THE UNIVERSITY OF HULL

DEVELOPMENT OF SYNTHETIC ROUTES TOWARDS BIS(MACROCYCLIC) COMPOUNDS FOR IMAGING AND THERAPY OF CANCER

Being a thesis submitted for the Degree of Doctor of Philosophy in the University of Hull

by

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Abstract

Porphyrin molecules are efficient generators of singlet oxygen and are used as photosensitisers in PDT. They are also known to accumulate in tumour tissue due to the hyperpermeability of tumour vasculature and poor lymphatic drainage, thus exhibiting a passive tumour targeting property.

Functionalised tetraazamacrocycles based around the cyclam and cyclen structures form thermodynamically stable and kinetically inert complexes with metal ions such as copper(II) and gadolinium(III), and are used *in vivo* for both imaging and radiotherapy (MRI, PET, radiotherapy).

Three convergent synthetic routes have been used to successfully synthesise novel molecules which combine these two elements, and therefore open up the possibility of combining imaging and therapy (PET-PDT and MRI-PDT) or dual therapy (radiotherapy-PDT) in one tumour avid compound.

A series of novel macrocyclic chelators based around the cyclen framework have been successfully synthesised, each containing a functional handle for further elaboration of the structure. The amino bearing derivatives have been successfully coupled to a porphyrin compound via reductive amination and Buchwald-Hartwig coupling reactions, giving four new examples of porphyrin-cyclen conjugates.

A Suzuki coupling methodology has been used to synthesise a porphyrin-cyclen conjugate with an aryl-aryl linkage which exhibits enhanced stability towards acidic conditions.

The compounds have been characterised by NMR, mass spectrometry and UV/vis spectroscopy.

Abbreviations

ATP	adenosine triphosphate
Boc	*tert*-butoxycarbonyl
Cyclam	1,4,8,11-tetraazacyclotetradecane
Cyclen	1,4,7,10-tetraazacyclododecane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	N,N'-dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	distortion enhancement by polarisation transfer
diTs	ditosyl
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DQF COSY	double quantum filtered correlation spectroscopy
DTPA	diethylenetriaminepentaacetic acid
ESI	electrospray ionisation
HIV	human immunodeficiency virus
HpD	hematoporphyrin derivative
IR	infrared
LUMO	lowest unoccupied molecular orbital
MALDI	matrix assisted laser desorption ionisation
MR	magnetic resonance
MRI	magnetic resonance imaging
NBS	N-bromosuccinimide
NMR	nuclear magnetic resonance
PDT	photodynamic therapy
PET	positron emission tomography
PS	photosensitiser
RT	room temperature

SPECT	single positron emission computed tomography
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSI	trimethylsilyliodide
TPP	5,10,15,20-tetraphenylporphyrin
UV	ultraviolet

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Chapter One: Introduction

1.1 Introduction

Cancer is an umbrella term used to describe many diseases characterised by the uncontrolled growth of abnormal cells. These cells invade adjoining parts of the body and may eventually spread to other areas via the lymphatic system or the blood – a process called metastasis. Cancer is one of the leading causes of death in the world and in 2007 was responsible for 7.9 million deaths worldwide. Metastases are a major cause of death from cancer.[1]

In light of this, early detection and treatment of cancer is of vital clinical importance. The development of non-invasive imaging techniques such as positron emission tomography (PET), single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI) have enabled clinicians to accurately locate and identify tumour masses throughout the human body, and hence allow more efficient application of the chosen treatment.

The three conventional treatments for cancer, radiotherapy, chemotherapy and surgery, are still the most common modalities used clinically to remove or destroy malignant tumour masses, while more selective treatments such as photodynamic therapy (PDT), hyperthermia, sonodynamic therapy and immunotherapy have been introduced. Often, the limitations of a particular treatment, or the side effects for the patient, limit the effectiveness of the therapy and this has led to combination therapy regimes, whereby the patient receives two or more modalities either concurrently or sequentially.[2]

A single bifunctional compound which could combine tumour detection and imaging with destruction of the malignant cells would be of great clinical benefit. A scenario could be envisaged whereby the tumours are located, imaged and treated all in one session, by administration of a single drug. The design and development of synthetic routes to such compounds is the general aim of this work.

1.2 Photodynamic therapy

Photodynamic therapy (PDT) is a treatment regime used primarily for cutaneous tumours and cancers of the head and neck, but also for cancers of the lung, digestive tract and genitourinary tract.[3] It is used both palliatively and curatively[4] as a single mode of therapy and in conjunction with other cancer treatments including surgery.[4] Treatment of cancer by PDT involves administration of a light activated drug called a photosensitiser (PS), which is non-toxic in the absence of light. The photosensitiser initially distributes to many different tissues, but is selectively retained longer in tumour masses. After a period of time (normally 24-96 hours),[4] the ratio of photosensitiser in cancerous versus healthy tissue reaches an optimal level, and the volume of the tumour is irradiated with light in the visible or near IR region, activating the photosensitiser. Once activated, a reaction between the excited state photosensitiser and molecular oxygen takes place within the tissue. These reactions produce reactive oxygen species, which subsequently attack biological molecules in the immediate locality of the photosensitiser, causing oxidative damage, and ultimately leading to cell death.[5]

1.2.1 Photochemistry of PDT

The main cytotoxic agent in PDT is thought to be singlet oxygen ($^1O_2$),[6] and the mechanism by which this is formed by the photosensitiser is shown in Figure 1.

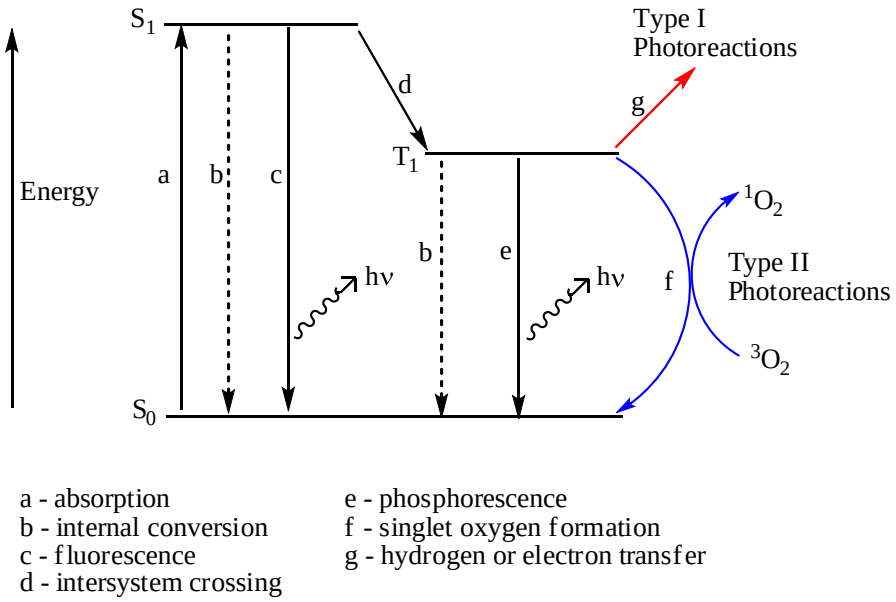
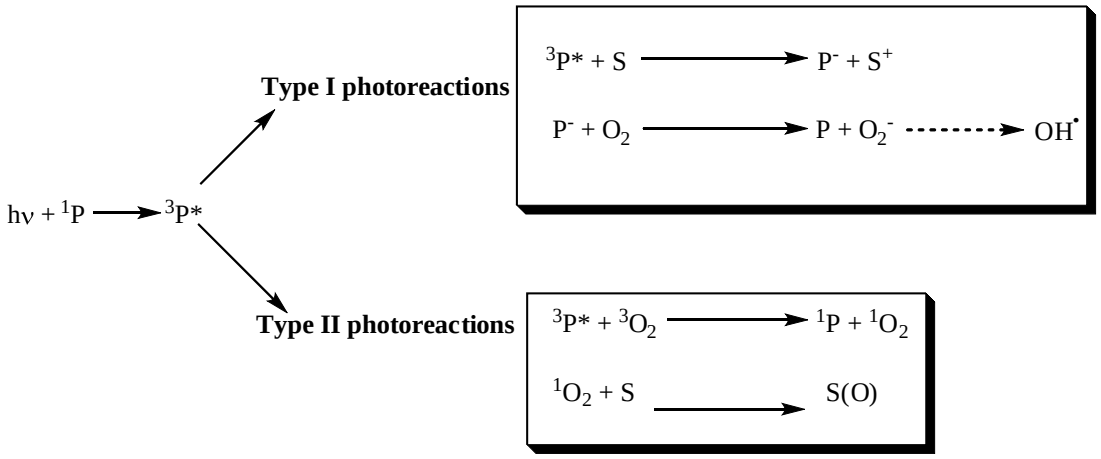


Figure 1: Modified Jablonski diagram depicting production of singlet oxygen

The photosensitiser sits in a singlet ground state (S_0), but upon absorption of a photon of light of the required energy (a), it is excited, populating a short lived (~10^{-6}s) singlet excited state (S_1).[7] This high energy state can relax by emission of a fluorescent photon (c) or by energy transfer upon collisions with nearby molecules in a non-radiative pathway called internal conversion (b). Since the lifetime of the singlet excited state is very short, fluorescent emission is statistically the most likely relaxation process. However, the molecule may undergo intersystem crossing (d) to an excited triplet state (T_1), and although this process is quantum mechanically forbidden, it does occur to a significant extent for many photosensitisers. Once in the triplet state, relaxation can again occur by radiative emission of a phosphorescent photon (e), or by interacting non-radiatively with another molecule in close proximity (c). Since the triplet state lifetime is much longer (10^{-2}s) than the singlet state,[7] the interaction with other molecules is more likely to occur. There are two types of reaction pathway that are possible from the triplet state and these are depicted in figure 2. Firstly, type I photoreactions (g) are redox processes involving direct electron or hydrogen transfer between the excited photosensitiser and oxygen or an organic biomolecule. These reactions lead to the production of the highly reactive hydroxyl radicals ($OH^{\cdot}$), via superoxide anions (O_2^{-}). Once formed the hydroxyl

radicals will react with organic biomolecules in the immediate vicinity, leading to cellular damage.[5]

Secondly, the triplet state photosensitiser can undergo type II photoreactions (f) which lead to production of singlet oxygen ($^1O_2$), the species that is usually the main cytotoxic agent in PDT. Molecular oxygen has a triplet ground state, giving it the correct symmetry to interact with the triplet photosensitiser. Thus, in aerobic environments, singlet oxygen is produced, which attacks local biomolecules, causing oxidative damage to cellular components, and leading to cell death.[5]



^1P -singlet (ground) state photosensitiser
$^3P^*$ - triplet (excited) state photosensitiser
P^- - reduced photosensitiser
S^+ - oxidised organic biomolecule
S(O) - oxidised organic biomolecule
S - organic biomolecule
O_2 / $^3O_2$ - ground state (triplet) oxygen
O_2^- - superoxide anion
$^1O_2$ - singlet oxygen

Figure 2: Summary of type I and type II photoreactions[5]

Type II photoreactions are thought to predominate in PDT,[7] although there is evidence that under certain circumstances, such as hypoxic environments, type I photoreactions may be dominant.[8]

1.2.2 Singlet oxygen ($^1O_2$)

Ground state (triplet) oxygen has two unpaired electrons in separate antibonding orbitals, a representation of which is shown in Figure 3. The first excited singlet state lies ~22 kcal.mol^{-1} higher in energy with the two outermost electrons having paired spins and occupying the same orbital. This is the state which is referred to in this report as singlet oxygen ($^1O_2$), and indeed is thought to be the cytotoxic species in PDT.[6] The high reactivity of this state is due to the two outer electrons filling a π antibonding orbital, destabilising the molecule. There is however a second excited state of oxygen, again a singlet state that is ~ 35 kcal.mol^{-1} above the ground state, in which the two outermost electrons reside in separate orbitals but with paired spins. This state is actually chemically unreactive because it decays to the lower singlet state by fast vibronic relaxation with surrounding molecules.[7] This is a spin allowed transition, conferring a lifetime of ~10^{-11} s (in aqueous environments) which is too short to allow chemical reaction with other molecules. In contrast, the lower singlet state cannot decay to the ground state as quickly since this involves a spin forbidden (singlet - triplet) transition, and is thus a relatively long lived species, with a lifetime of 1-3 μs in aqueous environments in the laboratory.[9] However, this is reduced dramatically in cellular systems[9] to 250 ns in the cytoplasm and 100 ns in lipid membranes. For PDT applications, this means that singlet oxygen cannot practically diffuse more than 45 nm in tissue and since this is considerably less than a single cell diameter, the initial site of production determines the site of photodamage and also which biological structures are attacked.

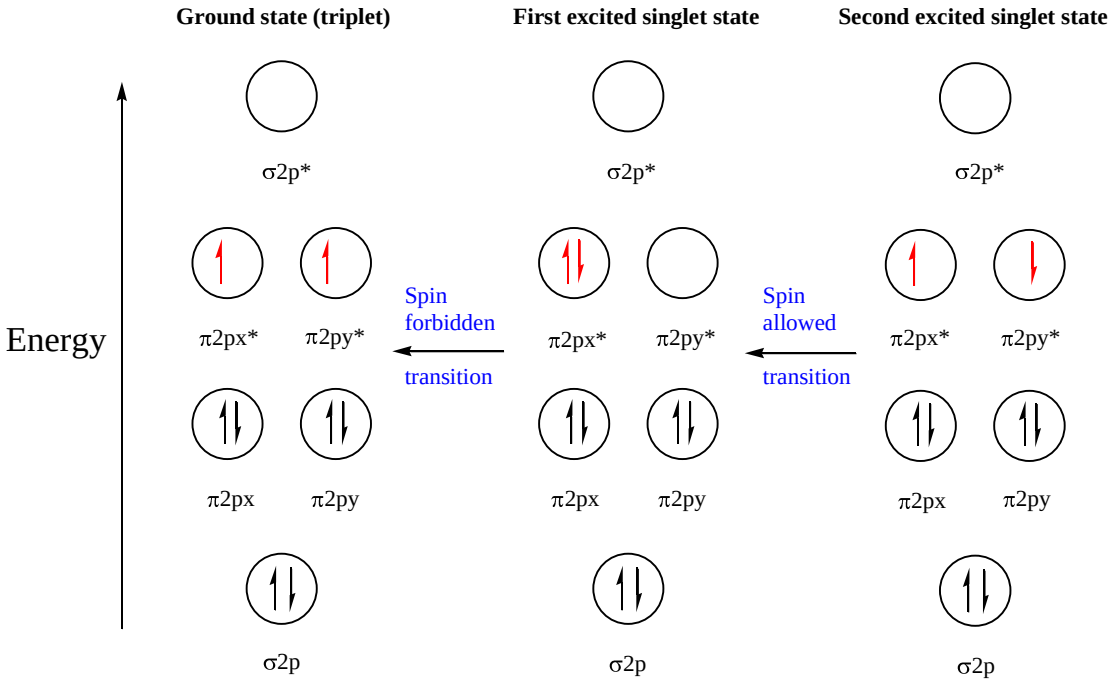


Figure 3: Electronic configuration of oxygen species

1.2.3 Biological targets of $^1O_2$

Singlet oxygen is known to attack many subcellular structures including nucleic acids, proteins, enzymes and membranes.[10] Nucleic acids are fragmented by attack at thymine or uracil containing sites,[11] and DNA repair enzymes are sensitive to oxidation.[12] Enzymes and proteins are damaged by photochemical reaction at binding/active sites resulting in loss of biological function.[11] Specifically, histidine, cysteine, methionine and tyrosine residues are attacked by singlet oxygen.[11] Cellular membranes, such as mitochondria and nuclear membranes are severely damaged by oxidation of unsaturated fatty acids and cholesterol. The reduction of the intracellular ATP pool has been followed by ^{31}P NMR in the first few hours after PDT treatment, indicating the degradation of the mitochondrial membrane.[13]

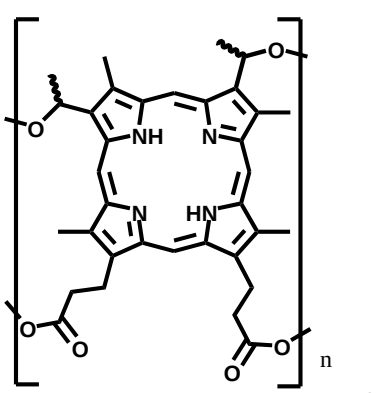
By attacking these targets $^1O_2$ mediates cell death in a direct manner by both necrosis and apoptosis. Necrosis is a degenerative process resulting in cell death from acute tissue injury. The plasma membrane becomes degraded and cannot control the flow of ions and water in and out of the cell, which leads to osmotic

swelling, and bursting of the cell.[11] Apoptosis, however, is a naturally occurring process also known as programmed cell death, and is part of the normal development of the body. Damage to the cellular components by photooxidation can cause a change in the delicate balance of positive and negative signalling that keeps cells healthy, and induce the apoptotic process. Additionally, PDT may destroy tumours by attacking the vasculature, effectively starving the tumour of nutrients and oxygen.[14]

1.2.4 Porphyrins as photosensitisers in PDT

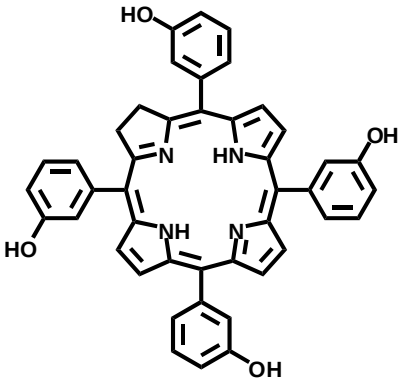
Porphyrins are aromatic, planar macrocycles consisting of four pyrrole units linked by carbon bridges. They are efficient producers of singlet oxygen in aerobic environments and hence, are good candidates for photosensitisers. The porphyrin core comprises a conjugated π system, containing 18 electrons and giving rise to strong absorption in the visible region. The uv/visible absorption spectrum of a typical free base porphyrin shows an intense band between 390-420 nm (Soret band) and four less intense bands at higher wavelengths (Q bands), conferring their characteristic deep red/purple colour. Transmittance of light through tissue at wavelengths below 600 nm is reduced due to strong absorption by endogenous biomolecules such as haemoglobin. Furthermore, the penetration depth of light through tissue increases with wavelength, from ~0.5 cm at 630 nm to ~1 cm at 800 nm. Photosensitisers that absorb above 800 nm are often inefficient at producing singlet oxygen due to insufficient energy of their triplet states. Thus, the optimal absorption range for a photosensitiser is between 600-800 nm for clinical efficacy. The highest wavelength absorption band in a porphyrin spectrum is usually between 600-700 nm and this, allied to their inherent non-toxicity in the absence of light, are two of the main reasons why they are used in PDT. Another reason is that porphyrins exhibit a higher affinity for malignant over healthy tissue. This was first reported in the 1940s by Auler[15] and Figge[14] who demonstrated that hematoporphyrin exhibited an affinity for neoplastic tissue over healthy tissue *in vivo*. Following this finding, Lipson *et al.*[16] developed a closely related compound called hematoporphyrin derivative (HpD) that showed an even higher affinity for tumour tissue, leading to its use in

the diagnosis of cancer by using the fluorescent emission of the porphyrin to detect tumours. In the 1960s it was realised by several groups[17-19] that the combination of HpD and light could destroy tumour cells, and this line of research developed over many years eventually leading to the use of porphyrin based molecules as photosensitisers in clinical PDT. In 1995, a purified version of HpD called Photofrin™ was licensed for clinical use against cancer and has since been approved in over forty countries for treatment of cancers of the lung, as well as the genitourinary and digestive tracts. As shown in Figure 4, Photofrin™ **1** consists of a complex mixture of oligomers. It has an absorption band at 630 nm that is used for activation during PDT, but there is considerable ongoing research in developing second generation photosensitisers with improved photochemical properties. One such compound is *meso*-tetra-(hydroxyphenyl)chlorin **2** (Figure 4) which has been approved for use in the European Union, Norway and Iceland for palliative treatment of head and neck cancers, under the generic name Temoporfin™. This has a chlorin core structure where one of the double bonds exocyclic to the core π-system has been reduced, resulting in a bathochromic shift and intensification of the longest wavelength absorption band to 652 nm. To date, these are the only two porphyrin based agents licensed for PDT of cancer, although benzoporphyrin derivative monoacid ring A **3** (Figure 4) is currently marketed under the name Visudyne and used for age related macular degeneration. Tin etiopurpurin **4** (Figure 4) is one of many other porphyrin photosensitisers currently undergoing clinical trials for cancer therapy.



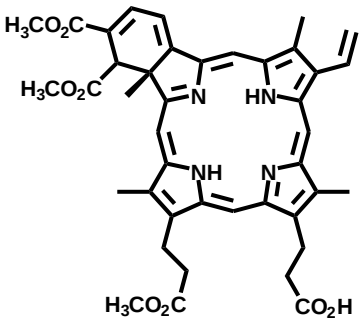
1

Photofrin™



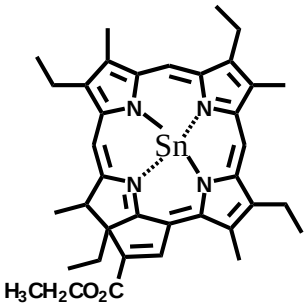
2

meso-tetra-(hydroxyphenyl) chlorin (Temoporfin™)



3

Benzoporphyrin Derivative Monoacid Ring A (Visudyne™)



4

Tin Etiopurpurin (Purlytin™)

Figure 4: Porphyrin based photosensitisers

1.2.5 Selectivity of porphyrin photosensitisers

There is a 'triple effect' associated with the selectivity of PDT treatment using porphyrin based photosensitisers. First, is the previously mentioned affinity of the porphyrin for tumour cells over healthy cells, second, the area/volume of irradiation can be very accurately controlled using fibre optics and endoscopy, and third, due to the high reactivity of $^1O_2$, tissue destruction occurs within a very narrow radius of production. In spite of this, the main side effect to PDT comes in the form of heightened skin sensitivity to light, which can last for many weeks after treatment.[5] This is due to healthy cells retaining a level of photosensitiser, up to and beyond the activation point of the treatment. The accumulation of porphyrin based photosensitisers in tumours is not yet fully understood, however, some of the commonly accepted factors that play a role are:

Leaky tumour vasculature and poor lymphatic drainage

The fast rate of growth of tumours leads to angiogenesis and new vasculature to supply them with nutrients. This fast rate of growth leads to a deficiency in pericyte function, and tumour blood vessels are usually leaky, thin walled and have an irregular diameter.[20] In addition to this, tumours are known to have a poor lymphatic clearance system, either due to obstructions or underdevelopment.[21] The combination of these factors is thought to cause the photosensitiser to leak out of the blood stream and be retained in the tumour stroma due to the inadequate lymphatic drainage.[22, 23]

pH of interstitial fluid in tumours.

Cancer cells require a lot of energy, thus high levels of aerobic glycolysis are maintained, which leads to production of lactic acid. This in turn lowers the pH of the interstitial fluid in the tumour, which is thought to lead to retention of weakly acidic photosensitisers.[24]

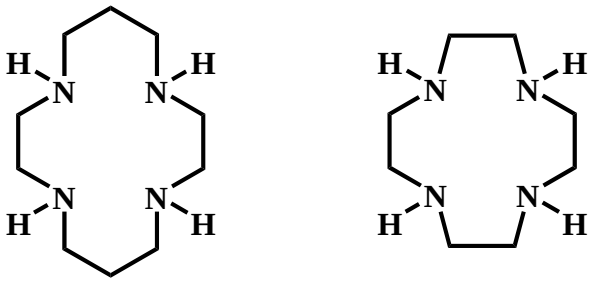
Serum protein binding.

Serum proteins such as albumin are known to be carriers of endogenous porphyrins in the blood[25-27] and it has been shown that amphiphilic photosensitisers also bind to these proteins.[28] In conjunction with the overexpression of the low density lipoprotein receptor on cancer cells, it is thought that serum proteins facilitate the transport and delivery of the photosensitiser to the cell via binding to this receptor.[28]

Many other theories have been proposed,[28-31] but the common theme is that the photosensitiser is not seeking out a specific target on the tumour cell, but is selectively retained inside the tumour. The photosensitiser is not selectively targeting tumour mass, so following administration, it is initially accumulated in all cells before being processed at different rates in healthy and malignant cells.

1.3 Tetraazamacrocycles

Functionalised tetraazamacrocycles based around the core structures 1,4,8,11-tetraazacyclotetradecane (cyclam) and 1,4,7,10-tetraazacyclododecane (cyclen), shown in Figure 5, are currently being used in many biomedical areas, including biomimetics,[32] radioactive diagnosis[33] and treatment of cancer,[34] anti-human immunodeficiency virus (HIV) agents,[35] and magnetic resonance imaging (MRI) contrast agents.[36] Underpinning the use of such compounds in these applications is the formation of stable complexes with a range of metal cations.



5
Cyclam

6
Cyclen

Figure 5: Structures of cyclam and cyclen

1.3.1 Factors for strong binding of metal ions

According to Busch,[37] there are two main characteristics of macrocycles required to bind a metal ion strongly, namely complementarity and constraint, and these are described together as multiple juxtapositional fixedness. For an optimally stable complex both factors should be maximised.

1.3.2 Complementarity

This can be described as how well the metal ‘fits’ into the macrocycle cavity, and encompasses size, shape and electronic properties of the ligand. For a metal complex to be at its most stable, there will be an optimal combination of the

following factors:

- number of donor atoms
- electronic properties of donors
- geometrical arrangement
- metal ion – donor atom bond distance

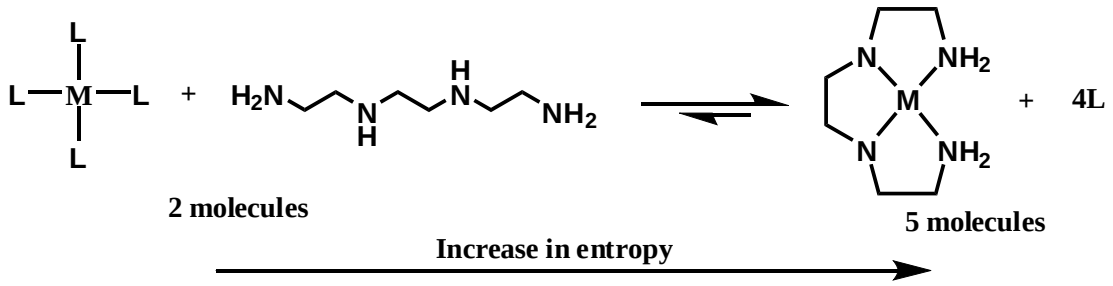
Due to the finite number of possibilities for these interactions, Busch described complementarity as a first order effect, implying it can only ultimately confer a finite amount of stability to the complex.[37]

1.3.3 Constraint

Constraint is concerned with the number of donor atoms and the flexibility between them, and deals with ligand rigidity and complexity. Two aspects have been identified and studied: topology and rigidity

1.3.3.1 Topology – "the interconnectedness of ligand donor atoms"[37]

It is known that the binding constant of a ligand increases with the number of donor atoms**,** which is known as the chelate effect.[37] Cyclam and cyclen each contain four nitrogen donors, and form metal ion complexes which are more thermodynamically stable than complexes comprising four monodentate ligands. This can be explained[37] by an increase in entropy of a system such as that shown in Scheme 1. As the four monodentate ligands are displaced from the metal ion by the polyamine ligand, there is an increase in disorder; there are two molecules on the left hand side of the reaction but five on the right, giving a favourable entropic increase in stability.



Scheme 1: Entropy and the chelate effect

Additionally, if the ligand is assumed to bind in a stepwise fashion, as each metal-ligand bond forms, the effective concentration of the remaining donor atoms increases.[38] It has been shown that in a nickel(II) system with a polydentate nitrogen donor ligand, the nickel-nitrogen bond cleavage rate is the same as for a monodentate ligand.[39] This leads to the conclusion that the second donor atom must bind more quickly than the first, or the chelate would be lost at a rate equal to the monodentate system. An accepted explanation is that the effective concentration of the second donor atom increases after the first one is bound to the metal ion. This is due to a tethering effect, bringing the second donor closer to the metal ion and making it more likely to bond than an unlinked donor. (Figure 6)

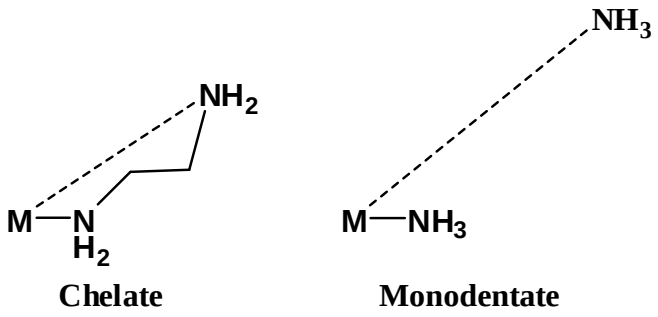


Figure 6: Tethering effect leading to increased effective concentration of the ligand[38]

Thus, coordination is said to be an associative process, with the first donor binding helping the second one to bind and so on. The reverse process of ligand dissociation is equally affected. When one donor atom has dissociated from the metal ion the tethering effect gives it a high effective concentration and hence the

rate of bond reformation is higher than the rate of dissociation of the second donor atom. The overall result is an extra kinetic stability conferred upon the complex when compared to the analogous monodentate complex.

When the donor atoms are part of a macrocycle, a further energy stabilisation is observed called the macrocyclic effect.[40] This can be rationalised by the difficulty of dissociation of the first donor atom from the metal ion. A polydentate acyclic ligand is able to dissociate from a metal by successive stepwise breaking of metal ligand bonds, beginning at a terminal donor atom. However, a macrocycle contains no end donor atom, thus ligand distortion must occur to facilitate cleavage of a ligand-metal bond as shown in Figure 7. The activation energy required for this process makes dissociation very slow, and it has been shown that the macrocyclic effect arises mainly from this extra kinetic stability gained.[37]

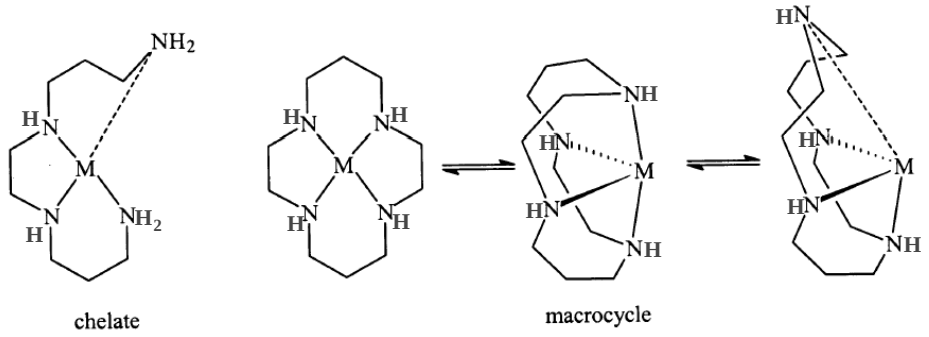


Figure 7: Comparison of first donor dissociation between a chelate and a macrocycle[38]

1.3.3.2 Rigidity – "how fixed in space the donor atoms are"[37]

It has been shown that rigid ligands with donor atoms fixed in a favourable orientation to bind the metal ion, form more stable complexes than those of more flexible ligands.[41] This is understood to stem from the rigidity holding the donor atom in position such that they cannot move and deform the ligand shape. This tightly restricted movement about the median bond values of distance and angle gives a lower possibility of bond deformation and hence, bond dissociation.

The cyclam and cyclen macrocycles provide four nitrogen donor atoms for

complexation of a host metal cation, however, substitution of the secondary amines can be undertaken to increase the number of donor atoms. This gives these macrocycles and their derivatives the ability to act as ligands for a range of metal cations which require coordination numbers of between four and eight, including the transition metals and the lanthanoids.

1.4 Use of cyclam and cyclen derivatives in medical imaging and therapy

1.4.1 Positron emission tomography (PET)

This technique is used to identify the position of a radioactive tracer within the human body. A positron is the antimatter equivalent of an electron, and positron emission occurs when a nuclear proton changes into a positron (β^+), a neutron and a neutrino.[42] Since it is the position of the tracer that is being detected, the radionuclide must be delivered to the neoplastic site before a diagnostic image can be obtained.

The imaging regimen requires the patient to be administered with a tracer which typically consists of a tumour avid biomolecule attached to a positron emitting radionuclide. After administration, a suitable time is allowed to elapse to allow the radionuclide to locate within the tumour mass, and the patient is then placed within a detector.

Inside the body, a positron travels a small distance (1-2mm) before it comes into contact with a tissue electron and the particles annihilate, with their combined mass being converted into energy (according to $E=mc^2$).[42] This leads to the emission of two γ ray photons of 511keV which travel away from each other at $180 \pm 0.25°$ as shown in Figure 8.[43]

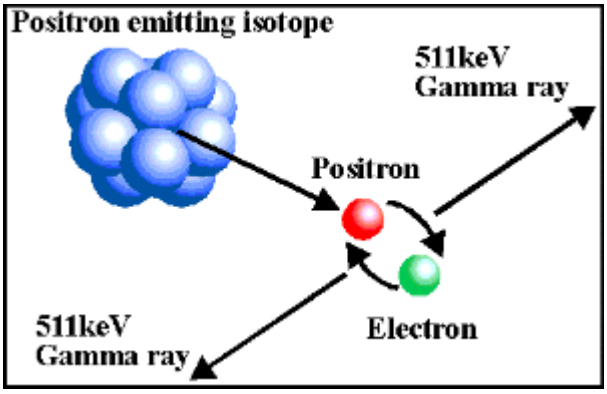


Figure 8: Schematic showing positron emission and subsequent annihilation with an electron[43]

The slight variation from 180° is caused by residual motion of the positron and electron at the point of annihilation. Some of the photons will be absorbed by surrounding nuclei and some will be scattered by tissue, but when two photons are detected simultaneously at 180°, the position of the annihilation event that generated them can be calculated (Figure 9). After 500000 detections, distribution of the tracer can be calculated by tomographic reconstruction.[42]

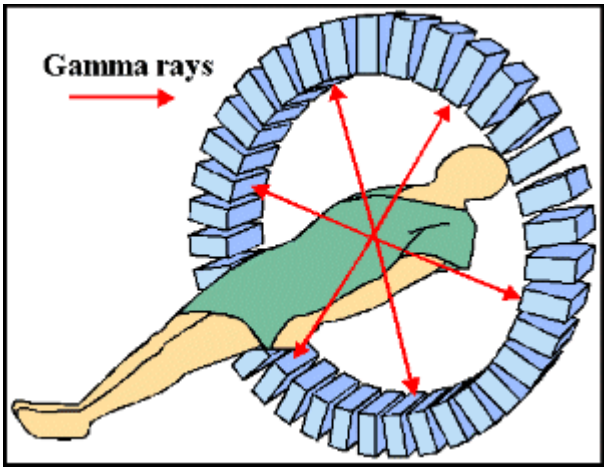


Figure 9: Schematic showing a PET scanner detecting divergent γ rays to generate a 3D image of the location of the PET tracer.[43]

There are many radionuclides which are used for PET imaging including several isotopes of copper.[44] The details of these radioactive copper isotopes are shown in Table 1.

