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Design and synthesis of small molecule probes for metabolic processes

Andrew G. Cairns

Supervisor: Dr Richard Hartley

Thesis submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy in chemistry

School of Chemistry
University of Glasgow
September 2013
Abstract

Synthesis of a photoactivated uncoupler I was completed and subsequently used by collaborators to demonstrate mitochondria uptake.

The synthesis of a ratiometric, targetable calcium sensor was completed up to intermediate II (9 steps), alongside a thiohydantoin heterocycle III synthesised in 5 steps. A co-worker has subsequently completed the probe synthesis based on this route, with the resulting probe showing good binding and optical responses in testing.

Numerous routes to 5,6-disubstituted phenanthridinium salts were investigated towards the synthesis of a mitochondrially targeted superoxide probe and hydroxylated standards. In the course of this work a novel cyclisation was developed based on intramolecular $S_N$Ar giving access to 9-benzyloxyphenanthridinium salt V.

Rapid and high-yielding access to 5,6-disubstituted phenanthridinium salts IX was then achieved through forming benzophenones VIII via Suzuki coupling and converting these to imines with the alkylamine. The nitrogen atom of the imine then undergoes cyclisation onto the aryl fluoride in an intramolecular $S_N$Ar upon heating. This transformation was shown to have good steric and electronic tolerance in the synthesis of 13 phenanthridinium analogues with 6 structural diversification points. Subsequent DFT calculations by a colleague showed this reaction proceeds in a concerted fashion and as such represents a considerable mechanistic novelty.
Efforts towards a new probe for mitochondrial superoxide led to the synthesis of 3-tertbutyl-dihydrophenanthridine X, which does not intercalate into DNA upon oxidation. This concept was refined and lead to the development of neopentyl ethidium XI and the targeted analogue MitoBNH XII and its deuterated analogue XIII.
Declaration

This thesis represents the original work of Andrew Gerard Cairns unless explicitly stated otherwise in the text. No part of this thesis has previously been submitted for a degree at the University of Glasgow or any other university. The research was carried out at the University of Glasgow (UK) in the Loudon laboratory under the supervision of Dr Richard C. Hartley during the period October 2009 to December 2012.
Acknowledgements

During the course of my PhD studies I have had the opportunity to work with, learn from, and exasperate many fantastic people. I would like to thank a few of those here, starting with my supervisor, Dr Richard Hartley, who has read bits of this document so many times I am not sure which of us is most pleased to see the back of it. I would like to thank him for the opportunity to undertake research in his group, but also for taking the time to guide my work and teach me so much. I should also thank him for tremendous patience and composure on the many occasions I am sure he was (metaphorically) tearing his hair out.

In a similar vein I would like to thank the venerable and learned Stuart, who was an invaluable source of advice, gently discouraging many of my worst ideas, and was always entertaining and easy to work with in addition - and who is definitely not grumpy, regardless of what Carlos says. Also, Stephen for useful advice but particularly for his entertaining company, and not so much for his singing, and Adam, for many extremely pedantic (and very entertaining) arguments on many an esoteric topic.

Thanks also to Rachel, who doesn’t visit often enough but is always engaging, Justyna, who I know doesn’t really drink that much vodka but tolerates my nonsense regardless, and Kishore. I would also like to mention the more recent additions in Asma, Masha, Carlos and Helmi, who make me feel a great deal older than I am but also always make me smile.

I should also thank our fellow inhabitants of the Loudon lab, the Sutherland group. In particular Lynne and Adele for listening to a great deal of whining about nearly every topic, Ewen for enduring a great deal of chemistry chat, and Grafto for never snapping and burying me in his garden. Also Filip, Fiona, Mark, Alastair, Lindsay and Lorna for their less recent but no less enjoyable company.

Outside the Loudon lab far too many names come to mind to list here, so in general thanks to all the many students and staff who have made my time here so pleasant. With regard to my work I would like to thank the technical staff who miraculously keep the place running and accommodated so many seemingly random requests, and in particular Stuart Mackay for allowing my lack of planning to constitute his emergency in the printing of this thesis.

I should reserve the final thanks for my family, and in particular my parents for their emotional support throughout my PhD and financial support during my writing up period - you will be delighted to hear I am never, ever doing anything like this again.
Abbreviations

(aq) aqueous solution
(l) liquid
(vac) in vacuum
°C degrees Celsius
µL microlitre
Å angstrom
Ac acetyl
Acetyl CoA Acetyl coenzyme A
ADP adenosine diphosphate
AMP adenosine monophosphate
APCI atmospheric pressure chemical ionisation
ATP adenosine triphosphate
APTRA o-aminophenol-N,N,O-triacetic acid
BAPTA 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid
BHT butylated hydroxytoluene
Bn benzyl
Boc tert-butyloxycarbonyl
br Broad (NMR signal)
Bu butyl
cAMP cyclic adenosine monophosphate
CCCP Carbonyl cyanide m-chlorophenylhydrazone
cGMP cyclic guanosine monophosphate
CICR calcium induced calcium release
COSY correlation spectroscopy
CRET chemiluminescence resonance energy transfer
DCM dichloromethane
DEPT distortionless enhancement by polarisation transfer
DFT density functional theory
DHP dihydropyran
DIBALH diisobutylaluminium hydride
DIPEA N,N-diisopropylethylamine
DME dimethylether
DMF dimethylformamide
DMNPE 1-(4,5-dimethoxy-2-nitrophenyl)ethyl
DMSO dimethylsulfoxide
DNA deoxyribonucleic acid
DNP 2,4-dinitrophenol
dppf 1,1'-bis(diphenylphosphino)ferrocene
EDCI 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EI electron impact
Eq equatorial
eq equivalent
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>ESI</td>
<td>electrospray ionisation</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>ETFQOR</td>
<td>electron transferring flavoprotein ubiquinone oxidoreductase</td>
</tr>
<tr>
<td>EWG</td>
<td>electron withdrawing group</td>
</tr>
<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
</tr>
<tr>
<td>FADH₂</td>
<td>flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FCCP</td>
<td>carboxylcyanide-p-trifluoromethoxyphenylhydrazone</td>
</tr>
<tr>
<td>FRET</td>
<td>fluorescence resonance energy transfer</td>
</tr>
<tr>
<td>FRTA</td>
<td>free radical theory of ageing</td>
</tr>
<tr>
<td>g</td>
<td>grams</td>
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<tr>
<td>GPDH</td>
<td>glycerol 3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>HMBC</td>
<td>heteronuclear multiple-bond correlation spectroscopy</td>
</tr>
<tr>
<td>HOMO</td>
<td>highest occupied molecular orbital</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<td>high resolution mass spectroscopy</td>
</tr>
<tr>
<td>HSQC</td>
<td>heteronuclear single-quantum correlation spectroscopy</td>
</tr>
<tr>
<td>IF</td>
<td>complex I NADH binding site</td>
</tr>
<tr>
<td>InsP₃</td>
<td>inositol triphosphate</td>
</tr>
<tr>
<td>IP₃R</td>
<td>inositol trisphosphate receptor</td>
</tr>
<tr>
<td>IPA</td>
<td>isopropyl alcohol</td>
</tr>
<tr>
<td>'Pr</td>
<td>isopropyl</td>
</tr>
<tr>
<td>IQ</td>
<td>complex I ubiquinone binding site</td>
</tr>
<tr>
<td>IRA</td>
<td>anionic ion exchange resin</td>
</tr>
<tr>
<td>J</td>
<td>joule</td>
</tr>
<tr>
<td>K</td>
<td>Kelvin</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LiHMDS</td>
<td>lithium hexamethyldisilazide</td>
</tr>
<tr>
<td>M</td>
<td>Mole(s) per litre</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MeCN</td>
<td>acetonitrile</td>
</tr>
<tr>
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<tr>
<td>MHz</td>
<td>megahertz</td>
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<tr>
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<tr>
<td>mmol</td>
<td>millimole(s)</td>
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<tr>
<td>Mol</td>
<td>mole(s)</td>
</tr>
<tr>
<td>MP</td>
<td>melting point</td>
</tr>
<tr>
<td>MRC</td>
<td>mitochondrial research centre</td>
</tr>
<tr>
<td>Ms</td>
<td>mesyl</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>mtDNA</td>
<td>mitochondrial deoxyribonucleic acid</td>
</tr>
<tr>
<td>NADH</td>
<td>Nictoinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NHC</td>
<td>N-heterocyclic carbene</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate receptor</td>
</tr>
</tbody>
</table>
NMR  nuclear magnetic resonance
NOESY  nuclear Overhauser effect spectroscopy
NSI  nanospray ionisation
Nu  nucleophile
o-  *ortho*
OGDH  2-oxo-glutarate dehydrogenase
p-  *para*
Pd/C  palladium on carbon
PDH  pyruvate dehydrogenase
PEPPSI  pyridine-enhanced precatalyst preparation stabilization and initiation
PET  photoinduced electron transfer
PG  protecting group
Ph  phenyl
PMCA  Plasma membrane Ca2+ ATPase
Pt/C  platinum on carbon
RET  reverse electron transfer
Rf  retention factor
RNA  ribonucleic acid
ROS  reactive oxygen species
RT  room temperature
RyR  Ryanodine receptor
SERCA  sarco/endoplasmic reticulum Ca2+-ATPase
SET  single electron transfer
SM  starting material
SN1  monomolecular nucleophilic substitution
SN2  bimolecular nucleophilic substitution
SNAr  nucleophilic aromatic substitution
SOD  superoxide dismutase
T  temperature
‘Bu  tertiary butyl
TEMPO  (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl
Tf  triflyl
TFA  trifluoroacetic acid
THF  tetrahydrofuran
TLC  thin layer chromatography
TMRE  tetramethyl rhodamine ester
TMS  trimethylsilyl
TMSCl  trimethylsilyl chloride
TPP  triphenylphosphonium
TS  transition state
UCP  uncoupling protein
UV  ultraviolet (wavelength)
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Chapter 1: Metabolism and the mitochondria

1.1 Introduction to metabolism

The etymology of the word ‘metabolism’ seems initially ironic; coming from the Greek ‘metaboile’, meaning ‘to change’, the term encompasses the chemical reactions which sustain living organisms, allowing our cells and bodies to function consistently. In reality, however, the story of life is one of continuous chemical change; the order which characterises our construction is actively maintained through a constant balance of competing and intertwined processes. Ultimately we rely on the transformation of raw materials (food, air) to replenish our supply of chemical building blocks, and provide the energy to utilise these. Life fundamentally relies upon increasing entropy and avoiding equilibrium, and metabolism is the continuous process which makes this possible.

The complexity, interconnectedness and dynamism of cellular chemistry makes interrogating and understanding metabolic systems tremendously challenging. Some simplifying classifications can be used, however. Metabolic processes can be broken into subdivisions (Figure 1). ‘Catabolism’ refers to the conversion of organic molecules into lower energy products and capturing the energy liberated. ‘Anabolism’ refers to processes which use this energy to construct complex molecules from simpler building blocks, such as in protein construction in the endoplasmic reticulum. Collections of enzymes and other active biomolecules can also be assigned to ‘pathways’, referring to their consecutive application to an initial substrate.

![Diagram of Metabolism](image)

**Figure 1** - The linking of catabolic and anabolic processes.

In order for the process of metabolism to work, an effective means of releasing and capturing energy and transporting it between these types of pathway must exist. In humans and other large organisms relatively diverse, complex chemical fuels such as proteins and lipids are broken down...
into their subunits, supplying amino acids, fatty acids, mononucleotides and sugars. These are then transported to cells, where further metabolism occurs.

1.2 The cellular organelles

![Figure 2 - Simplified schematic of a cell.](image)

Each cell contains a large number of specialised compartments (Figure 2), or organelles, which segregate metabolic pathways and chemical environments. Anabolic processes such as the synthesis of DNA (Nucleus) and proteins (Ribosomes) occur at a variety of specific sites, allowing regulation of their chemical environment. The major catabolic processes occur within the mitochondria (Figure 3).

1.3 Mitochondria

![Figure 3 - Basic mitochondrial anatomy, with the electron transport chain located in the inner membrane walls.](image)
Mitochondria are unusual organelles, and probably evolved from prokaryotic symbionts.\textsuperscript{15} They have their own DNA and RNA, along with the machinery required to produce some of their own proteins. On average typical animal cells have 1000 mitochondria, with up to 2000 found in some cells. They resemble bacteria in size and shape, and can be very mobile.\textsuperscript{16} The outer membrane contains a protein called porin, and is consequently permeable to molecules up to about 5000 daltons in mass.\textsuperscript{17} The inner membrane by contrast is impermeable except where specific transport proteins are involved. This membrane has a highly convoluted structure made up of cristae, providing a large surface area. The surface area can vary between cells; a cardiac muscle cell mitochondrion has triple the cristae of an equivalent mitochondrion in a liver cell.\textsuperscript{16} Within the inner membrane is the matrix, a dense gel composed of up to 50% protein.\textsuperscript{18}

It is within the mitochondrial matrix that many of the major metabolic cycles found in animals occur, including the catabolism by which energy is acquired to sustain the chemical and physical processes carried out within eukaryotic cells. There are many complexities, variations and interlinked processes,\textsuperscript{19} but the key processes are remarkably conserved between organisms,\textsuperscript{20} and can be simplified as described below (Figure 4). Carbohydrates and sugars are ultimately broken down to glucose and then further to pyruvate via glycolysis\textsuperscript{21} in the cytosol, producing adenosine triphosphate (ATP). ATP acts as an energy ‘currency’\textsuperscript{22} within the body by releasing energy via controlled degradation to either adenosine diphosphate (ADP) or adenosine monophosphate (AMP). Glycolysis also produces nicotinamide adenine dinucleotide (NADH), a redox active molecule which can transport high energy electrons. Within the mitochondria more ATP and NADH result from the oxidation of pyruvate to CO\textsubscript{2}, first through the pyruvate dehydrogenase complex to Acetyl CoA, then the Krebs cycle, with the electrons liberated being ‘stored’ in NADH and FADH\textsubscript{2} (flavin adenine dinucleotide).

![Summary of Cellular Respiration](image)

**Figure 4** - The link between glucose and ATP.\textsuperscript{23}
1.4 The electron transport chain

The electron transport chain is the series of complexes in which enzymes allow electron transfer from NADH and FADH$_2$ to carriers of successively lower energy, ending in reduction of cytochrome c. This complex is then re-oxidised by molecular oxygen to generate water.\textsuperscript{18} The energy liberated in these transfers is used to expel protons from the mitochondrial matrix, generating an increasing charge gradient (chemiosmotic potential) across the membrane until the energy required to expel protons against this gradient exceeds that produced by the reduction of oxygen with NADH (Figure 5).\textsuperscript{24}

![Figure 5 - The electron transfer chain, with the green ‘water level’ showing the relative electron free energy.\textsuperscript{25} Adapted by permission from Macmillan Publishers Ltd: Nature reviews. Molecular cell biology Moncada, S.; Erusalimsky, J. D. 3, 214–20. Copyright 2002.](image)

The complexes of the electron transport chain are all embedded within the impermeable inner mitochondrial membrane, and allow electrons to move via a mixture of redox active mobile electron carriers such as ubiquinone and cytochrome c and quantum tunnelling between metal centres.\textsuperscript{26} The physical barrier is vital, preventing the proton concentration equilibrating across the membrane and so allowing different environments to be established in the intermembrane space and the mitochondrial matrix. The charge and concentration difference across the membrane acts as potential energy store. This proton motive force is comprised of a membrane potential of 160 mV a pH difference of approximately half a unit.\textsuperscript{27} This electrochemical gradient couples the catabolic electron transport chain to the anabolic process of regenerating ATP from ADP and AMP when it is used to drive ATP synthase in a process termed ‘oxidative phosphorylation’.
1.5 Oxidative phosphorylation

Oxidative phosphorylation is catalysed by ATP synthase. This complex is highly conserved and very similar in form and function in all organisms in which oxidative phosphorylation occurs.\textsuperscript{28} The membrane-bound $F_0$ portion is associated with proton translocation, and the kinetic energy generated causes rotation of the $F_1$ subunit in a clockwise manner, catalysing ATP synthesis (Figure 6, a).\textsuperscript{29} It is estimated that each molecule of ATP synthesised correlates to the translocation of 3-4 protons.\textsuperscript{30} The reversible action of ATP synthase allows it to pump protons back out of the mitochondria (Fig. 6, b) when the membrane potential has been disrupted.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{ATP_Synthase.png}
\caption{The catalytic function of ATP synthase.\textsuperscript{31}}
\end{figure}

1.6 Mitochondria in ageing and disease: the importance of reactive oxygen species (ROS)

Given the crucial nature of respiration and the important role played by mitochondria in signalling and homeostasis, it is unsurprising that mitochondrial dysfunction is implicated in many important medical conditions, including cancer,\textsuperscript{32} diabetes\textsuperscript{33} and mitochondrial myopathies such as ragged red fibre disease.\textsuperscript{34} However, many of the most important effects of mitochondrial dysfunction arise indirectly from the generation of reactive oxygen species (ROS).

For many years it has been known that oxygen is both essential for life and toxic,\textsuperscript{35} for the same reasons. As a powerful oxidant it plays a fundamental role in being the final destination for electrons from the electron transport chain. Inevitably such a powerful oxidant is also capable of doing considerable damage within the cell. Oxygen levels within tissue are much lower than atmospheric concentrations, and the sites where it is reduced are highly evolved to contain dangerous radical by-products. Even so high concentrations reduce the lifespan of many species and oxidative damage in response to normal atmospheric concentrations is measurable.
Oxidative damage is a major topic in biomedical fields, often implicated in degenerative diseases and illnesses associated with ageing, in addition to various cardiovascular illnesses. Mitochondria are known to be a major source of reactive oxygen species (ROS), with many redox active centres and electron transfer components. Among the most common ROS are superoxide and hydrogen peroxide. Although among the less reactive oxidative species generated, both can be converted by redox active centres to the hydroxyl radical, which reacts with biomolecules at a nearly diffusion limited pace.

1.7 Free radical theory of ageing

Proposed by Denham Harman in 1954, the free radical theory of ageing proposes that the gradual damage which constitutes the ageing process arises ultimately from oxidative damage. Harman extended this in 1972 to suggest that superoxide radicals generated in metabolism are responsible for the most damage, and that maximum lifespan is ultimately determined by the rate of damage to the mitochondrial DNA. The theory is difficult to prove or disprove conclusively and remains a matter of considerable debate, with attempts to provide definitive answers hampered by the complexities of the system studied and the limitations of the tools available to do so.
Chapter 2: Mitochondrial uncoupling and MitoPhotoDNP

2.1 Mitochondrial uncouplers

2.1.1 Endogenous uncouplers

The term ‘mitochondrial uncoupling’ describes a mechanism which allows protons to leak across the membrane without passing through ATP synthase (therefore uncoupling proton influx from ATP synthesis, Figure 7). The transfer of a proton across the electrochemical gradient is accompanied by a loss of potential energy, and where this is not used to drive ATP synthesis such energy is released as heat.

Figure 7 - The electron transport chain with (b) and without (a) uncoupling protein (UCP2) which allows protons to leak across the membrane. Adapted by permission from Macmillan Publishers Ltd: Nat. Rev. Neurosci. Andrews, Z. B.; Diano, S.; Horvath, T. L., 6, 829-40, 2005. Copyright 2005.

Uncoupling is a naturally occurring phenomenon, and generating heat (hence maintaining homeostasis) is one important role. Another is to reduce the electrochemical gradient, and this impacts a range of other variables. The class of proteins involved (UCPs) have also been shown to be involved in fatty acid metabolism, insulin production and neuron function and are believed to have other roles. The uncoupling proteins have a specific role in regulation of the influx of calcium ions (an important signalling species within cells, Chapter 11) into mitochondria, via their ability to regulate membrane potential. By reducing mitochondrial membrane potential calcium influx is retarded or reversed and resulting cell death can be prevented.

Reduction of the membrane potential is also important in limiting generation of ROS. These are caused by electron leakage from the electron transport chain to nearby molecular oxygen, generating the superoxide radical (O$_2^-$) which is catalytically converted to a mix of molecular oxygen and hydrogen peroxide (H$_2$O$_2$). Superoxide (and hence hydrogen peroxide) formation is
dependent on membrane potential. Limited uncoupling as provided by the UCP proteins can therefore protect against oxidative damage (Figure 8).

**Figure 8** - Photomicrographs comparing tyrosine hydrolase neurons (green) wild type (a) and without UCP2 (b). ROS shown by red staining (ethidium, b). Higher exposure to the dye (c, d) compares wild type (c) with a transgenic cell containing human UCP2 (d). Republished with permission of the Society of Neuroscience from “Neurobiology of Disease Uncoupling Protein-2 Is Critical for Nigral Dopamine Cell Survival in a Mouse Model of Parkinson’s Disease”, Zane B. Andrews, Balazs Horvath, Colin J. Barnstable, John Elseworth, Lichuan Yang, M. Flynt Beal, Robert H. Roth, Russell T. Matthews, and Tamas L. Horvath, 25, 1, 2005. Permission conveyed through Copyright Clearance Center, Inc.
2.1.2 Synthetic uncouplers

Uncoupling proteins are vastly complex proteins with very specific functions, but it is possible to reproduce the mitochondrial uncoupling with much simpler molecules. There are several mechanisms by which this can be done, and the simplest is direct proton cycling. The requirements are simple; the uncoupler must have a protonated form and a non-protonated form with a Pka close enough to 7 for a significant amount to be in the protonated form in the intermembrane space and the non-protonated form in the matrix. Any prospective uncoupler must also be able to cross the inner mitochondrial membrane in both its protonated and non-protonated forms (so the non-protonated form should be uncharged or heavily delocalised) and prove resilient enough to have a sustained effect. Several of the most commonly used uncouplers are shown below (Fig. 9)

Of the compounds shown (Fig. 9), 2,4-dinitrophenol 1 (DNP) is perhaps the best known. The compound itself is a weak lipophilic acid (pKa = 3.96), and is partially protonated in the space between the inner and outer mitochondrial membranes. Upon crossing into the matrix (resting pH ~ 8) the DNP is deprotonated, leaving a DNP anion 5 (Fig. 10). Aromaticity and conjugation with the nitro groups make the negative charge delocalised and diffuse, so it does not prevent the anion crossing the membrane. DNP uncouples directly via proton cycling and has a narrow therapeutic window, causing hyperthermia in the case of a large dose.
Carbonyl cyanide m-chlorophenylhydrazone (CCCP) 2 and carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) 3 are similar in both structure and function, with pKa of ~6.0 and 6.2 respectively.\(^{47,48}\) In each case the acidic proton is the secondary amine attached to the aryl group. The deprotonation of CCCP 2 brings the entire molecule into conjugation, delocalising the charge over a large electronic surface and resulting in only a diffuse charge (Scheme 1). This allows CCCP 6 to cross membranes.

\[\text{Scheme 1 - Charge delocalisation upon deprotonation of CCCP 2 to give CCCP^- 6.}^{49}\]

Butylated hydroxytoluene (BHT) 4, works somewhat differently from DNP and FCCP. At low concentrations BHT induced uncoupling is through interaction with adenine nucleotide translocase (ANT)\(^{50}\) through an unknown mechanism to uncouple the mitochondria. This pathway has a limited capacity, and produces weak uncoupling even when fully activated. This ANT dependent mechanism is not unique to BHT 4; benzoic acid uncouples only through this mechanism. DNP 1 and CCCP 2 appear also to activate this pathway but this is overshadowed by their proton cycling properties, which are far better than BHT 4 and mask the effects of the ANT pathway. At higher concentrations BHT produces additional uncoupling through the proton cycling mechanism used by DNP 1, CCCP 2 and FCCP 3. The effect of combining a poor proton cycler such as BHT with the ANT mechanism is to give concentration dependent uncoupling over a much wider range of concentrations.\(^{44}\)

Although the criteria for an effective uncoupler appear specific, some of these requirements (membrane permeability for polar molecules) are requirements of drugs in general, and compounds designed for other uses can act as uncouplers. DNP itself was not used for this purpose until many years after discovery,\(^{51}\) and nimesulfide,\(^{52}\) a non-steroidal anti-inflammatory drug, is another example. There are some possibilities for uncoupler design which better take advantage of the peculiar properties of mitochondria; in particular the membrane potential which drives ATP synthesis.
2.2 Targeted photoactivated uncoupler design

Our aim was to use the membrane potential to target a caged uncoupler to the mitochondria and then release it by irradiation with light. Thus, combining mitochondria targeting and a photolinker could allow selective mitochondrial accumulation and photorelease of an uncoupler. This would allow a small dose of inactive caged uncoupler to accumulate at the mitochondrial membrane and then be selectively activated. The caged uncoupler could be useful for temporal and spatially resolved studies on the mitochondria with imaging techniques. DNP 1 was chosen as the uncoupler in the caged uncoupler MitoPhotoDNP 7 (Scheme 2); its advantages include its properties being well characterised, the compound stable and the uncoupling kinetics relatively simple. Targeting would be achieved using a lipophilic cation, and alkyltriphenylphosphonium group (TPP). Photoactivated release of a phenol from an o-nitrobenzyl linker is quite well known and efficient and the synthesis not intrinsically difficult. Therefore this photolinker was chosen on the basis that it should be easy to link with the other components of the molecule and o-nitrobenzyl group is tolerant of a wide range of groups and substituents. An added advantage for both DNP and o-nitrobenzyl is that both have been used heavily in the past, potentially providing a lot of directly comparable data for both uncoupling studies and photocleavage. The TPP targeting group and the photolinker will now be discussed in detail.

Scheme 2 - MitophotoDNP target 7. The caged uncoupler, o-nitrobenzyl linker and the TPP targeting group are colour coded.

2.3 Lipophillic cations

Cell membranes are composed of lipids, with polar groups on the surface interacting with water molecules and long saturated alkyl chains within the membrane. Simple membranes self-assemble, as water-alkyl interactions are weaker than those between water molecules and charged groups or other water molecules, and so the molecules assemble in order to give the arrangement with minimal contact between water and lipophilic groups. Compounds added to the system will be found in equilibrium between the aqueous cytoplasm and hydrophobic membranes, with the position of this equilibrium determined by the properties of the compound. The equilibrium constant is expressed as a partition coefficient. A drug or probe will generally have to diffuse through several layers of each environment, so a compound which splits reasonably evenly between the two media is usually preferred for these. Because the probe molecules are diffusing in this manner they will generally be found in places other than their target, and this dispersion is accounted for in dosing.
If the drug or probe target is within the mitochondrial matrix or inner mitochondrial membranes then the charge gradient across the inner mitochondrial membrane provides an opportunity to concentrate compounds in the inner mitochondrial membrane or matrix. Cations that are able to diffuse across the inner membrane will accumulate in accordance with the Nernst equation. The primary difficulty with such an approach is that highly charged or polar compounds often struggle to get through membranes, and molecules targeted at inner mitochondrial processes need to diffuse through a number of these. As such, a very hydrophilic compound is unlikely to reach its target. Fortunately charged groups with diffuse charges stabilised by resonance or induction have less favourable interactions with water and are therefore more lipophilic. Alternatively, cations could be attached to otherwise very hydrophobic groups or molecules in order to balance out the effect of the cation. These effects can be modelled as giving the ion a large hydrophobic surface.

Organophosphonium cations, particularly those with large lipophilic groups insulating the charge, are a good choice for targeting groups and there is literature precedence for use of alkyltriphenylphosphonium (TPP) groups for this purpose. Previous studies have shown that the phosphonium group itself integrates into the membrane surface, but is also able to travel through it (Fig 11). As a result concentrations on membranes surfaces are high but within the membrane the concentration is much lower, although a long alkyl chain between the charged head group and an attached drug may allow the active part of the drug to accumulate deeper in the membrane. Bioactive organic molecules bearing TPP groups accumulate several hundred-fold in the mitochondrial matrix. Furthermore, one such compound, MitoQ, has been taken orally by patients in Phase II clinical studies for up to 1 year without problems, so TPP is well validated for biological use.

2.4 Photolinkers

The difficulty with such a targeting strategy is that the TPP ions will inhibit the proton cycling if attached to an uncoupler. This was an issue with MitoDNP 9 (Figure 12). One way to overcome
this problem is to use a connection between the two components which can be broken by specific stimuli.

Figure 12 - MitoDNP

Selecting the stimulus in question is challenging; the mitochondria is integrated with the biochemical pathways of the cell in a vastly complex manner not yet fully understood and as such it is difficult to find a stimulus which will break up a linker without impacting surrounding systems. One strategy is to use a photolinker, which can be cleaved on application of relatively intense light of specific wavelength. A laser beam can provide the correct energy to a small area of the cell where the targeted uncoupler is present. The wavelength specific nature of photoexcitation means that cellular components caught within the beam are unlikely to be very badly damaged if the photolinker is designed to cleave at a wavelength different from those which excite groups in proteins, lipids and sugars.

Design considerations for photolinkers are outlined by Adams and Tsein, for whom the following properties are vital: the attached molecule should be unreactive when linked (or ‘caged’), the molecule should be released in high yield and sufficiently quickly by wavelengths of light non-detrimental to the biological system used, and any side products generated should be both unreactive and unable to interact with the biological system. A small selection of photolinkers extensively used are shown below (Fig. 13).

Figure 13 - A large range of photolinkers are used. The selection presented is comprised of ortho-nitrobenzyl derivatives, the related DMNPE analogues, phenacyl ethers and coumarins.

Some common features are apparent; all have highly conjugated and mostly rigid structures. Either of these properties could be coincidental as rigid structures tend to be highly conjugated and vice versa, but in reality both are important. UV absorption (usually the wavelength region used for ease of handling) is typically high among conjugated, aromatic compounds, and a molecule with a rigid excited state will relax to ground state less easily due to Condon’s rule (the limited geometries available restrict transitions available into lower energy levels). Many of the molecules are polar and some have highly polar excited states. Most are incapable of acting as acids or bases under physiological conditions. This is good as pH dependence would greatly complicate calculations of quantum yield and effective use.
Probably the most commonly used photocleavable linker is the \( o \)-nitrobenzyl group, which is optimally excited at 265 nm. A number of variants exist with a benzylic proton replaced. A general scheme for the photocleavage of such compounds is outlined below (Scheme 3).

Scheme 3 – General photocleavage of \( o \)-nitrobenzyl compounds.$^{61}$

The photoreaction starts with proton transfer to the nitro group in the excited state, at this point generating 2 geometrical isomers of the intermediate 15 if \( R \) and \( X \) are not identical. A proton is then transferred between the oxygens on the nitro group, giving \( E \) or \( Z \) aci-nitro intermediates 17 which can be observed and distinguished from one another, before forming a cyclic benzisoxazoline 18. Both isomers then decay through cyclic state 18 into nitroso compound 19 which loses \( X \) to form the carbonyl 20.

\( o \)-Nitrobenzyl groups have several advantages, such as being compatible with the functional groups for which photolinker are commonly used,$^{64}$ including primary amines which work poorly with other linkers. They are relatively easy to make and have reasonable photochemical properties,$^{62}$ which are unfortunately influenced by substituent, \( \text{pH} \), solvent polarity and a host of other environmental factors.$^{55}$ A major advantage of these compounds is that their kinetic behaviour is relatively well understood and characterised. The disadvantage of \( o \)-nitrobenzyl derivatives is the undesirable reactivity of the nitrosobenzaldehyde products 20.$^{63}$ DMNPE (11, \( R = \text{Me} \)) is an ortho-nitrobenzyl analogue with a longer photocleavage wavelength (365 nm), highlighting the impact of the methoxy groups adding electrons to the ring system. The release mechanism is analogous to that of other \( o \)-nitrobenzyl compounds.

Phenacyl ester derivatives such as 12 (Fig. 13) are used specifically where a carboxylic acid cleavage product is desired, and produce these in good yield and purity with a range of substrates.$^{64}$ These groups can also be used on phosphates$^{65}$ to good effect. They display high solubility in water and have been used as a photolinker in biotin/avidin systems.$^{66}$ 4-Methoxyphenacyl 12 is optimally excited at 280 nm, but can be excited at higher wavelengths.$^{50}$ High quantum yields are achieved for suitable substrates, and cleavage can be very fast.$^{64}$
The final group listed in Figure 13 are coumarin based. Low quantum yields are observed for carboxylic acids, but results are reasonable for uncaging cAMP and cGMP, and amines can also be caged. Coumaryls can also be used to release sulfates, alcohols and diols. The advantages of coumaryl chromophores is the high wavelength absorption maxima which are very substituent dependent, but can extend into the visible region. The group also gives rise to a fluorescent by-product, and gives fast release with the potential for two-photon excitation.

2.5 Iodo MitoPhotoDNP 7-I synthesis

Scheme 4 - Overview of the route to MitoPhotoDNP.

The planned synthetic route to MitoPhotoDNP 7-I uses a convergent synthesis following previous work within the group (Scheme 4). The ortho nitrobenyl photocleavable group 22 (green) and the mitochondria targeting triphenylphosphonium salt 24 (blue) are prepared and linked to form phosphonium iodide salt 25-I prior to addition of the DNP uncoupling moiety to form MitoPhotoDNP iodide 7-I. Caroline Quin had previously prepared MitoPhotoDNP by the same route except that an alkyl bromide had been used instead of alkyl iodide 24. Her last two steps had suffered from low yields and more compound was required for testing.

In my hands, the commercially available benzaldehyde 21 was reduced to benzylic alcohol 22 using NaBH₄ in MeOH in high yield (Scheme 5).

Scheme 5 - Reduction of benzaldehyde 21.

The TPP iodide targeting group and linker was synthesised by substituting an iodide from 1,4-diodobutane 23 with triphenylphosphine, taking advantage of the poor solubility of charged...
groups in PhMe to precipitate the TPP salt and give only the monosubstitution product 24 (Scheme 6).

![Scheme 6 - Targeting group synthesis](image)

The phenol 22 and targeting group 24 were then coupled under basic conditions (Scheme 7) to give phosphonium salt 25-I. Initially coupling in MeOH was problematic, with the difficulty of separating multiple phosphonium salt products exacerbated by the product proving unstable on silica. Switching the reaction solvent to dry MeCN and increasing the number of equivalents of phenol 22 from 1 to 1.7 resulted in near quantitative coupling.

![Scheme 7 - Coupling of the photoactivated and targeting groups](image)

The final step of the synthesis was an S$_{N}$Ar comprising attack by the benzylic alcohol 25-I onto 1-fluoro-2,4-dinitrobenzene 26 under mild conditions (Scheme 8).

![Scheme 8 - Addition of DNP uncoupling moiety](image)

The 1-fluoro-2,4-dinitrobenzene substrate 26 is ideal for this reaction as the ortho and para nitro groups stabilise the transition state leading to the Meisenheimer complex intermediate. Since the initial nucleophilic addition to generate this intermediate is usually the rate determining step, the highly electronegative fluorine also stabilises the transition state leading to this formal carbanion. Three solvents were tested for this transformation, with acetone proving the most effective, giving high conversion. The low solubility of phosphonium salt 25-I hindering reaction in Et$_2$O, and MeCN gave a complex mixture of side products. Chromatographic purification proved challenging, with the MitoPhotoDNP iodide 7-I eventually isolated in low yield.
2.6 MitoPhotoDNP Tetraphenylborate Salt synthesis

Following the difficulties experienced with the iodide salt 7-I, the more crystalline tetraphenylborate salt was synthesised as an alternative (Scheme 9).

Scheme 9 - Tetraphenylborate synthesis.

The first two steps gave the same products as previously isolated by Caroline Quin. Using dibromobutane 27 substitution is significantly slower than using diiodide 23 due to the poorer leaving group but also higher yielding. The bromide 28 was coupled to phenol 22 to give alcohol 25-Br then converted to the tetraphenylborate salt 25-BPh₄ prior to DNP addition to give MitoPhotoDNP tetraphenyl borate salt 7-BPh₄, which crystallised following column chromatography. Unfortunately Dr Murphy’s group in Cambridge showed that with this counterion MitoPhotoDNP is too hydrophobic and insoluble for uptake testing.
2.7 Alternate routes and variant compounds

Several alternate routes were investigated to circumvent the purification difficulties experienced in the synthesis of MitoPhotoDNP iodide 7-I. The p-xylyl linker was considered on the assumption that the more rigid final product would prove easier to purify, and substitution at the benzylic sites would be facile. Consequently, xylyl bromide 29 was converted into phosphonium salt 30. However a complex mixture resulted from the attempted coupling to the o-nitrobenzyl group to give triphenylphosphonium bromide 31 (Scheme 10).

Scheme 10 - Attempted synthesis of more rigid p-xylyl derivative.

2.8 Uptake Testing

MitoPhotoDNP iodide 7-I was tested for mitochondrial uptake by the group of Dr Michael P. Murphy (MRC, Cambridge), with the results shown below (Figure 14).

Figure 14 - Electrode response to the uptake of MitoPhotoDNP.

The response of an electrode selective for the triphenylphosphonium cation is shown on the y-axis. Using an appropriately buffered solution at pH 7.2 and 37 °C, five sequential additions of MitoPhotoDNP allowed calibration of a linear response. Next Complex I of the electron transport chain was inhibited by adding the pesticide rotenone, which prevents development of a membrane potential in the rat liver mitochondria subsequently added. This means the decrease in electrode response can be attributed solely to MitoPhotoDNP accumulating by diffusion and partitioning into the membranes of the mitochondria. Succinate is then added to stimulate Complex II (succinate dehydrogenase), which is part of both the Krebs cycle and the electron
transport chain. This and bypasses the blockage of Complex I by rotenone. The electron transport chain begins to function and the electrode response then drops as most of the remaining free MitoPhotoDNP partitions into the mitochondrial matrix. The final addition of FCCP 3 (Fig. 3) uncouples the mitochondria eliminating the membrane potential, causing free MitoPhotoDNP concentrations to return towards the previous equilibrium position. The process is only semi-quantitative and drift means that the minimum prior to addition of FCCP 3 and the restored levels following FCCP 3 addition are lower than expected.

2.9 Effectiveness in cells

Following the successful demonstrations of mitochondrial uptake a post-doc in the group, Dr Stuart Caldwell, developed an efficient route to the MitoPhotoDNP bromide. Susan Chalmers from the group of Prof. John G. McCarron then tested MitoPhotoDNP bromide salt 7-Br in colonic smooth muscle cells (Fig. 15).

Figure 15\textsuperscript{71} - Photolytic uncoupling of mitochondria in a whole cell - colours are artificial.

The cells were loaded with tetramethyl rhodamine ester (TMRE), a fluorescent dye which is dependent on membrane potential.\textsuperscript{72} Panel i shows the effect of brief UV laser irradiation (355 nm for 85 ms) in the absence of MitoPhotoDNP 7. 200 nM of MitoPhotoDNP 7-Br was added and allowed to equilibrate. The same irradiation protocol was then applied (panel ii), with a loss of TMRE fluorescence shown in red at the site of photolysis. Rotenone and oligomycin were added resulting in total depolarisation (panel iii). The cell without colouring is also shown (panel iv). These results (in conjunction with appropriate controls) proved the effectiveness of the probe, and the work was subsequently published.\textsuperscript{71}
Chapter 3: Design of a selective, quantitative superoxide probe

3.1 Types of small molecules probes used to study metabolism

3.1.1 Introduction to different types of probe

Limited information can be acquired from looking at the anatomy of cells, and the native chemical species are often engaged in multiple different pathways. The difficulties are exacerbated by the necessity of studying transient species and situations where a single species may engage in many processes dependent on timescale and concentration. The obvious solution to understanding a complex system is to break it down into smaller parts, such as single pathways or cascades. One way to do this is to induce effects via mechanisms alien to normal cellular chemistry, or to measure an artificial species known to behave in a particular way. In essence, this is the role of the small molecule probe.

Various elaborate designs abound, and ‘chemical probe’ is a descriptive rather than prescriptive term so over definition must be avoided, but some categorisation is possible. In particular, the distinction should be made between probes used to validate drug targets, and those used to interrogate cellular chemistry. The latter are the focus of the following discussion. It should be noted that the term ‘small molecule probe’ can apply to any species significantly below the masses of biological polymers such as protein and RNA strands, including some molecules which are very large by the usual standards of a synthetic chemist, and covers any conceivable sensing or reporting mechanism. Some general points can be made relating to their design nonetheless.

There are two essential functions any such probe must display. These are sensing, the interaction which allows the species or phenomena being studied to be recognised by the probe, and reporting, any process by which a sensing event can be recognised by the investigator. Additionally, with the occasional exception the response of probes to stimuli can be described as reversible, as in the case of ion chelators, or irreversible, usually where a chemical bond is formed.

Some of the same considerations applied in medicinal chemistry are important in probe design. Any probe intended to diffuse through the cytosol must have appropriate solubility, for example. Fortunately the requirements with respect to these properties are much less stringent than for drugs. Many probes are applied to cells or small fragments of organisms, and with techniques such as patch clamping not even membrane permeability is required. Toxicity is only a concern in so far as it occurs during the time course of the measurement. In order to reach the desired species on which it is to report a probe must have appropriate stability. Stability is contextual; a probe need only survive long enough to be useful. Often stability is related to selectivity for reaction with a particular species, especially in the case of irreversible reaction. However, selectivity might also be used to refer to different binding affinities for different ions. Kinetics is important and restricts the ability of the probe to report on dynamic and transient events. Sensitivity needs to be appropriate to the concentration of the species measured. Finally, in many cases it is desirable to localise probe distribution, to a particular compartment within the cell. To
better illustrate the interplay of these factors examples are described in the following sections (3.1.3 - 3.1.6). First, a brief explanation of fluorescence and associated phenomena is required.

3.1.2 FRET, PET and fluorescence

Many probes use fluorescence as a reporting mechanism. Excitation is caused by absorption of a photon corresponding to the difference between ground and excited state energies, leading to the promotion of an electron between levels corresponding to molecular orbitals (Figure 16). Within these energy levels numerous rotational and vibrational levels exist. The ‘S’ and ‘T’ markers refer to ‘singlet’ and ‘triplet’, the spin states of the system, which are maintained on initial excitation. UV absorption results in the $S_0 \rightarrow S_x$ transition, with excitation to $S_2$ or higher not forbidden. Fluorescence refers to a spin allowed relaxation to a lower energy state by emission of a photon through the lowest vibrational level of $S_1$ to $S_0$. This is because fluorescence is slow compared to relaxation through vibration or internal conversion. As the rate of stimulated emission is proportional to the cube of emission frequency, only relatively high energy transitions occur in this fashion, and so $S_2 \rightarrow S_1$ is less likely to be fluorescent.


Although fluorescent relaxation is from the lowest energy $S_1$ level, higher vibrational or rotational energy levels may receive the electron prior to further non-radiative relaxation. Likewise, excitation can be to any rotational or vibrational level, and so a range of wavelengths are
absorbed and emitted, although each individual event corresponds to a transition of precise energy (Figure 17).

Many of the same considerations relevant to photo uncaging groups (Section 2.4) apply also to fluorophore design. In particular, UV absorption energies usually correspond to $\pi \rightarrow \pi^*$ transitions and so conjugated systems have the correct energies. Particularly in extended systems, the conjugation of multiple bonds allows absorption across more of the molecules’ surface. These traits lend rigidity, limiting both the overlap of other energy levels which might allow non-radiative and limiting the excited state geometry available for such relaxation (see Section 2.4).

Because relaxation can also occur rapidly through interactions with neighbouring molecules, fluorescence has extraordinary environmental sensitivity. This has both positive and negative impacts on its use for detection. In Figure 18 excitation of an electron from $S_0$ to $S_1$ transfers electron density to the nitrogen, delocalising the charge over 33. This excited species then forms hydrogen bonds with methanol giving a vibrational relaxation pathway which competes with, and quenches, fluorescence. The outcome of this competition, and hence the fluorescence quantum yield, is determined by the acidity of the solvent hydrogen bond. However, the emission wavelength is equally dependent on solvent hydrogen bond acidity and the dipolarity of the system. As a result both variables can be defined from the fluorescence spectra of the compound.94

![Figure 18](image)

**Figure 18** - Excitation of a fluorophore in a polar, acidic environment. Reproduced from de Silva et al.94

Fluorescence resonance energy transfer (FRET) is a non-radiative energy transfer mechanism by which excitation can be passed from one chromophore to another in close proximity, so long as the emission spectra of the excited donor overlaps with the absorption spectra of the acceptor (Figure 19).81 The distance dependency is $r^{-6}$, so such mechanisms can be activated by a stimulus which changes the conformation of the molecule, giving a dramatic ‘turn on’ effect. They can also be used to modulate the wavelength of eventual emission. The first fluorophore can be common among a set of probes, allowing all to be excited simultaneously but with different subsequent emission wavelengths.82
Photoinduced electron transfer (PET) is a common quenching mechanism utilised in probe design (Figure 20). Excitation promotes an electron from the fluorophore HOMO into the fluorophore LUMO. An electron can then be transferred into the fluorophore HOMO from the PET donor HOMO, preventing radiative relaxation into the fluorophore HOMO. Changing the energy levels in such a way as to either make transfer from the donor HOMO geometrically impossible or reducing the energy of this level to make transfer unfavourable will remove the quenching mechanism and allow fluorescence.

For a successful probe the fluorescence emission must change upon the sensor portion interacting with the target species. There are 2 changes likely, change in emission wavelength and change in brightness, and ideally both are desirable. A change in emission or excitation wavelength allows activated and unactivated forms to be separately imaged, and so be observed more accurately, a process referred to as ratiometric imaging. An increase in brightness on binding is preferred: a decrease in brightness also works in principle but in practice the sensitivity of such probes is more limited. Increased brightness helps maximise spatial resolution with light...
microscopy, and is also important in order to minimise the quantity of dye required (and hence any disruption it may cause).

3.1.3 Reversible sensing of an abundant endogenous species: Calcium green FlAsH

The first example of a chemical probe involves sensing a vital and abundant species in living cells. The pivotal role of Ca\(^{2+}\) ions within and between cells is detailed later (Chapter 11), but for the present it is sufficient to understand that it is among the most vital of signalling species, and that transient, localised variations in [Ca\(^{2+}\)] are crucial cellular events. Numerous probes exist for the purpose of calcium imaging,\(^{84,85}\) and green flash (Figure 21) was designed specifically to report on transient microdomains of high calcium concentration.\(^{86,88}\) The arsenic groups allow selective binding to a tetracysteine sequence expressed in specific proteins known to occur close to the phenomenon studied. The soft Lewis acid arsenic atoms bind strongly to the soft Lewis base sulfur atoms of cysteine, and strong binding and selectivity are achieved through the chelate effect, resulting in nearly all expressed tetra cysteine moieties binding fluorophore. Prior to binding the free arsenic groups quench fluorescence through either vibrational relaxation or photoinduced electron transfer (PET),\(^{87}\) and so the restriction on movement and orbital geometry provided by tethering results in a four-fold increase in fluorescent emission, allowing successful tethering to be confirmed. Binding to Ca\(^{2+}\) then causes a further ten-fold increase in fluorescence, presumably restricting one or both of the aforementioned quenching mechanisms in the upper, ion chelating portion of the molecule. Lastly, excitation (488 nm) and emission (approx. 540 nm) are at wavelengths where few biomolecules absorb.

![Figure 21 - The bound, active form of the calcium green FlAsH probe.](image)

The calcium ion sensor component is based on the APTRA pentacoordinate magnesium chelator,\(^{89}\) known to have low calcium affinity. A carboxylate was replaced with a morpholine amide, reducing the Lewis basicity of the coordination site to improve selectivity over Zn\(^{2+}\) (\(K_d > 10\) mM). Low affinity (\(K_d = 100 \mu\text{M}\)) means a greater range of [Ca\(^{2+}\)] can be distinguished prior to
saturation, and fast dissociation kinetics allows resolution of multiple events within a short time frame (Figure 22).

**Figure 22** - The dynamic response range (A) and response times (B) demonstrate how low binding affinity produces ideal properties for measuring rapid concentration variations in an abundant species such as Ca2+. Adapted by permission from Macmillan Publishers Ltd: Nature Chemical Biology, Tour, O.; Adams, S. R.; Kerr, R. A.; Meijer, R. M.; Sejnowski, T. J.; Tsien, R. W.; Tsien, R. Y. 3, 423–431, copyright 2007.
3.1.4 Irreversible sensing of a toxic species at low concentration: Rhodamine based mercury probe

Quite different properties are desirable in a mercury probe. Where Ca\(^{2+}\) is ubiquitous and vital, Hg\(^{2+}\) is scarce and deadly. When looking for a rare species, an irreversible sensing approach is often desirable, allowing the reacted form concentration to build up over time and so improve detection. This is especially true in the case of an ion like mercury which does not perform a useful role; generally the role of a probe is to confirm the presence of Hg\(^{2+}\), rather than investigate its interactions. Fine tuning kinetics and providing mechanisms to localise the probe is not especially important; selectivity and sensitivity become much more so, and stability and toxicity concerns may be greater if longer observation periods are required. Several rhodamine based probes\(^{90,91}\) utilise cyclisation of thiosemicarbazide groups driven by the affinity of mercury for sulfur (Scheme 11). Mercury preferentially bonds with the thiocarbonyl following coordination in 36, increasing reactivity towards the favoured 5-exo-trig cyclisation from the amide, which is activated by the \(\alpha\) effect. Desulfurisation is then driven by the aromaticity of the furan product 38.

![Scheme 11 - The mechanism of the Hg\(^{2+}\) sensing desulfurisation reaction.](image)

**Figure 23** - Fluorescence of zebrafish before (left) and after (right) addition of HgCl\(_2\). Adapted from Ko *et al.*, J. Am. Chem. Soc., 128, 43, 2006.\(^{90}\)

The applications of the probe are reflected in design elements; the non-fluorescent to fluorescent transition limits quantification accuracy but improves contrast, and the rhodamine dye has strong absorbance and high quantum yield (\(\phi_f = 0.52\)), making rhodamine 38 detectable at 2 ppb.\(^{74}\)
an emission maximum of 577 nm biomolecule absorbance of the emission is negligible. Figure 23 also demonstrates that the probe equilibrates through the organism, and as xanthene 35 is stable from pH 4-14, it should be active in virtually all cellular environments. The cyclisation used is selective for Hg$^{2+}$, and the probe gave minimal responses in the presence of other metal ions.

### 3.1.5 Irreversible sensing of a low abundance species: Dicarbonyl hydrogen peroxide probe

H$_2$O$_2$ is less ubiquitous than Ca$^{2+}$ but of more subtle effect than Hg$^{2+}$. The most obvious reactivity of H$_2$O$_2$ is as an oxidising agent, a property shared by other ROS. Another property is used to distinguish it from other ROS. Many ROS are radicals, and less nucleophilic than the conjugate base of H$_2$O$_2$. The combination of high nucleophilicity and the presence of a hydroxide leaving group in H$_2$O$_2$ is exploited for selective detection. One fluorescein probe uses the rapid Baeyer-Villiger reaction of benzil moieties followed by hydrolysis to generate a fluorescein with a high quantum yield ($\phi_f = 0.8$), and long excitation (495 nm) and emission (525 nm) wavelengths (Scheme 12). The standout feature of this probe is a 150-fold increase in brightness from benzil 39 to fluorescein 40. While fluorescence and quenching observation is often serendipitous, in this case some rationale is given for the design: the reduction potential of the benzil group suggests a LUMO low enough to undergo donor PET quenching (Section 3.1.2) from the excited fluorophore.

![Scheme 12 - Activation of the fluorophore by H$_2$O$_2$.](image)

Reasonable kinetics are also required as $H_2O_2$ is created and destroyed both regularly and in a localised fashion, and a moderate rate ($k = 4.2 \times 10^{-3} s^{-1}$, pseudo-1st order with 1000 fold excess of $H_2O_2$) was ideal as it does not disturb the steady state $H_2O_2$ concentration by competing with endogenous processes. The probe also reacts with alkylhydroperoxides and peroxynitrite (Figure 24), although the latter is a product of $H_2O_2$ and NO, and so the reaction could be considered an indirect measurement of $H_2O_2$. Fortunately, $H_2O_2$ is far more abundant than these species so the error induced by these side reactions is insignificant.

### 3.1.6 A targeted, quantitative and non-fluorescent hydrogen peroxide probe

The three previous examples provided all report by means of fluorescence. There are major advantages to this approach; numerous fluorophores are well characterised and often synthetically easy to obtain, it is non-invasive and extremely sensitive, simple experiments are (relatively) easy to do, and the output is visual, intuitively understandable, and gives dynamic responses and spatial distributions. For all these reasons and others most biological probes rely on fluorescence. As demonstrated previously however, the ideal properties of a probe are dependent on the measurement required, and fluorescence has drawbacks. In particular, it is extraordinarily sensitive to environmental factors, and the cell is an extremely complex environment. Basic fluorescent measurements do not therefore allow quantification. Ratiometric probes can ameliorate some of the difficulties (Chapter 11), but a truly ratiometric probe is a non-trivial undertaking, and not a complete solution. Additionally, isolated mitochondria are known to be of limited comparative value when seeking to understand physiological systems (see Section 3.2). It is therefore necessary to examine the whole organism at once, which is not usually possible with optical techniques due to limited penetration. Where accurate quantification rather than spatial information is the goal alternatives to fluorescence should be considered.

One technique offering better quantification is LC/MS/MS, which was utilised for $H_2O_2$ quantification using MitoB, a probe developed and synthesised by the Hartley group and tested by the Murphy group at the MRC in Cambridge. The use of this probe is summarised in Figure 25. The arylboronic acid is attached to a triphenylphosphonium targeting moiety, which increases mitochondrial accumulation as described in Section 2.3. In drosophila calculations suggested 90% of the probe was within cells, and 98% of the cellular probe was within mitochondria. The boronic acid from MitoB is then selectively attacked by the hydroperoxide anion, which reacts substantially faster than $H_2O_2$, and is more prevalent within the mitochondrial matrix due to its higher pH in this environment. This results in four-fold faster conversion within the mitochondrial matrix versus the cytosol at the same concentration. Between the accumulation and preferential reaction of MitoB 41 the resulting MitoP/MitoB ratio is substantially indicative of mitochondrial $H_2O_2$ concentrations, with an error comparable with those inherent in such experiments regardless of detection method.

MitoB 41 and mitoP 42 diffuse freely across membranes and so can be administered to whole organisms and the MitoB/MitoP ratio determined by extraction and extrapolated to quantitative
data, with deuterated analogues of both added to the homogenised organism to correct for detection variations. The cationic nature of the probes improves mass spectrometry detection.

Figure 25 - The application and quantification of MitoB, reproduced with minor modifications from Cochemé, H. M.; Quin, C.; McQuaker, S. J.; Cabreiro, F.; Logan, A.; Prime, T. a; Abakumova, I.; Patel, J. V; Fearnley, I. M.; James, A. M.; Porteous, C. M.; Smith, R. a J.; Saeed, S.; Carré, J. E.; Singer, M.; Gems, D.; Hartley, R. C.; Partridge, L.; Murphy, M. P. *Cell metabolism* 2011, 13, 340–50.

3.2 Reactive oxygen species

3.2.1 What to measure?

Following the success of MitoB, my research involved using the same approach to measure another ROS, superoxide. Before describing the design of the probe it is necessary to consider the context, specifically to look in more detail at the generation and role of ROS.

The term ROS originally referred to a handful of oxygen-containing radicals, but the list of chemical species included has lengthened as it has become apparent that the sequences of biological reactions related to these radical species is very complex and often interlinked. The greater understanding of the cellular chemistry has resulted in a broadening of the definition to the point where both non-radicals and non-oxygen containing species are regularly described as ROS. Many researchers working in the area are now suggesting that, if the term itself has not outlived its usefulness as a descriptor it is not specific enough to describe a target of study. There are several reasons for this; one is that the term has now expanded to cover a range of very distinct chemical species with different specificity, different levels of reactivity and sometimes generated in different places. Therefore attributing an outcome to ROS is a poor description of
the likely actual pathway. Secondly, some ROS are now known to be useful or indeed essential, such as nitric oxide or H$_2$O$_2$, for signalling.$^{99}$

Broadly the term ROS covers species that are capable either of causing oxidative damage or being converted by redox chemistry to a species which can do so. The majority of these species come from chain reactions initiated by superoxide,$^{100}$ and the radical chain generated from a single superoxide radical may span thousands of events.$^{106}$ The full details of the interactions of the myriad of ROS are both extremely complex and a matter of ongoing study but for the purposes of this summary the basics may be shown diagrammatically (Figure 26).

---

**Figure 26**: This image was originally published in Biochemical Journal. Murphy, M. P. 2009. How mitochondria produce reactive oxygen species; Biochemical Journal, 417, 1-13 © Portland Press Ltd.

A rough summary is as follows. The respiratory chain generates various ROS through initial production of superoxide. These ROS can then damage lipids, DNA and enzymes and cause cell or tissue death, being implicated in over 100 human or animal diseases.$^{106}$ However, some of these species also play an important role in cellular signalling pathways. Some 90% of ROS originate in the mitochondria,$^{102}$ and many are too short lived to diffuse significantly (Figure 27).
Ideally biomarkers could be used to observe the effects of oxidation, and this is a common approach. Increasingly, however, it has been realised that the role of some ROS is considerably more complex than initially surmised, and may in fact signal stress and mobilise protective responses in some cases. Furthermore, some common biomarkers have been specifically shown to be problematic. For example, lipid peroxidation may be as much a measure of the antioxidant defence as anything else, and similarly protein carbonylation represents protein turnover rates.

An alternative would be to quantify the species causing individual types of oxidative damage events, but in many cases the terminal oxidant is the extremely reactive hydroxyl radical, which has the highest reduction potential of any known oxygen-centred radical (+2.31 V). This has an extremely low steady-state concentration owing to its ability to react at essentially diffusion limited pace with virtually any biomolecule, meaning there is almost nothing to measure and the terminal oxidation is hardly selective anyway. Other species such as hydroperoxyl and alkylperoxyl radicals are also capable of causing damage by lipid peroxidation, but these are difficult to distinguish from each other and transient themselves. Instead, the precursor to these reactive species, superoxide, was considered. This can lead directly to the hydroxyl radical via iron catalysed Haber-Weiss and Fenton chemistry (Scheme 13).

\[
\begin{align*}
(a) \quad & \text{Fe}^{3+} + O_2^- \rightarrow \text{Fe}^{2+} + O_2 \\
(b) \quad & \text{Fe}^{2+} + H_2O_2 \rightarrow \text{Fe}^{3+} + \cdot OH + \cdot OH \\
(c) \quad & \text{Overall reaction: } H_2O_2 + O_2^- \rightarrow \cdot OH + \cdot OH + O_2
\end{align*}
\]

Scheme 13 - Haber-Weiss and Fenton iron catalysed hydroxyl radical formation from $H_2O_2$ and $O_2^-$

While still difficult, it may be possible to measure superoxide. Superoxide is the product of one electron reduction of the diradical $O_2$, which has two partially filled degenerate orbitals and correspondingly accepts only one electron at a time, into an antibonding orbital. Although the standard reduction potential for this process is negative, superoxide formation is favourable under physiological conditions due to the relative concentrations of $O_2$ and $O_2^-$ within the matrix, where tentative estimates suggest a superoxide concentration of 10-200 pM. Superoxide is relatively unreactive, (although capable of damaging some Fe-S clusters), but matrix $O_2^-$ is
limited by superoxide dismutase, an enzyme which converts the radical into \( \text{H}_2\text{O}_2 \) extremely rapidly (\( k \) is approx. \( 2.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \)), a consideration not factored into the diffusion radius shown in Figure 27. When the effect of SOD, antioxidants and other defense mechanisms are considered alongside the difficulty of anionic superoxide crossing the inner mitochondrial membrane, multiple recent reviews have concluded that significant \( \text{O}_2^- \) efflux from the matrix is improbable.\(^{108,106} \) Since oxidative damage to mitochondrial DNA is postulated to be the limiting factor on human longevity according to the free radical theory of ageing, investigations can be limited to superoxide in the mitochondrial matrix. There is not currently any way of measuring matrix superoxide production or accurately determining its steady state concentration in the matrix of living organisms.\(^{101} \)

3.2.2 How is superoxide produced?

Several sources of superoxide are known to exist within the mitochondrial matrix (Figure 28), although the mechanisms and extent of their activity under physiological conditions is poorly understood.\(^{109,110} \)

![Figure 28](image_url) - The maximal rates of superoxide production at different sites in the mitochondria. It is not clear to what extent all of these are active in vivo. Superoxide production sites are pyruvate dehydrogenase (PDH), 2-oxo-glutarate dehydrogenase (OGDH), complex I NADH binding site (IF), complex I ubiquinone binding site (IQ), electron transferring flavoprotein ubiquinone oxidoreductase (ETFQOR), glycerol 3-phosphate dehydrogenase (GPDH) and complex III outer ubiquinone binding site (IIIQo).

The best understood of these are within the electron transport chain (see Section 1.4). Complex II produces superoxide in specific experimental conditions but is not believed to produce significant quantities \textit{in vivo}, while the affinity of complex IV for partially reduced oxygen prevents escape of superoxide.\(^{108} \) Complexes I and III are capable of superoxide production. An exhaustive description of these complexes is unnecessary, but as superoxide formation by complex I has been discussed in detailed reviews,\(^{101,112} \) a brief outline of the process is provided for illustrative purposes.
The reduction of oxygen to superoxide is believed to occur through an outer sphere mechanism, and so proximity is required. Only the entry (IF) and exit (IQ) of the complex I electron transport chain are accessible, as the Fe-S clusters are protected by the complex proteins (Figure 29).

The binding of NADH to the flavin (IF) at the peak of the complex I electron chain as drawn results in hydride transfer between the two, giving NAD\(^+\). Binding and transfer is extremely rapid and reversible, with electron transfer by quantum tunnelling also extremely quick, and the rate limiting step is reduction of the ubiquinone at the lower energy site (IQ). It is possible to treat NADH/NAD\(^+\) binding and hydride transfer as a pre-equilibrium, with the ratio essentially setting the relative levels of oxidised and reduced flavin. Superoxide production is favoured by a high NADH/NAD\(^+\) ratio, as a greater proportion of IF sites are reduced and so able to reduce oxygen in turn. Interestingly, increasing the absolute NADH concentration rather than changing the NADH/NAD\(^+\) ratio inhibits superoxide production by preventing access to the flavin site (oxygen reduction is very slow compared to NADH hydride transfer).
Until recently it was suspected that IQ was also a significant superoxide source, as shown in Figure 28. This was considered to be particularly relevant during Reverse Electron Transfer (RET), where the mitochondrial chemiosmotic potential is sufficiently high to drive reverse transfer from a pool of ubiquinol (the reduced form of ubiquinone, Scheme 14) up the complex I electron transport chain. The absence of ubiquinone to react with the reduced N2 cluster would allow oxygen to do so instead. Underlining the developing nature of our knowledge of this area, recent evidence\textsuperscript{113} appears to discount IQ as a superoxide source, showing superoxide reduction even under RET conditions occurs via the IF flavin.

The above description gives only a very simplified outline of the current understanding pertaining to complex I superoxide production. As the literature related to superoxide production is extremely extensive and the topic only tangentially related to the work undertaken no further description of superoxide production is attempted here, but may be found in both the recent reviews referenced throughout this section and in several others.\textsuperscript{114,115}

3.3 Designing a probe for matrix superoxide quantification in whole organisms
3.3.1 What tools are available?

Several of the previously cited reviews explicitly emphasise the need for more information on superoxide, in fact our ability to quantify this species in the matrix under physiological conditions is very limited.\textsuperscript{101} Most studies purporting to measure superoxide do so indirectly by measuring another species, usually H\textsubscript{2}O\textsubscript{2}. In many cases this is likely sufficient, but several assumptions are required. These include assuming that effectively all superoxide is converted to H\textsubscript{2}O\textsubscript{2}, and that all H\textsubscript{2}O\textsubscript{2} comes from superoxide within the matrix (or at least that we can accurately correct for side reactions), and that no significant H\textsubscript{2}O\textsubscript{2} degradation occurs prior to measurement.\textsuperscript{101} We know that all three of these assumptions are certainly incorrect at least some of the time.\textsuperscript{116,117,118} This does not of course mean that measuring H\textsubscript{2}O\textsubscript{2} as an indicator of superoxide levels is invalid; in many cases it will likely give accurate data, but given our limited understanding of the mitochondrial matrix under physiological conditions it would be beneficial to measure the species of interest directly. In this respect many tools relying on specific damage markers are similarly limited by our understanding of their native roles and interactions. For example, when using
aconitase oxidation as a marker it should be noted that the iron released will take part in Fenton chemistry and modify the ROS balance present.\textsuperscript{119}

Some tools for the direct measurement of superoxide do exist, and understanding the limitations of these is important in identifying new approaches to the problem. Many of the approaches have been reviewed\textsuperscript{120} and the good and bad points of various detection schemes detailed. It is sufficient to note here that generally these approaches either display low selectivity or are very obviously unsuitable for \textit{in vivo} application. A small selection of alternative superoxide probes are listed in a more recent review.\textsuperscript{121} All rely on optical approaches, which limits the tissues in which they can be utilised. As mentioned earlier (Section 3.1.5), use of fluorescence as a quantification tool is limited by environmental sensitivity. In addition, the possibility of reabsorption of emitted photons by nearby probe molecules is relevant where selective uptake strategies could result in high local probe concentrations. Because of the limitations of the reporting technique, the compounds described in the following section are unsuitable for quantification of superoxide levels in a whole organism \textit{in vivo}. However, the details of their function, and particularly their sensing moieties, can be used to design an alternative probe to do so.

### 3.3.2 A selective, oxidation resistant, ‘turn on’ fluorophore: Hydrocyanine dyes

The hydrocyanine probes developed by Murthy and coworkers\textsuperscript{122} (Scheme 15) feature an extended conjugated system in the oxidised cyanine form 44, which can be disrupted by reduction. The reduced form 45 is able to diffuse through membranes as it is uncharged. Subsequent oxidation by ROS results in a strongly fluorescent, membrane-impermeable probe.

