound **6**. Encouraged by the observations of **71** exploration into the effects of substituting the 5-position with a trifluoromethyl functional group was explored *via* the synthesis of **72**. It was previously observed that compound **7** has a greater binding affinity for the allosteric site *vs* compound **6**; however, it was observed that a switch in activity from NAM to PAM occurs as a consequence of this substitution (Figure 78). Following the observation of the tight SAR around the NAM/PAM binding mode of **71** and **72,** efforts were made to slightly expand the steric bulk in the 7-position whilst maintaining the identity of the 5-substituent (Cl) to afford the 7-ethynyl substituted indole compound **75,** which was observed not to possess any detectable affinity for the receptor. Unlike the tolerability of the 2 and 5 positions of the indole core (Table 3 and Table 1) the 7-position of the indole core appears to be at the crux of a subtle and sensitive interaction.

In completion of the pyrrolopyridine SAR in which the carbon of the indole framework is substituted for an aromatic nitrogen atom, compounds **82, 83** and **84** were synthesised. Compounds **83** and **84** were observed not to possess any measurable affinity during the drug-profiling intracellular Ca^{2+} assay; compound **82** was observed to possess a weak inhibitory effect (Figure 79). To determine the mode of interaction of **82** a competitive radio-ligand binding assay with ^{3}H-Granisetron was conducted and found that there was no displacement of the radio-labelled ligand, therefore **7e** is apparently a weak NAM.

2.9 Second pass SAR

Upon reflection of the results obtained from the initial SAR study It was observed that substitution at the 2-position with the 2-(2-hydroxy)ethyl moiety was found to be well tolerated with regard to there being little to no change in the binding affinity of **6** and **7** *vs* **25** and **26**. these findings in combination with the apparent PAM/NAM switch observed with 7-methylation (compound **72**) led to the design and synthesis of the tri-substituted indole **86a** (Table 8).

Entry	Compound	EC50	Observation
8a	2-(5-Chloro-7-methyl-1H-indol-2-yl)ethan-1-ol, **86a**	11 μM	NAM- 55% residual activity at 100 μM

Table 8-Summary of 2nd pass SAR by drug profiling intracellular Ca^{2+} assay

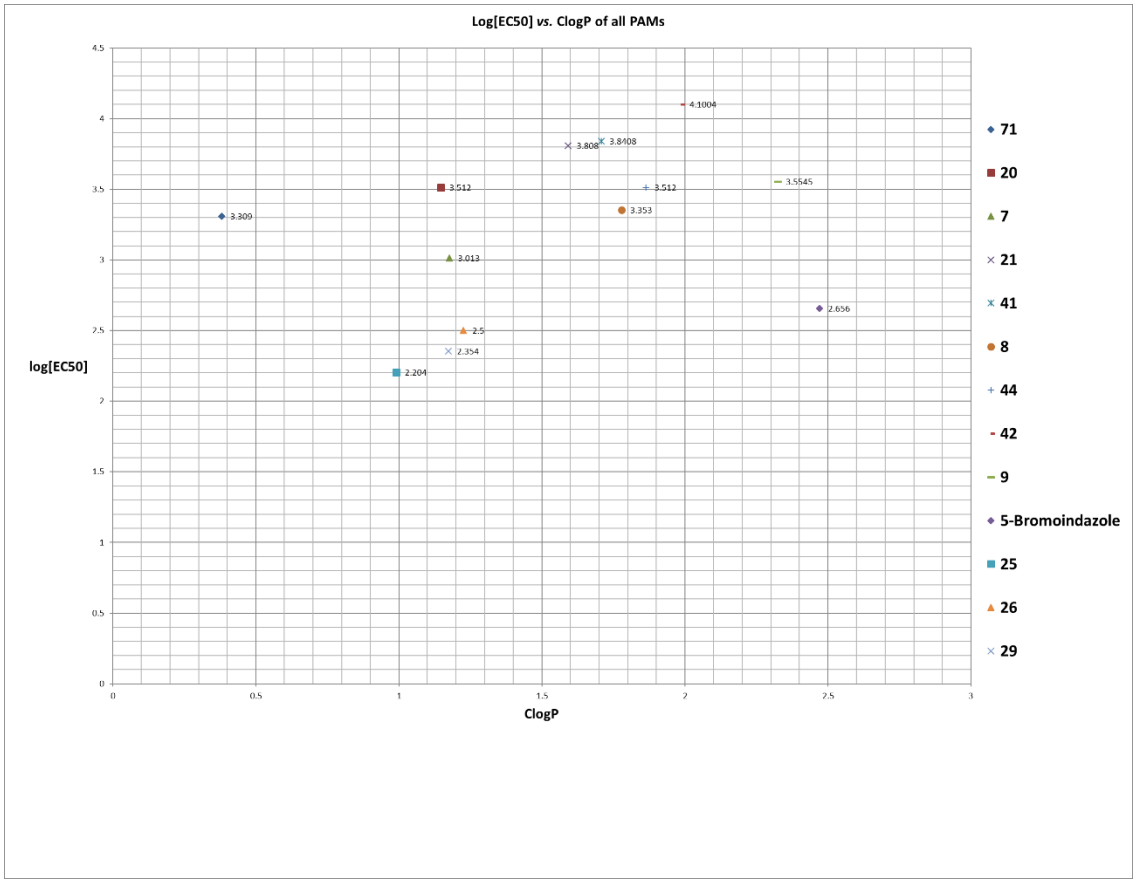
Pleasingly compound **86a** maintains the NAM functionality of **72** (confirmed by radio-ligand binding assay) whilst the binding affinity of **86a** is slightly increased (4-fold improvement *vs* **72***)* see Figure 80.

2.10 Conclusion of indole SAR study

I. Graph of ClogP vs. Log(EC_{50}) and analysis of observed affinity.

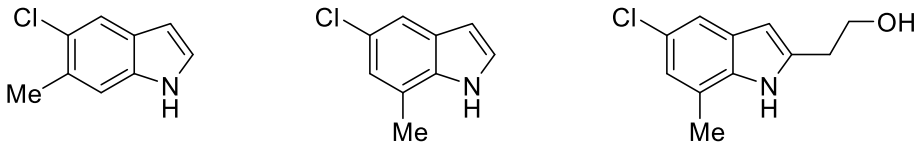
To correlate the observed EC_{50} values of each of the PAMs that have been identified in the SAR discussed above, each compound has been plotted on a graph of log[EC_{50}] *vs.* ClogP (Graph 1). As can immediately be seen from Graph 1 there is no correlation, between the potency (EC_{50}) of the compounds generated from the SAR, to ClogP. This suggests that there are more subtle structural differences between the PAMs that provide the variance in EC_{50} which is consistent with

observations around the apparent importance of the indole NH and the variable impact of increasing steric bulk in different regions of the molecule. The main concern with the biological data is the apparent differences observed in maximal effects between different PAMs; this variability may result from intrinsic differences in compound activity, but could also be impacted by load efficiency of the Ca^{2+} sensor between assays, as well as potential saturation of the calcium sensor (5-HT alone is able to elicit a maximal response close to the upper limit of detection of Fluo-4). Later chapters describe efforts towards a more robust calcium detection system, however compounds **8**, **20** and **44** did not show significant difference in maximum effects when tested on the same day with a dye capable of detecting a larger maximal response.



Graph 1- log[EC_{50}] *vs.* ClogP for all PAMs discussed in table 2 - table 7

A key aim of this study was to identify a potential switch in binding effects towards negative allosteric modulation. At the outset of the studies there was no evidence that this could be achieved with the indole framework; however, the results pleasingly indicate that this can be achieved as shown by compounds **63, 71** and **86a** (Figure 17).



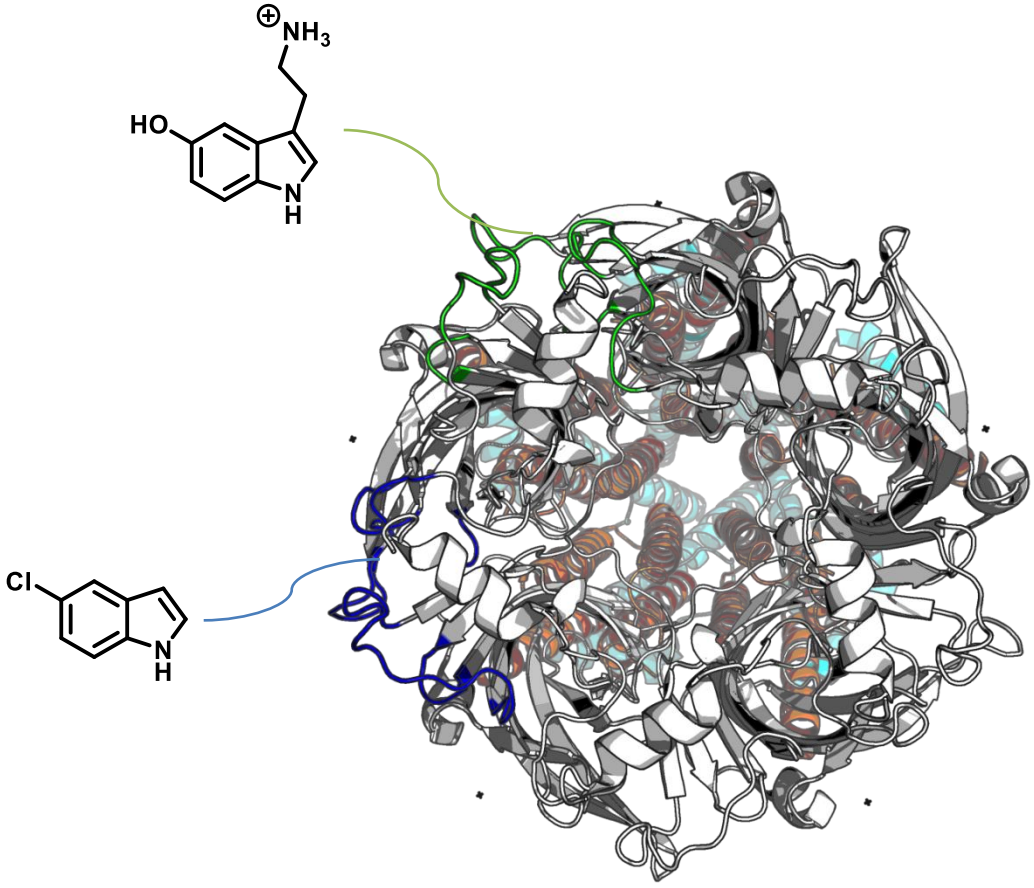
63 **71** **86a**

Figure 17- Structures of the three NAMs identified *via* the SAR study of the indole core.

II. Proposed identity of the allosteric site

The current hypothesis as to the identity of the allosteric site is that it is actually a non-ligand bound orthosteric site and the allosteric activation of the receptor occurs as follows: 1) 5-HT binds at the orthosteric site which creates a conformational change in the protein structure that alters the remaining interfaces of the pentameric subunits of the receptor, which make up the orthosteric sites. 2) This conformational change distorts the binding pocket at the non-ligand bound site creating an activated binding site which enables a molecule that, prior to this conformational change could not bind, to bind. 3) The bound allosteric molecule either potentiates or inhibits the signal and ion-flow depending on the interaction of the bound molecule with the binding loops which make up the activated binding domain. An attempt to illustrate this activation then modulation 2-step processes is included below (Figure 18). The change in the mode of the allosteric modulator, i.e. PAM to NAM, could be explained due to different steric interactions between the modulator and the activated non-ligand bound inter-

pentameric binding site binding loops. In the case of the 7-methylated indoles **63, 71** and **86a**, one of the complementary binding loops may be partially blocked by the presence of the 7-methyl functional group causing it to sit in a slightly perturbed state leading to an inhibitory signalling route for the rest of the receptor.



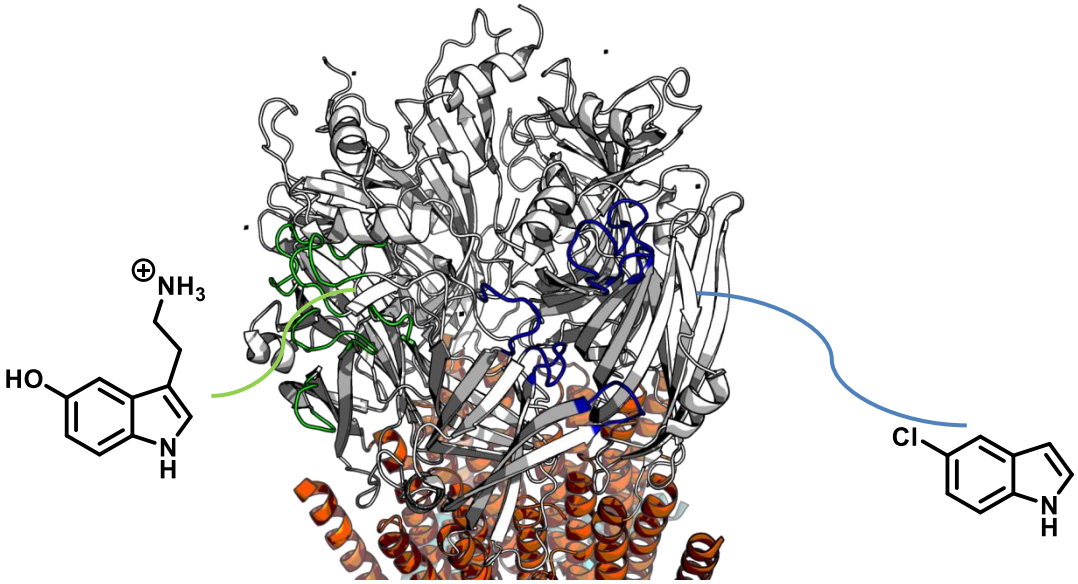
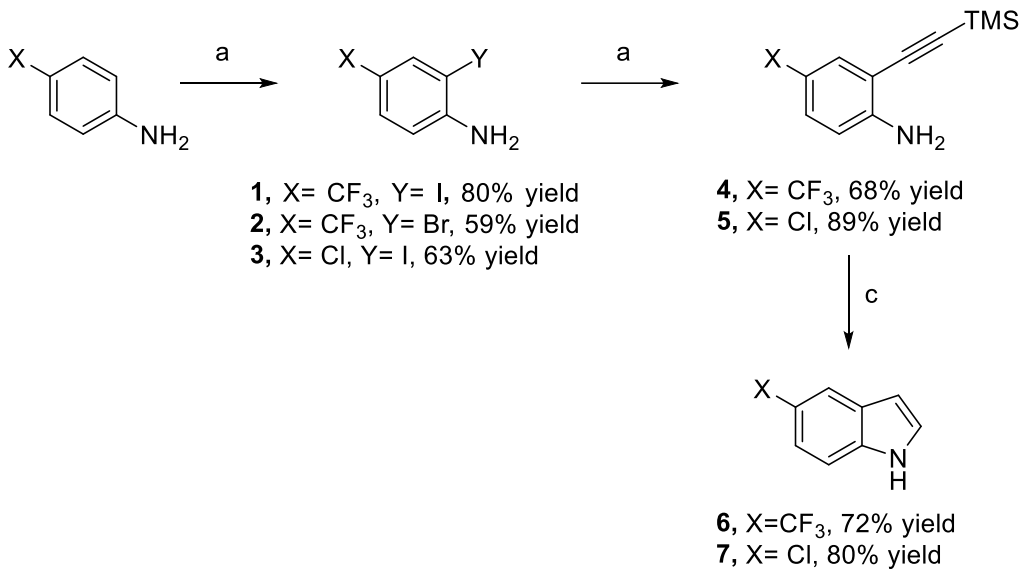


Figure 18- Diagram of 5-HT$_3$A receptor (Top) perspective down the ion-pore; (Bottom) perspective perpendicular to the axis of the ion channel; 5-HT-binding site (orthosteric site) shown in green, activated-unbound orthosteric site shown in blue; llama antibody proteins removed for clarity; (PDB= 4PIR).

3 R&D- SAR chemical synthesis and strategy

3.1 Synthesis of electron-poor 5-substituted indoles

5-(Trifluoromethyl)indole **6** and 5-chloroindole **7** were synthesised *via* a three step halogenation-Sonogashira-cyclisation sequence using the conditions reported by G*oldstein et al*[44]*. a*s shown in Scheme 2. This sequence was found to afford the products in moderate yields and avoids the use of toxic hydrazines used in the Fisher indole synthesis.

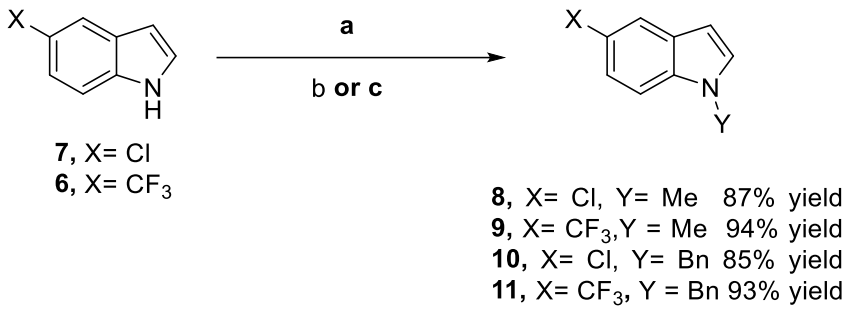


Scheme 2- Goldstein's synthesis of 5-substituted indoles; Conditions: a) Y=I, $Me_3NBnICl_2$, $CaCO_3$, MeOH/DCM, 6 h or Y= Br, NBS, MeCN 0 °C - r.t., 16 h; b) Ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3 r.t. 20 h; c) CuI, CaCO3, DMF 120 °C, 2 h.

3.2 Synthesis of 1-alkylindoles

The synthesis of indoles **8-11** was achieved in high yield, with the indole initially activated by use of the strong heterogeneous/non-nucleophilic base sodium hydride followed by alkylation

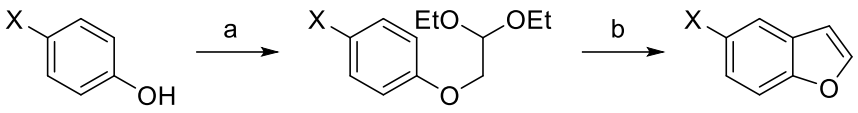
of the anion formed with the appropriate alkyl halide according to the synthesis of 1-methylated indoles reported by *Tatsumi et al.*[45]*;* and the 1-benzylated indoles via the synthesis of *Suna et al.*[46] as shown in Scheme 3



Scheme 3- Conditions: a) NaH, (Y = Bn, DMF) or (Y = Me, THF), 0 °C 1 h; b) (Y = Me) MeI, THF, 0 °C, 16 h; (Y = Bn) BnBr, DMF, 0 °C, 16 h

I. Synthesis of benzofurans

5-Chlorobenzofuran **14** was synthesised according to patent WO200406829-A1[47] where the alkylation of 4-substituted-phenols with bromoacetaldehyde diethylacetal affords tethered latent electrophiles **12** and **13** that. Upon heating under acidic conditions, **12** undergoes an intramolecular electrophilic aromatic cyclisation reaction followed by elimination to restore aromaticity and reveal the benzofuran ring to afford **14**. When these cyclisation conditions were applied to trfluoromethylated-analogue **13** however, no cyclisation was observed. This may be rationalised as the electron-withdrawing nature of the trifluoromethyl functional group reducing the availability of the π-electrons of the benzene ring to engage in electrophilic aromatic substitution (Scheme 4).



X= Cl
X= CF_3

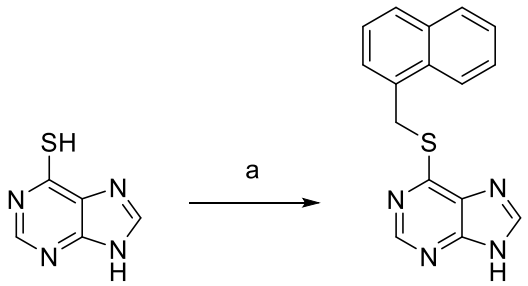
12, X= Cl 87% yield
13, X= CF_3 92% yield

14, X= Cl 42% yield

Scheme 4-Conditions: a) $CaCO_3$, Bromoacetaldehyde diethyl acetal, DMF 65 °C, 60 h; b) Polyphosphoric acid, PhMe, 90 °C, 16 h

II. Synthesis of PU-02

PU-02 is discussed by Jensen et al[43] in terms of its biological efficacy however there are no reported syntheses cited. **15** was successfully synthesised via the alkylation of 6-mercaptopurine under basic conditions with chloromethylnaphthalene (Scheme 5).



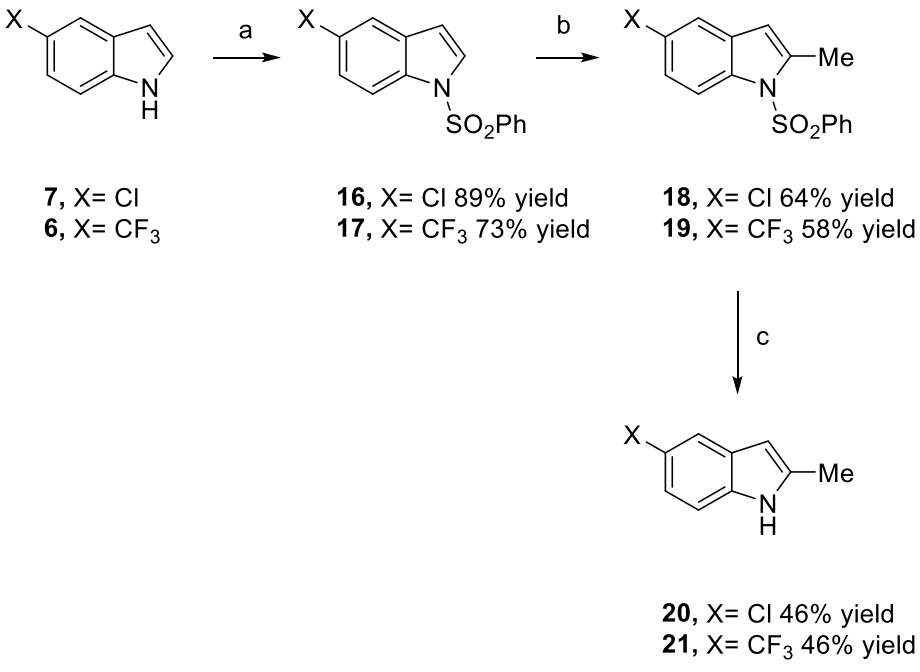
15
99% yield

Scheme 5-Conditions: K_2CO_3, 6-mercaptopurine, NMP, r.t., 16 h

3.3 Synthesis of 2-substituted indoles

I. 5-Halo-2-methyl-indoles

The synthesis of 5-chloro-2-methylindole **20** was achieved following the procedure reported by Disabre et. al.[48] where the indole nitrogen was protected as a phenylsulfonamide, which acts as a directing group for 2-lithiation with LDA. The same synthesis was then applied to 5-(trifluoromethyl)indole to afford **21** as described in Scheme 6

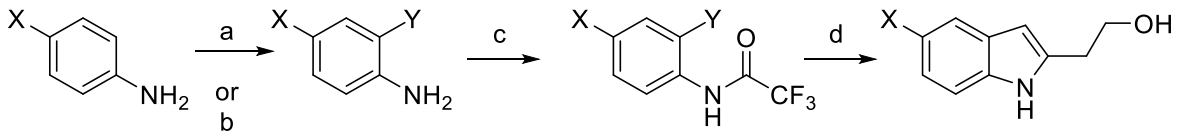


Scheme 6-Conditions: a) i) NaH, THF, 0 °C, ii) $PhSO_2Cl$, 0 °C; b) i) LDA, THF, −78 °C; c) K_2CO_3, MeOH, 70 °C

II. Synthesis of 2-(2-hydroxyethyl)-5-haloindoles

The synthesis of compound **25** was achieved following a procedure reported in patent WO2006128142[49] where bromide **23** undergoes Sonogashira cross-coupling followed by intramolecular copper (I)-catalysed cyclisation to form the 2-substituted indole **25** in one-pot. This

was found to also be a viable synthesis of **26** and with the modification of iodination, where bromination was used in the first two examples, **29** was also synthesised in this fashion although with a noticeably reduced yield (Scheme 7).



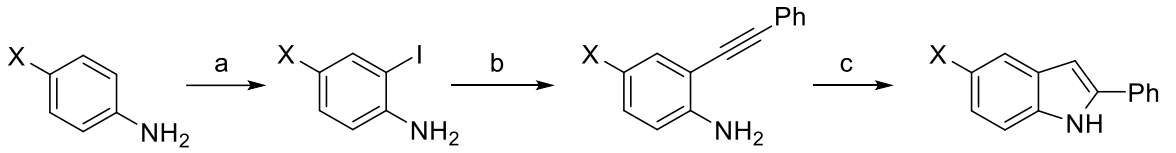
22, X = Cl, Y = Br 87% yield **23,** X = Cl, Y = Br 30% yield **25,** X = Cl 74% yield
2, X = CF_3, Y= Br 59% yield **24,** X = CF_3, Y = Br 88% yield **26,** X = CF_3 46% yield
27, X = Br, Y = I 53% yield **28,** X = Br, Y = I 86% yield **29,** X = Br 7% yield

Scheme 7- Conditions: a) NBS, MeCN, r.t., 16 h; b) Me_3BnICl_2, $CaCO_3$, MeOH, DCM, 6 h; c) TFAA, NEt_3, DCM, 0 °C, 2 h; d) 3-butyn-1-ol, $Pd(PPh_3)_2Cl_2$, CuI, Net_3, DMF, 120 °C, 8 h

The apparent reason for the significantly lower yield of **29** is due to the reactivity of the C-Br bond within **28** providing further cross-coupling as well as reduction to C-H. This was proven by the isolation of of *bis*-butyn-1-ol adducts being isolated along with a 5-hydro-product (see experimental section for details).

III. 2-Phenyl-5-haloindole synthesis.

The synthesis of 2-phenyl-5-haloindoles was initially attempted *via* an analogous synthetic sequence to that wereused to make 5-(trifluoromethyl)indole **6** (Scheme 2) using phenylacetylene as the coupling partner for the Sonogashira cross-coupling to afford **30-32**, which lead to, *via* Cu^I catalysed cyclisation, the successful synthesis of **33** and **34** (Scheme 8).



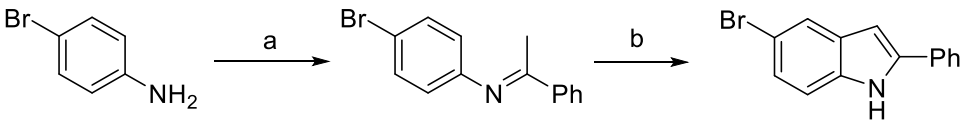
3, X= Cl, 63% yield
27, X = Br, 53% yield
1, X= CF_3, 80% yield

30, X= Cl, 47% yield
31, X= Br, 63% yield
32, X= CF_3, 70% yield

33, X= Cl, 38% yield
34, X= CF_3, 50% yield

Scheme 8-Conditions: a) Me_3BnICl_2, $CaCO_3$, MeOH, DCM, 6 h; b) phenylacetylene; $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, DMF, 50 °C; c) CuI, DMF, r.t., 48 h

However it was found that this method would not afford 5-bromo-2-phenyl-indole **35** which was instead synthesised *via* palladium-catalysed aerobic oxidative cyclisation of *N*-aryl-imines as described by *Yoshikai et al.*[50] (Scheme 9).

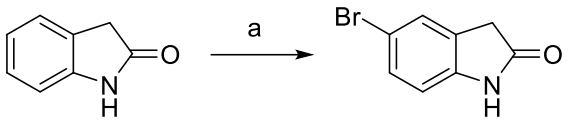


35
39% yield

Scheme 9- Conditions: a) acetophenone 4 Å molecular sieves, PhMe, 110 °C, 48 h; b) $Pd(OAc)_2$, $Cu(OAc)_2$, DMSO, 40 °C, 12 h; yield over 2 steps.

IV. Synthesis of 5-bromo-2-oxindole

5-Bromo-2-oxindole **36** was synthesised according to the procedure reported by *Zhang et. al.*[51] where oxindole undergoes an electrophilic aromatic substitution reaction at the 5-position with an electrophilic source of bromine (Scheme 10).

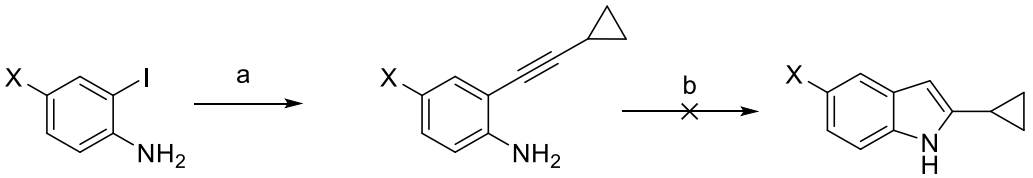


36
52% yield

Scheme 10- Conditions: a) NBS, MeCN, 0 °C 3 h

V. Synthesis of 2-cyclopropyl-5-haloindoles

Initial attempts to access the 2-cyclopropyl-5-haloindoles *via* an analogous synthetic procedure to that described in Scheme 2 was found to be unsuccessful (Scheme 11).



3, X= Cl
27, X= Br
1, X= CF_3

37, X= Cl, 40% yield
38, X= Br, 44% yield
39, X= CF_3, 65% yield

X= Cl
X= Br
X= CF_3

Scheme 11- Conditions: a) Cyclopropylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, Net_3, DMF, 50 °C, 16 h; b) CuI, $CaCO_3$, DMF, 120 °C, 2 h; OR CuI, $CaCO_3$, r.t. 48 h.

The Sonogashira cross-coupling reaction of the 2-iodo-4-haloanilines provided 2-(cyclopropylethynyl)-4-haloanilines **37–39** in moderate yields however the cyclisation reaction for **37–39** did not progress at the lower temperatures used in the synthesis of **6** and **7**. Increase in reaction temperature did not afford the desired indoles and lead to a complex mixture of degradation products. To overcome this, application of the synthetic conditions described in the synthesis of 2-(2-hydroxyethyl)-5-haloindoles (Scheme 7) with cyclopropyl acetylene in place of the 3-butyn-1-ol was found to afford **40** in reasonable yield (Scheme 12).

24 **40**
35% yield

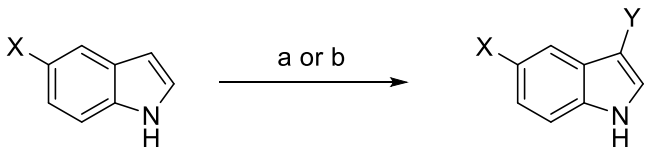
Scheme 12- Conditions: a) Cyclopropylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, Net_3, DMF, 120 °C, 8 h;

Due to the results of the Ca^{2+} intracellular assay process for **40** revealing orthosteric binding, the 5-chloro and 5-bromo-2-cyclopropylindoles were not synthesised.

3.4 Synthesis of 3-substituted indoles

I. 5-3-Dihaloindole synthesis

5,3-Dichloroindole **41** and 3-chloro-5-(trifluoromethyl)indole **42** were synthesised according to the procedure reported by Williams et al[52] in good yields (Scheme 13).



X= Cl **7**
X= CF_3 **6**

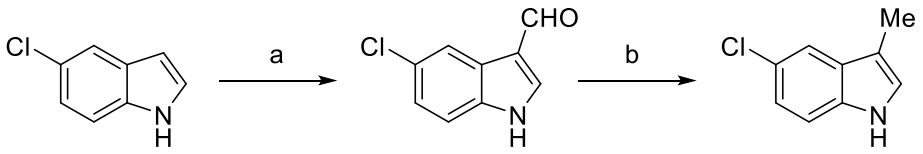
41, X= Cl, Y = Cl 75% yield
42, X= CF_3, Y = Cl 97% yield
43, X = Cl, Y = 60% yield

Scheme 13- Conditions: a) (Y = Cl) NCS, DMF, 0 °C – r.t. 16 h; b) (Y = Br) BNS, DMF, 0 °C – r.t., 16 h

In a related manner, **43** was synthesised *via* electrophilic bromination which proceeded in good yield (Scheme 13).

VI. 5-Halo-3-methylindoles

The synthesis of 5-chloro-3-methylindole **44** was achieved *via* the procedure reported by *Xiao* et al [53] whereby 5-chloroindole is formylated in the 3-positon *via* a Vilsmeir Haack formylation and the aldehyde formed undergoes a reduction and deoxygenation step with $LiAlH_4$; this deoxygenation step is presumably caused by anchimeric assistance from the indole nitrogen lone pair of electrons (Scheme 14).



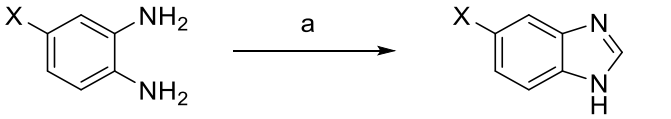
7 **44**
68% yield

Scheme 14- Conditions: a) $POCl_3$, DMF 0 – 40 °C, 2.5 h; b) $LiAlH_4$, THF 0 °C, 16 h

Following the synthesis of **44** the synthesis of 5-(trifluoromethyl)-3-methylindole was attempted in the same fashion, however the reduction and deoxygenation step did not proceed as it did with the chlorinated example in Scheme 14.Instead, degradation to multiple unidentified by-products was observed.

VII. Synthesis of 5-substituted benzimidazoles

Benzimidazoles **45** and **46** were synthesised according to *Raphael et al*.[54] in good yields from their corresponding 2-amino-4-haloanilines (Scheme 15).

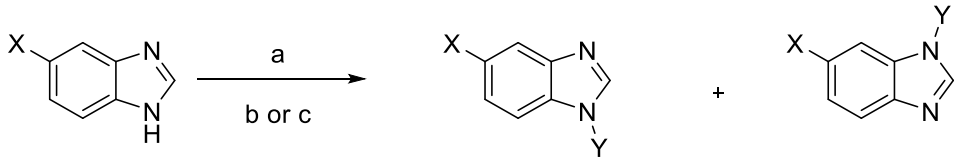


X= Br
X= CF_3

45, X= Br 94% yield
46, X= CF_3 92% yield

Scheme 15-Conditions: a) Formic acid, 4 N HCl (Aq.), 100 °C, 45 min.

Further derivatisation to form *N*-methylbenzimidazole **50** and *N*-benzylbenzimidazoles **47, 48** and **49** was achieved as described in Scheme 16, *via* a synthetic procedure inspired by *Tatsumi et al*[45] and *Suna et al.*[46] featured in the Synthesis of 1-alkylindoles above (Scheme 3). As expected there was no observed regio-selectivity for this reaction as described below (Scheme 16) however there was sufficiently different affinity to silica between **48** and **49** for their purification *via* careful column chromatography and the two isomers were disambiguated with the aid of a NOe NMR experiment.



45, X= Br
46, X= CF_3

47a, X = Br, Y = Bn 36% yield
48, X = CF_3, Y = Bn 30% yield
50, X = CF_3, Y = Me 93% yield

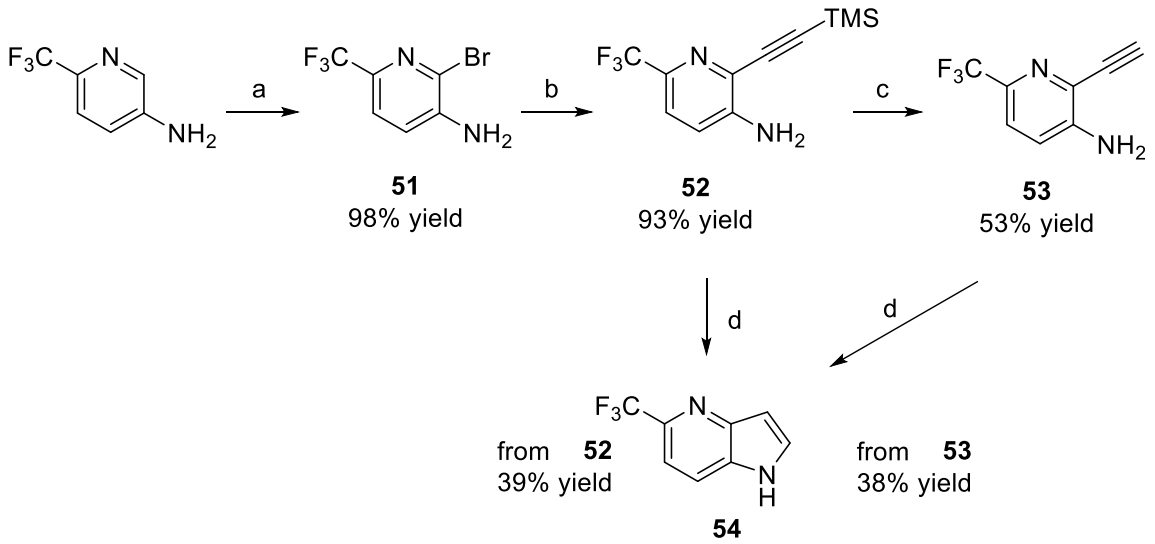
47a, X = Br, Y = Bn 36% yield
49, X = CF_3, Y = Bn, 33% yield

Scheme 16-Conditions: a) NaH, THF, 0 °C, 0.5 h; b) (Y = Bn) BnBr, 16 h, r.t.; c) (Y = Me) MeI, THF, 0 °C, 16 h

3.5 Synthesis of 5-substituted-pyrrolo[3,2-b]pyridines

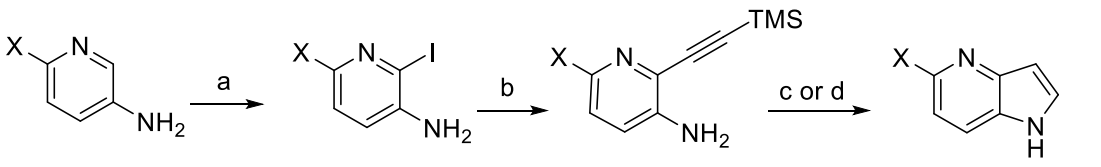
The synthesis of 5-(trifluoromethyl)-1H-pyrrolo[3,2-b]pyridine **54** was reported in patent EP2548876-A1 which was found to proceed in good yield; however it was found that the

methanolysis of the trimethylethynyl-silane was a redundant step and could be removed, *i.e.* base-catalysed cyclisation of **52,** with an overall improvement in yield as shown in Scheme 17.



Scheme 17- Conditions: a) NBS, MeCN, 0 °C – r.t., 3 h. b) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, THF, r.t., 16 h; c) K_2CO_3, MeOH, r.t., 2 h; d) ^{t}BuOK, NMP, r.t., 16 h.

The methodology outlined in Scheme 17 was applied to afford the 5-chloro and 5-bromopyrrolo[3,2-b]pyridines **59** and **60** from their respective halogenated aminopyridines; it is interesting to note that there is an intrinsic preference of each alkynyl substrate (**52**, **57** and **58**) to cyclising more efficiently under either base catalysed conditions (**52** and **57**) or copper (I) catalysed conditions (**58)** Scheme 18.

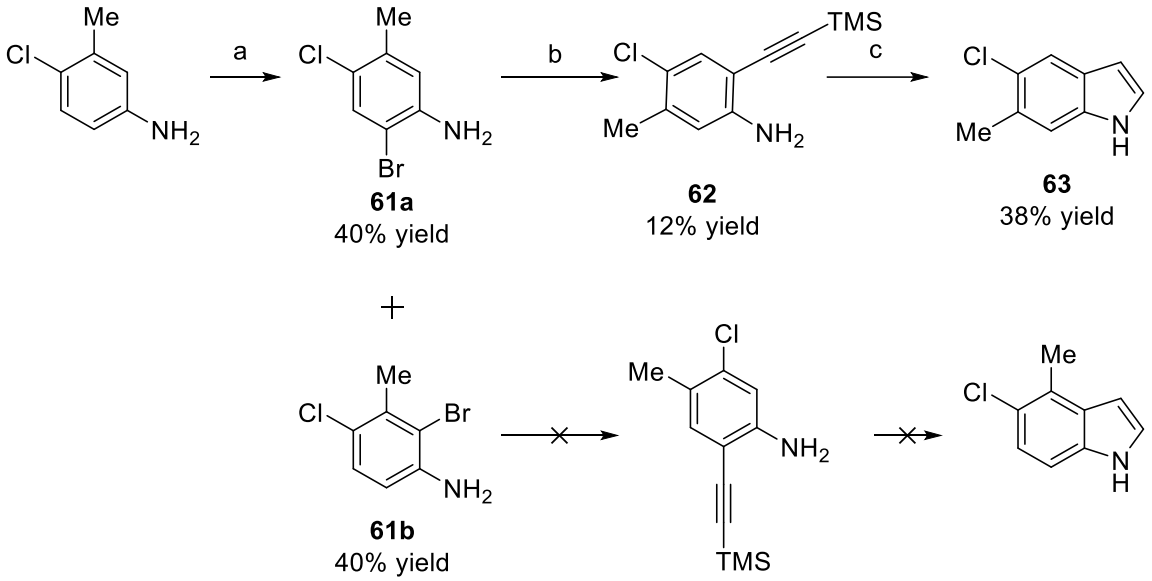


Scheme 18-Conditions: a) $Me_3NBn\ ICl_2$, $CaCO_3$, MeOH, DCM, r.t., 10 h; b) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, r.t., 16 h; c) (X = Cl) CuI, $CaCO_3$, DMF, 120 °C, 2 h; d) (X = Br) ^{t}BuOK, NMP, r.t., 16 h

Chloropyridine **57** was cyclised to pyrrolo[3,2-b]pyridine **59** in a 32% yield *via* Cu^{I} catalysed cyclisation conditions akin to that used in the Goldstein synthesis of 5-substituted indoles (Scheme 2). However, when these cyclisation conditions were applied to pyridine **58** the reaction yield fell significantly to afford only 5% yield of **60**. It was observed that under basic conditions with ^{t}BuOK **58** cyclised to **60** in a 41% yield, it is interesting to note that the substituent at the 6-position of the pyridine (**52, 57** and **58**) appears to influence the performance of the copper catalysed cyclisation.

3.6 Synthesis of 5-chloro-6-methylindole

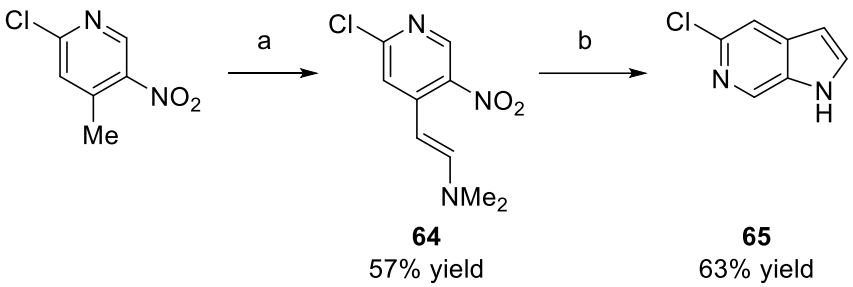
The synthesis of 5-chloro-6-methylindole **63** was designed to proceed in an analogous way to the synthesis of 5-(trifluoromethyl)indole **6** (Scheme 2), however no regio-selectivity for the electrophilic aromatic bromination of 4-chloro-3-methylaniline was observed. This reaction instead afforded a 1:1 mixture of inseperable brominated anilines that could potentially enable access to not only 5-chloro-6-methylindole but also 5-chloro-4-methylindole. Unfortunately, only 2-bromo-4-chloro-5-methylindole **61a** underwent the Sonogashira cross-coupling with ethynyltrimethylsilane to afford **62**. Cyclisation was achieved *via* copper (I)-mediated cyclisation conditions to afford 5-chloro-6-methylindole **63** in moderate yield (Scheme 19).



Scheme 19-Conditions: a) NBS, MeCN, r.t., 8 h; b) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, 70 °C, 16 h; c) CuI, $CaCO_3$, DMF, 120 °C, 16 h

VIII. Synthesis of 5-chloropyrrolo [2,3-c]pyridine

The synthesis of 5-chloropyrrolo[2,3-c]pyridine **64** *via* the conditions detailed in patent WO2010/42337 A1 which proceeds in a very similar way to that of a Leimgruber-Batcho indole synthesis[55], where alkylation at the benzylic 3-methyl position is observed to form the enamine **64** which was cyclised under acidic reductive conditions to afford **65** in good yield (Scheme 20).

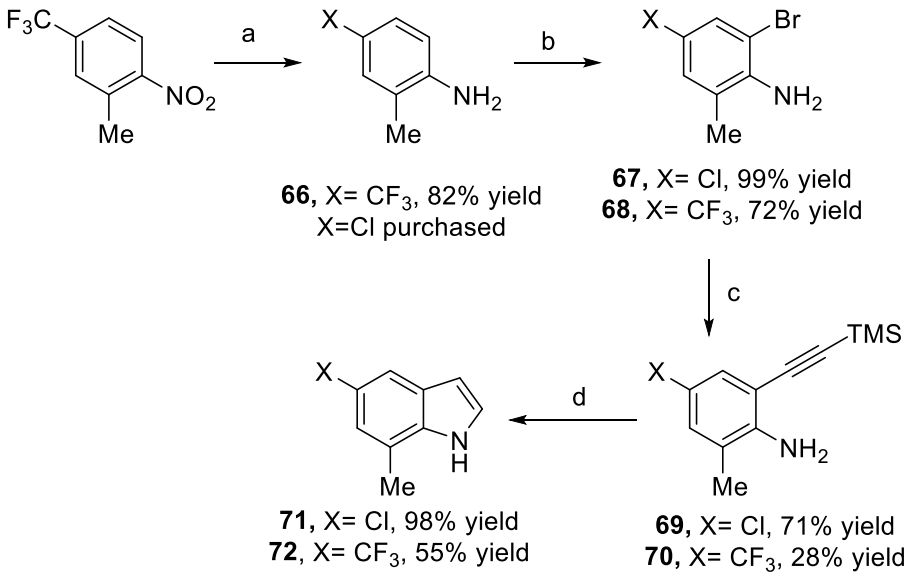


Scheme 20-Conditions: a) dimethylformamide dimethylacetal, DMF, 90 °C, 18 h; b) Zn, acetic acid, 118 °C, 16 h

3.7 Synthesis of 7-substituted indoles

II. 5-Chloro-7-methylindole

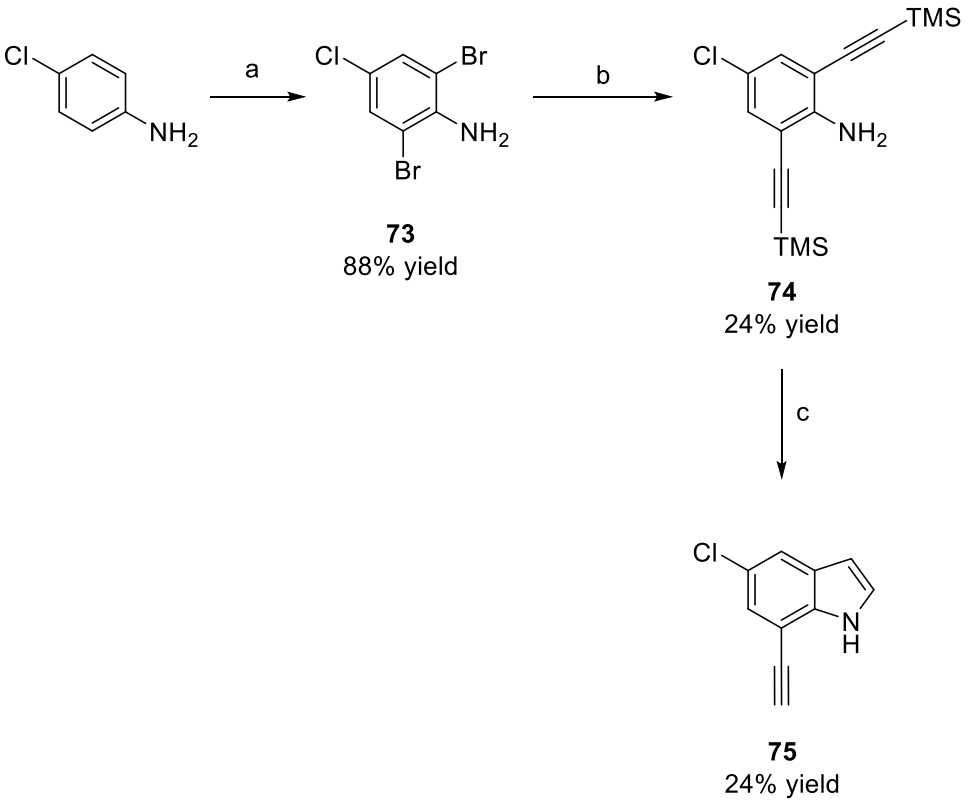
5-Chloro-7-methylindole **72** was synthesised *via* an analogous process to 5-(trifluoromethyl)indole **6**, starting with 4-chloro-2-methylaniline which was brominated in the 6-position to afford **67**. Sonogashira cross-coupling with ethynyltrimethylsilane afforded **69** in moderate yield then Cu^{I} catalysed cyclisation was achieved using microwave irradiation, which proceeded in very high yield to form **71**. Application of these conditions to **66,** which was accessed *via* the hydrogenolysis of 2-nitro-4-(trifluoromethyl)-nitrobenzene, afforded 5-(trifluoromethyl)-7-methylindole **72** in good yield (Scheme 21).



Scheme 21-Conditions: a) H_2, Pd/C, MeOH, r.t., 16 h; b) NBS, MeCN, 0 °C – r.t., 3 h; d) (X= Cl) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, 85 °C, 16 h; d) (× = CF_3) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, DMF, 120 °C, 4 h

IX. Synthesis of 5-chloro-7-ethynyl-1H-indole

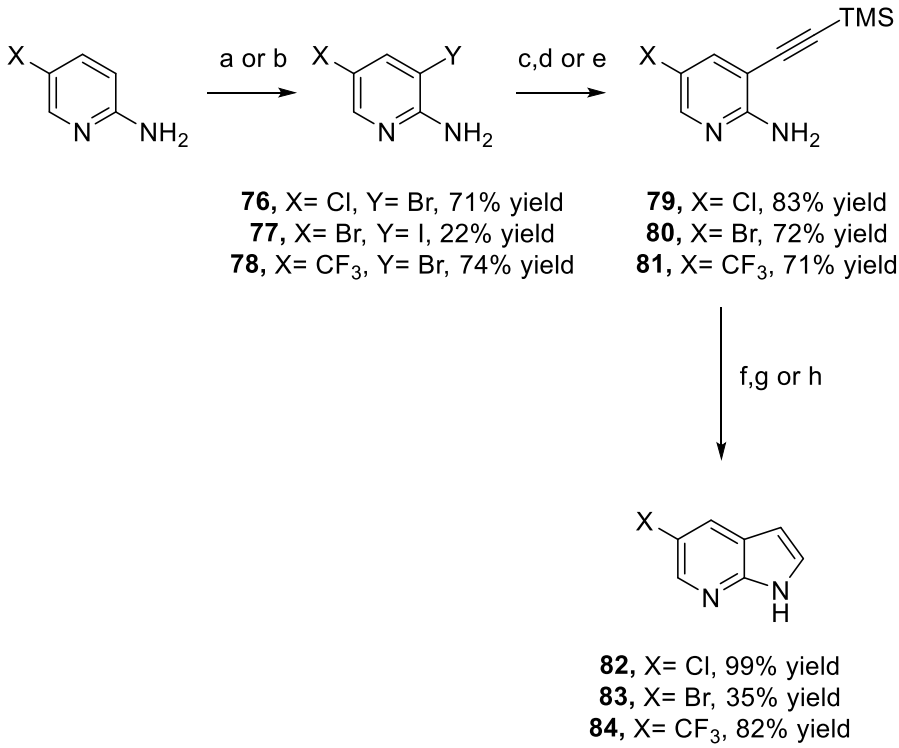
5-Chloro-7-ethynylindole **75** was synthesised *via* 2,6-dibromination of 4-chloroaniline with NBS to provide **73**, which underwent Sonogashira cross-coupling with ethynyltrimethylsilane to afford **74.** In this cross-coupling reaction significant degradation was observed leading to a decreased yield than observed in the synthesis of **7** (Scheme 2). The cyclisation of **74** was initially attempted with Cu (I) catalysed conditions yet found to only yield degradation. The cyclisation of **74** was successfully achieved under basic conditions to afford **75** in moderate yield (Scheme 22).



Scheme 22-Conditions: a) NBS, MeCN, 0 °C – r.t., 16 h; b) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, 80 °C, 16 h; c) ^tBuOK, NMP, 80 °C, 4 h.

X. Synthesis of 5-substituted-pyrrolo[2,3-b]pyridines

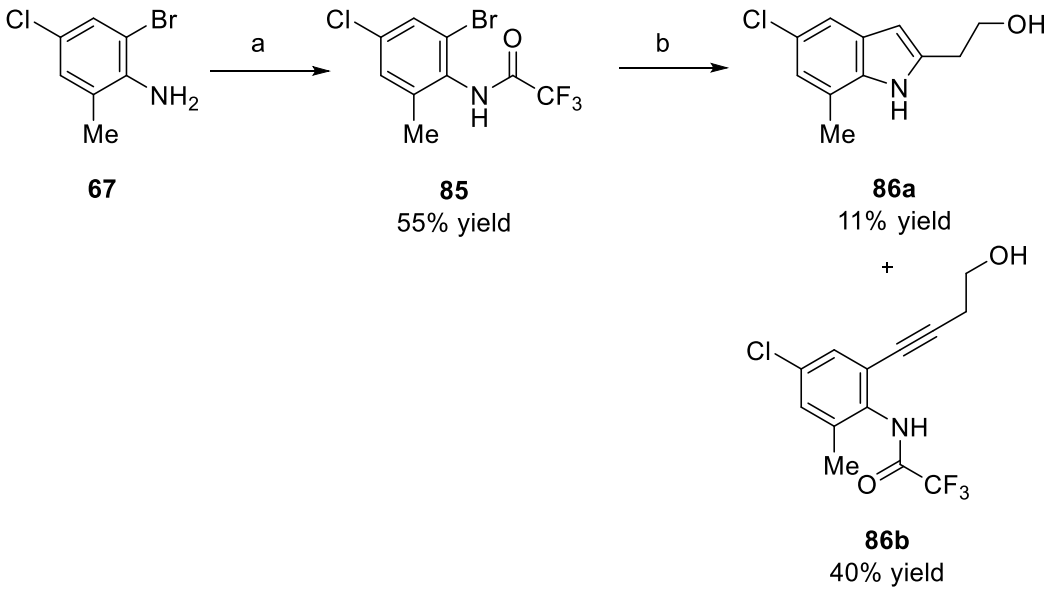
The synthesis of 5-bromo-pyrrolo[2,3-b]pyridine **83** was synthesised as reported in patent US2006/183758 A1 which afforded the target compound in reasonable yield. Synthesis of the chlorinated **82** and the trifluoromethylated **84** analogues were found to occur in a similar fashion however the synthesis was successfully performed starting with bromination instead of iodination, *via* bromopyridines **76** and **78**, which were found to improve the yields considerably (Scheme 23).



Scheme 23-Conditions: a) (X = Cl/CF_3) NBS, 0 °C – r.t., 3 h; b) (X = Br) HIO_4, I_2, acetic acid, MeCN, 50 °C, 4 h; c) (X = Cl) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, 70 °C, 16 h; d) (X = Br) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, r.t., 16 h; e) (X = CF_3) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, DMF r.t., 16 h; f) (X = Cl) CuI, $CaCO_3$, DMF, 120 °C, 16 h; g) (X = Br) ^tBuOK, DMF, r.t., 16 h; h) (X = CF_3) NaH, NMP, 80 °C, 4 h.

XI. Synthesis of Synthesis of 2-(5-chloro-7-methylindol-2-yl)ethan-1-ol

The synthesis of **86a** was achieved *via* similar conditions to those reported in patent WO2006128142[49] which were discussed in the synthesis of 2-substituted indoles **25** and **26**, described in Scheme 7; this instance however, starting the synthesis with 2-bromo-4-chloro-6-methylaniline **67** which was discussed in as an intermediate in the synthesis of 5-chloro-7-methylindole **71** described in Scheme 21. The synthesis was achieved with a somewhat reduced yield compared to that of the simpler 2-(5-chloroindol-2-yl)ethan-1-ol **25.** It is apparent that the reaction is much slower to cyclise the intermediate cross-coupled adduct, in the case of the 7-methylated species described below (Scheme 24), compared to that of the intermediate formed prior to the cyclisation of 25 described in Scheme 7, where the initial cross-coupling adduct is not isolated over the same time scale.



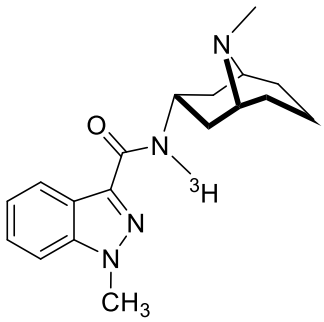
Scheme 24-Conditions: a) TFAA, NEt_3, DCM, 0 °C – r.t., 2 h; b) 3-butyn-1-ol, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, DMF, 120 °C, 4 h.

3.8 Summary of synthesis

Through the SAR study a variety of synthetic routes have been utilised to assemble indoles with different substitution patterns as well as several pyrrolopyridines. A general procedure of *ortho*-halogenation followed by Sonogashira cross coupling and cyclisation with either base or copper catalysis is shown to be a good synthetic approach to afford electron-poor examples of these structures.

4 Fluorescent drugs as alternatives for radio-ligand binding assay

Assessment of the binding mode of compounds that behave as antagonists of the 5-HT_3A receptor has relied upon radio-ligand binding studies where the compound of interest is dosed along with a [^{3}H]-labelled orthosteric agent that has high binding affinity at the orthosteric site, specifically [^{3}H]-Granisetron (Figure 19). Through measuring the level of radioactivity retained by HEK assay cells it is possible to determine the degree of ligand binding. If the test compound displaces the radio-labelled orthosteric agent this provides direct evidence of competitive binding, likely to result from the compound of interest interacting with the orthosteric site of the receptor. In this case it is deduced that the compound of interest is an orthosteric inhibitor. Alternatively, if the radio-ligand at the orthosteric site is not displaced, this demonstrates that the test compound is a non-competitive inhibitor, suggesting that it is binding at another site on the receptor and is therefore an allosteric modulator.



[3H]-Granisetron

Figure 19- [^{3}H]-Granisetron

Although the technique of radio-ligand binding has provided reliable results there are clear issues with its use including the expense of acquiring radio-labelled analogues of the orthosteric agents to be used, such as Granisetron, due to the safety and legislative issues arising from handling, synthesising and purifying radioactive compounds. Another issue is that the waste produced from any radio-labelled assay must be treated in a radioactive waste stream that, aside from also being costlier compared with normal contaminated solid waste, should be minimised where possible for ethical reasons. The limited availability and prohibitive cost of radiolabelled tool compounds also limits the scope of studies that can be undertaken. For example, to evaluate the impact of PAM/NAMs it would be ideal to be able to assess the impact on the binding of both orthosteric agonists and antagonists. Radio-labelled tool compounds for such studies must high affinity and/or slow off rate in order to allow retention of the labelled molecule during the wash stages, and hence 5-HT itself lacks sufficient intrinsic activity to be useful. Quipazine is a quinoline based agonist that is known to bind the 5-HT_3A receptor at the orthosteric site with a high binding affinity (~2 nM EC_{50})[56] and is a well-characterised 5-HT_3 agonist. Due to the emerging need to identify the interactions of compounds with the orthosteric site as well as to help clarify the identity of allosteric site, the design and synthesis of a fluorescent analogue of quipazine was embarked upon. It was hypothesised that a fluorescent analogue of quipazine that had suitably

similar pharmacological properties to quipazine could be used to determine modes of binding *via* the use of a fluorescence polarization experiment similar to that outlined with labelled granisetron analogues reported by *Jack et al.*[57]

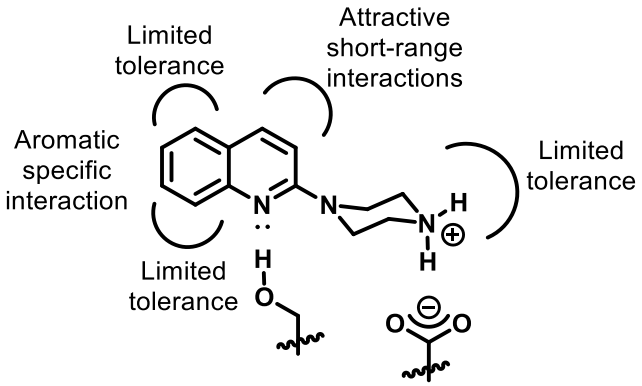
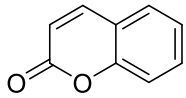
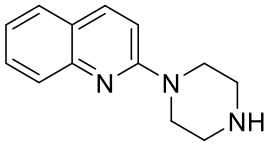


Figure 20- Summary of the SAR of quipazine reported by Langer et al[56]

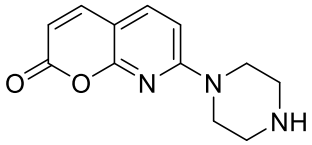
In order to make a fluorescent quipazine-derived drug with the best chance of maintaining the pharmacological activity of the parent compound the smallest changes as possible had to be made to the molecular structure to yield a fluorophore. As shown by Figure 20 above, which summarises the scope for derivatisation around quipazine based on the reported SAR of *Langer et al,* the key interactions are stemming from the quinoline nitrogen interacting with a serine O-H as well as the charged H-bond between the piperazine NH_2^+ and the carboxylate CO_2^-. It is suggested that there is only limited chemical space at the phenyl-end of the quinoline ring for derivatisation, with this information in hand use of large fluorophores as discussed in chapter 6 would almost certainly negatively affect the binding. Instead a conservative modification of the quinoline ring to an aza-coumarin was selected (Figure 21).



Coumarin



Quipazine



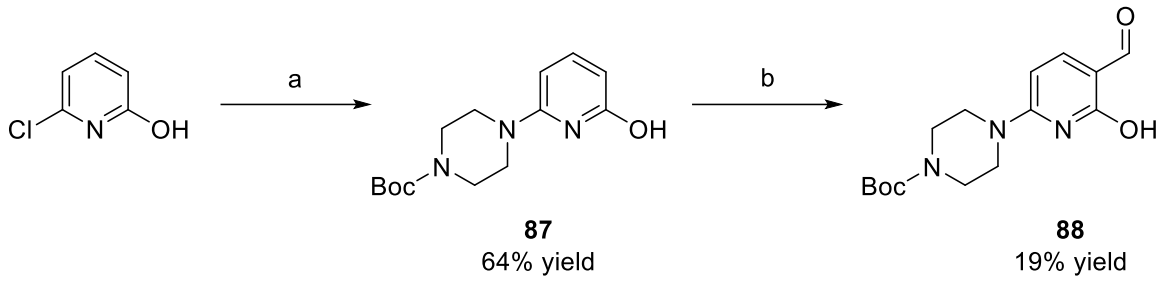
Aza-coumarin
FL-Quip
95

Figure 21-proposed fluorescent analogue of quipazine

Aza-coumarins have previously been reported in the literature in a number of applications utilising their fluorescent properties in fluorescent cellular-probes[58], laser-dyes[59] as well as their innate anti-microbial properties[60] and they all have very similar emission properties to that of Coumarin with a range from ~410-470 nm meaning they emit in the blue region of the visible spectrum.

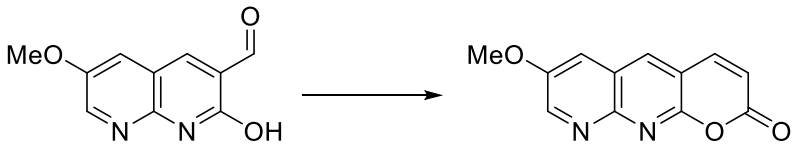
4.1 Synthesis of FL-Quip

The proposed synthesis of FL-Quip **95** was inspired by a patent for the synthesis of compounds designed for the treatment of Spinal Muscular Atrophy, as reported by *Meijler et al* [61], where compound **87** was accessed from S_NAr of 6-chloropyridin-2-ol with *N*-Boc-piperazine followed by an unusual *ortho*-formylation at the 3-position with anhydrous magnesium chloride and paraformaldehyde in the presence of triethylamine as a base[62] to afford **88**, as shown in Scheme 25.



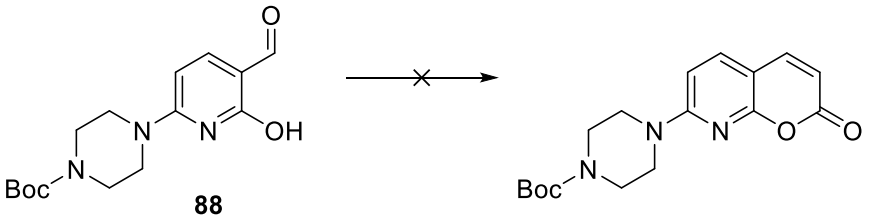
Scheme 25-Conditions: a) *N*-Boc-piperazine, *n*-butanol, 121 °C, 3 d; b) paraformaldehyde, $MgCl_2$, MeCN, 60 °C, 16 h.

From aldehyde **88** the pyranone ring system was to be constructed via the method described by Bhojya et al[63] as shown in Scheme 26 below.



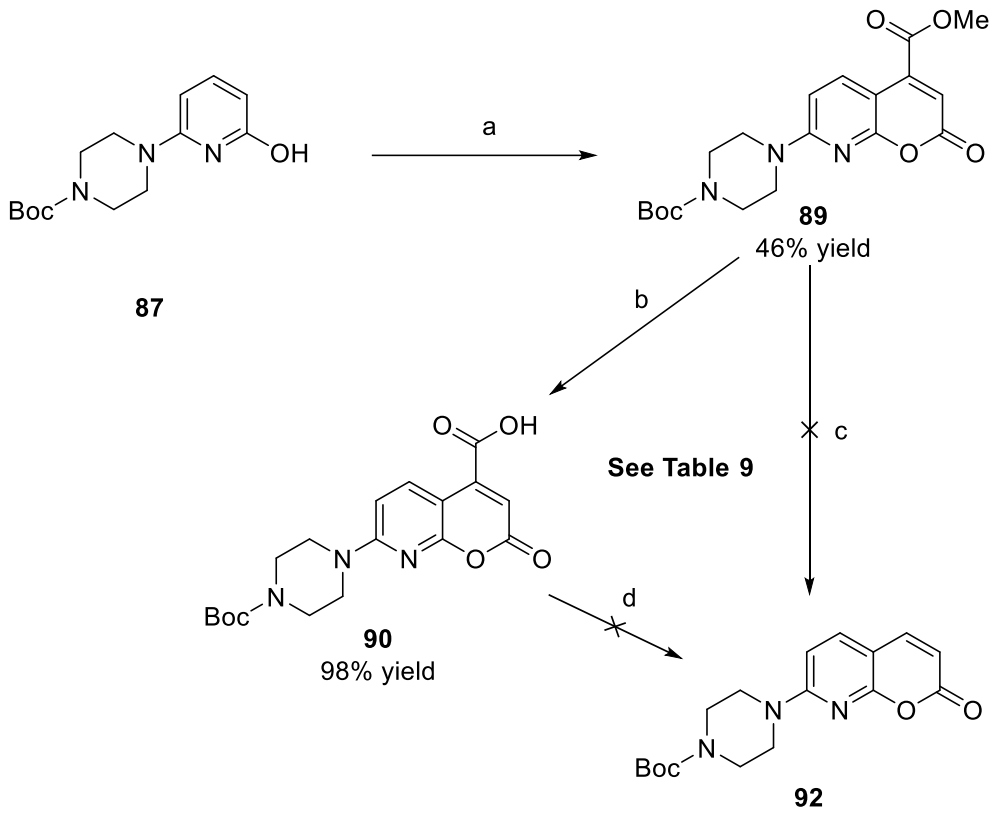
Scheme 26-*Bhojya et al's* synthesis of aza-coumarin ring; Conditions: NaOAc , Ac_2O, µW, 100 °C, 15 min.

However, despite several adaptions including changes to temperature, microwave irradiation conditions, classical heating in a variety of solvents as well as the addition of stronger bases such as ^{t}BuOK the desired product was not observed (Scheme 27).



Scheme 27- Application of Bhojya *et al's* conditions to 88; Conditions: NaOAc, Ac_2O, µW, 100 °C, 2 h.

An alternative synthesis was proposed utilising the reactivity of activated vinylphosphonium salt species reported originally by Yavari *et al*[64], which successfully formed the coumarin pyranone ring appended with a 4-methylcarboxylate (Scheme 28).



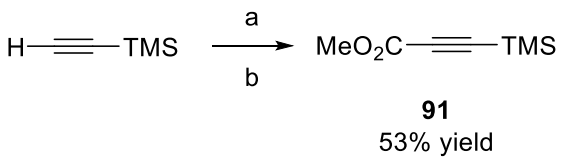
Scheme 28-Conditions: a) dimethylacetylene dicarboxylate, triphenylphosphine, PhMe, 0 – 110 °C, 20 h; conditions b), c) and d) are summarized in table 9.

Attempts to decarboxylate the pyranone **89** were unfortunately unsuccessful despite a variety of conditions being explored as summarized in Table 9 below.

Entry	Conditions	Comment	Conditions lit. source
1	i) KOH MeOH 65 °C (99% yield) ii) Cu_2O, 1,10-phenanthroline Quinoline, NMP (3:1) 180 °C 15 min	Step (ii) performed at lower temperatures (r.t. → 150°C). T<150°C no reaction observed T>150°C degradation observed	*Cahiez et al*[65]
2	Cu, Quinoline 180 °C 19h	1 hour-19 hours Consumption of starting material no intelligible products, Degradation observed	Litinas *et al*[66]
3	TFA DCM 40 °C 17 h	Successful decarboxylation, loss of piperazine ring observed	N/A

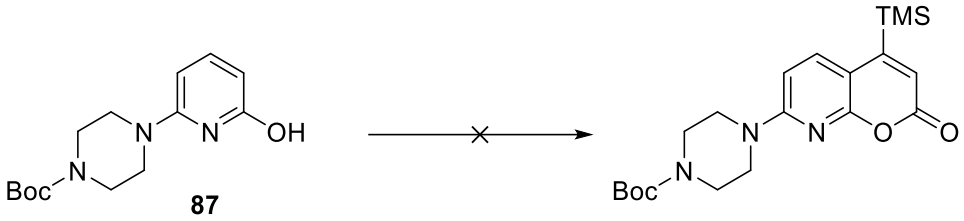
Table 9-Summary of decarboxylation conditions attempted

In an attempt to bypass the decarboxylation issues observed with **89** in the synthesis described above (Scheme 28), application of *Yavari et al's* conditions with ethyl-3-(trimethylsilyl)propiolate **91** which was synthesised according to *Belotti et al*[67] was attempted (Scheme 29).



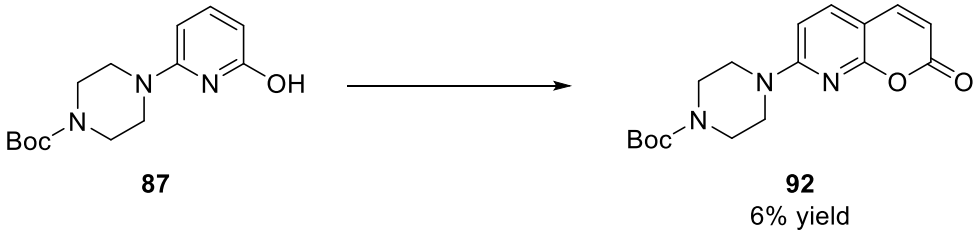
Scheme 29-Conditions: a) n-BuLi, −78 °C – r.t., THF, 40 min; b) ethyl chloroformate, 2 h.

Unfortunately, no aza-coumarin products were observed from the reaction of **91** under Yavari's conditions; presumably the acetylene derivative is not sufficiently activated in this example (Scheme 30) to react as it was observed to with DMAD above (Scheme 28).



Scheme 30-Conditions: ethyl-3-(trimethylsilyl)propiolate (91), triphenylphosphine, toluene, 0 – 110 °C, 20 h.

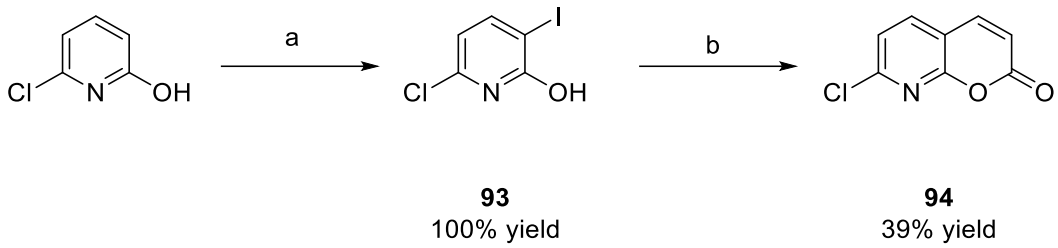
Due to this difficulty to selectively decarboxylate the coumarin **89** another route was attempted inspired by the work of Sharma et al[68] utilising palladium-catalysed C-H functionalisation where **87** was reacted with methylacrylate to form aza-coumarin **92** (Scheme 31).



Scheme 31- Conditions: methylacrylate, $Pd(OAc)_2$, $Cu(OAc)_2$, 1,10-phenanthroline, 1,2-DCE, NaOAc, 4 Å molecular sieves, 110 °C, 72 h

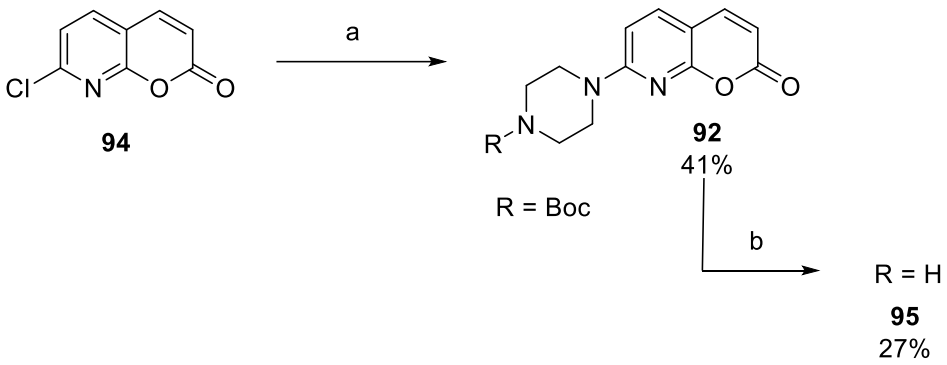
Upon optimisation, it was determined that the reaction performed with comparable yield when dichloromethane was substituted for 1,2-dichloroethane and the reaction was performed in a sealed tube. The yield however for this reaction was unacceptably low and so a more efficient reaction was still necessary. Considering the degradation observed when working with the compounds appended with the piperazine a new strategy was developed, approaching the

synthesis with the S_NAr reaction to install the piperazine last and instead focus on synthesis of the coumarin ring first *via* the route shown in Scheme 32 below.



Scheme 32-Conditions: a) I_2, K_2CO_3, H_2O, r.t., 1 h; b) methylacrylate, $Pd(OAc)_2$, NEt_3, MeCN, 82 °C, 4 h

The iodination of 2-chloro-6-hydroxypyridine was achieved in quantitative yield to afford **93** under basic conditions inspired by Hartz *et al*[69] followed by a Heck palladium-catalysed cross coupling reaction with methylacrylate to afford the chloro-aza-coumarin **94** in a 39% yield over two steps. The S_NAr reaction of *N*-Boc-piperazine with chloro-aza-couimarin **94** proceeded in a notably lower yield than in the synthesis of **87**, but never the less, provided the desired Boc-protected piperazinyl-aza-coumarin **92** which was deprotected under acidic conditions to afford our desired FL-quip **95**, (Scheme 33).



Scheme 33- Conditions: a) *N*-Boc-piperazine, *n*-butanol, 50 °C, 3 d; b) TFA, DCM, r.t., 1 h.

As expected, aza-coumarin **95** noticeably emitted in the blue region of the visible spectrum and very intensely so when excited with long-wave UV irradiation (Figure 22)**.**

Figure 22- Aza-coumarin 95, in NMR tube, dissolved in $CDCl_3$ irradiated at 365 nm

4.2 Results

Unfortunately, despite the very conservative structural change between quipazine and FL-Quip **95** FL-Quip is no longer observed to be an agonist of the 5-HT_3A receptor in any detectable way, which was determined *via* a competitive binding experiment with radio-labelled Granisetron. An aliquot from the cellular assay was taken and confirmed by mass spectrometry that **95** was present after the competitive binding experiment. This confirms that **95** was not simply degrading in solution or sequestering out of solution. The loss of binding may be explained by the binding mode postulated in the original quipazine literature, where they identify that there is a very-

limited tolerance for substitution at the 8-position of the quinoline ring and suggest that this position is close to a backbone amide carbonyl in the orthosteric site (Figure 23).

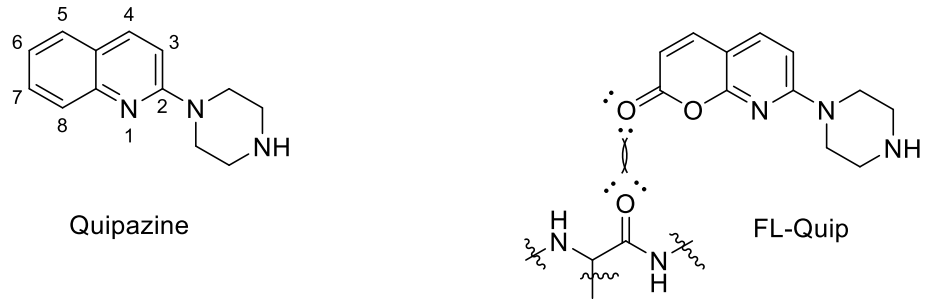


Figure 23- possible electronic-repulsion of carbonyl with receptor peptide

4.3 Conclusions

A novel quipazine fluorescent analogue, FL-Quip **95**, was successfully prepared and tested but did not retain the desired activity at the 5-HT_3A receptor. Further analogues of quipazine could be envisaged, potentially through adding a separate fluorophore to a suitable site on the agonist ligand, however given the tight SAR around the quipazine core this was deemed to be too high-risk to be useful. Separate attempts to label a known partial agonist s-zacopride led to a change in the functionality for the compound to a clean antagonist, (Sam Butterworth, Graziella Greco and Alexander Roberts, unpublished results).

5 Photo-affinity studies

The first descriptions of Photo-Affinity Labelling (PAL) appeared in the literature in the 1960's following the work of Westheimer *et al*[70] and the approach has since become an invaluable tool when exploring ligand-receptor interactions.[71] The key concept orientates around a photo-reactive group that can be activated with light to form a highly reactive species that will alkylate proximal functionality of the target to form a covalent bond. The reactive groups should be sufficiently reactive to rapidly/instantly quench in water, such that only protein-bound ligands lead to protein adducts, leading to specific labelling patterns generally identified by partial protein digestion and MS/MS. The pattern of residues alkylated are indicative of the ligand binding site. Commonly used PG's in PAL include aryl-azides,[72] benzophenones[73] and diazirines[71] (Figure 24).

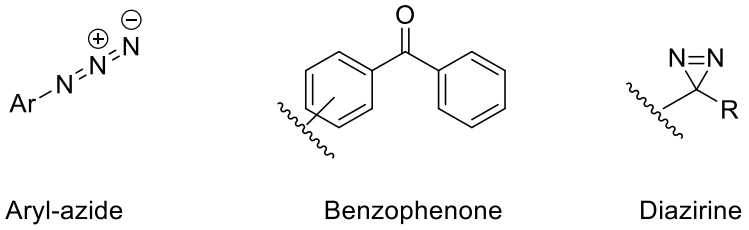
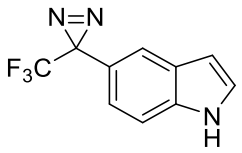


Figure 24-Commonly used PAL PG's

Diazirines have become an increasingly popular choice as a photo-reactive group for PAL studies due to their small size relative to the other PG's, which helps to retain as much structural similarity to the parent ligand (non-PAL ligand), as well as their ability to be electronically tailored to afford either a singlet or a triplet carbene (dependent upon electron withdrawing or donating functionality appended).[71] A recent publication by Hashimoto *et al.*[74] detailing the synthesis of 5 and 6-(3-trifluoromethyl)diazirinylindoles came to our attention and the synthesis of 5-(3-(trifluoromethyl)-3H-diazirin-3-yl)-1H-indole **101** (Figure 25) was persued in the hope that it may

behave as an allosteric modulator and thus provide some insight into the location of the allosteric site.



101

Figure 25 Hashimoto's (3-(trifluoromethyl)-3H-diazirin-3-yl)-1H-indole

To support the hypothesis that the allosteric binding site of the 5-HT_3A receptor is in fact a non-ligand bound inter-pentameric site that has been activated by the binding of 5-HT at another inter-pentameric site, determination of the functional activity of 5-(3-(trifluoromethyl)-3H-diazirin-3-yl)-1H-indole **101** was elucidated. If **101** proved to interact with the 5-HT_3A receptor in an allosteric fashion then it would enable photo-affinity studies *via* the irradiation of cells that express the 5-HT_3A receptor which have been dosed with **101** and then, *via* protein digestion followed by purification, identify which residues on the receptor the *in situ* formed carbene are reacting with in most abundance by mass spectrometry. What is hoped to be seen bu this experiment is the majority of the purified receptor proteins being alkylated within the same chemical space, as that is what would be associated with the diazirine-indole associating with the receptor in a specific interaction, not spread across a variety of sites upon the receptor, see Figure 26 below.

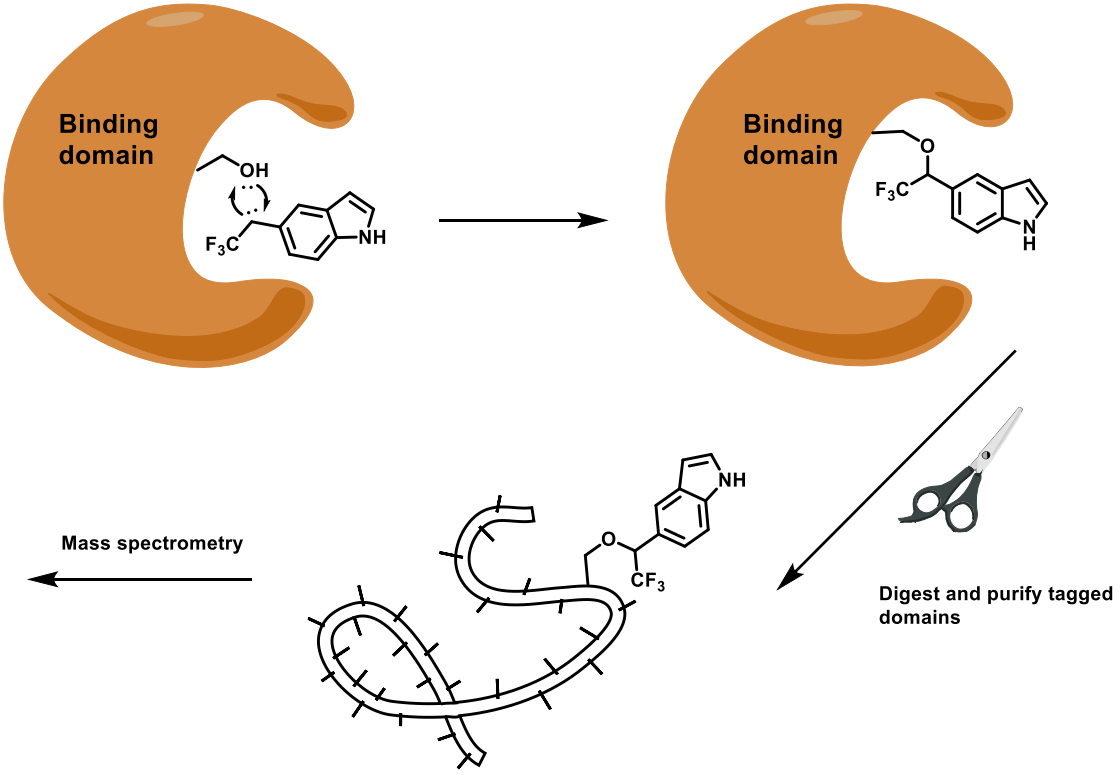
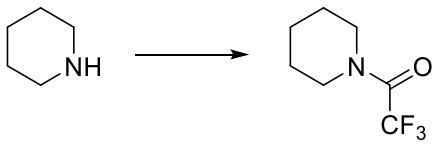


Figure 26- Schematic of the process involved in photo-affinity binding

From the SAR that had been conducted by this point it seemed likely that, as **101** is an indole with an electron withdrawing functionality in the 5-position, that this too would interact with the 5-HT_3A receptor as an allosteric modulator; and most likely as a PAM. 2,2,2-Trifluoro-1-(piperidin-1-yl)ethan-1-one is commercially available however the cost of purchasing it was prohibitive. Therefore **96** was synthesised according to *Brindisi et al*[75] as described below (Scheme 34).