Table 1: Radioactive isotopes of copper and their uses in medicine[44]

Isotope	Half Life	Imaging (emission energy, abundance)	Therapy (energy, range in tissue)
^{60}Cu	20 min	PET (β^+, 873keV, 93%	
^{61}Cu	3.3 hr	PET (β^+, 527keV, 62%	
^{62}Cu	9.7 min	PET (β^+, 1315keV, 98%	
^{64}Cu	12.7 hr	PET (β^+, 278keV, 19%	β^- 190keV, 0.95mm
^{66}Cu	5.4 min		β^- 1109keV, 5.6mm
^{67}Cu	62 hr	SPECT (91, 7%; 93keV, 16%; 185, 48%)	β^- 121keV, 0.61mm

^{60}Cu, ^{61}Cu, ^{62}Cu and ^{64}Cu are all positron (β^+) emitters which can be used for PET imaging.[45] ^{60}Cu, ^{61}Cu, ^{62}Cu are used to measure blood flow and hypoxia while the longer half life of ^{64}Cu has led to the development of ^{64}Cu labelled monoclonal antibodies and peptides for tumour imaging via active targeting.[45]

However, ^{64}Cu is also a β^- emitter which makes it suitable for radiotherapy of cancer and it is this dual functionality which has made this isotope especially worthy of study. ^{66}Cu and ^{67}Cu are also β^- emitters with ^{67}Cu having the most appropriate half life for therapeutic purposes.[45]

1.4.1.1 Tetraazamacrocycles in PET

The PET imaging technique relies on the radionuclide being delivered selectively to the target area (the tumour), and this can be achieved by using a bifunctional chelator which is covalently attached to a targeting biomolecule, such as a protein or antibody, while strongly binding the radionuclide. The tight binding of the radionuclide is required to achieve high thermodynamic stability *in vivo* whilst also being kinetically inert.[46]

Most bifunctional chelators used for $^{64/67}$Cu isotopes are based around the cyclam and cyclen macrocycles because in addition to the complexes meeting the previously stated requirements of kinetic and thermodynamic stability, such

ligands can rapidly coordinate metal ions, show aqueous solubility and have high resistance to transmetallation *in vivo*.[44,47,48] Cyclam and cyclen derivatives that have been investigated for use as copper chelators for PET imaging and radiotherapy are shown below (Figure 10).[44]

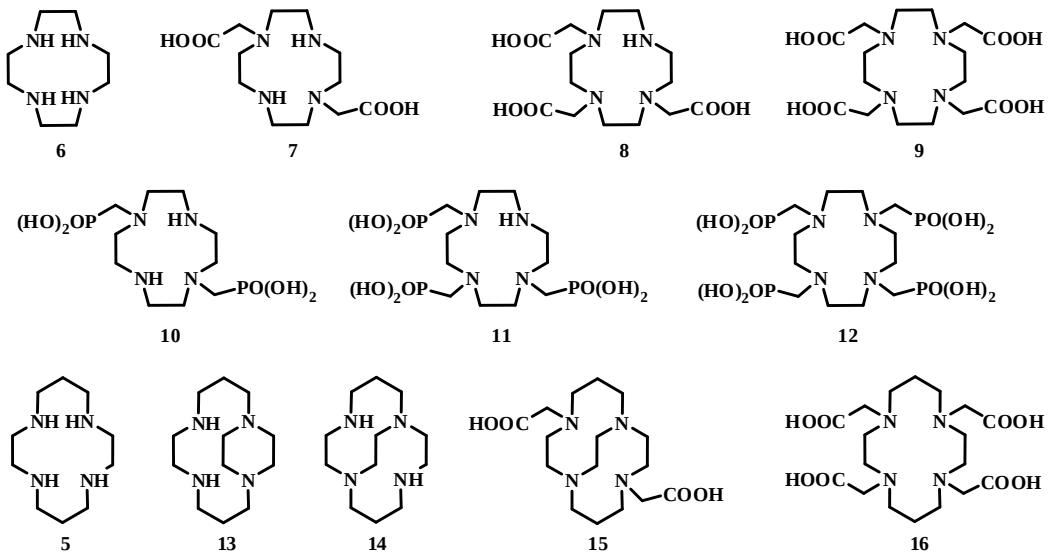


Figure 10: Cyclam and cyclen derivatives studied as $^{64/67}Cu^{2+}$ chelators for use in PET imaging and radiotherapy[44]

The compounds in Figure 10 show how the structure of the chelators has been changed in order to study the effect on biodistribution and overall efficacy in the search for an effective bifunctional chelator. The most stable oxidation state of copper is +2 and so the radionuclide is used in this form.[49] Since copper(II) can form complexes with coordination numbers between four and six,[49] the substitution of the macrocycles with coordinating pendant arms allows not only control of the denticity of the complex, but also the overall charge. The macrocycles without pendant arms form positively charged complexes as do the phosphonate ester derivatives. The carboxylate pendant arms are deprotonated upon complexation so the macrocycles containing these groups can form complexes which are neutral or overall negatively charged, depending on the number of carboxylate groups present. The overall charge on the complex has been shown to affect biodistribution and clearance rates *in vivo* and the numbers and types of donor atoms affects the kinetic and thermodynamic stability of the

complex.[44] The rigidified macrocycles have been shown to exhibit very high association constants as well as long half lives of dissociation, but the kinetics of complexation of Cu(II) are slower than the non-rigidified macrocycles.[44]

Figure 11 shows the structures of two bifunctional chelators which are currently undergoing trials for PET imaging and radiotherapy and illustrates the different approaches used to connect such chelators to targeting biomolecules.[50] Compound **17** has been conjugated to an anticolorectal carcinoma monoclonal antibody and radiolabelled with ^{64}Cu for evaluation as a PET tracer.[50] The carbon backbone of the macrocycle has been substituted with a linking group to connect to the monoclonal antibody. The common alternative is shown by the benzoic acid derivative **18** (Figure 11) in which the linking group is attached to one of the amino functionalities in the macrocycle.[51] This compound is undergoing evaluation as a ^{67}Cu based radiopharmaceutical for cancer therapy and has been attached to AB35, a monoclonal antibody directed against carcinoembryonic antigen.[51]

The point of attachment of the linking group is clearly dependent on many factors, such as synthetic considerations and the number of pendant arms required for complexation of the radiometal. Additionally, the linker group itself can have a direct effect on the pharmacokinetic properties of the bifunctional chelator as well as the stability of the metal complex.[46]

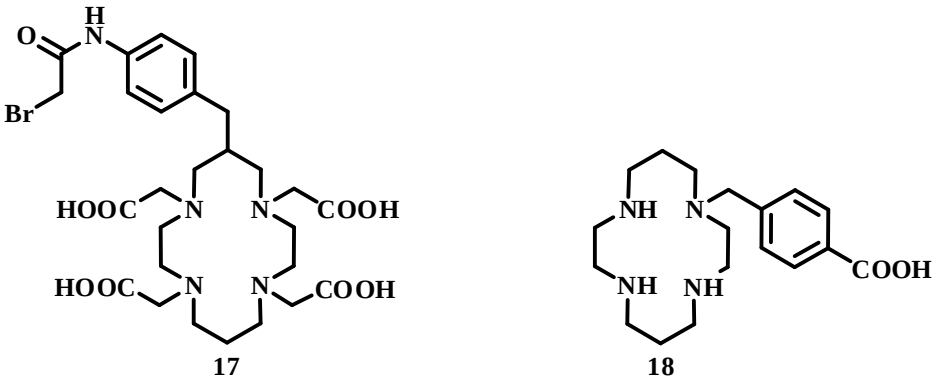


Figure 11: Two cyclam based chelators currently undergoing trials for PET imaging and radiotherapy[50, 51]

1.4.2 Magnetic resonance imaging (MRI)

MRI is a diagnostic medical technique used to identify pathological regions within the body. The fundamental property attenuated during an MRI scan is the magnetic moment of the hydrogen atom nuclei (protons) in tissue water. In the absence of a magnetic field, the magnetic moments of proton nuclei are oriented in a random fashion, but on application of a strong external magnetic field, they precess around an axis which is aligned parallel or antiparallel with the direction of the applied field.[52] This creates two states, a low energy state which is aligned with the field and a high energy state opposed to the field, where the energy difference between the two states is given by:

$\Delta E = \hbar\nu$ where $\hbar$=Planck's constant

ν=Larmor or resonance frequency

This frequency is unique for protons and proportional to the applied magnetic field. The lower energy state is more populated than the higher one, and this is in accordance with and described by Boltzmann statistics. A pulse of electromagnetic radiation at the Larmor frequency is then used to excite nuclei from the low to the high energy state. Subsequently, these protons relax back to the lower state by emission of a pulse of electromagnetic radiation, and it is this pulse that can be detected, measured and converted into a signal. More accurately, it is the difference in the total electromagnetic radiation absorbed (nuclei excited) and emitted (nuclei relaxing) that is measured, so the signal intensity is proportional to the population of the two energy states. The macroscopic property being visualised on a magnetic resonance image is the distribution and concentration of water in body tissues.[52] This gives a contrast between tissue of different types, such as bone and skin, but also differentiates between healthy and malignant tissues. An example of an image generated in this way is shown in Figure 12.

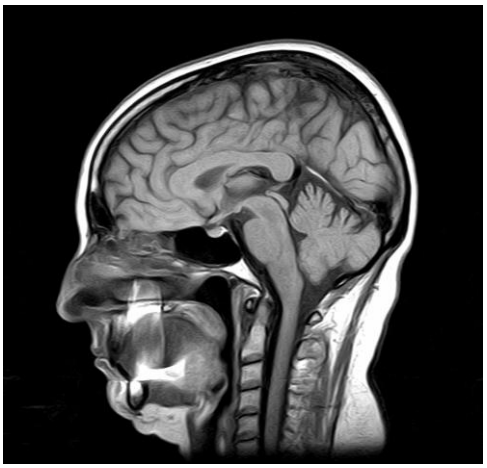


Figure 12: An example of an image created by MRI[52]

1.4.3 Contrast agents in MRI

Contrast agents are used in MRI to generate a more intense signal contrast between different types of tissues.[36] They are compounds that are administered before excitation of the protons, and perturb the natural rate of relaxation of water protons in their immediate vicinity, thus generating a brighter image. When a proton is in the high energy state, it relaxes via two mechanisms : spin-lattice and spin-spin relaxation.

- Spin-lattice relaxation is otherwise known as the longitudinal relaxivity and is effectively the transfer of energy from an excited water proton to nearby local molecules and can be measured as the T_1 relaxation time.[52]
- Spin-spin relaxation is also known as transverse relaxation and is the transfer of energy from the excited proton to another low energy proton, resulting in the excitation of the second proton. It is measured as the T_2 relaxation time.[52]

A contrast agent works by aiding the relaxation of the excited protons, increasing the rate of relaxivity and reducing either T_1 or T_2. The commercial gadolinium based contrast agents are known as T_1 agents because their mode of action is to increase the rate of spin-lattice relaxation and reduce T_1.

1.4.3.1 Tetrazamacrocycles in magnetic resonance imaging

A range of T_1 contrast agents based around the highly paramagnetic gadolinium(III) ion are currently being used for diagnostic imaging of the central nervous system (especially brain tumours), multiple sclerosis and breast cancer.[53] Some examples of compounds that are used clinically are shown in Figure 13.[36]

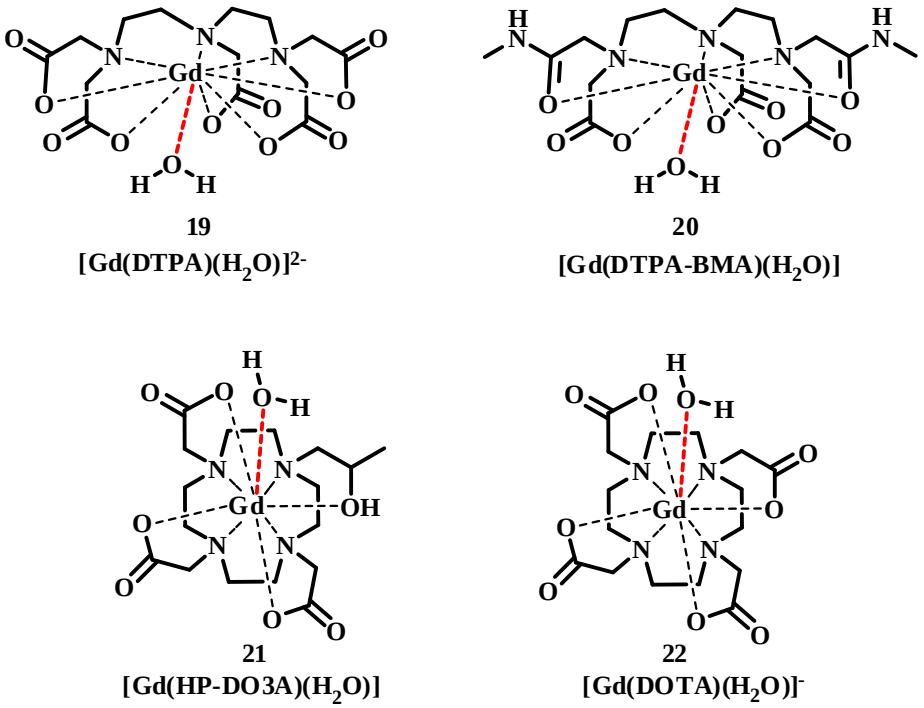


Figure 13: Examples of Gd(III) based contrast agents approved for use in the clinic[36]

The paramagnetism of gadolinium(III) created by seven unpaired *f* electrons is the property exploited in these contrast agents.[36,52,53] This facilitates the relaxation of excited protons through a hyperfine interaction with the unpaired electrons in the gadolinium valence shell. Effectively, the excess energy is transferred from the proton to the gadolinium (III) ion and this reduces the T_1 relaxation time, leading to a brighter image.[53] However, Gd^{3+} is highly toxic *in vivo*[52] and so needs to be bound strongly in a chemical complex in such a way that renders it unable to leach out, while allowing local water molecules to approach closely enough to facilitate their proton relaxation. In the commercial agents, as shown in Figure 13, the Gd^{3+} ion is held in nine coordinate geometry,

with the octadentate ligands leaving one free coordination site for the inner sphere water molecule. Both acyclic and macrocyclic (cyclen based) chelators are used, but the macrocyclic ligands show significantly enhanced kinetic stabilities.[36] In the macrocylic compounds the highly oxophilic Gd^{3+} ion is bound by four nitrogen donors in the macrocycle and four oxygen atoms from the pendant arms, conferring enough kinetic and thermodynamic stability for use *in vivo*. Due to the size of the metal ion, it sits just above the mean ligand plane, giving enough access for a local water molecule to bind in a dynamic reversible way.[36] Thus, the mode of action of the contrast agent is to aid relaxation of the excited protons in this bound water molecule (inner sphere water), which then exchanges with a local water molecule. This dynamic equilibrium allows the contrast agent to affect the signal intensity of surrounding tissue water.[52]

These types of low molecular weight gadolinium complexes are extracellular agents, distributing into the intravascular and interstitial space, and hence are known to be non-site specific agents.[54] They passively accumulate in body tissue, enhancing the relaxivity and hence the signal, of the local environment. Contrast agents that target certain types of tissue have been reported in the last few years, including agents that accumulate in the liver, lymph nodes and atherosclerotic plaques.[55-57]

1.5 Aims of this project

The aim of this work is to develop a synthetic route which allows access to molecules containing a porphyrin and a cyclam or cyclen derived macrocycle.
As already explained, porphyrin molecules are efficient generators of singlet oxygen and are used as photosensitisers in PDT. They are also known to accumulate in tumour tissue due to the hyperpermeability of tumour vasculature and poor lymphatic drainage. Due to this, porphyrins exhibit a passive tumour targeting property.

Tetraazamacrocycles based around cyclam and cyclen are excellent ligands for a variety of metal ions including copper(II) and gadolinium(III) among others. As already explained, the macrocycles can be substituted with pendant arms, giving them the correct denticity and electronic properties to coordinate these metal ions very strongly. The complexes formed with these metal ions are thermodynamically stable and kinetically inert and so can be used *in vivo* for both imaging and radiotherapy.

A molecule combining these two elements would therefore have the possibilities of combining imaging and therapy (PET-PDT and MRI-PDT) or dual therapy (radiotherapy-PDT) in one tumour avid compound.

Clinically, the advantages of having access to such compounds are :

- The optimisation of PDT treatment by more accurate location of the porphyrin using the imaging modality. This would allow more accurately directed light to the areas containing the photosensitiser.
- Longer residence times of the imaging modality in vivo due to the retention of the porphyrin, giving an enhanced window of opportunity for imaging the patient.
- Selective retention of the porphyrin in tumour mass would allow location and identification of metastases by the imaging component.

- Imaging of the accumulation of the porphyrin in tumour mass could be used to optimise the timing of activation of the photosensitiser, hence improving the efficacy of the treatment.
- The effects of PDT treatment could be observed by use of the imaging modality.
- A patient could be given a single drug for a combination of radiotherapy and PDT.

1.6 Porphyrins combined with imaging and therapy agents

In 1983, Wong *et al.* radiolabelled hematoporphyrin derivative (HpD) with ^{99m}Tc, with a view to using the known accumulation of HpD in tumour tissue to develop a targeted imaging agent.[58] Subsequent studies showed that the compound did indeed accumulate in mammary tumour adenocarcinomas of mice.[59] However, the compound was only stable for three hours and the ^{99m}Tc ion was shown to be coordinated by the carboxylic acid chains on the porphyrin periphery as opposed to the porphyrin macrocycle itself.[59] Based on this research, Murugesan *et al.* synthesised the carboxylic acid bearing porphyrins shown in Figure 14.[60, 61]

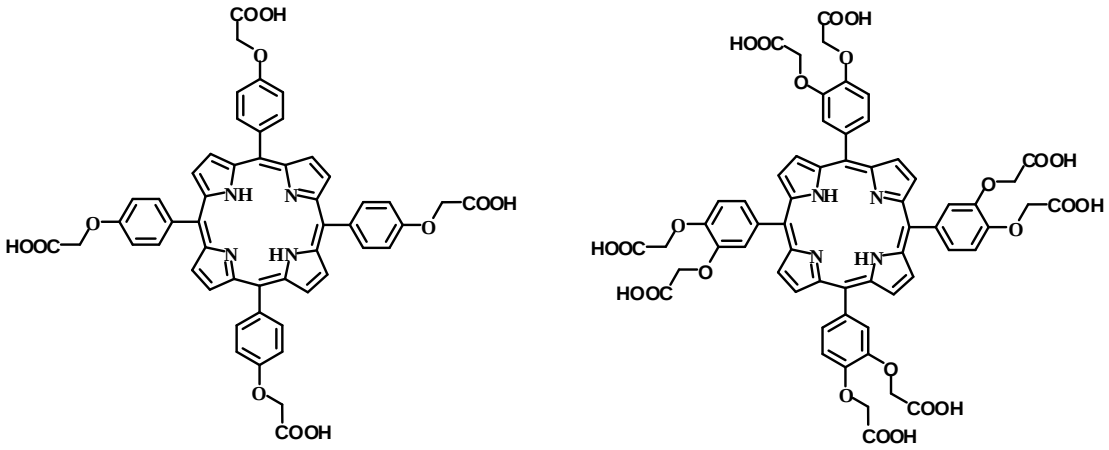
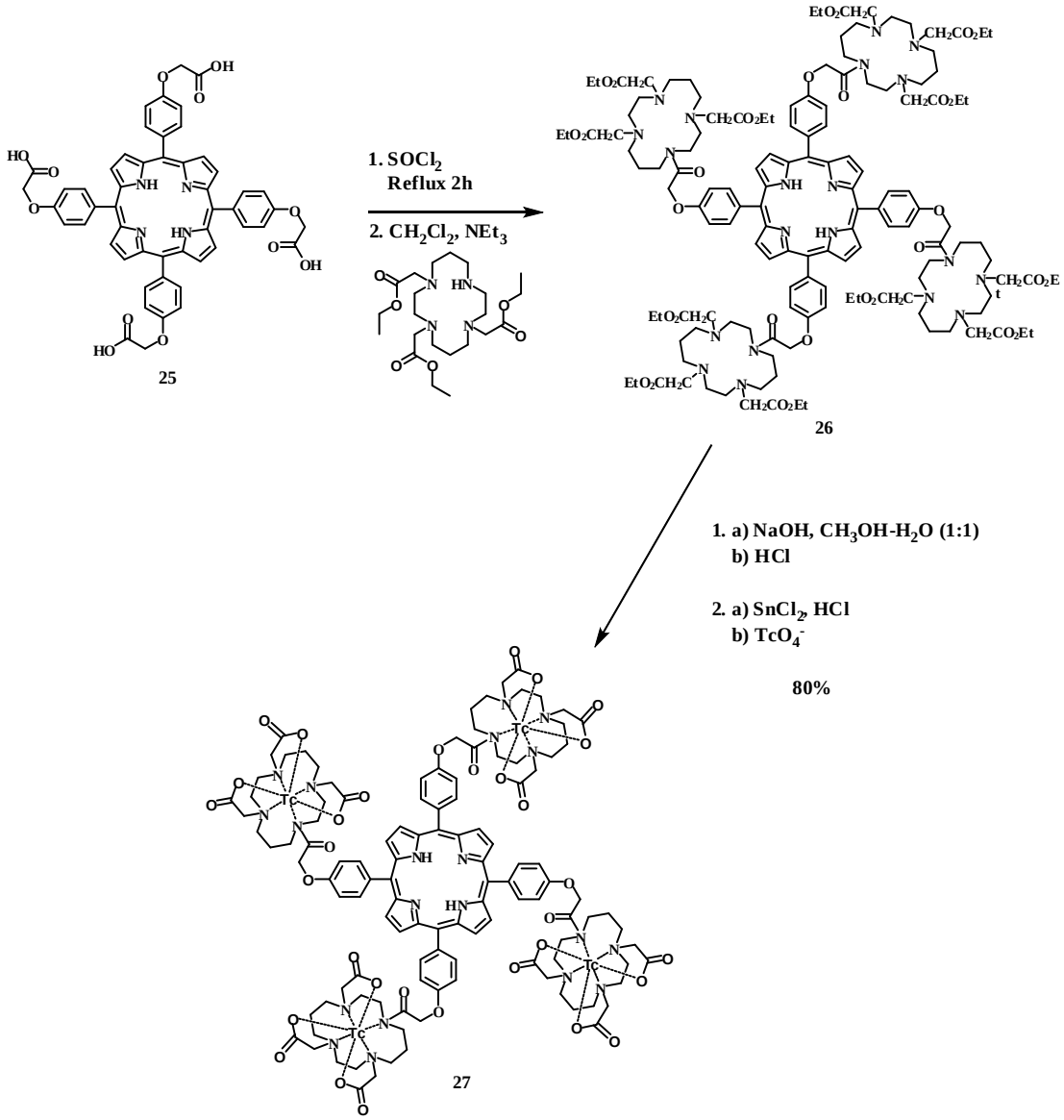


Figure 14: Examples of porphyrins used as ligands for ^{99m}Tc ions[60, 61]

These compounds were used to coordinate ^{99m}Tc and similar results were obtained with respect to their accumulation in abdominal carcinoma of mice and mammary tumours in rats. The ^{99m}Tc ion was once again shown to be coordinated by the carboxy chains on the porphyrin periphery and the compounds were stable for only four hours.[60, 61] This prompted the incorporation of more efficient ligands into the porphyrin structure to enhance the *in vivo* stability of the ^{99m}Tc ion.[62] Scheme 2 shows the synthetic route used to

synthesise a porphyrin bearing four cyclam coordinated ^{99m}Tc ions. *In vivo* distribution studies were carried out on compound **27** in mammary tumour bearing rats and tumour to muscle ratios compared favourably with a range of tumour seeking radiopharmaceuticals in current usage. The compound was also stable for more than five hours, giving a significant improvement over the previous studies. It is interesting to note that although the authors conclude that this compound shows potential for tumour imaging, no reference is made to the possibility of a targeted combined PDT-radiotherapy usage.[62]



Scheme 2: Cyclam bearing porphyrins synthesised to coordinate $^{99m}Tc^{3+}$ ions for targeted PET imaging[62]

Hindre *et al.* synthesised the porphyrin-Gd DTPA conjugate **28**, as shown in Figure 15, with a view to targeted MRI of tumour cells.[63]

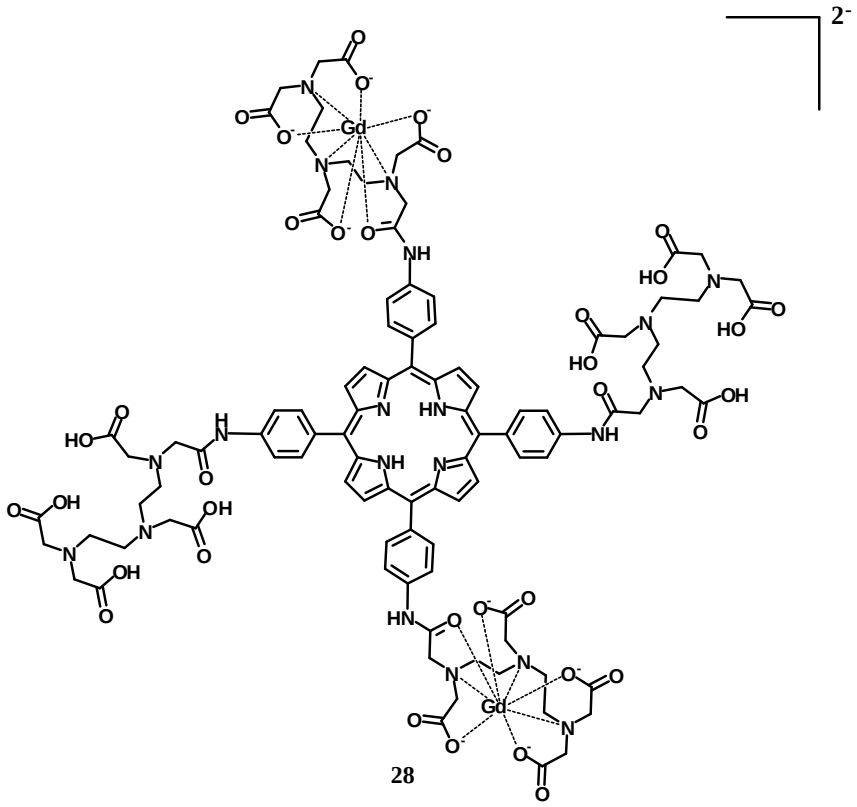
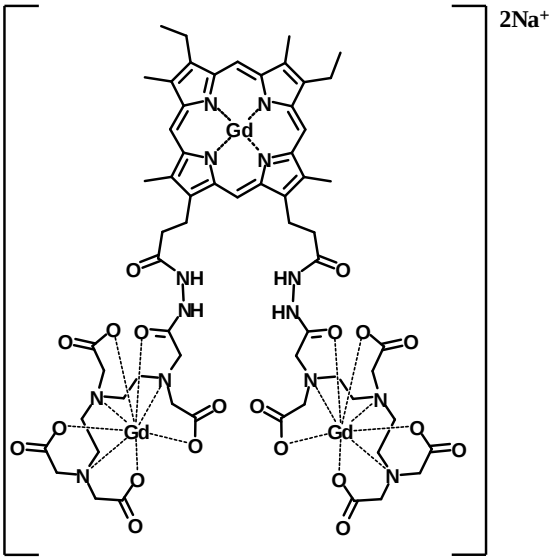


Figure 15: Structure of DTPA-porphyrin conjugate synthesised by Hindre *et al.* for tumour targeted MRI[63]

They compared the porphyrin conjugate **28** with free Gd DTPA in animal models and found that the compound was still selective retained in tumour mass, leading to reduction of T1 relaxation times in both lymphoblastic leukaemia cells and HT 29 human colonic cancer cells, 24 hours after administration. MR imaging showed an enhancement in contrast between the tumour and adjacent tissue after use of this compound. In addition, they measured an enhanced relaxivity in the conjugate compared to Gd DTPA which they suggest was caused by the larger size of the molecule reducing the rotational correlation time of the compound. Being published in a specialist MRI journal, the report includes only a brief discussion of the synthesis of this compounds. However, since four DTPA ligands are joined to the porphyrin yet only two are used to coordinate gadolinium ions, it seems logical that the original synthetic target was a porphyrin with all four chelators acting as ligands, although this is not discussed

in the paper.[63] Gadophrin 2 an analogous compound (Figure 16), has been synthesised by a different group with similar imaging results.[64]



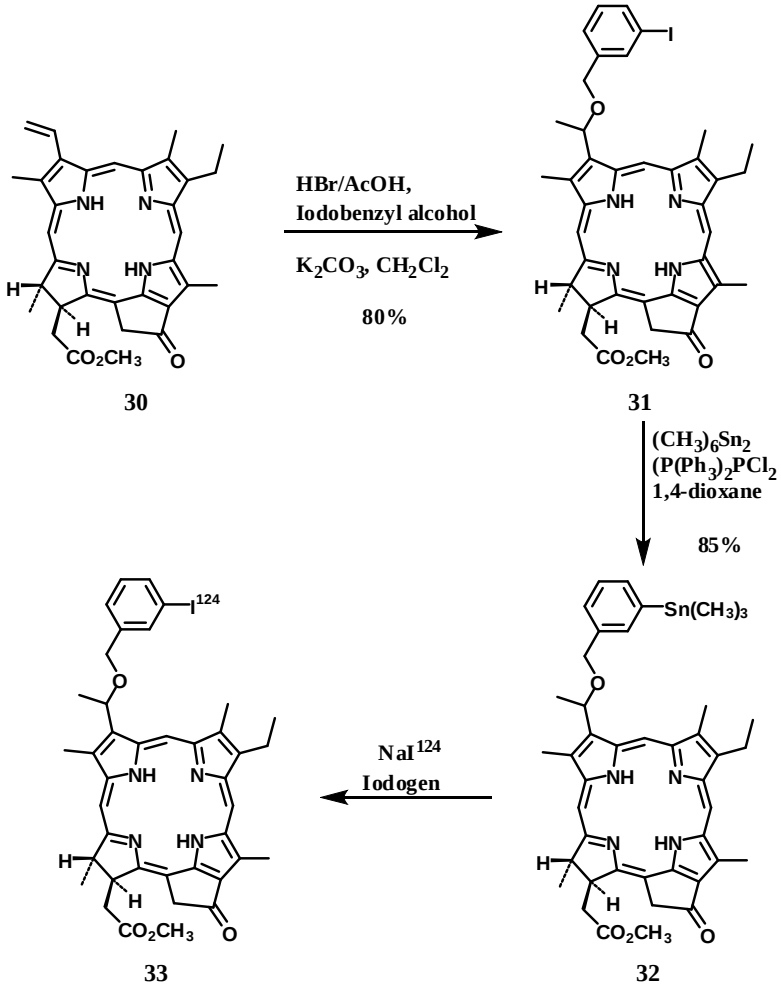
29

Figure 16: Structure of Gadophrin 2, a necrosis avid MRI contrast agent[64]

Later studies on both of these compounds have shown that they are avid only for non-viable (usually necrotic) tissues and not viable tumour cells,[64-66] although this has led to their use for imaging of myocardial infarction.[67-69]

Pandey and co-workers have synthesised several compounds for PDT-imaging purposes, similar to the aims of this project.[70] Their systems have all been based around derivatives of the naturally occurring chlorophyll a and so have absorption bands in the region 660-700nm, making them good candidates for PDT.[70]

To create a PDT-PET agent, they used this porphyrin template to attach a positron emitting radionuclide (^{124}I), synthesising compound **33** in three steps as shown in Scheme 3.[71] The behaviour exhibited by the porphyrin of selective retention in tumour tissue was used as the 'targeting' modality.



Scheme 3: Synthesis of a bifunctional PDT-imaging (PET) agent[71]

After administration to RIF tumour bearing CH3 mice, they found that the photosensitiser distribution could be imaged by PET and that the compound localised in mitochondria.[71] Although the maximum intensity occurred 24 hours after administration, the best image contrast was found at 48h due to rapid clearance of the compound from the liver, spleen and intestine compared to the tumour. The compound exhibited photosensitising properties, with 80% of mice tumour free after 60 days.[71]

In another study, the same group[72] synthesised compound **34** as shown in Figure 17 in order to create a dual PDT-MRI agent, once again exploiting the selective porphyrin retention in tumour tissue for 'targeting'. This was administered to tumour bearing mice in a liposomal formulation. The compound proved to be

tumour avid and enhanced the tumour image by 57% (24 hours postinjection) with no enhancement for fat and minimal enhancement for muscle tissue. This enhanced image remained at a measurable level for more than 24 hours post injection, whereas the commercial agent Gd DTPA (Magnavist®) clears the body within 1 hour and shows little contrast after 30 minutes. The T1 relaxivity was measured at 18.70 $mM^{-1}s^{-1}$ compared to 3.25 $mM^{-1}s^{-1}$ for Gd DTPA. The photosensitising ability of the porphyrin was not compromised by the proximity of the Gd^{3+} ions and C3H mice bearing RIF tumours were treated by PDT, with 80% being reported as tumour free after 90 days.[72]

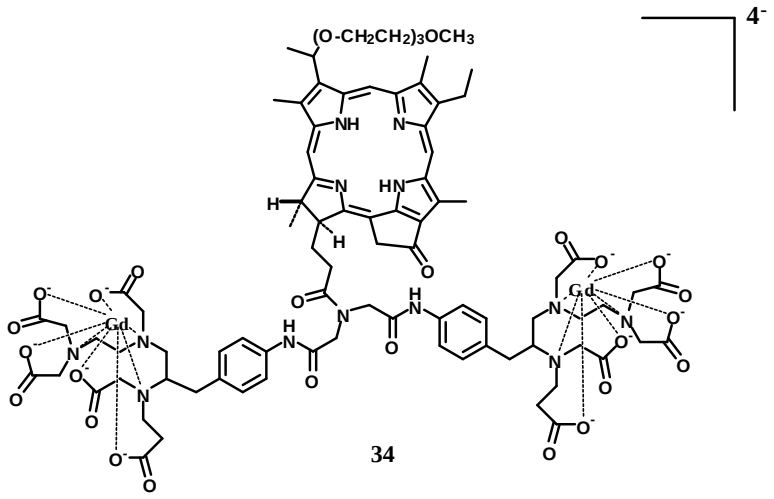
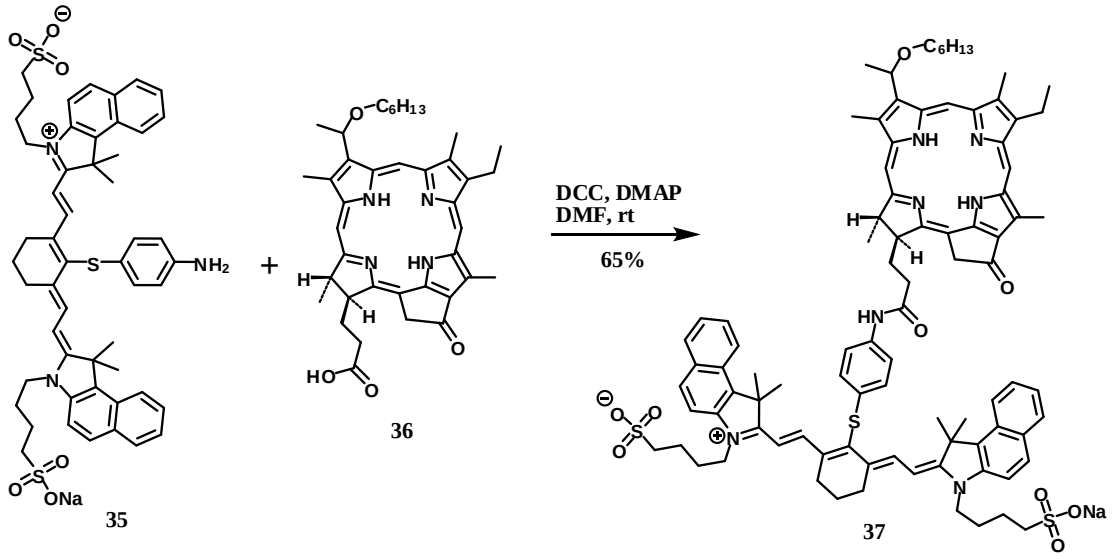


Figure 17: A porphyrin substituted with two Gd^{3+} acyclic chelators for targeted PDT-MRI[72]

Pandey *et al.* also combined the same porphyrin core **36** with the cyanine dye IR820 **35**, as shown in Scheme 4.[73] The cyanine dye has the required photophysical properties for fluorescence imaging but has no tumour specificity. Hence, the role of the porphyrin was as a tumour localising agent as well as a photosensitiser, giving a dual PDT-fluorescence imaging compound. The investigators found that compound **37** degraded when irradiated at wavelengths in the porphyrin absorption band, but it remained intact when irradiated at the correct wavelength for the cyanine dye to absorb. After administration to CH3 mice bearing RIF tumours, the conjugate showed uptake in tumour and skin up to 48 hours by reflectance spectroscopy, but after 72 hours had cleared from the skin to negligible levels while remaining in the tumour at high enough concentrations to be used for imaging the tumour mass. The photosensitising

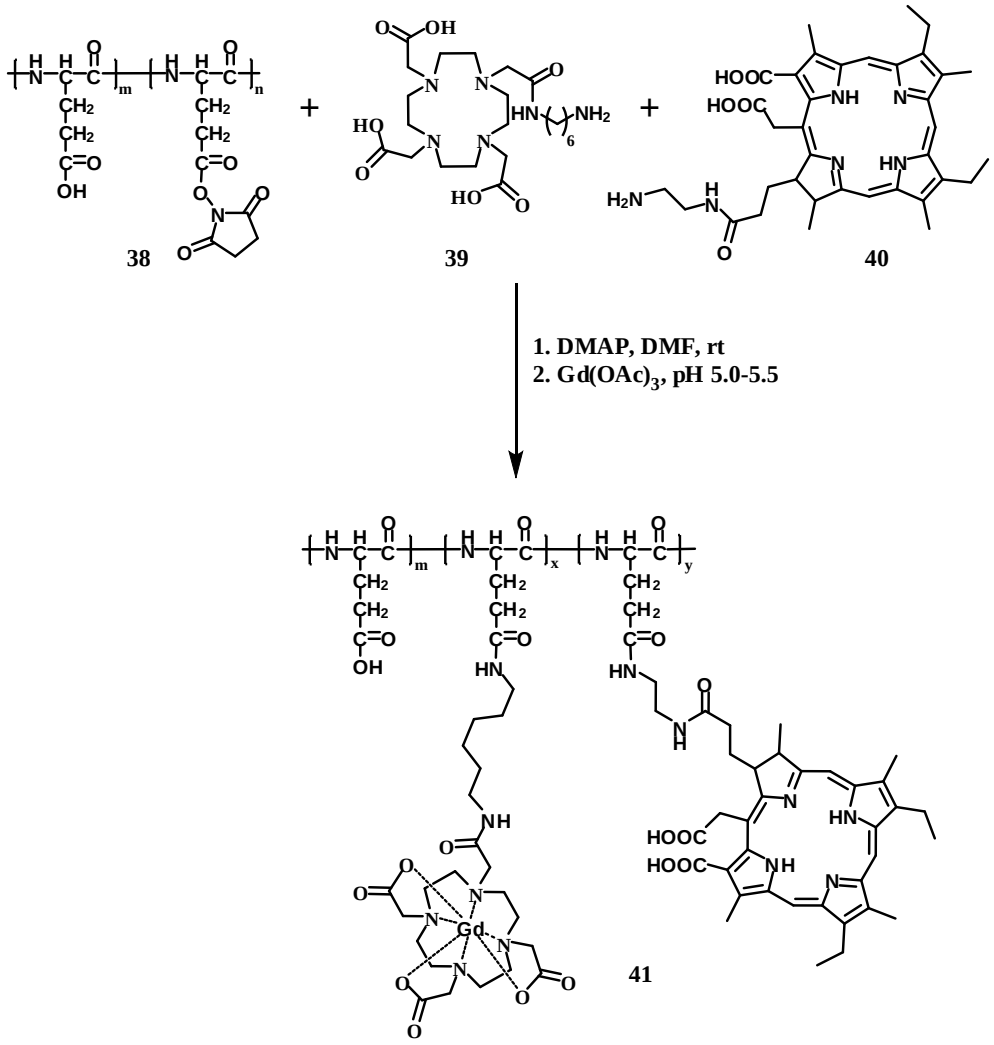
ability was retained with 8/10 mice tumour free after 90 days post irradiation.[73]



Scheme 4: Synthesis of a porphyrin-cyanine dye conjugate for PDT-fluorescence imaging[73]

More recently, the group of Lu synthesised the bifunctional polymer conjugate **41** containing a chlorin photosensitiser and a macrocyclic gadolinium contrast agent as shown in Scheme 5.[74]

Poly(*L*-glutamic acid) was used as the carrier molecule and was loaded via reaction of the free amines on the contrast agent and the chlorin photosensitiser with the activated esters on the polymer. The reaction was done stepwise with the relative ratio 16:2 (contrast agent:photosensitiser) in order to achieve the correct dosage for both imaging and therapy. Athymic nude mice bearing human breast carcinoma xenografts were administered with the polymer conjugate which preferentially accumulated in the solid tumour, an effect which is known to derive from the hyperpermeability of the tumour vasculature.[74] MRI showed a gradual accumulation of the conjugate in tumour, and a strong enhancement of inner tumour tissue was reported at 18 hours post injection. PDT was performed immediately after the MR images were taken and tumour regrowth was significantly delayed in comparison to a control group, leading the authors to conclude that the conjugate is effective for both tumour detection and minimally invasive therapy.[74]

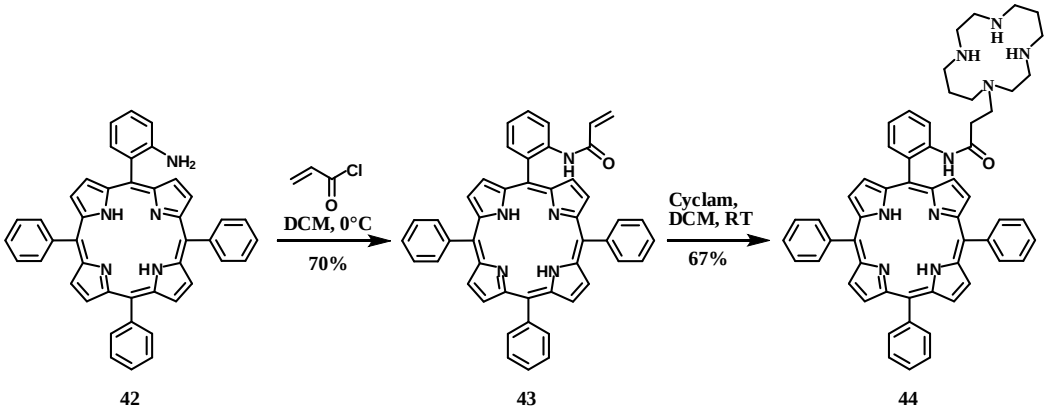


Scheme 5: Synthesis of a polymer loaded with photosensitiser and contrast agents for combined PDT-MRI[73]

1.7 Reported syntheses of linked porphyrin-cyclam/cyclen conjugates

Apart from the two examples of cyclam/cyclen derivatives appended to porphyrins already discussed, [62,73] there are several other instances of such molecules being reported in the literature.