![Scheme 15 - Interconversion of cyanine 44 (oxidised) and hydrocyanine 45 (reduced) by NaBH₄ or superoxide respectively.\textsuperscript{122}](image)

Cyanines 44 have several advantages as fluorophores. The extended conjugated system in the more fluorescent form 44 gives a long wavelength, low energy excitation (535-750 nm) and emission (560-830 nm), with the exact values dependent on the analogue used. The hydrocyanine form 45 is only weakly fluorescent, with oxidation producing a 100-fold increase in fluorescence intensity. The reduced moiety is quite resilient to auto-oxidation in aqueous environments, which is attributed to a lower driving force for oxidation compared to ethidium based probes (Section 3.3.4), for which this can be a limitation. Reasonably good selectivity for superoxide is reported (Figure 30) and believed to arise from two consecutive one electron oxidations. Importantly low reactivity with H₂O₂ is observed. Significant side reactions have been reported with indiscriminately reactive species such as the hydroxyl radical, but this is present only in...
vanishingly low concentration (see Section 3.2.1). These probes are sensitive to nanomolar levels of radical oxidants.

![Figure 30 - The selectivity of Hydro-Cy3 for various ROS. Republished with permission from Kundu et al. ©2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim](image)

### 3.3.3 A ROS sensitive chemiluminescent probe: KEIO-BODIPY-KBI

An alternative to fluorescence as an optical reporting tool is showcased in Suzuki and coworkers’s chemiluminescent probe 46 for ROS. The energy emitted in this case is liberated by oxidation of the probe itself, so no external stimulation is required. The chemiluminescent group was linked to an additional BODIPY fluorophore, with the idea of allowing transfer of excitation through chemiluminescence resonance energy transfer (CRET), which is functionally identical to FRET (see Section 3.1.2). Longer wavelength emission from the fluorophore is then less likely to be absorbed by biomolecules. This was the strategy originally pursued, but the probe synthesised emitted at a higher wavelength (545 nm) than the BODIPY fluorophore used, and so the emission was attributed to the whole probe acting as a single chromophore, exhibiting a strong response under superoxide generating conditions.

![Scheme 16 - The chemiluminescent reaction of KEIO-BODIPY-KBI probe 46.](image)

High specificity is claimed but not fully demonstrated for this probe. The reported response to \( \text{H}_2\text{O}_2 \) was relatively strong, although this was attributed to the higher concentration of this oxidant under test conditions. A lower response was observed in the presence of catalase, which destroys \( \text{H}_2\text{O}_2 \), suggesting a 30:1 selectivity for superoxide over \( \text{H}_2\text{O}_2 \) assuming direct comparison is possible.
3.3.4 Unusual product selectivity and a targeted probe: MitoSOX

The use of hydrophenanthridines as probes is not an especially recent development. The parent phenanthridinium salts had long been used for a variety of purposes (see Section 8.1.1), and ethidium was known to undergo metabolic interactions in vivo in the 1970s. By the mid 1980s oxidation of hydroethidine 48 (Scheme 17) was being used to infer the redox conditions in different areas of the cell. This oxidation was attributed to ROS in 1990, with sensitivity to both H$_2$O$_2$ and O$_2^-$ observed. In 2003 Kalyanaraman and coworkers observed during a study on redox sensitive dyes that superoxide addition appeared to lead to a different product to that produced from reaction with H$_2$O$_2$, hydroxyl radicals, peroxynitrite, cytochrome c and nitric oxide, although the actual structure was unknown. Later work clarified the structure and suggested a possible mechanism for this oxidation, shown in Scheme 17 and described below.

![Scheme 17 - Oxidation of hydroethidine 48 makes both ethidium 51 and 2-OH-E$^+$ 55.](image)

One electron oxidation of hydroethidine 48 gives aminyl radical 49 or radical cation 50, which can interconvert via gain or loss of a proton. This rate of this initial step varies with oxidant and may be very fast, but is usually rate determining. Further one electron oxidation gives the parent ethidium 51. Alternatively, in the presence of O$_2^-$ extremely rapid (k approx. 2 x 10$^9$ M$^{-1}$s$^{-1}$) reaction with another O$_2^-$ produces peroxy adduct 52, with water subsequently lost to form iminyl-quinone 53. Tautomerisation, possibly via a reaction with a hydride donor or acceptor gives phenoxide 54, which is strongly favoured by the formation of an extended aromatic system. The conjugated, electron poor phenoxide 54 has a pKa of 7.4 and the protonated 2-hydroxyethidium (2-OH-E$^+$) 55 and deprotonated form 54 are roughly equal in concentration.
within the cell. Two equivalents of oxidant are required in this pathway, however as many species can perform the original oxidation and O\(_2^-\) is present at low concentrations, it is reasonable to assume that one superoxide radical gives one molecule of 2-OH-E\(^+\) \(\text{55}\) under physiological conditions.

The key advantage of this mechanism is that extensive testing suggests that oxidation of hydroethidine \(\text{48}\) produces the 2-OH phenanthridinium product (2-OH-E\(^+\) \(\text{55}\)) only in the presence of O\(_2^•\); \(^{132,133}\) This selectivity is vital in detecting a species present only at low concentration. The Kalyanaraman group went on to suggest the red-shifted fluorescence of 2-OH-E\(^+\) \(\text{55}\) relative to ethidium \(\text{51}\) could be used to distinguish the two, \(^{130}\) and add a TPP cation to the end of a hexyl analogue to give MitoSOX \(\text{56}\) and ensure targeting to the mitochondria \(\text{Fig. 27}\). They abandoned this position on fluorescent quantification when it became clear that numerous fluorescent oxidation products were generated and interfered with quantification, and that the hydroethidium moiety was light sensitive, instead embracing HPLC quantification approaches. \(^{134}\) Various other detection methods exist, \(^{132,134}\) some of which may give sensitivity down to the attomolar level. \(^{135}\)

![Scheme 18](image)

Scheme 18 – The reaction of MitoSOX with superoxide gives rise to a product variously identified as the 2-OH phenanthridinium \(\text{57}\) or the 9-OH phenanthridinium \(\text{58}\).

Beckman and coworkers attempted to use lower wavelength excitation to measure selectively superoxide formation in the mitochondrial matrix with MitoSOX. \(^{136}\) Uptake and release in accordance with the membrane potential was demonstrated and the identity of the superoxide addition adduct was disputed. Beckman and co-workers claim the 9-OH analogue is produced, although it is labelled as the 2-OH compound, perhaps due to confusion surrounding the naming convention on phenanthridines. The different regioisomers have both recurred in the literature since, probably obscured by the related misnaming, and this uncertainty is further addressed in Chapter 10. Later work by the Beckman group uses MitoSOX for live cell imaging. \(^{137}\)

When the creation of a mass spectrometry probe analogous to MitoB was investigated it was therefore considered that the creation of a deuterated analogue of MitoSOX \(\text{56}\) to correct for variations in uptake would allow quantification by mass spectrometry of superoxide production within the mitochondrial matrix of whole organisms. Unfortunately, no synthesis of MitoSOX had previously been published. In planning a route, the various literature approaches to 5,6-disubstituted dihydrophenanthridines were therefore considered and will be discussed in the next chapter.
Chapter 4: Making 5,6-disubstituted dihydrophenanthridines and phenanthridinium salts

4.1 Introduction to the synthesis of 5,6-disubstituted dihydrophenanthridines

Electron-rich 5,6-disubstituted dihydrophenanthridines like MitoSOX 56 are susceptible to oxidation and are generally prepared by reduction of the more stable 5,6-disubstituted phenanthridinium salts 60 or less commonly, the 6-substituent is introduced by addition of a formal carbanion or equivalent reagent to an N-alkylphenanthridinium 62 that is not substituted at C-6. These two disconnections are shown in Scheme 19 and will be further discussed within the sections marked.

![Scheme 19](image)

Scheme 19 - The initial disconnections used for the synthesis of a 5,6-disubstituted phenanthridinium salt 59.

4.2 Disconnection one: the ubiquitous N-alkylation

Hydrogenation and most hydride reducing agents will reduce phenanthridiniums to dihydrophenanthridines, so the main challenge is accessing the parent 5,6-disubstituted phenanthridinium salt 60. Given the many uses of 5,6-disubstituted phenanthridinium derivatives 60 (see Section 8.1.1), and the large number which have been synthesised, it is striking that N-alkylation of a 6-substituted phenanthridine is almost invariably the final step. Phenanthridines 61 are poor nucleophiles, so the early examples of N-alkylation and preparation of phenanthridiniums 60 bearing simple short N-alkyl chain used excess alkyl tosylates, bromides and iodides at elevated temperatures. More recent syntheses mostly use triflates. Synthetic approaches to the phenanthridine precursors 61 are much more varied, and a brief discussion of literature examples is organised in sections according to the retrosynthesis shown below (Scheme 20). Probably the most common disconnection is between C6 and C7a (red, 4.2.2). Addition of a nucleophile to the C6 unsubstituted phenanthridine is also possible (green, 4.2.3), as is intramolecular imine formation from a C6 ketone and a C4a amine (pink, 4.2.4). A few recent methods use the more unusual N5-C4a disconnection (blue, 4.2.5), with the C1a-C10a strategy most commonly applied to the synthesis of benzophenanthridine natural products (orange, 4.2.6).
4.2.1 Forming the C6-C7a bond: intramolecular nucleophilic attack from an arene

The C6-C7a disconnection to give imine 63 is by far the most common retrosynthesis of 6-substituted phenanthridines 60. In the forward direction this is nucleophilic addition to a polarised C=N which is an extremely common transformation and compatible with several classic heterocycle synthesis strategies. These include the Bischler-Napieralski reaction, sometimes called the Hubert-Pictet reaction (ZnCl$_2$, 250-300 °C) or Morgan-Walls cyclisation (POCl$_3$, 80-200 °C) depending on conditions (Scheme 21). Nucleophilic attack from the aromatic ring on an iminium chloride generated in-situ from amide 69 gives phenanthidine 70, and is mechanistically similar to the Vilsmeier-Haack formylation (see Section 11.4.1).

Scheme 21 - An example of the Morgan-Walls reaction from Lion et al.$^{143}$
Another common way of forming phenanthridines is the Pictet-Spengler reaction (Scheme 22), e.g. imine (or iminium) formation from amine 71 and aldehyde 72 is followed by intramolecular nucleophilic attack in a 6-endo-trig cyclisation to give a dihydrophenanthridine. The dihydrophenanthridine oxidises under reaction conditions to give phenanthridine 73.

Scheme 22 - Phenanthidine synthesis by Pictet-Spengler reaction.

The Pictet-Spengler reaction is slightly different from the Bischler-Napieralski in that the C=N bond is formed in the same reaction as the C6-C7a bond. This is a relatively common theme among phenanthridine synthesis protocols. Intramolecular addition of an iminyl radical to an aromatic ring has also recently been used to effect the same transformation (Scheme 23) in a reaction between an aryl boronic or alkyl boronic acid 74 and a biaryl isocyanate 76. According to the mechanism proposed by Chatani and coworkers, reaction of boronic acid 74 with a one electron oxidant [Mn(III)] gave radical 75 which was trapped with isocyanobiphenyl 76 to give the carbon-centred radical 77, which reacts to form the better stabilised phenyl radical 78. Further oxidation to cation 79 followed by proton loss gives phenanthridine 80.

Scheme 23 - Mechanism proposed by Chatani and coworkers for the cyclisation of isocyanobiphenyl 76 to phenanthridine 80.
4.2.2 Addition of R3 to C6: nucleophilic attack on a phenanthidine

Nucleophilic addition to C6 is a common strategy for adding a range of R3 groups. This is not a phenanthidine synthesis strategy so much as a functionalization, as the starting material 64 is a phenanthidine. Treating phenanthidine 81 which has no R2 group with phenyllithium gives the 6-phenylphenanthidine 82, following oxidation in the work up, which can be further substituted to 6,6 disubstituted hydrophenanthidine 83 (Scheme 24) under harsh conditions. Addition to give the C6 phenyl derivative 82 can alternatively be achieved quantitatively under relatively mild conditions using a nickel catalysed arylzinc addition.

![Scheme 24 - C6 functionalisation of phenanthidine 81.](image)

Organometallic nucleophiles are not required, enolate addition also works well. Surprisingly, C6 addition of an amine occurs in preference to reaction with an alkyl bromide, as evidenced by the formation of phenanthridinium 87a by Cronin and coworkers. This mechanism was proven by opening the five-membered ring, with the failure of the subsequent product to re-cyclise ruling out the Baldwin disallowed 5-endo-trig cyclisation. An interesting disproportionation was observed, presumably from intermediate 86, which is reminiscent of the Cannizzaro reaction.

![Scheme 25 - Unexpected C6 addition of an amine to phenanthidine.](image)
4.2.3 N5-C6 condensation: cyclisation through imine formation

Compounds 92 closely related to phenanthridines can be made from biphenyls 91 with final intramolecular condensation of an amine onto a suitable ketone.\textsuperscript{149} Imine formation is preceded by C1a-C10a bond formation via Suzuki coupling between aryl halides 89 and boronic acids 90, while an acid sensitive amine protecting group allows deprotection and cyclisation to target 92 in one step (Scheme 26).

\begin{center}
\begin{tikzpicture}
\node[anchor=east] at (0,0) {Scheme 26 - Suzuki coupling to form C1a-C10a followed by combined deprotection and cyclisation.\textsuperscript{149}};
\end{tikzpicture}
\end{center}

Transition metal catalysed C-H activation followed by carboamination allows addition of more varied and complex C6 substituents and has been demonstrated with both free\textsuperscript{150} (Scheme 27) and protected amines.\textsuperscript{151}

\begin{center}
\begin{tikzpicture}
\node[anchor=east] at (0,0) {Scheme 27 - C-H activation and carboamination on unprotected biphenyl-2-ylamine 93.\textsuperscript{150}};
\end{tikzpicture}
\end{center}

An alternative formation of the imine is showcased in the work of Gallo and co-workers,\textsuperscript{152} who use azides 95 as amino surrogates and a combination of a ruthenium catalyst, white light and heating to achieve a C-H amination and oxidation tandem reaction with the release of N\textsubscript{2} to give phenanthridines 96 (Scheme 28). Yields were variable and may reflect the difficulty of removing the side products reported.

\begin{center}
\begin{tikzpicture}
\node[anchor=east] at (0,0) {Scheme 28 - A ruthenium catalysed benzylic C-H amination and oxidation process.\textsuperscript{152}};
\end{tikzpicture}
\end{center}
4.2.4 N5-C4a cyclisation: transition metal and radical induced cyclisation

Unlike the analogous C6-C7a bond formation on the other side of the heterocyclic B ring, N5-C4a cyclisation is comparatively rare. Presumably this reflects the ability of unsubstituted phenyl ring systems to undergo electrophilic substitution reactions, while leaving groups are generally required for the equivalent nucleophilic aromatic substitution. As a particularly electronegative element, nucleophilic attack on nitrogen is unusual and so formation of N5-C4a is rarely used to complete phenanthridine synthesis. Lautens and coworkers synthesise phenanthridines via an olefination-cyclisation cascade (Scheme 29), and propose formation of this bond by reaction of an imine with an aryl palladium (IV) species derived from the aryl triflate 97. A radical reaction not dissimilar to that used for C6-C7a bond formation has also been demonstrated as one outcome from photolysis of oxime carbamates.

Scheme 29 - Olefination-cyclisation, from Blanchot et al. Bonds formed shown in red.

4.2.5 C1a-C10a: benzyne cyclisation and C-H activation

An unusual strategy for forming the C1a-C10a bond has been used to synthesise phenanthidine natural products lacking the C6 substituent. The phenanthidine moiety is found in numerous alkaloids, and among these many are N-alkylated, although not necessarily found in the quaternary salt form. Naturally occurring benzophenanthridines are usually structurally and electronically different from ethidium-like phenanthridinium salts, and so different synthetic approaches are preferred. In particular, a benzyne cyclisation has been used in the synthesis of multiple benzophenanthridines alkaloids. One example is the conversion of aryl halide 100 into benzophenanthridine 101 (Scheme 30).

Scheme 30 - Synthesis of a benzophenanthridine through benzyne cyclisation.

The mechanistic steps of this type of cyclisation are shown in Scheme 31. First the amine 102 is deprotonated, then deprotonation of the CH of aryl halide 103 gives benzyne 104. Nucleophilic attack from C1a (ortho to the amino group) onto the benzyne forms the C1a-C10a bond, in carbanion 105. Protonation of anion 105 during the work up then gives the dihydrophenanthridine 106, which is then oxidised to give phenanthridine 106. Deprotonation of the amine 102 is required to activate ring A sufficiently to cyclise onto the benzene.
Subsequent investigation produced some mechanistic details: \( N \)-methylation prevented an analogous cyclisation, and deprotonation of only the amine alone did not produce cyclisation, showing that benzyne formation is necessary.\(^{161}\) Substitution in the 7 position shown improves yield by favouring a conformation where the carbons of the A ring ortho to the nitrogen anion are close in space to the benzyne generated. The presence of alkoxy groups on the C ring are noted to favour side reactions because inductively withdrawing groups increase benzyne electrophilicity.\(^{162}\) Changing conditions to LDA in THF at \(-78 °C\) improved the yield dramatically in some cases, and this was attributed to the bulkier base being prevented by steric hindrance from reacting further with the benzyne generated.\(^{163}\) An alternative cyclisation of similar substrates was demonstrated by Nakanishi \textit{et al} in the synthesis of drug candidate NK109, where the phenanthridine was closed by radical cyclisation instead.\(^{164}\) More recently Peng \textit{et al}\(^{165}\) report cyclisation by a C-H activation mechanism (Scheme 32). Takeda \textit{et al} have demonstrated this transformation occurring in tandem with prior \( N \)-arylation, however low to moderate yields resulted.\(^{166}\)

\textbf{Scheme 31} - Benzyne cyclisation to a phenanthridine. The proton abstracted is shown on precursor 105.

\textbf{Scheme 32} - C-H activation and intramolecular cyclisation to form C1a-C10a.

4.3 Disconnection two: addition of a nucleophile to an \( N \)-alkylphenanthridinium

Preparation of 6-substituted phenanthridines 61 and \( N \)-alkylation to give 5,6-disubstituted phenanthridinium 60 is the most common way of accessing these compounds (Scheme 19). However, addition of carbanion equivalents to \( N \)-alkylphenanthridinium salts 62 also works. The practical difficulty of separating multiple \( N \)-alkylphenanthridinium salts in any mixture reduces the appeal of this approach. Furthermore the solubility of \( N \)-alkylphenanthridinium salts is usually low in the types of non-polar solvent often used for organometallic reagents. However, a range of benzophenanthridines have been synthesised by Nakanishi \textit{et al} in this fashion (Scheme 33).\(^{167}\) The methoxy adduct 111 (a pseudobase) forms the phenanthridinium 112 reversibly in solution,
avoiding solubility problems and allowing reaction with the Grignard nucleophile to form dihydrophenanthridine 113. Even so, the authors note synthetic difficulties in preparing analogues in this way.

Scheme 33 - Grignard addition to a phenanthridinium salt.

The potential reversibility of nucleophilic additions to C6 can be utilised in sensor systems. Cronin and co-workers\textsuperscript{168} developed a pH sensor based on cyclisations from groups such as malononitrile (pKa approx. 11)\textsuperscript{169} which may or may not be nucleophiles in aqueous solution, depending on pH (Scheme 34). If the malononitrile is protonated the aromatic open form 114 is favoured. However, when the pH is reduced the neutral C6 adduct 115 is preferred despite the loss of aromaticity. This results in a fluorescent pH sensor, with change in absorbance and fluorescent properties upon acidification.

Scheme 34 - A pH dependent photoswitch based on reversible addition to the C6 position of the phenanthridinium.

Chen et al\textsuperscript{170} take advantage of reversible addition to phenanthridinium salts in a combined coumarin-phenanthridinium heterocycle 116 which displays thermochromic properties upon cooling in MeOH.

Scheme 35 - Coumarin-phenanthridinium combination 116 displays thermochromic properties.\textsuperscript{170}
4.4 Direct access to 5,6-disubstituted dihydrophenanthridines

As previously discussed, according to the literature it is almost invariably necessary to N-alkylate a phenanthridine in order to make the corresponding 5,6-disubstituted phenanthridinium. Two recent exceptions exist: a single N-methyl hydrophenanthridine analogue 118 has been made via the Pictet-Spengler reaction, but the electronics of the precursor biphenyl 117 have been optimised and it is unclear that this strategy would be more generally applicable (Scheme 36).171

Scheme 36 - Synthesis of an N-methyl hydrophenanthridine 118 by Pictet Spengler reaction.

Direct synthesis of dihydrophenanthridines with bulkier R groups has also been reported with what the authors describe as ‘a hydride transfer/cyclisation/Dötz benzannulation cascade’ (Scheme 37), but this requires R² and R³ to be identical, and the starting material would likely prove challenging and sensitive. Additionally, while technically impressive the mechanistic complexity of this process would likely make optimisation very difficult.172

Scheme 37 - Dihydrophenanthridine synthesis via cascade reaction.172
Chapter 5: Synthesis of 3,8-Diamino-6-phenylphenanthridinium salts

5.1 Preparation of 9-Hydroxyphenanthridine for N-alkylation

Utilisation of MitoSOX 56 as a mass spectrometry superoxide probe required both deuterated and non-deuterated standards for the reaction product, believed to be the 9-OH compound 58 (See Chapter 10). Late addition of the TPP component would allow a late common synthetic intermediate. Conveniently, N-alkylation is by far the most common way of making these salts anyway, so a synthetic route to a protected 9-hydroxy phenanthridine precursor 126 was designed.

![Synthesis of a precursor to 9-hydroxyphenanthridinium 126.](image)

The route (Scheme 38) is based on one originally attempted by Susan MacIntyre,173 who had prepared benzophenones 123 and 124 starting with nitration of dihydroxybenzophenone 122, benzyl protection of both phenols and then attempted selective deprotection. Disappointing yields resulting from low selectivity in nitration and deprotection steps were addressed by rearranging the synthesis.

The literature174 and experience within the group suggested mono protection of 2,4-dihydroxybenzophenone 122 should be possible due to the low acidity of the phenolic hydroxyl group ortho to the ketone. Hydrogen bonding between 2-OH and C=O allows the 4-OH to be preferentially deprotonated. A single deprotonation increases the ring electron density and consequently disfavours diphenoxide formation, allowing the more acidic phenol to be protected selectively. This allowed near quantitative conversion of diol 122 into para-benzyloxy protected compound 123. The same procedure cannot be used if C5 nitration precedes protection as the presence of the nitro group makes both phenols sufficiently acidic that either could be deprotonated, and like the ketone, the nitro group hydrogen bonds with an ortho phenol. Nitration of phenol 123 under mild conditions resulted in complete conversion of starting material 124 into a mixture of nitrobenzophenone 124 and an alternative nitrated side product in
a ratio of 4.2:1. The yield of the pure phenol 124 from the recrystallisation was limited due to the similarity of the side product.

Conversion of phenol 124 into aryl tosylate 125 was achieved overnight in pyridine. We reasoned that ketone 125 would react with 1,3-diaminobenzene to give phenanthridinium salt 126 when treated with acid. An initial attempt utilising Amberlyst 15 failed. When the mixture was heated under reflux with p-toluene sulfonic acid for 5 days under Dean-Stark conditions, a red solid with low solubility precipitated, the identity of which was eventually confirmed by washing with strong aqueous base, so that the $^1$H NMR spectrum was not complicated by signals from p-toluene sulfonate (Scheme 39). This revealed the 7.30 ppm doublet (H1), which correlated with peaks for H2 and H4 in the COSY spectrum confirming the identity of phenanthridine 127.

![Scheme 39 - Neutralisation of phenanthridinium tosylate 126.](image)

The mechanism of this transformation is believed to involve the formation of both imine isomers 128 and 129 by removing water. The sterically less favoured isomer 129 then cyclises by nucleophilic attack from the para position of the dianiline ring on the aryl tosylate, with the resulting Meisenheimer complex 130 stabilised by the electron withdrawing properties of the para nitro group. Aromaticity is restored by loss of the tosylate from this. Restoration of aromaticity across the ring system then provides a strong driving force for deprotonation of 131. Formation of phenanthridinines by this type of cyclisation is unprecedented (Scheme 40). The absence of a strong base rules out cyclisation via a benzyne as used in the synthesis of Fagaronine and recent analogues (see Section 4.2.5). Attempts to use a higher boiling solvent, chlorobenzene, in the hope that higher temperatures would encourage isomerisation of the imine 128 and drive the reaction to completion were unsuccessful.
Scheme 40 - Postulated mechanism for cyclisation from benzophenone 125 to phenanthridinium 126.
5.2 N-Alkylation of Phenanthridines

5.2.1 Introduction and phenanthridines

3,8-Diaminophenanthidine 133 is commercially available, so with 9-benzyloxy variant 127 in hand alkylation to give N-alkylphenanthridinium salts was attempted. Since the 3,8-diaminoprecursor is commercially available this is an ideal approach to MitoSOX. Avoiding synthesis of the heterocycle results in a short synthesis, with only protection of the 3,8-amino functionality and alkylation required (Scheme 41).

Scheme 41 - Alkylation strategy for synthesis of N-alkylphenanthridinium salts 135.

Commercially available phenanthridine 133 was protected as a variety of carbamates according to literature procedures (Scheme 42). The Boc protected phenanthridine 137 was previously prepared by Stephen McQuaker, who attempted the alkylation with a variety of dihalides without success. Of the three additional carbamate-protected phenanthridines the methyl carbamate 139 proved very insoluble but the benzyl and ethyl analogues 138 and 140 were tractable.

Scheme 42 - Carbamate protection of phenanthridine 136
5.2.2 Alkylating agents

![Scheme 43 - Synthesis of a monophosphonium triflate salt 143.](image)

Although there is literature precedent for use of alkyl dihalides, phenanthridines are very poor nucleophiles so triflate is the most commonly employed leaving group. Bis-triflate 142 was prepared according to a literature procedure from diol 141, with a distinctive 2H t at 4.5 ppm in the 'H NMR spectrum indicating success (Scheme 43). On treatment with triphenylphosphine the insolubility of phosphonium cations in Et₂O allowed the mono-TPP salt 143 to be prepared exclusively. 'H NMR spectroscopy showed yields in excess of 90% based on characteristic CH₂ groups adjacent to phosphonium and triflate groups, and material was used directly rather than isolated due to moisture sensitivity. This compound proved hugely more moisture sensitive than the parent bis-triflate 142. This problem was independently experienced by the group of Robin Smith and was attributed to the charge improving association with water molecules, making hydrolysis more probable. The sensitivity of phosphonium triflate 143 required fresh preparation and laborious handling conditions, so monobenzyl triflate 146 was synthesised instead (Scheme 44).

![Scheme 44 - Synthesis of a monobenzyloxy alkyl triflate 146.](image)

One end of 1,6-hexanediol was benzylated, with the waxy solid being dried by azeotrope with PhMe prior to protection using a modified literature procedure. No selectivity is achieved in this reaction and the statistical mixture results. It was quickly realised that most of the diol could be washed out, and the remainder would stick to the SiO₂ in the 1:1 petrol/Et₂O mixture which eluted the desired monobenzyl alcohol 145. A sevenfold excess of 1,6-hexanediol was used with the excess removed by washing and filtration through SiO₂. This avoids generation of the more difficult to remove dibenzylated side product, and the diol could be recovered and reused if desired. Triflate formation then gave monobenzyl triflate 146 in good yield.
5.2.3 Alkylation experiments

Alkylation (Scheme 45) was attempted under various conditions, summarised in Table 1.

![Scheme 45 - General scheme for the alkylation of protected 3,8-diaminophenanthridines](image)

Table 1

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Triflate</th>
<th>T (°C)</th>
<th>t (h)</th>
<th>Solv.</th>
<th>Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>137</td>
<td>142</td>
<td>RT - 120</td>
<td>20/3</td>
<td>PhNO₂</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>138</td>
<td>142</td>
<td>RT</td>
<td>1.5</td>
<td>THF</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>138</td>
<td>142</td>
<td>RT</td>
<td>72</td>
<td>THF</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>138</td>
<td>143</td>
<td>RT</td>
<td>17</td>
<td>DCM</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>138</td>
<td>143</td>
<td>RT</td>
<td>17</td>
<td>PhNO₂</td>
<td>NaHCO₃(aq)</td>
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<tr>
<td>VI</td>
<td>138</td>
<td>143</td>
<td>reflux</td>
<td>17</td>
<td>CD₃CN</td>
<td>Proton sponge</td>
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<tr>
<td>VII</td>
<td>138</td>
<td>143</td>
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<td>VIII</td>
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<td>143</td>
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<td>CD₃CN</td>
<td>-</td>
</tr>
<tr>
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<td>143</td>
<td>RT</td>
<td>17</td>
<td>d₆-DMSO</td>
<td>-</td>
</tr>
<tr>
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<td>146</td>
<td>RT</td>
<td>60</td>
<td>CD₃CN</td>
<td>-</td>
</tr>
<tr>
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<td>138</td>
<td>146</td>
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<td>60</td>
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<td>146</td>
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<td>18.5</td>
<td>PhNO₂</td>
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<td>22</td>
<td>PhNO₂</td>
<td>-</td>
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<tr>
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<td>PhNO₂</td>
<td>NaHCO₃(aq)</td>
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<tr>
<td>XVIII</td>
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<td>21</td>
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<td>-</td>
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<td>146</td>
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<tr>
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<td>146</td>
<td>70</td>
<td>16</td>
<td>PhNO₂</td>
<td>[a]</td>
</tr>
</tbody>
</table>

[a] 2,6-diterbutyl-4-methylpyridine

Nitrobenzene was the solvent of choice and is commonly used as the solvent for phenanthidine alkylation, being polar enough to solubilise starting materials, products and the S_N2 transition state (which involves charge separation in this case) while being inert to most electrophiles due to electron deficiency. One indicator of alkylation is a t at around 4.5 ppm in the ¹H NMR spectrum, distinguished from the CH2-1 peak at 4.5 ppm of triflates as a much broader signal.

The Boc protected phenanthidine 137 did not react with triflate 142 at RT; heating to 120 °C followed by cooling and addition of Et₂O resulted in precipitation of a mixture, possibly including...
phenanthridinium 147 as a minor component (I). Similar results were achieved regardless of reaction time with benzylcarbamate 138 in THF (II and III). For the alkylation of TPP triflate 143 DCM was tested (IV) as it is not very hygroscopic and phosphonium salts tend to be soluble in chlorinated solvents, but little or no reaction was observed. Since elimination was a plausible competing reaction a base was introduced to re-activate any subsequently protonated phenanthridine; NaHCO$_3$(aq) was tested based on literature precedent (V). Following reaction a precipitate was observed, but no product. To eliminate decomposition during work up reaction in CD$_3$CN was followed by $^1$H NMR spectroscopy. Heating to reflux caused decomposition (VI and VII), but entry VIII showed peaks at 4.5 and 4.1 ppm. These could be CH$_2$-1' and increase in size over 1 h, but heating overnight caused decomposition. Complicated mixtures uniformly resulted from these conditions at all time points. The procedure was repeated in d$_6$-DMSO due to the availability of literature data, allowing alkylation to be judged by comparison with the ethidium analogue prepared and assigned by Luedtke$^{180}$ - a shift to bring H7 upfield of H2/H9 would be considered characteristic of the phenanthridinium 148. DMSO also allows higher temperatures if needed and the dispersion of the $^1$H NMR peaks for the aromatic region in this solvent is helpful. No product was observed (IX).

The moisture sensitivity of triflate 143 was considered to be impeding progress. Repeating previous tests with triflate 146 (X - XIII) showed complete consumption of triflate, but no product 148. Room temperature alkylation with triflate 146 in PhNO$_2$ did not result in reaction with benzyl carbamate 138 (XIV), but with methyl carbamate 139 gave a minor side product with an ortho d at 10 ppm and a broad t at 4.7 ppm in the $^1$H NMR spectrum, suggesting phenanthridinium formation (XV). However, this was not successfully purified by chromatography or a Strata X-C ion exchange column.

Repetition with ethyl carbamate 140 and alkyl triflate 146 followed by repeated chromatography with both SiO$_2$ and C18 cartridges did give fractions which unambiguously contained an N-alkyl phenanthridinium 150 based on the characteristic broad t at 4.5 ppm (distinct in form from the sharp CH$_2$-OTf from triflate 146), and amide broad singlets at 10 ppm in d$_6$-DMSO in the $^1$H NMR spectrum (XVI). These amide peaks were present in duplicate in approximately 1:1 ratio, which was initially attributed to slow rotation of the amide giving rotamers on the NMR timescale as the benzyl CH$_2$ peak is present only once and integrates as if part of a single compound with the aromatic protons in the $^1$H NMR spectrum. Including NaHCO$_3$(aq) (XVII) afforded a fraction in which phenanthridinium 150 was the major component. This allowed more precise development of a TLC system, which was important as PhNO$_2$ is removed via initial chromatography of the reaction mixture. With evidence that the alkylation worked (if poorly), phosphonium triflate 143 was applied to the new conditions (XVIII - XIX). NaHCO$_3$(aq) was omitted as experience had shown the moisture sensitivity of the alkylation agent by this point, with moderate conversion observed regardless of reaction time. On analysing fractions collected during chromatography of entries XVIII - XIX additional amide peaks were revealed to be an impurity rather than a rotamer when a trace sample from which they were absent was isolated. Faced with this purification problem

Chapter 5: Synthesis of 3,8-Diamino-6-phenylphenanthridinium salts 65
triflate 146 was again used, to avoid the complication of separating phosphonium salts from phenanthridinium salts.

Although NaHCO$_3$(aq) appeared to improve conversion a base soluble in anhydrous PhNO$_2$ was considered preferable, with the caveat that very low nucleophilicity was required to prevent competition. 2,6-Di-tert-butyl-4-methylpyridine was used, and after 72 h the desired product 150 appeared favoured by approximately a 63:37 ratio over the side product (Scheme 46). Repeated chromatography gave a 7% yield at a purity estimated to be around >90% if residual DCM is ignored (XXI). Increasing the equivalents of triflate 146 and the temperature caused a loss of selectivity and a 1:1 ratio of the phenanthridinium 150 to side product (XXII).

**Scheme 46** - Alkylation and intended route to the MitoSOX precursor 152.

Mixtures of phenanthridinium 150 and the side product from alkylation were subjected to hydrogenolysis to remove the benzyl protecting group. Clean deprotection resulted, and subsequent chromatography gave a 17% yield of alcohol 151. Impure material was used for a small scale test of iodination (Appel reaction) and triphenylphosphine addition, confirming the conditions did not result in decomposition but unfortunately not that TPP salt 152 is formed. Small scale removal of ethyl carbamate by reflux in HBr(aq) gave better chromatographic separation, although unpredictable elution was still observed (see later). A single column yielded some fractions of approximately 90% purity, but the use of $d_6$-DMSO as an NMR solvent hid the CH$_2$ shift which would confirm the conversion from alcohol to bromide.

Particular note was made during the course of the above work of the difficulty inherent in purifying these compounds, with considerable streaking contributing to a situation where elution
rarely mimicked TLC. Acetone/EtOAc systems worked well on TLC but proved hopeless on silica column. Even the various DCM/MeOH systems often required washing with high percentages of MeOH and/or NEt₃ to recover the majority of material, which often adhered to the baseline but matched earlier fractions by NMR and TLC when recovered.

Scheme 47 - Outline of a reduction-alkylation based synthesis of phenanthridinium iodide 154.

When the difficulty of phenanthridine alkylation became apparent reduction of ethyl carbamate 140 to an amine and subsequent alkylation of hydrophenanthridine 153 was investigated (Scheme 47). Hydrogenation over Pd/C in AcOH (allowing reduction through the reactive iminium) was unsuccessful, so LiBH₄ was used. This is more reactive than NaBH₄ due to the higher Lewis acidity of Li⁺, and is soluble in ethereal solvents, from which N-alkylphenanthridinium 154 might be expected to precipitate were the steps combined. LiBH₄ does not reduce carbamates in numerous literature examples. Reduction was confirmed by TLC and colour change. Additional aromatic peaks for the hydrophenanthridine 153 in the ¹H NMR spectrum including those at 7.6 ppm d (J = 9.2 Hz, H1/H10) and 6.64 ppm dd (J = 8.4, 2.1 Hz, H2/H9) are considerably upfield of where equivalent peaks are expected in the oxidised form 140. Re-oxidation is rapid; at best a 3:1 ratio of 153:140 was observed by NMR spectroscopy (Scheme 31).

Alkylation was attempted on the mixture using iodobutane, but even with long (72 h) reaction times no alkylation was observed. It was increasingly apparent that the synthesis of N-alkylphenanthridinium salts was not trivial, so an alternative route was attempted.
5.3 Route to N-alkylphenanthridinium salts via Bischler-Napieralski cyclisation

An alternative synthesis attempts to build on a literature report of cyclisation directly to a phenanthridinium alkaloid precursor 155 via Bischler-Napieralski type cyclisation (Scheme 48).

This is analogous to the classic Morgan-Walls cyclisation to give phenanthridines (see Section 4.2.1).

![Scheme 48 - Literature example of a Bischler-Napieralski cyclisation to a phenanthridinium based alkaloid.](image)

Using the strategy outlined in Scheme 49 intermediate 158 is the key precursor for the cyclisation reaction. 4,4-Dinitrobiphen-2-ylamine 157 was prepared from biphenyl-2-ylamine 156, according to a literature procedure using KNO₃ in H₂SO₄, which provides a powerful nitrating agent with lower water content than HNO₃(aq)/H₂SO₄(aq).

![Scheme 49 - Synthesis of 3,8-diaminophenanthridinium salt 159 via Bischler-Napieralski reaction required access to key tertiary amide intermediate 158.](image)

Using a large excess of KNO₃ (8 eq.) resulted in a mixture of double and possible triple nitrated aromatic rings even at low temperature, but reducing the amount of KNO₃ to 2.2 eq. gave clean conversion with no purification aside from an aqueous wash. The regiochemistry of nitration on...
the aniline ring is consistent with the electron withdrawing effect of the aryl ammonium group generated in strongly acidic conditions. Amide formation by refluxing dinitro biphenyl-2-ylamine \( 156 \) with benzoyl chloride in chlorobenzene as in Lion et al resulted in a mixture of products.\(^{143}\) Mild alternative conditions\(^{185}\) gave good conversion (Scheme 50) to amide \( 160 \).

Amide \( 160 \) dissolved well only in DMSO or hot DMF, complicating the recovery of material. Quenching into water gave a very fine precipitate which did not reliably filter out, or stay settled enough to separate from the solution after centrifugation. Many attempts were made at the subsequent alkylation with increasingly harsh conditions, including heating amide \( 160 \) to 170 °C with 3 eq. sodium hydride and excess 1,6-dichlorohexane for 90 min. Use of iodide catalysis was tested, as were various alkylating agents including triflate \( 142 \), 1,6-diodohexane and an alkyl iodide bearing a THP-protected alcohol \( 162 \) synthesised via the Appel reaction (Scheme 51).

![Scheme 51](image)

Scheme 51 - Synthesis of a mono-THP protected iodohexyl compound \( 162 \).

Attempted alkylation of amide \( 160 \) resulted in various mixtures of numerous and diverse decomposition products under these conditions or indeed any more unusual procedures tested.\(^{186,187}\) The alkylation of amide \( 160 \) was expected to be problematic, being very electron poor and because the tertiary amide \( 158 \) would be sterically congested. However, it was not fully appreciated how strained the desired product would be until the atropisomerism displayed by a less hindered carbamate \( 245 \) (Scheme 77) was later identified.

The original literature cyclisation\(^{184}\) required heating to reflux in MeCN even when decorated with multiple donating groups (Scheme 52), so it was anticipated that reduction of the nitro groups on amide \( 160 \) would prove necessary. Reduction via transfer hydrogenation (Scheme 52) was tested on the dinitroamide \( 160 \).

![Scheme 52](image)

Scheme 52 - Reduction of dinitro biphenyl \( 160 \) to give more nucleophilic biphenyl \( 163 \).

The diamino compound \( 163 \) was confirmed by a uniform upfield shift in the aromatic protons in the \(^1H\) NMR spectrum and the appearance of a 4H broad singlet at 3.8 ppm, but not purified or further characterised. To overcome alkylation difficulties the steps were rearranged, with benzoyl
protection following reductive amination. Aldehyde 164 was synthesised by Swern oxidation of alcohol 161 (Scheme 53).

![Scheme 53 - Swern oxidation to aldehyde 164.](image)

Attempts at reductive amination to convert primary amine 157 into secondary amine 165, both in-situ and via isolation of imine 166 and reduction proved unsuccessful, as did an unusual procedure using the direct alkylation of the amine 157 with alcohol 161 (Scheme 54).^186

![Scheme 54 - Different reductive amination protocols failed to access secondary amine 165.](image)

The reactivity of the aniline was proving problematic, so the more circuitous route of reaction with an acid chloride and subsequent reduction was investigated, for which several acylating agents were prepared (Scheme 55). Synthesis and isolation of the 7-membered cyclic anhydride 169 from a literature procedure^188 starting with adipic acid 167 proved problematic. Double acid chloride 170 was synthesised by reaction with thionyl chloride and reacted immediately with aniline 157, resulting in a complex mixture of products. Mono methyl adipic acid was then used instead to generate acid chloride 171.

![Scheme 55 - Preparation of acylating agents 170 and 171.](image)
Amine 157 was then reacted with acid chloride 171 to give amide 172 in good yield (Scheme 56).

Ester 172 was hydrolysed to give acid 173.

Scheme 56 - Secondary amine 175 was accessed by an acylation-reduction approach, but tertiary amide intermediate 158 remained elusive.

The next step was reduction of the amide using the neutral and electrophilic THF-borane reagent. The borane coordinates to the more Lewis basic position prior to reduction, and in the case of carboxylic acids forms a triacyloxyborane (Scheme 57). Coordination to the acid generates complex 176, or a related structure, which decomposes to a structure 177 analogous to a mixed anhydride allowing rapid reduction. The expected result is that the aldehyde is generated then reduced in preference to the amide. A hydride such as DIBALH would preferentially reduce the amide, risking cyclisation from the amine when the aldehyde is produced. There is strong literature precedent for borane tolerating aromatic nitro groups, unlike reagents such as lithium aluminium hydride.

Scheme 57 - Triacyloxyborane 176 decomposes to a mixed anhydride 177.

An initial test of reduction of acid 173 with 4 eq. of borane and overnight reflux showed reduction of one nitro group in addition to the expected reaction. Reflux with 2 eq. overnight gave reduction of the acid to the alcohol without reaction at the amide. Returning to 4 eq. of borane and monitoring by TLC allowed for good conversion to alcohol 174. Attempts to generate the amide
precursor 158 lead to benzoyl protection of the alcohol giving benzoate 175, and subsequent deprotonation of this with LiHMDS and reaction with benzoyl chloride proved ineffective. Conversion directly to phenanthridinium was attempted via procedures reminiscent of the Pictet Spengler reaction, initially combining TMSCl with benzaldehyde 72 and alcohol 174, before applying harsher literature Pictet-Spengler cyclisation and spontaneous oxidation conditions reported for the cyclisation of amine 156 to give phenanthridine 179<sup>193</sup> using benzaldehyde in TFA at high temperature and in a sealed tube (Scheme 58).

![Scheme 58 - Literature Pictet-Spengler procedure.](image)

Although this literature example does not form an N-alkylphenanthridinium, the reaction occurs in neat TFA so it is reasonable to assume the reaction proceeds through an iminium intermediate. In reality only a TFA ester (identified by a downfield shift in the adjacent CH<sub>2</sub>) was isolated (Scheme 59), probably due to the low reactivity of the aniline of dinitrobiphenyl derivative 33 towards electrophiles.

![Scheme 59 - Pictet-Spengler attempt on secondary amine 174 gave only TFA ester 180.](image)
5.4 Summary

Having attempted multiple alkylation and cyclisation based routes, recurring difficulties were apparent. Alkylation of the consistently hindered and unreactive nitrogen was always problematic, exacerbated by the electron poor phenanthridines and biphenyl systems used.

Concurrent preliminary work on commercially available 3,8-diamino-6-phenylphenanthridinium salts by Dr Murphy’s group at MRC in Cambridge suggested that the DNA intercalation observed made reproducible extraction challenging. On this basis the target was changed and required a bespoke phenanthidine heterocycle. Without the benefit of the commercially available 3,8-Diaminophenanthridine as a starting material for the new route and in light of the considerable difficulties encountered the alkylation work was abandoned. Similarly the biphen-2-ylamine routes were deemed to be too dependent on difficult alkylations and to offer limited scope for analogue synthesis. Therefore, when considering a new target alternative synthetic routes were investigated instead.
Chapter 6: A new targeted superoxide sensor design

6.1 Design and retrosynthesis

6.1.1 Avoiding DNA intercalation

In order to avoid DNA intercalation, it was necessary to modify the target probe. The structure was tweaked by exchanging the 3-amino group for a tert-butyl moiety, as shown in blue in superoxide probe target 181 (Figure 32). This should avert intercalation because the bulky substituent will make intercalation between DNA base pairs energetically unfavourable.

![Figure 32 - Target phenanthridinium showing A, B, C labels for rings, with the blocking 'Bu group in blue.](image)

6.1.2 Synthetic route towards superoxide probe 181

The design of the new probe 181 imposed the requirement of selective substitution at the 3 and 8 positions. In addition, based on the failure of previous approaches a few guiding principles were identified for application in evaluating routes:

- Very electron poor aniline components should be avoided as they are unreactive, and sterically hindered versions even more so.
- The N-alkyl group should be appended prior to construction of the heterocyclic B-ring as N-alkylation of phenanthridines is a poor reaction.
- When constructing the B-ring, avoid loading one component of an intermolecular reaction with all the bulk of the C6- and N- substituents.
- The reaction to make the N-alkylphenanthridinium should be simple, clean, high yielding and close to the end of the synthesis, because the separation of different phenanthridinium compounds is difficult and their solubility is problematic.
- Demanding transformations that require extensive purification should occur prior to the construction of the N-alkylphenanthridinium for the same reasons.

Working from these principles, a new route was devised concluding the synthesis of phenanthridinium core 182 with intramolecular imine formation. This is a 6-exo-trig cyclisation to make a polyaromatic system, and should be highly favoured. Additionally, the phenanthridinium salt 182 would likely precipitate from a non-polar solvent allowing easy isolation. The precursor ketone 183 could be prepared with robust and reliable cross coupling chemistry such as the Suzuki reaction which would be used to form the C13-C10a bond. The cross coupling could use either the combination of an ortho-halobenzophenone 185 with ortho-amINOarylmetal 187 or a 2-metalobenzophenone 184 with ortho-amINOaryl halide 186. This route avoids tertiary amine or
amide formation, which had torpedoed previous routes. The R group can be introduced early, with a variety of methods available to access secondary amine 186 from aniline 188. The 6-Ph substituent could be included by taking advantage of the commercial availability of benzophenones and the extensive literature on these substrates.

Scheme 60 - Retrosynthesis for a proposed cross-coupling based synthesis. Unlike previous routes which relied upon C-N or C-C bond formation for key steps, C12-C10a is the critical transformation.

6.2 Suzuki cross coupling
6.2.1 Mechanism and ligand properties

Following assembly of the desired aromatic components they were to be linked to form the biaryl systems via palladium cross coupling chemistry, ideally the Suzuki coupling. The reaction itself was an obvious choice due to its ubiquity, robustness and substrate tolerance. An array of catalysts and conditions exist and three common catalysts which will be important in later discussion are Pd(PPh3)_4 (tetakis) 189 and (dpdf)PdCl2 190, which involve phosphine ligands, and PEPPSI-Pr 191, which contains a N-heterocyclic carbene ligand (Figure 33).

Figure 33 - A selection of common palladium cross coupling catalyst systems.
A discussion of the mechanism and the choice of conditions is appropriate (Scheme 61).

The precise nature of the catalytic Pd(0)ₙ species varies and is a matter of debate, with Pd(0)L₂ 193 and Pd(0)L both possible. The cycle shown above (Scheme 61) and described below is based on the tetrakis system (L = PPh₃). The metal has a full outer shell of d¹⁰ electrons in Pd(0)L₄ 189 with repulsion reducing the bond strength and increasing ligand lability. Once one triphenylphosphine is lost the L₂ 193 and L₃ 192 forms equilibrate,¹⁹⁴ with L₂ species 193 considered catalytically active. Bidentate ligands such as dppf 190 are conceptually similar but the active Pd(0) species is generated from the dihalide Pd(0) salt rather than dissociating an additional bidentate ligand.¹⁹⁵
both cases the active species is usually a 14-electron complex which becomes 4 coordinate upon oxidative addition.

It is currently believed the active Pd\(^{0}\)L\(_2\) species 193 undergoes oxidative addition inserting itself into the C-X bond of the aryl halide 194 via a concerted process to give a cis Pd\(^{0}\) species. A theoretical study suggests this process is favoured by kinetics compared to insertion into an alkyl halide due to back bonding in the transition state resulting from interaction of the aromatic π system with X.\(^{196}\) An exothermic isomerisation driven by the stability of the trans isomer\(^{197}\) and probably occurring via ligand addition to form a trigonal bipyramidal transition state\(^{200}\) leads to the observed trans\(^{198,199}\) Pd\(^{III}\) species 195. This can be the rate determining step, especially for aryl chlorides. Substrate electronics influence the efficiency of this step, with electron poor aryl halides reacting more rapidly due to the electron rich nature of the neutral palladium species 193, particularly where ligands are strongly donating.\(^{200}\)

The halide is reversibly displaced by a nucleophilic ligand, usually hydroxide generated by a base reacting with water as shown in Scheme 61 but fluoride is also frequently used.\(^{201}\) This gives complex 196, which undergoes faster transmetallation.\(^{202,204}\) In the case of hydroxide the oxophillicity of the boron allows coordination prior to transmetallation in complex 200 and as a result the identity of the base counterion is important due to the potential competitive coordination of Pd-OH complex 196 by cations to form non-productive complex 197.\(^{203}\)

Due to the electron deficient nature of Pd\(^{0}\) complexes transmetallation is a more facile process when an electron rich arylboronic acid is employed, and is disfavoured by donating ligands.\(^{200}\) The arylboronic acid 198 is necessarily in equilibrium with the unreactive trihydroxyborate 199, providing an upper limit to the acceleration provided by base.\(^{201,204}\) The trans complex formed 201 is relatively stable\(^{199,205}\) in the absence of base, with recent literature suggesting further coordination with hydroxide to give a pentacoordinate compound 202 which bypasses the requirement for trans/cis isomerisation prior to reductive elimination.\(^{201,206}\) Analogous acceleration by a fifth ligand is preceded by a styrene promoter,\(^{207}\) and fluoride is believed to behave similarly. Reductive elimination then occurs to give the biaryl product 203, and this is most rapid when the electronic properties of the aryl groups are markedly different.\(^{208}\) Electron rich ligands disfavour reductive elimination, while wide bite angle bidentate ligands sterically favour elimination.\(^{209}\)
6.2.2 Mono-ligated palladium species

The previously shown catalytic cycle assumes Pd\(^{0}\)\(_{2}\) is the active catalyst. However, Pd\(^{0}\)\(_{L}\) is also possible. Bulky monodentate ligands are believed to form tricoordinate\(^{210,211}\) intermediate \(\text{205}\) upon oxidative addition. The pathway to these intermediates is dependent mostly on the halide used (Figure 34).\(^{212}\)

Figure 34 - The effect of ligand size on the generation of monoligated palladium species.\(^{213}\)

The reaction coordinate diagram shows the dissociation of bicoordinate complex \(\text{193}\) to give monocoordinate complex \(\text{204}\) and oxidative addition to ArX \(\text{194}\) giving tricoordinate complex \(\text{205}\). Increasing ligand bulk favours this dissociation by increasing the energy of the bicoordinate complex \(\text{193}\) more than the dissociated form \(\text{204}\), as do specific interactions found in some ligands.\(^{214}\) The nature of this process depends mostly on the halide used.\(^{212}\)

In the case of aryl chlorides prior ligand dissociation from complex \(\text{193}\) gives \(\text{204}\), a 12 electron species which undergoes rapid oxidative addition.\(^{215,216}\) Aryl iodides react via the PdL\(_{2}\) species, with one ligand then dissociating to form tricoordinate species \(\text{205}\), which may then dimerise depending on ligand steric.\(^{217}\) Aryl bromides are variable; multiple mechanisms may occur in a substrate dependent fashion. Subsequent transmetallation may occur more easily to tricoordinate complexes,\(^{214,218}\) and calculations on C-C and related C-N systems suggest they undergo reductive elimination more readily.\(^{219,220,221}\)

6.2.3 N-Heterocyclic carbene catalysts

\(N\)-Heterocyclic carbene (NHC) ligands may also generate monocoordinate species \(\text{204}\). NHC ligands represent an alternative to phosphine ligands. Originally believed to form PdL\(_{2}\) complexes, it is now established that PdL complexes are the active species.\(^{200,222}\) One example is the PEPPSI-\(^{i}\)Pr catalyst \(\text{191}\) (Figure 33). The core of the ligands themselves is an aromatic,\(^{223}\) singlet,\(^{224}\) bent
carbene stabilised by σ withdrawing and π donating nitrogen atoms. While the reaction mechanism is broadly the same as when using phosphine ligands these complexes have important distinct properties. Bonding within NHC complexes varies depending on a range of subtleties.

![Figure 35 - The three major contributors to NHC-M bonding.](image)

There are three major bonding contributions to NHC metal complexes (Figure 35). Strong σ donation from the lone pair is the largest feature, providing more electron donation than phosphine ligands. Of the remaining interactions metal → π* backdonation is more important for d10 metal complexes. A significant electrostatic component exists, particularly for cationic Pd species, but has proven difficult to quantify. In the case of the PEPPSI family of commercially available catalysts activation is believed to occur through double transmetallation of the Pd(II) salt, and elimination to give Pd(0), then pyridine dissociation and oxidative addition, which is favoured by substituents increasing σ donation. Moderately bulky ligands improve either transmetallation or reductive elimination steps, with the effect of the pyridine ligand on the rate suggesting reattachment by this point. NHC ligand based catalysts display high stability, allowing low catalyst loading.

For testing purposes three catalytic systems were selected; the heavily used and studied Pd(PPh₃)₄, (dppf)PdCl₂, selected as one of the more versatile bidentate catalysts, and the most active of the available PEPPSI-NHC catalysts, PEPPSI-Pr (Figure 33).
6.3 Methods of constructing arylboron reagents

6.3.1 Miyaura Borylation:

Organometallics 185 and 186 are required for the cross-coupling and as discussed above organoboron compounds are ideal for this. The Miyaura reaction is a way of converting aryl halides 194 to arylboronate esters 206 which uses a catalytic cycle mechanistically analogous to the Suzuki reaction to achieve borylation without the use of powerfully basic organometallic reagents (Scheme 62).

Scheme 62 - The Miyaura reaction.

Much of the prior discussion of the Suzuki mechanism is applicable here. The key difference is that these borylations go through a $\text{L}_2\text{Pd}^{(II)}\text{OAc}$ complex. The hard Lewis base and soft Lewis acid combination may make this complex more reactive than the halide equivalent, with changes in geometry also implicated in some cases. Pinacolboron acetate is produced as a by product. The weak base used limits hydroxide formation when water is present suppressing Suzuki coupling, although dry conditions are often required for good conversion. Various related procedures exist using different metals, different boronic esters and in one case bis-boronic acid.

6.3.2 Lithium - halogen exchange followed by borylation

Organolithium and Grignard type reagents can be used to make arylboronates (Scheme 63). Rapid metal-halogen exchange on aryl halide 194 generates a highly nucleophilic organometallic species 207, which attacks a suitable boronic ester to give the arylboronate salt 199. Acidic workup then gives the boronic ester 198. Literature studies generally focus on one reagent, but in essence both share distinctive properties and observations made of one type of reagent is often extended to the other in literature. Here the general overview and more extreme chemical properties of organolithiates are described, followed by more specific discussion of ‘ate’ complex decomposition in organomagnesium species.

Scheme 63 - Metal halogen exchange followed by borylation.
The lithium-halogen exchange is an equilibrium process, with the position determined by the relative stability of the carbanions formed (Figure 36).\textsuperscript{234} Increasing s character lowers the energy of the HOMO orbital so when exchanging an aryl halide with an alkyl lithium species the transfer goes essentially to completion, because an aryllithium is more stable than an alkyl lithium.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure36.png}
\caption{Relative stability of carbanions}
\end{figure}

The mechanism of these exchanges is not completely understood. Complications include the aggregation of lithiated species, which is controlled by the steric of the alkyl portion and varies with temperature, solvent and concentration.\textsuperscript{235} In the absence of a Lewis base hexamers and tetrarmers predominate. Lithium ions (bonding is predominantly ionic)\textsuperscript{236,237} form \(L_3\) triangles.\textsuperscript{237} Each lithium has at least four contacts, and is electron deficient in nature. The \(\beta\)butyllithium often used forms discrete octahedrons.\textsuperscript{238} Addition of a Lewis base results in the positive lithium being stabilised by interaction with the base and reduces the aggregation, increasing reactivity.\textsuperscript{235,237}

The difference in reactivity is potentially enormous; Jones \textit{et al}\textsuperscript{239} show that dimeric \(\beta\)butyllithium is 8 orders of magnitude more reactive than tetramereric forms towards a terminal acetylene. In the same paper they estimate the monomer is at least 14 orders of magnitude more reactive than the tetramer towards the same substrate.\textsuperscript{239} Such differences in already rapid transformations make identifying the active species challenging. Depending on the nature of the arylhalide\textsuperscript{194} either a radical mechanism (SET) through radical anion \textsuperscript{209} then neutral radical \textsuperscript{211}, or an ‘ate’ complex such as \textsuperscript{213} may be involved (Figure 37).

![SET Mechanism](image)

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure37.png}
\caption{SET (radical) and ATE (nucleophilic) mechanisms}
\end{figure}

\[ X = \text{Halogen} \]

Based on EPR spectroscopy,\textsuperscript{240} cyclic radical probe studies,\textsuperscript{241} and the isolation of characteristic products\textsuperscript{242} radical mechanisms are known to occur, particularly at higher temperatures and where an alkyl bromide is the substrate.\textsuperscript{243} Some ‘ate’ complexes have been isolated and are effective nucleophiles.\textsuperscript{244} Studies on the effects of substituent electrons\textsuperscript{245,246} and substrate
The stoichiometry on the kinetics of aryl halide exchange suggest this is the dominant mechanism. Evidence for one mechanism does not necessarily preclude the other; detection of radicals does not disprove the involvement of an ‘ate’ complex. Some iodine ‘ate’ complexes generate radicals during decomposition.

Lithium - halogen exchange is a rapid process, occurring preferentially to deprotonation in some cases. Organolithium species are reactive and in particular very basic (the conjugate acid of C₂H₅Li has a pKa of around 50 relative to water). As such, low temperatures and short reaction times are preferable, and the exclusion of oxygen and water is vital.

Many of the mechanistic details pertaining to organolithium species are also relevant to magnesium halogen exchange processes; in particular an ‘ate’ complex is implicated. Some particular details have been elucidated by Hoffmann and co-workers.

Figure 38 - The proposed ‘ate’ complex route to the exchange product. Diagram and proposed structures amalgamated from Hoffmann et al.

If attached groups are sufficiently stabilising the ‘ate’ complex may take the form of an observable intermediate, either a solvent separated ion pair or dissociated ions (Figure 38, Situation C). Otherwise a ‘contact ion pair’ where the metal ion is more closely associated may be a transition state (Situation A). Alternatively, in Situation B ‘ate’ complex is present only as a reactive intermediate and decomposes too rapidly to be observed. Situation C is also favoured by higher electron density at the magnesium centre, and hence by coordinating solvents. However, even with very stable ‘ate’ complexes cationic species have a role in the final conversion to the Grignard reagent, with a bimolecular process 1st order with regard to total magnesium concentration observed. Introduction of an electrophile in the presence of an ‘ate’ complex of this type may catalyse a chain radical process leading to Grignard formation.
6.4 Synthesis of benzophenones components

6.4.1 Synthesis of ortho-halobenzophenone component

Availability of 2-aminobenzophenone amine 217 and precedent\(^{255}\) for synthesis of 2-iodobenzophenone 218 (Scheme 64) contributed to the decision to utilise Suzuki coupling.

Scheme 64 - Synthesis of benzophenone iodide 218.

Diazotisation and quenching with potassium iodide gave near quantitative conversion with a procedure modified from literature.\(^{255}\) The viscosity of the mixture and the quantity of gas generated meant mechanical stirring, a large flask and careful additions were necessary. The weak C-I bond and electron deficient aryl ring make this substrate ideal for the aryl halide component of Pd cross coupling reactions.

The synthetic plan was tested at this point by trial coupling reactions on iodobenzophenone 218, initially with 4-methoxyphenylboronic acid 219 which is electron rich and should undergo transmetallation readily, with reductive elimination also favoured due to the electronic complementarity with iodobenzophenone 218. The methoxybenzophenone 220 was isolated in an acceptable unoptimised yield (Scheme 65).

Scheme 65 - Test Suzuki coupling of iodobenzophenone 218 with a methoxyarylboronic acid 219.

In order to model the ortho substituent and establish the electronic of boronic acids or esters which could be used 2-nitrophenylboronic acid was coupled to benzophenone 218 (Scheme 66).

Scheme 66 - Test Suzuki coupling of iodobenzophenone 218 with a nitroarylboronic acid 221.
The coupled benzophenone 222 was not isolated but inferred from the crude $^1$H NMR spectrum by observation of H4 and H6, which were clustered together downfield of both H3 and their equivalent iodobenzophenone 218 peaks, a pattern consistent with benzophenone 222 and observed repeatedly in other analogues (Chapter 8).

Aryl bromide 223 was then used to test Negishi coupling conditions (Scheme 67). Successful lithiation of arylbromide 223 and transmetallation to Zn was followed by transfer into a reaction mixture with iodobenzophenone 218 and heating. A 64:36 ratio of starting iodobenzophenone 218 to 2-arylbenzophenone 225 was identified by $^1$H NMR. No quenched arylzinc 224 was observed as a result of its volatility. Although optimisation would be required this demonstrated Negishi coupling to be possible with iodobenzophenone 218.

6.4.2 ortho-metalobenzophenones

The alternative benzophenone-derived coupling partner would be an ortho-metalobenzophenone 185. Some confusion surrounded the literature conditions$^{255}$ for magnesiation of iodobenzophenone 218; the paper and the supporting information offer conflicting accounts of the reaction temperature. Testing first with iPrMgCl and PhMgCl at −40 °C (Scheme 68) a mixture of products was observed when either Grignard reagent was used followed by addition of borate 226.

Chromatography allowed some of these fractions to be investigated, and it was observed that the aromatics signals typically shift substantially upfield in the $^1$H NMR spectrum. This included a species with a $d$ ($J = 2.7$ Hz, H6) and $dd$ ($J = 8.5, 2.7$ Hz, H4) between 6.8 and 7.0 ppm, but the ortho $d$ (H3) at 7.8 ppm. These peaks are close together in the iodobenzophenone 218, probably because the H4 and H6 positions are conjugated to electron withdrawing groups, and the H3 position is ortho to iodine, which effects a downfield shift on the ortho position.$^{256}$ The opposite change in shift pattern would therefore be expected for successful magnesiation-borylation, and
this product appears more consistent with attack on a withdrawing group, to which both nitro and ketone groups are potentially susceptible. Broad singlets at 5.8 ppm and 4.9 ppm which exchanged upon D$_2$O shake were also observed in these mixtures. No dehalogenation product was observed.

Testing the more conservative SI conditions, exchange of iodine with iPrMgCl or PhMgCl at $-78 \, ^\circ\text{C}$ followed by quenching with MeOH demonstrated that little or no exchange had occurred under these conditions (Scheme 69).

![Scheme 69 - Metallation test of iodobenzophenone 218.](image)

With low temperature Grignard formation ineffective and side products apparently favoured over the formation of boronate ester 227 at higher temperature, Miyaura borylation was attempted instead (Scheme 70).

![Scheme 70 - Product mixture from the attempted Miyaura borylation of iodobenzophenone 218.](image)

A complex mixture resulted from all attempts at these reactions, but the major products were identified. Using 1.1 eq. of bispinacolatodiboron, dehalogenated benzophenone 228 was identified as a minor component by comparison with literature data,$^{257}$ and the major component isolated and characterised as dimer 229. This is believed to form through a Suzuki reaction between the iodide 218 and product 227, although dimer 229 was also observed in Scheme 107 where no borylated derivative could be formed. In order to favour the boronate ester 227, the bispinaocolatodiboron equivalence was increased to 2 and the equivalence of KOAc reduced from 5 to a more normal 3, the concentration doubled and the catalyst loading halved. This resulted in a mixture including fluorenone 230$^{258}$ (major) and dimer 229 (minor). The probable mechanism of fluorenone 230 formation can be ventured based on a very similar cyclisation with a palladacycle catalyst and fluorenone products, which is believed to occur through oxidative addition to Pd$^{0}$ followed by C-H activation, and reductive elimination.$^{259}$ The exact nature of such processes is a matter of ongoing debate, and a Heck-type mechanism is also conceivable.$^{260,261,262}$ The isolation of small, pure crystals of the fluorenone cyclisation product 230, appeared to indicate that oxidative addition is followed by competing borylation and C-H activation based cyclisation of the
oxidative addition product. Rapid Suzuki coupling also competes once the arylboronate ester is present in significant quantities. Reduction of the temperature to 60 °C and reaction time to 3 h showed iodobenzophenone 218, dimer 229 and fluorenone 230, demonstrating that the Suzuki coupling is faster than the initial borylation and hence one of these side reactions will always be preferred, making Miyaura borylation unfeasible.
6.5 Synthesis of the tert-butylaryl component
6.5.1 Synthesis of ortho-(N-alkylamino)aryl halides

Ortho-(N-alkylamino)aryl halides were potential coupling partners and precursors to ortho-(N-alkylamino)arylmetals 186 (Scheme 60). N-Alkylation of aniline 188 with an alkyl halide was considered so alkyl bromide 232 was prepared via the Appel reaction. Reductive amination also appeared to be a good approach to preparing N-alkylaniline derivatives, so previously prepared alcohol 145 was oxidised to give aldehyde 231. Numerous suitable procedures exist for oxidation of alcohol 145 to aldehyde 231; a TEMPO oxidation protocol was chosen as a mild, robust, cheap and quick approach (Scheme 71). The mechanism of this reaction is shown in Scheme 72.