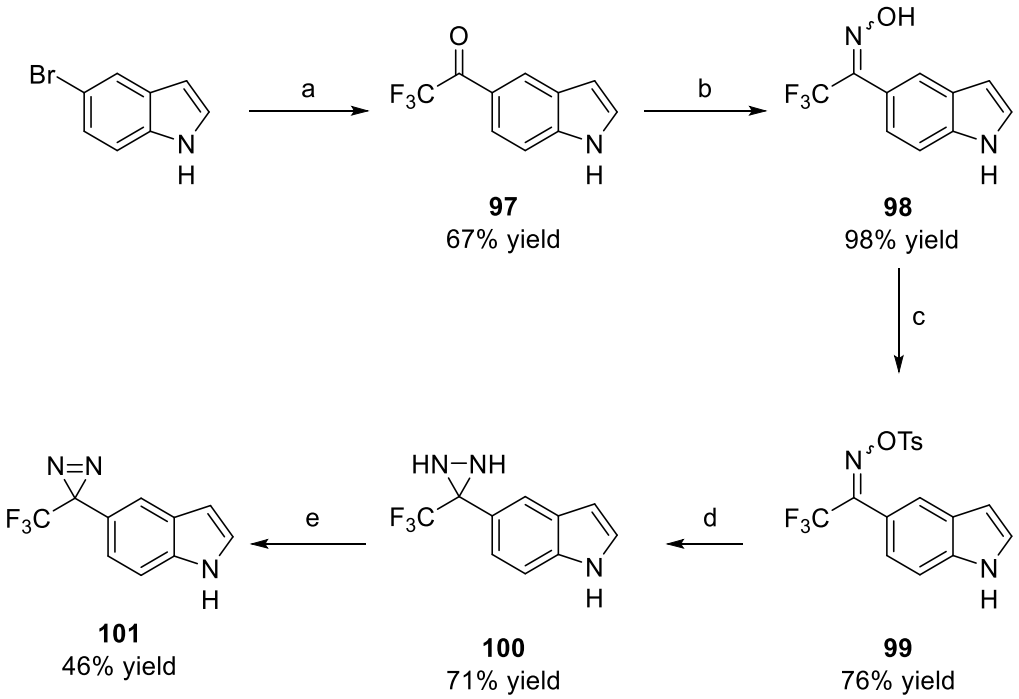


96
96% yield

Scheme 34-Conditions: TFAA, THF, 0 °C – r.t., 6h

5.1 Synthesis of Hashimoto's diazirinylindole, 101

Hashimoto's indole **101** was synthesised via their reported procedure[74] as shown in Scheme 35.



Scheme 35- Conditions: a) i) NaH, THF, 0 °C, 1 h; ii) ^{t}BuLi, −78 °C, 25 min; iii) 2,2,2-Trifluoro-1-(piperidin-1-yl)ethan-1-one, −78 °C - r.t., 6 h; b) hydroxylamine hydrochloride, pyridine, 80 °C, 4 h; c) TsCl, NEt_3, acetone, 0 °C – r.t., 6 h; d) NH_3, Et_2O, −78 °C – r.t. 150 PSIG, e) MnO_2, Et_2O, r.t. 16 h .

The synthesis began with the commercially available 5-bromoindole which undergoes lithium-halogen exchange with *tert*-butyl lithium, followed by reaction with 2,2,2,-trifluoro-1-(piperidin-1-yl)ethan-1-one, to install the trifluoromethylacetyl moiety at the 5-position affording **97**. Reaction of **97** with hydroxylamine afforded the oxime **98** in high yield which was transformed into the *O*-tosyl-oxime **99**, to serve as a latent leaving group. Treatment of *O*-tosyl oxime **99** with ammonia at room temperature afforded the diazirane compound **100** in moderate yield, which was oxidised with MnO_2 to the photo-labile diazirine **101** in moderate yield.

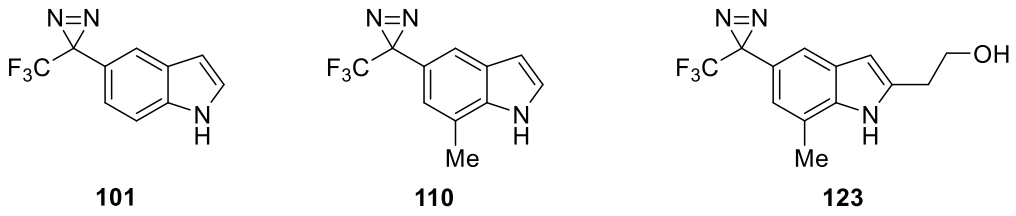
Pleasingly, **101** was as expected observed to behave as a PAM of the 5-HT_3 receptor with very similar affinity and potentiation to 5-chloroindole **7** (Figure 81 and Table 10).

Entry	Compound	EC_{50}	Observation
9a	5-(3-Trifluoromethyldiazirinyl)-indole, **101**	41 μM	PAM- 1000% potentiation at 100 μM
9b	2,2,2-Trifluoro-1-(1H-indol-5-yl)ethan-1-one oxime, **98**	18 μM	PAM- 625% potentiation at 100 μM
9c	5-(3-(Trifluoromethyl)diaziridin-3-yl)-1H-indole, **100**	58 μM	PAM- 265% potentiation at 100 μM
9d	2,2,2-Trifluoro-1-(1H-indol-5-yl)ethan-1-one, **97**	17 μM	PAM- 750% potentiation at 100 μM

Table 10-Summary of data for the drug profiling intracellular Ca^{2+} assay of Hashimoto's indole

5.2 Design and synthesis of novel photo-affinity allosteric modulators

Hashimoto's indole **101** provides a photo-affinity labelled PAM with which it may be possible to explore the identity of the allosteric site, that is, it could be used to identify if the allosteric site is in fact a non-5-HT bound orthosteric site. However, assuming that **101** does identify that the allosteric site is in fact a non-5-HT bound orthosteric site, it would not elucidate the location of binding of any of the compounds that have been identified as NAMs. To address this matter, the design of two potential NAMs **110** and **123** that include diazirine photo-affinity labels was performed as described in Figure 27.

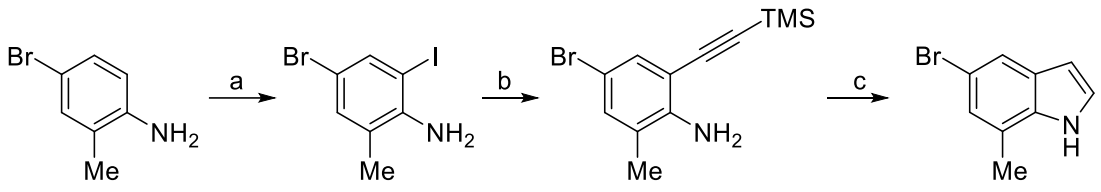


101 **110** **123**

Figure 27- structures of target diazirinyl-indoles

Application of the findings of the SAR performed in the previous section, it appears that in order to access the NAM binding mode inclusion of a methyl-group in the 7-position of the indole core is required (see **71**), which naturally leads to the design compound **110** as a potential photo-affinity labelled NAM. Furthermore, as was identified with the second pass of SAR, the combination of a 2-(2-hydroxyethyl) functional group along with the 7-methyl provided a NAM **86a** with an improved binding affinity; this logic was applied in the design of **123**. However, as was observed with the SAR described in the previous chapter, the only NAMs that have currently been observed possess a chlorine atom appended to the 5-position of the indole core and there are clearly steric and electronic differences between observed NAMs **71** and **86a** to potential photo-affinity labelled NAMs **110** and **123**.

The synthesis of **110** was performed utilising the readily available 4-bromo-2-methylaniline as a starting material, *via* an analogous synthetic route to that used to synthesise indoles **6** and **7.** *Ortho***-**iodination to afford **102** followed by a Sonogashira cross-coupling afforded 4-bromo-2-methyl-6-((trimethylsilyl)ethynyl)aniline **103** that was found to cyclise to afford 5-bromo-7-methylindole **105** under Cu (I) catalysis (Scheme 36).



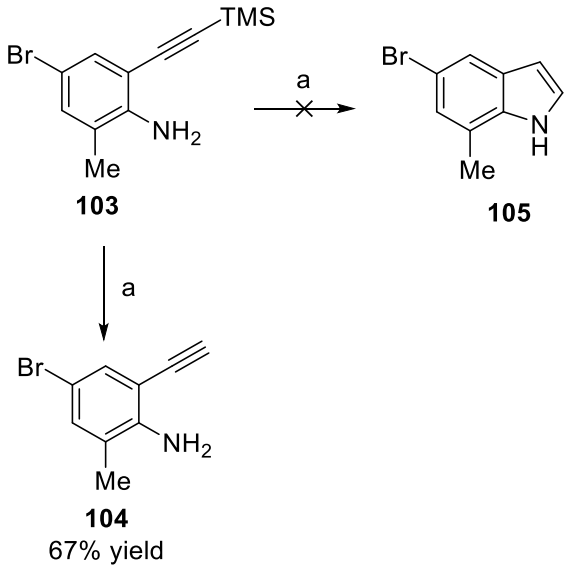
102
80% yield

103
85% yield

105
63% yield

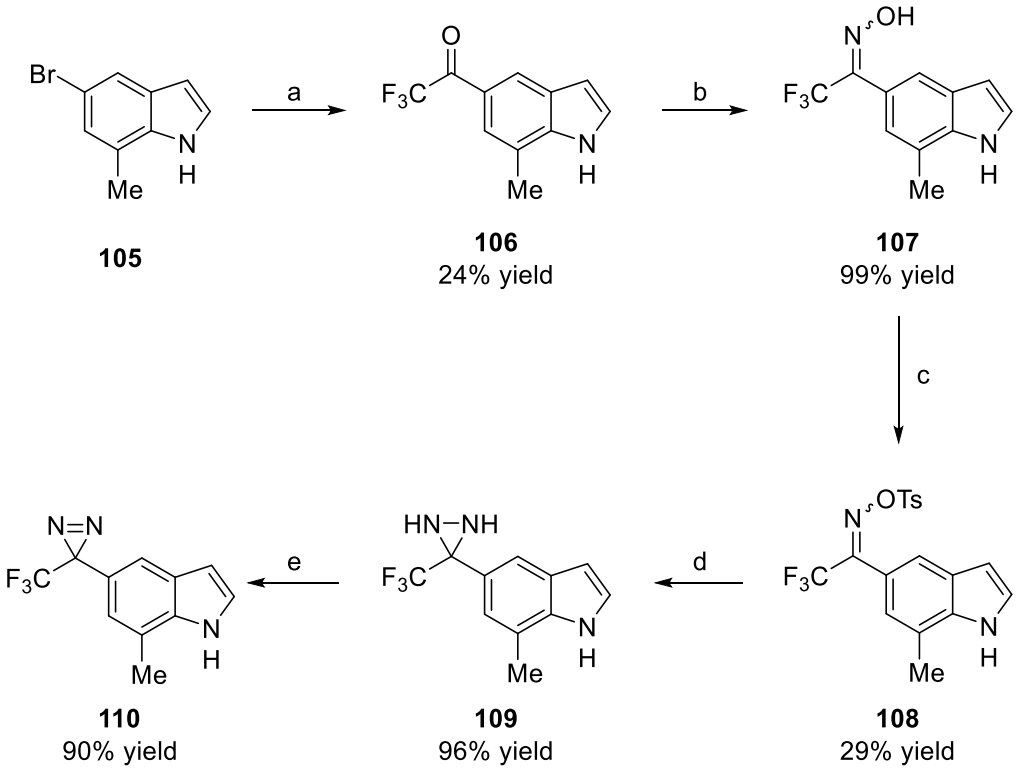
Scheme 36-Conditions: a) $Me_3NBn\ ICl_2$, $CaCO_3$, MeOH, DCM, r.t., 6 h; b) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, r.t., 18 h; c) CuI, NMP, 180 °C, 1.5 h

In an attempt to optimize the cyclisation of **103** to form **105** the base-catalysed conditions, that had previously been observed to yield superior results to copper (I) catalysed cyclisations, were employed to form pyrrolo[3,2-b]pyridines **83** and **84** (Scheme 23). Interestingly, no cyclisation occured under the basic conditions and instead only observed the formation of the de-silylation product **104** (Scheme 37).



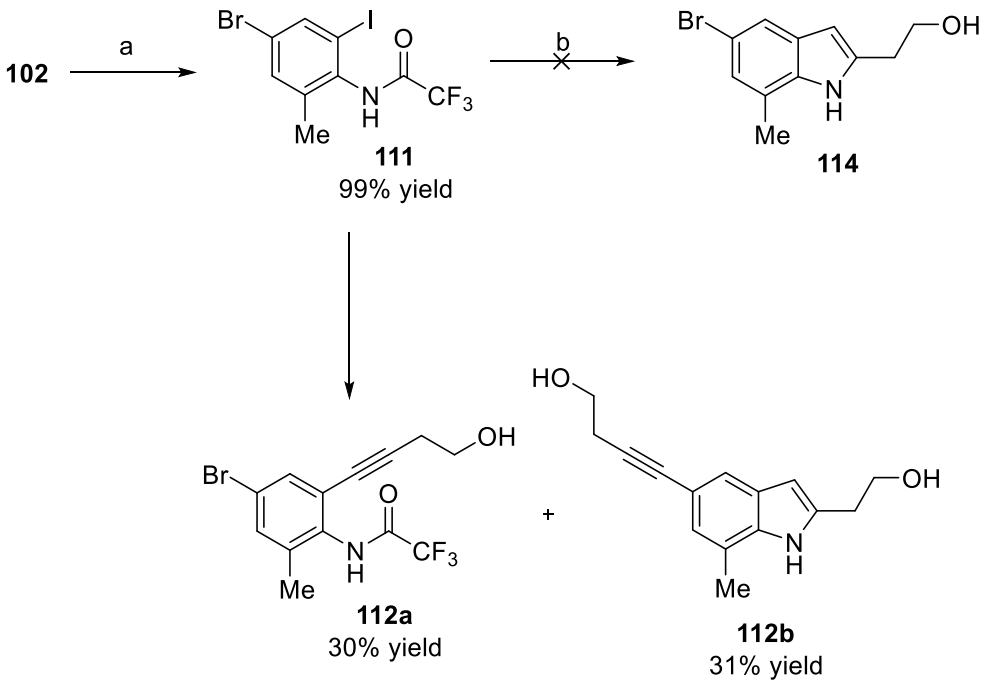
Scheme 37-Conditions: ^{t}BuOK, NMP, r.t., 2 h

5-Bromo-7-methylindole **105** was itself a compound of interest as it fits the SAR pattern of exploration described in chapter 2, however no observable activity was detected for this compound which is unusual as a toleration of a wide variety of groups at the 5-position has been oberved. Installation of the photo-active trifluoromethyldiazirinyl group at the 5-position was achieved *via* application of the conditions reported in *Hashimoto*'s synthesis, which afforded the novel diazirinylindole **110** in moderate yields (Scheme 38).



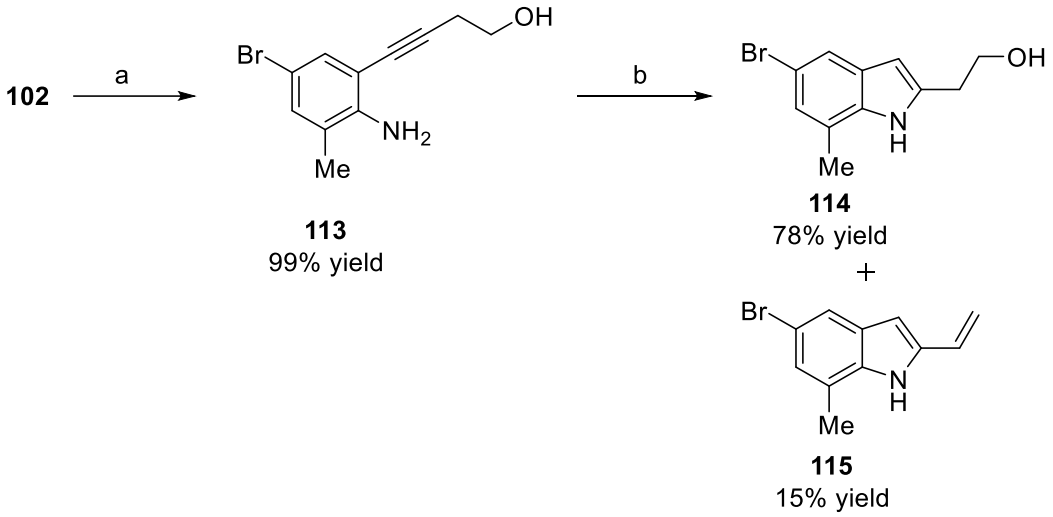
Scheme 38- Conditions: a) i) NaH, THF, 0 °C, 1 h; ii) ^{t}BuLi, −78 °C, 25 min; iii) 2,2,2-Trifluoro-1-(piperidin-1-yl)ethan-1-one, −78 °C - r.t., 6 h; b) hydroxylamine hydrochloride, pyridine, 80 °C, 4 h; c) TsCl, NEt_3, acetone, 0 °C – r.t., 6 h; d) NH_3, Et_2O, −78 °C – r.t. 150 PSIG, e) MnO_2, Et_2O, r.t. 16 h .

The formation of **123** was achieved from a common starting point, compound **102** and initially attempted *via* a similar synthetic sequence that was utilised in the synthesis of 2-(2-hydroxyethyl)-5-haloindoles (Scheme 7) outlined in scheme 39 below.



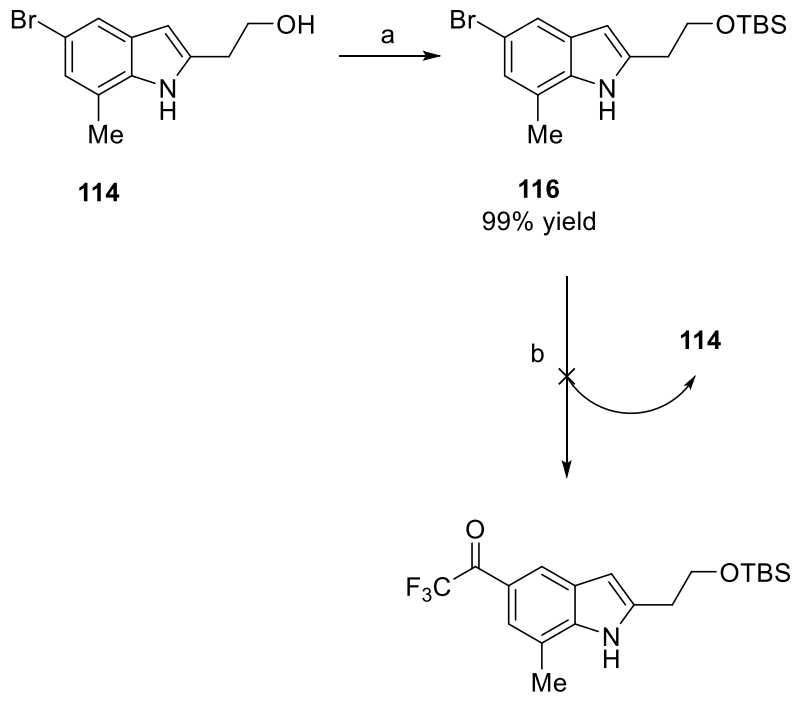
Scheme 39-Conditions: a) TFAA, NEt_3, DCM, 0 °C, 2 h; b) 3-butyn-1-ol, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, DMF, 50 °C, 5 h.

Unfortunately the reactivity of the *in situ* formed aryl bromide **112a** towards cross-coupling under these conditions following the initial desired cross-coupling taking place led to the formation of 4-(2-(2-hydroxyethyl)-7-methyl-1H-indol-5-yl)but-3-yn-1-ol **112b** and no observed desired indole **114**. Attempts to cyclise **112a** were unfortunately unsuccessful and so a milder step-wise approach to synthesising **114,** more akin to the synthesis of the other indoles, *via* the synthesis of **113** was performed at ambient temperature (Scheme 40)**.**



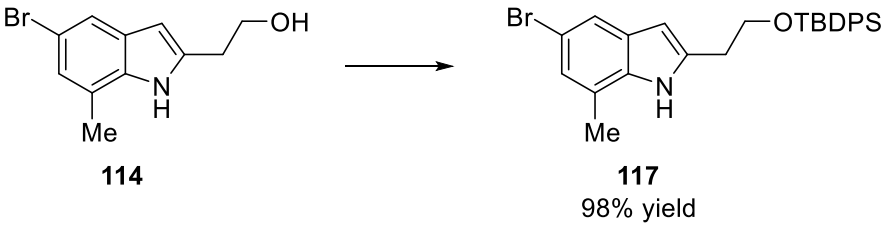
Scheme 40-Conditions: a) 3-butyn-1-ol, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, DMF, r.t., 13 h; b) ^tBuOK, NMP, r.t., 2 h

A small amount of **115** was formed as a by-product of the base-catalysed cyclisation of **113** which provided another testable compound for the SAR at the 2-position the indole core as well as a potential manifold for derivatisation. Recognising the importance of protecting the primary alcohol of **114** before proceeding with the lithium-halogen exchange, initially **114** was protected as a *tert*-butyldimethylsilyl ether. However, under the lithium-halogen exchange conditions, desilylation to reveal the primary alcohol was the dominating reaction pathway that occurred rather than halogen exchange; this is an observation reportedly due to the metalation of the methyl attached to silicon as a result of silicon's ability to stabilise alpha anions [76] *via* its d-orbital overlap with the formed alpha-anion and indeed has been utilised to synthetic advantage by other groups[77] (Scheme 41).

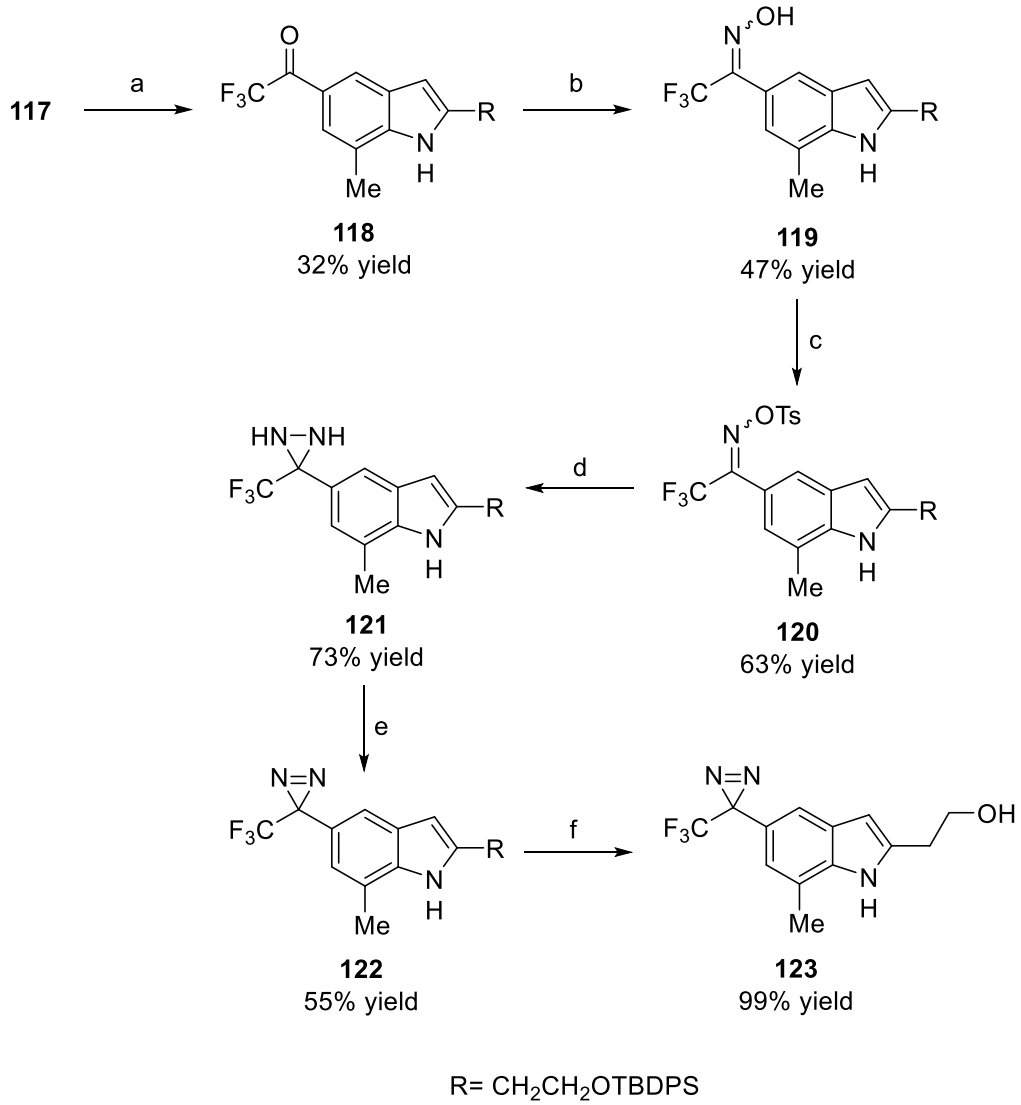


Scheme 41-Conditions: a) TBSCl, Imidazole, THF, 0 °C, 16 h; b) i) NaH, THF, 0 °C, 1 h; iii) ^{t}BuLi, –78 °C, 25 min; iii) 2,2,2-Trifluoro-1-(piperidin-1-yl)ethan-1-one, – 78 °C – r.t., 6 h.

To prevent this desilylation from occurring the more substantial TBDPS silyl-ether protecting group was employed to form **117** (Scheme 42), which is stable under the lithium halogen exchange conditions, and proceeded to obtain **123** in a related manor to **110** above (Scheme 43).



Scheme 42- Conditions: TBDPSCl, imidazole, THF, 0 °C, 16 h



Scheme 43- Conditions: a) i) NaH, THF, 0 °C, 1 h; ii) ^{t}BuLi, −78 °C, 25 min; iii) 2,2,2-Trifluoro-1-(piperidin-1-yl)ethan-1-one, −78 °C - r.t., 6 h; b) hydroxylamine hydrochloride, pyridine, 80 °C, 4 h; c) TsCl, NEt_3, acetone, 0 °C – r.t., 6 h; d) NH_3, Et_2O, −78 °C – r.t. 150 PSIG, e) MnO_2, Et_2O, r.t. 8 h ; f) TBAF, THF, 0 °C, 1.5 h.

5-Bromoindole derivative **117** was successfully transformed into ketone **118** *via* the same conditions reported by Hashimoto *et al*. with a noticeable decrease in yield. Reaction of **118** with hydroxylamine hydrochloride afforded the oxime **119** again in a lower yield that that of the related compounds **98** and **107,** the reason for this decrease in yield is not currently known and could possibly be improved upon repetition. **119** was successfully transformed into sulfonate **120** which was treated with ammonia at ambient temperature and high pressure to afford the

diazirane **121** in a moderate yield for each transformation. **121** was successfully oxidised with MnO_2 to afford the light sensitive silyl-ether **122**, which was found to undergo desilylation to provide the primary alcohol **123** in high yield.

5.3 Drug-profiling of intermediates formed in the synthesis of photo-affinity indoles

The above synthetic sequences afforded several intermediates that were tested for their effect upon the 5-HT_3A receptor *via* the drug-profiling intracellular Ca^{2+} assay, as described during the SAR chapter, the results of which are described in Table 11.

Entry	Compound	EC_{50}	Observation
10a	5-Bromo-7-methyl-1H-indole, **105**	N/A	No effect.
10b	7-Methyl-1H-indole, **106b**	19 μM	NAM- 48% residual activity at 100 μM
10c	2,2,2-Trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one, **106**	31 μM	NAM- 45% residual activity at 100 μM
10d	2,2,2-Trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one oxime, **107**	68 μM	PAM- 370% potentiation at 100 μM
10e	7-Methyl-5-(3-(trifluoromethyl)diaziridin-3-yl)-1H-indole, **109**	N/A	No effect
10f	5-Bromo-7-methyl-2-vinyl-1H-indole, **115**	26 μM	PAM- 340% potentiation at 100 μM

Table 11- Summary of Photo-affinity indole data by drug profiling intracellular Ca^{2+} assay

From the synthesis of **106** a by-product of the lithium halogen exchange reaction, 7-methyl-indole entry **106b**, was isolated and tested in the drug-profiling intracellular Ca^{2+} assay. Interestingly **106b** was found to behave as an inhibitor in the assay experiment (Figure 85). A subsequent radio-ligand competitive binding assay was performed which identified that **106b** doesn't compete with the radio-ligand thus identifying **106b** as a NAM. This result exemplifies the SAR that the 7-position of the indole core is key to the observation of a PAM to NAM switch.

The observation of an inhibitory profile for entry **106** in the drug-profiling intracellular Ca^{2+} assay (Figure 86) which was then followed by a radio-ligand competitive binding assay which confirmed that **10c** interacts as a NAM. This was an encouraging result as it supports the concept that **110** may also interact with the 5-HT_3A receptor as a NAM. Entry **107** appears to behave as a PAM in the drug-profiling intracellular Ca^{2+} assay (Figure 87). Compound **115** was observed to interact as a PAM in the drug-profiling intracellular Ca^{2+} assay consistent with the general observation that larger 5-substituents favouring positive modulation (Figure 88).

5.4 Summary of photo-affinity indole research

The synthesis of two novel diazirinyl-indoles **110** and **123** was performed (Figure 27) and are currently awaiting biological testing. The photoaffinity experiments have been delayed pending the characterisation of a His-tagged 5-HT_3A construct in the HEK cell systems, as the construct previously available showed a loss of functional activity. Further testing will confirm whether this work has led to useful photoaffinity probes of both positive and negative modulators from the indole series. If successful this should provide evidence of the location of the indole binding site(s) on 5-HT_3 receptor and whether it is a change of binding site or binding mode that causes the switch in activity in this series.

6 Photoinduced Electron Transfer sensors (PET)

The purpose of a chemosensor is to relay a chemical interaction, such as a change in pH, into a signal, such as fluorescence, that can be detected by analytical methods for interpretation. Some sensors are qualitative and simply confirm the presence of a chosen analyte whilst others can be used to quantify the analyte abundance and thus provide real-time insight into chemical and biological processes. Fluorescent indication and visualisation of biological analytes offers considerable advantages over other alternative analytical methods such as NMR or micro-electrodes; as well as generally offering high sensitivity and specificity, fluorescent sensors of intracellular processes offer good spatial and temporal sampling capability as well as providing relatively cheap and operationally simple detection methods which are generally non-destructive to the cell. Fluorescence is an experimentally versatile spectroscopic technique in which signals from sensors can be monitored as absorption or emission spectra where intensities, intensity ratios, lifetimes and fluorescence anisotropy (polarisation) can all be utilised to provide insight to biological and biophysical processes [78]. PET Sensors, that typically contain the general structure of [Fluorophore]-[Spacer]-[Receptor], [79] rely as the name suggests, upon PET quenching generating a non-radiative decay pathway for the excited state of the fluorophore. This can be exploited to generate a sensor by designing the receptor in its unbound state to have a HOMO that is higher in energy relative to the HOMO of the fluorophore (Figure 28). Upon excitation of an electron from the HOMO of the fluorophore, to generate the excited state, an internal electron transfer from the HOMO of the receptor to the SOMO of the fluorophore occurs (PET) and consequent internal electron transfer of excited state electron to the SOMO of the receptor returns the system to ground state in a process termed non-radiative decay (Figure 29); this affords the 'off state' (Figure 30). It is important that upon binding the analyte the HOMO of the receptor-analyte complex becomes lower in energy relative to the HOMO of the fluorophore and thus the internal

electron transfer no longer takes place leading to relaxation of the excited state electron back to the SOMO of the fluorophore (Figure 31) *via* release of energy in the form of fluorescence and in doing so returning the system back to its ground state and thus affords the 'on-state' of the system (Figure 32).

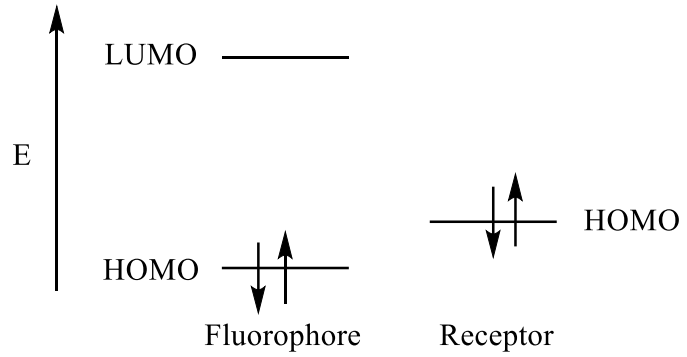


Figure 28-Ground state of PET system

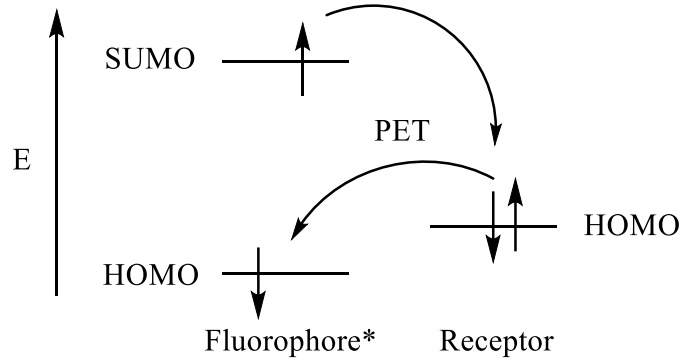


Figure 29- Excited state of PET system undergoing intramolecular electron transfer leading to a non-radiative decay of the excided state

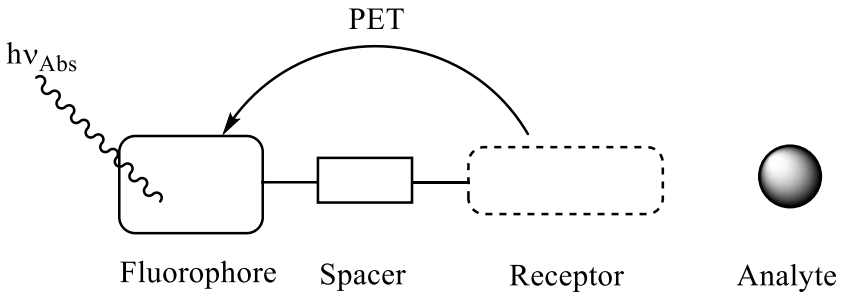


Figure 30-The "off-state" of the substrate unbound system

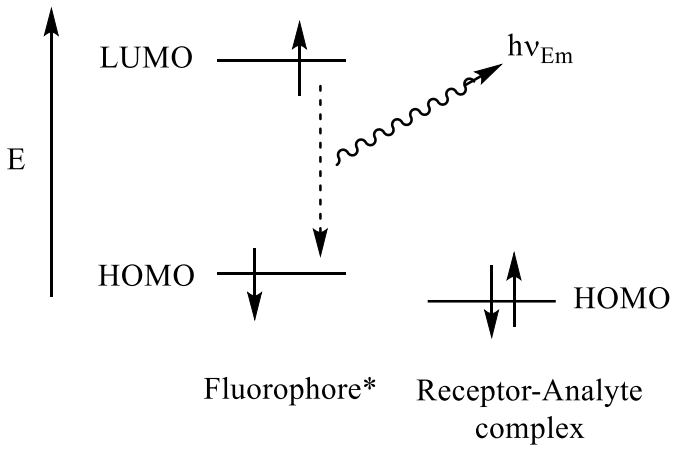


Figure 31-upon binding the PET quenching is silenced

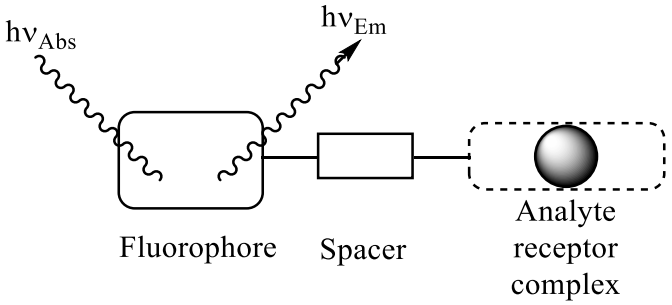


Figure 32- upon substrate binding the "on-state" is afforded

this general schematic can be used to describe PET fluorescent sensors of a variety of analytes including protons, [80] phosphate,[81] fluoride[82] and, most relevant to this research, biologically relevant metals such as calcium. [83] Calcium specific sensors were developed due to the necessity of being able to non-destructively quantify cellular calcium levels with an appropriate time resolution in living cells. The early development of UV-absorption sensors for Ca^{2+} suffered from several problems including poor selectivity of Ca^{2+} over competing endogenous cations such as H^{+}, Zn^{2+} or Mg^{2+} and general difficulty of modifying the sensors to have different affinities for the substrate and tuneable spectral properties.[84]

6.1 Early Ca^{2+} PET sensors

An early example of a calcium sensor is Arsenazo-III (Figure 33), however there were many issues associated with these early compounds such as poor selectivity for Ca^{2+} as well as the requirement for breaching of the plasma membrane in order to enable cellular uptake of the sensor molecules into the cytoplasm[85,86]

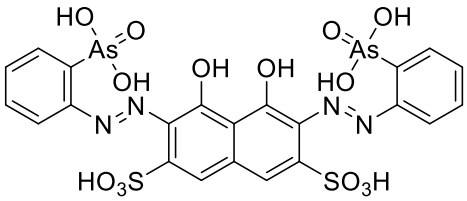


Figure 33-Arsenazo-III, an early example of a PET system used for Ca^{2+} sensing

The development of more selective calcium chelators designed for binding calcium was reported by Tsien *et. al.* in the form of (1,2-bis(*o*-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid) BAPTA which was inspired by the well-known chelator EGTA[84] (Figure 34)

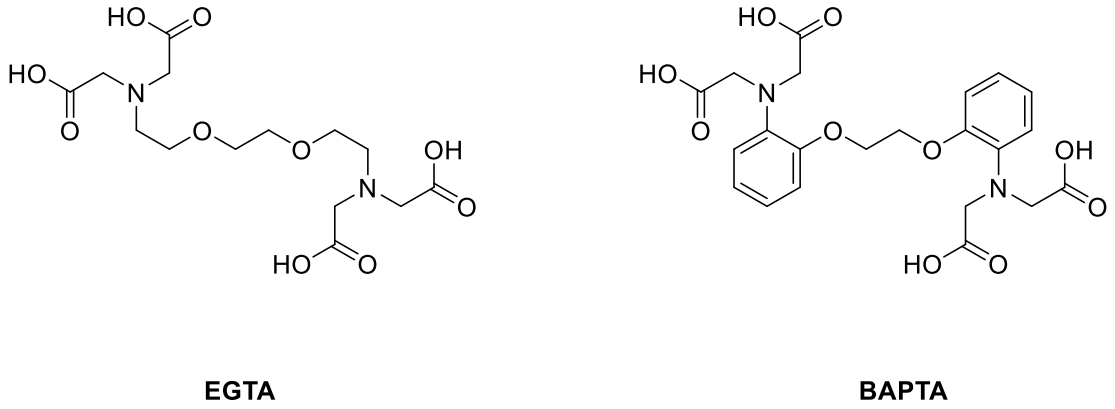


Figure 34- Chemical structures of EGTA and BAPTA chelators

BAPTA offers considerably greater selectivity for calcium over other metals such as magnesium, when compared to EGTA, due to a more rigid binding cavity as well as reduced variance in K_d with respect to pH (less inactive chelator due to *N*-protonation at pH 7) due to the lower nitrogen pKa values of 5-6 down from 9-10 (EGTA)[87]. Early calcium sensors that emit in the visible region of the electromagnetic spectrum include Quin-2 which could be loaded into intact mammalian cells in the form of a cell membrane-permeant ester (acetoxymethoxy ester, AM) derivative that due to its uncharged, hydrophobic form diffuses freely across the cell membranes but once encapsulated becomes hydrolysed *via* cytoplasmic esterases to reveal the tetra-anion that remains trapped in the cytosol[85] (Figure 35).

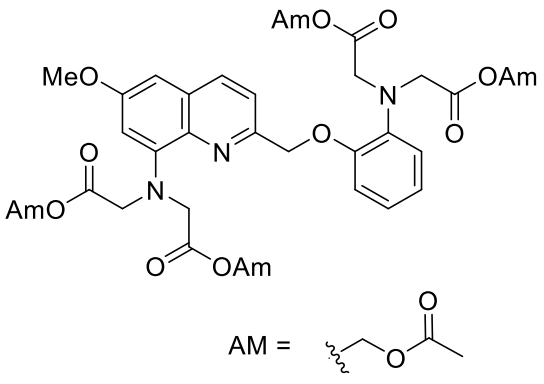
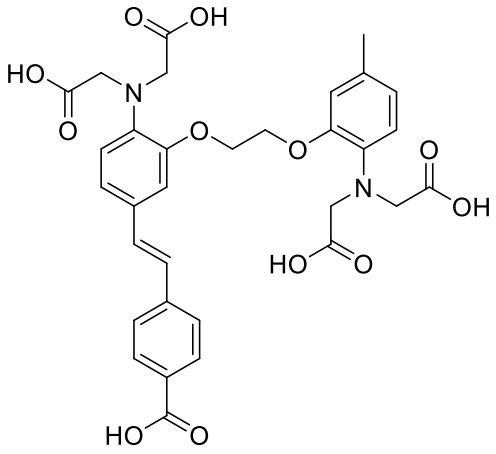
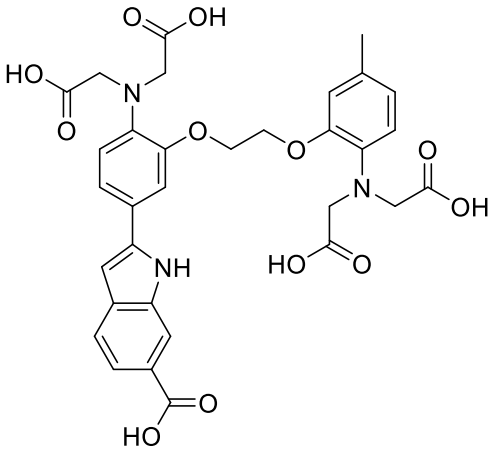


Figure 35- Quin-2-AM, a second generation Ca^{2+} PET sensor with cell-membrane permiant esters (OAm)

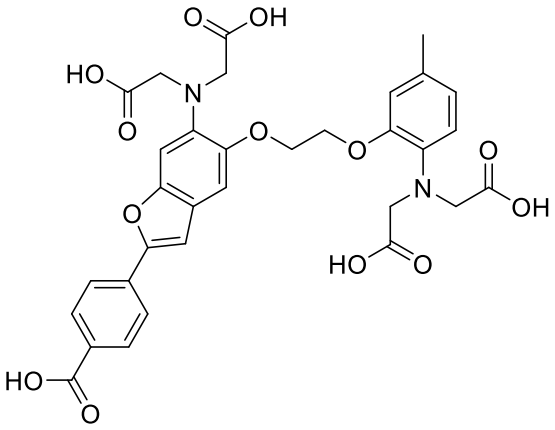
Despite the clear advances of Quin-2 its preferred excitation wavelength of 339 nm is too short and UV irradiation at this wavelength causes significant auto-fluorescence from the cells, leading to high signal to noise ratios within experiments as well as high variance between batches of data.[88] Furthermore the relatively low quantum yield and extinction coefficient (0.03 to 0.14 and ~5000 respectively) leads the amount of Quin-2 loaded onto cells needing to be very high (up to 10^{-4}M or more). This high cellular loading is shown to significantly buffer the free calcium within the cells, also the K_d of Quin-2 for calcium is in the order of 10^{-7} M, which is only useful for sensing low cellular levels of calcium and the sensor becomes saturated at micromolar levels or above somewhat limiting its application in a context that has large dynamic ranges of Ca^{2+}concentration.[83] Although BAPTA was originally designed with a view for UV-absorption spectroscopy it rather fortunately turns out that the oxidation potential of the HOMO of the system is indeed suitable for inclusion into a fluorophore-spacer-receptor system. BAPTA was soon shown to be a suitable receptor for PET fluorescent sensors by *Tsien et al*[83] in the generation of Ca^{2+} sensors that replaced Quin-2 which included stilbene based fluorophore systems such as Stil-1, indole based fluorophore system indo-1 and furan based fluorophore system such as Fura-1 (Figure 36).



Stil-1



Indo-1



Fura-1

Figure 36-BAPTA-derived flourescent PET Ca^{2+} sensors

Stil-1, indo-1 and Fura-1, despite having markedly higher selectivity for Ca^{2+} over Mg^{2+} compared to Quin-2 as well as a much improved (~30 fold) increase in fluorescence upon Ca^{2+} binding, suffer from severe compartmentalisation and protein binding that limits their effective use with cellular/ *in vivo* experiments.[88]

6.2 Current ratiometric PET fluorescent sensors

I. Fluo-4

Replacement of the fluorescent component of these early sensors by derivatives of the fluorescein core led to the Fluo-3 and Fluo-4 sensors. These have become the standard for ratiometric Ca^{2+} detection due to improved quantum yields and faster cellular loading times compared to previous sensors. Fluo-4 offers practically no emission in the visible spectrum when not bound to Ca^{2+}, and upon binding with a K_d=335 nM (pH=7.6), fluorescence occurs at 506 nm. Fluo-4 possesses around a 100-fold increase in fluorescence intensity upon binding Ca^{2+} as well as in improved excitation coefficient compared to Fluo-3 due to the substitution of the chlorine atoms for fluorine (Figure 37). [88]

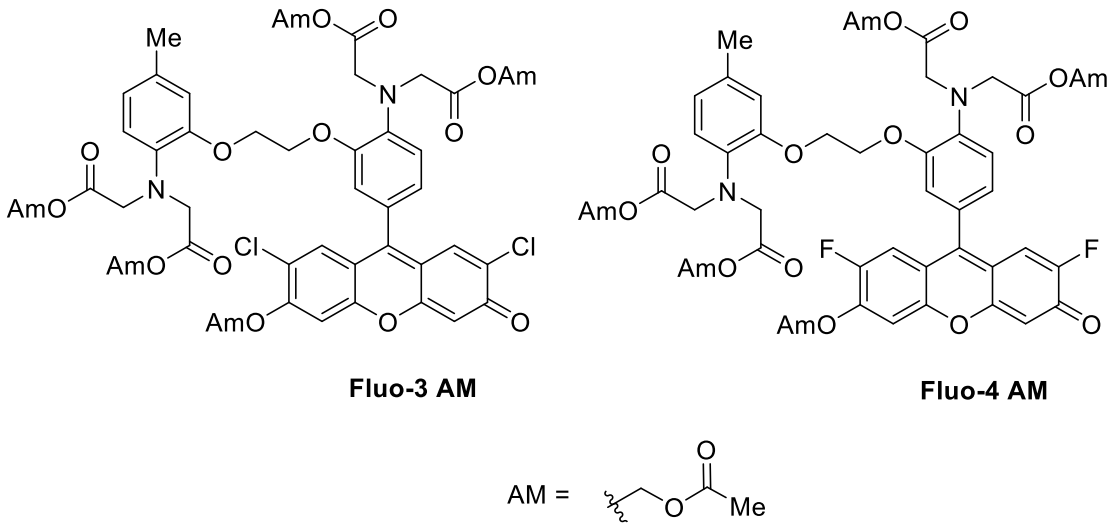
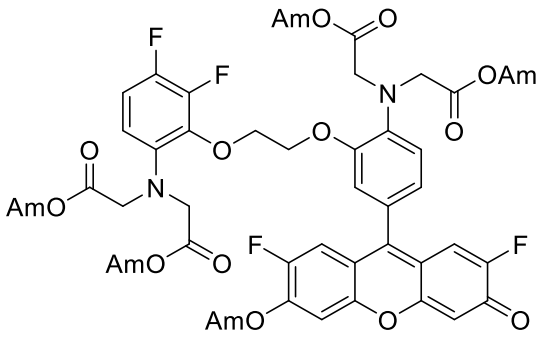


Figure 37-Ratiometric fluorescent PET sensors of Ca^{2+} Fluo-3 and Fluo-4 in cell permeant ester form (OAm)

XII. Fluo-4 FF

Due to the necessity to be able to monitorCa^{2+} concentration at levels greater than "resting-levels" sensors with lower affinity for Ca^{2+} were developed, most notably Fluo-4 FF (Figure 38). The BAPTA phenyl that normally supports a methyl substituent in a *para*-position to the nitrogen instead supports two fluorine atoms: one *para* to the nitrogen and one *ortho* to the phenyl oxygen. The introduction of the fluorine atoms upon the BAPTA phenyl ring system affords a more electron withdrawn system through the σ-framework; this in turn renders the carboxylates attached to that difluoro-aniline to have comparatively lower electron density with which to form electrostatic bonds to Ca^{2+}ions and yields a lower K_d of 9.7 µM for Fluo-4 FF compared to =335 nM (pH=7.6) for Fluo-4 as well as a slightly red-shifted emission wavelength of 516 nm.



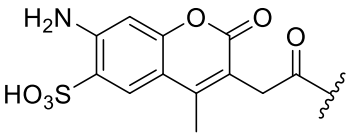
Fluo-4 FF AM

Figure 38-Low affinity PET fluorescent Ca^{2+} sensor Fluo-4FF in cell permeant ester form (OAm)

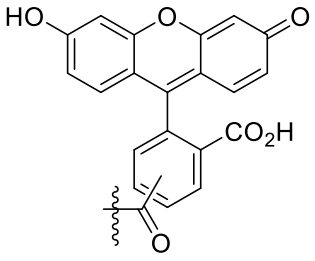
6.3 Fluorophores

The selection of which fluorophore to utilise in a PET fluorescent sensor largely comes down to the constraints imposed by the context of the experiment i.e. the cell type, pH at which

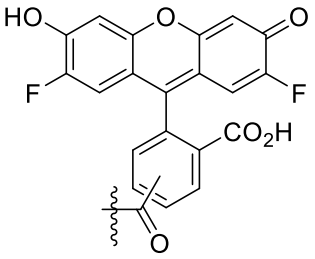
the sensor is required to operate in; as well as the instrument intended to observe the fluorescent signal upon.[89] Visible spectrum dyes have been developed into a number of PET sensors that span the whole visible spectrum. At the blue end of the spectrum, coumarin derived fluorophores are ubiquitous such as Alexa Fluor 350 (346/445 nm). [90] The most common green dyes include Fluorescein[91] as their fluorophore such as Fluorescein (494/520 nm) or a derivative thereof, often fluorinated derivatives, such as Oregon green (496/524 nm). [92] Dyes that emit in the red region of spectrum most commonly include Rhodamine as the fluorophore such as Rhodamine B (568/583 nm)[93], or derivatives thereof, such as Texas red-× (595/615 nm)[94] (Figure 39).



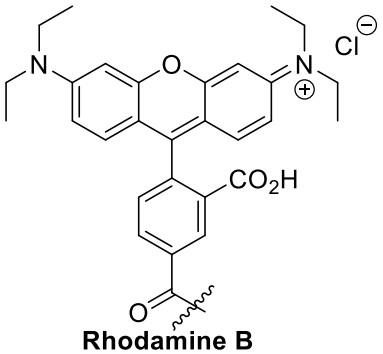
Alexa Fluor 350



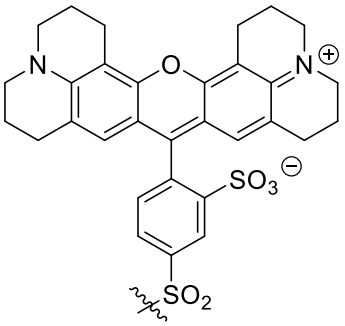
Fluorescein



Oregon green 488



Rhodamine B



Texas red-X

Figure 39- Commonly utilised commercially available fluorophores

I. BODIPY fluorophores

4,4-Difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacenes, more commonly referred to as BODIPY fluorophores, were originally synthesised in the late 1960's making them a comparatively new family of fluorophores.[95] Since the mid-1990's a considerable amount of interest and focus has been directed towards BODIPY based fluorophores for a number of applications ranging from biological labelling, [96] tuneable-laser dyes, [97] electroluminescent devices, [98] fluorescent switches[78,89] and last but not least as fluorophores in sensors.[78,99,100] BODIPY based fluorophores now span the visible spectrum (Figure 40) and in many cases they have surpassed the performance of the fluorescein and rhodamine based sensors; the BODIPY-FL fluorophore for example has excitation/emission maxima (503/512 nm) is now regularly used as an alternative to fluorescein. There are certain properties of BODIPY-FL that make it potentially superior to fluorescein in some applications, these include: very high extinction coefficient (>80,000 $cm^{-1}M^{-1}$ compared to fluorescein (74,000 $cm^{-1}M^{-1}$) in methanol, a narrower emission band and, in particular, greater photo-stability and resilience to photo-bleaching.[78,101]

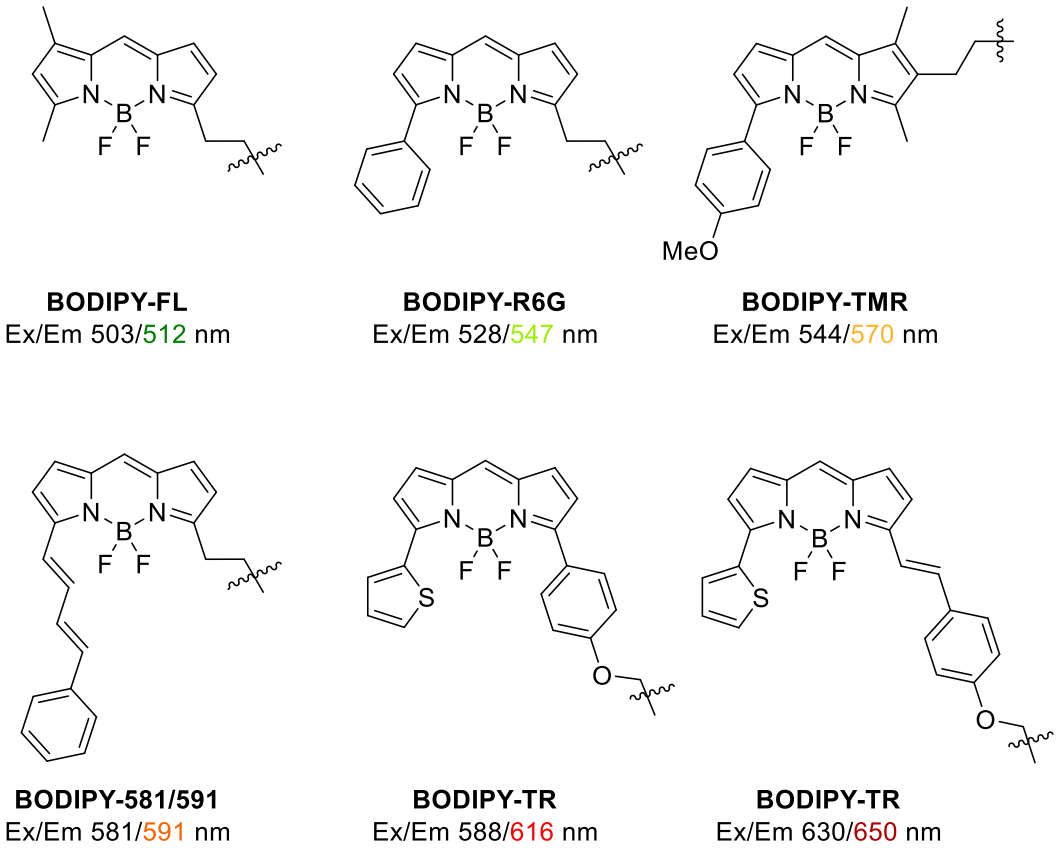
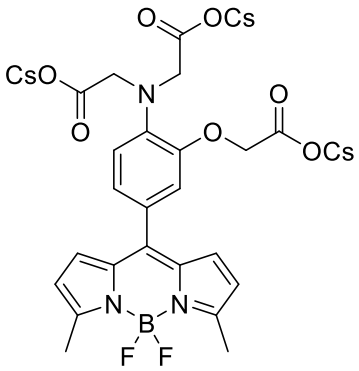


Figure 40-Commercially available BODIPY fluorophores from ThermoFisher scientific

In general, BODIPY fluorophores can be made to absorb and emit at higher wavelengths with an increase in conjugation.

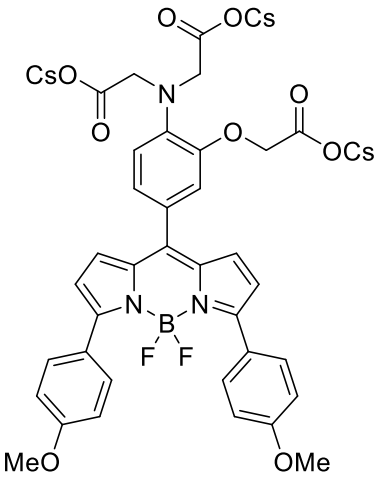
XIII. Ca^{2+} BODIPY PET sensors

Recently Boens *et al*[102] reported the synthesis and utility of low-affinity BODIPY-based PET sensors for Ca^{2+} where two sensors were synthesised to emit in either the green or red region of the visible light spectrum (Figure 41).



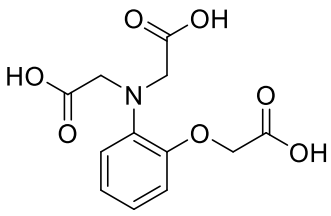
BODIPY APTRA-G

Ex/Em= 508/525



BODIPY APTRA-R

Ex/Em= 574/618

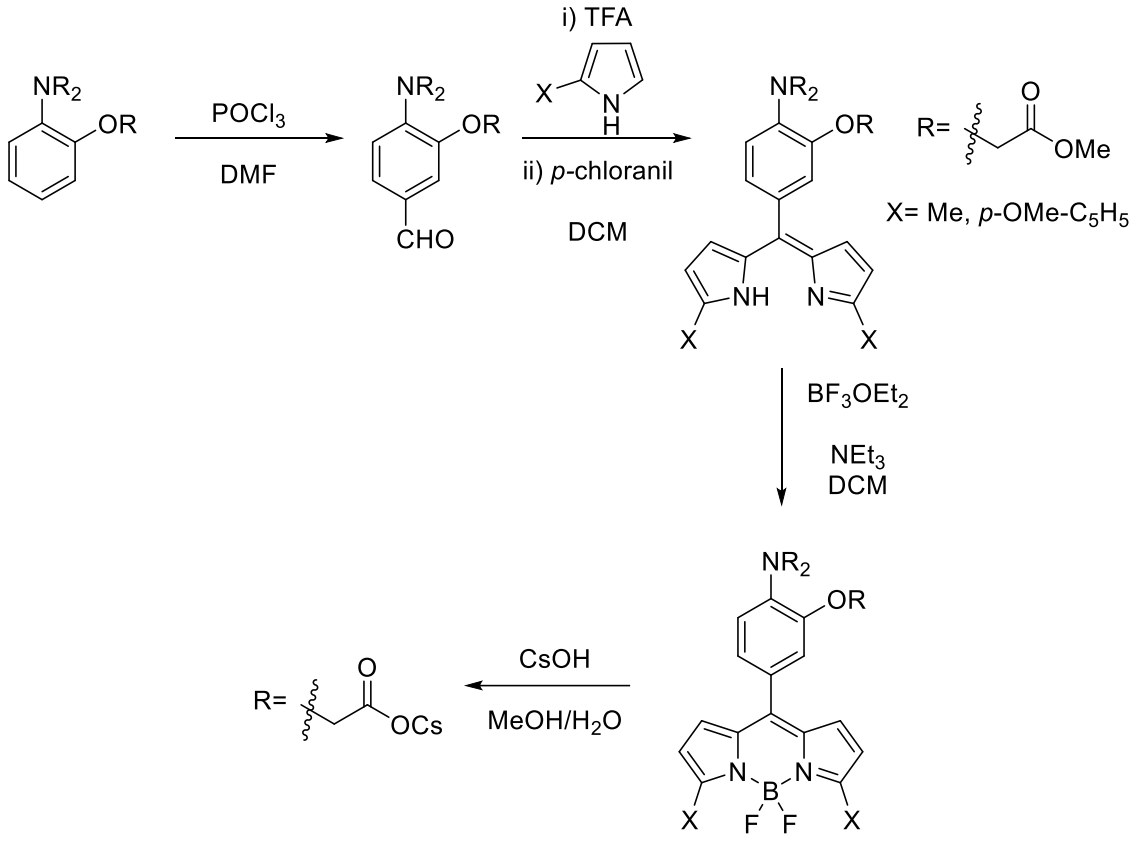


APTRA

Figure 41- BODIPY-APTRA-G (green visible emission) and BODIPY-APTRA-R (Red visible emission) reported by *Boens et al* and APTRA the metal chelator

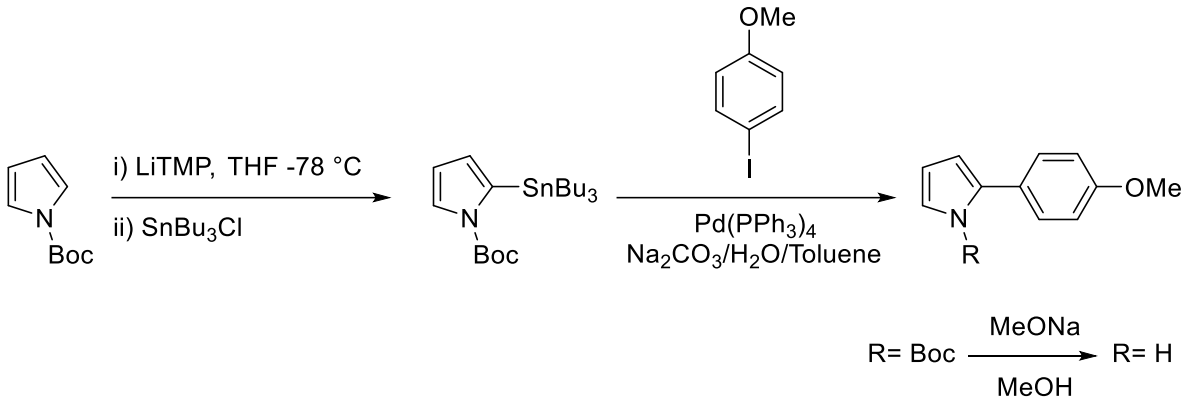
Both sensors were accessed by a synthetic pathway starting from APTRA-trimethyl ester which was formylated *via* the Vilsmeir Hack reaction followed by acid catalysed condensation and

oxidation of the formed aldehyde with the relevant pyrrole derivative and *p*-chloranil to form a dipyrrin-intermediate. The dipyrrin intermediate is then converted into the BODIPY APTRA tri-ester *via* the treatment with $BF_3.OEt_2$ and NEt_3 and then the esters were hydrolysed by saponification conditions (Scheme 44).



Scheme 44-Boen's synthesis of BODIPY-APTRA compounds

2-Methylpyrrole is a commercially available pyrrole derivative, 2-(4-methoxyphenyl)pyrrole however is not and Boen *et al* synthesised it according to a procedure reported by Kulyk *et al,* outlined in Scheme 45.[103]



Scheme 45-Kulyl *et al's* synthesis of 2-(4-methoxyphenyl)pyrrole

Unfortunately, despite showing ~150% increase in fluorescence upon binding calcium, the APTRA chelatory unit of the sensor also binds other biological metals ions with high affinity, such as Mg^{2+} and K^{+} which severely limits its application within the cellular context for ratiometric detection of calcium.[78,102]

7 Development of novel ratiometric BODIPY fluorescent sensors of Ca^{2+}

The SAR study of the 5-haloindole pharmacophore, described in chapter 2, identified several PAMs that potentiate the 5-HT_3A cellular signalling to the extent that it increases the intracellular calcium efflux beyond the linear dynamic range of the fluorescent assay used. This presents a problem in that once the linear region of the dose-response curve is exceeded it becomes impossible to accurately determine the cytosolic calcium level *via* the assay fluorescent signal, see

Figure 42**.**

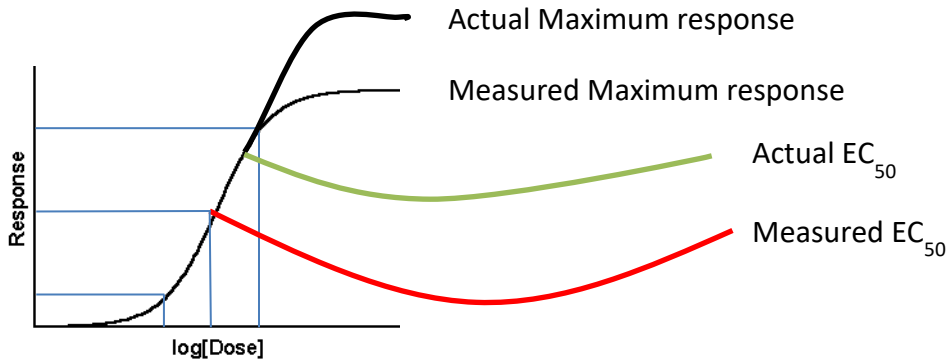


Figure 42-Diagram showing the observed effect of passing above linear dynamic range of fluorescent dose response assay

This relates to a fundamental issue associated with fluorescent calcium sensors such as Fluo-4 where there is a linear region of calcium dose *vs* fluorescent signal only within one Log unit of concentration around the K_d of the sensor. In the case of Fluo-4, which is the sensor used in the *in* vitro dose response assays used in this research, the observed K_d is 335 nM and linearity is observed from 35 nM up to 3.5 µM[88], with higher concentrations giving an increasingly small gain in signal. A lower-affinity sensor such as Fluo-4FF with a K_d = 9.7 µM can be used to quantify the calcium concentration at the higher concentrations (0.97 – 97 µM), where Fluo-4 would begin to saturate however the lower concentrations will not be in the linear dynamic range. Only one of these two sensors may be used at a given time within a given cell culture and as they both emit light at ~515 nm in the green region of the visible spectrum and therefore it will not be possible to observe the signals for the dye independently, disrupting the essential internal calibration to establish the maximal signal for each dye.

One solution to remedy this issue would be to include a second Ca^{2+} sensor within the assay that absorbs and emits in different regions of the visible spectrum to that of Fluo-4 as well as possessing a different affinity for calcium compared to Fluo-4. If implemented correctly this dye system would provide an increase in linear calcium response from 100-fold with a single dye, to

10,000 fold with the dual system. In the case of standard BAPTA derivative discussed to date this would allow detection from ~30 nM to ~100 µM (~3000-fold). Upon consideration of the available fluorescent calcium sensors, the low affinity red-emitting Rhodamine based high affinity Ca^{2+} sensor Rhod-FF combined with high affinity green emitting Fluo-4 was explored (Figure 44 and Figure 43Figure 43-Simulated absorption and emission spectra of Fluo-4 (green) and Rhod-FF (orange) (Generated using ThermoFisher Fluorescence SpectraViewer))

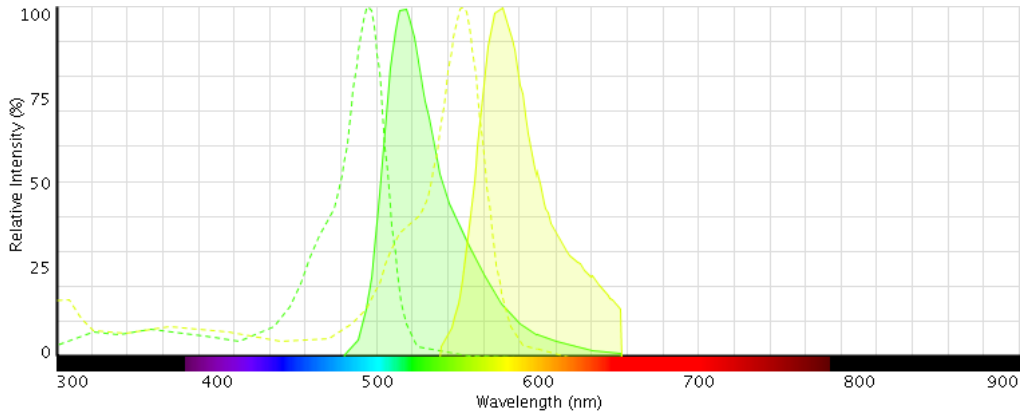


Figure 43-Simulated absorption and emission spectra of Fluo-4 (green) and Rhod-FF (orange) (Generated using ThermoFisher Fluorescence SpectraViewer)

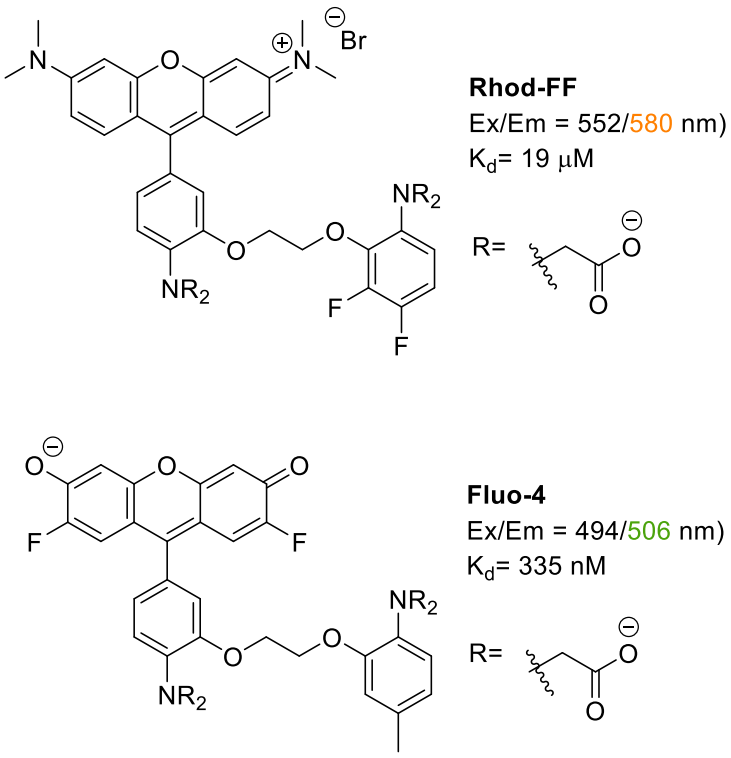


Figure 44- High affinity green and low affinity orange calcium sensors

Unfortunately, due to the altered charge state of Rhod-FF, the sensor is well known to become sequestered in the mitochondria of cells, an area that is particularly high in Ca^{2+} whereas the uncharged AM ester of Fluo-4 passes more easily into the cytosol. As a consequence, when used to detected calcium influx in whole cells the lower affinity and higher local concentration partially cancel out, and the dye preforms in a similar fashion to Fluo-4 (Barnes *et al*, unpublished data). Even if these effects were not as significant, that fact the dyes are detecting in different regions of the cell will inevitably lead to inaccurate readings[104]. As the key issue appears to be compartmentalisation of the two sensors, which locate at different parts of the cell and thus are exposed to different levels of Ca^{2+}, the design and synthesise novel ratiometric Ca^{2+} PET fluorescent sensors began. As outlined in chapter 3-I due to the necessity for the pair of fluorescent sensors (red and green pair) to have as closely related chemical structures as possible the BODIPY fluorophore was selected as the basis of the sensors coupled with the high and low

affinity chelatory (BAPTA and BAPTA-FF) units of Fluo-4 and Fluo-4FF; this led to the design of novel ratiometric PET fluorescent sensors that would be used as matched pairs (Figure 45).

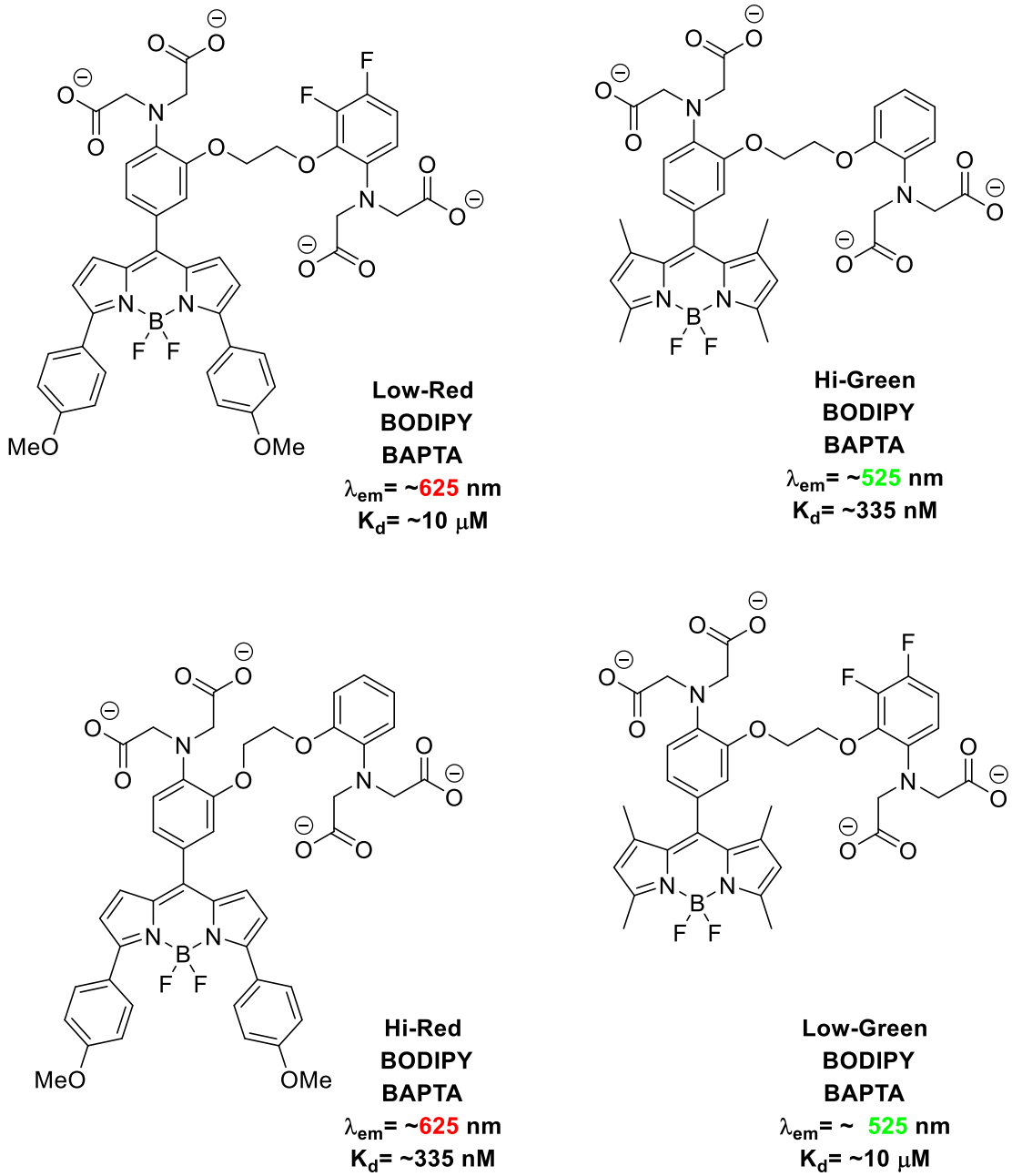


Figure 45- Novel ratiometric fluorescent Ca^{2+} PET BODIPY sensors

A matched pair of either Hi-Red-BODIPY-BAPTA with Low-Green-BODIPY-BAPTA or Low-Red-BODIPY-BAPTA with Hi-Green-BODIPY-BAPTA should provide a suitable tandem dye set. It is believed these pairs would be advantageous in the assay compared to the trialled pair which failed (Figure 44) due to both dyes being more closely chemically related *i.e.* same net charge and relative polarity. There is also likely to be a greater difference in wavelength between the pair of dyes, minimising the effect of potential inter-dye energy transfer leading to inaccurate data. A simulated tandem dye absorption and emission spectrum of a matched pair of green and red dyes discussed (Figure 45) is included below (Figure 46).

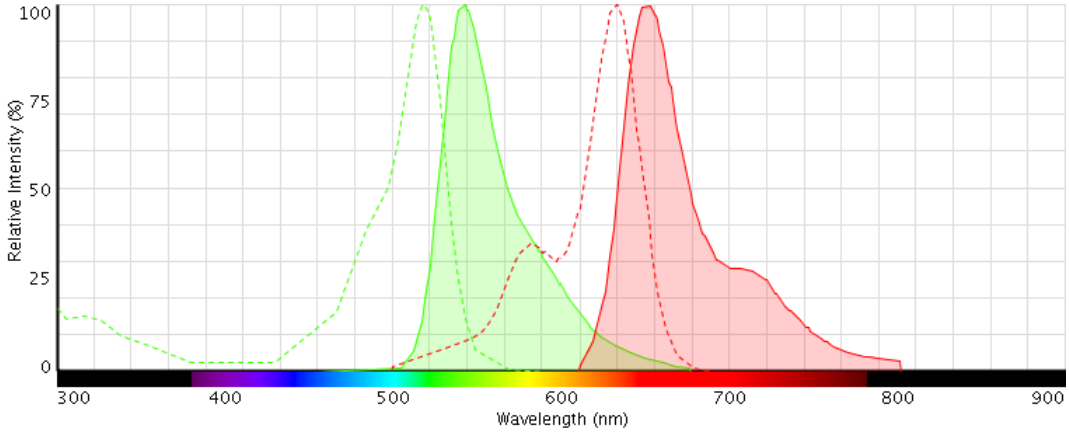


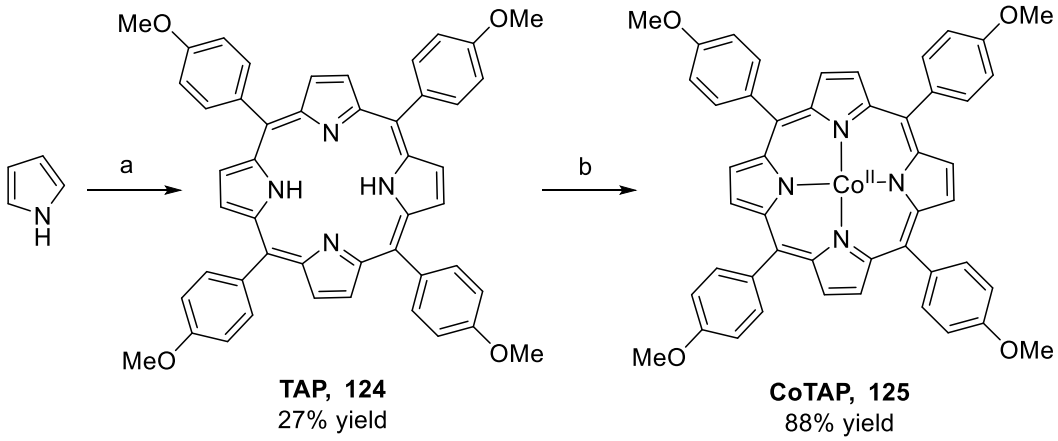
Figure 46- Simulated absorption and emission spectra of matched pair Hi-Green-BODIPY-BAPTA (green) and Low-Red-BODIPY-BAPTA (Red) (Generated using ThermoFisher Fluorescence SpectraViewer)

7.1 Synthesis of BODIPY sensors

I. Synthesis of 2-anisyl pyrroles

The green BODIPY fluorophores are assembled *via* the condensation of 2,4-dimethylpyrrole, a commercially available pyrrole, and an activated acyl equivalent of the BAPTA. For the red fluorophores to be synthesised by this method however the required 2-(4-

methyoxyphenyl)pyrrole **127,** which at the time of this research, was not commercially available and so needed to by synthesised. Rather than utilising the synthesis reported by Kulyk *et al*[103] (Figure 41) a synthetic route was sought that did not require the use of stannanes as they are undesirably toxic. Initially the synthesis of 2-(4-methoxyphenyl)pyrrole *via* the radical arylation of pyrrole with 4-iodoanisole **126** (synthesised according to *Morita et al.*[105]) as reported by *Chan et al*[106]using the CoTAP catalyst **125** synthesised according to the procedure reported by Rieger *et* al[107](Scheme 47) was explored. The suggested mechanism for the CoTAP catalysed radical arylation is shown Figure 47. The synthesis of the CoTAP catalyst **125** was achieved in moderate yield *via* the condensation of *p*-anisaldehyde with pyrrole under acidic conditions followed by oxidation under ambient conditions to afford the porphyrin **124** which was used to chelate cobalt (Scheme 46).



Scheme 46-Conditions: a) 4-Methoxybenzaldehyde, propionic acid, 140 °C, 2 h; b) $Co(OAc)_2$, DMF, 110 °C

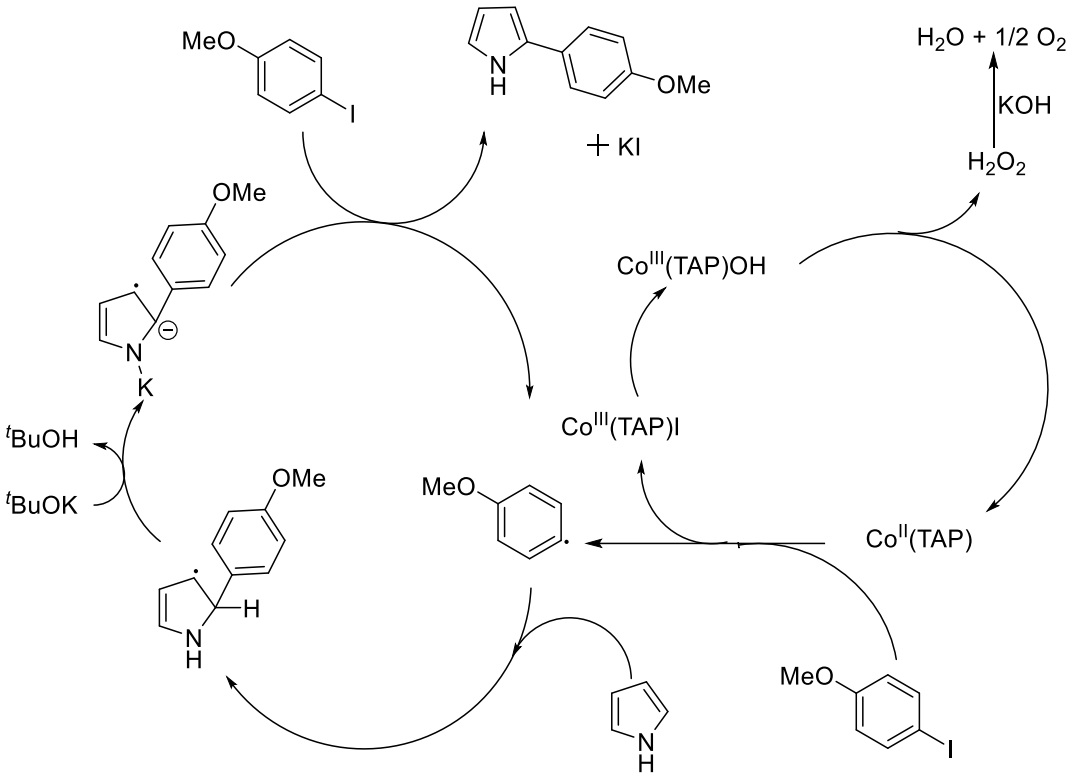
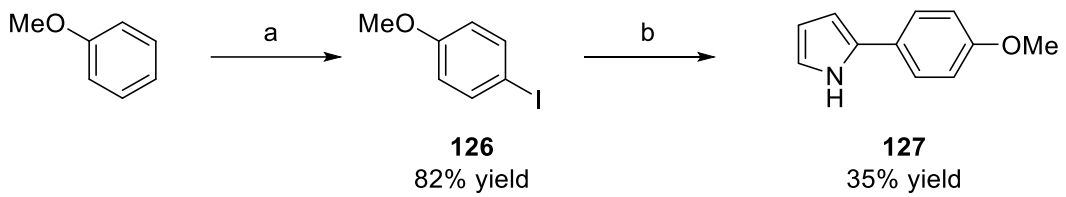


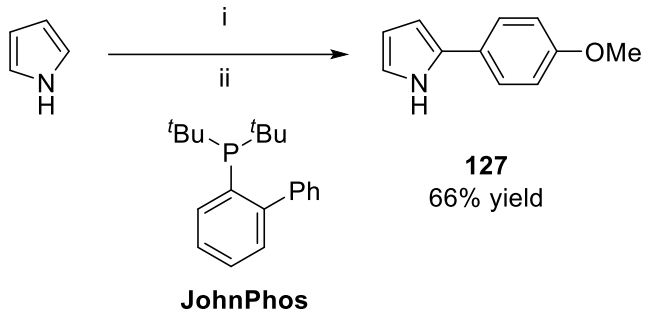
Figure 47- Proposed mechanism of the CoTAP radical arylation of pyrrole

4-Methoxyiodobenzene **126** was reacted with pyrrole in the presence of CoTAP catalyst **125** to afford **127** in moderate yield (Scheme 47). Despite the reaction being relatively rapid, the purification of the product is rather labour intensive, largely due to the vast excess of pyrrole that must be evaporated combined with the inherent instability of the product **127** under ambient conditions.