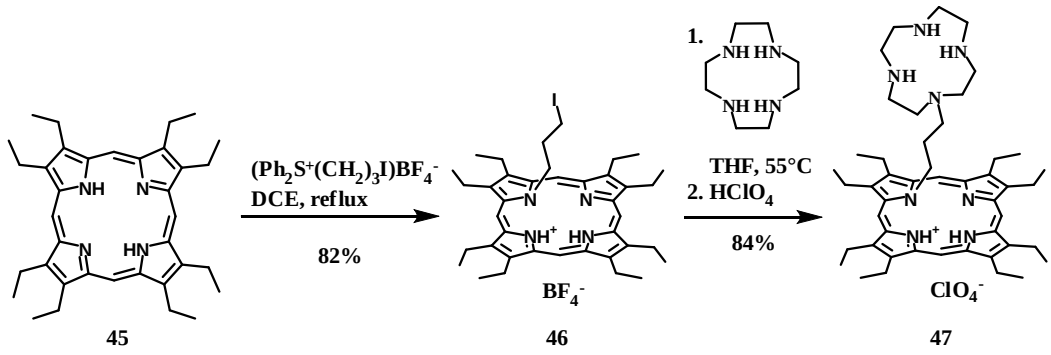
Weiss and co-workers[75] synthesised the porphyrin-cyclam compound **44** for use as a heterobimetallic mimic for cytochrome c oxidase by the route shown in Scheme 6. For a magnetic interaction between the two metal centres to occur, the requirement was for a cofacial arrangement of the two macrocycles with a flexible linking group. The route chosen involved condensing porphyrin **42**, containing an aromatic amine on the *ortho* position of a phenyl ring, with acrolyl chloride to leave an available activated olefin. This was subsequently reacted with the ring amine of cyclam in a conjugate addition. Both reactions gave acceptable yields although the authors could not selectively form the porphyrin complex of iron(III) from this bis ligand. The iron(III) was complexed by the activated olefin porphyrin before reaction with the cyclam macrocycle. The copper(II) was finally inserted into the cyclam ligand at the end of the synthesis.[75]



Scheme 6: Synthesis of a cyclam appended porphyrin as a cytochrome oxidase c model[75]

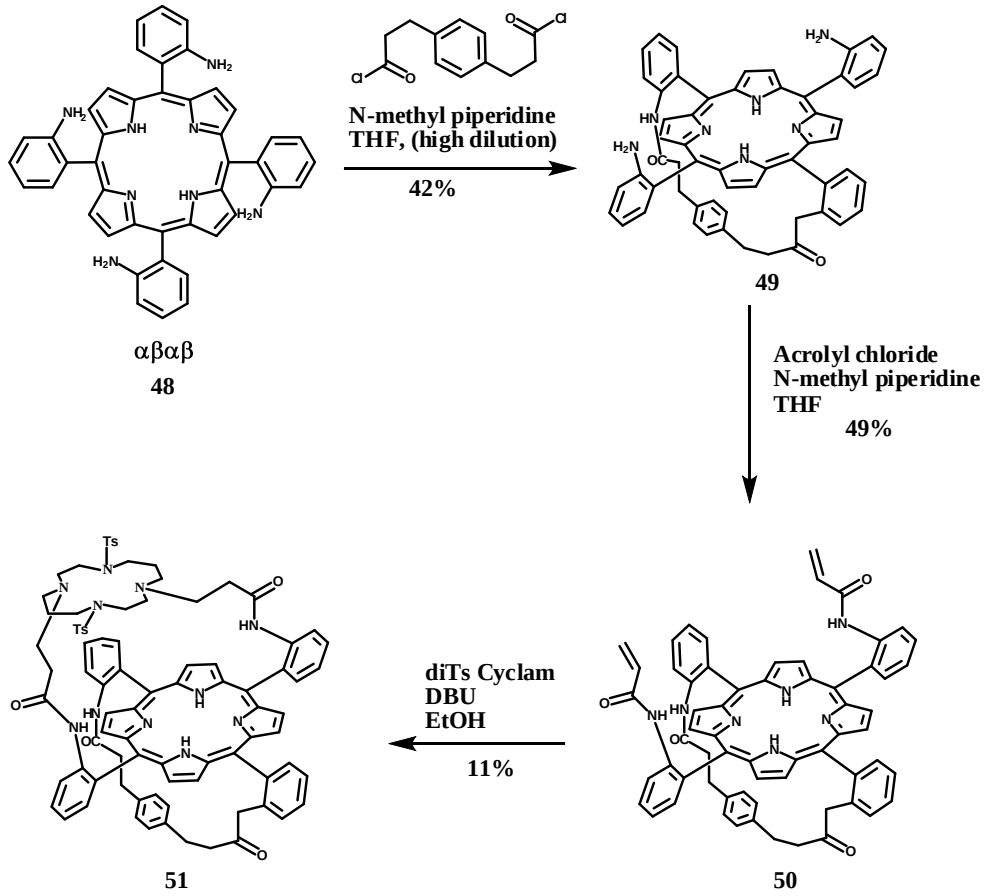
Takeda and Setsune[76] were also interested in a dinucleating system with a cofacial arrangement of the macrocycles for biomimetic models. They used the route shown in Scheme 7 to synthesise porphyrin-cyclen conjugate **47**. Alkylation of an inner nitrogen group in the porphyrin macrocycle was chosen in order to force the macrocycles into the desired cofacial position. This was

achieved by nucleophilic substitution of diphenyl sulfonium tetrafluoroborate by 2,3,7,8,12,13,17,18-octaethyl porphyrin **45**, followed by anion exchange. The desired product **47** was then obtained by reaction of cyclen with the aliphatic iodide protruding from the porphyrin core. Good yields were reported for both reactions.[76]



Scheme 7: Alkylation of the inner amine of a porphyrin with cyclen in order to furnish a cofacial arrangement of the two macrocycles[76]

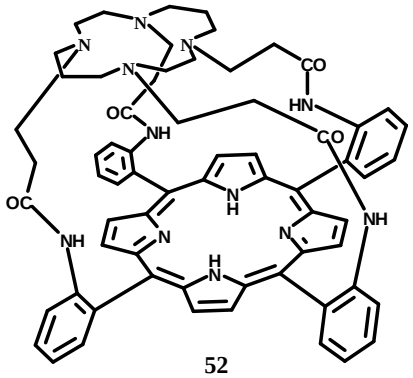
Studies of compound **44** synthesised by Weiss *et al.*[75] had shown that there was no interaction between the two metal centres and so Guilard and Boitrel[77] designed compound **51** as shown in Scheme 8. This molecule comprises a cyclam ligand strapped to a porphyrin molecule in order to force the macrocycles to have a cofacial arrangement. The reaction sequence uses the αβαβ atropoisomer of the tetraaminophenyl porphyrin **48** to selectively react two amines with the acyl chloride functionalities on the phenylene linking group. The orientation of the phenyl rings on the porphyrin leads to the selectivity of this reaction - the reactive amine groups have a *trans* arrangement to one another with respect to the porphyrin core. Having protected two amines, the remaining ones are then reacted in a sequence analogous to that used by Weiss *et al.*[75] Treatment with acrolyl chloride gave compound **50** - a porphyrin with two activated ester groups which were then reacted with cyclam to afford the desired porphyrin **51**.[77]



Scheme 8: Synthesis of a cyclam strapped porphyrin[77]

Analogous compounds were synthesised by the group of Collman[78] and were reported around the same time as the Boitrel paper.[77] They began from the αααα-atropoisomer of the tetraaminophenyl porphyrin and condensed this with acrolyl chloride to give four activated esters all protruding upwards from the porphyrin macrocycle. These were reacted with cyclam and cyclen to give compound **52**, comprising cofacial macrocycles but with each of the amino groups on the cyclen/cyclam attached to the porphyrin (Figure 18).[78]

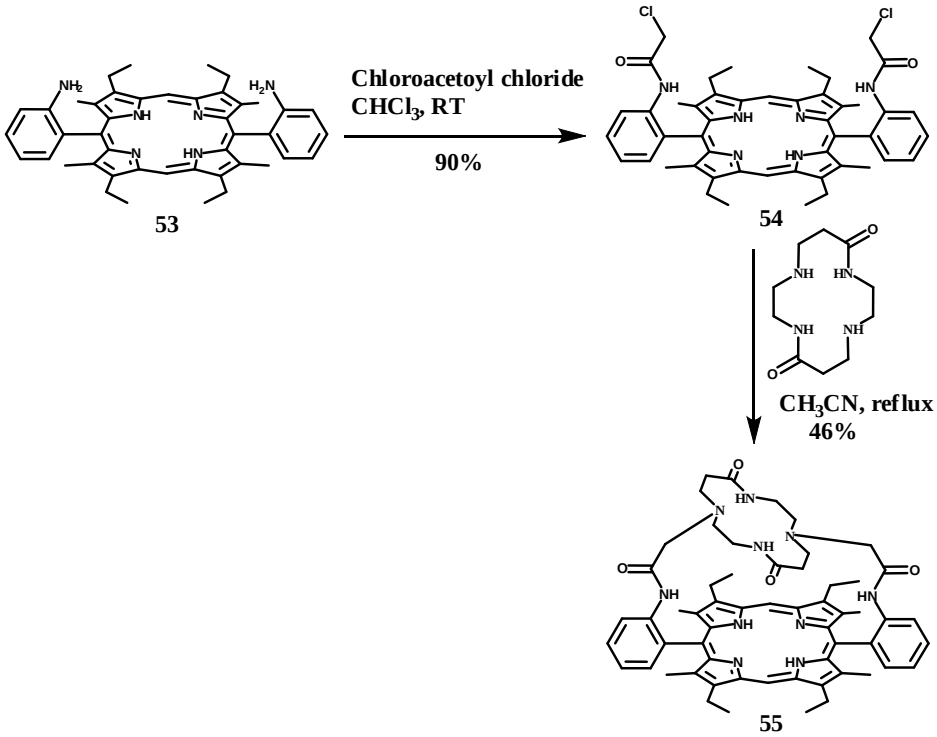
Similar methodology was used by Rose *et al.*[79] to synthesise so called 'barrel' porphyrins which contained two cyclen macrocycles strapped in the same way (4 positions), one on either face of the porphyrin.



52

Figure 18: Strapped porphyrin-cyclam compound synthesised by Collman *et al.*[78]

Smith *et al.*[80] used a diamino substituted diphenyl porphyrin **53** as their starting material, a molecule which showed no atropoisomerism at room temperature and so facilitated easier synthesis (scheme 9). Treatment with chloroacetoyl chloride gave the activated chloromethyl porphyrin **54** which was subsequently reacted with dioxocyclam to yield compound **55**. The dioxocyclam was chosen due to the lower reactivity of the amide nitrogens, thus negating the need to protect two of the the amine groups of cyclam in a *trans* fashion.[80]



Scheme 9: Synthesis of a strapped dioxocyclam porphyrin[80]

1.8 Summary

Porphyrin molecules are efficient generators of singlet oxygen when activated by light of an appropriate wavelength and are used as photosensitisers in photodynamic therapy. They exhibit a passive tumour targeting property, accumulating in tumour tissue due to the hyperpermeability of tumour vasculature and poor lymphatic drainage.

Cyclam and cyclen macrocycle derivatives are excellent ligands for metal ions such as copper(II) and gadolinium(III) among others. The complexes formed with these metal ions are thermodynamically stable and kinetically inert and are used *in vivo* for PET, MR imaging and radiotherapy.

The aim of this work was to develop a synthetic route which allows access to compounds containing a porphyrin photosensitiser and a cyclam/cyclen based macrocyclic ligand. Such a compound would have uses in tumour targeted imaging and therapy (PET-PDT and MRI-PDT) or dual therapy (radiotherapy-PDT).

1.9 Synthetic overview

The overall aim of this project is to develop a compound that contains a cyclen or cyclam based macrocyclic chelator and a porphyrin as shown in Figure 19, for use as a combination imaging-therapy agent.

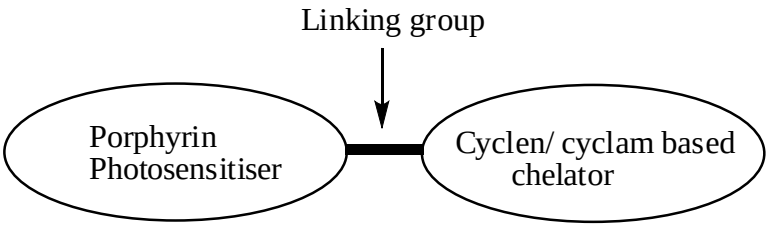


Figure 19: Schematic showing the aim of the synthesis

As already discussed, porphyrin compounds exhibit the physicochemical properties required to be used as photosensitisers in PDT. Cyclen macrocyclic chelators are form stable and inert complexes with gadolinium(III) and are used as MRI contrast agents, while cyclam complexes of copper(II) exhibit a similarly high level of thermodynamic and kinetic stability and are used in PET.

Based on a convergent synthetic strategy, the porphyrin and the cyclam/cyclen macrocycle must contain compatible functional groups to link them together. Additionally, the cyclen macrocycle must contain carboxylate containing pendant arms to offer the correct denticity for complexation of gadolinium(III).

The synthetic route must be general enough to allow coupling of both porphyrins and cyclam/cyclen macrocycles with differing substitution patterns. This is important because it will allow substituents to be changed, altering properties such as solubility or the balance between hydro- and lipophilicity.

Chapter 2: Synthesis of cyclen and cyclam macrocyclic chelators

2.1 Synthesis of cyclen based bifunctional chelators

2.1.1 Aims

The aim was to synthesise the bifunctional macrocycles **56**, **57**, **58** and **59** as shown below (Figure 20).

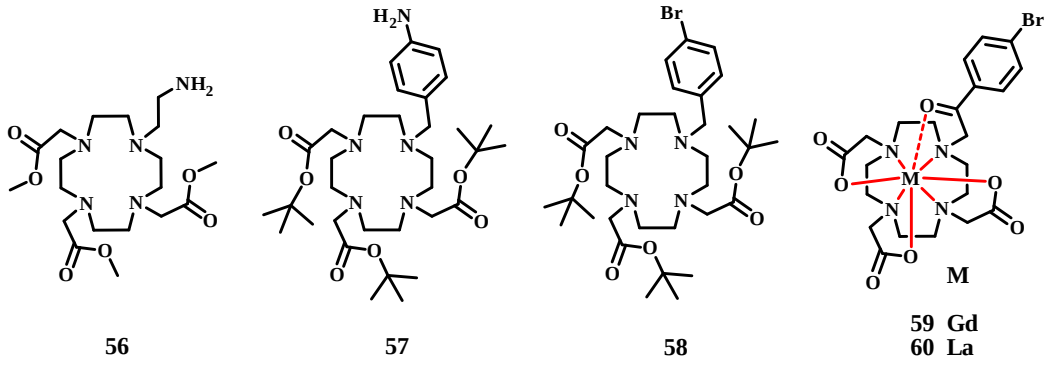


Figure 20: Bifunctional chelator target molecules

The target compounds **56**, **57**, **58**, **59** and **60** are all cyclen macrocycles functionalised with a reactive group on a pendant arm for subsequent attachment of a porphyrin moiety. In order to leave several reaction possibilities open for the porphyrin attachment, functional groups with different reactivity were chosen. In the case of **56** and **57** this is an alkyl and an aromatic amine respectively, which can be used as a nucleophilic species, whereas **58**, **59** and **60** contain an aromatic bromide group, useful for palladium mediated coupling reactions. Cyclen based macrocycles are known to form kinetically inert and thermodynamically stable complexes with metal ions, such as gadolinium(III). Carboxylate containing pendant arms are often used as the additional donor groups, so the target molecules **59** and **60** contain three such arms in order to facilitate formation of a stable, neutral metal complex. (Figure 20). However, these groups cause solubility problems during organic synthesis and so in targets **56**, **57** and **58** these groups are protected as *tert*-butyl or methyl esters. Deprotection of the esters to form the metal ion complex could then be carried out.

As an alternative strategy, compounds **59** and **60** contain the same linking functionality (aromatic bromide) as **58** but have already been complexed with a lanthanoid metal prior to being attached to the porphyrin.

2.1.2 Asymmetric substitution of cyclen/ cyclam macrocycles

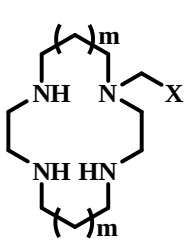
In order to access the target molecules, the four equivalent amine groups in the symmetric macrocycle cyclen must be functionalised in an asymmetric manner. The ways in which this has been achieved in the chemical literature can be broken down into three main categories:

1. Direct selective functionalisation of one amine, followed by derivatisation of the remaining three.
2. Direct functionalisation of three of the amines followed by substitution of the remaining one.
3. Protection – selective derivatisation – deprotection routes.

2.1.2.1 Direct substitution of one amine group on free base cyclam and cyclen

This is usually done with a large excess of macrocycle over the electrophile to ensure the major product is mono-N-substituted. There are many examples of cyclam and cyclen which have been monoalkylated in this way, incorporating a wide variety of functionality within the substituents.[81-85] Some examples of these are shown in Figure 21. Meunier *et al.*[81] used such a method to incorporate carboxylic acid and pyridyl side chains onto both cyclam and cyclen. The reactions used electrophilic species incorporating halide leaving groups, a five to tenfold excess of macrocycle and were heated to 110 °C in DMF. The choice of solvent played a key role by causing the unreacted cyclam/cyclen to precipitate out of solution upon cooling and therefore facilitated easier isolation of products **61** and **62**, which were obtained in yields between 40%-80% based on the

electrophile. Helps *et al.*[82] synthesised the aromatic derivatives **63-65** shown in Figure 21 using the same tenfold excess of macrocycle and an excess of inorganic base (potassium carbonate) in chloroform at ambient temperature. The polarity difference of the unreacted cyclam and the product was exploited to wash out the starting material from the crude reaction mixture using ethereal solvent. Yields were between 70-80% and the excess macrocycle was recovered. Similar methods have been used to incorporate an alkyl chain **66**[83] and a bipyridyl group **67**[84] using ethanol and aqueous methanol as solvent respectively along with a fivefold excess of macrocycle. Duimstra *et al.*[85] have used this method to derivatise cyclen with a pendant arm containing a sugar residue in a base free reaction at ambient temperature in DMSO (compound **68**).



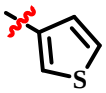
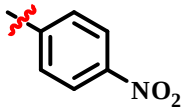
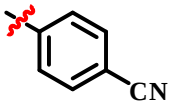
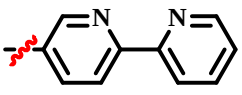
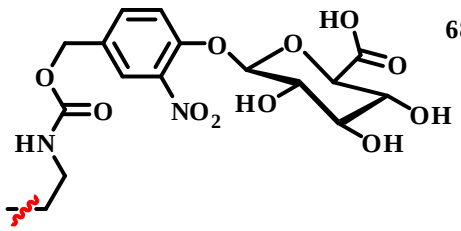
m	X	
0	CH_2COOH	61
1	CH_2COOH	62
1		63
1		64
1		65
1	$C_{15}H_{31}$	66
1		67
0		68

Figure 21: Examples of monofunctionalised cyclam / cyclen derivatives[81-85]

In an attempt to produce cyclen based lanthanide chelators with a functional handle, Kruper and co-workers[86] noticed an unexpected selectivity towards mono alkylation when using a stoichiometric ratio of macrocycle to electrophile. The reactions were carried out in anhydrous, non polar, aprotic solvents such as dichloromethane or chloroform stabilised with pentene. Their highest reported yields were obtained when using sterically hindered secondary bromide electrophiles in a 1:1 ratio with free base cyclen – Figure 22. It is noteworthy that the products **70**, **72** and **74** were isolated as the monohydrobromide salts, and indeed the selectivity in these reactions appears to stem from the relative basicity of the secondary amines in the macrocyclic ring. Table 2 shows the pK_a values for the four amines on the cyclam and cyclen molecules.[87] The first two protonation constants are significantly higher than the last two, for both macrocycles. Hancock and co-workers[87] assign this drop to the build up of strain energy as three protons are placed within the macrocyclic ring. This is comprised of both electrostatic repulsion and van der Waals repulsion between the protons. The net result is that the first two amines on cyclam and cyclen are basic but the third and fourth are not and Kruper *et al.*[86] used this trend to interpret their highly selective reactions. Since the reactions were performed in the absence of base, the first alkylation is followed by protonation of the second amine. The remaining amines are non-basic and also non-nucleophilic and so the crude products show mainly mono-substituted products with only small amounts of dialkyl species present. These are removed by column chromatography and the pure hydrogen bromide salts **70**, **72** and **74** were isolated. Indeed the authors state that performing the reactions with base present reduced the selectivity and yields. The steric hindrance present in the electrophiles **69**, **7**1 and **73** is clearly a factor in the success of the reactions, probably slowing the second alkylation down considerably in comparison to the competing protonation. This is confirmed by the much lower yield of the last entry in Figure 22 which uses an unhindered alkyl bromide **75**, with a resulting loss of yield of product **76**. The solvent plays an important role with nonpolar aprotic solvents preferred to polar solvents which they report as promoting proton transfer and subsequent loss of selectivity.[86]

Table 2: Successive pK_a values for cyclam and cyclen[87]

	pK_1	pK_2	pK_3	pK_4
Cyclen	10.6	9.6	1.5	0.7
Cyclam	11.6	10.6	1.6	2.4

The pK values in the table refer to the following equilibria:

pK_1 : $L \leftrightarrow LH^+$ pK_2 : $LH^+ \leftrightarrow [LH_2]^{2+}$

pK_3 : $[LH_2]^{2+} \leftrightarrow [LH_3]^{3+}$ pK_4 : $[LH_3]^{3+} \leftrightarrow [LH_4]^{4+}$ L = free base cyclam/cyclen

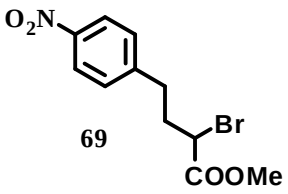
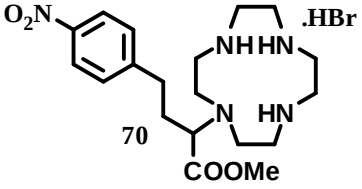
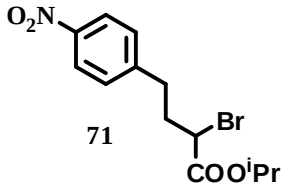
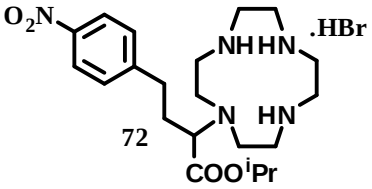
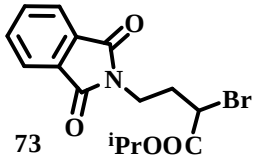
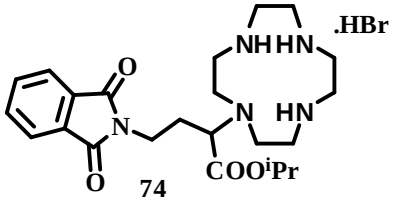
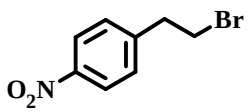
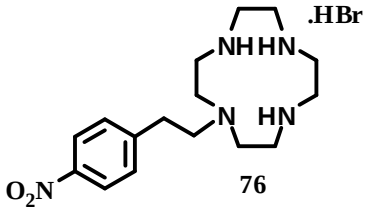
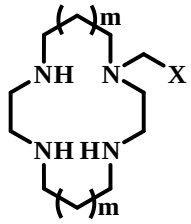
Electrophile	Product	Yield
 69	 70	84%
 71	 72	72%
 73	 74	79%
 75	 76	39%

Figure 22: Cyclen derivatives synthesised by Kruper *et al.*[86]

In 2002, Li and co-workers[88] found that the results reported by Kruper *et al.*[86] could not be maintained when the electrophile is unhindered or containing reactive functional groups.[88] They reported a method which is compatible with a wide range of functional groups such as ethers, esters, alcohols, alkenes and larger moieties such as sugars and crown ethers as shown in Figure 23. Their synthesis proceeds by slow addition of the electrophile to a double excess of macrocycle and a five-fold excess of inorganic base, in anhydrous acetonitrile at 60 °C. After workup and flash chromatography on silica gel they report yields of monosubstituted cyclam and cyclen macrocycles **77**-**86** in the range 66-93% .



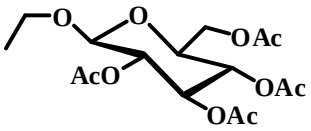
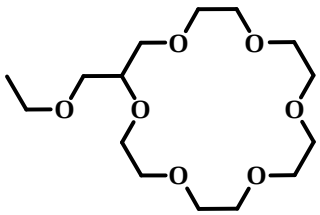
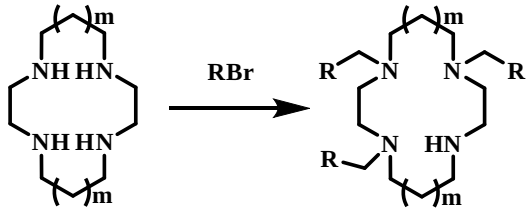
X		m	Yield / %
CH_2COOEt	**77**	**0**	**79**
	78	**1**	**85**
$CH_2CH{=}CH_2$	**79**	**0**	**84**
	80	**1**	**91**
CH_2CH_2OH	**81**	**0**	**83**
	82	**1**	**90**
	**83**	**0**	**75**
	84	**1**	**83**
	**85**	**0**	**66**
	86	**1**	**78**

Figure 23: Monofunctionalised cyclam/cyclen compounds synthesised by Li and co-workers[88]

2.1.2.2 Direct substitution of three amine groups on free base cyclam and cyclen

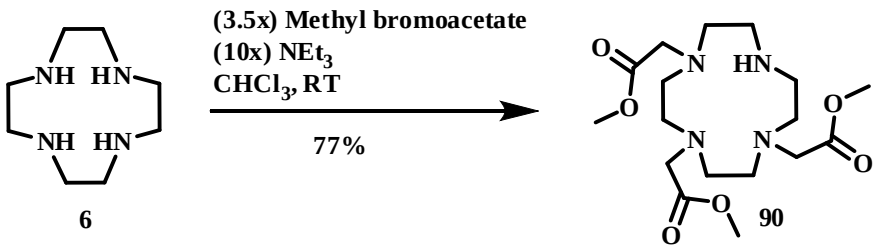
Free base cyclam and cyclen can be directly alkylated on three of the amine groups by careful control of stoichiometry and reaction conditions to maximise the yield (Figure 24). The Mishra group[89] developed a methodology to substitute both macrocycles with ethyl ester bearing pendant arms on three of the available amines by slow addition of 2.24 equivalents of electrophile in dichloromethane solution at ambient temperature. They reported yields of 72% and 74% for compounds **87** and **88** respectively and it is interesting to note that the reaction is base free. Faulkner and co-workers[90] alkylated cyclen with three *tert*iary butyl containing pendant arms using 3.3 equivalents of electrophile and excess inorganic base in a similar procedure, to give **89** in an isolated yield of 42%. It is noteworthy that neither of these procedures required column chromatography to isolate the product, which was obtained by recrystallisation.



R		m	Yield / %
CH_2COOEt	**87**	**0**	**72**
	88	**1**	**74**
$CH_2COOC(CH_3)_3$	**89**	**0**	**42**

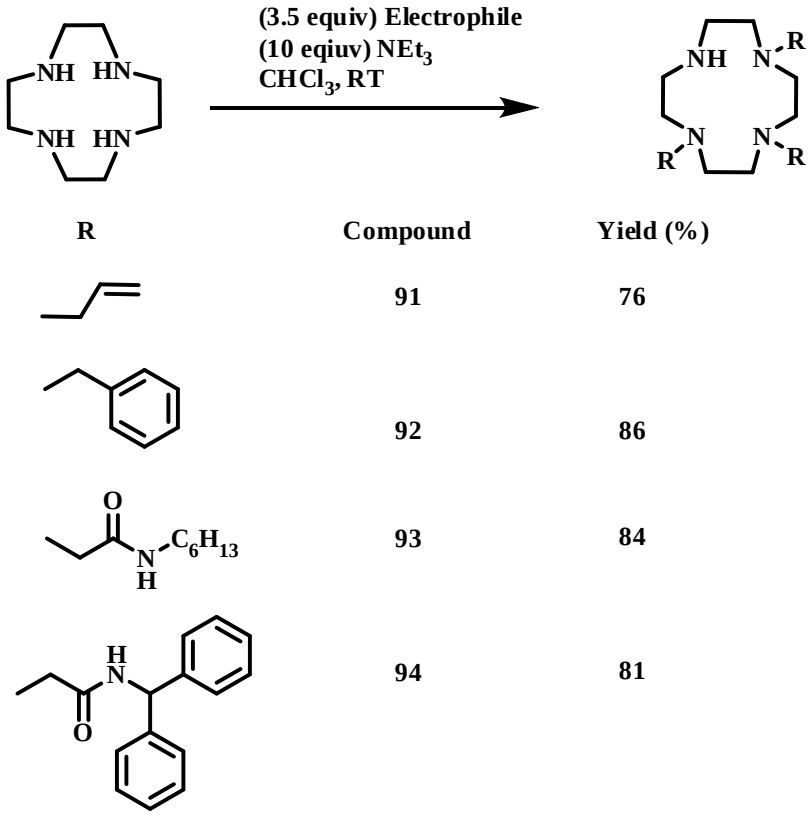
Figure 24: Cyclam / cyclen compounds synthesised by Mishra *et al.*[89] and Faulkner *et al.*[90]

A comprehensive study by Li *et al.*[91] led to the development of a more general method of selectively trialkylating cyclen, with improved isolated yields of products. They examined the effect of solvent and base on the synthesis of the *tris*-*tert*-butyl ester shown in Scheme 10.



Scheme 10: Optimised synthesis of 1,4,7-(*tris*-methoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane[91]

The optimum conditions were found to be chloroform solvent, 3.5 equivalents of electrophile and ten equivalents of triethylamine as auxiliary base. Although this method is just an optimisation of the previous work, the yield of 77% of **90** is significantly higher than previously reports. The generality of the method was illustrated by using a series of electrophiles to synthesise compounds **91-94** as shown in Figure 25.



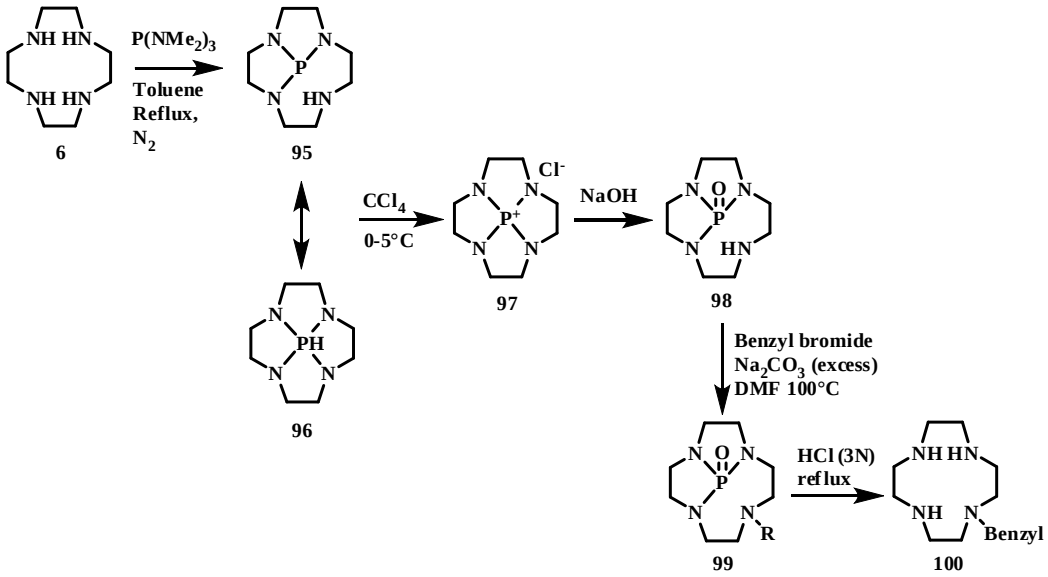
R	Compound	Yield (%)
	91	76
	92	86
	93	84
	94	81

Figure 25: Trialkylated cyclen derivatives synthesised by Li *et al.*[91]

2.1.3 Protection – selective derivatisation – deprotection routes

2.1.3.1 Protection of three amine functionalities by trivalent species

Handel *et al.*[92] developed a synthetic methodology for monoalkylation of cyclam and cyclen by using a phosphoryl group to temporarily block three of the amine groups on the macrocycle. This was followed by reaction of the fourth amine with an electrophile before removal of the protecting group, Scheme 11.