Scheme 71 - Synthesis of aldehyde 231 and bromide 232.

Scheme 72 - Postulated TEMPO oxidation mechanism.264

Oxidation of the TEMPO radical 233 generates a nitrosonium cation 235 which undergoes nucleophilic attack from the alcohol 234, with deprotonation forming complex 236. The resulting oxidation occurs by deprotonation to form the aldehyde 237 and eject the reduced cyclic hydroxylamine 238. Re-oxidation occurs first to nitroxy radical 233 then nitrosonium 235. In the protocol NaOCl was added as the oxidant, but HOBr formed with KBr contributes significantly to
the oxidation rate. Oxygen can be used as the oxidant with the correct co-catalyst. Reaction is faster at pH 8.6 than at 12.7 (probably due to OBr partitioning mostly into the aqueous phase) and faster at 0 °C than RT (due to nitrosonium stability). Benzyl ethers can oxidise to benzoates in systems containing NaOCl, and the benzyl ester was observed as a side product in the 1H NMR spectrum at levels of up to 15 - 20%, as identified by a 2H t at 4.35 ppm and 3 aromatic signals consistent is splitting and shift pattern to a phenyl group with an electron withdrawing substituent. Careful TLC monitoring and restricted reaction time and NaOCl equivalence eliminated this problem and allowed isolation in quantitative yield.

With these in hand the necessary amine 240 was prepared (Scheme 73). Literature conditions allowed near quantitative nitration of tert-butyl aryl bromide 223 to give nitroaryl 239. Dithionite reduction was attempted based on literature conditions. This was initially successful, but proved unreliable. Reduction with an iron acid mixture proved more robust, giving aniline 240 in high yield.

![Scheme 73 - Bromide tBu coupling partner 240 synthesis.](image)

Imine formation by stirring amine 240 and aldehyde 231 with Na2SO4 then reduction with LiBH4 was ineffective, as was alkylation with alkyl bromide 232 at 80 °C in DMF with Cs2CO3, and at RT with NaH. Reductive amination with 2 eq. TFA produced a mixture of compounds. NaBH(OAc)3/DCE gave very slow conversion and side products. An alternative TFA based procedure utilising much higher equivalence of TFA and later addition of aldehyde 231 in DCM gave rapid conversion to 241 in good yield.

### 6.5.2 Synthesis of ortho-(N-alkylamino)arylmetals

Attempts were then made to use metal - halogen exchange and nucleophilic attack from the subsequent organometallic species to form a boronic ester Suzuki precursor 242 (Scheme 74).

![Scheme 74 - Borylation by metal halogen exchange on amine 241 was not successful.](image)
Magnesium - halogen exchange \textit{ortho} to a primary amine had previously been achieved by first deprotonating the amine.\textsuperscript{274} Metallation of secondary aniline \textit{241} was first attempted both with a combination of Grignard reagents; PhMgCl is used to effect deprotonation to form a ‘magnesium amide’. The more reactive iPrMgCl is then used to transmetallate to give the aryl Grignard reagent\textsuperscript{274} which can then be used for nucleophilic attack on a borate to give the Suzuki precursor. Use of the literature conditions returned starting material and a small quantity of a side product. Allowing the mixture of aryl bromide and Grignard reagents to stir at RT for 3 h prior to cooling and quenching with a boronic ester gave a 63:37 ratio of aniline \textit{241} to the side product. From the mixture the $^1\text{H}$ NMR spectrum of this side product was observed to have an ABX coupling system shifted substantially downfield. Both the CH$_2$ s of a benzyl group at 4.5 ppm and CH$_2$ groups consistent with 1’ (at 3.3 ppm) and 6’ (at 3.5 ppm) positions were associated with this side product. The amino proton which is well defined and coupled to CH$_2$-1’ in aniline \textit{241} is missing however. Following chromatographic purification none of this side product was isolated. Both literature\textsuperscript{275} and the experience of colleagues confirm that chromatographic purification of pinacolboronate esters is possible. Indeed complete hydrolysis of a pinacolboronate ester is a difficult transformation.\textsuperscript{276} While it must be allowed that the electron-donation from the \textit{ortho} group would accelerate protodeboronation, the coupling patterns observed in the reaction mixture and column fractions do not suggest this. On this basis, while the identity of the side product was not ascertained it was not believed to be the desired product.

When lithium halogen exchange was attempted no reaction was observed at $-78^\circ\text{C}$ with less than 3 equivalents. It is possible that benzylic lithiation or directed lithiation occur. Warming a solution of aniline \textit{241} and excess $^1\text{butyllithium}$ from $-78^\circ\text{C}$ to $-55^\circ\text{C}$ was noted to produce a green colour. Subsequent addition of boronate ester produced the same impurity as previously noted and numerous others. At this point the metal - halogen exchange was abandoned, and the Miyaura borylation reaction attempted instead (Scheme 75).

![Scheme 75 - Miyaura borylation of secondary amine 241 gave dehalogenation product 243 among other side products.](image)

Miyaura borylation of aryl bromide \textit{241} resulted in complete conversion giving two products. The major one was identified as dehalogenated amine \textit{243} on the basis of an apparent $t$ with an \textit{ortho} coupling constant ($J = 8.1$ Hz) which would represent the position \textit{meta} to the amine, and a \textit{meta} apparent $t$ ($J = 1.8$ Hz) consistent with the position between the amine and the \textit{tert}-butyl group. $^{11}\text{B}$ NMR confirmed the absence of boron from either product, proving the reaction to be unsuccessful.
Deviating from the original synthetic plan, a reductive amination of iodobenzophenone 218 with aniline 241 was then attempted (Scheme 76). Successful transformation to tertiary aniline 244 would give the desired trialkyl system which could then be linked, by Ullman coupling for example.

Scheme 76 - Reductive amination to give substrate 244.

No reaction was observed, with starting materials 241 and 218 isolated. Alternatives to aniline 241 were then investigated.
6.5.3 Synthesis of $N$-alkyl-$N$-arylamides as precursors to the Suzuki Reaction

In order to simplify the transmetallation the aniline $\mathbf{241}$ was protected with Boc to give a carbamate (Scheme 77). This removes the most acidic hydrogen, and preventing formation of an anion at this position should improve the reactivity of the aryl bromide. Very hindered Suzuki couplings are well preceded.\textsuperscript{210}

![Scheme 77 - Synthesis of tertiary amide 245.](image)

No reaction was observed when aniline $\mathbf{241}$ was heated to reflux with NEt$_3$, DMAP and di-tert-butyl dicarbonate in DCM. Instead various strong bases were tested to deprotonate the aniline $\mathbf{241}$ prior to dicarbonate addition. Using 1.05 eq. of PhMgCl produced no reaction, however the equivalent reaction with 1.12 eq. $^6$butyllithium gave good conversion, with some residual starting material but little decomposition. The procedure was refined by exchanging the $^6$butyllithium with LiHMDS.\textsuperscript{278} This allowed use of extra equivalents to drive the reaction to completion without lithiation giving rise to side products, and the isolation of the tertiary amide $\mathbf{245}$ in good yield. Minor side peaks were observed, alongside substantial broadening and inequivalent CH$_2$-$1'$. It was unclear if these represented an impurity or NMR timescale inequivalence due to sterics, so $^1$H NMR at elevated temperature was compared to the RT data (Figure 39).

![Figure 39 - Temperature controlled $^1$H NMR spectra in CDCl$_3$ of tertiary amide 245, showing RT (top) and 55 °C (bottom).](image)
Two effects were identified. Axial chirality resulting in diastereotopic protons (green), which still do not interconvert at 55 °C, and geometrical isomers (red), which do. The origins of these effects are shown in Figure 40.

Figure 40 - Two types of asymmetry are present on the NMR timescale; axial chirality (a) along the C2-N bond, and geometrical isomers (b) where rotation around the amide bond is observed. Note the amide C2-N bond and the N-C1’ bonds are out of the aryl ring plane (probably at approximately 90°).

The diastereotopic protons arise from axial chirality. The relation of protons H_{1A}' and H_{1B}' to the ring and bromide atom differ and the rotation is too slow for these to interconvert on the NMR timescale, remaining so even at 90 °C. H_{1A}' and H_{1B}' therefore represent protons on one species inequivalent due to differences in environment. Geometrical isomerism in the form of slow rotation around the amide bond is the origin of the side peaks mentioned, and in this case these signals are the same proton in different species. These geometrical isomers 246 and 247 interconvert in a slow equilibrium process. Thus, unlike H_{1A}' and H_{1B}' the integrals of these signals are not required to be equal to one another. In this case one form is clearly favoured, but heating to 50 °C is sufficient to eliminate this effect, confirming one compound is present.

Both effects will in principle be eliminated if sufficient energy is provided to increase rotation speed until interconversion occurs on the NMR timescale. In the case shown the coalescence temperatures (T_c) cannot be established from the data obtained. If this was possible the difference in chemical shift can be related to a rate constant for rotation through the Gutowsky equation and the energetic barrier to the given rotation further approximated through the Eyring equation.\(^{279}\)

Following confirmation that the tertiary amide 245 had been isolated pure, metal - halogen exchange of this substrate was investigated. Lithiation conditions were effective for amide 245 and this was confirmed by quenching the aryllithium into MeOH, giving dehalogenated amide 248 in good yield (Scheme 78).
From the equivalent magnesiation with iPrMgCl at −30 °C only starting material was recovered. Repeat of the lithiation with nbutyllithium and quenching with a boronate ester (Scheme 79) gave mostly the dehalogenation product 248 rather than arylboronate 249, but chromatography showed several other side products, including one in which the 1H NMR spectrum combined a broad 2H t at 3.65 ppm for CH₂N and a dd (1H, J = 8.5, 2.4 Hz) at 7.2 ppm suggesting a 1,2,4-substitution pattern for the aromatic ring. The lack of a large methyl s for a pinacol ester and the equivalence of H₁₅A and H₁₅B ruled out the desired product however. Another product observed as part of the mixture showed an aromatic t at 7.8 ppm (J = 8.1 Hz), consistent with the position meta to the amine in a dehalogenated product, and diastereotopic protons for the CH₂⁻¹’ position. Repeat reactions with trimethylborate as the quenching agent resulted in dehalogenation product.

Although some precedent exists for crowded positions adjacent to Boc groups failing to lithiate at −78 °C, the dehalogenation product would appear to discount this possibility. Instead it was concluded that the reaction with borate esters is disfavoured by steric repulsion.
It was decided that replacing the bromine with a zinc chloride would by comparison clash far less with the ortho substituents, so a Negishi coupling was attempted (Scheme 80).

![Scheme 80 - Unsuccessful transmetallation and Negishi coupling.](image)

Treating aryl bromide 245 with n-butyllithium then transmetallation to zinc and using the reaction mixture for a cross-coupling reaction with aryl iodide 218 gave only benzophenone starting material 218 and quenched amide 248. The possibility of instead using tertiary amide 245 as the halide component was then investigated (Scheme 81). This was treated with 2-nitrophenylboronic acid 221, in an attempt to synthesise biphenyl 252. No reaction was observed and amide 245 was consequently abandoned.

![Scheme 81 - Unsuccessful test of Suzuki coupling with tertiary amide 245.](image)

Instead of using an additional protecting group, a secondary amide would provide a less strained alternative. This would prevent the amine interfering with Miyaura borylation, and secondary amides are surprisingly effective at directing lithiation. Reduction could then be effected prior to cross-coupling, or if this proved problematic reasonable precedent exists for amide reduction in the presence of a ketone. First a suitable acid chloride 254 was prepared from acid 253 (Scheme 82).

![Scheme 82 - Preparation of acid chloride 254.](image)
Acid chloride 254 was used directly (Scheme 83) following preparation.

Scheme 83 - Synthesis of phosphonium amide 256.

Synthesis of amide 255 proceeded in disappointing yield. However, subsequent formation of phosphonium salt 256 proved efficient. Miyaura borylation\(^{283}\) then resulted in generation of side products; the most prominent appeared to lack the phosphonium salt as judged by the lack of the characteristic 2H \(m\) at 3.8 ppm in the \(^1\)H NMR spectrum resulting from the CH\(_2\) coupling to the adjacent \(^31\)P. The aryl peaks were all subsumed into a broad \(m\) and so no coupling pattern could be deduced, but the significant downfield shift is inconsistent with conversion from a bromide to a boronate ester substituent. A variant without the phosphonium present was investigated (Scheme 84).

Scheme 84 - Alternate approaches to the synthesis of carboxylic acid 259 and unsuccessful formation of amide 260.

The synthesis of amide 260 was approached by first producing carboxylic acid 259, originally by Jones’ oxidation\(^{284}\) of the alcohol 145 in good yield. This was improved upon by an alternative 2 step procedure\(^{285}\) from caprolactone 258 with initial hydrolysis and benzyl protection of both acid and alcohol groups, followed by ester hydrolysis for selective deprotection to acid 259. Subsequent formation of an acid chloride and amide formation from amine 240 resulted in a
Later EDCI coupling conditions appeared to give slow reaction. A 1:1 mixture of starting material 240 and a side product was obtained. The side product gave a downfield shift of around 0.3 ppm in the $^1$H NMR spectrum in the dd signal assigned para to the amine but a much smaller shift of 0.1 ppm in the d assigned meta to the amine. These integrated reasonably with peaks assigned as CH$_2$-1’ and CH$_2$-6’. This is consistent with the amide 260, although the material was not isolated or further pursued as this approach was abandoned in favour of $S_N$Ar, an alternative route which had been performed in parallel and which was felt to offer more novelty and versatility. This will be discussed in the next chapter.
Chapter 7: Phenanthridinium-based sensor by nucleophilic aromatic substitution

7.1 New synthetic plan

During the development of the Suzuki coupling an alternative approach was conceived (Scheme 85). This retained nitro reduction of phenanthridinium 262 to give the aniline in the 8 position of probe 261 but envisaged an 6-exo-trig cyclisation by $S_N$Ar from a benzophenone derived imine (N5) to a halogen-substituted C4a. The electronics of imine 263 are appropriate as the rate-determining step of $S_N$Ar is nucleophilic addition to form a carbanion, which is stabilised by electron withdrawing groups such as the 7a imine and especially the 8 nitro group, both of which are conjugated to the 4a position. Fluorine was used as the halide because the carbanion is inductively stabilised by the leaving group, and fluorine provides more effective stabilisation due to its greater electronegativity relative to the other halides. The alkylamine 264 is separately synthesised allowing the chain and triphenylphosphonium targeting group to be introduced by formation of imine 263 from biphenyl 265. The aryl-aryl bond of biphenyl 265, which becomes C1a-C10a in probe 261 would be formed by Suzuki coupling of benzophenone 218 with ortho-fluoroarylboronate 266, previously formed by borylation of aryl fluoride 267.

Scheme 85 – Retrosynthesis of $S_N$Ar route, atoms numbered as positioned in probe 261.

The route outlined in Scheme 85 has several advantages. The late addition of alkylamine 264 results in a more convergent synthesis, and imine formation is well preceded on a huge range
of substrates, various benzophenones included. The 2-fluoroarylboron component 266 is also a far less sterically demanding substrate than the previously synthesised alkyl anilines (Chapter 6).
7.2 Aryl fluoride synthesis

Synthesis of the appropriate aryl halides 267 was required in order to test this route. Aryl fluorides are an obvious choice of electrophile for $S_N$Ar reactions. Aromatic fluorination is a relatively challenging transformation at a laboratory scale, due to harsh conditions and hazardous, difficult to manage reagents and side products. Taking advantage of the commercial availability of aryl fluorides a route using Friedel-Crafts alkylation of 2-fluorohalobenzenes 268 and 269 was investigated. Consideration of both Hammett parameters and the influence of the halides on $^{13}$C NMR shifts suggested C5 is the more nucleophilic position, but the differences between C4 and C5 are small. It was believed that steric interactions may favour formation of the C4 substituted aryl fluoride 271. A variety of Friedel-Crafts alkylation conditions were used to probe the regiochemistry of the reaction (Scheme 86).

\[ \text{Scheme 86 - Friedel-Crafts alkylation, possible regiochemistry.} \]

Rapid conversion was achieved with a 3% loading of AlCl$_3$ in $^t$BuCl at 0 °C. When applied to 1-bromo-2-fluorobenzene 268 the C5 substituted arylbromide 272 was the major product, but the 4-tert-butylaryl bromide product was observed as a minor product (Scheme 87).

\[ \text{Scheme 87 - Friedel-Crafts alkylation on the bromoaryl substrate 268 gave mostly C5 selectivity.} \]

When repeated with the 1-iodo-2-fluorobenzene 269 the procedure gave near quantitative conversion to the C5 substituted aryliodide 273 (Scheme 88).

\[ \text{Scheme 88 - Friedel-Crafts alkylation on the iodoaryl substrate 269 gave complete C5 selectivity.} \]

These results prove the desired regiochemistry to be the less favoured outcome for the Friedel-Crafts alkylation, due to the influence of the fluoride group on electrophilic substitution. On this basis it was decided that the fluoride should be introduced after the tert-butyl group. Effective electrophilic fluorination protocols rely on the formation of organometallic nucleophiles, difficult to do selectively in the presence of an aryl bromide. With the electron rich aryl bromide 223 unsuitable for $S_N$Ar reactions and with regiocontrol of fluorination vital the Balz-Schiemann
reaction appeared to be the most viable approach. Aniline 240 was used to provide the desired regiochemistry (Scheme 89).

\[
\begin{align*}
\text{Br} & \quad \text{NH}_2 \\
\rightarrow & \quad \text{NOBF}_4, \text{DCM} \\
\quad 0^\circ \text{C} - \text{RT} & \quad \rightarrow \quad \text{Br} \\
\text{240} & \quad \text{274} \\
& \quad \text{PhMe} \\
& \quad \Delta \\
\rightarrow & \quad \text{Br} \\
& \quad \text{F} \\
\text{274} & \quad \text{275} 72\% \\
\end{align*}
\]

**Scheme 89** - Synthesis of the fluoroaryl substrate 275.

The Balz-Schiemann reaction actually involves two steps, with diazonium tetrafluoroborate 274 formed and then thermally decomposed. Initially a combination of HBF\(_4\)(aq) and NaNO\(_2\) was reacted with aniline 240 to form the diazonium tetrafluoroborate 274 in 51% yield after washing with HBF\(_4\)(aq) and Et\(_2\)O. Due to the low solubility of diazonium salt 274 subsequent pyrolysis under reflux in PhMe gave only a low yield of aryl fluoride 275 (24%). DMSO was tested as a solvent for pyrolysis, giving clean conversion to a non-fluorinated product assumed to be the phenolic derivative. This is consistent with a literature study showing incorporation of the oxygen from DMSO, forming phenols due to a preassociation of the solvent molecule prior to generation of the aryl cation.\(^{291}\)

Although diazonium salt 274 had been isolated in reasonable yield, the combination of poor solubility in PhMe, toxic reagents and the potentially explosive intermediate made this procedure undesirable. Instead, the diazonium tetrafluoroborate salt 274 was formed with NOBF\(_4\) in DCM, then PhMe added and the DCM removed under vacuum before pyrolysis to give aryl fluoride 275 in good yield.\(^{292,293}\) Scale up was carried out in batches for safety reasons. Various details of the reaction mechanism remain obscure. There are two alternate pathways for decomposition broadly accepted in the literature (Scheme 90).\(^{294}\)

\[
\begin{align*}
\text{N} & \quad \text{F} \\
\quad 276 & \quad \text{F} \\
& \quad \text{BF}_4^- \\
& \quad \text{X} \\
\end{align*}
\]

**Scheme 90** - Alternate dediazonation pathways.\(^{295}\) D is a radical donor, X = electrophile.

Balz-Schiemann products arise from the heterolytic pathway (I), where the C-N bond of the diazonium tetrafluoroborate ion pair 276 is broken, resulting in an ion pair between nitrogen and the reactive phenyl cation\(^{296}\) 277 which reacts with a nucleophile within a local solvation sphere to give aryl fluoride 278.\(^{297}\) The nucleophile has been shown to be BF\(_4^-\) rather than F\(^-\).\(^{298}\) Heterolytic decomposition is favoured by electron donating substituents which destabilise the arenediazonium 276 and stabilise the cation 277. These reactions are favoured by oxygen rich
atmospheres which suppress the homolytic pathway (II), in which the aryldiazonium salt 276 decomposes to the phenyl radical 279, which reacts with an electrophile and undergoes SET to give neutral species 280. Many reactions of this type exist, with some mechanistic variation. The homolytic pathway can result from solvent acting as a nucleophile prior to decomposition to the phenyl radical 279. Since the homolytic pathway can result in protodediazoniation and aryl homocoupling in addition to formation of the desired product non-nucleophilic solvents are preferred, and basic conditions avoided.

Evidence for the Balz-Schiemann going through the heterolytic pathway (I) can be seen in the biaryl product composition seen when the reaction takes place in the presence of other aryl groups, with regiochemistry defined by ring electronics and influenced by substituents (Scheme 91). The Gomberg - Bachmann reaction uses diazonium salts to produce biphenyls under neutral or basic conditions via a radical mechanism and correspondingly does not show the same selectivity in product composition. One study of diazonium tetrafluoroborate pyrolysis in the solid phase observed the Balz-Schiemann reaction accelerates in a fashion reminiscent of a chain reaction, leading the authors to propose a mechanism where radicals are generated following decomposition to an ion pair.

Scheme 91 - Products observed from pyrolysis of diazonium tetrafluoroborate 276 (top) and chloride 282 (bottom).

![Scheme 91](image-url)
7.3 Borylation of 2-fluoroarylhalides

The aryl bromide 275 was to be converted to a boronate using a lithium-halogen exchange. Conditions for the lithiation and conversion to the potassium trifluoroborate salt were tested on 1-bromo-2-fluorobenzene 268 (Scheme 92).

Scheme 92 - Lithiation condition testing

The aryllithium species is generated at low temperature from ortho arylbromide 268 to avoid side reactions, including solvent decomposition. Triisopropylborate was used as the boronate source, forming the arylboronate ester which was converted to the stable trifluoroborate salt 285 by stirring in KHF$_2$(aq). Trifluoroborate salt 285 was crystallised from acetone. Formation of the aryltrifluoroborate 285 avoids the formation of boronate trimers sometimes seen in boronic acid synthesis.\(^{213}\) Although these react similarly to the monomers, analysis and yield calculation is less precise where multiple species are present. The trifluoroborate salts undergo slow hydrolysis to the boronic acid prior to cross-coupling, with side reactions suppressed by the low concentration of the boronic acid.\(^{306}\) The procedure was repeated with aryl bromide 275 (Scheme 93). Lithiating aryl bromide 275 and borylating then quenching into a KHF$_2$ solution gave clean conversion to trifluoroborate salt 266 without the need for recrystallisation.

Scheme 93 - Synthesis of the potassium trifluoroborate coupling partner

7.4 Suzuki coupling to form ortho-arylbenzophenone 265

The coupling of arylmetal 266 and benzophenone 218 was expected to be challenging due to both having ortho substituents, so time was spent identifying appropriate cross-coupling conditions. For testing purposes three catalytic systems were selected; the heavily used and studied Pd(PPh$_3$)$_4$ (tetrakis) 189, one of the more versatile bidentate catalysts (dppf)PdCl$_2$ 190,\(^{230}\) and the most active\(^{231}\) of the available PEPPSI-NHC catalysts, PEPPSI-IrPr 191 (Figure 33). The need for alcoholic solvents was anticipated based on the solubility of the potassium trifluoroborate salts 266 and 285. Since hydrolysis to the boronic acid precedes transmetallation low water content could inhibit reaction; use of a hygroscopic solvent also eliminated this concern. EtOH was used over MeOH allowing a greater thermal range without changing solvents. The temperature (78 °C) and catalyst loading (3%) are within the normal range for Suzuki coupling. DIPEA was used as the base with the exception of the PEPPSI-IrPr example where K$_2$CO$_3$ was initially used in line with the
The model potassium aryl trifluoroborate salt 285 was initially used as a test substrate and coupled to iodobenzophenone 218 with all three catalysts with the intention of making ketone 286 (Scheme 94).

![Scheme 94 - Cross coupling test reaction.](image)

Little reaction was observed using Pd(PPh$_3$)$_4$, and as this catalyst was also the least stable it was not further tested. Both dpf 190 and PEPPSI-iPr ligands consumed the starting material, giving one spot by TLC. Subsequent $^1$H NMR spectra showed two compounds, one of which could be readily identified as benzophenone 286 due to $^{19}$F coupling on the fluorinated aromatic ring. These proved inseparable by flash chromatography. HPLC was used to isolate the other product as the dehalogenated compound 228.

Isolation of the dehalogenated material suggested problems with the transmetallation or reductive elimination steps. Trifluoroboronate 266 is more electron rich (aiding transmetallation) and more electronically divergent from iodobenzophenone 218 (aiding reductive elimination) than the test substrate 285. It was decided that N,N-diisopropylethylamine (DIPEA) should be used as the base for both catalysts in the hope that β-hydride elimination would aid reduction of Pd(II) salts to Pd(0). DIPEA is also more soluble and less likely to reduce the available water content, and the conjugate acid is less likely to interact with Pd-OH prior to transmetallation. PEPPSI-iPr was tested in the coupling of iodobenzophenone 218 and trifluoroborate salt 266 and the biaryl 265 was isolated in good yield (Scheme 95). The dpf catalyst 190 also worked but the reaction was marginally less clean.

![Scheme 95 - The Suzuki coupling of iodobenzophenone 218 and potassium aryltrifluoroborate 266.](image)
7.5 Imine formation and S\textsubscript{N}Ar to complete phenanthridinium core

7.5.1 Imine formation

With the key C1a-C10a bond made the remaining key transformations were the formation of the imine and intramolecular nucleophilic displacement of the fluoride ion to give the phenanthridinium core. The sequence was tested using benzophenone 265 and hexylamine to make imine 287, which could then be cyclised (Scheme 96). Heating in MeCN at 80 °C or at 150 °C in a sealed tube under microwave irradiation gave no reaction, and heating benzophenone 265 to 90°C in neat hexylamine also gave only starting material. However, using an excess of hexylamine with titanium tetrachloride converted benzophenone into imine 287 in acceptable yield following purification.

![Scheme 96 - Formation of imine 287 followed by heating gives cyclisation to phenanthridinium salt 288.](image)

The imine retained the dual 1,2,4-trisubstituted systems of benzophenone 265 in the \textsuperscript{1}H NMR spectrum (Figure 41). H4 and H6 showed a significant upfield shift, while H3 scarcely moved. This suggests a change in the electronics of the group at C1.

![Figure 41 - Benzophenone 265 (top) vs imine 287 (bottom), with key shifts highlighted.](image)
a different conformation relative to the other ring of the benzophenone. The H-F couplings in the $^1$H NMR spectrum of the imine 287 were confirmed using $^{19}$F decoupled $^1$H NMR spectroscopy and showed that the fluoride had been retained in the same position as in the starting benzophenone 265. Interestingly, the ortho F appears to couple with both C3 and H3 of the neighbouring C ring, demonstrated by the small additional C(H) - F coupling in the $sp^2$ region of the $^{13}$C NMR spectrum and the loss of the smaller splitting of the $dd$ assigned to H3 in the $^{19}$F decoupling experiment. Imine 287 shares structural features with literature compounds exhibiting this phenomenon, which in those cases was attributed to N lone pair - F repulsion locking the F in close proximity to C/H to which it couples.309 This is consistent with the observation of a pair of ddd at 3.0 - 3.5 ppm that were identified as diastereotopic protons at CH$_2$N resulting from hindered rotation about the bond linking the two rings of the biaryl unit.
7.5.2 Cyclisation

There are very few examples of imines displacing an aryl halide intramolecularly. However, an intramolecular S_{N}Ar was attempted by heating the crude imine 287 with 3 eq. TMSCl in CDCl₃ at 60 °C for 1 h prior to ^1H NMR analysis. The resulting ^1H NMR spectrum showed a few interesting features:

- Additional methylsilane peaks were apparent in the 0.0 - 0.5 ppm region.
- The aromatic region matched the pattern of the imine, but the benzophenone rings in particular showed strong downfield shifts. Also, peaks assumed to be H5'' and H6'' of the A-ring were separated.
- The broad peak at 4.5 - 5.0 ppm characteristic of CH₂N⁺ in the phenanthridinium salts was not observed.

These were considered consistent with TMS-iminium salt 289, although hardly conclusive evidence (Figure 42).

![Figure 42 - Possible TMS-iminium adduct 289.](image)

Following work up and short-path vacuum distillation of this reaction mixture in a kugelrohr apparatus, ^1H NMR spectroscopy showed peaks characteristic of N-alkyl phenanthridinium salts, including a broad singlet at 5 ppm. Attempting to cyclise pure imine 287 at 200 °C in nitrobenzene resulted in isolation of phenanthridine 290 (a small amount of which was isolated for characterisation purposes) and no phenanthridinium 288 (Scheme 97).

![Scheme 97 - High temperature cyclisation of imine 287.](image)

Cyclisation of imine 287 (Scheme 97) was next investigated by heating the solid to 100 °C in a kugelrohr apparatus for 1 h, causing the material to discoulour. Addition of CDCl₃ followed by removal by distillation and heating for a further hour gave complete conversion of starting material to a mixture containing no ^19F-^1H coupling in the ^1H NMR spectrum and no ^19F according
to $^{19}$F decoupled $^1$H NMR spectroscopy (Figure 43). These clearer spectra resolved a peak corresponding to CH$_2$N at 3.3 ppm as a 2H t. This was inconsistent with a phenanthridinium 288, but consistent with amine 292, which would result from hydroxide attack to give a pseudobase 291 (see Section 4.3) and opening. COSY analysis shows H6’’ to be considerably downfield of H3’’ and H5’’ (Figure 43), which is as expected for this position, meta to both donating groups and conjugated to a stronger withdrawing group than before in the form of the ketone. Based on this evidence, these peaks were assigned to the hydrolysis product 292.

Imine 287

Amine 292

Phenanthridinium 288

Figure 43 - heating imine (top) results in a mixture containing predominantly amine 292 (middle), which then cyclises to the N-alkylphenanthridinium salt 288 on addition of TFA.

Scheme 98 - The cyclisation of imine 287 and subsequent products. Protons are colour coded to the $^1$H NMR spectra shown above (Figure 43).

As loss of fluorine had been observed, the eventual fate of the fluoride anion was of interest. It was considered that formation of fluoro silicates in the glass may drive the hydrolysis reaction, providing water and destabilising the salt. To test this theory the kugelrohr cyclisation experiment was repeated twice in the presence of chromatography grade SiO$_2$. On both occasions the imine 287 reacted somewhat more slowly, as expected in the presence of an acidic additive. Trace
quantities of phenanthridinium 288 were formed alongside a greater range of side products. Comparison of the results with and without SiO₂ (Figure 44) indicated that omission of SiO₂ was preferable.

![Comparison of cyclisation for 1 h from CHCl₃, with and without SiO₂. Green boxes indicate phenanthridinium peaks, red is the open form and blue the SM.](image)

**Figure 44** - Comparison of cyclisation for 1 h from CHCl₃, with and without SiO₂. Green boxes indicate phenanthridinium peaks, red is the open form and blue the SM.

It was expected that acidic conditions would push the equilibrium to favour phenanthridinium 288 over pseudobase 291 and ketone 292 by favouring dehydration and limiting availability of hydroxide or neutral water for hydrolysis to the amine 292. Electron-rich phenanthridiniums would show greater hydrolytic stability as the electrophilicity of the iminium would decrease so it was decided to treat with acid after the cyclization and reduce the nitro group under acidic conditions.
7.6 Scale up and completion of the probe

The Suzuki coupling of trifluoroborate salt 266 and iodobenzophenone 218 was repeated on a large scale and benzophenone 265 isolated in a 95% crude yield (Scheme 99). This unpurified material was used to prepare imine 287, which was not purified following work-up, but was heated in a kugelrohr at 100 °C for 1 h. The resulting mixture was treated with TFA to ensure conversion to the phenanthridinium salt 288, and immediately hydrogenated over Pd/C using TFA as solvent. The mixture was neutralised and dried with K₂CO₃. An ion exchange procedure converted the material to mesylate. This gave the target aminophenanthridinium salt 293 in 47% yield from benzophenone 265 and a 45% overall yield from iodobenzophenone 218.

Scheme 99 - The final synthesis of the 'Bu superoxide probe model 293.

Phenanthridinium 293 was a precursor to a potential untargeted superoxide sensor 294, so reduction to the latter was tested and reacted rapidly with NaBH₄. Re-oxidation proved very rapid and so isolation of the reduced form required development of a specific protocol (Scheme 100).
Scheme 100 - Reduction of superoxide probe precursor 293 to superoxide probe 294.

The oxidised form 293 was added to a degassed biphasic system of H₂O and Et₂O through which argon is constantly bubbled. Excess NaBH₄ was added and the bright orange colour of the aqueous phase was lost, then the system sealed and shaken for 10 min. Without opening the vessel, the Et₂O layer is carefully syringed out into another vessel sealed and under argon, then additional degassed Et₂O used to repeat the process. A high flow of argon was then used to evaporate the Et₂O. When taken up and in degassed CDCl₃ ¹H NMR spectroscopy showed complete reduction, although rapid re-oxidation subsequently occurred. Since the dihydrophenanthridine 294 could be prepared from phenanthridinium 293, the phenanthridinium 294 was submitted for testing by the group of Dr Michael P. Murphy at the MRC, Cambridge. The results of these tests will be discussed in Chapter 9. Since we had now developed a new route to N-alkylphenanthridiniums, we decided to exemplify this methodology, and this will be discussed in the next chapter.
Chapter 8: Methodology exemplification

8.1 Why exemplify this methodology?

8.1.1 The uses of \( N \)-alkylphenanthridinium salts

\( N \)-Alkylphenanthridinium heterocycles have a considerable multitude of uses aside from their potential as superoxide probes, such as DNA intercalating dyes (such as ethidium bromide) and trypanocidal drugs \(^{311} \) e.g. Samorin. The combination of appropriate redox properties and intercalation allows phenanthridinium salts to be used in the study of the redox and electron transport properties of DNA.\( ^{176,139} \) Phenanthridinium salts function as allosteric inhibitors of acetylcholine esterase.\( ^{312} \) Related alkaloids display potential anti-tumour properties. These are reliant on the planar, cationic nature of the heterocycles,\( ^{313} \) and can occur through Topoisomerase inhibition.\( ^{314,315} \) They have also been shown to exhibit antibiotic properties.\( ^{316,317} \)

8.1.2 Methodology scope and novelty

In addition to the previously mentioned features, the route described in Chapter 7 also has considerable chemical novelty - \( S_{N}Ar \) reactions utilising an \( N \)-alkyl imine nucleophile are extremely rare, but a few examples do exist.\( ^{318,319} \) The same reaction with an acyclic \( N \)-alkyl imine nucleophile, however, is almost unprecedented - only one literature example was found.\( ^{320} \)

Despite their many applications, extant synthetic routes to phenanthridinium salts are limited, and rely on \( N \)-alkylation almost without exception (see Sections 4.1-4.3). A synthetic route to structural analogues without this often messy and slow transformation would allow the properties of phenanthridinium salts to be investigated and tuned more easily to meet the range of applications mentioned, and is therefore of considerable practical value.

Following optimisation the imine cyclisation in Chapter 7 gave near quantitative conversion to the \( N \)-alkylphenanthridinium salt \( 288 \). The efficiency of this transformation was gratifying, and further work was undertaken to illustrate the electronic and steric tolerance of the cyclisation with a view towards providing a practical, general and convergent route to these important substrates. Scheme 101 outlines how our strategy allows access to a phenanthridinium salt \( 295 \) in just three steps from an arylboron \( 296 \), alkylamine \( 297 \) and benzophenone \( 298 \). Many analogues of each of these components are available commercially, and far more are identified in the literature. Furthermore, the chemistry used to access the precursor imines (Suzuki coupling, imine formation) is well understood, well precedented and generally robust.

Scheme 101 - A generalised approach to the synthesis of 5,6-disubstituted \( N \)-alkylphenanthridinium salts.
8.2 Target selection for methodology exemplification

Subsequently targets were selected to exemplify the reaction and explicate its utility (Figure 45), focusing particularly on showing variation in electronics, steric and substitution pattern while starting from a small palette of available or readily synthesised compounds.

![Selected exemplification targets](image)

**Figure 45** - Selected exemplification targets

The 6 points of variation in the core structure give a diversity of objective compounds commensurate to the scope and utility to be demonstrated; compounds 299, 302, 303, 304, 305 and 307 to probe the electronics of the system, 308 and 309 the steric tolerance, 306 and 311 the importance of the phenyl substituent. The remaining targets show either diversification routes (300 and 310) or in the case of 301 access to a novel structure which would require a convoluted synthetic route if constructed via alkylation protocols.
8.3 Precursor synthesis
8.3.1 Synthesis of precursor arylboron 296 components

Maximising the use of commercially available components such as the range of 2-fluorophenylboronic acids and previously synthesised components such as iodobenzophenone 218 was also considered during target selection. As such, the 2-haloaryl boronic acids used to couple with the precursor benzo and acetophenones 298 were mostly purchased from commercial sources (Figure 46).

![Figure 46 - The arylmetal components. With the exception of 266 and 315, these are commercially available.](image)

As potassium trifluoroborate salt 266 had already been synthesised, only pinacolborate 315 required preparation. Grignard formation with PhMgBr then borylation was attempted according to a literature procedure but this gave a mixture of products (Scheme 102).

![Scheme 102 - Attempted synthesis of arylboronate 315 by Grignard formation and quenching with a boronate electrophile.](image)

Instead pinacolboronate 315 was synthesised via Miyaura borylation (Scheme 103), using a modified literature procedure. 0.15 eq. of NEt₃ is added to activate the catalyst by β-hydride elimination. Quantitative conversion was observed; however repeated chromatography proved
necessary to remove the boronate byproducts. Recrystallisation of the pinacolboronate ester gave clean material in a lower yield.

The potassium trifluoroborate salt 320 was also synthesised, by subjecting the crude reaction mixture from Miyuara borylation to KHF$_2$(aq) and recrystallising from acetone (Scheme 104). Unfortunately this gave the salt 320 in low yield.

![Scheme 104 - Palladium catalysed borylation giving the potassium trifluoroborate salt 320.](image)

8.3.2 Synthesis of precursor arylhalide component

For the most part 2-iodo-4-nitrobenzophenone 218 was used as the aryl halide component, supplemented by 2-bromoacetophenone 321, and 2-iodobenzophenone 322, and 2-bromo-4-nitroacetophenone 323 (Figure 47).

![Figure 47 - Selected benzophenone precursors.](image)

The synthesis of 2-iodo-4-nitrobenzophenone 218 has been described already (see Chapter 5) and ketones 322 and 323 were purchased. 2-Bromonitroacetophenone 321 was synthesised by nitration of 2-bromoacetophenone 323. Three sets of conditions were tested: KNO$_3$/H$_2$SO$_4$(aq), Ac$_2$O/HNO$_3$(aq) and AcOH/HNO$_3$(aq). Acetophenone 323 proved too deactivated for the milder conditions so the H$_2$SO$_4$(aq)/KNO$_3$ conditions were scaled up, and stopped at around 85-90% completion due to side product formation. Chromatography gave the desired compound in moderate yield (Scheme 105).

![Scheme 105 - Synthesis of 4-nitroacetophenone 321.](image)
8.3.3 Suzuki cross coupling to give biaryl precursors

A range of biaryls 324 - 333 (Figure 48) were then prepared by Suzuki coupling (Scheme 106 and Table 2), with conditions used initially based on those previously used for benzophenone 265 (Section 7.4). Although the reagents are the same the conditions used varied (Table 2). These reactions can be divided into 2 categories: microwave and non-microwave reactions. The former was developed as a response to difficulties in getting particularly electron-poor arylboronates such as nitroarene 315 to cross couple, where higher temperatures are necessary. Transmetallation is usually the rate limiting step in the case of the NHC catalysts used, and electron poor arylboronates undergo this reaction less readily. The microwave is a fast and efficient way of achieving higher temperatures, and reaction was further aided by higher concentrations. More specific microwave effects have previously been claimed, but a recent review suggests there is no evidence of this.323

![Chemical structures of biaryls 324 - 333](image)

Figure 48 - Cross coupling aryl halide and transmetallation partners.
Scheme 106 - Generalised Suzuki coupling outline.

Table 2 - Summary of Suzuki reaction conditions and product yields.

<table>
<thead>
<tr>
<th>Target</th>
<th>[ArX]</th>
<th>t (h)</th>
<th>ArBX, eq.</th>
<th>cat.</th>
<th>T (˚C)</th>
<th>µwave</th>
<th>Purification</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>265</td>
<td>0.12</td>
<td>4</td>
<td>1.12</td>
<td>6%</td>
<td>78</td>
<td>N</td>
<td>Chromatography</td>
<td>78%</td>
</tr>
<tr>
<td>324</td>
<td>0.41</td>
<td>0.17</td>
<td>1.03</td>
<td>5%</td>
<td>125</td>
<td>Y</td>
<td>Chromatography</td>
<td>40%</td>
</tr>
<tr>
<td>325</td>
<td>0.28</td>
<td>1</td>
<td>1.00</td>
<td>5%</td>
<td>125</td>
<td>Y</td>
<td>Chromatography</td>
<td>53%</td>
</tr>
<tr>
<td>326</td>
<td>0.10</td>
<td>22</td>
<td>1.11</td>
<td>3%</td>
<td>78</td>
<td>N</td>
<td>Chromatography</td>
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The conditions described for 4-buty1 benzophenone 265 were used as a starting point. The 6% catalyst loading appeared to give near quantitative conversion, but chromatography entailed substantial loses. Purification difficulties were observed with several substrates.

Nitroacetophenone 321 reacted readily under microwave conditions, giving a 3 - 4:1 ratio of the 3,8-Dinitroacetophenone 324 to an acetophenone derived impurity. Purification proved difficult, giving a moderate yield. Coupling to give heterocyclic benzophenone derivative 325 was attempted under thermal conditions, but only unreacted benzophenone 218 was recovered in large quantities. As the pyridylboronic acid 312 has a reported melting point of 172 ˚C and no aqueous wash was utilised in work up it was assumed that deboronation had destroyed the transmetalating component as pyridyl boronic acids are prone to decomposition. It is also possible that the pyridyl 312 competes with the weakly binding 3-chloropyridine ligand. Raising the reaction temperature with the microwave showed the Suzuki coupling to be faster than decomposition at 125 ˚C, allowing pyridylbenzophenone 325 to be isolated, although chromatography proved challenging.

The coupling of bromoarylboronic acid 313 with 2-ido-5-nitrobenzophenone 218 to give bromobenzophenone 326 posed a challenge; although electron poor 2-ido-5-nitrobenzophenone 218 was likely to undergo oxidative addition more rapidly than the bromide, the bromoarylbenzophenone 326 is also a good Suzuki coupling substrate and is present in higher concentrations as the reaction nears completion. Originally an excess of the iodobenzophenone 218 was used, but it was established that this starting material was chromatographically...
inseparable from the bromobenzophenone 326. Some success was achieved by sonicating the chromatographed mixture in an Et₂O/Pet. ether mixture in which the product 326 was sparingly soluble, resulting in precipitation of the product as a fine white solid. Multiple failures to repeat this procedure with a later batch of material suggested that the conditions required are very precise and difficult to achieve reproducibly. Instead it proved necessary to use an excess of arylboronic acid 313 to consume fully the 2-ido-5-nitrobenzophenone 218. This generated another impurity, identified as benzophenone dehalogenation product 228 (Scheme 70), which could be removed by chromatography.

Coupling to give unsubstituted 2-arylbenzophenone 327 worked under thermal (76%) conditions. The equivalent acetophenone 329 required harsher conditions, producing a good yield only after extensive microwave heating.

Since arylboronate 315 was synthesised by the Miyaura reaction, an attempt was made to combine Miyaura and Suzuki reactions to synthesise dinitrobenzophenone 328 by changing the Miyaura catalyst to PEPPSI-iPr and then adding the Suzuki reagents and a small amount of water after 16 h, but a complicated mixture containing little or no product resulted. Suzuki coupling of isolated arylboronate 315 with iodobenzophenone 218, under thermal conditions proved sluggish. Microwave irradiation of a 0.1 M solution of each component to 120 °C for 10 mins gave rapid conversion and mostly the dinitrobenzophenone 328. Dropping to 100 °C for 30 min reduced side product generation. Adjusting the conditions to 100°C/30 min at 0.2 M concentration of iodobenzophenone 218 gave higher conversion and a yield of 99% after chromatography.

Synthesis of aryl chloride 330 was problematic. The PEPPSI-iPr catalyst can affect oxidative addition on C - Cl bonds, and the electronics of the product 330 favour this and disfavour transmetallation from the arylboronate 316. Standard thermal conditions proved ineffective while microwave heating to 125 °C for 1 h with 1.1 eq. 2-chloro-5-nitrophenylboronic acid 316 gave side products in addition to chlorobenzophenone 330. Reducing the number of equivalents of chloroboronic acid 316 to 0.9 and reaction time to 10 min gave little purity change. A ratio of 3:1 for product to homocoupled benzophenone 229 resulted (Scheme 107). This suggests transmetallation is sufficiently slow for competitive Pd(II) reduction. Increasing the number of equivalents substantially to counteract this would risk further cross coupling of the chlorobenzophenone 330 with arylboronic acid 316, so a moderate yield of 56% was accepted.

Scheme 107 - Products of chloroarylboronic acid 316 and iodobenzophenone 218 cross coupling.
Synthesis of 2-arylbenzophenone 331 from iodobenzophenone 218 and 2-fluorophenylboronic acid 314 proved easier. Thermal conditions gave near quantitative conversion to mononitrobenzophenone 331 on a small scale. An unexpected shortage of 2-fluorophenylboronic acid 314 meant only 0.9 equivalents could be used, and subsequent Suzuki coupling resulted in a mixture of compounds. Separation proved challenging, with chromatography followed by precipitation as described for bromobenzophenone 326 giving a moderate yield of mononitrobenzophenone 331. Both methylbenzophenone 332 and methoxybenzophenone 333 were synthesised by Suzuki coupling in a near quantitative fashion thermally, being recrystallised from CHCl₃ to remove inorganic impurities prior to the methodology steps.

8.3.4 Nitration of 2-(5''-methoxy-2''-fluorophenyl)-5-nitrobenzophenone 333

To investigate the interplay of electron donating and withdrawing groups and to produce a precursor to the 2-hydroxyphenanthridinium MitoSOX standard desired for the superoxide probe project (Chapter 10) the dinitro compound 334 above was among the target precursors for the phenanthridiniums. Rather than modify the Suzuki precursor it was felt that the electronics of the ring systems might be distinct enough to allow regioselective nitration (Scheme 109). Although the major features of electrophilic aromatic substitutions and nitration in particular are understood, some details are recently discovered or disputed. Preassociation of the nitronium ion and the arene substrate occurs, through an ionic (π) complex or via SET to give two radicals, depending on the nature of the reactants. Either then give the σ-complex which precedes product. The ease of rearranging these associated states explains the observed high regioselectivity but low substrate selectivity. Various nitration conditions were attempted.

Initial attempts to nitrate methoxyarene 333 with AcOH/HNO₃(aq) proved this system to be insufficiently reactive, with no reaction observed after 1 h. Nitration in a biphasic mixture using H₂SO₄(aq)/KNO₃ resulted in spontaneous decomposition to an insoluble black residue, believed to be composed of sulfonylation products.

The nitronium salt NO₂BF₄ is a common, powerful nitrating agent. This was tested on the rationale that addition of 1 equivalent at −42 °C in MeCN followed by slow warming would result in preferential reaction at the more electron rich site and give dinitro compound 334. Practical and theoretical problems became apparent - NO₂BF₄ is sufficiently hygroscopic to be extremely difficult to weigh on a sub 50 mg scale, and sparingly soluble. The reaction with methoxyarene 333 is rapid and exothermic, so the degree of local temperature control achieved with an external dry ice bath is debatable. The handling properties of NO₂BF₄ made using the correct equivalence challenging, and excesses resulted in further nitration and isolation of trinitro arene 335 showed it to have been formed in a 2:1 ratio (¹H NMR) to the dinitrobenzophenone target 334, although the absence of methoxybenzophenone 333 from the mixture was considered encouraging. The regiochemistry of trinitrobenzophenone 335 was confirmed through analysis of C-F coupling in the ¹³C NMR spectra; in this case the C(H)-F coupling on the penta-substituted aryl ring was 28 ppm, too large to be any position but adjacent to F. When addition in MeCN solution was
attempted fewer equivalents of the reagent were added due to the low solubility of NO$_2$BF$_4$; a mixture of methoxybenzophenone 333 and dinitrobenzophenone 334 resulted (Scheme 108).

\[ \text{Scheme 108 - Trinitroaryl 335 was observed in a 2:1 ratio to desired dinitroaryl 334 when NO}_2\text{BF}_4 \text{ was used as the nitrating agent.} \]

These practical difficulties motivated the use of an Ac$_2$O/HNO$_3$(aq) system. Ac$_2$O/HNO$_3$(aq) is known to be a more powerful nitrating combination than AcOH/HNO$_3$(aq). Inclusion of DCM aided the solubility of methoxyarene 333 in acetic anhydride prior to HNO$_3$(aq) and optimisation of the reaction time to 2 h at 0 °C allowed isolation of the dinitrobenzophenone 334 in 72% yield (Scheme 109).

\[ \text{Scheme 109 - Selective nitration of lower benzophenone ring.} \]
8.3.5 Alternate alkylation groups

Having assembled a range of 2-arylbenzophenones for imine formation a number of amines and amine precursors were selected as condensation partners (Figure 49).

![Figure 49](image)

**Figure 49** - Alternative alkyl groups investigated. Compound 338 was provided by Stuart Caldwell.

Compounds 336, 337 and 344 are commercially available. To produce the final superoxide probe target 261 either the $^3$PPh$_3$ or a surrogate should be substituted on the end of the alkyl chain. Unsurprisingly, therefore 4-aminobutylphosphonium iodide 339 was the first substrate investigated. Nucleophilic substitution using NH$_3$(aq) in MeCN was performed on the iodobutylphosphonium iodide 24 synthesised previously (Section 2.5, Scheme 6). This gave a mixture, with a colleague having a similar result using condensed NH$_3$(aq). Instead using sodium azide as the nucleophile 4-azidophosphonium salt 345 was prepared quantitatively. Reduction of this alkyl azide proved difficult, with Staudinger reduction using PPh$_3$ in DCM appearing to result in several products. Investigation showed iminophosphorane in the $^1$H NMR spectrum alongside side products when hydrolysis was omitted. When reduction with LiAlH$_4$ in THF was tested azidoalkylphosphonium salt 345 was destroyed. Hydrogenation with Pd/C/H$_2$(g) showed amine 339 in the $^1$H NMR spectrum, but the initial choice of AcOH prevented precipitation and complicated isolation. Switching to MeOH and using a high catalyst loading (0.24 eq. of 10% Pd/C) with the azidobutylphosphonium salt 345 gave near quantitative conversion to the aminobutylphosphonium salt 339 (Scheme 110).

![Scheme 110](image)

**Scheme 110** - Synthesis of aminobutylphosphonium salt 339.

6-Azidohexylphosphonium iodide 340 was synthesised through the same nucleophilic substitution as used to synthesise phosphonium salt 345 from the iodoalkyl precursor 348 acquired from Stephen McQuaker (Scheme 52). The simple hexylazide 343 and 1,6-diazidohexane 349 were similarly prepared from the alkyl bromides 346 and 347.

![Scheme 111](image)

**Scheme 111** - Synthesis of azidoalkyl compounds 340, 343 and 349. 6-Azidohexylphosphonium iodide 348 was provided by Stephen McQuaker.
The 1,6-azidohexane 349 was reduced (Scheme 53) with the two-phase Staudinger procedure which allows monoreduction as previously discussed in Section 11.4.3, Scheme 142.

Scheme 112 - Monoreduction of 1,6-diazohexane 349.

Difficulties were encountered in the synthesis of alkyne 341, where use of hydrazine produced alkene side products. An alternative procedure use AcOH and NaBH₄ was successful, but purification by ion exchange gave a poor yield (Scheme 113).

Scheme 113 - Deprotection to yield alkyne 341 proved low yielding.

With a range of simple amines in hand, imine formation was investigated.

8.3.6 Imine formation

Imine 287 had been prepared from benzphenone 265 using TiCl₄ in DCM or PhMe. TiCl₄ is a powerful dehydrating agent due to the oxophilicity of the Ti⁴⁺ ion and the favourability of TiO₂ formation. The high Lewis acidity of TiCl₄ allows it to coordinate to carbonyls activating these to nucleophilic attack. It has been suggested the active species in imine formation is an alkylamino complex of titanium (IV), certainly a colour change is observed on combination with hexylamine in PhMe or DCM.

A series of N-hexylimine derivatives (Scheme 114, Figure 50) were synthesised under conditions similar to those used for the original benzphenone imine 287. Use of PhMe was convenient as 1 M TiCl₄ in PhMe is bright orange, so the quality of the reagent is easily assessed. The excess of amine, which was usually around 7 eq., was reduced where the amine required synthesis. Between 1.5 and 2.0 eq. TiCl₄ was used. The variation in TiCl₄ and concentration were decided pragmatically: where problems with stirring were observed PhMe was added, incomplete conversion after approx. 24 h was taken to mean more TiCl₄ was required. The original substrate 265 gave several products in the imine formation procedure, which required chromatography to separate. However, the unpurified mixture reacted to produce the desired phenanthridinium products cleanly, so the imines were not purified following work up. More electron rich ketones gave cleaner conversion to the imine, while electron poor ketones cyclised rapidly following imine formation to produce a mixture. As the overlapping signals in the ¹H NMR spectrum often obsfuscated important aromatic peaks, reaction completion was confirmed by loss of starting
material peaks, including movement of H4/H6 (Section 7.5, Figure 41). The diastereotopic CH\textsubscript{2}-1 peaks at 3.0 - 3.5 ppm were also considered a useful indicator.

Scheme 114 - Imine formation outline.

Figure 50 - The imine precursors targets.

The work up procedure developed with experience, as the quantity of TiO\textsubscript{2} produced in quenching, the residual hexylamine and the vulnerability of the product to hydrolysis made identification of the correct procedure challenging.

The conversion of acetophenone 329 to imine 363 did not proceed well and there were large quantities of SM present after work up, but it is not known if this represents incomplete conversion or facile hydrolysis.

Overall the imines 353 - 362 formed from hexylamine could be prepared efficiently using TiCl\textsubscript{4}, but the TiCl\textsubscript{4} solutions have short shelf lives, and the moisture sensitivity makes a usually routine
transformation the most technically demanding of the route. Although effective in the previous synthesis, TiCl₄ has limited functional group tolerance, so the range of amines that could be used was explored briefly. Bulky cyclohexylmethylamine reacted with benzophenone 265 to give imine 364 (Scheme 114). Similarly, the azido functionality in amine 342 was tolerated and the imine 365 formed easily (Scheme 115). On the other hand imine formation with amines 336 - 341, 343 and 344 failed when TiCl₄ was used as the Lewis acid. A range of other conditions including MgSO₄, PhMe/TsOH, BF₃.OEt₂, Ti(OiPr)₄ and CH(OEt)₃ were then investigated. However, all were unsuccessful.

Attempts were also then made to form the imine with an aza-Wittig reaction using alkyl azides 340 and 343 with PPh₃ or PBu₃ and benzophenone 265 (Scheme 117). Following a trial with benzophenone 265, azide 343 and PPh₃ in THF in the microwave at 100 °C, the iminophosphorane product of the Staudinger reaction was identified by a signal at 3.08 ppm, which was a dt (J = 18.3, 7.4 Hz) and is consistent with literature values for the methylene unit of Ph₃P=NCH₂. This was observed in the presence of benzophenone 265, showing the iminophosphoranes generated to be insufficiently reactive with the ketone to produce imine 287.

Chapter 8: Methodology exemplification
8.4 Cyclisation
8.4.1 Solvent free thermal cyclisation

With the crude imines 287 and 353 - 365 in hand, the limits of the cyclisation procedure were established. The procedure developed for the preparation of the superoxide probe precursor 288 involved heating under an inert atmosphere in a kugelrohr apparatus at atmospheric pressure. At the beginning of the reaction the imine is loaded as a solution in chloroform, which quickly evaporates leaving a layer of imine on the hot glass surface.

The utility of these conditions were tested using the substrates believed to be most electronically and sterically demanding, methoxybenzophenone imine 361 and 3’’-methylbenzophenone imine 362, respectively. The conjugation of methoxy group in imine 361 disfavs reaction para to this group. This is demonstrated by considering the contribution of minor resonance form 366, which displays the increase in electron density at C2’’ in exaggerated fashion (Scheme 118). Unsurprisingly the reaction is slower than with the benzophenone imine 287. However, forcing conditions (2 h 45 min at 100 °C, then 3 h at 125 °C) were successful and gave the phenanthridinium 304 in a 3:1 ratio over an impurity. Chromatography using 2% MeOH, 1% NEt₃ in DCM gave the N-hexylphenanthridinium 304 in a yield of 47% over two steps from the benzophenone 366. The ¹H NMR spectrum of the impurity displayed a pattern for the aromatic protons that is characteristic of a phenanthridinium salt and so it was assumed to be elimination product 367.

Scheme 118 - The cyclisation of 5’’-methoxybenzophenone imine 361, with the contributing resonance form 366, and suspected elimination product phenanthridinium 367.

When imine formation was tested with sterically challenging 3’’-methylbenzophenone 332 two products resulted, with the expected benzophenone imine 362 favoured 3:1 over a related fluorinated compound which did not show inequivalent protons at CH₂N. Initial heating (100 °C, 1 h) in the kugelrohr increased the ratio of imine 362 to the unidentified side product of imine formation, but did not give significant conversion to the N-alkylphenanthridinium salt. When the reaction temperature was increased to 150 °C for 2 h 30 min, cyclisation and elimination of the N-alkyl side chain occurred together to give phenanthridine 368, and this elimination was driven to completion by heating for 3 h at 200 °C (Scheme 119). A small quantity was purified for characterisation purposes.
Scheme 119 - The steric hindrance to cyclisation of 3”'-methylbenzophenone imine 362, as shown by the red arcs.

8.4.2 Microwave-assisted cyclisations to give phenanthridinium salts in aprotic solvents

It was clear that the method of cyclisation was not appropriate for imine 362 and had to be improved. Furthermore, solid material heats unevenly and chars, and this would be problematic for many of the the imines 353-365 as they would not be liquid at 100 °C given that most of the benzophenones melted at a higher temperature than this. It was also thought that other researchers would be more likely to make use of a more conventional procedure.

Consequently, microwave heating in a range of solvents was tested. 1 mg/mL solutions of imine 287 were heated in sealed tubes at 100 °C for 1 h using a 50 W microwave. No protic solvents were investigated because most of these are moderately nucleophilic and may compete with the desired reaction. The reactions in acetone and in toluene gave side products. Reactions in dichloromethane and THF did not go to completion, but the THF mixture contained only starting imine and product. The reaction in MeCN gave complete conversion, but had traces of side product, so the procedure was optimised to 2 h in THF at 100 °C. It is not surprising that reaction in the most polar solvent gave fastest conversion since the conjectured transition state (SNAr) is more polar than the ground state.
8.4.3 Optimisation of individual cyclisations and results

With the start point for optimisation chosen, 20 mg portions of benzophenone imines were weighed by difference (due to the oily or foam-like nature of the imines) into 1 mL solvent and sonicated into solution or suspension. Small variations in molar concentration are unimportant considering the high dilution and intramolecular reaction. Following microwave heating the solvent was removed under vacuum, then a sample taken up in deuterated solvent and approx. 0.05 mL d-TFA added. Conditions were assessed based on conversion as observed by $^1$H NMR spectroscopy, with final conditions then applied to the conversion of 100 mg in 5 mL of solvent.

The work up procedure used for the scaled up microwave conditions involved removing the reaction solvent under reduced pressure and re-dissolving the residue in dry Et$_2$O, then precipitating the phenanthridinium salt with excess ethereal HCl. Difficulties achieving reasonable spectra were common features of the phenanthridinium work, due to broad aromatic peaks attributed to aggregation effects such as π stacking, so the solvent used for the definitive spectra vary with the substrate. The conditions for the final cyclisations and the combined yields over imine formation and cyclisation steps are summarised in Table 3.

![Scheme 120 - Imine formation/cyclisation overview.](image)

**Table 3 - Imine formation/cyclisation combined yield - conditions refer to cyclisation.**

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<th>R$_4$</th>
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* Original kugelrohr conditions.

# Cyclisation complete on work up of imine formation.

† Compound isolated as enamine.
Upon scale up of the test cyclisation in THF at 100 °C for 2 h imine 287 gave good conversion to phenanthridinium 288. Cyclisations to electronically similar 8-nitrophenanthridiniums 299 and 300 gave good yields under these conditions, with the variation in the yield dictated mostly by the imine formation step.

The 2"'-fluoro-3"'-azabenzophenone imine 353 gave rapid reaction; most of the material cyclised during work up, with 30 min of microwave heating ensuring complete conversion. Ascertaining the nature of this compound posed a quandary; isolation of the double chloride salt was envisaged on addition of HCl. No sign of this species was observed by mass spectrometry suggesting that the final isolated form is the single salt. Chlorobenzophenone imine 358 rapidly cyclised to give phenanthridinium 302, despite the poorer chloride leaving group. This salt proved hygroscopic and required additional drying, resulting in a small loss in yield.

The cyclisation to 2-methoxy-3-nitro phenanthridinium 303 proceeded in good yield under the THF/100 °C conditions. This is consistent with the summation of the Hammett parameters. Even though the methoxy group is electron-donating and directly conjugated to the site of nucleophilic attack, the inductive withdrawing effect of the meta NO₂ group dominates. No impurities were observed so the lower yield is probably the result of losses on handling and drying. In the absence of the electron withdrawing group, the electron-donation of the methoxy dominates and the reaction is demanding. An attempt was also made to replace the kugelrohr method for synthesis of C2-OMe substrate 304, but similar conversion was achieved under microwave heating.

Both the dinitroacetophenone 324 and analogous 3,8-dinitrobenzophenone 328 gave quantitative conversion to product without an explicit cyclisation step; for dinitroacetophenone 324 the neutral product enamine 369 is easier to isolate, although the expected phenanthridinium 306 is formed in acid (Scheme 121).

![Diagram of Scheme 121](image)

Scheme 121 - Enamine 369 is isolated from imine formation, but reversibly forms phenanthridinium 306 on acidification.

Cyclisation of imine 356 derived from benzophenone 327 to phenanthridinium 307 with unsubstituted A and C rings demonstrated the unsuitability of using THF at 100 °C for less activated substrates. Longer reaction times in this solvent resulted in decomposition. Testing at an increased temperature of 110 °C in THF and MeCN revealed the MeCN to be superior for slower cyclisations. High conversion was observed, with a 97% yield over the cyclisation step.
Following the earlier failure to form 4-methylphenanthridinium 308 by heating imine 362 formed from benzophenone 332 under the solvent-less conditions, this substrate was re-investigated under microwave conditions. No product was observed at 100 °C in THF, but increasing the temperature to 125 °C did give sluggish conversion and decomposition. Using MeCN as the solvent at 110 °C provided a breakthrough, converting the majority of the imine 362 into 4-methylphenanthridinium 308 in 1 h. Increasing the temperature to 120 °C produced impurities, and longer reaction times at 110 °C gave a mixture of the starting imine 362, 4-methylphenanthridinium 308 and phenanthridine 368. Driving the reaction to completion (14 h, MeCN, 110 °C) proved detrimental; more of phenanthridine 368 was observed (see Scheme 119, Section 8.4.1). Iterative testing showed that 10 h at 110 °C in MeCN gave the best ratio of product to starting imine 362 and phenanthridine 308. Purification on an ion exchange column gave the 4-methylphenanthridinium 308 in a high yield.

By contrast the other sterically challenging substrate N-cyclohexylmethyl imine 364 cyclised with relative alacrity in MeCN at 110 °C. The alternative of using THF at 100 °C was sluggish compared to the N-hexyl derivative 287, and significant impurities were generated. Loading the crude mixture from the MeCN reaction onto SiO₂ and subsequent washing with DCM allowed impurities to be removed while the acidic silica prevented hydrolysis of the phenanthridinium salt 309. Addition of 0.5% NEt₃ to DCM then allowed rapid elution of the open form, which was cyclised with HCl to give the phenanthridinium chloride in good yield. Azido-phenanthridinium 310 required similar chromatographic purification due to decomposition of the imine 365 derived from the benzophenone 327 (perhaps via a process analogous to the Schmidt rearrangement). The incorporation of an azido group and would allow functionalization via the Huisgen cycloaddition.
8.5 Further modifications of phenanthridinium products

The synthesis of 3-bromophenanthridinium 300 offered the possibility of synthesising a DNA targeting H₂O₂ probe 370 based on the group’s previous work. Conceptually the hydrolysis of a boronic ester 370 would give a small group in plane with the ring and capable of H bonding, mimicking the 3-NH₂ group important to intercalation. Reaction with H₂O₂ at DNA could be monitored by fluorescence (Scheme 122). The empty orbital of the boron may well quench the fluorescence of the phenanthridinium probe 371, but even if it did not formation of the phenol would give a considerable Stokes shift, allowing ratiometric measurement.