Scheme 47-Condtions: a) NCS, NaI, AcOH, 50 °C, 2 h; b) CoTAP (10 mol %), ^{t}BuOH, KOH, pyrrole, 200 °C, 45 min

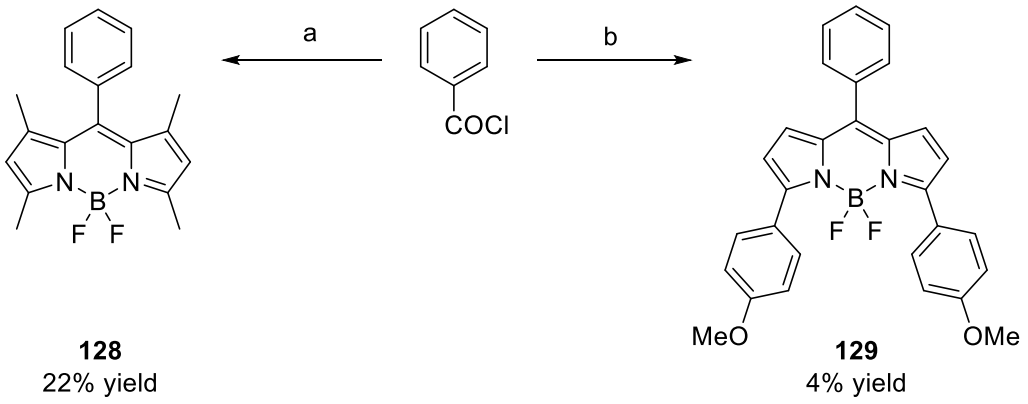
The reaction proceeds with a relatively low yield of 35% and is difficult to scale up as it is performed in a sealed tube at ~130 PSIG. Due to the combination of the above issues with this approach, an alternative synthesis of **127** was persued**.** The palladium-catalyesed cross-coupling of a zinc-pyrrole complex with 4-bromoanisole as reported by Sadighi *et al* was found to afford **127** in high yield at the scale required (Scheme 48).[108]



Scheme 48-Conditions: i) NaH, THF, 0 °C, 30 min; ii) $ZnBr_2$, $Pd(OAc)_2$, JohnPhos, 4-bromoanisole, THF, 65 °C, 48 h

II. BODIPYs *via* acid chlorides

Initially, simplified Red and green BODIPY dyes **128** and **129** were synthesised to explore the merits of synthesising BODIPYs from acid chlorides as reported by Boyer *et al* [109] (Scheme 49).



Scheme 49-Conditions: a) i) 2,4-dimethylpyrrole, DCM, 16 h; ii) BF_3OEt_2, NEt_3, DCM, 1 h; b) 2-(4-methoxyphenyl)pyrrole (127), DCM, 16 h; ii) BF_3OEt_2, NEt_3, DCM, 1 h

The synthesis of BODIPY fluorophores in this manner proceeds *via* the synthesis of a dipyrrin species as shown in Figure 48. The dipyrrin formed in this manner is not typically isolated but is reacted *in situ* with BF_3OEt_2 under basic conditions to insert the boron difluoride moiety of the BODIPY as above (Scheme 49).

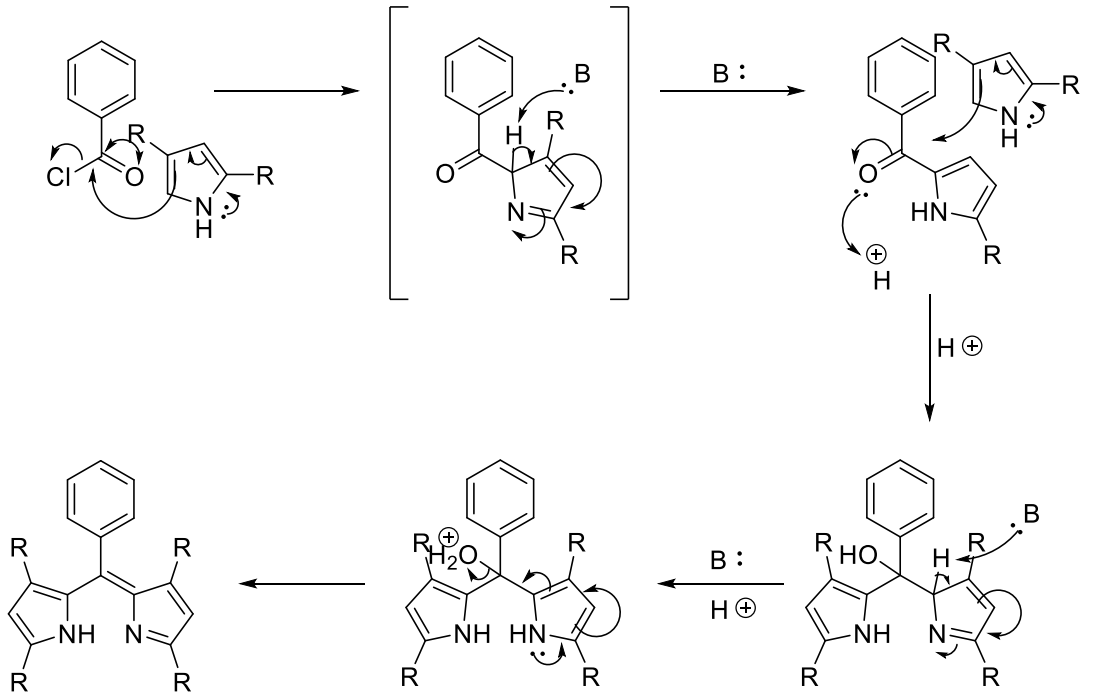
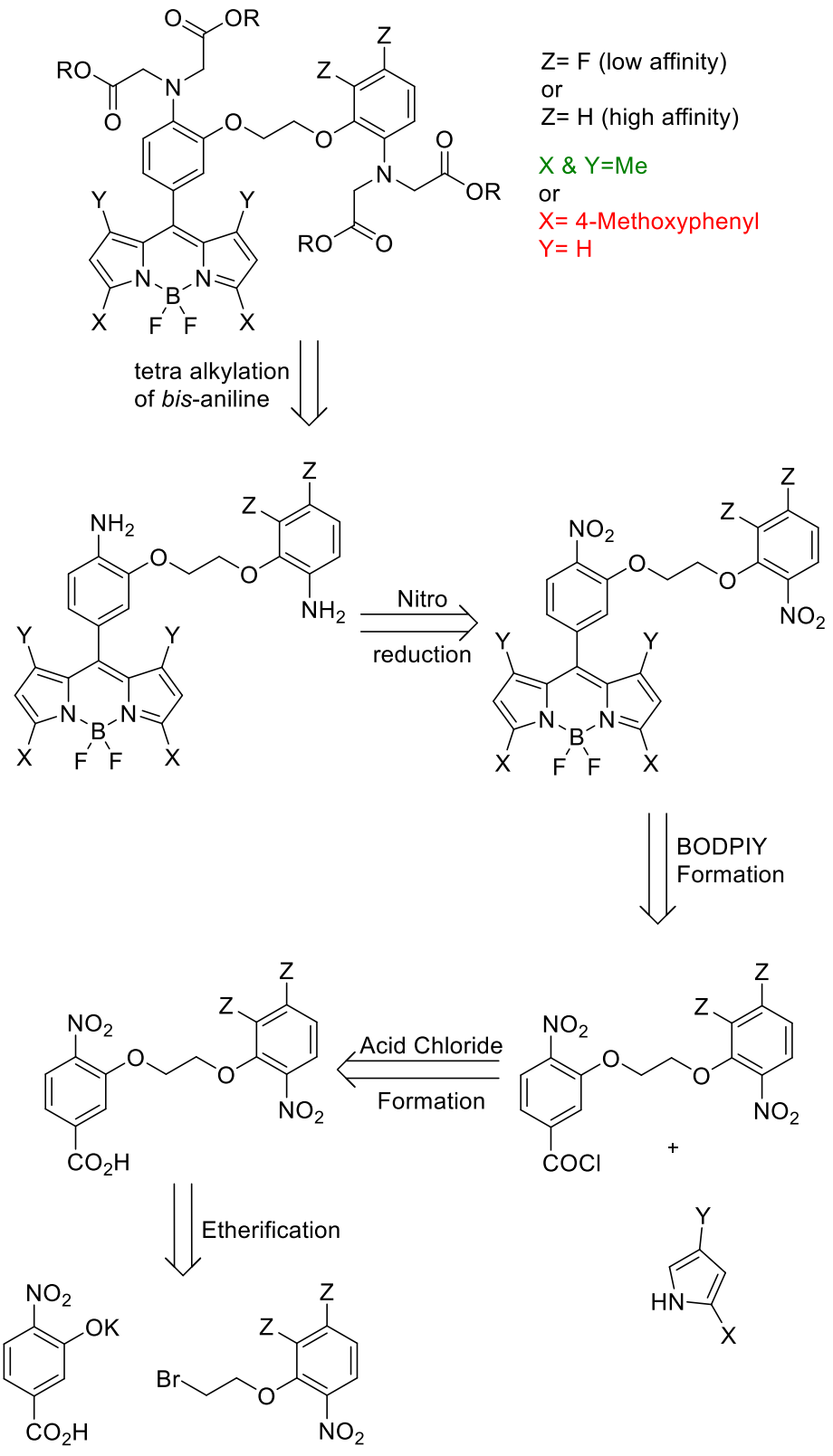


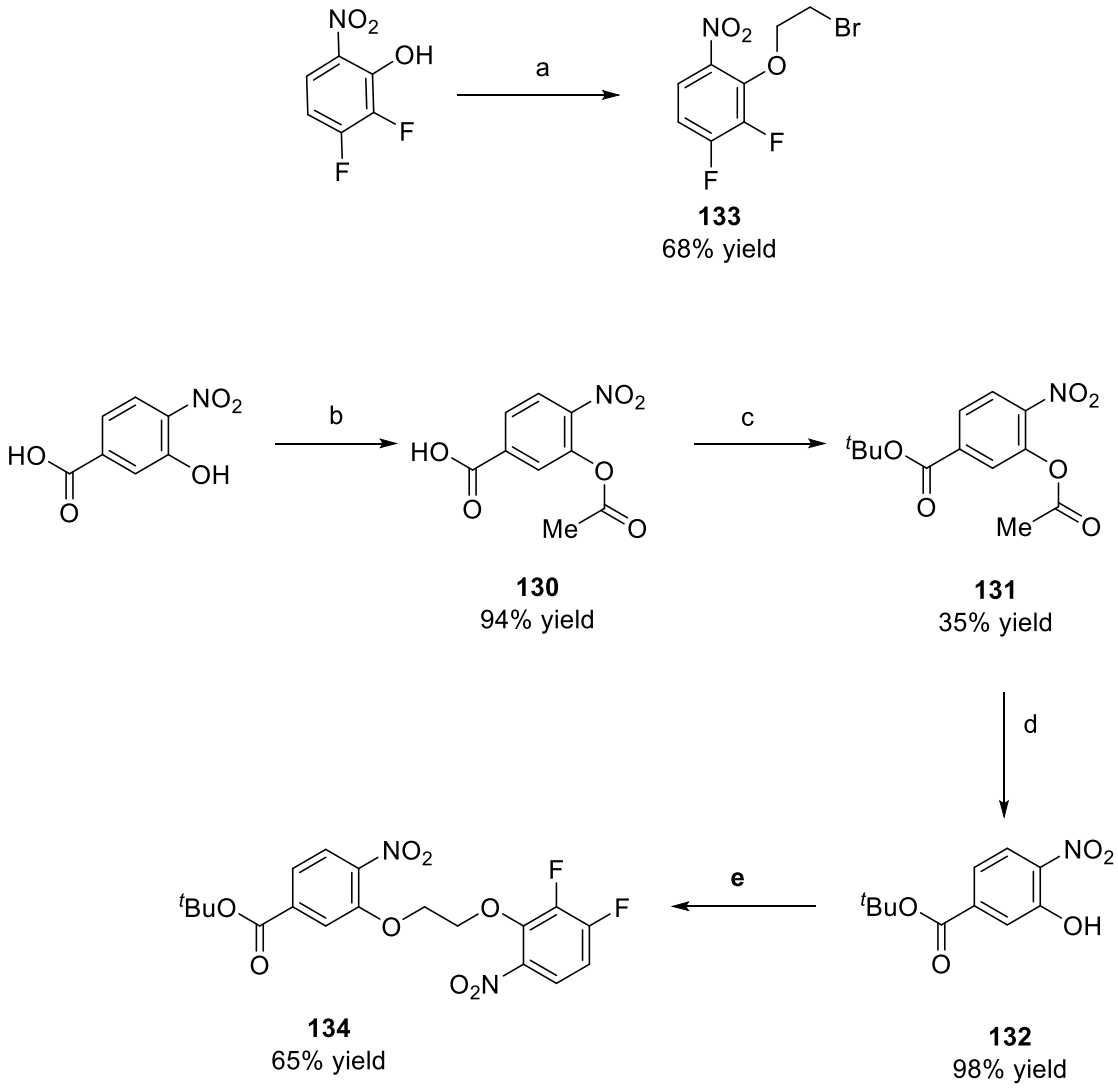
Figure 48-Proposed mechanism for formation of dipyrrins from acid chlorides

The synthesis of the sensors (Figure 45) was initially designed around the BODIPY fluorophores being assembled onto the nitro-phenyl precursor to BAPTA *via* reaction of the required pyrrole derivative and and acid chloride of the BAPTA-precursor. This would then provide access to the sensors following reduction of the nitro groups to anilines followed by alkylation to assemble the BAPTA as a tetra-ethyl ester and later hydrolysed to afford the sensors, in their sensory active form, as a tetra-carboxylate. The tetra-carboxylate form could then be converted into the cell permeant tetra AM-ester for cell loading, see Scheme 50 below.



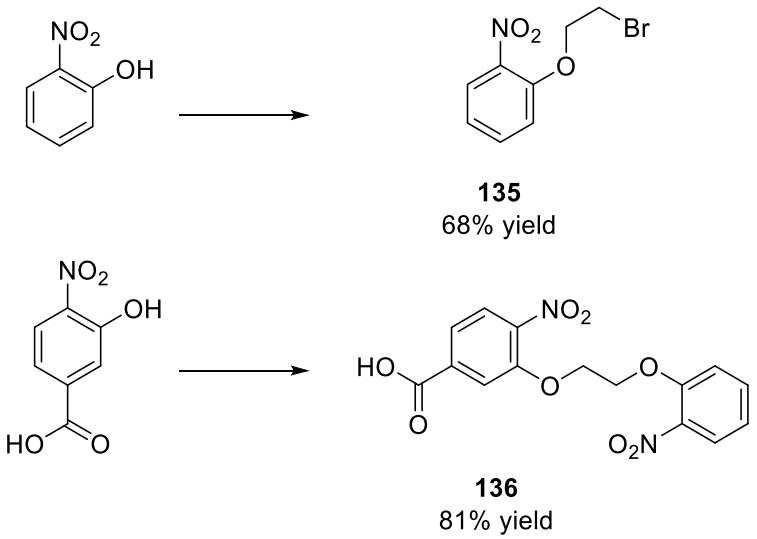
Scheme 50-Retrosynthetic scheme for the synthesis of novel BODIPY-BAPTA sensors

Acetylation of commercially available 3-hydroxy-4-nitrobenzoic acid afford **130** which then underwent Ag^{I} catalysed alkylation with *tert*-butyl bromide to afford *tert*-butyl ester **131.** Hydrolysis of the acetyl group affordednitrophenol coupling partner **132** which was reacted with **133** to form an advanced intermediate containing our BAPTA-carbon framework **134** in moderate yield (Scheme 51).



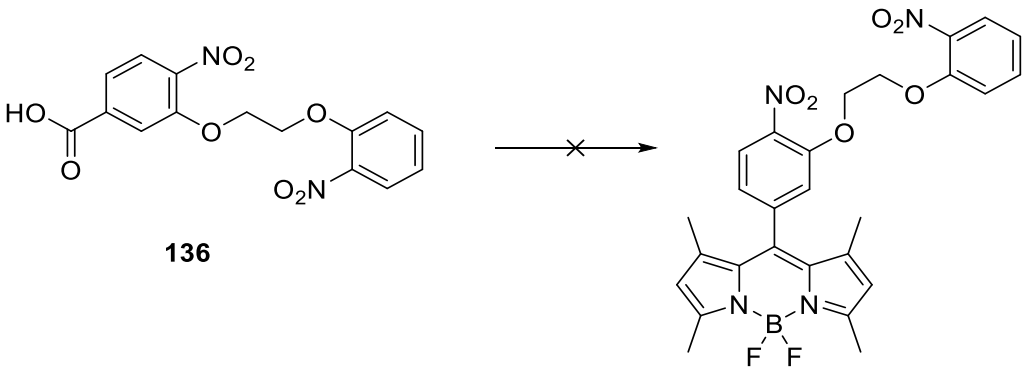
Scheme 51-Conditions: a) i) NaH, DMF, 0 °C, 15 min, ii) 1,2-dibromoethane, r.t. – 140 °C, 2 h; b) Ac_2O, pyridine, 115 °C, 4 h; c) ^{t}BuBr, Ag_2O, MeCN/H_2O; d) KOH, MeOH, H_2O, 50 °C, 30 min; e) 133, K_2CO_3, NMP, 140 °C, 10 min.

It was later determined that it is possible to assemble the BAPTA framework without the need for protection of the carboxylic acid **130** as it does not appear to cause any competing reactions during the alkylation step (Scheme 51).



Scheme 52- Synthesis of advanced intermediate 136 for high affinity sensors ; Conditions: 1,2-dibromoethane, K_2CO_3, MeCN, 80 °C, 2 h

Unfortunately, the condensation reaction of the acid chloride of **136** with 2,4-dimethylpyrrole was not observed to occur (Scheme 53).



Scheme 53- The attempted formation of green BODIPY fluorophore from nitro-BAPTA aryl-acid chloride; Conditions: i) $(COCl)_2$ Cat. DMF, DCM, 0 °C, 2 h; ii) 2,4-dimethylpyrrole, r.t., 19 h; iii) BF_3OEt_2, NEt_3, DCM, 6 h

The formation of the acid chloride was achieved by the reaction of distilled oxalyl chloride with catalytic DMF, and carboxylic acid derivative **136** in DCM. After 2 h an aliquot was taken from the reaction mixture and quenched with HPLC grade methanol, ESI + mass spectrometry confirmed the formation of the methyl ester and, consequently, the acyl chloride. The reaction of the acid chloride with 2,4-dimethylpyrrole was followed by TLC, which revealed a complex mixture of products and disappointingly no fluorescent spots were observed as had been the case in the formation of simplified model systems **128** and **129**. Despite several attempts no evidence of forming either the dipyrrin or the desired BODIPY in this reaction were detected.

III. **BODIPYs *via* aldehydes**

Another common synthetic approach to BODIPYs, initially described by Daub et al[110] is *via* the condensation of the chosen pyrrole derivative with an aldehyde under acidic conditions to form a dipyrrole species, which is typically not stable enough for purification, then an oxidation is performed, often using DDQ or p-chloranil, to afford the dipyrrin. This may be purified or reacted in situ to form a BODIPY with boron trifluoride diethyletherate under basic conditions (Figure 49).

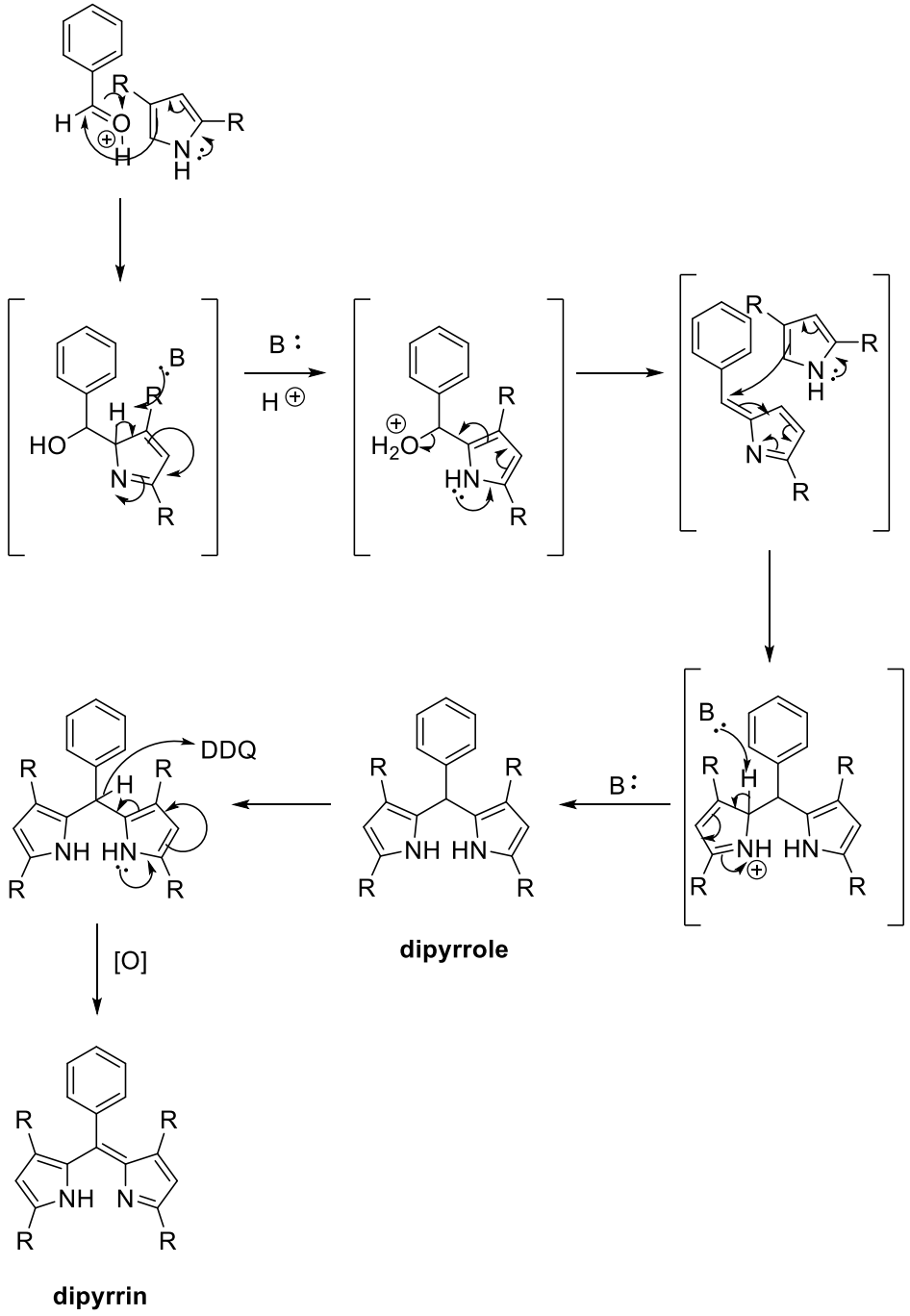
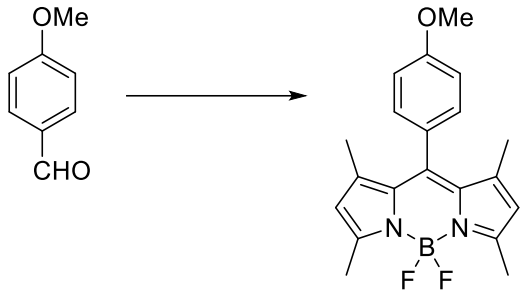


Figure 49- Proposed mechanism for the formation of dipyrrins *via* dipyrroles

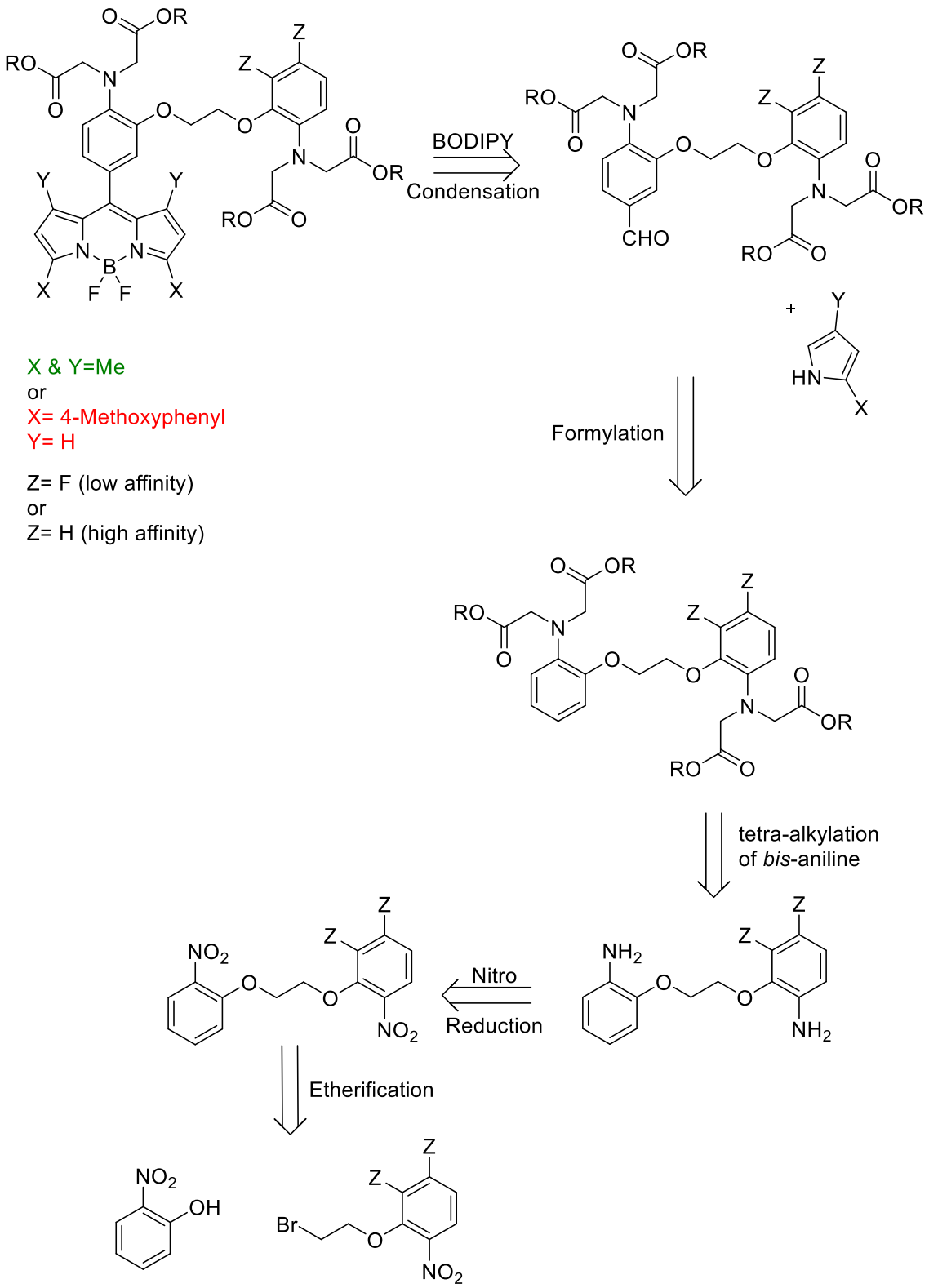
This method appears to be favoured when working with aryl carbonyls, and indeed afforded an improved yield of BODIPY **137**(Scheme 54).



137
33% yield

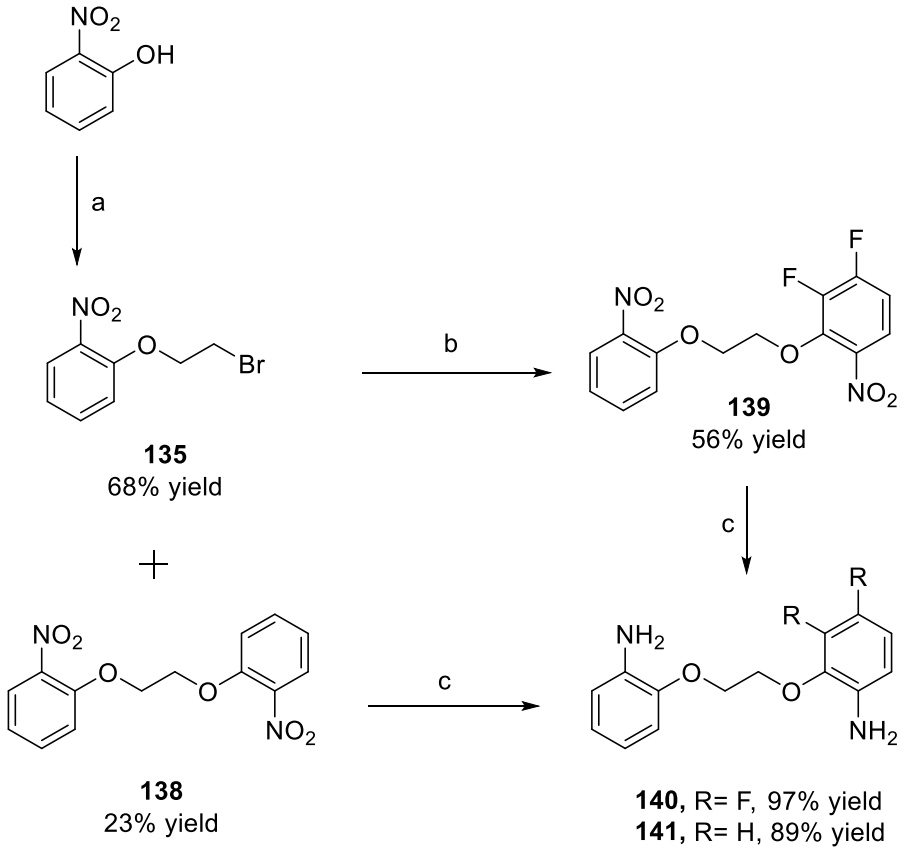
Scheme 54-Synthesis of green BODIPY from 4-methoxybenzaldehyde; Conditions: i) 2,4-dimethylpyrrole, TFA, DCM, r.t., 16 h; ii) DDQ, 0 °C, 2 h; iii) BF_3OEt_2, NEt_3, 16 h

Encouraged by these findings, the synthesis of the BODIPY-BAPTA sensors were more closely aligned with previous BAPTA-based PET fluorescent sensors, such as Indo-1, Fura-1 and Stil-1 reported by Tsien *et al*[83] described in Figure 36. The BAPTA chelatory part of the sensor is assembled first, masking the binding units as alkyl esters then proceeding to formylate the BAPTA aryl ring. The formed aldehyde may then be used to form the BODIPY fluorophore in an analogous manner to that above as described in the retrosynthetic Scheme 55 below.



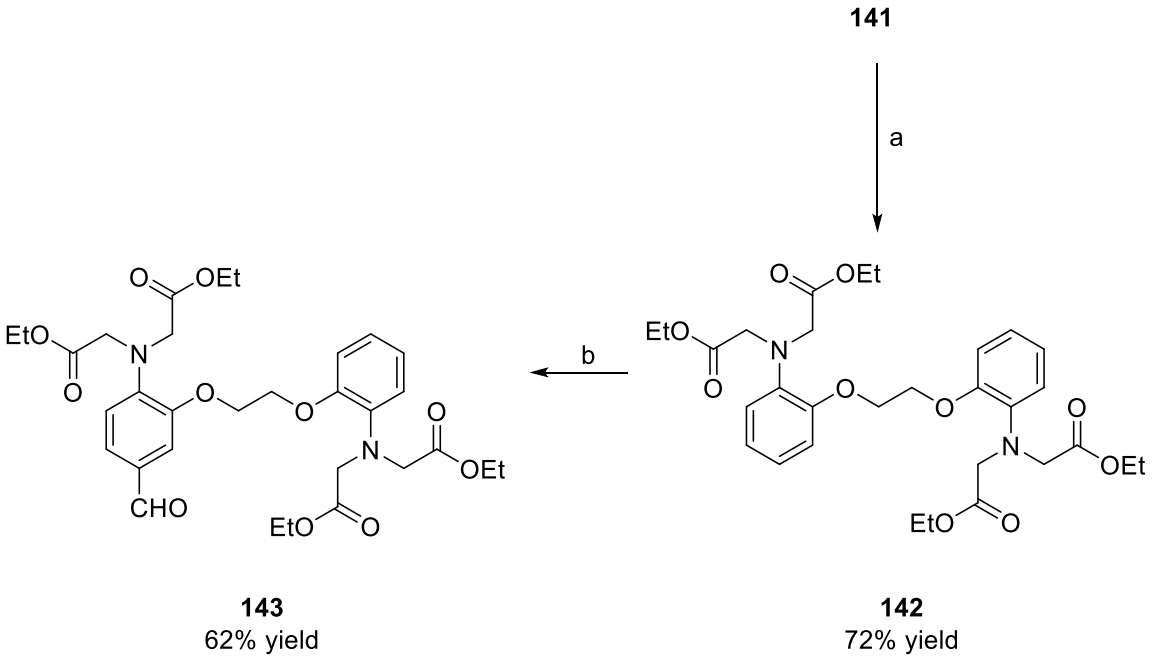
Scheme 55- Retrosynthetic scheme for the synthesis of BODIPY BAPTA sensors from BAPTA aldehydes.

The synthesis began from 2-nitrophenol which was alkylated with 1,2-dibromoethane under basic conditions to afford a mixture of **135** and **138,** the ratio of which can be tailored by controlling the stoichiometry to afford one or the other in greater abundance. This is useful as **138** provides a direct route to the high-affinity BODIPY sensors and **135** provides a route *via* displacement of the bromide with sodium 2,3-difluoro-6-nitrophenoxide to the low-affinity sensors. The corresponding nitroaryl-ethers were then reduced utilising hydrogenolysis to afford *bis*-anilines **140** and **141** which were found to rapidly degrade when exposed to atmospheric conditions (Scheme 56). At this stage it was decided to pilot the remaining steps in the synthesis using the symmetric *bis*-aniline **141** with a view to demonstrate formation of the fluorophores, thus preserving the expensive fluorinated compound **140**, should an alternative route be required.

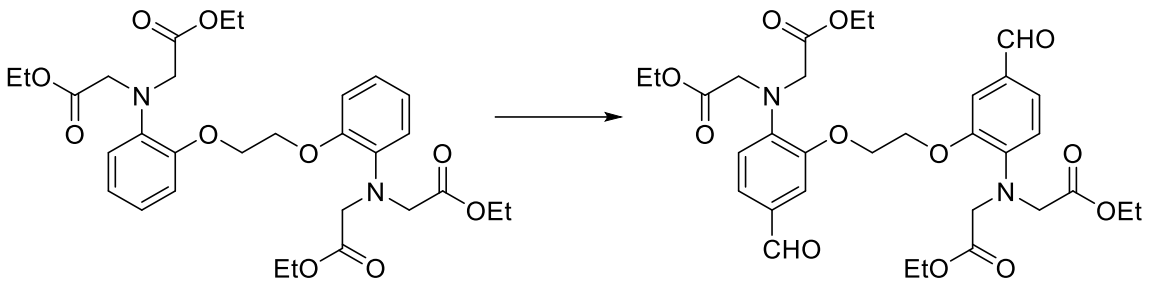


Scheme 56-The synthesis of BAPTA-precursor anilines 140 and 141; Conditions: a) 1,2-dibromoethane, K_2CO_3, MeCN, 60 °C, 16 h; b) 2,3-difluoro-6-nitrophenol; NaH, DMF, 140 °C, 8 h; c) H_2, Pd/C (5 mol%), EtOAc, r.t., 6 - 16 h

The alkylation of *bis*-aniline **141** was performed using ethyl bromoacetate catalysed with sodium iodide and proton sponge as a base. It was observed that rigorously dried reagents are necessary for these alkylation reactions to afford **142**; if non-dried reagents and solvents were used hydrolysis of the esters was observed to predominate. Formylation of **142** with the Vilsmeir Haack reaction proceeded in moderate yield (Scheme 57) however the stoichiometry of $POCl_3$ is crucial. Where an excess was used, a symmetric *bis*-formylation product was observed as the predominating or sole outcome of the reaction (Scheme 58).



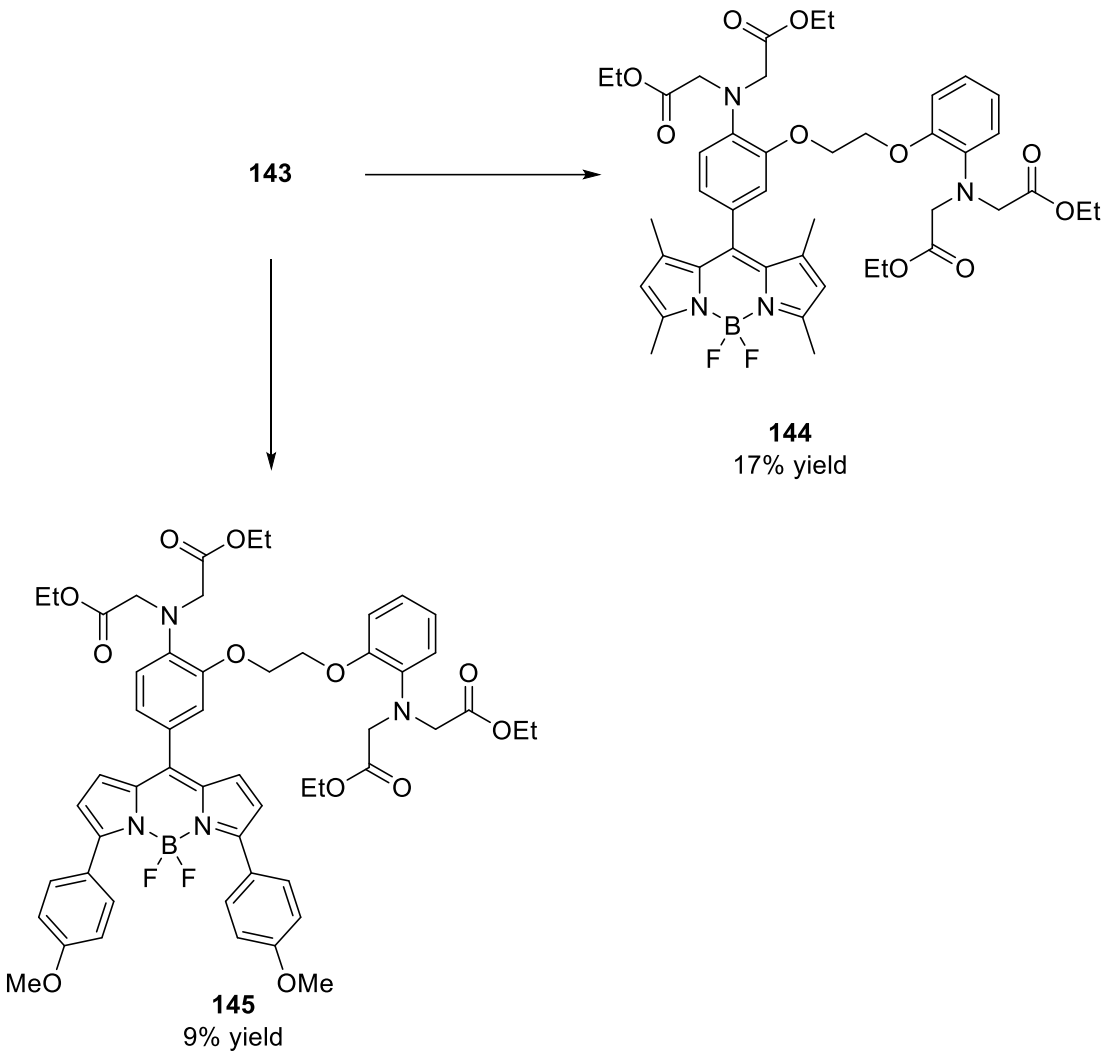
Scheme 57- Synthesis of BAPTA-CHO-tetra-ethyl-ester; Conditions: a) Ethyl bromoacetate, *N*,*N*,*N'*,*N'*-tetramethylnaphthalene-1,8-diamine, NaI, 60 °C, 48 h; b) $POCl_3$, DMF, 0 – 70 °C, 16 h



142

Scheme 58-Resulting over-formylation where excess electrophile is formed; Conditions: $POCl_3$, DMF, 0 – 70 °C, 16 h

Syntheses of both the green and red BODIPY fluorophores **144** and **145** *via* the conditions described by Daub et al[110] were successfully achieved (Scheme 59). Pleasingly TLC of the reaction reveals fluorescent spots when irradiated with a longwave UV TLC-lamp and indeed upon purification, whilst the compounds are dissolved in polar aprotic solvents, fluorescence is very clearly observed (Figure 50).



Scheme 59- Synthesis of novel BODIPY sensors as tetra-ethyl esters *via* Daub *et al*'s conditions [110]; Conditions: a) i) 2,4-dimethylpyrrole, TFA, DCM, r.t., 16 h; ii) DDQ, DCM, 0 °C, 2 h; iii) BF_3OEt_2, NEt_3, 2 h; b) as above with 2-(4-methoxyphenyl)-pyrrole in the place of 2,4-dimethylpyrrole.

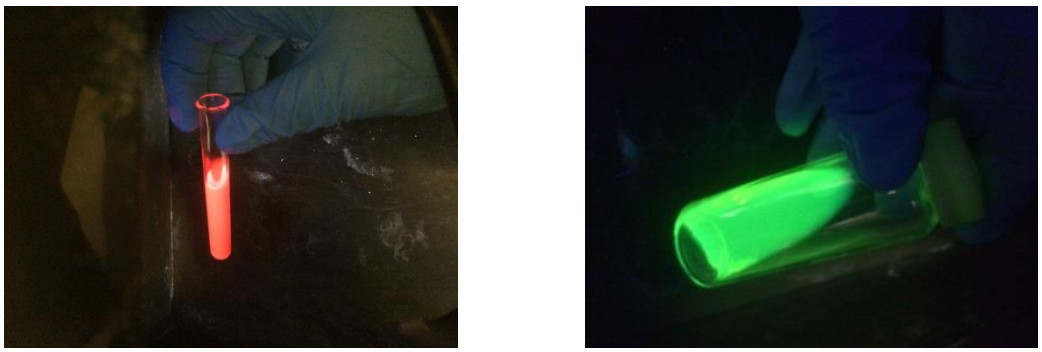
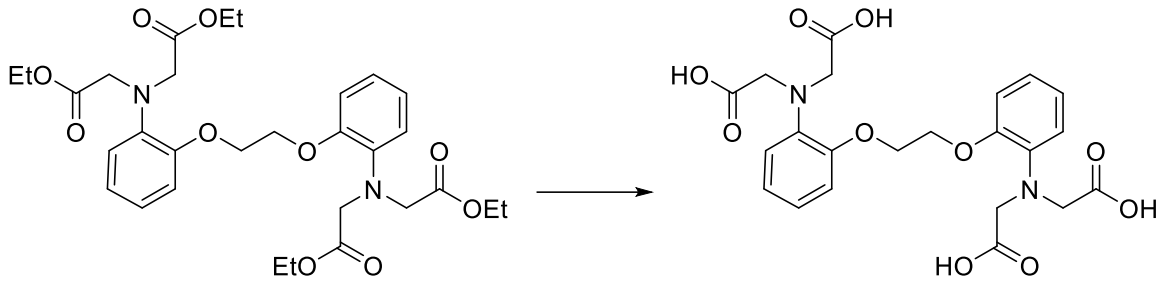


Figure 50-Samples of 145 (left) and 144 (right) dissolved in acetone irradiated with longwave UV (λmax~365 nm)

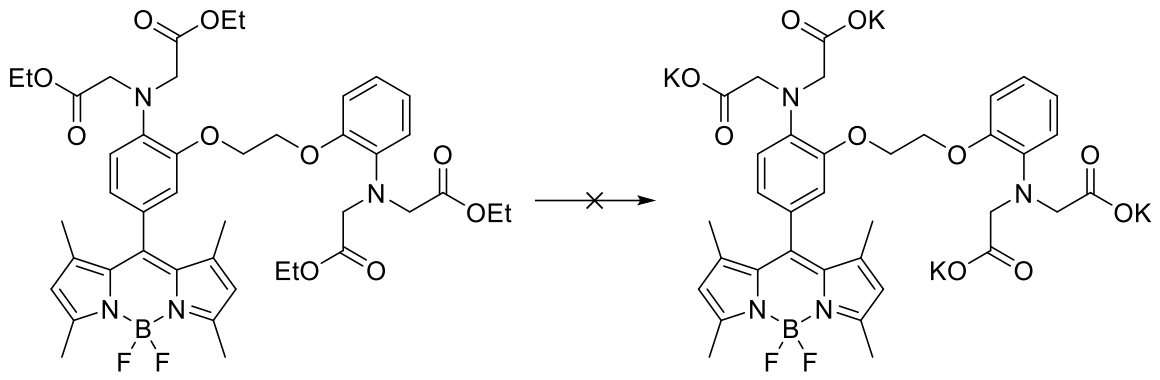
It was observed that **144** appears to be relatively stable to ambient conditions and light with no degradation observed over 1 month when monitored by TLC. However **145** appeared to degrade over 5 days unless stored under an argon atmosphere. The mechanism for this degradation is not currently known but the degradation products appear to be non-fluorescent. Mass spectrometry suggests that the boron is not present in the degradation products (i.e. a loss of boron isotopic pattern as a result of ^{10}B and ^{11}B). Deprotection of **144** to the functionally active sensor was attempted *via* saponification conditions as described by Tsien *et al* in their work on BAPTA-derived fluorescent probes and sensors. [83] The saponification conditions were initially trialled utilising **142** as a model system and the reaction was found to proceed in moderate yield (Scheme 60).



142 **146** 72% yield

Scheme 60-Saponification of BAPTA-TEE as a model system for BODIPY-BAPTA-TEE; Conditions: i) KOH, EtOH, H_2O, 80 °C, 2 h.

Unfortunately, severe degradation was observed when these conditions were applied to **144**; from TLC it appeared that the starting material was consumed within 15 minutes and a product formed that is significantly more polar which still fluoresced under longwave UV irradiation(Scheme 61).



144

Scheme 61-Attempted saponification of Hi green BODIPY-BAPTA-TEE; Conditions: KOH, EtOH, H_2O, 80 °C, 2 h

Analytical reverse phase HPLC revealed multiple compounds as well as some remaining unreacted **144** that presumably was not visibly by TLC due to it becoming sequestered along with the degradation products (Figure 51).

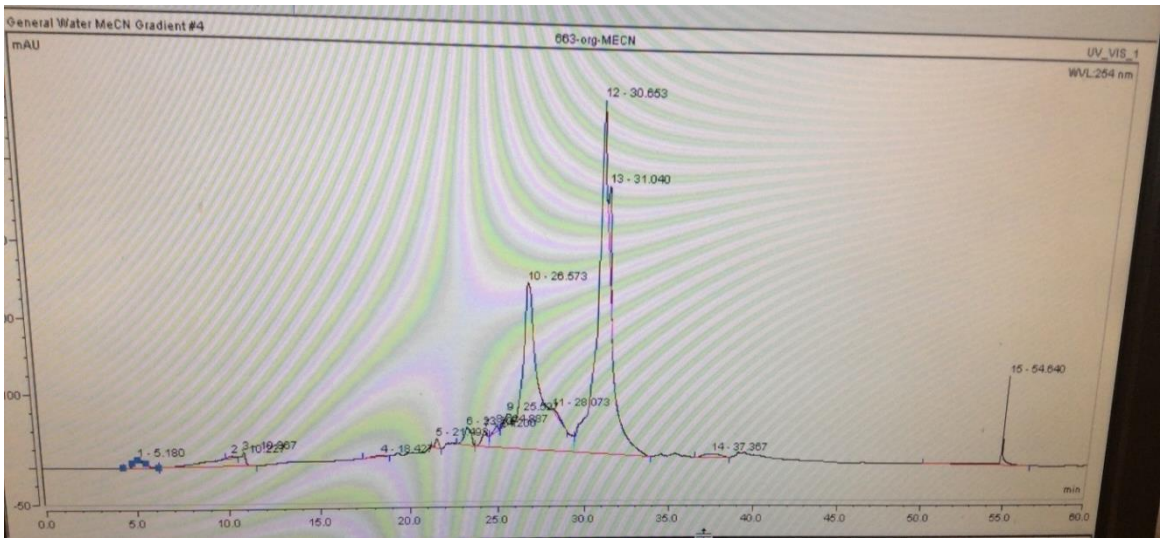


Figure 51- Analytical reverse phase HPLC trace (C18, 0-100% MeCN : H_2O); unreacted Hi-Green-BODIPY-BAPTA-TEE 144, retention time 55.54 minutes

In the hope that one of the peaks observed in the analytical HPLC trace between 25-35 minutes of retention time was the desired saponification product purification *via* semi-prep-HPLC

using the same conditions as used with the analytical HPLC. Unfortunately, despite being able to isolate three distinct peaks with retention times at 26.7, 29.8 and 33.4 minutes, none of these fractions were found to contain the desired product (Figure 52).

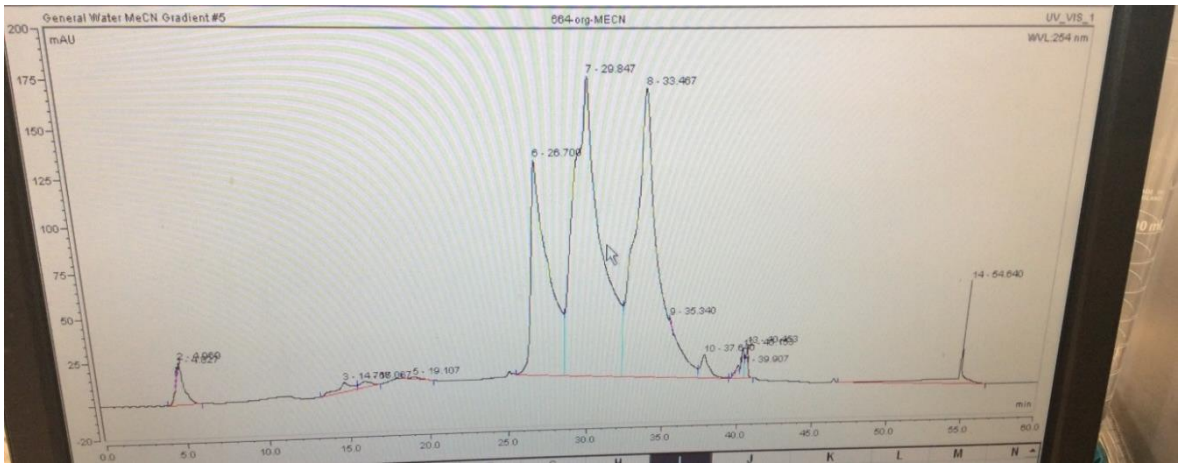


Figure 52- Prep-HPLC trace of attempted purification of saponification of Hi-Green-BODIPY-BAPTA-TEE, 144

It is clear that this method of hydrolysis is unsatisfactory and due to the inherent instability of BODIPYs in acidic conditions, acid catalysed hydrolysis is also fundamentally unsuitable.

I. BAPTA-tetra-benzyl esters

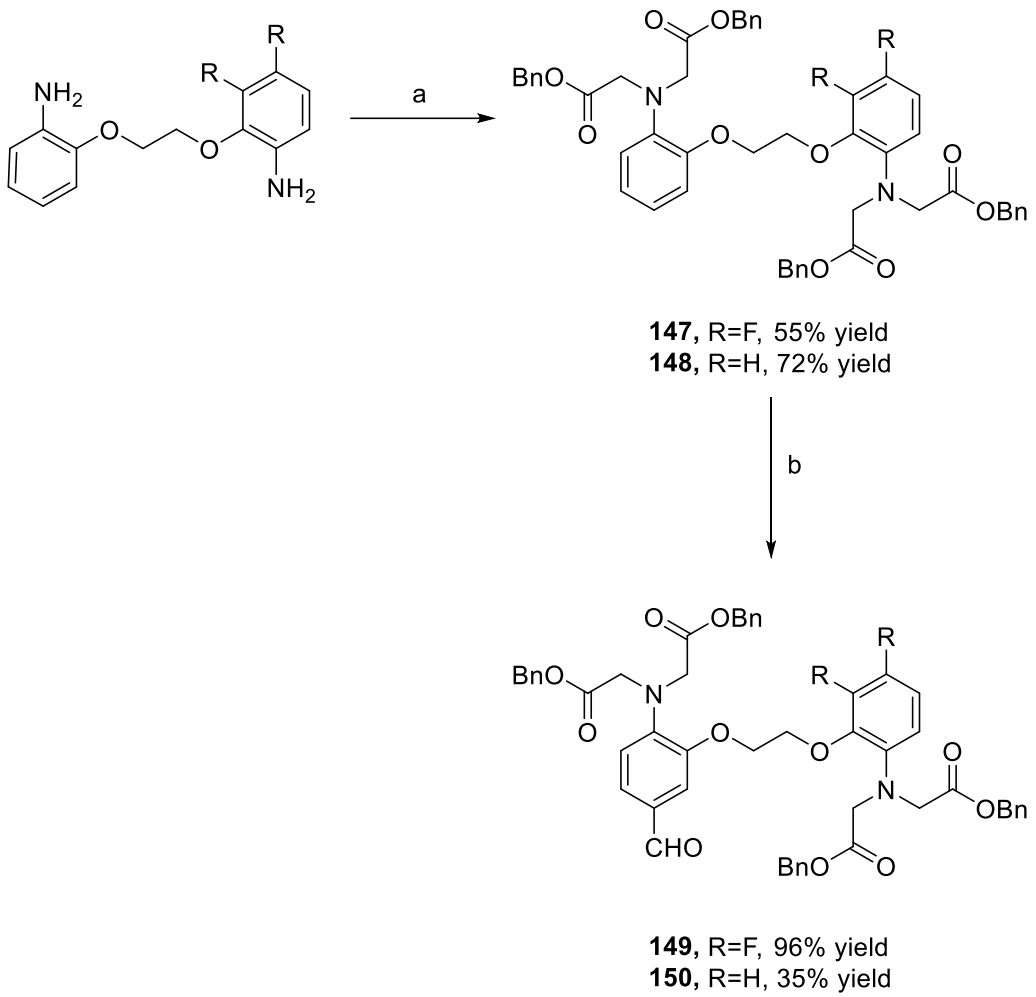
Due to apparent instability of the BODIPY fluorophore to saponification conditions and the purification that followed them, the use of an alternative ester with milder conditions for conversion into the free tetra-carboxylic acid was clearly needed. The use of tetra-benzyl ester derivatives of BAPTA were explored as the functionally active sensor could be revealed under hydrogenolysis conditions that, based on the reported work of Kellam *et al,*[111] should be compatible with the BODIPY core. The synthesis of BAPTA-TBE **148** was achieved in an analogous way to BAPTA-TEE **142** using benzyl bromoacetate in place of ethyl bromoacetate as the alkylating

agent of the *bis*-anilines in a moderate yield (Scheme 62). However, in the case of the fluorinated *bis*-aniline **140,** it was observed that the *N*-alkylation does not reach completion and appears to stop after the third substitution. This is suggested to be due to the increase in steric bulk when going from ethyl to benzyl groups at the ester increasing the energy barrier for the final alkylation beyond that achieved when heated to reflux in acetonitrile. To remedy this, the reaction was trialled with different solvents and temperatures in both a conventional round-bottom flask as well as in a CEM microwave reactor sealed vessel (Table 12)**.**

Entry	Reagents	Solvent [molarity of aniline]	Temperature (Heating method)	Time	Observation
1	**140** (1 eq.) Benzylbromo acetate (6 eq.) Proton Sponge ® (6 eq.) NaI (2 eq.)	MeCN [0.1 M]	(RBF/heat mantle)	7 d	Mixture of double and triple alkylation products only a trace of tetra-substitution products.
2	As above	MeCN [0.1 M]	150 °C (µW)	2 h	Multiple unidentified degradation products
3	As above	DMF [0.05 M]	153 °C (RBF/heat mantle)	5 h	Multiple unidentified degradation products, <10% desired product
4	As above	DMF [0.05 M]	200 °C (µW)	2.5 h	Multiple unidentified degradation products, <10% desired product
5	As above	BuCN [0.25 M]	115 °C (RBF/heat mantle)	14 d	55% product, majority of remaining material at triple alkylation.

Table 12- summary of conditions trialled for the alkylation of fluorinated *bis*-aniline; RBF –Round Bottom Flask, µW- CEM microwave reactor and vessel.

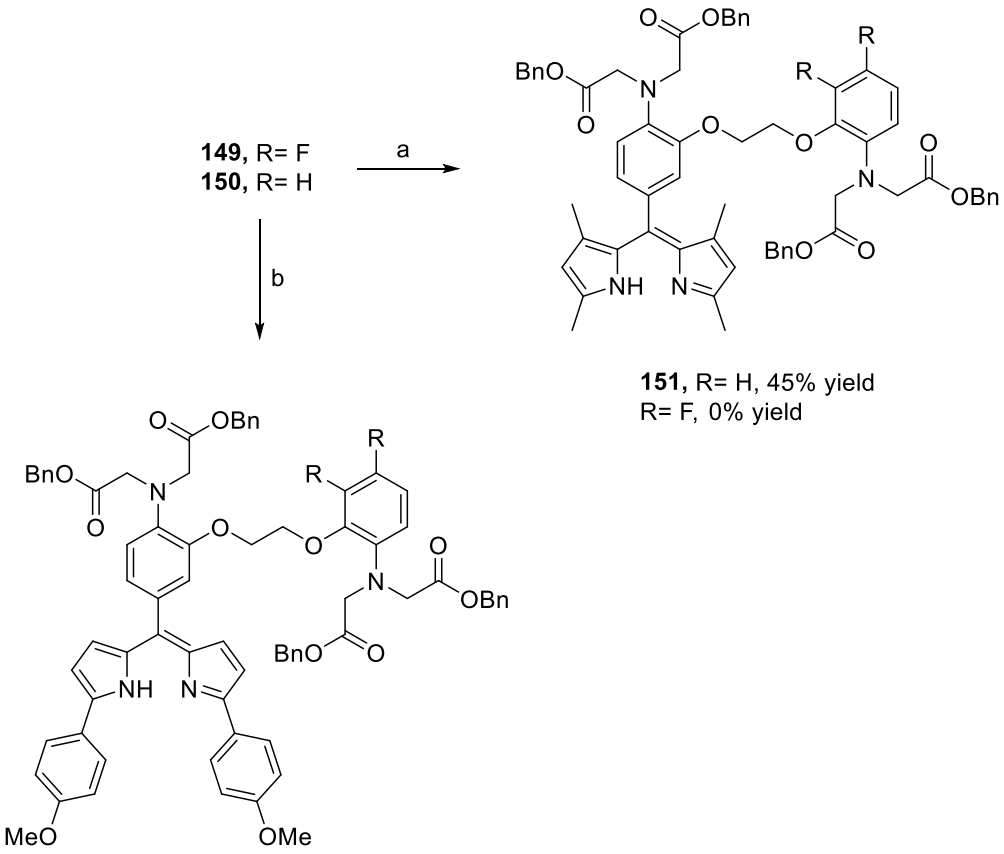
This screen of conditions identified suitable conditions in the form of a 14-day reflux in butyronitrile (Table 12, entry **5**), which afforded the product **147** in reasonable yield on gram scale (Scheme 62).



Scheme 62- Formation of BAPTA-TBE and BAPTA-TBE-CHO Hi and Low affinity chelators; Conditions: a) (R = H) benzyl bromoacetate, NaI, *N*,*N*,*N'*,*N'*-tetramethylnaphthalene-1,8-diamine, MeCN, 60 °C, 48 h; (R = F) benzyl bromoacetate, NaI, *N*,*N*,*N'*,*N'*-tetramethylnaphthalene-1,8-diamine, BuCN, 60 °C, 14 d; b) $POCl_3$, DMF, 0 – 70 °C, 16 h.

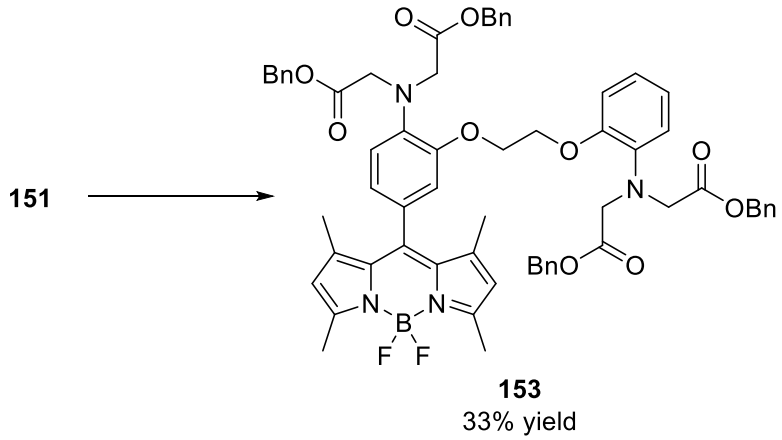
Following the successful tetra-alkylation of the bis anilines, **147** and **148** were formylated *via* the Vilsmeir-Haack reaction in the same fashion as **143**. The formylation performed with high yield to provide difluoro-bapta **149** as only a single formylation can occur due to the crowded nature of the fluorinated ring system; **150** was afforded in reasonable yield with a significant amount of **148** being recovered. In the case where more than 1.1 equivalents of $POCl_3$ was used, the *bis*-formylated symmetrical system **150b** is observed to dominate the reaction products; for this reason, the stoichiometry of $POCl_3$ was kept at 1.1 equivalents so that the major outcome of the reaction was **150** and recoverable, thus recyclable, **149.**

BODIPY synthesis from the BAPTA-tetra-ester-aldehydes **149** and **150** was attempted utilising methodology reported by Thompson et al[112] whereby, upon formation of an aryl-dipyrrin, a lithiation of the dipyrrin-N-H is performed with LiHMDS at −78 °C followed by addition of BF_3OEt_2. It is suggested that the lithiation of the dipyrrin-acidic nitrogen is stablising and improves both the reaction rate and yield [112]. These conditions were used in the place of those used in the synthesis of **144** and **145** with a view to increase the yield, aldehyde **150** was then reacted with 2,4-dimethyl pyrrole to afford dipyrrin **151** as well as 2-(4-methoxyphenyl)pyrrole **127** to afford **152** but unfortunately this was not observed for **149** where the low affinity-dipyrrins were not detected to form (Scheme 63)



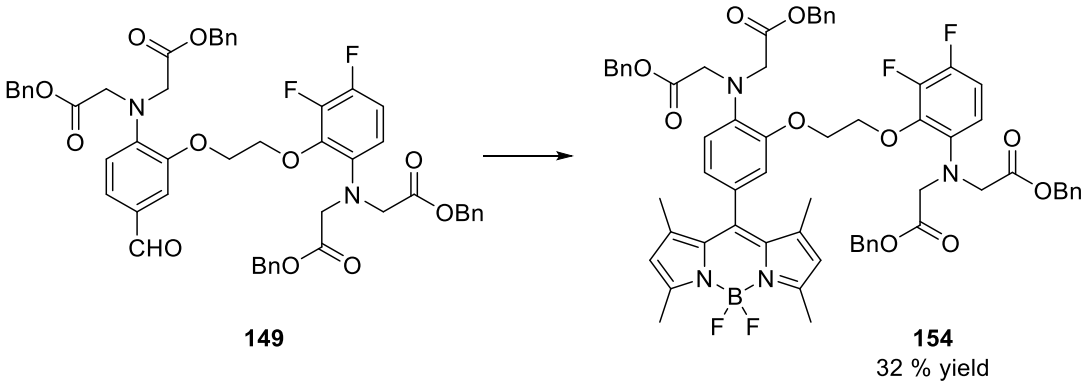
Scheme 63- Synthesis of dipyrrins formed from BAPTA-TBE-CHO and BAPTA-TBE-CHO-FF; Conditions: a) i) 2,4-dimethylpyrrole, TFA, DCM, r.t., 8 h; ii) DDQ, DCM, 0 °C, 2 h; b) i) 2(-4-methoxyphenyl)pyrrole, TFA, DCM, r.t., 8 h

Application of *Thompson et al's* conditions[112] were found to afford a slightly improved yield when comparing the synthesis of Hi-Green-BODIPY-BAPTA-TBE **153** *vs.* Hi-Green-BODIPY-BAPTA-TEE **144** as the yield for the one-pot formation of **144** (TEE) was 17% whereas the stepwise process yielded dipyrrin **151** in 45% yield and then BODIPY **153** also in a 45% yield, with a combined yield over both steps of 20% (Scheme 64).



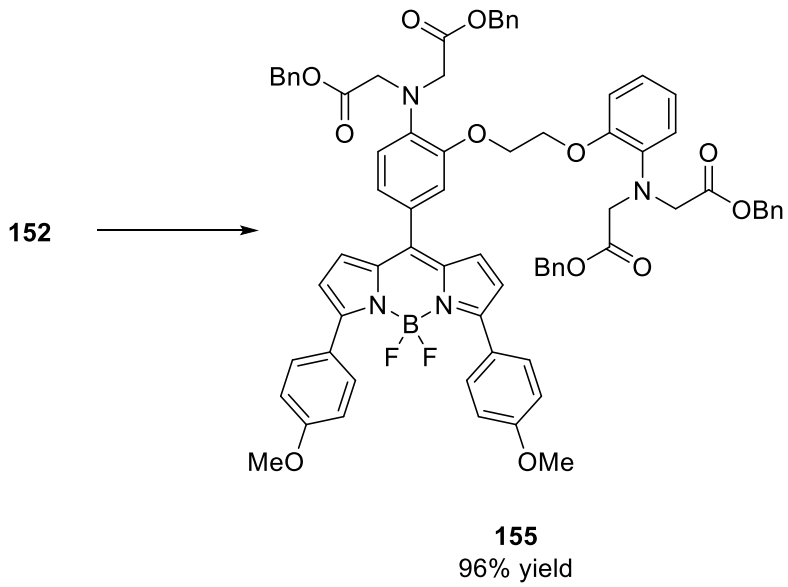
Scheme 64-Application of *Thompson et al's* lithiation-boration conditions; Conditions: i)LiHMDS, THF, −78 °C, 1 h; ii) BF_3OEt_2, −78 °C – r.t., 16 h.

Synthesis of low-affinity-BODIPY-BAPTA-TBE **154** was achieved with a 32% yield applying the one-pot conditions of Daub et al[110] to aldehyde **149** (Scheme 65).



Scheme 65- Synthesis of low-affinity-BODIPY-BAPTA-FF-TBE; Conditions: i) 2,4-dimethylpyrrole, DCM, r.t., 8 h; ii) DDQ, DCM, 0 °C, 2 h; iii) BF_3OEt_2, NEt_3, 16 h

The synthesis of hi-affinity-Red-BODIPY-BAPTA-TBE **155** was achieved in a very high yield of 96% applying Thompson *et al's* conditions as shown in Scheme 66 below. At this stage there is no hypothesis to explain the inconsistent pattern of results that have been observed for the synthesis of these BODIPY compounds.

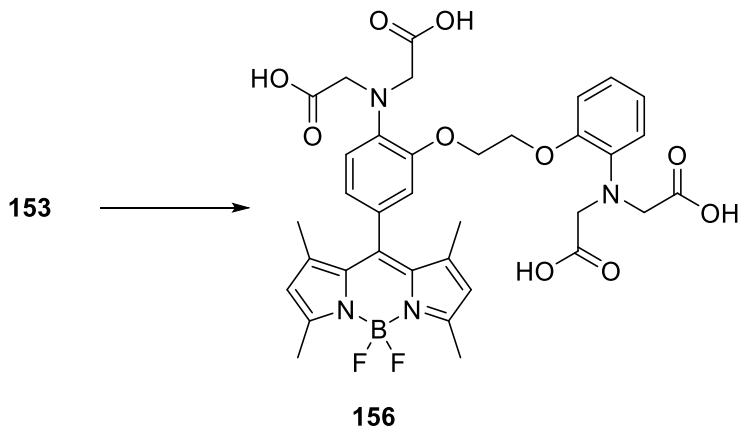


Scheme 66-Synthesis of Hi-Red-BODIPY-BAPTA-TBE *via* Thompson *et al's* conditions; Conditions: i) LiHMDS, THF, −78 °C, 1 h; ii) BF_3OEt_2 −78 °C – r.t., 16 h.

Unfortunately, stability issues were obserevd with the Hi-Red-BODIPY-BAPTA-TBE **155** as was the case with Hi-Red-BODIPY-BAPTA-TEE **145**. As mentioned previously, degradation to more polar compounds that no longer show $^{10}B/^{11}B$ isotopic patterns in the mass spectrum was observed. It is postulated that the boron is not ligated well by this particular dipyrrin moiety and over time, *via* an unknown mechanism, the boron appears to disassociate from the BODIPY. Given the instability of some products and the likely sensitivity of all reactions to quality of reagents and solvents a larger number of repetitions would be required to draw clear conclusions upon the ideal route to these compounds.

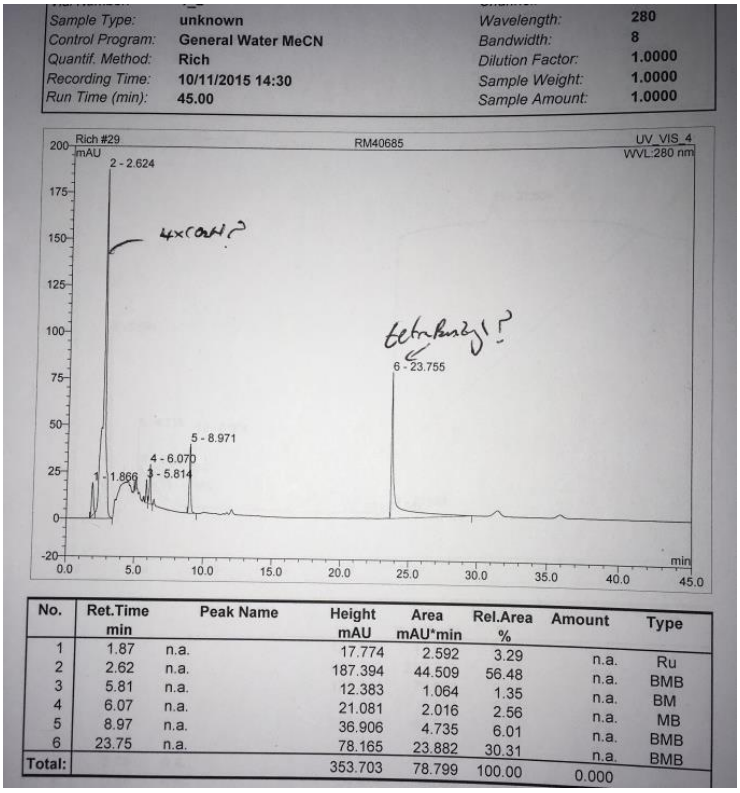
II. Debenzylation by hydrogenolysis of Hi-Green-BODIPY-BAPTA-TBE

Proceeding with the both the green sensors **153** and **154** and the high-affinity red sensor **155** protected as tetra-benzyl esters, the deprotection of the esters *via* hydrogenolysis was explored; Initially, the hydrogenolysis of **153** with 2.5 mol% palladium on activated carbon was attempted (Scheme 67).



Scheme 67- Hydrogenolysis of Hi-green-BODIPY-BAPTA-TBE 153 with Pd/C; Conditions: $H_{2\,(g)}$, Pd/C (2.5 mol%), MeOH/EtOH, r.t., 6 h

Analytical reverse-phase HPLC of the crude product of the reaction (Figure 53) was performed; pleasingly a significant product of this reaction was observed with retention time of 2.62 minutes which, due to its great increase in polarity upon deprotection, tentatively assigned as the product **156**. There also appeared to be some unreacted **153** remaining after 2 h of reaction (retention time of 23.7 minutes). However, despite several attempts to purify this compound with HPLC only severe degradation was observed.



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.87	n.a.	17.774	2.592	3.29	n.a.	Ru
2	2.62	n.a.	187.394	44.509	56.48	n.a.	BMB
3	5.81	n.a.	12.383	1.064	1.35	n.a.	BM
4	6.07	n.a.	21.081	2.016	2.56	n.a.	MB
5	8.97	n.a.	36.906	4.735	6.01	n.a.	BMB
6	23.75	n.a.	78.165	23.882	30.31	n.a.	BMB
Total:			353.703	78.799	100.00	0.000	

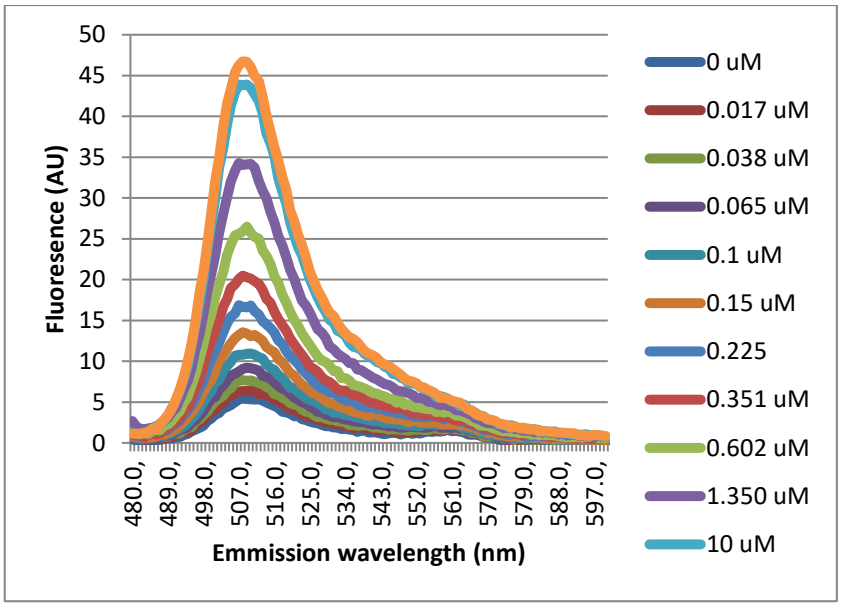
Figure 53- Analytical HPLC trace for the hydrogenolysis of Hi-Green-BODIPY-BAPTA-TBE 153 with Pd/C (2.5 mol%), 6 h

Due to the inability to purify the product of this hydrogenolysis qualitative assessment of the fluorescent properties of the crude material was performed. Initially a sample was dissolved in 20% methanol in deionised water and found to possess a very faint green emission when irradiated with longwave UV light but upon addition of $CaCl_2$ the emission to become noticeably more intense.

7.2 Ca^{2+} fluorescent dose-response of Hi-Green-BODIPY-BAPTA

To quantify this observation the Biotium ™ Calcium calibration buffer kit was purchased; the kit comprises two components: Component A (zero free Ca^{2+}: 10 mM K_2EGTA, 100 mM KCl and 10 mM MOPS at pH 7.2) and component B (40 µM free Ca^{2+}: 10 mM CaEGTA, 100 mM KCl and

10 mM MOPS at pH 7.2). The method described in the product information protocol PI-59100, derived from the work of Stokes *et al*was implemented [113]. Using a 750 µL quartz cuvette and a Shimadzu RF5301PC spectrofluorophotometer, a solution of 1 µM crude sensor was tested with the above method of reciprocal dilution to measure the fluorescence of the sensor at Ca^{2+} concentrations between 0 – 39.8 µM (Graph 2).

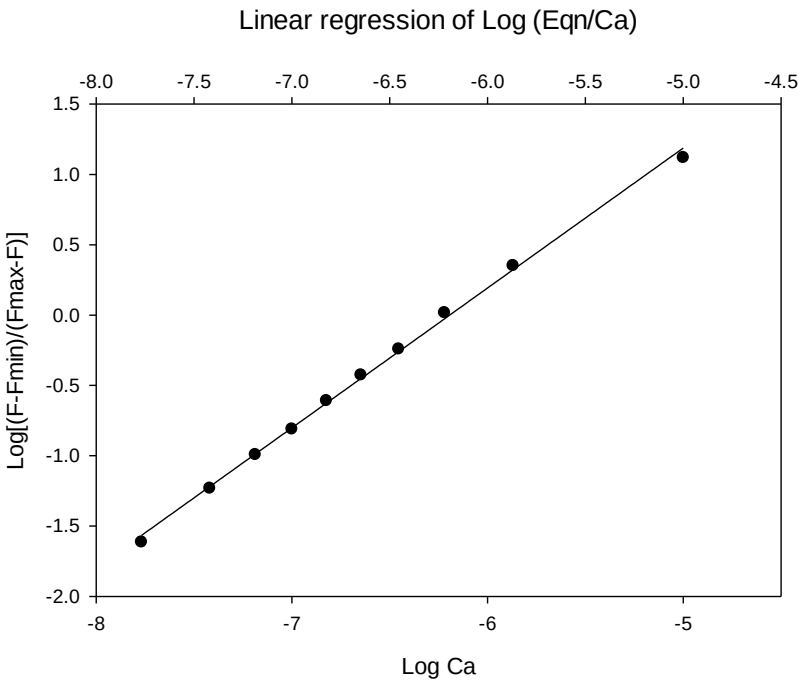


Graph 2- Ca^{2+} fluorescent dose-response of Hi-Green-BODIPY-BAPTA 156 (1 µM; Biotium calcium calibration buffer kit and Shimadzu RF-5301PC spectrofluorophotometer

The fluorescent intensity and free Ca^{2+} has the following relationship:

$$\text{Log}\{(F-F_{min})/(F_{max}-F)\} = -\log K_d + \log[Ca^{2+}]$$

This relationship allows the creation of a plot of $\log\{(F-F_{min})/(F_{max}-F)$ *vs.* $\log[Ca^{2+}]$, where $[Ca^{2+}]$ is expressed in moles, and the x-intercept from the plot is $\log K_d$ thus $10^x = K_d$.

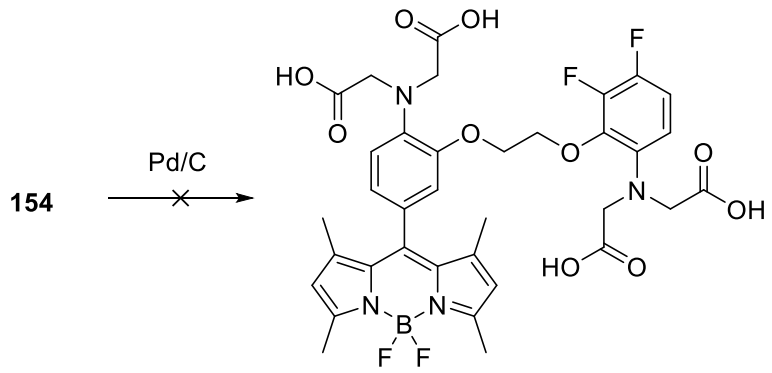


Graph 3- Linear regression of Hi-Green-BODIPY-BAPTA; log[(F-F_{min})/(F_{max}-F)] *vs*. log[Ca^{2+}]

The x-intercept of the above linear regression of log[(F-F_{min})/(F_{max}-F)] *vs*. log[Ca^{2+}] is found to be −6.19, thus K_d of Hi-Green-BODIPY-BAPTA **156** is equal to 0.64 µM, which is consistent with the closely related Fluo-4 which has K_d 0.34 µM.

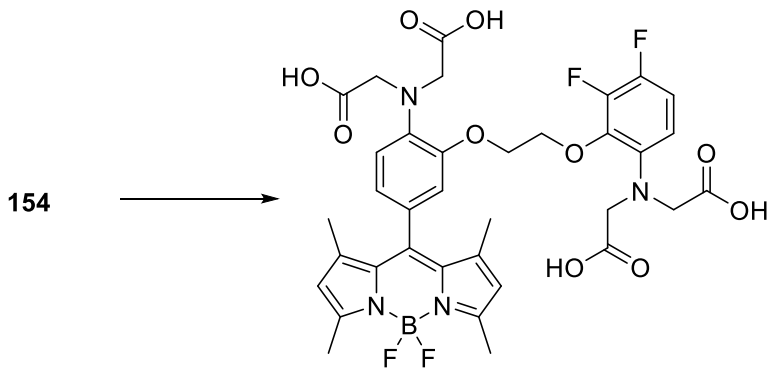
7.3 Debenzylation by hydrogenolysis of Low-Green-BODIPY-BAPTA-TBE

Unfortunately, severe degradation to non-fluorescent, non-boron containing compounds (mass spectrum showed no boron isotopic pattern) was observed when hydrogenolysis using palladium on activated carbon was used under a hydrogen atmosphere upon low green-BODIPY-BAPTA-FF-TBE **154** (Scheme 68).



Scheme 68- Observed degradation for the hydrogenolysis of Low-Green-BODIPY-BAPTA-FF-TBE; Conditions: $H_{2\,(g)}$, Pd/C (2.5 mol%), MeOH, 6 h

The hydrogenolysis was performed again using 5 mol% palladium (II) hydroxide as the catalyst, and the reaction was followed by HPLC at 2 h (Scheme 69).