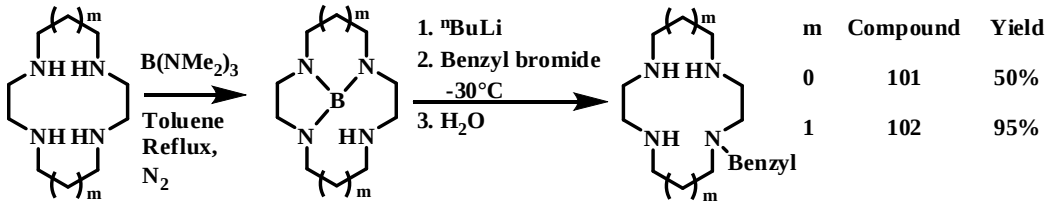


Scheme 11: Monoalkylation of cyclen using a phosphoryl protecting group[92]

The phosphoryl species was inserted into the free base macrocycle 6 by reaction with hexamethyl phosphoric triamide. This lead to the tautomeric equilibrium of 95 and 96 (phosphorus(III) and (V) oxidation states) which was then oxidised by CCl_4 at low temperature (0-5 °C) to give the phosphonium chloride salt, 97. After basic hydrolysis, the resulting phosphonium oxide species, 98 was treated with benzyl bromide in a stoichiometric ratio in DMF at 100 °C to yield 99 followed by acidic removal of the phosphoryl protecting group to give 100. Although there are several steps, the overall yield was reported as 90%, but the drawback to this method is intolerance of many functional groups within the electrophile due to the harsh acidic conditions used to remove the protecting group. The same group[93] also developed an analogous method using a boron

protecting group by using the reagent tris(dimethylamino) borane. The reaction sequence is applicable to both cyclen and cyclam but a higher yield was reported for the cyclam derivative 101. Ultimately, though the scope is limited due to the use of strong base (^{n}butyl lithium) required to facilitate alkylation of the final amine in the macrocylic ring.(

Scheme **12**)

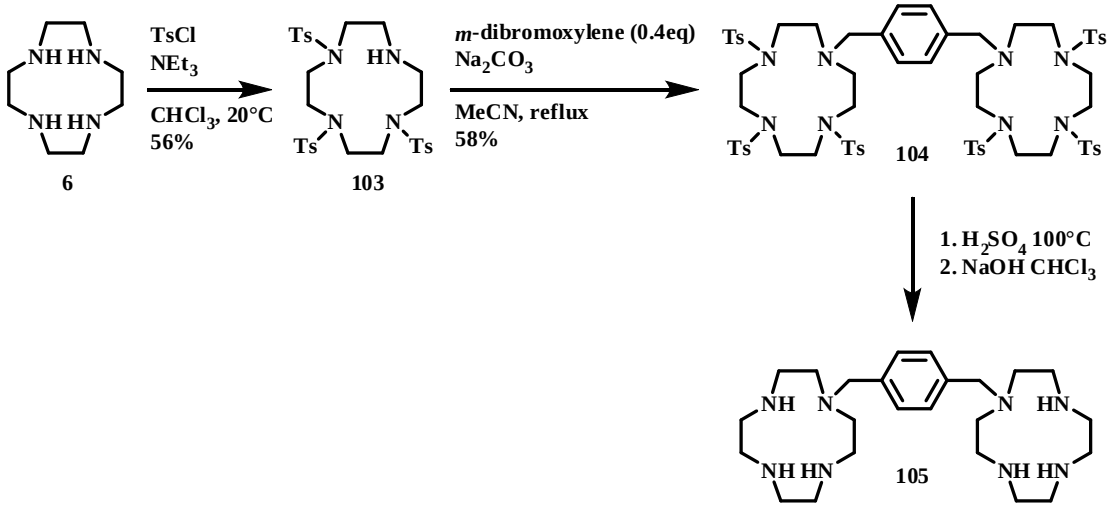


m	Compound	Yield
0	101	50%
1	102	95%

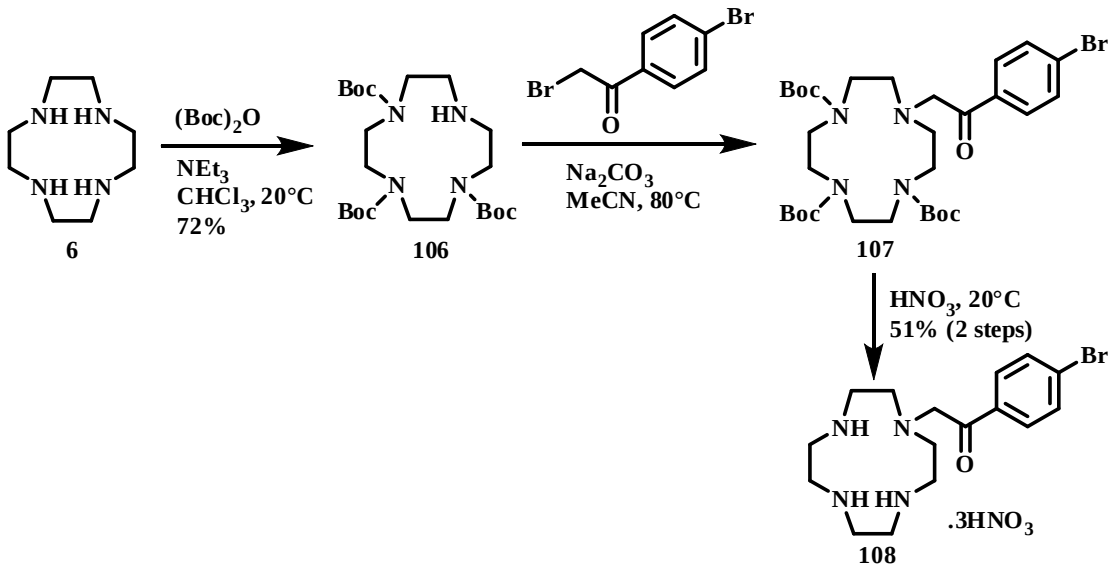
Scheme 12: Use of a boron protecting group for monoalkylation of cyclam/cyclen[93]

Another type of protection-alkylation-deprotection sequence is possible by direct substitution of three of the ring amines by protecting groups. Two common functionalities used for this purpose are tosyl[94] and *tert*butoxycarbonyl.[95] As shown in Scheme 13, compound **103** has been synthesised in 56% yield by slow addition of a double excess of *p*-toluenesulphonyl chloride to a chloroform solution of cyclen **6** with excess triethylamine present.[94] A double excess of the electrophile was found to give the optimum yield since this ratio diminishes formation of the tetra-N-substituted macrocycle which caused the separation of the desired product to be much more difficult. As shown in Scheme 14, tri-N-*tert*butoxycarbonyl protected cyclen **106** has been synthesised using a similar procedure, again with slightly less than the expected 3:1 ratio of electrophile to macrocycle.[95] Both of these procedures give a reliable route to monoalkylated cyclen and cyclam and some examples of their use are shown in Scheme 13 and Scheme 14. Their scope is ultimately limited by the nature of the deprotection conditions required to furnish the free macrocycle. The tosyl deprotection conditions involve using boiling concentrated sulphuric acid or 48% HBr solution under reflux conditions. The *tert*butoxycarbonyl deprotection did not require heating but still involves strongly acidic conditions (trifluoroacetic acid

or 48% HBr solution) and so both of these routes are only possible for electrophiles containing functionalities which are compatible with strong acids.



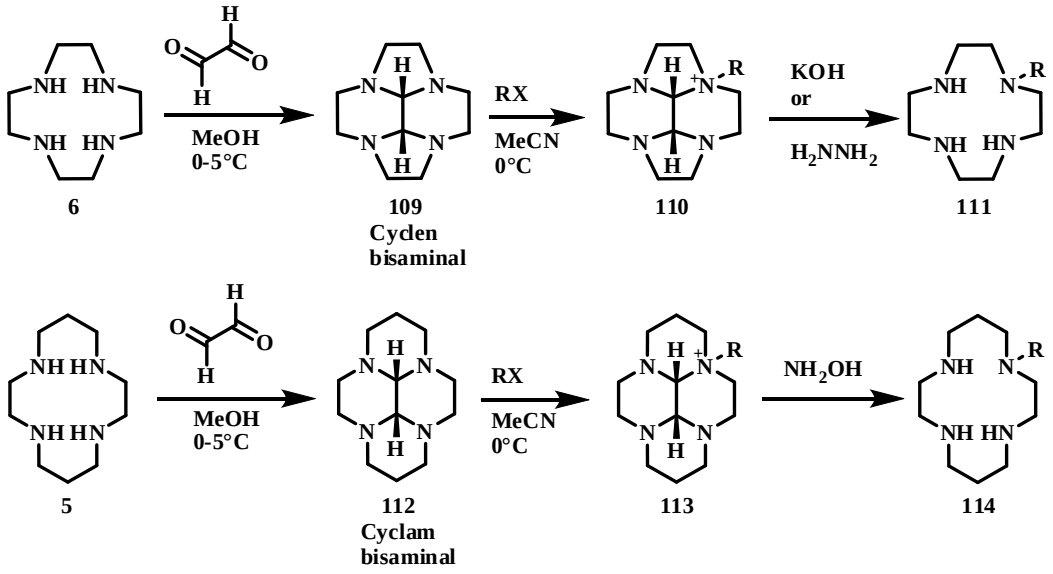
Scheme 13: Asymmetric substitution of cyclen using tosyl protecting groups[94]



Scheme 14: An example of N-Boc protecting groups used in the synthesis of asymmetrically substituted cyclen[96]

2.1.3.2 Protection – selective derivatisation – deprotection using bisaminal methodology

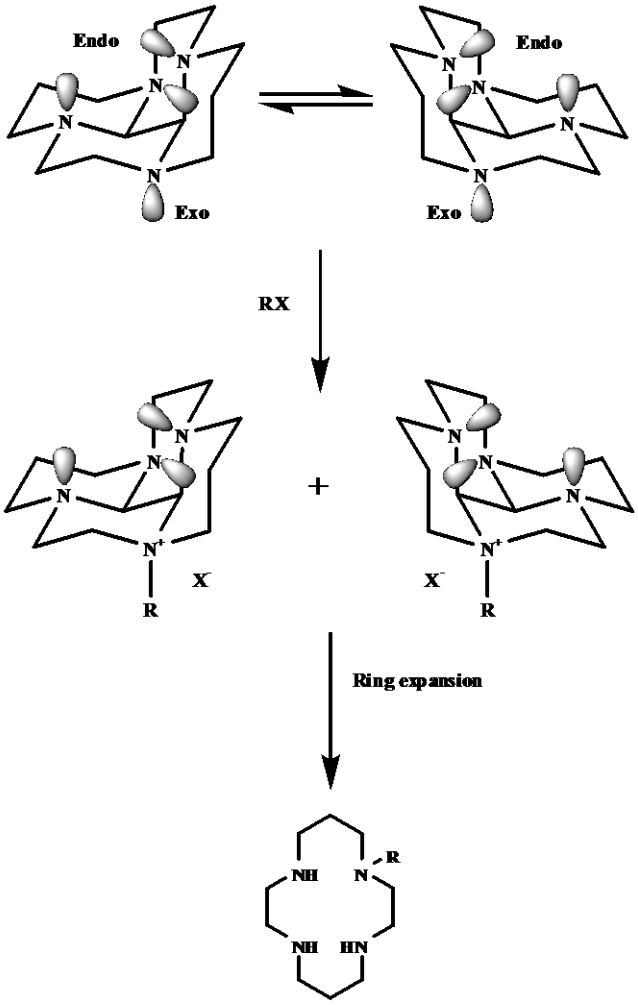
This methodology inserts a two carbon bridge into the free base macrocycle, rigidifying the structure and effectively protecting three of the amines so that one can be selectively alkylated. The bridge is then removed to leave the free base macrocycle with only one functionalised amine group-Scheme 15. The glyoxal adducts of cyclam **112** and cyclen **109**, were first reported by Weisman *et al.* in 1980.[97] Since then the syntheses of these molecules has been optimised and they are now prepared by adding a solution of one equivalent of glyoxal (40% aq) to a methanolic solution of the free base macrocycle at 0-5 °C.[98] This methodology gave access to **109** and **112** in high yields (85-95%) as shown in Scheme 15.



Scheme 15: Use of bisaminals to monofunctionalise cyclam and cyclen macrocycles[98]

The tetracyclic structures have *cis* configuration about the central two carbon bridge, which has been confirmed by temperature dependent NMR experiments.[97, 99] The result of this configuration is that the molecule sits in a folded geometry giving rise to a concave and a convex face. This leaves two non-adjacent nitrogen lone pairs facing away from the concave face (*endo*) and the other two pointing away from the convex face (*exo*) and their reactivity towards electrophiles is governed by this alignment.[100] The two *exo* lone pairs are much less sterically hindered than the *endo* ones and they act as nucleophiles

whereas the two *endo* ones do not. The cyclam bisaminal is chiral due to a C_2 symmetry axis orthogonal to the central carbon-carbon bridge, and hence exists as a pair of enantiomers which interconvert at ambient temperature.[99] Alkylation is typically carried out in dry acetonitrile with a slight excess of electrophile at room temperature.[98] The products of this reaction are two enantiomeric quaternary ammonium salts. Dialkylation is a much slower process and isolation of the monosubstituted product is aided by precipitation from solution, facilitating isolation by a simple filtration / washing procedure. After successful monosubstitution of one amine, the two carbon bridge can be removed by treatment with hydrazine or inorganic base such as potassium hydroxide which gives just the single monoalkylated macrocycle as the product (Scheme 16)

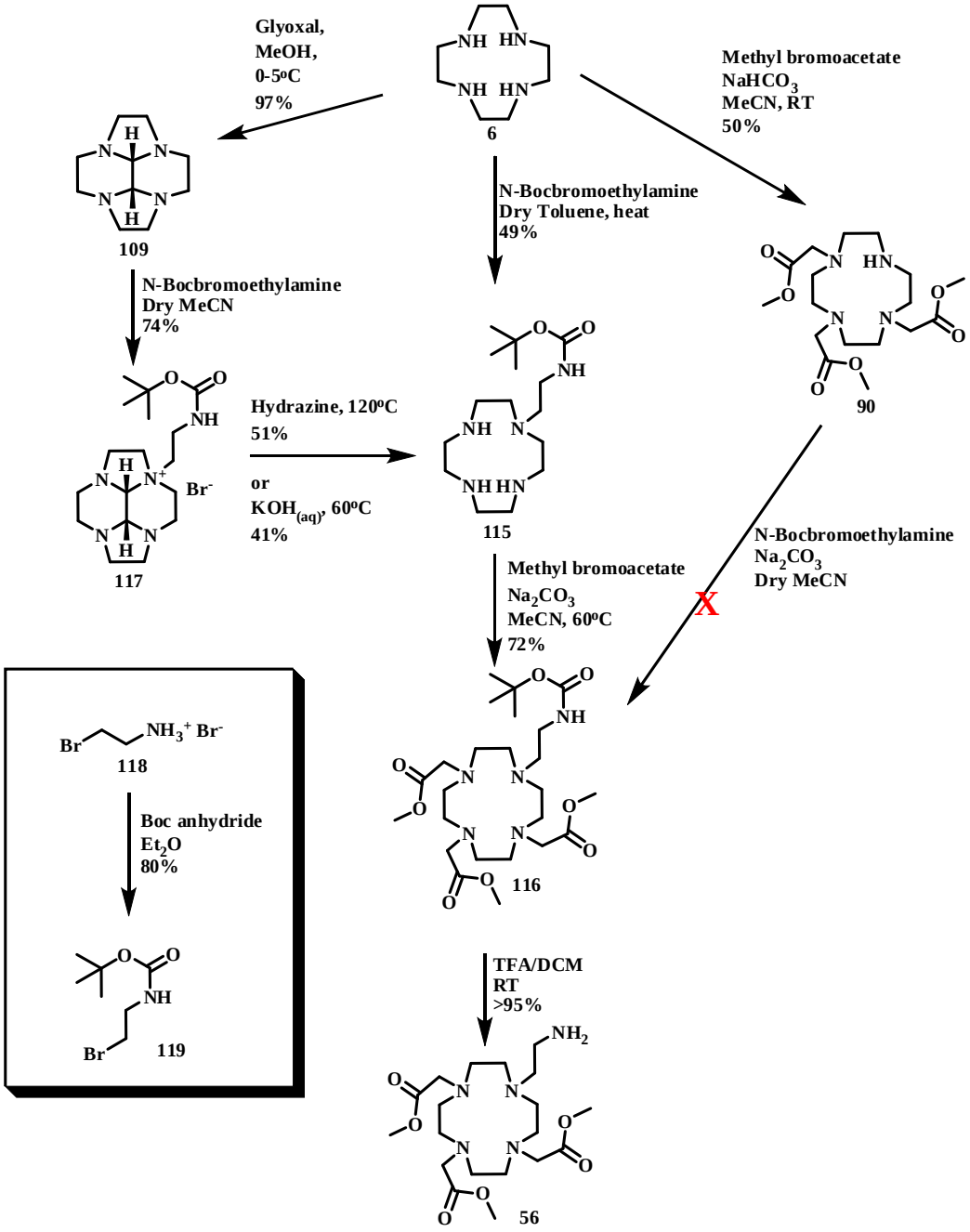


Scheme 16: Schematic showing the *cis* configuration about the central two carbon bridge, and the effect on the orientation of the nitrogen lone pairs

2.1.4 Synthesis of 1-aminoethyl-4,7,10-(tris-methoxycarbonylmethyl)-(1,4,7,10-tetraazacyclododecane) (56)

2.1.4.1 Synthetic strategy

The synthetic routes used to obtain compound **56** are shown in Scheme 17 below.



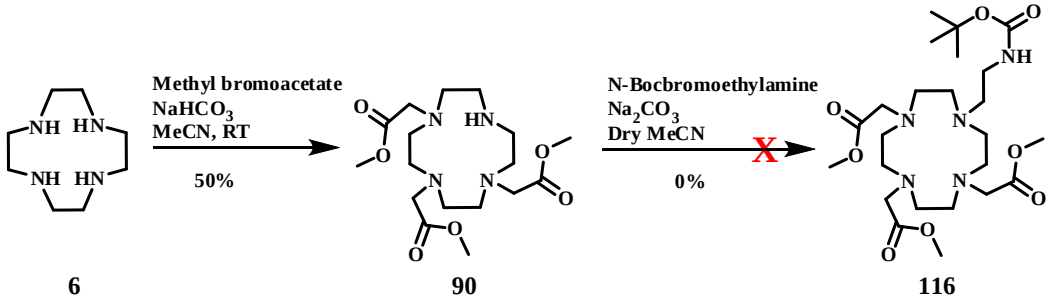
Scheme 17: Synthetic routes for the synthesis of compound 56

Compound **56** is comprised of a cyclen macrocycle substituted with three ester containing pendant arms and one ethyl amine arm, and so the free base cyclen macrocycle needed to be asymmetrically functionalised.
The amine pendant arm needed to be introduced onto the macrocycle in a protected form so that the nucleophilic amine did not interfere with subsequent reactions. The acid labile *tert*-butoxycarbonyl (Boc) group was chosen as the protecting group. To facilitate selective unmasking of the amine once the macrocycle had been functionalised, the type of esters needed to be chosen so that they were less susceptible to acidic conditions than the Boc group and for this purpose methyl ester arms were chosen.

The initial approach to obtaining macrocycle **56** was to alkylate three of the amines with the ester arms, isolating the product **90** and then subsequently alkylating the fourth amine with the N-Boc protected arm. However, this route was unsuccessful and so the sequence of reactions was reversed, monoalkylating cyclen with the N-Boc pendant arm followed by alkylating the remaining three amines with the ester pendant arms. A literature search showed that this reaction sequence was already known[101], and it was indeed successful in obtaining compound **116**. However, the drawback to this route was its inefficiency with respect to the starting macrocycle cyclen, which is relatively expensive and used in excess. So an alternative route was explored using the selective alkylation of the bisaminal intermediate **109**. Compound **109** was selectively monoalkylated with the N-Boc amine arm **119**, and the rigidifying bridge removed to furnish compound **115**, hence providing an alternative route to the target compound **56**.

2.1.4.2 Results and discussion

The initial attempts to synthesise the aminoethyl cyclen derivative **56** were via the trifunctionalised cyclen intermediate **90**, as shown in Scheme 18 below.

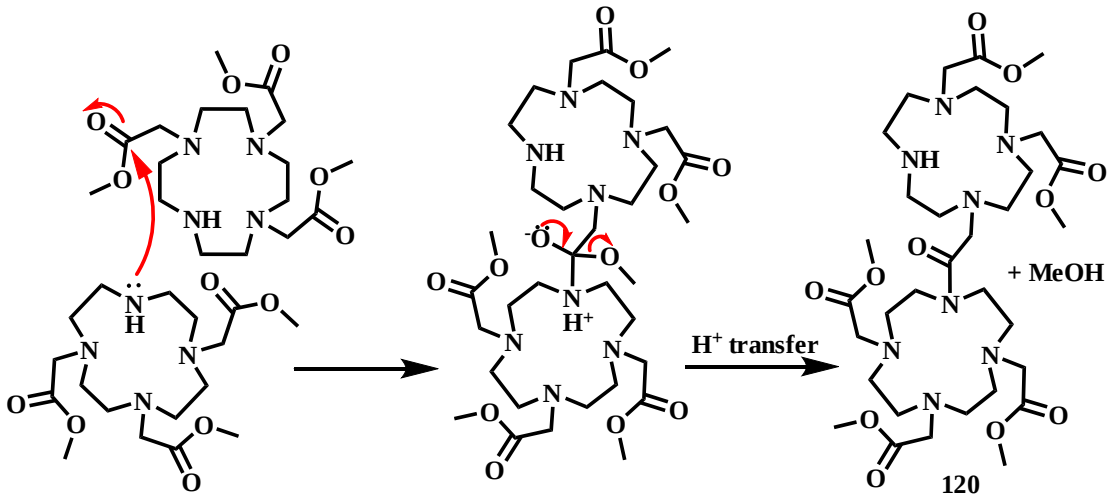


Scheme 18: Attempted synthesis of compound 116

Compound **90** was synthesised by dropwise addition of methyl bromoacetate to a solution of cyclen in acetonitrile with $NaHCO_3$ present. A triple excess of the electrophile was used and after three days of stirring at room temperature, compound **90** was isolated, after aqueous work up and chromatography, in 50% yield. Bromoethylamine hydrobromide **118** was then treated with Na_2CO_3(aq) followed by Boc anhydride to protect the amine and furnished compound **119** in 80% yield.[102]

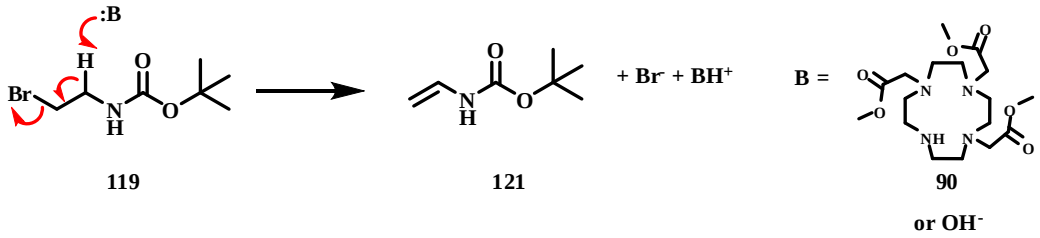
Subsequent attempts at alkylating the remaining free amine on the macrocycle with N-Bocbromoethylamine were, however, unsuccessful. Initially a one to one molar ratio of macrocycle to electrophile was used, with one equivalent of sodium carbonate as base and the reaction was attempted at room temperature. Close monitoring by TLC showed no appreciable reaction occurring even when left for four days, so the reaction was heated gently to 60 °C. This caused one main product to be formed, which was duly isolated by column chromatography. The compound exhibited a complex NMR spectrum, but the lack of peaks characteristic of the *tert*iary butyl group in both the ^{1}H and ^{13}C spectra clearly indicated it was not the desired compound **116**. The proton spectrum showed very broad, unassignable signals in the region where the macrocyclic ring protons were expected and typical ring carbon signals were also present in the

^{13}C spectrum, indicating that the compound did contain the macrocyle unit. However, the complexity of the signals gave rise to the possibilities that either a mixture was still present or more than one macrocycle could be present in the compound. Careful analysis by TLC was consistent with a single product and showed that the compound had not degraded during work up and isolation. An intense new signal was also observed in the ^{13}C spectrum at 160ppm, alongside the original ester signals which was also consistent with the macrocycle dimerising/polymerising, a possible route for which is shown in Scheme 19 below.



Scheme 19: Postulated mechanism of dimer formation

The amide bond linking the two macrocycles in **120** would be expected to give a signal around 160-170ppm in the ^{13}C spectrum. Although this product was not identified, the clear implication was that the macrocycle was not reacting with the electrophile to give the desired product. Since the electrophile was consumed in the reaction, there remains a possibility of a competing elimination mechanism as depicted in Scheme 20. The base involved could be either the starting macrocycle **90** or the inorganic base added to the reaction. The product would be nucleophilic and hence unreactive to any remaining compound **90.**

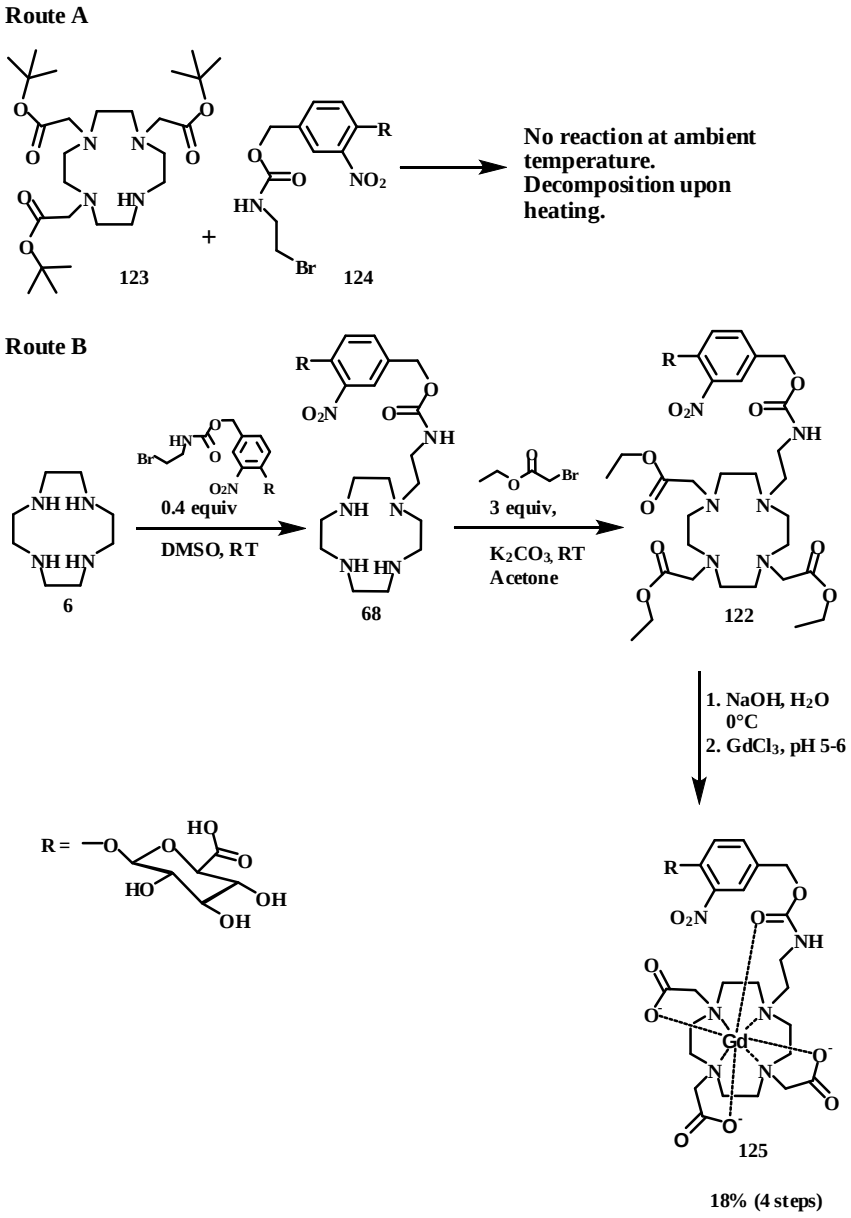


Scheme 20: Proposed elimination reaction between compounds 119 and 90

Further attempts at alkylation were carried out at room temperature using an excess of the electrophile and also using a small amount of KI to improve the reactivity of compound **119**, but none of these were successful.

A similar problem was encountered by Duimstra *et al.*[85] when they were attempting to synthesise the cyclen derivative **122** (Scheme 21). As shown in route a, the initial approach was to react the trisubstituted cyclen **123** with the sugar appended alkyl bromide **124**. However, the reaction was still incomplete after four days at ambient temperature, and byproducts were beginning to form. On applying heat, the sugar containing arm degraded and they were forced to change their synthetic route.

As shown in route B, alkylation of the free base macrocycle with compound **124** was successful and although compound **68** could not be isolated, the desired product **122** was obtained after subsequent alkylation of the remaining secondary ring amines. The ester groups were hydrolysed and the metal ion complex **125** formed in 18% yield over four steps.

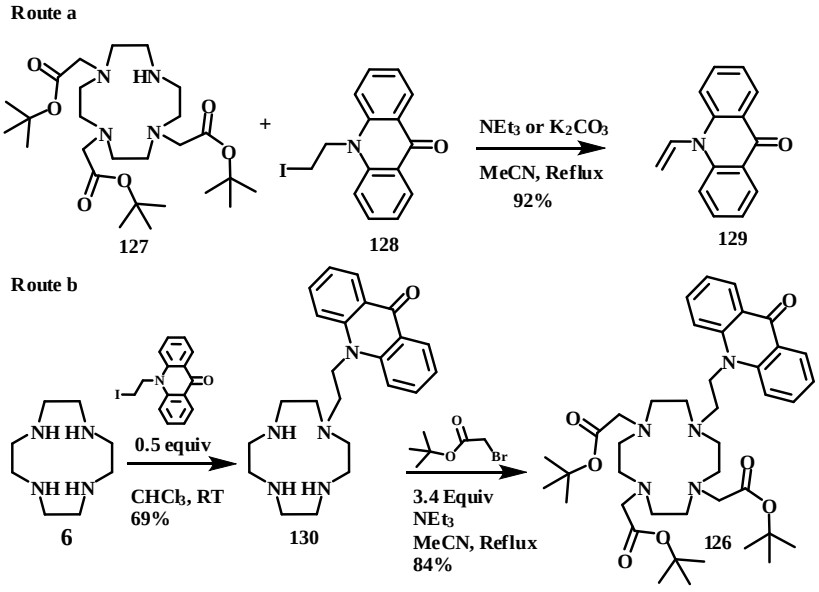


Scheme 21: Literature report showing two approaches to asymmetric substitution of amines on a cyclen macrocycle[85]

An analogous problem was encountered by Sammes *et al.*[90] as shown in Scheme 22. They were aiming to synthesise the target molecule **126** by reacting trisubstituted macrocycle **127** with the N-iodoethyl substituted acridone **128**. Compound **127** was dissolved in acetonitrile and K_2CO_3 added, followed by slow addition of the electrophilic species **128**, and gentle heating for 36 hours. However, the product isolated was the elimination product **129** which was formed in very high yield. They do not discuss this failure in the paper, but turn to a route analogous to that used by Duimstra et al.[85] and successfully obtain **126**

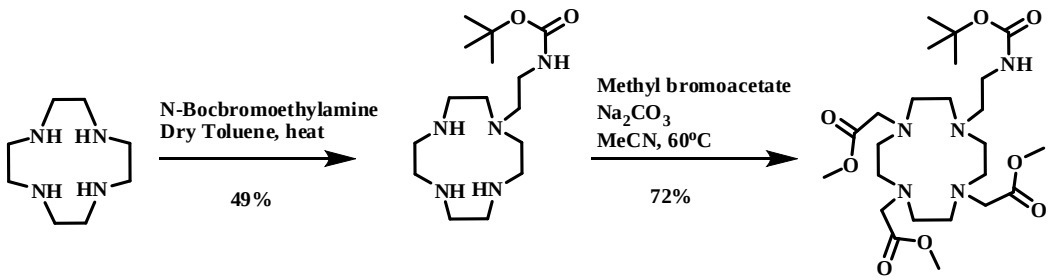
by monoalkylating free base cyclen with the acridone derivative **128** followed by trialkylation of the remaining amines on the macrocycle with the *tert*iary butyl containing arms (Scheme 22 – route b). This time the yields were reported and they isolated monoalkylated intermediate species **130** in 69% yield and the target compound **126** in 84% yield in the subsequent reaction.

These two examples show that alkylation of the fourth amine on the macrocycle is not a trivial procedure even with reactive electrophiles such as in the latter case with iodinated species **128**.



Scheme 22: Literature report of an elimination reaction by the free amine on a cyclen macrocycle[90]

Hence a new route was required to synthesise the required aminoethyl substituted cyclen **56**, and like the two groups discussed, the first obvious option was to reverse the synthetic steps in order to put the single N-Boc protected amine arm onto the macrocycle before the ester arms (Scheme 23). Indeed, a search of the literature found that the desired compound **116** had been synthesised previously by Mishra *et al.*[101]



6 **115** **116**

Scheme 23: Synthetic route to compound 116[101]

Using the reported method[101] a two and a half fold excess of free base cyclen **6** was dissolved in anhydrous toluene and heated under reflux with one equivalent of the electrophile **119**. This methodology uses the stoichiometry of the reagents i.e the excess of macrocycle to facilitate the mono substituted cyclen **115** being the main product. Indeed **115** was isolated in 49% yield without recourse to chromatographic separation. The product has much higher solubility in organic solvents such as dichloromethane and much lower solubility in water compared to cyclen (both free base and protonated), allowing isolation of the product by the simple procedures of extraction and washing.

Again following the procedure reported[101] compound **115** was dissolved in anhydrous acetonitrile with 3.5 equivalents of methyl bromoacetate and sodium carbonate as base. The mixture was heated to 60 °C under a nitrogen atmosphere and was monitored by TLC. A single product formed with the expected R_f value and was duly isolated by column chromatography. However, both ^{1}H and ^{13}C NMR analysis indicated that a mixture was present. As mentioned previously, these types of macrocyclic compounds often exhibit broadened signals in their ^{1}H spectra, so the ^{13}C spectra are generally used as a primary resource for identification purposes. Figure 26 shows two areas of the ^{13}C spectra for the mixture initially isolated and the pure sompound **116** as a comparison. It can be clearly seen that the carbonyl (ester) region shows four peaks in the mixture and only the expected two in the pure compound. At lower chemical shift values, two peaks can be seen in the mixture which correspond to the carbonyl peak within the Boc protecting group, whereas only one is expected and indeed seen in the

pure compound. This indicated that the reaction was not complete and the isolated material was probably a mixture of the dialkylated macrocycle and the desired product, even though it appeared as a single spot by TLC analysis. Hence, the material was treated with a further 1.5 equivalents of methyl bromoacetate in the same manner as before which after workup and isolation, furnished a pure sample of compound **116** in 72% yield.