Scheme 122 - The hydrolysis of boronic ester 370 to potential DNA-localised hydrogen peroxide probe 371, and reaction with hydrogen peroxide to form 3-hydroxyphenanthridinium 372.

The bromophenanthridinium salt 300 was reduced using an Iron/acid procedure under mild conditions at 40 °C (Scheme 123), to give 8-aminophenanthridinium 373, a versatile precursor due to the aryl bromide functionality.

Scheme 123 - Iron reduction of the 3-bromophenanthridinium 300.

Trial Miyaura borylation reactions were unsuccessful; in dioxane solubility proved low. Reaction in DMSO gave difficulties in recovery of the material. Given time and thorough screening of conditions, testing of alternative procedures, and investigation of options such as protecting the aniline or borylation before cyclisation, it is anticipated that synthesis of hydrogen peroxide probe 370 should be achievable.

Small scale reduction of 3-azaphenanthridinium 301 gave a 60% yield of the 8-aminophenanthridinium 374 (Scheme 124), identified by ¹H NMR spectroscopy (Figure 51).
Scheme 124 - Small scale reduction of azaphenanthridinium 301.

Figure 51 - Comparison between pre and post reduction material.

The overall upfield shift is consistent with the nitro reduction, but the most characteristic change is the large upfield shift of H7 (red), which is particularly pronounced in 8-amino substrates, and is mirrored in the 13C spectrum. Time constraints did not allow this reaction to be repeated and further characterisation data to be acquired.

Ename 369 was reduced under similar conditions to the aza-phenanthridinium 301, then the mixture was acidified so that the phenanthridinium partitioned into the aqueous layer. Neutralising the aqueous layer, and then reducing the mixture in a two-phase system of water and CHCl3 using NaBH4 led to isolation of dihydrophenanthridine 375 in low yield (Scheme 125). In CDCl3 the reduced form proved stable for several days.
Scheme 125 - Enamine reduction. The product is isolated in the reduced form due to NaBH₄ in the work up.

In addition to modifying the products of the cyclisation, some more thought was given to the mechanism. In the original plan an $S_N$Ar mechanism had been assumed to operate, and some features of this mechanism are now described in greater detail.
8.6 Nucleophilic aromatic substitution mechanism

Nucleophilic aromatic substitution (S_{n}Ar) is a reasonably well understood process, with the accepted mechanism shown in **Figure 52**. The nucleophile 377 approaches the aromatic electrophile and attacks the δ⁺ carbon attached to the ‘X’ group, breaking the aromaticity of the ring system 376. This forms species 378, which is the first transition state. The formation of TS1 is usually (but not invariably)³³⁸ the rate determining step. A conjugated electron withdrawing group is generally considered necessary³³⁹ except perhaps in the presence of a very powerful nucleophile³⁴⁰ or perhaps with the aid of an alternate single electron transfer (SET) mechanism.³⁴³ This withdrawing group stabilises the formally anionic ring system, and the capacity of this group to stabilise charge will strongly affect TS1. The degree of polarisation of C-X and the capacity of the leaving group to stabilise the anion will also contribute, as does the nucleophilicity of the attacking group.³⁴¹ Intermediate 379, known as the Meisenheimer complex, is formed with a full bond to both nucleophile and leaving group. These have pyramidal geometry at C1 and in some cases can be isolated as stable salts.³⁴² A further (usually) lower energy barrier exists to TS2, 380, which represents the dissociation of the leaving group as an anion, allowing the complex to re-aromatise and give aromatic product 381.

**Figure 52** - Generic S_{n}Ar reaction coordinate diagram.

That nucleophilic attack as the rate limiting step is an inversion of the order normally encountered in both S_{n}1 and S_{n}2 mechanisms, and as such the leaving group characteristics which favour these (such as weak C-X bonding) do not apply. The greater polarisation and charge stabilisation of TS1 offered by fluorine means that it is favoured over Cl as a leaving group in S_{n}Ar (k_F/k_Cl may be as high as 1300), although it is probably poorer with regard to the actual leaving step TS2.³⁴³ Complicating this simplistic picture is the importance of solvent and hydrogen bonding, particularly with amine nucleophiles, with ortho-nitro groups increasing reactivity through
hydrogen bonding with the nucleophile in TS1.\textsuperscript{344} In some cases specific solvent hydrogen bonding effects may also be relevant.\textsuperscript{345}

The addition of hydrogen bonding and additional solvent molecules does produce a more complex picture of SN\textsubscript{Ar}, and these systems are less understood. One particular situation outlined in Figure 53 arises where amine nucleophiles are used in the presence of a weak base and formation of TS2 is rate-limiting. In this case general base catalysis is observed (Figure 53).\textsuperscript{345,346} Nucleophilic attack from the amine 382 on arene 376 generates an ammonium species 384. Both the ammonium and hydrogen bonding interaction with the departing nucleophile will stabilise 383 and 384, so deprotonation is required to increase electron density at the pyramidal carbon and proceed to the rate determining TS2. The involvement of multiple molecules of amine in a cyclic arrangement with the zwitterionic Meisenheimer complex is suggested by Isanbor and Babatunde\textsuperscript{346} with George and coworkers also implicating an additional molecule of ethanolamine in Meisenheimer complex decomposition in SN\textsubscript{Ar} reactions on fluoronitrobenzenes.\textsuperscript{347}

![Figure 53 - TS2 limited, base catalysed SN\textsubscript{Ar.}](image)

Both models shown (Figure 52 and Figure 53) feature a Meisenheimer complex, and theoretical studies consistently find this intermediate in a variety of conditions.\textsuperscript{341,345,347,348,349} A few exceptions exist, with a brief communication\textsuperscript{350} by Iwata and coworkers implicating a concerted process through a transition state equivalent to TS2 in Scheme 126 in the formation of the minor para regioisomer in reaction of ammonia with a polyfluorobenzene.

![Scheme 126 - Calculations by Iwata and co-workers suggest that formation of para isomer 389 is concerted.](image)
Goryunov et al. were able to take advantage of the favourable formation of Si-F bond in a leaving group to give concerted reaction between Me₃SiPMe₂ and polyfluoroarenes (Scheme 127).\(^\text{351}\)

![Scheme 127 - Typical example of the concerted SₙAr conditions given by Goryunov et al.\(^\text{351}\)](image)

Some calculations support the idea of concerted displacement in particular circumstances, for non-first row leaving groups,\(^\text{352}\) with the observation that Meisenheimer complexes formed from iodoarenes are unstable,\(^\text{348}\) and that gas phase calculations suggest heavier halides require multiple withdrawing groups to form Meisenheimer complexes.\(^\text{353}\) It should be noted that even relatively favourable SₙAr processes often require considerable heating; reaction of 2,4-dichloropyridine with aqueous ammonia requires heating to 150-175 °C. As pyridine provides a level of activation analogous to a nitro substituent,\(^\text{343}\) the theoretical reactivity of weakly activated aryl bromides and iodides is probably unimportant as reaction is unlikely to occur; at least when using aryl fluorides or favourable, activated substrates a Meisenheimer complex should be assumed.
8.7 The mechanism of imine cyclisation: a computational study

8.7.1 Mechanistic uncertainty

Originally envisaged as an $S_N$Ar, another mechanistic pathway is hypothetically possible for the cyclisation shown; 6π electrocyclisation.\(^{354}\)

Scheme 128 - Literature 6π electrocyclisation example.\(^{354}\)

Curly-arrow representations of both mechanisms result in resonance structures of the Meisenheimer complex (Figure 54), illustrating the similarity of the pathways. The fundamental difference is the orbitals involved; $S_N$Ar originating from the imine lone pair, electrocyclisation from the C=N π bond. This difference has repercussions for the transition state geometry but cannot be convincingly distinguished experimentally. Dr Hans Senn investigated the reaction computationally to ascertain the correct mechanism, and provided all of the data and much of the interpretation for the following section.

Figure 54 - Suggested $S_N$Ar (top) and electrocyclisation (bottom) mechanisms give rise to the same Meisenheimer complex-like structure 395 according to a curly arrow model.
8.7.2 The calculated pathway and energy values

The N-alkyl chain was modelled as an ethyl group to reduce the complexity of the calculations. Structures 397a-h for a number of structural analogues (Table 4) were calculated using DFT modelling, with possible intermediates identified. With the exception of c, d and h these analogues had substituents on the A and C rings that matched compounds synthesised. The pathway considered involved conversion of the imine 396 into Meisenheimer complex 398 via transition state 397. The Meisenheimer complex then collapses to a close ion pair 399, which can then dissociate to solvent separated ion pair 401 or recombine to give the adduct of 400 of counterion addition to the iminium ion. The last structure is analogous to the pseudobase discussed in Section 4.3, Scheme 35.

![Diagram](image)

**Scheme 129** - The species and pathway identified by DFT calculations.

**Table 4** - The range of substrates investigated in the computational study

<table>
<thead>
<tr>
<th>Substrate</th>
<th>X</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
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<tr>
<td>a</td>
<td>F</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>b</td>
<td>F</td>
<td>H</td>
<td>H</td>
<td>NO₂</td>
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<tr>
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<td>F</td>
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<tr>
<td>d</td>
<td>F</td>
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<td>F</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
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<tr>
<td>f</td>
<td>F</td>
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<td>NO₂</td>
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<tr>
<td>g</td>
<td>F</td>
<td>OMe</td>
<td>H</td>
<td>NO₂</td>
</tr>
<tr>
<td>h</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>NO₂</td>
</tr>
</tbody>
</table>
The pathway identified was shown to be an $S_N$Ar process (Scheme 129) based on transition state geometry, but with a twist. The Meisenheimer complex 398 is unstable and dissociates spontaneously into ion pair 399. Depending on the interplay of ion solvation enthalpy and C-X bond strength, the ion pair 399 can separate to form a solvent separated ion pair 401 (an entropic rather than enthalpically favoured process), or form an unusual C6-X adduct 400.

Table 5 - DFT calculation results. The N5-C4a and C4a-X distances refer to TS 397, and are numbered as would be normal for the product phenanthridinium salts 401.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta G$ / (kJ mol$^{-1}$)</th>
<th>$d^i$ / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>396</td>
<td>TS 397</td>
<td>399</td>
</tr>
<tr>
<td>a</td>
<td>103</td>
<td>−65</td>
</tr>
<tr>
<td>b</td>
<td>98</td>
<td>−57</td>
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<tr>
<td>c</td>
<td>93</td>
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<td>g</td>
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<td>−61</td>
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<tr>
<td>h</td>
<td>120</td>
<td>−126</td>
</tr>
<tr>
<td>a$_{vac}$ [c]</td>
<td>112</td>
<td>80[b]</td>
</tr>
</tbody>
</table>

[a] Calculated at M06-2X/def2-TZVP+ level in polarizable continuum MeCN ($\varepsilon_r = 22.5$) for $T = 383$ K, $p = 320$ kPa. [b] The 6-chlorophenanthridine 400g is not a stable minimum, but dissociates into the ion pair 399g during structure optimization. [c] Calculated in vacuum. [d] Energy of 398$_{vac}$. In vacuum, the ion pair 399$_{vac}$ is not stable, but collapses to 398$_{vac}$.

Although the mechanistic pathway is consistent over the substrates, the stability of the solvent separated ion pair 401 versus halide adduct 400, transition state energy and bond lengths vary (Table 5). For those substrates synthesised activation energies (90 - 112 kJmol$^{-1}$) correlate well to the reaction conditions. Figure 55 shows a reaction coordinate diagram based on the calculated values for f.
Figure 55 - A Reaction coordinate diagram for cyclisation of benzophenone imine \textbf{396f} to phenanthridinium salt \textbf{401f}, based on DFT calculations.
The calculated structures of 396-401 will now be considered in order and related to the experimental observations, and the task of distinguishing between the electrocyclisation and SNAr mechanisms.

8.7.3 The Calculated Conformations of Imines 396a-h

The calculated conformation of the imine 396a corresponds well with the $^1$H NMR data (Figure 56). The orientation of the phenyl ring face on to the C-ring is consistent with the upfield $^1$H NMR shift of H6 (8.1 ppm vs 8.3 ppm for H4). The electrophilic fluoroarene A ring is out of plane of the C ring by -54° in 396a, which limits conjugation between the rings and in more substituted analogues, reduces the effect that groups on one ring have on the other. Considerable steric congestion suggests a high barrier to rotation about the N-CH$_2$ bond, demonstrated by the inequivalent methylene protons. Comparing Hirschfeld charges for a and h show the C-Cl to be less polarised revealing a less δ$^+$ C2”. Thus, the more electronegative halogen atom lowers σ$^*$$_{C\chi}$ so that the aryl fluoride is more reactive.
8.7.4 The Calculated Structure of Transition State 397a

The TS structure 397a represents an ‘early’ transition state as it is closer in structure to 396a than product phenanthridinium 401a, with the electrophilic A ring still -37° out of plane of the C ring (Figure 57). A shorter N-C4a distance suggests the vacuum transition state $397a_{\text{vac}}$ is later. The key feature is that the lone pair of N5 points at C4a at 125° from the ring plane of ring A (numbering see Scheme 129), which is consistent with the $S_n$Ar mechanism and inconsistent with an electrocyclisation. This will be discussed in more detail in Section 8.7.7. The F is shifted out of plane of the A ring by 36° giving pyramidal geometry, and the C-F bond lengthened due to loss of conjugation, although the ‘early’ nature of the TS is underlined by the average C4a-F bond length of around 1.39 Å for transition state structures 397a-h being equivalent to a full $sp^3$ C-F bond. The nearby C=C bond is lengthened from 1.39 Å to 1.42 Å, but C=N is almost unchanged (1.28 Å from 1.27 Å), which is again consistent with the $S_n$Ar mechanism and inconsistent with an electrocyclisation. Nitro groups on the electrophilic A ring have a stronger effect than those on the C ring even if meta to F (397b vs 397c, Table 5), consistent with their strong inductive effect. Comparing 397d and 397f shows the extent of the TS stabilisation provided by resonance from nitro groups on the different rings, with the long N5-C4a bond length in 397d suggesting this stabilisation allows an especially early TS. These results show the extent to which the biphenyl torsion angle reduces the effect of conjugated groups from the C ring on the TS energy.
8.7.5 The Calculated Structure of ion pairs 399a and 401a

The structure shown (Figure 58) represents both ion pairs, 399a and 401a. These are stabilised by aromaticity across the A, B and C phenanthridinium rings, providing a planar structure (Figure 58). Separation of the close ion pair 399 to give solvent separated ion pair 401 is enthalpically disfavoured; however the entropic contribution from dissociating the ions is large enough to favour ion pair separation in solution. The conformation shows the C6-phenyl ring to be at an angle of close to 90˚ from the plane of the phenanthridinium, is characteristic of these salts. This conformation is consistent with the 1H NMR spectra of the product chloride salt 307, where H2” and H6” are upfield of H3” and H5”. This is due to the loss of conjugation and mesomeric interaction with the electron poor phenanthridinium caused by the lack of orbital overlap between the respective π systems of the phenanthridinium and the phenyl ring in the conformation shown. In contrast to the transition state 397, the product 401 is destabilised by electron poor substituents. The position of electron donating or withdrawing substituents appears to have less influence on the energies of these planar, conjugated species than in 397 or 400.

8.7.6 The Calculated Structure of fluoride pseudo-base 400a

Figure 59 - Unexpected halide intermediate 400a.

Halide addition at C6 of the ion pair 399 gives an adduct analogous to the pseudobases proposed in Section 4.3, Scheme 35. This unusual C6-halide adduct (Figure 59) is enthalpically favoured, and in some cases this preference overcomes the entropic drive for solvent separated ion pair 401. This
tends to occur where electron withdrawing substituents destabilise the phenanthridinium and make C6 more δ+. Phenanthridinium substituents are very influential; dinitrophenanthridinium fluoride adduct 400f is quite strongly favoured compared to solvent separated ion pair 401f (−29 kJmol⁻¹). In comparison the 2-methoxy phenanthridinium fluoride adduct 400e is less favoured than the solvent separated ion pair 401e (+ 12 kJmol⁻¹). The pseudobase species neutralises charge and relieves the strain caused by the clashing 5 and 6 substituents, but the most important stabilising element is C-F bond strength. Chloride adduct 400g is unstable and dissociates to 399g. The solvation of the Cl⁻ anion is weaker than the equivalent solvation of F⁻, however the difference between the strength of the C-Cl bond and a C-F bond in the halide adduct more than offsets the differences in anion solvation energy. During the synthesis a chloride counter ion is added on work up, so the isolation of solely the ion pair product 401 is consistent with the results of these calculations. In addition, the chloride anion is added to the work up as HCl, which forms the much weaker acid HF. The relatively weak acidity of HF is a reflection of the favourability of the H-F bonds, and the enthalpic gain from generating this relatively low energy side product would also favour the formation of the solvent separated ion pair 401.
8.7.7 Natural Bond Orbital analysis:

The pyramidal geometry and the bond lengths of transition state 397 leave little doubt this is a $S_N$Ar type process. Further evidence was acquired through Natural Bond Orbital analysis, producing a model with localised orbitals rather molecular orbitals. This is displayed in Figure 60.

![Figure 60 - Imine lone pair ($n_n$) interacting with $\pi^*$ and $\sigma^*$ orbitals.](image)

Both of the interactions shown in Figure 60 are highly favourable; donation of 0.4e from the lone pair to the $\pi^*$ (C4=C4a) aryl antibonding orbital is the largest interaction. This is clear in Figure 60 from the degree of overlap. Donation primarily to the aryl $\pi^*$ orbital denotes an $S_N$Ar mechanism. The donation to the C4a-F antibonding $\sigma^*$ orbital is also significant, and weaker when $X = Cl$ than when $X = F$. None of the $\pi$ C=N affiliated orbitals have significant overlap with the electrophilic A ring. The transition state represents formation of the C4a-N5 bond and is essentially a Meisenheimer complex, with no intermediates between this and close ion pair 399. The reaction is therefore concerted, which represents a considerable mechanistic novelty, with extant studies showing unstable Meisenheimer complexes doing so only where additional stabilisation of $F^-$ reduces the barrier to the C-F bond breaking.351,359

The relative energies of the calculated transition states 397a-g correspond well with the experimental conditions for these cyclisations. The $S_N$Ar mechanism and calculated structures perhaps hint at why 3''-methylbenzophenone imine 362 was more difficult to cyclise than N-methylcyclohexane imine derivative 364 - $S_N$Ar allows for the R substituent of the imine to be directed away from the reaction site in a way impossible in 6π electrocyclisation, and for a C3 methyl substituent in either mechanism.
Chapter 9: A novel mitochondria-targeted probe for superoxide

9.1 Testing of the 8-amino-3-‘butyl-6-phenyldihydrophenanthridine probe

Testing of the precursor 293 to superoxide probe 294 was carried out by Dr Murphy’s group at the MRC in Cambridge. Following the procedure developed in Glasgow, Angela Logan reduced the phenanthridinium 293 with NaBH₄ in Et₂O-water so that the product dihydrophenanthridine 294 partitioned into the Et₂O layer. Separation of layers and removal of Et₂O gave a single product by HPLC. Dissolving the dihydrophenanthridine in EtOH, diluting with buffer and then reaction with superoxide generated from xanthine from xanthine oxidase gave no oxidation products. Neither phenanthridinium 293 nor hydroxyphenanthridinium 402 were produced.

Scheme 130 - The reaction of model probe 293 with superoxide, with the expected substitution shown in red, the redox active iminium in orange and the ‘Bu group which prevents intercalation in green.

Based on the work of collaborators it was believed that the regiochemistry of superoxide reaction would give the 9-OH compound 402. On consulting the literature it was apparent that the regiochemistry of the superoxide reaction was disputed and attack at the C-2 was proposed by some authors. A detailed description of this issue and the assignment of phenanthridinium salts generally is provided in Chapter 10, but the potential site of reactivity at C-2 was blocked in dihydrophenanthridine 294.
9.2 A new probe design

Having considered the available literature (Chapter 10) it was decided that a new probe design should allow reaction at 2 and 9 positions, while preferably being electronically similar to MitoSOX and preventing intercalation. We envisaged that aniline alkylation with a bulky group such as tBu would achieve this. The $N$-tBu analogue was discounted as it might block both sites and would have low stability in acid; a robust molecule would allow easy handling and increase the variety of conditions available for work up and purification. It was desirable to have an electronic rich substrate to improve the challenging alkylation. The small tweak of inserting a CH$_2$ into the design (using neopentyl groups) gave a much more robust molecule (Figure 61), while decreasing the steric hindrance to reaction at C2.

![Figure 61](image-url) - The improved targeted superoxide probe 404, MitoBNH and its phenanthridinium precursor 403 are shown. Potential substitution sites are shown in red, the targeting group in blue, the bulky neopentyl groups in green and the redox active iminium site in orange.
9.3 Synthesis of the model phenanthridinium

Prior difficulties had emphasised the difficulty of N-alkylation of electron-poor phenanthridines and purification of their phosphonium analogues. The model untargeted bisneopentylethidium (BNE) analogue 405 was synthesised as a model compound (Scheme 131).

Scheme 131 - Synthesis of a model neopentyl ethidium superoxide probe 406.

Reductive amination was investigated for alkylation of 3,8-diaminophenanthridine 133 using Pd/C and H2 according to a literature procedure. Imine π bonds are weaker than comparable carbonyls and undergo reversible iminium formation, allowing their reduction where Pd/H2 alone is usually insufficient for carbonyl reduction. Less than 10% conversion was observed. Instead the TFA/acetoxyborohydride procedure used previously for used in synthesis of secondary aniline 241 (Section 6.5.1) was applied to 3,8-diaminophenanthridine 133, giving quantitative conversion to neopentyl derivative 405. Alkylation with EtOTf followed by chromatography gave ethidium derivative 406 in 50% yield. The key feature here is that the N-alkylamino groups at C-3 and C-8 are strongly electron-donating making the N-alkylation of the phenanthridine using it favourable, but the neopentyl groups discourage alkylation of the pendant amines. Purification via recrystallisation in IPA also gives ethidium derivative 406, which is intensely purple.

Although synthesised to test conditions, phenanthridinium 406 has potential uses as a non-intercalating ethidium analogue. The oxidised version can be detected easily by mass spectrometry so the corresponding dihydrophenanthridine could function as an untargeted probe for ROS which react with phenanthridiniums in a characteristic fashion, such as nitric oxide. It is anticipated that the group will investigate the utility of this compound in future.
9.4 Synthesis of MitoBNH

Given previous difficulties with the phosphonium compound 142, the triflate 408 of 1-bromohexan-1-ol 407 was selected as the alkylating agent of choice and synthesised (Scheme 132) using the same procedure as for triflates 142 and 146.

Scheme 132 - Synthesis of bromohexyltriflate 408.

The synthesis of the final probe is shown below (Scheme 133). Dry Et₂O was chosen for the alkylation reaction as experience suggested that the phenanthridinium cationic products would precipitate. Alkylation of bisneopentylphenanthridine 405 worked, giving bisneopentylhexidium alkyl bromide 409. Unfortunately the material proved partially soluble in Et₂O and so chromatography was necessary but insufficient for complete purification, giving 90 - 95% purity on a small scale. On the expectation that the dication produced by replacement of the bromide with phosphonium would result in phenanthridinium 403 being insoluble in PhMe or Et₂O, less pure bromide 409 was used in the scaled up addition to form the phosphonium salt 403. Chromatography, ion exchange and precipitation did not give complete purification and so HPLC was employed to isolate the phenanthridinium 403, giving a disappointing yield.

Scheme 133 - Synthesis of MitoBNH precursor 403.

The d-15 phosphonium analogue 410 (Figure 62) was required as an internal standard for mass spectrometry calibration, and was synthesised on a smaller scale via the same route but using d₁₅-PPh₃. This compound was isolated (4%) by HPLC, but a small of excess methanesulfonic acid was present following ion exchange and the material was contaminated on washing.

Figure 62 - The deuterated mass spectrometry standard 410.
Chapter 10: Assignment of the proton NMR of phenanthridinium salts

10.1 Why spectral assignment is important

Review of the literature associated with MitoSOX 56 suggests some confusion exists as to the regiochemistry of superoxide addition. Chang\textsuperscript{363} names the product as the 2-hydroxyphenanthridinium 57 while displaying the 9-hydroxy analogue 58 (Figure 63). The original literature suggests attack at the 2-position, and some spectral evidence has been offered for this conclusion by Zhao et al.\textsuperscript{129} The site of addition critically affects the synthesis of the MS standards and the design of analogues of MitoSOX.

![Figure 63 - MitoSOX and potential oxidation products.]

The issue was therefore re-examined. A paper by Beckman and co-workers\textsuperscript{136} compares the COSY correlation spectrum obtained from MitoSOX 56 to the spectrum of the superoxide oxidation product. The COSY of the superoxide addition product allows assignment of one spin system unambiguously, which is either H1-H2-H4 for product 58 or H10-H9-H7 for product 57 depending on the regiochemistry of addition (Table 6).

![Table 6 - The potential assignments of the 1,2,4 spin system identified by COSY. All other aromatic peaks are within a 7.6 - 8.0 ppm range.]

<table>
<thead>
<tr>
<th>δ (ppm)</th>
<th>Multiplicity</th>
<th>Assignment:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>57 58</td>
</tr>
<tr>
<td>6.2</td>
<td>d</td>
<td>H7 H4</td>
</tr>
<tr>
<td>7.4</td>
<td>s</td>
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</tr>
<tr>
<td>7.5</td>
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<td>H9 H2</td>
</tr>
<tr>
<td>8.4</td>
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<td>H10 H1</td>
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</tbody>
</table>

It is then stated that the signal from either H2 or H9 of dihydrophenanthridine 56 has been lost and that definitive assignment of the regiochemistry is limited by the impossibility of distinguishing the A and C ring systems. As H9 was believed to be the more reactive position, the superoxide product was identified as 9-hydroxyphenanthridinium 58. Although logical within the scope of the work presented the more illuminating comparison of the addition product to the generic oxidation product 411 is missed; these systems are more electronically comparable. It was anticipated that if A and C rings were distinguished in a more definitive fashion then the regiochemistry of superoxide addition could likewise be ascertained by comparison with literature data.
10.2 Assignment of 3,8-dinitrophenanthridinium salt 305

The steps used to assign phenanthridinium ring protons are detailed below alongside some commentary on the electronic features of phenanthridinium salts as inferred from the NMR spectroscopy data. The trends and assignments observed are in agreement with the detailed study presented by Leudkte et al.\textsuperscript{180}

![Figure 64 - Numbering of 3,8-dinitrophenanthridinium 305.](image)

Phenanthridinium salts show characteristic NMR features which aid assignment of the protons of the A and C rings. \( ^1 \text{H} \) NMR shifts are variable depending on conditions, but the order of the peaks is consistent. An assignment of 3,8-dinitrophenanthridinium 305 (Figure 64) illustrates these (Figure 65).

![Figure 65 - \( ^1 \text{H} \) NMR spectra in CD\(_3\)OD, assigned with the aid of the 2D spectra presented in Figure 66 - Figure 68.](image)

The H7 proton signal is important in assignment of the C ring spin system, and as a result key to differentiating the A and C spin systems. This is because it displays a large upfield shift relative to other phenanthridinium signals. The presence of an 8-amino moiety exaggerates this shift in other compounds, which may be as far upfield as 6.0 ppm in more electron rich phenanthridinium salts than 3,8-dinitrophenanthridinium 305. One explanation for this behaviour is that the 6-Ph ring sits at an angle close to 90° to the plane of the phenanthridinium heterocycle, allowing the ring π system to shield H7.
The assignment of H7 itself can in the case of phenanthridinium 305 be confirmed by HMBC correlation to a quaternary $^{13}$C peak at 168 ppm, which is too far downfield to credibly represent any position but C6, and which correlates further to H2''/H6'' itself (Figure 66).

**Figure 66** – HMBC of dinitrophenanthridinium 305 in CD$_3$OD. The H7 – C6 and C6 – H2''/H6'' correlations are highlighted. f1 corresponds to the 1D $^{13}$C spectrum, while f2 corresponds to the 1D $^1$H NMR spectrum.

Having identified H7, the attached carbon C7 can be identified from HSQC (Figure 67). There are a few distinctive features worthy of comment. Firstly, as demonstrated in Figure 67, many of the phenanthridinium $^1$H NMR shifts suggest very electronically distinct protons, yet the $^{13}$C NMR spectra suggest the attached carbons are fairly similar electronically. The strong downfield shift of H1/H10 is a recurring feature of the phenanthridinium core regardless of substitution pattern, and can probably be attributed to the influence of the neighbouring A/C ring π system through space. Similarly, the upfield shift of the C4 in the $^{13}$C NMR is characteristic. Indeed in more electron rich examples this may be below 100 ppm, giving values rarely seen in aromatic systems in the absence of metallic substituents or the heavy atom effect.

**Figure 67** – HSQC of dinitrophenanthridinium 1 in CD$_3$OD.
Having distinguished H7 from H4, COSY spectra can then be used to link the other signals to these and hence differentiate the large ortho d of H1 from that of H10 and the dd of H2 from that of H9 by following the correlations around the ring systems (Figure 68).

Figure 68 – COSY spectrum of 3,8-dinitrophenanthridinium 305 in CD$_3$OD. The assignment of H9 and H10 from H7 is illustrated.

Following analysis of the 2D spectra shown the A and C ring systems can therefore be unambiguously differentiated. In practice COSY correlation spectroscopy is usually sufficient as the position of H7 and H4 are often readily apparent. Similarly, placement of H1 relative to H10 and H2 relative to H9 are general, excepting where the electronic effect of a substituent exerts a disproportionate influence on one or the other ring system.
10.3 The assignment of 3,8-diaminophenanthridinium salt 406

It was desirable that the $^1$H NMR of the phenanthridinium precursor 403 to the final superoxide probe be described as thoroughly and accurately as possible in order to provide an analogue for identifying the superoxide reaction product with final probe 404 and any other potential side products. For this neopentylethidium 406 (Table 7) was employed as a model because the ring system is extremely similar to that of the phenanthridinium precursor to the final probe, and was characterised by COSY, HMBC and HSQC correlation spectroscopy. In addition to the correlations already described HMBC correlations from the CH$_2$-1’ triplet at around 4.5 ppm to H4 allowed the identity of H4 to be assigned. Accordingly the identity of H7, already acknowledged within the literature for electronically similar systems, was established unambiguously. The $^1$H (Figure 69) and $^{13}$C NMR assignments are presented below (Table 7).

Table 7 - Structure and $^{13}$C assignment of bisneopentylethidium 406.

<table>
<thead>
<tr>
<th>Pos.</th>
<th>ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>124.17</td>
</tr>
<tr>
<td>C1a</td>
<td>117.43</td>
</tr>
<tr>
<td>C2</td>
<td>117.30</td>
</tr>
<tr>
<td>C3</td>
<td>152.30</td>
</tr>
<tr>
<td>C4</td>
<td>98.30</td>
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<tr>
<td>C4a</td>
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<tr>
<td>C6</td>
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<tr>
<td>C7a</td>
<td>124.73</td>
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<tr>
<td>C7</td>
<td>105.50</td>
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<tr>
<td>C8</td>
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<tr>
<td>C11</td>
<td>49.49</td>
</tr>
<tr>
<td>C12</td>
<td>14.48</td>
</tr>
</tbody>
</table>

Figure 69 - Assigned $^1$H NMR spectrum of bisneopentylethidium 406.

One unusual feature of this spectrum is the two neat 1H t observed for the amines, probably due to steric hindrance slowing exchange. The 2 ppm shift difference illustrates the difference in
electronics between the A and C ring systems. With H7 identified, re-examination of both Beckman\textsuperscript{136} and Zhao’s\textsuperscript{129} 2D NMR data demonstrate that attack occurs in the 2 position, and the correct structure of the product is phenanthridinium 57.

10.4 General features of the $^1$H NMR spectra of phenanthridinium salts

10.4.1 Orientation of the C6-Ph ring

The conformation adopted by the C6-Ph group relative to the phenanthridinium unit is reflected in both $^1$H and $^{13}$C NMR spectra, in the unusual order of the signals. In a ring system conjugated to an electron poor iminium substituent such as a phenanthridinium core, it is reasonable to expect the ortho (H2''/H6'') and para (H4'') signals to have similar chemical shifts and both to be downfield of the meta protons (H3''/H5'') due to the mesomeric contribution of the electron poor substituent. Instead H4'' is the most downfield shifted signal, followed by H3''/H5'', with the ortho H2''/H6'' signals invariably displaying the lowest chemical shift of these phenyl group protons, because the phenyl group is twisted out of conjugation (Figure 70). This is consistent with the unconjugated phenanthridinium core influencing these shifts in a predominantly inductive fashion.

![Figure 70 - Poor p orbital overlap from the C6-phenyl to the phenanthridinium core.](image)

10.4.2 Assigning protons of an unsubstituted A ring

Substrates which do not display the ABX (3,8 substitution) patterns provided additional challenges, making distinguishing H1/H4 and H2/H3 difficult. NOESY correlations allow these signals to be distinguished by correlating H1/H10 and H4/CH$_2$-1’.

10.5 Substituent Effects

The existence of strong substituent effects can disrupt the established order of peaks; these are consistent with the known donating or withdrawing capability of the groups in question. A few substrates with difficult to interpret electronic influences are discussed in the following pages.
10.5.1 Assigning H1 and H4 of 2-Methoxy-3,8-dinitro-5-(hex-1'-yl)-6-phenylphenanthridinium chloride 303

One exotic example is the 2-methoxy-3-nitrophenanthridinium 303. In the absence of substituents H1 would be the more downfield signal, however 2-OMe and 3-NO2 move the H1 and H4 peaks upfield and downfield respectively. Basing an assignment on the balance of two unquantified, competing, and variable effects is dubious, so NOESY correlation spectroscopy was used to acquire a definitive answer.

![Figure 71 - NOESY spectrum in CDCl3, with ‘real’ correlations (negative signals) to other protons in blue. The observed correlations are shown on the structure.](image)

The correlation from H10 (9.2 ppm, ortho coupling) and 2-OMe (4.2 ppm, s) identify H1 as the 4th signal from the left (approx. 8.5 ppm), and correlation from CH2-1’ (4.7 ppm) gives additional confirmation by identifying H4. Correlation between H2’’/H6’’ and CH2-1’ is also visible.
10.5.2 Assigning H1 and H3 of 4-aza-5-(hex-1'-yl)-6-phenyl-8-nitrophenanthridinium chloride 301

The pyridine ring system in 4-azaphenanthridinium 301 means many of the previously identified patterns would not necessarily be expected to apply. If it is assumed that the chemical shift patterns observed in simple pyridines is replicated, H3 would be shifted up to 1 ppm downfield relative to H1 before the influence of the larger conjugated system is considered. H1 itself would be expected still to display a downfield shift. The rationale for this observation is unlikely to be abolished by a change in the nature of the ring aromatic system, although the magnitude of the shift may vary. Again, even if numbers could be placed on these two effects a simple additive approach does not necessarily account for larger changes to the heterocycle electronics induced by the pyridine. NOESY correlation spectroscopy allowed these protons to be identified.

![Figure 72 - NOESY of 4-azaphenanthridinium 301 in CD3OD with 'real' correlations (negative signals) to other protons in blue. The observed correlations are shown on the structure.](image)

H10 is readily identified (9.4 ppm, $d$, ortho coupling) and gives a correlation to H1, more than 0.3 ppm downfield of H3. As expected H2 is shifted upfield (1.2 ppm from H1), and is upfield even of H7. This is supported by the $^1$H NMR spectrum coupling pattern as relatively small ortho couplings of 4 - 6 Hz are common from the position (H3) adjacent to the nitrogen of the pyridine (shown by the red arrow in Figure 72).
10.5.3 Coalescence of 3-tertbutyl-5-(cyclohexylmethylamino)-6-phenyl-8-nitrophenanthridinium chloride

Figure 73 - Fluxional behaviour on the $^1$H NMR timescale based on slow rotation around N5-CH-1' resulting in slow interconversion of H'$_{1A}$ and H'$_{1B}$ is observed for phenanthridinium 309.

Hindered phenanthridinium 309 showed distinctive fluxional behaviour, due to slow rotation around N5-C1'. CH$_2$-1' was variably present as two broad singlets H'$_{1A}$ and H'$_{1B}$ (Figure 74) or absent in room temperature spectra, a result of rotation interchanging the position of these protons on a timescale close to that of the NMR relaxation. The signal was resolved by increasing the temperature, resulting in coalescence (Figure 74). The $^{13}$C spectra also showed an unusually broad aromatic signal believed to represent C2''/C6'' - higher temperatures (70 °C in d$_6$-DMSO) producing an acceptable spectra.

Figure 74 - $^1$H spectra of phenanthridinium 309 in CD$_3$OD from RT to 50 °C with the appearance of CH$_2$-1' highlighted.
Chapter 11: A targeted and ratiometric fluorescent calcium sensor

11.1 Calcium and the mitochondria

11.1.1 Why is calcium relevant?

As with many components within the cell, the mitochondria orchestrate a number of other processes in addition to their vital role in providing energy for cellular processes. Of particular interest and discussed here is the role of the mitochondria in calcium signalling. The calcium dication is responsible for the regulation of a remarkable variety of cellular and extra-cellular processes. Calcium is the single most abundant metal in many animals; in humans it is responsible for bone structure, muscle contraction (including heartbeat), regulation of ATP synthesis in some cells and signalling for a vast number of different processes, possibly including cell mitosis. \(^{366}\) Signals may be local, through the cell or indeed between cells. Concentration curves over the timespan of a signal can assume different shapes and the lifetime of a signal itself can vary greatly depending on the process; exocytosis can be triggered in microseconds but transcription may take minutes or even hours. \(^{367}\) Calcium signalling is therefore a topic of considerable depth and discussion here is limited to two specific contexts: the role of mitochondria in calcium signalling and the importance of such signalling in smooth muscle contraction.

The specific functions of mitochondria in ion handling in general and calcium signalling specifically is a result of the chemiosmotic potential and resulting charge gradient across the inner mitochondrial membrane creating conditions different from those experienced elsewhere in the cell. Mitochondria have been identified as crucial elements in calcium signalling control in nearly all cell types. \(^{368}\) The formation of ATP and control of calcium ion concentration are intrinsically linked, with calcium ions being maintained in steep concentration excesses in organelles such as the endoplasmic reticulum and Golgi apparatus via ATP powered calcium pumps. \(^{369,370}\)
11.1.2 Calcium regulation within the mitochondria

A concise (if simplified) summary of calcium regulation within the mitochondria is illustrated in Figure 75 and is interpreted as follows; a sodium proton exchanger pumps out sodium ions, driven by proton influx due to the chemiosmotic potential. This sodium can then re-enter via an exchanger coupled to calcium efflux. This calcium removal is balanced by a uniporter transporting calcium ions into the mitochondria. Within the matrix the calcium is present in at least two forms: free calcium, often referred to as \([\text{Ca}^{2+}]_m\), and as an insoluble phosphate salt (believed to be either hydroxyapatite or tricalcium phosphate). The phosphate ions are transferred to the matrix in the form of phosphoric acid. The solubility of this salt is strongly pH dependent and addition of a protonophore leads to acidification and dramatic rises in \([\text{Ca}^{2+}]_m\). The formation of this salt acts as a buffer on matrix calcium ion concentration.

Figure 75 - Illustration of calcium homeostatis regulation within the mitochondria. Reproduced from Szabadkai and Duchen, 2008.

The contributions of the antiports and buffering is shown in Figure 76. Calcium ion matrix \([\text{Ca}^{2+}]_m\) concentration can rise in a linear fashion at low concentration, acting as a messenger to stimulate enzymes such as NADH dehydrogenases and the Kreb’s cycle, increasing ATP output. Since extramitochondrial calcium rises as a result of many processes which consume ATP, this calcium feedback mechanism is regulating metabolism. Above this concentration range phosphate buffering ensues, and is so effective that a 50 fold increase in total matrix calcium causes an increase of \([\text{Ca}^{2+}]_m\) of less than 50%. Calcium efflux within this range is dependent on phosphate concentration and pH rather than calcium concentration, as shown by the relatively flat activity of the antiport. The uniporter by contrast increases in activity as a 3.5 power of extramitochondrial...
concentration ([Ca$^{2+}$]$_{e}$), combining with the buffering to allow the mitochondria to control the local cytosol calcium concentration ([Ca$^{2+}$]$_{c}$), maintaining a concentration known as the ‘set point’, with a value of 0.78 µM under normal conditions.\textsuperscript{371}

In the 3\textsuperscript{rd} ‘overload’ phase the buffering capacity of the phosphate salts is exceeded, leading to linear rises in [Ca$^{2+}$]$_{m}$. When this occurs calcium is released alongside low molecular weight ions and compounds, and additional solute enters, causing swelling and rupturing of the membrane. This is known as the permeability transition and has been investigated as a possible trigger for cell death via release of cytochrome c and other proteins with similar effects, through opening of a non-selective pore in both mitochondrial membranes.\textsuperscript{368} Loss of calcium homeostasis is an importantstep in cell death.\textsuperscript{371} This includes apoptotic death as well as necrotic (traumatic) destruction of cells. There is some evidence that in some cases even within the cell death process the majority of mitochondria continue to function in order to provide the energy for the programmed cell death.\textsuperscript{377}

An interesting example of Ca$^{2+}$ mediated cell death occurs where glutamate has accumulated in response to anoxia or ischemia. In this case depolarising mitochondria to prevent uptake of calcium in the mitochondria has been shown to protect neurons.\textsuperscript{42} In this case the relationship between the cytosolic calcium ion concentration [Ca$^{2+}$]$_{c}$, and cell death is not obvious, since similar concentrations from other sources do not trigger cell death. There is some evidence that the specific entry point for calcium in this case (NMDA gated channels) results in higher localised concentrations near receptors which cause nitrous oxide production (NO), which combines with the calcium concentration to trigger depolarisation and cell death.\textsuperscript{378} This highlights the importance of transient and local Ca$^{2+}$ concentration variations, and the interactions of mitochondria and other organelles are vital in these events. Mitochondria also support calcium ion signalling by allowing the generation of microdomains of differing calcium ion concentration. This involves both active uptake and calcium release and the provision of ATP allowing other organelles to modulate their calcium uptake. Examples of direct involvement include the reduction of calcium ion concentration following depolarisation in signalling, which is achieved through the sodium/calcium exchange at the inner mitochondrial membrane.\textsuperscript{368}
11.2 Calcium signalling in the sarcoplasmic reticulum of smooth muscle

11.2.1 Smooth muscle

As a tissue where energy consumption and the associated metabolic rate are directly tied to function and vary accordingly, muscle is of particular interest in the study of metabolism. Three types of muscle exist, and these are different at the cellular level: skeletal, cardiac and smooth muscle. Both skeletal and cardiac muscle produce short, powerful contractions (albeit repetitively in the case of cardiac muscle), by contrast smooth muscle produces a longer, sustained contraction. We have no conscious control over smooth muscle contraction, which respond to autonomic nervous signals and local stimuli, and are flexible, with a fusiform shape. This dependence on local stimulus and different mode of action allows smooth muscle to perform different roles, such as lining most blood vessels (not large arteries) and regulating their diameter to maintain pressure or contracting rhythmically in a peristaltic fashion to force matter through the digestive tract. Smooth muscle also plays an important structural role, helping to maintain the positioning and shape of internal organs. Calcium concentration plays an important role in muscle contraction through ‘excitation contraction coupling’, and so variation in patterns of contraction are likely to be reflected in the pattern of calcium signalling, in which a specialised organelle called the sarcoplasmic reticulum is heavily involved.

11.2.2 Calcium signalling and the sarcoplasmic reticulum

Both the sarcoplasmic reticulum, which is a type of endoplasmic reticulum found in smooth muscle, and the endoplasmic reticulum are in general strongly associated with mitochondria. There are two types of endoplasmic reticulum (rough and smooth) and their combined roles include numerous functions including synthesis of membranes, lipids, steroids and proteins and detoxification of drugs in addition to calcium storage and signalling functions. Calcium related interaction between mitochondria and the endoplasmic reticulum is believed to have an important role in stress related cell death. The sarcoplasmic reticulum is a more specialised organelle, with a primary role in calcium storage and signalling and is found in muscle tissue, with calcium release being a major component of the ‘excitation contraction coupling’ which stimulates muscle contraction. The calcium gradient is maintained via active transport (Ca\(^{2+}\)-ATPase enzymes such as SERCA) which requires a constant supply of ATP; for this reason mitochondria are often found close to the sarcoplasmic reticulum, and release ATP at specific sites, generating local high concentrations of ATP. In these regions the calcium releasing channels from both organelles are directly linked. Calcium concentrations also control the shape and position of mitochondria, for example causing migration of mitochondria onto the surface of the endoplasmic reticulum. Although present in all muscle, the role of the sarcoplasmic reticulum may vary somewhat between muscle cell types.

Referring to the diagram shown (Figure 77), the interplay of calcium and the sarcoplasmic reticulum in smooth muscle contraction can be described as follows. The sarcoplasmic reticulum uses active transport to contribute to calcium regulation by removing it from the surrounding cytoplasm (1), which allows muscle relaxation (2). Calcium signals are then stimulated, modified
or amplified by this calcium being released in response to stimuli (3). The excitability of the membrane to further stimulus is then modified via calcium sensitive channels for other ions (4), and calcium released directly to extrusion pumps in the plasma membrane (5). The sarcoplasmic reticulum interacts strongly with mitochondria in smooth muscle (99.4% of mitochondria is within 30 nm of the sarcoplasmic reticulum, with 82% totally surrounded by it), producing various microdomains of different calcium ion concentration and exchanging calcium with the mitochondria depending on the situation (6).

![Figure 77 - Calcium and the sarcoplasmic reticulum in smooth muscle contraction.](image)

The proximity of the mitochondria and the release points for calcium ions in the sarcoplasmic reticulum leads to the mitochondrial calcium transporter taking up calcium ions much faster than would normally be expected, due to high local concentrations. It has been suggested that by decreasing feedback from the calcium sensors in the sarcoplasmic reticulum stimulating the release the calcium efflux is maximised. Release channels include IP$_3$R and RyR receptors, which release calcium ions in response to messenger concentration and in a fashion mediated by a number of other factors. IP$_3$R features a channel activated by binding of inositol triphosphate and mediated in activity by calcium ion concentration, providing a feedback mechanism, and are also found outside of the sarcoplasmic reticulum. IP$_3$R channels are not randomly distributed but are clustered in groups across the sarcoplasmic reticulum, allowing various local concentrations to exist in a cell on a transient basis. The RyR (ryanodine sensitive release) channels are much larger and bind less specifically, being activated and deactivated directly by calcium binding in the protein. As a result the channel is stimulated by micromolar concentrations of calcium ions but deactivated at millimolar concentrations. Of these, RyR1 is the more sensitive, possibly amplifying the calcium signal until RyR2 is activated, leading to a
large calcium efflux known as a ‘spark’. A third RyR3 is far more evenly distributed and may be an inhibitor under normal physiological conditions, although it can function as a release channel in the correct circumstances.

11.2.3 Calcium induced calcium release in smooth muscle contraction

The collection of positive feedback mechanisms referred to as ‘calcium induced calcium release’ (CICR) are important in numerous cell types. They are believed to be involved in smooth muscle contraction, although the contribution from different Ca$^{2+}$ sources probably varies somewhat. Various permutations are set out in Jaggar et al and discussed in a review by Berridge. An outline of the mechanisms involved is illustrated in Figure 78 as follows. (1) An external messenger such as acetylcholine activates a membrane receptor releasing the signalling molecule inositol triphosphate (labelled InsP$_3$ in Figure 78) into the cytosol. (2) Synchronously through various mechanisms high extra-cellular Ca$^{2+}$ leaks into the cytoplasm, reducing membrane polarisation and increasing [Ca$^{2+}$]$_c$, which is sequestered into the sarcoplasmic reticulum (3). The InsP$_3$ channel IP$_3$R precipitates a Ca$^{2+}$ ‘spark’, (4), which is then amplified by positive feedback cycles from both IP$_3$R and RyR channels (5). Increasing Ca$^{2+}$ results in contraction of the muscle (6), with SERCA and the functionally analogous PMCA pumping Ca$^{2+}$ into the SR and extracellular space respectively (7). Local increases in Ca$^{2+}$ also activate potassium channels, which hyperpolarise the cell to prevent further excitation in a negative feedback mechanism limiting contraction (8). In some cells Ca$^{2+}$ may also activate chloride channels which depolarise the cell and allow further Ca$^{2+}$ accumulation from the extracellular space (9). It should be noted that while these mechanisms are all known to exist in some form, their relative importance or even involvement is variable and not well understood.

In some cells it is possible that the final chloride induced Ca$^{2+}$ influx initiates contraction, however it is not clear that global (full cytoplasm) increase in calcium ion concentration is required to open

![Figure 78 - Calcium induced contraction in smooth muscle. ©2008 The Author. Journal compilation. ©2008 The Physiological Society](image-url)
chloride channels, with both major ion channel types able to generate substantial local increases in calcium ion concentration. These local increases allow for selective activation of calcium sensors based on a number of factors. Because loss of the high local concentration is rapid due to diffusion, selective activation of receptors with high association constants over slower receptors with comparable dissociation constants is possible. The localised nature of these sparks also allows spatial resolution.\textsuperscript{395}

The interaction of mitochondria and the sarcoplasmic reticulum is expressed through various channels in carefully defined positions themselves modulated by several variables. Localised microdomains selectively activate and in turn activate further receptors and organelles. These are based on the selectivity of receptors which can be modulated up or down by both calcium ion concentration and other factors. Coupling this to the potential for receptors to be selectively activated based on association constant and calcium signalling within cells clearly constitutes an immensely complicated system. This is only very roughly elucidated here and varies considerably between cell types. The remarkable control and regulation of calcium within the smooth muscle cell allows a single species to act as a messenger selectively for a huge variety of processes and highlights the challenges and potential of designing sensors for such a system.
11.3 Design of a ratiometric intracellular ion probe

11.3.1 Probe design features

With the importance of probes for calcium ions clear, there is some potential for combining a mitochondria-targeting group with a calcium ion sensor in the same way as targeting DNP decouplers is potentially useful. However, with calcium ions involved in so many ion channels, organelles and individual signalling pathways there is also great utility in other targeting strategies. For this reason the synthesis of a fluorescent calcium sensor which can be easily conjugated to various targeting groups was planned. The fluorophore and binding groups are based on extant probes \(^{396,397}\) modified to allow easy attachment of different targeting groups (Scheme 134).

![Scheme 134 - Ion sensor target divided roughly into components; the ionophore (red), fluorophore (green) and targeting handle (purple).](image)

Sensor precursor 412 has an azide for attachment to different targeting groups by click chemistry. \(^{398}\) It is based on the benzofuran fluorophore Fura red due to the large stokes shift on \(\text{Ca}^{2+}\) observed with this fluorophore, which allows ratiometric imaging. \(^{399}\) This is attached through a conjugated system to a polydentate (octacoordinate) calcium chelator. Binding to calcium changes the bond angles from the coordinating groups to the tertiary amine, removing these from conjugation and changing the electronic properties of the ion sensor. Through conjugation this electronic change is passed to the fluorophore (Scheme 134), and here a difference in electronics translates to different excitation and fluorescence wavelengths.

The chelating section of the molecule is based on Tsein’s 1,2-bis(o-aminophenoxy)ethane-\(N,N,N',N'\)-tetraacetic acid (BAPTA) \(\text{Ca}^{2+}\) sensors \(^{396}\) as used in the Fura type indicators, \(^{73}\) although with the important addition of a nitro group on one ring. The reasoning for this is the dramatic increase in dissociation constant observed upon addition of an electron withdrawing substituent to Fluo-4. \(^{400}\) The intention is that the probe should have high selectivity but relatively low affinity to achieve a large dynamic reporting range and image specifically high local, transient \(\text{Ca}^{2+}\) concentrations. The probe is also ratiometric, due to the different absorption wavelength exhibited by chelating and non-chelating fura indicators.
11.3.2 Planned forward synthesis

The modular synthesis illustrated below shares many common features with other syntheses of BAPTA type probes, in particular that of Grynkiewicz, Poenie and Tsein. The two core rings A and B form the basis of the fluorophore and the binding modulating group respectively and are constructed by applying well established chemistry to hydroquinone 414 through a series of protection, nitration, and deprotection then linked by alkylation with nitroarene 415 to form BAPTA precursor 416. Reduction of the nitro groups in this precursor is followed by alkylation to add the chelating arms, followed by formylation of the more electron-
rich ring and deprotection to give aldehyde 417. The placement of the last nitration step is restricted to quite late in the synthesis due to electronics, in particular the need for the formylation of ring B to deactivate this ring and improve nitration selectivity for ring A. Alkylation of aldehyde 417 with an aldehyde surrogate and condensatory cyclisation to give fura red precursor 418, with further condensation to connect thiohydantoin 419 forming ring C. Hydrolysis of the ester groups to give calcium sensor 412 as a tetra-acid would then complete then initial synthesis. An appropriate attachment point for the targeting group is also simultaneously attached with the thiohydantoin. There are a range of options for this group, with the typical example being an azido group as a precursor to copper-catalysed Huisgen cycloaddition. 398

11.4 Synthesis of a ratiometric Ca$^{2+}$ ion sensor
11.4.1 Synthesis and linking of phenol components

The synthesis of the phenolic components (Scheme 136) and formation of the BAPTA skeleton were all carried out on large scale, allowing purification via non-chromatographic techniques such as crystallisation.

![Scheme 136 - BAPTA skeleton construction](image)

Benzyl protection of hydroquinone 414 to give dibenzylated phenol 420 works with benzyl chloride or bromide, although mechanical stirring proved necessary due to the viscosity of the suspension. The ethanol used was distilled from KOH prior to use due to the observation of a brown residue in a small scale trial which was attributed to additives in this solvent.
The benzyl ether 420 is sufficiently electron rich for nitration using nitric and acetic acid with modest heating to give nitroarene 421. This approach is considerably milder than more common nitric/sulphuric acid mixes as the rate equation for such an electron rich ring system would be zeroth order with respect to the aromatic component. The rate is dependent only on nitronium ion formation and this species is immediately consumed. Consequently the mixture is less oxidising. This may be important in avoiding oxidation to a quinone-type structure. The acetic acid also serves to moderate the acidity of nitric acid.

Deprotection to give phenol 422 using TFA and chloroform proved slow, unreliable and difficult to purify. An alternative was investigated which made use of the ortho-nitro group to chelate a strong Lewis acid with an adjacent ether and so promote the deprotection of the phenol in this position through complex 424 (Scheme 137). The result is benzyl chloride and the aluminium phenoxy 425, which subsequently hydrolyses in acidic solution to the desired phenol product.

![Scheme 137 - Lewis acid catalysed deprotection.](image)

The conditions used are modified from a literature procedure, an initial small scale trial showed that the reaction worked well and gave good conversion. On scale up concentration was increased and the temperature was lowered to compensate for the change in heat loss characteristics of the exothermic reaction with the increased scale and concentration. The starting material was completely consumed and selectivity for the phenol 422 is about 5:1 over alternate phenolic products. The yield was limited by the repeated recrystallisation needed to remove side products, which had similar solubilities to the product.

The bromide 415 was synthesised in moderate yield by reacting phenol 423 with dibromoethane in DMF, with similar conditions then used to link bromide 415 to phenol 422 to give biaryl 416. The initial attempt at this reaction contained dibromoethane and dimer 426 was isolated as a byproduct (Scheme 138).

![Scheme 138 – Small quantities of dimer 426 resulted from leftover dibromoethane in the coupling of nitroarenes 415 and 422.](image)
Removal of leftover dibromoethane on scale up by distillation under reduced pressure from the product 415 allowed the subsequent coupling to form 416 to proceed quantitatively.

Material generated from the large scale early synthesis was then transformed over a number of steps to the fluorophore precursor (Scheme 139). These steps proved more problematic and required more development.

\[
\text{Scheme 139 - Continuation of the synthesis towards the fluorophore}
\]

The reduction of the dinitro precursor 416 is complicated by the need to preserve the benzyl ether. Several conditions were evaluated in search of a replacement for hydrogenation over the Pt/C reagent which was used in the synthesis of Tsein’s original ion sensor due to the expense of using this reagent on a large scale. Sodium dithionite in THF was tested, but no reaction was observed. Palladium on carbon with ammonium formate as a hydrogen source was also tested because it was hoped the reactivity of the nitro and benzyl groups would be sufficiently different as to allow reduction of the nitro group without deprotection. Small scale experiments quickly showed that complete reduction without deprotection was impossible under the conditions used.
As an alternative, iron in aqueous acid is a well-established reagent for reduction of nitroarenes. Initial tests in ethanol based on procedures previously used within the group proved unsuccessful, but replacement of the ethanol with an acetone/water mix gave clean conversion to dianiline and acceptable yields after the solvent ratio was modified to better suit the substrate.

The ‘arms’ of the molecule were then attached to dianiline, which is a potentially problematic reaction due to the need to ensure complete reaction and thus avoid difficult to remove impurities without risking generation of quaternary ammonium salts. Some variability in yields was seen in earlier attempts at this reaction, which may be explained by the subsequent observation that product is somewhat sensitive to moderate heat and possibly prolonged exposure to light. Use of a large excess of reagents and careful drying of all reagents used allowed for eventual quantitative conversion to tetra-ester.

Formylation was achieved with the Vilsmeier-Haack reaction, for which the mechanism is shown below (Scheme 140).

Scheme 140 - The Vilsmeier-Haack formylation mechanism.
First the Vilsmeier reagent 433 is generated through nucleophilic attack by the oxygen atom of DMF 431 on the phosphorus atom, which subsequently loses a chloride. The resulting iminium phosphoryl chloride 432 is attacked by the chloride to form the iminium chloride 433, aided by the energetically favourable formation of the strong phosphorus-oxygen double bond. Once formed the reagent undergoes nucleophilic attack from the more electron rich aromatic ring of tetraester 428. The regiochemistry of addition is controlled by the ortho and para directing amino group and benzyl ether on the A ring, which both donate most strongly to the same positions. Since the position ortho to the amino group is sterically crowded selective reaction para to this group is favourable. The amine then ejects the chloride to reform the iminium structure 435, and a hydrogen ion is readily lost from the site of reaction due to the lower energy of the resulting aromatic system. The iminium ion 435 is hydrolysed in aqueous work up to give the desired aldehyde 429.

In Tsein’s synthesis the major alternative formylation site on the B ring is blocked by a methyl group absent in our target, so formylation of the tetraester compound 428 required optimisation of conditions. Variation of conditions and equivalents showed that it was possible to formylate in only the desired position using additional equivalents of POCl₃ for a short period of time (monitored by TLC), giving aldehyde 429 in high yield.

Deprotection of benzyl ether 429 proved difficult; initially transfer hydrogenation with ammonium formate appeared effective, but proved unreliable on a larger scale and lead to decomposition. This was attributed to the acidic ammonium and an alternative hydrogen source was investigated, 1,4-cyclohexadiene, but no reaction was observed.

Lewis acids were also investigated, starting with a procedure using magnesium bromide and reputed to be quite specific for benzyl groups next to an aldehyde in a similar manner to the earlier aluminium trichloride deprotection. However benzyl ether 429 showed poor solubility in the specific solvent system used for this reaction, and no reaction was observed. Aluminium trichloride gave deprotection but other phenolic side products. The ability of this reagent to generate HCl(aq) in the presence of moisture and potentially cleave ether links meant that generation of impurities was difficult to control. Nevertheless some material was brought through with this system.

Replacing the earlier transfer hydrogenation procedures with hydrogen gas and using a purer batch of starting material and a rigorous freeze/thaw degassing procedure prior to saturating the solvent with H₂(g) gave phenol 430 in good yield.
11.4.2 Nitration: addition of the binding modulating group

Scheme 141 - Attempted nitration of the B ring.

The addition of a nitro group \( \text{para} \) to the amino group on ring B, which was on the less substituted ring system was a vital part of the initial design of the synthesis, but the exact position of the step in the synthesis is somewhat flexible. Since literature precedent for nitration at this stage exists the procedure was tested.\(^{407}\)

Given the earlier experience with acidic reagents for the deprotection, it was not surprising to observe considerable decomposition of the starting material when heated in a nitric acid/acetic acid mixture. Compared to the literature synthesis this reaction was of small scale, and so controlling the equivalents of nitric acid was problematic. Following this result the nitration step was postponed to later in the synthesis.

11.4.3 Thiohydantoin heterocycle synthesis

Scheme 142 - Synthesis of the thiohydantoin heterocycle \( 419 \).

The thiohydantoin coupling partner \( 419 \) for later in the sequence was completed (Scheme 142), beginning from 1,7-dibromoheptane \( 436 \) and following a literature route to the amine \( 438 \) in which the diazo compound \( 437 \) is formed by reaction with sodium azide in water. Although most
literature routes make use of DMF to improve mixing this proved unnecessary. This was followed by Staudinger reduction of one azido group featuring a biphasic Et₂O/aqueous acid system. The amine formed is protonated and thus dragged into the acidic aqueous layer, preventing over-reduction.

![Scheme 143 – Mechanism of the Staudinger reduction.](image)

The Staudinger reaction mechanism (Scheme 143) has been investigated computationally in the literature.⁴⁰⁹,⁴¹⁰ These studies suggest the phosphine nucleophile attacks the α nitrogen atom of the azide 437 on the same side as the γ nitrogen atom with the attached R group to give a cis phosphazide due to stabilising interactions between the phosphine and the γ nitrogen atom. The resulting triphenylphosphonium salt 441 is subsequently attacked by the γ negative nitrogen atom to form a 4-membered intermediate 442,⁴⁰⁹ which decomposes, extruding N₂ to give the iminophosphorane. It is noted⁴⁰⁹,⁴¹⁰ that the energies of these transition states and intermediates are very close in the pathway and the reaction may be concerted in some cases. The resulting iminophosphorane can then undergo hydrolysis to give the amine 438.

The isothiocyanate 439 was then formed in good yield by gradual addition of thiophosgene to a stirring mixture of mono azide 438. It was hoped that reaction with glycine methyl ester hydrochloride would lead directly to closed ring system 419,⁴¹¹ but instead the open chain compound 440 was isolated. Different ring closing strategies were attempted, with stirring in DCM with SiO₂ proving unsuccessful, and p-toluenesulfonic acid in toluene giving lower yields than the aqueous acid/EtOH system shown in the scheme. The desired heterocycle was then isolated in good yield by recrystallisation. The completed heterocycle was used by Dr Stuart Caldwell and Laura Martin to make the intended fluorophore in advancing the calcium sensor synthesis and a separate project, respectively.
11.4.4 Benzofuran formation

![Scheme 144](image)

**Scheme 144** - Routes investigated towards formation of the benzofuran.

Following the decision to delay nitration, benzofuran formation was instead attempted. The combination of the diethyl acetal alkylating agent and the subsequent acid catalysed condensation are well precedent in literature. However, this is often a low yielding reaction. Initial alkylation proved difficult, perhaps due to thermal decomposition of the starting material 430. A slower reaction at lower temperature appeared to yield some product 444 as part of a complex mixture, identified by a set of symmetrical multiplets at 3.9-3.6 ppm and a t at 4.8 ppm in the $^1$H NMR spectrum (CDCl$_3$). Cyclisation was attempted both by stirring in acetic acid and using HCl$_{(aq)}$/MeOH, but neither gave any of the desired product 446. Consequently, this route to the benzofuran product 446 was abandoned.

Failure of the cyclisation represented a considerable difficulty; the substrate 430 is unsuitable for adaptation to a route mimicking the synthesis of related ion sensors. An alternative strategy of allylation to give allyl ether 445 then dihydroxylation and oxidative cleavage was investigated (Scheme 144). The first step was attempted on small scale (0.07 mmol) and the correct product was identified as part of a mixture due to the distinctive allyl signals (Figure 79), with the H$_C$-H$_B$ geminal coupling of very similar size to the $J_4$ coupling between H$_A$ and H$_C$ and H$_B$ giving the appearance of a dq structure, consistent with literature examples. The yield was estimated by $^1$H NMR to be around 44%. No further work was carried out on this route.
11.4.5 Further work

Work on the above route was halted at this point as Dr Stuart Caldwell pursued an alternative route, before eventually building on the route outlined to complete the synthesis of the final probe with a KDEL peptide sequence for retention in the sarcoplasmic reticulum attached (Figure 80).

The structure and route are mostly as previously described, with the exception of the heterocycle where a shorter chain has been utilised to aid purification.
Chapter 12: Thesis summary and future work

12.1 Summary

Synthesis of a photoactivated uncoupler 7-I was completed (Figure 81) and subsequently used by collaborators to demonstrate mitochondria uptake. The probe was then shown to allow selective uncoupling of mitochondria on UV stimulation.

![Figure 81 - MitophotoDNP iodide salt](image)

The synthesis of a ratiometric, targetable calcium sensor was completed up to intermediate 430 (9 steps), alongside a thiohydantoin heterocycle 419 synthesised in 5 steps (Figure 82). A co-worker has subsequently completed the probe synthesis based on this route, with the resulting probe showing good binding and optical responses in testing.