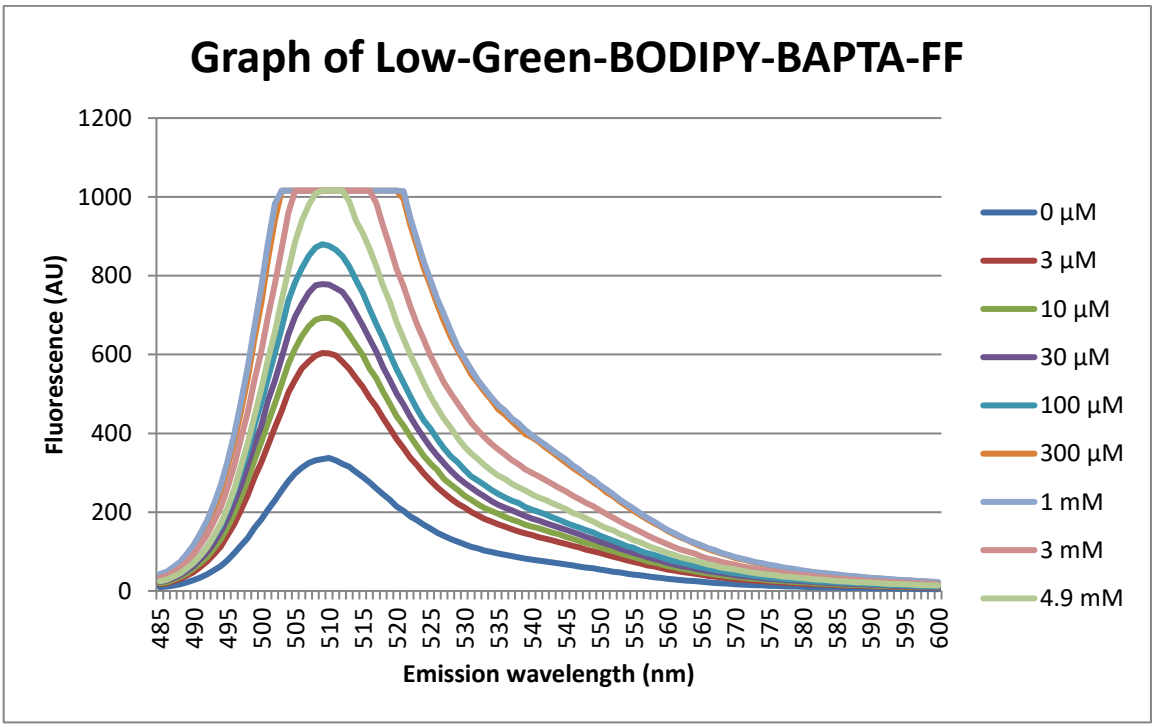


Scheme 69- Debenzylation of Low-Green-BODIPY-BAPTA-FF-TBE *via* hydrogenolysis catalysed with $Pd(OH)_2$; Conditions: $H_{2(g)}$, $Pd(OH)_2$ (5 mol%), MeOH, 2 h

After 2 hours of reaction under these conditions, no remaining starting material was detected *via* analytical reverse phase HPLC (C18, 50% MeCN : H_2O) and the appearance of two significantly more polar products were observed (Figure 90). Attempts to purify this reaction using reverse phase preparatory HPLC, using the same method used for the analytical runs that afforded the traces in the figures above, unfortunately proved unsuccessful and it appears that the product degraded whilst undergoing chromatography. Once again, due to a lack of methodology with which to purify **157**, the fluorescent properties of the crude material were assessed.

Unfortunately, unlike with the high affinity sensor **156**, there is no commercially available low affinity Ca^{2+} calibration kit available to calibrate low affinity sensors, so, in order to gain preliminary data for the fluorescent dose response a solution of low-Green-BODIPY-BAPTA-FF (10 µM) in milliQ water buffered to pH 7.2 with MOPS; to serve as the zero free Ca^{2+} component in the experiment was created. The high-concentration free Ca^{2+} component was achieved by making up a solution of low-Green-BODIPY-BAPTA-FF **157** (10 µM) with $CaSO_4$ (4.9 mM) in miliQ water buffered to pH 7.2 with MOPS.

Using a 750 µL quartz cuvette, Shimadzu RF5301PC spectrofluorophotometer and the solutions mentioned above, the low-Green-BODIPY-BAPTA-FF **157** was tested and the results shown in Graph 4 below.

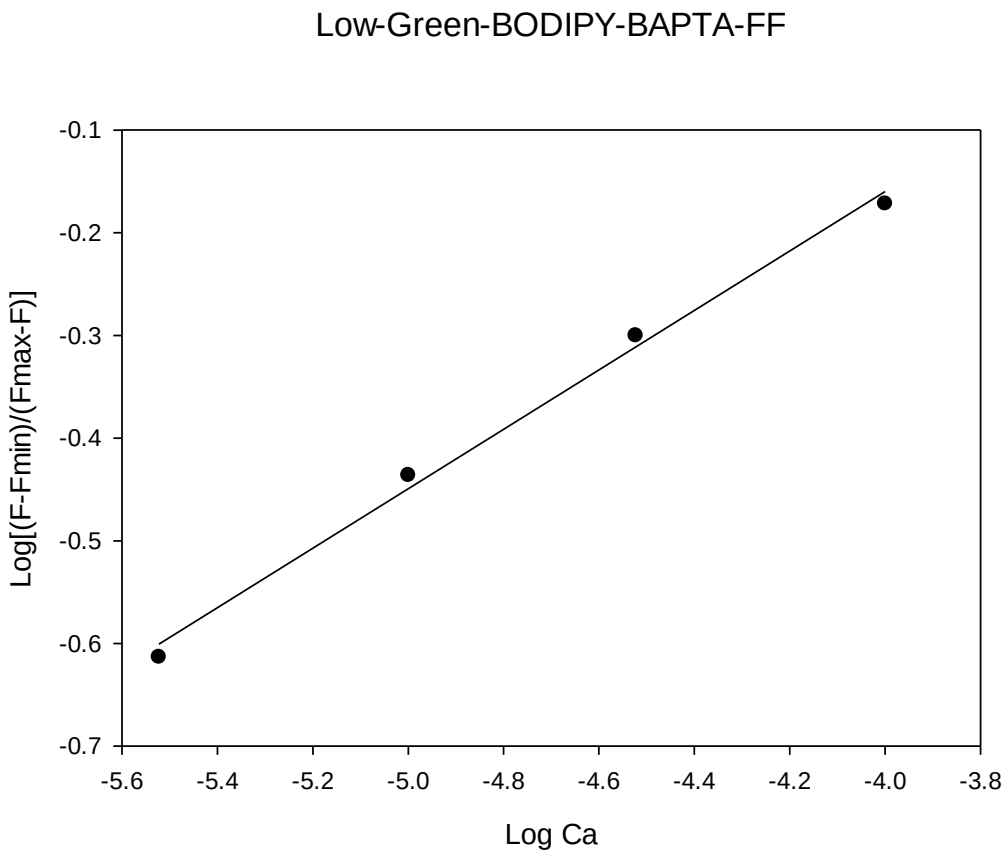


Graph 4-Ca^{2+} fluorescent dose-response of Low-Green-BODIPY-FF-Tetra-Acid 157 (10 µM MOPS buffered miliQ water/$CaSO_4$ and Shimadzu RF-5301PC spectrofluorophotometer

From the preliminary results, it is clear that the Low-Green-BODIPY-BAPTA-FF **157** sensor is functioning, as intended, as a Ca^{2+} PET quenching fluorescent sensor. However, the graph above presents a few issues: 1) the higher free Ca^{2+} doses (namely 300 µM, 1 mM, 3 mM and 4.9 mM) have emission maxima greater than the detection limit of the spectrofluorophotometer and thus the maximal fluorescence of the sensor cannot be determined at these Ca^{2+} concentrations. 2) A lower fluorescence signal is observed for the two highest Ca^{2+} doses which is counter-intuitive and unexpected.

The first issue may be corrected by instrumental calibration with a decreased slit width or a decreased concentration of the sensor in the cuvette as the slit width used was 5 nm for excitation and 5 nm for emission and the concentration of the sensor was 10 µM. The unusual results record for the two highest concentrations could be due to aggregation of the sensors in solution causing non-radiative energetic decay, although more study would be required to confirm this.

A preliminary graphical representation of the linear section of the fluorescent emission data is shown (Graph 5).



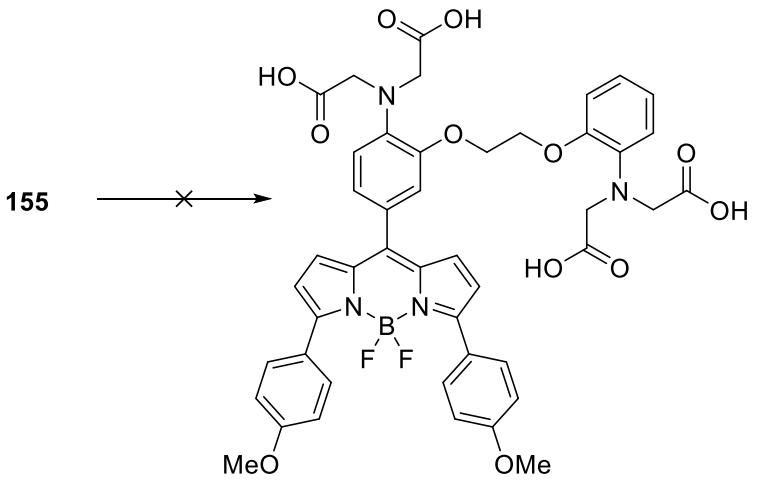
Graph 5- Linear regression of Low-Green-BODIPY-BAPTA-FF 157; log[(F-F_{min})/(F_{max}-F)] *vs*. log[Ca^{2+}]

The x-intercept of the above linear regression of $\log[(F-F_{min})/(F_{max}-F)]$ *vs*. $\log[Ca^{2+}]$ is found to be −4.52, thus the K_d of Hi-Green-BODIPY-BAPTA is estimated to be 30.2 μM.

The reported K_d of Fluo-4FF for Ca^{2+} which, as described in the introduction to PET-fluorescent sensors is the current standard low-affinity Ca^{2+} sensor, is 9.7 μM. The results of this initial experiment have a calculated the K_d of **157** for Ca^{2+} to be within the same order of magnitude as the commercially available standard, Fluo-4FF.

I. Debenzylation by hydrogenolysis of Hi-Red-BODIPY-BAPTA-TBE

Following the success of debenzylation of both **153** and **154**, the same conditions were applied to Hi-Red-BODIPY-BAPTA-TBE **155** (Scheme 70).



Scheme 70- degradation of Hi-Red-BODIPY-BAPTA 155; Conditions: $H_{2(g)}$, $Pd(OH)_2$, MeOH, 2 h

Unfortunately, the reaction resulted in severe degradation to a complex mixture of non-boron containing compounds. It appears that the chosen 2-(4-methoxyphenyl)pyrrole-based red-BODIPY fluorophore is not stable enough for use in the context of a BAPTA-based PET-fluorescent sensor.

7.4 Synthesis of higher coordination number BODIPY red sensors

As is clear from the results discussed above, the BODIPYs synthesised from 2-(4-methoxyphenyl)pyrrole **127,** appear to be relatively unstable when compared to those synthesised from 2,4-dimethylpyrrole. After discovering a recent publication by Nabashima *et al*[114] which details a variety of N_2O_2-tetra-dendate dipyrrin-based fuorophores of aluminium and boron (Figure 54) the synthesis of similar N_2O_2-tetradendate systems was performed.

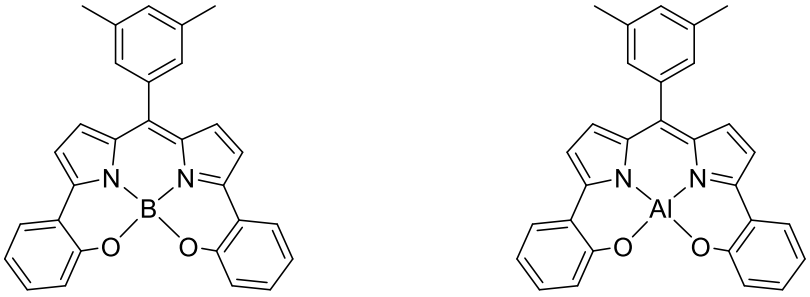
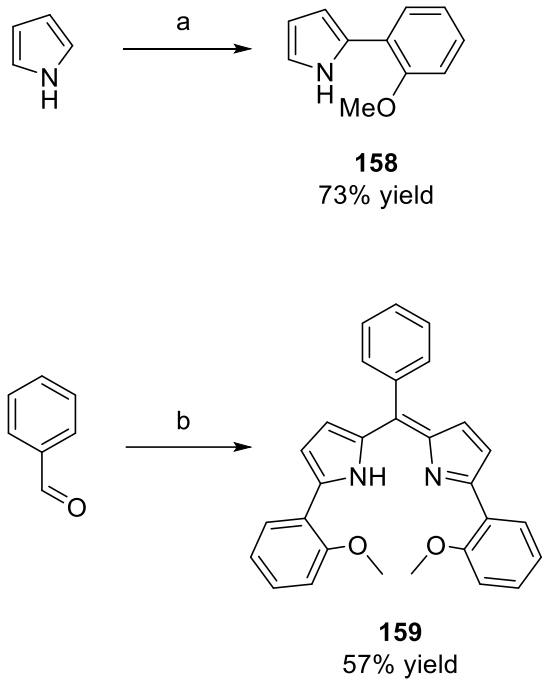


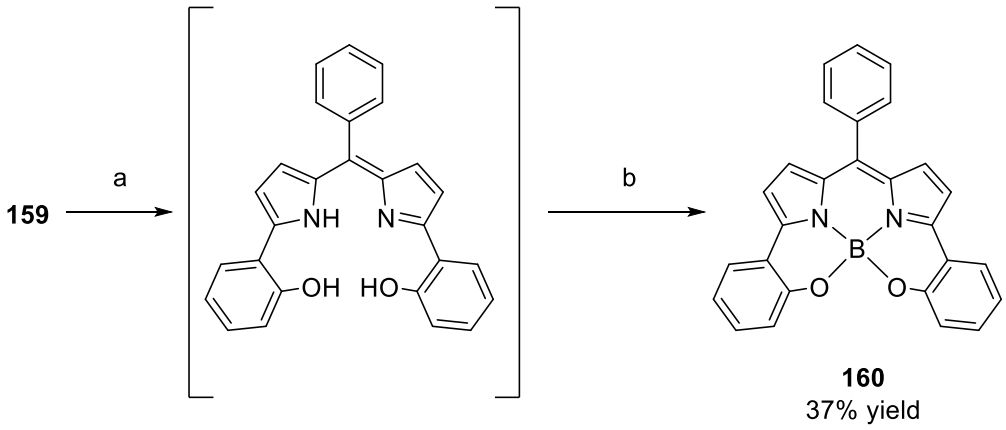
Figure 54- Nabeshima's N_2O_2-tetra-dendate metallodipyrrins

The hypothesis was that the higher coordination of these tetra-dendate dipyrrin-complexes would improve the stability of the fluorophore when compared to the anisyl-BODIPY fluorophore that were utilised in this whilst still providing an emission in the red region of the electromagnetic spectrum Pleasingly, it was found that the conditions reported by Sadighi *et al*[108] afforded 2-(2-methoxyphenyl)pyrrole **158** in a moderate yield; and the dipyrrin **159** was formed *via* the conditions reported by Daub *et al*[110] in a good yield (Scheme 71).



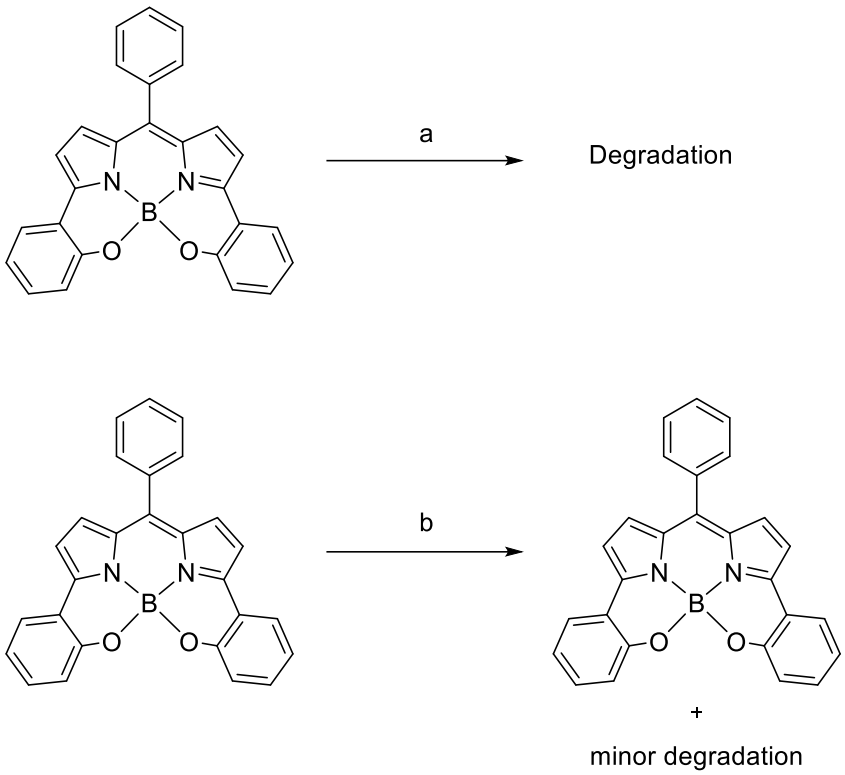
Scheme 71-Synthesis of 2-(2-methoxyphenyl)pyrrole and dipyrrin 159; Conditions: a) i) NaH, THF, 0 °C, 25 min; ii) $ZnBr_2$, 15 min; iii) 2-bromomethoxybenzene, $Pd(OAc)_2$, JohnPhos, THF, 65 °C, 48 h; b) i) 2-(2-methoxyphenyl)pyrrole, TFA, DCM, r.t., 16 h, ii) DDQ, 0 °C, 2 h

N_2O_2-BODIPY **160** was synthesised as reported by Nabashima *et al.*[114] where the purified dipyrrin **159** was demethylated with boron tribromide to reveal the tetra-dentate dipyrrin which undergoes boration with trimethyl borate (Scheme 72).



Scheme 72-One-pot de-methylation boration of 159; Conditions: a) BBr_3, DCM, 0 °C, 3 h; b) $B(OMe)_3$, MeOH, $CHCl_3$, 65 °C, 3 h.

To determine the stability of the N_2O_2-tetra-dentate system toward hydrogenolysis, **160** was subjected to the conditions used to reveal the BAPTA in the previous section as shown in Scheme 73.

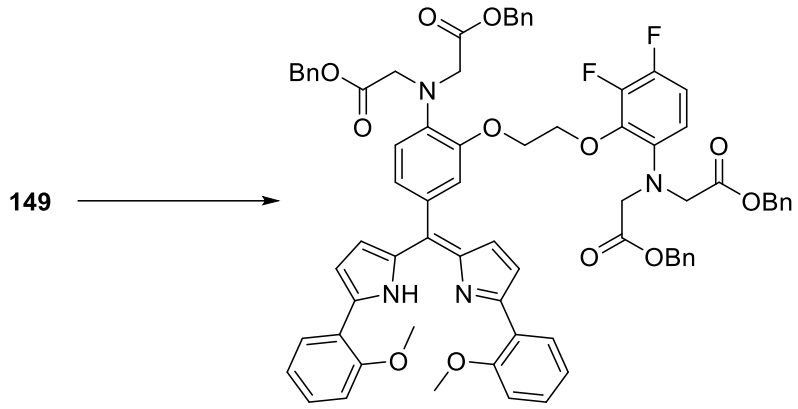


Scheme 73- Stability test of tetra-dentate BODIPY fluorophore 160 to hydrogenolysis; Conditions: a) $H_{2\,(g)}$, Pd/C (5 mol%), MeOH, 6 h; b) $H_{2\,(g)}$, $Pd(OH)_2$ (5 mol%), MeOH, 6 h.

In correlation with previous observations of the majority of the BODIPY compounds described in this research, complete degradation of **160** was observed after 6 hours under hydrogenolysis conditions with 10% palladium on activated carbon as the catalyst. When the experiment was repeated with $Pd(OH)_2$ however, only slight degradation was observed over the same time-scale, this suggests that these tetra-dentate N_2O_2-based dipyrrin systems may be more suitable red-fluorophores for this application.

I. Synthesis of tetra-dentate-BODIPY-BAPTA systems

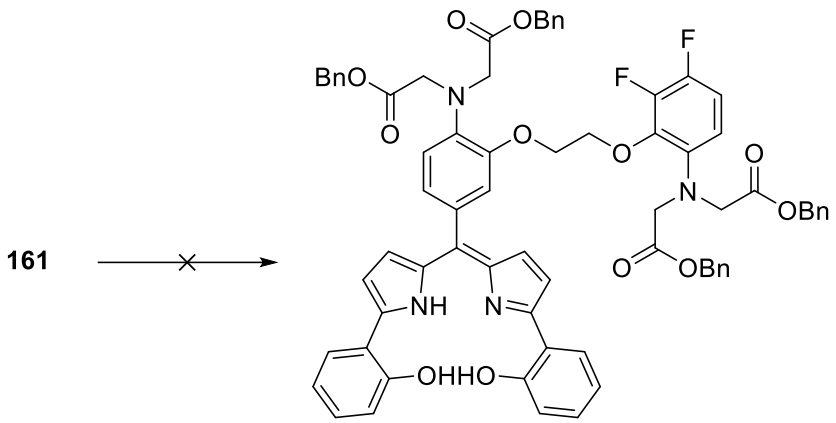
Incorporation of this N_2O_2 tetra-dentate BODIPY fluorophore into a low affinity BAPTA-FF system was achieved *via* Daub *et al's* conditions, [110] affording the dipyrrin **161** in moderate yield (Scheme 74).



161
35% yield

Scheme 74-Synthesis of dipyrrin 161 as a precursor to tetra-dentate Low-Red-BODIPY-BAPTA-FF; Conditions: i) 2-(2-methoxyphenyl)pyrrole (158), TFA, DCM, r.t., 16 h; ii) DDQ, 0 °C, 2 h.

Unfortunately, on application of the reported conditions of Nabashima *et al* [114] to demethylate the phenyl ethers of **161** to reveal the tetra-dentate boron-binding moiety, led only to severe degradation and no isolation of the desired compound (Scheme 75).



Scheme 75- Unsuccessful selective demethylation of 161 leading to severe degradation; Conditions: BBr_3, DCM, 0 °C.

It was difficult to characterise the products of this reaction, as a complex mixture of products was formed. It appeared that the conditions led to debenzylation of the benzyl esters of BAPTA as well as cleavage of the BAPTA-OCH_2CH_2O carbon oxygen bonds.

8 Future work

8.1 Continuation of SAR around indole core

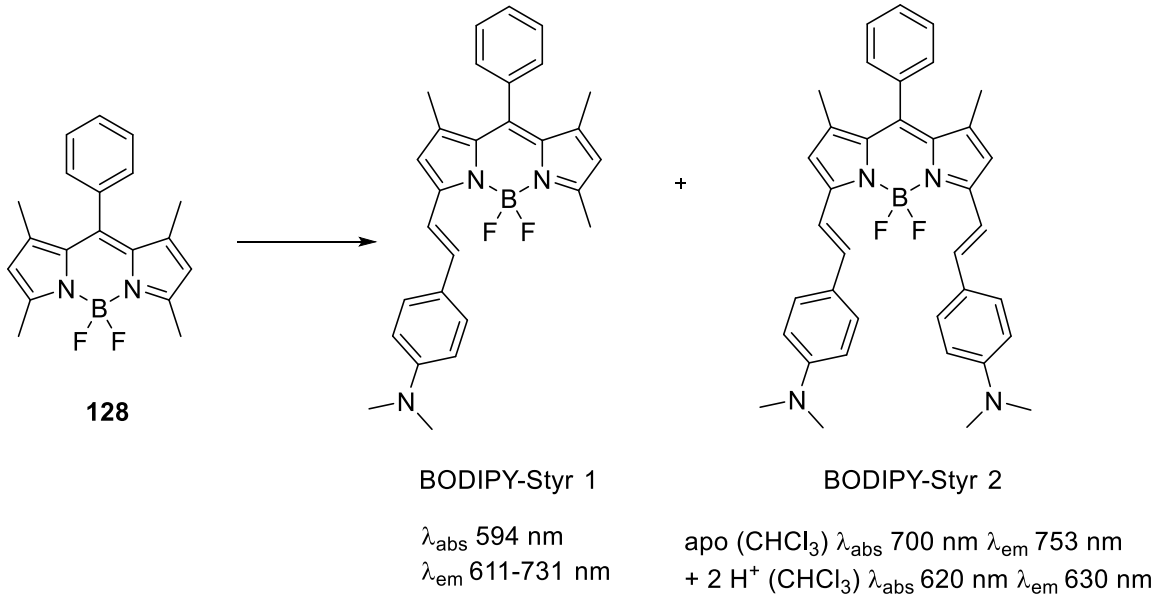
From the NAMs that were identified in this research, **63** and **71** and **86a** (Figure 17), further SAR should be conducted to attempt to increase the affinity for the receptor to nM concentrations as this would be considered potent enough to become a drug lead for development. It would be useful, and indeed interesting, to perform a modified version of the fluorescent assay process with HEK-293 cells which have been transfected with hetero-pentameric 5-HT_3 receptors comprising combinations of the other most widely expressed subunits. In an assay where there is only a single type-A subunit, as it is known that type-A subunits form the orthosteric site; The hypothesis that the identity of the allosteric site being a non-5-HT bound orthosteric domain may be tested as the allosteric agents that have been discussed above would not interact with this receptor. Equally the inverse argument may be proven and allosteric activity may prevail which suggests that the allosteric site is in fact not a non-ligand bound orthosteric site.

8.2 Fluorescent Quipazine derivative FL-Quip

Despite having no observed effect for the intended purpose which FL-Quip was designed, it is reported that 8-azacoumarins possess potent anti-bacterial and anti-microbial properties and therefore FL-Quip may show utility in these applications.

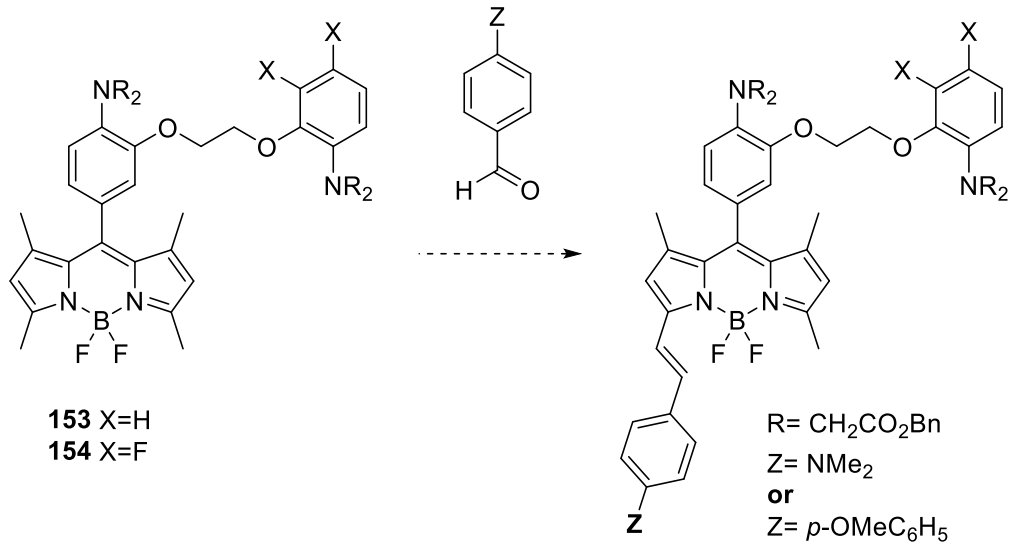
8.3 Synthesis of red BODIPY-BAPTA PET fluorescent sensors

Daub et al.[115] reported the synthesis of styrenyl-BODIPY derivatives that emit in the red to near-IR region of the spectrum via the condensation between **128** and *N,N-4*-dimethylaminobenzaldehyde (Scheme 76).



Scheme 76-*Daub et al's* synthesis of styrenyl BODIPY dyes derived from 128; Conditions: 4-dimethylaminobeznaldehyde, AcOH, piperidine, toluene, 110 °C.

These conditions could be applied directly toward the synthesis of red BODIPY sensors utilising **153** and **154** as starting materials (Scheme 77).



Scheme 77-Synthesis of novel mono-styr-BODIPY-BAPTA-TBE; Conditions: AcOH, piperidine, toluene, 100 °C

If the reactions described in Scheme 77 were to afford the styrenyl-BODIPY fluorophores from **153** and **154** it is possible that these compounds will be robust enough to survive the hydrogenolysis deprotection methods (Scheme 69), however the presence of the styrene moiety may be sensitive to reduction under these conditions. It is possible that the basic dimethylaniline derivative may introduce problems in a cellular context so the *p*-anisyl-derivative may be more suitable.

9 Experimental Chapter

I. General experimental information

^{1}H and ^{13}C NMR data were recorded on a Bruker AVIII 300, Bruker AVIII 400 spectrometer and GHOSY data recorded on DRX500. Spectra were recorded in deuterated-chloroform referenced to residual solvent and reported downfield from TMS for $^{1}H/^{13}C$ and $CFCl_3$ for ^{19}F; coupling constants (*J*) are reported in Hz. The following abbreviations are used to describe multiplicity; s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, br.-broad, ap. apparent. Mass spectra were recorded on a LCT spectrometer utilizing electrospray ionization (recorded in the positive mode) with a methanol mobile phase, or electron impact ionization, HRMS were recorded on a LCT spectrometer using lock-mass incorporated into the mobile phase. IR spectra were recorded neat on Perkin Elmer 100-series FT-IR spectrometer and reported in cm^{-1}. Melting points were determined using open glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. Analytical TLC was carried out on Merck 60 F245 aluminium backed silica gel plates. Short wave or long wave UV-radiation (245/365 nm), vanillin stain or basic $KMnO_4$ stain were used to visualize TLC plates. Compounds were purified by flash column chromatography using Merck silica gel 60 (0.040-0.063 nm). THF, toluene, DCM, MeOH and MeCN were dried by passing through activated alumina columns. Pyridine and triethylamine were distilled from calcium hydride. All other reagents and solvents were purchased from Aldrich, Alfa Aesar, Fisher Scientific or Fluorochem UK and were used without further purification. The following cooling baths were used; 0°C (ice/water) and -78 °C (dry ice/acetone). All reactions in non-aqueous solvents were carried out under argon in glassware, which was flame-dried under high-vacuum.

II. Dose-response curves from Ca^{2+} intracellular assay

×-axis= Log concentration of compound; Y-axis = % of maximal fluorescence observed with just 5-HT.

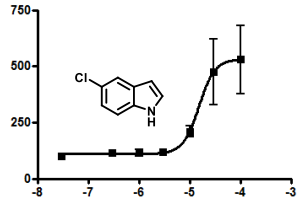


Figure 55-Dose-response curve for 5-Chloroindole, 1e

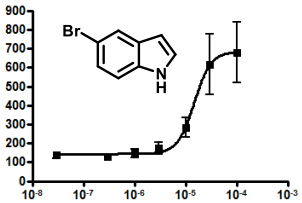


Figure 56-Dose-response curve for 5-bromoindole, 1c

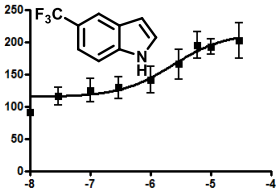


Figure 57-Dose-response curve for 5-(trifluoromethyl)indole, 1a

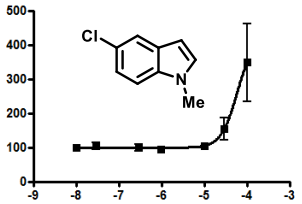


Figure 58-Dose-response curve for 5-chloro-1-methylindole, 2a

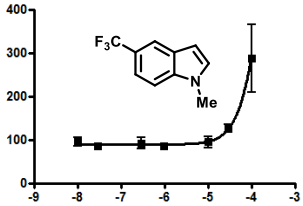


Figure 59-Dose response curve for 5-(trifluoromethyl)-1-methylindole, 2b

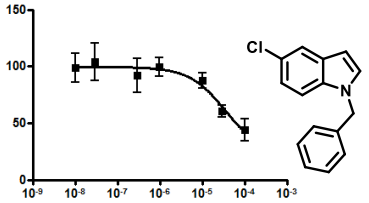


Figure 60- Dose-response curve for 5-chloro-1-benzylindole, 2e

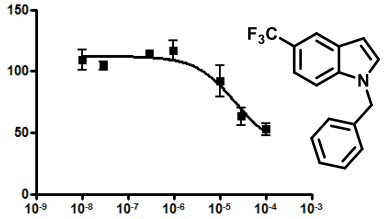


Figure 61-Dose-response curve for 5-(trifluoromethyl)-1-benzylindole. 2d

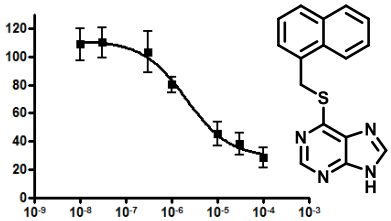


Figure 62- Dose-response curve for PU-02, 2f

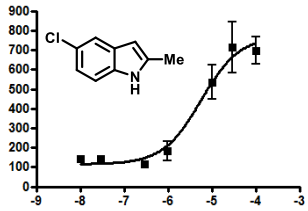


Figure 63-Dose-response curve for 5-chloro-2-methylindole, 3a

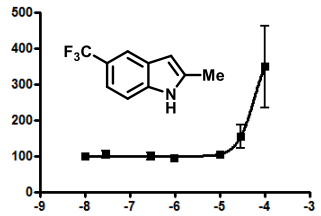


Figure 64-Dose-response curve for 5-(trifluoromethyl)-2-methylindole, 3b

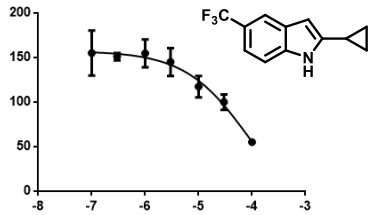


Figure 65-Dose-response curve for 2-cyclopropyl-5-(trifluoromethyl)indole, 3g

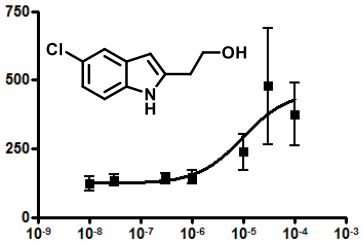


Figure 66-Dose response curve for 2-(5-chloro-1H-indol-2-yl)ethan-1-ol, 3c

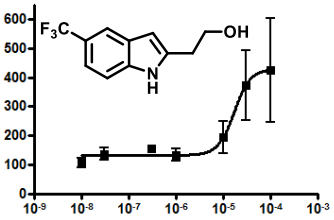


Figure 67- Dose-response curve for 2-(5-(trifluoromethyl)-1H-indol-2-yl)ethan-1-ol, 3d

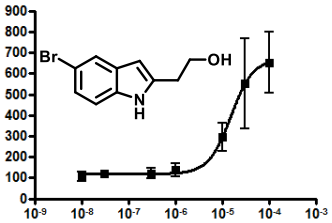


Figure 68-Dose-response curve for 2-(5-bromo-1H-indol-2-yl)ethan-1-ol, 3e

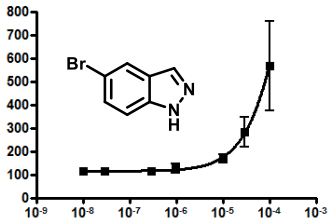


Figure 69- Dose-response curve for 5-bromoindazole, 3j

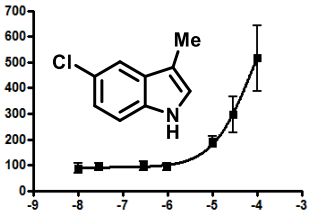


Figure 70-Dose-response curve for 5-chloro-3-methylindole, 4d

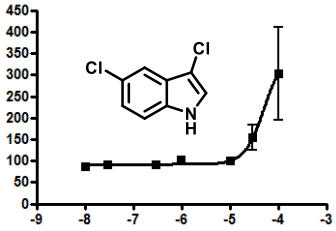


Figure 71-Dose-response curve for 5,3-dichloroindole, 4c

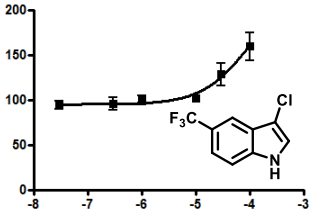


Figure 72-Dose-response curve for 5-(trifluoromethyl)-3-chloroindole, 4e

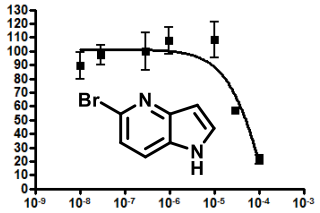


Figure 73-Dose-response curve for 5-Bromo-1H-pyrrolo[3,2-b]pyridine, 5a

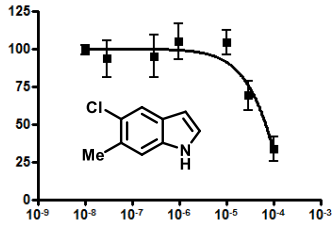


Figure 74- Dose-response curve for 5-chloro-6-methylindole

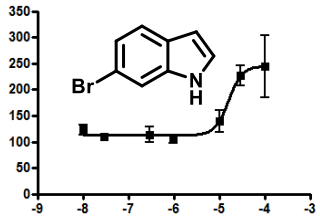


Figure 75-Dose-response curve for 6-bromoindole, 6d

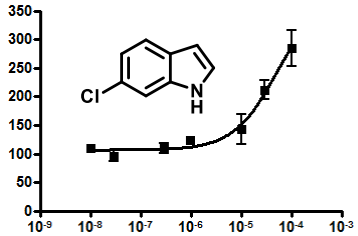


Figure 76-Dose-response curve for 6-chloroindole, 6e

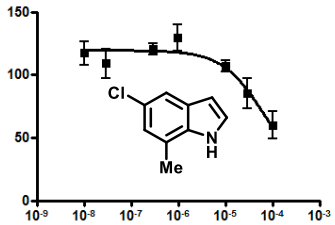


Figure 77-Dose-response curve for 5-chloro-7-methylindole, 7a

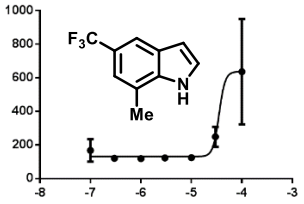


Figure 78-Dose-response curve for 5-(trifluoromethyl)-7-methylindole, 7b

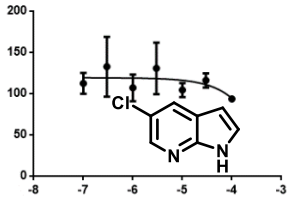


Figure 79-Dose-response curve for 5-chloro-1H-pyrrolo[2,3-b]pyridine, 7e

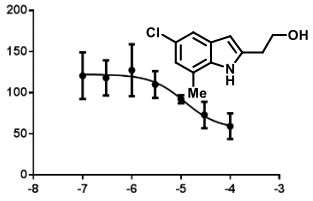


Figure 80-Dose-response curve for 2-(5-chloro-7-methylindo-l2-yl)ethan-1-ol, 8a

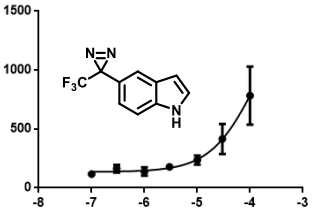


Figure 81-Dose-response curve for Hashimoto's indole, 9a

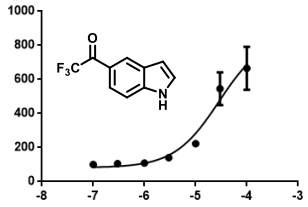


Figure 82-Dose-response curve for 2,2,2-trifluoro-1-(1H-indol-5-yl)ethan-1-one, 9d

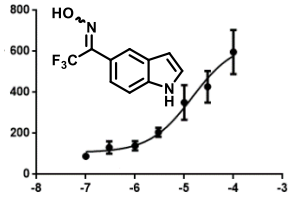


Figure 83-Dose-response curve for 2,2,2-trifluoro-1-(1H-indol-5-yl)ethan-1-one oxime, 9b

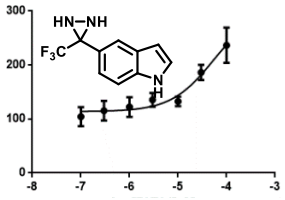


Figure 84-Dose-response curve for 5-(3-(trifluoromethyl)diaziridin-3-yl)-1H-indole, 9c

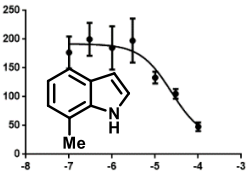


Figure 85-Dose-response curve of 7-methylindole, 10b

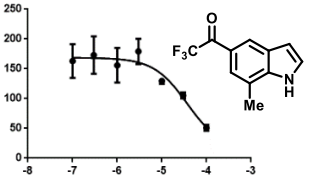


Figure 86-Dose-response curve for 2,2,2-trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one, 10c

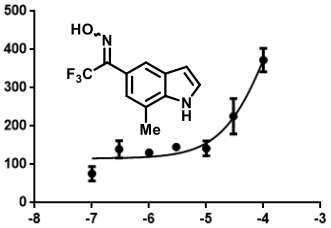


Figure 87-Dose-response curve of 2,2,2-trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one oxime, 10d

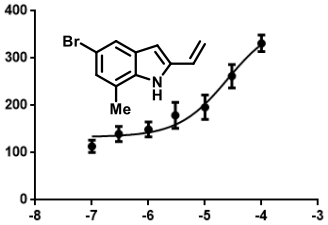
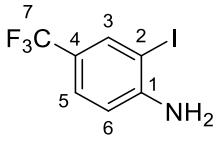


Figure 88-Dose-response curve for5-bromo-7-methyl-2-vinyl-1H-indole, 10f

2-Iodo-4-(trifluoromethyl)aniline, 1



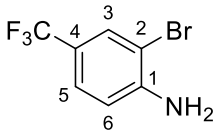
A known compound synthesised according to a literature procedure.[44]

To a stirred suspension of 4-(trifluoromethyl)aniline (0.39 mL, 2.42 mmol) and $CaCO_3$(27 mg, 0.26 mmol) in MeOH (1.1 mL) and DCM (0.4 mL) under an argon atmosphere at room temperature, benzyltrimethylammonium dichloroiodate (96 mg, 0.26 mmol) was added portion-wise over 2 hours whilst the reaction was shielded from light with aluminum foil. The reaction was stirred for a further 4 hours after the final addition and then filtered, concentrated under vacuum and purified by column chromatography (50% DCM in hexane) to afford the product (555 mg, 80% yield) as a brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, *J* = 1.2 Hz, 1H, H3), 7.37 (dd, *J* = 8.4, 1.2 Hz, 1H, H5), 6.74 (d, *J* = 8.4, 1H, H6), 4.41 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 149.7 (C1), 136.4 (q, *J* = 4.0 Hz, C3), 126.7 (q, *J* = 3.0 Hz, C5), 123.7 (q, *J* = 271.0 Hz (C7), 122.3 (q, *J* = 33.0 Hz, C4), 113.7 (C6), 82.1 (C2); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -61.34 (s, CF_3).

Analytical data in agreement with literature values. [44]

2-Bromo-4-(trifluoromethyl)aniline, 2



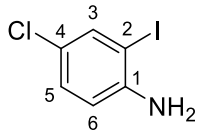
A known compound[116] synthesised *via* an unreported procedure.

To a stirred solution of 4-(trifluoromethyl)aniline (0.4mL, 3.1 mmol) in MeCN (31 mL) under an argon atmosphere cooled to 0 °C; NBS (0.55g, 3.1 mmol) was added in a single portion and the reaction allowed to reach room temperature over 16 hours. Water (40 mL) was added and the reaction mixture was extracted with EtOAc (3 × 30 mL) and the combined organic dried over $MgSO_4$. Solvents were removed under vacuum and purification was achieved by column chromatography (20% EtOAc in hexane) to afford the product (439 mg, 59% yield) as an orange crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, *J* = 1.3 Hz, 1H, H3), 7.39 – 7.29 (m, 1H, H5), 6.76 (d, *J* = 8.4 Hz, 1H, H6), 4.35 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 147.1 (C1), 130.0 (q, *J* = 7.4 Hz, C3), 125.7 (q, *J* = 3.5 Hz, C5), 124.0 (q, *J* = 271.0 Hz, C7), 121.1 (q, *J* = 33.3 Hz, C4), 114.8 (C6), 108.2 (C2); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -61.3; **TOF M/Z (ES+)** 239.2 ($C_7H_6{}^{79}BrF_3N$) 100%, 241.2 ($C_7H_6{}^{81}BrF_3N$) 40%; **M.P**. (From EtOAc) : 28-30 °C.

Analytical data in agreement with literature values. [116]

4-Chloro-2-iodoaniline, 3



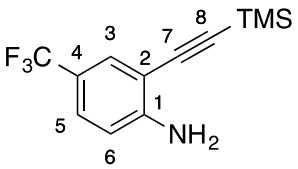
A known compound[117] synthesised *via* an unreported procedure.

To a stirred suspension of 4-chloroaniline (500 mg, 3.92 mmol) and $CaCO_3$(435 mg, 4.31 mmol) in MeOH (6 mL) and DCM (12 mL) under an argon atmosphere at room temperature, benzyltrimethylammonium dichloroiodate (1.36 g, 3.92 mmol) was added and the shielded from light with aluminum foil and the reaction was stirred for 4 hours. The reaction was then filtered, concentrated *in vacuo* and purified by column chromatography (50% DCM in hexane) to afford the product (621 mg, 63% yield). R_f = 0.45 (40% EtOAc in hexane) as an orange solid

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, *J* = 2.4 Hz, 1H, H3), 7.10 (dd, *J* = 8.6, 2.4 Hz, 1H, H5), 6.67 (d, *J* = 8.6 Hz, 1H, H6), 4.07 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.7 (C1), 137.9 (C3), 129.4 (C5), 123.3 (C4), 115.1 (C6), 83.6 (C2); **TOF M/Z (ES+)** 253.9 [^{35}Cl-M+H] 100%, 255.9 [^{37}Cl-M+H] 40%.

Analytical data in agreement with literature values. [117]

4-(Trifluoromethyl)-2-((trimethylsilyl)ethynyl)aniline, 4



A known compound synthesised according to a literature procedure.[44]

Via 2-Iodo-4-(trifluoromethyl)aniline

A solution of 2-iodo-4-(trifluoromethyl)aniline (555 mg, 1.93 mmol) in triethylamine (0.56 mL) was added to a suspension of ethynyltrimethylsilane (0.32 mL, 2.21 mmol), CuI (33.2 mg, 1.93 mmol) and $Pd(PPh)_2Cl_2$ (12 mg, 0.22 mmol) in triethylamine (5 mL) under an argon atmosphere with stirring. The reaction mixture was stirred at room temperature for 20 hours, diluted with water (6 mL) and DCM (6 mL), filtered through Celite; the organic layer was separated and the aqueous extracted into DCM (3 × 5 mL) then the combined organic layers dried over $MgSO_4$ and concentrated under vacuum. Purification was achieved by column chromatography (50% DCM in hexane) to afford the product (240 mg, 68% yield) as a brown oil.

Via 2-bromo-4-(trifluoromethyl)aniline

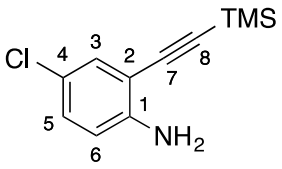
A solution of 2-bromo-4-(trifluoromethyl)aniline (435 mg, 1.82 mmol) in triethylamine (3 mL) was added to a suspension of ethynyltrimethylsilane (0.26 mL, 1.82 mmol), CuI (18 mg, 0.09 mmol) and $Pd(PPh)_2Cl_2$ (64 mg, 0.09 mmol) in triethylamine (5 mL) under an argon atmosphere with stirring. The reaction mixture was stirred at room temperature for 20 hours, diluted with water (6 mL) and DCM (6 mL), filtered through Celite; the organic layer was separated and aqueous extracted into DCM (3 × 5 mL) then the combined organic layers dried over $MgSO_4$ and concentrated under vacuum. Purification was achieved by column chromatography (50% DCM in hexane) to afford the product (95 mg, 20% yield) as a brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 – 7.52 (m, 1H, H3), 7.32 (dd, *J* = 8.6, 2.1 Hz, 1H, H5), 6.71 (d, *J* = 8.6 Hz, 1H, H6), 4.49 (s, 2H, HN_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 150.7 (C1), 129.9 (C3), 126.9 (C5),

125.8 (q, *J* = 33.2 Hz, C4), 124.3 (q, *J* = 271.4 Hz, C9), 113.7 (C6), 107.5 (C2), 101.4 (C7), 100.3 (C8), 0.16 ($SiMe_3$); **^{19}F NMR**(282 MHz, $CDCl_3$) δ -61.5; **TOF M/Z (ES+)** Found 224.0665 ($C_{11}H_{15}{}^{35}ClNSi$) Calc. 224.0662, 204.1 [$M+H^+$+H_2O] 100%, 186.0 [M+H] 20%; **FTIR** (Neat) 3435, 3368, 3327, 2961, 1626, 1588, 1329, 1101.

Analytical data in agreement with literature values. [44]

4-Chloro-2-((trimethylsilyl)ethynyl)aniline, 5



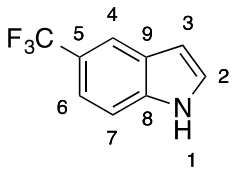
A known compound synthesised according to a literature procedure.[118]

To a stirred suspension of 2-iodo-4-chloroaniline (313 mg, 1.24 mmol), ethynyltrimethylsilane (160 µL, 1.24 mmol), CuI (25 mg, 0.13 mmol) and triethylamine (5 mL) under an argon atmosphere; $Pd(PPh_3)_2Cl_2$ (44 mg, 0.062 mmol) was added in a single portion and the reaction mixture was stirred at room temperature for 16 hours. DCM (10 mL) and water (10 mL) were added to the reaction mixture, which was then filtered through Celite. The organic layer was separated and the aqueous phase extracted into DCM (3 × 10 mL), the combined organic layers were dried over $MgSO_4$ and solvents removed under vacuum, purification was achieved by column chromatography (20% EtOAc in hexane) to afford the product (140 mg, 89% yield) as a brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, *J*=2.5 Hz, 1H, H3), 7.05 (dd, *J* = 8.7, 2.5 Hz, 1H, H5), 6.61 (d, *J* = 8.7 Hz, 1H, H6), 4.22 (s, 2H, NH_2), 0.26 (s, 9H, $SiMe_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 147.0 (C1), 131.6 (C3), 130.0 (C5), 122.2 (C4), 115.4 (C6), 109.3 (C2), 101.2 (C7), 100.5 (C8), 0.2 (C8); **TOF M/Z** (ES+) Found 224.0665 ($C_{11}H_{15}{}^{35}ClNSi$) Calc. 224.0662, 224.1 [^{35}Cl-M+H] 100%, 226.1 [^{37}Cl-M+H] 25%.

Analytical data in agreement with literature values. [118]

5-(Trifluoromethyl)-1H-indole, 6



A known compound synthesised according to a literature procedure.[44]

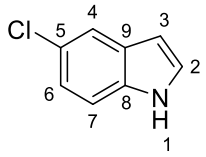
4-(Trifluoromethyl)-2-((trimethylsilyl)ethynyl)aniline (384 mg, 1.49 mmol) $CaCO_3$ (150 mg, 1.49 mmol) and CuI (142 mg, 0.75 mmol) were suspended in DMF (8 mL) under an argon atmosphere and heated to 120 °C with stirring for 2 hours. The reaction was cooled to room temperature and diluted with brine (15 mL) and extracted with Et_2O (3 × 15 mL). The combined organics were dried over $MgSO_4$ and solvents removed under vacuum. Purification was achieved by column chromatography (50% DCM in Hexane) to afford the product (215 mg, 78% yield) as a brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H, NH), 8.00 (s, 1H, H4), 7.52 – 7.40 (m, 2H, H, H6 & H7), 7.33 – 7.27 (m, 1H, H2), 6.73 – 6.59 (m, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.2 (C8), 127.3 (C9),

125.9 (C2), 125.4 (q, *J* = 271 Hz, C7), 122.3 (q, *J* = 31.6 Hz, C5), 118.8 (q, *J* = 3.1 Hz,C4), 118.5 (q, *J* = 4.0 Hz, C6) 111.4 (C7), 103.6 (C3); **^{19}F NMR** (282 MHz, $CDCl_3$) δ −60.04 (s, CF_3); **TOF M/Z (ASAP+)** 185.0 ($C_9H_6F_3N$) 100%.

Analytical data in agreement with literature values. [44]

5-Chloroindole, 7



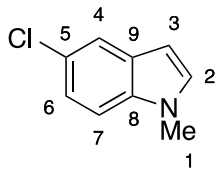
A known compound synthesised according to a literature procedure.[119]

4-Chloro-2-((trimethylsilyl)ethynyl)aniline (200 mg, 0.89 mmol) $CaCO_3$ (89 mg, 0.89 mmol) and CuI (86 mg, 0.45 mmol) were suspended in DMF (5 mL) under an argon atmosphere and heated to 120 °C with stirring for 2 hours. The reaction was cooled to room temperature and diluted with brine (15 mL) and extracted with Et_2O (3 × 15 mL). The combined organics were dried over $MgSO_4$ and solvents removed under vacuum. Purification was achieved by column chromatography (50% DCM in Hexane) to afford the product (108 mg, 80% yield) as a brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (s, 1H, H1), 7.61 (d, *J* = 2.0 Hz, 1H, H4), 7.32 (d, *J* = 8.6 Hz, 1H, H7), 7.25 – 7.23 (m, 1H, H2), 7.15 (dd, *J* = 8.6, 2.0 Hz, 1H, H6), 6.53 – 6.48 (m, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 133.4 (C8), 129.5 (C9), 126.2 (C5), 125.8 (C6), 124.4 (C2), 119.6 (C4), 104.8 (C7), 103.8 (C3).

Analytical data in agreement with literature values. [119]

5-Chloro-1-methyl-1*H*-indole, 8



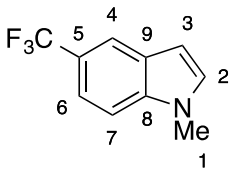
A known compound prepared according to the literature[45]

5-Chloro-1*H*-indole (50 mg, 0.33 mmol) was dissolved in THF (3.5 mL) and cooled to 0 °C under an argon atmosphere. NaH (20 mg, 0.51 mmol, 60% mineral oil dispersion,) was added in a single portion and the reaction was warmed to room temperature over 1 hour. The reaction was re-cooled to 0 °C and iodomethane (27 µL, 0.44 mmol) was added dropwise via syringe over 15 minutes. The reaction was stirred for 16 hours and quenched with saturated ammonium chloride solution and extracted with diethyl ether (3 × 5 mL). The combined organic layers were washed with brine (5mL) and dried over $MgSO_4$ and solvents were removed *in vacuo,* purification was achieved by column chromatography (20% EtOAc in Hexane) to afford the product (49 mg, 87% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, *J* = 2.0 Hz, 1H, H4), 7.24 (d, *J* = 8.7 Hz, 1H, H7), 7.16 (dd, *J* = 8.7, 2.0 Hz, 1H, H6), 7.07 (d, *J* = 3.1 Hz, 1H, H2), 6.42 (d, *J* = 3.1 Hz, 1H, H3), 3.78 (s, 3H, 1-Me); **TOF M/Z (EI+)** Found 165.0342 ($C_9H_8{}^{35}ClN$) Calc. 165.0345, [^{35}Cl-M+H] 100%, 167.0 [^{37}Cl-M+H] 25%.

Analytical data in agreement with literature values. [45]

1-Methyl-5-(trifluoromethyl)-1*H*-indole, 9



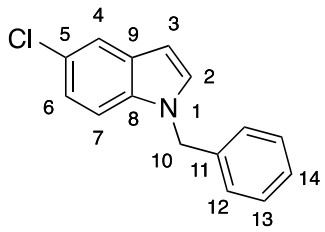
A known compound prepared according to the literature[120]

5-(Trifluoromethyl)-1*H*-indole (50mg, 0.27 mmol) was dissolved in THF (3 mL) and cooled to 0 °C under an argon atmosphere. NaH (60% mineral oil dispersion, 16.5 mg, 0.41 mmol) was added in a single portion and the reaction allowed to warm to room temperature over 1 hour. The reaction was re-cooled to 0 °C and iodomethane (22 µL, 0.36 mmol) was added dropwise via syringe over 15 minutes. The reaction was stirred for 16 hours and quenched with ammonium chloride (sat. 5 mL) and extracted with diethyl ether (3 × 5 mL). The combined organics were washed with brine (5 mL) and dried over $MgSO_4$ and solvents were removed *in vacuo.* Purification was achieved by column chromatography (20% EtOAc in Hexane) to afford the product (51 mg, 94% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 – 7.91 (m, 1H, H4), 7.44 (dd, *J* = 8.9, 1.6 Hz, 1H, H6), 7.40 (d, 8.9 Hz, 1H, H7), 7.16 (d, *J* = 3.1 Hz, 1H, 2H), 6.58 (dd, *J* = 3.1, 0.6 Hz, 1H, H3), 3.84 (s, 3H, 1-Me); **TOF M/Z (EI+)** Found 199.0608 ($C_{10}H_8F_3N$) Calc. 199.0609, 199.01 [M+H] 100%, 200.1 [^{13}C-M+H] 10%.

Analytical data in agreement with literature values. [120]

1-Benzyl-5-chloro-*1H*-indole, 10



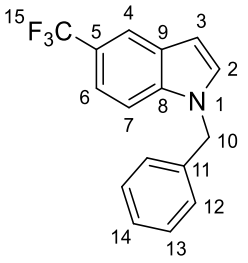
A known compound synthesised according to a literature procedure.[46]

To a stirred solution of 5-chloro-1*H*-indole (50 mg, 0.33 mmol) in DMF (3.3 mL) under an argon atmosphere; NaH (60% oil dispersion, 21 mg, 0.53 mmol) was added as a single portion and the reaction mixture was stirred at 0 °C for 1 hour. Benzyl bromide (47 µL, 0.40 mmol) was added dropwise over 15 minutes and the reaction mixture was allowed to warm to room temperature over 16 hours. Brine (5 mL) was added and the reaction mixture was extracted with Et_2O (3 × 5 mL), combined organics dried over $MgSO_4$ and solvents removed under vacuum. Purification was achieved by column chromatography (25% DCM in Hexane) to afford the product (68 mg, 85% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, *J* = 1.5 Hz, 1H, H4), 7.40 – 7.28 (m, 3H), 7.23 – 7.05 (m, 5H), 6.52 (dd, *J* = 3.2, 0.7 Hz, 1H, H3), 5.31 (s, 2H, H10; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.0 (C11), 130.1,(C8), 129.1 (C9), 128.2 (C2), 128.0 (C13), 126.9 (C5), 125.2 (C14), 118.9 (C12), 118.6 (C7), 110.1(C6), 102.9 (C3), 50.5 (C10); **TOF M/Z (AP+)** 242.0784 (^{35}Cl-M+H) 100%, 244.1 (^{37}Cl-M+H) 25%; **FTIR** (Neat) 3033, 2925, 1623, 1496, 1326, 1107, 714.

Analytical data in agreement with literature values. [46]

1-Benzyl-5-(trifluoromethyl)-1*H*-indole, 11



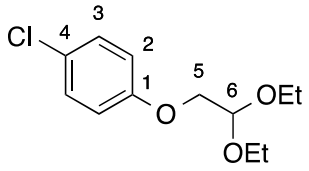
A known compound synthesised according to a literature procedure.[121]

To a stirred solution of 5-(trifluoromethyl)-1*H*-indole (50 mg, 0.27 mmol) in DMF (2.7 mL) under an argon atmosphere; sodium hydride (60% oil dispersion, 17.3 mg, 0.43 mmol) was added as a single portion and the reaction mixture was stirred at 0 °C for 1 hour. Benzyl bromide (39 µL, 0.32 mmol) was added dropwise over 15 minutes and the reaction mixture was allowed to warm to room temperature over 16 hours. Brine (5 mL) was added and the reaction mixture was extracted with Et_2O (3 × 5 mL), the combined organic layers dried over $MgSO_4$ and solvent removed under vacuum. Purification was achieved by column chromatography (25% DCM in Hexane) to afford the title compound (69 mg, 93% yield) as a white solid.

^{1}H NMR) (300 MHz, $CDCl_3$) δ 7.89 (s, 1H, H4), 7.42 – 7.27 (m, 5H, H6, H7, H12 & H14)), 7.24 (d, *J* = 3.2 Hz, 1H, H2), 7.09 (dd, *J* = 7.6, 1.8 Hz, 2H, H13), 6.53 (d, *J* = 3.2 Hz, 1H, H3) 5.36 (s, 2H, H10); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.2 (C11), 134.8 (C8), 129.9 (C9), 129.0 (C2), 128.9 (C13), 128.05 (q, *J* = 276.9 Hz, C15) 127.9 (C5), 126.8 (C14), 125.4 (C12), 122.1 (q, J=33.1 Hz, C5), 120.5 (q, J = 4.5 Hz, C6), 120.0 (q, *J* = 3.4 Hz, C4) 110.9 (C7), 101.5 (C3), 50.4 (C10); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -60.1; **TOF M/Z (AP+)** 276.1 [M+H] 100%, 277.1 [^{13}C-M+H] 15%, 292.1 [M+Na] 5%; **FTIR** (Neat) 3109, 3061, 3030, 2926, 2860, 1743, 1710, 1507, 1495, 722.

Analytical data in agreement with literature values. [121]

4-Chloro-1-(2,2-diethoxyethoxy)benzene, 12



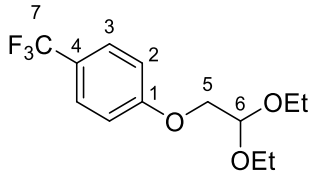
A known compound synthesised according to a literature procedure.[47]

4-Chlorophenol (500 mg, 3.89 mmol) and caesium carbonate (1.78 g, 5.45 mmol) were suspended in DMF (10 mL) with stirring under an argon atmosphere. 2-Bromoacetaldehyde diethylacetal (0.67 mL, 4.47 mmol) was added in a single portion and the reaction was heated at 60 °C for 48 hours. The reaction mixture was cooled to room temperature and diluted with (25 mL) and extracted with Et_2O (3 × 25 mL) the combined organic layers were dried over $MgSO_4$ and solvents removed *in vacuo*. Purification was achieved by column chromatography (10% EtOAc in Hexane) to afford the product (827 mg, 87% yield) as a clear-yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (d, *J* = 9.0 Hz, 2H, H3), 6.85 (d, *J* = 9.0 Hz, 2H, H2), 4.82 (t, *J* = 5.2 Hz, 1H, H6), 3.97 (d, *J* = 5.2 Hz, 2H, H5), 3.82 – 3.69 (m, 2H, OC**H$_2$**CH_3), 3.69 – 3.54 (m, 2H, OC**H$_2$**CH_3'), 1.24 (t, *J* = 7.1 Hz, 6H, 2 × OCH_2**CH_3**); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 157.4 (C1), 129.4 (C3), 125.9 (C4), 116.1 (C2), 100.6 (C6), 69.0 (C5), 62.8 (O**CH$_2$**CH_3), 62.6 (O**CH$_2$**CH_3'), 15.5 (OCH_2**CH_3**).

Analytical data in agreement with literature values. [47]

1-(2,2-diethoxyethoxy)-4-(trifluoromethyl)benzene, 13



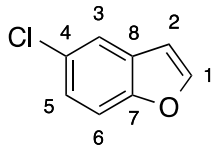
A known compound synthesised according to a literature procedure.[47]

4-(Trifluoromethyl)phenol (500 mg, 3.89 mmol) and cesium carbonate (1.78 g, 5.45 mmol) were suspended in DMF (10 mL) with stirring under an argon atmosphere. 2-Bromoacetaldehydediethylacetal (0.67 mL, 4.47 mmol) was added in a single portion and the reaction was heated at 60 °C for 48 hours. The reaction mixture was cooled to room temperature and diluted with brine (25 mL) then extracted with Et_2O (3 × 25 mL), the combined organics were dried over $MgSO_4$ and solvents were removed under vacuum. Purification was achieved by column chromatography (10% EtOAc in Hexane) to afford the product (790 mg, 92% yield) as a clear yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.5 Hz, 2H, H3), 6.98 (d, *J* = 8.5 Hz, 2H, H2), 4.84 (t, *J* = 5.2 Hz, 1H, H6), 4.04 (d, *J* = 5.2 Hz, 2H, H5), 3.84 – 3.71 (m, 2H, O**CH_2**CH_3), 3.70 – 3.57 (m, 2H, O**CH_2**CH_3'), 1.25 (t, *J* = 7.1 Hz, 6H, 2 × OCH_2**CH_3**); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 161.2 (C1), 127.0 (q, *J* = 3.8 Hz, C3), 124.7 (q, *J* = 239.3 Hz, C7), 123.2 (q, *J* = 30.2 Hz, C4), 114.8 (C2), 101.6 (C6), 68.7 (C5), 62.9 (O**CH_2**CH_3), 63.0 (O**CH_2**CH_3'), 15.5 (OCH_2**CH_3**).

Analytical data in agreement with literature values. [47]

5-Chlorobenzofuran, 14



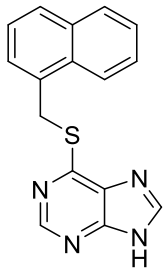
A known compound synthesised according to a literature procedure.[47]

2-(4-Chlorophenoxy)acetaldehyde dimethylacetal (900 mg, 3.68 mmol) and polyphosphoric acid (0.5 mL, 10.5 mmol) were dissolved in toluene (5 mL) with stirring under an argon atmosphere and heated at 90°C for 16 hours. The reaction was then cooled to room temperature and diluted with water (25 mL), the organic layer was extracted with EtOAc (3 × 25 mL), the combined organics were dried over $MgSO_4$ and solvents removed under vacuum. Purification was achieved by column chromatography (100% hexane), to afford the product (236 mg, 42% yield) as a clear yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (d, *J* = 2.2 Hz, 1H, H1), 7.57 (d, *J* = 2.1 Hz, 1H, H6), 7.43 (d, *J* = 8.7 Hz, 1H, H3), 7.26 (dd, *J* = 8.7, 2.1 Hz, 1H, H5), 6.73 (dd, *J* = 2.2 Hz, 1H, H2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 153.5 (C7), 146.4 (C1), 128.9 (C5), 128.5 (C3), 124.3 (C6), 120.9 (C4), 112.5 (C7), 106.4 (C2); **TOF M/Z (EI+)** Found 152.0028 ($C_8H_5{}^{35}ClO$) Calc. 152.0029, 152.0 [^{35}Cl-M+H] 100%, 154.0 [^{37}Cl-M+H] 20%.

Analytical data in agreement with literature values. [47]

6-((naphthalen-1-ylmethyl)thio)-9H-purine (PU-02), 15

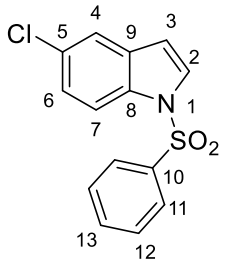


A Known yet unreported compound.

To a stirred suspension of 9H-purine-6-thiol (100 mg, 0.59 mmol) and 1-(chloromethyl)naphthalene (124 mg, 0.71 mmol) in NMP (6 mL) K_2CO_3 (98 mg, 0.71 mmol) was added as a single portion and the reaction stirred for 16 hours. The reaction was poured into water (50 mL) then filtered and the filtrate washed with water (2 × 10 mL) and the filtrand was dried *via* co-evaporation with acetone to afford the title compound (169 mg, 99% yield) as a white crystalline solid.

^{1}H NMR (400 MHz, DMSO) δ 12.86 (s, 1H), 8.81 (s, 1H), 8.44 (s, 1H), 8.19 (d, *J* = 8.1 Hz, 1H), 7.99 – 7.95 (m, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.72 (d, *J* = 6.9 Hz, 1H), 7.63 – 7.51 (m, 1H), 7.50 – 7.42 (m, 1H), 5.16 (s, 2H); **^{13}C NMR** (101 MHz, DMSO) δ 151.5, 133.5, 133.0, 131.1, 128.7, 128.2, 127.7, 126.4, 126.0, 125.5, 123.8, 108.9; **TOF M/Z (ES+)** Found 293.0856 ($C_{16}H_{13}N_4S$) Calc. 293.0861, 293.1 [M+H] 100%, 294.1 [^{13}C-M+H] 30%; **M.P.** (From water) 196-198 °C.

5-Chloro-1-(benzenesulfonyl)-1*H*-indole, 16



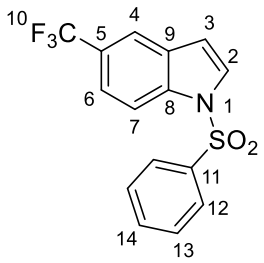
A known compound prepared according to a literature procedure.[48,122]

To a stirred solution of 5-chloro-1*H*-indole (100 mg, 0.66 mmol) in THF (1.5 mL) cooled to 0 °C under an argon atmosphere; NaH (60% oil dispersion, 35 mg, 0.86 mmol) was added as a single portion and the suspension was stirred for 45 minutes. Benzenesulfonyl chloride (110 µL, 0.86 mmol) was added dropwise over 20 minutes and stirred for 16 hours and warmed to room temperature. Ammonium chloride (3 mL, Sat. Aq.) was added to the reaction mixture and the liquor extracted with EtOAc (3 × 5 mL), the combined organic layers were washed with brine then dried over $MgSO_4$ and then concentrated *in vacuo.* Purification was achieved by column chromatography (25% DCM in hexane), to afford the product (204 mg, 89% yield) R_f = 0.4 (20% EtOAc in hexane) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 – 8.02 (m, 1H,), 7.97 – 7.82 (m, 3H), 7.69 – 7.40 (m, 4H), 7.27 (d, *J* = 3.0 Hz, 1H, H2), 6.61 (dd, *J* = 3.0, 0.7 Hz, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.1 (C10), 134.2 (C13), 132.1 (C8), 129.8 (C12), 129.5 (C9), 127.8 (C11), 127.1 (C5), 126.9 (C6), 125.1 (C4), 121.2 (C7), 114.7 (C3), 108.8 (C2); **TOF M/Z (AP+)** 292.0 ($C_{14}H_{11}{}^{35}ClNO_2S$) 100%, 294.0 ($C_{14}H_{11}{}^{37}ClNO_2S$) 25%; **FTIR** (Neat) 3143, 3115, 3069, 2978, 2901, 1440, 1370, 1170, 1142.

Analytical data in agreement with literature values. [122]

5-(Trifluoromethyl)-1-(phenylsulfonyl)-1*H*-indole, 17



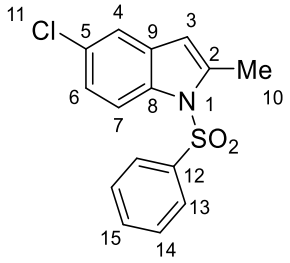
A novel compound synthesised according to a literature procedure for a closely related compound.[123]

To a stirred solution of 5-(trifluoromethyl)-1*H*-indole (100 mg, 0.54 mmol) in THF (1.2 mL) cooled to 0 °C under an argon atmosphere; NaH (60% oil dispersion, 28 mg, 0.70 mmol) was added as a single portion and the suspension was stirred for 45 minutes. Benzenesulfonyl chloride (90 µL, 0.7 mmol was added dropwise over 20 minutes and stirred for 16 hours and warmed to room temperature. Ammonium chloride (sat. aq. 3 mL) was added to the reaction mixture and the liquor extracted with EtOAc (3 × 5 mL), the combined organics were washed with brine and dried over $MgSO_4$. The solvent was removed under vacuum, purification was achieved by column chromatography (25% DCM in hexane), to afford the title compound (180 mg, 73% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 – 8.00 (m, 2H, H12), 7.97 – 7.42 (m, 7H), 6.75 (dd, *J* = 3.7, 0.7 Hz, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.1 (C8), 135.4 (C11), 134.4 (C14), 130.6 (C13), 129.8 (C12), 129.6 (C9), 128.1 (C2), 127 .2 (q, *J* = 4.3 Hz, C4), 125.9 (q, *J* = 34.7 Hz, C5), 124.4 (q, *J* = 259.6

Hz, C10), 121.6 (q, *J* = 3.4 Hz,C6), 119.1 (C7), 113.9 (C2), 109.3 (C3); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -61.19 (s); **TOF M/Z (ES+)** 277.2 ($C_9H_6F_3NO_2SNa$) 100%, 208.0 ($C_9H_6F_3NNa$) 40%.

5-Chloro-2-methyl-1-(benzenesulfonyl)-1*H*-indole, 18



A known compound synthesised according to a literature procedure.[48]

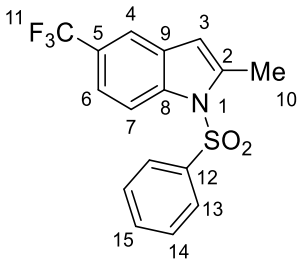
To a stirred solution of 5-chloro-1-(benzenesulfonyl)-1*H*-indole (172 mg, 0.59) in THF (2.5 mL) cooled to -78 °C under an argon atmosphere; LDA (1.51 M, 507 µL, 0.77 mmol) was added dropwise over 20 minutes. The reaction mixture was stirred for 2 hours followed by a dropwise addition of iodomethane (40 µL, 0.65 mmol) over 15 minutes. The reaction mixture was allowed to warm to room temperature over 16 hours, at which time, water (5 mL) was added. The reaction liquor was acidified to pH 6 with HCl (1M) and extracted with EtOAc (3 × 5 mL), the combined organics were dried over $MgSO_4$ and solvents removed under vacuum. Purification was achieved by column chromatography (25% EtOAc in Hexane), to afford the product (115 mg, 64% yield) R_f = 0.45 (20% EtOAc in hexane) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.6 Hz, 1H, H7), 7.76 (dd, *J* = 8.6, 1.3 Hz, 2H, H13), 7.61 – 7.36 (m, 4H, H14, H4 and H6), 7.22 (dd, *J* = 8.9, 2.1 Hz, 1H, H15), 6.33 – 6.22 (m, 1H, H3), 2.59 (s, 3H, H10); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.9 (C12), 134.2 (C8), 134.0 (C2), 132.1 (C15), 131.0

(C14), 129.5 (C9), 126.8 (C5), 126.3 (C13), 124.0 (C6), 119.7 (C4), 115.6 (C7), 109.1 (C3), 15.8 (C10**); TOF M/Z (ES+)** 299.9 ($C_9H_7{}^{35}ClNO_3S$ + Na) 100%, 445.1 ($C_{15}H_{12}{}^{35}ClNO_2S$+ H_2O+ Na) 60%, 294.0 ($C_{15}H_{12}{}^{35}Cl\ NO_2S$+ H_2O+ Na) 15%; **FTIR** (Neat) 3068, 2928, 1590, 1443, 1370, 1176, 732.

Analytical data in agreement with literature values. [48]

5-(Trifluoromethyl)-2-methyl-1-(benzenesulfonyl)-1*H*-indole, 19

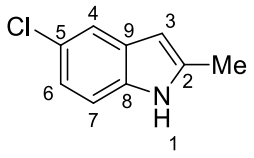


A novel compound.

To a stirred solution of 5-(trifluoromethyl)-1-(benzenesulfonyl)-1*H*-indole (128 mg, 0.39) in THF (2 mL) cooled to -78 °C under an argon atmosphere; LDA (1.51 M, 406 µL, 0.51 mmol) was added dropwise over 20 minutes. The reaction mixture was stirred for 2 hours followed by a dropwise addition of iodomethane (27 µL, 0.43 mmol) over 15 minutes. The reaction mixture was allowed to warm to room temperature over 16 hours, at which time, water (5 mL) was added. The reaction liquor was acidified to pH 6 with HCl (1M) and extracted with EtOAc (3 × 5 mL), the combined organic layers were dried over $MgSO_4$ and then concentrated *in vacuo*. Purification was achieved by column chromatography (25% EtOAc in hexane) to afford the product (78 mg, 58% yield) R_f = 0.65 (25% EtOAc in hexane) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, *J* = 8.4 Hz, 1H, H7), 7.79 (dd, *J* = 8.4, 1.0 Hz, 2H, H13), 7.71 – 7.67 (m, 1H, H4), 7.63 – 7.40 (m, 4H, H14, H15 and H6), 6.42 (s, 1H, H3), 2.62 (s, 3H, H10); **^{13}C NMR (**101 MHz, $CDCl_3$) δ 139.4 (C8), 139.1 (C12), 134.2 (C2), 129.6 (C15), 129.5 (C14), 128.1 (C9), 127.6 (d, *J* = 248.5 Hz, C11), 126.9 (C13), 126.1, 125.9 (q, *J* = 32.3 Hz, C5), 120.7 (q, *J* = 3.3 Hz, C4), 117.6 (q, *J* = 3.9 Hz, C6) 114.7 (q, *J* = 3.4 Hz, C7), 109.5 (C3), 15.8 (C10); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -61.2; **M/Z (AP+)** 340.1 [M+H] 100%, 341.1 [^{13}C-M+H] 10%.

5-Chloro-2-methyl-1*H*-indole, 20



A known compound synthesised according to a literature procedure.[48]

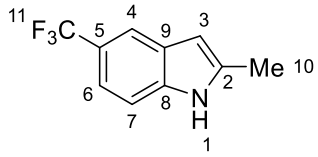
To a stirred solution of 5-chloro-2-methyl-1-(benzenesulfonyl)-1*H*-indole (112 mg, 0.37 mmol) in methanol (7 mL) K_2CO_3 (152 mg, 1.1 mmol) was added in a single portion and the reaction mixture was heated at reflux for 16 hours. The reaction mixture was cooled to room temperature, water (10 mL) was added and the reaction liquor extracted with DCM (3 × 10 mL). The combined organics were washed with brine then dried over $MgSO_4$ and solvents were removed under vacuum. Purification was achieved by column chromatography (100% toluene) to afford the product (28 mg, 46% yield) R_f = 0.5 (10% Acetone in toluene) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (s, 1H, NH), 7.48 (d, *J* = 2.0 Hz, 1H, H4), 7.17 (d, *J* = 8.6 Hz, 1H, H7), 7.06 (dd, *J* = 8.6, 2.0 Hz, 1H, H6), 6.22 – 6.13 (m, 1H, H3), 2.43 (d, *J* = 0.8 Hz, 3H, 2-Me); **^{13}C**

NMR (101 MHz, $CDCl_3$) δ 154.1 (C2), 149.3 (C8), 136.7 (C9), 136.2 (C5), 135.0 (C4), 131.9 (C6), 128.7 (C7), 118.4 (C3), 52.8 (Me); **TOF M/Z (ES+)** Found 166.0430 ($C_9H_9{}^{35}ClN$) Calc. 166.0424, 166.0 [^{35}Cl-M+H] 95%, 168.0 [^{37}Cl-M+H] 25%.

Analytical data in agreement with literature values. [48]

5-(Trifluoromethyl)-2-methyl-1*H*-indole, 21



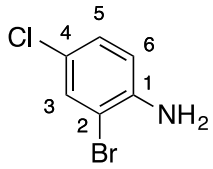
A compound that is identified in the literature yet is not characterized.[124,125]

To a stirred solution of 5-(trifluoromethyl)-2-ethyl-1-(phenylsulfonyl)-1*H*-indole (76 mg, 0.23 mmol) in methanol (5 mL) K_2CO_3 (95 mg, 0.69 mmol) was added in a single portion and the reaction mixture was heated at reflux for 16 hours. The reaction mixture was cooled to room temperature, water (10 mL) was added and the reaction liquor extracted with DCM (3 × 10 mL). The combined organics were washed with brine then dried over $MgSO_4$ and solvents were removed under vacuum. Purification was achieved by column chromatography (100% toluene), to afford the product (21 mg, 46% yield) R_f = 0.75 (50% DCM in hexane) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H, H1), 7.80 (s, 1H, H4), 7.41 – 7.29 (m, 2H, H6 & H7), 6.31 (s, 1H, H3), 2.47 (d, *J* = 0.6 Hz, 3H, 2-Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.1 (C8), 128.6 (C2), 126.9 (C9), 125.5 (q, *J* = 271.4 Hz, C11), 124.3 (C5), 122.0 (q, *J*= 30.2 Hz, CF_3), 117.9 (C4), 117.4 (C7),

110.4 (C6), 101.4 (C3), 13.9 (C10); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -60.18 (s, CF_3); **TOF M/Z (ES+)** Found 200.0679 ($C_{10}H_9F_3N$) Calc. 200.0687, 200.1 [M+H] 100%, 201.1 [^{13}C-M+H] 10%; **FTIR** (Neat) 3403.6, 2978.2, 2901.4, 1699.5, 1628.3, 1603.8, 1311.4, 1282.7, 1266.7, 1234.3, 1161.9, 1100, 1074.2, 1046.0, 989.1, 708.7, 626.5.

2-Bromo-4-chloroaniline, 22



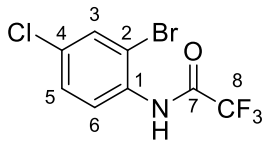
A known compound synthesised according to a literature procedure.[126]

To a stirred solution of 4-chloroaniline (0.5g, 3.92 mmol) in MeCN (39 mL) under an argon atmosphere cooled to 0 °C; *N*-bromosuccinimide (0.7g, 3.92 mmol) was added in a single portion and the reaction allowed to warm to room temperature over 16 hours. Water (40 mL) was added and the reaction mixture was extracted with EtOAc (3 × 40 mL) and the combined organic layers were dried over $MgSO_4$. Solvents were removed under vacuum and purification was achieved by column chromatography (40% EtOAc in hexane) to afford the title compound (706 mg, 87% yield) R_f = 0.65 (40% EtOAc in hexane) as a red solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, *J* = 2.3 Hz, 1H, H3), 7.07 (dd, *J* = 8.6, 2.3 Hz, 1H, H5), 6.69 (d, *J* = 8.6 Hz, 1H, H6), 4.05 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 143.0 (C1), 132.0 (C3), 128.5 (C5), 123.2 (C4), 116.3 (C6), 109.3 (C2); **TOF M/Z (ES+)** 208.0 ($C_6H_6{}^{81}Br^{35}ClN$ & $C_6H_6{}^{79}Br^{37}ClN$ 100%), 205.9 ($C_6H_6{}^{79}Br^{35}ClN$) 80%, 210.0 ($C_6H_6{}^{81}Br^{37}ClN$) 25%; **M.P.**(From EtOAc) 48-50 °C.

Analytical data in agreement with literature values. [126]

N-(2-Bromo-4-chlorophenyl)-2,2,2-trifluoroacetamide, 23



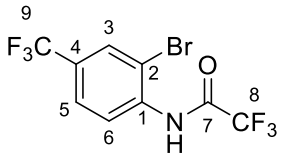
A known compound prepared according to a literature procedure.[49]

To a stirred solution of 2-bromo-4-chloroaniline (352 mg, 1.705 mmol) and NEt_3 (272 µL, 1.88 mmol) in dry DCM (7 mL) cooled to 0 °C under an argon atmosphere; trifluoroacetic anhydride (261 µL, 1.88 mmol) was added dropwise over 5 minutes then the reaction stirred for 2 hours. The reaction was then diluted with DCM (100 mL) and washed with HCl (2 × 50 mL, 0.1 M, Aq.) and the organic phase was dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (5% acetone in toluene) to afford the title compound (332 mg, 64% yield) R_f = 0.75 (5% acetone in toluene) as a white crystalline solid as well as recovered 2-bromo-4-chloroaniline (105 mg, 30%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.24 (d, *J* = 8.9 Hz, 1H), 7.60 (d, *J* = 2.3 Hz, 1H), 7.36 (dd, *J* = 8.9, 2.3 Hz, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 154.9 (q, 33.0 Hz, C7), 133.3 (C1), 132.2 (C4), 131.9 (C3), 128.9 (C6), 115.6 (q, 238. Hz, C8), 114.4 (C2); **TOF M/Z (ES+)** 302.3 ([^{35}Cl^{79}Br-M]$^+$) 100% 304.3 [^{35}Cl^{81}Br-M]$^+$; **FTIR** (Neat) 3273.5, 3109.5, 1710.3, 1583.3, 1537.4, 1469.4, 1276.9, 1198.5, 1157.9, 1135.8, 1097.5, 818.6, 754.7; **M.P.** (From EtOAc) 38-40 °C.