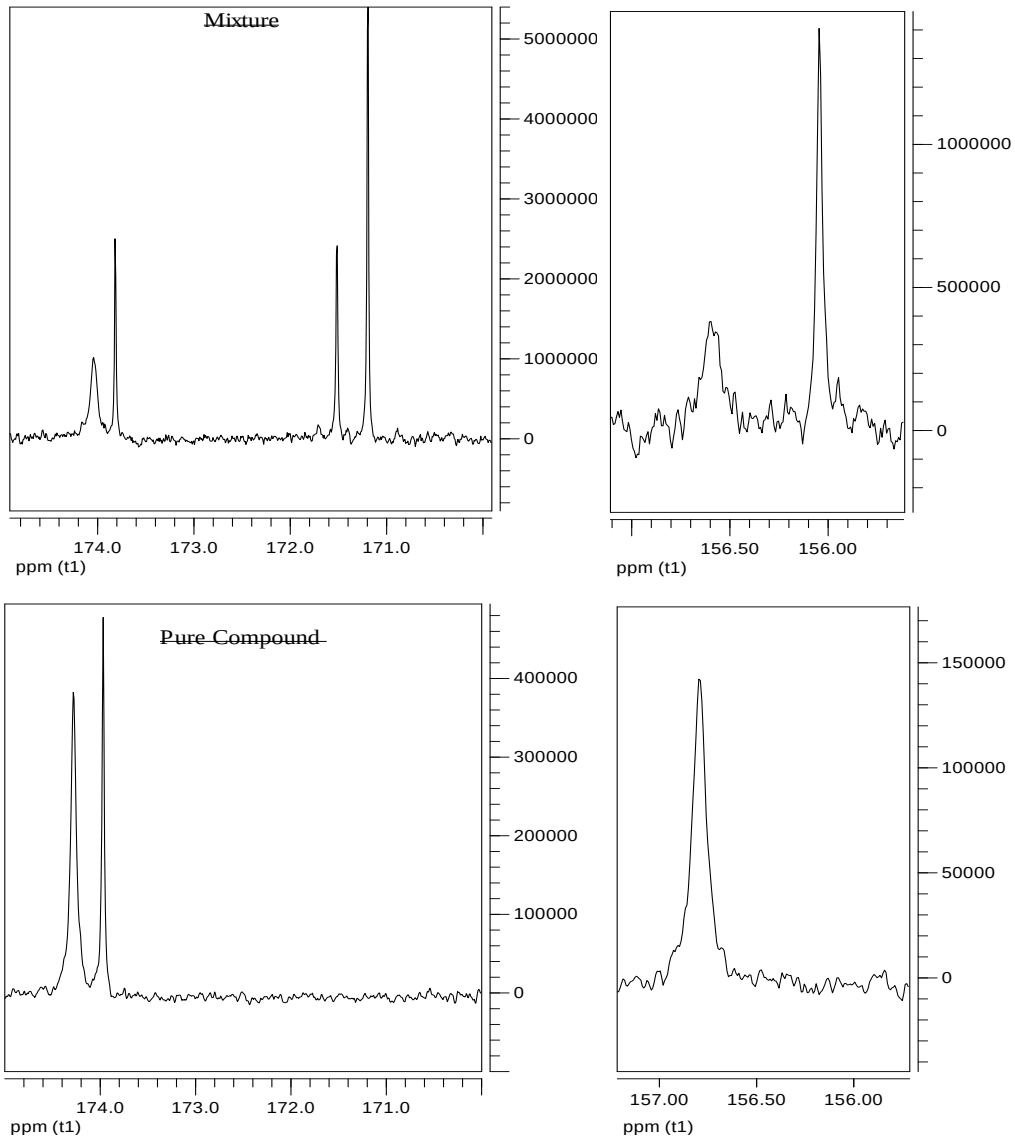
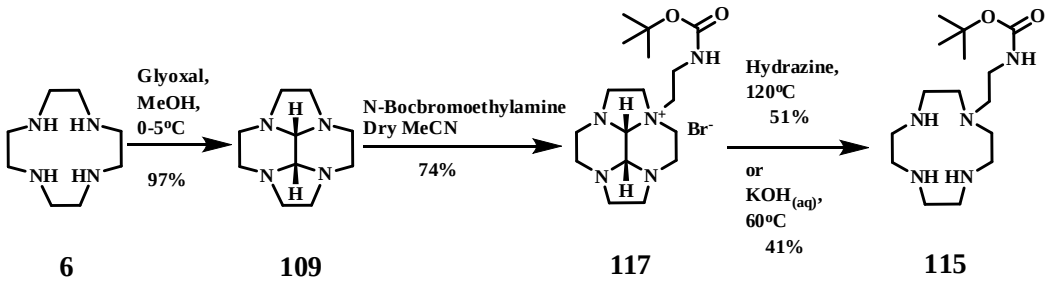


Figure 26: ^{13}C spectra of reaction mixture and pure compound 116

Although the route to compound **116** was successful, it required the use of a large excess of the starting macrocycle cyclen. This is a relatively expensive reagent and although some of the excess was recovered, an alternative route

which gave the product in a similar overall yield without using any excess cyclen was desirable. Hence a synthetic route using the bisaminal methodology described earlier was attempted (Scheme 24).

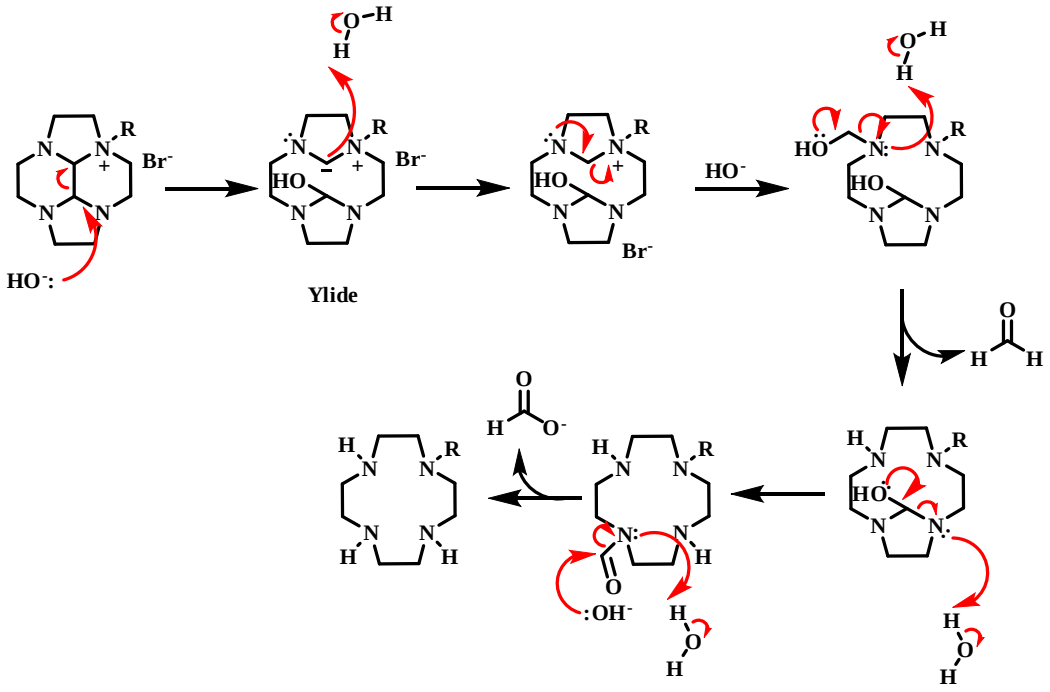


Scheme 24: Synthetic route to compound 115 using bisaminal methodology

Cyclen was rigidified with a two carbon bridge in the *cis* configuration by reaction with glyoxal at 0-5 °C following literature methods.[98] This is an almost quantitative reaction yielding the rigidified macrocycle **109** with only two of the amine groups remaining nucleophilic as discussed previously in this report. Compound **117** was formed by treatment of **109** with one equivalent of compound **119** in dry acetonitrile at room temperature.[103] The alkylation of such macrocycles must be carried out at room temperature since they are susceptible to degradation when heated. The reaction was monitored by TLC and after 14 days athough some starting materials remained, no further reaction appeared to be taking place. The product was easily isolated in 74% yield by successive washing and trituration due to its vastly differing solubility in diethyl ether when compared to both starting materials. Since the symmetry of the macrocycle has now been broken and compound **117** also exists as a pair of optical isomers both the ^{1}H and ^{13}C spectra are very complex. However, the spectrum could be partially assigned by combining using 2D NMR experiments alongside literature interpretation[103] of analogous compounds. Mass spectrometry was used to confirm the identity of the molecule.

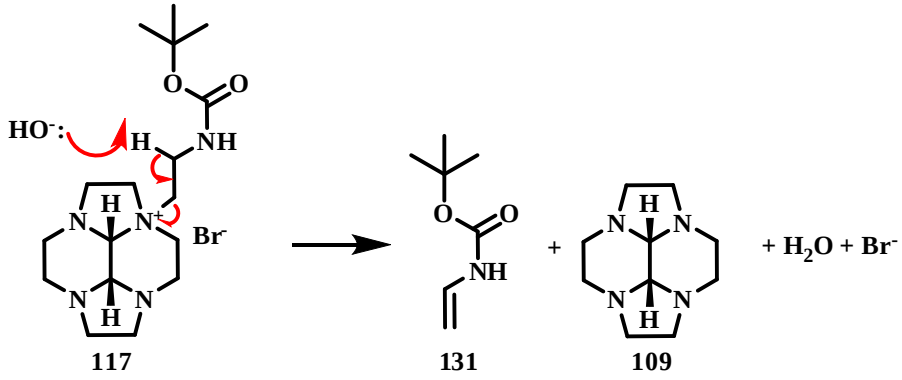
Compound **117** then required deprotection to form the free alkylated macrocycle. This was initially attempted using aqueous KOH (20% w/v) with heating to 60 °C under a nitrogen atmosphere, using the method described by Petillo *et al.*[103]

This conversion proceeded smoothly and after workup the product **115** was isolated in 41% yield by column chromatography on neutral alumina. The NMR analysis could be compared directly with that of compound **115** synthesised by the previous route. The mechanism for this reaction has not yet been elucidated but Petillo *et al.* have proposed the mechanism shown in Scheme 25.



Scheme 25: Proposed mechanism of bisaminal deprotection[103]

They reported high yields when the R group was a simple alkyl chain, but in a reaction where the side arm had acidic protons they reported no ring expansion, but an elimination product and the glyoxal bridged macrocycle were recovered. They conclude that the formation of the ylide is competing with any possible elimination pathways. This may help to explain the relatively low yield in the reaction as although the only product isolated was the desired compound **115**, the elimination pathway shown in Scheme 26 is consistent with the findings of their report.[103]



Scheme 26: Proposed competing reaction in the synthesis of compound 115 via bisaminal protection

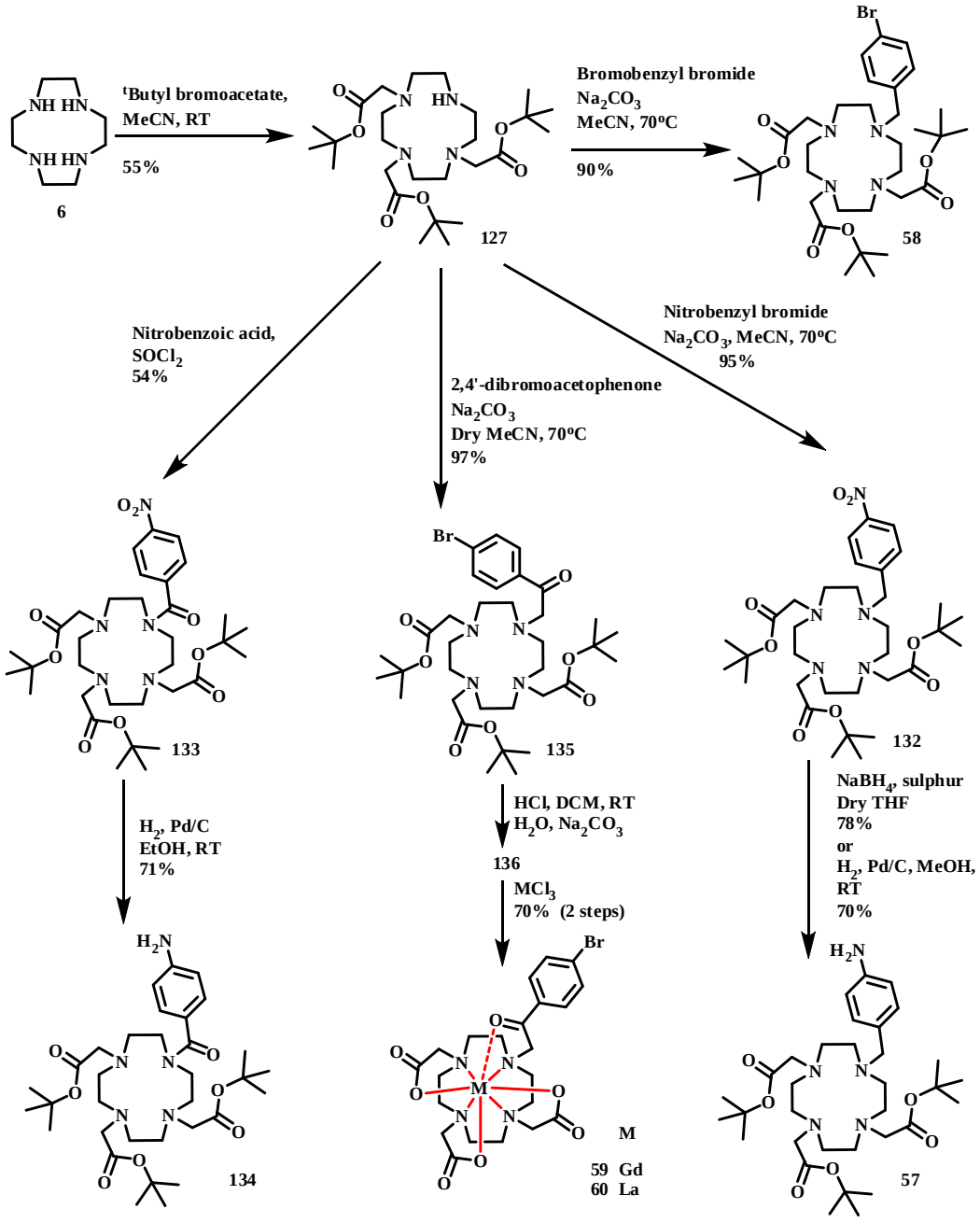
As an alternative to the ring expansion of compound **117** using aqueous KOH, the macrocyclic salt was dissolved in hydrazine monohydrate and heated to 120 °C. This yielded the desired product **115** in a higher yield (51%) but this time without any need for chromatographic separation. The overall yield from free base cyclen to compound **115** via this protection-deprotection strategy was 37%. Thus it gave a lower yield than the direct monoalkylation route (49%) but it was much less wasteful of the expensive starting material cyclen.

Comopund **116** was then treated with trifluoroacetic acid in dichloromethane in order to remove the N-Boc group and restore the free primary amine on the pendant arm. This procedure was fairly unremarkable and deprotected the amine in 95% yield without interfering with the methyl ester groups also present in the molecule.

2.1.5 Synthesis of aryl bromide and aryl amine bearing macrocycles, 57, 58, 59 and 60

2.1.5.1 Synthetic strategy

The synthetic routes used to obtain compound **57, 58**, **59 and 60** are shown in Scheme 27 below.



Scheme 27: Synthetic routes used to prepare substituted cyclam macrocycles

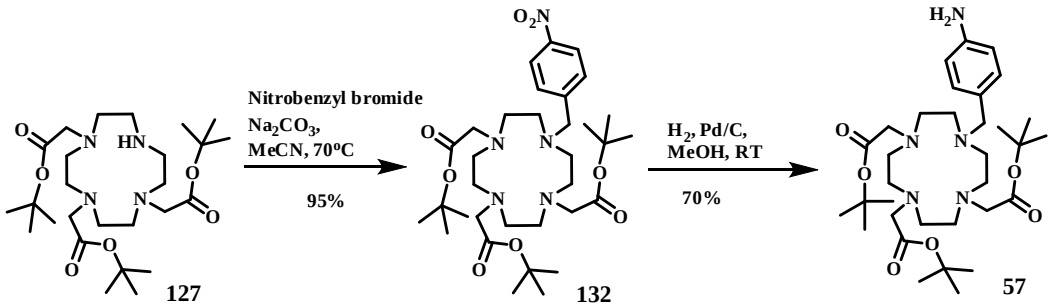
The three target compounds were once again asymmetrically substituted cyclen macrocycles, with one arm containing a reactive functional group to be used to attach a porphyrin moiety. The aim was to obtain one compound with an aromatic amine (compound **57**) and one with an aromatic bromide (compound **58**), both in the free base form macrocycle, and as the metal ion complexes (compounds **59** and **60**).
The key intermediate in their syntheses was the known compound **127**[104], which was then alkylated with the relevant electrophile successfully at the remaining reactive ring amine to give compounds **58**, **132** and **135**.

Compound **57** was already reported in the literature and the aromatic amine was furnished by substituting compound **127** with nitrobenzyl bromide followed by reduction of the aromatic nitro group.[105] However, following problems with the published method, an alternative reducing reagent ($NaBH_2S_3$) was found and ultimately used for this transformation. The problems with this route lead to the synthesis of compound **134**, which although not an original target molecule, is a close analogue of compound **57,** just differing by an amide bond on the aromatic amine pendant arm.

Compound **58** was obtained in a straightforward manner from compound **127** by alkylation with p-bromobenzylbromide, as was compound **135** from alkylation of **127** with 2,4-dibromoacetophenone. The metallation of compound **135** was done by standard procedures used in our group to give the gadolinium(III) and lanthanum(III) complexes.

2.1.5.2 Results and discussion

Compound **127** was synthesised from free base cyclen by a known literature method.[104] Slow addition of a threefold excess of the electrophile to a solution of cyclen at room temperature maximised the yield of the trialkylated product, which was isolated by column chromatography in a yield of 55%. The stock of this compound was synthesised prior to the improved method by Li *et al.*[91] coming to the author's attention. This key intermediate was then used to ultimately prepare compounds **57**, **58**, **59**, **60** and **134**. (Scheme 28).



Scheme 28: Synthetic route to compound 57[105]

The route to the aminobenzyl derivatised cyclen **57** (Scheme 28) was already known in the literature, and indeed the published procedure[105] was used to obtain compound **132** in almost quantitative yield (95%). The subsequent reduction of the aromatic nitro group in compound **132** to the free amine was much more problematic however. Initial attempts were made using a 5% loaded Pd/C catalyst, methanol solvent and hydrogen delivered via a balloon as described in the published report[105] but this method appeared to have no effect on the starting material when monitored by TLC (the product was expected to be significantly more polar than the starting material). A change to ethanol as solvent gave two products by TLC, one was non-polar with respect to the starting material and still UV active, the other was more polar but not UV active. This appeared to show that N-debenzylation was occurring, instead of / as well as the reduction of the aromatic nitro group. This is consistent with the literature since amines are often protected using benzyl groups and one of the commonly used deprotection procedures is catalytic hydrogenation.[106] However, as shown by the successful

literature report[105] it should be feasible to hydrogenate the aromatic nitro group without removing the benzyl arm. Further attempts were made using combinations of 5-10% loaded Pd/C catalysts and methanol/ethanol as solvents but none were successful. The working hypothesis at this time was that the catalyst was perhaps too active. A search of the literature found a report involving the synthesis of a Pd/C catalyst poisoned with ethylenediamine reported by Hirota *et al.*[107] Their work showed that this catalyst could be used to chemoselectively hydrogenate nitro groups (among many others) in the presence of O-benzyl and N-carboxybenzyloxy functionalities. Although N-benzyl groups were not mentioned it their report, it seemed feasible that this catalyst could provide the chemoselectivity required. The catalyst was duly prepared and used but appeared to produce several different products when analysed by TLC and when the reaction mixture was analysed by ^{1}H NMR - the aromatic region showed several sets of doublets. Repeated attempts using differing amounts of this catalyst and changing solvent systems did not give the required transformation either. So, the reaction was then repeated using 10% Pd/C and ethanol as solvent and this time analysed at regular intervals by ^{1}H NMR. Figure 27 shows the aromatic region of the reaction mixture over a period of 30 hours.

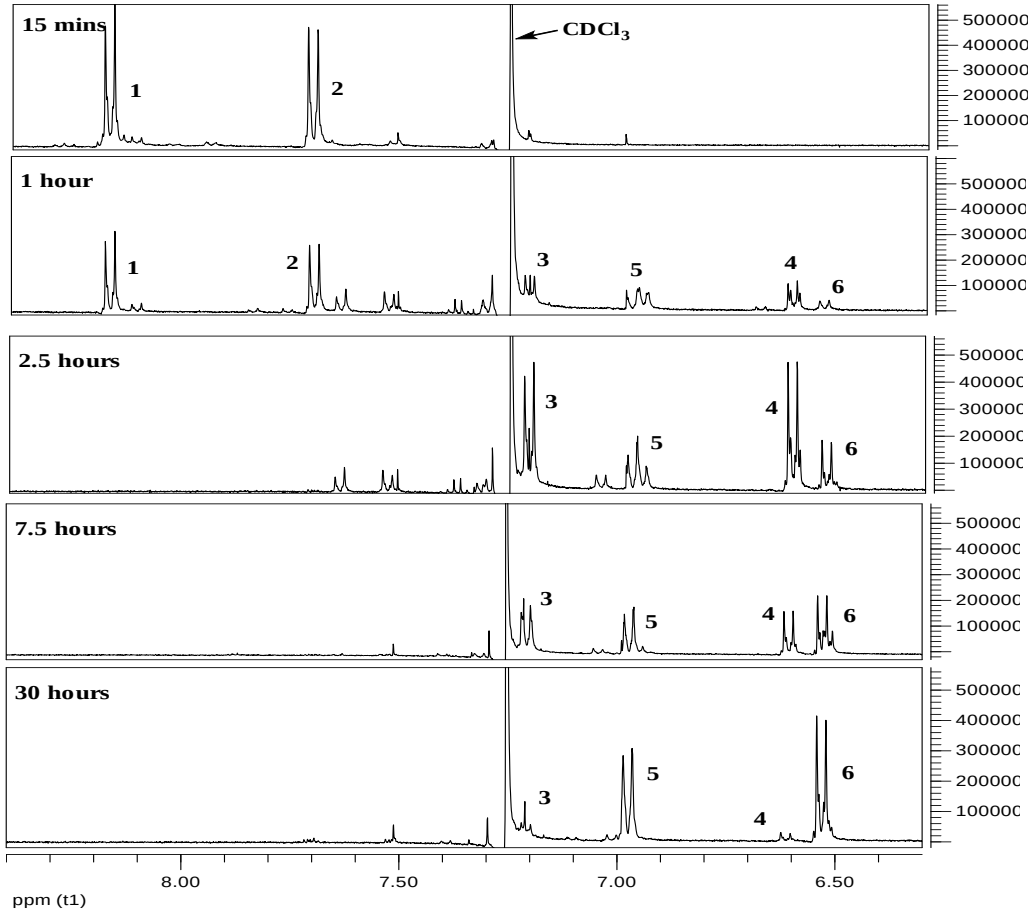


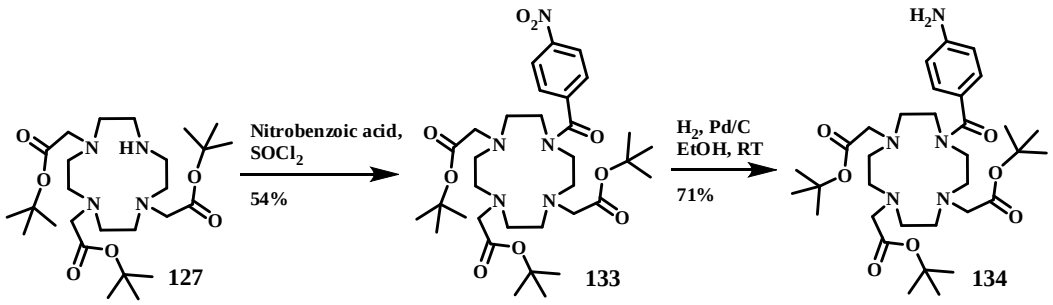
Figure 27: ^{1}H NMR of reaction mixture showing aromatic nitro group reduction over 30 hours

It can be seen that the doublets 1 and 2 from the starting material are consumed and initially two main product peaks (3 and 4) are produced. These are subsequently consumed and eventually the peaks 5 and 6 are formed. No further change occurred after this time. The interpretation of this was that peaks 3 and 4 were the desired product (the aromatic amine) and that peaks four and five belonged to the aromatic species formed after debenzylation. Careful analysis of the reaction by TLC was consistent with this hypothesis. Since the NMR shows that the desired product and the debenzylation were being formed at similar times, the problem appeared to be stopping the reaction at the optimal time to give the highest yield of the desired product.

The reaction was therefore repeated under the same conditions and stopped after 3 hours when the initial product seemed to be at its maximum yield. After work up and separation by silica gel chromatography the desired product **57** was

finally isolated in 70% yield, its identity being confirmed by ^{1}H and ^{13}C NMR in conjunction with mass spectral analysis.

While this reaction was being investigated and optimised, compound **133** was synthesised to give an analogous compound with an amide linking group that was more robust under the conditions used for catalytic hydrogenation. (Scheme 29)



Scheme 29: Synthetic route to compound 134

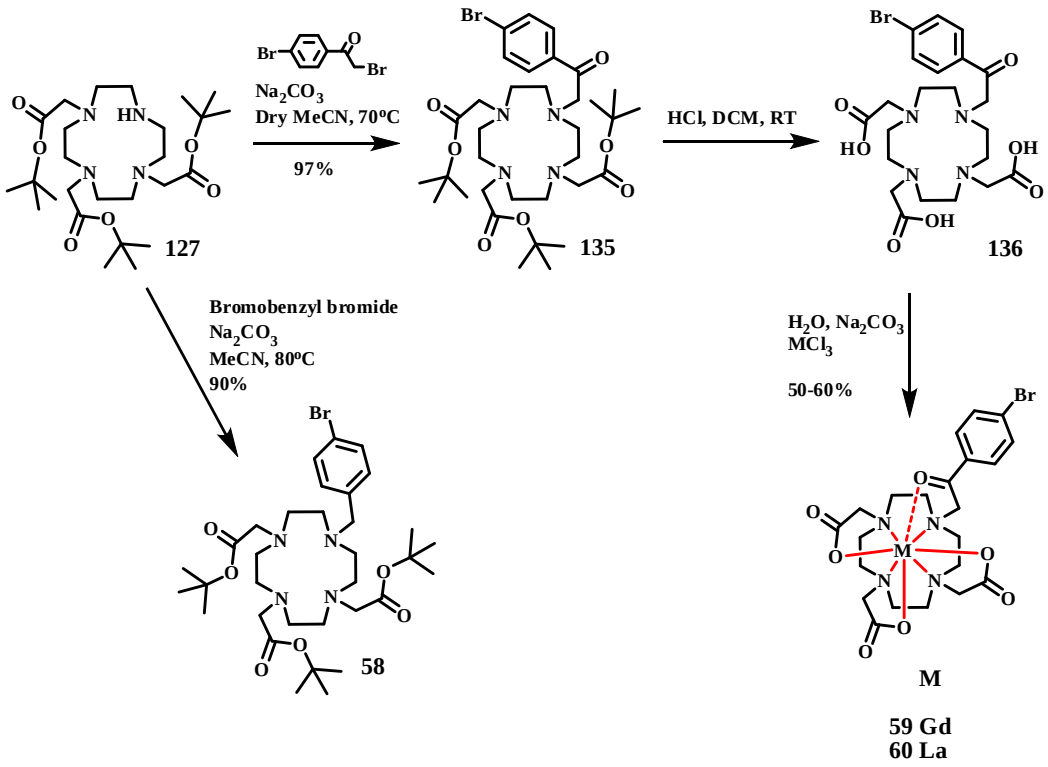
Compound **133** was formed by treatment of *p*-nitrobenzoyl chloride (made *in situ*) with compound **127** and triethylamine in dry dichloromethane. After work up, and column chromatography, the product was isolated in 54% yield as a yellow solid.

When this compound was hydrogenated under the same conditions used for compound **132** (5% Pd/C, EtOH solvent), the reaction produced one main compound after only two hours. Allowing the reaction proceed for longer had no further effect. The isolated product, obtained in 71% yield, was identified as compound **134** by ^{1}H and ^{13}C NMR and confirmed by mass spectometry, which reinforces the likelihood that compound **132** was cleaving at the benzylic position when hydrogenated.

Although compound **57** had been successfully synthesised, another method for the desired transformation was investigated from a report by Laliberté *et al.*[108] They described a method for preparing the reducing agent $NaBH_2S_3$ (sodium trithioborate) and investigated its chemoselectivity. This reagent was reported to reduce aromatic nitro groups while leaving other groups untouched except for

aldehydes, ketones, lactones and epoxides – none of which were present in this reaction scheme. Initially $NaBH_2S_3$ was prepared *in situ* and treated with compound **132** in a 1:1 ratio as described in the report.[108] TLC analysis appeared promising but showed an incomplete reaction, even after 24 hours. So the reaction conditions were changed and it was found that a double excess of the reducing agent gave the highest yield of the desired compound. Macrocycle **57** was isolated after basic work up and chromatography in 78% yield, without the problems inherent in the catalytic hydrogenation route.

A small modification to the procedure used to form macrocycle **132** gave the previously unreported compound **58** in 90% yield after chromatographic separation. (Scheme 30). The maximum yield of **58** was obtained when a slight excess of the bromobenzyl bromide (1.1x) was used instead of a stoichiometric amount as used in the synthesis of **132**. This same adapted procedure was used to give access to the previously unreported bromo-acetophenone derivatised cyclen **135**, in a yield of 97%. The ester groups in compound **135** were hydrolysed by treatment of a dichloromethane solution of **135** with concentrated HCl, followed by neutralisation with base. The product was subsequently extracted into water and heated with an excess of the lanthanide trichloride salt to give the gadolinium complex **59** in a yield of 60% and the lanthanum complex **60** in 52% yield. Mass spectral analysis of these complexes showed characteristic isotopic patterns for the lanthanoid ions in the molecular envelope.



Scheme 30: Synthetic routes to compounds 58, 59 and 60

2.2 Synthesis of a rigidified cyclam macrocyclic chelator

2.2.1 Aims

The aim of this synthetic scheme was to obtain the rigidified macrocycles 1,5,8,12-tetraaza-bicyclo[10.2.2]hexadec-5-yl)-acetic acid *tert*-butyl ester **137** and 4-carbo-*tert*-butoxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane **138** as shown in Figure 28.

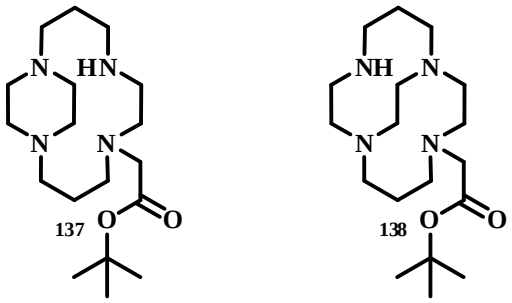


Figure 28 : Target compounds 137 and 138

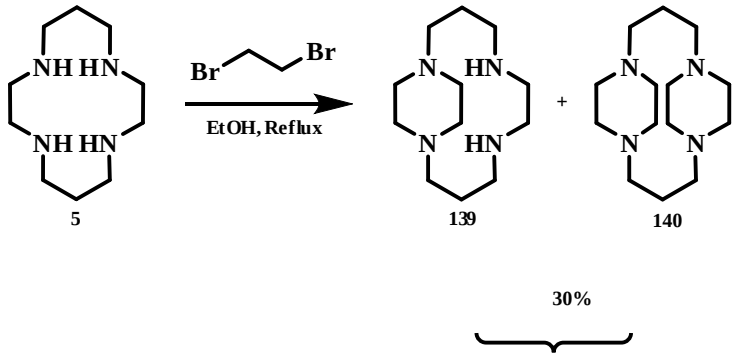
Compound **137** contains a two carbon bridge between two adjacent nitrogen atoms which is commonly referred to as a 'side bridge' and compound **138** has the same ethane bridge but this time between two non-adjacent nitrogen atoms – this is commonly referred to as a 'cross bridge'. As discussed in the introduction of this report these bridges confer upon them less conformational flexibility than the parent macrocycle and hence improves the kinetic and thermodynamic stability of their metal complexes towards metal ions.[109, 110] Both compounds and some of their variants are known as good chelators for copper (II) ions.[109-112]

Compounds **137** and **138** each contain a free secondary amine functionality that can be exploited for further derivatisation, specifically in this case for attachment to a porphyrin moiety.

Both target molecules contain a *tert*-butyl containing pendant arm which, once hydrolysed to the parent carboxylic acid will form a point of ligation for a metal centre.

2.2.2 Side Bridged Macrocycles

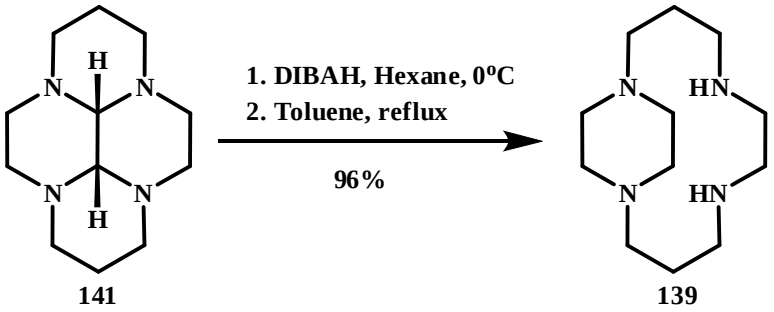
The synthesis of the side bridged cyclam **139** was first reported in 1980 by Wainwright (Scheme 31).[113] Treatment of free base cyclam with a double excess of 1,2 dibromopropane resulted in the isolation of compounds **139** and **140** in 30% yield (Scheme 31). They report that the mono bridged compound **139** was about 90% abundant in this mixture.



Scheme 31: The first reported synthesis of 'side bridged cyclam'[113]

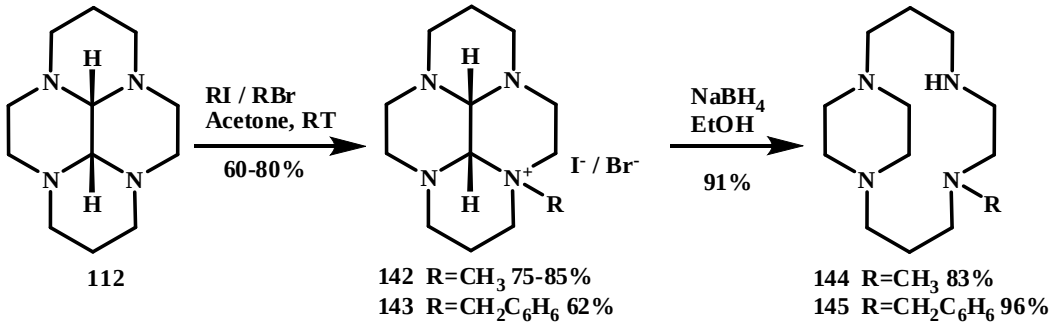
The low yield was attributed to competing reactions, such as elimination and also the fact that both substitution and elimination reactions create acid as a side product which immediately quaternises the remaining amines, rendering them unreactive. The attempts to solve this by use of 'acid scavengers' were reported to be unsuccessful.[113]

One year later, the group of Yamamoto[114] improved the synthesis of compound **139** by reductive ring expansion of the tetracyclic amine **141** (Scheme 32)Scheme 32: Reduction of glyoxal bridged cyclam to give the side bridged derivative**[114]** By treatment of **141** with diisobutylaluminium hydride they obtained compound **139** in almost quantitative yield (96%).



Scheme 32: Reduction of glyoxal bridged cyclam to give the side bridged derivative[114]

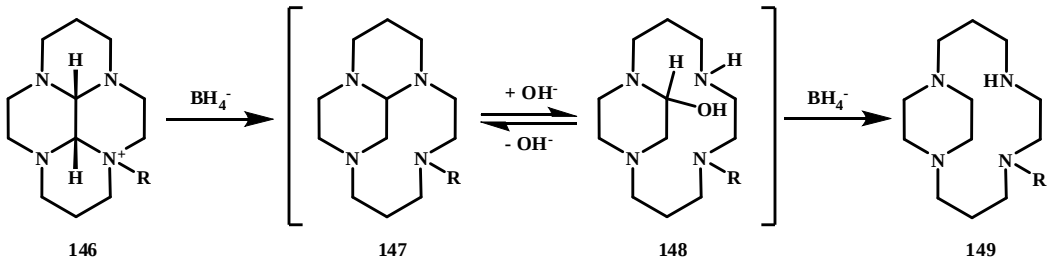
However, compound **139** still has two secondary amine groups which are equally reactive, so the problem of chemoselective alkylation of one of these remained until Kolinski[115] published such a route in 1995 (Scheme 33).