![Figure 82 - The BAPTA intermediate 430 and thiohydantoin heterocycle 419.](image)

Numerous routes to 5,6-disubstituted phenanthridinium salts were investigated towards the synthesis of a mitochondrially targeted superoxide probe and hydroxylated standards. In the course of this work a novel cyclisation was developed based on intramolecular S$_{N}$Ar giving access to 9-benzylxyphenanthridinium salt 126.

![Scheme 145 - Novel cyclisation to 9-OH phenanthridine 126.](image)

Rapid and high-yielding access to 5,6-disubstituted phenanthridinium salts 289 and 299-310 was then achieved through forming benzophenones 265, 324-333 via Suzuki coupling and converting these to imines with the alkylamine. The nitrogen atom of the imine then undergoes cyclisation onto the aryl fluoride in an intramolecular S$_{N}$Ar upon heating. This transformation was shown to
have good steric and electronic tolerance in the synthesis of 13 phenanthridinium analogues with 6 structural diversification points (Scheme 146). Subsequent DFT calculations by a colleague showed this reaction proceeds in a concerted fashion and as such represents a considerable mechanistic novelty.

Scheme 146 - Precursors 448 and 449 allow access to benzophenones 265 and 324 - 333. The imine formation and cyclisation by intramolecular S$_{N}$Ar gives the 5,6-disubstituted phenanthridinium salts 289 and 299 - 310 in high yield.

Efforts towards a new probe for mitochondrial superoxide led to the synthesis of 3-tertbutyl-dihydrophenanthridine 294, which does not intercalate into DNA upon oxidation. This concept was refined and lead to the development of neopentyl ethidium 406 and the targeted analogue MitoBNH 404 and its deuterated analogue 410.

Figure 83 - The non DNA-intercalating probes synthesised.

12.2 Future work

With regard to MitoPhotoDNP 7 and the ratiometric Ca$^{2+}$ sensor it is hoped these will be appropriately utilised by collaborators, and the designs could be used to deliver alternative payloads or target different organelles, respectively.

The cyclisation to the 9-benzylxyphenanthridinium 126 may provide rapid access to substituted 6-phenylphenanthridines, if higher yields and milder conditions could be achieved. The key to improving this reaction is likely to be found in reducing the imine isomerisation energy, and to this end an N-coordinating additive may prove helpful.
Numerous diversifications and expansions may be possible on the fruitful imine S$_n$Ar cyclisation. The C6 functionalisation opportunities offered by the enamine 369 may be worthy of investigation, allowing additional flexibility in decorating these important heterocycles. Many analogues are likely to be accessible via the methodology developed, including C6 unsubstituted variants. Improving or replacing the TiCl$_4$ imine formation would be desirable, especially if the aza-Wittig reaction could be made to work with a less stabilised phosphine or without the C6 substituent in order to consolidate the imine formation step into the cyclisation procedure. Replacing the alkylamine with hydrazine or hydroxylamine may allow low temperature cyclisation due to the α effect, and the products may give interesting optical and particularly photolytic properties.

Most obviously, the methodology will be utilised to access further probe systems, and to this end work is ongoing within the group to complete the DNA targeted H$_2$O$_2$ probe 371.

Some further work is required to re-synthesise the MitoBNH deuterated standard, and then access the 2-hydroxymitoBNH controls. It is hoped that when these are available collaborators will be able to use these probes for accurate in vivo quantification of superoxide. The neopentylethidium model 406 is independently of interest, with the intention being to make a deuterated standard and use the combination for quantifying superoxide more generally.
Chapter 13: Experimental

$^1$H NMR were obtained using Bucker-ACS 60 spectrometers operating at 500 and 400 MHz respectively, $^{13}$C NMR spectra at 126 and 101 MHz accordingly. All coupling constants are recorded in Hz and reported uncorrected. DEPT was used to assign C, CH, CH$_2$ and CH$_3$. 2D techniques including COSY, HSQC, HMBC, NOESY and $^{19}$F equivalents and decoupled protons were used to aid assignment. The $^{19}$F spectra for trifluoroborurate salt 266 was corrected using CF$_3$CO$_2$H as an external standard. EI and CI mass spectra were obtained using the (M Station) JEOL JMS-700 spectrometer. Some ESI spectra were collected on a Bruker MicroTOF-Q in Glasgow, others by an LTQ Orbitrap XL by the National Mass Spectrometry Service Centre in Swansea. IR Spectra were obtained with a Shimadzu FTIR 8400S. Alumina for chromatography was deactivated by saturation with deionised water followed by drying at 150 °C for 72 h. Ethanol for the synthesis of 1,4-bis(benzyloxy)-2-nitrobenzene was distilled from KOH. Acetone was dried by stirring with 4 angstrom molecular sieves or distilled from CuSO$_4$. All dry triethylamine was distilled from CaH$_2$ and stored over KOH. Dry solvents Hexylamine was distilled from CaH$_2$ and stored over KOH. Pyridine was distilled from CaH$_2$. All glassware for reactions involving anhydrous conditions was dried in an oven at 250 °C or flame dried under vacuum, with dry solvents collected from a Puresolv solvent purification system. A CEM Explorer PSL set to a maximum power of 50W, maximum ramp time of 20 min (never reached) and a 15 atm pressure cut off was used for the microwave reactions. All NEt$_3$ was distilled from CaH$_2$ and stored over KOH. Pyridine was distilled from CaH$_2$. Allyl bromide was distilled MgSO$_4$ in a dark environment and kept covered in storage to protect from light. NaI used was previously dried by heating under vacuum at 100 °C for 6 h.

5-(Hydroxy-2-nitrobenzyl)methanol 22

NaBH$_4$ (2.38 g, 62.9 mmol, 1.90 eq.) was added over 45 min to a stirred solution of 5-hydroxy-2-nitrobenzaldehyde (5.06 g, 33.0 mmol, 1.00 eq.) in MeOH (100 mL) at 0 °C under argon. After 17 h the reaction was quenched into H$_2$O. MeOH was removed and the aqueous concentrated under reduced pressure, leaving a yellow solid. This was partitioned between Et$_2$O and 0.5 M HCl$_{aq}$, then the aqueous re-extracted with Et$_2$O (x 3). The combined organic extracts were dried (MgSO$_4$) and filtered, then the solvent was removed under reduced pressure and the solid dried under high vacuum to give benzylic alcohol 22 as a solid (4.68 g, 91%). $\delta$$_H$ (400 MHz, CD$_3$OD): 8.08 (1H, d, $J$ = 9.0 Hz, H3), 7.26 (1H, s, H6), 6.78 (1H, dd, $J$ = 8.9, 2.4 Hz, H4), 4.94 (2H, s, CH$_2$OH). In agreement with values from Caroline Quin’s thesis.414
4-Iodobut-1-yltriphenylphosphonium iodide 24

![Chemical Structure](image)

Triphenylphosphine (9.18 g, 35.0 mmol, 1.00 eq.) was dissolved in dry PhMe (40 mL). This was added slowly to a stirred solution of 1,4-diiodobutane (5.0 mL, 38 mmol, 1.1 eq.) in dry PhMe (160 mL) under argon which was then heated to 100 °C in the dark for 4 h. A white solid was formed which was then vacuum filtered and washed with Et₂O then hot PhMe and dried under vacuum to give the phosphonium salt 24 as a powder (15.4 g, 26.9 mmol, 77%).

δ\(_H\) (400 MHz, CDCl\(_3\)): 7.92 - 7.78 (9H, m, \(\text{^3PPh}_3\)), 7.74 - 7.68 (6H, m, \(\text{^3PPh}_3\)), 3.90 - 3.80 (2H, m, CH\(_2\)CH\(_3\)^+PPh\(_3\)), 3.35 (2H, t, \(J = 6.1\) Hz, IC\(_2\)CH\(_2\)), 2.24 (2H, quintet, \(J = 6.6\) Hz, ICH\(_2\)CH\(_2\)CH\(_2\)), 1.83 (2H, sextet, \(J = 7.8\) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)PPh\(_3\)).

δ\(_C\) (CDCl\(_3\), 100 MHz): 135.25 (d, \(J = 3.0\) Hz, CH), 133.65 (d, \(J = 10.1\) Hz, CH), 130.61 (d, \(J = 12.6\) Hz, CH), 117.68 (d, \(J = 86.2\) Hz, C), 32.58 (d, \(J = 17.0\) Hz, CH\(_3\)), 21.85 (d, \(J = 51.4\) Hz, CH\(_3\)), 7.29 (CH\(_2\)).

IR (ATR cm\(^{-1}\)): 2890 (CH), 2859 (CH), 1588 (C\(_{Ar}\)=C\(_{Ar}\)), 1484 (C\(_{Ar}\)=C\(_{Ar}\)), 1433 (P-Ph). MS (NSI\(^+\)): 445 (M\(^+\), 100%), 289 (M\(^+\)–HI–H\(_2\)–C\(_2\)H\(_4\)).

{4-[3’-(Hydroxymethyl)-4’-nitrophenoxy]butyl}triphenylphosphonium iodide 25-I

![Chemical Structure](image)

Phenol 22 (1.18 g, 7.21 mmol, 1.00 eq.), phosphonium salt 24 (2.49 g, 4.35 mmol, 0.60 eq.), and Cs\(_2\)CO\(_3\) (1.48 g, 4.53 mmol, 0.63 eq.) were stirred and heated under reflux and argon in dry MeCN (20.0 mL) for 72 h. After cooling DCM was added, then the solution decanted off. The residual solvent was removed under reduced pressure, and the material dissolved in DCM and washed with 1 M NaOH\(_{aq}\). The aqueous washings were extracted with DCM (x 2). The combined organics were then dried (MgSO\(_4\)), then filtered and the solvent removed to give phosphonium salt 25-I as a yellow foam (2.57 g, 97%).

δ\(_H\) (400 MHz, CDCl\(_3\)): 8.03 (1H, d, \(J = 9.1\) Hz, H5’), 7.81 - 7.75 (9H, m, \(\text{^3PPh}_3\)), 7.71 - 7.65 (6H, m, \(\text{^3PPh}_3\)), 7.48 (1H, d, \(J = 2.8\) Hz, H2’), 6.73 (1H, dd, \(J = 9.1, 2.8\) Hz, H6’), 4.70 (2H, s, CH\(_2\)OH), 4.29 (2H, t, \(J = 6.1\) Hz, OCH\(_2\)CH\(_2\)), 3.75 - 3.65 (2H, m, CH\(_2\)CH\(_2\)^+PPh\(_3\)), 2.19 (2H, quintet, \(J = 6.6\) Hz, OCH\(_2\)CH\(_2\)CH\(_2\)), 1.85 (2H, sextet, \(J = 7.8\) Hz, CH\(_2\)CH\(_2\)CH\(_2\)^+PPh\(_3\)).

δ\(_C\) (CDCl\(_3\), 100 MHz): 163.12 (C), 142.71 (C), 139.45 (C), 135.27 (d, \(J = 2.9\) Hz, CH), 133.66 (d, \(J = 10.0\) Hz, CH), 130.63 (d, \(J = 12.6\) Hz, CH), 127.35 (CH), 117.80 (d, \(J = 86.2\) Hz, C), 113.60 (CH), 67.21 (CH\(_3\)), 61.66 (CH\(_3\)), 29.03 (d, \(J = 16.7\) Hz, CH\(_3\)), 22.33 (d, \(J = 51.3\) Hz, CH\(_3\)), 18.95 (d, \(J = 3.8\) Hz, CH\(_3\)). IR (ATR cm\(^{-1}\)): 3320 (OH), 3053 (C\(_{Ar}\)-H), 2932 (CH), 2864 (CH), 1613 (C\(_{Ar}\)=C\(_{Ar}\)), 1584 (C\(_{Ar}\)=C\(_{Ar}\)), 1576 (C\(_{Ar}\)=C\(_{Ar}\)), 1506 (NO\(_2\)), 1433 (P-Ph). MS (FAB/NOBA), m/z: 486 [M\(^+\) (phosphonium cation), 100%], 317 (C\(_{29}\)H\(_{22}\)P\(^{+}\), 83), 262.1 (^3PPh\(_3\), 53). HRMS: 486.1834. C\(_{29}\)H\(_{22}\)O\(_4\)NP requires 486.1829.
Benzylic alcohol 25-I (712 mg, 1.16 mmol, 1.00 eq.), 1-fluoro-2,4-dinitrobenzene 26 (0.60 mL, 5.0 mmol, 4.3 eq.) were combined and stirred in dry acetone (15.0 mL) under argon. Dry triethylamine (0.40 mL, 2.9 mmol, 2.5 eq.) was then added and the mixture heated to 45 °C for 72 h in the dark. The solvent was removed under reduced pressure and the material dissolved in DCM and washed with 0.5 M NaOH (aq) (x 3). The DCM layer was then dried (MgSO₄), filtered and the solvent removed under reduced pressure. The material was then dissolved in minimal DCM and precipitated with addition of Et₂O (x 2), then this process repeated to give a brown foam (706 mg). Column chromatography on 244 mg of this material using gradient elution [MeCN – EtOAc (10:3 - 10.1), then MeCN – MeOH (20:1 - 1:1)] on deactivated alumina gave the DNP derivative 7-I as a yellow foam (13 mg, 5%).

δ_H (400 MHz, CDCl₃): 8.87 (1H, d, J = 2.8 Hz, H3”), 8.52 (1H, d, J = 9.3, 2.8 Hz, H5”), 8.27 (1H, d, J = 9.2 Hz, H6”), 7.94 - 7.84 (6H, m, °PPh3), 7.82 - 7.76 (3H, m, °PPh3), 7.74 - 7.65 (6H, m, °PPh3), 7.58 (1H, d, J = 2.7 Hz, H2’), 7.50 (1H, d, J = 9.3 Hz, H5’), 7.06 (1H, dd, J = 9.1, 2.8 Hz, H6’), 5.79 (2H, s, CH₂OAr”), 4.37 (2H, t, J = 6.2 Hz, OCH₂CH₂), 4.15 - 4.00 (2H, m, CH₂CH₂°PPh₃), 2.28 (2H, quintet, J = 6.8 Hz, OCH₂CH₂), 1.96 - 1.85 (2H, m, CH₂CH₂°PPh₃), 1.76 - 1.62 (2H, m, CH₂CH₂°PPh₃), 1.58 - 1.46 (2H, m, CH₂CH₂°PPh₃), 1.38 (2H, m, CH₂CH₂°PPh₃).

δ_C (CDCl₃, 100 MHz): 163.93 (C), 156.00 (C), 140.57 (C), 139.07 (C), 138.45 (C), 135.00 (d, J = 3.0 Hz, CH), 133.72 (d, J = 10.0 Hz, CH), 130.44 (d, J = 12.5 Hz, CH), 129.85 (CH), 128.43 (CH), 122.42 (CH), 118.30 (d, J = 85.9 Hz, C), 115.30 (CH), 114.50 (CH), 114.07 (CH), 69.41 (CH₂), 68.02 (CH₂), 29.07 (d, J = 17.0, CH₂), 21.95 (d, J = 50.5 Hz, CH₂), 19.18 (d, J = 4.0 Hz, CH₂). IR (NaCl, DCM cm⁻¹): 3054 (C Ar-H), 2987 (CH), 2924 (CH), 2854 (CH), 1606 (C=O=C), 1519 (C=O=C), 1438 (P-Ph). MS (NSI⁺): 652 [M⁺ (phosphonium cation), 100%]. HRMS: 652.1834. C₃₅H₃₁O₈N₃P requires 652.1843.

4-Bromobut-1-yltriphenylphosphonium bromide 28

A stirred mixture of triphenylphosphine (2.73 g, 10.4 mmol, 1.00 eq.) and 1,4-dibromobutane (1.50 mL, 12.6 mmol, 1.21 eq.) in dry PhMe (35 mL) was then heated to reflux under argon for 72 h. The mixture was allowed to cool and filtered. The solid collected was washed with Et₂O and hot PhMe, then dissolved in MeOH. The solvent was removed under reduced pressure to give solid 28 (4.30 g, 100%). δ_H (400 MHz, CDCl₃): 7.91 - 7.85 (6H, m, °PPh₃), 7.83 - 7.77 (3H, m, °PPh₃), 7.74 - 7.68 (6H, m, °PPh₃), 4.02 - 3.94 (2H, m, CH₂CH₂°PPh₃), 3.59 (2H, m, J = 5.9 Hz, BrCH₂CH₂), 2.33 (2H, quintet, J = 6.5 Hz, BrCH₂CH₂), 1.86 (2H, sextet, J = 7.8 Hz, CH₂CH₂°PPh₃). Compound is commercially available.415
{4-[3’-(Hydroxymethyl)-4’-nitrophen oxy]butyl}triphenylphosphonium bromide 25-Br

Phenol 22 (829 mg, 5.08 mmol, 1.00 eq.), phosphonium salt 28 (1.81 g, 4.50 mmol, 0.89 eq.), and Cs₂CO₃ (1.48 g, 4.53 mmol, 0.89 eq.) were stirred under reflux and argon in dry MeCN (20.0 mL) for 72 h. The mixture was partitioned between H₂O and EtOAc, then the aqueous was re-extracted with EtOAc (x 4). The mixture was then dried (MgSO₄) and the solvent removed under reduced pressure. The material was then dissolved in minimal DCM and precipitated with Et₂O. Repeating this process gave triphenylphosphonium salt 25-Br (706 mg, 28%) which was used without further purification.

{4-[3’-(Hydroxymethyl)-4’-nitrophen oxy]butyl}triphenylphosphonium tetraphenylborate 25-BPh₄

Benzylic alcohol 25-Br (706 mg, 1.26 mmol, 1.00 eq.) and NaBPh₄ (450 mg, 1.31 mmol, 1.04 eq.) were dissolved in a biphasic mixture of DCM (50 mL) and H₂O (50 mL). The layers were separated then the DCM layer was partitioned with H₂O. The organic layer was dried (MgSO₄), filtered and the solvent removed under reduced pressure. The sample was dissolved in minimal DCM and precipitated with MeOH, giving salt 25- BPh₄ (574 mg, 55%) as a brown foam sufficiently pure for the next step. δ H (400 MHz, CDCl₃): 8.14 (1H, d, J = 9.1 Hz, H5’), 7.46 - 7.23 (23H, m, Ph₄B⁺, ‘PPh₃), 7.11 (1H, d J = 2.7 Hz, H2’), 6.98 - 6.86 (8H, m, Ph₃B⁻), 6.82 - 6.74 (4H, m, Ph₃B⁻), 6.70 (1H, dd, J = 9.1, 2.8 Hz, H6’), 5.02 (2H, s, CH₂OH), 3.76 (2H, t, J = 5.4 Hz, OCH₃CH₂), 2.12 - 2.00 (2H, m, CH₂CH₂PPh₃), 1.65 - 1.35 (4H, m, OCH₂CH₂CH₂CH₂PPh₃).

{4-[3’-(2”,4”-Dinitrophenoxymethyl)-4’nitrophenoxy]butyl}triphenylphosphonium tetraphenylborate 7-BPh₄

A stirred mixture of benzylic alcohol 25-BPh₄ (574 mg, 0.71 mmol, 1.00 eq.), 1-fluoro-2,4-dinitrobenzene 26 (0.5 mL, 4.0 mmol, 5.6 eq.) and dry NEt₃ (0.15 mL, 1.1 mmol, 1.5 eq.) in dry acetone (10.0 mL) under argon was heated to 45 °C for 72 h in the dark. The solvent was removed under reduced pressure and the material dissolved in DCM and washed with 0.2 M NaOH (aq) (x 3). The DCM layer was then dried (MgSO₄), filtered and the solvent removed under reduced pressure. The material was redissolved in minimal DCM and precipitated with excess MeOH. The residual solution was decanted off and the solid dried under vacuum. Following column chromatography [SiO₂, dry loaded with DCM, EtOAc/MeOH (10:1)] the solvent mix was allowed to partially
evaporate over 4 days, resulting in formation of needle shaped brown crystals (104 mg, 15%) of probe 7-BPh₃. δH (400 MHz, CDCl₃): 8.86 (1H, d, J = 2.9 Hz, H3”), 8.59 (1H, dd, J = 9.3, 2.9 Hz, H5”), 8.29 (1H, d, J = 9.2 Hz, H6”), 7.94 - 7.87 (3H, m, ’PPh₃”), 7.86 - 7.72 (13H, ’PPh₃, H5”), 7.34 (1H, d, J = 2.6 Hz, H2”), 7.22 - 7.16 (8H, m, Ph₄B –), 7.13 (1H, dd, J = 9.2, 2.8 Hz, H6’) 6.93 (8H, t, J = 6.9 Hz, Ph₄B –), 6.80 (4H, t, J = 6.9 Hz, Ph₄B –), 5.88 (2H, s, CH₂OAr”), 4.23 (2H, t, J = 6.0 Hz, OC₂H₂CH₂), 3.74 - 3.61 (2H, m, CH₂C₂H₂+PPh₃), 1.98 (2H, quintet, J = 6.4 Hz, OCH₂CH₂), 1.76 (2H, sextet, J = 7.6 Hz, CH₂CH₂CH₂+PPh₃). MS (NSI +): 652 [M+ (phosphonium cation), 100%]. HRMS: 652.1836. C₃₅H₃₁O₈N₃P requires 652.1843. MS (NSI –): 319 [M– (tetraphenyl borate anion), 100%]. HRMS: 319.1664. C₂₄H₂₀B requires 319.1664.

4-Bromomethyltriphenylphosphonium bromide 30

A solution of triphenylphosphine (307 mg, 1.17 mmol, 1.00 eq.) in dry PhMe (2.0 mL) which was added slowly to a stirring solution of α,α’-dibromoxylene 29 (616 mg, 2.34 mmol, 2.00 eq.) in dry PhMe (10.0 mL) at 100 °C under argon. After 20 h the mixture was allowed to cool and the precipitate was collected and washed with Et₂O and hot PhMe. After drying under vacuum salt 30 was collected as a solid (379 mg, 63%). δH (400 MHz, CDCl₃): 7.82 - 7.71 (9H, m, +PPh₃), 7.69 - 7.60 (6H, m, ’PPh₃), 7.18 - 7.08 (4H, m, H2,3,5,6), 5.50 (2H, d, J = 14.2 Hz, ArC₂H₂+PPh₃), 4.40 (2H, s, ArCH₂Br). Data matches previous spectra by Stephen McQuaker.416

2-Benzoyl-5-(benzyloxy)phenol 123

2,4-dihydroxybenzophenone 122 (5.00 g, 23.4 mmol, 1.00 eq.), KI (5.84 g, 35.2 mmol, 1.50 eq.) and NaHCO₃ (3.05 g, 36.3 mmol, 1.55 eq.) were combined and stirred under argon in dry MeCN (30 mL). Benzyl chloride (3.3 mL, 29 mmol, 1.2 eq.) was added, followed by additional dry MeCN (30 mL). The reaction mixture was then heated under reflux and argon for 48 h. The contents of the flask were washed out with additional DCM, and the solvent removed from all the material. The solid was then washed with DCM and the organic extracts dried (MgSO₄) then filtered and the solvent removed under reduced pressure to give benzyl ether 123 (6.74 g, 95%). A small portion was crystallised for analysis (acetone/water), giving needles. δH (400 MHz, CDCl₃): 12.68 (1H, s, PhOH), 7.64 (2H, d, J = 7.6 Hz, H2’, H6”), 7.60 - 7.46 (4H, m, H3, H3’, H4’, H5”), 7.46 - 7.32 (5H, m, Ph), 6.61 (1H, d, J = 2.5 Hz, H6), 6.49 (1H, dd, J = 9.0, 2.4, H4), 5.13 (2H, s, OCH₂Ph). δC (100 MHz, CDCl₃): 200.04 (C), 166.27 (C), 165.29 (C), 138.26 (C), 135.85 (C), 135.32 (CH), 131.48 (CH), 128.85 (CH), 128.73 (CH), 128.34 (CH), 128.30 (CH), 127.54 (CH), 113.33 (C), 107.89 (CH), 102.12 (CH), 70.29 (CH₂). IR (ATR cm⁻¹): 3032 (C=Ar-H), 2091, 1643 (C=O), 1597 (C₆H₅=C₆H₅), 1505 (C₆H₅=C₆H₅), 1342 (Ph-
OH). MS (El⁺): 304 (M⁺, 95%), 91 (C₇H₇⁺, 100). HRMS: 304.1098. C₂₀H₁₆O₃ requires 304.1099. MP: 118 °C.

### 2-Benzoyl-5-benzyloxy-4-nitrophenol 124

Phenol 123 (1.04 g, 3.41 mmol, 1.00 eq.) was suspended stirring in AcOH (6.6 mL) under argon, then HNO₃(aq) (0.42 mL, 6.6 mmol, 1.9 eq., 70%) was added dropwise. After 4 h 30 min stirring under argon the reaction mixture was heated to 50 °C and developed a red colour. After a further 17 h the mixture was poured into water, then extracted with chloroform (x 2) and the combined organics dried by azeotrope in PhMe and the solvent removed under reduced pressure. A mixture of 4-nitro and 6-nitro compounds (4.2:1) resulted, from which dropwise addition of water to the compound dissolved in acetone caused precipitation to give 4-nitrophenol 124 (517 mg, 45%). δH (400 MHz, CDCl₃): 12.95 (1H, s, PhOH), 8.39 (1H, s, H3), 7.69 - 7.65 (2H, m, H2′, H6′), 7.65 - 7.62 (1H, m, H4′), 7.55 (2H, t, J = 7.5 Hz, H3′, H5′), 7.49 (2H, d, J = 7.3 Hz, H2″, H6″), 7.42 (2H, t, J = 7.1 Hz, H3″, H4″, H6″), 6.70 (1H, s, H6), 5.27 (2H, s, OCH₂Ph). ¹³C NMR (101 MHz, CDCl₃) δ 199.60, 168.65, 158.57, 150.49, 146.21, 136.18, 134.28, 133.61, 131.12, 129.95 (CH), 129.90 (CH), 128.94 (CH), 128.73 (CH), 128.50 (CH), 128.43 (CH), 128.33 (CH), 127.28 (CH), 124.98 (C), 110.48 (CH), 71.92 (CH₃), 21.73 (CH₃). Data matches previous characterisation by Susan Macintyre.¹⁷³

### 2'-Benzoyl-5'-benzyloxy-4'-nitrophenyl 4-methylbenzene-1-sulfonate 125

Nitrophenol 124 (2.01 g, 5.73 mmol, 1.00 eq.) and para-methylbenzenesulfonyl chloride (1.42 g, 7.47 mmol, 1.30 eq.) were combined stirring in dry pyridine (10.0 mL), then any residual solid washed in with additional dry pyridine (23.0 mL). The reaction mixture was then stirred at RT for 17 h. The solvent was removed under reduced pressure, and the solids dissolved in DCM and washed with water (x 2), then the solvent was removed from the collected organics which were then dried by azeotrope in PhMe. Tosylate 125 was isolated (2.86 g, 99%). δH (400 MHz, CDCl₃): 7.98 (1H, s, H3′), 7.61 - 7.53 (3H, m, H2″, H4″, H6″), 7.53 - 7.38 (9H, m, H2, H6, H3″, H5″, OCH₂Ph), 7.29 (1H, s, H6″), 7.14 (2H, d, J = 8.1 Hz, H3, H5), 5.31 (2H, s, OCH₂Ph), 2.36 (3H, s, CH₃Ar). δC (100 MHz, CDCl₃): 190.36 (C), 154.43 (C), 150.49 (C), 146.21 (C), 136.18 (C), 134.28 (C), 133.61 (CH), 131.12 (C), 129.95 (CH), 129.90 (CH), 128.94 (CH), 128.73 (CH), 128.50 (CH), 128.43 (CH), 128.33 (CH), 127.28 (CH), 124.98 (C), 110.48 (CH), 71.92 (CH₃), 21.73 (CH₃). Data matches previous characterisation by Susan Macintyre.¹⁷³
9-(Benzyloxy)-8-nitro-6-phenylphenanthridin-3-amine 127

Tosylate 125 (303 mg, 0.60 mmol, 1.00 eq.) was combined with 1,3-phenylenediamine (408 mg, 3.77 mmol, 6.29 eq.) and p-toluenesulfonic acid (96 mg, 0.50 mmol, 0.84 eq.) in PhMe (30 mL) within a dean stark apparatus, and heated under reflux stirring under argon for 120 h. The material was then filtered, and the filter cake washed with organic solvents (MeOH x 3, DCM, x 1), isolating the salt form 126 (130 mg, 28%), which was then washed with 7M NaOH (aq) to give phenanthridine 127 as a red solid for characterisation purposes.

\[ \delta H (400 MHz, d_6-DMSO): 7.94 (1H, s, H7), 7.69 - 7.62 (3H, m, H2', H4', H6'), 7.59 (1H, s, H10), 7.53 (1H, d, J = 7.9 Hz, H2'', H6''), 7.48 - 7.41 (4H, m, H3', H5', H3'', H5''), 7.38 - 7.33 (1H, t, J = 7.4 Hz, H4''), 7.30 (1H, d, J = 9.3 Hz, H1), 7.0 (1H, dd, J = 9.3, 2.2 Hz, H2), 6.93 (1H, d, J = 2.2 Hz, H4), 6.52 (2H, d, br, D\textsubscript{2}O, NH\textsubscript{2}), 5.46 (2H, s, OC\textsubscript{2}H\textsubscript{5}Ph). \]

\[ \delta C (100 MHz, d_6-DMSO): 153.03 (C), 150.55 (C), 152.52 (C), 150.05 (C), 148.22 (C), 137.89 (C), 136.02 (C), 134.32 (C), 130.05 (CH), 128.87 (CH), 128.71 (CH), 128.53 (CH), 128.05 (CH), 128.01 (CH), 127.29 (CH), 125.53 (CH), 121.06 (CH), 118.45 (C), 115.92 (C), 109.05 (CH), 102.58 (CH), 102.53 (CH), 70.38 (CH\textsubscript{2}). IR (ATR cm\textsuperscript{-1}): 3341 (NH), 3171 (NH), 1636 (C=\textsubscript{N}), 1597 (C\textsubscript{Ar}=C\textsubscript{Ar}), 1528 (NO\textsubscript{2}). MS (FAB): 422.4 [(M+H)+, 100%], 329.2 [(M – C\textsubscript{6}H\textsubscript{7}N)+, 73], 307.2 [(M – C\textsubscript{7}H\textsubscript{7}–NO\textsubscript{2} + Na)+, 74]. HRMS: 422.1501. C\textsubscript{26}H\textsubscript{19}N\textsubscript{3}O\textsubscript{3} requires 422.1505. MP (as tosylate salt): 245 - 248 °C.

3,8-Bis(carbobenzylamino)-6-phenylphenanthridine 138

3,8-Diamino-6-phenylphenanthridine 133 (108 mg, 0.378 mmol, 1.00 eq.) was dissolved in THF (2.0 mL) and cooled to 0 °C stirring under argon. NaHCO\textsubscript{3} (84 mg, 1.0 mmol, 2.6 eq.) was added followed by dropwise addition of benzyl chloroformate (3.0 mL, 21 mmol, 2.3 eq.), after which the mixture was allowed to warm to RT and stirred under argon for 72 h. The mixture was quenched into water then extracted with Et\textsubscript{2}O (x 2). The organic layer was washed repeatedly with water then dried (MgSO\textsubscript{4}), filtered and the solvent removed under reduced pressure. Column chromatography [SiO\textsubscript{2}, dry loaded in THF, Pet. ether/EtOAc (1:1)] gave phenanthridine 138 as a solid (172 mg, 82%). R\textsubscript{f} = 0.64 in Pet. ether/EtOAc (1:1). \(^1\)H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 8.45 (d, \(J = 9.1\) Hz, H10), 8.37 (d, \(J = 9.0\) Hz, H11), 8.01 (1H, d, br, J = 8.3 Hz, H9), 7.98 (1H, d, J = 2.2 Hz, H4), 7.92 (1H, d, br, J = 8.7 Hz, H2), 7.87 (1H, d, J = 2.2 Hz, H7) 7.65 (2H, dd, J = 7.8, 1.5 Hz, H2'', H6''), 7.50 - 7.43 (3H, m, H3'', H4'', H5''), 7.41 (2H, dd, J = 7.8, 1.3 Hz, H2''', H6'''), 7.39 - 7.29 (8H, m, H3''', H4''', Ph'), 7.13 (2H, s, br, NH), 5.23 (2H, s, 8-NHCO\textsubscript{2}CH\textsubscript{2}), 5.19 (2H, s, 3-NHCO\textsubscript{2}CH\textsubscript{2}). \(^13\)C NMR (126 MHz, CDCl\textsubscript{3}) \(\delta\) 161.28 (C), 153.31 (C), 143.83 (C), 139.39 (C), 138.05 (C), 136.39 (C), 135.94
3,8-Bis(carboxethoxyamino)-6-phenylphenanthridine 139

3,8-Diamino-6-phenylphenanthridine 133 (510 mg, 1.79 mmol, 1.00 eq.) in dry pyridine (3.0 mL) was cooled to 0 °C stirring under argon. Methyl chloroformate (0.55 mL, 7.1 mmol, 4.0 eq.) was added dropwise then the mixture heated to RT and stirred under argon for 20 h. The mixture was filtered, washed with water then the solid filtered and washed with MeOH. The solvent was removed under reduced pressure then the solid residue washed with 1 M NaOH (aq), filtered and acidified with 1 M HCl (aq) then dissolved in MeOH and the solvent removed under reduced pressure. The solid was dried by azeotrope with PhMe to give methylcarbamate 139 as the orange hydrochloride salt (649 mg, 83%). For analysis a sample (21 mg) of the salt was loaded onto a strata WCX ion exchange resin in MeOH and washed with further MeOH, then eluted with 7 M NH₃/MeOH solution and removing the solvent under reduced pressure (19 mg, quant.). ¹H NMR (500 MHz, CD₃OD) δ 8.48 (1H, d, J = 8.9 Hz, H10), 8.40 (1H, d, J = 8.9 Hz, H1), 8.12 (1H, d, J = 2.2 Hz, H2), 7.67 - 7.62 (2H, m, H2’, H6’), 7.60 - 7.54 (3H, m, H3’, H4’, H5’), 3.80 (3H, s, 8-NHCO₂CH₃), 3.73 (3H, s, 3-NHCO₂CH₃). ¹³C NMR (126 MHz, CD₃OD) δ 161.49 (C), 154.94 (C), 154.91 (C), 142.90 (C), 139.18 (C), 139.07 (C), 137.64 (C), 129.35 (CH), 128.90 (C), 128.58 (CH), 128.02 (CH), 124.81 (C), 123.01 (CH), 122.45 (CH), 119.20 (CH), 119.09 (CH), 115.96 (CH), 51.24 (CH₃). IR (ATR cm⁻¹, as HCl salt): 3221 (N-H), 3056 (C=H), 2954 (C-H), 1729 (C=O), 1701 (C=O), 1622 (C=N), 1586 (C₆=C₆), 1575 (C₆=C₆), 1525 (C₆=C₆), 1071 (C-O). MS (NSI⁺): 1204 [(3M+H)⁺, 9%], 803 [(2M+H)⁺, 49], 402 [(M+H)⁺, 100]. HRMS: 402.1446. C₃₃H₂₈N₄O₄ requires 402.1448. MP (as HCl salt): Decomposition above 325 °C.

3,8-Bis(carboxethoxyamino)-6-phenylphenanthridine 140

3,8-Diamino-6-phenylphenanthridine 133 (4.05 g, 14.2 mmol, 1.00 eq.) was dissolved in THF (112 mL) and cooled to –11 °C stirring under argon. NaHCO₃ (2.92 g, 34.8 mmol, 2.45 eq.) was added followed by dropwise addition of ethyl chloroformate (3.3 mL, 35 mmol, 2.4 eq.), after which the
mixture was stirred for 3 h then allowed to warm to RT and stirred under argon for 44 h. The mixture was quenched into MeOH then the solvent removed under reduced pressure. The residue was partitioned between ETOAc and 1 M NH₃(aq), and the organic layer washed with water (x 3) and concentrated under reduced pressure, then 1 M HCl(aq) was added and the mixture cooled to ~5 °C for 16 h. The mixture was filtered and washed with EtOAc, then the filter cake dissolved in DCM/MeOH/1 M NH₃(aq) and the solvent removed under reduced pressure. The residue was dissolved in EtOAc and washed with water (x 2), dried (MgSO₄), filtered and the solvent removed under reduced pressure, giving bisethyl carbamate 140 as a yellow solid (5.82 g, 95%). ¹H NMR (400 MHz, DMSO) ¹H NMR (400 MHz, DMSO) δ 10.02 (1H, s, br, 8-NHCO₂Et), 8.98 (1H, s, 3-NHCO₂Et), 8.73 (1H, d, J = 9.1 Hz, H10), 8.63 (1H, d, J = 9.1 Hz, H1), 8.31 (1H, d, br, J = 1.6 Hz, H4), 8.22 (1H, d, J = 2.1 Hz, H7), 7.96 (1H, dd, J = 9.0, 2.0 Hz, H9), 7.81 (1H, dd, J = 8.9, 2.2 Hz, H2), 7.74 - 7.67 (2H, m, H2’, H6’), 7.65 - 7.53 (3H, m, H3’, H4’, H5’), 4.19 (2H, q, J = 7.1 Hz, 8-NHCO₂CH₂CH₃), 4.11 (2H, q, J = 7.1 Hz, 3-NHCO₂CH₂CH₃), 1.29 (3H, t, J = 7.1 Hz, 8-NHCO₂CH₂CH₃), 1.22 (3H, t, J = 7.1 Hz, 8-NHCO₂CH₂CH₃). ¹³C NMR (101 MHz, DMSO) δ 160.38 (C=O), 153.59 (C), 153.55 (C), 143.29 (C), 139.51 (C), 139.28 (C), 138.79 (C), 129.54 (CH), 128.58 (CH), 128.20 (CH), 124.39 (C), 123.11 (CH), 122.79 (CH), 122.70 (CH), 118.92 (CH), 118.46 (C), 116.44 (CH), 115.05 (CH), 60.35 (CH₃), 14.50 (CH₃), 14.43 (CH₃). IR (ATR cm⁻¹): 3293 (N-H), 2917 (C=O), 2852 (C-H), 2852 (C-H), 2852 (C-H), 1707 (C=O), 1620 (C=O), 1590 (C=O), 1549 (C=O), 1522 (C=O), 1071 (C-O). MS (EI⁺): 429 (M⁺, 70%), 383 (M⁺ - C₃H₂OH, 100), 336 [(M – H)⁺ – 2C₂H₅OH – CO, 63], 295 (37). HRMS: 429.1688. C₂₆H₂₃O₄N₃ requires 429.1689. MP: 128 - 132 °C. This compound is known in literature.⁴¹⁷

1,6-Hexanetrifluoromethanesulfonyl 142

Triflic anhydride (10.0 g, 35.4 mmol, 1.00 eq.) was weighed under argon then dissolved in dry DCM (30 mL) and stirred at 0 °C under argon. Dry pyridine (2.8 mL, 35 mmol, 0.98 eq.) was added followed by 1,6-hexanediol 141 (1.70 mg, 14.4 mmol, 0.41 eq.). After stirring under argon at RT for 35 min the reaction mixture was quenched and washed with water (x 2) and the organic layer dried (MgSO₄), filtered and the solvent removed under reduced pressure. The residue was redissolved in Et₂O and filtered through a combination of SiO₂ and MgSO₄. Product 142 was isolated as a yellow solid (3.44 g, 63%). δ_H (400 MHz, CDCl₃): 4.55 (4H, t, J = 6.28 Hz, CH₂OTf), 1.94 - 1.79 (4H, m, CH₂CH₂OTf), 1.55 - 1.48 (4H, m, CH₂CH₂CH₂OTf) δ_C (100 MHz, CDCl₃): 118.64 (q, J = 254 Hz), 29.04, 24.58. ¹H NMR data agrees with literature.⁴¹⁷

Chapter 13: Experimental
6-Trifluoromethylsulfonylhex-1-yltriphenylphosphonium trifluoromethylsulfonate 143

Trflate 142 (197 mg, 0.515 mmol, 1.00 eq.) was dissolved in dry Et₂O (2.6 mL) and stirred under argon. Triphenylphosphine (124 mg, 0.43 mmol, 0.83 eq., previously dried by azeotrope in PhMe) was added and the mixture stirred for 1 h. The solvent was then removed under reduced pressure. The resulting solid 143 (318 mg) was of >90% estimated purity by ¹H NMR. ¹H NMR (500 MHz, CDCl₃) δ 7.91 - 7.61 (15H, m, PPh₃), 4.61 - 4.52 (2H, m, CH₂-6), 3.48 - 3.30 (2H, m, CH₂-1), 1.93 - 1.76 (2H, m, CH₂-5), 1.75 - 1.32 (6H, m, CH₂-2, CH₂-3, CH₂-4). The stability of this compound was too poor to collect further data.

6-Benzylxoyhexan-1-ol 145

1,6-Hexanediol 141 (49.34 g, 417.4 mmol, 7.075 eq.) was melted and dried by azeotrope with PhMe, which was removed under reduced pressure. Dry DMF (120 mL) was added and the reaction cooled to 0 °C stirring under argon. NaH (60% dispersion in mineral oil, 2.52 g, 63.0 mmol, 1.07 eq.) was added portionwise, then after 30 min stirring followed by dropwise addition of benzyl bromide (7.0 mL, 59 mmol, 1.0 eq.). After 10 min stirring the mixture was heated to 60 °C for 48 h. The solvent was then removed under reduced pressure. Column chromatography [SiO₂, loaded with Pet. ether, Pet. ether/EtOAc (3:1)] gave alcohol 145 as an oil (11.71 g, 95%). Rᵣ = 0.11 Pet. ether/EtOAc (3:1). ¹H NMR (400 MHz, CDCl₃) δ 7.39 - 7.24 (5H, m, Ph), 4.50 (2H, s, CH₂Ph), 3.64 (2H, t, J = 6.4 Hz, CH₂OH), 3.47 (2H, t, J = 6.6 Hz, CH₂OBn), 1.70 - 1.52 (4H, m, CH₂-2, CH₂-5), 1.47 - 1.33 (4H, m, CH₂-3, CH₂-4), 1.22 (1H, s, br, OH). ¹³C NMR (126 MHz, CDCl₃) δ 138.64, 128.36, 127.64, 127.51, 72.89, 70.33, 62.97, 32.72, 29.72, 26.02, 25.58. Data is consistent with literature values.⁴¹⁸

6-Benzyloxyhex-1-yltrifluoromethanesulfonate 146

Trifluoromethanesulfonic anhydride (10 g, 35 mmol, 1.3 eq.) was dissolved with stirring in dry DCM (55 mL) and cooled to 0 °C under argon. Addition of dry pyridine (2.8 mL, 35 mmol, 1.3 eq.) was followed by allowing the mixture to warm to RT and adding alcohol 145 (5.58 g, 26.8 mmol, 1.00 eq.), then stirring for 2 h under argon. The mixture was diluted with DCM and washed with water (x 2). The washings were extracted with DCM and the combined organics dried (MgSO₄), filtered, the solvent removed under reduced pressure and the residue washed through a small plug of SiO₂ with dry Et₂O, then the solvent removed under reduced pressure to give trifluoromethanesulfonyl 146 as a brown oil (7.15 g, 78%). ¹H NMR (400 MHz, CDCl₃) δ 7.39 - 7.27
phenanthridinium trifluoromethanesulfonate 150

Phenanthridine 140 (208 mg, 0.484 mmol, 1.00 eq.) and 2,6-diterbutyl-4-methylpyridine (283 mg, 1.38 mmol, 2.87 eq.) were combined under argon then trifluoromethanesulfonate 146 (704 mg, 2.07 mmol, 4.31 eq.) was added as a solution in dry PhNO_2 (3.0 mL). After 72 h stirring under argon the mixture was transferred to SiO_2 in DCM and washed with additional DCM, followed by elution with DCM/MeOH (9:1). Repeated column chromatography [SiO_2, loaded in DCM, DCM/MeOH gradient elution (33:1 - 3:2)] gave a soft yellow residue which was 2:1 Phenanthridinium 150 to DCM by weight (26 mg, 7%). 1H NMR (400 MHz, CDCl_3) δ 9.08 (1H, s, br, 3-NHCOEt), 8.55 (1H, d, br, J = 1.2 Hz, 8-NHCOEt), 8.34 (1H, d, J = 9.4 Hz, H1), 8.31 (1H, s, br, H4), 8.28 (1H, d, J = 9.3 Hz, H10), 8.13 (1H, dd, br, J = 9.2, 1.4 Hz, H2), 8.06 (1H, dd, J = 9.1, 2.1 Hz, H9), 7.75 (1H, d, J = 1.8 Hz, H7), 7.72 - 7.62 (3H, m, H3\'', H4\'', H5\''), 7.38 (2H, dd, J = 7.5, 1.7 Hz, H2\'', H6\''), 7.29 - 7.13 (5H, m, OCH_2Ph), 4.62 (2H, s, br, CH_2-1\''), 4.37 (2H, s, OCH_3Ph), 4.11 (2H, q, J = 7.1 Hz, 3-NHCOCH_2CH_3), 4.02 (2H, q, J = 7.1 Hz, 8-NHCOCH_2CH_3), 3.31 (2H, t, J = 6.4 Hz, CH_2-6\'''), 1.83 (2H, s, br, CH_2-2\''), 1.48 - 1.38 (2H, m, CH_2-5\''), 1.29 - 1.10 (4H, m, CH_2-3\'', CH_2-4\''), 1.23 (3H, t, J = 7.1 Hz, 3-NHCOCH_2CH_3), 1.14 (3H, t, J = 7.1 Hz, 8-NHCOCH_2CH_3). 13C NMR (126 MHz, CDCl_3) δ 161.94 (C), 154.07 (C), 153.58 (C), 143.36 (C), 140.03 (C), 138.63 (C), 133.92 (C), 131.83 (CH), 130.77 (C), 130.59 (C), 130.39 (CH), 128.83 (CH), 128.27 (CH), 128.03 (CH), 127.54 (CH), 127.44 (CH), 127.39 (CH), 125.02 (C), 124.38 (CH), 122.70 (CH), 120.89 (C), 120.67 (q, J = 320.3 Hz, CF_3), 118.07 (CH), 106.65 (CH), 72.87 (CH_2), 70.03 (CH_2), 61.61 (CH_2), 61.51 (CH_2), 54.79 (CH_2), 29.53 (CH_3), 29.24 (CH_3), 26.09 (CH_3), 25.28 (CH_3), 14.24 (CH_3), 14.21 (CH_3). IR (ATR cm\(^{-1}\)): 3259 (N-H), 2943 (C-H), 2858 (C-H), 1727 (C=O), 1622 (C=N), 1596 (C_\text{ar}=C_\text{ar}), 1552 (C_\text{ar}=C_\text{ar}), 1519 (C_\text{ar}=C_\text{ar}). MS (NSI\(^+\)): 620 [M\(^+\) (phenanthridinium cation), 100%]. HRMS: 620.3104. C_{38}H_{42}N_4O_5 requires 620.3119. MP: 73 - 85 °C.
3,8-Bis(carboethoxyamino)-5-(1'-hexan-6-ol)-6-phenylphenanthridinium trifluoromethanesulfonate 151

Phenanthridinium salt 150 (260 mg, 0.338 mmol, 1.00 eq.) was combined in MeOH (3.0 mL) with 10% palladium on carbon (50 mg, 0.047 mmol, 0.14 eq.), then the solution frozen in N$_2$(g), exposed to vacuum and then sealed and warmed to until the mixture melted. This process was repeated (x 2), then H$_2$(g) bubbled through the solution for 20 min and the reaction stirred at RT under H$_2$(g) for 20 h. The mixture was filtered through celite, eluting with DCM and MeOH, then dried (MgSO$_4$), filtered and the solvent removed under reduced pressure. Column chromatography [SiO$_2$] gave alcohol 151 as a yellow foam (40 mg, 17%). R$_f$ = 0.25 DCM/MeOH (17:3). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.57 (1H, s, br, 3-NHCO$_2$Et), 10.28 (1H, s, br, 8-NHCO$_2$Et), 9.08 (1H, d, $J = 9.4$ Hz, H1), 9.02 (1H, d, $J = 9.2$ Hz, H10), 8.68 (1H, s, br, H4), 8.26 (1H, dd, $J = 9.3$, 2.0 Hz, H2), 8.14 (1H, dd, $J = 9.2$, 1.9 Hz, H9), 7.86 - 7.73 (6H, m, H7, Ph), 4.53 (2H, s, br, CH$_2$-1’), 4.32 (1H, t, $J = 5.1$ Hz, OH), 4.26 (2H, q, $J = 7.1$ Hz, 3-NHCO$_2$CH$_2$CH$_3$), 4.09 (2H, q, $J = 7.1$ Hz, 8-NHCO$_2$CH$_2$CH$_3$), 1.97 (2H, s, br, CH$_2$-2’), 1.51 - 1.36 (2H, s, br, CH$_2$-5’), 1.35 - 1.09 (4H, m, CH$_2$-3’, CH$_2$-4’), 1.32 (3H, t, $J = 7.1$ Hz, 3-NHCO$_2$CH$_2$CH$_3$), 1.20 (3H, t, $J = 7.1$ Hz, 8-NHCO$_2$CH$_2$CH$_3$). (ATR cm$^{-1}$): 3058 (C=Ar-H), 2939 (C-H), 2870 (C=O), 1729 (C=O), 1623 (C=N), 1596 (C=Ar-H), 1553 (C=Ar=H), 1519 (C=Ar=H). MS (ESI$^+$): 530 [M$^+$ (phenanthridinium cation), 100%]. HRMS: 530.2664. C$_{32}$H$_{36}$N$_2$O$_5$ requires 530.2649.

5-Nitro-2-(4'-nitrophenyl)aniline 157

Biphenyl-2-ylamine 156 (13.8 g, 81.3 mmol, 1.00 eq.) was dissolved in concentrated H$_2$SO$_4$(aq) (98%, 170 mL) at 0 °C with mechanical stirring. After the initial heat from neutralisation had dissipated KNO$_3$ (16.2 g, 161 mmol, 1.98 eq.) was added portion wise over 20 min. The mixture was then kept at 0 °C stirring for 4 h 30 min before being quenched slowly into ice water then basified with NaOH$_{aq}$ (7 M). The suspension formed was filtered. The filter cake was dissolved in acetone and solvent removed under reduced pressure. The solid was washed repeatedly with water to remove inorganic impurities, then transferred in acetone and dried by azoetric in PhMe (x 3) before being dried under vacuum at 80 °C for 6 h. To give amine 157 as an orange solid (17.1 g, 84%). $\delta$$_A$ (400 MHz, d$_5$-DMSO): 8.33 (2H, d, $J = 8.8$ Hz, H3’, H5’), 7.76 (2H, d, $J = 8.8$ Hz, H2’, H6’), 7.65 (1H, d, $J = 2.4$ Hz, H6), 7.45 (1H, dd, $J = 8.4$, 2.4 Hz, H4), 7.29 (1H, d, $J = 8.4$ Hz, H3), 5.76 (2H, s, NH$_2$). $\delta$$_C$ (400 MHz, d$_5$-DMSO): 148.21 (C), 146.75 (C), 146.66 (C), 144.61 (C), 131.24 (CH), 129.95 (CH), 129.03 (C), 123.95 (CH), 110.36 (CH), 109.03 (CH). IR (ATR cm$^{-1}$): 3470 (NH), 3381 (NH), 3106 (C=Ar-H), 3086 (C=Ar-H), 1632 (C=Ar=CN$_2$), 1597 (C=Ar=CN$_2$), 1501 (NO$_2$), 1337 (NO$_2$). MS (Cl) 260 [(M+H$^+$)$^+$, 9%], 230, 200 [(M+H$^+$)$^+$ – H$_2$N$_2$O$_2$, 27], 113, (47), 85 (73), 71 (100). HRMS: 260.0672.
\[ \text{C}_{12}\text{H}_{10}\text{O}_{4}\text{N}_{3} \text{ requires 260.0671. MP: 199 - 205 °C. Compound has been previously synthesised but limited data was reported.}^{143} \]

N-[5'-Nitro-2'-(4''-nitrophenyl)phenyl]benzamide 160

\[
\begin{align*}
\text{O}_2\text{N} & \quad \text{H} \\
\text{NH} & \quad \text{O} \\
\text{O} & \quad \text{O}
\end{align*}
\]

Adapted from a literature procedure.\textsuperscript{185} Dry NEt\textsubscript{3} (0.15 mL, 1.1 mmol, 1.1 eq.) was added to a solution of amine 157 (295 mg, 0.99 mmol, 1.00 eq.) in dry DCM (8.4 mL) stirring under argon, followed by dropwise addition of benzoyl chloride (180 µL, 1.55 mmol, 1.57 eq.). After 1 h stirring under argon the mixture was quenched into 0.5 M HCl\textsubscript{(aq)}, then extracted into DCM and washed (0.5 M HCl\textsubscript{(aq)} x 1, 0.5 M NaOH\textsubscript{(aq)} x 2). The collected organic layer was dried (MgSO\textsubscript{4}), filtered, then the solvent removed under reduced pressure and the solid dried by azeotrope in PhMe and then further evaporated with CHCl\textsubscript{3}. The material was crystallised (acetone/water) to give the amide 160 (267 mg, 74%) as a solid.

\[ \text{δ}_1 (400 MHz, d_6\text{-DMSO}): 10.52 (1H, s, br, NHCO), 8.59 (1H, d, J = 2.4 Hz, H6'), 8.41 (2H, d, J = 8.8 Hz, H3'', H5''), 8.36 (1H, dd, J = 8.6, 2.4, H4'), 7.94 - 7.88 (4H, m, H2'', H2, H6, H6''), 7.88 (1H, d, J = 8.6 Hz, H3'), 7.69 (1H, tt, J = 7.4 Hz, 1.3 Hz, H4) 7.64 - 7.56 (2H, m, H3, H5). \text{δ}_2 (400 MHz, d_6\text{-DMSO}): 165.76 (C), 147.42 (C), 147.04 (C), 144.52 (C), 141.61 (C), 136.49 (C), 133.79 (C), 131.89 CH), 131.71 (CH), 129.82 (CH), .128.44 (C), 127.64 (CH), 123.63 (CH), 121.98 (CH), 121.02 (CH). IR (ATR cm\textsuperscript{-1}): 3214 (CON-H), 1645 (C=O), 1597 (C\text{-Ar} = C\text{-Ar}), 1510 (NO\textsubscript{2}), 1341 (NO\textsubscript{2}). MP: 232 °C. MS (ESI\textsuperscript{-}): 362 [(M-\text{H})\textsuperscript{-}], 100]. HRMS: 362.0767. \text{C}_{19}\text{H}_{12}\text{N}_{3}\text{O}_{5} \text{ requires 362.0782.}

6-(Tetrahydropyran-2'-yloxy)hexan-1-ol 161

Previously dried by azeotrope with PhMe, 1,6-hexanediol 141 (40.0 g, 338 mmol, 4.02 eq.) was melted at 60 °C stirring under argon. Addition of para toluenesulfonic acid monohydrate (3.0 g, 16 mmol, 0.2 eq.) appeared to cause some precipitation, so the mixture was heated to 90 °C and allowed to cool back to 50 °C prior to addition of 3,4-dihydropyran (7.7 mL, 84 mmol, 1.0 eq.) and stirring for 72 h at this temperature under argon. The reaction mixture was washed repeatedly with water then the solvent removed under reduced pressure. Column chromatography [SiO\textsubscript{2}, Pet. ether/EtOAc (1:1)] gave the alcohol 161 as an oil (10.3 g, 61%). Pet. ether/EtOAc (1:1) R\textsubscript{i} = 0.39. \text{δ}(400 MHz, CDCl\textsubscript{3}): 4.57 (1H, dd, J = 4.5, 2.8 Hz, H2'), 3.86 (1H, ddd, J = 11.1, 7.3, 3.6 Hz, H6'\text{ax}), 3.78 - 3.69 (1H, m, CH\textsuperscript{4}H\textsuperscript{6}OTHP), 3.68 - 3.61 (2H, m, CH\textsubscript{2}OH), 3.54 - 3.47 (1H, m, H6'\text{eq}), 3.39 (1H, m, CH\textsuperscript{4}H\textsuperscript{6}OTHP), 1.89 - 1.76 (1H, m, H3') 1.76 - 1.67 (1H, m), 1.67 - 1.47 (8H, m, CH\textsubscript{2}), 1.47 - 1.34 (4H, m, CH\textsubscript{2}), 1.28 (1H, s, br, OH). \textsuperscript{1}H NMR data agrees with literature.\textsuperscript{420}

\[ \text{C}_{19}\text{H}_{12}\text{N}_{3}\text{O}_{5} \text{ requires 362.0767.} \]
2- (6'-iodohex-1'-yloxy)tetrahydropyran 162

Triphenylphosphine (2.62 g, 10.0 mmol, 2.13 eq.) and imidazole (664 mg, 9.75 mmol, 2.07 eq.) were dried by azeotrope in PhMe then dissolved in dry DCM (30 mL). Iodine (2.55 g, 9.96 mmol, 2.12 eq.) was added at 0 °C to the stirring solution. After 15 min the mixture was warmed to RT and alcohol 161 (950 mg, 4.70 mmol, 1.00 eq.) was added dropwise in DCM (1.0 mL). The mixture was then stirred under argon for 4 h, then quenched into water and extracted in DCM. The solvent was removed under reduced pressure. Column chromatography [SiO₂, Pet. ether/EtOAc (19:1)] gave iodide 162 as an oil (620 mg, 42%). Rᵣ = 0.58 Pet. ether/EtOAc (19:1). δ_H (400 MHz, CDCl₃): 4.57 (1H, dd, J = 4.4, 2.8 Hz, H1), 3.92 - 3.81 (1H, ddd, J = 11.0, 7.3, 3.4 Hz, H₆⁻ax), 3.74 (1H, dt, J = 9.6, 6.7 Hz, C₇H₇H₈OTHP), 3.54 - 3.47 (1H, m, H₆⁻eq), 3.39 (1H, dt, J = 9.6, 6.5 Hz, CH₃H₈OTHP), 3.19 (2H, t, J = 7.1 Hz, CH₂), 1.90 - 1.78 (3H, m, CH₂CH₂J, 1 of CH₂), 1.76 - 1.67 (1H, m, 1 of CH₂), 1.66 - 1.48 (6H, m, 3 x CH₂), 1.48 - 1.35 (4H, m, 2 x CH₂). δ_C (100 MHz, CDCl₃): 98.80, 67.31, 62.29, 33.37, 30.67, 30.22, 29.43, 25.40, 25.14, 19.60, 6.97. ¹_H and ¹³_C NMR match literature data.⁴²¹

6-(Tetrahydropyran-2’-yloxy)hexanal 164

Oxalyl chloride (10.0 mL, 120 mmol, 2.9 eq.) was added at –78°C to DCM (160 mL) stirring under argon. After 10 min DMSO (8.5 mL, 120 mmol, 2.9 eq.) was syringed into the mixture which was then stirred for a further 40 min. Alcohol 161 (8.3 g, 41 mmol, 1.0 eq.) was then added as a DCM solution (80 mL) and the reaction allowed to stir at –78 °C under argon for 2 h. NEt₃ (30.0 mL, 215 mmol, 5.24 eq.) was then added and the reaction allowed to warm to RT overnight. The mixture was washed with 0.5 M HCl(aq), which was back extracted with DCM. The combined organics were washed with water, then dried (MgSO₄), filtered and the solvent removed under reduced pressure. The residue was washed through SiO₂ with Et₂O, then dried (MgSO₄), filtered and the solvent removed under reduced pressure, giving aldehyde 164 as an oil (7.7 g, 93%). (400 MHz, CDCl₃): 9.77 (1H, t, J = 1.7 Hz, C(O)H), 4.60 - 4.54 (1H, m, H⁻2'), 3.92 - 3.81 (1H, m, H⁻6'), 3.80 - 3.69 (1H, m, CH₃H₈OTHP) 3.58 - 3.45 (1H, m, H⁻6'), 3.43 - 3.35 (1H, m, CH₃H₈OTHP), 2.45 (2H, td, J = 7.3, 1.8 Hz, CH₂C=O), 1.9 - 1.3 (12H, m, CH₂). Data consistent with literature.⁴²²
Methyl 5-[[5'-nitro-2'-(4''-nitrophenyl)phenyl]carbamoyl]pentanoate 172

6-Methoxy-6-oxohexanoic acid 171 (1.0 mL, 5.8 mmol, 2.0 eq.) was dissolved in SOCl₂ (3.0 mL, 41.2 mmol, 7.1 eq.) and heated stirring under reflux and argon for 13 h. The SOCl₂ was then removed under reduced pressure. Some of this material (1.0 g, 5.6 mmol, 1.9 eq.) was dissolved in dry DCM (3.0 mL) and added dropwise to a stirring solution of amine 157 (772 mg, 2.98 mmol, 1.00 eq.) in dry DCM (30 mL) under argon. After 17 h stirring the mixture was quenched into water, then the layers separated and the organic layer washed with 0.5 M HCl (aq) (x 1) and 0.5 M NaOH (aq) (x 2), then dried (MgSO₄) and filtered. The solvent was evaporated off at atmospheric pressure then traces removed under vacuum. The mixture was then dry loaded onto celite in DCM. Column chromatography [SiO₂, Pet. ether/EtOAc, gradient elution (7:3 to 3:7)] gave 1.02 g (86%) gave amide as a solid 172. δₜ (400 MHz, CDCl₃): 9.10 (1H, d, J = 1.8 Hz, H6'), 8.40 (2H, d, J = 8.8, H3'', H5''), 8.09 (1H, dd, J = 8.4 Hz, H4''), 7.76 (2H, d, J = 8.8 Hz, H2'', H6''), 7.43 (1H, d, J = 8.4 Hz, H3'), 7.24 (1H, s, br, NHCO), 3.65 (3H, s, CO₂C₃H₃), 2.33 (2H, t, J = 7.0 Hz, CH₂CO₂Me) 2.28 (2H, t, J = 6.9 Hz, CH₂CONH), 1.72 - 1.59 (4H, m, NCOCH₂C₃H₂C₃H₂CH₂CO₂Me). δₐ (100 MHz, CDCl₃): 173.83 (C), 170.99 (C), 148.46 (C), 148.16 (C), 143.05 (C), 136.45 (C), 135.68 (C), 130.70 (CH), 130.06 (CH), 124.59 (CH), 119.54 (CH), 118.03 (CH), 51.66 (CH₃), 36.96 (CH₃), 33.42 (CH₂), 24.59 (CH₂), 24.01 (CH₂), 17.24 (1H, s, br, NHCO), 3.70 [(M+H)+ – MeOH, 13], 154 (100). HRMS (FAB): 402.1298 requires 402.1301 C₁₉H₂₀O₇N₃.

MP: 154 - 156 °C.

Methyl 5-N-[[5'-nitro-2'-(4''-nitrophenyl)phenyl]carbamoyl]pentanoic acid 173

Ester 172 (199 mg, 0.50 mmol, 1.00 eq.) was suspended in ethanol (2.5 mL) and 7 M KOH (aq) was added (0.25 mL, 1.8 mmol, 3.5 eq.). The solution was then stirred for 17 h at RT under argon and quenched into water. The mixture was extracted with Et₂O and EtOAc (x 2). The aqueous layer was acidified with 0.5 M HCl (aq) and the precipitate filtered off. After drying the solid was dissolved in acetone then dried (MgSO₄) and filtered. After removal of solvent under reduced pressure the product 173 was collected as a beige solid (147 mg, 77%). δₜ (400 MHz, CD₂OD): 8.50 (1H, d, J = 2.3 Hz, H6'), 8.35 (2H, d, J = 8.8 Hz, H3'', H5''), 8.20 (1H, dd, J = 8.5, 2.3 Hz, H4''), 7.67 (2H, d, J = 8.8 Hz, H2'', H6''), 7.63 (1H, d, J = 8.5 Hz, H3''), 2.30 (2H, t, J = 7.1 Hz, CH₂CO₂H), 2.27 (2H, t, J = 7.1 Hz, CH₂CONH), 1.66 - 1.49 (4H, m, CH₃CH₂CH₂CH₂O₂H). δₐ IR (ATR cm⁻¹): 3233 (NH), 3086 (C₉H₇), 3032 (C₉H₇-
193

H), 2940 (C-H), 2870 (C-H), 1705 (C=O), 1528 (C=O), 1505 (NO2), 1335 (NO2). MP: 187 °C. Used without further analysis.

6-[(5'-Nitro-2'-(4''-nitrophenyl)phenyl)amino]hexan-1-ol 174

BH3-THF (24.6 mL of a 1 M solution, 24.6 mmol, 4.01 eq.) was added dropwise to a stirred solution of carboxylic acid 173 (2.38 g, 6.14 mmol, 1.00 eq.) in dry THF (30 mL) under argon. The mixture was then heated to reflux for 2 h. On cooling the solution was quenched into MeOH and filtered through MgSO4 eluting with DCM and CHCl3. The solvent was then removed under reduced pressure and the material dried by azeotrope with PhMe. Column chromatography [SiO2, Pet. ether/EtOAc (1:1)] and drying under high vacuum gave 174 as a red solid (1.53 g, 69%). Rf = 0.41 in Pet. ether. ether/EtOAc (1:1).

1H NMR (400 MHz, CDCl3): 8.36 (2H, d, J = 8.8 Hz, H3'', H5''), 7.61 (2H, d, J = 8.8 Hz, H2'', H6''), 7.63 - 7.60 (1H, m, H4''), 7.50 (1H, d, J = 2.2 Hz, H6''), 7.18 (1H, d, J = 8.2 Hz, H3''), 3.95 (1H, t, D2O ex., J = 5.1 Hz, NH), 3.65 (2H, td, J = 6.4, 5.3 Hz, CH2OH), 3.18 (2H, td, J = 7.1, 5.4 Hz, CH2NHR), 1.65 - 1.52 (4H, m, CH2CH2NH, CH2CH2OH), 1.45 - 1.35 (4H, m, CH2CH2CH2-CH3), 8.36 (1H, t, J = 5.3 Hz, OH).