Analytical data in agreement with literature values. [49]

N-(2-Bromo-4-(trifluoromethyl)phenyl)-2,2,2-trifluoroacetamide, 24

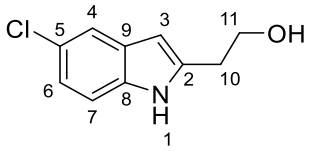


A novel compound prepared *via* a literature procedure.[49]

To a stirred solution of 2-bromo-4-(trifluoromethyl)aniline (383 mg, 1.56 mmol) and NEt_3 (246 µL, 1.76 mmol) in dry DCM (6.5 mL) cooled to 0 °C under an argon atmosphere; trifluoroacetic anhydride (244 µL, 1.76 mmol) was added dropwise over 5 minutes then the reaction stirred for 2 hours. The reaction was then diluted with DCM (100 mL) and washed with HCl (2 × 50 mL, 0.1 M, Aq.) and the organic phase was dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (5% acetone in toluene) to afford the title compound (474 mg, 88% yield) R_f = 0.75 (5% acetone in toluene) as a clear yellow oil

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (br. s, 1H, NH), 8.50 (d, *J* = 8.7 Hz, 1H, H5), 7.88 (d, *J* = 1.3 Hz, 1H, H3), 7.67 (d, *J* = 8.7 Hz, 1H, H6); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.9 (q, 32.2 Hz, C7), 136.2 (C1), 129.8 (C3), 129.7 (C5), 126.0, (C4) 124.9 (q, *J* = 270.8 Hz, C9), 121.6 (C6), 113.8 (C2), 115.8 (q, 286 Hz, C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -62.61, -75.84; **TOF M/Z (ES+)** 764.7 (2[M]$^+$) 100%, 765.7 ([2M]+H) 50%; **FTIR** (Neat) 3301.5, 3068.1, 1713.2, 1611.7, 1589.7, 1540.2, 1319.9, 1281.0, 1173.2, 1114.8, 1078.1, 912.7, 892.3, 829.3, 733.4, 686.7; **M.P.** (From EtOAc) 44-46 °C.

2-(5-Chloro-1H-indol-2-yl)ethan-1-ol, 25



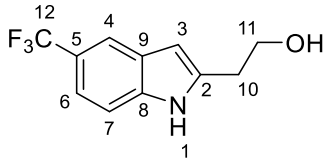
A known compound synthesised according to a literature procedure.[49]

To a stirred solution of *N*-(2-Bromo-4-chlorophenyl)-2,2,2-trifluoroacetamide (179mg, 0.59 mmol), but-3-yn-1-ol (90 µL, 1.18 mmol), CuI (11.4 mg, 0.07 mmol) and NEt_3 (248 µL, 1.78 mmol) in DMF (3 mL, argon degassed) in a sealed tube (15 mL Ace-tube); $Pd(PPh_3)_2Cl_2$ (21 mg, 0.03 mmol) was added as a single portion and the reaction heated to 120 °C for 8 hours. The reaction was diluted with EtOAc (100 mL) then filtered through Celite and the filtrate then washed with brine (5 × 100 mL) then the organic phase was dried over $MgSO_4$ and concentrate *in vacuo*. Purification was achieved *via* column chromatography (2% MeOH in DCM) to afford the title compound (86 mg, 74% yield) R_f = 0.25 (2% MeOH in DCM) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H, H1), 7.49 (d, *J* = 2.0 Hz, 1H, H4), 7.17 (d, *J* = 8.6 Hz, 1H, H6), 7.06 (dd, *J* = 8.6, 2.0 Hz, 1H, H5), 6.19 (dd, *J* = 1.9, 0.8 Hz, 1H, H3), 3.90 (t, *J* = 5.7 Hz, 2H, H11), 2.93 (t, *J* = 5.7 Hz, 2H, H10), 2.68 (br. s, 1H, OH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.1, 134.5, 129.7, 125.1, 121.3, 119.2, 111.6, 99.9, 62.1, 31.2; **TOF M/Z (ES+)** Found 196.0532 ($C_{10}H_{11}NO^{35}Cl$) Calc. 196.0529, 196.1 [M+H] 100%, 178.1 ([M- HO]$^+$) 50%, 198.1 ([^{13}C-M+H]) 10%; **FTIR** (Neat) (br.) 3404.1 (OH), 3288.9 (NH), 2925.8, 1654.4, 1615.4, 1578.0, 1467.5, 1447.6, 1413.7, 1308.5, 1263.6, 1170.6, 1059.8, 1048.1, 913.5, 864.1, 791.7, 735.8, 690.3.

Analytical data in agreement with literature values. [49]

2-(5-(Trifluoromethyl)-1*H*-indol-2-yl)ethan-1-ol, 26

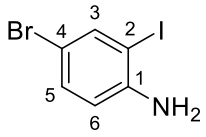


A novel compound.

To a stirred solution of *N*-(2-bromo-4-(trifluoromethyl)phenyl)-2,2,2-trifluoroacetamide (200mg, 0.592 mmol), but-3-yn-1-ol (90 µL, 1.19 mmol), CuI (12 mg, 0.06 mmol) and NEt_3 (250 µL, 1.78 mmol) in DMF (3 mL, argon degassed) in a sealed tube (15 mL Ace-tube); $Pd(PPh_3)_2Cl_2$ (22 mg, 0.03 mmol) was added as a single portion and the reaction heated to 120 °C for 8 hours. The reaction was diluted with EtOAc (100 mL) then filtered through Celite and the filtrate then washed with brine (5 × 100 mL) then the organic phase was dried over $MgSO_4$ and concentrate *in vacuo*. Purification was achieved *via* column chromatography (50% acetone in toluene) to afford the title compound (62 mg, 46% yield) R_f = 0.8 (50% acetone in toluene) as a brown oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (br. s, 1H, H1), 7.82 (s, 1H, H4), 7.38 – 7.30 (m, 2H, H6 + H7), 6.33 (s, 1H, H3), 3.95 (t, *J* = 5.7 Hz, 2H, H11), 2.99 (t, *J* = 5.7 Hz, 2H, H10), 2.78 (br. s, 1H, OH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.5 (C8), 137.6 (C2), 132.1 (d, *J* = 9.8 Hz, C9), 129.0 (d, *J* = 11.9 Hz, C6), 125.5 (q, *J* = 271.4 Hz, C12), 122.0 (q, *J* = 31.6 Hz, C5), 118.0 (d, *J* = 3.4 Hz, C4), 110.8 (C7), 100.9 (C3), 62.2 (C11), 31.1 (C10); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -60.1; **TOF M/Z (ES+)** Found 230.0797 ($C_{11}H_{11}NOF_3$) Calc. 230.0793, 230.1 [M+H] 100%, 231.1 [^{13}C-M+H] 10%; **FTIR** (Neat) br 3266.4 (OH), 2933.2, 2878.8, 165839, 1630.4, 1438.4, 1329.1, 1260.0, 1156.9, 1104.6, 1052.5, 810.1.

4-Bromo-2-iodoaniline, 27



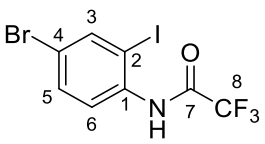
A known compound synthesised according to a literature procedure.[127]

To a stirred suspension of 4-bromoaniline (500 mg, 2.91 mmol) and $CaCO_3$(333 mg, 3.2 mmol) in MeOH (6 mL) and DCM (12 mL) under an argon atmosphere at room temperature, benzyltrimethylammonium dichloroiodate (1.012 g, 2.91 mmol) was added and the shielded from light with aluminium foil and the reaction was stirred for 4 hours. The reaction was then filtered, concentrated *in vacuo* and purified by column chromatography (50% DCM in hexane) to afford the product (462 mg, 53% yield) R_f = 0.38 (50% DCM in hexane) as a light brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, *J* = 2.2 Hz, 1H, H3), 7.22 (dd, *J* = 8.5, 2.2 Hz, 1H, H5), 6.62 (d, *J* = 8.5, 1H, H6), 4.11 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.1 (C1), 140.5 (C3), 132.2 (C5), 115.7 (C6), 110 (C4), 84.2 (C2); **M.P.**(From EtOAc) 48-50 °C.

Analytical data in agreement with literature values. [127]

N-(4-Bromo-2-iodophenyl)-2,2,2-trifluoroacetamide, 28



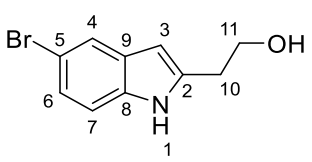
A known compound[128] synthesised *via* an unreported procedure.

To a stirred solution of 4-bromo-2-iodoaniline (420 mg, 1.41 mmol) and NEt_3 (216 µL, 1.55 mmol) in dry DCM (6 mL) cooled to 0 °C under an argon atmosphere; trifluoroacetic anhydride (216 µL, 1.55 mmol) was added dropwise over 5 minutes then the reaction stirred for 2 hours. The reaction was then diluted with DCM (100 mL) and washed with HCl (2 × 50 mL, 0.1 M, Aq.) and the organic phase was dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (5% acetone in toluene) to afford the title compound (478 mg, 86% yield) R_f = 0.8 (5% acetone in toluene) as an off-white crystalline solid as well as recovered 4-bromo-2-iodoroaniline (42 mg, 10%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (br. s, 1H, NH), 8.12 (d, *J* = 8.8 Hz, 1H, H6), 7.98 (d, *J* = 2.2 Hz, 1H, H3), 7.55 (dd, *J* = 8.8, 2.2 Hz, 1H, H5); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.2 (q, 32.4 Hz, C7), 141.0 (C1), 134.9 (C3), 132.7 (C5), 122.8 (C6), 119.8 (C4), 115.3 (q, 286 Hz, C8) 96.6 (C2); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -75.77; **TOF M/Z (ES+)** Found 415.8361 ($C_8H_4NO^{79}BrINaF_3$) Calc. 415.8371; **FTIR** (Neat) 3269.9, 3089.7, 1700.3, 1574.4, 1530.5, 1376.7, 1275.6, 1188.3, 1159.3, 1090.6, 823.6, 741.4; **M.P.** (From EtOAc) 75-77 °C.

Analytical data in agreement with literature values. [128]

2-(5-Bromo-1H-indol-2-yl)ethan-1-ol, 29

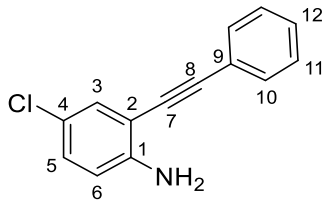


A novel compound.

To a stirred solution of *N*-(4-bromo-2-iodophenyl)-2,2,2-trifluoroacetamide (200mg, 0.51 mmol), but-3-yn-1-ol (77 µL, 1.01 mmol), CuI (10 mg, 0.05 mmol) and NEt_3 (213 µL, 1.52 mmol) in DMF (2.5 mL, argon degassed) in a sealed tube (15 mL Ace-tube); $Pd(PPh_3)_2Cl_2$ (19 mg, 0.03 mmol) was added as a single portion and the reaction heated to 120 °C for 8 hours. The reaction was diluted with EtOAc (100 mL) then filtered through Celite and the filtrate then washed with brine (5 × 100 mL) then the organic phase was dried over $MgSO_4$ and concentrate *in vacuo*. Purification was achieved *via* column chromatography (50% acetone in toluene) to afford the title compound R_f = 0.8 (50% acetone in toluene) as a clear brown oil (8 mg, 7% yield). N.B. substantial degradation observed.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (br. s, 1H, H1), 7.71 – 7.62 (m, 1H, H6), 7.19 (d, *J* = 1.7 Hz, 1H, H4), 7.18 (s, 1H, H7), 6.21 (d, *J* = 1.3 Hz, 1H, H3), 3.96 (t, *J* = 5.7 Hz, 2H, H11), 2.99 (t, *J* = 5.7 Hz, 2H, H10), 1.99 (br. s, 1H, OH); **TOF M/Z (ES+)** Found 240.0028 ($C_{10}H_{11}NO^{79}Br$) Calc. 240.0024, 240.0 [^{79}Br-M+H] 100%, 242.0 [^{81}Br-M+H] 100%, 241.0 [$^{79}Br^{13}$C-M+H] 10%, 243.0 [$^{81}Br^{13}$C-M+H] 10%; **FTIR** (Neat) (br.) 3665.3 (OH), 2992.9, 2972.3, 2899.8, 1705.4, 1624.0, 1396.0, 1254.9, 1066.7, 1050.7, 726.6.

4-Chloro-2-(phenylethynyl)aniline, 30



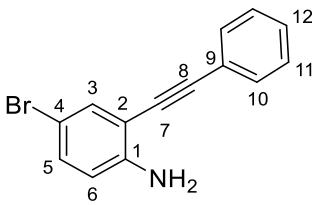
A known compound synthesised according to a literature procedure.[129]

To a stirred solution of 2-iodo-4-chloroaniline (117 mg, 0.46 mmol), phenylacetylene (56 µL, 0.51 mmol) NEt_3 (1 mL) and CuI (9 mg, 0.05 mmol) in DMF (2.5 mL,); $Pd(PPh_3)_2Cl_2$ (17 mg, 0.02 mmol) was added as a single portion with stirring under and argon atmosphere and the reaction was heated to 50 °C for 16 h. The reaction was then diluted with DCM (50 mL), filtered through Celite and the filtrate was washed with brine (3 × 50 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated *in vacuo*. The crude brown resin was purified *via* column chromatography (30% DCM in hexane) to afford the title compound (49 mg, 47% yield) R_f = 0.45 (50% DCM in hexane) as an off-white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.26 (s, br, 2H, NH_2), 6.64 (d, *J*=8.7, 1H,), 7.08 (dd, *J*=8.7, *J*=2.4Hz, 1H), 7.3–7.4 (m, 4H), 7.5–7.55 (2H); **^{13}C-NMR**(75 MHz, $CDCl_3$): δ 146.2 (C1), 131.5 (C10), 131.3 (C3), 129.6 (C5), 128.5 (C12), 128.4 (C11), 122.4 (C9), 122.8 (C4), 115.4 (C6), 109.3 (C2), 95.6 (C7), 84.6 (C8); **TOF M/Z (ES+)** Found 228.0582 ($C_{14}H_{11}N^{35}Cl$) Calc. 228.0580, 228.1 [^{35}Cl-M+H] 100%, 230.1 [^{37}Cl-M+H] 20%.

Analytical data in agreement with literature values. [129]

4-Bromo-2-(phenylethynyl)aniline, 31



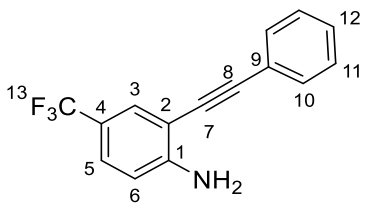
A known compound synthesised according to a literature procedure.[130]

To a stirred solution of 2-iodo-4-bromoaniline (231 mg, 0.78 mmol), phenylacetylene (94 µL, 0.85 mmol) NEt_3 (1.5 mL) and CuI (15 mg, 0.08 mmol) in DMF (7.5 mL,); $Pd(PPh_3)_2Cl_2$ (27 mg, 0.04 mmol) was added as a single portion with stirring under and argon atmosphere and the reaction was heated to 50 °C for 16 h. The reaction was then diluted with DCM (50 mL), filtered through Celite and the filtrate was washed with brine (3 × 50 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated *in vacuo*. The crude brown resin was purified *via* column chromatography (20% DCM in hexane) to afford the title compound (132 mg, 63% yield) R_f = 0.5 (50% DCM in hexane) as an off-white solid.

^{1}H-NMR (300 MHz; $CDCl_3$) δ 7.39-7.46 (m, 3 H, H11 + H12), 7.25-7.30 (m,3 H, H3 + H10), 7.12 (1 H, dd, *J*=8.6, 2.3 Hz, H5), 6.50 (d,1 H, *J*= 8.6 Hz, H6), 4.20 (s, 2 H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.9 (C1), 140.4 (C9), 134.3 (C5), 132.5 (C10), 131.6 (C3), 128.7 (C11), 128.5 (C12), 115.9 (C6), 109.4 (C4), 109.1 (C8), 95.9 (C7), 84.6 (C2); **TOF M/Z (ES+)** Found 272.0070 ($C_{14}H_{11}N^{79}Br$) Calc. 272.0075, 272.0 [^{79}Br-M+H] 100%, 274.0 [^{81}Br-M+H] 100%, 273.0 [^{79}Br-^{13}C-M+H] 10%, 275.0 [^{81}Br-^{13}C-M+H]10%; **M.P.**(From EtOAc) 76-78 °C.

Analytical data in agreement with literature values. [130]

2-(Phenylethynyl)-4-(trifluoromethyl)aniline, 32



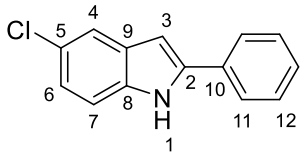
A known compound synthesised according to a literature procedure.[131]

To a stirred solution of 2-iodo-4-(trifluoromethyl)aniline (280 mg, 0.98 mmol), phenylacetylene (118 µL, 1.07 mmol) NEt_3 (1.5 mL) and CuI (19 mg, 0.1 mmol) in DMF (2.5 mL,); $Pd(PPh_3)_2Cl_2$ (35 mg, 0.05 mmol) was added as a single portion with stirring under and argon atmosphere and the reaction was heated to 50 °C for 16 h. The reaction was then diluted with DCM (50 mL), filtered through Celite and the filtrate was washed with brine (3 × 50 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated *in vacuo*. The crude brown resin was purified *via* column chromatography (30% DCM in hexane) to afford the title compound (177 mg, 70% yield) R_f = 0.75 (50% DCM in hexane) as an orange crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, *J* = 1.7 Hz, 1H, H3), 7.60 – 7.53 (m, 2H, H5 + H12), 7.43 – 7.37 (m, 4H, H10 + H11), 6.74 (d, *J* = 8.5 Hz, 1H, H6), 4.60 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 150.3 (C1), 131.6 (12), 129.6 (C3), 128.8 (C10), 128.6 (C11), 126.7 (C5), 124.5 (q, *J* = 270.7 Hz (C13), 122.8 (C9), 119.9 (q, *J* = 33.2 Hz, C4), 113.8 (C6), 107.6 (C2), 95.8 (C8), 84.5 (C7); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -61.3; **TOF M/Z (ES+)** Found 262.0838 ($C_{15}H_{11}NF_3$) Calc. 262.0844, 262.1 [M+H] 100%, 263.1 [^{13}C-M+H] 10%; **FTIR** (Neat) 3457.3, 3358.5, 3196.2, 2968.1, 1981.8, 1905.8, 1618.6, 1506.7, 1488.8, 1429.1, 1328.1, 1262.7, 1102.8, 1069.9, 907.8, 825.9, 759.0, 689.8; **M.P.** (From DCM) 67-69 °C.

Analytical data in agreement with literature values. [131]

5-chloro-2-phenyl-1H-indole, 33



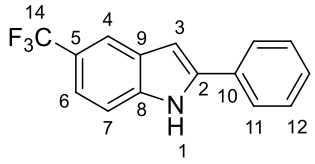
A known compound[132] synthesised via an unreported procedure.

To a stirred suspension of 4-chloro-2-(phenylethynyl)aniline (37 mg, 0.16 mmol), $CaCO_3$ (16 mg, 0.16 mmol) in DMF (0.5 mL) under an argon atmosphere; CuI (15.6 mg, 0.08 mmol) was added as a single portion and the reaction stirred at r.t. for 48 hours. The reaction was diluted with EtOAc (20 mL), filtered through Celite then washed with brine (5 × 20 mL). The organic phase was dried over $MgSO_4$ then concentrated *in vacuo* and purified *via* column chromatography (50% DCM in hexane) to afford the title compound (14 mg, 38% yield) R_f = 0.5 (50% DCM in hexane) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (br s, 1H, H1), 7.61 (d, J = 7.3 Hz, 2H, H11), 7.54 (s, 1H, H4), 7.41-7.35 (m, 2H, H12), 7.33-7.27 (m, 1H, H6), 7.27-7.25 (m, 1H, H7), 7.12 (m, 1H, H13), 6.74 (s, 1H, H3); **^{13}C NMR** (100 MHz, $CDC1_3$): δ 139.2 (C8), 135.0 (C2), 131.7 (C10), 130.2 (C12), 129.1 (C9), 128.1 (C13), 125.7 (C11), 125.3 (C5), 122.6 (C6), 119.9 (C4), 111.7 (C7), 99.5 (C3); **TOF M/Z (ES+)** 227.0 [^{35}Cl-M+H] 100%, 229.0 [^{37}Cl-M+H] 30%; **FTIR** (Neat) 3345.8, 3063.1, 1763.9, 1726.4, 1615.7, 1579.1, 1470.5, 1445.7, 1255.5, 1163.2, 803.6, 688.9; **M.P.** (From EtOAc) 195-197 °C.

Analytical data in agreement with literature values. [132]

2-phenyl-5-(trifluoromethyl)-1H-indole, 34



A known compound[133] synthesised *via* an unreported procedure[50]

Via Palladium-catalyzed aerobic oxidative cyclization of N-aryl imines

To a stirred suspension of 4-(trifluoromethyl)aniline (314 µg, 2.5 mmol) and 4Å molecular sieves (500 mg, powdered) in dry toluene (10 mL) acetophenone (234 µL, 2 mmol) was added as a single portion under an argon atmosphere and the reaction heated to reflux for 48 hours. The reaction was then cooled to r.t. and filtered under an argon atmosphere, the filtrand was washed with Et_2O and the combined organics were concentrated *in vacuo.* Purification was achieved *via* column chromatography (5% EtOAc in hexane) to afford the product R_f=0.95 (20% EtOAc in hexane) as a clear yellow oil (110 mg, 0.42 mmol) that was found to degrade under atmospheric conditions. To the oil and $Cu(OAc)_2$ (228 mg, 1.25 mmol) in DMSO (2 mL) $Pd(OAc)_2$ (10 mg, 0.04 mmol) was added as a single portion and the reaction heated to 40 °C for 12 hours. The reaction was cooled to r.t., diluted with EtOAc (50 mL) then filtered through Celite. The filtrate was washed with brine (5 × 50 mL) then the organic phase was dried over $MgSO_4$ then concentrate *in vacuo*. Purification was achieved *via* column chromatography (50% DCM in hexane) to afford the title compound (5 mg, 5% yield) R_f = 0.4 (50% DCM in hexane) as a clear yellow oil.

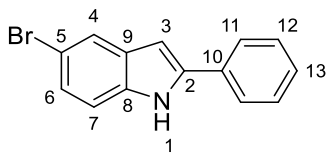
Via copper (I) mediated cyclisation

To a stirred suspension of 4-(trifluoromethyl)-2-(phenylethynyl)aniline (48 mg, 0.18 mmol), $CaCO_3$ (9.5 mg, 0.09 mmol) in DMF (0.5 mL) under an argon atmosphere; CuI (18 mg, 0.09 mmol) was added as a single portion and the reaction stirred at r.t. for 48 hours. The reaction was diluted with EtOAc (20 mL), filtered through Celite then washed with brine (5 × 20 mL). The organic phase was dried over $MgSO_4$ then concentrated *in vacuo* and purified *via* column chromatography (50% DCM in hexane) to afford the title compound (24 mg, 50% yield) R_f = 0.4 (50% DCM in hexane) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (brs, 1H), 7.91 (s, 1H), 7.64 (d, J = 7.3 Hz, 2H), 7.47-7.42 (m, 4H), 7.36 (t, J = 7.3 Hz, 1H), 6.87 (d, J = 1.9 Hz, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 140.1 (C8), 138.0 (C2), 131.7 (C10), 129.2 (C13), 128.8 (C12), 128.5 (C11), 125.4 (q, *j* = 276 Hz, C14), 125.4 (C9), 122.9(q, *J* = 34.0 Hz, C5), 119.0 (C4) , 118.4 (C6), 111.2 (C7), 100.7 (C3); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -60.5.

Analytical data in agreement with literature values. [133]

5-Bromo-2-phenyl-1H-indole, 35



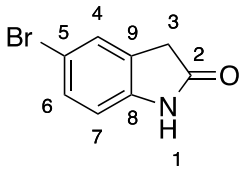
A known compound[134] synthesised *via* an unreported procedure[50]

To a stirred suspension of 4-bromoaniline (430 mg, 2.5 mmol) and 4Å molecular sieves (500 mg, powdered) in dry toluene (10 mL) acetophenone (234 µL, 2 mmol) was added as a single portion under an argon atmosphere and the reaction heated to reflux for 48 hours. The reaction was then cooled to r.t. and filtered under an argon atmosphere, the filtrand was washed with Et_2O and the combined organics were concentrated *in vacuo* to afford the product R_f = 0.95 (20% EtOAc in hexane) as a clear yellow oil (257 mg, 0.98 mmol), that was found to degrade under atmospheric conditions, and was used without further purification. To a stirred solution of the oil and $Cu(OAc)_2$ (532 mg, 2.93 mmol) in DMSO (10 mL) $Pd(OAc)_2$ (22 mg, 0.10 mmol) was added as a single portion and the reaction heated to 40 °C for 12 hours. The reaction was cooled to r.t., diluted with EtOAc (100 mL) then filtered through Celite. The filtrate was washed with brine (5 × 50 mL) then the organic phase was dried over $MgSO_4$ then concentrate *in vacuo*. Purification was achieved *via* column chromatography (50% DCM in hexane) to afford the title compound (92 mg39% yield) R_f = 0.25 (50% DCM in hexane) as brown crystalline solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (br. s, 1H, H1), 7.75 (d, *J* = 0.6 Hz, 1H, H4), 7.67 – 7.65 (m, 2H, H11), 7.48 – 7.43 (m, 12H), 7.38 – 7.32 (m, 1H, H13), 7.27 (m, 2H, H6 + H7), 6.76 (d, *J* = 2.1 Hz, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.3 (C8), 135.5 (C9), 131.9 (C10), 131.1 (C2), 129.3 (C12), 128.3 (C9), 125.4 (C11), 125.3 (C7), 123.2 (C4), 113.6 (C5), 112.4 (C6), 99.6 (C3); **TOF M/Z (ES+)** 272.0 [^{79}Br-M+H] 100% 274.0 [^{81}Br-M+H] 100%; **FTIR** (Neat) 3434.7, 3006.8, 1855.9, 1453.5, 1309.8, 913.3, 877.7, 793.3, 760.8, 738.4, 688.8, 677.7; **M.P.** (From DCM) 172-175 °C.

Analytical data in agreement with literature values. [134]

5-Bromo-2-oxindole, 36



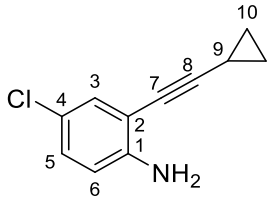
A known compound synthesised according to a literature procedure.[51]

To a stirred solution of 2-oxindole (50 mg, 0.38 mmol) in MeCN (2.5 mL) NBS (66 mg, 0.38 mmol) was added under an argon environment at 0 °C. After three hours the reaction was diluted with water (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and solvents were removed under vacuum. Purification was achieved by column chromatography (10-30% EtOAc in hexane) to afford the product (42 mg, 52% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (br. s, 1H, NH), 7.36 (s, *J* = 4.9, 4.0 Hz, 2H, H4 & H6), 6.76 (d, *J* = 8.9 Hz, 1H, H7), 3.55 (s, 2H, 2 × H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 177.6 (C2), 139.4 (C8), 130.8 (C4), 127.9 (C6), 127.3 (C7), 111.0 (C5), 115.0 (C9), 36.0 (C3); **FTIR** (Neat) 3152, 3082, 2955, 2854, 2736, 1694 (C=O stretch), 1614, 1471; **TOF M/Z** (ES+) Found 211.97189 ($C_8H_7NO^{79}Br$) Calc. 211.9711, 212.0 [^{79}Br-M+H] 100%, 214.0 [^{81}Br-M+H] 100%.

Analytical data in agreement with literature values. [51]

4-Chloro-2-(cyclopropylethynyl)aniline, 37

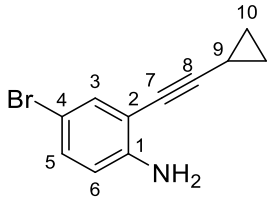


A novel compound synthesised *via* a procedure modified from the literature.[44]

To a solution of 2-iodo-4-chloroaniline (150 mg, 0.59 mmol), cyclopropylacetylene (55 µL, 0.65 mmol) NEt_3 (600 µL, 4.74 mmol) and CuI (12 mg, 0.06 mmol, 10 mol%) in DMF (6 mL, 0.1M); $Pd(PPh_3)_2Cl_2$ (21 mg, 5 µMol, 5 mol%) was added as a single portion with stirring under and argon atmosphere and the reaction was heated to 50 °C for 16 h. The reaction was then diluted with EtOAc (25 mL) and filtered through a Celite pad, the filtrate was washed with brine (3 × 25 mL) then organics dried over $MgSO_4$ and concentrated *in vacuo*. The crude brown resin was purified by column chromatography in (20% DCM in hexane) to afford the title product (45 mg, 40 yield) R_f = 0.25 (20% DCM in hexane) as a faintly yellow clear oil with 2-iodo-4-chloroaniline R_f = 0.45 (20 mg, 14 %).

^{1}H-NMR (300 MHz; $CDCl_3$) δ 7.18 (1 H, d, *J* = 2.5 Hz, H3), 7.00 (1 H, dd, *J* = 8.6, 2.5 Hz, H5), 6.58 (1 H, d, *J* = 8.6 Hz, H6), 4.15 (2 H, s, NH2), 1.49 (1 H, m, H9), 0.85-0.94 (2 H, m, H10 ax/eq), 0.78-0.83 (2 H, m, H10 Cis/Trans); **^{13}C-NMR** (101 MHz; $CDCl_3$) δ 146.6 (C1), 131.5 (C3), 128.9 (C5), 122.2 (C4), 115.3 (C6), 110.3 (C2), 100.1 (C8), 71.2 (C7), 9.1 (C9), 0.4 (C10); **TOF M/Z (ES+)** Found 192.0572($C_{11}H_{11}N^{35}Cl$) Calc. 192.0580, 192.1 [^{35}Cl-M+H] 100%, 194.1 [^{37}Cl-M+H] 30%; **FTIR** (Neat) 3675 (N-H), 3393 (C≡C), 3091-2901 (C_{ar}-H), 2222(C_{alk}-H), 1705, 1576, 1445, 1406, 1314, 1063, 780, 692, 680.

4-Bromo-2-(cyclopropylethynyl)aniline, 38

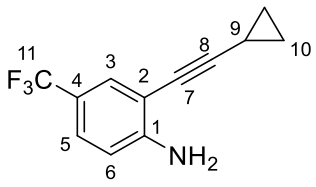


A novel compound

To a suspension of 4-bromo-2-iodoaniline (231 mg, 0.78 mmol), cyclopropylacetylene (73 µL, 0.853 mmol) and CuI (15 mg, 0.08 mmol) in NEt_3 (1 mL) under an argon atmosphere $PdPPh_3Cl_2$ (27 mg, 0.04 mmol) was added in a single portion and the reaction was heated to 50 °C with stirring for 16 h. The reaction was cooled to room temperature, diluted with EtOAc (10 mL) and filtered through Celite then the filtrate was concentrated *in vacuo* and the crude brown resin was purified by column chromatography (30% EtOAc in hexane) to afford the title compound (81 mg, 44% yield) R_f = 0.45 (30% EtOAc in hexane) as a faintly brown clear oil.

^{1}H-NMR (300 MHz; $CDCl_3$) δ 7.32 (1 H, d, *J* = 2.3, H3), 7.14 (1 H, dd, *J* = 8.6, 2.3 Hz, H5), 6.54 (1 H, d, *J* = 8.6 Hz, H6), 4.16 (2 H, s, NH_2), 1.49 (1 H, m, H9), 0.85-0.94 (2 H, m, H10 (Cis/Trans)), 0.77-0.83 (2 H, m, H10 (Cis/Trans)) **^{13}C–NMR** (101 MHz; $CDCl_3$) δ 146.6 (C1), 134.0 (C3), 131.8 (C5), 131.3 (C4), 115.2 (C6), 109.2 (C2), 99.9 (C8), 70.6 (C7), 8.7 (C9), 0.0 (C10); **TOF MS (ES+)** Found 236.0076 ($C_{11}H_{11}N^{79}Br$) calc. 236.0075, 236.0 (M+H ^{79}Br) 40%, 238.0 (M+H ^{81}Br) 40%; **FTIR** (Neat) 3446.0, 3373.9, 2988.8, 2901.8, 1612.0, 1475.1, 1388.0, 1051.7, 867.3, 809.9, 667.3.

2-(Cyclopropylethynyl)-4-(trifluoromethyl)aniline, 39

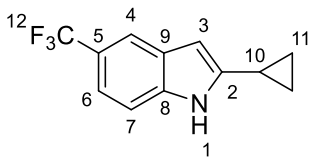


A Novel compound.

To a solution of 2-iodo-4-(trifluoromethyl)aniline (279 mg, 0.98 mmol), cyclopropylacetylene (91 µL, 1.07 mmol) NEt_3 (1.5 mL) and CuI (19 mg, 0.1 mmol) in DMF (6 mL); $Pd(PPh_3)_2Cl_2$ (35 mg, 0.05 mmol) was added as a single portion with stirring under and argon atmosphere and the reaction was heated to 50 °C for 16 h. The reaction was then diluted with EtOAc (50 mL) and filtered through a Celite pad, the filtrate was washed with brine (3 × 25 mL) then organics dried over $MgSO_4$ and concentrated *in vacuo*. The crude brown resin was purified by column chromatography in (40-80% DCM in hexane) to afford the product (143 mg, 65% yield) R_f = 0.7 (DCM) as a brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, *J* = 1.7 Hz, 1H, H3), 7.28 (d, *J* = 8.6 Hz, 1H, H6), 6.68 (dd, *J* = 8.5, 1.7 Hz, 1H, H5), 4.46 (s, 2H, NH_2), 1.55 – 1.45 (m, 1H, H9), 0.96 – 0.86 (m, 4H, H10), 0.86 – 0.78 (m, 1H, H10'); **^{13}C-NMR** (100 MHz, $CDCl_3$) δ 150.1 (C1), 129.3 (q, J = 7.7 Hz, C3), 125.4 (q, J = 3.4 Hz, C5), 124.4 (q, J = 271.4 Hz, C11), 119.3 (q, *J*= 34.1 Hz (C4), 113.2 (C6), 110.7 (C2), 99.8 (C8), 70.6 (C7), 8.7 (C10), -0.1 (C9); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -61.4; **TOF M/Z (ES+)** Found 226.0839 ($C_{12}H_{11}NF_3$) Calc. 226.0844, 226.1 [M+H] 100%, [^{13}C-M+H] 10%; **FTIR** (Neat) 3493.8, 3446.9, 3390.1, 2988.8, 227.9, 1618.9, 1434.3, 1361.2, 1322.7, 1146.6, 1105.1, 1067.9, 1026.8, 880.8, 818.4.

2-Cyclopropyl-5-(trifluoromethyl)-1H-indole, 40

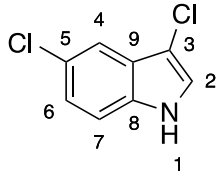


A Novel compound.

To a stirred solution of *N*-(2-bromo-4-(trifluoromethyl)phenyl)-2,2,2-trifluoroacetamide (220 mg, 0.66 mmol), cyclopropylacetylene (112 µL, 1.31 mmol), CuI (14 mg, 0.07 mmol) and NEt_3 (238 µL, 1.96 mmol) in DMF (3.6 mL, argon degassed) in a sealed tube (15 mL Ace-tube); $Pd(PPh_3)_2Cl_2$ (24 mg, 0.03 mmol) was added as a single portion and the reaction heated to 120 °C for 8 hours. The reaction was diluted with EtOAc (100 mL) then filtered through Celite and the filtrate then washed with brine (5 × 100 mL) then the organic phase was dried over $MgSO_4$ and concentrate *in vacuo*. Purification was achieved *via* column chromatography (50% DCM in hexane) to afford the title compound (52 mg, 35% yield) R_f = 0.4 (50% DCM in hexane) as a clear yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H, H1), 7.80 (d, *J* = 0.7 Hz, 1H, H4), 7.35 (d, *J* = 1.6 Hz, 1H, H6), 7.33 (d, *J* = 0.7 Hz, 1H, H7), 6.25 – 6.22 (m, 1H, H3), 2.03 – 1.92 (m, 1H, H10), 1.06 – 0.98 (m, 2H, 2 × H11), 0.84 – 0.77 (m, 2H, 2 × H11'); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 143.8 (C8), 137.2 (C2), 128.3 (C9), 125.6 (q, *J* = 271.3 Hz, C12), 122.2 (q, *J* = 31.5 Hz, C5), 117.9 (q, *J* = 3.7 Hz, C4), 117.4 (q, *J* = 3.7 Hz, C6), 110.4 (C7), 98.8 (C3), 9.0 (C10), 7.7 (C11); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -60.2; **TOF M/Z (ES+)** Found 226.0847 ($C_{12}H_{11}NF_3$) Calc. 226.0844, 226.1 [M+H] 100%, 227.1 [^{13}C-M+H] 10%; **FTIR** (Neat) 3315.1, 298.6, 2900.2, 1683.7, 1629.9, 15263.7, 1320.8, 1292.2, 1259.7, 1112.8, 1048.2, 958.4.

3,5-Dichloro-1*H*-indole, 41



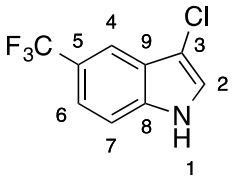
A known compound synthesised according to a literature procdure.[52]

5-Chloro-1*H*-indole (30 mg, 0.20 mmol) was dissolved in DMF (2 mL) and cooled to 0 °C under an argon atmosphere. NCS (27 mg, 0.20 mmol) was added as a single portion and the reaction was stirred at 0 °C and allowed to reach room temperature over 16 hours. The reaction mixture was diluted with brine (5 mL) and extracted with Et_2O (3 × 5 mL), the combined organic layers were dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude oil was purified by flash column chromatography (10% EtOAc in Hexane) to afford the product (28 mg, 75% yield) as a pink crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H, H1), 7.64 (d, *J* = 2.1 Hz, 1H, H4), 7.30 (d, *J* = 8.5 Hz, 1H, H7), 7.22 (dd, *J* = 8.5, 2.1 Hz, 1H, H6), 7.2 (s, 1H, H3); **TOF M/Z (ES-)** Found 195.9795 ($C_8H_4{}^{35}Cl_2N$) Calc. 197.9884, 195.98 [$^{35}Cl_2$-M-H] 100%, 197.99 [^{35}Cl-^{37}Cl-M-H] 50%, 199.99 [^{37}Cl-^{37}Cl-M-H] 10%.

Analytical data in agreement with literature values. [52]

3-Chloro-5-(trifluoromethyl)-1*H*-indole, 42

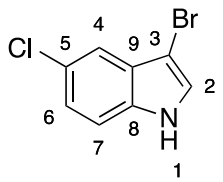


A novel compound.

5-(Trifluoromethyl)-1*H*-indole (30 mg, 0.16 mmol) was dissolved in DMF (2 mL) and cooled to 0 °C under an argon atmosphere. NCS (27 mg, 0.20 mmol) was added as a single portion and the reaction was stirred at 0 °C and allowed to reach room temperature over 16 hours. Following this time the reaction mixture was diluted with brine (5 mL) and extracted with Et_2O (3 × 5 mL), the organic layers were dried over $MgSO_4$ and solvent was removed *in vacuo*. The crude oil was purified by flash column chromatography (10% EtOAc in Hexane) to afford the product (34 mg, 97% yield) R_f = 0.3 (10% EtOAc in Hexane) as a red crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (s, 1H, H1), 7.97 (q, *J* = 0.8 Hz, 1H, H3), 7.51 (dd, *J* = 8.7, 2.1 Hz, 1H, H6), 7.47 (d, *J* = 8.6 Hz, 1H, H7), 7.32 (d, *J* = 2.1 Hz, 1H, H4).

3-Bromo-5-chloro-1*H*-indole, 43



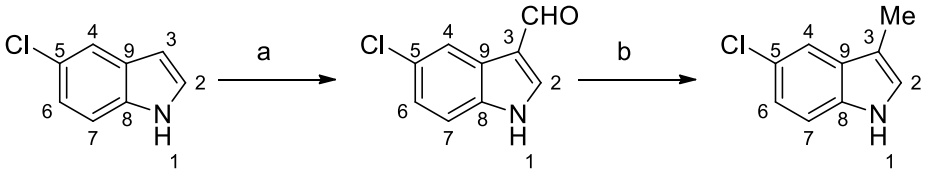
A known compound[135] synthesised *via* an unreported procedure.

5-Chloro-1*H*-indole (18 mg, 0.12 mmol) was dissolved in DMF (1 mL) and cooled to 0 °C under an argon atmosphere. NBS (21 mg, 0.12 mmol) was added as a single portion and the reaction was stirred at 0 °C and allowed to reach room temperature over 16 hours. The reaction mixture was diluted with brine (3 mL) then extracted with Et_2O (3 × 3 mL), the combined organic layers were dried over $MgSO_4$ and solvent removed *in vacuo*. Purification was achieved via column chromatography (20% EtOAc in Hexane) to afford the product (27 mg, 60% yield) as a light sensitive white solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.22 (br. s, 1H, H1), 7.57 (d, *J* = 2.0 Hz, 1H, H4), 7.30 (d, *J* = 8.7 Hz, 1H, H7), 7.25 (d, *J* = 2.6 Hz, 1H, H2), 7.20 (dd, *J* = 8.7, 2.0 Hz, 1H, H6); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 133.8 (C8), 128.1 (C9), 126.7 (C5), 124.8 (C2), 123.8 (C6), 118.9 (C4), 112.6 (C7), 91.3 (C3).

Analytical data in agreement with literature values. [135]

5-Chloro-3-methyl-1*H*-indole, 44



5 **26** **27**

A known compound synthesised according to a literature procedure.[53]

5-Chloro-1*H*-indole **5** (50 mg, 0.33 mmol) was dissolved in DMF (5 mL) and cooled to 0 °C with stirring under an argon atmosphere. Phosphorus (V) oxychloride (37 µL, 0.40 mmol) was added dropwise over 30 minutes and once addition was complete the reaction was warmed to 40 °C and stirred for 2 hours. Following this time NaOH (2M aq., 0.3 mL, excess) was added and the reaction mixture was heated at 90 °C for one hour and then allowed to cool to room temperature. Once the reaction had cooled, the residue was extracted with EtOAc (3 × 15 mL) and the combined organic layers were washed with brine (15 mL) then dried over $MgSO_4$; solvents were removed under vacuum and product identified as **26** by ^{1}H-NMR and used straight away without further purification

III. 5-Chloro-1H-indole-3-carbaldehyde

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.03 (s, 1H, CHO), 8.87 (br. s, 1H, H1), 8.33 (d, *J* = 2.0 Hz, 1H, H7), 7.87 (d, *J* = 2.1 Hz, 1H, H6), 7.35 (d, *J* = 0.5 Hz, 1H, H4), 7.30 (d, *J* = 2.0 Hz, 1H, H2).

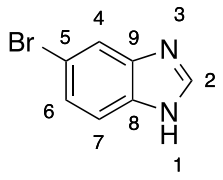
IV. 5-Chloro-3-methyl-1H-indole, 42

5-Chloro-1*H*-indole-3-carbaldehyde **26** (60 mg, 0.33 mmol) was dissolved in THF (1.5 mL) and cooled to 0 °C with stirring under an argon atmosphere. $LiAlH_4$ (20 mg, 0.5 mmol) was added as a single portion to the solution and the reaction was allowed to warm to room temperature over 16 hours. Excess $LiAlH_4$ was quenched using the Steinhardt workup procedure, the crude organic residue was concentrated under vacuum and purified by column chromatography (5% Et_2O in Hexane) to afford the product **27** (37 mg, 68% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (br. s, 1H, H1), 7.58 (d, *J* = 2.0 Hz, 1H, H7), 7.26 (s, 1H, H4), 7.18 (d, *J* = 2.0 Hz, 1H, H6), 7.03 – 6.99 (m, 1H, H2), 2.33 (d, *J* = 1.1 Hz, 3H, Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 134.7 (C8), 129.6 (C9), 125.0 (C5), 123.1 (C6), 122.2 (C4), 118.5 (C7), 112.1 (C2), 111.7 (C3), 9.7 (2Me); **TOF M/Z (EI+)** Found 165.0346 ($C_9H_8{}^{35}ClN$) calculated 165.0345, 165.0 [^{35}Cl-M^+] 100%, 167.0 [^{37}Cl-M^+] 25%.

Analytical data in agreement with literature values. [53]

5-Bromo-1*H*-benzimidazole, 45



A known compound synthesised according to a literature procedure.[136]

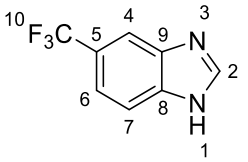
4-Bromobenzene-1,2-diamine (50 mg, 0.28 mmol) and formic acid (22 µL, 0.58 mmol) were dissolved in HCl (272 µL, 4M Aq.) and heated to reflux for 45 minutes. The reaction was cooled to room temperature and quenched with NH_4OH (2 mL, 28% w/v) and the aqueous layer decanted off to afford a black solid that was dissolved in Et_2O (5 mL), dried over $MgSO_4$ and solvent removed under vacuum to afford the product (52mg, 94% yield) as a black solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.21 (br. s, 1H, H1), 8.20 (s, 1H, H2), 7.82 (d, *J* = 1.7 Hz, 1H, H4), 7.54 (d, *J* = 8.6 Hz, 1H, H7), 7.40 (dd, *J* = 8.6, 1.7 Hz, 1H, H6); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 141.8 (C2), 139.1 (C8), 136.8 (C9), 126.4 (C5), 118.6 (C6), 116.8 (C7), 116.2 (C4); **TOF M/Z** (ES+) Found

196.9718 ($C_7H_6{}^{79}BrN_2$) Calc. 196.9714, 197.0 [^{79}Br-M+H] 100%, 199.0 [^{81}Br-M+H] 100%, **FTIR** (Neat) 3289, 2966, 1652, 1593, 1523, 1492.

Analytical data in agreement with literature values. [136]

5-(Trifluoromethyl)-1*H*-benzimidazole, 46



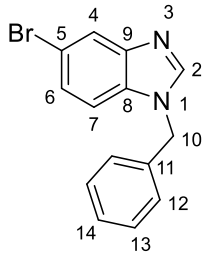
A known compound synthesised according to a literature procedure.[47]

4-(Trifluoromethyl)benzene-1,2-diamine (50 mg, 0.28 mmol) and formic acid (22 µL, 0.58 mmol) were dissolved in HCl (272 µL, 4M Aq.) and heated at reflux for 45 minutes. The reaction was cooled to room temperature and quenched with NH_4OH (2 mL, 28 w/v %) and the aqueous layer decanted off to afford a black solid that was dissolved in Et_2O (5 mL), dried over $MgSO_4$ and the solvent removed *in vacuo* to afford the product (48 mg, 92% yield) as a brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.11 (br. s, 1H, NH), 8.35 (s, 1H, H2), 7.99 (d, *J*=1.0 Hz, 1H, H4), 7.75 (d, *J* = 8.5 Hz, 1H, H7), 7.56 (dd, *J* = 8.5, 1.0 Hz, 1H, H6); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 143.0 (C2), 139.3 (C8), 137.8 (C9), 125.8 (q, 30.2 Hz, C5), 124.8 (q, *J* = 271.8 Hz, C10), 120.2 (q, J = 3.2 Hz, C6), 115.6 (C7), 113.9 (q, *J* = 4.2 Hz, C4); **TOF M/Z (ES+)** Found 187.0490 ($C_8H_6F_3N_2$) Calc. 187.0483, 187.0 [M+H] 100%, 188.0 [^{13}C-M+H] 10%; **FTIR** (Neat) 3003.6, 2913.2, 2595.2, 1663.2, 1477.6, 1421.5, 1092.7, 1050.6, 956.3, 943.2, 914.4, 869.2, 803.8, 670.2, 662.2.

Analytical data in agreement with literature values. [47]

1-Benzyl-5-bromo-1H-benzo[d]imidazole, 47



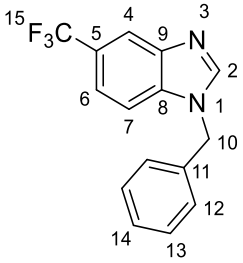
A known compound that is poorly described in the literature.[137]

To a stirred solution of 5-(trifluoromethyl)-1H-benzo[d]imidazole (100 mg, 0.51 mmol) in dry THF (4 mL) cooled to 0 °C, under an argon atmosphere, NaH (28 mg, 0.70 mmol, 60% mineral oil; dispersion) was added as a single portion. The reaction was stirred for 30 minutes then a solution of benzyl bromide (42 µL, 0.35 mmol) in THF (6 mL) was added as a single portion at 0 °C and then the reaction was warmed to r.t. and stirred for 16 hours. NH_4Cl (5 mL, Sat. Aq.) was added and mixture extracted with EtOAC (3 × 15 mL), the combined organic extracts were dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (30-50% EtOAc in hexane) to afford the title compound (104 mg, 72% yield) R_f = 0.25 as a light-brown crystalline solid as a 1:1 mixture with the regioisomer 1-benzyl-6-bromo-1H-benzo[d]imidazole.

^{1}H NMR 5-Bromo-isomer (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 1.6 Hz, 1H, H4), 7.93 (s, 1H, H2), 7.36 – 7.31 (m, 4H, H6, H7 and H12), 7.17 – 7.10 (m, 3H, H13 and H14), ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 1.6 Hz, 1H), 7.93 (s, 1H), 7.36 – 7.31 (m, 4H), 7.17 – 7.10 (m, 3H), 5.32 (s, 2H, H10); **6-Bromo-isomer**: δ 7.91 (s, 1H, H2), 7.68 (d, J = 8.6 Hz, 1H, H4), 7.46 – 7.37 (m, 2H, H7 and H5), 7.37

– 7.30 (m, 3H, H12 and H14), 7.21 – 7.11 (m, 2H, H13), 5.30 (s, 2H, H10); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.4, 144.2, 143.8, 143.0, 135.0, 134.9, 129.2, 129.1, 128.5, 128.5, 127.1, 127.1, 127.0, 126.9, 126.2, 125.7, 123.3, 121.7, 116.4, 115.4, 113.1, 111.3, 48.9; **TOF M/Z (ES+)** 287.0[^{79}Br-M+H] 100%, 289.0[^{81}Br-M+H] 100%, 288.0[^{79}Br-^{13}C-M+H] 40%, 290.0[^{81}Br-^{13}C-M+H] 40%.

1-benzyl-5-(trifluoromethyl)-1H-benzo[d]imidazole, 48



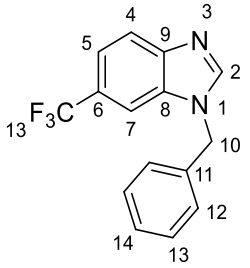
A novel compound.

To a stirred solution of 5-(trifluoromethyl)-1H-benzo[d]imidazole (87 mg, 0.47 mmol) in dry THF (8 mL) cooled to 0 °C, under an argon atmosphere, NaH (25.2 mg, 0.63 mmol, 60% mineral oil; dispersion) was added as a single portion. The reaction was stirred for 30 minutes then a solution of benzyl bromide (38 µL, 0.32 mmol) in THF (6 mL) was added as a single portion at 0 °C and then the reaction was warmed to r.t. and stirred for 16 hours. NH_4Cl (5 mL, Sat. Aq.) was added and mixture extracted with EtOAC (3 × 15 mL), the combined organic extracts were dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (30-50% EtOAc in hexane) to afford the title compound (38 mg, 30% yield) R_f = 0.35 as a white crystalline solid as well as the other regioisomer 1-benzyl-6-(trifluoromethyl)-1H-benzo[d]imidazole (43 mg, 33% yield) R_f = 0.32 as a white crystalline solid.

1-benzyl-5-(trifluoromethyl)-1H-benzo[d]imidazole, 48

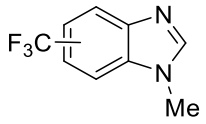
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H, H4), 8.06 (s, 1H, H2), 7.49 (dd, *J* = 8.6, 1.2 Hz, 1H, H6), 7.37 (d, *J* = 3.7 Hz, 1H, H7), 7.36 – 7.32 (m, 2H, 2 xH13), 7.20 – 7.15 (m, 2H, 2 × H12), 5.39 (s, 2H, 2 × H10); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.1 (C2), 143.6 (C5), 136.0 (C4), 134.9, 129.3, 128.7, 127.2, 125.05 (q, *J* = 32.2 Hz, C5), 124.89 (q, *J* = 271.8 Hz, C15), 120.18 (q, *J* = 3.4 Hz, C4), 118.36 (q, *J* = 4.0 Hz, C6), 110.7, 49.3; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -60.7; **TOF M/Z (ES+)** Found 277.0954 ($C_{15}H_{12}N_2F_3$) Calc. 277.0953, 277.1 [M+H] 100%, 278.1[^{13}C-M+H] 25%; **FTIR** (Neat) 3068.8, 3032.7, 2927.9, 1629.5, 1502.9, 1487.4, 1455.9, 1441.8, 1341.8, 1326.9, 1310.2, 1287.3, 1160.7, 1046.7, 915.8, 869.9, 810.9, 749.0, 698.4, 658.4, 631.2.

1-Benzyl-6-(trifluoromethyl)-1H-benzo[d]imidazole, 49



^{1}H NMR(300 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.91 (d, *J* = 8.5 Hz, 1H), 7.59 (s, 1H), 7.54 (dd, *J* = 8.5, 1.2 Hz, 1H), 7.40 – 7.33 (m, 3H), 7.24 – 7.16 (m, 2H), 5.40 (s, 2H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.6 (C2), 145.1 (C9), 134.8 (C8), 129.4 (C12), 128.8 (q, *J* = 3.2 Hz, C7), 127.3 (C13), 125.6 (q, *J* = 32.4 Hz, C6), 124.8 (q, *J* = 272.0 Hz, C15), 121.1 (q, *J* = 4.2 Hz, C5), 119.5 (C14), 110.7 (C11), 107.9 (C4), 49.2 (C10); **TOF M/Z (ES+)** 277.1 [M+H] 100%, 278.1[^{13}C-M+H] 25%.

1-Methyl-(5/6)-(trifluoromethyl)-1H-benzo[d]imidazole, 50



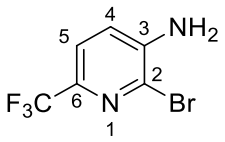
A known compound[138] synthesised *via* an unreported route.

To a stirred solution of 5-(trifluoromethyl)-1H-benzo[d]imidazole (201 mg, 1.08 mmol) in dry THF (5.5 mL) under an argon atmosphere NaH (66 mg, 1.65 mmol, 60% mineral oil dispersion) was added as a single portion at 0 °C and the reaction stirred for 15 minutes before being warmed to r.t. for a further 15 minutes. The reaction was cooled back to 0 °C and MeI (97 µL, 1.57 mmol) was added as a single portion and the reaction warmed to r.t. over 16 hours. NH_4Cl (5 mL, sat. Aq.) was added and the reaction mixture stirred for 10 minutes before being extracted with EtOAc (3 × 50 mL). The combined organic fractions were dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (10% acetone in toluene) to afford the title compounds (200 mg, 93% yield, 1:1 mixture) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.59 (s, 1H), 7.47 (d, *J* = 8.5 Hz, 1H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.21 – 7.16 (m, 1H), 7.10 (d, *J* = 7.3 Hz, 1H), 3.77 (s, 3H, Me), 3.76 (s, 3H, Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.9 (C2/C2'), 145.5 (C2/C2'), 143.1, 137.8, 128.9, 128.2, 125.3, 124.9 (q, *J* = 271.7 Hz, *C*F_3), 124.51 (q, *J* = 32.2 Hz, CF_3-*C*), 120.6, 119.8, 119.7, 119.0, 118.9, 117.9, 117.8, 109.9, 107.3, 107.2, 31.1 (Me); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -60.5, -60.6; **TOF M/Z (ES+)** Found 201.0647 ($C_9H_8N_2F_3$) Calc. 201.0640, 201.1 [M+H] 100%, 202.1 [^{13}C-M+H] 20%.

Analytical data in agreement with literature values. [138]

3-Amino-2-bromo-6-(trifluoromethyl)pyridine, 51



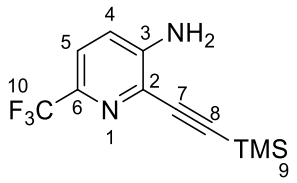
A known compound synthesised according to a literature procedure.[139]

To a stirred solution of 6-(trifluoromethyl)-3-aminopyridine (100 mg, 0.62 mmol) in MeCN (4 mL) at 0 °C under an argon atmosphere, NBS (111 mg, 0.62 mmol) was added as a single portion. The reaction mixture was allowed to warm to room temperature over 3 hours, after which, water (5 mL) was added and the reaction liquor extracted with EtOAc (3 × 5 mL). The combined organics were dried over $MgSO_4$ and solvents were removed under vacuum, purification was achieved by column chromatography (5-20% EtOAc in hexane) to afford the title compound (147 mg, 98% yield) R_f = 0.4 (20% EtOAc in hexane) as pale brown bladed-crystals.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.2 Hz, 1H, H5), 7.04 (dd, *J* = 8.2, 0.5 Hz, 1H, H4), 4.44 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.5 (C2), 143.9 (C3), 130.6 (d, J = 274.2 Hz, C7), 128.8 (q, *J* = 35.3 Hz, C6), 121.0 (q, *J* = 11.6 Hz, C5), 120.8 (q, *J* = 2.0 Hz, C4); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -66.50 (s, CF_3).

Analytical data in agreement with literature values. [139]

3-Amino-6-(trifluoromethyl)-2-((trimethylsilyl)ethynyl)pyridine, 52



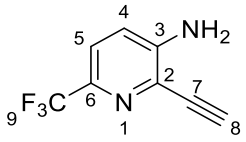
A known compound synthesised according to the literature.[139]

To a stirred suspension of 3-amino-2-bromo-6-(trifluoromethyl)pyridine (133 mg, 0.55 mmol), ethynyltrimethylsilane (93 µL, 0.66 mmol), CuI (5.5mg, 0.03 mmol) in triethylamine (6 mL) and THF (7 mL) under an argon atmosphere, $Pd(PPh_3)_2Cl_2$ (20 mg, 0.03 mmol) was added in a single portion and the reaction mixture was stirred at room temperature for 16 hours. EtOAc (10 mL) was added and the reaction mixture filtered through Celite. The filtrate was separated and the aqueous phase extracted into DCM (3 × 10 mL); the combined organic layers were dried over $MgSO_4$ and solvents removed*in vacuo.* Purification was achieved by column chromatography (50% DCM in hexane) to afford the title compound 132 mg, 93% yield) R_f = 0.15 (50% DCM in hexane) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.5 Hz, 1H, H5), 7.07 (dd, *J* = 8.5, 0.5 Hz, 1H, H4), 4.75 (s, 2H, NH_2), 0.24 (s, *J* = 3.6 Hz, 9H, $SiMe_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.8 (C3), 137.36 (q, *J* = 35.3 Hz, C6), 134.09 (q, *J* = 11.6 Hz, C5), 121.76 (q, *J* = 272.8 Hz, C10), 121.16 (q, *J* = 2.0 Hz, C4), 120.5 (C2), 102.6 (C7), 99.1 (C8), -0.2 (C9); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -66.5.

Analytical data in agreement with literature values. [139]

3-Amino 2-ethynyl-6-(trifluoromethyl)-pyridine, 53



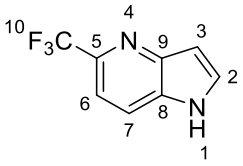
A known compound synthesised according to a literature procedure.[140]

To a stirred solution of 6-(trifluoromethyl)-2-((trimethylsilyl)ethynyl)-3-aminopyridine (132 mg, 0.51 mmol) in methanol (5 mL) under an argon atmosphere; K_2CO_3 (106 mg, 0.77 mmol) was added as a single portion and the reaction was stirred at room temperature for 2 hours. Following this time the reaction was diluted with water (5 mL), extracted with EtOAc (3 × 5 mL) and the combined organics washed with brine (5 mL) then dried over $MgSO_4$. Solvent was removed under vacuum and purification achieved by column chromatography (50% EtOAc in hexane) to afford the product (50 mg, 53% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.5 Hz, 1H, H5), 7.08 (dd, *J* = 8.5, 0.4 Hz, 1H, H4), 4.63 (s, 2H, NH_2), 3.55 (s, 1H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.8 (C3), 136.8 (C2), 137.1 (q, *J* = 35.3 Hz, C6), 127.1 (q, *J* = 11.6 Hz, C5), 121.6 (q, *J* = 272.8 Hz, C9), 120.8 (q, *J* = 2.0 Hz, C4), 84.1 (C7), 78.7 (C8);**^{19}F NMR** (282 MHz, $CDCl_3$) δ -66.7.

Analytical data in agreement with literature values. [140]

5-(Trifluoromethyl)-1*H*-pyrrolo[3,2-*b*]pyridine, 54



A known compound synthesised according to a literature procedure[140]

V. Via 3-Amino 2-ethynyl-6-(trifluoromethyl)-pyridine

To a stirred solution of 3-amino 2-ethynyl-6-(trifluoromethyl)-pyridine (50 mg, 0.27 mmol) in *N*-Methyl-2-pyrrolidone (1mL), ^{t}BuOK (60 mg, 0.54 mmol) was added under an inert atmosphere at room temperature. After 16 hours the reaction was diluted with brine (2 mL), extracted into EtOAc (3 × 3 mL) and combined organic layers were dried over $MgSO_4$. The solvents were removed under vacuum and purification achieved by column chromatography (50% EtOAc in hexane) to afford the title compound (19 mg, 38% yield) as a white solid.

VI. Via 3-Amino-6-(trifluoromethyl)-2-((trimethylsilyl)ethynyl)pyridine

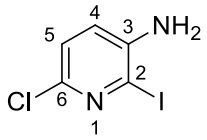
To a stirred solution of 3-amino-6-(trifluoromethyl)-2-((trimethylsilyl)ethynyl)pyridine (20 mg, 0.11 mmol) in *N*-Methyl-2-pyrrolidone (1.1 mL) under an argon atmosphere; ^{t}BuOK (24 mg, 0.22 mmol) was added as a single portion at room temperature. The reaction mixture was stirred for 16 hours, then quenched with brine (3 mL), extracted into Et_2O (3 × 3 mL) and the combined organic layers were dried over $MgSO_4$. Solvents were removed under vacuum and purification

achieved by column chromatography (25% EtOAc in hexane) to afford the product as a white solid (5mg, 39% yield).

^{1}H NMR (300 MHz, MeOD) δ 7.98 (d, *J* = 8.5 Hz, 1H, H7), 7.77 (d, *J* = 3.3 Hz, 1H, H3), 7.55 (d, *J* = 8.5 Hz, 1H, H6), 6.72 (dd, *J* = 3.3, 0.9 Hz, 1H, H2); **^{13}C NMR** (101 MHz, MeOD) δ 147.2 (C9), 141.4 (q, *J* = 33.5 Hz, C5), 133.2 (C2), 131.8 (C8), 124.0 (q, *J* = 272.6 Hz, C10), 120.7 (C7), 113.9 (q, *J* = 2.7 Hz, C6), 103.0 (C3); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -65.67 (s, CF_3); **TOF M/Z (ES+)** 187.1 [M+H] 100%, 188.1 [^{13}C-M+H] 10%.

Analytical data in agreement with literature values. [140]

3-Amino-6-chloro-2-Iodopyridine, 55



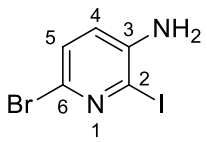
A known compound[139] synthesised *via* an unreported procedure.

To a stirred suspension of 3-amino-6-chloropyridine (100mg, 0.78 mmol) and $CaCO_3$ (87 mg, 0.86 mmol) in methanol (3.6 mL) and DCM (1.2 mL) under an argon atmosphere; benzyltrimethylammonium dichloroiodate (298 mg, 0.86 mmol) was added in 4 portions over 2 hours whilst the reaction was shielded from light with aluminum foil. Following the final addition, the reaction was stirred at room temperature for a further 6 hours, filtered, dried over $MgSO_4$ and solvents were removed under vacuum. Purification was achieved with column chromatography (50% DCM in Hexane) to afford the product (75 mg, 39% yield) as purple crystals.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (d, *J* = 8.3 Hz, 1H, H5), 6.90 (d, *J* = 8.3 Hz, 1H, H4), 3.84 (s, 2H, NH_2); **TOF M/Z (ES+)** 255.1 [^{35}Cl-M+H] 100%, 257.1 [^{37}Cl-M+H] 30%.

Analytical data in agreement with literature values. [139]

3-Amino-6-bromo-2-iodopyridine, 56



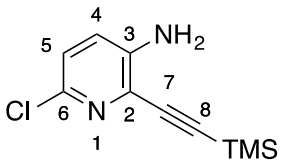
A known compound[141] synthesised *via* an unreported procedure.

To a stirred suspension of 6-Bromo-3-aminopyridine (100mg, 0.58 mmol) and $CaCO_3$ (64 mg, 0.64 mmol) in methanol (3.6 mL) and DCM (1.2 mL) under an argon atmosphere; benzyltrimethylammonium dichloroiodate (221 mg, 0.64 mmol) was added in 4 portions over 2 hours whilst the reaction was shielded from light with aluminum foil. Following the final addition, the reaction was stirred at room temperature for a further 6 hours, filtered, dried over $MgSO_4$ and solvents were removed under vacuum. Purification was achieved with column chromatography (50% DCM in hexane) to afford the product (54 mg, 31%) as a brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, *J* = 8.3 Hz, 1H, H3), 6.81 (d, *J* = 8.3 Hz, 1H, H4), 3.72 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 144.3 (C3), 127.7 (C5), 127.2 (C4), 122.7 (C6), 105.9 (C2); **FTIR** (Neat) 3297, 2938, 2852, 1645, 1586, 1545, 1501, 1026.

Analytical data in agreement with literature values. [141]

3-Amino-6-chloro-2-((trimethylsilyl)ethynyl)pyridine, 57



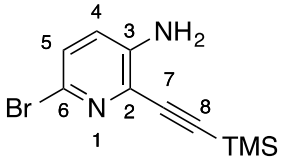
A known compound prepared according to a literature procedure.[139]

To a stirred suspension of 3-Amino-6-chloro-2-Iodopyridine (75 mg, 0.30 mmol), ethynyltrimethylsilane (34 µL, 0.35 mmol), CuI (5.7 mg, 0.03 mmol) triethylamine (3.2 mL) and THF (3.5 mL) under an argon atmosphere, $Pd(PPh_3)_2Cl_2$ (21 mg, 0.03 mmol) was added in a single portion and the reaction mixture was stirred at room temperature for 16 hours. EtOAc (5 mL) was added and the mixture filtered through Celite. The organic layer was separated and the aqueous phase extracted into DCM (3 × 5 mL), the combined organic layers were dried over $MgSO_4$ and the solvents removed *in vacuo*. Purification was achieved by column chromatography (50% DCM in hexane) to afford the title compound (28 mg, 42% yield) as a dark brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (d, *J* = 8.5 Hz, 1H, H5), 6.99 (d, *J* = 8.5 Hz, 1H, H4), 4.09 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 144.0 (C3), 139.2 (C5), 127.3 (C4), 124.9 (C6), 124.3 (C2), 102.4 (C7), 99.3 (C8), -0.07 ($SiMe_3$); **TOF M/Z (ES+)** 279.1 [^{35}Cl-M+H+$(H_2O)_3$] 100%, 225.1 [^{35}Cl-M+H] 25%, 227.1 [^{37}Cl-M+H] 10%.

Analytical data in agreement with literature values. [139]

3-Amino-6-Bromo-2-((trimethylsilyl)ethynyl)pyridine, 58

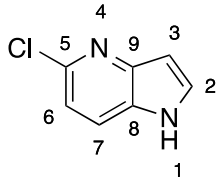


A novel compound.

To a stirred suspension of 3-Amino-6-bromo-2-iodopyridine (96 mg, 0.32 mmol), ethynyltrimethylsilane (49 µL, 0.35 mmol), CuI (3.5 mg, 0.02 mmol) in triethylamine (3.5 mL) and THF (3.3 mL) under an argon atmosphere $Pd(PPh_3)_2Cl_2$ (11 mg, 0.02 mmol) was added and the reaction mixture was stirred at room temperature for 16 hours. EtOAc (5 mL) was added to the reaction mixture and it was filtered through Celite. The organic layer was separated and the aqueous phase extracted into DCM (3 × 5 mL), the combined organic layers were dried over $MgSO_4$ and then concentrated *in vacuo.* Purification was achieved by column chromatography (50% DCM in hexane) to afford the title compound (83 mg, 97% yield) R_f = 0.65 (40% EtOAc in hexane) as a pale brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, *J* = 8.5 Hz, 1H, C5), 6.92 (d, *J* = 8.5 Hz, 1H, C4), 4.50 (s, 2H, NH_2), 0.19 (s, 9H, $SiMe_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 144.4 (C3), 132.5 (C5), 128.3 (C6), 120.7 (C4), 103.9 (C2), 101.1 (C7), 99.8 (C8), -0.1 ($SiMe_3$)102.4 (C7), 99.3 (C8), -0.1 ($SiMe_3$).

5-Chloro-1*H*-pyrrolo[3,2-*b*]pyridine, 59



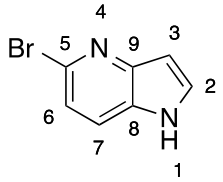
A known compound synthesised according to a literature procedure.[140]

To a stirred suspension of 3-Amino-6-chloro-2-((trimethylsilyl)ethynyl)pyridine (100 mg, 0.45 mmol) and $CaCO_3$ (45 mg, 0.45 mmol) in DMF (2.2 mL) under an argon atmosphere; CuI (43 mg, 0.22 mmol) was added in a single portion and the reaction mixture was heated to 120 °C. After 2 hours, the reaction was cooled to room temperature, diluted with brine (10 mL), extracted into Et_2O (3 × 10 mL) and combined the organic layers were dried over $MgSO_4$. The solvent was removed *in vacuo* and purification achieved by column chromatography (50% DCM in hexane), to afford the title compound (22 mg, 32% yield) R_f = 0.5 (50% DCM in hexane) as a yellow solid.

^{1}H NMR (300 MHz, MeOD) δ 7.80 (dd, *J* = 8.5, 0.8 Hz, 1H, H7), 7.60 (d, *J* = 3.3 Hz, 1H, H3), 7.13 (d, *J* = 8.5 Hz, 1H, H6), 6.53 (dd, *J* = 3.3, 0.8 Hz, 1H, H2); **^{13}C NMR** (101 MHz, MeOD) δ 146.9 (C9), 144.3 (C5), 131.5 (C7), 129.2 (C8), 123.1 (C6), 117.2 (C2), 102.1 (C1); **TOF M/Z (ES+)** 100% 176.1 [^{35}Cl-M+Na], 30% 178.1 [^{37}Cl-M+Na]; **FTIR** (Neat): 3183, 3105, 3038, 2917, 1612, 1548, 1399, 1319, 1110, 898.

Analytical data in agreement with literature values. [140]

5-Bromo-1*H*-pyrrolo[3,2-*b*]pyridine, 60



A known compound that is completely uncharacterized in the literature.

VII. Copper (I) mediated- cyclisation method

To a stirred suspension of 3-amino-6-bromo-2-((trimethylsilyl)ethynyl)pyridine (83 mg, 0.30 mmol) and $CaCO_3$(81 mg, 0.30 mmol) in DMF (6 mL) under an argon atmosphere; CuI (29 mg, 0.15 mmol) was added in a single portion and the reaction mixture was heated at 120 °C. After 2 hours, the reaction was cooled to room temperature, diluted with brine (15 mL), extracted into Et_2O (3 × 15 mL) and combined organic layers were dried over $MgSO_4$. The solvent was removed *in vacuo* and purification achieved by column chromatography (50% DCM in Hexane), to afford the title compound (3 mg, 5% yield) R_f= 0.1 (5% acetone in toluene) as a white solid.

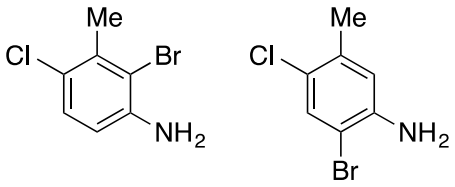
VIII. Base-mediated cyclisation method

To a stirred solution of 3-amino-6-bromo-2-((trimethylsilyl)ethynyl)pyridine (32 mg, 0.12 mmol) in *N*-Methyl-2-pyrrolidone (1.2 mL), ^{t}BuOK (27 mg, 0.24 mmol) was added under an inert atmosphere at room temperature. After 16 hours the reaction was diluted with brine (5 mL), extracted into EtOAc (3 × 5 mL) and the combined organics were dried over $MgSO_4$. The solvent

was removed *in vacuo* and purification achieved by column chromatography (25% EtOAc in Hexane) to afford the product (10 mg, 41% yield) as a white solid.

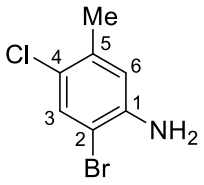
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.73 (br. s, 1H, NH), 7.88 (d, *J* = 8.5 Hz, 1H, H7), 7.55 (d, *J* = 8.5 Hz, 1H, H6), 7.47 (d, *J* = 8.3 Hz, 1H, H2), 7.16 (d, *J* = 8.3 Hz, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 148.3 (C9), 144.3 (C5), 131.8 (C7), 123.7 (C8), 120.0 (C2), 119.4 (C6), 113.6 (C3); **TOF M/Z (ES+)** 214.9 [^{79}Br-M+H^++H_2O] 100%, 216.9 [^{81}Br-M+H^++H_2O] 100%.

2-Bromo-4-chloro-3-methylaniline and 2-bromo-4-chloro-5-methylaniline, 61



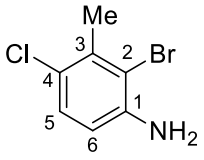
A mixture of known yet un-characterised compounds.

To a stirred solution of 3-chloro-2-methylaniline (200 mg, 1.41 mmol) in MeCN (14 mL) under an argon atmosphere cooled to 0 °C; N-bromosuccinimide (251 mg, 1.41 mmol) was added as a single portion. The reaction mixture was allowed to warm to room temperature over 8 hours and then diluted with water (15 mL). The reaction mixture was extracted with EtOAc (3 × 15 mL), combined organics washed with brine and then dried over $MgSO_4$. Solvent was removed under vacuum and purification achieved by column chromatography (20% DCM in hexane) to afford the title compounds (249 mg, 80% yield, 1:1 ratio of isomers) as a brown oil.



61a

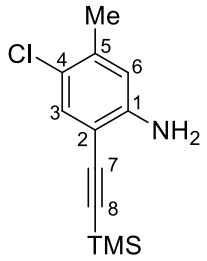
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (s, 1H, H3), 6.64 (d, *J* = 0.5 Hz, 1H, H6), 3.99 (s, 2H, NH_2), 2.24 (s, 3H, Me); **^{13}C-NMR** (101 MHz, $CDCl_3$) δ142.8 (C1), 136.2 (C5), 128.4 (C3), 123.7 (C4), 113.7 (C6), 106.4 (C2), 19.9 (Me); **TOF M/Z (ES+)** Found 219.9523 ($C_7H_8{}^{79}Br\ {}^{35}ClN$) Calc. 219.9529, 221.9 [^{35}Cl-^{81}Br-M+H] 100%, 220.0 [^{35}Cl-^{79}Br-M+H] 80%, 223.9 [^{37}Cl-^{81}Br-M+H] 15%; **FTIR** (Neat) 3412, 3315, 3187, 2920, 1608, 1586, 1454, 1413, 802.



61b

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (d, *J* = 8.6 Hz, 1H, H5), 6.58 (d, *J* = 8.6 Hz, 1H, H6), 4.12 (s, 2H, NH_2), 2.48 (s, 3H, Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 143.3 (C1), 136.3 (C3), 132.0 (C5), 123.4 (C4), 117.5 (C6), 112.7 (C3), 21.2 (3-Me).

4-Chloro-5-methyl-2-((trimethylsilyl)ethynyl)aniline, 62

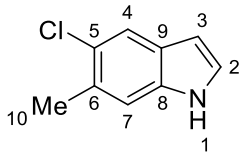


A novel compound

To a stirred suspension of 2-bromo-4-chloro-3-methylaniline and 2-bromo-4-chloro-5-methylaniline (1:1 mix of regioisomers, 250 mg, 1.13 mmol), ethynyltrimethylsilane (157 µL, 1.13 mmol), CuI (11mg, 0.057 mmol) in triethylamine (23 mL) under an argon atmosphere; $Pd(PPh_3)_2Cl_2$ (40 mg, 0.057mmol) was added in a single portion and the reaction mixture was stirred at 70 °C for 16 hours. The reaction was cooled to room temperature filtered through Celite followed by DCM wash (3 × 25 mL) to the Celite bed and reaction flask. The combined organic layers were washed with brine (50 mL), then combined organic layers were dried over $MgSO_4$ and solvents removed under vacuum. Purification was achieved by column chromatography (25% DCM in hexane) to afford the title compound (40 mg, 12% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, *J* = 2.4 Hz, 1H, H3), 6.98 (d, *J* = 2.0 Hz, 1H, H6), 4.19 (s, 2H, NH_2), 2.13 (s, 3H, Me), 0.26 (s, 9H, $SiMe_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.3 (C1), 132.2 (C5), 130.8 (C3), 123.3 (C4), 121.8 (C6), 108.8 (C7), 101.0 (C8), 17.6 (5-Me), 0.2 ($SiMe_3$); **TOF M/Z (ES+)** Found 238.0826 ($C_{12}H_{17}N^{35}ClSi$) Calc. 238.0819, 238.1 [^{35}Cl-M+H] 100%, 240.1 [^{37}Cl-M+H] 25%; **FTIR**: 3469, 3347, 2958, 2901, 2146, 1612, 1488, 1246, 1004, 833.

5-Chloro-6-methyl-1*H*-indole, 63



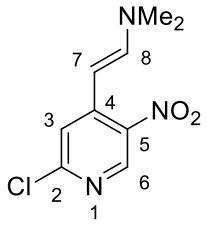
A known compound[142] synthesised via a novel procedure.

To a stirred suspension of 4-chloro-5-methyl-2-((trimethylsilyl)ethynyl)aniline (71 mg, 0.3 mmol), $CaCO_3$ (30 mg, 0.30 mmol) in DMF (3 mL) at room temperature under an argon atmosphere; CuI (28 mg, 0.15 mmol) was added as a single portion. The reaction mixture was heated to 120 °C for 16 hours and then cooled to room temperature. Brine (5 mL) was added and the reaction mixture extracted with Et_2O (3 × 5 mL), the combined organic layers were dried over $MgSO_4$ and then concentrated *in vacuo*. Purification was achieved by column chromatography (30% EtOAc in hexane), to afford the product (19 mg, 38% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.44 (s, 1H, H4), 7.33 – 7.27 (m, 1H, H2), 7.24 – 7.15 (m, 2H, H3 & H7), 2.47 (s, 3H, Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 134.5 (C8), 131.0 (C6), 129.3 (C5), 128.9 (C2), 127.7 (C9), 120.8 (C4), 112.7 (C7), 109.9 (C3), 20.7 (C10); **TOF M/Z (ES+)** 323.0 [^{35}Cl-M+H] 100%, 325.0 [^{37}Cl-M+H] 25%; **FTIR** (Neat) 3398, 2976, 2902, 2339, 1452, 1333, 1091, 686.

Analytical data in agreement with literature values. [142]

(*E*)-2-(2-Chloro-5-nitropyridin-4-yl)-*N*,*N*-dimethylethen-1-amine, 64



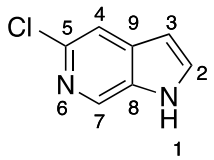
A known compound synthesised according to a literature procedure.[143]

To a stirred solution of 2-chloro-4-methyl-5-nitropyridine (400 mg, 2.32 mmol) in DMF (23 mL) under an argon atmosphere; dimethylformamide-*N,N*-dimethylacetal (0.68 mL, 5.1 mmol) was added as a single portion and reaction mixture was heated to 90 °C for 18 hours. Brine (40 mL) was added and the reaction liquor was extracted with Et_2O (3 × 40 mL) and combined organic layers were dried over $MgSO_4$. The solvents were removed *in vacuo* and purification achieved by column chromatography (40% EtOAc in hexane) to afford the product (303 mg, 57% yield) as a brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (s, 1H, H6), 7.33 (d, *J* = 13.2 Hz, 1H, H8), 7.25 (s, 1H, H3), 5.96 (d, *J* = 13.2 Hz, 1H, H7), 3.06 (s, 6H, NMe_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 167.6 (C2), 153.7 (C6), 149.0 (C4), 148.3 (C4), 134.6 (C3), 115.4 (C8), 88.3 (C7), 41.3 ($N(CH_3)_2$); **TOF M/Z (ES+)** 228.1 [^{35}Cl-M+H] 100%, 230.1 [^{37}Cl-M+H] 25%; **FTIR** (Neat) 3391, 3106, 2917, 1663, 1625, 1574, 1212, 1045, 939, 762.

Analytical data in agreement with literature values. [143]

5-Chloro-1*H*-pyrrolo[2,3-c]pyridine, 65



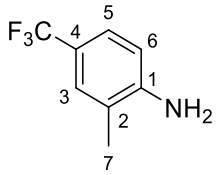
A known compound synthesised according to a literature procedure.[143]

To a stirred solution of (*E*)-2-(2-chloro-5-nitropyridin-4-yl)-*N*,*N*-dimethylethen-1-amine (300 mg, 1.32 mmol) in acetic acid (glacial, 15 mL); zinc dust (500 mg, 7.65 mmol) was added as a single portion and the reaction was heated to 118 °C for 16 hours. The reaction was cooled to room temperature then filtered through Celite, the reaction liquor was concentrated under reduced pressure, washed with NaOH (1M, 10 mL) and extracted into EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ then concentrated *in vacuo* and purification was achieved *via* column chromatography (50% EtOAc in hexane) to afford the product (127 mg, 63% yield) R_f = 0.15 (40% EtOAc in hexane) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.28 (br. s, 1H, NH), 8.62 (s, 1H, H7), 7.57 (s, 1H, H4), 7.53 – 7.45 (m, 1H, H2), 6.54 (d, *J* = 2.1 Hz, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 140.6 (C8), 136.2 (C5), 133.1 (C9), 132.5 (C6), 130.5 (C2), 114.9 (C4), 102.1 (C3); **TOF M/Z (ES+)** Found 153.0216 ($C_7H_6{}^{35}ClN_2$) Calc. 153.0220, 153.0 [^{35}Cl-M+H] 100%, 155.0 [^{35}Cl-M+H] 40%; **FTIR** (Neat) 3185, 3126, 3053, 2998, 2894, 2864, 1573, 1283, 877, 732.

Analytical data in agreement with literature values. [143]

2-Methyl-4-(trifluoromethyl)aniline, 66



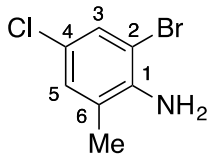
A known compound synthesised *via* an unreported procedure.

To a stirred solution of 2-methyl-1-nitro-4-(trifluoromethyl)benzene (316 mg, 1.46 mmol) in MeOH (10 mL, argon degassed) Pd/C (100 mg, 0.06 mmol, 10 wt. %) was added under an argon atmosphere. The suspension was then subjected to a flow of H_2 bubbles introduced *via* a B Braun Sterican needle (0.8 × 120 mm), inserted *via* septum from the top to the bottom of the flask attached to a balloon containing H_2, whilst the septum was vented *via* another B Braun Sterican needle. The introduction of H_2 gas in this way accelerates the displacement of the argon dissolved in the solution and promotes faster reaction times. N.B. it is wise not to stir at this point as the sediment will likely block the needle. Following *circa* 10 minutes of H_2 bubbling, the gas injection needle is withdrawn from the solution meniscus and the reaction allowed to stir for 48 hours. N.B. the hydrogen balloon was refilled once per day due to deflation. The reaction was followed by LC/MS until the presence of nitro and hydroxylamine compounds were no longer detectable then the H_2 inlet was replaced with argon and the solution again degassed with argon for 5 minutes. Following this time the reaction was filtered through Celite and concentrated *in vacuo* to afford the title compound, (209 mg, 82% yield) without the need for further purification, as a clear yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (s, H3), 7.28 (d, *J* = 8.5 Hz, 1H, H5,), 6.68 (d, *J* = 8.5 Hz, 1H, H6), 3.88 (s, 2H, NH_2), 2.19 (s, 3H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 147.7 (C1), 127.6 (q, *J* = 3.9 Hz, C5), 125.01 (q, *J* = 270.5 Hz, CF_3), 124.4 (q, *J* = 3.4 Hz C3), 121.9 (C2), 120.26 (q, *J* = 32.2 Hz,C4), 114.1 (C6), 17.4 (C7); **TOF M/Z (EI+)** Found 175.0603 ($C_8H_8NF_3$) Calc. 175.0609; **FTIR** (Neat) 3493.4, 3404.8, 2937.9, 1627.4, 1518.4, 1325.3, 1297.9, 1194.3, 1143.8, 1099.1, 1078.0, 994.4, 900.8, 820.3, 736.9.