Scheme 33: Chemoselective alkylation of glyoxal bridged cyclam[115]

Their synthetic route selectively mono-alkylated the tetracyclic bisaminal **112** in good yield with either methyl iodide or benzyl bromide to give compounds **142** and **143**. With this mono-alkylation successfully achieved, reductive ring expansion of these compounds with $NaBH_4$ in ethanol furnished the monoalkylated bridged cyclam species **144** and **145** in yields of 83 and 96%.

The reaction pathway proposed for this reduction is shown in Scheme 34.[115] The initial site of reduction is the bridge carbon bonded to the quaternary amine. The tricyclic tetraamine **147** formed is unstable with respect to hydrolysis and this creates an equilibrium with the corresponding carbinolamine **148**. Subsequent further reduction then generates the side bridged macrocycle product **149**.



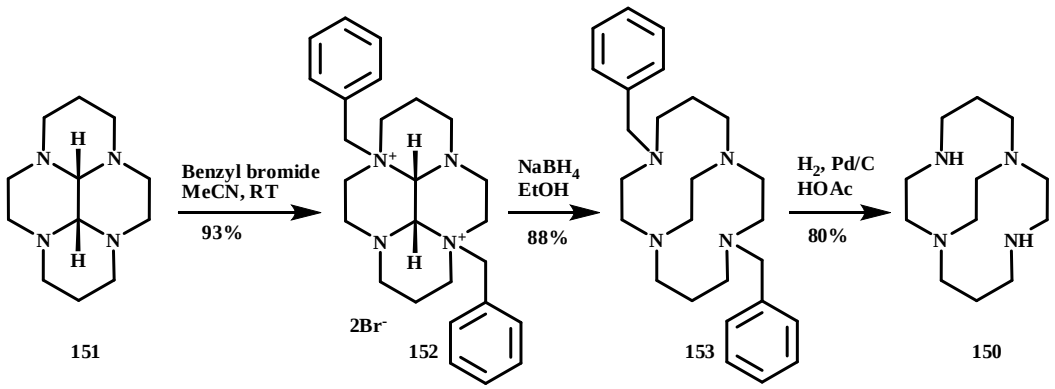
Scheme 34: Proposed mechanism of glyoxal bridge reduction in the cyclam macrocycle[115]

This method has since been used in our group to produce a variety of this type of macrocycle[110, 116] and the remaining free secondary amine subsequently used to successfully functionalise the compounds further.

2.2.3 Cross Bridged Macrocycles

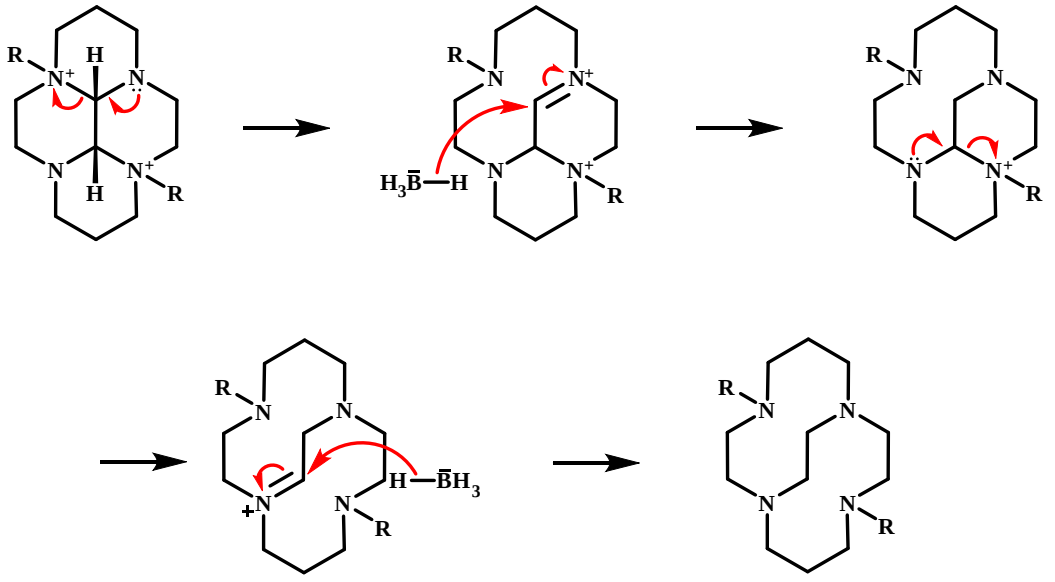
Incorporation of a two carbon bridge between non-adjacent nitrogen atoms in the cyclam ring to form the cross bridge species **150** cannot be achieved by nucleophilic substitution. As shown by Wainwright[113], this route results in the bridge being inserted between two adjacent nitrogen atoms – forming the side bridged cyclam macrocycle. Similarly, reductive ring expansion of the monoalkylated bisaminal **146** also leads to the side bridged product **149** as shown by the Kolinski group.[115]

However, the group of Weisman[117, 118] discovered that by dialkylating the bisaminal **151**, the subsequent reduction of the carbon bridge occurred at both quaternary amines thus facilitating the production of cross bridged species such as compound **153** (Scheme 35).



Scheme 35: Synthesis of cross bridged cyclam.[117, 118]

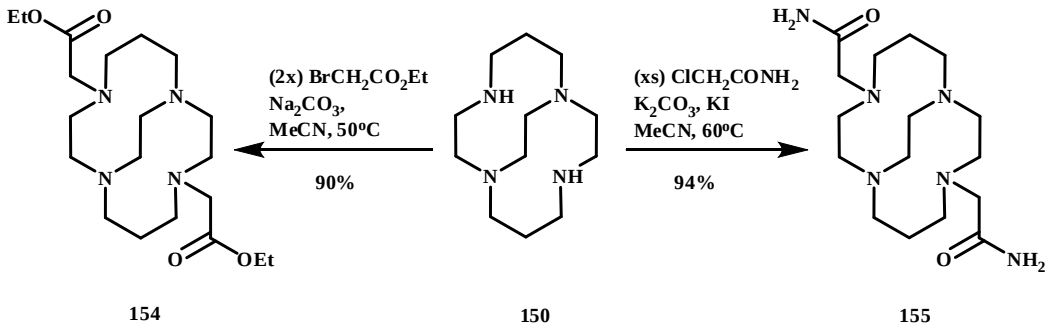
The mechanism they proposed for the reductive ring expansion of the dialkylated bisaminal **152** is shown in Scheme 36. It depicts initial iminium ion formation leading to N-C bond cleavage followed by reduction of the ion. This is then repeated on the other end of the two carbon bridge, ultimately forming the cross bridged product.



Scheme 36: Proposed mechanism of reductive cleavage of the glyoxal bridge to give cross bridge cyclam[118]

Compound **153** was then debenzylated to form the free base macrocycle **150**, which was subsequently dialkylated to give the two species **154** and **155** shown in Scheme 37. Thus, the benzyl arms were used to protect the non-adjacent

amines and therefore direct the reductive ring expansion so that it formed the cross bridge product.



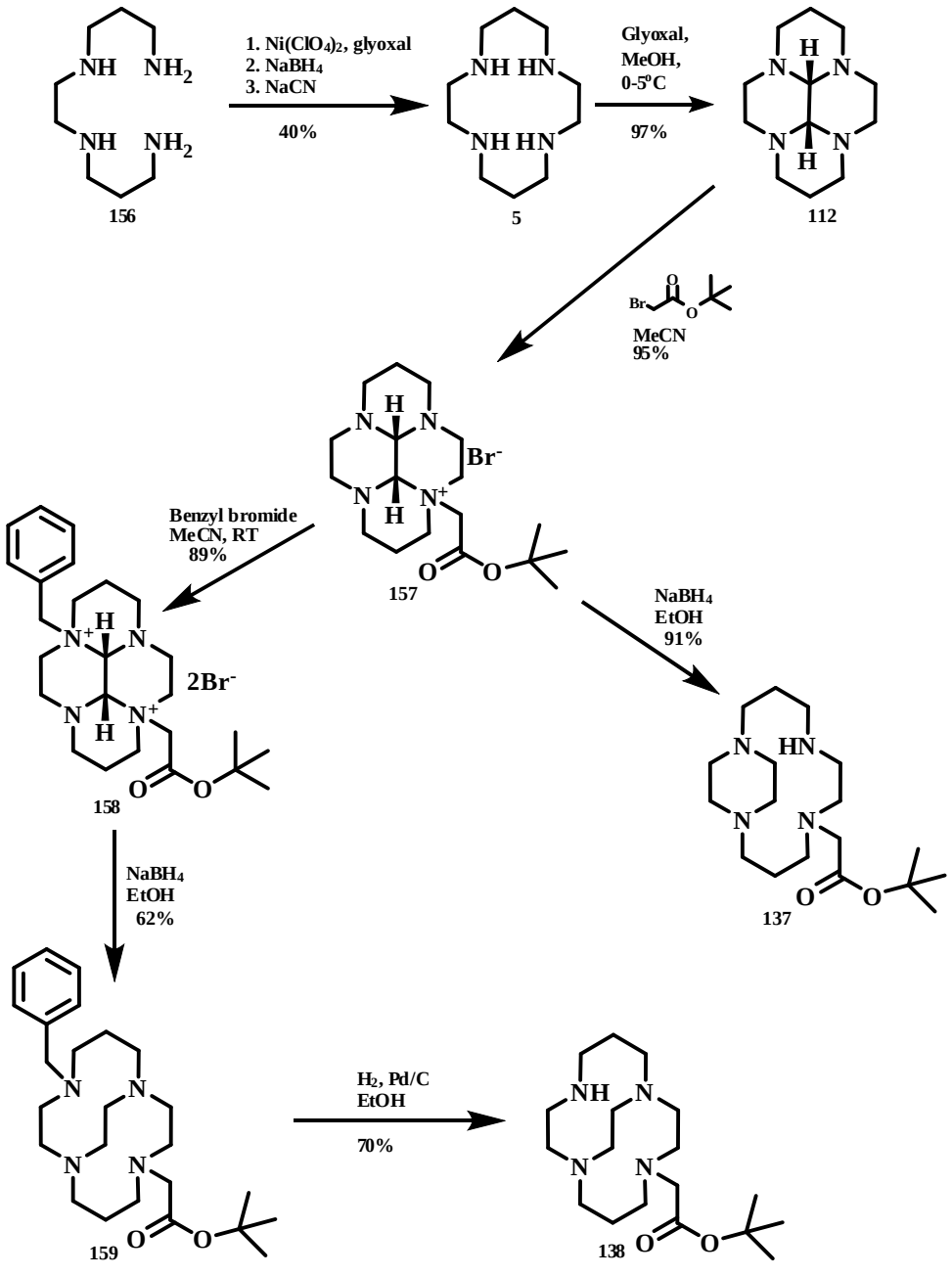
154 **150** **155**

Scheme 37: Examples of dialkylation of cross bridge cyclam.[118]

Subsequent work in our group has adapted this method to funtionalise the bisaminal **112** with two different protecting groups that can both survive the conditions for ring expansion. Once the cross bridged species has been formed, one of these groups can be selectively removed and the uncovered amine funtionalised. This can then be repeated for the other protected amine, thus giving access to a cross bridged species with two different pendant arms.[119]

2.2.3.1 Synthetic strategy

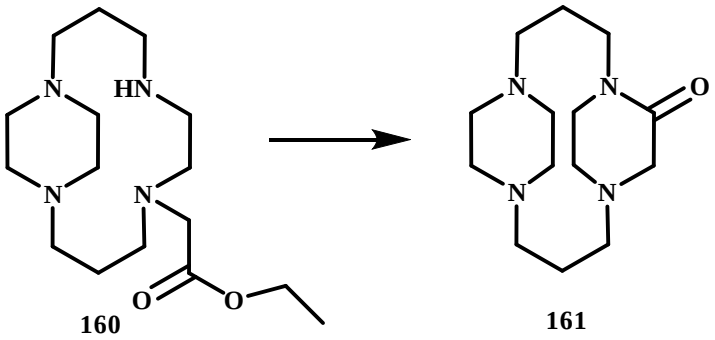
The synthetic routes to compounds **137** and **138** are shown in Scheme 38 below.



Scheme 38: Synthetic routes to side bridged and cross bridged cyclam derivatives 137 and 138

Compounds **137** and **138** both contain a *tert*-butyl based pendant arm. This is a

protected carboxylic acid, which will be unmasked and used as a point of coordination for a metal ion. The acid was protected throughout the synthesis to improve solubility in organic solvents and hence make manipulation and isolation of these compounds easier. The *tert*-butyl ester was chosen because it has proven to be stable to $NaBH_4$[110] which is required for reductive ring expansion to obtain **137** and **159**. These esters are also resistant to attack by bases and nucleophiles which was also a consideration as intramolecular lactamisation has been reported in side bridged cyclam macrocycles containing an ethyl ester pendant arm (Scheme 39).[82, 120, 121]



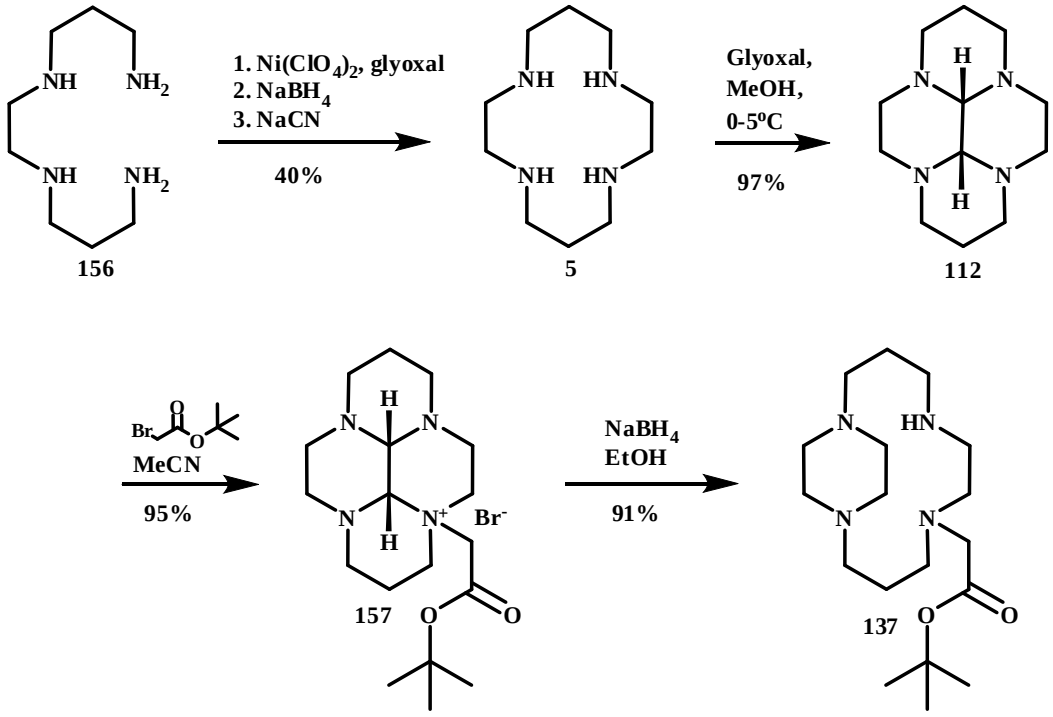
Scheme 39: Schematic showing reported intramolecular lactamisation in a side bridges cyclam derivative[82, 120, 121]

For both target molecules, the key intermediate in their synthesis is the glyoxal bridged cyclam **112**. The restricted flexibility and the folded nature of this compound provides the chemoselectivity required to alkylate only one of the amine groups with the *tert*-butyl ester containing arm, as discussed previously in this report. This mono-substitution creates the tetracyclic salt **157** which can then undergo reductive ring expansion as described by Kolinski[115] to give the side bridged target, compound **137**.

A modification of the procedure of Weisman *et al.*[117] was used to obtain the cross bridged target molecule **138**. Further alkylation of compound **157** can also be achieved with the reaction occurring on the nitrogen non-adjacent to the one previously substituted. The creation of a second quaternary amine causes the subsequent reductive ring expansion to generate the cross bridge instead of the

side bridge. Hence, the second pendant arm is 'directing' the placement of the required bridge and in this case needs to be easily removed after the cross bridge has been formed. Hence, a benzyl arm was used as a protecting group for this amine and was chosen because it can be removed in good yield by reagents and conditions under which the *tert*-butyl ester arm would remain intact. This was the route chosen to obtain compound **138**.

2.2.3.2 Synthesis of side bridge macrocycle 137

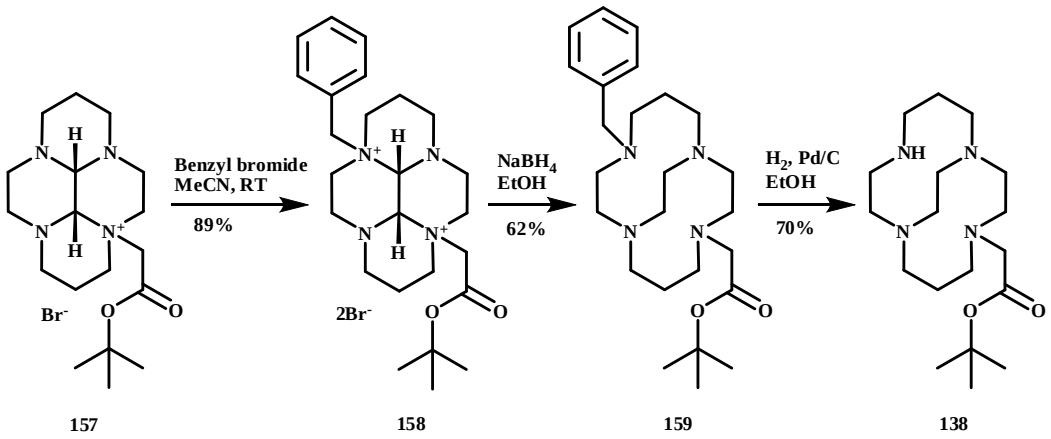


Scheme 40: Synthetic route to side bridged cyclam derivative 137

The macrocycle **5** (cyclam) was synthesised by condensing the nickel(II) complex of 1,5,8,12-tetraazadodecane with glyoxal.[122] (Scheme 40). The α-diimine product of this reaction was then reduced ($NaBH_4$) and treated with cyanide to remove the metal and furnish the free base cyclam **5** in a yield of 40%. In a route analogous to the synthesis of compound **117**, the macrocycle was rigidified with a two carbon bridge fixed in the *cis* configuration in order to effectively protect three of the amine groups.[98] As described earlier in this report, the folded geometry allows differentiation of the amines groups in the macrocycle. Two non-adjacent amines are rendered effectively unreactive due to

steric hindrance by their fixed orientation. The mono-alkylated compound **157** was obtained by treating **112** with only one equivalent of the electrophile using the same procedure as for compound **117**.[103] The dramatic change in solubility from reactant to product caused precipitation and hence facilitated easy isolation as well as preventing dialkylation and compound **157** was obtained in high yield (95%).The carbon bridge was then reduced by the method of Kolinski[115] to give the partially rigidified macrocycle **137** in 91% yield.

2.2.3.3 Synthesis of cross bridged compound 138



Scheme 41: Synthetic route to cross bridged cyclam derivative 138

Compound **157** was substituted for a second time by benzylation of the remaining reactive amine (Scheme 41). This reaction was significantly slower than the original alkylation that produced **157** and took 6 days to reach completion with compound **158** being isolated in 89% yield. Previous studies[119] have shown that the dialkylated salt **158** can undergo ring expansion with $NaBH_4$ despite having an ester group that is seemingly also susceptible to reduction. The *tert*-butyl ester group is reduced slowly in comparison with the reduction of the carbon bridge, allowing the reaction to be controlled to give the optimal yield of the desired compound **159**. Thus, the dialkylated salt was treated with $NaBH_4$ in EtOH for 7 days giving the ring expanded cross bridge cyclam species **159** in 62% yield after chromatographic isolation. Finally the N-debenzylation of compound **159** was achieved by catalytic hydrogenation to give the target compound **138** in 70% yield.[118]

2.3 Summary

The cyclen based macrocycles **56**, **57**, **58**, **59**, **60** and **134** and the cyclam based macrocycles **137** and **138** have all been successfully synthesised (Figure 29).

As already described, cyclen macrocycles functionalised with carboxylate containing pendant arms form stable and inert complexes with gadolinium(III) ions, and the rigidified cyclam based chelators form stable and inert complexes with copper(II). Hence, these macrocycles have potential uses in biomedical imaging (MRI and PET).

The compounds also contain a functional handle which can be exploited for diversification of the structure.

The alkylamino bearing compound **56** was already known in the literature but a new route via bisaminal methodology was designed and demonstrated for the synthesis of this compound.

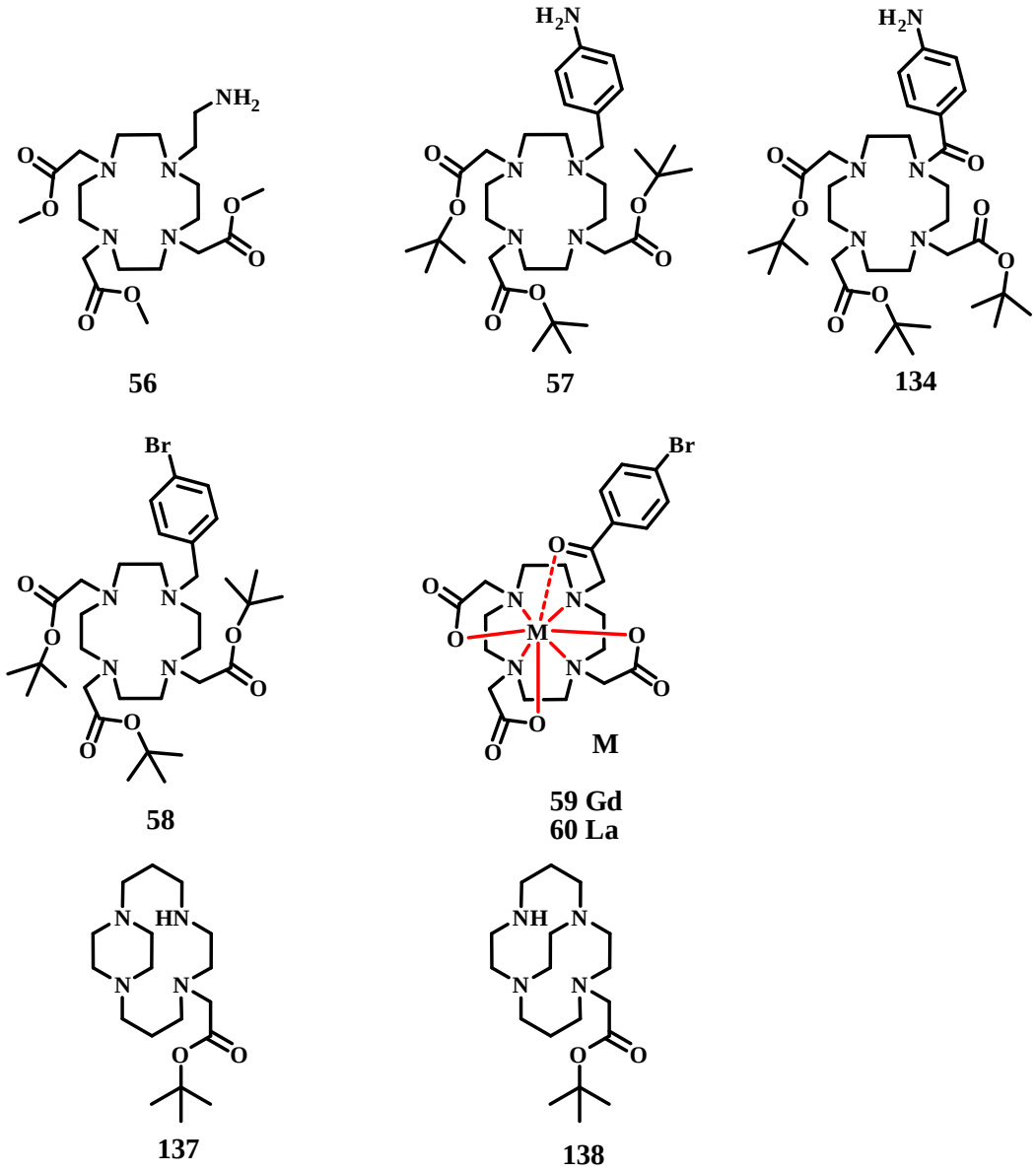
Although the yield was lower than the literature procedure (37% compared to 49%), the route provides more control over the monoalkylation and therefore offers an improved scope for such reactions involving the cyclen macrocycle.

Compound **57** had also been reported previously. However, difficulties were encountered in the synthesis of this compound, and a new method for the last transformation provided a cleaner reaction and an improved yield (78% compared to 70%). This method also led to the synthesis of compound **134**, a previously unreported compound.

Along with compounds **137** and **138**, these macrocycles contain amino groups which can be utilised for further diversification of their structure.

The aryl bromide bearing compounds **58**, **59** and **60** were all previously unreported, and have been synthesised in good yields. This functional handle opens the possibility of using transition metal catalysed coupling reactions for further elaboration.

The metal ion complexes **59** and **60** have been synthesised to allow an alternative approach to the desired bismacrocyclic compounds. These will allow the coupling reaction to be the last stage in the synthesis, removing the need for the harsh conditions required in the ester deprotection step.



56 **57** **134**

58 **59 Gd** **60 La**

137 **138**

Figure 29: List of compounds successfully synthesised

Chapter 3: Coupling the cyclam/cyclen moieties to a porphyrin via the β-position on the porphyrin ring

3.1 Introduction

With the cyclen and cyclam based compounds **56**, **57**, **58**, **59**, **60**, **134**, **137**, and **138** already synthesised, the next requirement was to develop a route by which these compounds could be attached to a porphyrin macrocycle.

This clearly required the synthesis of a porphyrin containing one functional group complementary to the reactive groups on the cyclen/cyclam compounds.

Synthesis of an asymmetric porphyrin can be achieved using two main strategies:

1. Synthesis of a symmetrically substituted porphyrin followed by subsequent mono-functionalisation
2. Incorporation of an asymmetric substitution pattern during the initial porphyrin formation reaction

Synthesis of symmetric porphyrins has the advantages of higher yielding reactions and less tedious and time consuming isolation than the synthesis of asymmetric porphyrins. However, this is counterbalanced by the more limited options for monofunctionalisation of the highly symmetric porphyrin molecule.

This chapter covers the routes established via mono-functionalisation of a symmetric porphyrin.

3.2 Porphyrins

3.2.1 Physicochemical properties

Porphyrins are aromatic macrocycles containing 22 π electrons, 18 of which are involved in the delocalisation pathway as shown in Figure 30.

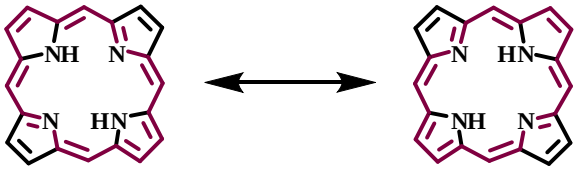
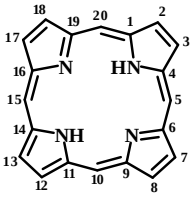


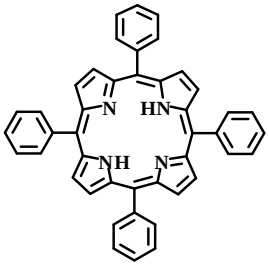
Figure 30: Delocalisation pathways in the porphyrin macrocycle

The aromaticity of the porphyrin molecule defines the majority of its organic chemistry, with electrophilic substitution being one of the most common reactions this type of molecule undergoes. The porphyrin core is numbered as shown in Figure 31. Generally the most electron rich, and therefore reactive, sites are the 5, 10, 15 and 20 positions (meso positions) but the 2, 3, 7, 8, 12, 13, 17 and 18 positions (β positions) are favoured when the meso positions are substituted. When the meso positions are substituted by phenyl based groups the greater reactivity of the porphyrin ensures that electrophilic substitution occurs predominantly on the periphery of the macrocycle as opposed to the substituent rings.



2, 3, 7, 8, 12,13,17,18 = β positions

5,10,15,20 = meso positions



5,10,15,20-Tetraphenylporphyrin (TPP) 162

Figure 31: Numbering of the porphyin core

Free base porphyrins have characteristic absorption spectra consisting of one very intense band at ~420nm (Soret band) and four less intense bands between 500 and 700nm (Q bands).

3.2.3 Transition metal complexes of porphyrin ligands

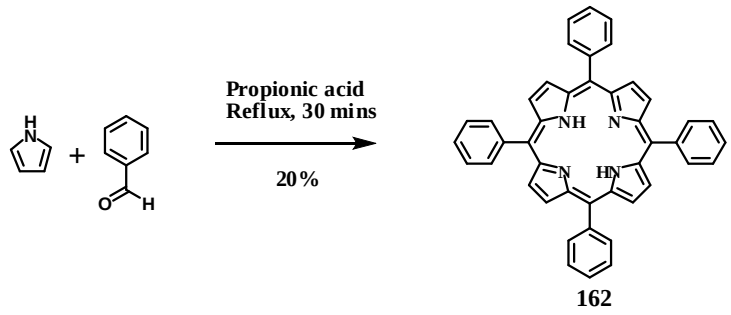
Porphyrins readily form metal complexes with a range of metal cations including divalent magnesium(II) and calcium(II) as well as many transition metal ions.[123] The metal ions increase electron density on the porphyrin macrocycle by an inductive effect, activating the porphyrin periphery towards electrophilic attack. However, the porphyrin ring is a π donor ligand and can donate electron density into any empty *d* orbitals present on the metal centre. Thus, the balance between these two effects determines the amount of electron density on the macrocycle periphery and its ultimate reactivity. Cations such as Zn(II), Cd(II) and Mg(II) have no available *d* orbitals to receive back donation of electron density and therefore activate the porphyrin macrocycle the most. Ions such as Ni(II) and Cu(II) have partially filled *d* orbitals (d^8 and d^9 respectively) and accept backdonation from the ligand, hence the activation of the porphyrin is somewhat reduced. By comparison, transition metal ions with empty *d* orbitals (electronic configuration d^1-d^5) deactivate the ring considerably towards electrophilic attack. Reactions involving functionalisation of the porphyrin ring are often done by using a transition metal ion complex instead of the free base porphyrin, mainly for this very reason but also to protect the inner nitrogen atoms from reaction. Common metals used for this purpose are nickel(II), copper(II) and zinc(II), but zinc(II) is very acid labile so in many cases the other two ions are used instead due to their greater stability under acidic conditions.[123]

The insertion of a metal ion into the porphyrin macrocycle changes their absorption spectra compared to the free base macrocycle. The spectra contain an intense Soret band at ~420nm but they show only two Q bands due to the higher symmetry of the molecule.

3.3 Formation of symmetric meso-tetraarylporphyrins

3.3.1 Alder-Longo method[124]

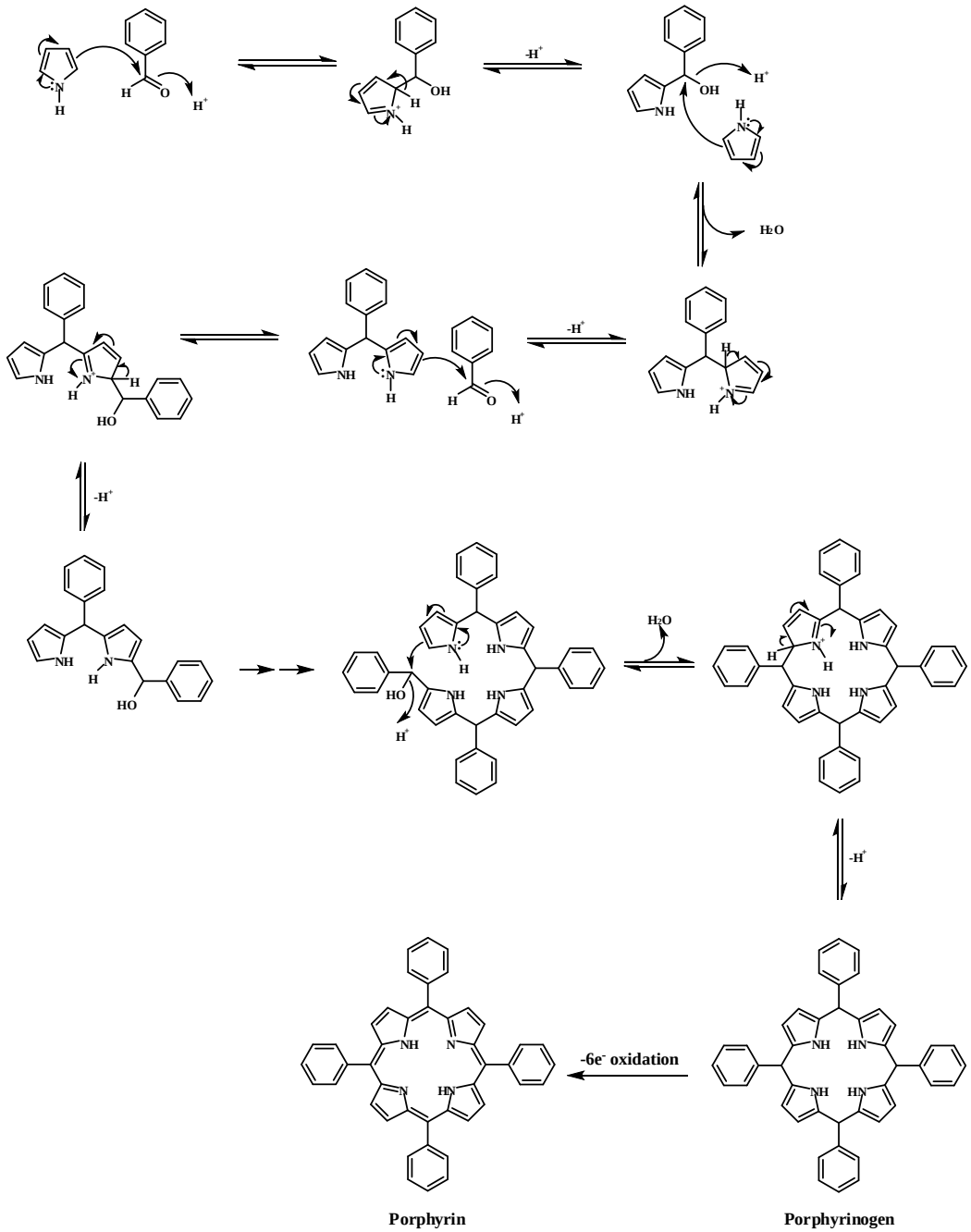
In 1964, the group of Adler studied the formation reaction of tetraaryl porphyrins by condensation of aromatic aldehydes with pyrrole under acidic conditions.[125] Shortly afterwards they published a synthesis of meso-tetraphenyl porphyrin[124] (TPP) **162** which was a significant improvement on the previous synthetic route to this molecule.[126] Now commonly referred to as the Adler or Adler Longo method, an equimolar ratio of pyrrole and benzaldehyde were added to boiling propionic acid and the solution heated under reflux, open to the atmosphere, for 30 minutes. Upon cooling, the porphyrin crystallised from the acidic solution, facilitating easy isolation by a sequence of filtration and washing. The isolated yield of TPP was reported as 20% as shown in Scheme 42.