IR (ATR cm⁻¹): 3626 (OH), 3356 (NH), 3109 (CAr-H), 2932 (C-H), 2862 (C-H), 1597 (CAr=CAr), 1512 (NO2), 1335 (NO2). MS (FAB): 360 [(M+H)+, 100%], 154 (44). HRMS (FAB): 360.1560. C18H22O5N requires 360.1559. MP: 75 - 76 °C.

6'-[(5''-Nitro-2''-(4'''-nitrophenyl)phenyl)amino]hexan-1'-yl benzoate 175

Alcohol 174 (542 mg, 1.51 mmol, 1.00 eq.) was dissolved in dry DCM (22 mL) stirring under argon. Dry NEt3 (0.25 mL, 1.8 mmol, 1.2 eq.) was added, followed by benzoyl chloride (160 µL, 1.38 mmol, 0.914 eq.). The mixture was stirred for 20 h at RT under argon, then quenched into 1 M NaOH(aq) and extracted with DCM. The organic layer was washed with 1 M HCl(aq) and 1 M NaOH(aq), dried (MgSO4), filtered and the solvent removed under reduced pressure. Column chromatography [SiO2, loaded in minimal DCM, gradient elution with Pet. ether/EtOAc (4:1) - (1:1)] gave ester 175 as an orange solid (572 mg, 89%). Rf = 0.8 in Pet. ether/EtOAc (1:1). 1H NMR (500 MHz, CDCl3) δ 8.37 (2H, d, J = 8.9 Hz, H3'''', H5'''''), 8.04 (dd, J = 8.4, 1.3 Hz, H2, H6), 7.66 - 7.60 (3H, m, H4'', H2'', H6'''''), 7.57 (1H, tt, J = 7.4, 1.4 Hz, H4), 7.52 (1H, d, J = 2.2 Hz, H6'''''), 7.45 (2H,
dd, \( J = 7.9, 7.5 \text{ Hz}, \text{H}3, \text{H}5 \), 7.20 (1H, d, \( J = 8.2 \text{ Hz}, \text{H}3'\)), 4.34 (2H, t, \( J = 6.6 \text{ Hz}, \text{CH}2-1'\)), 3.97 (1H, t, \( J = 5.2 \text{ Hz}, \text{NH} \)), 3.21 (2H, dt, \( J = 5.5, 7.1 \text{ Hz}, \text{CH}2-6'\)), 1.86 - 1.76 (2H, quin., \( J = 6.6 \text{ Hz}, \text{CH}2-2'\)), 1.69 - 1.61 (2H, quin., \( J = 7.3 \text{ Hz}, \text{CH}2-5'\)), 1.56 - 1.41 (4H, m, \text{CH}2-3', \text{CH}2-4'\)). \(^{13}C\) NMR (126 MHz, CDCl\(_3\)) \& 166.61 (C), 149.44 (C), 147.70 (C), 145.79 (C), 144.32 (C), 132.91 (CH), 130.54 (CH), 130.34 (C), 129.97 (CH), 129.49 (CH), 128.36 (CH), 124.56 (CH), 111.72 (CH), 104.95 (CH), 64.66 (CH\(_2\)), 43.74 (CH\(_3\)), 28.87 (CH\(_3\)), 28.61 (CH\(_2\)), 26.66 (CH\(_2\)), 25.68 (CH\(_2\)). IR (ATR cm\(^{-1}\)): 3405 (N\(\equiv\)H), 2934 (C=O), 2856 (C\(\equiv\)H), 1784 (C=O), 1722, 1614 (C\(_A\)=C\(_A\)), 1585 (C\(_A\)=C\(_A\)), 1516 (NO\(_2\)), 1344 (NO\(_2\)). MS (NSI\(^{+}\)): 949 [(2M+Na\(^{+}\), 16%), 502 [(M+K\(^{+}\), 29), 486 [(M+Na\(^{+}\), 65), 464 [(M+H\(^{+}\)), 100]. HRMS: 464.1814. C\(_{25}\)H\(_{30}\)N\(_3\)O\(_6\) requires 465.1848. MP: 83 - 85 °C.

6-[[5'-nitro-2'-(4''-nitrophenyl)phenyl]amino]hexan-1-yl trifluoroethanoate 180

Alcohol 174 (49 mg, 0.13 mmol, 1.0 eq.) and benzaldehyde (30 µL, 0.29 mmol, 2.2 eq.) were combined in TFA (0.3 mL) then sealed in an argon flushed tube and heated to 120 °C for 2 days, then 125 °C for a further 3 days. The solvent was then removed under reduced pressure. Column chromatography gave trifluoroacetic ester 180 as a brown oil (13 mg, 21%). \(^1H\) NMR (500 MHz, CDCl\(_3\)) \& 8.38 (2H, d, \( J = 8.9 \text{ Hz}, \text{H}3''\), H5''), 7.67 - 7.60 (3H, m, H2'', H6'', H4''), 7.52 (1H, d, \( J = 2.2 \text{ Hz}, \text{H}6'\)), 7.21 (1H, d, \( J = 8.2 \text{ Hz}, \text{H}3'\)), 4.37 (2H, t, \( J = 6.6 \text{ Hz}, \text{CH}2-1\)), 3.97 (1H, s, NH), 3.21 (2H, t, \( J = 7.1 \text{ Hz}, \text{CH}2-6\)), 1.83 - 1.74 (2H, m, CH2-2), 1.68 - 1.60 (2H, m, CH2-5), 1.50 - 1.39 (4H, m, CH2-3,4). \(^{13}C\) NMR (126 MHz, CDCl\(_3\)) \& 157.52 (q, \( J = 42.2 \text{ Hz}, \text{C} \)), 149.44 (C), 147.72 (C), 145.73 (C), 144.30 (C), 130.57 (CH), 129.97 (CH), 124.55 (CH), 114.50 (q, \( J = 285.6 \text{ Hz}, \text{CF}3 \)), 111.80 (CH), 104.94 (CH), 67.91 (CH\(_3\)), 43.71 (CH\(_3\)), 28.83 (CH\(_2\)), 28.01 (CH\(_2\)), 26.54 (CH\(_2\)), 25.29 (CH\(_2\)). IR (ATR cm\(^{-1}\)): 2934 (C-H), 2856 (C-H), 1784 (C=O), 1722, 1614 (C\(_A\)=C\(_A\)), 1597 (C\(_A\)=C\(_A\)), 1516 (NO\(_2\)), 1341 (NO\(_2\)). MS (APCI\(^{+}\)): 456 [(M+H\(^{+}\), 100%), 356 [(M+H\(^{+}\) – CF\(_3\)CO\(_2\)H], 260 [(M+H\(^{+}\) – CF\(_3\)CO\(_2\)H – C\(_{2}H_{12}\)]. HRMS: 456.1369. C\(_{25}\)H\(_{30}\)F\(_{3}\)N\(_3\)O\(_6\) requires 456.1377.

2-Iodo-5-nitrobenzophenone 218

2-amino-5-nitrobenzophenone 217 (15.0 g, 42.5 mmol, 1.00 eq.) was dissolved in DMSO (300 mL). H\(_2\)SO\(_4\)(aq) (30%, 300 mL) was added to the stirring mixture at 0 °C over 5 min and the mixture stirred at this temperature for 30 min. A 3.7 M solution of NaN\(_2\)(aq) (70 mL, 260 mmol, 6.1 eq.) was added dropwise and the mixture stirred for 3 h at 0 °C. K\(_{2}\)(aq) was added dropwise as an 5.6 M solution (90 mL, 500 mmol, 12 eq.) and the mixture allowed to warm to RT and stir for 48 h. The
reaction mixture was then diluted (H$_2$O) and extracted with EtOAc. The combined organics were washed sequentially with saturated NaHCO$_3$(aq), saturated Na$_2$SO$_3$(aq) and water before drying (MgSO$_4$), filtering and removal of solvent under reduced pressure. This gave benzophenone 218 as a yellow solid (21.4 g, 98%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.16 (1H, d, $J = 8.6$ Hz, H3), 8.12 (1H, d, $J = 2.6$ Hz, H6), 8.02 (1H, dd, $J = 8.6$, 2.7 Hz, H4), 7.82 - 7.78 (2H, dd, $J = 8.4$, 1.3 Hz, H2', H6'), 7.68 (1H, ddt, $J = 7.8$, 7.0, 1.3 Hz, H4), 7.52 (2H, t, $J = 7.8$ Hz, H3', H5'). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 195.10 (C), 147.88 (C), 146.04 (C), 141.28 (C), 134.71 (C), 134.65 (C), 130.60 (CH), 129.21 (CH), 125.32 (CH), 123.00 (CH), 100.81 (C). Consistent with literature data.  

2-(4''-Methoxybenzene)-5-nitrobenzophenone 220

Benzophenone 218 (101 mg, 0.286 mmol, 1.00 eq.), 4-methoxyphenylboronic acid 219 (68 mg, 0.45 mmol, 1.5 eq.), NaHCO$_3$ (83 mg, 0.99 mmol, 3.4 eq.), PhMe (2.8 mL) and MeOH (0.3 mL) were combined and under argon and frozen with N$_2$(l), then placed under vacuum and melted. This process was repeated twice, then Pd(PPh$_3$)$_4$ was added (34 mg, 0.030 mmol, 0.10 eq.) and the mixture heated to 90 °C stirring under argon for 16 h. The mixture was partitioned between Et$_2$O and water, then the aqueous further extracted with Et$_2$O and the combined organics dried (MgSO$_4$), filtered and the solvent removed under reduced pressure. Column chromatography [SiO$_2$, Pet. ether/EtOAc (7:1)] gave the coupled biaryl 220 as a thick yellow oil (41 mg, 43%). R$_f$ = 0.24, Pet. ether/EtOAc (7:1). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.39 (1H, dd, $J = 8.5$, 2.4 Hz, H4), 8.35 (1H, d, $J = 2.3$ Hz, H6), 7.64 (3H, apparent d, $J = 8.6$ Hz, H3, H2', H6'), 7.48 (1H, tt, $J = 7.5$, 1.3 Hz, H4'), 7.33 (1H, t, $J = 7.8$ Hz, H3', H5'), 7.22 (2H, d, $J = 8.8$ Hz, H2'', H6''), 6.78 (2H, d, $J = 8.8$ Hz, H3'', H5''). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 196.50 (C), 160.20 (C), 147.08 (C), 146.50 (C), 139.82 (C), 136.34 (C), 133.78 (CH), 131.11 (CH), 130.47 (C), 130.32 (CH), 130.00 (CH), 128.63 (CH), 124.99 (CH), 124.02 (CH), 114.35 (CH), 55.40 (CH$_3$). (ATR cm$^{-1}$): 3065 (C=H), 2961 (C=H), 2934 (C-H), 2837 (C-H), 1663 (C=O), 1604 (C$_{Ar}$=C$_{Ar}$), 1579 (C$_{Ar}$=C$_{Ar}$), 1516 (NO$_2$), 1342 (NO$_2$). MS (Cl$^+$): 334 [(M+H)$^+$, 100%], 304 [(M+H)$^+$ – OCH$_3$, 62]. HRMS: 334.1081. C$_{20}$H$_{16}$O$_4$N requires 334.1079.
3-Nitrobenzophenone 228

Iodobenzophenone 218 (107 mg, 0.300 mmol, 1.00 eq.), trifluoroborate salt 285 (70 mg, 0.35 mmol, 1.2 eq.), PEPPSI-iPr catalyst (4.0 mg, 0.0059 mmol, 0.02 eq.) and K₂CO₃ (124 mg, 0.900 mmol, 3.00 eq.) were combined under argon then the flask evacuated and refilled with argon (x 2). EtOH (degassed with argon, 4 mL) was added and the mixture stirred under reflux and argon for 18 h. The mixture was then diluted with Et₂O and filtered through celite then dried (MgSO₄), filtered and the solvent removed under reduced pressure. Nitrobenzophenone 228 was estimated to be 65% of the crude by ¹H NMR; a sample was separated from the residue, which included dimer 229, by HPLC [C12, gradient elution; MeOH/0.1% HCO₂H (aq) (60:40) for 8 min, then gradient to (95:5) by 30 min.] for characterisation purposes. RT = 19.2 min. Rᵣ = 0.24 in Pet. ether/EtOAc (5:1). ¹H NMR (500 MHz, CDCl₃) δ 8.66 - 8.59 (1H, m, H₂), 8.48 - 8.42 (1H, m, H₆), 8.15 (1H, dd, J = 7.7, 1.0 Hz, H₄), 7.84 - 7.78 (2H, m, H₂', H₆'), 7.71 (1H, t, J = 7.9 Hz, H₅), 7.67 (1H, t, J = 7.5 Hz, H₄'), 7.54 (2H, t, J = 7.8 Hz, H₃', H₅'). ¹³C NMR (126 MHz, CDCl₃) δ 194.30 (C), 148.27 (C), 139.25 (C), 136.44 (C), 135.56 (CH), 133.51 (CH), 130.16 (CH), 129.78 (CH), 128.89 (CH), 126.86 (CH), 124.87 (CH). MS (El⁺): 227 (M⁺, 65%), 105 (C₆H₅CO⁺, 100). HRMS: 227.0584. C₁₃H₉O₃N requires 227.0582. Consistent with literature data.

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2,2'-Dibenzoyl-4,4'-dinitrobiphenyl 229

Iodobenzophenone 218 (1.00 g, 2.83 mmol, 1.12 eq.), PEPPSI-iPr catalyst (96 mg, 0.14 mmol, 0.05 eq.) and arylboronic acid 316 (510 mg, 2.53 mmol, 1.00 eq.) were divided evenly between two microwave tubes (10.0 mL) under argon. The tubes were opened to vacuum, then refilled with argon. This process was repeated twice then N,N-diisopropylethylamine (1.45 mL, 8.32 mmol, 3.29 eq.) and degassed ethanol (10.0 mL) were split between the tubes. Each tube was heated to 125 °C for 10 min. After cooling the mixtures were combined and partitioned between DCM and 1 M HCl (aq) then the aqueous layer extracted (DCM x 2). The combined organics were dried (MgSO₄),
filtered and the solvent removed under reduced pressure. Column chromatography [SiO₂, Pet.
Ether/EtOAc (15:1), after drying loading onto celite] gave dimer 229 as a yellow solid (24 mg, 2%).

¹H NMR (500 MHz, CDCl₃) δ 8.36 (2H, dd, J = 8.5, 2.4 Hz, H5, H5′), 8.27 (2H, d, J = 2.3 Hz, H3, H3′),
7.64 (4H, dd, J = 8.4, 1.2 Hz, H2″, H6″), 7.61 (2H, d, J = 8.5 Hz, H6, H6′), 7.40 (2H, tt, J = 7.5, 1.3 Hz,
H4″), 7.31 (4H, dd, J = 8.2, 7.5 Hz, H3″, H5″).

¹³C NMR (126 MHz, CDCl₃) δ 194.35 (C), 146.83 (C), 145.02 (C), 139.09 (C),
135.64 (C), 133.98 (CH), 132.41 (CH), 130.31 (CH), 128.57 (CH), 125.01 (CH), 124.22 (CH). (ATR cm⁻¹): 1667 (C=O), 1597 (C=Ar=Ar), 1526 (NO₂), 1346 (NO₂).


6-Benzylxyhexen-1-1 231

Alcohol 145 (11.5 g, 55.0 mmol, 1.00 eq.) was dissolved in DCM (550 mL) and cooled to 0 °C then
KBr (1.30 g, 10.9 mmol, 0.198 eq.) added, followed by TEMPO (88 mg, 0.56 mmol, 0.010 eq.) and
a combination of NaOCl (aq) (assumed 4%, 100 mL, 56.9 mmol, 1.03 eq.) and NaHCO₃ (aq) (140 mL,
160 mmol, 2.91 eq.). The mixture was stirred for 2 h, then additional NaOCl (aq) (23.0 mL, 13.1
mmol, 0.238 eq.) was added. The mixture was stirred for 2 h with addition of NaOCl (aq) (9.0 mL
5.12 mmol, 0.0936 eq. in total), then the biphasic mixture separated and the aqueous extracted
with DCM. The combined organics were dried (MgSO₄), filtered and the solvent removed under
reduced pressure to give aldehyde 231 as an oil (11.05 g, 97%).

¹H NMR (400 MHz, CDCl₃) δ 9.79 (1H, t, J = 1.8 Hz, CH₂C), 7.40 - 7.30 (5H, m, Ph), 4.52 (2H, s,
OC₂H₂Ph), 3.50 (2H, t, J = 6.5 Hz, CH₂-5), 2.46 (2H, td, J = 7.4, 1.7 Hz, CH₂-1), 1.74 - 1.60 (4H, m, CH₂-2, CH₂-4), 1.50 - 1.39 (2H, m,
CH₂-3). ¹³C NMR (126 MHz, CDCl₃) δ 202.83 (C), 138.67 (C), 128.51 (CH), 127.78 (CH), 127.68
(CH), 73.08 (CH₂), 70.16 (CH₂), 43.98 (CH₂), 29.65 (CH₂), 25.97 (CH₂), 22.03 (CH₂). Consistent with
literature data.⁴²³

6-Benzylxy-1-bromohexane 232

Alcohol 145 (1.50 g, 7.17 mmol, 1.00 eq.) was dissolved in dry DCM (26 mL) with stirring under
argon and cooled to 0 °C. CBr₄ (3.05 g, 9.18 mmol, 1.28 eq.) and PPh₃ (2.44 g, 9.30 mmol, 1.30 eq.)
were added successively, then the mixture stirred under argon for 20 min before being allowed to
warm to RT and stir for a further 17 h. The mixture was washed with brine and water, dried
(MgSO₄), filtered and the solvent removed under reduced pressure. Column chromatography
[SiO₂, Pet. ether/Et₂O (99:1)] followed by vacuum distillation to remove CHBr₃ gave alkyl bromide
232 as an oil (1.76 g, 91%). ¹H NMR (500 MHz, CDCl₃) δ 7.37 - 7.26 (5H, m, Ph), 4.50 (2H, s,
OCH₂Ph), 3.47 (2H, t, J = 6.5 Hz, CH₂-6), 3.40 (2H, t, J = 6.8 Hz, CH₂-1), 1.86 (2H, tt, J = 6.9, 6.8 Hz,
CH₂-5), 1.63 (2H, tt, J = 7.0, 7.0 Hz, CH₂-2), 1.50 - 1.36 (4H, m, CH₂-3, CH₂-4). ¹³C NMR (126 MHz,
CDCl₃) δ 138.74 (C), 128.51 (CH), 127.78 (CH), 127.67 (CH), 73.07 (CH₂), 70.34 (CH₂), 34.05 (CH₂),
32.90 (CH₂), 29.73 (CH₂), 28.15 (CH₂), 25.57 (CH₂). Data is consistent with literature.⁴²⁴
2-Nitro-4-tertbutylbromobenzene 239

A solution of HNO$_3$(aq) (1.0 mL, 16 mmol, 1.4 eq.) in H$_2$SO$_4$(aq) (98%, 2mL) was added to stirring 4-tertbutylbenzene at 0 °C. The mixture was allowed to gradually warm to RT and stirred under argon for 14 h. The reaction mixture was then partitioned with Pet. ether, and the aqueous re-extracted with Pet. ether. The combined organics were washed with brine then dried (MgSO$_4$), filtered and the solvent removed under reduced pressure to give nitroarene 239 as a yellow oil (2.82 g, 95%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.83 (1H, d, $J$ = 2.4 Hz, H3), 7.64 (1H, d, $J$ = 8.5 Hz, H6), 7.48 – 7.42 (1H, dd, $J$ = 8.5, 2.4 Hz, 1H), 1.34 [9H, s, C(CH$_3$)$_3$]. Consistent with literature data.

2-Amino-4-tertbutylbromobenzene 240

Bromobenzene 239 (3.5 g, 14 mmol, 1.0 eq.) was dissolved in a mixture of AcOH (60 mL) and EtOH (60 mL), and iron filings (4.06 g, 72.6 mmol, 5.34 eq.) added, then the mixture stirred and heated to 100 °C for 2 h under argon. The mixture was cooled then basified with NaOH$_{aq}$ (7 M) and extracted with EtOAc (x 2). The combined organics were washed with brine then dried (MgSO$_4$), filtered and the solvent removed under reduced pressure. The brown oil was dried by azeotrope with PhMe then dissolved in Pet. ether and dry loaded onto SiO$_2$, washed with pet. ether and eluted with Et$_2$O, then dried (MgSO$_4$), before filtering and removal of the solvent under reduced pressure, giving aniline 240 as a red oil (2.80 g, 91%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.31 (1H, d, $J$ = 8.4 Hz, H6), 6.79 (1H, d, $J$ = 2.3 Hz, H3), 6.66 (1H, dd, $J$ = 8.4, 2.3 Hz, H5), 4.02 (2H, s, br, NH$_2$), 1.27 [9H, s, C(CH$_3$)$_3$]. $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 151.97 (C), 143.59 (C), 132.10 (CH), 117.17 (CH), 113.27 (CH), 106.41 (C), 34.56 (C), 31.31 (CH$_3$). Consistent with literature data.

2-(6'-Benzyloxyhexylamino)-tertbutylbromobenzene 241

Aniline 240 (6.0 g, 26 mmol, 1.0 eq., dried by azeotrope with PhMe) was cooled to –15 °C under argon then dry TFA (34 mL) added and the material agitated and warmed until the material dissolved, then cooled to –15 °C. NaBH(OAc)$_3$ (12.0 g, 56.6 mmol, 2.18 eq.) was added and the mixture stirred for 30 min, then aldehyde 231 (10.1 g, 48.6 mmol, 1.87 eq., dried by azeotrope
with PhMe) was added portion wise as a solution in dry DCM (135 mL). After 20 min the mixture was quenched into NaOH (aq) (7 M) and extracted with DCM (x 2), then the combined organics dried (MgSO₄), filtered and the solvent removed under reduced pressure. Column chromatography [SiO₂, Pet. ether/Et₂O (20:1)] gave secondary amine 241 as an orange oil (7.86 g, 72%). Rᵣ = 0.30 in Pet. ether/Et₂O (10:1). ¹H NMR (500 MHz, CDCl₃) δ 7.36 - 7.26 (5H, m, Ph”), 7.31 (1H, d, J = 8.5 Hz, H6), 6.64 (1H, d, J = 2.2 Hz, H3), 6.59 (1H, dd, J = 8.3, 2.3 Hz, H5), 4.51 (2H, s, OCH₃Ph), 4.18 (1H, t, J = 5.0 Hz, NH), 3.48 (2H, t, J = 6.5 Hz, CH₂-6”), 3.16 (2H, dt, J = 7.0, 5.3 Hz, CH₂-1”), 1.75 - 1.61 (4H, m, CH₂-2”, CH₂-5”), 1.48 - 1.41 (4H, m, CH₂-3”, CH₂-4”), 1.29 [9H, s, C(CH₃)₃]. ¹³C NMR (126 MHz, CDCl₃) δ 151.87 (C), 144.60 (C), 138.68 (C), 131.69 (CH), 128.36 (CH), 127.64 (CH), 127.51 (CH), 114.92 (CH), 108.69 (CH), 106.69 (C), 72.94 (CH₂), 70.30 (CH₂), 43.84 (CH₂), 34.73 (C), 31.32 (CH₃), 29.73 (CH₃), 29.31 (CH₃), 27.02 (CH₂), 26.07 (CH₂). (ATR cm⁻¹): 3408 (N-H), 2933 (C-H), 2854 (C-H), 1588 (C₅=CH₃), 1511 (C₅=CH₃). MS (Cl⁻): 420 [⁸¹BrM+H]⁺, 96%, 418 [¹⁹BrM+H]⁺, 100%. HRMS: 420.1711 and 418.1748. C₂₃H₃₅ON⁸¹Br and C₂₃H₃₅ON⁷⁹Br require 420.1728 and 418.1746 respectively.

2-[N-(6’-benzyloxyhexamino)-tert-butylcarbamate]-4-tert-butylbromobenzene 245

Aniline 241 (5.425 g, 12.79 mmol, 1.000 eq.) and di-tert-butyl dicarbonate (5.66 g, 25.9 mmol, 2.02 eq.) were combined in dry THF (65 mL) at 0 °C under stirring under argon. Lithium hexamethyldisilazide (1.0 M in THF, 20.0 mL, 20 mmol, 1.6 eq.) was added over 10 min, then the mixture was allowed to warm to RT and stirred for 3 h. The reaction was cooled back to 0 °C and additional Lithium hexamethyldisilazide (5.0 mL, 5.0 mmol, 0.4 eq.) and di-tert-butyl dicarbonate (3.0 g, 14 mmol, 1.1 eq.) added, then the reaction allowed to warm to RT and stirred under argon for 17 h. The solvent was removed under reduced pressure, then the residue triturated with warm Et₂O and filtered. The combined organics were washed with NaHCO₃ (aq) and brine, then dried (MgSO₄), filtered and the solvent removed under reduced pressure. Hexamethylsilazide was distilled out of the product under high vacuum at 50 °C. Column chromatography [SiO₂, Pet. ether/Et₂O gradient from (20:1) - (10:1), using ceric ammonium molybdate solution to visualise residual hexamethylsilazide] gave carbamate 245 as a pink oil (5.42 g, 82%). Rᵣ = 0.17 in Pet. ether/Et₂O (5:1). ¹H NMR (400 MHz, CDCl₃) δ 7.54 - 7.45 (1H, m, H6), 7.37 - 7.26 (5H, m, Ph), 7.22 - 7.11 (2H, m, H5, H3), 4.51 - 4.46 (2H, m, OCH₃Ph), 3.81 - 3.63 (1H, m, CH₂-1”, 3.50 - 3.40 (2H, m, CH₂-6”), 3.34 - 3.21 (1H, m, CH₂-1’), 1.67 - 1.46 (8H, m, CH₂-2’, CH₂-3’, CH₂-4’, CH₂-5’), 1.43 - 1.24 (18H, m, C(CH₃)₂ x 2). Spectrum is complex due to the appearance of atropisomers on an NMR timescale - limited coalescence seen at 90 °C. ¹³C NMR (101 MHz, DMSO, recorded at 90 °C) δ 153.39, 151.32, 148.46, 140.71, 138.85, 132.31, 127.95, 127.69, 127.13, 127.01, 125.45, 119.61, 83.62, 79.01, 71.96, 69.69, 34.11, 30.76, 29.14, 27.92, 27.26, 26.15, 25.41. (ATR cm⁻¹): 2967 (C-H), 2936 (C-H), 2862 (C-H), 1697 (C=O). MS (Cl⁻): 520 [⁸¹BrM+H]⁺, 17%, 518 [⁷⁹BrM+H]⁺, 18%, 464
[\textsuperscript{\textit{81}}\text{BrM}+\text{H}]^+ - \text{C(CH}_3\text{)}_3 \text{, 100}, 462 \ [\textsuperscript{\textit{79}}\text{BrM}+\text{H}]^+ - \text{C(CH}_3\text{)}_3 \text{, 100}, 420 \ [\textsuperscript{\textit{81}}\text{BrM}+\text{H}]^+ - \text{C(CH}_3\text{)}_3 - \text{CO}_2 \text{, 30}, 418 \ [\textsuperscript{\textit{79}}\text{BrM}+\text{H}]^+ - \text{C(CH}_3\text{)}_3 - \text{CO}_2 \text{, 32}, 382 \ [\textit{M-H}]^+ - \text{Br} - \text{C(CH}_3\text{)}_3 \text{, 22}. \text{HRMS: } 520.2263 \text{ and } 518.2258. \text{C}_{28}\text{H}_{41}\text{O}_3\text{N}_{81}\text{Br and C}_{28}\text{H}_{41}\text{O}_3\text{N}_{79}\text{Br require } 520.2285 \text{ and } 518.2270.

2-[\textit{N-(6')-benzyloxyhexylamino}]-\text{tert-butylicarbamate]-4-\text{tert-butylibenzene} 248

Bromobenzene 245 (96 mg, 0.19 mmol, 1.00 eq.) was dissolved in dry THF (1.0 mL) under argon and the stirring solution cooled to \(-78\) °C. \textit{Butyllithium} (2.5 M in hexane, 90 µL, 0.23 mmol, 1.2 eq.) was added and the mixture stirred under argon for 1 h. MeOH (0.3 mL) was added then the mixture partitioned between Et\textsubscript{2}O and water then the organic layer dried (MgSO\textsubscript{4}), filtered and the solvent removed under reduced pressure. Column chromatography [SiO\textsubscript{2}, Pet. ether/Et\textsubscript{2}O (5:1)] gave carbamate 248 as an oil (61 mg, 75%). R\textsubscript{f} = 0.43 in Pet. ether/Et\textsubscript{2}O (2:1).

\textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 7.29 - 7.13 (7H, m, Ph, H\textsubscript{1}, H\textsubscript{3}), 7.10 (1H, s, br, H\textsubscript{5}), 6.91 (1H, d, \(J = 7.3\) Hz, H\textsubscript{6}), 4.42 (2H, s, OC\textsubscript{2}H\textsubscript{5}Ph), 3.54 (2H, apparent t, \(J = 7.5\) Hz, CH\textsubscript{2}-6'), 3.38 (2H, t, \(J = 6.6\) Hz, CH\textsubscript{2}-1'), 1.57 - 1.44 (4H, m, CH\textsubscript{2}-2', CH\textsubscript{2}-5'), 1.41 - 1.19 (22H, m, CH\textsubscript{2}-3', CH\textsubscript{2}-4', C(CH\textsubscript{3})\textsubscript{3} \times 2).

\textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \(\delta\) 170.61 (C), 152.20 (C), 135.27 (C), 131.65 (CH), 122.65 (CH), 119.54 (CH), 110.24 (C), 36.86 (CH\textsubscript{2}), 35.01 (C), 33.15 (CH\textsubscript{2}), 32.09 (CH\textsubscript{2}), 31.31 (CH\textsubscript{3}), 24.04 (CH\textsubscript{2}).

2-(5'-bromopentanamide)-\textit{tert}butylbromobenzene 255

5-Bromopentanoic acid 253 (900 mg, 4.97 mmol, 1.13 eq.) was dissolved in SOCl\textsubscript{2} and heated to reflux stirring under argon for 70 h. The SOCl\textsubscript{2} was removed by distillation then the residue dissolved in dry THF (10 mL). The mixture was cooled to 0 °C then a solution of bromoaniline 240 (1.0 g, 4.4 mmol, 1.0 eq.) in dry THF (30 mL) was added. The mixture was allowed to warm gradually to RT and stirred under argon for 17 h. The mixture was then quenched into NaHCO\textsubscript{3}(aq) and extracted into Et\textsubscript{2}O. The organic layer was washed with NaHCO\textsubscript{3}(aq), brine and water then dried (MgSO\textsubscript{4}), filtered and the solvent removed under reduced pressure. Column chromatography [SiO\textsubscript{2}, dry loaded onto celite with Et\textsubscript{2}O, dry loaded onto celite with Et\textsubscript{2}O (10:1)] to give amide 255 as an oil (621 mg, 36%). R\textsubscript{f} = 0.08 Pet. ether/Et\textsubscript{2}O (5:1). 

\textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 8.43 (1H, s, br, H\textsubscript{3}), 7.58 (1H, s, br, NH), 7.43 (1H, d, \(J = 8.5\) Hz, H\textsubscript{6}), 7.01 (1H, dd, \(J = 8.5, 2.2\) Hz, H\textsubscript{5}), 3.45 (2H, t, \(J = 6.5\) Hz, CH\textsubscript{2}-5'), 2.48 (2H, t, \(J = 7.1\) Hz, CH\textsubscript{2}-2'), 2.04 - 1.84 (4H, m, CH\textsubscript{2}-3', CH\textsubscript{2}-4'), 1.31 (9H, s, C(CH\textsubscript{3})\textsubscript{3}).

\textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \(\delta\) 170.61 (C), 152.20 (C), 135.27 (C), 131.65 (CH), 122.65 (CH), 119.54 (CH), 110.24 (C), 36.86 (CH\textsubscript{2}), 35.01 (C), 33.15 (CH\textsubscript{2}), 32.09 (CH\textsubscript{2}), 31.31 (CH\textsubscript{3}), 24.04 (CH\textsubscript{2}).
5-[(2-Bromo-5-tert-butylbenzene)carbamoyl]butyltriphenylphosphonium bromide 256

Bromide 255 (543 mg, 1.39 mmol, 1.00 eq.) was dried by azeotrope with PhMe then dissolved in dry MeCN (3.0 mL) and transferred into triphenylphosphine (dried by azeotrope with PhMe, 720 mg, 2.75 mmol, 1.98 eq.) under argon, then additional dry MeCN (2.0 mL) added and the mixture stirred under reflux and argon for 64 h. The mixture was cooled, the solvent removed under reduced pressure and the residue dissolved in DCM and precipitated by addition to Et₂O, then the solvent decanted off and the process repeated. The residue was loaded onto SiO₂ in DCM and washed with further DCM, then eluted with DCM/MeOH, dried (MgSO₄), filtered and the solvent removed under reduced pressure, giving triphenylphosphonium bromide 256 as a white solid (700 mg, 77%).

6-Benzyloxyhexanoic acid 259

Based on the procedure of Lermer et al., ε-caprolactone 258 (5.0 mL, 45 mmol, 1.0 eq.) was dissolved stirring in dry PhMe (120 mL) under argon, then KOH (12.5 g, 223 mmol, 4.95 eq.) and benzyl bromide (26.5 mL, 223 mmol, 4.95 eq.) added and the mixture heated under reflux for 48 h. After cooling the mixture was diluted and partitioned between Et₂O and water, then the aqueous further extracted with Et₂O (x 2). The solvent was removed from the combined organics under reduced pressure, then PhMe (15 mL) and NaOH₂₄₅₆₇₈₉₀ (5.0 M, 45 mL, 225 mmol, 5.0 eq.) added and the mixture heated with stirring under reflux for 18 h. After cooling the mixture was partitioned with Et₂O and water, then the aqueous further extracted with Et₂O (x 3), acidified with a mixture of 1 M H₂SO₄ and ice then extracted with DCM (x 3). The chlorinated extracts were combined, dried (MgSO₄), filtered and the solvent removed under reduced pressure to give acid 259 as a yellow oil (9.46 g, 95%).
(2H, m, OCH$_2$Ph), 3.54 - 3.44 (2H, m, CH$_2$-6), 2.43 - 2.32 (2H, m, CH$_2$-2), 1.75 - 1.59 (4H, m, CH$_3$-3, CH$_3$-5), 1.52 - 1.39 (2H, m, CH$_3$-4). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 178.68 (C), 138.69 (C), 128.51 (CH), 127.78 (CH), 127.67 (CH), 73.07 (CH$_2$), 70.21 (CH$_2$), 33.87 (CH$_2$), 29.54 (CH$_2$), 25.87 (CH$_2$), 24.65 (CH$_3$). Data consistent with literature values.

$^{2}$Fluoro-$^-$tert-butylphenyliodide 273

\[
\text{\includegraphics{273.png}}
\]

2-fluorophenyliodide 269 (260 µL, 2.23 mmol, 1.00 eq.) and tert-butyl chloride (372 µL, 0.338 mmol, 1.47 eq.) were combined and cooled to 0 °C stirring under argon. AlCl$_3$ (9 mg, 0.07 mmol, 0.03 eq.) was added, then the mixture stirred for 15 min and quenched into ice water, then combined with Na$_2$S$_2$O$_3$(aq) and shaken until colourless then extracted with DCM (x 2). The organics were washed with brine, dried (MgSO$_4$), filtered and the solvent removed under reduced pressure, giving aryl iodide 273 as an oil (610 mg, 98%). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.71 (1H, dd, $J$ = 6.0, 2.4 Hz, H6), 7.31 (1H, ddd, $J$ = 8.7, 4.8, 2.4 Hz, H4), 6.98 (1H, dd, $J$ = 8.6, 7.8 Hz, H3), 1.29 [9H, s, C(CH$_3$)$_3$]. $^{13}$C NMR (126 MHz, CDCl$_3$) δ 159.80 (d, $J$ = 243.5 Hz, CF), 149.05 (d, $J$ = 3.5 Hz, C), 136.37 (d, $J$ = 1.2 Hz, CH), 127.20 (d, $J$ = 6.9 Hz, CH), 115.04 (d, $J$ = 23.3 Hz, CH), 81.08 (d, $J$ = 24.9 Hz, C), 34.44 (C), 31.48 (CH$_3$). $^{19}$F NMR (377 MHz, CDCl$_3$) δ −99.20 - −99.25 (m). (ATR cm$^{-1}$): 2963 (C-H), 2907 (C-H), 2870 (C-H), 1493 (C$_9$H$_9$F). MS (El$^+$): 278 (M$^+$, 98%), 263 (M$^+$ – CH$_3$, 100), 235 (M$^+$ – CH$_3$ x 3, 35), 136 (C$_9$H$_9$F$^+$), 39). HRMS: 277.9965. C$_{10}$H$_{12}$FI requires 277.9968.

$^2$Bromo-$^-$tert-butylphenyldiazonium tetrafluoroborate 274

\[
\text{\includegraphics{274.png}}
\]

2-amino-$^-$4-tert-butilbromobenzene 240 (976 mg, 4.28 mmol, 1.00 eq.) was added dropwise to a stirring solution of HBF$_4$(aq) (48%, 5.0 mL, 38 mmol, 8.9 eq.). After 30 min the mixture was cooled to 0 °C then NaNO$_2$(aq) (5.1 M, 2.0 mL, 10 mmol, 2.4 eq.) was added dropwise. After 30 min stirring the mixture was shaken then allowed to warm to RT and stirred for 2 h. Precipitation with Et$_2$O followed by vacuum filtration, washing with HBF$_4$(aq), then drying under vacuum gave the diazonium salt 274 as a beige solid (719 mg, 51%). $^1$H NMR (400 MHz, d$_6$-DMSO) δ 8.94 (1H, d, $J$ = 1.9 Hz, H6), 8.25 (1H, dd, $J$ = 8.8, 2.3 Hz, H4), 8.22 (1H, d, $J$ = 8.6 Hz, H3), 1.33 [9H, s, C(CH$_3$)$_3$]. The very low solubility and probable explosive nature of this compound hindered further characterisation.

Chapter 13: Experimental

202
2-Fluoro-4-tert-butylphenylbromide 275

\[
\begin{align*}
\text{Br} & \quad \text{F} \\
\text{275}
\end{align*}
\]

NOBF₄ (313 mg, 2.70 mmol, 1.32 eq.) was dissolved in stirring, dry DCM (10.0 mL), at 0 °C under argon. After 30 min 2-Amino-4-tertbutylphenylbromide 240 (471 mg, 2.04 mmol, 1.00 eq.) was added dropwise and the mixture allowed to warm to RT under argon for 13 h. PhMe (10.0 mL) was added then the DCM removed carefully under vacuum. The suspension was heated to reflux for 2 h stirring under argon, then cooled and the solvent removed under reduced pressure. Column chromatography (SiO₂, Pet. ether) gave aryl fluoride 275 as an oil (341 mg, 72%).

\[\begin{align*}
^1\text{H NMR} & (500 \text{ MHz}, \text{CDCl}_3) \delta 7.46 (1\text{H, apparent t, } J = 7.9 \text{ Hz, H6}), 7.16 (1\text{H, dd, } J = 10.9, 2.2 \text{ Hz, H3}), 7.06 (1\text{H, dd, } J = 8.4, 2.1 \text{ Hz, H5}), 1.32 [9\text{H, s, C(CH}_3)_3]. \\
^{13}\text{C NMR} & (126 \text{ MHz, CDCl}_3) \delta 159.01 (d, J = 245.9 \text{ Hz, CF}), 153.49 (d, J = 5.9 \text{ Hz, C}), 132.95 (CH), 122.49 (d, J = 3.2 \text{ Hz, CH}), 113.94 (d, J = 22.4 \text{ Hz, CH}), 105.51 (d, J = 20.8 \text{ Hz, C}), 34.88 (d, J = 1.4 \text{ Hz, C}), 31.21 (CH). \\
^{19}\text{F NMR} & (470 \text{ MHz, CDCl}_3) \delta −108.22 (dd, 10.8, 7.4 \text{ Hz}).
\end{align*}\]


Potassium 2-Fluorophenyltrifluoroborate 285

\[
\begin{align*}
\text{BF}_3\text{K} & \quad \text{F} \\
\text{285}
\end{align*}
\]

2-Fluorophenylbromide 268 (0.62 mL, 5.7 mmol, 1.0 eq.) was dissolved in stirring in dry THF (15.0 mL) under argon and cooled to −77 °C. "butyllithium (2.5 M in hexane, 2.50 mL, 6.25 mmol, 1.10 eq.) was added over 20 min, then the mixture stirred for 65 min. Triisopropoxyborate (2.65 mL, 11.5 mmol, 2.01 eq.) was added and the mixture stirred for a further 30 min before warming to RT and stirring for a further 30 min. The mixture was poured into KH₂F₂(aq) (0.85 M, 30.0 mL, 25.5 mmol, 4.48 eq.) and stirred for 17 h under argon. The solvent was removed under reduced pressure, triturated with Et₂O and filtered. The filter cake was dissolved in acetone then dried (MgSO₄), filtered and the solvent removed under reduced pressure. Crystallisation from acetone gave trifluoroborate salt 285 as a solid (317, 28%).

\[\begin{align*}
^1\text{H NMR} & (400 \text{ MHz, DMSO}) \delta 7.34 (1\text{H, t, } J = 5.3 \text{ Hz, H6}), 7.11 - 7.03 (1\text{H, m, H4}), 6.91 (1\text{H, td, } J = 7.2, 0.7 \text{ Hz, H5}), 6.79 (1\text{H, t, } J = 8.6 \text{ Hz, H3}). \\
^{13}\text{C NMR} & (126 \text{ MHz, DMSO}) \delta 165.60 (dd, J = 239.0, 0.8 \text{ Hz, CF}), 134.08 (dq, J = 13.4, 2.6 \text{ Hz, CH}), 127.06 (d, J = 8.0 \text{ Hz, CH}), 122.35 (d, J = 2.4 \text{ Hz, CH}), 113.59 (d, J = 25.9 \text{ Hz, CH}). (\text{ATR cm}^{-1}): 3078 (C₆H₅), 3057 (C₆H₅), 1616 (C₆H₅), 1566 (C₆H₅). \text{Data is consistent with literature values.}
\end{align*}\]
Potassium 2-Fluoro-4-tert-butylphenyltrifluoroboronate 266

Aryl bromide 275 (5.88 g, 25.5 mmol, 1.00 eq.) was dissolved in dry THF (100 mL) and cooled to −77 °C. 1.6 M "butyllithium (21.0 mL, 33.6 mmol, 1.32 eq.) was added to the stirring mixture dropwise over 50 min, then the mixture allowed to stir for 90 min. Triisopropylborate (12.0 mL, 50.5 mmol, 1.98 eq.) was added dropwise over 10 min and the mixture allowed to warm to RT and stir for 14 h. The mixture was quenched into a KHF₂ (10.0 g, 128 mmol, 5.0 eq.) solution [MeOH/H₂O (1:1)] and allowed to stir for 55 h then the solvent removed under reduced pressure. The residue was dissolved in acetone and filtered, then the filtrate concentrated. Crystallisation from acetone gave Potassium trifluoroborate salt 266 (4.22 g, 64%). ¹H NMR (500 MHz, Acetone) δ 7.45 (1H, apparent t, J = 7.3 Hz, H6), 7.03 (1H, ddd, J = 7.7, 1.7, 0.9 Hz, H5), 6.87 (1H, dd, J = 11.8, 1.6 Hz, H3) [C(CH₃)]₃. ¹³C NMR (126 MHz, Acetone) δ 168.23 (d, J = 27.3 Hz, CH), 135.87 (d, J = 13.2, 2.6 Hz, CH), 121.19 (CH), 112.49 (d, J = 27.3 Hz, CH), 35.86 (d, J = 1.8 Hz, C), 32.71 (CH₃), 314.19 (CH₃). ¹⁹F NMR [470 MHz, (CD₃)₂CO] δ −107.63 ~ −107.79 (m), −137.84 ~ −138.49 (m). ν_max(AR)cm⁻¹: 2965 (C=O), 1624, 1551 (C=O). HRMS: 219.0971. C₂₀H₂₀BF₃ requires 219.0974. MP: > 250 °C.

2-(2"-Fluorophenyl)-5-nitrobenzophenone 286

Iodobenzophenone 218 (1.29 g, 3.65 mmol, 1.00 eq.), PEPPSI-²Pr catalyst (72 mg, 0.11 mmol, 0.03 eq.) and arylboronic acid 314 (450 mg, 3.65 mmol, 1.00 eq.) were combined under argon and the reaction vessel was opened to vacuum, then refilled with argon. This process was repeated twice then N,N-diisopropylethylamine (1.90 mL, 10.9 mmol, 2.99 eq.) and degassed ethanol (18.0 mL) added. The mixture was heated to reflux stirring for 16 h. After cooling the mixture was filtered through celite eluting with DCM and the solvent removed under reduced pressure. After column chromatography [SiO₂, Pet. ether/Et₂O gradient (99:1) to (9:1)] the solvent was removed under reduced pressure and the material suspended in Pet. ether/Et₂O (9:1) then sonicated. Ketone 286 precipitated as an amorphous solid (570 mg, 49%). ¹H NMR (400 MHz, CDCl₃) δ 8.44 (1H, dd, J = 8.4, 2.4 Hz, H4), 8.41 (1H, dd, J = 2.4, 0.5 Hz, H6), 7.71 ~ 7.67 (3H, m, H3, H2', H6'), 7.52 (1H, ddt, J = 8.0, 6.9, 1.3 Hz, H4'), 7.40 ~ 7.35 (2H, m, H3', H5'), 7.30 ~ 7.23 (2H, m, H4'', H6''), 7.11 (1H, td, J = 7.8, 1.1 Hz, H5''), 6.95 (1H, ddd, J = 10.2, 8.9, 1.0 Hz, m, H3'). ¹³C NMR (101 MHz, CDCl₃) δ 195.11 (d, J = 1.1 Hz, C), 158.91 (d, J = 247.8 Hz, CF), 147.18 (C), 141.69 (C), 140.57 (d, J = 1.0 Hz, C), 136.19 (C), 133.68 (CH), 132.53 (d, J = 1.4 Hz, CH), 131.18 (d, J = 2.6 Hz, CH), 130.99 (d, J = 8.3 Hz, CH), 129.99 (CH), 128.58 (CH), 126.05 (d, J = 14.8 Hz, C), 125.09 (CH), 124.60 (d, J = 3.7 Hz, CH), 204
Iodobenzophenone 218 (431 mg, 1.22 mmol, 1.00 eq.), PEPPSI-Pr catalyst (51 mg, 0.08 mmol, 0.06 eq.) and potassium trifluoroborate salt 266 (353 mg, 1.37 mmol, 1.12 eq.) were combined under argon then the reaction vessel was opened to vacuum, then refilled with argon. This process was repeated twice then N,N-diisopropylethylamine (0.74 mL, 4.2 mmol, 3.4 eq.) and degassed ethanol (10.0 mL) added. The mixture was heated to reflux stirring for 4 h. After cooling the mixture was partitioned between Et₂O and H₂O, then the aqueous re-extracted with Et₂O. The combined organics were washed with brine, then dried (MgSO₄), filtered through celite eluting with Et₂O and the solvent removed under reduced pressure. Column chromatography [SiO₂, Pet. Ether/EtOAc (8:1), loaded in CHCl₃] gave ketone 265 as a pale straw coloured oil (357 mg, 78%).

\[ R_f = 0.26 \text{ in Pet. ether/EtOAc (5:1).} \]

\[ \text{^1H NMR (500 MHz, CDCl₃) } \delta 8.44 - 8.39 (2H, m, H₄, H₆), 7.67 (3H, d, br, J = 7.8 Hz, H₃, H₂', H₆'), 7.49 (1H, apparent t, J = 7.4 Hz, H₄'), 7.35 (2H, apparent t, J = 7.7 Hz, H₃', H₅'), 7.18 (1H, apparent t, J = 8.1 Hz, H₆''), 7.11 (1H, dd, J = 8.1, 1.8 Hz, H₅''), 6.92 (1H, dd, J = 12.4, 1.7 Hz, H₃''), 1.23 [9H, s, C(CH₃)₃]. \]

\[ \text{^13C NMR (126 MHz, CDCl₃) } \delta 195.21 (d, J = 1.1 Hz, C), 158.66 (d, J = 246.7 Hz, CF), 155.27 (d, J = 6.9 Hz, C), 146.85 (C), 141.83 (C), 140.31 (C), 136.24 (C), 133.31 (CH), 132.33 (d, J = 1.2 Hz, CH), 130.55 (d, J = 3.1 Hz, CH), 129.79 (CH), 128.33 (CH), 124.98 (CH), 124.11 (CH), 122.73 (d, J = 15.5 Hz, C), 121.43 (d, J = 3.0 Hz, CH), 112.80 (d, J = 22.4 Hz, CH), 34.82 (d, J = 1.4 Hz, C), 30.94 (CH₃). \]

\[ \text{^19F NMR (470 MHz, CDCl₃) } \delta -115.11 - -115.20 (m). \]

\[ \nu_{\text{Max}}(\text{ATR}) \text{cm}^{-1}: 2964 (CH), 1670 (C=O), 1606 (C₆=CH₂), 1582 (C₆=CH₂), 1523 (NO₂), 1349 (NO₂). \]

\[ \text{MS (EI')}: 377 (M^+, 64%), 362 (M^+ - CH₃, 100). \]

\[ \text{HRMS (EI')}: 377.1426. \]

\[ C_{19}H_{12}O_{3}NF \text{ requires M}^+ 377.1427. \]
**N-hexyl imine of 2-(2''-Fluoro-4''-tertbutylphenyl)-5-nitrophenyl(phenyl)benzophenone 287**

Benzophenone 265 (1.01 g, 2.66 mmol, 1.00 eq.) and dry hexylamine (2.45 mL, 18.6 mmol, 6.97 eq.) were combined stirring under argon at 0 °C in dry PhMe (20.0 mL) then titanium tetrachloride (0.44 mL, 4.0 mmol, 1.5 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 17 h. The mixture was quenched into H₂O, with the residue washed out with Et₂O. The layers were separated and the aqueous was then extracted (Et₂O, x 2) then the combined organics washed (brine) and dried (MgSO₄), then filtered and the solvent removed under reduced pressure. The imine 287 was purified by chromatography [SiO₂, Pet. ether/Et₂O (20:1)] and isolated as a solid (665 mg, 54%). R_f = 0.26 Pet. Ether/Et₂O (20:1).

1H NMR (500 MHz, CDCl₃) δ 8.34 (1H, d, J = 8.5, 2.4 Hz, H₄), 8.09 (1H, d, J = 2.4 Hz, H₆), 7.67 (1H, dd, J = 8.5, 1.8 Hz, H₃), 7.52 - 7.44 (2H, m, H₂', H₆'), 7.34 (1H, tt, J = 7.3, 1.3 Hz, H₄'), 7.02 (1H, dd, J = 12.4, 1.7 Hz, H₃''), 6.98 (1H, dd, J = 8.1, 1.8 Hz, H₅''), 6.94 (1H, apparent t, J = 8.0 Hz, H₆''), 3.26 - 3.18 (1H, m, H₁A'''), 3.08 (1H, d, J = 13.6, 7.8 Hz, H₁B'''), 1.63 - 1.53 (1H, m, H₂A'''), 1.45 - 1.33 (1H, m, H₂B'''), 1.32 - 1.15 (15H, m, H₃'', H₄'', H₅'', tBu), 0.85 (3H, t, J = 7.1 Hz, C(CH₃)₃).

13C NMR (126 MHz, CDCl₃) δ 164.11 (C), 159.13 (d, J = 247.1 Hz, CF), 155.28 (d, J = 6.8 Hz, C), 147.26 (C), 141.92 (C), 139.50 (C), 138.16 (C), 132.48 (d, J = 2.6 Hz, CH), 130.13 (CH), 129.94 (d, J = 2.9 Hz, CH), 128.29 (C₄H₅), 128.15 (C₄H₅), 124.05 (CH), 123.36 (CH), 122.72 (d, J = 14.6 Hz, C), 121.20 (d, J = 3.1 Hz, CH), 113.12 (d, J = 22.5 Hz, CH), 54.39 (CH₃), 34.97 (d, J = 1.4 Hz, C), 31.80 (CH₃), 30.93 (CH₃), 27.29 (CH₃), 22.72 (CH₃), 14.19 (CH₃).

19F NMR (377 MHz, CDCl₃) δ -116.01 (dd, J = 12.4, 7.8 Hz). \( \nu_{\text{Max}}(\text{ATR}) \text{cm}^{-1} \): 2958 (C-H), 2932 (C-H), 2859 (C-H), 1624 (C=N), 1578 (C₆N=C₆N), 1524 (NO₂), 1436 (NO₂). MS (EI⁺): 460 (M⁺, 7%), 441 (C₂₉H₂₃N₂O₂⁺), 7), 403 (C₂₅H₂₄F₂N₂O₂⁺), 22), 84 (C₆H₁₂⁺, 100). HRMS: 460.2527. C₂₉H₂₃F₂N₂O₂ requires 460.2526. MP: decomp.

**3-tert-Butyl-6-phenyl-8-nitrophenanthridine 290**

Imine 287 (10 mg, 0.02 mmol, 1.00 eq.) was heated to 200 °C stirring in dry nitrobenzene (1.0 mL) for 1 h, then cooled and the solvent removed under reduced pressure. The resulting mixture was dissolved in MeOH and HCl(aq) (1 M, approx. 1:1), and the solvents removed under reduced pressure. The salt was loaded onto a Strata SCX ion exchange column, then washed with additional MeOH. The material was then eluted with 7 M NH₄ in MeOH, and the solvent removed.
under reduced pressure to give traces of phenanthridine 290 (2 mg, 26%). $^1$H NMR (500 MHz, CDCl$_3$) δ 9.01 (1H, d, J = 2.3 Hz, H7), 8.81 (1H, d, J = 9.1 Hz, H10), 8.63 (1H, dd, J = 9.1, 2.4 Hz, H9), 8.58 (1H, d, J = 8.7 Hz, H1), 8.30 (1H, d, J = 2.0 Hz, H4), 7.86 (1H, dd, J = 8.7, 2.1 Hz, H2), 7.77 - 7.73 (2H, m, H2', H6'), 7.65 - 7.57 (3H, m, H3', H4', H5'), 1.48 [s, 9H, C(CH$_3$)$_3$]. $^{13}$C NMR (126 MHz, CDCl$_3$) δ 161.51 (C), 155.02 (C), 145.97 (C), 145.23 (C), 138.70 (C), 137.58 (C), 129.84 (CH), 129.60 (CH), 129.07 (CH), 126.93 (CH), 126.46 (CH), 125.21 (CH), 124.52 (C), 124.29 (CH), 124.02 (CH), 122.65 (CH), 120.24 (C), 35.48 (C), 31.40 (CH$_3$). $\nu_{\text{max}}$(ATR)cm$^{-1}$: 2965 (C-H), 1616 (C=N), 1589 (C$_{3}$=C$_{a}$), 1537 (C$_{a}$=C$_{a}$), 1516 (NO$_2$), 1341 (NO$_2$). MS (EI$^+$): 356 (M$^+$, 59%), 341 (M$^+$ – CH$_3$), 100, 326 (M$^+$ – C$_7$H$_6$ 49), 311 [(M$^+$ – NO$_2$), 60], 295 (M$^+$ – CH$_3$, HNO$_2$), 34. HRMS: 356.1526. C$_{23}$H$_{20}$N$_2$O$_2$ requires 356.1525.

3-tert-Butyl-5-(hex-1'-yl)-6-phenyl-8-nitrophenanthridinium chloride 288

\[ \text{NO}_2 \]

\[ \text{CH} \]

\[ \text{N} \]

\[ \text{Cl} \]

Benzophenone 265 (500 mg, 1.33 mmol, 1.00 eq.) and dry hexylamine (1.05 mL, 9.31 mmol, 7.00 eq.) were combined stirring under argon at 0 °C in dry PhMe (5.0 mL) then a 1 M solution of titanium tetrachloride in dry PhMe (2.00 mL, 2.00 mmol, 1.50 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 40 h. The mixture was quenched into 1 M K$_2$CO$_3$(aq) then filtered through celite and extracted into DCM (x 3). The combined organics were acidified (0.55 M HCl(aq)). The acidic aqueous extracts were further extracted (DCM, x 2), then the organic extracts recombined and basified in 1 M K$_2$CO$_3$(aq). The organic layers were dried (MgSO$_4$), then filtered and the solvent removed under reduced pressure. From the crude yield of 623 mg of extremely viscous brown oil, a portion (100 mg, 0.22 mmol, 1.00 eq.) was weighed by difference onto a spatula and sonicated into a microwave tube (10 mL) containing dry THF (5.0 mL). The tube was filled with argon and sealed then heated to 100 °C for 2 h. After cooling the material was transferred to a round bottom flask with DCM and the solvent removed under reduced pressure. The residue was dissolved in dry Et$_2$O then precipitated with 2 M ethereal HCl (1.00 mL, 2.00 mmol, 9.09 eq.). The precipitate was allowed to settle, then the bulk of the solvent syringed out and the remainder removed under reduced pressure, isolating the phenanthridinium chloride 288 as a brown foam (87 mg, 85%). $^1$H NMR (400 MHz, 0.75 mL CD$_3$CN + 0.05 mL CF$_3$CO$_2$D) δ 8.96 (d, J = 9.2 Hz, H10), 8.86 (1H, d, J = 9.1 Hz, H1), 8.71 (1H, dd, J = 9.2, 2.3 Hz, H9), 8.16 - 8.05 (3H, m, H2, H4, H7), 7.72 - 7.67 (1H, m, H4''), 7.67 - 7.61 (2H, m, H3'', H5''), 7.51 - 7.46 (2H, m, H2'', H6''), 4.68 - 4.56 (2H, m, CH$_2$-1''), 1.85 - 1.76 (2H, m, CH$_2$-2''), 1.34 [s, 9H, C(CH$_3$)$_3$], 1.20 - 1.09 (2H, m, CH$_2$-3''), 1.00 (4H, m, CH$_2$-4', CH$_2$-5'), 0.63 (3H, t, J = 7.0 Hz, CH$_3$-6'). $^{13}$C NMR (126 MHz, 0.75 mL CD$_3$CN + 0.05 mL CF$_3$CO$_2$D) δ 164.32 (C), 158.62 (C), 147.28 (C), 138.16 (C), 134.94 (C), 132.03 (CH), 129.95 (CH), 129.68 (CH), 129.62 (CH), 128.22 (CH), 127.91 (CH), 125.60 (CH), 125.38 (C), 125.19 (CH), 123.51 (C), 117.03 (CH), 116.30 (C), 55.26 (CH$_2$), 35.86 (C), 30.33 (CH$_3$), 29.89 (CH$_3$), 28.82 (CH$_2$).
25.66 (CH$_2$), 21.79 (CH$_2$), 12.84 (CH$_3$). $\nu_{\text{Max (ATR)}}$ cm$^{-1}$: 2956 (C=H), 2927 (C=H), 2870 (C=H), 1620 (C=N), 1588 (C$_{ar}$=C$_{ar}$), 1539 (C$_{ar}$=C$_{ar}$), 1342 (NO$_2$). MS (ESI$^+$): 441 [M$^+$ (phenanthridinium cation), 100%]. HRMS: 441.2516. C$_{29}$H$_{33}$N$_2$O$_2$ requires 441.2537.

3-tert-Butyl-5-(hex-1'-yl)-6-phenyl-8-aminophenanthridinium chloride 293

Benzophenone 265 (500 mg, 1.33 mmol, 1.00 eq.) and dry hexylamine (1.23 mL, 9.31 mmol, 7.16 eq.) were combined stirring under argon at 0 ºC in dry DCM (10.0 mL) then titanium tetrachloride (0.22 mL, 2.0 mmol, 1.5 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 15 h. The remaining mixture was quenched into H$_2$O and partitioned with DCM then the aqueous layer was re-extracted (DCM x 2). The combined organics were washed with brine and dried by filtration through a hydrophobic frit, then the solvent removed under reduced pressure. The residue was dissolved in dry CHCl$_3$ and transferred to a kugelrohr bulb. The CHCl$_3$ was evaporated at atmospheric pressure and the oil allowed to heat at 100 ºC under argon for 110 min. The oil was then dissolved in CHCl$_3$ and TFA (10:1), which were removed under reduced pressure to give the cyclised nitro product. This was dissolved in TFA (18.0 mL) and 10% Pd/C (349 mg, 0.33 mmol, 0.25 eq.) added, then the mixture stirred while a balloon of H$_2$ was used to degas the solution. The mixture was allowed to stir for 2 h at RT under a H$_2$(gl) atmosphere, then concentrated. The residue was diluted and neutralised by addition of DCM and K$_2$CO$_3$ respectively, then filtered through a combination of celite and K$_2$CO$_3$ with additional DCM. The material was purified by chromatography (SiO$_2$, loaded and washed with DCM, then eluted with DCM/MeOH). The counterion was then exchanged to mesylate on an Amberlite IR-401 column, giving the final salt 293 as a red foam (315 mg, 47%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.60 (1H, d, $J$ = 9.0 Hz, H1), 8.43 (1H, d, $J$ = 9.1 Hz, H10), 8.03 (1H, d, $J$ = 1.4 Hz, H4), 7.96 (1H, dd, $J$ = 8.8, 1.5 Hz, H2), 7.84 (1H, dd, $J$ = 8.9, 1.7 Hz, H9), 7.76 - 7.68 (3H, m, H3'', H4'', H5''), 7.47 - 7.41 (2H, m, H2'', H6''), 6.34 (1H, d, $J$ = 2.2 Hz, H7), 5.43 (2H, s, br, NH$_2$), 4.80 - 4.60 (2H, m, br, CH$_2$-1’), 2.59 (3H, s, O$_3$CH$_3$), 1.96 - 1.79 (2H, m, br, CH$_2$-2’), 1.46 [9H, s, C(CH$_3$)$_3$], 1.32 - 1.10 (6H, m, CH$_2$-3’, CH$_2$-4’, CH$_2$-5’), 0.79 (3H, t, $J$ = 7.0 Hz, CH$_2$-6’). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 160.63 (C), 153.78 (C), 150.31 (C), 131.65 (CH), 131.61 (C), 131.29 (C), 129.97 (CH), 129.40 (CH), 128.67 (CH), 128.16 (CH), 127.03 (C), 126.32 (C), 125.03 (C), 123.60 (CH), 123.51 (CH), 115.13 (CH), 110.53 (CH), 54.30 (CH$_3$), 39.46 (CH$_3$), 35.80 (C), 31.15 (CH$_3$), 30.89 (CH$_3$), 29.72 (CH$_3$), 26.28 (CH$_3$), 22.27 (CH$_3$), 13.84 (CH$_3$). $\nu_{\text{Max (ATR)}}$ cm$^{-1}$: 3333 (NH), 3210 (NH), 2959 (C-H), 2932 (C-H), 2866 (C-H), 1609 (C=N). MS (ESI$^+$): 411 [M$^+$ (phenanthridinium cation), 100%], 327 [(M+H)$^+$ – C$_6$H$_{12}$, 25]. HRMS: 411.2803. C$_{29}$H$_{33}$N$_2$O$_2$ requires 411.2795.
2-Fluoro-4-nitrophenyl-4,4,5,5-tetramethyl-1,3,2-dioxoborolane 315

![Chemical Structure](image)

Aryl bromide 319 (1.88 g, 8.55 mmol, 1.00 eq.), bis(pinacolato)diboron (2.24 g, 8.82 mmol, 1.03 eq.), anhydrous potassium acetate (2.71 g, 27.6 mmol, 3.23 eq.) and (dppf)PdCl₂·DCM (150 mg, 0.18 mmol, 2 mol%) were combined and opened to vacuum, then refilled with argon. The cycle was repeated (x 2) then dry 1,4-dioxane (65 mL) added and the reaction heated to 100 °C and stirred under argon for 3 h. The mixture was filtered through celite, dried (MgSO₄), filtered and the solvent removed under reduced pressure. Column chromatography [SiO₂, gradient from Pet. ether to Pet. ether/EtOAc (6.7:1)] gave impure boronate ester (2.01 g), of which a portion (1.81 g) was chromatographed again [SiO₂, gradient from Pet. ether to Pet. ether/EtOAc (6.7:1)] to give boronate ester 315 as a solid (1.27 g, 61%).

1H NMR (500 MHz, CDCl₃) δ 8.00 (1H, dd, J = 8.2, 2.0 Hz, H5), 7.92 (1H, dd, J = 8.2, 5.8 Hz, H6), 7.88 (1H, dd, J = 8.7, 2.0 Hz, H3), 1.38 (12H, s, CH₃ x 4).

13C NMR (101 MHz, CDCl₃) δ 166.69 (d, J = 255.5 Hz, CF), 150.99 (d, J = 9.2 Hz, C), 137.82 (d, J = 8.5 Hz, CH), 118.42 (d, J = 3.6 Hz, CH), 110.93 (d, J = 29.4 Hz, CH), 84.84 (C), 24.88 (CH₃).

19F NMR (377 MHz, CDCl₃) δ −98.70 (dd, J = 8.6, 5.8 Hz).

Max (ATR) cm⁻¹: 2978 (C-H), 2932 (C-H), 1611 (C₆Ar=CHAr), 1524 (NO₂), 1341 (NO₂). MS (APCI⁺): 268 [(M+H)⁺, 100%]. HRMS: 268.1149. C₁₂H₁₆BFNO₄ requires 268.1151. MP: 73 - 77 °C.