Analytical data in agreement with literature values.

2-Bromo-4-chloro-6-methylaniline, 67



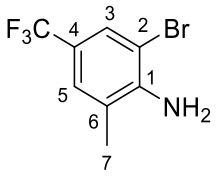
A known compound synthesised according to a literature procedure.[144]

To a stirred solution of 4-chloro-2-methylaniline (300 mg, 2.12 mmol) in MeCN (22 mL) at 0 °C under an argon atmosphere, NBS (377 mg, 2.12 mmol) was added as a single portion. The reaction mixture was allowed to warm to room temperature over three hours, after which, water (22 mL) was added and the reaction liquor extracted with EtOAc (3 × 40 mL). The combined organic layers were dried over $MgSO_4$ and solvents were removed *in vacuo*, purification was achieved by column chromatography (30% EtOAc in Hexane) to afford the product (434 mg, 99% yield) R_f = 0.85 (40% EtOAc in hexane) as an orange crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (d, *J* = 2.1 Hz, 1H, H5), 6.99 (d, *J* = 2.1 Hz, 1H, H3), 4.04 (s, 2H, NH_2), 2.19 (s, 3H, Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 141.2 (C1), 129.6 (C6), 129.4 (C5), 124.6 (C3), 122.6 (C4), 109.2 (C2), 18.4 (Me); **TOF M/Z (ES+)** Found 219.9530 ($C_7H_8{}^{79}Br^{35}ClN$) Calc. 219.9529, 222.0 [^{79}Br-^{35}Cl-M+H] 100%, 224.0 [^{81}Br-^{35}Cl-M+H] 100%.

Analytical data in agreement with literature values. [144]

2-Bromo-6-methyl-4-(trifluoromethyl)aniline, 68



A known compound prepared *via* an unreported synthesis.

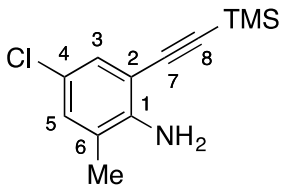
To a stirred solution of 2-methyl-4-(trifluoromethyl)aniline (189 mg, 1.08 mmol) in MeCN (7.5 mL) NBS (139 mg, 0.78 mmol) was added as a single portion and the reaction was stirred at r.t. for 16 hours under an argon atmosphere. The reaction was diluted with EtOAc (30 mL) then washed with $Na_2S_2O_3$ (2 × 25 mL, Sat. Aq.) then the organic phase dried over $MgSO_4$ and concentrated *in vacuo*. Purification was achieved *via* column chromatography (20% EtOAc in hexane) to afford the title compound (196 mg, 72% yield) R_f = 0.75 (20% EtOAc in hexane) as a waxy white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, 1H, H3), 7.24 (s, 1H, H5), 4.37 (br. s, 2H, NH_2), 2.24 (s, 3H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.3 (C1), 127.7 (C6), 126.4 (q, *J* = 3.3 Hz, C3), 124.1 (q, *J* = 271.0 Hz, CF_3), 122.9 (q, *J* = 3.9 Hz, C5), 120. 6 (q, *J* = 32.9 Hz, C4), 108.2 (C2), 18.4 (C7); **^{19}F NMR** (282 MHz,

$CDCl_3$) δ -61.2; **TOF M/Z (EI+)** Found 252.9716 ($C_8H_7NF_3{}^{79}Br$) Calc. 252.9714, 252.95 [^{79}Br-M^+] 100%, 254.95 [^{81}Br-M^+] 95%; **FTIR** (Neat) 3500.1, 3401.7, 2983.9, 2932.6, 2857.8, 1622.8, 1329.0, 1319.8, 1287.8, 1182.3, 1152.3, 1096.9, 881.7, 762.0, 664.3.

Analytical data in agreement with literature values.

4-Chloro-2-methyl-6-((trimethylsilyl)ethynyl)aniline, 69



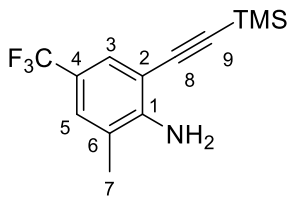
A known compound[145]synthesised *via* an unreported procedure

To a stirred suspension of 2-bromo-4-chloro-6-methylaniline (398 mg, 1.98 mmol), ethynyltrimethylsilane (274 µL, 1.98 mmol), CuI (38 mg, 0.20 mmol) triethylamine (9 mL) under an argon atmosphere, $Pd(PPh_3)_2Cl_2$ (139 mg, 0.20 mmol) was added in a single portion and the reaction mixture was stirred at 85 °C for 16 hours. EtOAc (15 mL) was added to the reaction mixture and it was filtered through Celite and water (15 mL) added to the filtrate. The organic layer was separated and the aqueous phase extracted (3 × 15 mL EtOAc), the combined organic layers were dried over $MgSO_4$ and solvents removed under vacuum; purification was achieved by column chromatography (20% EtOAc in Hexane) to afford the product (336 mg, 71% yield) R_f = 0.8 (20% EtOAc in hexane) as a brown crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, *J* = 2.4 Hz, 1H, H3), 6.98 (d, *J* = 2.4 Hz, 1H, H5), 4.19 (s, 2H, NH_2), 2.13 (s, 3H, 6-Me), 0.26 (s, 9H, $SiMe_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.3 (C1), 132.2 (C5), 130.8 (C6), 129.2 (C3), 123.3 (C4), 121.8 (C2), 108.8 (C7), 101.0 (C8), 17.6 (6-Me), 0.2 ($SiMe_3$); **TOF M/Z (ES+)** Found 238.0804 ($C_{12}H_{17}{}^{35}ClNSi$) Calc. 238.0819, 238.1 [^{35}Cl-M+H] 100%, 240.1 [^{37}Cl-M+H] 25%.

Analytical data in agreement with literature values. [145]

2-Methyl-4-(trifluoromethyl)-6-((trimethylsilyl)ethynyl)aniline, 70

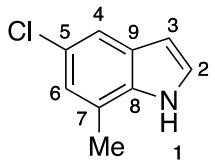


A novel compound.

To a solution of 2-bromo-6-methyl-4-(trifluoromethyl)aniline (241 mg, 0.95 mmol), ethynyltrimethylsilane (263 µL, 1.9 mmol) NEt_3 (398 µL, 2.85 mmol) and CuI (19 mg, 0.10 mmol) in DMF (4.7 mL; $Pd(PPh_3)_2Cl_2$ (34 mg, 0.04 mmol) was added as a single portion with stirring under and argon atmosphere and the reaction was heated to 120 °C for 4 h. The reaction was then diluted with EtOAc (50 mL) and filtered through a Celite pad, the filtrate was washed with brine (3 × 50 mL) then organics dried over $MgSO_4$ and concentrated *in vacuo*. The crude brown resin was purified by column chromatography in (10% EtOAc in hexane) to afford the title product (71 mg, 28% yield) R_f = 0.45 (10% EtOAc in hexane) as a brown solid as well as a brown oil R_f=0.15 (10% EtOAc in hexane) which is a complex mixture of several compounds (115 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (s, 1H, H3), 7.23 (s, 1H, H5), 4.52 (s, 2H, NH_2), 2.18 (s, 3H, H7), 0.28 (s, 9H, TMS); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 149.1 (C1), 127.5 (C6), 127.5 (q, *J* = 3.4 Hz, C3), 124.6 (q, *J* = 270.8 Hz, CF_3), 121.4 (q, *J* = 4.2 Hz, C5), 119.3 (q, *J* = 32.8 Hz, C4), 107.1 (C2), 101.0 (C8), 100.8 (C9), 17.7 (C7), 0.2 ($SiMe_3$); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -61.4; **TOF M/Z (EI+)** Found 271.1005 ($C_{13}H_{16}NF_3Si$) Calc. 271.1004, 256.10 [M^+ -Me] 100%, 271.12 [M^+] 80%, **FTIR** (Neat) 3517.8, 3403.5, 2967.1, 2137.3, 1618.8, 1349.9, 1219.9, 1146.2, 1098.1, 908.1, 836.6, 759.1, 750.8, 707.6, 651.9; **M.P.** (From EtOAc) 48-50 °C.

5-Chloro-7-methyl-1*H*-indole, 71



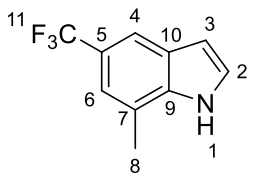
A known compound[146] synthesised *via* an unreported procedure.

To a stirred solution of 4-Chloro-2-methyl-6-((trimethylsilyl)ethynyl)aniline (216 mg, 0.91 mmol) in NMP (1 mL) under an argon atmosphere; CuI (43 mg, 0.23 mmol) was added in a single portion and the reaction mixture was heated to 180 °C in a CEM 5 mL sealed microwave vessel. After 6 hours, the reaction was cooled to room temperature, diluted with brine (40 mL), extracted into Et_2O (3 × 40 mL) and combined organic layers were dried over $MgSO_4$. The solvent was removed under vacuum and purification achieved by column chromatography (20% EtOAc in hexane), to afford the product (147 mg, 98% yield) R_f = 0.4 (20% EtOAc in hexane) as a brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (s, 1H, NH), 7.51 (dd, *J* = 3.2, 0.5 Hz, 1H, H2), 7.23 – 7.16 (m, 1H, H6), 7.01 (d, *J* = 0.8 Hz, 1H, H4), 6.53 (dd, *J* = 3.2, 2.1 Hz, 1H, H3), 2.45 (s, 3H, Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 133.9 (C8), 128.4 (C9), 125.4 (C5), 125.2 (C2), 122.7 (C7), 121.8 (C6), 117.8 (C4), 102.9 (C3), 16.5 (Me); **TOF M/Z (EI+)** Found 165.0339 ($C_9H_8N^{35}Cl$), Calc. 165.1345, 165.0 [^{35}Cl-M^+] 100%, 167.0 [^{37}Cl-M^+] 50%; **FTIR** (Neat) 3399.0, 2977.9, 2911.6, 1609.6, 1588.7, 1469.9, 1448.3, 1432.1, 1411.6, 1393.6, 1377.9, 1333.6, 1286.5, 1120.9, 1082.1, 881.6, 849.3, 805.6, 753.5; **M.P.** (From EtOAc) 106-108 °C.

Analytical data in agreement with literature values. [146]

7-Methyl-5-(trifluoromethyl)-1H-indole, 72



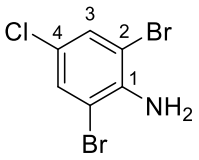
A novel compound.

To a stirred solution of 2-methyl-4-(trifluoromethyl)-6-((trimethylsilyl)ethynyl)aniline (75 mg, 0.28 mmol) and $CaCO_3$ (28 mg, 0.28 mmol) in DMF (1.3 mL) under an argon atmosphere; CuI (26 mg, 0.14 mmol) was added in a single portion and the reaction mixture was heated *via* microwave irradiation to 120 °C in a CEM 5 mL sealed microwave vessel. After 16 hours, the reaction was cooled to room temperature, diluted with brine (40 mL), extracted into Et_2O (3 × 40 mL) and combined organic layers were dried over $MgSO_4$. The solvent was removed under vacuum and

purification achieved by column chromatography (10% EtOAc in hexane), to afford the product (30 mg, 55% yield) R_f = 0.25 (10% EtOAc in hexane) as a brown oil.

^{1}H NMR(400 MHz, $CDCl_3$) δ 8.24 (br. s, 1H, H1), 7.82 (s, 1H, H4), 7.34 – 7.29 (m, 1H, H2), 7.25 (s, 1H, H6), 6.66 (dd, *J* = 3.2, 2.1 Hz, 1H, H3), 2.54 (s, 3H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 136.9 (C9), 126.8 (C10), 125.5 (C2), 125.54 (q, *J* = 271.3 Hz, C11), 122.58 (q, *J* = 31.6 Hz, C5), 120.9 (C7), 119.2 (q, *J* = 3.4 Hz, C6), 116.5 (q, *J* = 4.2 Hz, C4), 104.3 (C3), 16.8 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -60.2; **TOF M/Z (EI+)** Found 199.0604 ($C_{10}H_8NF_3$) Calc. 199.0609, 199.07 [M^+] 100%, 200.09 [^{13}C-M^+] 10%.

2,6-Dibromo-4-chloroaniline, 73



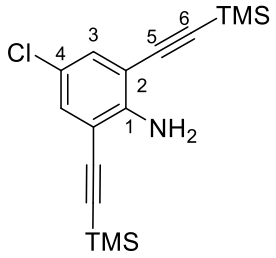
A known compound[147] synthesised *via* an unreported route

To a stirred solution of 4-chloroaniline (200 mg, 1.57 mmol) in MeCN (15 mL) at 0 °C under an argon atmosphere, NBS (559 mg, 3.14 mmol) was added as a single portion. The reaction mixture was allowed to warm to room temperature over 16 hours, after which, water (30 mL) was added and the reaction liquor extracted with EtOAc (3 × 50 mL) and the combined organics were dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (25% EtOAc in hexane) to afford the title compound (395 mg, 88% yield) R_f = 0.8 (25% EtOAc in hexane) as dark brown bladed-crystals.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (s, 2H, H3), 4.55 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 140.1 (C1), 131.4 (C3), 122.9 (C4), 108.6 (C2); **TOF M/Z (AP+)** 284.9 [^{35}Cl-^{81}Br-M+H] 100%, 286.9 [^{37}Cl-^{81}Br-M+H] 80%, 282.9 [^{35}Cl-^{79}Br-M+H] 30%.

Analytical data in agreement with literature values. [147]

4-Chloro-2,6-bis((trimethylsilyl)ethynyl)aniline, 74

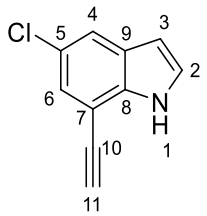


A novel compound.

To a stirred suspension of 2,6-dibromo-4-chloroaniline (395 mg, 1.91 mmol), ethynyltrimethylsilane (556 µL, 4.02 mmol), CuI (37 mg, 0.191 mmol) triethylamine (20 mL) under an argon atmosphere, $Pd(PPh_3)_2Cl_2$ (134 mg, 0.19 mmol) was added in a single portion and the reaction mixture was stirred at 80 °C for 16 hours. EtOAc (15 mL) was added to the reaction mixture and it was filtered through Celite and water (15 mL) added to the filtrate. The organic layer was separated and the aqueous phase extracted into EtOAc (3 × 15 mL), the combined organic layers were dried over $MgSO_4$ and the solvents were removed under vacuum, purification was achieved *via* column chromatography (10% EtOAc in hexane) to afford the product (146 mg, 24% yield) R_f = 0.85 (25 % EtOAc in hexane) as a brown oil.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.21 (s, 2H, H3), 4.81 (br. s, 2H, NH_2), 0.25 (s, 18H, 2 × $SiMe_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 148.6 (C1), 132.2 (C3), 121.0, (C4) 108.7 (C2), 101.7 (C5), 99.9 (C6), 0.1 ($SiMe_3$); **TOF M/Z (ES+)** Found 320.1046 ($C_{16}H_{23}Si_2{}^{35}Cl$) Calc. 320.1058, 320.1 [^{35}Cl-M+H] 100%, 322.1 [^{37}Cl-M+H] 25%; **FTIR** (Neat) 3388.9, 2963.2, 2901.8, 2066.9, 1713.7, 1248.0, 838.4, 781.5, 703.4.

5-Chloro-7-ethynyl-1H-indole, 75



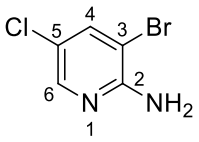
A novel compound

To a solution of 4-chloro-2,6-bis((trimethylsilyl)ethynyl)aniline (107 mg, 0.26 mmol) in NMP (1 mL) ^{t}BuOK (58 mg, 0.51 mmol) was added in a single portion then the reaction was heated to 80 °C and stirred under an argon atmosphere for 4 h. The reaction was cooled to room temperature, water (15 mL) was added and the mixture was extracted with EtOAc (3 × 10 mL). the combined organic fractions were dried over $MgSO_4$ then concentrated *in vacuo* and the crude brown solid was purified by column chromatography (40% EtOAc in Petrol (40-60 °C)) to afford the title compound (23 mg, 62% yield) R_f= (50% DCM in hexane) as a clear yellow oil.

^{1}H-NMR (300 MHz; $CDCl_3$) δ 8.48 (1 H, s, H1), 7.63 (1 H, d, *J* = 1.8 Hz, H6), 7.33 (1 H, d, *J* = 1.8 Hz, H4), 7.27 (1 H, d, *J* = 2.7 Hz, H2), 6.53 (1 H, t, *J* = 2.7 Hz, H3), 3.41 (1 H, s, H11); **^{13}C-NMR** (101 MHz;

$CDCl_3$) δ 135.5 (C8), 128.6 (C9), 125. 9 (C5), 125.8 (C2), 125.2 (C6), 121.7 (C4), 106.1 (C7), 103.3 (C3), 82.3 (C10), 79.1 (C11); TOF MS (ES+) Found 175.0191 ($C_{10}H_6$ ^{35}ClN) calc. 175.0189; **FTIR** (Neat) 3675.3 (NH_2), 2969.5, 2169.3 (C≡C sharp, C-H), 1644.9, 1473.1, 1456.6, 1391.2, 1361.2, 1287.0, 1220.6, 900.85, 855.5, 720.

2-Amino-3-bromo-5-chloropyridine, 76



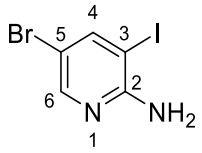
A known compound synthesised *via* an unreported procedure[148]

To a stirred solution of 2-amino-5-chloropyridine (100 mg, 0.78 mmol) in MeCN (8 mL) at 0 °C under an argon atmosphere, *N*-bromosuccinimide (138 mg, 0.78 mmol) was added in a single portion. The reaction mixture was allowed to warm to room temperature over 3 hours, after which water (8 mL) was added and the reaction liquor extracted into EtOAc (3 × 8 mL). The combined organic layers were dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via*column chromatography (15% EtOAc in hexane) to afford the product (115 mg, 71% yield) R_f = 0.6 (40% EtOAc in hexane) as a brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, *J* = 2.2 Hz, 1H, H6), 7.66 (d, *J* = 2.2 Hz, 1H, H4), 4.92 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.6 (C2), 141.8 (C6), 139.7 (C4), 120.6 (C5), 104.2 (C3); **TOF M/Z (ES+)** 208.9 [^{81}Br-^{35}Cl-M+H] 100%, 206.9 [^{79}Br-^{35}Cl-M+H] 80%, 210.9 [^{81}Br-^{37}Cl-M+H] 20%; **FTIR** (Neat) 3443, 3426, 3361, 3323, 3062, 2891, 2911, 1613, 1583, 1558, 1467, 854.

Analytical data in agreement with literature values. [148]

5-Bromo-3-iodopyridin-2-amine, 77



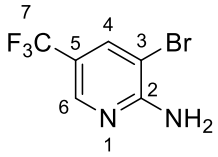
A known compound synthesised according to a literature procedure.[149,150]

To a solution of 5-bromopyridin-2-amine (332 mg, 1.88 mmol) and periodic acid (127 mg, 0.56 mmol) in MeCN/AcOH (20 mL, 50% AcOH) under an argon atmosphere iodine (212 mg, 0.84 mmol) was added as a single portion and the reaction heated to 50 °C for 4 hours. NaOH (10 mL, 28% w/v) was added and the mixture stirred for 10 minutes; the resulting suspension was filtered to afford a brown solid. The filtrand was washed with MeCN/H_2O (3 × 10 mL, 50% MeCN) which afforded the title compound (124 mg, 22% yield) R_f = 0.65 (40% EtOAc in hexane) as a brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, *J* = 1.8 Hz, 1H, H4), 7.94 (d, *J* = 1.8 Hz, 1H, H6), 5.01 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 156.6 (C2), 148.7 (C6), 148.3 (C4), 107.4 (C5), 77.7 (C3); **TOF M/Z (ES+)** Found 298.8686 ($C_5H_5N_2I^{79}Br$) Calc. 298.8681, 298.9 [^{79}Br-M+H] 100%, 300.8 [^{81}Br-M+H] 100%; **FTIR** (Neat) 3676.0, 3445.8, 3278.3, 3126.6, 2988.8, 2901.6, 1626.6, 1566.3, 1453.9, 1238.0, 1021.2, 898.6, 741.7, 676.5; **M.P.** (From EtOAc) 112-114 °C.

Analytical data in agreement with literature values. [150]

3-Bromo-5-(trifluoromethyl)pyridin-2-amine, 78



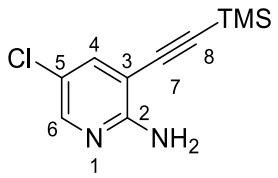
A known compound[151] synthesised *via* an unreported route.

To a stirred solution of 5-(trifluoromethyl)-2-aminopyridine (200 mg, 1.23 mmol) in MeCN (12 mL) at 0 °C under an argon atmosphere, NBS (220 mg, 1.23 mmol) was added as a single portion. The reaction mixture was allowed to warm to room temperature over 3 hours, after which, water (15 mL) was added and the reaction liquor extracted with EtOAc (3 × 50 mL) and the combined organics were dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (5-20% EtOAc in hexane) to afford the title compound (220 mg, 74% yield) R_f = 0.4 (20% EtOAc in hexane) as pale brown bladed-crystals.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, *J* = 1.4 Hz, 1H, H6), 7.86 (d, *J* = 1.4 Hz, 1H, H4), 5.32 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 158.7 (C2) ,144.8 (C4), 137.5 (C6), 127.9 (q, *J* = 34.5 Hz, C5), 120.3 (q, *J* = 298.5 Hz, C7), 103.6 (C3); **TOF M/Z (ES+)** Found 240.9597 ($C_6H_5N_2F_3{}^{79}Br$) Calc. 240.9588, 243.0 [^{81}Br-M+H] 100%, 241.0 [^{79}Br-M+H] 80%, 243.9 [^{81}Br-^{13}C-M+H] 10%; **FTIR** (Neat) 3486.9, 3295.9, 3159.3, 1633.3, 1598.3, 1497.6, 1316.5, 1291.5, 1260.5, 1154.9, 1086.2, 1031.2, 914.6, 940.3, 914.6, 754.56, 686.9; **M.P.** (From EtOAc) 74-76 °C.

Analytical data in agreement with literature values. [151]

5-Chloro-3-((trimethylsilyl)ethynyl)pyridin-2-amine, 79



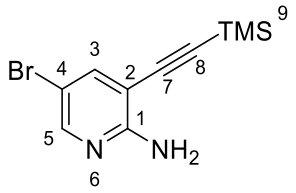
A known compound synthesised according to a literature procedure.[152]

To a stirred suspension of 2-amino-3-bromo-5-chloropyridine (329 mg, 1.76 mmol), ethynyltrimethylsilane (240 µL, 1.76 mmol), CuI (34 mg, 0.18 mmol) triethylamine (8 mL) under an argon atmosphere, $Pd(PPh_3)_2Cl_2$ (124 mg, 0.18 mmol) was added in a single portion and the reaction mixture was stirred at 70 °C for 16 hours. EtOAc (15 mL) was added to the reaction mixture and it was filtered through Celite and water (15 mL) added to the filtrate. The organic layer was separated and the aqueous phase extracted into EtOAc (3 × 15 mL), the combined organic layers were dried over $MgSO_4$ and the solvents were removed under vacuum, purification was achieved by column chromatography (15%→20%→25%→30% EtOAc in hexane) to afford the product (331 mg, 83% yield) as a brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 2.4 Hz, 1H, H6), 7.50 (d, *J* = 2.4 Hz, 1H, H4), 5.05 (s, 2H, NH_2); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 157.6 (C2), 146.7 (C6), 139.4 (C4), 120.0 (C5), 104.2 (C3), 102.7 (C7), 99.0 (C8), 0.0 ($SiMe_3$); **TOF M/Z (ES+)** Found 225.0610 ($C_{10}H_{14}{}^{35}ClN_2$) Calc. 225.0615, 225.1 [^{35}Cl-M+H] 100%, 227.1 [^{37}Cl-M+H] 35%; **FTIR** (Neat) 3456, 3289, 3159, 2960, 2150 (C-C), 1623, 1555, 1457, 1249, 929; **M.P.** From EtOAc) 62-64 °C.

Analytical data in agreement with literature values. [152]

5-Bromo-3-((trimethylsilyl)ethynyl)pyridin-2-amine, 80



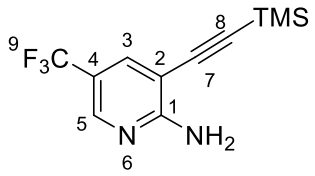
A known compound synthesised according to a literature procedure.[153]

To a degassed suspension of 5-bromo-3-iodopyridin-2-amine (124 mg, 0.42 mmol), ethynyltrimethylsilane (58 µL, 0.46 mmol), CuI (8 mg, 0.04 mmol) and triethylamine (1 mL, freshly distilled) under an argon atmosphere; $Pd(PPh_3)_2Cl_2$ (15 mg, 0.02 mmol) was added as a single portion and the reaction stirred at r.t. for 16 hours. The reaction mixture was diluted with DCM (20 mL), filtered through Celite and the combined organic fractions concentrated *in vacuo*. Purification was achieved *via* column chromatography (20% EtOAc in hexane) to afford the title compound (80 mg, 72% yield) R_f = 0.8 (20% EtOAc in hexane) as an off white solid.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 8.04 (d, *J* = 2.4 Hz, 1H, H3), 7.63 (d, *J* = 2.4 Hz, 1H, H5), 5.10 (br s, 2H, NH_2), 0.26 (s, 9H, $SiMe_3$); **^{13}C-NMR** ($CDCl_3$, 75 MHz): δ 157.8 (C1), 148.6 (C5), 142.1 (C3), 106.9 (C4), 105.0 (C2), 103.0 (C7), 98.8 (C8), -0.0 ($SiMe_3$); **TOF M/Z (ES+)** 271.1 [^{81}Br-M+H] 100%, 269.0 [^{79}Br-M+H] 90%, 272.1 [^{81}Br-^{13}C-M+H] 20%, 270.0 [^{79}Br-^{13}C-M+H] 15%; **M.P.** (From EtOAc) 131 – 132 °C.

Analytical data in agreement with literature values. [153]

5-(Trifluoromethyl)-3-((trimethylsilyl)ethynyl)pyridin-2-amine, 81



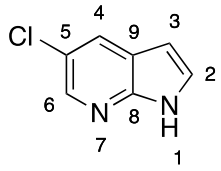
A Known compound prepared according to a literature procedure.[154]

To a degassed suspension of 3-bromo-5-(trifluoromethyl)pyridin-2-amine (250 mg, 0.95 mmol), ethynyltrimethylsilane (132 µL, 1.05 mmol), CuI (19 mg, 0.1 mmol) and triethylamine (1 mL, freshly distilled) in DMF (0.5 mL) under an argon atmosphere; $Pd(PPh_3)_2Cl_2$ (34 mg, 0.05 mmol) was added as a single portion and the reaction stirred at r.t. for 16 hours. The reaction mixture was diluted with EtOAc (100 mL), filtered through Celite then the filtrate washed with brine (5 × 50 mL) and the combined organic fractions were dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (86 mg, 35% yield) R_f = 0.8 (20% EtOAc in hexane) as an off white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (s, 1H, C5), 7.73 (d, *J* = 2.2 Hz, 1H, C3), 5.58 (s, 2H, NH_2), 0.27 (s, 9H, TMS); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 160.9 (C1), 145.6 (q, *J* = 4.2 Hz, C5), 137.3 (q, *J* =3.9 Hz C3), 124.0 (q, *J* = 270.9 Hz, (C9), 102.9 (C7), 102.8 (C2), 116.5 (q, *J* = 33.5 Hz, C4), 98.7 (C8), -0.1 ($Si(CH_3)_3$; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -61.4; **TOF M/Z (ES+)** Found 259.0872 ($C_{11}H_{14}N_2F_3Si$) Calc. 259.0878, 259.1 [M+H] 100%, 260.1 [^{13}C-M+H] 10%; **FTIR** (Neat) 3472.4, 3453.5, 3293.2, 3148.9, 2156.2, 1633.3, 1567.7, 1566.6, 1549.8, 1328.6, 1252.2, 1102.5, 1087.2, 839.2, 760.7; **M.P.** (From EtOAc) 108-110 °C.

Analytical data in agreement with literature values. [154]

5-Chloro-1*H*-pyrrolo[2,3-b]pyridine, 82



A known compound[155] synthesised *via* an unreported procedure.

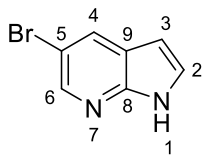
To a stirred suspension of 2-amino-5-chloro-3-((trimethylsilyl)ethynyl)pyridine (330 mg, 1.47 mmol) and $CaCO_3$ (148 mg, 1.47 mmol) in DMF (28 mL) under an argon atmosphere; CuI (140 mg, 0.74 mmol) was added in a single portion and the reaction mixture was heated to 120 °C. After 16 hours, the reaction was cooled to room temperature, diluted with brine (40 mL), extracted into Et_2O (3 × 40 mL) and the combined organic layers were dried over $MgSO_4$. The solvents were removed *in vacuo* and purification achieved by column chromatography (10%→15%→20%→25%→30% EtOAc in hexane), to afford the product (221 mg, 99% yield) R_f = 0.3 (40% EtOAc in hexane) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.16 (s, 1H, NH), 8.29 (d, *J* = 2.1 Hz, 1H, H6), 7.94 (d, *J* = 2.1 Hz, 1H, H4), 7.42 (d, *J* = 3.3 Hz, 1H, H2), 6.47 (d, *J* = 3.3 Hz, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 147.1 (C8), 141.2 (C6), 128.4 (C4), 127.0 (C2), 123.9 (C5), 121.4 (C9), 100.7 (C3); **TOF M/Z (ES+)** Found 153.0216 ($C_7H_6N_2{}^{35}Cl$) Calc. 153.0220, 153.0 [^{35}Cl-M+H] 100%, 155.0 [^{37}Cl-M+H] 40%, 154.0 [^{35}Cl-^{13}C-M+H] 10%, 156.0 [^{37}Cl-^{13}C-M+H] 2.5%; **FTIR** (Neat) 3184.8, 3126.9, 3053.4, 2997.7, 2894.4,

2863.7, 1573.4, 1469.7, 1402.8, 1337.7, 1283.5, 1109.8, 877.1, 732.9, 689.3; **M.P**.(From EtOAC) 152-154 °C.

Analytical data in agreement with literature values. [155]

5-Bromo-1H-pyrrolo[2,3-b]pyridine. 83



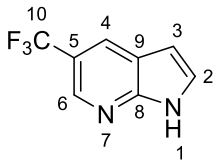
A known compound synthesised according to a literature procedure.[156]

To a stirred solution of 5-bromo-3-((trimethylsilyl)ethynyl)pyridin-2-amine (62 mg, 0.23 mmol) in DMF (1.5 mL) under an argon atmosphere ^{t}BuOK (52 mg, 0.46 mmol) was added as a single portion and the reaction stirred at r.t. for 16 h. The reaction was then diluted with EtOAc (50 mL) and washed with brine (5 × 50 mL) then the combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo.* Purification was achieved *via* column chromatography (10% acetone in toluene) to afford the title compound (16 mg, 35% yield) R_f = 0.25 (10% acetone in toluene) an off white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.85 (bs. 1 H, H1), 8.37 (s, 1 H, H9), 8.08 (d, 1 H, C4), 7.38 (m, 1 H, C2), 6.47 (m, 1 H, C3); **^{13}C-NMR** (300 MHz, $CDCl_3$): δ 147.2 (C8), 143.3 (C6), 131.3 (C4), 126.9 (C2), 122.3 (C9), 111.5 (C5), 100.8 (C3); **TOF M/Z (ES+)** [^{81}Br-M+H] 100%, 197.0 [^{79}Br-M+H] 90%.

Analytical data in agreement with literature values. [156]

5-(Trifluoromethyl)-1H-pyrrolo[2,3-b]pyridine, 84



A known compound synthesised *via* an unreported procedure.

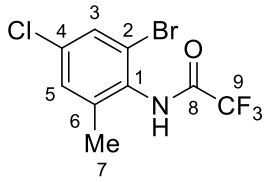
To a stirred solution of 5-(Trifluoromethyl)-3-((trimethylsilyl)ethynyl)pyridin-2-amine (42 mg, 0.16 mmol) in NMP (0.8 mL) under an argon atmosphere NaH (8 mg, 0.2 mmol, 60% mineral oil dispersion) was added as a single portion and the reaction stirred for 10 minutes. The reaction was then heated to 80 °C for 4 hours then cooled to r.t., diluted with EtOAc (20 mL) and quenched with NH_4Cl (5 mL, sat. Aq). The mixture was washed with brine (5 × 25 mL) and the organic phase dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (25 mg, 82% yield) R_f = 0.41 (40% EtOAc in hexane) as a brown crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.38 (s, 1H, H1), 8.63 (d, *J* = 1.0 Hz, 1H, H6), 8.25 (s, 1H, H4), 7.53 (dd, *J* = 3.4, 2.2 Hz, 1H, H2), 6.64 (dd, *J* = 3.4, 2.2 Hz, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 149.9 (C8), 139.8 (q, *J* = 3.2 Hz,C6), 127.4(q, *J* = 3.5 Hz, C4), 126.8 (C2), 125.0 (q, *J* = 271.5 Hz, C10), 119.7 (C9) 119.4 (q, *J* = 32.5 Hz, C5), 101.9 (C3); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -59.9; **TOF M/Z (ES+)** Found 187.0487 ($C_8H_6N_2F_3$) Calc. 187.0483, 187.0 [M+H] 100%, 188.1 [^{13}C-M+H] 15%; **FTIR** (Neat) 3143.5,

3002.0, 2885.2, 1614.9, 1588.1, 1337.3, 1310.7, 1254.2, 1187.7, 1145.2, 1095.1, 1068.8, 941.4, 911.6, 781.9, 734.5, 663.9; **M.P.** (From EtOAc) 155-157 °C.

Analytical data in agreement with literature values.

N-(2-Bromo-4-chloro-6-methylphenyl)-2,2,2-trifluoroacetamide, 85



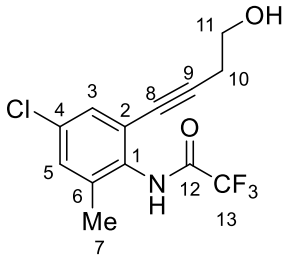
A novel compound.

To a stirred solution of 2-bromo-4-chloro-6-methylaniline (480 mg, 2.2 mmol) and NEt_3 (458 µL, 3.3 mmol) in dry DCM (6.5 mL) cooled to 0 °C under an argon atmosphere; trifluoroacetic anhydride (458 µL, 3.3 mmol) was added dropwise over 5 minutes then the reaction stirred for 2 hours. The reaction was then diluted with DCM (100 mL) and washed with HCl (2 × 50 mL, 0.1 M, Aq.) and the organic phase was dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (10% EtOAc in hexane) to afford the title compound (376 mg, 55% yield) R_f = 0.8 (10% EtOAc in hexane) as a brown solid as well as recovered 2-bromo-4-chloro-6-methylaniline (196 mg, 41% recovered).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (br. s, 1H, NH), 7.48 (d, *J* = 2.0 Hz, 1H, H3), 7.23 (d, *J* = 2.0 Hz, 1H, H5), 2.24 (s, 3H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.5 (q, *J* = 37.8 Hz, C8), 139.4 (C6), 134.9 (C1),

130.5 (C4), 130.4 (C5), 129.8 (C3), 122.3 (C2), 115.9 (q, *J* = 288.6 Hz, C9), 18.9 (C7); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -72.5; **TOF M/Z (EI+)** Found 314.9280 ($C_9H_6NOF_3{}^{35}Cl^{79}Br$) Calc. 314.9273, 236.01 [^{35}Cl-M-Br] 100%, 238.02 [^{37}Cl-M-Br] 60%, 316.94 [$^{35}Cl^{81}$Br-M+] 55%, 314.94 [$^{35}Cl^{79}$Br-M+] 50%, 318.94 [$^{37}Cl^{81}$-M+] 15%; **FTIR** (Neat) 3216.8, 3058.8, 2871.1, 1716.6, 1535.8, 1462.8, 1713.8, 1152.7, 1096.6, 857.3, 832.9, 762.7, 695.6; **M.P.** (From EtOAc) 122-124 °C.

N-(4-Chloro-2-(4-hydroxybut-1-yn-1-yl)-6-methylphenyl)-2,2,2-trifluoroacetamide, 86b



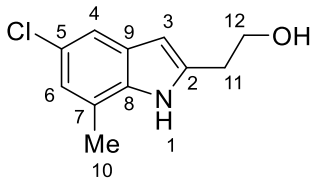
A novel compound.

To a stirred solution of *N*-(2-bromo-4-chloro-6-methylphenyl)-2,2,2-trifluoroacetamide (150mg, 0.47 mmol), but-3-yn-1-ol (72 µL, 0.95 mmol), CuI (5 mg, 0.05 mmol) and NEt_3 (198 µL, 3 mmol) in DMF (2.4 mL, argon degassed) in a sealed tube (15 mL Ace-tube); $Pd(PPh_3)_2Cl_2$ (9 mg, 0.03 mmol) was added as a single portion and the reaction heated to 120 °C for 4 hours. The reaction was diluted with EtOAc (100 mL) then filtered through Celite and the filtrate then washed with brine (5 × 100 mL) then the organic phase was dried over $MgSO_4$ and concentrate *in vacuo*. Purification was achieved *via* column chromatography (20% acetone in toluene) to afford the title compound (57 mg, 40% yield) R_f = 0.4 (20% acetone in toluene) as a yellow solid as well as 2-(5-chloro-7-

methyl-1H-indol-2-yl)ethan-1-ol (11 mg, 11% yield) R_f= 0.35 (20% acetone in toluene) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (br. s, 1H, NH), 7.28 (d, *J* = 2.3 Hz, 1H, H3), 7.18 (d, *J* = 2.3 Hz, 1H, H5), 3.76 (t, *J* = 6.1 Hz, 2H, H11), 2.64 (t, *J* = 6.1 Hz, 2H, H10), 2.48 (br. s, 1H, OH), 2.19 (s, 3H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.7 (q, *J* = 37.6 Hz, C12), 137.3 (C1), 133.6 (C6), 132.1 (C4), 130.9 (C5), 129.9 (C3), 122.7 (C2), 116.1 (q, *J* = 288.5 Hz, C13), 94.8 (C9), 77.0 (C8), 60.8 (C11), 23.7 (C10), 18.4 (C7); **TOF M/Z (ES+)** Found 328.0329 ($C_{13}H_{11}NO_2Na^{35}ClF_3$) Calc. 328.0328, 328.03 [^{35}Cl-M+Na] 100%, 330.02 [^{37}Cl-M+Na] 20%, **FTIR** (Neat) 3211.0, 304937, 2918.1, 2231.2, 1698.7, 1542.3, 1377.7, 1242.1, 1203.9, 1225.7, 1149.0, 1035.7, 898.2, 858.1, 713.9, 671.9; **M.P.** (from EtOAc) 66-68 °C.

2-(5-Chloro-7-methyl-1H-indol-2-yl)ethan-1-ol, 86a

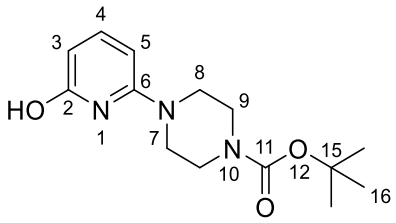


A novel compound.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (br s, 1H, H1), 7.35 (d, *J* = 1.7 Hz, 1H, H6), 6.92 (dd, *J* = 1.7, 0.8 Hz, 1H, H4), 6.25 – 6.22 (m, 1H, H3), 3.98 (t, *J* = 5.7 Hz, 2H, H12), 3.01 (t, *J* = 5.7 Hz, 2H, H11), 2.44 (s, 3H, H10); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.4 (C8), 134.2 (C2), 129.0 (C9), 125.3 (C5), 122.1 (C7), 121.2 (C6), 117.1 (C4), 100.6 (C3), 62.4 (C12), 31.2 (C11), 16.7 (C10); **TOF M/Z (ES+)** Found 210.0690 ($C_{11}H_{13}NO^{35}Cl$) Calc. 210.0686, 210.06 [^{35}Cl-M+H] 100%, 212.06 [^{37}Cl-M+H] 30%; **FTIR**

(Neat) 3422.2, 3299.2, 2916.7, 1684.0, 1587.6, 1471.7, 1454.9, 1316.9, 1231.6, 1150.9, 1038.3, 888.2, 846.8, 789.2, 744.8.

tert-Butyl 4-(6-hydroxypyridin-2-yl)piperazine-1-carboxylate, 87



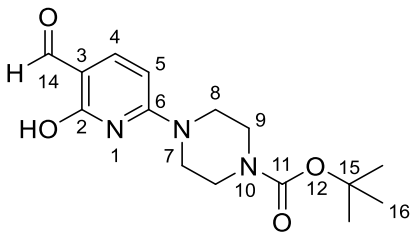
Prepared according to a literature procedure.[157]

To a stirred solution of 2-chloro-6-hydroxypyridine (6.15g, 47.5 mmol) in n-BuOH (25 mL) *N*-Boc-piperazine (22.1 g, 119 mmol) was added as a single portion. The reaction mixture was heated to 121 °C for 3 days at which point TLC showed no remaining 2-chloro-6-hydroxypyridine. The reaction mixture was evaporated to dryness *in vacuo*, the residue then suspended in ethyl acetate (250 mL) and washed with water (2 × 250 mL). The organic fraction was dried over $MgSO_4$ and dried *in vacuo* to afford an oil that was recrystalised from Petroleum spirit (40-60 °C) and DCM to afford the product (8.13 g, 64% yield) as an off-white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 12.51 (s, 1H, OH), 7.31 (dd, *J* = 8.5, 7.8 Hz, 1H, H4), 5.96 (d, *J* = 8.7 Hz, 1H, H3), 5.51 (d, *J* = 7.6 Hz, 1H, H5), 3.60 – 3.52 (m, 4H, H9), 3.30 (m, 4H, H8), 1.46 (s, 9H, H16); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.5 (C2), 154.7 (C11), 153.5 (C6), 142.9 (C4), 107.8 (C3), 90.9, (C5) 80.2 (C15), 47.7 (C8), 43.16 (d, *J* = 95.0 Hz, C9) 28.5 (C16); **M.P.** (From EtOAc) 145-147 °C)

Analytical data in agreement with literature values. [157]

tert-Butyl-4-(5-formyl-6-hydroxypyridin-2-yl)piperazine-1-carboxylate, 88



Prepared according to a literature procedure.[61]

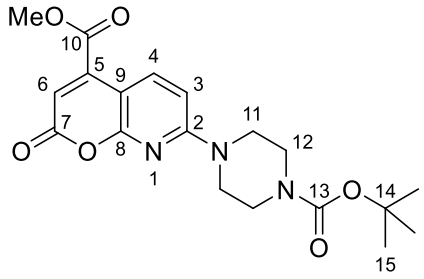
To a mixture of *tert*-butyl 4-(6-hydroxypyridin-2-yl)piperazine-1-carboxylate (1 g, 3.58 mmol) and anhydrous $MgCl_2$ (0.69 g, 7.16 mmol) in dry MeCN was added NEt_3 (3 mL, 17.90 mmol) and the mixture stirred at room temperature for 15 minutes under an argon atmosphere. Para-formaldehyde (1.071 g, 35.8 mmol, anhydrous) was added as a single portion and the reaction was heated to 60 °C for 18 hours then cooled to room temperature. The reaction mixture was diluted with EtOAc (60 mL) and Rochelle's salt (25 mL, 1M aq.) then stirred for 15 minutes. The organic layer was separated then washed with NH_4Cl (60 mL, sat. aq.) then dried over $MgSO_4$, concentrated *in vacuo* and purified by column chromatography (50% EtOAC in petroleum spirit 40-60 °C) to afford the product (0.2 g, 19% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 12.32 (s, 1H, OH), 9.53 (s, 1H, CHO), 7.62 (d, *J* = 8.7 Hz, 1H, H5,), 6.18 (d, *J* = 8.7 Hz, 1H, H4), 3.80 – 3.69 (m, 4H, H9), 3.57 – 3.46 (m, 4H, H8), 1.46 (s, 9H, H16); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 190.6 (C14), 166.8 (C2), 154.7 (C11), 143.3 (C6), 98.7 (C3), 80.5 (C5), 60.5

(C15), 44.7 (C8), 43.1 (C9), 28.5 (C16); **TOF M/Z (ES+)** Found 330.1434 [M+Na] ($C_{15}H_{21}N_3O_4Na$) Calc. 330.1430, 330.1 [M+Na] 100%, 352.1 [M+Na+MeOH] 20%; **M.P.** (From EtOAc) 137-139 °C.

Analytical data in agreement with literature values. [61]

Methyl-7-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-oxo-2H-pyrano[2,3-b]pyridine-4-carboxylate, 89

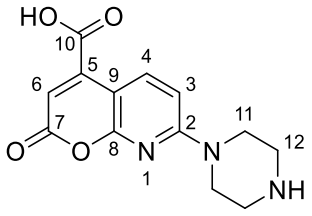


A novel compound.

To a solution of tert-butyl 4-(6-hydroxypyridin-2-yl)piperazine-1-carboxylate (200 mg, 0.72 mmol) and triphenylphosphine (188 mg, 0.72 mmol) in toluene (6mL) cooled to 0 °C under an argon atmosphere, acetylenedimethyldicarboxylate (DMAD) (97 µL, 0.79 mmol) was added dropwise over 10 minutes. The reaction was then stirred for 30 minutes at 0 °C, then warmed to room temperature over 30 minutes and then heated to 110 °C for 20 hours. The reaction was cooled to room temperature, the solvent removed *in vacuo* and the crude brown oil purified *via* column chromatography in 40% EtOAc in hexane to afford the title compound (141 mg, 46% yield) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, *J* = 9.0 Hz, 1H, H3), 6.65 (s, 1H, H6), 6.55 (d, *J* = 9.0 Hz, 1H, H4), 3.93 (s, 3H, OMe), 3.78 – 3.65 (m, 4H, H11), 3.61 – 3.46 (m, 4H, H12), 1.46 (s, 9H, H15); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.2 (C10), 164.5 (C7), 160.7 (C8), 159.5 (C2), 158.3 (C5), 154.7 (C13), 141.4 (C9), 137.6 (C3), 113.9 (C6), 104.2 (C4), 80.4 (C14), 53.1 (OMe), 44.5 (C11), 43.2 (C12), 28.5 (C15); **TOF M/Z (ES+)** Found 412.1484 [M+Na] ($C_{19}H_{23}N_3O_6Na$) Calc. 412.1485, 412.1 [M+Na] 100%, [^{13}C-M+Na] 20%; **FTIR** (Neat) 2974.9, 2927.7, 2860.8, 1707, 1683.2, 1618.8, 1583.0, 1533.0, 1412.3, 1364.4, 1283.7, 1240.6, 1168.0, 1142.4, 1079.9, 1005.0, 931.9, 868.3, 857.2, 815.6, 761.6, 679.0, 652.9.

2-Oxo-7-(piperazin-1-yl)-2H-pyrano[2,3-b]pyridine-4-carboxylic acid, 90



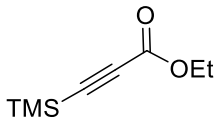
A novel compound.

To a stirred solution of methyl 7-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-oxo-2H-pyrano[2,3-b]pyridine-4-carboxylate (21 mg, 0.05 mmol) in MeOH (10 mL) Potassium hydroxide (61 mg, 1.08 mmol) was added as a single portion and the reaction mixture was heated to 65 °C for 1 ¾ hours. The reaction was then cooled to room temperature and stirred for 18 hours then filtered to remove the precipitate. The filtrate was dried *in vacuo* and the resulting residue then suspended in acetone then filtered and the precipitates collected and combined with the initial filtrand. The

combined solids were dried *in vacuo* to afford the title compound (19 mg, 98% yield) as bright-yellow crystals.

^{1}H NMR (300 MHz, DMSO) δ 9.57 (s, 1H, CO_2H), 8.38 (d, *J* = 9.0 Hz, 1H, H4), 7.00 (d, *J* = 9.0 Hz, 1H, H3), 6.56 (s, 1H, H6), 3.93 (s, 4H, H11), 3.18 (s, 3H, H12); **TOF M/Z (ES+)** Found 276.0987 [M+H] ($C_{13}H_{14}N_3O_4$) Calc. 276.0984.

Ethyl 3-(trimethylsilyl)propiolate, 91



Prepared according to a literature procedure.[67]

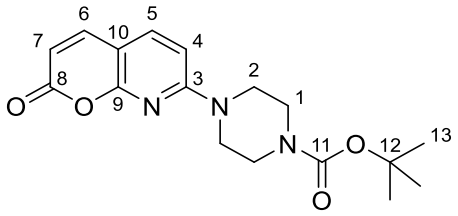
To a solution of ethynyltrimethylsilane (1.13 mL, 8 mmol) in dry THF (12 mL) cooled to −78 °C under an argon atmosphere n-BuLi (6.1 mL, 1.45 M, 8.8 mmol) was added dropwise over 10 minutes with stirring. The reaction was stirred at this temperature for a further 30 minutes then warmed to r.t. and ethylchloroformate (freshly distilled, 8.41 µL, 8.8 mmol) was added dropwise over 5 minutes. The reaction was stirred at r.t. for a further 2 hours then quenched by the addition of NH_4Cl (10 mL, Sat. Aq.) then organics extracted into EtOAc (50 mL). The combined organics were concentrated *in vacuo* and the residue then distilled *via* a Kugelrohr fractional distillation under an argon atmosphere starting at 70 °C for 30 minutes (B.P. ethynyltrimethylsilane = 53 °C, 1 atm.) then increasing the temperature 10 °C every 3 minutes until at 100 °C (B.P. ethylchloroformate = 93°C) where the temperature was held for 20 minutes.

The apparatus was then allowed to cool to room temperature and the pure product (657 mg, 53 % yield) as a clear, faintly-orange oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.23 (q, *J* = 7.1 Hz, 2H, CH_2), 1.31 (t, *J* = 7.1 Hz, 3H, CH_3), 0.24 (s, 6H, 2 × SiMe), 0.20 (s, 3H, 1 × SiMe); **TOF M/Z (EI+)** 155.1 [M-CH_3] ($C_7H_{11}O_2Si$) 100%, 125.1 [M-(3 × Me)] ($C_5H_5O_2Si$) 60%.

Analytical data in agreement with literature values. [67]

tert-Butyl-4-(2-oxo-2H-pyrano[2,3-b]pyridin-7-yl)piperazine-1-carboxylate, 92



A novel compound synthesised from a literature procedure.[68]

Prepared *via* tert-butyl 4-(6-hydroxypyridin-2-yl)piperazine-1-carboxylate

To a degassed solution of methylacrylate (purified before use, 45 µL, 0.5 mmol), $Cu(OAc)_2$ (100 mg, 0.5 mmol), 1,10-phenanthroline (20 mg, 0.1 mmol) and sodium acetate (123 mg, 1.5 mmol) with 4 Å molecular sieves in dry 1,2-dichloroethane (4 mL) $Pd(OAc)_2$ (11.2 mg, 0.05 mmol) was added as a single portion and the reaction was heated to 110 °C under an argon atmosphere for 24 hours. The reaction was then cooled to r.t. and diluted with DCM (25 mL) then filtered through

Celite and then concentrated *in vacuo.* Purification was achieved *via* column chromatography to afford the title compound (18 mg, 6% yield) R_f = 0.45 (10% acetone in toluene) as a white solid.

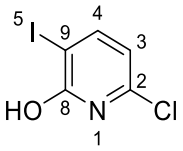
N.B. upon repeat 1,2-dichloroethane was successfully replaced with DCM and the reaction was performed in a sealed tube, in the place of a round-bottom flask, to achieve the desired temperature.

Prepared from 7-chloro-2H-pyrano[2,3-b]pyridin-2-one

To a stirred solution of 7-chloro-2H-pyrano[2,3-b]pyridin-2-one (25 mg, 0.14 mmol) in n-BuOH (2 mL) tert-butyl piperazine-1-carboxylate 77 mg, 0.41 mmol) was added as a single portion and the reaction heated to 50 °C for 2 hours under an argon atmosphere. The reaction was then diluted with EtOAc (25 mL) then washed with water (3 × 15 mL) then the organic fractions were dried over $MgSO_4$ and concentrated *in vacuo.* Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound R_f = 0.1 (31 mg, 41% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.6 Hz, 1H, H6), 7.52 (d, *J* = 9.3 Hz, 1H, H5), 6.53 (d, *J* = 8.6 Hz, 1H, H7), 6.14 (d, *J* = 9.3 Hz, 1H. H4), 3.80 – 3.65 (m, 4H, H2), 3.63 – 3.47 (m, 4H, H1), 1.48 (s, 9H, H13); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 161.4 (C8), 158.4 (C9), 154.7 (C11), 142.6 (C5), 138.1 (C6), 129.0 (C3), 125.3 (C10), 111.2 (C4), 103.9 (C7), 103.6, 80.3 (C12), 44.5 (C1), 43.2 (C2), 28.4 (C13); **TOF M/Z (ES+)** Found 354.1427 [M+Na] ($C_{17}H_{21}N_3O_4Na$) Calc. 354.1430; **M.P.(**From EtOAc) 133-135 °C.

6-chloro-3-iodopyridin-2-ol, 93



A commercially available compound prepared *via* an adaption of a literature procedure[69] as well as novel method.

To a stirred solution of 6-chloropyridin-2-ol (389 mg, 3 mmol) in MeOH (10 mL) and DCM (20 mL) Benzyltrimethylammonium dichloroiodate (1.044 g, 3 mmol) was added as a single portion with stirring. The reaction mixture was shielded from light with the aid of aluminium foil and stirred for 3 hours then the solvents were removed *in vacuo*. The residue was dissolved in DCM (100 mL) and washed with $NaHCO_3$ (2x 100 mL, Sat. Aq.) then brine (100 mL) and then dried over $MgSO_4$ and concentrated *in vacuo.* Purification *via* column chromatography (10% acetone in toluene) afforded the title compound (323 mg, 42% yield) as a white solid.

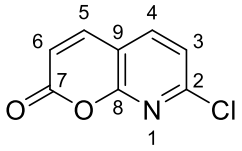
Modified literature procedure[69]

To a stirred suspension of 6-chloropyridin-2-ol 100 mg, 0.77 mmol) and K_2CO_3 (213 mg, 1.54 mmol) in water (10 mL) iodine (196 mg, 0.77 mmol) was added and the reaction stirred for 2 hours. Following this time the initial brown solution had become clear and colourless and the reaction was extracted acidified with HCl (1 M, Aq.) and then extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried over $MgSO_4$ then the solvent was removed *in vacuo* to afford the product (196 mg, 99% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.8 Hz, 1H, H4), 6.45 (d, *J* = 7.8 Hz, 1H, H3); **TOF M/Z (ESI+**) 309.93 [^{35}Cl-M+Na+MeOH] ($C_6H_7{}^{35}ClINO_2Na$) 100%, 311.96 [^{37}Cl-M+Na+MeOH] ($C_6H_7{}^{37}ClINO_2Na$) 35%.

Analytical data in agreement with literature values.

7-chloro-2H-pyrano[2,3-b]pyridin-2-one, 94



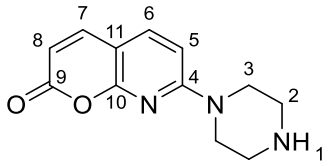
A novel compound.

To a stirred solution of 6-chloro-3-iodopyridin-2-ol (97 mg, 0.38 mmol), triethylamine (159 µL, 1.14 mmol) and methylacrylate (103 µL, 1.14 mmol) in dry MeCN (10 mL) under an argon atmosphere, $Pd(OAc)_2$ (9 mg, 0.1 mmol) was added as a single portion and the reaction was heated to 82 °C for 4 hours. Due to the appearance of several products observed *via* TLC, the reaction was cooled to r.t. then filtered through Celite, which was rinsed with EtOAc (2x 10 mL), and then solvent removed *in vacuo.* Purification was achieved *via* column chromatography (10% acetone in toluene) to afford the title compound (27 mg, 39% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, *J* = 7.5 Hz, 1H, C5), 7.54 (d, *J* = 7.5 Hz, 1H, H4), 6.73 (d, *J* = 7.5 Hz, 1H, H3), 6.68 (d, *J* = 7.7 Hz, 1H, H6); **TOF M/Z (ESI+)** 204.0 [M+Na] ($C_8H_4{}^{35}ClNO_2Na$) 100%,

206.0 [M+Na] ($C_8H_4{}^{37}ClNO_2Na$) 35%, 204.0 [M+Na+MeOH] ($C_9H_8{}^{35}ClNO_3$) 40%, 206.0 [M+Na+MeOH] ($C_9H_8{}^{37}ClNO_3$) 15%.

7-(Piperazin-1-yl)-2H-pyrano[2,3-b]pyridin-2-one, 95

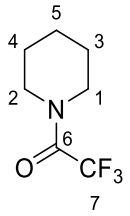


A novel compound.

To a stirred solution of *tert*-butyl 4-(2-oxo-2H-pyrano[2,3-b]pyridin-7-yl)piperazine-1-carboxylate (31 mg, 0.09 mmol) in DCM (1 mL) TFA (147 µL, 1.92 mmol) was added as a single portion and the reaction was stirred for 2 hours. The reaction was diluted with DCM (10 mL), neutralised and the pH then brought to ~9 with NaOH (1 M, Aq.) then the organic phase was separated and the aqueous phase then extracted with DCM (3 × 5 mL) and the combined organic phases were dried over $MgSO_4$ and then concentrated *in vacuo* to afford the title compound (6 mg, 27% yield) as a clear colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, *J* = 8.7 Hz, 1H, H7), 7.52 (d, *J* = 9.3 Hz, 1H, H6), 6.53 (d, *J* = 8.7 Hz, 1H, H8), 6.13 (d, *J* = 9.3 Hz, 1H, H5), 3.74 – 3.68 (m, 4H, H3), 3.02 – 2.95 (m, 4H, H2), 1.91 (br. s, 1H, H1); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.2 (C9), 161.7 (C10), 146.6 (C4), 142.8 (C7), 138.0 (C6), 112.3 (C8), 111.0 (C11), 103.7 (C5), 45.9 (C3), 29.9 (C2); **TOF M/Z (ES+)** Found 232.1087 ($C_{12}H_{14}N_3O_2$) Calc. 232.1086, 232.1 [M + H] ($C_{12}H_{15}N_3O_2$) 100%, 233.1 [^{13}C-M + H] ($C_{12}H_{15}N_3O_2$) 10%.

2,2,2-Trifluoro-1-(piperidin-1-yl)ethan-1-one, 96



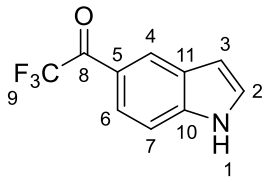
A known compound prepared according to a literature procedure.[158]

To a stirred solution of piperidine (2 mL, 20.3 mmol) and NEt_3 (2.82 mL, 20.3 mmol) in dry THF (20 mL) cooled to 0 °C under an argon atmosphere, trifluoroacetic anhydride (2.82 mL, 20.3 mmol) was added dropwise over 10 minutes and the reaction was allowed to warm to r.t over 6 hours. The reaction was diluted with Et_2O (150 mL) then washed with $NaHCO_3$ (150 mL, Sat. Aq.) then washed with water (150 mL) then brine 150 mL) and the organic phase was dried over $MgSO_4$ then concentrated *in vacuo*. The crude material was distilled (90 °C, 25 mmHg) to afford the title compound (3.53 g, 96% yield) as a clear and colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.61 – 3.45 (m, 4H, H1 + H2), 1.69 – 1.52 (m, 6H, H3-H5); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.3 (q, *J* = 35.3 Hz, C6), 116.6 (q, *J* = 288.0 Hz, C7) 46.8 (d, *J* = 3.3 Hz, C1), 44.5 (C2), 26.3 (C3), 25.3 (C4), 24.1 (C5); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -69.0; **TOF M/Z (EI+)** 181.1 [M+] ($C_7H_{10}F_3NO$) 100%, 182.1 [M+H] ($C_7H_{11}F_3NO$) 15%.

Analytical data in agreement with literature values. [158]

2,2,2-Trifluoro-1-(1H-indol-5-yl)ethan-1-one, 97



A known compound prepared according to a literature procedure.[74]

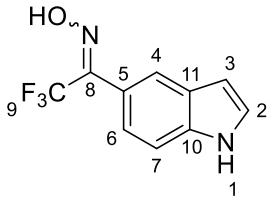
To a stirred solution of 5-bromoindole (1.57 g, 8.00 mmol) in dry THF (24 mL) cooled to 0 °C under and argon atmosphere NaH (320 mg, 8.00 mmol) was added in portions (1/4 total mass) over 5 minutes then the reaction was allowed to warm to r.t. over 1 hour. The reaction was cooled to -78 °C and ^{t}-BuLi (17 mL, 17.00 mmol, 1 M in heptane) was added dropwise over 15 minutes and the reaction stirred at -78 °C for 15 minutes. 2,2,2-Trifluoro-1-(piperidin-1-yl)ethan-1-one (2.4 mL, 17.00 mmol) was added as a single portion and the reaction stirred at -78 °C for 15 minutes then removed from the coolant dewar and allowed to warm to r.t. and stirred for 16 hours. The reaction was quenched with MeOH under an argon atmosphere then the reaction mixture was poured into NH_4Cl (50 mL, Sat. Aq.) and extracted with Et_2O (4 × 50 mL) and the combined organics were dried over $MgSO_4$ then concentrated *in vacuo.* Purification was achieved *via* column chromatography, (25% DCM in hexane) to afford the title compound co-eluted with *N*-trifluoroacetylpiperidine, then distillation (90 °C, 25 mmHg) to afford the pure title compound (1.14 g ,67% yield) as a thick yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 – 8.50 (m, 1H, H1), 8.46 (s, 1H, H4), 7.96 (d, *J* = 8.7 Hz, 1H, H6), 7.50 (d, *J* = 8.7 Hz, 1H, H7), 7.34 (dd, *J* = 3.2, 2.4 Hz, 1H, H2), 6.80 – 6.70 (m, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 180.56 (q, *J* = 33.8 Hz, C8), 139.7 (C10), 127.7 (C9), 126.7 (C2), 125.6 (H4), 123.6

(H6), 122.2 (C5), 117.3 (q, *J* = 291.7 Hz, (C9), 111.9 (C7), 104.8 (C3); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -70.1.; **TOF M/Z (EI+)** 213 (M^+) ($C_{10}H_6F_3NO$) 100%, 214 (^{13}C-M^+) ($C_{10}H_6F_3NO$) 20%.

Analytical data in agreement with literature values. [74]

2,2,2-Trifluoro-1-(1H-indol-5-yl)ethan-1-one oxime, 98



A known compound synthesised according to a literature procedure.[74]

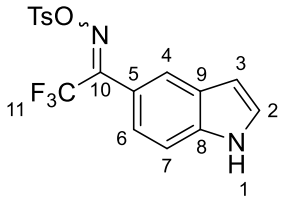
To a stirred solution of 2,2,2-Trifluoro-1-(1H-indol-5-yl)ethan-1-one (96 mg, 0.45 mmol) in pyridine (1 mL) $NH_2OH.HCl$ (39 mg, 0.563 mmol) was added as a single portion and the reaction was heated to 80 °C for 3 hours. The reaction was cooled to r.t. then diluted with Et_2O (50 mL) and washed with $CuSO_4$ (5 × 50 mL, 0.1 M, Aq.) then the organic phase was dried over $MgSO_4$ then concentrated *in vacuo* to afford the product (101 mg, 98% yield) R_f = 0.5 (50% EtOAc in hexane) as a white crystalline solid.

^{1}H NMR (Mixture of Oxime isomers) (400 MHz, MeOD) δ 7.74 (s, 1H, H4), 7.67 (s, 1H, H4'), 7.42 (2 × d, *J*=, 8.5 Hz, 2H, H6 + H6'), 7.31 – 7.18 (m, 4H, H7 + H7' + H2 +H2'), 6.51 (t, *J* = 3.7 Hz, 2H, H3 + H3'); **^{13}C NMR** (101 MHz, MeOD) δ 148.98 – 147.84 (m, C8), 138.2, 137.9, 129.1, 128.9, 126.8 (C2'), 126.7 (C2), 122.0 (C4'), 122.5 (C4), 122.3 (C6'), 121.9 (C6), 120.37 (q, *J* = 249.1 Hz, C9).

118.9, 112.0 (C7'), 111.9 (C7), 103.1 (C3'), 103.0 (C3); **^{19}F NMR** (282 MHz, MeOD) δ -63.6, -67.2; **TOF M/Z (ES-)** 227.0 ($C_{10}H_6F_3N_2O$) (M-H) 100%, 228.0 ($C_{10}H_6F_3N_2O$) (^{13}C- M-H) 10%.

Analytical data in agreement with literature values. [74]

2,2,2-Trifluoro-1-(1H-indol-5-yl)ethan-1-one *O*-tosyl oxime, 99



A known compound synthesised according to a literature procedure.[74]

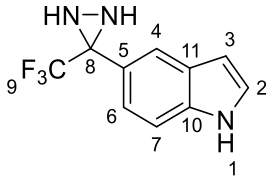
To a stirred solution of 2,2,2-Trifluoro-1-(1H-indol-5-yl)ethan-1-one oxime (112 mg, 0.49 mmol) and NEt_3 75 µL, 0.54 mmol) in dry acetone (10 mL) cooled to 0 °C under an argon atmosphere p-toluenesulfonyl chloride mono-hydrate (103 mg, 0.54 mmol) was added as a single portion and the reaction was warmed to r.t. over 6 hours. The reaction was concentrated *in vacuo* then suspended in Et_2O (50 mL), washed with citric acid (25 mL, 0.1 M, Aq.) then dried over $MgSO_4$ and concentrated *in vacuo*. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (143 mg, 76% yield) R_f = 0.5 (40% EtOAc in hexane) as an off-white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H, H1), 7.90 (d, *J* = 8.3 Hz, 2H, Ts), 7.80 (d, *J* = 8.3 Hz, 2H, Ts), 7.75 (s, 1H, H4), 7.42 (d, *J* = 8.6 Hz, 1H, H6), 7.29 – 7.27 (m, 1H, H2), 7.22 (dd, *J* = 8.5, 1.3 Hz, 1H, H7), 6.63 – 6.55 (m, 1H, H3), 2.42 (s, 3H, Ts); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.3 (q, *J* = 32.7 Hz,

C10), 139.2, 137.2, 131., 129.9, 129.9, 129.4 (C5), 127.6 (C9), 126.5 (C2), 126.1 (C4), 122.2 (C7), 121.2 (q, *J* = 206.5 Hz C11), 111.6 (C6), 103.8 (C3), 21.6 (Ts); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -66.1; **TOF M/Z (ESI+)** 437.1 (M + Na + MeOH) 100%; 405.0 (M + Na) 100%.

Analytical data in agreement with literature values. [74]

5-(3-(Trifluoromethyl)diaziridin-3-yl)-1H-indole, 100



A known compound synthesised according to a literature procedure.[74]

To a stirred solution of 2,2,2-trifluoro-1-(1H-indol-5-yl)ethan-1-one O-tosyl oxime (105 mg, 0.28 mmol) in dry Et_2O (3 mL) cooled to -78 °C in an Ace-tube (25 mL capacity, fitted with a subaseal septum); NH_3(g) was condensed *via* a B Braun Sterican needle (0.8 × 120 mm) (approximately 5 mL condensed in this way). The septum was removed and the gasket-sealed PTFE screw-lid was fitted and the gasket was reinforced with cable ties (*N.B.* This helps prevent gasket failure due to corrosive atmosphere, see figure 1).

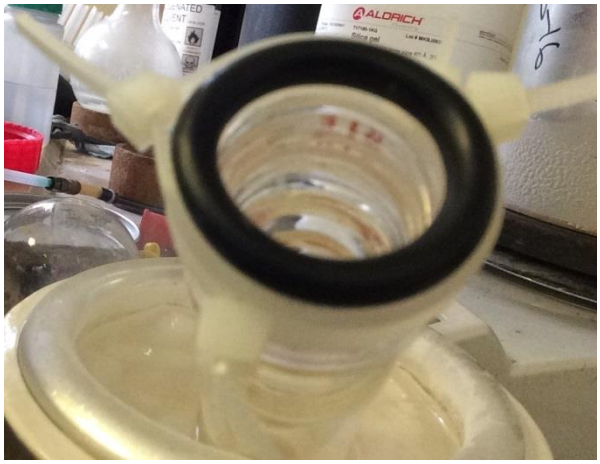


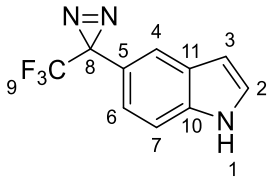
Figure 89- Cable tie reinforced sealed tube gasket in Dewar.

The sealed tube was now removed from the coolant Dewar and placed in a steel container fitted with a lid and the reaction warmed to 20 °C over 16 hours. After this time had passed liquid nitrogen (*ca*. 50 mL) was added to the steel container and after 3 minutes the sealed-tube removed and the PTFE lid loosened to allow gas venting upon warming. The reaction was allowed to gradually warm to r.t. over 1 hour then was placed under an argon flow (introduced *via* a B Braun Sterican needle (0.8 × 120 mm) to ensure residual NH_3 had dissipated. The reaction was then concentrated *in vacuo* and purified *via* column chromatography (30% EtOAc in hexane) to afford the title compound (44 mg, 71% yield) R_f = 0.4 (30% EtOAc in hexane) as a white crystalline solid.

^{1}H NMR (400 MHz, THF) δ 10.34 (s, 1H, H1), 7.82 (s, 1H, H4), 7.51 – 7.30 (m, 2H, H6 + H7), 7.29 – 7.23 (m, 1H, H2), 6.48 – 6.45 (m, 1H, H3), 3.33 (d, *J* = 8.7 Hz, 1H, Diazirane NH), 3.05 (d, *J* = 8.7 Hz, 1H, Diazirane NH); **TOF M/Z (AP+)** 228.1 (M+H) 100%, 229.1 (M+H (^{13}C)) 10%.

Analytical data in agreement with literature values. [74]

5-(3-(Trifluoromethyl)-3H-diazirin-3-yl)-1H-indole, 101



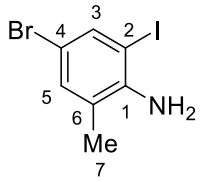
A known compound synthesised according to a literature procedure.[74]

To a stirred solution of 5-(3-(trifluoromethyl)diaziridin-3-yl)-1H-indole (22 mg, 0.1 mmol) in dry Et_2O (10 mL) under an argon atmosphere shielded from light with aluminium foil; MnO_2 (100 mg, activated ~85%, Sigma Aldrich) was added as a single portion and the reaction was stirred for 8 hours. The reaction was filtered through Celite then the filtrate was concentrated *in vacuo*. Purification was achieved *via* column chromatography (50% DCM in hexane) to afford the title compound (10 mg, 46 % yield) R_f = 0.95 (DCM) as a light sensitive clear yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.54 (d, *J* = 0.6 Hz, 1H, H4), 7.41 (d, *J* = 8.6 Hz, 1H, H7), 7.28 (d, *J* = 2.8 Hz, 1H, H3), 7.08 (dd, *J* = 8.6, 0.7 Hz, 1H, H6), 6.61 – 6.55 (m, 1H, H3); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 136.2 (C10), 128.1 (C5), 125.8 (C11), 122.70 (q, *J* = 274.9 Hz, C9), 120.5 (C2), 120.2 (C4), 118.6 (C6), 111.7 (C7), 103.3 (C3), 86.0 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -65.5; **TOF M/Z (EI+)** ($C_{10}H_6F_3N$) $^{\cdot+}$ 100%; 226.0 (M+H, $C_{10}H_6F_3N_3$) 10%.

Analytical data in agreement with literature values. [74]

4-Bromo-2-iodo-6-methylaniline, 102



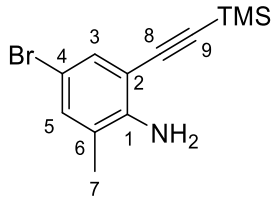
A known compound[159] synthesised *via* an unreported procedure.

To a stirred suspension of 4-bromo-2-methylaniline (0.127 g, 6.81 mmol) and $CaCO_3$(789 mg, 7.49 mmol) in MeOH (20 mL) and DCM (25 mL) under an argon atmosphere at room temperature, benzyltrimethylammonium dichloroiodate (2.487 g, 7.15 mmol) was added portion-wise over 2 hours whilst the reaction was shielded from light with aluminum foil. The reaction was stirred for a further 4 hours after the final addition and then filtered, concentrated under vacuum and purified by column chromatography (25% EtOAc in hexane) to afford the product (1.697 g, 80% yield) R_f=0.6 (25% EtOAc in hexane) as a dark brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, *J* = 1.9 Hz, 1H, H3), 7.14 (d, *J* = 1.9 Hz, 1H, H5), 4.08 (s, 2H, NH_2), 2.19 (s, 3H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 144.2 (C1), 138.3 (C3), 133.1 (C5), 123.9 (C6), 109.9 (C4), 84.4 (C2), 18.9 (C7).

Analytical data in agreement with literature values. [159]

4-Bromo-2-methyl-6-((trimethylsilyl)ethynyl)aniline, 103

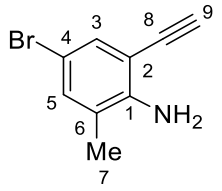


A novel compound.

A solution of 4-bromo-2-iodo-6-methylaniline (840 mg, 2.69 mmol) in triethylamine (10 mL) was added to a suspension of ethynyltrimethylsilane (0.4 mL, 2.82 mmol), CuI (51.5 mg, 0.27 mmol) and $Pd(PPh)_2Cl_2$ (94 mg, 0.14 mmol) in triethylamine (5 mL) and DMF (5 mL) under an argon atmosphere with stirring. The reaction mixture was stirred at room temperature for 20 hours, diluted with EtOAc (250 mL) and filtered through Celite. The organic filtrate was washed with brine (5 × 200 mL) then the combined organic layers dried over $MgSO_4$ and concentrated *in vacuo*. Purification was achieved *via* column chromatography (20% EtOAc in hexane) to afford the product (662 mg, 85% yiield) as a brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, *J* = 1.9 Hz, 1H, H3), 7.11 (d, *J* = 1.9 Hz, 1H, H5), 4.21 (s, 2H, NH_2), 2.13 (s, 3H, H7), 0.26 (s, 9H, TMS); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.7 C1), 133.6 (C4), 132.1 (C3), 123.7 (C6), 109.3 (C4), 108.6 (C2), 101.0 (C8), 100.8 (C9), 17.6 (C7), 0.2 (TMS); **TOF M/Z (EI+)** Found 281.0248 ($C_{12}H_{16}NSI^{79}Br$) Calc. 281.0235; **FTIR** (Neat) 3484.6, 3387.5, 2958.5, 2141.7, 1611.8, 1468.5, 1436.2, 1248.3, 1236.8, 940.2, 867.2, 839.9, 758.4, 700.0, 654.6.

4-Bromo-2-ethynyl-6-methylaniline, 104

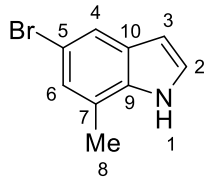


A novel compound.

To a stirred solution of 4-bromo-2-methyl-6-((trimethylsilyl)ethynyl)aniline (352 mg, 1.25 mmol) in NMP (12 mL) ^{t}BuOK (280 mg, 2.5 mmol) was added as a single portion and the reaction stirred at r.t. for 2 hours. The reaction was diluted with EtOAc (200 mL) then washed with citric acid (100 mL, 0.1 M, Aq.) then brine (5 × 200 mL) and the organic phase and the organic phase dried over $MgSO_4$ then concecntrated *in vacuo*. Purification was achieved *via* column chromatography (50% EtOAc in hexane) to afford the title compound (174 mg, 67% yield) R_f = 0.8 (50% EtOAc in hexane) as a brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (d, *J* = 2.0 Hz, 1H, H5), 7.14 (d, *J* = 2.0 Hz, 1H, H3), 4.23 (s, 2H, NH_2), 3.42 (s, 1H, H9), 2.12 (s, 3H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.0 (C1), 133.8 (C5), 132.3 (C3), 123.8 (C6), 108.4 (C4), 107.9 (C2), 83.4 (C8), 79.7 (C9), 17.5 (C7); **TOF M/Z (EI+)** Found 208.9846 ($C_9H_8N^{79}Br$) Calc. 208.9840; **FTIR** (Neat) 3415.6, 3335.1, 3279.4, 2094.4 (Alkyne C-H), 1740.8, 1615.9, 1466.5, 1438.2, 1233.9, 1004.9, 867.1, 857.5, 667.2; **M.P.** (From EtOAc) 38-40 °C.

5-bromo-7-methyl-1H-indole, 105

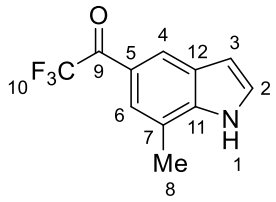


A novel compound.

To a stirred solution of 4-bromo-2-methyl-6-((trimethylsilyl)ethynyl)aniline (1.35 g, 6.44 mmol) in NMP (6.5 mL) in a CuI (306 mg, 1.61 mmol) was added in a single portion and the reaction mixture was heated to 180°C in a CEM 25 mL sealed microwave vessel for 1.5 hours. The reaction was cooled to r.t. then diluted with EtOAc (400 mL) then filtered through Celite; the filtrate was washed with brine (5 × 200 mL) then the organic phase was dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (842 mg, 63% yield) R_f = 0.45 (40% EtOAc in hexane) as a brown oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H, H1), 7.71 – 7.64 (m, 1H, H4), 7.20 – 7.16 (m, 1H, H2), 7.16 – 7.13 (m, 1H, H6), 6.53 (dd, *J* = 3.2, 2.1 Hz, 1H, H3), 2.44 (s, 3H, H8); **13C NMR** (75 MHz, CDCl3) δ 130.6 (C9), 128.9 (C10), 125.2(C7), 125.1 (C6), 122.2 (C2), 120.9 (C5), 113.0 (C4), 102.7 (C3), 16.5 (C8); **TOF M/Z (AP+)** Found 208.9843 ($C_9H_8N^{79}Br$) Calc. 208.9840; **FTIR** (Neat) 3422.3 (br. N-H), 2974.9, 2921.8, 2857.8, 1699.3, 1578.4, 1452.0, 1416.9, 1317.7, 1108.4, 846.4, 843.3, 765.2, 725.1.

2,2,2-Trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one, 106



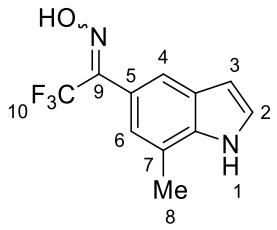
A novel compound.

To a stirred solution of 5-bromo-7-methylindole (842 mg, 4.01 mmol) in dry THF (8 mL) cooled to 0 °C under and argon atmosphere; NaH (160 mg, 4.01 mmol) was added as a single portion and the reaction stirred at 0 °C for 1 hour. The reaction was cooled to −78 °C then ^{t}BuLi (5.72 mL, 8.52 mmol, 1.49 M in pentane) was added dropwise over 15 minutes and the reaction was then stirred for a further 10 minutes after which 2,2,2-trifluoro-1-(piperidin-1-yl)ethan-1-one (1.26 mL, 8.52 mmol) was added dropwise over 2 minutes and the reaction stirred at −78 °C for a further 30 minutes then allowed to warm to r.t. over 4 hours. The reaction was quenched *via* slow addition of NH_4Cl (4 mL, Sat. Aq.) at 0 °C then the suspension was extracted with EtOAc (250 mL), the aqueous phase then separated and washed with a EtOAc (2 × 50 mL) and the organic phases then combined then dried over $MgSO_4$ and concentrated *in vacuo.* Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (224 mg, 24% yield) R_f = 0.45 (40% EtOAc in hexane) as an orange crystalline solid in a as well as 7-methylindole (398 mg, 76% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H, H1), 8.34 (s, 1H, H4), 7.76 (s, 1H, H6), 7.37 – 7.26 (m, 1H, H2), 6.72 (d, *J* = 1.9 Hz, 1H, H3), 2.54 (s, 3H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 180.8 (q, *J* = 33.7 Hz, C9), 139.7 (C11), 127.3, 126.5 (C2), 123.9 (C4), 123.6 (C6), 122.4, 121.6 (C5), 117.5 (q, *J* = 291.8 Hz)

(C10), 105.4 (C3), 16.6 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -69.7; **TOF M/Z (EI+)** Found 227.0558 ($C_{11}H_8NOF_3$) Calc. 227.0558, 227.0 (M+H) 100%; **FTIR** (Neat) 3318.1 (br. N-H), 2930.3, 1680.9, 1589.4, 1442.0, 1353.8, 1238.8, 1181.0, 1127.4, 1106.8, 1065.7, 1003.2, 889.6, 762.9, 727.1, 703.2, 679.4; **M.P.** (From EtOAc) 106-108 °C.

2,2,2-Trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one oxime, 107



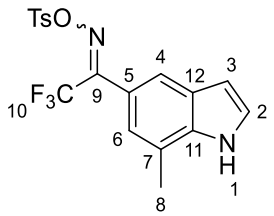
A novel compound.

To a stirred solution of 2,2,2-trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one (140 mg, 0.62 mmol) in pyridine (1.5 mL) $NH_2OH.HCl$ (54 mg, 0.77 mmol) was added as a single portion and the reaction was heated to 80 °C for 3 hours. The reaction was cooled to r.t. then diluted with Et_2O (50 mL) and washed with $CuSO_4$ (5 × 50 mL, 0.1 M, Aq.) then the organic phase was dried over $MgSO_4$ then concentrated *in vacuo* to afford the product (148 mg, 99% yield) R_f = 0.5 (50% EtOAc in hexane) as a faintly yellow crystalline solid.

^{1}H NMR (Mix of oxime isomers) (400 MHz, $CDCl_3$) δ 10.01 (s, 2H, Oxime-H), 8.29 (br. s, 2H, H1 + H1'), 7.60 (s, 1H, H4'), 7.54 (s, H, H4), 7.07 (t, *J* = 2.8 Hz, 2H, H2 + H2'), 7.03 (s, 1H, H6'), 6.99 (s, 1H, H6), 6.50 – 6.43 (m, 2H, H3 + H3'), 2.34 (s, 3H, H8'), 2.32 (s, 3H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 149.74 – 147.61 (m, C10 + C10'), 136.4, 136.3, 127.2, 127.1, 125.2, 125.1, 122.4, 122.3,

121.9, 120.7, 120.6, 119.6, 119.4, 117.6, 103.8, 103.7, 16.6, 16.5; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -62.2, -66.3.; **TOF M/Z (EI+)** Found 242.0676 ($C_{11}H_9N_2OF_3$) Calc. 242.0667; **FTIR** (Neat) 3443.3 (Indole N-H), 3237.8 (O-H), 2929.9, 1604.6, 1425.7, 1184.4, 1136.6, 1109.3, 979.8, 957.7, 855.2, 771.2, 722.1, 654.3; **M.P.** (From EtOAc) 93-95 °C.