Scheme 42: Adler-Longo synthesis of meso-tetraphenylporphyrin[124]

Their studies showed that no reaction occurred without acid and that the yield was significantly reduced to around 5% when a nitrogen atmosphere was used.[127] The reaction is known to proceed through a porphyrinogen intermediate which is then oxidised by oxygen in the air, forming the porphyrin.[128] The proposed mechanism for this process is shown in Scheme 43. They showed that changing the solvent to acetic acid gave a higher yield (40-45%) over a longer reaction time but the porphyrin was more soluble in this solvent and hence, more difficult to purify, whereas butyric acid gave a much lower yield. It was also found that the porphyrin product contained around 2-10% of chlorin contaminant, which can be removed by oxidation[129] or chromatography. The low yield is caused by

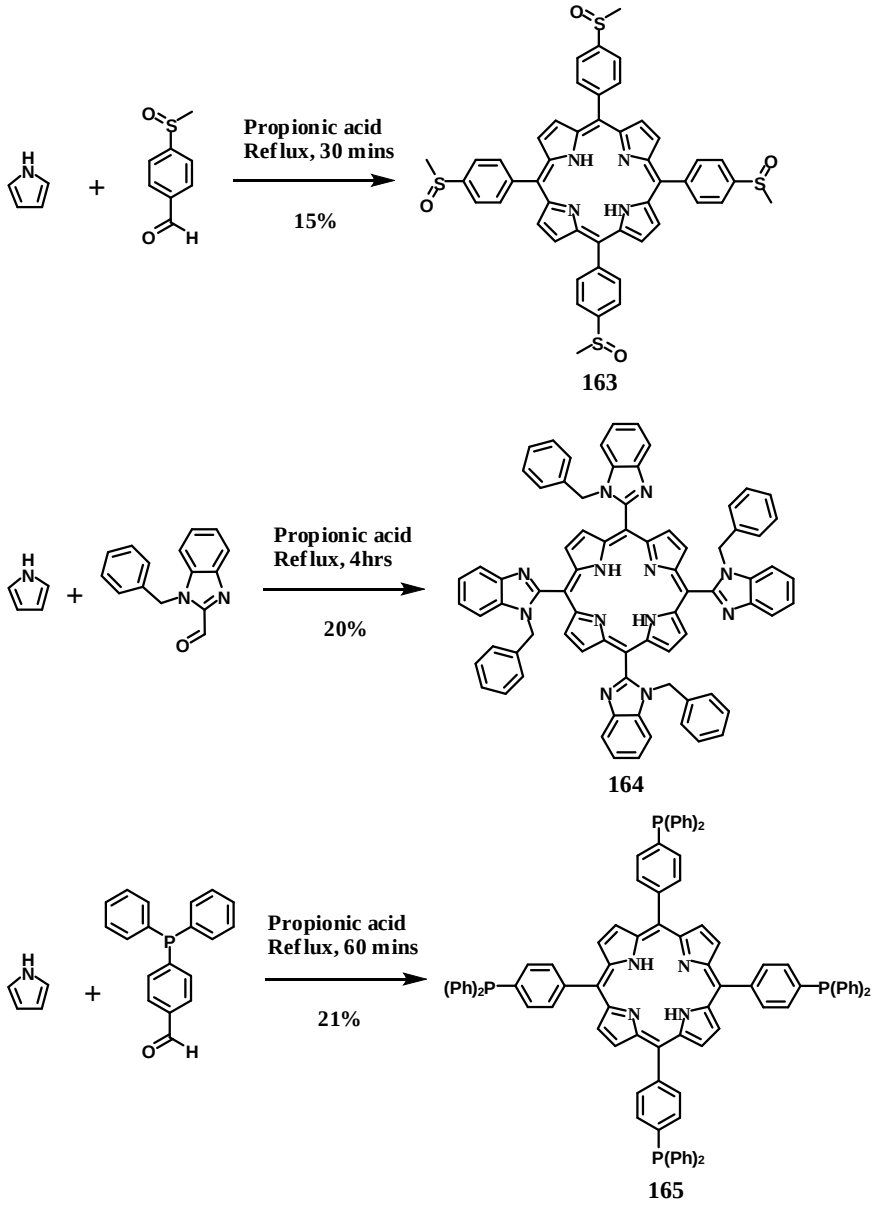
the formation of pyrrolic oligomers which do not cyclise to form the porphyrinogen and form a dark tarry residue from which the porphyrin must be separated. The method is scaleable and hence allows access to porphyrins on multigram scales.



Scheme 43: Proposed mechanism of porphyrin formation

The boiling propionic acid solvent used in the Adler method facilitates solvation

of a wide variety of aldehydes, giving the reaction a wide scope. However, this also makes the methodology unsuitable for aldehydes which are sensitive to these conditions. Despite this, a large range of porphyrins have been synthesised by this method with diverse functionality, three of which (**163**-**165**) are shown in Scheme 44.[130-132] Porphyrins have been synthesised with substituents on the ortho, meta and para positions of the phenyl rings, including both electron donating and electron withdrawing groups.[133-136] In addition, aldehydes containing macrocyclic and organometallic functionalities have been successfully condensed via the Adler method.[137, 138]

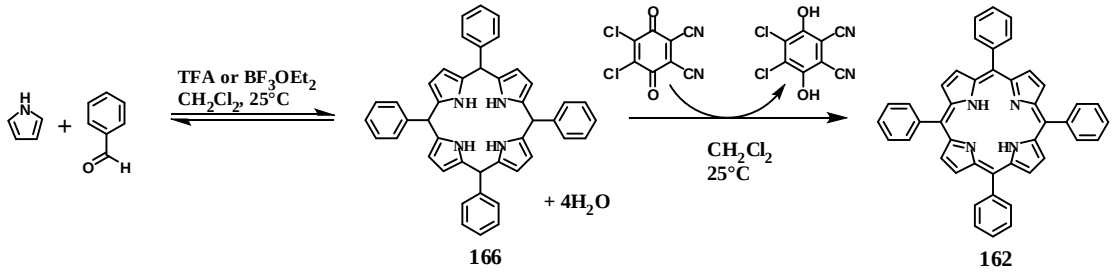


Scheme 44: Some examples of porphyrins successfully synthesised using Adler-Longo conditions[130-132]

To summarise, the Adler synthesis allows access to tetraaryl porphyrins with a wide range of substituents on a relatively large scale in ~20% yield. However, the harsh conditions used limit its usefulness with acid sensitive benzaldehydes and isolation and purification of the porphyrin from the tar laden propionic acid can be very difficult and extremely time consuming. In addition to this, reproducibility of yield from one reaction to the next is often unreliable.

3.3.2 Lindsey method[139, 140]

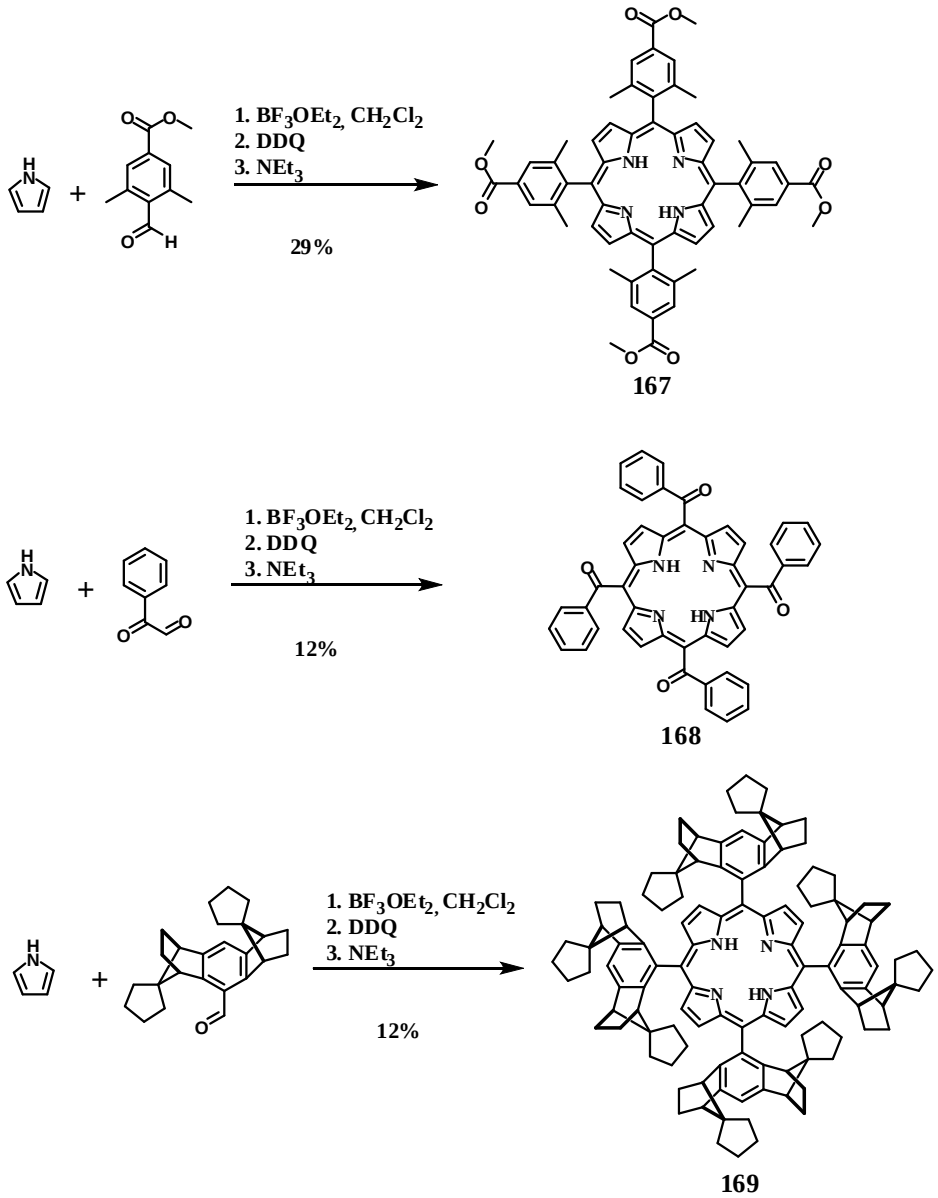
In 1986, the Lindsey group[139] reported a new synthesis of tetraaryl porphyrins developed as a result of attempts to circumvent the limitations of the Adler[124] method. Specifically, they were interested in developing a route which would give access to porphyrins in a higher yield and which was also more tolerant of sensitive benzaldehydes.[140] The porphyrinogen was known to be a key intermediate of porphyrin synthesis[128] and was formed by a series of equilibria from the starting materials of pyrrole and benzaldehyde, as shown in Scheme 43. The Lindsey method establishes this equilibrium under mild conditions (CH_2Cl_2 or $CHCl_3$ solvent, ambient temperature, acid catalysis, inert atmosphere) followed by addition of an oxidant to irreversibly convert the porphyrinogen **166** into the porphyrin **162**.[139, 140] (Scheme 45)



Scheme 45: The Lindsey synthesis of meso-tetraphenylporphyrin[139, 140]

A series of extensive investigations have been undertaken to understand the reaction and the optimum conditions required are often dependent upon the

structure of the desired porphyrin.[141-146] However, a general conclusion was that the reaction is most efficient when concentrations of both starting materials were 10 mM, leading to symmetric porphyrins in yields as high as 50%.[140] This methodology has been used for the synthesis of a wide range of meso-substituted porphyrins, and has proved to be mild enough to tolerate many functional groups that would not withstand the Adler conditions.[140, 147-150] Reports of the synthesis of porphyrins bearing electron withdrawing and electron releasing substituents on all positions of the phenyl rings demonstrate the wide scope and general utility of this methodology.[79, 150-153] Some examples of porphyrins synthesised by this method are shown in Scheme 46.[152, 154]

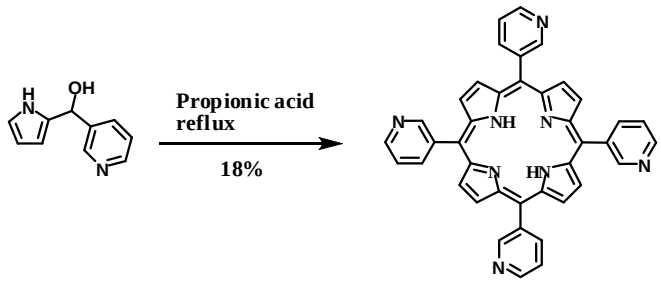


Scheme 46: Examples of porphyrins successfully synthesised using Lindsey conditions[152, 154]

The Lindsey conditions fail when the aldehyde is insoluble in either CH_2Cl_2 or $CHCl_3$ and the main practical drawback is that due to the high dilution conditions required, it is only of realistic value for making porphyrins on a scale of less than 1g.

3.3.3 Porphyrins from pyrrole-carbinols

As shown in Scheme 43, a key intermediate in the formation of the porphyrin is a pyrrole-carbinol, and symmetric porphyrins can be synthesised by self condensation of these compounds under acidic conditions, as shown in Scheme 47.[133]



170

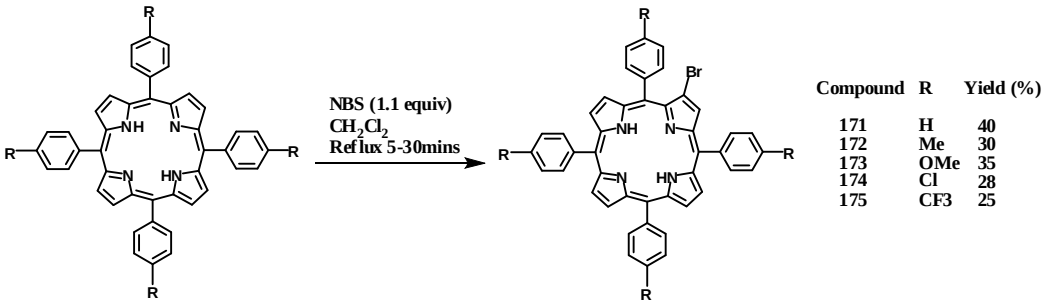
Scheme 47: An example of a porphyrin synthesised by the pyrrole-carbinol route[133]

This drawback to this methodology is the requirement of functionalisation of pyrrole prior to condensation. However, several ways of synthesising pyrrole-carbinols have been reported along with the porphyrins resulting from their subsequent condensation.[155-159] In comparison with the Adler[124] and Lindsey[139] routes, this methodology forms the porphyrin in two steps as opposed to one. Hence, as long as the benzaldehyde is available and stable, one of the previous routes would be the route of choice to a symmetric porphyrin.

3.4 Selective mono functionalisation of 5, 10, 15, 20-tetraaryl porphyrins at the β-position

3.4.1 Halogenation

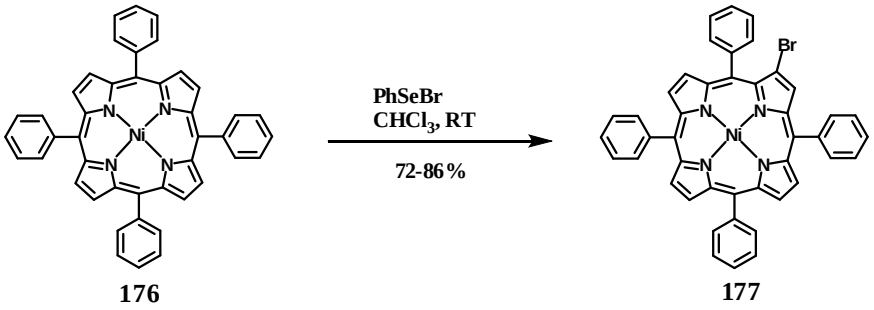
Tetraphenyl porphyrin (TPP) **162** can be brominated at the β-position by treatment with NBS in $CHCl_3$ as shown by Callot[160]. Despite the use of only 1.1 equivalents of the brominating agent, the macrocycle undergoes polysubstitution and the mono-brominated porphyrin is only isolated after lengthy chromatographic separation in low yield (30-40%). This method is applicable with many substituents on the phenyl rings and was used by Liu *et al.*[161] to synthesise the bromo porphyrins (**171-175**) shown in Scheme 48.



Scheme 48: Mono-bromination of TPP[161]

Chlorination of TPP using NCS is a slower reaction than bromination and hence the mono chlorinated product can be obtained in a slightly higher yield. Callot *et al.*[162] heated a dichloroethane solution of TPP and NCS under reflux conditions for two hours and isolated the mono-substituted product in 47% after chromatographic separation.

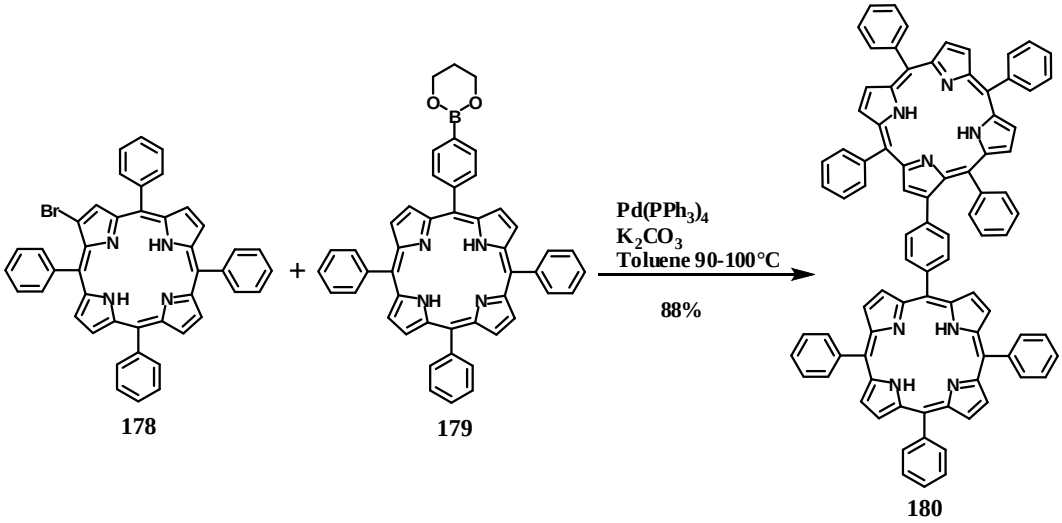
These relatively low yields can be improved by using the method of van Lier *et al.*[163] who described the treatment of the nickel complex of TPP **176** with PhSeBr. This reagent afforded the mono brominated product **177** in an isolated yield of 72-86%, Scheme 49.



176 **177**

Scheme 49: Mono bromination of Ni-TPP[163]

These β-brominated porphyrins allow access to a range of reactions for further functionalisation and manipulation of the structure. Chan and Zhou[164, 165] used the free base 2-bromo-TPP **178** in a palladium catalysed coupling reaction with the boronic ester appended porphyrin **179** to obtain the dimeric porphyrin **180** product in good yield as shown in Scheme 50.



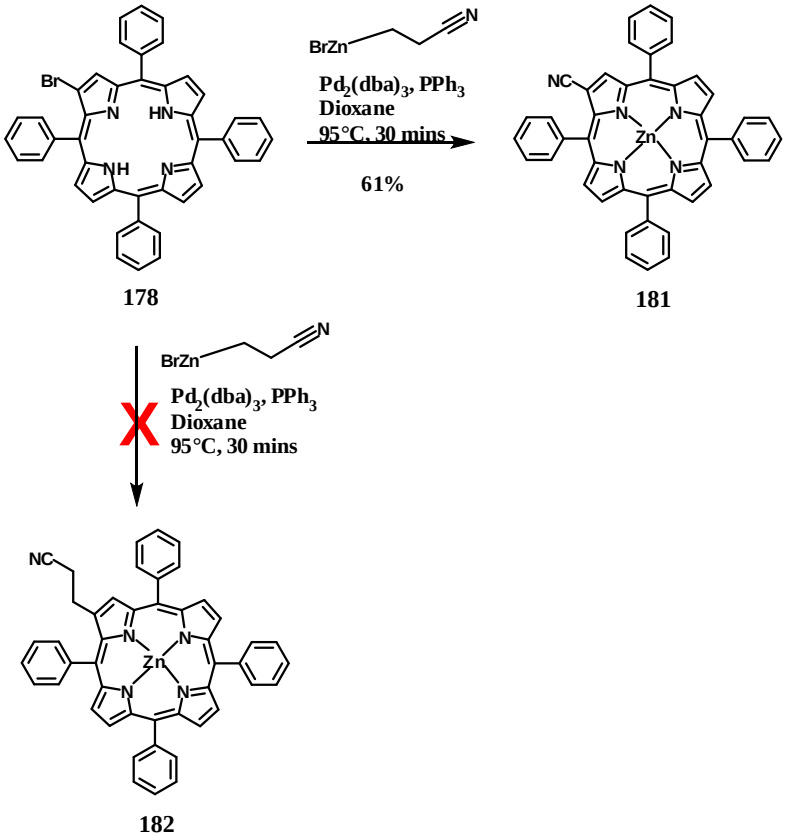
178 **179** **180**

Scheme 50: Porphyrin dimer obtained via palladium catalysed aryl coupling reaction[164, 165]

It is notable that although they used free base porphyrins, under their reaction conditions the porphyrin did not form a metal ion complex with the palladium(II) cation.

The zinc(II) complex of 2-bromo-TPP has been shown to undergo coupling reactions with both alkyl and aryl zinc organometallic reagents, using the same $Pd(PPh_3)_4$ catalyst.[166] Suda *et al.*[167] found that when they reacted cyanoethylzinc bromide with 2-bromo-TPP **178** the product was the cyano substituted zinc(II)

complex of TPP **181** and not the expected alkylated product **182** as shown in Scheme 51.



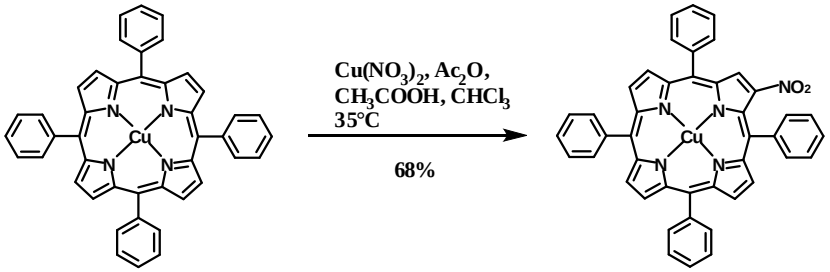
Scheme 51: 2-cyano-Zn-TPP formed when cyano ethyl zinc was reacted with with 2-bromo-TPP[167]

This unexpected reaction gave access to tetraphenyl porphyrin monosubstituted at the β position with a cyano group in good yield.

The nickel(II) complex of 2-bromo-TPP has also been shown to undergo nucleophilic substitution with *E*-benzaldoxime in DMSO at 80 °C.[168] The product is the nickel complex of 2-hydroxy-TPP and is isolated in 83% yield. The nickel(II) complex was used to protect the inner nitrogen atoms from protonation and also because nickel(II) does not induce enough electron density on the porphyrin ring to render it unreactive towards nucleophiles. According to the authors the reaction proceeds via a typical S_NAr mechanism with the halide ion acting as a leaving group.[168]

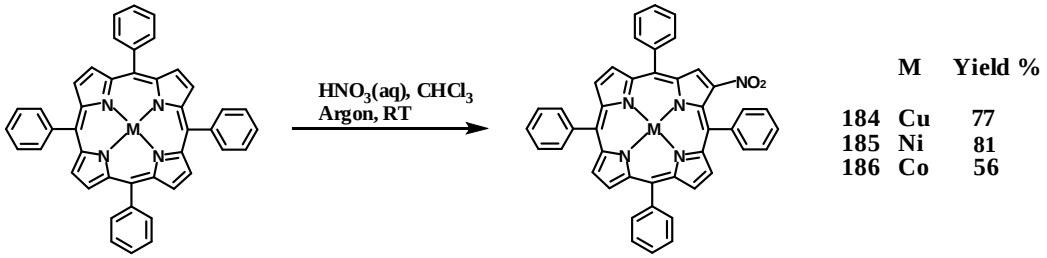
3.4.2 Nitration

Copper(II) TPP, **183** can be mono-nitrated at the β-position by treatment with $Cu(NO_3)_2$ and acetic anhydride as shown in Scheme 52[162]. Once substituted, the electron withdrawing nature of the nitro group deactivates the porphyrin to further nitration thus giving the mono nitro porphyrin **184** as the major product.



Scheme 52: Nitration of copper(II) TPP[162]

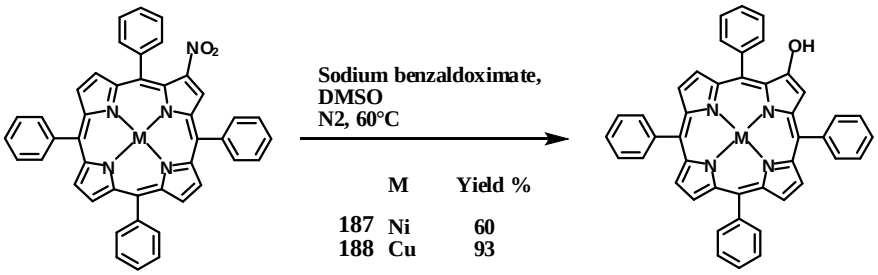
An alternative reaction was developed by Crossley *et al.*[169] who used 2.05 equivalents of NO_2 in CH_2Cl_2 to mononitrate a range of tetraaryl porphyrins and this method has been further optimised to give access to 2-nitro-TPP in 88% isolated yield.[170] Although the isolation of the product does not involve chromatography, the drawback to this method is the generation and handling of the gaseous nitrating reagent, a fact which was acknowledged by Ostrowski and co-workers[171] who developed a new method using aqueous nitric acid at room temperature (Scheme 53).



Scheme 53: Nitration of M(II) TPP with nitric acid[171]

Copper(II) 2-nitro-TPP **184** can be reduced to the corresponding amine by treatment with Sn/HCl or $NaBH_4$ in methanol with a 10% Pd/C catalyst, thus giving easy access to β–amino porphyrins.[172-174]

Free base β-nitro-tetraaryl porphyrins have been shown to undergo nucleophilic displacement with thiophenolate and ethylthiolate anions[174], while their copper(II) and nickel(II) complexes react with sodium benzaldoximate, forming the corresponding 2-hydroxy porphyrin derivatives.[175] (Scheme 54)

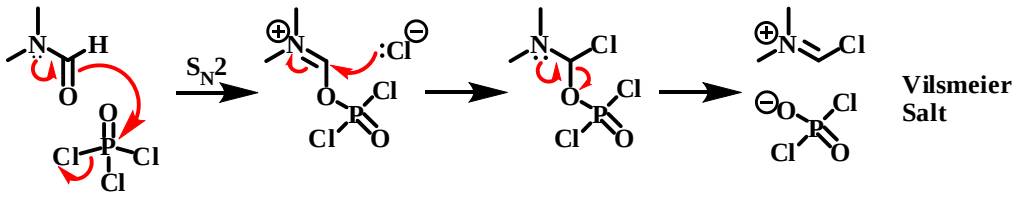


Scheme 54: 2-hydroxy porphyrin synthesis[175]

Michael type addition reactions are also well known, with metal complexes of β–nitro porphyrins being attacked by a variety of nucleophiles. [176-178]

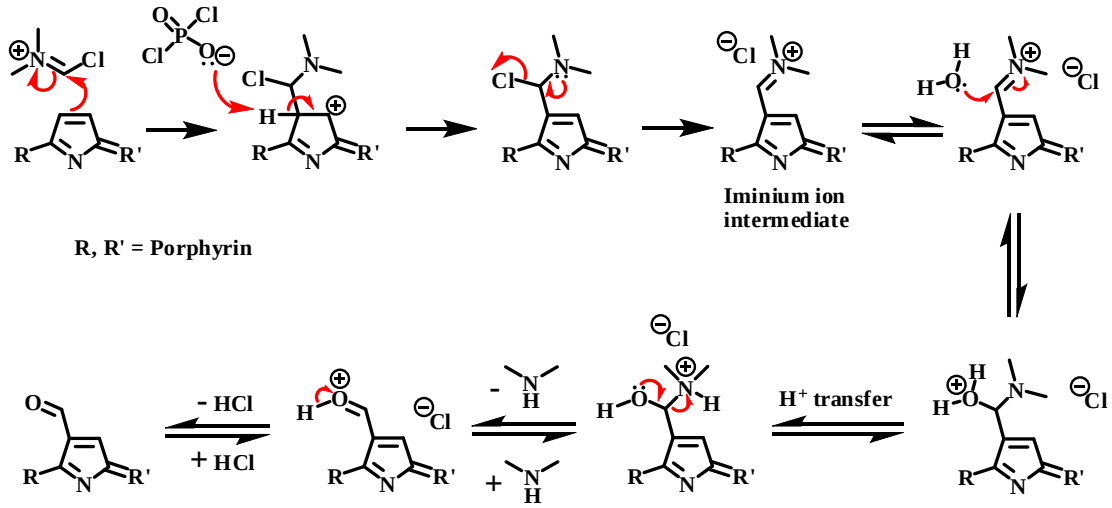
3.4.3 Formylation

The introduction of an aldehyde functional group at the β position of TPP can be achieved by treatment of a metal complex of the porphyrin with a mixture of phosphorus oxychloride and DMF at 0 °C. The overall mechanism of formylation of aromatic systems using $POCl_3$ and DMF is well known and proceeds via formation of a chloroiminium salt, known as the Vilsmeier reagent[179], as shown in Scheme 55.



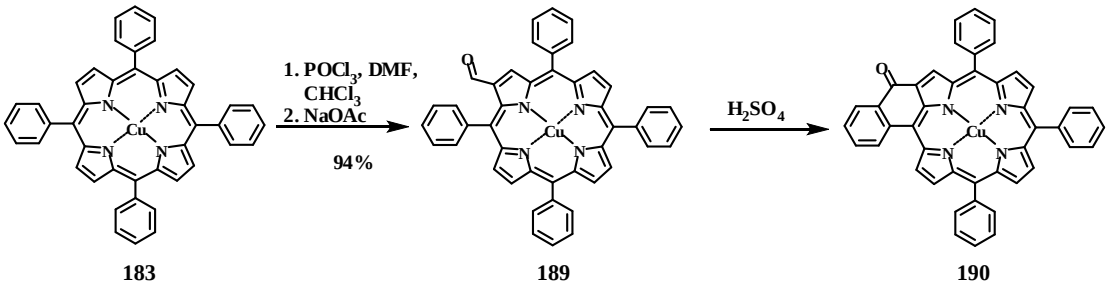
Scheme 55: Vilsmeier salt formation

This weakly electrophilic salt is then attacked by the aromatic system, in this case the porphyrin, as shown in Scheme 56.



Scheme 56: Mechanism of porphyrin formylation

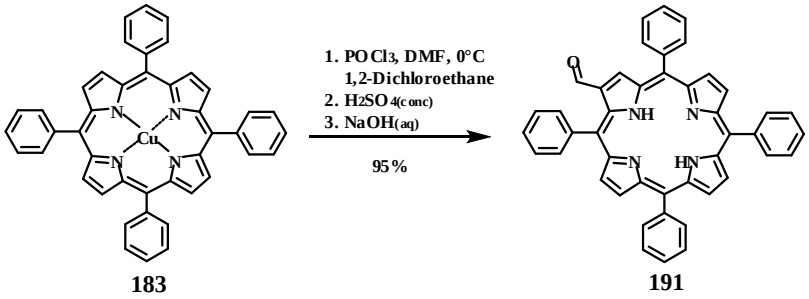
The metal ion complex is required to push electron density to the porphyrin periphery, thus making it nucleophilic enough to react with the Vilsmeier salt. This sequence generates an iminium ion which can then be hydrolysed to give the formyl group. The choice of metal ion is crucial to the reaction. It must activate the porphyrin periphery enough for the reaction to occur and be stable under the acidic conditions of the formylation yet be easily removed to yield the free base product. With these requirements in mind, it has been shown that the copper(II) complex of TPP **183** can be forrmylated in high yield[180]. However, the authors found that attempted demetallation of the porphyrin caused an intramolecular cyclisation reaction between the formyl carbon with the meta carbon of the nearby phenyl ring as shown in Scheme 57.[180]



183 **189** **190**

Scheme 57: Formation of 2-formyl-CuTPP followed by acid treatment[180]

Clearly the formyl group is more reactive towards the acid than the inner nitrogen atoms, but this problem was solved by Maxwell *et al.*[181] who found that because the intermediate iminium ion was stable to acidic conditions they could demetallate the porphyrin *in situ*, by treatment with sulphuric acid, prior to basic hydrolysis of the iminium ion to reveal the aldehyde group[181] (Scheme 58).

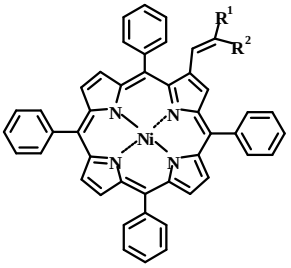


183 **191**

Scheme 58: Optimised synthesis of 2-formyl TPP[181]

3.4.3.1 Reactions of 2-formyl-TPP

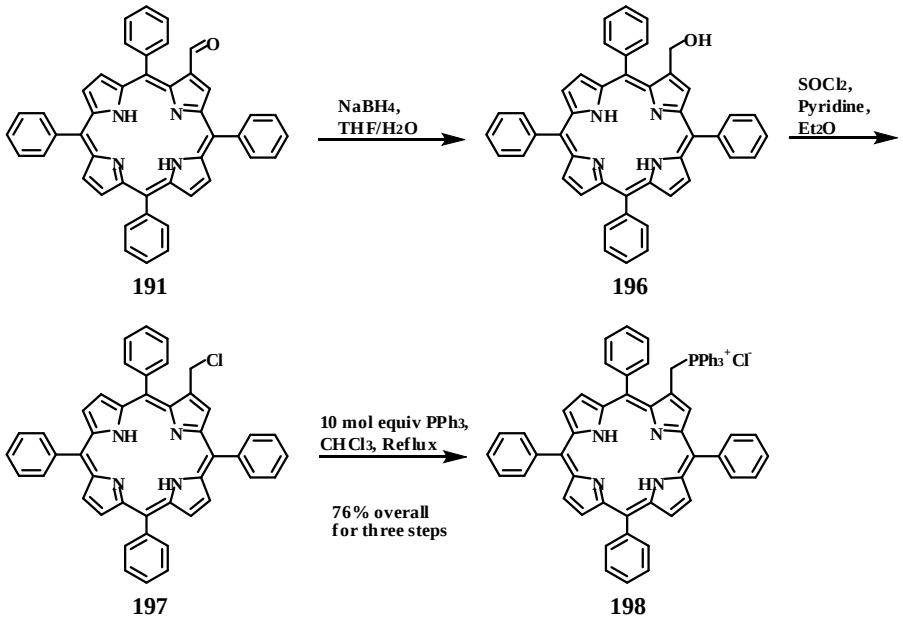
2-Formyl TPP **191** can undergo Wittig type reactions as demonstrated by Callot[182] who treated the nickel(II) complex of 2-formyl-TPP with $Ph_3P{=}CH_2$ (formed *in situ*) to give the vinyl porphyrin analogue. The same methodology utilising different Wittig reagents has been used to synthesise the compounds shown in Figure 32.[182, 183]



	R^1	R^2	Yield (%)
192	H	H	52
193	H/Br	H/Br	71 (cis+trans)
194	COOMe	H	41
195	H	COOMe	30

Figure 32: Products from Wittig reaction of nickel(II)-2-Formyl-TPP[182, 183]

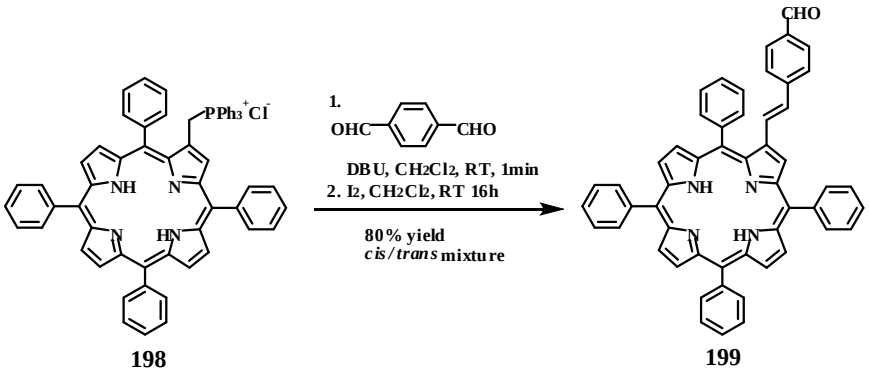
In addition to these reactions where 2-formyl-TPP **191** was reacted with a Wittig reagent, it has also been converted into a Wittig reagent.[184] Reduction with $NaBH_4$ followed by chlorination gives the chloroalkyl species **197** which was then treated with PPh_3 in $CHCl_3$ to give the Wittig porphyrin **198** in 76% overall yield (3 steps)[184] Scheme 59.