Potassium 2-fluoro-4-nitrophenyltrifluoroborate 320

![Chemical Structure](image)

Bromide 319 (2.00 g, 9.09 mmol, 1.00 eq.), bis(pinacolato)diboron (2.58 g, 10.1 mmol, 1.12 eq.), dry KOAc (2.78 g, 28.3 mmol, 3.12 eq.) and (dppf)PdCl₂·DCM (150 mg, 0.184 mmol, 0.0202 eq.) were placed under vacuum then the vessel refilled with argon. This process was repeated (x 2), then dry 1,4-dioxane (65 mL) was added and the mixture heated with stirring under argon to 100 °C for 6 h, with dry NEt₃ (0.20 mL, 1.4 mmol, 0.16 eq.) added at 60 °C. After cooling to 50 °C the mixture was vacuum filtered through celite and MgSO₄ eluting with CHCl₃, then the solvent removed from the filtrate under reduced pressure. The residue was dissolved in MeOH/water (1:1) then KHF₂ added (16 g, 205 mmol, 22.5 eq.) and the solution stirred for 20 h. The mixture was concentrated under reduced pressure and the residue dissolved in acetone and filtered. The filtrate was concentrated, and the potassium trifluoroborate salt 320 crystallised from hot acetone as a beige solid (367 mg, 16%).

1H NMR (500 MHz, Acetone) δ 7.82 (1H, ddd, J = 8.1, 2.1, 0.7 Hz, H5), 7.72 (1H, apparent t, br, J = 6.3 Hz, H6), 7.62 (1H, d, J = 8.6, 1.8 Hz, H3). 13C NMR (101 MHz, DMSO) δ 164.79 (d, J = 243.7 Hz, CF), 146.93 (d, J = 9.2 Hz, C), 134.85 (dq, J = 13.9, 2.6 Hz, CH), 117.67 (d, J = 2.8 Hz, CH), 109.11 (d, J = 31.7 Hz, CH). (ATR cm⁻¹): 1528 (NO₂), 1360 (NO₂).
Chapter 13: Experimental

2-Bromo-5-nitroacetophenone 321

![Structural formula of 2-Bromo-5-nitroacetophenone 321](image)

2-Bromoacetophenone 323 (2.00 g, 10.1 mmol, 1.00 eq.) was added to a solution of KNO3 (1.26 g, 12.5 mmol, 1.23 eq.) in H2SO4(aq) (10 mL) stirring at 0 °C. The mixture was allowed to stir warming to RT for 70 min, and was then quenched (H2O) and extracted with DCM (x 2). The combined organics were dried (MgSO4), filtered and the solvent removed under reduced pressure. Column chromatography [SiO2, Pet. ether/EtOAc (7:1)] gave nitroacetophenone 321 as a solid (1.34 g, 55%). Rf = 0.34 in Pet. Ether/EtOAc (7:1).

1H NMR (500 MHz, CDCl3) δ 8.28 (1H, d, J = 2.7 Hz, H6), 8.12 (1H, dd, J = 8.7, 2.7 Hz, H4), 7.82 (1H, d, J = 8.7 Hz, H3), 2.67 (3H, s, COCH3).

13C NMR (101 MHz, CDCl3) δ 198.87 (C), 147.06 (C), 142.39 (C), 135.27 (CH), 126.22 (C), 125.98 (CH), 123.83 (CH), 30.19 (CH3).

Max (ATR)cm⁻¹: 3095 (CAr-H), 3073 (CAr-H), 1709 (C=O), 1604 (CAr=CAr), 1568 (CAr=CAr), 1523 (NO2), 1355 (NO2). MS (EI+): 245 ([81BrM+], 22%), 243 ([79BrM+], 26), 230 ([81BrM+H] – CH3, 82], 228 ([79BrM+H] – CH3, 100], 184 ([81BrM+H] – CH3 – NO2, 24], 182 ([79BrM+H] – CH3 – NO2, 26].

HRMS: 244.9514. C8H681BrNO3 requires 244.9511. MP: 85 - 87 °C.

2-(2'-Fluoro-4'-nitrophenyl)-5-nitroacetophenone 324

![Structural formula of 2-(2'-Fluoro-4'-nitrophenyl)-5-nitroacetophenone 324](image)

Bromoacetophenone 321 (1.00 g, 4.10 mmol, 1.00 eq.), PEPPSI-Pr catalyst (140 mg, 0.21 mmol, 0.05 eq.) and arylboronic acid 315 (1.13 g, 4.22 mmol, 1.03 eq.) were divided evenly between two microwave tubes (10 mL) under argon. The tubes were opened to vacuum, then refilled with argon. This process was repeated twice then N,N-diisopropylethylamine (2.00 mL, 11.4 mmol, 2.79 eq.) and degassed ethanol (10.0 mL) were split between the tubes. Each tube was heated to 125 °C for 10 min. After cooling the mixtures were filtered through celite eluting with DCM and the solvent removed under reduced pressure. The residue was redissolved then partitioned (DCM/1 M HCl(aq)) then the aqueous layer extracted (DCM x 2). The combined organics were dried (MgSO4), filtered and the solvent removed under reduced pressure. Additional boronic acid (200 mg, 0.75 mmol, 0.18 eq.), N,N-diisopropylethylamine (1.00 mL, 5.74 mmol, 1.40 eq.), PEPPSI-Pr catalyst (50 mg, 0.07 mmol, 0.02 eq.) and ethanol (10.0 mL) was added and the mixture divided then the previous processes repeated. Column chromatography [SiO2, Pet. Ether/ EtOAc (5:1)] gave ketone 324 as a solid (499 mg, 40%). Rf = 0.23 in Pet. Ether/ EtOAc (5:1). 1H NMR (500 MHz, CDCl3) δ 8.65 (1H, d, J = 2.3 Hz, H6), 8.46 (1H, dd, J = 8.4, 2.3 Hz, H4), 8.17 (1H, dd, J = 8.4, 1.9 Hz,
Iodobenzophenone 218 (1.00 g, 2.83 mmol, 1.00 eq.), PEPPSI-iPr catalyst (100 mg, 0.15 mmol, 0.05 eq.) and arylboronic acid 312 (400 mg, 2.84 mmol, 1.00 eq.) were divided evenly between two microwave tubes (10 ml) under argon. The tubes were then opened to vacuum, then reﬁlled with argon. This process was repeated twice then N,N-diisopropylethylamine (1.46 mL, 8.38 mmol, 2.96 eq.) and degassed ethanol (10 mL) were split between the tubes. Each tube was heated to 125 °C for 1 h in a microwave. After cooling the mixtures were combined and ﬁltered through celite with DCM and the solvent removed under reduced pressure. The residue was partitioned between DCM and 1 M HClaq then the aqueous layer extracted (DCM x 2). The combined organics were dried (MgSO4), ﬁltered and the solvent removed under reduced pressure. Column chromatography [SiO2, Pet. Ether/EtOAc gradient from (7:1) to (5:1). The benzophenone 325 was isolated as a solid (480 mg, 53%). Rf = 0.14 in Pet ether/EtOAc (5:1). 1H NMR (400 MHz, CDCl3) δ 8.46 (1H, dd, J = 8.4, 2.4 Hz, H4), 8.42 (1H, d, J = 2.2 Hz, H6), 8.15 (1H, ddd, J = 4.9, 1.9, 1.2 Hz, H4”), 7.77 - 7.65 (4H, m, H3, H6”, H2’, H6’), 7.56 (1H, ddt, J = 8.0, 6.8, 1.3 Hz, H4”), 7.45 - 7.38 (2H, m, H3’, H5’), 7.20 (1H, ddd, J = 7.4, 4.9, 1.8 Hz, H5”). 13C NMR (101 MHz, CDCl3) δ 194.67 (C), 159.30 (d, J = 238.7 Hz, CF), 148.26 (d, J = 14.8 Hz, CH), 147.43 (C), 141.47 (d, J = 3.5 Hz, CH), 140.45 (C), 139.85 (d, J = 4.5 Hz, C), 135.85 (C), 134.05 (CH), 132.44 (d, J = 0.8 Hz, CH), 129.99 (CH), 128.80 (CH), 125.35 (CH), 124.29 (CH), 121.73 (d, J = 4.4 Hz, CH), 121.01 (d, J = 30.4 Hz, C). 19F NMR (377 MHz, CDCl3) δ -68.76 (d, J = 9.5 Hz). νmax(ATR) cm⁻¹: 3069 (C=H), 3062 (C=H), 1668 (C=O), 1607 (C=H=C=C), 1583 (C=H=C=C), 1523 (NO2), 1351 (NO2). MS (EI⁺): 322 (M⁺, 100%), 245 (M⁺ – Ph, 20), 121 (C6H3NO2⁺, 40), 105 (PhCO⁺, 87), 77 (Ph⁺, 37). HRMS: 322.0750. C18H15O3N2F requires 322.0754. MP: 125 - 127 °C.

2-2′′-(Fluoro-3′′-pyridyl)-5-nitrobenzophenone 325
2-(2''-Fluoro-4''-bromophenyl)-5-nitrobenzophenone 326

Iodobenzophenone 218 (1.45 g, 4.11 mmol, 1.00 eq.), PEPPSI-Pr catalyst (94 mg, 0.14 mmol, 0.03 eq.) and arylboronic acid 313 (1.00 g, 4.57 mmol, 1.11 eq.) were combined under argon and the reaction vessel was opened to vacuum, then refilled with argon. This process was repeated twice then N,N-diisopropylethylamine (2.4 mL, 14 mmol, 3.4 eq.) and degassed ethanol (40 mL) added. The mixture was heated to reflux stirring for 22 h. After cooling the mixture was filtered through celite eluting with DCM and the solvent removed under reduced pressure. The residue was partitioned between DCM and 1 M HCl (aq) then the aqueous layer further extracted (DCM x 2). The combined organics were dried (MgSO₄), filtered and the solvent removed under reduced pressure. Column chromatography [SiO₂, Pet. ether/EtOAc (15:1)]. Gave ketone 326 as a yellow solid (896 mg, 55%). Rf = 0.32 in Pet. ether/EtOAc (5:1).

1H NMR (400 MHz, CDCl₃) δ 8.43 (1H, dd, J = 8.4, 2.4 Hz, H4), 8.39 (1H, d, J = 2.3 Hz, H6), 7.75-7.68 (2H, m, H2', H6'), 7.65 (1H, d, J = 8.4 Hz, H3), 7.57 (1H, ddt, J = 8.0, 1.3 Hz, H4'), 7.41 (2H, t, J = 7.7 Hz, H3', H5'), 7.28 (1H, dd, J = 8.3, 1.6 Hz, H5''), 7.19-7.12 (2H, m, H3', H6').

13C NMR (101 MHz, CDCl₃) δ 194.82 (d, J = 0.7 Hz, C), 158.56 (d, J = 252.3 Hz, C), 147.20 (C), 140.69 (C), 140.36 (C), 135.94 (C), 133.96 (C), 132.42 (d, J = 0.6 Hz, CH), 132.02 (d, J = 3.3 Hz, CH), 130.06 (CH), 128.74 (CH), 128.07 (d, J = 3.7 Hz, CH), 125.29 (CH), 125.21 (d, J = 15.1 Hz, C), 124.21 (CH), 123.64 (d, J = 9.5 Hz, C), 119.62 (d, J = 25.1 Hz, CH).

19F NMR (470 MHz, CDCl₃) δ -112.25 (apparent t, J = 8.7 Hz). νmax(ATR)cm⁻¹: 3088 (C-Ar-H), 3071 (C=C), 1668 (C=O), 1605 (C=C), 1582 (C=C), 1524 (NO₂), 1350 (NO₂). MS (EI⁺): 401 (⁸¹BrM⁺, 26%) 399 (⁷⁹BrM⁺, 30), 353 (M⁺ – NO₂, 30), 105 (PhCO⁺, 100), 77 (Ph⁺, 34). HRMS: 398.9902 and 400.9877. C₁₈H₁₁O₃NBrF requires 398.9906 and 400.9888. MP: 101-105 °C.

2-(2''-Fluorophenyl)-benzophenone 327

Iodobenzophenone 218 (1.41 g, 4.57 mmol, 1.00 eq.), PEPPSI-Pr catalyst (76 mg, 0.11 mmol, 0.03 eq.) and arylboronic acid 314 (563 mg, 4.55 mmol, 1.00 eq.) were combined under argon and the reaction vessel was opened to vacuum, then refilled with argon. This process was repeated twice then N,N-diisopropylethylamine (1.91 mL, 11.0 mmol, 2.75 eq.) and degassed ethanol (18.0 mL) added. The mixture was heated to reflux stirring for 16 h. After cooling the mixture was filtered through celite eluting with DCM and the solvent removed under reduced pressure. The residue was taken up in Et₂O then dried (MgSO₄), filtered and the solvent removed under reduced pressure. Column chromatography [SiO₂, Pet. ether/EtOAc (12:1)] gave ketone 327 as a clear oil.
(958 mg, 76%). $R_t = 0.24\ \text{Pet. ether/EtOAc (12:1)}$. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.71 - 7.67 (2H, m, H2', H6'), 7.62 - 7.55 (2H, m, H6, H4), 7.51 - 7.47 (2H, m, H3, H5), 7.43 (1H, ddt, $J = 7.9, 6.8, 1.3$ Hz, H4'), 7.35 - 7.29 (2H, t, $J = 7.7$ Hz, H3', H5'), 7.24 (apparent td, $J = 7.6, 1.8$ Hz, H6''), 7.17 - 7.13 (1H, m, H4''), 7.05 (1H, apparent td, $J = 7.5, 1.3$ Hz, H5''), 6.88 (1H, ddd, $J = 10.1, 8.4, 1.2$ Hz, H3''). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 197.70 (d, $J = 11.1$ Hz, C), 159.20 (d, $J = 246.3$ Hz, CF), 130.84 (d, $J = 0.8$ Hz, C), 137.49 (C), 135.39 (C), 132.83 (CH), 131.60 (d, $J = 3.2$ Hz, CH), 131.24 (d, $J = 1.2$ Hz, CH), 130.65 (CH), 130.06 (CH), 128.72 (CH), 128.17 (CH), 127.66 (CH), 124.19 (d, $J = 3.7$ Hz, CH), 115.53 (d, $J = 22.3$ Hz, CH). $^{19}$F NMR (470 MHz, CDCl$_3$) $\delta$ -115.75 (dd, $J = 10.0, 7.7, 5.2$ Hz). $\nu_{\text{Max}}$ (ATR) cm$^{-1}$: 3061 (C=H), 3030 (C=H), 1667 (C=O), 1597 (C=C=C), 1582 (C=C=C), 1501 (C=C=C). MS (EI$^+$): 276 (M$^+$, 89%), 257 (M$^+$ – F; 38), 199 (M$^+$ – Ph, 100), 170 (C$_9$H$_{10}$O$^-$, 62). HRMS: 276.0946. C$_{19}$H$_{13}$O$_2$F requires 276.0950.

2-(2'-Fluoro-4'-nitrophenyl)-5-nitrobenzophenone 328

Iodobenzophenone 218 (995 mg, 2.82 mmol, 1.00 eq.), PEPPSI-Pr catalyst (105 mg, 0.15 mmol, 0.05 eq.) and arylboronate 315 (706 mg, 2.64 mmol, 0.94 eq.) were divided evenly into three microwave tubes (10 mL) and each reaction vessel was opened to vacuum, then refilled with argon. This process was repeated twice then N,N-diisopropylethylamine (1.56 mL, 8.96 mmol, 3.18 eq.) and degassed ethanol (15.0 mL) divided between them. Each tube was sealed and heated to 100 °C for 30 min in a microwave. After cooling the material was combined in DCM and filtered through a celite/K$_2$CO$_3$ mixture and the solvent removed from the filtrate under reduced pressure. The material was dry loaded onto celite in DCM then column chromatography [SiO$_2$, Pet. Ether/DCM, (1:1)] gave ketone 328 as a solid (962 mg, 99%). $R_t = 0.14$ DCM/Pet. ether (6:4). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.50 (1H, dd, $J = 8.4, 2.4$ Hz, H4), 8.44 (1H, d, $J = 2.3$ Hz, H6), 8.07 (1H, dd, $J = 8.4, 2.1$ Hz, H5''), 7.87 (1H, dd, $J = 9.4, 2.2$ Hz, H3''), 7.74 (2H, d, $J = 8.5$ Hz, H2', H6'), 7.69 (d, $J = 8.4$ Hz, H3), 7.63 - 7.58 (1H, tt, $J = 7.5, 1.2$ Hz, H4'), 7.52 (1H, dd, $J = 8.4, 7.4$ Hz, H6''), 7.46 (2H, m, H3', H5'). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 194.07 (d, $J = 1.1$ Hz, C), 158.42 (d, $J = 252.3$ Hz, CF), 148.95 (d, $J = 8.6$ Hz, C), 147.76 (C), 140.49 (d, $J = 0.9$ Hz, C), 139.37 (C), 135.77 (C), 134.02 (CH), 132.86 (d, $J = 15.6$ Hz, C), 132.22 (d, $J = 1.3$ Hz, CH), 131.74 (d, $J = 3.2$ Hz, CH), 129.92 (CH), 128.74 (CH), 125.30 (CH), 124.24 (CH), 119.47 (d, $J = 3.8$ Hz, CH), 111.62 (d, $J = 27.4$ Hz, CH). $^{19}$F NMR (470 MHz, CDCl$_3$) $\delta$ -110.18 (dd, $J = 9.3, 7.7$ Hz). $\nu_{\text{Max}}$ (ATR) cm$^{-1}$: 3090 (C=H), 1667 (C=O), 1597 (C=C=C), 1522 (NO$_2$), 1346 (NO$_2$). MS (Cl$^+$): 367 [(M+H)$^+$, 100%], 228 [(PhCOC$_6$H$_4$NO$_2$+H)$^+$, 92]. HRMS: 367.0722. C$_{19}$H$_{13}$O$_2$NF requires 367.0730. MP: 143 -144 °C.

Chapter 13: Experimental

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Bromoacetophenone 323 (800 mg, 4.02 mmol, 1.00 eq.), PEPPSI-Pr catalyst (136 mg, 0.20 mmol, 0.05 eq.) and aryl boronic acid 314 (562 mg, 4.02 mmol, 1.00 eq.) were divided evenly between two microwave tubes (10 mL) under argon. The tubes were opened to vacuum, then refilled with argon. This process was repeated twice then N,N-disopropylethylamine (2.10 mL, 15.6 mmol, 3.88 eq.) and degassed ethanol (12.0 mL) were split between the tubes. Each tube was heated to 125 °C for 1 h. After cooling the mixtures were filtered through celite with DCM and the solvent removed under reduced pressure. Column chromatography [SiO₂, Pet. Ether/EtOAc (15:1)] gave ketone 329 as a clear oil (725 mg, 84%). Rᵣ = 0.32 in 7:1 Pet. Ether/EtOAc. ¹H NMR (400 MHz, CDCl₃) δ 7.69 (1H, ddd, J = 7.6, 1.4, 0.5 Hz, H6), 7.55 (1H, td, J = 7.5, 1.4 Hz, H4), 7.46 (1H, td, J = 7.6, 1.3 Hz, H5), 7.40 - 7.31 (2H, m, H3, H4'), 7.29 (1H, dd, J = 7.6, 1.8 Hz, H6'), 7.22 (td, J = 7.5, 1.2 Hz, H5'), 7.15 - 7.08 (1H, m, H3'). ¹³C NMR (101 MHz, CDCl₃) δ 202.17 (C), 159.27 (d, J = 245.8 Hz, CF), 140.28 (C), 134.36 (C), 131.48 (CH), 131.17 (CH), 131.12 (d, J = 3.3 Hz, CH), 129.72 (d, J = 8.1 Hz, CH), 128.72 (d, J = 15.5 Hz, C), 128.39 (CH), 128.08 (d, J = 2.0 Hz, CH), 124.52 (d, J = 3.6 Hz, CH), 115.64 (d, J = 22.3 Hz, CH), 29.14 (CH₃). ¹⁹F NMR (377 MHz, CDCl₃) δ -116.23 - -116.33 (m). v_max(ATR) cm⁻¹: 3063 (C=O), 1688 (C=O), 1595 (C₆=CH₂), 1568 (C₆=CH₂), 1499 (C₆=CH₂). MS (EI⁺): 214 (M⁺, 57%), 199 (C₁₃H₈FO⁺, 100), 170 (C₁₂H₁₀O⁺, 42). HRMS: 214.0791. C₁₄H₁₄FO requires 214.0794.

2-(2′′-Chloro-5′′-nitrophenyl)-5-nitrobenzophenone 330

Iodobenzophenone 218 (1.00 g, 2.83 mmol, 1.12 eq.), PEPPSI-Pr catalyst (96 mg, 0.14 mmol, 0.05 eq.) and arylboronic acid 316 (510 mg, 2.53 mmol, 1.00 eq.) were divided evenly between two microwave tubes (10.0 mL) under argon. The tubes were opened to vacuum, then refilled with argon. This process was repeated twice then N,N-disopropylethylamine (1.45 mL, 8.32 mmol, 3.29 eq.) and degassed ethanol (10.0 mL) were split between the tubes. Each tube was heated to 125 °C for 10 min. After cooling the mixtures were combined and partitioned between DCM and 1 M HCl(aq) then the aqueous layer extracted (DCM x 2). The combined organics were dried (MgSO₄), filtered and the solvent removed under reduced pressure. Column chromatography [SiO₂, Pet. Ether/EtOAc (15:1), after drying loading onto celite]. Ketone 330 was isolated as a solid (540 mg, 56%). ¹H NMR (500 MHz, CDCl₃) δ 8.50 (1H, ddd, J = 8.4, 2.4 Hz, H4), 8.48 (1H, d, J = 2.3 Hz, H6),
2-(2’-Fluoro-3’-methylphenyl)-5-nitrobenzophenone 332

Iodobenzophenone 218 (688 mg, 1.95 mmol, 1.00 eq.), PEPSSI-Pr catalyst (41 mg, 0.06 mmol, 0.03 eq.) and aryllboronic acid 318 (300 mg, 1.95 mmol, 1.00 eq.) were combined under argon then the reaction vessel was opened to vacuum, then refilled with argon. This process was repeated twice then N,N-diisopropylethylamine (1.02 mL, 5.85 mmol, 3.00 eq.) and degassed ethanol (12.0 mL) added. The mixture was heated to reflux stirring for 22 h. After cooling the mixture was filtered through celite eluting with DCM and the solvent removed under reduced pressure. The material was redissolved in Et₂O, dried with MgSO₄ and ethereal HCl (2 M) added to precipitate the diisopropylethylamine as a salt, which was then removed by filtration. The solvent was then removed from the filtrate under reduced pressure and the solid recrystallised from CHCl₃ and washed with CHCl₃ to give ketone 332 as a brown solid (430 mg, 66%). Rf = 0.29 (SiO₂, Pet. ether/EtOAc (9:1)). ¹H NMR (500 MHz, CDCl₃) δ 8.42 (1H, dd, J = 6.2, 2.4 Hz, H₄), 8.41 (1H, d, J = 2.5 Hz, H₆), 7.69 - 7.64 (3H, m, H₃, H₂’, H₆’), 7.50 (1H, ddt, J = 7.9, 7.0, 1.3 Hz, H₄’), 7.35 (2H, apparent t, J = 8.0 Hz, H₃’, H₅’), 7.10 - 7.02 (2H, m, H₄”, H₆”), 6.97 (1H apparent t, J = 7.5 Hz, H₅”), 2.14 (3H, d, J = 2.2 Hz, CH₃). ¹³C NMR (126 MHz, CDCl₃) δ 195.24 (C), 157.34 (d, J = 246.3 Hz, CF), 147.08 (C), 142.13 (C), 140.46 (C), 136.27 (C), 133.53 (CH), 132.49 (CH), 132.45 (CH), 129.87 (CH), 128.58 (d, J = 2.5 Hz, CH), 128.44 (CH), 125.71 (d, J = 15.7 Hz, C), 125.40 (d, J = 17.7 Hz, C), 125.03 (CH), 124.18 (CH), 124.07 (d, J = 4.3 Hz, CH), 14.56 (d, J = 4.3 Hz, CH₃). ¹⁹F NMR (377 MHz, CDCl₃) δ −119.51 (apparent td, J = 6.9, 2.2 Hz). ν_max(ATR)cm⁻¹: 1670 (C=O), 1597 (C=NC₃), 1582 (C=NC₃), 1524 (NO₂), 1348 (NO₂). MS (EI⁺): 335 (M⁺, 97%), 320 (M⁺ – CH₃, 55), 258 (M⁺ – Ph, 43), 183 (C₁₃H₁₄F⁺, 42), 105 (PhCO⁺, 85), 86 (100). HRMS (EI⁺): 335.0954. C₂₀H₁₄FNO₃ requires M⁺ 335.0958. MP: 140 - 145 °C.
2-(2’”-Fluoro-5’”-methoxyphenyl)-5-nitrobenzophenone 333

Iodobenzophenone 333 (625 mg, 1.77 mmol, 1.00 eq.), PEPPSI-Pr catalyst (36 mg, 0.05 mmol, 0.03 eq.) and arylboronic acid 317 (301 mg, 1.77 mmol, 1.00 eq.) were combined under argon. Then the reaction vessel was opened to vacuum, then refilled with argon. This process was repeated twice then N,N-diisopropylethylamine (0.93 mL, 5.3 mmol, 3.0 eq.) and degassed ethanol (12.0 mL) added. The mixture was heated to reflux stirring with DCM and the solvent removed under reduced pressure. The material was then redissolved in Et₂O, dried with MgSO₄ and ethereal HCl (2 M) added to precipitate the diisopropylethylamine as a salt, which was then removed by filtration. The solvent was then removed from the filtrate under reduced pressure and the solid recrystallised from CHCl₃ then washed with CHCl₃ to give ketone 333 as a brown solid (517 mg, 83%).

Rᵣ = 0.20 [Pet. ether/EtOAc (9:1)]. ¹H NMR (500 MHz, CDCl₃) 8 8.43 (1H, dd, J = 8.4, 2.4 Hz, H₄), 8.40 (1H, d, J = 2.3 Hz, H₆), 7.70 (3H, apparent t, J = 8.7 Hz, H₃, H₂’, H₆”), 7.53 (1H, tt, J = 7.4, 1.3 Hz, H₄’), 7.39 (2H, apparent t, J = 7.8 Hz, H₃’, H₅’), 6.89 - 6.84 (1H, m, H₃’), 6.78 - 6.74 (2H, m, H₄”, H₆”), 3.72 (s, 3H).

¹³C NMR (101 MHz, CDCl₃) 8 195.13 (C), 155.80 (d, J = 2.0 Hz, C), 153.25 (d, J = 240.3 Hz, CF), 147.13 (C), 141.66 (C), 140.54 (C), 136.14 (C), 133.74 (CH), 132.46 (d, J = 1.0 Hz, CH), 130.00 (CH), 128.60 (CH), 126.32 (d, J = 16.5 Hz, C), 125.12 (CH, 124.10 (CH), 116.58 (d, J = 23.9 Hz, CH), 116.13 (d, J = 8.0 Hz, CH), 115.70 (d, J = 2.5 Hz, CH), 55.93 (CH₃). ¹⁹F NMR (377 MHz, CDCl₃) 8 -126.3 (dt, J = 9.6, 4.8 Hz). ν₅max(ATR)cm⁻¹: 2951 (CH), 2934 (CH), 2845 (CH), 1672 (C=O), 1595 (C=C₆), 1578 (C=C₆), 1518 (C=C₆), 1499 (NO₂), 1346 (NO₂). MS (EI⁺): 351 (M⁺, 90%), 279 (M⁻ – NO₂ – C₆H₅, 30), 274 (M⁻ – Ph, 25), 227 (PhCOCH₃, 25), 167,149 (O₂NC₆H₄CO⁺, 100) 105 (PhCO⁺, 77) (Ph⁺, 45). HRMS: 351.0902. C₂₀H₁₄O₄NF requires 351.0907.

MP: 100 - 102 °C.

Benzophenone 218 (749 mg, 2.13 mmol, 1.00 eq.), acetic anhydride (20 mL) and DCM (10.0 mL) were combined and cooled to 0 °C. Concentrated nitric acid (2.0 mL, 44 mmol, 21 eq.) was added and the mixture allowed to stir for 2 h then quenched into NaOH(aq) (1 M) and extracted (DCM, x 2). The organic layer was dried (MgSO₄), filtered and the solvent removed under reduced pressure. The residue was then dissolved in minimal hot EtOAc and precipitated by addition of pet. ether, then left to settle (12 h). The solvent was decanted off and the solid triturated with hot
EtOAc. Nitrobenzophenone 334 was isolated as a brown solid (604 mg, 72%). \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.48 (1H, dd, \( J = 8.4, 2.4 \) Hz, H4), 8.42 (1H, d, \( J = 2.3 \) Hz, H6), 7.76 - 7.68 (3H, m, H3, H2', H6'), 7.60 (1H, dtd, \( J = 8.0, 7.0, 1.3 \) Hz, H4'), 7.55 (1H, d, \( J = 8.8 \) Hz, H3''), 7.49 - 7.41 (2H, m, H3', H5'), 7.00 (1H, d, \( J = 5.9 \) Hz, H6''), 3.89 (s, 3H). \( ^13C \) NMR (101 MHz, CDCl\(_3\)) \( \delta \) 194.56 (C), 151.45 (d, \( J = 244.8 \) Hz, CF), 149.60 (d, \( J = 2.7 \) Hz, C), 147.75 (C), 140.75 (C), 139.48 (d, \( J = 0.9 \) Hz, C), 139.06 (d, \( J = 8.1 \) Hz, C), 135.78 (C), 134.37 (CH), 132.14 (d, \( J = 135.53 \) Hz, H3'), 130.01 (d, \( J = 16.7 \) Hz, C), 128.98 (CH), 125.40 (CH), 124.18 (CH), 116.20 (d, \( J = 2.5 \) Hz, CH), 113.66 (d, \( J = 28.3 \) Hz, CH), 57.29 (CH\(_3\)). \(^{19}F\) NMR (377 MHz, CDCl\(_3\)) \( \delta \) -122.91 (dd, \( J = 8.8, 6.0 \) Hz). \( \nu\_\text{Max}(\text{ATR}) \text{cm}^{-1} \): 3069 (C=H), 1668 (C=O), 1595 (C=O), 1522 (NO\(_2\)), 1346 (NO\(_2\)). MS (EI\(^+\)): 396 (M\(^+\), 100%), 105 (PhCO\(^+\), 59), 77 (Ph\(^+\), 31). HRMS: 396.0751. C\(_{20}\)H\(_{13}\)O\(_4\)N\(_2\)F requires 396.0758. MP: 143 - 144 °C.

2-(2''-Fluoro-4''-nitro-5''-methoxy-6''-nitrophenyl)-5-nitrobenzophenone 335

![Structure of 335](image)

Benzophenone 218 (100 mg, 0.284 mmol, 1.00 eq.) was dissolved in MeCN (2.0 mL) and stirred under argon and cooled to -42 °C. NOBF\(_4\) was added (approx. 2 eq.) and the reaction warmed to 0 °C then stirred for 10 min. The mixture was quenched into water then extracted with DCM. The organic layer was dried (MgSO\(_4\)), filtered and the solvent removed under reduced pressure. \(^1H\) NMR showed the major product to be the double nitrated benzophenone 335, and a characterisation sample was acquired by column chromatography [SiO\(_2\), Pet. ether/EtOAc (7:1)]. \( R_t \) = 0.20 in Pet. ether/EtOAc (7:1). \(^1H\) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.49 (1H, dd, \( J = 8.3, 2.3 \) Hz, H4), 8.47 (1H, dd, \( J = 2.3, 0.5 \) Hz, H6), 7.86 (1H, d, \( J = 8.0 \) Hz, H3''), 7.75 (2H, d, \( J = 8.4, 1.3 \) Hz, H2', H6''), 7.68 (1H, dtd, \( J = 7.9, 7.0, 1.4 \) Hz, H4''), 7.64 (1H, dd, \( J = 8.3, 0.5 \) Hz, H3), 7.56 - 7.50 (2H, m, \( J = 7.7 \) Hz, H3', H5'), 4.00 (3H, s, OCH\(_3\)). \(^13C\) NMR (101 MHz, CDCl\(_3\)) \( \delta \) 193.14 (C), 153.84 (d, \( J = 252.1 \) Hz, CF), 148.23 (C), 146.39 (C), 143.27 (d, \( J = 8.6 \) Hz, C), 143.01 (d, \( J = 4.3 \) Hz, C), 139.75 (C), 135.53 (C), 134.15 (CH), 133.82 (C), 132.14 (CH), 128.89 (CH), 126.89 (C), 126.10 (CH), 125.43 (CH), 114.45 (d, \( J = 28.2 \) Hz, CH), 65.32 (CH\(_3\)). \(^{19}F\) NMR (377 MHz, CDCl\(_3\)) \( \delta \) -110.49 (d, \( J = 8.0 \) Hz). \( \nu\_\text{Max}(\text{ATR}) \text{cm}^{-1} \): 3077 (C=H), 1668 (C=O), 1595 (C=O), 1522 (NO\(_2\)), 1346 (NO\(_2\)). MS (EI\(^+\)): 442 [(M+H\(^+\), 100%), 412 [(M+2H\(^+\)-OCH\(_3\)), 49]. HRMS: 442.0694. C\(_{20}\)H\(_{13}\)O\(_4\)N\(_2\)F requires 442.0687.

4-Azidobutyltriphenylphosphonium iodide 345

![Structure of 345](image)

Phosphonium iodide 24 (955 mg, 1.67 mmol, 1.00 eq.) and NaN\(_3\) (404 mg, 6.22 mmol, 3.72 eq.) were dissolved in a combination of MeCN (3.5 mL) and water (0.9 mL) then stirred and heated to 80 °C for 2 h. The mixture was then cooled and partitioned between DCM and water, then the aqueous re-extracted with DCM and the combined organics washed with brine then the solvent
removed under reduced pressure to give triphenylphosphonium salt 345 as a solid (820 mg, quant.). $^1$H NMR (400 MHz, CDCl$_3$) δ 7.83 - 7.70 (9H, m, $^3$PPh$_3$), 7.70 - 7.60 (6H, m, $^3$PPh$_3$), 3.81 - 3.68 (2H, m, CH$_2$-1), 3.37 (2H, t, $J = 6.2$ Hz, CH$_2$-4), 1.96 (2H, quin., $J = 6.9$ Hz, CH$_2$-2), 1.75 - 1.62 (2H, m, CH$_2$-3). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 135.28 (d, $J = 3.1$ Hz, CH), 133.83 (d, $J = 10.0$ Hz, CH), 130.68 (d, $J = 12.6$ Hz, CH), 118.11 (d, $J = 86.1$ Hz, C), 50.71 (d, $J = 1.2$ Hz, CH$_3$), 29.31 (d, $J = 16.7$ Hz, CH$_3$), 22.71 (d, $J = 51.0$ Hz, CH$_3$), 19.96 (d, $J = 3.9$ Hz, CH$_3$). (ATR cm$^{-1}$): 3075 (C$_{Ar}$=H), 3055 (C$_{Ar}$=H), 2945 (C$_{Ar}$-H), 2936 (C$_{Ar}$-H), 2893 (C$_{Ar}$-H), 2868 (C$_{Ar}$-H), 2097 (N$_3$), 1587 (C$_{Ar}$=C$_{Ar}$). MS (ESI$^+$) 289 [M$^+$ (phosphonium cation), 100%]. HRMS: 360.1627. C$_{22}$H$_2$I$^+$$^3$P requires 360.1624. MP: 142 - 144 °C.

4-Aminobutyltriphenylphosphonium iodide 339

Phosphonium salt 345 (836 mg, 1.72 mmol, 1.00 eq.) and 10% Pd/C (220 mg, 0.207 mmol, 0.120 eq.) were combined in MeOH (8.0 mL) and degassed by bubbling H$_2$(g) through the solution. The mixture was then stirred under a H$_2$(g) atmosphere for 14 h. The mixture was filtered through celite eluting with MeOH, then the solvent removed under reduced pressure. The residue was re-dissolved in DCM then dried (MgSO$_4$), filtered and the solvent removed under reduced pressure to give amine 339 as a solid (736 mg, 93%). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.88 - 7.57 (15H, m, $^3$PPh$_3$), 4.05 (2H, s, br, NH$_2$), 3.74 - 3.58 (2H, m, CH$_2$-1), 2.81 (2H, t, $J = 6.7$ Hz, CH$_2$-4), 2.02 - 1.89 (2H, m, CH$_2$-2), 1.78 - 1.64 (2H, m, CH$_2$-3). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 135.07 (d, $J = 2.8$ Hz, CH), 133.78 (d, $J = 10.0$ Hz, CH), 130.57 (d, $J = 12.5$ Hz, CH), 118.02 (d, $J = 86.0$ Hz, C), 39.97 (CH$_2$), 31.14 (CH$_3$), 22.66 (d, $J = 50.4$ Hz, CH$_3$), 20.06 (CH$_3$). (ATR cm$^{-1}$): 3378 (N-H), 3051 (C$_{Ar}$-H), 3008 (C$_{Ar}$-H), 2989 (C$_{Ar}$-H), 2867 (C$_{Ar}$-H), 1587 (C$_{Ar}$=C$_{Ar}$). MS (ESI$^+$): Matches data acquired by a colleague.$^{416}$

1-Azidohexane 343

Bromohexane 346 (2.0 g, 12 mmol, 1.0 eq.) was dissolved in MeCN (25 mL), then NaN$_3$ (2.35 g, 36.2 mmol, 3.01 eq.) and water (6 mL) added and the reaction stirred under reflux for 17 h. The mixture was partitioned between brine and Et$_2$O then the organic layer washed with water (x 3) and dried (MgSO$_4$), filtered and the solvent removed under reduced pressure to give azide 343 as an oil (1.40 g, 92%). $^1$H NMR (500 MHz, CDCl$_3$) δ 3.26 (2H, t, $J = 7.0$ Hz, CH$_2$-1), 1.60 (2H, quin., $J = 7.2$ Hz, CH$_2$-2), 1.45 - 1.27 (6H, m, CH$_2$-3, CH$_2$-4, CH$_2$-5), 0.91 (3H, t, $J = 7.0$ Hz, CH$_2$-6). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 51.75 (CH$_3$), 31.51 (CH$_2$), 29.01 (CH$_3$), 26.58 (CH$_2$), 22.65 (CH$_2$), 14.04 (CH$_3$). Consistent with literature data.$^{428}$
1,6-Diazidohexane 349

\[
\begin{align*}
\text{N}_3 & \quad \text{N}_3 \\
\end{align*}
\]

1,6-Dibromohexane 347 (1.90 mL, 12.4 mmol, 1.00 eq.) was added to a stirring solution of NaN\textsubscript{3} (4.96 g, 76.3 mmol, 6.15 eq.) in water (50 mL) and heated to reflux for 68 h. After cooling the mixture was extracted with Et\textsubscript{2}O (x 2), and the combined organic extracts dried (MgSO\textsubscript{4}), filtered and the solvent removed under reduced pressure. Azide 349 was isolated as a clear oil (2.10 g, quant.). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ 3.28 (4H, t, \(J = 6.9\) Hz, CH\textsubscript{2}-1, CH\textsubscript{2}-6), 1.67 - 1.57 (4H, m, CH\textsubscript{2}-2, CH\textsubscript{2}-5), 1.46 - 1.38 (4H, m, CH\textsubscript{2}-3, CH\textsubscript{2}-4). \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) δ 51.55 (CH\textsubscript{2}), 28.92 (CH\textsubscript{2}). Consistent with literature data.\textsuperscript{429}

6-Azidoheptyltriphenylphosphonium Iodide 348

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\begin{align*}
\text{P} & \quad \text{Ph} \quad \text{P} \\
& \quad \text{N}_3 \\
\end{align*}
\]

Triphenylphosphonium iodide 348 (100 mg, 0.167 mmol, 1.00 eq.) and NaN\textsubscript{3} (45 mg, 0.69 mmol, 4.2 eq.) were combined in a mixture of MeCN (0.5 mL) and water (0.1 mL) and heated to 80 °C with stirring for 2 h. The mixture was partitioned with water and CHCl\textsubscript{3} then the organic layer dried (MgSO\textsubscript{4}), filtered and the solvent removed under reduced pressure, giving azide 340 as a solid (89 mg, quant.). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ 7.92 - 7.54 (15H, m, +PPh\textsubscript{3}), 3.66 - 3.50 (2H, m, CH\textsubscript{2}-1), 3.17 (2H, t, \(J = 6.8\) Hz, CH\textsubscript{2}-6), 1.72 - 1.53 (4H, m, CH\textsubscript{2}-2, CH\textsubscript{2}-5), 1.52 - 1.41 (2H, m, CH\textsubscript{2}-4), 1.38 - 1.28 (2H, m, CH\textsubscript{2}-3). \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) δ 135.11 (d, \(J = 3.0\) Hz, CH), 133.54 (d, \(J = 10.0\) Hz, CH), 130.52 (d, \(J = 12.6\) Hz, CH), 117.90 (d, \(J = 86.0\) Hz, C), 51.16 (CH\textsubscript{2}), 29.75 (d, \(J = 16.0\) Hz, CH\textsubscript{2}), 28.33 (CH\textsubscript{2}), 26.10 (CH\textsubscript{2}), 22.96 (d, \(J = 50.4\) Hz, CH\textsubscript{2}), 22.39 (d, \(J = 4.3\) Hz, CH\textsubscript{2}). (ATR cm\textsuperscript{-1}): 3053 (C\textsubscript{Ar}-H), 2936 (C-H), 2859 (C-H), 2091 (N\textsubscript{3}), 1587 (C\textsubscript{Ar}=C\textsubscript{Ar}). Matches data acquired by a colleague.\textsuperscript{416}

1-Azido-6-aminohexane 342

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\begin{align*}
\text{N}_3 & \quad \text{NH}_2 \\
\end{align*}
\]

1,6-Diazidohexane 349 (7.47 g, 44.4 mmol, 1.00 eq.) was dissolved in Et\textsubscript{2}O (150 mL), and combined with 1 M HCl\textsubscript{(aq)} (150 mL). Triphenylphosphine (13.2 g, 50.3 mmol, 1.13 eq.) was added and the mixture stirred for 24 h. The layers were then separated and the aqueous basified with 7 M NaOH\textsubscript{(aq)} and extracted with Et\textsubscript{2}O (x 2). The combined organics were dried (MgSO\textsubscript{4}), then filtered and the solvent removed under reduced pressure to give amine 342 as a clear oil (4.74 g, 75%). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ 3.26 (2H, t, \(J = 6.9\) Hz, CH\textsubscript{2}-1), 2.70 (2H, t, \(J = 6.9\) Hz, CH\textsubscript{2}-6), 1.67 - 1.56 (2H, apparent quin. \(J = 7.1\) Hz, CH\textsubscript{2}-1), 1.46 (2H, quin., \(J = 7.0\) Hz, CH\textsubscript{2}-5), 1.42 - 1.32 (4H, m, CH\textsubscript{2}-3, CH\textsubscript{2}-4), 1.15 (2H, s, br, NH\textsubscript{2}). \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) δ 51.64 (CH\textsubscript{2}), 42.29 (CH\textsubscript{2}), 33.86 (CH\textsubscript{2}), 29.00 (CH\textsubscript{2}), 26.79 (CH\textsubscript{2}), 26.61 (CH\textsubscript{2}). Consistent with literature data.\textsuperscript{429}
Phthalimide 350 (5.0 g, 22 mmol, 1.0 eq.) was dissolved in IPA (25 mL) and water (15 mL) then NaBH₄ added (4.17 g, 110 mmol, 5.00 eq.) and the mixture stirred for 18 h. AcOH (100 mL) was then added and the mixture heated to 80 °C for 2 h. The material was loaded onto a previously acidified column of IR-120 resin (65 g), washed with water then eluted with NH₃(aq). The aqueous was extracted into Et₂O, then from the organic into 1 M HCl(aq) then the solvent removed under reduced pressure and the residue recrystallised from IPA to give amine 341 (300 mg, 14%). ¹H NMR (500 MHz, CD₃OD) δ 3.00 - 2.94 (2H, m, CH₂-1), 2.31 - 2.25 (3H, m, CH₂-2, CH₂-6), 1.85 - 1.76 (2H, m, CH₂-3), 1.66 - 1.58 (2H, m, CH₂-4). ¹³C NMR (126 MHz, CD₃OD) δ 84.00, 70.39, 40.34, 27.65, 26.39, 18.56. Consistent with literature data. ⁴³⁰

4-Methyl-6-phenyl-8-nitrophenanthridine 368

Imine 362 (100 mg, 0.24 mmol, 1.00 eq.) was weighed and sonicated into dry MeCN (5.0 mL) in a microwave tube (10 mL), which was sealed and heated to 110 °C in a microwave for 10 h. After cooling the solvent was removed under reduced pressure then the residue dissolved in dry Et₂O and precipitated with 2 M ethereal HCl (1.00 mL, 2.00 mmol, 8.33 eq.). The solvent was removed under reduced pressure then the mixture dissolved in MeOH and loaded onto a Strata WCX ion exchange column. The column was washed with additional MeOH before elution with NaOAc/MeOH (15 mg/mL). The solvent was removed under reduced pressure and replaced with dry CHCl₃ then the solution filtered and the solvent removed under reduced pressure. The product 368 was sequentially recrystallised from MeOH and dry CHCl₃, then isolated as a white solid (8 mg, 11%). ¹H NMR (500 MHz, CDCl₃) δ 9.11 (1H, d, J = 2.3 Hz, H7), 8.83 (1H, d, J = 9.1 Hz, H10), 8.61 (1H, dd, J = 9.1, 2.4 Hz, H9), 8.50 (1H, d, J = 8.1 Hz, H1), 7.86 - 7.79 (2H, m, H2', H6'), 7.75 (1H, d, J = 7.0 Hz, H3), 7.69 - 7.57 (4H, m, H2, H3', H4', H5'), 2.90 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 159.49 (C), 146.08 (C), 143.93 (C), 139.05 (C), 138.06 (C), 131.74 (CH), 130.35 (CH), 129.54 (CH), 128.87 (CH), 127.61 (CH), 124.98 (CH), 124.51 (CH), 124.23 (C), 123.89 (CH), 122.31 (C), 120.66 (CH), 18.51 (CH₃). \(v_{\text{Max}}(\text{ATR})\text{cm}⁻¹:\) 2918 (C=CH), 1614 (C=N), 1602 (C=CH), 1590 (C=C=C), 1571 (C=C=C), 1531 (NO₂), 1336 (NO₂). MS (APCI): 315 ([M+H]⁺, 100%). HRMS: 315.1129. C₂₀H₁₄N₂O₂ requires 315.1128. MP: 242 -243 °C.
Benzophenone 286 (253 mg, 0.79 mmol, 1.00 eq.) and dry hexylamine (0.73 mL, 5.5 mmol, 7.0 eq.) were combined stirring under argon at 0 °C in dry PhMe (3.0 mL) then 1 M titanium tetrachloride in dry PhMe (1.18 mL, 1.18 mmol, 1.49 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 72 h. The mixture was quenched into brine then filtered and extracted into Et₂O (x 1) and DCM (x 2). The combined organics were dried (MgSO₄), then filtered and the solvent removed under reduced pressure. From the crude yield of 272 mg of extremely viscous brown oil, a portion (101 mg, 0.25 mmol, 1.00 eq.) was weighed by difference onto a spatula and sonicated into a microwave tube (10 mL) containing dry THF (5.0 mL). The tube was filled with argon and sealed then heated in a microwave to 100 °C for 2 h. The solution was filtered and the residue eluted with CHCl₃ and MeOH, then the solvent removed under reduced pressure, then the residue triturated with pet. ether, which was filtered. The residue and filter cake were recombined in CHCl₃ and the solvent removed under reduced pressure. The residue was then redissolved in dry Et₂O and precipitated with ethereal HCl (1.00 mL, 2.00 mmol, 7.69 eq.), then allowed to settle for 15 h. The solid was partitioned between Et₂O and 1 M NaOH(aq) then the aqueous re-extracted (Et₂O x 1, CHCl₃ x 2). The organic fractions were recombined, then ethereal HCl (1.00 mL, 2.00 mmol, 7.69 eq.) and PhMe were added and the solvent removed under reduced pressure, isolating the phenanthridinium chloride 299 as brown solid (103 mg, 84%).

1H NMR (500 MHz, CD₃CN + 0.05 mL CF₃CO₂D): 9.12 (1H, t, J = 9.0 Hz, H10), 9.07 (1H, d, J = 7.9 Hz, H1), 8.87 (1H, d, J = 8.6 Hz, H9), 8.48 (1H, d, J = 8.3 Hz, H4), 8.26 (1H, s, H7), 8.21 (1H, apparent t, br, J = 7.2 Hz, H3), 8.14 (1H, apparent t, br, J = 7.1 Hz, H2), 7.83 (1H, t, J = 7.2 Hz, H4”), 7.77 (2H, t, J = 6.8 Hz, H3”, H5”), 7.63 (2H, d, J = 6.9 Hz, H2”, H6”), 4.71 (2H, s, CH₂-1’), 2.00 - 1.89 (2H, m, CH₂-2’), 1.30 - 1.04 (6H, m, CH₂-3’, CH₂-4’, CH₂-5’), 0.75 (3H, t, J = 6.9 Hz, CH₃-6’).

13C NMR (101 MHz, CD₃CN + 0.05 mL CF₃CO₂D) δ 166.13 (C), 149.03 (C), 140.13 (C), 136.62 (C), 135.59 (CH), 133.53 (CH), 132.71 (CH), 131.58 (C), 131.45 (CH), 131.07 (CH), 130.03 (CH), 129.10 (CH), 127.37 (C), 127.35 (CH), 127.31 (C), 127.04 (CH), 122.28 (CH), 57.07 (CH₃), 31.94 (CH₂), 30.71 (CH₂), 27.20 (CH₂), 23.35 (CH₂), 14.17 (CH₃).

ν_max(ATR) cm⁻¹: 3067 (C₆H₅-H), 2953 (C-H), 2926, (C-H), 2857 (C-H), 1620 (C=N), 1589 (Cₓ=Cₓ), 1537 (NO₂), 1341 (NO₂). MS (NSI⁺): 385 [M⁺ (phenanthridinium cation)], 100%; 301 (M⁺ – C₆H₁₀O₂, 15). HRMS: 385.1916. C₂₅H₂₅N₂O₅⁺ requires 385.1911. MP: 190 - 192 °C.
Benzophenone 326 (530 mg, 1.32 mmol, 1.00 eq.) and dry hexylamine (1.75 mL, 13.2 mmol, 10.0 eq.) were combined stirring under argon at 0 °C in dry PhMe (5.0 mL) then 1 M titanium tetrachloride in dry PhMe (1.86 mL, 1.86 mmol, 1.41 eq.) was added. The reaction was allowed to warm to RT and allowed to stir for 15 h, at which point additional 1 M titanium tetrachloride in PhMe was added (0.50 mL, 0.50 mmol, 0.38 eq.). After stirring for an additional 20 h the mixture was quenched into 1 M K₂CO₃(aq) then filtered and extracted into DCM (x 3). The combined organics were acidified (0.55 M HCl(aq)). The acidic aqueous extracts were further extracted (DCM, x 2), then the organic extracts recombined and basified in 1 M K₂CO₃(aq). The organic layers were dried (MgSO₄), then filtered and the solvent removed under reduced pressure. From the crude yield of 656 mg of extremely viscous brown oil, a portion (102 mg, 0.20 mmol, 1.00 eq.) was weighed by difference onto a spatula and sonicated into a microwave tube (10 mL) containing dry THF (5.0 mL). The tube was filled with argon and sealed then heated in a microwave to 100 °C for 2 h. After cooling the material was transferred to a round bottom flask with CHCl₃ and the solvent removed under reduced pressure. The residue was dissolved in dry Et₂O then precipitated with 2 M ethereal HCl (1.00 mL, 2.00 mmol, 10.0 eq.). The precipitate was allowed to settle, then the bulk of the solvent syringed out and the remainder removed under reduced pressure, then the solid triturated with dry Et₂O, CHCl₃ and MeOH consecutively. The solvent was removed under reduced pressure, isolating the phenanthridinium chloride 300 was isolated as an off-white solid (103 mg, 100%). ¹H NMR (400 MHz, CD₃OD) δ 9.25 (1H, d, J = 9.2 Hz, H10), 9.09 (1H, d, J = 8.9 Hz, H1), 8.93 (1H, dd, J = 9.2, 2.2 Hz, H9), 8.79 (1H, d, J = 1.4 Hz, H4), 8.33 – 8.27 (2H, m, H2, H6), 7.85 (1H, t, J = 7.5 Hz, H4’’), 7.79 (2H, t, J = 7.3 Hz, H3’’, H5’’), 7.68 (2H, d, J = 7.4 Hz, H2’’, H6’’), 4.87 - 4.72 (2H, m, CH₂-1’), 2.00 - 1.86 (2H, m, CH₂-2’), 1.31 - 1.03 (6H, m, CH₂-3’, CH₂-4’, CH₂-5’), 0.81 - 0.72 (3H, t, J = 7.2 Hz, CH₂-6’). ¹³C NMR (126 MHz, 0.75 mL CD₃OD + 0.05 mL CF₃CO₂D) δ 167.34 (C), 149.26 (C), 139.82 (C), 137.15 (C), 136.11 (CH), 133.68 (CH), 131.89 (CH), 131.19 (C), 131.06 (CH), 129.97 (C), 129.91 (CH), 129.29 (CH), 128.83 (CH), 127.30 (C), 127.02 (CH), 126.36 (C), 124.82 (CH), 57.10 (CH₃), 31.76 (CH₃), 30.55 (CH₃), 27.00 (CH₃), 23.15 (CH₃), 13.92 (CH₃). vmax(ATR)cm⁻¹: 2955 (C-H), 2930 (C-H), 2857 (C-H), 1618 (C=N), 1603 (C=Ar=C₆H₅), 1589 (C=Ar=C₆H₅), 1533 (NO₂), 1518 (C=Ar=C₆H₅), 1341 (NO₂). MS (ESI⁺): 465 [³⁷BrM⁺] (phenanthridinium cation), 100%, 463 [³⁹BrM⁺] (phenanthridinium cation), 98%. HRMS: 463.1011 and 465.0986. C₂₅H₂₄BrN₂O₂ requires 463.1016 and 465.0996. MP: 160 - 165 °C.
4-Aza-5-(hex-1'-yl)-6-phenyl-8-nitrophenanthridinium chloride 301

Benzophenone 325 (520 mg, 1.61 mmol, 1.00 eq.) and dry hexylamine (1.50 mL, 11.4 mmol, 7.05 eq.) were combined stirring under argon at 0 °C in dry PhMe (5.0 mL) then 1 M titanium tetrachloride in dry PhMe (2.40 mL, 2.40 mmol, 1.49 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 20 h. The mixture was quenched into H2O, then filtered through celite with DCM and partitioned between DCM and 1 M HCl (aq), then the aqueous layer was re-extracted (DCM x 2). The combined organics were washed with 1 M HCl (aq) then NaHCO3 (aq) and dried (MgSO4) then filtered and the solvent removed under reduced pressure.

From the crude yield of 631 mg of brown solid a portion (100 mg, 0.25 mmol, 1.00 eq.) was weighed by difference onto a spatula and sonicated into a microwave tube (10 mL) containing dry THF (5.0 mL). The tube was filled with argon and sealed then heated in a microwave to 100 °C for 30 min then cooled and transferred to a round bottomed flask in dry CHCl3, then the solvent removed under reduced pressure. The residue was dissolved in dry Et2O then precipitated with 2 M ethereal HCl (1.00 mL, 2.00 mmol, 8.13 eq.). The solvent was removed under reduced pressure, then the solid triturated with pet. ether and dried under reduced pressure. The salt was isolated as a solid (106 mg, 98%).

1H NMR (400 MHz, CD3OD) δ 9.71 (1H, dd, J = 8.5, 1.7 Hz, H1), 9.41 (1H, d, J = 9.1 Hz, H10), 9.38 (1H, dd, J = 4.4, 1.7 Hz, H3), 9.08 (1H, dd, J = 9.1, 2.3 Hz, H9), 8.49 (1H, d, J = 2.0 Hz, H7), 8.28 (1H, dd, J = 8.4, 4.4 Hz, H2), 7.97 (1H, t, J = 7.4 Hz, H4”), 7.91 (2H, m, CH2-1’), 7.85 - 7.79 (2H, d, J = 7.4 Hz, H2””, H6”), 5.12 - 5.02 (2H, m, CH2-1’), 2.08 - 1.97 (2H, m, CH2-2’), 1.40 - 1.16 (6H, m, CH2-3’, CH2-4’, CH2-5’), 0.87 (3H, t, J = 7.1 Hz, CH3-6’).

13C NMR (126 MHz, CDCl3) δ 167.29 (C), 153.69 (CH), 147.66 (C), 144.54 (C), 138.75 (C), 136.83 (CH), 132.35 (CH), 130.23 (CH), 129.73 (CH), 129.62 (C), 128.77 (CH), 128.02 (CH), 127.77 (CH), 126.80 (C), 126.32 (C), 121.51 (C), 54.80 (CH2), 30.65 (CH2), 29.78 (CH2), 26.47 (CH2), 22.28 (CH2), 13.86 (CH3). νmax(ATR)cm-1: 3064 (C=H), 2956 (C-H), 2931 (C-H), 2926 (C-H), 1623 (C=N), 1604 (C=CH), 1592 (C=CH), 1538 (NO2), 1518 (C=CH), 1343 (NO2). MS (ESI+): 418 [M+ (phenanthridinium cation) + MeOH, 62%], 386 [M+ (phenanthridinium cation), 100], 302 [(M+ (phenanthridinium cation) – C6H12, 69)]. HRMS: 386.1857. C24H24N3O2+ requires 386.1863. MP: 188 - 189 °C.
Benzophenone 330 (300 mg, 0.78 mmol, 1.00 eq.) and dry hexylamine (0.60 mL, 4.6 mmol, 5.9 eq.) were combined stirring under argon at 0 °C in dry PhMe (3.0 mL) then 1 M titanium tetrachloride in dry PhMe (0.98 mL, 0.98 mmol, 1.3 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 20 h. A sample (0.1 mL) was quenched into H₂O, partitioned between DCM and water and the organic separated and the solvent removed under reduced pressure. Proton NMR confirmed the reaction was complete. The remaining mixture was quenched into H₂O, then filtered through celite with DCM and partitioned between DCM and 1 M HCl (aq), then the aqueous layer was re-extracted (DCM x 2). The combined organics were washed with 1 M HCl (aq) then NaHCO₃(aq) and dried (MgSO₄) then filtered and the solvent removed under reduced pressure. From the crude yield of 336 mg of brown solid a portion (100 mg, 0.21 mmol, 1.00 eq.) was weighed by difference onto a spatula and sonicated into a microwave tube (10 mL) containing dry THF (5.0 mL). The tube was filled with argon and sealed then heated in a microwave to 100 °C for 30 min then cooled and transferred to a round bottomed flask in dry CHCl₃, then the solvent removed under reduced pressure. The solid was dissolved in dry Et₂O then precipitated with 2 M ethereal HCl (1.00 mL, 2.00 mmol, 9.35 eq.). The solvent was removed under reduced pressure. The salt 302 was isolated as a solid (91 mg, 86%).

\[ \text{1H NMR } \{500 \text{ MHz, CD}_2\text{CN/CF}_3\text{CO}_2\text{D (10:1)} \} \delta 9.82 (1H, d, J = 2.3 Hz, H1), 9.27 (1H, d, J = 9.1 Hz, H10), 8.95 (1H, dd, J = 9.1, 2.2 Hz, H9), 8.84 (1H, dd, J = 9.5, 2.2 Hz, H3), 8.66 (1H, d, J = 9.5 Hz, H4), 8.29 (1H, d, J = 2.2 Hz, H7), 7.86 (1H, t, J = 7.5 Hz, H4''), 7.79 (2H, t, J = 7.5 Hz, H3'', H5''), 7.63 (2H, d, J = 7.3 Hz, H2'', H6''), 4.79 - 4.66 (2H, m, CH₂-1''), 1.99 - 1.88 (2H, m, CH₂-2''), 1.30 - 1.03 (6H, m, CH₂-3', CH₂-4', CH₂-5''), 0.75 (3H, t, J = 7.2 Hz, CH₂-6''). \]

\[ \text{13C NMR } \{126 \text{ MHz, CD}_2\text{CN/CF}_3\text{CO}_2\text{D (10:1)} \} \delta 167.94 (C), 148.39 (C), 148.15 (C), 138.14 (C), 137.68 (C), 132.56 (CH), 131.23 (CH), 129.83 (CH), 129.38 (C), 128.56 (CH), 128.09 (CH), 127.42 (CH), 126.64 (C), 126.35 (C), 126.25 (CH), 123.34 (CH), 121.76 (CH), 56.45 (CH₂), 30.31 (CH₂), 28.88 (CH₂), 25.61 (CH₂), 21.89 (CH₃), 12.99 (CH₃).
\]

\[ \nu_{\text{Max}}(\text{ATR}) \text{cm}^{-1}: 3099 (C=O-H), 2957 (C=H), 2927 (C-H), 2857 (C-H), 1601 (C=N), 1578 (C₆=c₆), 1526 (NO₂), 1494 (NO₂), 1342 (NO₂), 1326 (NO₂). \]

\[ \text{MS (NSI): } 430 \text{ [M}^+\text{ (phenanthridinium cation), 100%].} \]

\[ \text{HRMS: } 430.1752. \text{ C}_{25}\text{H}_{24}\text{N}_3\text{O}_4^+ \text{ requires } 430.1761. \text{ MP: } 260 - 263 ^\circ \text{C.} \]
2-Methoxy-3,8-dinitro-5-(hex-1'-yl)-6-phenylphenanthridinium chloride 303

Benzophenone 334 (501 mg, 1.26 mmol, 1.00 eq.) and dry hexylamine (1.17 mL, 8.86 mmol, 7.03 eq.) were combined stirring under argon at 0 °C in dry PhMe (5.0 mL) then a 1 M solution of titanium tetrachloride in dry PhMe (1.9 mL, 1.90 mmol, 1.51 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 40 h. The mixture was quenched into H₂O, filtered through celite with DCM and partitioned between DCM and 0.5 M HCl (aq), then the aqueous layer was re-extracted (DCM x 2). The combined organics were dried (MgSO₄) then filtered and the solvent removed under reduced pressure. From the crude yield of 572 mg of brown solid a portion (100 mg, 0.21 mmol, 1.00 eq.) was weighed by difference onto a spatula and sonicated into a microwave tube (10 mL) containing dry THF (5.0 mL). The tube was filled with argon and sealed then heated in a microwave to 100 °C for 2 h. The solvent was removed under reduced pressure, then the residue dissolved in dry Et₂O and precipitated with 2 M ethereal HCl (1.00 mL, 2.00 mmol, 9.52 eq.). The precipitate was allowed to settle, then the bulk of the solvent syringed out and the process repeated. After the remaining solvent was removed under reduced pressure the phenanthridinium salt 303 was isolated as a powder (80 mg, 74%).