2,2,2-Trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one O-tosyl oxime, 108

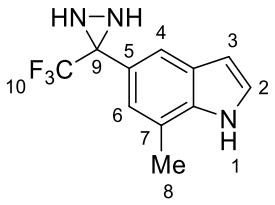


A novel compound.

To a stirred solution of 2,2,2-Trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one oxime (133 mg, 0.55 mmol) and NEt_3 85 µL, 0.60 mmol) in dry acetone (10 mL) cooled to 0 °C under an argon atmosphere p-toluenesulfonyl chloride mono-hydrate (116 mg, 0.60 mmol) was added as a single portion and the reaction was warmed to r.t. over 6 hours. The reaction was concentrated *in vacuo* then suspended in Et_2O (50 mL), washed with citric acid (25 mL, 0.1 M, Aq.) then dried over $MgSO_4$ and concentrated *in vacuo*. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (63 mg, 29% yield) R_f=0.6 (40% EtOAc in hexane) as pale-green crystalline solid as well as recovered 2,2,2-Trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one oxime (29 mg, 22% recovered).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H, H1), 7.79 (d, *J* = 8.2 Hz, 2H, OTs), 7.49 (s, 1H, H4), 7.27 (d, *J* = 8.2 Hz, 2H, OTs), 7.15 (t, *J* = 2.8 Hz, 1H, H2), 6.88 (s, 1H, H6), 6.48 (dd, *J* = 2.8, 1.3 Hz, 1H, H3), 2.37 (s, 3H, C8), 2.33 (s, 3H, Ts-*CH_3*); **^{13}C NMR** (101 MHz, CDCl3) δ 155.5 (q, *J* = 32.4 Hz, C9), 146.0, 136.8, 131.4, 129.8 (OTs), 129.3 (OTs), 127.0, 125.5 (C2), 122.0 (C4), 121.0, 120.02 (q, J = 277.9 Hz, C10), 120.0 (C6), 116.0, 104.1 (C3), 21.8 (OTs), 16.61 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -66.2; **TOF M/Z (EI+ TIC)** 451.3 ($C_{19}H_{19}F_3N_2NaO_4S$, M + Na + MeOH) 100%; 419.2 ($C_{18}H_{15}F_3N_2NaO_3S$, M + Na) 50%; **FTIR** (Neat) 3400.7, 3190.0, 30481, 2924.0, 1695.0, 1597.1, 1383.8, 1293.8, 1192.2, 1176.4, 1133.4, 1033.7, 1009.3, 881.8, 813.0, 762.5, 732.2, 708.1; **M.P.** (From EtOAc) 142-144 °C.

7-Methyl-5-(3-(trifluoromethyl)diaziridin-3-yl)-1H-indole, 109



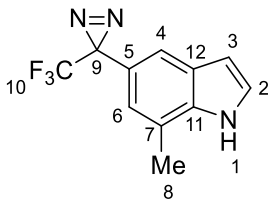
A novel compound.

To a stirred solution of 2,2,2-Trifluoro-1-(7-methyl-1H-indol-5-yl)ethan-1-one-*O*-tosyl oxime (60 mg, 0.15 mmol) in dry Et_2O (3 mL) cooled to −78 °C in an Ace-tube (25 mL capacity, fitted with a subaseal septum); NH_3(g) was condensed *via* a B Braun Sterican needle (0.8 × 120 mm) (approximately 5 mL condensed in this way). The septum was removed and the gasket-sealed PTFE screw-lid was fitted and the gasket was reinforced with cable ties (*N.B.* This helps prevent gasket failure due to corrosive atmosphere, see figure 1). The sealed tube was now removed from the coolant Dewar and placed in a steel container fitted with a lid and the reaction warmed to 20 °C over 16 hours. After this time had passed liquid nitrogen (*ca*. 50 mL) was added to the steel

container and after 3 minutes the sealed-tube removed and the PTFE lid loosened to allow gas venting upon warming. The reaction was allowed to gradually warm to r.t. over 1 hour then was placed under an argon flow (introduced *via* a B Braun Sterican needle (0.8 × 120 mm) to ensure residual NH_3 had dissipated. The reaction was then concentrated *in vacuo* and purified *via* column chromatography (30% EtOAc in hexane) to afford the title compound (35 mg, 96% yield) R_f = 0.5 (30% EtOAc in hexane) as a brown crystalline solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (br. s, 1H, H1), 7.79 (s, 1H, H4), 7.33 – 7.27 (m, 1H), 6.62 (dd, *J* = 3.1, 2.1 Hz, 1H), 2.83 (d, *J* = 7.8 Hz, 1H, NH-NH), 2.55 (s, 3H), 2.31 (d, *J* = 7.8 Hz, 1H, NH-NH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 136.2 (m, C10), 127.3, 125.4, 125.2 (C2), 123.5, 122.1 (C6), 120.8, 119.1 (C4), 103.8 (C3), 58.8 (q, J = 35.4 Hz, C9)), 16.8 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -75.71.; **TOF M/Z (EI+)** Found 241.0826 ($C_{11}H_{10}N_3F_3$) Calc. 241.0827; **FTIR** (Neat) 3293.5 (diazirane N-H stretch), 3195.9 (indole N-H), 2927.4, 1736.6, 1598.6, 1437.3, 1377.7, 1349.4, 1213.2, 1102.2, 1133.5, 941.5, 889.2, 877.0, 833.6, 731.1, 681.2; **M.P.** (from EtOAc) 85-87 °C.

7-Methyl-5-(3-(trifluoromethyl)-3H-diazirin-3-yl)-1H-indole, 110

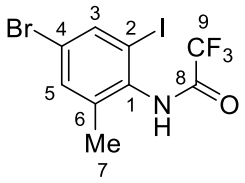


A novel compound.

To a stirred solution of 7-methyl-5-(3-(trifluoromethyl)diaziridin-3-yl)-1H-indole (35 mg, 0.145 mmol) in dry Et_2O (3 mL) under an argon atmosphere shielded from light with aluminium foil; MnO_2 (100 mg, activated ~85%, Sigma Aldrich) was added as a single portion and the reaction was stirred for 8 hours. The reaction was filtered through Celite then the filtrate was concentrated *in vacuo*. Purification was achieved *via* column chromatography (30% EtOAc in hexane) to afford the title compound (31 mg, 90% yield) R_f = 0.6 (30% EtOAc in hexane) as a light sensitive clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H, H1), 7.42 (s, 1H, H4), 7.27 (d, *J* = 2.9 Hz, 1H, H2), 6.87 (s, 1H, H6), 6.60 – 6.57 (m, 1H, H3), 2.50 (s, 3H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 136.0, 127.6, 125.4 (C2), 122.7 (q, *J* = 274.4 Hz, C10), 121.2, 120.7, 120.6 (C7), 118.0 (C4), 103.8 (C3), 30.4 – 28.36 (m, C9) 16.8 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -65.5; **TOF M/Z (AP+)** Found 240.0758 ($C_{11}H_9N_3F_3$) Calc. 240.0749.

N-(4-Bromo-2-iodo-6-methylphenyl)-2,2,2-trifluoroacetamide, 111



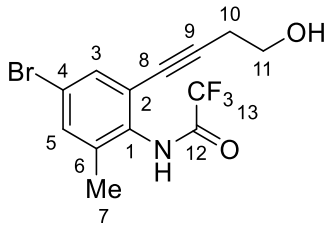
A novel compound.

To a stirred solution of 4-bromo-2-iodo-6-methylaniline (840 mg, 2.69 mmol) and NEt_3 (488 µL, 3.5 mmol) in dry DCM (30 mL) cooled to 0 °C under an argon atmosphere; trifluoroacetic anhydride (488 µL, 3.5 mmol) was added dropwise over 5 minutes then the reaction stirred for 2

hours. The reaction was then diluted with DCM (250 mL) and washed with HCl (2 × 100 mL, 0.1 M, Aq.) and the organic phase was dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (25% EtOAc in hexane) to afford the title compound (1.09 g, 99% yield) R_f = 0.8 (25% EtOAc in hexane) as an orange crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, *J* = 1.8 Hz, 1H, H3), 7.65 (s, 1H, NH), 7.42 (d, *J* = 1.7 Hz, 1H, H5), 2.26 (s, 3H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.3 (q, *J* = 37.7 Hz, C8), 139.3 (C1), 134.3 (C6), 133.4 (C3), 123.2 (C5), 115.9 (C4) (q, *J* = 288.4 Hz, C9), 98.7 (C2), 19.2 (C7); **TOF M/Z (EI+)** Found 406.8636 ($C_9H_6NOF_3{}^{79}BrI$) Calc. 406.8630; **FTIR** (Neat) 3241.9, 3062.6, 2859.9, 1706.8, 1528.9, 1161.7, 856.9, 745.4, 680.4, 695.2; **M.P.** (from EtOAc) 118-120 °C.

N-(4-Bromo-2-(4-hydroxybut-1-yn-1-yl)-6-methylphenyl)-2,2,2-trifluoroacetamide, 112a



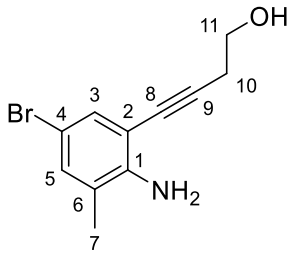
A novel compound.

To a stirred solution of N-(4-bromo-2-iodo-6-methylphenyl)-2,2,2-trifluoroacetamide (393 mg, 0.97 mmol), but-3-yn-1-ol (73 µL, 0.97 mmol), CuI (9.2 mg, 0.05 mmol) and NEt_3 (404 µL, 2.9 mmol) in DMF (4.5 mL) under an argon atmosphere in a CEM 25 mL sealed microwave vessel; $Pd(PPh_3)_2Cl_2$ (17 mg, 0.02 mmol) was added as a single portion and the reaction was heated to 50

°C for 4 hours. The reaction was cooled to r.t. then diluted with EtOAc (150 mL) then filtered through Celite and the filtrate was washed with brine (5 × 150 mL) then the organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (103 mg, 30% yield) R_f= 0.3 (40% EtOAc in hexane) as a faintly yellow crystalline solid as well as 4-(2-(2-hydroxyethyl)-7-methyl-1H-indol-5-yl)but-3-yn-1-ol oil (75 mg, 31% yield) R_f = 0.1 (40% EtOAc : hexane) as a brown.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H, NH), 7.42 (d, *J* = 1.7 Hz, 1H, H3), 7.33 (d, *J* = 1.7 Hz, 1H, H5), 3.73 (t, *J* = 6.0 Hz, 2H, H11), 2.72 (br.s, 1H, OH), 2.62 (t, *J* = 6.0 Hz, 2H, H10), 2.17 (s, 3H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.7 (q, *J* = 37.4 Hz, C12), 137.6 (c1), 133.7 (C5), 132.8 (C3), 132.7 (C6), 123.0 (C2), 121.5 (C4), 116.1 (q, *J* = 288.4 Hz, C13)), 94.9 (C9), 76.8 (C8), 60.8 (C11), 23.7 (C10), 18.2 (C7); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -75.3; **TOF M/Z (ES+)** Found 371.9818 ($C_{13}H_{11}NO_2{}^{23}Na^{79}BrF_3$) Calc. 371.9823; **M.P.** (From EtOAc) 95-97 °C.

4-(2-Amino-5-bromo-3-methylphenyl)but-3-yn-1-ol, 113

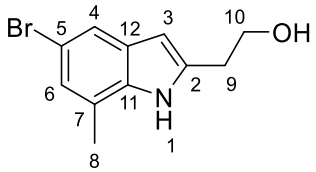


A novel compound.

To a stirred suspension of 4-bromo-2-iodo-6-methylaniline (1 g, 3.21 mmol), 3-butyn-1-ol (243 µL, 3.21 mmol), CuI (31 mg, 0.16 mmol) and triethylamine (1.35 mL, 9.63 mmol) in DMF (16 mL) under an argon atmosphere; $Pd(PPh_3)_2Cl_2$ (56 mg, 0.08 mmol) was added in a single portion and the reaction mixture was stirred at room temperature for 13 hours. EtOAc (250 mL) was added to the reaction mixture, which was then filtered through Celite. The organic layer was separated and the aqueous phase extracted into EtOAc (2 × 50 mL), the combined organic layers were washed with brine (5 × 200 mL) then dried over $MgSO_4$ and concentrated *in vacuo*. Purification was achieved by column chromatography (40% EtOAc in hexane) to afford the product (810 mg, 99% yield) R_f = 0.15 (40% EtOAc in hexane) as an orange crystalline solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 2.2 Hz, 1H, H3), 7.10 (d, *J* = 2.2 Hz, 1H, H5), 4.18 (s, 2H, NH_2), 3.88 – 3.79 (m, 3H, H10), 2.74 (t, *J* = 6.2 Hz, 2H, H11), 2.13 (s, 3H, H7), 1.61 (s, 1H, OH); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 145.3 (C1), 132.9 (C5), 131.9 (C3), 123.5 (C6), 109.5 (C4), 108.6(C2), 92.8 (C9), 78.1 (C8), 61.2 (C11), 23.9 (C10), 17.4 (C7); **TOF M/Z (ES+)** Found 254.0179 ($C_{11}H_{13}NO^{79}Br$) Calc. 254.0181; **FTIR** (Neat) 3407.1 (br. O-H), 3325 (br. N-H), 2917.2, 1644.6, 1603.7, 1577.2, 151.0, 1463.6, 1435.2, 1315.9, 1039.9, 874.4, 745.5; **M.P.** (From EtOAc) 45-47 °C.

2-(5-Bromo-7-methyl-1H-indol-2-yl)ethan-1-ol, 114

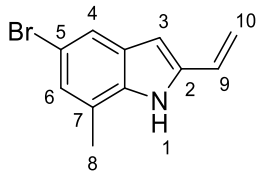


A novel compound.

To a stirred solution of 4-(2-amino-5-bromo-3-methylphenyl)but-3-yn-1-ol (37 mg, 0.15 mmol) in NMP (1.5 mL) under an argon atmosphere, ^{t}BuOK (33 mg, 0.29 mmol) was added as a single portion, and the reaction was stirred for 2 hours at r.t. The reaction was then diluted with EtOAc (50 mL) then washed with citric acid (50 mL, 0.1 M, Aq.) followed by brine (5 × 25 mL) then the combined organic phases were dried over $MgSO_4$ and then concentrated *in vacuo*. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (29 mg, 78% yield) R_f = 0.15 (40% EtOAc in hexane) as a clear yellow oil in a as well as 5-bromo-7-methyl-2-vinyl-1H-indole (5mg, 15% yield) R_f = 0.5 (40% EtOAc in hexane) as a clear brown oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H, H1), 7.51 (d, *J* = 1.8 Hz, 1H, H6), 7.06 (d, *J* = 1.8 Hz, 1H, H4), 6.25 (d, *J* = 1.8 Hz, 1H, H3), 4.39 (t, *J* = 6.5 Hz, 2H, H10), 3.11 (t, *J* = 6.5 Hz, 2H, H9), 2.44 (s, 3H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 136.3 (C11), 134.4 (C12), 129.8 (C2), 124.9 (C6), 121.6 (C7), 120.3 (C4), 113.1 (C5), 101.0 (C3), 63.5 (C10), 28.0 (C9), 16.5 (C8); **TOF M/Z (AP+)**Found 254.0180 ($C_{11}H_{13}NO^{79}Br$) Calc. 254.0181; **FTIR** (Neat) 3331.8 (br. O-H), 2925.8, 2851.8, 1686.0, 1602.5, 1472.3, 1263.4, 1163.6, 1119.5, 1041.6, 877.0, 850.1, 731.4.

5-Bromo-7-methyl-2-vinyl-1H-indole, 115

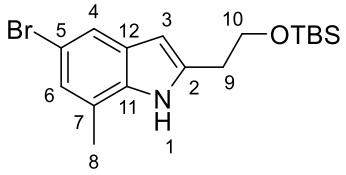


A novel compound.

^{1}H NMR ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H, H1), 7.54 (s, 1H, H4), 7.09 (d, *J* = 0.8 Hz, 1H, H6), 6.72 (dd, *J* = 17.8, 11.2 Hz, 1H, H9), 6.45 (d, *J* = 2.1 Hz, 1H, H3), 5.60 (d, *J* = 17.8 Hz, 1H, H10'), 5.31

(d, *J* = 11.2 Hz, 1H, H10), 2.47 (s, 3H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.1, 133.4, 130.1, 127.5 (C9), 126.2 (C4), 121.8, 121.0 (C6), 113.4, 113.1 (C10), 103.1 (C3), 16.6(C8); **TOF M/Z (EI+)** Found 234.9995 ($C_{11}H_{10}N^{79}Br$) Calc. 234.9997, 100% 235.0 (^{79}Br-M+H), 100% 237.0 (^{81}Br-M +H; **FTIR** (Neat) 3313.0 (N-H), 2924.3, 2848.8, 1726.5, 1460.1, 1373.4, 1230.4, 872.7, 746.4.

5-Bromo-2-(2-((tert-butyldimethylsilyl)oxy)ethyl)-7-methyl-1H-indole, 116



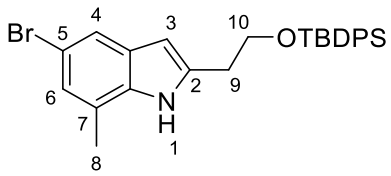
A novel compound.

To a stirred solution of 2-(5-Bromo-7-methyl-1H-indol-2-yl)ethan-1-ol (47 mg, 0.19 mmol) and imidazole (15 mg, 0.22 mmol) in dry THF (2 mL) under an argon atmosphere at 0 °C; *tert*-butylchlorodimethylsilane (34 mg, 0.22 mmol) was added as a single portion and the reaction allowed to warm to r.t. over 8 hours. The reaction was concentrated *in vacuo* then suspended in Et_2O (25 mL) and washed with citric acid (25 mL, 0.1 M, Aq.) then the organic phase was dried over $MgSO_4$ and then concentrated *in vacuo*. Purification was achieved *via* filtration through a short plug of SiO_2 eluted with Et_2O to afford the title compound (67 mg, 99% yield) as clear colourless oil.

^{1}H NMR 400 MHz, $CDCl_3$) δ 8.79 (s, 1H, H1), 7.51 (d, *J* = 1.2 Hz, 1H, H4), 7.04 (d, *J* = 0.9 Hz, 1H, H6), 6.19 – 6.16 (m, 1H, H3), 3.95 (t, *J* = 5.5 Hz, 2H, H10), 2.97 (t, *J* = 5.5 Hz, 2H, H9), 2.42 (s, 3H), 0.97

(s, 9H, Si^{t}Bu), 0.11 (s, 6H, Si*CH$_3$*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.4 (C11), 132.6 (C12), 129.4 (C3) 124.2 (C6), 121.3 (C7), 119.9(C5), 112.6 (C4), 99.8 (C3), 62.9(C10), 30.9 (Si*C*(CH_3)), 25.9 (Si*C(CH$_3$)*), 18.1 (C9), 16.5 (C8), -5.4 ($SiCH_3$); **TOF M/Z (EI+)** Found 367.0964 ($C_{17}H_{26}NO^{79}BrSi$) Calc. 367.0967.

5-Bromo-2-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)-7-methyl-1H-indole, 117



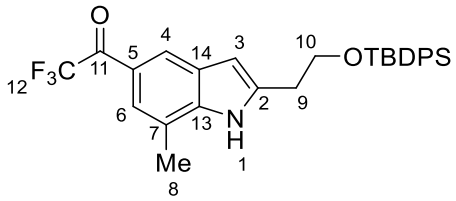
A novel compound.

To a stirred solution 2-(5-bromo-7-methyl-1H-indol-2-yl)ethan-1-ol (185 mg, 0.73 mmol) and imidazole (65 mg, 0.95 mmol) in dry THF (10 mL) under an argon atmosphere at 0 °C; *tert*-butylchlorodiphenylsilane (246 µL, 0.95 mmol) was added as a single portion and the reaction allowed to warm to r.t. over 8 hours. The reaction was concentrated *in vacuo* then suspended in Et_2O (100 mL) and washed with citric acid (100 mL, 0.1 M, Aq.) then the organic phase was dried over $MgSO_4$ and then concentrated *in vacuo*. Purification was achieved *via* filtration through a short plug of SiO_2 eluted with Et_2O to afford the title compound (356 mg, 98% yield) as clear orange oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H, H1), 7.63 (dd, *J* = 7.7, 1.7 Hz, 4H, TBDPS-*Ph*), 7.55 (dd, *J* = 7.7, 1.7 Hz, 4H, TBDPS-*Ph*), 7.43 (d, *J* = 1.8 Hz, 1H, H4), 6.95 (s, 1H, H6), 6.07 (d, *J* = 1.8 Hz, 1H, H3), 3.89 (t, *J* = 5.5 Hz, 2H, H10), 2.89 (t, *J* = 5.5 Hz, 2H, H9), 2.26 (s, 3H, H8), 1.05 (s, 9H, TBDPS-C*CH$_3$*);

^{13}C NMR (101 MHz, $CDCl_3$) δ 135.7 (TBDPS-*Ph)*, 135.6, 135.3 (TBDPS-*Ph)*, 134.9 (TBDPS-*Ph)*, 134.4 (TBDPS-*Ph),* 133.1, 130.1 (TBDPS-*Ph)*, 124.4 (C6), 121.6 (C4), 120.1 (C7), 112.8 (C5), 100.4 (C3), 64.1 (C10), 31.0 (C9), 26.9 (TBDPS-*C*CH_3), 26.7 (TBDPS-C*CH₃)*, 16.6 (C8); **TOF M/Z (ES+)** Found 492.1359 ($C_{27}H_{31}NOSi^{79}Br$) Calc. 492.1358; **FTIR** (neat) 3438.1 (N-H), 3070.8, 2929.9, 2856.9, 1686.7, 1588.7, 1470.3, 1427.4, 1361.6, 1109.0, 820.46, 737.3, 699.0.

1-(2-(2-((*tert*-Butyldiphenylsilyl)oxy)ethyl)-7-methyl-1H-indol-5-yl)-2,2,2-trifluoroethan-1-one, 118



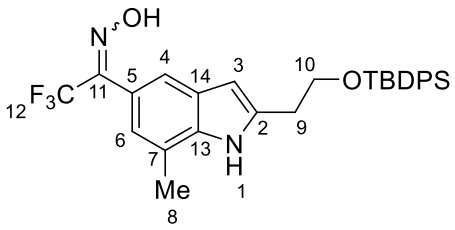
A novel compound.

To a stirred solution of 5-bromo-2-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)-7-methyl-1H-indole (484 mg, 0.98 mmol) in dry THF (4 mL) cooled to 0 °C under and argon atmosphere; NaH (41 mg, 1.03 mmol)was added as a single portion and the reaction stirred at 0 °C for 1 hour. The reaction was cooled to -78 °C then ^{t}BuLi (725 µL, 1.08 mmol, 1.49 M in pentane) was added dropwise over 15 minutes and the reaction was then stirred for a further 10 minutes after which 2,2,2-trifluoro-1-(piperidin-1-yl)ethan-1-one (218 µL, 1.48 mmol) was added dropwise over 2 minutes and the reaction stirred at -78 °C for a further 30 minutes then allowed to warm to r.t. over 4 hours. The reaction was quenched *via* slow addition of NH_4Cl (4 mL, Sat. Aq.) at 0 °C then the suspension was extracted with EtOAc (150 mL), the aqueous phase then separated and washed with a EtOAc (2 ×

50 mL) and the organic phases then combined then dried over $MgSO_4$ and concentrated *in vacuo.* Purification was achieved *via* column chromatography (10% Et_2O in hexane) to afford the title compound (156 mg, 32% yield) R_f = 0.15 (10% Et_2O in hexane) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1H, H1), 8.20 (s, 1H, H4), 7.69 (s, 1H, H6), 7.63 (dd, *J* = 7.9, 1.3 Hz, 4H, TBDPS-*Ph*), 7.47 – 7.32 (m, 6H, Ar-H (TBDPS-*Ph*), 6.39 (d, *J* = 1.7 Hz, 1H, H3), 4.01 (t, *J* = 5.4 Hz, 2H, H10), 3.03 (t, *J* = 5.4 Hz, 2H, H9), 2.42 (s, 3H, H8), 1.14 (s, 9H, TBDPS-C*CH$_3$*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 203.9 (C11), 140.3, 139.8, 135.4 (TBDPS-*Ph)*, 132.8, 130.1 (TBDPS-*Ph)*, 127.9 (TBDPS-*Ph)*, 123.2 (C4), 122.8 (C6), 102.7 (C3), 63.9 (C10), 31.9 (TBDPS-*C*CH$_3$), 30.8 (C9), 27.0 (TBDPS-C*CH$_3$*), 16.6 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -69.8; **TOF M/Z (ES+)** Found 532.1895 ($C_{29}H_{30}NO_2F_3{}^{23}NaSi$) Calc. 532.1896; **FTIR** (Neat) 3383.1 (N-H), 2933.3, 2854.8, 1680.2 (C=O), 1590.12, 1473.9, 1131.3, 1107.5, 1089.0, 1022.5, 914.8, 743.6, 701.3, 672.2.

1-(2-(2-((*tert*-Butyldiphenylsilyl)oxy)ethyl)-7-methyl-1H-indol-5-yl)-2,2,2-trifluoroethan-1-one oxime, 119



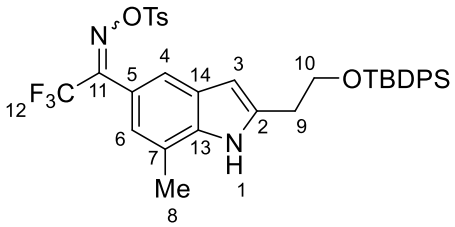
A novel compound.

To a stirred solution of 1-(2-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-7-methyl-1H-indol-5-yl)-2,2,2-trifluoroethan-1-one (120 mg, 0.24mmol) in pyridine (2 mL) $NH_2OH.HCl$ (25 mg, 0.35 mmol) was

added as a single portion and the reaction was heated to 80 °C for 3 hours. The reaction was cooled to r.t. then diluted with Et_2O (50 mL) and washed with $CuSO_4$ (5 × 50 mL, 0.1 M, Aq.) then the organic phase was dried over $MgSO_4$ then concentrated *in vacuo* to afford the product (57 mg, 47% yield) R_f = 0.3 (20% Et_2O in hexane) as a clear yellow oil.

^{1}H NMR (mix of the two oxime isomers) (400 MHz, $CDCl_3$) δ 8.91 (s, 1H, H1), 8.87 (s, 1H, H1), 7.71 – 7.33 (m, 22H, Ar-H), 7.09 (s, 1H, H4), 7.06 (s, 1H, H4), 6.30 (s, 1H, H3), 6.29 (s, 1H, H3), 4.01 (t, *J* = 5.1 Hz, 4H, H10), 3.03 (t, *J* = 5.1 Hz, 4H, H9), 2.44 (s, 3H, H8), 2.42 (s, 3H, H8), 1.15 (s, 24H, TBDPS-C*CH$_3$*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 149.2 (C11), 139.3, 136.8, 136.7, 135.6, 133.1, 130.1, 128.0, 127.8, 127.8, 121.9, 121.7, 121.6, 120.1, 120.0, 119.44 (q, J = 284.4 Hz, C12), 118.9, 118.7, 117.1, 101.5 (C4), C3, 101.4 (C3), 64.2 (C10), 31.1 TBDPS-*C*CH$_3$, 27.1 TBDPS-C*CH$_3$*, 19.3 (C9), 16.9 (C8), 16.8 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -62.1, -66.5; **TOF M/Z (ES+)** Found 525.2192 ($C_{29}H_{32}N_2O_2F_3Si$) Calc. 525.2185; **FTIR** (Neat) 3434.2 (br. (O-H/N-H)), 3072.4, 2962.9, 2931.5, 2859.0, 1700.8 (N=O), 1616.0, 1563.9, 1472.3, 1428.1, 1188.3, 1137.9, 1112.1, 1044.3, 967.4, 914.3, 869.4, 822.5, 785.0, 734.3, 702.0.

1-(2-(2-((*tert*-Butyldiphenylsilyl)oxy)ethyl)-7-methyl-1H-indol-5-yl)-2,2,2-trifluoroethan-1-one *O*-tosyl oxime, 120

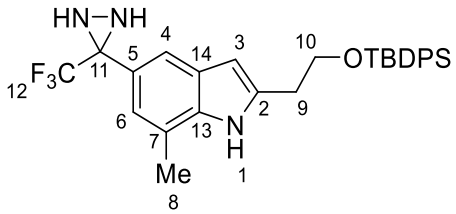


A novel compound.

To a stirred solution of 1-(2-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-7-methyl-1H-indol-5-yl)-2,2,2-trifluoroethan-1-one oxime (56 mg, 0.11 mmol) and NEt_3 21 µL, 0.14 mmol) in dry acetone (1 mL) cooled to 0 °C under an argon atmosphere p-toluenesulfonyl chloride mono-hydrate (27 mg, 0.14 mmol) was added as a single portion and the reaction was warmed to r.t. over 6 hours. The reaction was concentrated *in vacuo* then suspended in Et_2O (50 mL), washed with citric acid (25 mL, 0.1 M, Aq.) then dried over $MgSO_4$ and concentrated *in vacuo*. Purification was achieved *via* column chromatography (30% Et_2O in hexane) to afford the title compound (46 mg, 63% yield) R_f = 0.6 (40% EtOAc in hexane) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (br. s, 1H, H1), 7.91 (d, *J* = 8.3 Hz, 2H), 7.75 – 7.27 (m, 13H), 6.98 (s, 1H, H6), 6.29 (d, *J* = 1.8 Hz, 1H, H3), 4.01 (t, *J* = 5.5 Hz, 2H, H10), 3.02 (t, *J* = 5.5 Hz, 2H, H9), 2.48 (s, 3H, H8), 2.40 (s, 3H, OTs-CH_3), 1.15 (s, 9H, TBDPS-C*CH_3*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.43 (q, *J* = 32.4 Hz, C11) 145.8, 142.9, 139.6, 137.1, 135.5, 132.9, 131.7, 130.0, 129.8, 129.3, 127.9, 121.4 (C4), 119.2, 101.6 (C3), 64.0 (C10), 42.0 (TBDPS-*C*CH_3), 30.9 (C9), 27.0(TBDPS-C*CH_3*), 21.8, 19.2 (H8), 16.7(OTs-*CH_3*); **^{19}F-NMR** (282 MHz, $CDCl_3$) δ -66.08; **TOF M/Z (ES+)** Found 701.2092 ($C_{36}H_{37}N_2O_4SiF_3Na$) Calc. 701.2093; **FTIR** 3417.1, 3071.7, 2961.0, 2931.4, 2858.6, 1696.0, 1599.0, 1428.2, 1383.6, 1360.8, 1295.5, 1193.4, 1179.8, 1135.7, 1109.4, 1035.7, 1009.8, 938.2, 913.1, 885.1, 820.8, 768.0, 737.7, 700.6, 685.4.

2-(2-((*tert*-Butyldiphenylsilyl)oxy)ethyl)-7-methyl-5-(3-(trifluoromethyl)diaziridin-3-yl)-1H-indole, 121

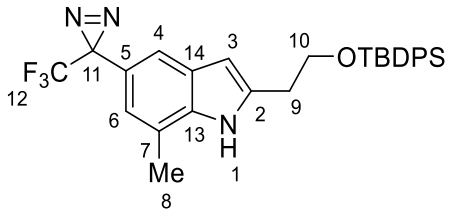


A novel compound.

To a stirred solution 1-(2-(2-((*tert*-Butyldiphenylsilyl)oxy)ethyl)-7-methyl-1H-indol-5-yl)-2,2,2-trifluoroethan-1-one O-tosyl oxime (46 mg, 0.07 mmol) in dry Et_2O (3 mL) cooled to −78 °C in an Ace-tube (25 mL capacity, fitted with a septum); NH_3 (g) was condensed *via* a B Braun Sterican needle (0.8 × 120 mm) (approximately 5 mL condensed in this way). The septum was removed and the gasket-sealed PTFE screw-lid was fitted and the gasket was reinforced with cable ties (*N.B.* This helps prevent gasket failure due to corrosive atmosphere, see figure 1). The sealed tube was now removed from the coolant Dewar and placed in a steel container fitted with a lid and the reaction warmed to 20 °C over 16 hours. After this time had passed liquid nitrogen (*ca*. 50 mL) was added to the steel container and after 3 minutes the sealed-tube removed and the PTFE lid loosened to allow gas venting upon warming. The reaction was allowed to gradually warm to r.t. over 1 hour then was placed under an argon flow (introduced *via* a B Braun Sterican needle (0.8 × 120 mm) to ensure residual NH_3 had dissipated. The reaction was then concentrated *in vacuo* and purified *via* column chromatography (40% Et_2O in hexane) to afford the title compound (26 mg, 73% yield) R_f = 0.45 (40% Et_2O in hexane) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (br. s, 1H, H1), 7.67 – 7.62 (m, 4H,TBDPS-*Ph*), 7.47 – 7.33 (m, 7H), 7.16 (s, 1H, H6), 6.27 (d, *J* = 1.9 Hz, 1H, H3), 3.99 (t, *J* = 5.5 Hz, 2H, H10), 3.02 (t, *J* = 5.5 Hz, 2H, H9), 2.78 (d, *J* = 8.4 Hz, 1H, diazirane NH), 2.42 (s, 3H, H8), 2.27 (d, *J* = 8.4 Hz, 1H, diazirane NH), 1.13 (s, 9H, TBDPS-C*CH$_3$*); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.4, 136.4, 135.6 (TBDPS-Ph), 133.1, 130.1, 129.7, 128.0, 127.8, 127.2, 123.1, 121.4, 120.2 (C4), 118.1 (C6), 101.1 (C3), 64.2 (C10), 42.1 (TBDPS-*C*CH_3), 31.1 (C9), 27.1 (TBDPS-C*CH$_3$*), 19.3 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -75.74; **TOF M/Z (ES+)** Found 524.2339 ($C_{29}H_{33}N_3OF_3Si$) Calc. 524.2345; **FTIR** 3436.8, 3255.7, 2932.9, 2861.0, 1691.8, 1598.2, 1468.8, 1426.8, 1385.6, 1326.1, 1141.9, 1109.6, 913.0, 822.0, 738.14, 703.6.

2-(2-((*tert*-Butyldiphenylsilyl)oxy)ethyl)-7-methyl-5-(3-(trifluoromethyl)-3H-diazirin-3-yl)-1H-indole, 122



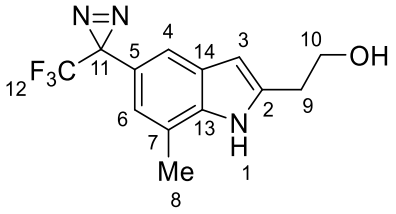
A novel compound.

To a stirred solution of 2-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-7-methyl-5-(3-(trifluoromethyl)diaziridin-3-yl)-1H-indole (26 mg, 0.05 mmol) in dry Et_2O (5 mL) under an argon atmosphere shielded from light with aluminium foil; MnO_2 (50 mg, activated ~85%, Sigma Aldrich) was added as a single portion and the reaction was stirred for 8 hours. The reaction was filtered through Celite then the filtrate was concentrated *in vacuo*. Purification was achieved *via* column

chromatography (40% Et_2O in hexane) to afford the title compound (14 mg, 55% yield) R_f = 0.55 (Et_2O in hexane) as a light sensitive clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (br. s, 1H,H1), 7.63 (dd, *J* = 8.0, 1.3 Hz, 4H, TBDPS-Ph), 7.46 – 7.28 (m, 7H, Ar-H), 6.78 (s, 1H, H6), 6.24 (d, *J* = 1.8 Hz, 1H, H3), 3.98 (t, *J* = 5.5 Hz, 2H, H10), 3.00 (t, *J* = 5.5 Hz, 2H, H9), 2.39 (s, 3H, H8), 1.13 (s, 9H, TBDPS-^{t}Bu); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 139.7, 136.2, 135.6 (TBDPS-Ph), 133.0, 130.2, 130.1 (m, C12), 129.7, 128.1, 128.0, 127.2, 120.6, 120.2, 119.9 (C4), 117.1 (C6), 101.1 (C3), 64.2 (C10), 31.1 (TBDPS-*C*CH_3), 29.9 (C9), 27.1(TBDPS-C*CH_3*), 19.3 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -65.46; **TOF M/Z (AP+)** Found 522.2183 ($C_{29}H_{31}N_3OF_3Si$) Calc. 522.2188.

2-(7-methyl-5-(3-(trifluoromethyl)-3H-diazirin-3-yl)-1H-indol-2-yl)ethan-1-ol, 123



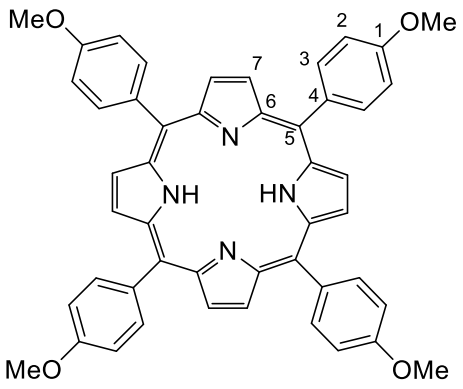
A novel compound.

To a stirred solution of 2-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-7-methyl-5-(3-(trifluoromethyl)diaziridin-3-yl)-1H-indole (14 mg, 0.03 mmol) in dry THF (1 mL) cooled to 0 °C under and argon atmosphere shielded from light with aluminium foil, TBAF (30 µL, 0.03 mmol, 1M in THF) was added dropwise over 2 minutes and the reaction stirred for 1.5 hours. The reaction

was then warmed to r.t. then concentrated *in vacuo* and the residue purified *via* column chromatography (40% EtoAc in hexane) to afford the title compound (8 mg, 99% yield) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (br. s, 1H, NH), 7.29 (s, 1H, H4), 6.79 (s, 1H,H6), 6.30 (s, 1H, H3), 3.99 (d, *J* = 5.6 Hz, 2H, H10), 3.03 (t, *J* = 5.6 Hz, 2H, H9), 2.47 (s, 3H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 138.8, 136.2, 134.5, 128.1, 120.6, 120.3, 120.1 (C6), 118.8 (CF_3), 117.0 (C4), 101.2 (C3), 62.5 (C10), 31.1 (C9), 16.8 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -65.49; **TOF M/Z (AP+)** Found 284.1012 ($C_{13}H_{13}N_3OF_3$) Calc. 284.1011, 256.1 ($C_{13}H_{12}F_3NO^{\cdot+}$) 100%.

5,10,15,20-tetrakis(4-methoxyphenyl)porphyrin (TAP), 124



Known compound synthesised according to the literature.[107]

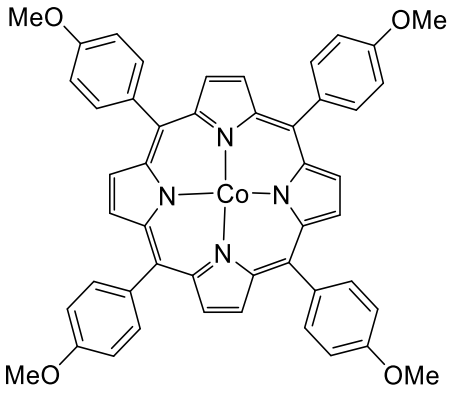
To stirring propionic acid (30 mL) pyrrole (0.97 mL, 1.01 mmol) and p-anisaldehyde (1.7 mL, 1.01 mmol) were added dropwise over 5 minutes at an equal rate with stirring and the resulting mixture was heated to 140 °C for 2 hours under an ambient atmosphere. The reaction was then cooled to r.t. then filtered to afford a purple crystalline solid. The crystals were sequentially

washed with water (20 mL) then ice-cold MeOH (10 mL) and allowed to dry under air. The solid was recrystallized with $CHCl_3$/MeOH to afford the title compound (200 mg, 27% yield) as a bright-purple crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.86 (s, 8H, H7), 8.13 (d, *J* = 8.5 Hz, 8H, H3), 7.29 (d, *J* = 8.5 Hz, 8H, H2), 4.10 (s, 12H, OMe); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 164.3 (C6), 159.5 (C1), 135.7 (C3), 134.8 (C5), 119.9 (C4), 112.3 (C2), 55.7 (OMe); **TOF M/Z (ES+)** 734.8 [M+H] 100%, 735.8 [^{13}C-M+H] 10%.

Analytical data in agreement with literature values. [107]

Cobalt(II)-5,10,15,20-tetrakis(4-methoxyphenyl)porphyrin-21,23-diide, 125



A known compound synthesised according to a literature procedure.[107]

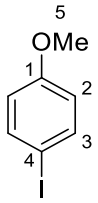
To a stirred solution of 5,10,15,20-tetrakis(4-methoxyphenyl)porphyrin (200 mg, 0.27 mmol) in DMF (5 mL, degassed with argon) under an argon atmosphere $Co(OAc)_2$ (73 mg, 0.41 mmol) was added and the reaction heated to 110 °C for 2 hours then cooled to 0 °C. water (25 mL, 0 °C) was

added, the suspension filtered and the filtrand washed with water (2 × 10 mL) to afford the title compound (188 mg, 88% yield) that was dried *via* co-evaporation *in vacuo* (acetone) as a purple solid.

TOF M/Z (ES+) Found 791.2078 ($C_{48}H_{36}N_4O_4{}^{59}Co$) Calc. 791.2069 791.2 [M (Co^{III})$^+$] 100%, 368.7 $[M+2H]^{2+}$ 90%, 792.2 [M (Co^{II})$^+$] 80%; **FTIR** (Neat) 3337.5, 2973.7, 2891.6, 1742.6, 1653.7, 1448.9, 1380.8, 1085.5, 1044.5, 878.8, 738.7.

Analytical data in agreement with literature values. [107]

4-Iodomethoxybenzene, 126



Prepared following a literature procedure.[105]

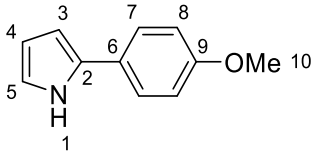
To a solution of 4-methoxybenzene (504 µL, 4.62 mmol) and NaI (693 mg, 4.85 mmol) in AcOH (20 mL,) *N*-chlorosuccinimide (617 mg mL, 4.85 mmol) was added as a single portion and the reaction was heated to 50 °C for 4h. The reaction was then cooled to r.t., poured into a separating funnel and EtOAc (200 mL) was added followed by careful addition of $NaHCO_3$ (250 mL, sat. aq.). Once the initial effervescence subsided the separating funnel was stoppered and carefully agitated then vented. After the effervescence subsides to a low level the organic layer was separated and the aqueous layer washed with EtOAc (2 × 50 mL). The organic layers were combined and washed

with $NaHCO_3$ (3 × 200 mL, sat. aq.) then dried over $MgSO_4$ then concentrated *in vacuo*. The crude orange waxy-oil was purified by column chromatography (30% EtOAc in hexane) to afford the title compound (883 mg, 82% yield) as a white crystalline solid.

^{1}H-NMR δ (300 MHz; $CDCl_3$) 7.53-7.58 (2 H, d, *J* = 9.0 Hz, H3), 6.65-6.71 (2 H, d, *J* = 9.0 Hz, H2), 3.77 (3 H, s, H5); ^{13}C-NMR δ (101 MHz; $CDCl_3$) 159.5 (C1), 138.3 (C3), 116.5 (C2), 82.8 (C4), 55.4 (C5); **TOF MS AP+**, 220.0 $[M\text{-}Me]^+$ 100%**,** 234.0 $[M^+]$ 80%, 205 235.0 $[^{13}C\text{-}M^+]$; **FTIR**, (Neat) 3081.1, 3006.9, 2967.5, 2939.0, 2838.5, 1585.3, 1567.9, 1483.0, 1454.7, 1285.6, 1238.9, 1174.8, 1026.5, 998.2, 829.4, 806.9, 785.0, 699.1.

Analytical data in agreement with literature values. [105]

2-(4-methoxyphenyl)-1H-pyrrole, 127



Prepared following a literature procedures[106,108] with modified purification.

Via the Co^{II}TAP radical arylation of 4-iodoanisole[108]

In an Ace-tube fitted with a septum a solution of 4-iodomethoxybenzene (506 mg, 2.16 mmol), ^{t}BuOH (2.07 mL, 21.6 mmol) and KOH (1.212 g, 21.6 mmol) in pyrrole (10 mL, 144 mmol) was degassed via passing argon through a submerged syringe needle for 10 minutes. To the degassed

suspension $Co^{II}TAP$ (171 mg, 0.22 mmol) suspended in degassed pyrrole (1 mL, 14.4 mmol) was added via syringe under an inert atmosphere, then the septum carefully removed and quickly replaced with the Ace-tube lid and the reaction mixture heated to 200 °C for 45 minutes in an aluminium heating block. The reaction was allowed to cool to room temperature then transferred into a Büchi kuglrohr flask with the aid of the minimum amount of MeOH, the methanol was then removed via rotary evaporation and the flask attached to a Glass Oven B-585 Kugelrohr. The liquids were distilled at 0.1 torr with heating sequentially increasing from r.t. to 60 °C with a gradient of 10 °C every 5 minutes to afford a green solid residue in the main chamber. The residue was dissolved in the minimum amount of MeOH then adsorbed onto silica (40-60 mesh) and purified *via* flash column chromatography (30% EtOAc in hexane) to afford the title compound (209 mg, 56% yield) as an off white crystalline solid.

Via palladium-catalysed cross- coupling of pyrrole [160]

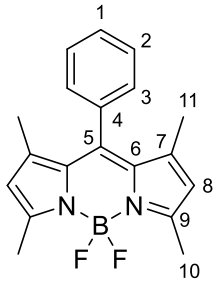
To a stirred solution of pyrrole (1 mL, 14.4 mmol, freshly distilled) in dry, degassed THF (5 mL) at 0 °C NaH (576 mg, 14.4 mmol, 60% mineral oil dispersion) was added under an argon atmosphere as a single portion and the suspension stirred for 30 minutes then warmed to r.t. $ZnBr_2$ (3.24 g, 14.4 mmol, anhydrous) was dissolved in dry, degassed THF (28 mL) and added to the reaction dropwise over 10 minutes at 0 °C. $Pd(OAc)_2$ (20 mg, 0.09 mmol), di-tert-butyl-o-biphenylphosphine (32 mg, 0.11 mg) and 4-bromoanisole (1.13 mL, 9.01 mmol) were added sequentially as single portions and the reaction heated to 65 °C for 48 h. After cooling to r.t. Et_2O (200 mL) and water (200 mL) were added and stirring continued for a further 15 minutes followed by filtration through Celite. The filter cake was repeatedly washed with Et_2O (5 × 50 mL) and the filtrate was transferred into a separatory funnel. After separation of the organic phase the aqueous phase was extracted with Et_2O (2 × 50 mL) and the combined organic phases washed

with brine then dried over $MgSO_4$ and concentrated in vacuo. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (1.027 g, 66% yield) R_f = 0.55 (40% EtOAc in hexane) as a faintly purple crystalline solid.

^{1}H-NMR (300 MHz; $CDCl_3$) δ 8.34 (1 H, bs, H1), 7.38-7.43 (2 H, m, H7), 6.90-6.95 (2 H, m, H8), 6.82 (1 H, td, *J* = 2.6 Hz, 1.5 Hz, H3), 6.43 (1 H, ddd, *J* = 3.5 Hz, 2.6 Hz, 1.5 Hz, H4), 6.29 (1 H, dt, *J* = 3.5 Hz, 2.6 Hz, H5), 3.83 (3 H, s, H10); **^{13}C-NMR** (101 MHz; $CDCl_3$) δ 125.4 (C7), 118.3 (C5), 114.5 (C8), 110.0 (C4), 105.0 (C3), 55.4 (C10); **TOF M/Z (EI+)** 173.1 [M^+] 100%, 174.1 [^{13}C-M^+] 10%**; M.P**. 144-146°C (from EtOAc).

Analytical data in agreement with literature values. [108]

5,5-Difluoro-1,3,7,9-tetramethyl-10-phenyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine, 128



A known compound synthesised according to a literature procedure.[161]

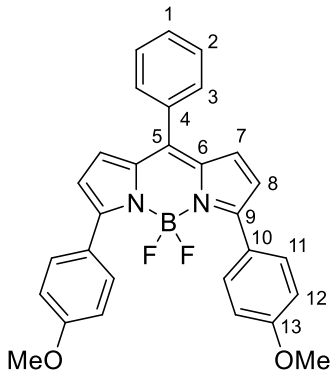
To a stirred solution of benzoyl chloride (83 µL, 0.711 mmol) in dry DCM (15 mL) cooled to 0 °C under an argon atmosphere; 2,4-dimethylpyrrole (160 µL, 1.57 mmol) was added dropwise over 2

minutes then the reaction was warmed to r.t. over 16 hours shielded from light with aluminium foil. The reaction was cooled to 0 °C then NEt_3(694 µL, 4.98 mmol) was added dropwise over 2 minutes then allowed to stir for 10 miutes, after-which $BF_3.OEt_2$ (678 µL, 5.4 mmol) was added as a single portion and the reaction was allowed to warm to r.t. over 16 hours. The reaction was diluted with DCM (100 mL) then washed with water (2 × 50 mL) and HCl (25 mL, 0.1 M, Aq.) then the organic phase was dried over Na_2SO_4 then concentrated *in vacuo.* The corresponding purple/red viscous oil was purified *via* column chromatrography (50% DCM in hexane) to afford the title compound (51 mg, 22% yield) as an orange crystalline solid with a green lustre.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 – 7.44 (m, 3H, Ph-H), 7.30 – 7.26 (m, 2H, Ph-H), 5.98 (s, 2H, 2 × BODIPY-Pyrrole-C-H), 2.55 (s, 6H, 2 × BODIPY-Me), 1.37 (s, 6H, 2 × BODIPY-Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.6 (C9), 143.3 (C4), 135.1 (C3), 134.7 (C7), 130.7 (C2), 129.2 (C5), 129.1 (C1), 128.1 (C6), 121.3 (C8), 14.7 (C10), 14.5 (C11); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -149.96 – -150.23 (m); **TOF M/Z (ES+)** 347.2 [^{11}B-M+Na] 100%, 346.2 [^{10}B-M+Na] 20%, 325.2 [^{11}B-M+H] 10%.

Analytical data in agreement with literature values. [161]

5,5-Difluoro-3,7-bis(4-methoxyphenyl)-10-phenyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine, 129



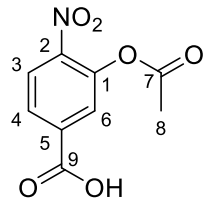
A novel compound.

To a stirred solution of Benzoyl chloride (31 µL, 0.263 mmol, freshly distilled) in dry DCM (6 mL) under an argon atmosphere excluded from light; 2-(4-methoxyphenyl)-1H-pyrrole (100 mg, 0.58 mmol) was added as a single portion and the reaction stirred at r.t. for 16 hours. The reaction was cooled to 0 °C and NEt_3 (257 µL, 1.84 mmol) was added dropwise over 5 minutes followed by the dropwise addition of BF_3OEt_2 (251 µL, 2 mmol) over 5 minutes. The reaction was warmed to r.t. over 1 hour then diluted with DCM (50 mL) and poured into water (100 mL). The organic phase was separated and the aqueous phase extracted with DCM (2 × 10 mL) then the combined organic phases were dried over Na_2SO_4 and then concentrated *in vacuo*. Purfication was achieved *via* column chromatography (10-20% EtOAc in hexane) to afford the title compound (10 mg, 4% yield) R_f = 0.7 (40% EtOAc in hexane) as a blue crystalline solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (dd, *J* = 8.2, 1.2 Hz, 4H, H11), 7.93 – 7.86 (m, 1H, H1), 7.66 – 7.51 (m, 6H, H7 + H2+H3), 7.51 – 7.45 (m, 4H, H10), 7.00 – 6.93 (m, 2H, H8), 3.87 (s, 6H, OMe); **^{13}C**

NMR (101 MHz, $CDCl_3$) δ 171.4 (C13), 145.5 (C9), 135.9 (C4), 133.9 (C5), 131.3 (C8), 130.7 (C11), 130.3 (C3), 128.6 (C2), 126.8 (C7), 125.7 (C1), 114.7 (C7), 113.9 (C12), 108.3 (C6), 55.5 (OMe); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -151.0; **TOF M/Z (ES+)** Found 503.1716 [^{11}B-M+Na] ($C_{29}H_{23}N_2O_2NaF_2{}^{11}B$) Calc. 503.1718, 503.2 [^{11}B-M+Na] 100%, 504.2 [^{11}B,^{13}C-M+Na] 25%, 502.2 [^{10}B-M+Na] 20%; **FTIR** (Neat) 3072.2, 2922.7, 2850.4, 2666.7, 2554.4, 1682.1, 1601.6, 1549.2, 1513.2, 1453.3, 1422.4, 1290.1, 1277.5, 1255.1, 1179.9, 1026.6, 931.1, 882.8, 834.6, 800.3, 705.9, 683.9, 666.4.

3-Acetoxy-4-nitrobenzoic acid, 130



A known compound synthesised according to a literature procedure.[162]

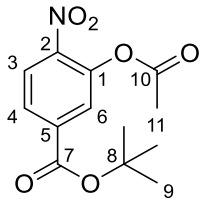
To a stirred solution of 3-hydroxy-4-nitrobenzoic acid (1 g, 5.46 mmol) in dry pyridine (2 mL) acetic anhydride (2 mL, 21.2 mmol) was added dropwise over 5 minutes then the reaction was heated to 115 °C for 4 hours. The reaction was cooled to r.t. then poured over ice water (100 mL) and the mixture was extracted into EtOAc (100 mL), the organic phase was washed with HCl (2 × 25 mL, 0.5 M Aq.). The combined organics were dried over $MgSO_4$ then concentrated *in vacuo* to afford the title compound (1.15 g, 94% yield) R_f = 0.3 (40% EtOAc, 1% AcOH in hexane) as a faintly yellow crystalline solid.

^{1}H NMR (300 MHz, DMSO) δ 13.78 (br. s, 1H, CO_2H), 8.23 (d, *J* = 8.5 Hz, 1H, H3), 8.02 (dd, *J* = 8.5, 1.8 Hz, 1H, H4), 7.96 (d, *J* = 1.7 Hz, 1H, H6), 2.34 (s, 3H, H8); **^{13}C NMR** (101 MHz, DMSO) δ 168.4

(C9), 165.0 (C7), 144.1 (C2), 143.0 (C1), 136.7 (C5), 127.7 (C4), 126.0 (C3), 125.9 (C4), 20.5 (C8); **TOF M/Z (ES-)** Found 224.0201 ($C_9H_6NO_6$) Calc. 224.0195, 224.02 [M-H] 100%, 471.02 [(2M-H) +Na] 40%; **FTIR** (Neat) 3063.9, 2838.9, 2557.8, 1781.3, 1690.9, 1594.5, 1531.3, 1431.8, 1353.6, 1292.9, 1175.8, 1078.2, 1010.9, 952.6, 912.9, 837.3, 774.0, 704.2; **M.P.** (From EtOAc) 174-176 °C.

Analytical data in agreement with literature values. [162]

tert-Butyl 3-acetoxy-4-nitrobenzoate, 131



A known compound[162] synthesised *via* an unreported procedure modified from the literature.[163]

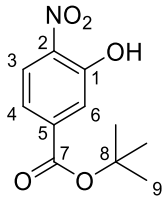
To a stirred solution of 3-Acetoxy-4-nitrobenzoic acid (275 mg, 1.22 mmol) and 2-bromo-2-methylpropane (413 µL, 3.66 mmol) in MeCN (12 mL, 90% Aq.) Ag_2O (565 mg, 2.44 mmol) was added as a single portion and the reaction stirred in darkness for 2 hours. The reaction was filtered and the filtrand repeatedly washed with Et_2O (3x 25 mL) until the washings were clear, the combined organics were dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (10% EtOAc in hexane) to afford the title compound (120 mg, 35% yield) R_f = 0.4 (10% EtOAc in hexane) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.5 Hz, 1H, H3), 7.98 (dd, *J* = 8.5, 1.8 Hz, 1H, H4), 7.82 (d, *J* = 1.7 Hz, 1H, H6), 2.39 (s, 3H, H11), 1.60 (s, *J* = 4.5 Hz, 9H, H9); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.6

(C10), 162.9 (C7), 144.1 (2), 144.0 (C5), 137.9 (C1), 127.6 (C4), 126.5 (C3), 125.8 (C6), 83.1 (C8), 28.2 (C9), 20.9 (C11); **FTIR** (Neat) 2980.3, 2931.8, 1781.4, 1719.2, 1594.9, 1529.4, 1368.8, 1298.8, 1158.8, 1111.7, 1078.9, 1010.2, 959.3, 841.3, 776.5, 740.2.

Analytical data in agreement with literature values. [162]

tert-Butyl-3-hydroxy-4-nitrobenzoate, 132



A known compound[162] synthesised *via* an unreported procedure modified from the literature.[163]

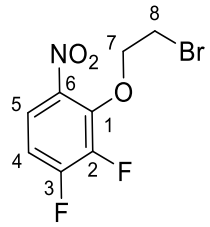
To a stirred solution of t*ert*-butyl 3-acetoxy-4-nitrobenzoate (80 mg, 0.28 mmol) in MeOH (5 mL) KOH (45% w/v, Aq.) was added until pH = 11 then the reaction was heated to 50 °C for 30 minutes. The reaction was cooled to r.t. and water was added until the mixture became homogenous then AcOH (glacial.) was added dropwise until pH = 7, the organic solvents were removed *in vacuo.* The resulting solid was dissolved in water then precipitated by acidifying with HCl (0.1 M, Aq.) to pH = 5 then dried *in vacuo* to afford the title compound (66 mg, 98% yield) as an orange crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, *J* = 8.8 Hz, 1H, H3), 7.75 (d, *J* = 1.7 Hz, 1H, H6), 7.56 (dd, *J* = 8.8, 1.7 Hz, 1H, H4), 1.60 (s, 9H, H9); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.5 (C7), 154.8 (C1), 140.2

(C2), 135.6 (C5), 125.2 (C3), 121.5 (C4), 120.7 (C6), 82.9 (C8), 28.2 (C9); **TOF M/Z (AP+)** 183.0 [M ^{t}Bu +H] ($C_7H_5NO_5$) 100%, [M+H] ($C_{11}H_{14}NO_5$) 20%.

Analytical data in agreement with literature values. [162]

2-(2-bromoethoxy)-3,4-difluoro-1-nitrobenzene, 133



A known compound synthesised according to a literature procedure.[164]

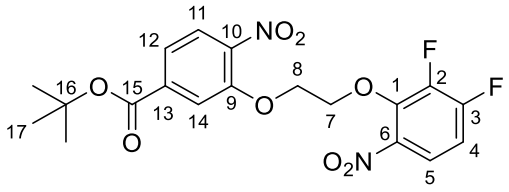
To a stirred solution of 2,3-difluoro-6-nitrophenol (105 mg, 0.6 mmol) in DMF (0.6 mL) cooled to 0 °C under an argon atmosphere; NaH (37 µL, 0.9 mmol, 60% mineral oil dispersion) was added as a single portion and the reaction warmed to r.t. over 15 minutes. 1,2-dibromoethane (207 µL, 2.4 mmol) was added as a single portion and the reaction was heated to 140 °C for 2 hours. The reaction was then cooled to r.t., diluted with Et_2O (25 mL) and washed with brine (5 × 25 mL) and the organic phases were dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (115 mg, 68% yield) R_f = 0.85 (40% EtOAc in hexane) as a clear yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 – 7.67 (m, 1H, H4), 7.13 – 7.00 (m, 1H, H5), 4.55 (dt, *J* = 6.4, 1.0 Hz, 2H, H7), 3.69 (t, *J* = 6.4 Hz, 2H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 154.1 (dd, *J* = 259.6, 11.4 Hz,

C3), 145.1 (dd, *J* = 253.7, 14.2 Hz, C2), 143.1 (dd, *J* = 11.6, 2.9 Hz, C1), 140.3 (C6), 120.6 (dd, *J* = 8.9, 3.9 Hz, C5), 111.9 (d, *J* = 19.3 Hz, C4), 74.9 (d, *J* = 5.1 Hz, C7), 28.6 (C8) ; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -124.8 – -125.0 (m), -148.2 – -148.4 (m); **TOF M/Z (EI+)** Found 280.9508 ($C_8H_6NO_3F_2{}^{79}Br$) Calc. 280.9499.

Analytical data in agreement with literature values. [164]

tert-Butyl-3-(2-(2,3-difluoro-6-nitrophenoxy)ethoxy)-4-nitrobenzoate, 134

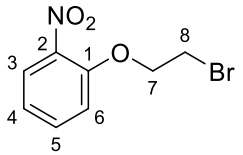


A novel compound.

To a stirred suspension of *tert*-butyl 3-hydroxy-4-nitrobenzoate (89 mg, 0.37 mmol) and K_2CO_3 (31 mg, 0.22 mmol) in NMP (0.4 mL), 2-(2-bromoethoxy)-3,4-difluoro-1-nitrobenzene (115 mg, 0.41 mmol) was added as a single portion dissolved in NMP (0.5 mL) and the reaction was heated to 140 °C for 10 minutes. The reaction was cooled to r.t. and diluted with EtOAc (20 mL) then extracted with brine (5 × 20 mL) and the organic phase was dried over $MgSO_4$ then concentrated *in vacuo.* Purificaiton was achieved *via* column chromatography (20% EtOAc in hexane) to afford the title compound (106 mg, 65% yield) R_f = 0.9 (40% EtOAc in hexane) as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, J = 8.4 Hz, 1H, H11), 7.71 (d, J = 1.4 Hz, 1H, H14), 7.70 – 7.66 (m, 1H, H5), 7.64 (dd, J = 8.4, 1.4 Hz, 1H, H12), 7.10 – 7.00 (m, 1H, H4), 4.73 – 4.66 (m, 2H, H7), 4.57 – 4.50 (m, 2H, H8), 1.60 (s, 9H, H17); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.7 (C15), 155.6 (C2), 155.5 (C3), 153.0 (C1), 152.9 (C9), 151.2 (C6), 146.4 (C10), 146.2 (C13), 143.7 (C11), 137.1 (C4), 125.1 (C12), 122.1 (C5), 115.6 (C14), 82.8 (C16), 71.4 (C7), 69.1 (C8), 28.2 (C17); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -124.50 – -125.26 (m), -148.01 – -148.77 (m); **TOF M/Z (ES+)** Found 463.0927 ($C_{19}H_{18}N_2O_8NaF_2$) Calc. 463.0929, 463.1 [M+Na] 100%, 464.1 [^{13}C-M+Na] 15%, 407.0 [M-^{t}Bu] 10%; **FTIR** (Neat) 2979.3, 1715.7, 1607.7, 1530.8, 1492.9, 1422.2, 1351.1, 1296.6, 1245.5, 1160.7, 115.5, 1062.1, 975.4, 888.1, 841.2, 811.1, 744.8, 689.7.

1-(2-Bromoethoxy)-2-nitrobenzene, 135



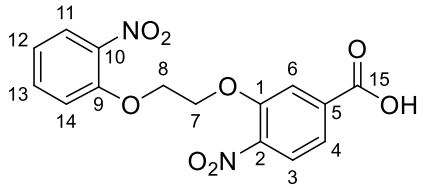
A known compound synthesized according to a literature procedure.[165]

The filtrate of the above reaction procedure for the synthesis of 1,2-bis(2-nitrophenoxy)ethane was concentrated *in vacuo* and the crude material was then dissolved in EtOAc (250 mL),washed with NaOH (3 × 250 mL,0.1 M) and then water (3 × 250 mL) at which point the extracts become colourless. The organic fraction was then dried over $MgSO_4$ and concentrated *in vacuo*. The viscous oily residue was then recrystallized from hexane/DCM to afford the title compound (3.326 g, 63% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 – 7.81 (m, 1H, H6), 7.60 – 7.50 (m, 1H, H4), 7.14 – 7.04 (m, 2H, H3 & H5), 4.42 (t, *J* = 6.5 Hz, 2H, H7), 3.67 (t, *J* = 6.5 Hz, 2H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 151.5 (C1), 134.2 (C2), 125.8 (C5), 121.5 (C3), 115.9 (C4), 115.4 (C6), 69.7 (C7), 28.1 (C8); **TOF M/Z (ES+)** 265.0 [^{79}Br-M+H^++H_2O] 50%, 267.0 [^{81}Br-M+H^++H_2O] 50%.

Analytical data in agreement with literature values. [165]

4-Nitro-3-(2-(2-nitrophenoxy)ethoxy)benzoic acid, 136



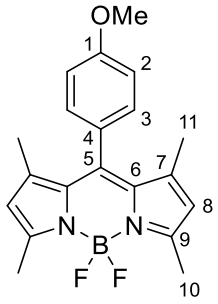
A novel compound.

To a stirred suspension of 1-(2-bromoethoxy)-2-nitrobenzene (100 mg, 0.41 mmol) and K_2CO_3 (67 mg, 0.49 mmol) in NMP (1.5 mL) 3-hydroxy-4-nitrobenzoic acid (82 mg, 0.45 mmol) was added as a single portion and the reaction was heated to 140 °C for 15 minutes. The reaction was then cooled to r.t., diluted with EtOAc (20 mL) then washed with brine (5 × 20 mL); the organic phase was dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (115 mg, 81% yield) as a green crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.49 (s, 1H), 8.18 (d, *J* = 8.8 Hz, 1H, H3), 7.84 (dt, *J* = 4.0, 1.7 Hz, 2H, H12+H13), 7.63 (dd, *J* = 8.8, 1.7 Hz, 1H, H4), 7.59 – 7.51 (m, 1H, H11), 7.15 – 7.05 (m, 2H, H6 +

H14); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 175.3 (C15), 164.2 (C1+C9), 154.6 (C2), 151.6 (C10), 137.5 (C13), 134.0 (C11), 125.6 (C3), 125.0 (C4), 121.8 (C12), 121.4 (C14), 115.2 (C6), 67.6 (C8), 63.6 (C7); **TOF M/Z/ (ES+)** Found 371.0489 [M+Na] ($C_{15}H_{12}N_2O_8Na$) Calc. 371.0491, 371.1 [M+Na] 100%, 372.1 [^{13}C-M+Na] 10%; **FTIR**(Neat) 3308.6, 3021.4, 2970.5, 2941.9, 1731.6, 1623.8, 1610.9, 1585.9, 1522.0, 136538, 1351.5, 1326.8, 1229.2, 1217.2, 1148.4, 1053.4, 926.8, 741.3, 688.9, 668.8; **M.P.** (From EtOAc) 94-96 °C.

5,5-Difluoro-1,3,5,7-tetramethyl-8-(*p*-methoxyphenyl)-4-bora-3a,4a-diaza-*s*-indacene, 137



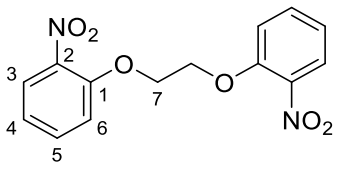
A novel compound.

To a stirred solution of 4-methoxybenzaldehyde (122 mg, 1 mmol) 2,4-dimethylpyrrole (232 µL, 2.25 mmol) in dry DCM (20 mL) under an argon atmosphere trifluoroacetic acid (10 uL) was added as a single addition and the reaction was stirred in darkness for 16 hours. The reaction was then cooled to 0 °C and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (227 mg, 1 mmol) was added as a single portion and the reaction was stirred for a further 10 minutes at 0 °C then warmed to r.t. and stirred for 2 hours. NEt_3 (2 mL, 14.4 mmol) and BF_3OEt_2 (2 mL, 16.6 mmol) were added dropwise, at the same time, over 10 minutes and the reaction then stirred for 16 hours. The

reaction was diluted with DCM (200 mL), washed with NaOH (2 × 100 mL, 0.1M, Aq.) then water (2 × 250 mL) and the organic fractions were dried over Na_2SO_4 then concentrated *in vacuo.* Purification was afforded *via* column chromatography (10% EtOAc in hexane) to afford the product (115 mg, 33% yield) as an orange crystalline solid with a green lustre.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 – 7.14 (m, 2H, H3), 7.04 – 6.98 (m, 2H, H2), 5.98 (s, 2H, H8), 3.87 (s, 3H, OMe), 2.55 (s, 6H, H10), 1.43 (s, 6H, H11); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 160.3 (C1), 155.4 (C4), 143.3 (C9), 141.9 (C5), 132.0 (C6), 129.3 (C7), 127.2 (C2), 121.2 (C3), 114.7 (C8), 55.5 (OMe), 29.8 (C10), 14.7 (C11); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -145.92 – -146.70 (m); **TOF M/Z (ES+)** 307.2 [M-BF_2+H] 100%, 377.2 [^{11}B-M+Na] 40%, 355.2 [^{11}B-M+H] 30%.

1,2-*bis*(2-Nitrophenoxy)ethane, 138



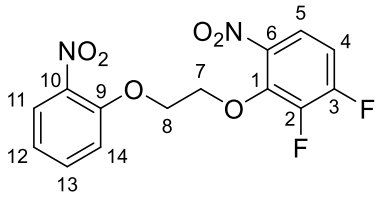
A known compound prepared by a method adapted from the literature.[83]

To a stirred suspension of 2-nitrophenol (3 g, 21.6 mmol) and K_2CO_3 (6 g, 43.4 mmol) in MeCN (90 mL) 1,2-dibromoethane (5.7 mL, 43.4 mmol) was added as a single portion and the reaction was heated to 80 °C for 16 hours under an argon atmosphere. The reaction was then cooled to r.t., concentrated *in vacuo* then suspended in Et_2O (200 mL) filtered through sintered glass, and the filtrand washed with Et_2O (2 × 100 mL) to afford the title (2.3g, 35% yield) compound as a white crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (dd, *J* = 8.1, 1.7 Hz, 2H, H3), 7.57 (ddd, *J* = 8.5, 7.5, 1.7 Hz, 2H, H5), 7.24 (dd, *J* = 8.5, 1.1 Hz, 2H, H4), 7.12 – 7.05 (m, 2H, H6), 4.54 (s, 4H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 152.0 (C1), 134.4 (C3), 134.2 (C2), 125.7 (C5), 121.5 (C4), 116.0 (C6), 68.8 (C7); **TOF M/Z (ES+)** Found 327.0598 ($C_{14}H_{12}N_2O_6Na$) Calc. 237.0593.

Analytical data in agreement with literature values. [83]

1,2-Difluoro-4-nitro-3-(2-(2-nitrophenoxy)ethoxy)benzene, 139

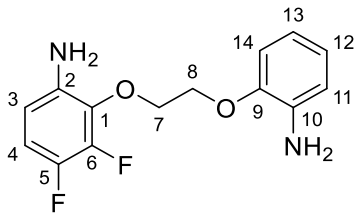


A novel compound.

To a stirred solution of 2,3-difluoro-6-nitrophenol (470 mg, 2.68 mmol) ind dry DMF (20 mL) cooled to 0 °C under an argon atmosphere; NaH (108 mg, 2.68 mmol, 60% mineral oil dispersion) was added as a single portion and the reaction stirred for 15 minutes then warmed to r.t.. A solution of 1-(2-bromoethoxy)-2-nitrobenzene (550 mg, 2.24 mmol) in dry DMF (15 mL) was added dropwise over 2 minutes then the reaction was heated to 130 °C for16 hours. The reaction was cooled to r.t. then diluted with EtOAc (200 mL) and washed with water (5 × 200 mL) and the organic phase dried over $MgSO_4$ then concentrated *in vacuo*. Purification was achieved *via* column chromatography (50% DCM in hexane) to afford the title compound (425 mg, 56% yield) R_f = 0.15 (50% DCM in hexane) as a yellow crystalline solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (dd, *J* = 8.1, 1.7 Hz, 1H, H11), 7.69 (ddd, *J* = 9.4, 5.2, 2.4 Hz, 1H, H5), 7.57 – 7.51 (m, 1H, H13), 7.12 (dd, *J* = 8.5, 0.8 Hz, 1H, H12), 7.09 – 7.00 (m, 2H, H4 & H14), 4.73 – 4.68 (m, 2H, H8), 4.52 – 4.47 (m, 2H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 154.3 (dd, *J* = 259.5, 11.5 Hz, C3), 151.8 (C9), 145.1 (dd, *J* = 253.3, 14.3 Hz, C2), 143.7 (C10), 140.1 (C1), 134.4 (C6), 125.9 (C13), 121.2 (C11), 120.6 (dd, *J* = 9.0, 3.9 Hz, C5), 114.9 (C12 & C14), 111.7 (d, *J* = 19.3 Hz, C4), 73.6 (d, *J* = 5.2 Hz, C7), 69.0 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -124.9 – -125.0 (m), -148.3 – -148.5 (m); **TOF M/Z (ES+)** Found 363.0402 ($C_{14}H_{10}N_2O_6F_2Na$) Calc. 363.0405, 363.3 [M+Na] 100%, 364.3 [^{13}C-M+Na] 15%; **FTIR** (Neat) 3103.7, 2948.6, 2878.8, 1626.1, 1607.3, 1522.0, 1473.3, 1491.3, 1449.1, 1348.0, 1302.5, 1276.4, 1245.3, 1213.8, 1166.6, 1064.4, 1049.6, 924.3, 851.6, 811.4, 742.8; **M.P.** (From DCM) 52-54 °C.

2-(2-(2-Aminophenoxy)ethoxy)-3,4-difluoroaniline, 140



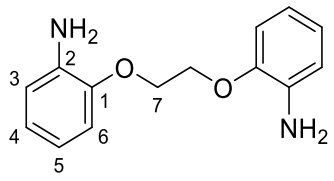
A novel compound.

To a stirred solution of 1,2-Difluoro-4-nitro-3-(2-(2-nitrophenoxy)ethoxy)benzene (475 mg, 1.4 mmol) in EtOH (200 mL, 95% Aq., argon degassed) Pd/C (88 mg, 0.05 mmol, 10 wt. %) was added under an argon atmosphere. The suspension was then subjected to a flow of H_2 bubbles introduced *via* a B Braun Sterican needle (0.8 × 120 mm), inserted *via* septum from the top to the bottom of the flask attached to a balloon containing H_2, whilst the septum was vented *via* another

B Braun Sterican needle. The introduction of H_2 gas in this way accelerates the displacement of the argon dissolved in the solution and promotes faster reaction times. N.B. it is wise not to stir at this point as the sediment will likely block the needle. Following *circa* 10 minutes of H_2 bubbling, the gas injection needle is withdrawn from the solution meniscus and the reaction allowed to stir for 48 hours. N.B. the hydrogen balloon was refilled once per day due to deflation. The reaction was followed by LC/MS until the presence of nitro and hydroxylamine compounds were no longer detectable then the H_2 inlet was replaced with argon and the solution again degassed with argon for 5 minutes. Following this time the reaction was filtered through Celite and concentrated *in vacuo*. N.B. the vacuum on the rotary evaporator was backfilled with argon introduced *via* a balloon due to the rapid degradation of the title compound in the presence of air, and Shlenk manifold used to the same ends for Hi-vacuum. The title compound (330 mg, 84% yield) was afforded, without the need for further purification, as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 – 6.80 (m, 2H), 6.77 – 6.62 (m, 3H), 6.37 (ddd, *J* = 9.0, 4.8, 2.3 Hz, 1H), 4.49 – 4.43 (m, 2H), 4.30 – 4.25 (m, 2H), 3.86 (s, 4H); **^{13}C NMR** (101 MHz, $CDCl_3$) 145.8 (dd, *J* = 80.9, 12.8 Hz, C6), 146.2 (C9), 143.37 (dd, *J* = 73.2, 12.8 Hz, C5),137.4 (C10), 136.8 (C2), 122.1 (C13), 118.6 (C12), 115.6 (C11), 112.5 (C14), 111.5 (d, *J* = 17.8 Hz, C4), 108.9 (dd, *J* = 7.0, 3.4 Hz, C3), 72.5(d, *J* = 4.4 Hz, C7), 67.7 (C8); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -150.1 (d, *J* = 20.7 Hz), -154.6 (d, *J* = 20.7 Hz); **TOF M/Z (ES+)** Found 281.1105 ($C_{14}H_{15}N_2O_2F_2$) Calc. 281.1102, 281.1 [M+H] 100%, 282.1 [^{13}C-M+H] 10%; **FTIR** (Neat) 3421.2, 3400.4, 3320.5, 3189.0, 3067.0, 2934.5, 2886.5, 1601.4, 1503.6, 1492.3, 1451.8, 1459.1, 1263.8, 1217.3, 1049.1, 979.3, 923.4, 733.4; **M.P.** (From DCM) 106-108 °C.

2,2'-(Ethane-1,2-diylbis(oxy))dianiline, 141



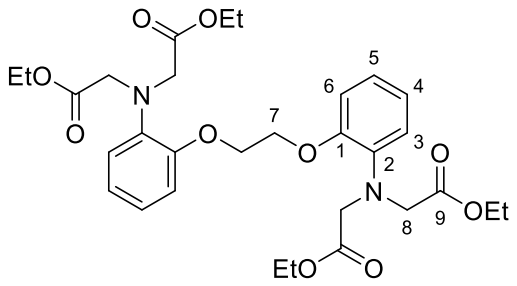
A known compound[166] prepared *via* an unreported procedure.

To a stirred solution of 1,2-bis(2-nitrophenoxy)ethane (1.3 g, 4.27 mmol) in argon-degassed EtOAc (50 mL) Pd/C (178 mg, 0.11 mmol, 10 wt. %) was added under an argon atmosphere. The suspension was then subjected to a flow of H_2 bubbles introduced *via* a B Braun Sterican needle (0.8 × 120 mm), inserted *via* septum from the top to the bottom of the flask attached to a balloon containing H_2, whilst the septum was vented *via* another B Braun Sterican needle. The introduction of H_2 gas in this way accelerates the displacement of the argon dissolved in the solution and promotes faster reaction times. N.B. it is wise not to stir at this point as the sediment will likely block the needle. Following *circa* 10 minutes of H_2 bubbling, the gas injection needle is withdrawn from the solution meniscus and the reaction allowed to stir for 48 hours. N.B. the hydrogen balloon was refilled once per day due to deflation. The reaction was followed by LC/MS until the presence of nitro and hydroxylamine compounds were no longer detectable then the H_2 inlet was replaced with argon and the solution again degassed with argon for 5 minutes. Following this time the reaction was filtered through Celite and concentrated *in vacuo*. N.B. the vacuum on the rotary evaporator was backfilled with argon introduced *via* a balloon due to the rapid degradation of the title compound in the presence of air, and Shlenk manifold used to the same ends for Hi-vacuum. The title compound (1.298 g, 99% yield) was afforded, without the need for further purification, as a fluffy white crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.89 – 6.79 (m, 4H, H3 + H6), 6.76 – 6.69 (m, 4H, H4+ H5), 4.37 (s, 4H, H7); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 146.4 (C1), 136.9 (C2), 122.1 (C5), 118.5 (C4), 115.5 (C3), 112.7 (C6), 67.6 (C7); **TOF M/Z (ES+)** Found 245.1296 ($C_{14}H_{17}N_2O_2$) Calc. 245.1290, 245.13 [M+H] 100%, 246.13 [^{13}C-M+H] 10%; **FTIR** (Neat) 3443.7, 3362.1, 3062.7, 2952.8, 1607.5, 1501, 1459.2, 1340.2, 1270.9, 1209.8, 1081.2, 944.1, 744.0, 734.4; **M.P**. (from EtOAc) 130-132 °C.

Analytical data in agreement with literature values. [166]

Tetraethyl-2,2',2'',2'''-(((ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(azanetriyl))tetraacetate, 142



Prepared according to a literature procedure.[83]

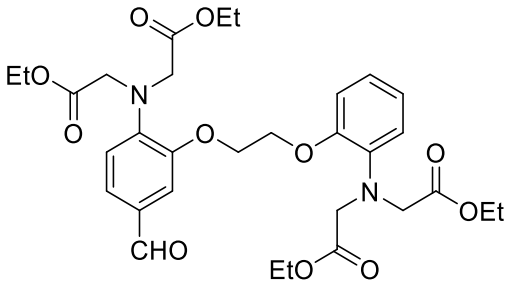
To a stirred solution of 2,2'-(ethane-1,2-diylbis(oxy))dianiline (281 mg, 1.15 mmol), N,N,*N*,*N*-tetramethylnaphthalene-1,8-diamine (1.232 g, 5.75 mmol) and sodium iodide (259 mg, 1.73 mmol) suspended in dry MeCN (25 mL) under an argon atmosphere Ethyl bromoacetate (544 µL, 5.75 mmol) was added and the reaction heated to 60 °C for 48 hours. The reaction was then cooled to room temperature and the solvent removed *in vacuo*. The residue was suspended in EtOAc (100 mL) and washed with phosphate buffer (pH 7.4, 3 × 100 mL) then brine (100 mL) and

then the organic fractions dried over $MgSO_4$ and concentrated *in vacuo*. Purification *via* column chromatography afforded the title compound (487 mg, 72% yield) R_f = 0.7 (30% EtOAc in hexane) as a white crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94 – 6.77 (m, 8H, H1-4), 4.26 (s, 4H, H7), 4.14 (s, 8H, H8), 4.02 (q, *J* = 7.1 Hz, 8H, $CO_2CH_2CH_3$), 1.12 (t, *J* = 7.1 Hz, 12H, $CO_2CH_2CH_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.7 (C9), 150.4 (C1), 139.5 (C2), 122.2 (C5), 121.5 (C4), 119.0 (C3), 113.2 (C6), 67.2 (C7), 60.8 (C8), 53.6 ($CO_2CH_2CH_3$), 14.1 ($CO_2CH_2CH_3$)**; TOF M/Z (ES+)** Found 611.2573 ($C_{30}H_{40}N_2O_{10}Na$) Calc. 611.2581, 611.1 [M+Na] 100%, 612.1 [^{13}C-M+Na] 40%; **FTIR** (Neat) 3068, 2986.3, 2933.0, 1737.3, 1595.5, 1504.9, 1240.0, 1170.4, 1123.7, 1061.2, 1023.4, 973.9, 942.8, 911.4, 730.7; **M.P.(**From EtOAc) 53-55 °C.

Analytical data in agreement with literature values. [83]

Diethyl-2,2'-((2-(2-(2-(bis(2-ethoxy-2-oxoethyl)amino)-5-formylphenoxy)ethoxy)phenyl)azanediyl)diacetate, 143



Synthesised with modification to the literature.[167]

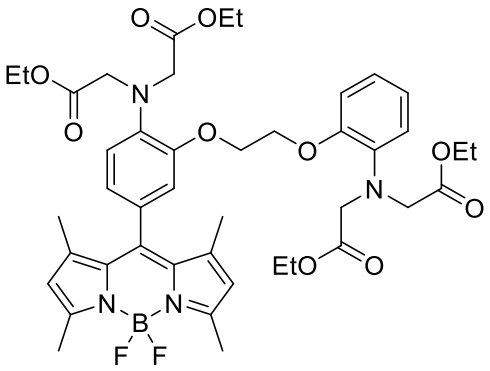
To a solution of tetraethyl-2,2',2'',2'''-(((ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(azanetriyl))tetraacetate (170 mg, 0.29 mmol) in dry DMF (10 mL) cooled to 0 °C under an argon atmosphere $POCl_3$ (37 µL, 0.29 mmol) was added in a dropwise fashion over 5 minutes with stirring. The reaction mixture was allowed to warm to r.t. and stirred for a further 2 hours then heated to 65 °C for 18 h. A 100 µL aliquot of the reaction mixture was taken, diluted with 0.5 mL EtOAc and partitioned with NaOH (0.5 mL, 0.1 M, aq.) in a 2 mL glass vial as a mini-workup for TLC. Despite the presence of unreacted starting material, the reaction was then diluted in DCM (100 mL) and poured into a beaker full of ice and NaOH (100 mL, 0.1 M, aq.) to quench. The mixture was allowed to quench for 1 h then the organic layer separated and the aqueous layer extracted with DCM (2 × 100 mL). The combined organic fractions were combined then dried over $MgSO_4$ and the solvent removed *in vacuo.* The residue is dissolved in EtOAc (250 mL) and washed with brine (4 × 250 mL) then dried over $MgSO_4$ and concentrated *in vacuo.* Purification was achieved via column chromatography (40% EtOAc in hexane) to afford the title compound (95 mg, 062% yield) R_f = 0.3 as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.79 (s, 1H, CHO), 7.36 (s, 1H, BAPTA-C-H), 6.92 – 6.79 (m, 5H, BAPTA-C-H), 6.75 (d, *J* = 8.3 Hz, 1H, BAPTA-C-H), 4.34 – 4.29 (m, 2H, $ArOCH_2\mathit{CH_2}OAr$), 4.29 – 4.24 (m, 2H, $ArO\mathit{CH_2}CH_2OAr$), 4.22 (s, 4H, 2 × $N\mathit{CH_2}CO_2CH_2CH_3$), 4.14 (s, 4H, 2 × $N\mathit{CH_2}CO_2CH_2CH_3$), 4.04 (q, *J* = 7.1 Hz, 8H, 4 × $NCH_2CO_2\mathit{CH_2}CH_3$), 1.14 (t, *J* = 7.1 Hz, 6H, 2 × $NCH_2CO_2CH_2\mathit{CH_3}$), 1.13 (t, *J* = 7.1 Hz, 6Hm 2 × $NCH_2CO_2CH_2\mathit{CH_3}$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 190.6 (CHO), 171.6 ($NCH_2\mathit{CO_2}OEt$), 170.9 ($NCH_2\mathit{CO_2}OEt$), 150.3 (BAPTA-C-N), 149.8 (BAPTA-C-N), 145.3 (BAPTA-C-O), 139.6 (BAPTA-C-O), 130.0 (BAPTA-*C*-CHO), 126.7 (BAPTA-C-H), 122.3 (BAPTA-C-H), 121.9 (BAPTA-C-H), 119.2 (BAPTA-C-H), 116.8 (BAPTA-C-H), 113.5 (BAPTA-C-H), 111.1 (BAPTA-C-H), 67.5 ($ArOCH_2CH_2OAr$), 66.9 ($ArOCH_2CH_2OAr$), 61.3 ($N\mathit{CH_2}CO_2CH_2CH_3$), 60.9 ($N\mathit{CH_2}CO_2CH_2CH_3$), 53.9 ($NCH_2CO_2\mathit{CH_2}CH_3$), 53.6 ($NCH_2CO_2\mathit{CH_2}CH_3$), 14.2 ($NCH_2CO_2CH_2\mathit{CH_3}$), 14.1 ($NCH_2CO_2CH_2\mathit{CH_3}$); **TOF M/Z (ES+)** Found 639.2532

[M+Na] Calc. 639.2530, 639.25 [M+Na] 100%, 640.26 [^{13}C-M+Na] 20%; **FTIR** (Neat) 3354.1, 2978.9, 1740.3, 1682.5, 1593.8, 1507.5, 1417.3, 1398.7, 1372.6, 1258.4, 1241.6, 1174.5, 1135.8, 1021.3, 973.8, 801.8, 744.5; **M.P.** (From EtOAc) 84-86 °C.

Analytical data in agreement with literature values. [167]

Diethyl-2,2'-((2-(2-(2-(bis(2-ethoxy-2-oxoethyl)amino)-5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenoxy)ethoxy)phenyl)azanediyl)diacetate, 144



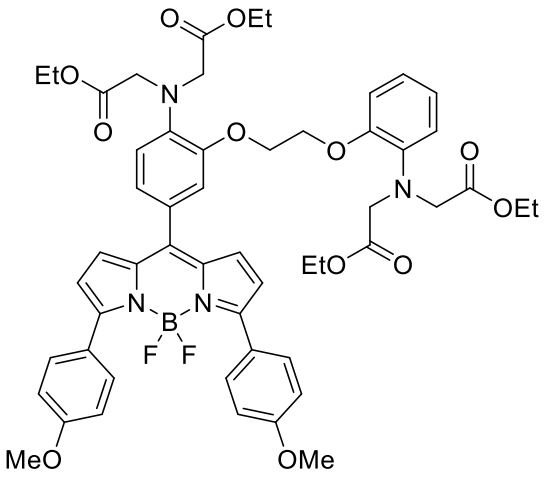
A novel compound

To a stirred solution of diethyl 2,2'-((2-(2-(2-(bis(2-ethoxy-2-oxoethyl)amino)-5-formylphenoxy)ethoxy)phenyl)azanediyl)diacetate (100 mg, 0.19 mmol) and 2,4-dimethyl-1H-pyrrole (44 µL, 0.43 mmol) in dry DCM (4 mL) under an argon atmosphere trifluoroacetic acid (2 uL) was added as a single addition and the reaction was stirred in darkness for 16 hours. The reaction was then cooled to 0 °C and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (47 mg, 0.21

mmol) was added as a single portion and the reaction was stirred for a further 40 minutes at 0 °C then warmed to r.t. and stirred for 2 hours. NEt_3 (380 µL, 2.73 mmol) and BF_3OEt_2 (380 µL, 2.73 mmol) were added dropwise over 10 minutes and the reaction stirred for 16 hours. The reaction was diluted with DCM (100 mL), washed with NaOH (2 × 25 mL, 0.1 M, Aq.) then water (2 × 25 mL) and the organic fractions were dried over Na_2SO_4 then concentrated *in vacuo.* Purification was afforded *via* column chromatography (40% EtOAc in hexane) followed by a second column (0-10% acetone in toluene) to afford the product (27 mg, 27% yield) as an orange crystalline solid with a green lustre.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.94 – 6.74 (m, 7H, BAPTA-C-H), 5.97 (s, 2H, 2 × BODIPY-Pyrrole-C-H), 4.30 – 4.21 (m, 4H, ArO*CH$_2$CH$_2$*OAr), 4.20 (s, 4H, 2 × N*CH$_2$*CO$_2$Et), 4.12 (s, 4H, 2 × N*CH$_2$*CO$_2$Et), 4.11 – 4.04 (m, 8H, 4 × NCH$_2$CO$_2$*CH$_2$*CH$_3$), 2.54 (s, 6H, BODIPY-Me), 1.48 (s, 6H, BODIPY-Me), 1.22 – 1.14 (m, 12H 4 × NCH$_2$CO$_2$CH$_2$*CH$_3$*); ^{19}F-NMR δ_F(282 MHz; $CDCl_3$): -146.28 (m); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.4 (NCH$_2$*CO$_2$*Et), 171.3 (NCH$_2$*CO$_2$*Et), 155.3 (BAPTA-C-N), 150.8 (Ar-C), 150.3 (Ar-C), 143.1 (Ar-C), 141.5 (Ar-C), 140.1 (Ar-C), 139.6 (Ar-C), 131.7 (Ar-C), 127. 9 (Ar-C), 122.4 (BAPTA-C-H), 121.9 (BAPTA-C-H), 121.2 (BAPTA-C-H), 121.1 (BAPTA-C-H), 119.4 (BAPTA-C-H), 118.9 (BAPTA-C-H), 114.1 (BAPTA-C-H), 112.9 (BODIPY-Pyrrole-*C*-H), 67.5 (ArOCH$_2$CH$_2$OAr), 67.2 (ArOCH$_2$CH$_2$OAr), 60.9 (N*CH$_2$*CO$_2$CH$_2$CH$_3$), 60.7 (N*CH$_2$*CO$_2$CH$_2$CH$_3$), 53.65 (NCH$_2$CO$_2$*CH$_2$*CH$_3$), 53.6 (NCH$_2$CO$_2$*CH$_2$*CH$_3$), 14.6 (BODIPY-Me), 14.2 (NCH$_2$CO$_2$CH$_2$*CH$_3$*), 14.1 (NCH$_2$CO$_2$CH$_2$*CH$_3$*); **TOF M/Z (ES+)** Found 857.3726 [^{11}B-M+Na] ($C_{43}H_{53}N_4O_{10}NaF_2{}^{11}B$) Calc. 857.3721, 857.4 [^{11}B-M+Na] 100%, 858.4 [^{13}C-^{11}B-M+Na] 30%, [^{10}B-M+Na] 10%; **FTIR** (Neat) 2979.4, 2923.9, 2869.8, 2042.9, 1741.8, 1728.5, 1601.1, 1541.8, 1504.5, 1410.1, 1371.1, 1182.8, 1155.1, 1064.1, 1025.4, 972.9, 941.4, 854.1, 807.9, 761.4, 741.1, 668.3; **M.P.**(From acetone) 106-108 °C.

Diethyl-2,2'-((2-(2-(2-(bis(2-ethoxy-2-oxoethyl)amino)-5-(5,5-difluoro-3,7-bis(4-methoxyphenyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenoxy)ethoxy)phenyl)azanediyl)diacetate, 145



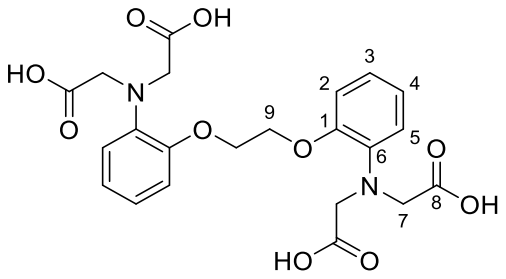
A novel compound.

To a stirred solution of diethyl 2,2'-((2-(2-(2-(bis(2-ethoxy-2-oxoethyl)amino)-5-formylphenoxy)ethoxy)phenyl)azanediyl)diacetate (95 mg, 0.18 mmol) and 2-(4-methoxyphenyl)-1H-pyrrole (70 µL, 0.40 mmol) in dry DCM (4 mL) under an argon atmosphere trifluoroacetic acid (2 uL) was added as a single addition and the reaction was stirred in darkness for 16 hours. The reaction was then cooled to 0 °C and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (45 mg, 0.2 mmol) was added as a single portion and the reaction was stirred for a further 40 minutes at 0 °C then warmed to r.t. and stirred for 2 hours. NEt_3 (360 µL, 2.58 mmol) and BF_3OEt_2 (360 µL, 2.99 mmol) were added dropwise over 10 minutes and the reaction stirred for 16 hours. The reaction was diluted with DCM (100 mL), washed with NaOH (2 × 25 mL, 0.1M, Aq.) then water (2 × 25 mL) and the organic fractions were dried over Na_2SO_4 then concentrated *in vacuo.* Purification was

afforded *via* column chromatography (40% EtOAc in hexane) followed by a second column (0-10% acetone in toluene) to afford the product (10 mg, 6% yield) as dark purple crystalline solid with a green lustre.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.9 Hz, 4H,4 × BODIPY-*p*-(OMe)*Ph-H*), 7.13 (s, 1H, BAPTA-C-H), 6.98 – 6.93 (m, 6H, BODIPY-4 × *p*-(OMe)*Ph-H +* BAPTA-C-H), 6.92 – 6.81 (m, 6H, BAPTA-C-H), 6.61 (d, *J* = 4.3 Hz, 2H, BODIPY-pyrrole-C-H), 4.35 – 4.27 (m, 4H, ArOCH_2CH_2OAr), 4.25 (s, 4H, 2 × NCH_2CO_2Et), 4.15 (s, 4H, 2 × NCH_2CO_2Et), 4.09 (q, *J* = 7.1 Hz, 4H, 2 × $CO_2CH_2CH_3$), 4.03 (q, *J* = 7.1 Hz, 4H, 2 × $CO_2CH_2CH_3$), 3.84 (s, 6H, 2 × OMe), 1.20 – 1.13 (m, 12H, 4 × $CO_2CH_2CH_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.6($CO_2CH_2CH_3$), 171.4 ($CO_2CH_2CH_3$), 160.7 (Ar-*C*-OMe), 157.9 (BAPTA-*C*-N), 150.4 (BAPTA-C-O), 149.5 (BAPTA-C-O), 142.6 (BODIPY-*C*-*p*(OMe)Ph), 141.4 (Ar-C), 139.6 (Ar-C), 136.2 (Ar-C), 131.2 (BODIPY-*p*-(OMe)*Ph-H*, 130.3 (Ar-C), 127.9 (Ar-C), 125.5 (Ar-C), 124.8 (BAPTA-C-H), 122.4 (BODIPY-pyrrole-C-H), 121.8 (BAPTA-C-H), 120.4 (BODIPY-pyrrole-C-H), 119.3 (BAPTA-C-H), 117.6 (BAPTA-C-H), 115.7 (BAPTA-C-H), 113.9 (BAPTA-C-H), 113.5 (BODIPY-*p*-(OMe)*Ph-H)*, 67.5 ($ArOCH_2CH_2OAr$), 67.1 ($ArOCH_2CH_2OAr$), 61.2 ($CO_2CH_2CH_3$), 60.8 ($CO_2CH_2CH_3$), 55.4 (OMe), 53.7 (NCH_2CO_2Et), 53.7 (NCH_2CO_2Et), 14.25, 14.2 ($CO_2CH_2CH_3$); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -151.3; **TOF M/Z (ES+)** Found 1013.3959 [^{11}B-M+Na] ($C_{53}H_{57}{}^{11}BN_4O_{12}F_2Na$) Calc. 1013.3932, 1013.4 [M+Na] 100%, [^{13}C-M+Na] 40%, [^{10}B-M+Na] 10%; **FTIR** (Neat) 3002.0, 2931.9, 2866.8, 2042.8, 1741.4, 1604.4, 1544.8, 1509.2, 1467.5, 1256.6, 1182.4, 1143.5, 1067.5, 1025.9, 797.3, 667.1; **M.P.**(From acetone) 113-115 °C.

2,2',2'',2'''-(((ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(azanetriyl))tetraacetic acid, 146



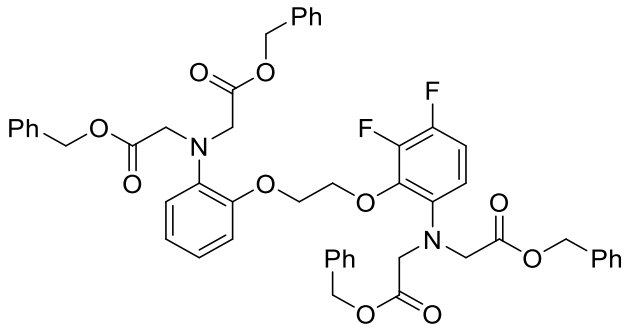
A known compound synthesised according to a literature procedure.[168]

To a stirred solution of tetraethyl-2,2',2'',2'''-(((ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(azanetriyl))tetraacetate (50 mg, 0.09 mmol) in EtOH (3.5 mL, 95% Aq.) at r.t.; KOH (85 µL, 0.85 mmol, 10 M Aq.) was added as a single portion and the reaction was heated to 80 °C for 2 hours. The reaction was cooled to r.t. then Amberlyst resin beads were added until the acidity of the reaction mixture was pH= ~3 then the reaction was filtered and the filtrate was concentrated *in vacuo* to afford the title compound (29 mg,72% yield) as a white solid.

^{1}H NMR (300 MHz, DMSO) δ 6.99 – 6.92 (m, 2H, H2,), 6.85 – 6.77 (m, 4H, H3 + H4), 6.72 – 6.66 (m, 2H, H5), 4.24 (s, 4H, H9), 3.99 (s, 4H, H7); **^{13}C NMR** (101 MHz, DMSO) δ 173.8 (C8), 149.3 (C6), 138.7 (C1), 121.3 (C4), 120.3 (C3), 117.9 (C5), 114.3 (C2), 67.4 (C9), 56.7 (C7); **TOF M/Z** (ES-) 474.9 [M-H] ($C_{22}H_{23}N_2O_{10}$) 100%, 475.9 [^{13}C-M-H] 40%.

Analytical data in agreement with literature values. [168]

Dibenzyl-2,2'-((2-(2-(6-(bis(2-(benzyloxy)-2-oxoethyl)amino)-2,3-difluorophenoxy)ethoxy)phenyl)azanediyl)diacetate, 147



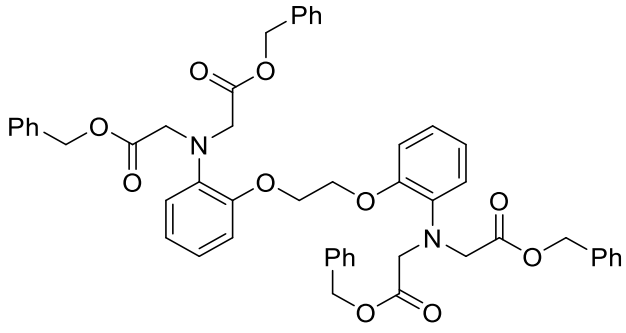
A novel compound.

Benzylbromoacetate (7.2 mL, 45.4 mmol) was added as a single portion to a mixture of 2-(2-(2-aminophenoxy)ethoxy)-3,4-difluoroaniline (1.455g, 5.19 mmol), N,N,*N*,*N*-tetramethylnaphthalene-1,8-diamine (6.65, 31.1 mmol) and sodium iodide (1.56 g, 10.38 mmol) suspended in dry butyronitrile (20 mL) under an argon atmosphere. The reaction mixture was heated to 115 °C with stirring for 3 days after which a further addition of benzylbromoacetate (1.63 mL, 10.38 mmol) and N,N,*N*,*N*-tetramethylnaphthalene-1,8-diamine (2.24g, 10.38 mmol) were added as a solution in butyronitrile (7 mL) and the reaction continually heated for a further 11 days. On the 14th day the reaction was cooled to r.t. and pyrrolidine was added and the mixture stirred for 15 minutes to destroy any unreacted electrophiles present, after which the reaction mixture was diluted with EtOAc (250 mL then filtered and the filtrand washed with EtOAc (250 mL). The combined organic fractions were washed with citric acid (2 × 250 mL, 0.5M) then dried over $MgSO_4$ and solvents removed *in vacuo* to afford a dark brown oil. Purificaiton was achieved via gradient column chromatography from 10-40% EtOAc in Petroleum spirit (40-60°C)

to afford the product (2.5 g, 55% yield) R_f = 0.2 (40% EtOAc in hexane) as a clear faintly yellow-brown oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.18 (m, 20H, 4 × $NCH_2CO_2CH_2$*Ph*), 6.92 – 6.70 (m, 5H, 5 × BAPTA-C-H), 6.66 – 6.58 (m, 1H, BAPTA-C-H), 5.09 (s, 4H, 2 × NCH_2CO_2*CH_2*Ph), 5.02 (s, 4H, 2 × NCH_2CO_2*CH_2*Ph), 4.37 (t, *J* = 4.8 Hz, 2H, ArO*CH_2*CH_2OAr), 4.29 (t, *J* = 4.8 Hz, 2H, ArOCH_2*CH_2*OAr)), 4.22 (s, 4H, 2 × N*CH_2*CO_2CH_2Ph), 4.13 (s, 4H, 2 × N*CH_2*CO_2CH_2Ph); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.3 (NCH_2*CO_2*CH_2Ph), 170.5 (NCH_2*CO_2*CH_2Ph), 150.8 (BAPTA-C-N), 139.5 (BAPTA-C-O), 139.3 (m, BAPTA-C-F), 137.4 (m, BAPTA-C-F) 135.8 ($NCH_2CO_2CH_2$*Ph*), 135.5 ($NCH_2CO_2CH_2$*Ph*), 128.7 ($NCH_2CO_2CH_2$*Ph-H*), 128.6 ($NCH_2CO_2CH_2$*Ph-H*), 128.6 ($NCH_2CO_2CH_2$*Ph-H*), 128.5 ($NCH_2CO_2CH_2$*Ph-H*), 128.4 ($NCH_2CO_2CH_2$*Ph-H*), 127.8, 127.1, 122.7 (BAPTA-C-H), 121.8 (BAPTA-C-H), 120.1 (BAPTA-C-H), 114.5 (BAPTA-C-H), 111.2 (BAPTA-C-H), 110.9 (BAPTA-C-H), 71.9 (ArO*CH_2*CH_2OAr), 67.9 (ArOCH_2*CH_2*OAr), 66.7 (NCH_2CO_2*CH_2*Ph), 66.5 (NCH_2CO_2*CH_2*Ph), 53.95 (N*CH_2*CO_2CH_2Ph), 53.90 (N*CH_2*CO_2CH_2Ph); ^{19}F NMR (282 MHz, $CDCl_3$) δ -143.3 (d, *J* = 21.4 Hz), -143.8 (d, *J* = 21.4 Hz); **TOF M/Z/ (ES+)** Found 895.3021 [M+Na] ($C_{50}H_{46}N_2O_{10}NaF_2$) Calc. 895.3018, 895.3 [M+Na] 100%, 896.3 [^{13}C-M+Na] 40%.

Tetrabenzyl-2,2',2'',2'''-(((ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(azanetriyl))tetraacetate, 148

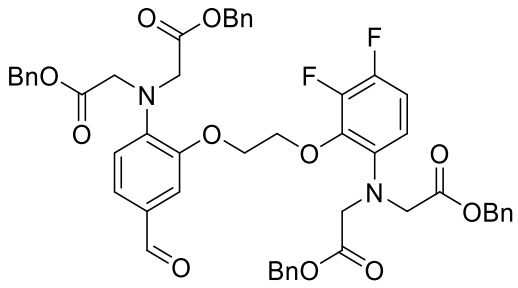


A Novel compound.

Benzylbromoacetate (7.2 mL, 45.4 mmol) was added as a single portion to a mixture of 2,2'-(ethane-1,2-diylbis(oxy))dianiline (1.849 g, 7.57 mmol), N,N,*N*,*N*-tetramethylnaphthalene-1,8-diamine (9.72g, 45.4 mmol) and sodium iodide (2.27g, 15.15 mmol) suspended in dry MeCN (75 mL) under an argon atmosphere. The reaction mixture was heated to 80 °C with stirring for 48 h after which it was cooled to room temperature. The reaction solvent was removed *in vacuo* and the residue suspended in ethyl acetate (250 mL), washed with HCl (250 mL, 0.5 M aq.) then washed with water (2 × 250 mL). The organic phase was separated and dried *in vacuo* and the clear yellow oil was recrystallized from petroleum spirit (40-60°C) and diethyl ether to afford the title compound, (5.747 g, 91% yield) as white crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.24 (m, 20H, $NCH_2CO_2CH_2$*Ph-H*), 6.98 – 6.86 (m, 8H, BAPTA-C-H), 5.05 (s, 8H, NCH_2CO_2*CH_2*Ph), 4.24 (s, 4H, ArO*CH_2CH_2*OAr), 4.22 (s, 8H, N*CH_2*CO_2CH_2Ph); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.3 (NCH_2*CO_2*CH_2Ph), 150.6 (BAPTA-C-N), 139.3 (BAPTA-C-O), 135.7 ($NCH_2CO_2CH_2$*Ph*), 128.6 ($NCH_2CO_2CH_2$*Ph-H)*, 128.4 ($NCH_2CO_2CH_2$*Ph-H)*, 128.3 ($NCH_2CO_2CH_2$*Ph-H)*, 122.5 (BAPTA-C-H), 121.7 (BAPTA-C-H), 119.6 (BAPTA-C-H), 113.9 (BAPTA-C-H), 67.3 (ArO*CH_2CH_2*OAr), 66.4 (NCH_2CO_2*CH_2*Ph), 53.8 (N*CH_2*CO_2CH_2Ph); **TOF M/Z (ES+)** Found 859.3206 [M+Na] ($C_{50}H_{48}N_2O_{10}Na$) Calc. 859.3207, 859.3 [M+Na] 100%, 860.3 [^{13}C-M+Na] 50%; **FTIR** (Neat) 3063.4, 3031.8, 2956.1, 1740.0, 1594.9, 1502.0, 1453.5, 1241.6, 1214.5, 1171.2, 1153.5, 1131.8, 1061.4, 985.6, 734.4, 694.6; **M.P.** (From hexane) 59-61 °C.

Dibenzyl-2,2'-((2-(2-(6-(bis(2-(benzyloxy)-2-oxoethyl)amino)-2,3-difluorophenoxy)ethoxy)-4-formylphenyl)azanediyl)diacetate, 149



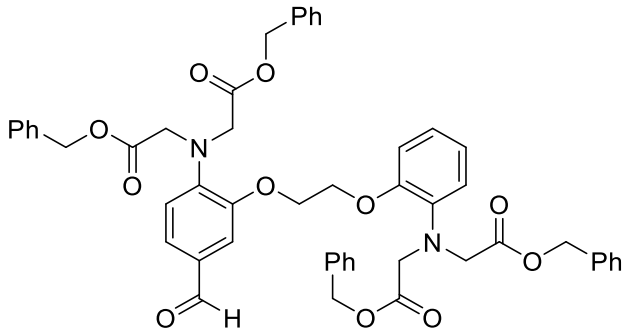
A novel a compound.

To a solution of dibenzyl 2,2'-((2-(2-(6-(bis(2-(benzyloxy)-2-oxoethyl)amino)-2,3-difluorophenoxy)ethoxy)phenyl)azanediyl)diacetate (75 mg, 0.09 mmol) in DMF (1 mL) cooled to 0 °C under an argon atmosphere $POCl_3$ (8 µL, 0.09 mmol) was added in a dropwise fashion over 5 minutes with stirring. The reaction mixture was stirred for a further 15 minutes then heated to 65 °C for 18 h. The reaction was then diluted in DCM (100 mL) and poured into a beaker full of ice and NaOH (20 mL, 0.1 M, aq.) to quench. The mixture was allowed to quench for 1 h then the organic layer separated and the aqueous layer extracted with DCM (2 x25 mL). The combined organic fractions were combined then dried over $MgSO_4$ and the solvent removed *in vacuo.* The residue is dissolved in EtOAc (50 mL) and washed with brine (4 × 50 mL) then dried over $MgSO_4$ and concentrated *in vacuo.* Purification was achieved via column chromatography (40% EtOAc in hexane) to afford the title compound (88 mg, 96% yield) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (s, 1H, CHO), 7.28 – 7.16 (m, 20H, CO_2CH_2*Ph*), 7.17 – 7.11 (m, 3H, BAPTA-C-H), 6.74 – 6.53 (m, 2H, BAPTA-C-H), 5.05 (s, 4H, 2 × CO_2*CH_2*Ph), 4.95 (s, 4H, 2 × CO_2*CH_2*Ph), 4.21 (s, 4H, 2 × N*CH_2*CO_2Bn), 4.20 – 4.16 (m, 2H, OC*H_2*CH_2O), 4.03 – 3.96 (m, 6H, 2 ×

N*CH*$_2$CO$_2$Bn + OC*H*$_2$CH$_2$O); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 190.7 (CHO), 170.6 (NCH$_2$*CO*$_2$Bn), 170.3 (NCH$_2$*CO*$_2$Bn), 149.8 (BAPTA-C-N), 148.5(BAPTA-C-N), 148.4, 146.6, 146.5, 146.0, 145.9, 144.9, 144.2, 144.0, 140.8, 140.7, 139.4, 137.3, 135.6, 135.5, 135.4, 130.2, 128.7 (CO$_2$CH$_2$*Ph*), 128.6 (CO$_2$CH$_2$*Ph* + BAPTA), 128.5 (CO$_2$CH$_2$*Ph* + BAPTA), 128.55 (CO$_2$CH$_2$*Ph* +BAPTA), 126.6 (CO$_2$CH$_2$*Ph*), 117.3, 114.7 (m, BAPTA-C-F), 111.9, 111.3 (m, BAPTA-C-F), 111.2, 71.3 (OCH$_2$CH$_2$O), 67.7 (OCH$_2$CH$_2$O), 66.9 (CO$_2$*CH*$_2$Ph), 66.8 (CO$_2$*CH*$_2$Ph), , 54.2 (N*CH*$_2$CO$_2$Bn), 53.9 (N*CH*$_2$CO$_2$Bn); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -142.9 (d, *J* = 21.5 Hz), -152.5 (d, *J* = 21.5 Hz); **TOF M/Z (ES+)** Found 923.2974 ($C_{51}H_{46}N_2O_{11}F_2Na$) Calc. 923.2967, 923.29 [M+ Na] 100%, 924.29 [^{13}C-M + Na] 40%; **FTIR** (Neat) 3033.5, 2942.2, 1738.5, 1680.1, 1593.3, 1502.8, 1453.9, 1502.8, 1256.7, 1156.5, 1135.2, 1048.2, 970.9, 805.9, 735.1, 695.8.

Dibenzyl-2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-formylphenoxy)ethoxy)phenyl)azanediyl)diacetate, 150



Novel compound

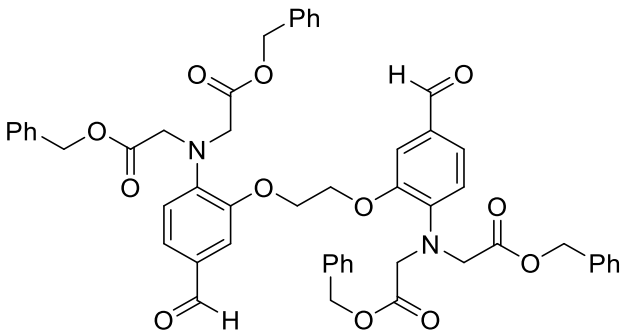
To a solution of tetrabenzyl-2,2',2'',2'''-(((ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(azanetriyl))tetraacetate (850 mg, 1.01 mmol) in DMF cooled to 0 °C under an argon atmosphere (10 mL) $POCl_3$ (105 µL, 1.12 mmol) was added in a dropwise fashion over 5

minutes with stirring. The reaction mixture was stirred for a further 15 minutes then heated to 65 °C for 18 h then a 100 µL aliquot of the reaction mixture was taken, diluted with 0.5 mL EtOAc and partitioned with NaOH (0.5 mL, 0.1 M, aq.) in a 2 mL glass vial as a mini-workup for TLC. Despite the presence of unreacted starting material, the reaction was then diluted in DCM (100 mL) and poured into a beaker full of ice and NaOH (100 mL, 0.1 M, aq.) to quench. The reason the reaction was quenched at this stage is due to the formation of the double formylation product (tetrabenzyl 2,2',2'',2'''-(((ethane-1,2-diylbis(oxy))bis(4-formyl-2,1-phenylene))bis(azanetriyl))tetraacetate) predominating the reaction outcome if further Vilsmeir' agent is introduced. The mixture was allowed to quench for 1 h then the organic layer separated and the aqueous layer extracted with DCM (2 × 100 mL). The combined organic fractions were combined then dried over $MgSO_4$ and the solvent removed *in vacuo.* The residue is dissolved in EtOAc (250 mL) and washed with brine (4 × 250 mL) then dried over $MgSO_4$ and concentrated *in vacuo.* Purification was achieved via column chromatography (40% EtOAc in petroleum-spirit (40-60 °C)) to afford the desired mono-aldehyde (309 mg, 35% yield) R_f=0.3 as a clear faintly brown oil as well as the bis-aldehyde (tetrabenzyl-2,2',2'',2'''-(((ethane-1,2-diylbis(oxy))bis(4-formyl-2,1-phenylene))bis(azanetriyl))tetraacetate) R_f = 0.15 (127 mg, 0.14 mmol) and unreacted tetrabenzyl 2,2',2'',2'''-(((ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(azanetriyl))tetraacetate (300 mg, 35% recovered) R_f = 0.65.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (s, 1H, CHO), 7.31 – 7.16 (m, 20H, $NCH_2CO_2CH_2Ph$-H), 6.90 – 6.77 (m, 6H, BAPTA-C-H), 6.67 (d, *J* = 8.2 Hz, 1H, BAPTA-C-H), 4.98 (s, 4H, $NCH_2CO_2\mathit{CH_2}Ph$), 4.97 (s, 4H, $NCH_2CO_2\mathit{CH_2}Ph$), 4.19 (s, 4H, $N\mathit{CH_2}CO_2CH_2Ph$), 4.16 – 4.09 (m, 8H, $N\mathit{CH_2}CO_2CH_2Ph$ + $ArO\mathit{CH_2CH_2}OAr$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 190.6 (CHO), 171.2 ($NCH_2\mathit{CO_2}CH_2Ph$), 170.6 ($NCH_2\mathit{CO_2}CH_2Ph$), 150.5 (BAPTA-C-N), 149.8 (BAPTA-C-N), 144.9 (BAPTA-C-O), 139.4 ($NCH_2CO_2CH_2\mathit{Ph}$), 135.7 ($NCH_2CO_2CH_2\mathit{Ph}$), 135.5 (BAPTA-*C*-CHO), 130.1, 128.7 ($NCH_2CO_2CH_2\mathit{Ph}$-*H*),

128.6 ($NCH_2CO_2CH_2$*Ph-H*), 128.5 ($NCH_2CO_2CH_2$*Ph-H*), 128.4 ($NCH_2CO_2CH_2$*Ph-H*), 126.7 (BAPTA-C-H), 122.6 (BAPTA-C-H), 122.1 (BAPTA-C-H), 119.9 (BAPTA-C-H), 117.0 (BAPTA-C-H), 114.3 (BAPTA-C-H), 111.4 (BAPTA-C-H), 67.4 ($ArOCH_2CH_2OAr$), 67.1 ($ArOCH_2CH_2OAr$), 66.8 ($NCH_2CO_2CH_2Ph$) , 66.5 ($NCH_2CO_2CH_2Ph$), 54.1 ($NCH_2CO_2CH_2Ph$), 53.8 ($NCH_2CO_2CH_2Ph$); **TOF M/Z (ES+)** 887.3167 [M+Na] ($C_{51}H_{48}N_2O_{11}Na$) Calc. 887.3156, 887.3 [M+Na] 100%, 888.3 [^{13}C-M+Na] 40%; **FTIR** (Neat) 3065.0, 3035.0, 2947.9, 2887.8, 1728.8, 138.1, 1679.2, 1592.3, 1506.5, 1455.9, 1234.9, 1164.1, 1122.9, 969.9, 934.4, 740.2, 732.8, 694.7.

Tetrabenzyl-2,2',2'',2'''-(((ethane-1,2-diylbis(oxy))bis(4-formyl-2,1-phenylene))bis(azanetriyl))tetraacetate, 150b

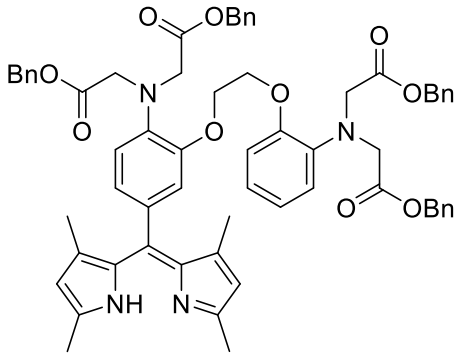


A novel compound.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.76 (s, 2H, CHO), 7.34 – 7.28 (m, 20H, $NCH_2CO_2CH_2$*Ph-H*), 7.26 – 7.20 (m, 4H, BAPTA-C-H), 6.71 (d, *J* = 8.6 Hz, 2H, BAPTA-C-H), 5.02 (s, 8H, $NCH_2CO_2CH_2Ph$-H), 4.23 (s, 8H, $NCH_2CO_2CH_2Ph$-H), 4.17 – 4.12 (m, 4H, $ArOCH_2CH_2OAr$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 190.5 (CHO), 170.5 ($NCH_2CO_2OCH_2Ph$), 149.7 (BAPTA-C-N), 145.0 (BAPTA-C-O), 135.4 ($NCH_2CO_2OCH_2Ph$), 130.1 (BAPTA-*C*-CHO), 128.7 ($NCH_2CO_2OCH_2Ph$-H), 128.5 ($NCH_2CO_2OCH_2Ph$-H), 128.4 ($NCH_2CO_2OCH_2Ph$-H), 126.9 (BAPTA-C-H), 117.2 (BAPTA-C-H), 111.4 (BAPTA-C-H), 67.1

(ArO*CH*$_2$*CH*$_2$OAr), 66.8 (NCH$_2$CO$_2$O*CH*$_2$Ph), 54.1 (N*CH*$_2$CO$_2$OCH$_2$Ph); **TOF M/Z (ES+)** Found 915.3076 [M+Na] ($C_{52}H_{48}N_2O_{12}Na$) Calc. 915.3105, 915.3 [M+Na] 100%, [^{13}C-M+Na] 45%; **FTIR** (Neat) 3074.0, 3035.0, 2932.8, 2818.8, 2725.7, 1744.5, 1676.5, 1589.4, 1517.8, 1500.1, 1454.4, 1415.6, 1400.7, 1381.9, 1262.5, 1246.4, 1196.3, 1157.0, 1137.2, 1060.9, 1001.3, 970.3, 903.7, 870.2, 791.1, 732.1, 695.5.

Dibenzyl-2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-((3,5-dimethyl-1H-pyrrol-2-yl)(3,5-dimethyl-2H-pyrrol-2-ylidene)methyl)phenoxy)ethoxy)phenyl)azanediyl)(Z)-diacetate, 151



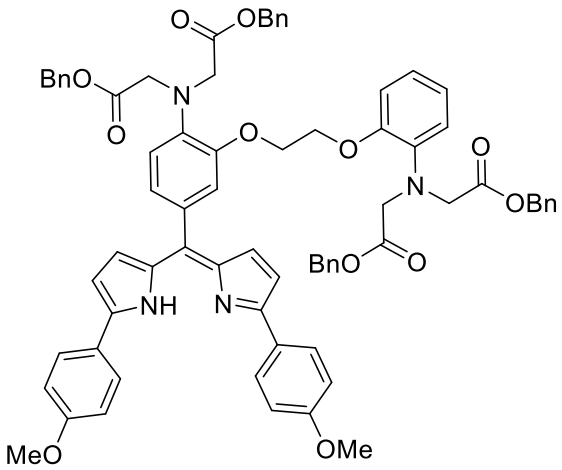
A novel compound

To a stirred solution of dibenzyl 2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-formylphenoxy)ethoxy)phenyl)azanediyl)diacetate (827 mg, 0.95 mmol) and 2,4-dimethyl-1H-pyrrole (219 µL, 2.13 mmol) in dry DCM (15 mL) under an argon atmosphere trifluoroacetic acid (9.5 uL) was added as a single addition and the reaction was stirred in darkness for 16 hours. The reaction was then cooled to 0 °C and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (237 mg, 1.05

mmol) was added as a single portion and the reaction was stirred for a further 40 minutes at 0 °C then warmed to r.t. and stirred for 2 hours. The reaction was then diluted with DCM (150 mL) and washed with water (2 × 150 mL) then organic fractions were dried over Na_2SO_4 and concentrated *in vacuo*. Purification was achieved *via* flash column chromatography utilising a stationary phase of Al_2O_3 (neutralised Brockman I) eluted with 0-50% EtOAc in DCM to afford the title compound (440 mg, 45% yield) R_f = 0.4 (50% EtOAc in DCM) as a brown-orange viscous oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.17 (m, 20H, 4 × CO_2CH_2*Ph*), 6.92 – 6.72 (m, 7H, BAPTA-C-H), 5.86 (s, 2H, BODIPY-pyrrole-C-H), 5.03 (s, 2H, CO_2*CH_2*Ph), 5.01 (s, 2H, CO_2*CH_2*Ph), 4.22 (s, 4H, 2 × N*CH_2*CO_2Bn), 4.19 – 3.99 (m, 8H, OCH_2CH_2O + 2 × N*CH_2*CO_2Bn), 2.30 (s, 6H, BODIPY-Me), 1.36 (d, *J* = 9.0 Hz, 6H, BODIPY-Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.2, 171.1, 151.5, 150.7, 150.7, 140.4, 139.5, 138.5, 136.7, 135.7, 135.7, 131.8, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 122.7 (BAPTA-Ar-H), 122.0 (BAPTA-Ar-H), 119.9 (BAPTA-Ar-H), 119.6 (BODIPY-pyrrole-C-H), 119.0 (BAPTA-Ar-H), 115.0 (BAPTA-Ar-H), 67.6 (OCH_2CH_2O), 66.5 (2 × CO_2*CH_2*Ph), 66.4 (2 × CO_2*CH_2*Ph), 54.0 (2 × N*CH_2*CO_2Bn), 16.1 (BODIPY-Me), 14.8 (BODIPY-Me); **TOF M/Z (ES+)** Found 1035.4545 ($C_{63}H_{63}N_4O_{10}$) Calc. 1035.4544; **FTIR** (neat) 3065, 3033.9, 2926.4, 2869.8, 1738.2, 1592.5, 1502.7, 1454.8, 1415.5, 1373.2, 1346.5, 1240.9, 1156.2, 1063.5, 969.9, 940.8, 909.9, 813.9, 729.9, 695.8.

Dibenzyl-2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-((5-(4-methoxyphenyl)-1H-pyrrol-2-yl)(5-(4-methoxyphenyl)-2H-pyrrol-2-ylidene)methyl)phenoxy)ethoxy)phenyl)azanediyl)(Z)-diacetate, 152



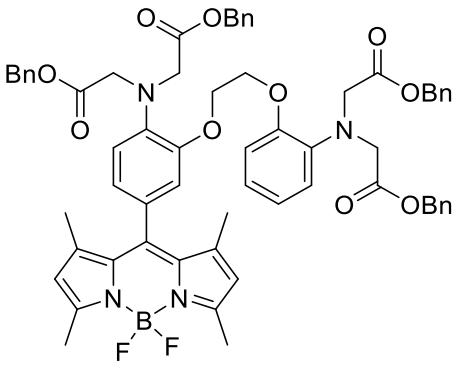
A novel compound.

To a stirred solution of dibenzyl 2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-formylphenoxy)ethoxy)phenyl)azanediyl)diacetate (178 mg, 0.21 mmol) and 2-(4-methoxyphenyl)-1H-pyrrole (80 mg, 0.46 mmol) in dry DCM (4 mL) under an argon atmosphere trifluoroacetic acid (2 uL) was added as a single addition and the reaction was stirred in darkness for 16 hours. The reaction was then cooled to 0 °C and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (51 mg, 0.23 mmol) was added as a single portion and the reaction was stirred for a further 40 minutes at 0 °C then warmed to r.t. and stirred for 2 hours. The reaction was then diluted with DCM (20 mL) and washed with water (2 × 15 mL) then organic fractions were dried over Na_2SO_4 and concentrated *in vacuo*. Purification was achieved *via* flash column chromatography utilising a

stationary phase of Al_2O_3 (neutralised Brockman I) eluted with DCM to afford the title compound (64 mg, 26% yield) R_f = 0.85 as a red oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.9 Hz, 4H, *p*-OMe-*Ph*), 7.32 – 7.15 (m, 24H, Bn-Ar-H + BAPTA-Ar-H), 7.01 (d, *J* = 8.9 Hz, 4H, *p*-OMe-*Ph*), 6.86 – 6.76 (m, 3H, Bapta-AR-H), 6.73 – 6.68 (m, 4H, BODIPY-Pyrrole-C-H), 5.05 (s, 4H, 2 × CO_2CH_2Ph), 4.97 (s, 4H, CO_2CH_2Ph), 4.26 (s, 4H, 2 × NCH_2CO_2Bn), 4.20 – 4.12 (m, 8H, 2 × NCH_2CO_2Bn + ArOCH_2CH_2OAr), 3.89 (s, 6H, 2 × OMe); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.3 (CO_2CH_2Ph), 160.3, 153.5, 150.7, 149.3, 141.7, 139.9, 139.5, 138.5, 135.7, 131.2, 129.8, 128.7, 128.6, 128.5, 128.4, 128.3, 127.7 (*p*-OMe-*Ph)*, 126.4, 124.9, 122.6, 121.9, 119.8, 117.9, 116.6, 114.9, 114.6 *(p*-OMe-*Ph)*, 114.4, 67.4 (ArOCH_2CH_2OAr)), 66.7 (2 × CO_2CH_2Ph), 66.5 (2 × CO_2CH_2Ph), 55.6 (2 × OMe), 54.0 (2 × NCH_2CO_2Bn), 53.9 (2 × NCH_2CO_2Bn); **TOF M/Z (ES+)** 1191.5 (M+H) 100%, 1192.5 ((^{13}C)M+H) 50%, 1213.4 (M+H+MeOH) 30%, 1214.4 ((^{13}C)M+H+MeOH) 20%; **FTIR** (Neat) 3029.3, 2970.4, 2932.6, 1740.9, 1603.8, 1504.6, 1455.6, 1370.3, 1230.6, 1216.9, 1168.6, 996.5, 833.3, 793.5, 697.9.

Dibenzyl-2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenoxy)ethoxy)phenyl)azanediyl)diacetate, 153



A novel compound.

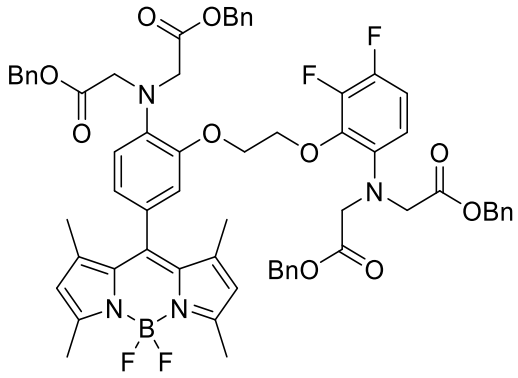
Via dibenzyl 2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-((3,5-dimethyl-1H-pyrrol-2-yl)(3,5-dimethyl-2H-pyrrol-2-ylidene)methyl)phenoxy)ethoxy)phenyl)azanediyl)(Z)-diacetate

To a stirred solution of dibenzyl 2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-((3,5-dimethyl-1H-pyrrol-2-yl)(3,5-dimethyl-2H-pyrrol-2-ylidene)methyl)phenoxy)ethoxy)phenyl)azanediyl)(Z)-diacetate (439 mg, 0.42 mmol) in dry THF (8 mL) at −78 °C under an argon atmosphere LiHMDS (930 µL, 0.84 mmol, 0.9 M in THF/ ethylbenzene) was added dropwise over 2 minutes. The solution was stirred at −78°C for 1 hour then BF_3OEt_2 (101 µL, 0.42 mmol) was added and the reaction was stirred for a further 1 hour before being allowed to warm to r.t over 16 hours. The reaction was then diluted with EtOAc (250

mL), washed with NH_4Cl (150 mL, Sat. Aq.) then water (2 × 250 mL) then the organic fractions were dried over Na_2SO_4 and then concentrated *in vacuo*. Purification *via* column chromatography (30%-50% Et_2O in hexane) afforded the title compound (290 mg, 33% yield) R_f = 0.1 (40% Et_2O in hexane) as an orange crystalline solid with a green lustre. The column was then flushed with acetone to afford unreacted dibenzyl 2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-((3,5-dimethyl-1H-pyrrol-2-yl)(3,5-dimethyl-2H-pyrrol-2-ylidene)methyl)phenoxy)ethoxy)phenyl)azanediyl)(Z)-diacetate (85 mg, 19% recovered yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 – 7.74 (m, 20H, CO_2CH_2*Ph*), 7.48 – 7.28 (m, 7H, BAPTA-Ar-H), 6.51 (s, 2H, BODIPY-Pyrrole-H), 5.60 (d, *J* = 7.9 Hz, 8H, CO_2*CH_2*Ph), 4.79 (s, 4H, N*CH_2*CO_2Bn), 4.76 – 4.71 (m, 2H, OCH_2CH_2O), 4.70 (s, 4H, N*CH_2*CO_2Bn), 4.64 – 4.58 (m, 2H, OCH2CH2O), 3.10 (s, 6H, BODIPY Me), 2.00 (s, 6H, BODIPY Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.1 (NCH$_2$*CO_2*Bn), 171.1 (NCH$_2$*CO_2*Bn), 155.4, 151.0, 150.6, 143.2, 141.6, 140.0, 139.5, 135.7, 135.6, 131.7, 128.6, 128.6, 128.4, 128.3, 128.3, 122.7, 122.2, 121.2, 120.1, 119.3, 115.0, 113.4, 67.6 (OCH_2CH_2O), 67.5 (OCH_2CH_2O), 66.6 (CO_2*CH_2*Ph), 66.4 (CO_2*CH_2*Ph), 54.0 (N*CH_2*CO_2Bn), 14.7 (Me); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -146.13; **TOF MS (ES+)** Found 1105.4370 ($C_{63}H_{61}{}^{11}BN_4O_{10}F_2Na$) Calc. 1105.4347; **FTIR** (Neat) 3065.2, 3032.0, 2950.9, 2920.9, 1736.8 (CO2Bn), 1595.6, 1542.9, 1542.9, 1506, 1407.8, 1373.9, 1308.3, 1261.9, 1233.2, 1194.5, 1062.7, 976.6, 943.9, 811.6, 745.3, 731.5, 696.6; **M.P.(**From EtOAc) 55-57 °C.

Dibenzyl-2,2'-((2-(2-(6-(bis(2-(benzyloxy)-2-oxoethyl)amino)-2,3-difluorophenoxy)ethoxy)-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenyl)azanediyl)diacetate, 154



A novel compound.

To a stirred solution of dibenzyl 2,2'-((2-(2-(6-(bis(2-(benzyloxy)-2-oxoethyl)amino)-2,3-difluorophenoxy)ethoxy)-4-formylphenyl)azanediyl)diacetate (88 mg, 0.01 mmol) and 2,4-dimethyl-1H-pyrrole (23 µL, 0.22 mmol) in dry DCM (1 mL) under an argon atmosphere trifluoroacetic acid (2 uL) was added as a single addition and the reaction was stirred in darkness for 16 hours. The reaction was then cooled to 0 °C and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (25 mg, 0.108 mmol) was added as a single portion and the reaction was stirred for a further 40 minutes at 0 °C then warmed to r.t. and stirred for 2 hours. NEt_3 (196 µL, 1.41 mmol) and BF_3OEt_2 (196 µL, 1.63 mmol) were added dropwise over 5 minutes and the reaction stirred for 16 hours. The reaction was diluted with DCM (100 mL), washed with NaOH (2 × 25 mL, 0.1M, Aq.) then water (2 × 25 mL) and the organic fractions were dried over Na_2SO_4 then concentrated *in vacuo.* Purification was afforded *via* column chromatography (30% EtOAc in hexane) to afford the

product (69 mg, 32% yield) R_f = 0.65 (30% EtOAc in hexane) as an orange crystalline solid with a green lustre.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.10 (m, 20H, 4 × $NCH_2CO_2CH_2$*Ph*), 6.84 – 6.56 (m, 5H, BAPTA-C-H), 5.90 (s, 2H, 2 × BODIPY-Pyrrole-C-H), 5.10 (s, 4H, 2 × NCH_2CO_2*CH_2*Ph), 4.94 (s, 4H, 2 × NCH_2CO_2*CH_2*Ph), 4.23 (s, 2H, N*CH_2*CO_2Bn), 4.11 – 4.04 (m, 4H, O*CH_2CH_2O* + N*CH_2*CO_2Bn), 3.99 – 3.93 (m, 2H, O*CH_2CH_2O*), 2.48 (s, 6H, 2 × Me), 1.39 (s, 6H, 2 × Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.2 (NCH_2*CO_2*CH_2Ph), 170.3 (NCH_2*CO_2*CH_2Ph), 155.4 (BAPTA-Ar-C-O), 151.0 (BAPTA-Ar-C-O), 143.4 (BAPTA-Ar-C-N), 141.8 (BAPTA-Ar-C-N), 141.0, 139.9, 139.5, 135.7, 135.4, 131.8, 128.7 ($NCH_2CO_2CH_2$*Ph)*, 128.6 ($NCH_2CO_2CH_2$*Ph)*, 128.5 ($NCH_2CO_2CH_2$*Ph)*, 128.4 ($NCH_2CO_2CH_2$*Ph)*, 127.8, 127.1, 121.2 (BAPTA-C-H), 121.0 (BODIPY-Pyrrole-C-H), 119.8 (BAPTA-C-H), 114.7 (m, BAPTA-C-H) 113.2 (BAPTA-C-H), 111.4 (BAPTA-C-H), 111.3 (BAPTA-C-H), 71.5 (O*CH_2CH_2O)*, 67.6 (O*CH_2CH_2O)*, 66.8 (NCH_2CO_2*CH_2*Ph), 66.7 (NCH_2CO_2*CH_2*Ph), 53.9 (N*CH_2*CO_2Bn), 14.7 (BODIPY-ME, 14.5(BODIPY-Me); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -143.00 (d, *J* = 21.5 Hz), -145.81 – -146.34 (m), -152.53 (d, *J* = 21.5 Hz); **TOF M/Z (ES+)** 1141.4 [^{10}B-M+Na] ($C_{63}H_{59}{}^{10}BF_4N_4O_{10}Na$) 100%, 1142.4 [^{11}B-M+Na] ($C_{63}H_{59}{}^{11}BF_4N_4O_{10}Na$) 50%; **FTIR** (Neat) 3034.1, 2956.9, 2933.4, 1738.7, 1502.9, 1476.4, 1411.9, 1372.1, 1305.2, 1189.3, 1155.2, 976.1, 807.1, 730.0, 696.8.

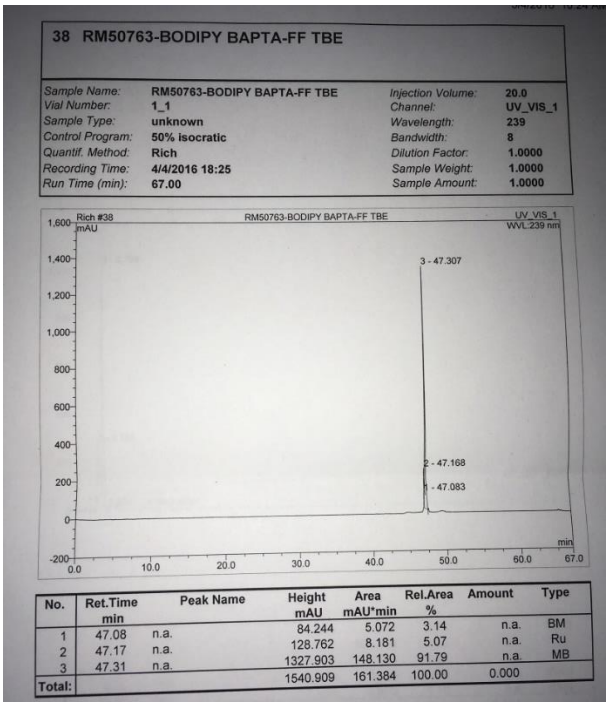
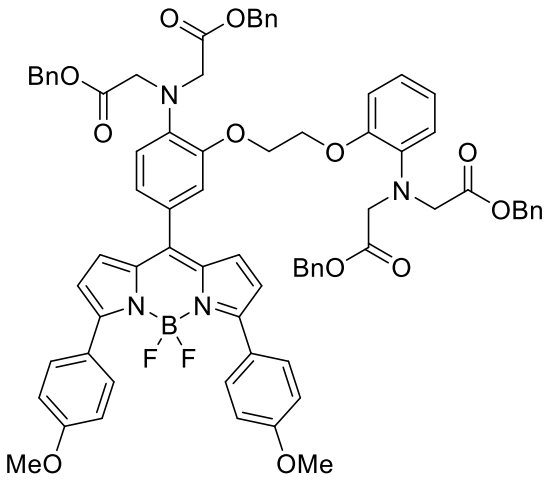


Figure 90- Reverse phase HPLC trace for Low-Green-BODIPY-BAPTA-FF-TBE 154, C18 0-50% MeCN 67 min method

dibenzyl 2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-(5,5-difluoro-3,7-bis(4-methoxyphenyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenoxy)ethoxy)phenyl)azanediyl)diacetate, 155



A novel compound

Via dibenzyl-2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-formylphenoxy)ethoxy)phenyl)azanediyl)diacetate

To a stirred solution of dibenzyl 2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-formylphenoxy)ethoxy)phenyl)azanediyl)diacetate (155 mg, 0.18 mmol) and 2-(4-methoxyphenyl)-1H-pyrrole (70 mg, 0.40 mmol) in dry DCM (4 mL) under an argon atmosphere trifluoroacetic acid (2 uL) was added as a single addition and the reaction was stirred in darkness for 16 hours. The reaction was then cooled to 0 °C and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (45 mg, 0.20 mmol) was added as a single portion and the reaction was stirred for a further 40 minutes at 0 °C then warmed to r.t. and stirred for 2 hours. NEt_3 (335 µL, 2.53 mmol) and BF_3OEt_2

(353 µL, 2.53 mmol) were added dropwise over 10 minutes and the reaction stirred for 16 hours. The reaction was diluted with DCM (100 mL), washed with NaOH (2 × 25 mL, 0.1M, Aq.) then water (2 × 25 mL) and the organic fractions were dried over Na_2SO_4 then concentrated *in vacuo.* Purification was afforded *via* column chromatography (40% EtOAc in hexane) followed by a second column (0-10% acetone in toluene) to afford the product (102 mg, 46% yield) as an opaque purple vitreous solid.

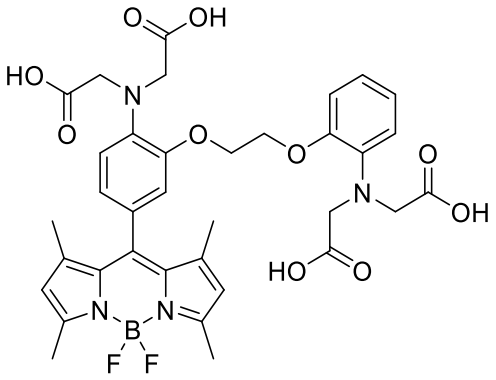
Via dibenzyl-2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-((5-(4-methoxyphenyl)-1H-pyrrol-2-yl)(5-(4-methoxyphenyl)-2H-pyrrol-2-ylidene)methyl)phenoxy)ethoxy)phenyl)azanediyl)(Z)-diacetate

To a stirred solution of dibenzyl 2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-((5-(4-methoxyphenyl)-1H-pyrrol-2-yl)(5-(4-methoxyphenyl)-2H-pyrrol-2-ylidene)methyl)phenoxy)ethoxy)phenyl)azanediyl)(Z)-diacetate (372 mg, 0.31 mmol) in dry THF (10 mL) at −78 °C under an argon atmosphere LiHMDS (406 µL, 0.41 mmol, 1 M in THF/ ethylbenzene) was added dropwise over 30 seconds. The solution was stirred at −78°C for 1 hour then BF_3OEt_2 (41 µL, 0.34 mmol) was added and the reaction was stirred for a further 1 hour before being allowed to warm to r.t. The reaction was then diluted with EtOAc (200 mL), washed with NH_4Cl (100 mL, Sat. Aq.) then water (2 × 200 mL) then the organic fractions were dried over Na_2SO_4 and then concentrated *in vacuo*. Purification *via* column chromatography (30%-100% EtOAc in hexane) afforded the title compound (369 mg, 96% yield) R_f = 0.8 (40% EtOAc in hexane) as a red vitrous crystalline solid with a purple lustre.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.8 Hz, 4H, *p*-(OMe)*Ph*), 7.27 – 7.13 (m, 20H, 4 × $NCH_2CO_2CH_2$*Ph*), 7.13 – 7.05 (m, 7H, BAPTA-C-H), 6.93 (d, *J* = 8.8 Hz, 4H, *p*-(OMe)*Ph*), 6.85 – 6.69

(m, 4H, BODIPY-Pyrrol-C-H), 4.97 (s, 4H, 2 × $NCH_2CO_2CH_2Ph$), 4.89 (s, 4H, 2 × $NCH_2CO_2CH_2Ph$), 4.20 (s, 4H, 2 × $NCH_2CO_2CH_2Ph$), 4.12 – 4.08 (m, 4H, $ArOCH_2CH_2OAr$), 4.07 (s, 4H, 2 × $NCH_2CO_2CH_2Ph$), 3.80 (s, 6H, 2 × OMe); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.3 (NCH_2CO_2Bn), 160.3 (Ar-*C*-OMe), 153.5 (BODIPY-*C*-(*p*-OMe)Ph), 150.7 (BAPTA-*C*-N), 149.3 (BAPTA-*C*-N), 141.6 (BAPTA-*CO)*, 139.9, 139.5, 138.5, 137.9, 135.7, 131.2, 129.8, 129.2, 128.7 ($NCH_2CO_2CH_2Ph$), 128.6 ($NCH_2CO_2CH_2Ph$), 128.5 ($NCH_2CO_2CH_2Ph$), 128.4 ($NCH_2CO_2CH_2Ph$), 127.6 (*p*-OMe*Ph)*, 126.4, 125.4, 124.8 (BAPTA), 122.6, 121.9, 119.7 (BAPTA), 117.8 (BAPTA), 116.6, 114.9, 114.6 (*p*-OMe*Ph)*, 67.4 ($ArOCH_2CH_2OAr$), 66.7 ($NCH_2CO_2CH_2Ph$), 66.5 ($NCH_2CO_2CH_2Ph$), 55.5 (OMe), 53.95 ($NCH_2CO_2CH_2Ph$), 53.9 ($NCH_2CO_2CH_2Ph$); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -151.4; **TOF M/Z (ES+)** 1191.5 [M-BF_2+H] 100%, 1192.5 [^{13}C-M-BF_2+H] 80%, 1261.5 [M+Na] 10%; **FTIR** (Neat) 3033.7, 2925.2, 2853.4, 1735.8, 1603.2, 1491.9, 1455.4, 1437.3, 1381.1, 1246.7, 1163.1, 1022.8, 160.9, 997.5, 953.1, 911.5, 832.6, 793.2, 734.3, 695.4; **M.P**. (From acetone) 52-54 °C.

2,2'-((2-(2-(2-(*bis*(Carboxymethyl)amino)-5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenoxy)ethoxy)phenyl)azanediyl)diacetic acid, 156



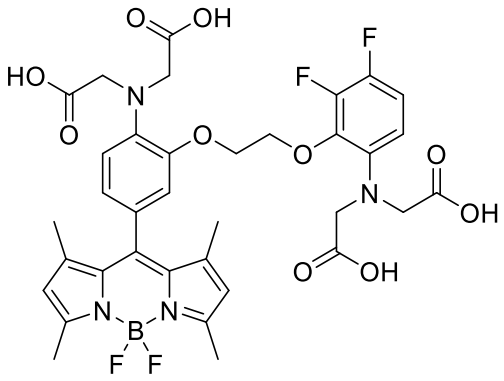
A novel compound.

To a stirred solution of dibenzyl 2,2'-((2-(2-(2-(bis(2-(benzyloxy)-2-oxoethyl)amino)-5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenoxy)ethoxy)phenyl)azanediyl)diacetate (145 mg, 0.13 mmol) in MeOH (5 mL, argon degassed) and EtOAc (5 mL, argon degassed) $Pd(OH)_2$ 9.5 mg, 0.01 mmol, 20% w/w on activated carbon) was added as a single portion. The solution was then subjected to a flow of H_2 bubbles introduced *via* a B Braun Sterican needle (0.8 × 120 mm), inserted *via* septum from the top to the bottom of the flask attached to a balloon containing H_2, whilst the septum was vented *via* another B Braun Sterican needle. The introduction of H_2 gas in this way accelerates the displacement of the argon dissolved in the solution and promotes faster reaction times. N.B. it is wise not to stir at this point as the sediment will likely block the needle. Following *circa* 10 minutes of H_2 bubbling, the gas injection needle is withdrawn from the solution meniscus and the reaction allowed to stir for 1 hour. The reaction was degassed with argon then filtered through Celite and the filtrate concentrated *in vacuo* to afford the title compound (79 mg, 81% yield) as an orange crystalline solid with a green lustre.

^{1}H NMR (400 MHz, d6-MeOD) δ 7.05 – 6.70 (m, 7H), 6.02 (s, 2H, pyrrole C-H), 4.28 (s, 4H, O*CH$_2$CH$_2$O*), 4.11 (s, 4H, N*CH$_2$*COOH), 3.99 (s, 4H, N*CH$_2$*COOH), 2.46 (s, 6H, Me), 1.50 (s, 6H, Me); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 175.9 (NCH$_2$*C*OOH), 175.7 (NCH$_2$*C*OOH), 156.4, 152.3, 151.9, 144.9, 143.4, 141.3, 140.2, 132.9, 129.4, 124.0, 122.5, 122.3, 122.1, 120.3, 114.8, 68.8 (O*CH$_2$CH$_2$O)*, 68.2 (O*CH$_2$CH$_2$O)*, 56.3 (N*CH$_2$*COOH), 56.0 (N*CH$_2$*COOH), 14.9 (2 × Me), 14.6 (2 × Me); **^{19}F NMR**(282 MHz, MeOD) δ -146.26 – -147.12 (m); **TOF M/Z ES(-)** Found 721.2494 ($C_{35}H_{36}{}^{11}BF_2N_4O_{10}$) (100%) Calc. 721.2493; **FTIR** (neat) 3425.2, 2959.9, 2926.8, 2879, 1673.6, 1604.7, 1505.9, 1543.1, 1468.5,

1409.7, 1306.3, 1263.2, 1193.9, 1156.8, 1059.2, 980.4, 817.5, 752.5; **M.P.** (From MeOH) 153-155 °C.

2,2'-((2-(2-(6-(Bis(carboxymethyl)amino)-2,3-difluorophenoxy)ethoxy)-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenyl)azanediyl)diacetic acid, 157

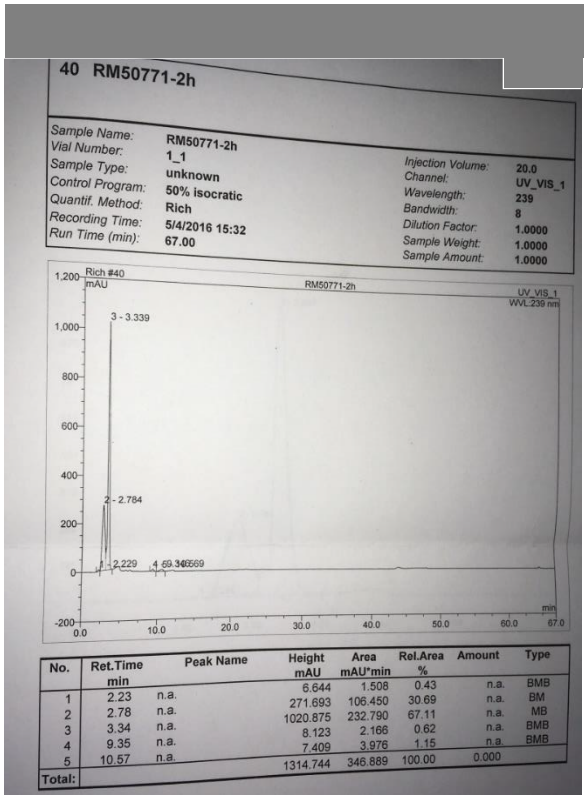


A novel compound.

To a stirred solution of dibenzyl-2,2'-((2-(2-(6-(bis(2-(benzyloxy)-2-oxoethyl)amino)-2,3-difluorophenoxy)ethoxy)-4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenyl)azanediyl)diacetate (56 mg, 0.05 mmol) in MeOH (10 mL) and EtOAc (30 mL) under an argon atmosphere; $Pd(OH)_2$ 2 mg, 2.5 µMol, 20% w/w on activated carbon) was added as a single portion. The solution was then subjected to a flow of H_2 bubbles introduced *via* a B Braun Sterican needle (0.8 × 120 mm), inserted *via* septum from the top to the bottom of the flask attached to a balloon containing H_2, whilst the septum was vented *via* another B Braun Sterican needle. The introduction of H_2 gas in this way accelerates the displacement of

the argon dissolved in the solution and promotes faster reaction times. N.B. it is wise not to stir at this point as the sediment will likely block the needle. Following *circa* 10 minutes of H_2 bubbling, the gas injection needle is withdrawn from the solution meniscus and the reaction allowed to stir for 2 hours. The reaction was degassed with argon then filtered through Celite and the filtrate concentrated *in vacuo* to afford the title compound (38 mg, 100% yield) as an orange crystalline solid with a green lustre.

^{1}H NMR (400 MHz, MeOD) δ 8.62 – 8.22 (m, 4H, Ar-H), 6.45 (s, 2H, BODIPY-Pyrrole-C-H), 6.07 – 5.99 (m, 2H, OCH_2), 5.90 – 5.83 (m, 2H, OCH_2), 5.75 (s, 2H NCH_2), 5.63 (s, 2H, NCH_2), 4.03 (s, 3H, BODIPY-Me), 3.08 (s, 3H, BODIPY-Me); **^{13}C NMR** (101 MHz, MeOD) δ 176.4 (C=O), 175.5 (C=O), 156.3 (Ar*C*-NR_2), 152.2, 144.8, 143.6, 141.2, 132.9, 129.0, 122.2 (ArC-F), 122.1 (BODIPY-Pyrrole-C-H), 120.5 (BAPTA-Ar-H), 115.1 (BAPTA-Ar-H), 111.9 (ArC-F), 73.0 (OCH_2), 69.5 (OCH_2), 56.8 (NCH_2), 56.0 (NCH_2), 30.7, 14.7 (Me), 14.5 (Me); **^{19}F NMR** (282 MHz, MeOD) δ -154.7 (Ar-F), -154.8 (Ar-F), -146.79 – -147.5 (m, BF_2); **TOF M/Z (ES-)** Found 757.2293 ($C_{35}H_{34}{}^{11}BN_4O_{10}F_4$) Calc. 757.2304, 757.2 (^{10}B-M-H) 100%, 758.2 (^{11}B-M-H) 20%; **M.P.** (From MeOH) 138-140 °C.



40 RM50771-2h

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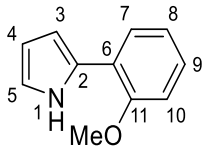
Vial Number:	1_1	Channel:	UV_VIS_1
Sample Type:	unknown	Wavelength:	239
Control Program:	50% isocratic	Bandwidth:	8
Quantif. Method:	Rich	Dilution Factor:	1.0000
Recording Time:	5/4/2016 15:32	Sample Weight:	1.0000
Run Time (min):	67.00	Sample Amount:	1.0000

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
			6.644	1.508	0.43	n.a.	BMB
1	2.23	n.a.	271.693	106.450	30.69	n.a.	BM
2	2.78	n.a.	1020.875	232.790	67.11	n.a.	MB
3	3.34	n.a.	8.123	2.166	0.62	n.a.	BMB
4	9.35	n.a.	7.409	3.976	1.15	n.a.	BMB
5	10.57	n.a.				0.000	
Total:			1314.744	346.889	100.00		

2-(2-Methoxyphenyl)-1H-pyrrole, 158



A known compound synthesised according to a procedure.[108]

Via the Co^{II}TAP radical arylation of 2-iodoanisole[108]

In an Ace-tube fitted with a septum a solution of 2-iodomethoxybenzene (143 µL, 1.49 mmol), ^{t}BuOH (1.05 mL, 11 mmol) and KOH (618 mg, 11 mmol) in pyrrole (3 mL, 43.2 mmol) was degassed *via* passing argon through a submerged syringe needle for 10 minutes. To the degassed

suspension $Co^{II}TAP$ (87 mg, 0.11 mmol) suspended in degassed pyrrole (1 mL, 14.4 mmol) was added via syringe under an inert atmosphere, then the septum carefully removed and quickly replaced with the Ace-tube lid and the reaction mixture heated to 200 °C for 45 minutes in an aluminium heating block. The reaction was allowed to cool to room temperature then transferred into a Büchi kuglrohr flask with the aid of the minimum amount of MeOH, the methanol was then removed via rotary evaporation and the flask attached to a Glass Oven B-585 Kugelrohr. The liquids were distilled at 0.1 torr with heating sequentially increasing from r.t. to 60 °C with a gradient of 10°C every 5 minutes to afford a green solid residue in the main chamber. The residue was dissolved in the minimum amount of MeOH then adsorbed onto silica (40-60 mesh) and purified *via* flash column chromatography (30% EtOAc in hexane) to afford the title compound (67 mg, 35% yield) as a faintly pink crystalline solid.

Via palladium-catalysed cross- coupling of pyrrole [160]

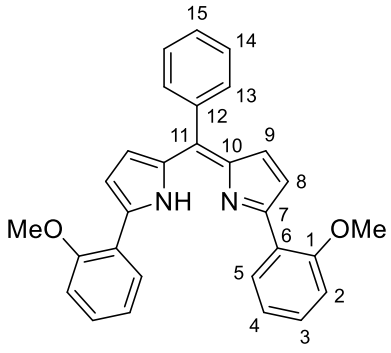
To a stirred solution of pyrrole (1 mL, 14.4 mmol, freshly distilled) in dry, degassed THF (5 mL) at 0 °C NaH (576 mg, 14.4 mmol, 60% mineral oil dispersion) was added under an argon atmosphere as a single portion and the suspension stirred for 30 minutes then warmed to r.t. $ZnBr_2$ (3.24 g, 14.4 mmol, anhydrous) was dissolved in dry, degassed THF (28 mL) and added to the reaction dropwise over 10 minutes at 0 °C. $Pd(OAc)_2$ (20 mg, 0.09 mmol), di-*tert*-butyl-*o*-biphenylphosphine (32 mg, 0.11 mg) and 2-iodoanisole (1.172 mL, 9.01 mmol) were added sequentially as single portions and the reaction heated to 65 °C for 48 h. After cooling to r.t. Et_2O (200 mL) and water (200 mL) were added and stirring continued for a further 15 minutes followed by filtration through Celite. The filter cake was repeatedly washed with Et_2O (5 × 50 mL) and the filtrate was transferred into a separatory funnel. After separation of the organic phase the aqueous phase was extracted with Et_2O (2 × 50 mL) and the combined organic phases washed

with brine then dried over $MgSO_4$ and concentrated *in vacuo*. Purification was achieved *via* column chromatography (40% EtOAc in hexane) to afford the title compound (1.13 g, 73% yield) R_f = 0.55 as a faintly purple crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.10 (s, 1H, H1), 7.96 (dd, *J* = 7.7, 1.7 Hz, 1H, H7), 7.50 – 7.38 (m, 1H, H9), 7.32 – 7.21 (m, 2H), 7.15 (td, *J* = 2.5, 1.5 Hz, 1H, H5), 7.06 (dd, *J* = 4.4, 2.5 Hz, 1H, H4), 6.97 – 6.90 (m, 1H, H3), 4.20 (s, 3H, OMe); **TOF M/Z (EI+)** 173.1 [M^+] ($C_{11}H_{11}NO$) 100%, 158.1 [M^+ -CH_3] ($C_{10}H_8NO$) 50%, 174.1 [^{13}C-M^+] ($C_{11}H_{11}NO$) 10%.

Analytical data in agreement with literature values. [108]

(Z)-2-(2-Methoxyphenyl)-5-((5-(2-methoxyphenyl)-2H-pyrrol-2-ylidene)(phenyl)methyl)-1H-pyrrole, 159



A known compound prepared according to a literature procedure.[114]

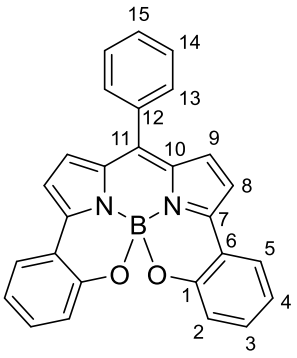
To a stirred solution of benzaldehyde (30 µL, 0.3 mmol), 2-(2-methoxyphenyl)-1H-pyrrole (117 mg, 0.68 mmol) in dry DCM (6 mL) under an argon atmosphere trifluoroacetic acid (3 µL) was added as a single addition and the reaction was stirred in darkness for 16 hours. The reaction was

then cooled to 0 °C and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (75 mg, 0.33 mmol) was added as a single portion and the reaction was stirred for a further 10 minutes at 0 °C then warmed to r.t. and stirred for 2 hours. The reaction was diluted with DCM (100 mL), washed with NaOH (2 × 50 mL, 0.1M, Aq.) then water (2 × 50 mL) and the organic fractions were dried over Na_2SO_4 then concentrated *in vacuo.* Purification was afforded *via* column chromatography (Al_2O_3, 40% EtOAc in DCM) to afford the title compound (74 mg, 57% yield) R_f = 0.9 (40% EtOAc in DCM) as red crystalline solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (dd, *J* = 7.7, 1.7 Hz, 2H, H5), 7.50 – 7.43 (m, 2H, H14), 7.40 – 7.35 (m, 3H, H4 + H15), 7.27 – 7.20 (m, 2H, H13), 6.98 – 6.93 (m, 2H, H3), 6.91 (d, *J* = 8.6 Hz, 2H, H2), 6.85 (d, *J* = 4.3 Hz, 2H, H9), 6.54 (d, *J* = 4.3 Hz, 2H, H8), 3.76 (s, 6H, 2 × OMe); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 157.4 (C1), 152.2 (C7), 139.5 (C10), 137.9, 131.0, 131.5 (C14), 129.7 (C5), 129.1 (C13), 128.6 (C8), 128.5 (C4), 127.6 (C15), 120.9 (C2), 118.4 (C9), 111.6 (C3), 55.9 (OMe); **TOF M/Z (ES+)** 433.2 [M+H] ($C_{29}H_{24}N_2O_2$) 100%, 434.2 [^{13}C-M+H] ($C_{29}H_{24}N_2O_2$) 30%.

Analytical data in agreement with literature values. [114]

(3Z,7E)-3-Phenyl-12,13-dioxa-2a1,18-diaza-12a-bora-4,7-(metheno)benzo[g]benzo[5,6]cycloundeca[1,2,3-cd]indene, 160

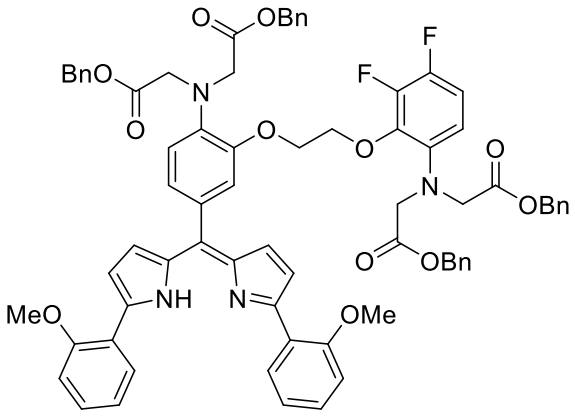


A novel compound synthesised *via* a related procedure.[114]

To a stirred solution of (Z)-2-(2-Methoxyphenyl)-5-((5-(2-methoxyphenyl)-2H-pyrrol-2-ylidene)(phenyl)methyl)-1H-pyrrole (54 mg, 0.13 mmol) in dry DCM (2 mL) cooled to 0 °C under an argon atmosphere; BBr_3 (1.25 mL, 1.25 mmol, 1 M in DCM) was added dropwise over 10 minutes and the reaction warmed to r.t. over 16 hours. The reaction was cooled to 0 °C and MeOH (5 mL) was added dropwise over 10 minutes to quench the reaction then $NaHCO_3$ (Sat. Aq.) was added to neutralize the solution the mixture and the organic phase was separated. The Aqueous phase was extracted with DCM (3 × 10 mL) and the organic phases were combined, dried over $MgSO_4$ and then concentrated *in vacuo*. The crude material was then dissolved in 50% MeOH in $CHCl_3$ (5 mL) under an argon atmosphere and trimethylborate (70 µL, 0.63 mmol) was added as a single portion and the reaction heated to 65 °C for 3h. The reaction was cooled to r.t. then concentrated *in* vacuo. Purification was achieved *via* column chromatography 50% EtOAc in hexane) to afford the title compound (19 mg, 37% yield) R_f = 0.75 (50% EtOAc in hexane) as a green crystalline solid with a red lustre.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (dd, *J* = 7.7, 1.5 Hz, 1H, H5), 7.75 (dd, *J* = 7.7, 1.5 Hz, 2H, H14), 7.61 – 7.52 (m, 3H, H15 + H4), 7.38 – 7.31 (m, 2H, H3), 7.09 (d, *J* = 4.4 Hz, 2H, H9), 7.06 (dt, *J* = 7.7, 1.0 Hz, 2H, H13), 6.97 (d, *J* = 8.2 Hz, 2H, H2), 6.91 (d, *J* = 4.4 Hz, 2H, H8); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 154.2 (C1), 149.9, (C7), 138.7, 134.3, 133.9, 132.2 (C3), 130.8, 130.6 (C14 & C15), 130.5, 130.2 (C9), 128.7 (C4), 125.8 (C5), 120.2 (C13), 119.8 (C2), 116.5 (C8); **TOF M/Z (ES+)** 435.1 [^{10}B-M+Na] ($C_{27}H_{17}BN_2O_2Na$) 100%, 413.1 [^{11}B-M+H] ($C_{27}H_{18}BN_2O_2$) 90%, [^{10}B-M+H] ($C_{27}H_{18}BN_2O_2$) 30%, [^{11}B-M+Na] ($C_{27}H_{17}BN_2O_2Na$) 15%.

Dibenzyl-2,2'-((2-(2-(6-(bis(2-(benzyloxy)-2-oxoethyl)amino)-2,3-difluorophenoxy)ethoxy)-4-((5-(2-methoxyphenyl)-1H-pyrrol-2-yl)(5-(2-methoxyphenyl)-2H-pyrrol-2-ylidene)methyl)phenyl)azanediyl)(Z)-diacetate, 161



A novel compound.

To a stirred solution of dibenzyl-2,2'-((2-(2-(6-(bis(2-(benzyloxy)-2-oxoethyl)amino)-2,3-difluorophenoxy)ethoxy)-4-formylphenyl)azanediyl)diacetate (253 mg, 0.28 mmol), 2-(2-

methoxyphenyl)-1H-pyrrole (110 mg, 0.63 mmol) in dry DCM (6 mL) under an argon atmosphere trifluoroacetic acid (3 uL) was added as a single addition and the reaction was stirred in darkness for 16 hours. The reaction was then cooled to 0 °C and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (70 mg, 0.31 mmol) was added as a single portion and the reaction was stirred for a further 10 minutes at 0 °C then warmed to r.t. and stirred for 2 hours. The reaction was diluted with DCM (100 mL), washed with NaOH (2 × 50 mL, 0.1M, Aq.) then water (2 × 50 mL) and the organic fractions were dried over Na_2SO_4 then concentrated *in vacuo.* Purification was afforded *via* column chromatography (40% EtOAc in hexane) to afford the title compound (118 mg, 35% yield) R_f = 0.6 (40% EtOAc in hexane) as red crystalline solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 – 7.96 (m, 2H, Ar-H), 7.31 – 7.23 (m, 20H, 4 × CO_2CH_2*Ph*), 7.22 – 7.15 (m, 6H, BAPTA-Ar-H), 7.11 – 6.86 (m, 6H), 6.79 – 6.63 (m, 4H Ar-H), 5.12 (s, 4H, 2 × CO_2*CH_2*Ph), 2 × CO_2*CH_2*Ph), 4.29 – 4.23 (m, 4H, 2 × N*CH_2*CO_2Bn), 4.11 – 4.00 (m, 8H, 2 × N*CH_2*CO_2Bn + O*CH_2**CH_2*O), 3.81 – 3.76 (m, 6H, 2 × OMe); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.3 (*CO_2*CH_2Ph), 170.6 *CO_2*CH_2Ph), 157.8, 152.01, 149.8, 149.2, 144.9, 141.0, 139.5, 135.7, 135.4, 131.7, 130.2, 129.7, 129.1, 128.7, 128.6, 128.5, 128.4, 126.6, 124.9, 122.7, 121.0, 118.3, 117.9, 117.3, 117.2, 114.7 (dd, *J* = 25.2, 7.9 Hz, Ar-C-F), 111.7, 67.7 (CO_2*CH_2*Ph), 66.8 (CO_2*CH_2*Ph), 66.4 (CO_2*CH_2*Ph), 66.3 (CO_2*CH_2*Ph), 54.2 (OMe); **^{19}F NMR** (282 MHz, $CDCl_3$) δ -142.9 (d, *J* = 21.5 Hz), -143.2 (d, *J* = 21.5 Hz); **TOF M/Z (ES+)** 1227.5 [M+H] ($C_{73}H_{65}F_2N_4O_{12}$) 100%, 1228.5 [^{13}C-M+H] ($C_{73}H_{65}F_2N_4O_{12}$) 80%; **FTIR** (Neat) 3385.9, 3032.9, 2943.2, 1737.4, 1596.1, 1502.1, 1454.8, 1228, 1160.9, 1053.5, 1000.9, 922.2, 802.2, 743.9, 696.3; **M.P.**(From EtOAc) 40-42 °C.

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