¹H NMR (400 MHz, 0.75 mL CD₃CN + 0.05 mL CF₃CO₂D) δ 9.31 (1H, d, J= 9.2 Hz, H₁₀), 9.02 (1H, dd, J = 9.2, 2.2 Hz, H₉), 8.90 (1H, s, H₄), 8.64 (1H, s, H₁), 8.36 (1H, d, J = 2.2 Hz, H₇), 7.94 (1H, t, J = 7.5 Hz, H₄”), 7.87 (2H, t, J = 7.3 Hz, H₃”, H₅”), 7.71 (2H, d, J = 7.1 Hz, H₂”, H₆”), 4.79 - 4.71 (2H, m, CH₂-1’), 4.36 (3H, s, OCH₃), 2.06 - 1.91 (2H, m, CH₂-2’), 1.39 - 1.10 (6H, m, CH₂-3’, CH₂-4’, CH₂-5’), 0.84 (3H, t, J = 7.1 Hz, CH₂-6’). ¹³C NMR (126 MHz, 0.75 mL DMSO + 0.05 mL CF₃CO₂D) δ 163.79 (C), 151.64 (C), 147.82 (C), 143.26 (C), 136.31 (C), 131.79 (CH), 129.70 (C), 129.63 (CH), 129.28 (CH), 129.06 (C), 128.44 (C), 128.36 (CH), 127.13 (CH), 127.06 (CH), 126.81 (C), 117.75 (CH), 109.21 (CH), 58.14 (CH₃), 55.44 (CH₃), 29.90 (CH₃), 28.53 (CH₂), 25.19 (CH₂), 21.47 (CH₂), 13.16 (CH₃). ν_max(ATR)cm⁻¹: 3018 (C₆H₅-H), 2954 (C-H), 2922 (C-H), 2917 (C-H), 2853 (C-H), 1621 (C=N), 1595 (C₆H₅=C₆H₅), 1537 (NO₂), 1521 (C₆H₅=C₆H₅), 1501 (C₆H₅=C₆H₅), 1350 (NO₂). MS (ESI⁺): 460 [M⁺ (phenanthridinium cation), 100%]. HRMS: 460.1846. C₂₆H₂₆N₃O₅⁺ requires 460.1867. MP: 195 - 198 °C.
Benzophenone 333 (102 mg, 0.29 mmol, 1.00 eq.) and dry hexylamine (0.27 mL, 2.0 mmol, 7.1 eq.) were combined stirring under argon at 0 °C in dry PhMe (1.0 mL) then titanium tetrachloride (0.42 mL, 0.4 mmol, 1.5 eq.) was added. The reaction was allowed to warm to RT and allowed to stir for 20 h. The mixture was quenched into H₂O and extracted into diethyl ether (x 2). The combined organics were dried (MgSO₄), then filtered and the solvent removed under reduced pressure. The crude yield of 114 mg was transferred in dry CHCl₃ into a kugelrohr bulb and the chloroform distilled out, then the oil heated at 100 °C for 2 h 45 min, then at 125 °C for 3 h. TFA was added then evaporated off under reduced pressure. The mixture was partitioned from a water/TFA mixture with DCM (x 3), and CHCl₃ (x 3). The organics were combined and the solvent removed under reduced pressure. Following column chromatography [SiO₂, loaded and washed in DCM then eluted with DCM/MeOH/NEt₃ (97:2:1)]. The solvent was removed under reduced pressure and replaced with CHCl₃ then the compound dried (MgSO₄). The mixture was filtered, then TFA was added then and the solvent removed under reduced pressure. The phenantridinium trifluoroacetate 304 was isolated as brown oil (70 mg, 47%). \(^1\)H NMR (500 MHz, CDCl₃) \(\delta\) 9.08 (1H, d, \(J = 9.1\) Hz, H10), 8.77 (1H, dd, \(J = 9.1, 2.1\) Hz, H9), 8.39 (1H, d, \(J = 9.6\) Hz, H4), 8.34 (1H, d, \(J = 2.2\) Hz, H7), 8.27 (1H, d, \(J = 2.5\) Hz, H1), 7.86 - 7.82 (1H, m, H4’’), 7.78 (2H, t, \(J = 7.8\) Hz, H3’’, H5’’), 7.74 (1H, dd, \(J = 9.6, 2.6\) Hz, H3), 7.65 (2H, d, \(J = 7.7\) Hz, H2’’, H6’’), 4.94 - 4.79 (2H, m, CH₂-1’), 4.10 (3H, s, OCH₃), 1.93 (2H, quin, \(J = 7.9\) Hz, CH₂-2’), 1.34 - 1.08 (6H, m, CH₂-3’, CH₂-4’, CH₂-5’), 0.81 (3H, t, \(J = 7.2\) Hz, CH₂-6’). \(^{13}\)C NMR (126 MHz, CDCl₃) 6 161.75 (C), 160.61 (C), 147.64 (C), 137.85 (C), 132.43 (CH), 130.11 (C), 129.73 (C), 129.57 (CH), 128.84 (CH), 128.05 (C), 127.74 (CH), 126.39 (CH), 125.70 (C), 124.60 (CH), 122.64 (CH), 106.64 (CH), 56.82 (CH₃), 56.09 (CH₃), 30.76 (CH₂), 30.05 (CH₂), 26.19 (CH₃), 22.26 (CH₃), 13.74 (CH₃). \(\nu_{\text{max}}\) (ATR) cm⁻¹: 2958 (C-H), 2920 (C-H), 2850 (C-H), 1777 (TFA), 1731 (TFA), 1614 (C=N), 1541 (C₆H₅=), 1592 (C₆H₅=), 1348 (NO₂). MS (NSI⁺): 415 [M⁺ (phenanthridinium cation), 100%]. HRMS: 415.2005, \(\text{C}_{26}\text{H}_{27}\text{N}_{2}\text{O}_{3}⁺\) requires 415.2016.
3,8-Dinitro-5-(hex-1'-yl)-6-phenylphenanthridinium chloride 305

Benzophenone 328 (390 mg, 1.06 mmol, 1.00 eq.) and dry hexylamine (1.00 mL, 7.57 mmol, 7.14 eq.) were combined stirring under argon at 0 °C in dry PhMe (5.5 mL) then a 1 M solution of titanium tetrachloride in dry PhMe (1.60 mL, 1.60 mmol, 1.51 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 48 h. The mixture was quenched into H₂O, and the resulting precipitate washed with Et₂O, CHCl₃, DCM, MeCN and MeOH and the organics combined and washed with brine and extracted DCM (x 1), Et₂O (x 1), and CHCl₃ (x 4). The combined organics were dried (MgSO₄) then filtered and the solvent removed under reduced pressure. From the crude yield of 504 mg of brown solid a portion (100 mg, 0.22 mmol, 1.00 eq.) was transferred to a round bottom flask with DCM and the solvent removed under reduced pressure. The residue was dissolved in dry Et₂O then precipitated with 2 M ethereal HCl (1.00 mL, 2.00 mmol, 9.09 eq.). The precipitate was allowed to settle, then the bulk of the solvent syringed out and the remainder removed under reduced pressure, isolating the phenanthridinium chloride 305 as a powder (95 mg, 97%).

1H NMR (500 MHz, CD₃OD) δ 9.58 (1H, d, J = 9.2 Hz, H1), 9.52 (1H, d, J = 9.1 Hz, H10), 9.44 (1H, d, J = 1.9 Hz, H4), 9.15 (1H, dd, J = 9.1, 2.3 Hz, H9), 9.01 (1H, dd, J = 9.1, 1.9 Hz, H2), 8.49 (1H, d, J = 2.2 Hz, H7), 8.01 (1H, dd, J = 9.1, 1.9 Hz, H2''), 7.96 (2H, t, J = 7.5 Hz, H3'''), 7.86 (2H, d, J = 7.8 Hz, H2''', H6'''), 5.10 - 4.99 (2H, m, CH₂-1'), 2.20 - 2.08 (2H, m, CH₂-2'), 1.49 - 1.40 (2H, m, CH₂-3'), 1.36 - 1.23 (4H, m, CH₂-4', CH₂-5'), 0.92 (3H, t, J = 7.1 Hz, CH₂-6').

13C NMR (126 MHz, CD₃OD) δ 170.05 (C), 152.37 (C), 150.81 (C), 139.67 (C), 137.15 (C), 134.72 (CH), 133.09 (CH), 132.01 (C), 131.97 (CH), 131.93 (C), 130.41 (CH), 130.32 (CH), 130.17 (CH), 129.44 (C), 128.82 (CH), 126.82 (CH), 118.76 (CH), 58.12 (CH₂), 32.68 (CH₂), 31.39 (CH₂), 27.95 (CH₃), 24.16 (CH₃), 15.01 (CH₃). ν_max(ATR) cm⁻¹: 2955 (C-H), 2928 (C-H), 2859 (C-H), 1618 (C=N), 1591 (C₆=C₆), 1537 (C₆=C₆), 1518 (NO₂), 1343 (NO₂). MS (NSI⁺): 430 [M⁺ (phenanthridinium cation), 100%]. HRMS: 430.1754. C₂₅H₂₄N₃O₄ requires 430.1761. MP: 189 °C.

3,8-Dinitro-5-(hex-1'-yl)-6-methylene-5-hydrophenanthidine 369

Benzophenone 324 (426 mg, 1.40 mmol, 1.00 eq.) and dry hexylamine (1.45 mL, 11.0 mmol, 7.58 eq.) were combined stirring under argon at 0 °C in dry PhMe (5.0 mL) then a 1 M solution of
titanium tetrachloride in dry PhMe (2.70 mL, 2.70 mmol, 1.93 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 19 h. The mixture was quenched into H₂O, then filtered through celite with DCM and partitioned between DCM and 1 M HCl(aq). Then the aqueous layer was re-extracted (DCM x 2). The combined organics were washed with 1 M HCl(aq) then NaHCO₃(aq) and dried (MgSO₄) then filtered and the solvent removed under reduced pressure. The enamine 369 was isolated as a purple solid (523 mg, quant.). ¹H NMR (400 MHz, CDCl₃) δ 8.62 (1H, d, J = 2.3 Hz, H7), 8.22 (1H, dd, J = 8.9, 2.3 Hz, H9), 8.03 (1H, d, J = 8.9 Hz, H10), 7.96 (1H, d, J = 8.7 Hz, H1), 7.74 (1H, dd, J = 8.7, 2.2 Hz, H2), 7.69 (1H, d, J = 2.1 Hz, H4), 4.96 (1H, d, J = 3.1 Hz, NC=CHtrans), 4.39 (1H, d, J = 3.2 Hz, NC=CHcis), 3.83 - 3.69 (2H, m, CH₂-1’), 1.83 (2H, quint., J = 7.8 Hz, CH₂-2’), 1.56 - 1.47 (2H, m, CH₂-3’), 1.47 - 1.36 (4H, m, CH₂-4’, CH₂-5’), 0.95 (3H, t, J = 7.1 Hz, CH₃-6’). ¹³C NMR (101 MHz, CDCl₃) δ 149.91 (C), 148.41 (C), 142.18 (C), 141.83 (C), 133.65 (C), 132.01 (C), 125.05 (CH), 124.37 (CH), 123.72 (C), 123.54 (CH), 120.68 (CH), 113.94 (CH), 108.01 (CH), 86.80 (CH₆), 48.23 (CH₃), 31.50 (CH₃), 26.69 (CH₃), 24.43 (CH₂), 22.75 (CH₂), 14.11 (CH₃). ṽ(ATR)cm⁻¹: 2957 (C=O), 2856 (C=H), 1603 (C₆H₅), 1578 (C₆H₅), 1520 (NO₂), 1339 (NO₂). MS (APCI⁺): 368 [(M+H)⁺, 100]. HRMS: 368.1603. C₂₀H₁₃N₂O₄⁺ requires 368.1605. MP: 169 - 171 °C.

5-((Hex-1’-yl)-6-phenylphenanthridinium chloride 307

Benzophenone 327 (594 mg, 2.15 mmol, 1.00 eq.) and dry hexylamine (2.00 mL, 15.1 mmol, 7.04 eq.) were combined stirring under argon at 0 °C in dry PhMe (5.0 mL) then titanium tetrachloride (0.35 mL, 3.2 mmol, 1.5 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 72 h. The mixture was quenched into brine, and extracted with diethyl ether (x 1), EtOAc (x 1) and DCM (x 5). The combined organics were dried (MgSO₄) then filtered and the solvent removed under reduced pressure. From the crude yield of 537 mg of brown oil a portion (100 mg, 0.28 mmol, 1.00 eq.) was weighed by difference onto a spatula and sonicated into a microwave tube (10 mL) containing dry MeCN (5.0 mL). The tube was filled with argon and sealed then heated in a microwave to 110 °C for 90 min. After cooling the material was transferred to a round bottom flask with DCM and the solvent removed under reduced pressure. The residue was dissolved in dry Et₂O then precipitated with 2 M ethereal HCl (1.00 mL, 2.00 mmol, 7.14 eq.) The precipitate was allowed to settle, then the bulk of the solvent syringed out and the remainder removed under reduced pressure, isolating the phenanthridinium salt 307 as a white solid (102 mg, 68%). ¹H NMR (500 MHz, 0.75 mL CD₃CN + 0.05 mL CF₃CO₂D) δ 9.01 (1H, d, J = 7.5 Hz, H1), 8.94 (1H, d, J = 8.1 Hz, H10), 8.40 (1H, d, J = 8.0 Hz, H4), 8.21 (1H, apparent t, J = 7.3 Hz, H9), 8.12 - 8.00 (2H, m, H2, H3), 7.80 - 7.67 (4H, m, H8, H2””, H3””, H5””), 7.61 - 7.50 (3H, m, H7, H2””, H6””), 4.74 - 4.55 (2H, m, CH₂-1’), 2.00 - 1.85 (2H, m, CH₂-2’), 1.30 - 1.18 (2H, m, CH₂-3’), 1.16 - 1.00 (m, CH₂-4’, CH₂-5’), 0.74 (3H, t, J = 7.0 Hz, CH₃-6’). ¹³C NMR (101 MHz, 0.75 mL CD₃CN + 0.05 mL
4- Methyl-5-(hex-1-yl)-6-phenyl-8-nitrophenanthridinium chloride 308

Benzophenone 332 (526 mg, 1.57 mmol, 1.00 eq.) and dry hexylamine (1.45 mL, 11.0 mmol, 7.01 eq.) were combined stirring under argon at 0 °C in dry PhMe (5.0 mL) then titanium tetrachloride (0.26 mL, 2.4 mmol, 1.5 eq.) was added. The reaction was allowed to warm to RT and allowed to stir for 72 h. The mixture was quenched into brine then filtered and extracted into diethyl ether (x 1) and DCM (x 2). The combined organics were dried (MgSO₄), then filtered and the solvent removed under reduced pressure. From the crude yield of 695 mg of extremely viscous brown oil, a portion (100 mg, 0.24 mmol, 1.00 eq.) was weighed by difference onto a spatula and sonicated then the compound dissolved in dry Et₂O then precipitated with 2 M ethereal HCl (1.00 mL, 2.00 mmol, 8.33 eq.). The precipitate was allowed to settle, then the bulk of the solvent syringed out and the remainder removed under reduced pressure, then the residue loaded onto a Strata WCX column in MeOH, then the column washed with additional MeOH before elution with NaOAc/MeOH (15 mg/mL). The solvent was removed under reduced pressure and replaced with CHCl₃ then the compound dried (MgSO₄) and the solution filtered then the solvent removed under reduced pressure. The phenanthyridinium chloride 308 was isolated as brown oil (77 mg, 79%). ¹H NMR (500 MHz, CD₂OD) δ 9.31 (1H, d, J = 9.2 Hz, H10), 9.10 (1H, dd, J = 7.6, 1.9 Hz, H1), 9.00 (1H, dd, J = 9.2, 2.3 Hz, H9), 8.48 (1H, d, J = 2.3 Hz, H7), 8.14 - 8.06 (2H, m, H2, H3), 8.00 - 7.94 (1H, m, H4′′′), 7.93 - 7.85 (4H, m, H2′′′, H3′′′, H5′′′, H6′′′), 5.05 (2H, t, J = 6.9 Hz, CH₂-1′), 3.07 (3H, s, ArCH₃), 1.54 (2H, quintet, J = 7.7 Hz, CH₂-2′), 1.08 - 1.00 (2H, m, CH₂-3′), 0.99 - 0.84 (4H, m, CH₂-4′, CH₂-5′), 0.71 (3H, t, J = 7.2 Hz, CH₂-6′). ¹³C NMR (126 MHz, CD₂OD) δ 169.05 (C), 148.88 (C), 140.64 (C), 139.63 (CH), 137.15 (C), 134.20 (CH), 133.25 (C), 132.45 (C), 132.11 (CH), 131.72 (CH), 131.43 (CH), 131.07 (CH), 129.26 (CH), 128.59 (C), 126.88 (CH), 126.82 (C), 125.11 (CH), 61.15 (CH₃), 31.75 (CH₃), 31.02 (CH₃), 26.66 (CH₃), 24.53 (CH₃), 23.02 (CH₂), 14.00 (CH₃). ν₅max(ATR)cm⁻¹ (TFA salt): 2961 (C-H), 2930 (C-H), 2862 (C-H), 1778 (TFA), 1732 (TFA), 1622 (C=N), 1586 (C₆H₅=NO₂), 1539 (C₆H₅=NO₂), 1348 (NO₂).
3-tert-Butyl-5-(cyclohexylmethylamino)-6-phenyl-8-nitrophenanthridinium chloride 309

Benzophenone 265 (360 mg, 0.95 mmol, 1.00 eq.) and cyclohexylmethylamine 336 (0.90 mL, 6.9 mmol, 7.3 eq.) were combined stirring under argon at 0 °C in dry PhMe (5.0 mL) then a 1 M solution of titanium tetrachloride in dry PhMe (1.50 mL, 1.50 mmol, 1.50 eq.) was added over 5 min. The reaction was allowed to warm to RT and allowed to stir for 20 h. The mixture was quenched into H₂O, then filtered through celite with DCM and partitioned between DCM and 0.5 M HCl(aq), then the aqueous layer was re-extracted (DCM x 2). The combined organics were washed with 1 M HCl(aq) then NaHCO₃(aq) and dried (MgSO₄) then filtered and the solvent removed under reduced pressure. From the crude yield of 445 mg of brown solid four portions (total of 80 mg, 0.16 mmol, 1.00 eq.) were weighed by difference onto a spatula and sonicated into microwave tubes (10 mL) containing dry MeCN (1.0 mL). The tubes were filled with argon and sealed then heated in a microwave to 110 °C for 90 min then cooled and the solvent removed under reduced pressure. Following column chromatography of the combined residues [SiO₂, loaded in and washed with DCM and eluted with DCM/NEt₃ (200:1)] the combined organics were concentrated under reduced pressure then washed (1 M HCl(aq) x 2), and dried (MgSO₄). After filtration 2 M ethereal HCl (1.00 mL, 2.00 mmol, 12.5 eq.) was added to the filtrate and the solvent removed under reduced pressure, to give phenanthridinium salt 309 as a brown solid (63 mg, 75%).

**Characterization Data**

**1H NMR (400 MHz, CD₃OD, 50°C)**

δ 9.32 (1H, d, J = 9.2 Hz, H₁₀), 9.20 (1H, d, J = 8.8 Hz, H₁), 8.99 (1H, dd, J = 9.2, 2.2 Hz, H₉), 8.49 - 8.43 (2H, m, H₄, H₇), 8.37 - 8.39 (2H, m, H₄, H₇), 8.37 (1H, t, J = 7.3 Hz, H₃′′), 7.98 (2H, t, J = 7.3 Hz, H₃′′, H₅′′), 7.79 (2H, d, J = 7.0 Hz, H₂′′, H₆′′), 5.00 (2H, s, br, CH₂-N), 2.08 (1H, m, CH₂-1′), 1.73 - 1.49 (2H, m, CH₂-2′ₐ), 1.59 [9H, s, C(CH₃)]], 1.40 - 0.82 (8H, m, CH₂-2′ₐ, CH₂-4′, CH₂-5′).

**13C NMR (126 MHz, DMSO, 70°C)** δ 164.57 (C), 157.00 (C), 146.73 (C), 137.58 (C), 135.08 (C), 131.65 (CH), 129.94 (C), 129.49 (CH), 129.31 (CH), 128.89 (CH), 128.82 (CH), 127.30 (CH), 125.55 (C), 125.31 (CH), 125.03 (CH), 123.00 (C), 117.19 (CH), 59.46 (CH₃), 38.29 (CH), 35.65 (C), 30.32 (CH₃), 29.88 (CH₃), 24.82 (CH₃).

**ν_max(ATR)cm⁻¹:** 2926 (C-H), 2853 (C-H), 1621 (C=N), 1587 (C₆H₅=C₆H₅), 1540 (C₆H₅=C₆H₅), 1343 (NO₂). MS (ESI⁺): 453 [M⁺ (phenanthridinium cation), 100]. HRMS: 453.2523. C₃₀H₂₇N₂O₂⁺ requires 453.2537.
Benzophenone 327 (414 mg, 1.49 mmol, 1.00 eq.) and 6-azidohex-1-ylamine (1.00 g, 7.86 mmol, 5.28 eq.) were combined stirring under argon at 0 °C in dry PhMe (7.0 mL) then 1 M titanium tetrachloride in dry PhMe (1.70 mL, 1.70 mmol, 1.14 eq.) was added over 5 min. After 15 h a sample (0.1 mL) was quenched into H₂O, partitioned between DCM and water and the organic separated and the solvent removed under reduced pressure. This process was repeated at 21 h, at which point the starting material was shown to be consumed by proton NMR. The mixture was quenched into water then filtered through celite with DCM, then partitioned between DCM and 1 M HCl(aq). The aqueous was extracted with DCM (x 2) then the collected organics washed with 1 M HCl(aq), followed by NaHCO₃(aq). The organic layers were dried (MgSO₄), then filtered and the solvent removed under reduced pressure. From the crude yield of 571 mg of brown oil a portion (100 mg, 0.25 mmol, 1.00 eq.) was weighed by difference onto a spatula and sonicated into a microwave tube (10 mL) containing dry MeCN (5.0 mL). The tube was filled with argon and sealed then heated in a microwave to 110 °C for 90 min. After cooling the residue was dissolved in MeOH and loaded onto a Strata SCX ion exchange column and washed (MeOH) then eluted (15 mg/mL NaOAc in MeOH). The solution was partitioned between CHCl₃ and 1 M HCl(aq), then the organic layer washed (1 M HCl(aq) x 2). The organic layer was dried (MgSO₄), then filtered and ethereal HCl (1.00 mL, 2.00 mmol, 7.75 eq.) was added, then the solvent removed under reduced pressure. The phenanthridinium chloride 310 was isolated as a brown oil (79 mg, 78%). ¹H NMR (400 MHz, 0.75 mL CDCl₃ + 0.05 mL CF₃CO₂D) δ 8.99 (1H, dd, J = 8.1, 1.7 Hz, H1), 8.93 (1H, d, J = 8.5 Hz, H10), 8.40 (1H, d, J = 8.3 Hz, H4), 8.30 (1H, ddd, J = 8.5, 7.2, 1.5 Hz, H9), 8.20 - 8.11 (2H, m, H2, H3), 7.81 - 7.67 (4H, m, H8, H3”, H4”, H5”), 7.68 (1H, d, J = 7.7 Hz, H7), 7.60 - 7.53 (2H, m, H2”, H6”), 4.89 - 4.79 (2H, m, CH₂-1’), 3.26 (2H, t, J = 6.7 Hz, CH₂-6’), 2.01 (2H, s, br, CH₂-2’), 1.58 - 1.49 (2H, m, CH₂-3’), 1.42 - 1.23 (4H, m, CH₂-4’, CH₂-5’). ¹³C NMR (101 MHz, 0.75 mL CDCl₃ + 0.05 mL CF₃CO₂D) δ 163.82 (C), 138.43 (C), 135.27 (C), 133.35 (C), 133.21 (CH), 133.09 (CH), 132.37 (CH), 131.21 (CH), 130.71 (CH), 130.05 (CH), 127.62 (CH), 126.38 (C), 125.10 (C), 124.83 (CH), 122.75 (CH), 119.35 (CH), 54.47 (CH₃), 51.02 (CH₃), 29.75 (CH₃), 28.06 (CH₃), 25.84 (CH₃), 25.62 (CH₃). v_max(ATR) cm⁻¹ (as TFA salt): 2928 (C-H), 2856 (C-H), 1779 (TFA), 1738 (TFA), 1622 (C=N), 1588 (C₆H₅=C₆H₅), 1538(C₆H₅=C₆H₅). MS (NSI⁺): 381 [M⁺ (phenanthridinium cation)], 38%], 256 (M⁺ – C₆H₁₂, 100). HRMS: 381.2073. C₂₅H₂₅N₄⁺ requires 381.2074.
Nitrophenanthridinium 300 (250 mg, 0.50 mmol, 1.00 eq.) was dissolved in a 1:1 mixture of AcOH and EtOH (15.0 mL) then iron powder (346 mg, 6.20 mmol, 10.7 eq.) added and the mixture heated stirring to 40 °C for 90 min. The mixture was then poured into 1 M HCl (aq) and extracted with DCM (x 2). The combined organics were then washed with 1 M HCl (aq) and NaHCO$_3$ (aq) (x 2) sequentially, before drying (MgSO$_4$), filtering and removing the solvent under reduced pressure. The amine 373 was isolated as a red powder (200 mg, 85%). $^1$H NMR (500 MHz, CDCl$_3$) δ 8.57 (1H, d, J = 8.9 Hz, H1), 8.48 (1H, d, J = 8.6 Hz, H10), 8.24 (1H, s, H4), 7.98 (1H, d, J = 8.7 Hz, H2), 7.83 - 7.68 (4H, m, H9, H3”, H4”, H5”), 7.49 (2H, d, J = 6.8 Hz, H2”, H6”), 6.52 (1H, s, H7), 4.95 (2H, s, br, NH$_2$), 4.63 (2H, t, J = 8.4 Hz, CH$_2$-1’), 1.95 - 1.83 (2H, m, CH$_2$-2’), 1.36 - 1.11 (6H, m, CH$_2$-3’, CH$_2$-4’, CH$_2$-5’), 0.84 (3H, t, J = 7.2 Hz, CH$_2$-6’). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 162.20 (C), 151.19 (C), 133.51 (CH), 132.28 (C), 131.91 (CH), 131.01 (C), 130.11 (CH), 129.92 (CH), 128.22 (CH), 127.55 (C), 126.08 (C), 125.78 (C), 125.45 (CH), 123.85 (C), 123.55 (CH), 122.14 (CH), 111.20 (CH), 54.75 (CH$_3$), 30.74 (CH$_3$), 29.67 (CH$_3$), 26.26 (CH$_3$), 22.34 (CH$_3$), 13.91 (CH$_3$). $\nu_{Max}$(ATR)cm$^{-1}$: 3277 (N-H), 3133 (N-H), 2952 (C-H), 2929 (C-H), 2859 (C-H), 1609 (C=N), 1579 (C=$\equiv$C$_{Ar}$). MS (ESI$^+$): 435 [M$^{81}$Br$^+$ (phenanthridinium cation), 100%], 433 [M$^{79}$Br$^+$ (phenanthridinium cation), 100%]. HRMS: 435.1242, 433.1255. C$_{25}$H$_{26}^{81}$BrN$_2$ requires 435.1253, C$_{25}$H$_{26}^{79}$BrN$_2$ requires 433.1274. MP: 153 - 157 °C.

3,8-Diamino-5-(hex-1’-yl)-6-methyl-5,6-dihydrophenanthidine 375

Enamine 369 (100 mg, 0.27 mmol, 1.00 eq.) was dissolved in a 1:1 mixture of AcOH and EtOH (4.0 mL) then iron powder (151 mg, 2.71 mmol, 10.0 eq.) added and the mixture heated stirring to 60 °C for 1 h. The mixture was then poured into 1 M HCl (aq), then the acidity reduced with NaHCO$_3$ (aq). Excess NaBH$_4$ was added and the aqueous extracted with CHCl$_3$ which was then dried (MgSO$_4$) and filtered, then the solvent removed under reduced pressure. The phenanthidine 375 was isolated as a solid (15 mg, 18%). $^1$H NMR (500 MHz, CDCl$_3$) δ 7.41 (1H, d, J = 8.2 Hz, H10), 7.40 (1H, d, J = 8.1 Hz, H1), 6.62 (1H, dd, J = 8.3, 2.4 Hz, H9), 6.39 (1H, d, J = 2.4 Hz, H7), 6.15 (1H, dd, J = 8.1, 2.2 Hz, H2), 6.03 (1H, d, J = 2.1 Hz, H4), 4.26 (1H, q, J = 6.5 Hz, H6), 3.60 (4H, s, br, NH$_2$), 3.37 (1H, dt, J = 13.9, 7.0 Hz, CH$_2$-1’), 3.04 (1H, dt, J = 13.8, 7.7 Hz, CH$_2$-1’), 1.71 - 1.59 (2H, m, CH$_2$-2’), 1.41
- 1.19 (6H, m, CH$_2$-3', CH$_2$-4', CH$_2$-5'), 1.10 (3H, d, J = 6.5 Hz, C-6CH$_3$), 0.88 (3H, t, J = 7.0 Hz, CH$_3$-6').

$^1$C NMR (101 MHz, CDCl$_3$) δ 146.27 (C), 144.38 (C), 144.16 (C), 137.87 (C), 123.44 (CH), 122.89 (CH), 122.60 (C), 114.93 (C), 114.74 (CH), 111.81 (CH), 104.81 (CH), 100.11 (CH), 58.31 (CH), 49.59 (CH$_3$), 31.86 (CH$_3$), 28.00 (CH$_3$), 27.13 (CH$_3$), 22.80 (CH$_3$), 18.57 (CH$_3$), 14.21 (CH$_3$). vmax (ATR) cm$^{-1}$: 3453 (N=CH), 1495 (N=C), 13453 (N=NH), 2026 (C=O). MS (EI): m/z 425 (M$^+$, 100%), 342 (M$^+$ - CH$_3$), 308 (M$^+$ - 2CH$_3$), 294 [(M+H)$^+$ - CH$_2$]$_2$. HRMS: 425.2837.

3,8-Diamino(2,2-dimethylpropyl)-6-phenylphenanthridine 405

3,8-Diamino-6-phenylphenanthridine 133 (650 mg, 2.28 mmol, 1.00 eq.) and NaBH(OAc)$_3$ (1.45 g, 6.83 mmol, 3.00 eq.) were mixed in TFA (0.81 mL, 11 mmol, 4.7 eq.). The mixture was cooled to −15 °C under argon with an IPA/CO$_2$ bath. DCM (6.5 mL) was added and the mixture stirred. 2,2-dimethylpropanal (0.54 mL, 5.0 mmol, 2.2 eq.) was then added by syringe and the mixture allowed to stir for 2 h, then the mixture was partitioned between DCM and H$_2$O, then the aqueous extracted with DCM. The combined organic layers were washed with brine, then extracted with DCM and the organics combined and solvent removed under reduced pressure. The material was dissolved in Et$_2$O and precipitated with excess ethereal HCl (2 M), then allowed to settle overnight. The solid was filtered, washed with Et$_2$O and dissolved in DCM then washed with NaHCO$_3$(aq). The aqueous was re-extracted with DCM, then the combined organics dried (MgSO$_4$), filtered and the solvent removed under reduced pressure to give phenanthridine 405 as a foam (1.08 g, 99%).

$^1$H NMR (CDCl$_3$, 500 MHz): 8.28 (1H, d, J = 9.0 Hz, H10), 8.19 (1H, d, J = 8.9 Hz, H11), 7.68 – 7.63 (2H, m, H2'', H6''), 7.53 – 7.43 (3H, m, H3'', H4'', H5''), 7.26 (1H, d, J = 2.3 Hz, H4), 7.16 (1H, dd, J = 8.9, 2.5 Hz, H9), 7.02 – 6.97 (2H, m, H7, H2), 3.91 (2H, s, br, 3-NHR, 8-NHR), 3.02 (2H, s, 8-NHCH$_2$), 2.87 (2H, s, 3-NHCH$_2$), 1.00 [9H, s, 8-NHCH$_2$C(CH$_3$)$_3$], 0.96 [9H, s, 3-NHCH$_2$C(CH$_3$)$_3$]. $^1$C NMR (126 MHz, CDCl$_3$) δ 159.91 (C), 148.65 (C), 146.73 (C), 143.19 (C), 139.57 (C), 129.51 (CH), 128.56 (CH), 128.25 (CH), 126.56 (C), 125.12 (C), 122.36 (CH), 121.94 (CH), 120.51 (CH), 117.04 (CH), 116.17 (C), 107.43 (CH), 107.33 (CH), 55.88 (CH$_3$), 55.84 (CH$_3$), 32.04 (C), 31.97 (C), 27.58 (CH$_3$), 27.56 (CH$_3$). IR (ATR) cm$^{-1}$: 3424 (N-H), 3285 (N-H), 2953 (C-H), 2864 (C-H), 1620 (C=N), 1568 (C$_A$=C$_A$), 1512 (C$_A$=C$_A$). MS (EI$^+$): 425 (M$^+$, 72%), 368 [M$^+$ – C(CH$_3$)$_3$, 100]. HRMS: 425.2837. C$_{29}$H$_{35}$N$_3$ requires 425.2831.
3,8-Diamino(2,2-dimethylpropyl)-5-N-ethyl-6-phenylphenanthridinium methanesulfonate

Phenanthridine 405 (100 mg, 0.23 mmol, 1.00 eq.) dissolved in Et₂O (1.0 mL), then stirred under argon at RT. Ethyl triflate (34 μL, 0.26 mmol, 1.1 eq.) was added by syringe followed by a further 0.2 mL Et₂O. After 20 h stirring at RT the reaction was quenched into H₂O, extracted with DCM (x 2) then the organic layers dried (MgSO₄), filtered and the solvent removed under reduced pressure. Column chromatography [SiO₂, gradient from DCM/MeOH/NEt₃ (200:2:1) to (100:2:1)] was followed by ion exchange to mesylate and salt 406 was isolated as purple foam (65 mg, 50%).

¹H NMR (CDCl₃, 400 MHz): 8.24 (1H, d, J = 9.3 Hz, H1), 8.05 (1H, d, J = 9.3 Hz, H10), 7.89 (1H, s, H4), 7.78 - 7.64 (3H, m, H3″, H4″, H5″), 7.48 (1H, dd, J = 9.2, 2.5 Hz, H9), 7.40 - 7.31 (3H, m, H2″, H6″, H2), 7.13 (1H, t, J = 6.0 Hz, NH), 5.97 (1H, d, J = 2.4 Hz, H7), 4.87-4.75 (3H, m, NCH₂CH₃, NH), 3.12 (2H, d, J = 6.1 Hz NHCH₂), 2.81 (3H, s, CH₃SO₂⁻), 2.65 (2H, d, J = 6.1 Hz, NHCH₂), 1.52 (3H, t, J = 7.1 Hz, NCH₂CH₃), 1.09 [9H, s, C(CH₃)₃], 0.86 [9H, s, C(CH₃)₃]. ¹³C (101 MHz, CDCl₃) δ 157.40 (C), 152.30 (C), 147.64 (C), 134.79 (C), 132.08 (C), 131.07 (CH), 129.70 (CH), 128.99 (C), 128.15 (CH), 128.03 (CH), 124.73 (C), 124.17 (CH), 122.06 (CH), 117.78 (CH), 117.43 (C), 105.05 (CH), 98.30 (CH), 55.15 (CH₂), 54.96 (CH₂), 49.49 (CH₃), 32.65 (C), 32.39 (C), 27.63 (CH₃), 27.55 (CH₃), 14.48 (CH₃). IR (ATR cm⁻¹): 3350 (N–H), 2957 (C–H), 2864 (C–H), 1622 (C=O), 1535 (C₆=C₆). MS (as CF₃SO₂⁻ salt, NSI⁻): 454 (M⁺, 100%). HRMS: 454.3211. C₃₁H₄₀N₃ requires 454.3217. MS (as CF₃SO₂⁻ salt, NSI⁻): 148 (CF₃SO₂⁻, 100%). HRMS: 148.9530. CF₃SO₃ requires 148.9526. MP: Decomp > 180 °C.

1-Bromohexyltrifluoromethanesulfonate 408

1-Bromohexan-6-ol 407 (1.00 g, 5.52 mmol, 1.00 eq.) was added to a 0 °C stirring solution of dry DCM (5.0 mL), dry pyridine (0.4 mL, 6.1 mmol, 1.1 eq.) and triflic anhydride (1.02 mL, 6.08 mmol, 1.10 eq.) under argon. After 90 min the mixture was quenched into DCM/H₂O, then the organic washed (x 2) with H₂O and dried (MgSO₄) then filtered and the solvent removed under reduced pressure. The triflate 408 was isolated as a brown oil (1.318 g, 76%). δ₁H (CDCl₃, 400 MHz): 4.55 (2H, t, J = 6.4 Hz, CH₂OTf), 3.41 (2H, t, J = 6.6 Hz, CH₂Br), 1.93 - 1.81 (4H, m, CH₂CH₂OTf, CH₂CH₂Br), 1.57 - 1.42 (4H, m, CH₂CH₂CH₂CH₃), δ₂ (CDCl₃, 100 MHz): 119.02 (q, J = 319.7 Hz, CF₃), 77.29 (CH₃), 33.66 (CH₂), 32.69 (CH₂), 29.47 (CH₂), 27.76 (CH₂), 24.71 (CH₂).
3,8-Diaminoneopentyl-5(6'-bromohexyl)-6-phenylphenanthridinium triflate 409

Triflate 408 (413 mg, 1.32 mmol, 1.02 eq) was dissolved in dry Et₂O (6.6 mL) stirring under argon and cooled to −30 °C, then phenanthridine 405 (549 mg, 1.29 mmol, 1.00 eq.) was added and the mixture allowed to warm to RT then stirred for 17 h. The mixture was quenched into H₂O and extracted into DCM, then dried (MgSO₄), filtered and the solvent removed under reduced pressure. Column chromatography [SiO₂, loaded in DCM, DCM/MeCN/NEt₃, gradient from (100:7.5:1) to (100:15:2)]. The fractions were dried (MgSO₄), filtered and the solvent was removed under reduced pressure. A mixture containing mostly the desired product 409 was obtained (450 mg, 50%) and used directly without further purification.

3,8-Diaminoneopentyl-5(6'-triphenylphosphoniohexyl)-6-phenylphenanthridinium mesylate 403

Bromophenanthridinium 409 (300 mg, 0.41 mmol, 1.00 eq.) was transferred to a flask in DCM which was then removed under reduced pressure. Triphenylphosphine was dried by azeotrope with PhMe, then added (1.07 g, 4.08 mmol, 10.0 eq.), followed by dry PhMe (6.0 mL). The reaction was heated to reflux for 15 h under argon stirring. The mixture was cooled to 70 °C then poured into PhMe. The residue was dissolved in CHCl₃, then the solvent removed under reduced pressure. The material was converted to chloride with an anion exchange resin [loaded in MeOH, eluted with MeOH/H₂O (1:1)]. Following column chromatography (SiO₂, loaded with CHCl₃, CHCl₃/MeOH/NH₃(aq) gradient from (100:15:1) to (100:25:2)) product fractions were combined, then the solvent removed under reduced pressure. The material was dissolved in CHCl₃ and precipitated with Et₂O and Pet. ether. The resulting mixture was purified using HPLC in 20 mg/10 ml aliquots (9:1 H₂O/MeCN).

<table>
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<tr>
<th>Time: (min)</th>
<th>0.1% TFA(aq): (%)</th>
<th>MeCN: (%)</th>
<th>Flow: (ml/min)</th>
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Column: C18 synergi Max-RP 10 µm 250 x 21.2 mm  RT: 48.5 min
The clean fractions from the aliquots were combined and the solvent removed under reduced pressure. The counter ion was then exchanged (IRA 401 resin in mesylate form, loaded and eluted in 1:1 MeOH/H2O) to the mesylate form. The double salt 403 was isolated as a purple glass (38 mg, 9%). δH (CDCl3, 500 MHz): 8.34 (1H, d, J = 9.6 Hz, H1), 8.21 (1H, d, J = 9.2 Hz, H10), 7.97 (1H, apparent s, H4), 7.80 - 7.62 (19H, m, CH2-6'), 7.34 - 7.30 (2H, m, CH3'), 7.29 (1H, J = 9.4 Hz, 1.7 Hz, H2), 7.11 (1H, t, J = 6.1 Hz, C3-NH), 5.96 (1H, d, J = 2.4 Hz, H7), 5.18 (1H, s, CH2-1'), 4.68 (2H, apparent s, broad, CH2-6'), 3.60 - 3.48 (2H, m, CH2-6'), 3.08 (2H, d, J = 6.1 Hz, 3-NHC6H5), 2.70 (6H, s, C6H5SO3-2'), 2.63 (2H, d, J = 6.1 Hz, 8-NHC6H5), 1.83 (2H, apparent s, broad, CH2-3'), 1.65 - 1.44 (6H, m, CH2-3', CH2-4', CH2-5'), 1.01 [9H, s, 3-CH2C(CH3)], 0.83 [9H, s, 8-CH2C(CH3)]. δC (CDCl3, 125 MHz): 157.52 (C), 152.91 (C), 148.40 (C), 135.29 (d, J = 2.7 Hz, CH), 135.18 (C), 133.90 (d, J = 10.0 Hz, CH), 132.49 (C), 131.31 (CH), 130.78 (d, J = 12.6 Hz, CH), 129.97 (CH), 129.32 (CH), 129.14 (C), 128.28 (CH), 124.92 (C), 124.53 (CH), 122.14 (CH), 118.74 (d, J = 86.1 Hz, C), 117.43 (C), 116.32 (CH), 104.70 (CH), 100.06 (CH), 55.46 (CH2), 55.12 (CH2), 53.65 (CH2), 53.65 (CH2), 39.95 (CH2), 33.10 (C), 32.87 (C), 29.41 (CH2), 28.97 (CH2), 25.04 (CH2), 22.09 (d, J = 4.2 Hz, CH2). 13C NMR (101 MHz, CDCl3) δ = 153.26 (C), 137.37 (C), 128.68 (CH), 128.02 (CH), 127.60 (CH), 115.90 (CH), 70.74 (CH2). Consistent with literature data. 1,4-Bis(benzyloxy)benzene 420 4,1-Bis(benzyloxy)-2-nitrobenzene 421

Protected quinol 420 (65.0 g, 224 mmol, 1.00 eq.) was suspended in glacial acetic acid (240 mL) and a solution of nitric acid in glacial acetic acid (HNO3aq 70% aqueous solution, 17 mL, 270 mmol, 1.2 eq. Made up to 75 mL with glacial acetic acid) was added to the mixture and
mechanically stirred for 2 h, then additional AcOH (300 mL) added and the reaction stirred at 50 °C for 18 h. The material was cooled to 0 - 5 °C for 4 h, then filtered. The filtrate was diluted (H₂O) and further precipitate collected, then the remaining solution concentrated under reduced pressure and the process repeated. Remaining H₂O and AcOH were removed by azeotrope with PhMe, which was then removed under reduced pressure. Nitro compound 421 was isolated as a yellow solid (73.2 g, 97%). δ_H (400 MHz, CDCl₃): 7.49 (1H, d, J = 3.0, H3), 7.46 - 7.30 (10H, m, OCH₂Ph), 7.12 (1H, dd, J = 9.2, 3.1 Hz, H5), 7.05 (1H, d, J = 9.2 Hz, H6), 5.18 (2H, s, CH₂), 5.05 (2H, s, CH₂). 13C NMR (101 MHz, CDCl₃) δ 152.30 (C), 146.39 (C), 135.97 (C), 135.88 (C), 128.75 (CH), 128.70 (CH), 128.37 (CH), 128.22 (CH), 127.59 (CH), 127.15 (CH), 121.54 (CH), 117.24 (CH), 111.18 (CH), 117.24 (CH). IR (ATR cm⁻¹): 1520 (NO₂), 1497 (C₅Ar=C₅Ar), 1341 (NO₂). MS (ESI⁺): 358 [(M+Na)⁺, 100]. HRMS: 358.1034. C₂₀H₁₇NNaO₄ requires 358.1050. Compound known but not previously fully characterised.

Nitroaryl 421 (4.50 g, 13.4 mmol, 1.00 eq.) was dissolved in CHCl₃ (65 mL) and stirred at 0 °C under argon. To the mixture a suspension of AlCl₃ (2.07 g, 15.5 mmol, 1.16 eq.) in CHCl₃ (10 mL) was added portionwise with additional CHCl₃ (20 mL). The reaction was monitored by TLC and was complete after 15 min. The mixture was quenched into HCl (aq) (1 M) and extracted with DCM (x 3). The combined organics were dried (MgSO₄), filtered and the solvent removed under reduced pressure. The residue was crystallised from boiling MeOH, then recrystallised from boiling MeOH with 5% H₂O added. The resulting crystals were washed (H₂O), then partitioned between H₂O and DCM. The organic layer was separated and the solvent removed under reduced pressure, giving nitrophenol 422 as a solid (1.88 g, 57%). δ_H (400 MHz, CDCl₃): 10.36 (1H, s, ArO), 7.61 (1H, d, J = 3.1 Hz, H3), 7.46 - 7.33 (5H, m, OCH₂Ph), 7.29 (1H, dd, J = 9.2, 3.1 Hz, H5), 7.10 (1H, d, J = 9.2 Hz, H6). 13C NMR (101 MHz, CDCl₃) δ 151.64 (C), 150.20 (C), 135.86 (C), 132.97 (C), 128.76 (CH), 128.42 (CH), 127.88 (CH), 127.65 (CH), 120.93 (CH), 107.19 (CH), 70.92 (CH₂). IR (ATR cm⁻¹): 3231 (O-H), 3123 (C₅Ar-H), 3106 (C₅Ar-H), 1584 (C₅Ar=C₅Ar), 1530 (NO₂), 1501 (C₅Ar=C₅Ar), 1485 (C₅Ar=C₅Ar), 1316 (NO₂). MS (El⁺): 245 (M⁺, 18%), 91 (C₇H₇⁺, 100). HRMS: 245.0696. C₁₃H₁₂N₂O₄ requires 245.0688. Compound known but not previously fully characterised.

1-(2-bromoethoxy)-2-nitrobenzene 415

Following the method of Tsein and coworkers, 415 2-nitrophenol 423 (3.99 g, 28.7 mmol, 1.00 eq.), 1,2-dibromoethane (7.5 mL, 87 mmol, 3.0 eq.) and K₂CO₃ (4.36 g, 31.6 mmol, 1.10 eq.) were combined in DMF (6.0 mL) and stirred at 120 °C for 3 h under argon. The precipitate was filtered off and washed with DCM and H₂O. The filtrate layers were separated and the organics were
washed with 0.5 M NaOH(aq) and saturated brine solution. The organics were dried (MgSO₄) and filtered, then the solvent was removed under reduced pressure to give the bromide 415 (4.53 g, 65%) as a solid. δH (400 MHz, CDCl₃): 7.85 (1H, dd, J = 8.3, 1.7 Hz, H6), 7.55 (1H, ddd, J = 8.4, 7.5, 1.7 Hz, H4), 7.12 - 7.06 (2H, m, H3, H5), 4.42 (2H, t, J = 6.5 Hz, OCH₂CH₂Br), 3.68 (2H, t, J = 6.5 Hz, OCH₂CH₂Br). This material is commercially available and ¹H NMR is consistent with literature data.332

2-Nitro-1-{2"-(4"-benzyloxy-2"-nitrophenoxy)ethoxy}benzene 416

Following the method of Tsein and coworkers396 1-(2-bromoethoxy)-2-nitrobenzene 415 (10.9 g, 44.3 mmol, 1.47 eq.), nitrophenol 422 (7.0 g, 30 mmol, 1.0 eq.) and K₂CO₃ (3.0 g, 22 mmol, 0.7 eq.) were combined in dry DMF (17.0 mL) and stirred at 140 °C for 2 h under argon, then allowed to stir at RT for 12 h. The material was heated to 50 °C, and the precipitate was dissolved with dropwise addition of water. After 24 h stirring precipitate had formed and was collected by filtration. The material was then washed out in a mixture of acetone and hexane. The solvent was removed under reduced pressure and the compound dissolved in DCM then washed with water (x 3) and 5% LiCl(aq) solution, then the solvent removed from the combined organics to give the ether 416 in quantitative yield (12.4 g, quant.). δH (400 MHz, CDCl₃): 7.84 (1H, dd, J = 8.1, 1.7 Hz, H3), 7.57 (1H, ddd, J = 8.5, 7.4, 1.7 Hz, H5), 7.47 - 7.32 (6H, m, OCH₂Ph, H3"), 7.23 - 7.19 (3H, m, H6', H5', H6''), 7.12 - 7.05 (1H, m, H4), 5.07 (2H, s, OC₆H₄Ph), 4.52 - 4.46 (4H, m, OC₆H₄C₆H₄O). δC (CDCl₃, 100 MHz): 152.95 (C), 151.956 (C), 146.37 (C), 140.63 (C), 140.20 (C), 135.90 (C), 134.34 (CH), 128.76 (CH), 128.38 (CH), 127.59 (CH), 125.68 (CH), 121.73 (CH), 121.24 (CH), 118.75 (CH), 115.61 (CH), 111.04 (CH), 70.96 (CH₂), 69.99 (CH₂), 68.77 (CH₂). IR (ATR cm⁻¹): 3094 (CH), 2960 (CH), 2929 (CH), 2871 (CH), 1606 (C=O), 1518 (NO₂), 1497 (C=O), 1487 (NO₂), 1451 (C=O). MS (EI⁺): (410 M⁺, 45%), 166 (O₃NCC₆H₄OCH₂CH₃, 38), 122 (C₆H₄NO₂⁺, 44), 91 (PhCH₂⁺, 100). HRMS: 410.1114. C₂₁H₁₈N₂O₇ requires M⁺ 410.1114. MP: 110-111 °C.

1-Nitro-2-{2"-(2"-nitrophenoxy)ethoxy}benzene 426

Dimer 426 (321 mg, 4%) was isolated as a side product of an earlier synthesis of ether 416, and separated from ether 416 by column chromatography [SiO₂, Pet. ether/EtOAc (5:2)]. δH (400 MHz, d₆-DMSO): 7.86 (2H, dd, J = 8.0, 1.7 Hz, H6, H3''), 7.66 (2H, ddd, J = 8.4, 7.5, 1.7 Hz, H4, H5''), 7.44 (2H, J = 8.5, 1.0 Hz, H3, H6''), 7.15 (2H, m, H5, H4''), 4.54 (4H, s, OCH₃). δC (100 MHz, d₆-DMSO): 150.89 (C), 139.75 (C), 134.30 (CH), 124.83 (CH), 120.98 (CH), 115.55 (CH), 68.02 (CH₂). IR (ATR cm⁻¹): 2931 (C-H), 2878 (C-H), 1605 (C=O), 1512 (NO₂), 1358 (NO₂). MS (EI⁺): 304 (M⁺ 28%), 166
Dinitro compound 416 (100 mg, 0.24 mmol, 1.00 eq.) was dissolved in acetone (4.0 mL) and added to a stirring solution of NH₄HCO₂ (123 mg, 1.95 mmol, 8.13 eq.) in deionised water (1.0 mL). Iron filings (141 mg, 2.52 mmol, 10.5 eq.) were added and the reaction mixture was then stirred at 70 °C under argon for 17 h. The mixture was filtered through celite with acetone, then the solvent removed under reduced pressure. The residue was dissolved in DCM, then washed with brine and dried (MgSO₄), filtered and the solvent removed under reduced pressure, giving diamine 427 as a solid (52 mg, 61%).

Dinitro compound 416 (100 mg, 0.24 mmol, 1.00 eq.) was dissolved in acetone (4.0 mL) and added to a stirring solution of NH₄HCO₂ (123 mg, 1.95 mmol, 8.13 eq.) in deionised water (1.0 mL). Iron filings (141 mg, 2.52 mmol, 10.5 eq.) were added and the reaction mixture was then stirred at 70 °C under argon for 17 h. The mixture was filtered through celite with acetone, then the solvent removed under reduced pressure. The residue was dissolved in DCM, then washed with brine and dried (MgSO₄), filtered and the solvent removed under reduced pressure, giving diamine 427 as a solid (52 mg, 61%).


Aniline 427 (1.80 g, 5.14 mmol, 1.00 eq.) was dried by azeotrope twice in PhMe then combined under argon with NaI (2.12 g, 14.1 mmol, 2.75 eq., dried under vacuum at 100 °C for 6 h) and proton sponge (also dried by azeotrope in PhMe, 7.50 g, 35.0 mmol, 6.81 eq.) dry MeCN (10.0 mL) and ethyl bromoacetate (5.00 mL, 46.3 mmol, 9.01 eq.) were added to the stirring mixture which was then heated under reflux for 24 h. The solvent was then removed under reduced pressure and the residue was dissolved in PhMe and filtered. The filtrate was washed 5 times with dilute HCl(aq), then the organics were then combined and the solvent removed under vacuum. Crystallisation (Pet. ether/EtOAc) gave the diammine tetraester 428 (4.00 g, quant.) as needles. δH

Tetraester 428 (1.02 g, 1.28 mmol, 1.00 eq.) was dried by azeotrope twice in PhMe then dissolved in dry DMF (62 mL) and stirred under argon at 0 °C. Dry pyridine (1.56 mL, 19.0 mmol, 14.8 eq.) was added followed by slow addition of POCl₃ (1.56 mL, 16.7 mmol, 13.4 eq.). After 30 min the reaction mixture was allowed to warm to RT, and the reaction was followed by TLC. After 1 h 10 min the mixture was quenched into cold 1 M NaOH(aq). After vacuum filtration the filtrate was extracted with PhMe (x 3), which was then removed under reduced pressure. The sample was dissolved in DCM and precipitated with hexane, the solid collected and the process repeated, giving aldehyde 429 (700 mg, 77%) as an amorphous solid. δn (400 MHz, CDCl₃): 10.31 (1H, s, CHO) 7.42 - 7.31 (5H, m, Ph), 7.29 (1H, s), H6''''), 6.93 - 6.79 (4H, m, H3''', H4''', H5''', H6'''''), 6.30 (1H, s, H3'''''), 5.10 (2H, s, OCH₂Ph), 4.27 - 4.20 (4H, m, OCH₂CH₂O), 4.18 (4H, s, NCH₂), 4.14 (4H, s, NCH₂), 4.06 (4H, q, J = 7.1 Hz, CO₂CH₂), 4.03 (4H, q, J = 7.1 Hz, CO₂CH₂), 1.16 (6H, t, J = 7.2 Hz, CH₃CH₂), 1.14 (6H, t, J = 7.1 Hz, CH₂CH₂), δc (100 MHz, CDCl₃): 187.49 (CH), 171.51 (C), 170.7051 (C), 157.34 (C), 150.19 (C), 146.44 (C), 143.96 (C), 139.48 (C), 136.32 (C), 128.71 (CH), 128.24 (CH), 127.29 (CH), 122.10 (CH), 121.66 (CH), 119.10 (CH), 117.98 (C), 113.32 (CH), 110.62 (CH), 102.67 (CH), 71.10 (CH₂), 67.61 (CH₂), 66.90 (CH₂), 61.22 (CH₃), 60.76 (CH₃), 53.86 (CH₂), 53.50 (CH₂), 14.07 (CH₃), 14.02 (CH₃). IR (ATR cm⁻¹): 2975 (C-H), 2938 (C-H), 2928 (C-H), 2873 (C-H), 1745 (C=O), 1718 (C=O), 1662, 1598 (C₆=C₆), 1517 (C₆=C₆), 1508 (C₆=C₆). MS (NSI): 1462 [2M+Na⁺], 6%, 761 [(M+K⁺), 3], 745 [(M+Na⁺), 60], 740 [(M+NH₄⁺), 45], 723 [(M+H⁺), 100]. HRMS: 723.3121 requires 723.3129, C₃₀H₄₂O₁₂N₂. MP: 135 - 136 °C.
Ethyl-2-[[2'-(2''-bis(2'''-ethoxy-2'''-oxoethyl)amino)-5'''-formyl-4'''-(prop-2'''-en-1'''-yloxy)phenoxy)ethoxy]phenyl][2'-ethoxy-2'-oxoethyl]amino]acetate 430

Aldehyde 429 (500 mg, 0.62 mmol, 1.00 eq.) was combined in acetic acid (6.0 mL) with palladium on carbon (10% loading by weight, 160 mg, 0.15 mmol, 0.24 eq.). After an initial 10 min flush with argon, the solution was frozen in N_2 (l) and the gas removed under vacuum. The mixture was then melted under H_2 (g), which was bubbled through the solution for 5 min. This procedure was repeated twice, then the mixture allowed to stir under H_2 (g) for 2 h 20 min. The mixture was then filtered through celite eluting with DCM then dried over (MgSO_4) filtered and the solvent removed under reduced pressure. Azeotrope with PhMe then removal of the solvent under reduced pressure was used to remove AcOH. This gave the desired phenol 430 (387 mg, 87%) as a solid.

\[ \delta_1H (400 MHz, CDCl_3): 11.21 (1H, s, PhO_H), 9.61 (1H, s, CHO), 6.92 (1H, s, H6'''''), 6.94 - 6.82 (4H, m, H3'', H4'', H5'' H6'''), 6.15 (1H, s, H3'''''), 4.23 (4H, s, OCH_2CH_2O), 4.22 (4H, s, NCH_2), 4.15 (4H, s, NCH_2), 4.08 (4H, q, J = 7.1 Hz, CO_2CH_2), 4.08 (4H, q, J = 7.1 Hz, CO_2CH_2), 1.19 (6H, t, J = 7.2 Hz, CH_3CH_2), 1.16 (6H, t, J = 7.2 Hz, CH_3CH_2), \delta_1C (100 MHz, CDCl_3): 193.22 (CH), 171.49 (C), 170.37 (C), 158.82 (C), 150.17 (C), 148.32 (C), 142.72 (C), 139.48 (C), 122.25 (CH), 121.78 (CH), 119.23 (CH), 115.90 (CH), 113.38 (CH), 113.06 (C), 104.22 (CH), 68.23 (CH_2), 66.92 (CH_2), 61.34 (CH_2), 60.78 (CH_3), 53.85 (CH_2), 53.48 (CH_2), 14.09 (CH_3), 13.97 (CH_3). IR (ATR cm\(^{-1}\)): 2977 (C_Ar-H), 2960 (C_Ar-H), 2937 (C_Ar-H), 2970 (C_Ar-H), 2977 (C_Ar-H), 2877 (C_Ar-H), 1739 (CO), 1630 (CO), 1507 (C_Ar=C_Ar). MS (NSI): 671 [(M+K')], 655 [(M+Na'), 62], 650 [(M+NH_4^+), 22], 633 [(M+H')], 100]. HRMS: 633.2652. (M+H')

C_{31}H_{41}O_{12}N_2 requires 633.2659. MP: 78 - 80 °C.

Ethyl-2-[[2'-(2''-bis(2'''-ethoxy-2'''-oxoethyl)amino)-5'''-formyl-4'''-(prop-2'''-en-1'''-yloxy)phenoxy)ethoxy]phenyl][2'-ethoxy-2'-oxoethyl]amino]acetate 445

Phenol 430 (49 mg, 0.07 mmol, 1.0 eq.) was dried by azeotrope in PhMe and diluted in dry DMF (1.5 mL). This mixture was syringed into a dry vial containing a stirrer bar, K_2CO_3 (37 mg, 0.3 mmol, 3.7 eq.) and tetrabutylammonium bromide (2 mg, 6 µmol, 0.09 eq.), all of which had previously been dried by azeotrope in PhMe. The mixture was cooled with stirring to 0 °C under argon in the dark. Allyl bromide (10 µL, 0.11 mmol, 1.5 eq.) was added and the mixture warmed...
to RT and allowed to stir for 48 h. The mixture was then filtered eluting with DCM and the filtrate was washed with water, saturated brine solution and then NaHCO₃(aq) (saturated), and dried (MgSO₄). The mixture was filtered and the solvent removed under reduced pressure, to give phenol **445** impure (54 mg, estimate 44% purity from NMR). Data derived from the spectrum of the mixture.

$\delta^1 H$ (400 MHz, CDCl₃): 10.30 (1H, s, CHO), 6.93 - 6.78 (5H, m, H3””, H4””, H5””, H6””, H6’’’’), 6.26 (1H, s, H3’’’’), 6.03 (1H, ddt, $J$ = 17.3, 10.5, 5.2 Hz, OCH₂CH₂), 5.41 (1H, dq, $J$ = 17.3, 1.4 Hz, CH₂CHCH₂), 5.31 (1H, dq, $J$ = 10.5, 1.4 Hz, CH₂CHCH₂), 4.56 (2H, dt, $J$ = 5.2, 1.5 Hz), 4.23 - 4.19 (8H, m, OCH₂CH₂O, NCH₂), 4.07 (4H, q, $J$ = 7.2 Hz, CO₂CH₂CH₃), 4.04 (4H, q, $J$ = 7.2 Hz), 1.16 (6H, t, $J$ = 7.2 Hz, CH₂CH₃), 1.14 (6H, t, $J$ = 7.2 Hz, CH₂CH₃). MS (NSI): 711 ([M+Na]+, 73%), 673 ([M+H]+, 100%). HRMS: 673.2964 requires 673.2972, C₃₃H₄₅O₁₂N₂.

1,7-Diazododecane **437**

1,7-dibromomethane **436** (4.0 mL, 30 mmol, 1.0 eq.) and NaN₃ (11.4 g, 176 mmol, 5.87 eq.) were combined in deionised water (130 mL) and stirred at reflux under argon for 13 h 40 min. The desired product was then extracted into DCM. This solution was dried (MgSO₄), filtered, then the solvent removed under reduced pressure to give diazide **437** as an oil (4.5 g, 88%).

$\delta^1 H$ (400 MHz, CDCl₃): 3.27 (4H, t, $J$ = 6.9 Hz, N₃CH₂CH₂), 1.65 - 1.56 (4H, m, N₃CH₂CH₂), 1.45 - 1.31 (6H, m, N₃CH₂CH₂CH₂CH₂CH₂). $\delta^1 C$ (400 MHz, CDCl₃): 51.41 (CH₂), 28.75 (CH₂), 28.70 (CH₂), 26.59 (CH₂). IR (ATR cm⁻¹): 2935 (C-H), 2876 (C-H), 2860 (C-H), 2091 (N₃), 1464, 1456. MS (APCI⁺): 183 [(M+H)⁺, 100%). HRMS: 183.1348. C₇H₁₇N₆ requires 183.1353. Consistent with literature data.³⁴

7-Azidohept-1-amine **438**

1,7-diazododecane **437** (4.5 g, 20 mmol, 1.0 eq.) was dissolved in Et₂O (180 mL). Dilute HCl(aq) (1M, 135 mL) was added, followed by triphenylphosphine (6.0 g, 23 mmol, 1.1 eq.). The reaction mixture was then allowed to stir for 36 h at RT under argon, then the aqueous layer was extracted with Et₂O (100 mL, x 4) and DCM (100 mL, x 3), with NaOH(aq) (7 M) used to basify the aqueous between extractions. The organic layers were combined and dried (MgSO₄), then filtered and the solvent removed under reduced pressure to give amine **438** as an oil (2.98 g, 75%). $\delta^1 H$ (400 MHz, CDCl₃): 3.26 (2H, t, $J$ = 6.9 Hz, N₃CH₂CH₂), 2.69 (2H, t, $J$ = 6.9 Hz, N₃CH₂CH₂), 1.66 - 1.54 (2H, m, N₃CH₂CH₂), 1.50 - 1.26 (8H, m, 4 x CH₂). $\delta^1 C$ (400 MHz, CDCl₃): 51.60 (CH₂), 42.35 (CH₂), 33.89 (CH₂), 28.93 (CH₂), 26.89 (CH₂), 26.84 (CH₂). IR (ATR cm⁻¹): 2928 (C-H), 2876 (C-H), 2860 (C-H), 2091 (Nₛ), 1464, 1456. MS (APCI⁺): 157 [(M+H)⁺, 100%). HRMS: 157.1444. C₇H₁₅N₄ requires 157.1448.
Azido compound 438 (2.8 g, 14 mmol, 1.0 eq.) and CaCO₃ (2.9 g, 29 mmol, 2.1 eq.) were combined stirring under argon in DCM (62.2 mL) and deionised water (62.2 mL). Thiophosgene (1.6 mL, 22 mmol, 1.5 eq.) was then added slowly to the stirring mixture. After 1 h 30 min the reaction mixture was quenched into water, then extracted with DCM (100 mL, x 3), dried (MgSO₄), then filtered and the solvent removed under reduced pressure to give product 439 (88%).

\[ \delta^H (400 \text{ MHz, CDCl}_3): 3.52 (2H, t, J = 6.6 \text{ Hz, SCNC}_2\text{H}_2\text{CH}_2), 3.27 (2H, t, J = 6.9 \text{ Hz, N}_3\text{C}_2\text{H}_2\text{CH}_2), 1.76 - 1.66 (2H, m, SCNCH}_2\text{C}_2\text{H}_2), 1.66 - 1.56 (2H, m, N}_3\text{CH}_2\text{CH}_2), 1.50 - 1.31 (3 x \text{CH}_2, 6\text{H}). \delta^C (400 \text{ MHz, CDCl}_3): 204.39 (\text{C}), 51.38 (\text{CH}_2), 45.01 (\text{CH}_2), 29.85 (\text{CH}_2), 28.73 (\text{CH}_2), 28.38 (\text{CH}_2), 26.53 (\text{CH}_2), 26.46 (\text{CH}_2). \]

Methyl-2-{{(7-azideoheptyl)carbamothioyl}amino}acetate 440

Isothiocyanate 439 (3.12 g, 15.8 mmol, 1.00 eq.) was combined in EtOAc (90 mL) with NEt₃ (2.50 mL, 17.7, 1.12 eq.) and glycine methyl ester hydrochloride (2.22 g, 17.7 mmol, 1.12 eq.) and heated stirring under reflux and argon. The reaction mixture was partitioned between DCM and water and the organic layer washed with water and brine solution then dried (MgSO₄), filtered and the solvent removed under reduced pressure to give the product 440 (3.83 g, 85%), which was used without purification.

1-(7-Azideoheptyl)thiohydantoin 419

Carbamothioyl 440 (3.83 g, 14.7 mmol, 1.00 eq.) was refluxed stirring in a mixture of ethanol (190 mL) and 0.5 M HCl(sol) (25.9 mL) heated under argon. The solvent was then removed under reduced pressure and the mixture partitioned between DCM and water, then extracted with DCM (3 x 100 mL). The combined organics were dried (MgSO₄), then filtered and the solvent removed under reduced pressure. The material was then crystallised (Pet. ether/EtOAc) to give thiohydantoin 419 (2.58 g, 76%) as yellow needles. \( \delta^H (400 \text{ MHz, CDCl}_3): 4.06 (2H, d, J = 1.12 \text{ Hz, R(CO)NC}_2\text{H}_2\text{CO}), 3.81 (2H, t, J = 7.54 \text{ Hz, (CS)RNC}_2\text{H}_2), 3.26 (2H, t, J = 6.93 \text{ Hz, N}_3\text{CH}_2\text{CH}_2), 1.75 - 1.54 (4H, m, N}_3\text{CH}_2\text{CH}_2, \text{HNCH}_2\text{C}_2\text{H}_2), 1.44 - 1.28 (6H, m, other \text{CH}_2). \delta^C (100 \text{ MHz, CDCl}_3): 185.23 (\text{C}), 171.38 (\text{C}), 51.41 (\text{CH}_2), 48.30 (\text{CH}_2), 41.32 (\text{CH}_2), 28.73 (\text{CH}_2), 28.64 (\text{CH}_2), 27.40 (\text{CH}_2), 26.54 (\text{CH}_2). \]

IR (ATR cm\(^{-1}\)): 1740 (CO), 3177 (NH), 2933 (C-H), 2854 (C-H), 2090 (N=), 1538 (HNC), 1467, 1445 (CH\(_2\)), 1353 (HCN), 1273 (N=). MS (Cl\(^+\)): 256 [(M+H\(^+\)], 100%. HRMS: 256.1236 requires 256.1232, C\(_{10}\)H\(_{16}\)ON\(_5\).MP: 66°C
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