191 **196** **197** **198**

Scheme 59: Synthesis of a Wittig porphyrin[184]

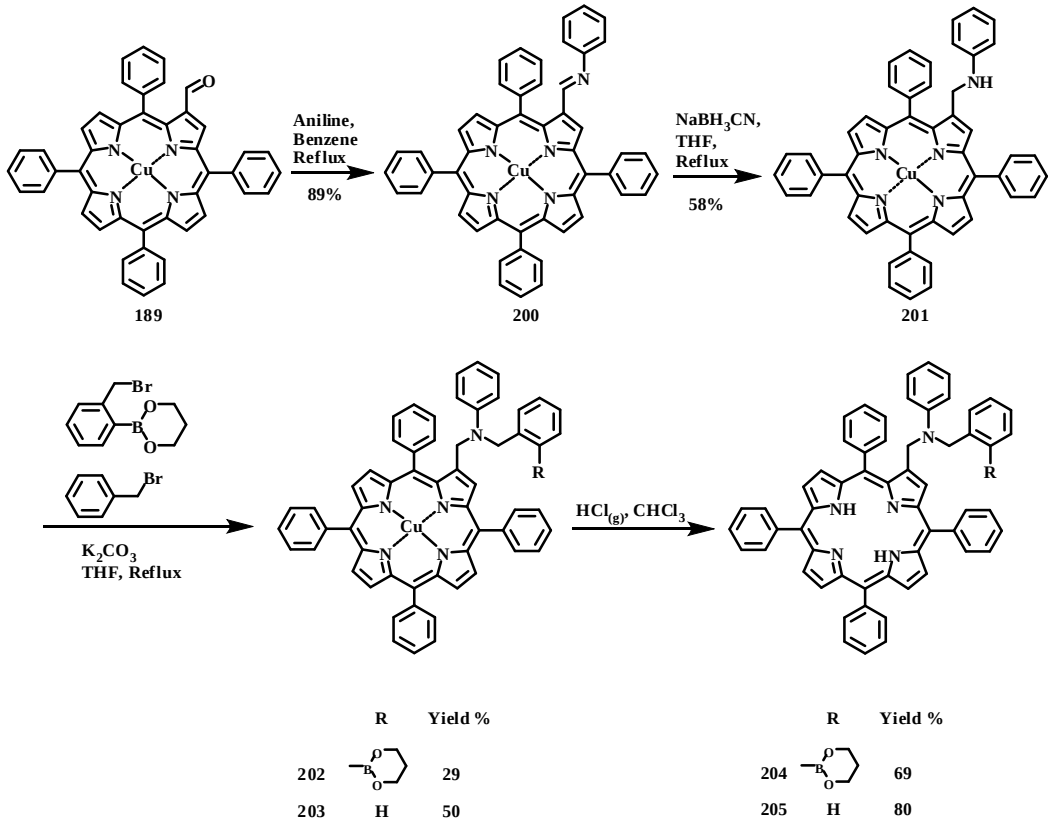
This methodology was used by Reid *et al.*[185] to form the conjugated aryl aldehyde appended porphyrin **199** as shown in Scheme 60.



198 **199**

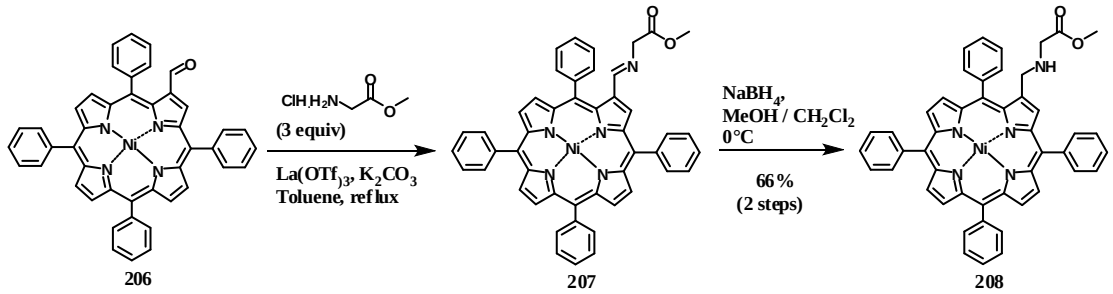
Scheme 60: Reaction of the Wittig porphyrin[185]

Shinkai *et al.*[186] used a reductive amination sequence to further functionalise the copper(II) complex of 2-formyl-TPP **189** with two different groups as shown in Scheme 61. Amination of the aldehyde **189** gave the imine **200** in good yield and they isolated this compound before reducing it with $NaBH_3CN$ in a lower yield of 58%. Thus, the amine **201** was synthesised from the starting material in 52% over the two steps. The secondary amine was subsequently alkylated with two different groups – benzyl bromide and a boronic ester substituted analogue in 50% and 29% yield respectively. Finally, the metal centre was removed from the porphyrin in the last step. The synthesis is not discussed in the paper, so it is not clear why the metal centre was kept inside the porphyrin throughout this sequence. NMR details are not reported and the paramagnetic copper(II) centre would make the analysis by ^1H NMR difficult. The low yield of the boronic ester appended compound **202** is likely to be due to the Lewis acid nature of this reagent.



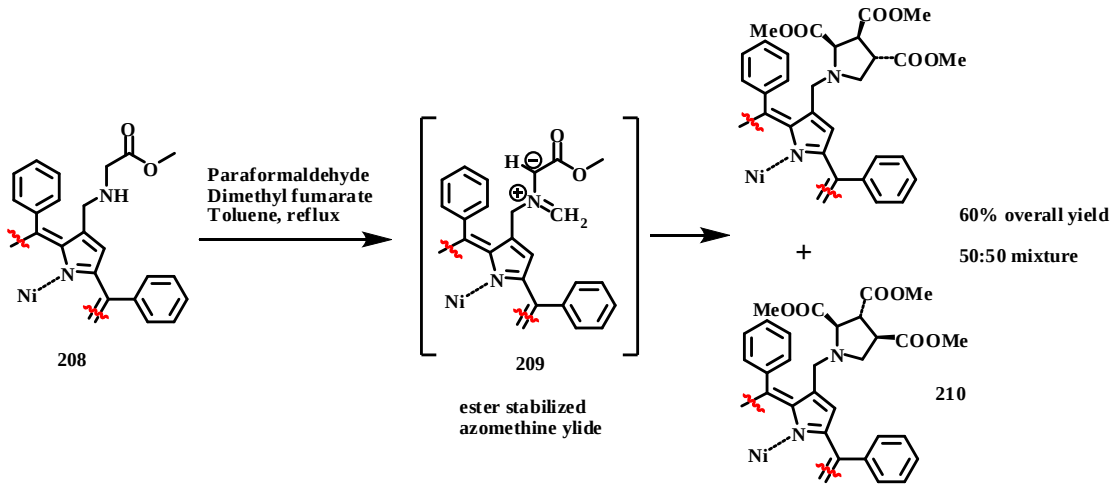
Scheme 61: Reductive amination using Cu(II)-2-formyl-TPP[186]

Cavaleiro *et al.*[187] used a similar methodology to synthesise **208** as shown in Scheme 62. They used a triple excess of amine and $La(OTf)_3$ as a Lewis acid to form the imine **207**, which was then reduced using $NaBH_4$ to yield the amine **208** in 66% over the two steps.



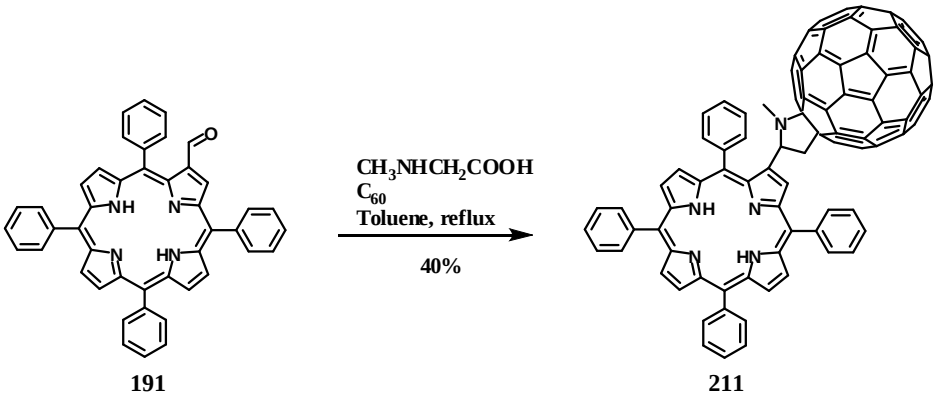
Scheme 62: Reductive amination of Ni(II)-2-Formyl-TPP[187]

Compound **208** was treated with paraformaldehyde, creating the ester stabilized azomethine ylide **209**, [188] followed by the dipolarophile dimethyl fumarate, to give compounds **210** (a 50:50 steroisomeric mixture) in 60% yield.[187] (Scheme 63)



Scheme 63: Formation and cycloaddition reaction of a porphyrin substituted azomethine ylide.[187]

A similar methodology has been used to append a C_{60} functionality onto the β-position of TPP.[189] As shown in Scheme 64, free base 2-formyl-TPP **191** was condensed with N-methylglycine to form an unstabilised azomethine ylide[190], which was then trapped with the dipolarophile C_{60} to give **211** in 40% yield. Note that in contrast to the above work, the porphyrin was used as the formyl component in the reaction.[189]



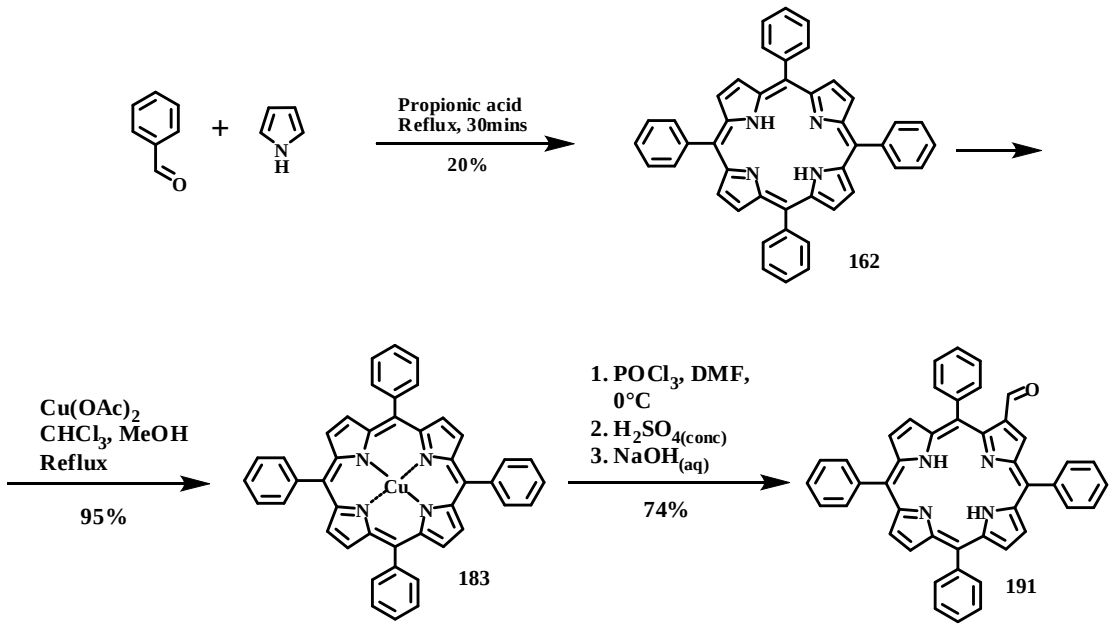
Scheme 64: Appending C_{60} onto the β-position of TPP via 1,3-dipolar cycloaddition[189]

3.5 Synthetic strategy

Taking the literature procedures described above and the requirements of this project into account, it was decided to explore a route to a coupled cyclam/cyclen-porphyrin conjugate via 2-formyl-TPP **191**. As already shown, this is a known compound which can be synthesised on a multigram scale using a reliable literature procedure. Since five of the cyclam/cyclen compounds already synthesised contain an amino group (**56**, **57**, **134**, **137** and **138**), the ubiquitous reductive amination reaction appeared to offer a direct synthetic route to the desired cyclam/cyclen-porphyrin target. Additionally, although 2-formyl-TPP **191** is a well known molecule, reductive amination onto the porphyrin ring has not been reported in any significant depth in the current literature on porphyrin synthesis and modification.

3.6 Synthesis of 2-formyl TPP

The synthetic route used to synthesise 2-formyl-TPP **191** is shown in Scheme 65.



Scheme 65: Synthesis of 2-Formyl-TPP[181]

5,10,15,20-Tetraphenylporphyrin (TPP) **162** was synthesised in 20% isolated yield by the method of Adler and Longo[124] and then converted into the copper(II) complex by adding a methanolic excess of copper(II) acetate to a heated solution (70 °C) of **162** in chloroform. After work up, the copper(II) complex **183** was obtained in high yield (95%). This complex was then formylated according to the method of Bonfantini *et al.*[181] As described earlier, this procedure follows three clear steps:-

1. treatment of the porphyrin with $POCl_3$ and DMF forms an intermediate iminium ion which is resistant to acidic conditions
2. at this point, $H_2SO_{4(conc)}$ is added to remove the copper(II) ion from the porphyrin
3. basic hydrolysis of the intermediate iminium ion yields the free base mono-formylated porphyrin in good yield.

The copper (II) metal ion was utilised to increase the reactivity of the porphyrin by increasing electron density at the periphery, whilst also protecting the inner nitrogen atoms from protonation. The yield of this reaction was 74% which was somewhat lower than the reported yield of 94%, but the three steps represent a very satisfactory route to a monofunctionalised, unsymmetrically substituted porphyrin. The downfield region of the ^{1}H NMR spectrum of compound **191**, where the β protons appear, is shown in Figure 33.

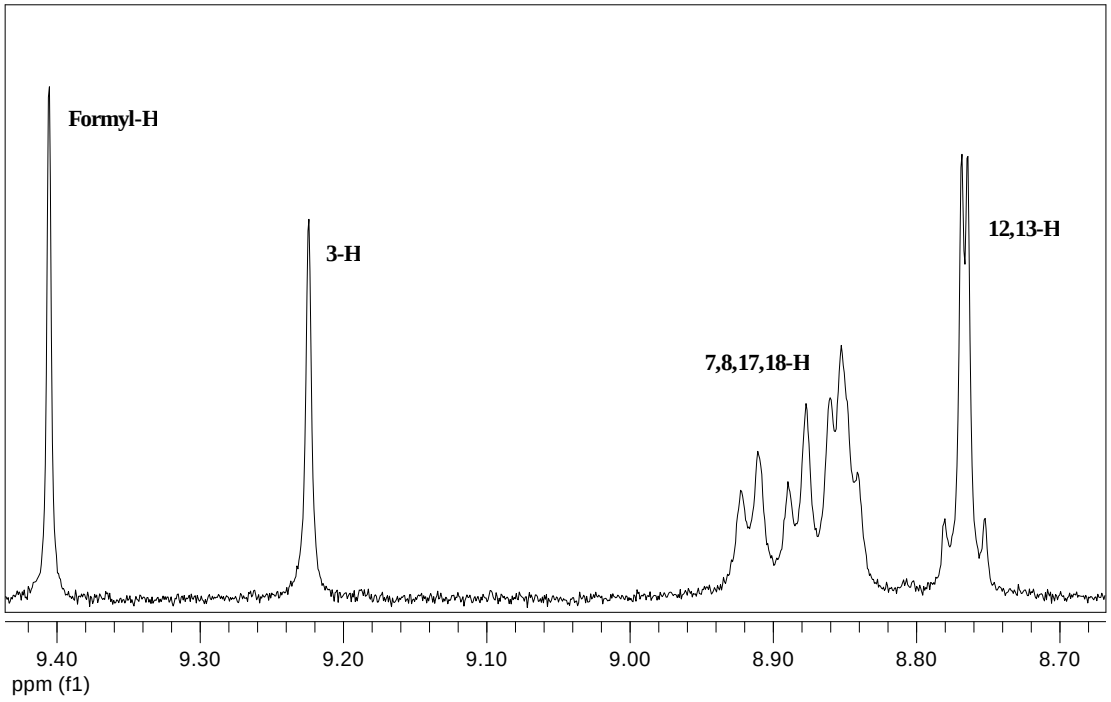


Figure 33: ^{1}H NMR of 2-formyl-TPP

The proton in the 3-position (α to the formyl substituent) is seen as a singlet and at 9.22ppm is the highest of the β protons, as expected. The protons in positions 12 and 13 on the porphyrin ring are almost equivalent magnetically and appear as the AB system around 8.70-8.80, since these are the furthest from the formyl substituent they appear at the lowest δ value. The other four protons appear in the multiplet between 8.82 and 8.94ppm. The δ values and integrations are consistent with the literature, but the fine structure is seen more clearly than was previously reported.[181]

With a stock of compound **191** in hand, the objective was to utilise this species to develop a method by which a cyclen or cyclam macrocycle could be attached to the porphyrin via the formyl functionality. Several cyclen / cyclam macrocycles had already been synthesised bearing a mixture of 1°, 2°, alkyl and aromatic free amines as shown in Figure 34. Since 1° and 2° amines are known to react with aldehydes directly to form imines, a one pot reductive amination sequence was proposed to link these two macrocycles together.

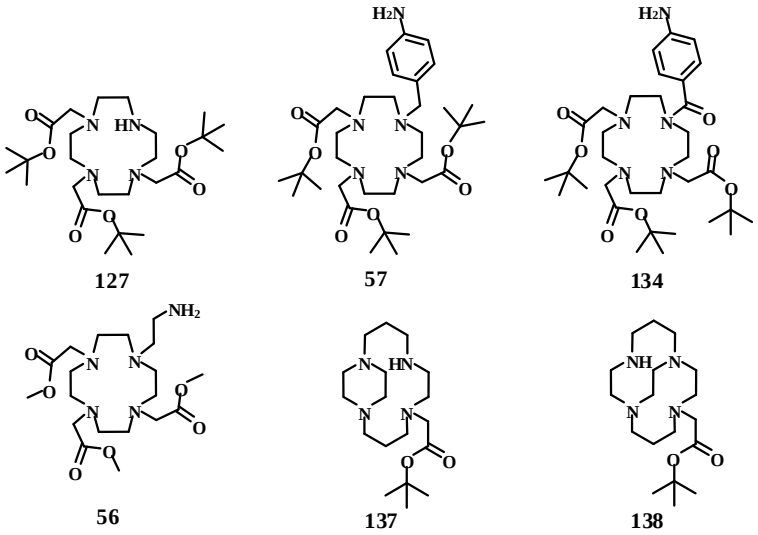
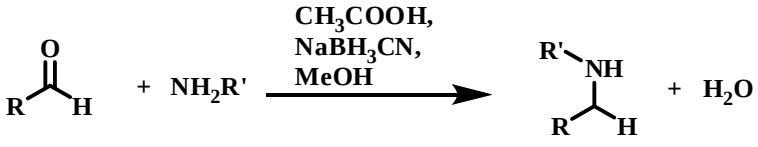


Figure 34: Cyclen and cyclam macrocycles bearing free amine functional groups

3.7 Reductive amination

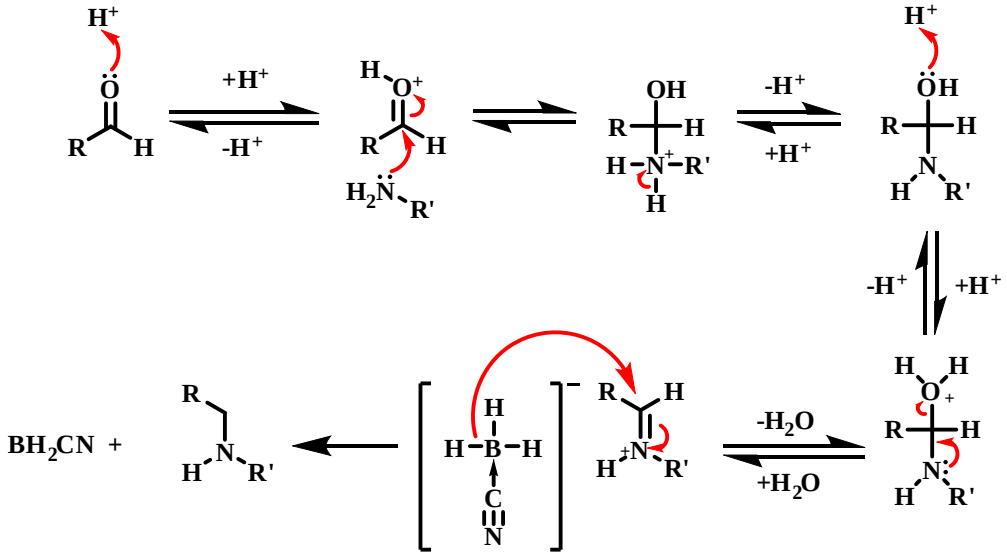
Reductive amination is a well known reaction[191] involving the condensation of an aldehyde or ketone with ammonia, or 1° and 2° amines as shown in Scheme 66.



Scheme 66: Reductive amination

As shown in the mechanism below (Scheme 67), the reaction involves acid catalysed iminium ion formation with subsequent reduction to the amine occurring in the final step. Imine formation is an equilibrium process favoured by acidic conditions. Acetic acid is often used to protonate the carbonyl compound, thus activating it to nucleophilic attack. However, if the concentration of acid is too high the amine will be protonated too, rendering it unreactive, and hence inhibiting the reaction. Due to this, a catalytic amount of acid is used to set up an equilibrium between protonated carbonyl and protonated amine, leaving some of

the amine free to act as a nucleophile. The reducing agent needs to be stable under acidic conditions and able to react with imines/iminium ions, but not with the carbonyl containing starting material. Sodium cyanoborohydride is often used as the reductant because, in addition to being acid stable, it reduces imines more quickly than aldehydes or ketones. Since the reduction step is the only non-equilibrium step, the reductant is effectively driving the reaction forward.



Scheme 67: Reductive amination mechanism

3.7.1 Synthetic strategy

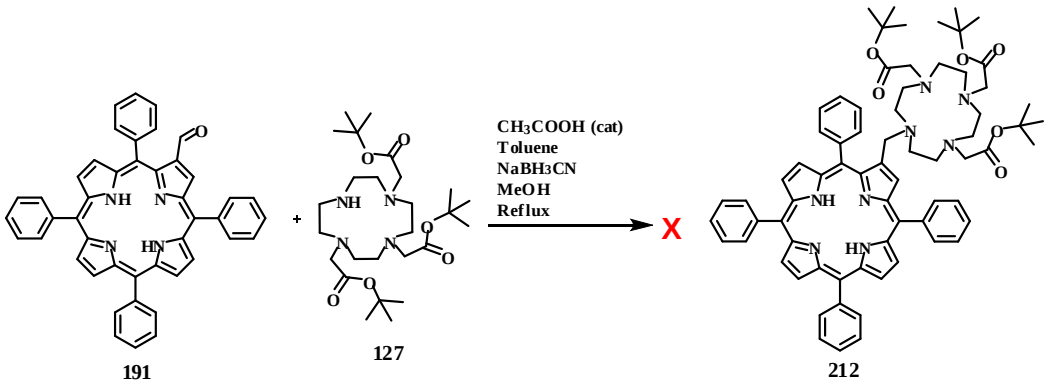
Both literature reports of reductive aminations on tetraphenyl porphyrin derivatives have used a two step procedure whereby the imine is generated and then isolated before being reduced, although neither paper explains why this methodology was used[186, 187]. The initial aim was to improve this protocol and try to generate the amine product in a one pot reaction. The two obvious ways to do this are:

1. allow the imine to form in high yield, then add the reductant *in situ* (one pot, two steps)
2. mix all the reactants, including the reducing agent, at the beginning of the reaction (one pot, one step)

The first option needs a method of driving the equilibrium formation of the imine to obtain as high a yield as possible, such as removal of water from the reaction. In the second case, the reductant is actually driving the equilibrium forward by removing the imine from the mixture. However, this method also relies on the reducing agent being stable to the acidic conditions of the reaction and the elevated temperatures used.

3.7.2 Attempted direct attachment of compound 127

The first reaction attempted is shown in Scheme 68.



Scheme 68: Attempted reductive amination of 2-formyl-TPP with DO3A tris-*tert*-butyl ester

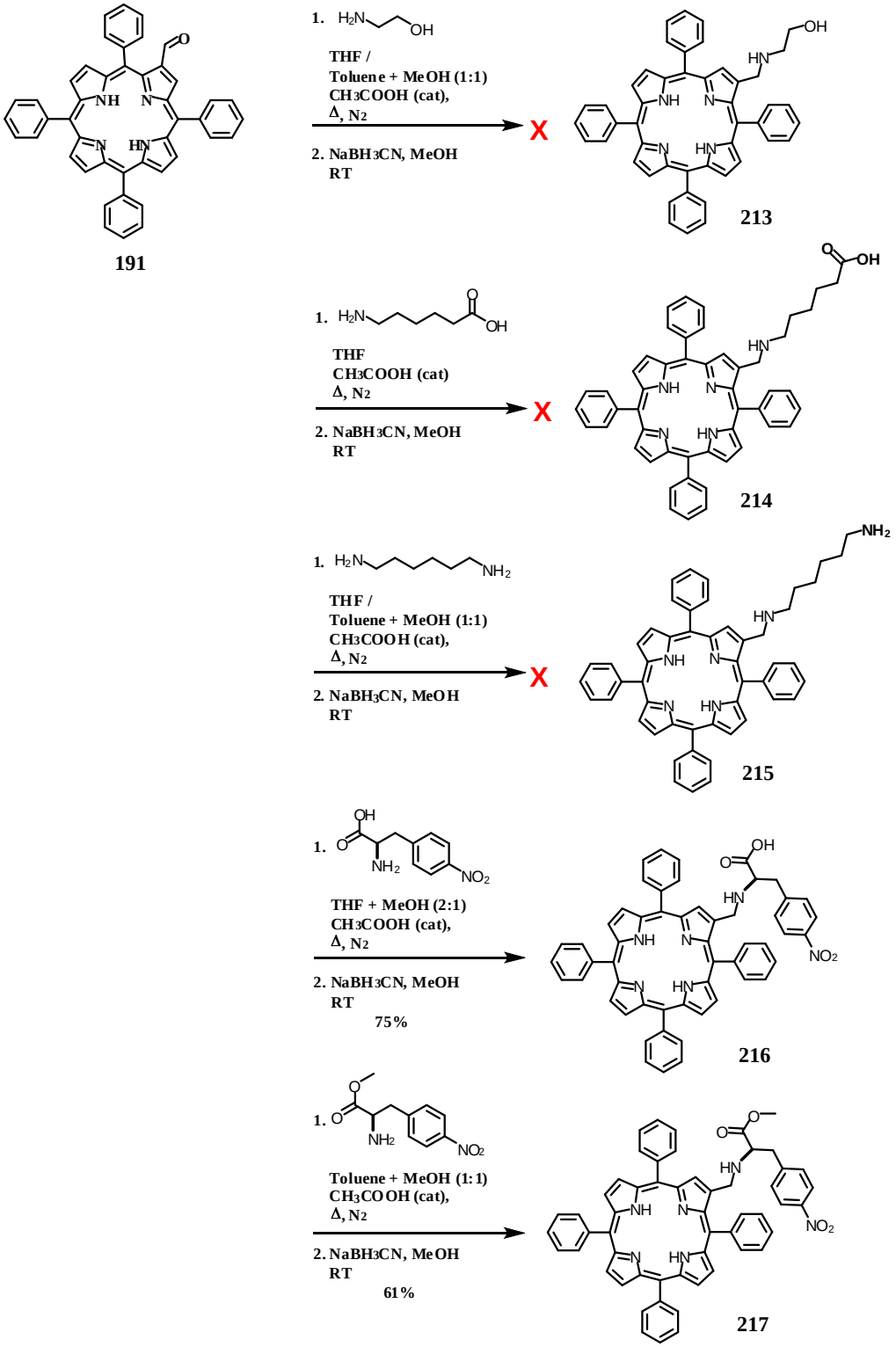
The cyclen based compound **127**, bearing a secondary amine on the macrocyclic ring was initially reacted with compound **191**. Both compounds were dissolved in toluene with **127** in a twofold excess, a few drops of acetic acid were added and the solution was heated under reflux conditions. The porphyrin starting material is non-polar compared to **127**, thus making it very easy to monitor the reaction by TLC (the imine formed would be very polar in comparison to porphyrin **191**). However, after several hours of heating, TLC analysis showed no consumption of starting materials. The reaction was cooled to ambient temperature, a Dean-Stark trap was fitted and activated 4Å molecular sieves

added to try to drive the equilibrium imine formation forward, and heating was resumed. After heating for a further three hours no change was seen. The reaction was repeated using anhydrous THF in place of toluene and heated under an inert (N_2) atmosphere. This time the amine **127** was washed with aqueous $NaHCO_3$ solution to ensure it was not protonated, before being added to the reaction mixture. After several hours of heating no imine formation could be detected. Since the macrocycle **127** had been used in several reactions previously where the 2° amine had proved nucleophilic (see previous chapter), the hypothesis was that there was too much steric hindrance between the macrocycles for the reaction to occur. A similar attempt using macrocycle **137** had the same ineffectual result. Hence it was decided to try to attach a spacer group to the porphyrin to which the cyclen/cyclam macrocycles could be appended in a subsequent step.

To this end, a set of readily available alkyl and aromatic amines were used to allow investigation of the reductive amination reaction with porphyrin **191**. Each amine also contained a second functional group to which the cyclam/cyclen macrocycles could potentially be attached.

3.7.3 Reductive amination with alkyl amines

The reactions attempted with alkyl amines are shown below (Scheme 69).



Scheme 69: Reductive amination attempts with alkyl amines

3.7.3.1 Reaction using 2-aminoethanol, attempted synthesis of 213

A twofold excess of 2-aminoethanol and compound **191** were reacted together using anhydrous THF as the solvent, a catalytic amount of acetic acid, 4Å molecular sieves to absorb water and the heating was carried out under a nitrogen atmosphere. However, no change could be detected over a period of several hours when monitoring by TLC. Thus it appeared once again that imine formation was not taking place. A methanolic solution of $NaBH_3CN$ was added in case the TLC result had been misinterpreted, but this had no effect. An aliquot was taken from the reaction mixture and the porphyrin fraction collected by preparative TLC. It was identified as the starting material **191**, hence the reaction appears to be completely ineffective. The reaction was repeated using a toluene / methanol (1:1) mixed solvent system, but this also failed.

3.7.3.2 Reaction using 6-aminohexanoic acid, attempted synthesis of 214

A twofold excess of 6-aminohexanoic acid and compound **191** were dissolved in anhydrous THF, three drops of CH_3COOH and activated 4Å molecular sieves added and the solution was heated under an inert atmosphere (N_2). TLC monitoring showed a change after one hour, with one main porphyrin product appearing which was considerably more polar than the starting porphyrin **191**. This was consistent with the expected imine formation. Heating was allowed to continue until, after approximately 5 hours, no further change could be seen, but the mixture still contained a considerable amount of the porphyrin starting material. At this point the reaction was left to cool to ambient temperature, still under the nitrogen atmosphere. When cool, a methanolic solution of sodium cyanoborohydride was added in one portion and the reaction mixture stirred for 2 hours. Further monitoring by TLC showed no discernable change. However, since the polarity difference of the imine intermediate and the final amine was unknown, this in itself did not necessarily indicate a failure of the reduction step. Additionally, if the reduction step had failed, it could be reattempted if the imine could be isolated. After work up, TLC showed the same two spots still present (starting porphyrin and the proposed imine/amine), and the mixture was subjected to column chromatography in order to isolate the more polar fraction.

However, during this process, the spot could clearly be seen degrading as it travelled down the column and no product was could be collected. This was consistent with failure of the reduction step, since imine compounds are known to be more stable when both sides of the imine double bond are substituted with an aryl group.

3.7.3.3 Reaction using 1,6-diaminohexane, attempted synthesis of 215

The initial reaction with 1,6-diaminohexane was carried out using the same reaction conditions as for 6-aminohexanoic acid, except a larger excess (5 equivalents) of the diamine was used to try to inhibit dimer formation. This time, two spots appeared on the TLC plate after only ten minutes and over the course of five hours, the starting porphyrin reduced in concentration while the more polar product appeared to increase in concentration. After no further change was detectable, the reaction mixture was again cooled to ambient temperature and then treated with a methanolic solution of $NaBH_3CN$. After stirring for ten minutes, TLC showed a mixture of seven porphyrin spots. Column chromatography was used to try to isolate the fractions, but degradation appeared to occur, with most of the material sticking to the top of the column, and it could not be isolated. The reaction was repeated using a toluene / methanol (1:1) mixed solvent system and the same initial two spots could be seen clearly by TLC. An aliquot was removed from the reaction mixture and the remainder was treated with the methanolic reductant solution as previously described. The aliquot which had not been reduced was subjected to preparative TLC in order to try to isolate the proposed product, but once again the compounds appeared to degrade very quickly and no pure fraction could be isolated. After aqueous work up, some of the remaining solution was loaded onto a preparative TLC plate in an attempt to obtain enough material for analysis and to gain some insight into the reaction. Although the compound degraded quickly, a very small amount (~1mg) was obtained and was analysed by ^{1}H NMR. Although this turned out to be impure, it did appear to have some of the desired product present. Figure 35 shows the aromatic region and two parts of the alkyl region. The aromatic region was quite clean, with peaks a-d showing the β-protons in a pattern typical of a mono-β-substituted porphyrin. Peaks e and f are assigned to the 2,6-protons on

the phenyl rings while the signals g and h represent the 3,4 and 5-protons. Moving to the alkyl regions, the spectrum was not well resolved but three signals could clearly be identified. Peaks j and k are clearly non-first order signals, which are consistent with the inner protons of the diamino chain connected to the porphyrin. Peak i is in the correct region to be the two protons directly attached to the porphyrin 2-position. Although this analysis is only partial and the sample was clearly a mixture, it suggested that the reaction was probably working, but the product was unstable when subjected to silica gel chromatography.

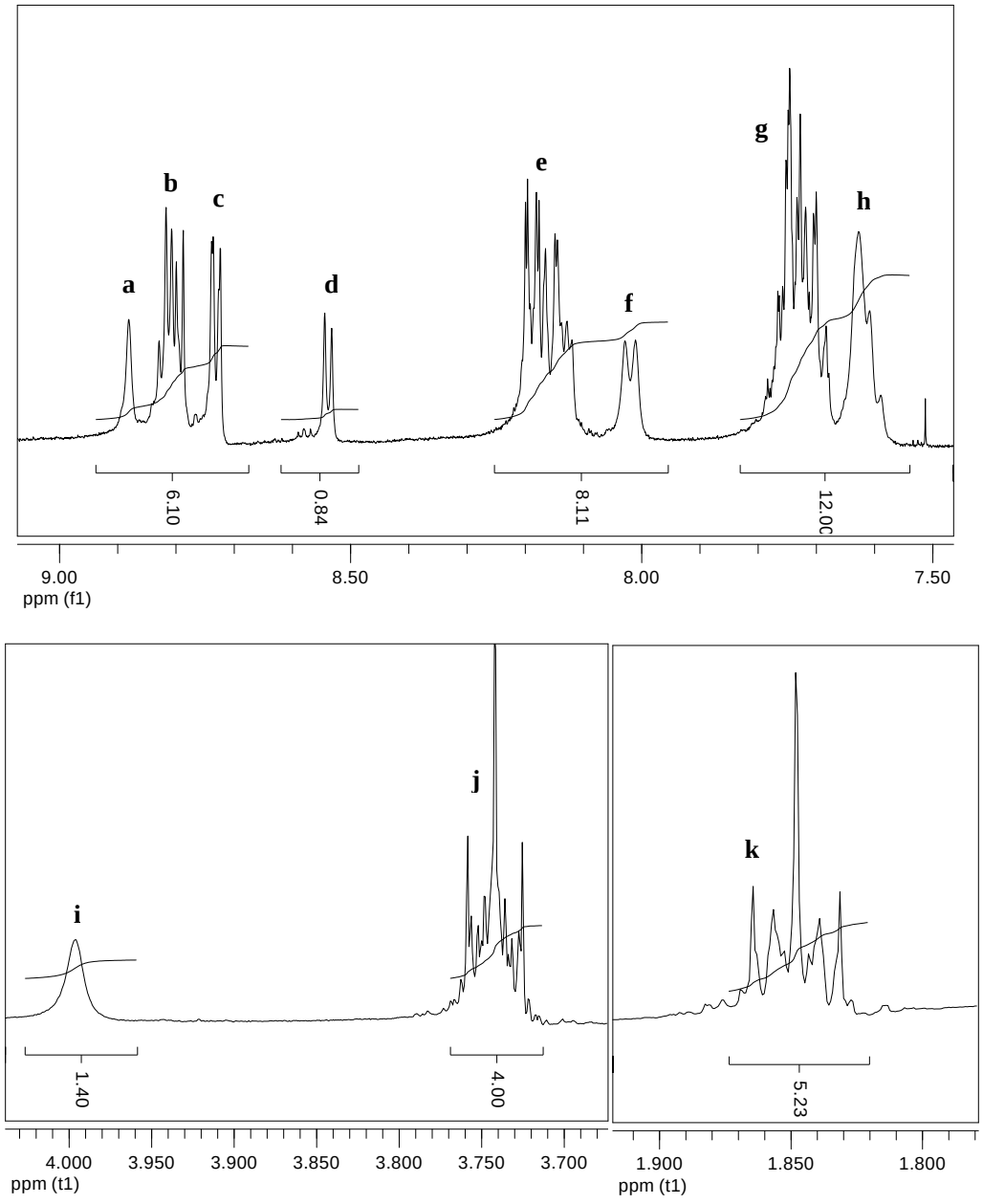


Figure 35: ^{1}H NMR of mixture formed from attempted reaction between 2-formyl-TPP and 1,6-diaminohexane

3.7.3.4 Reaction using S-4-nitrophenylalanine-synthesis of compound 216

The amino acid starting material had very low solubility in most organic solvents, but was soluble in methanol and sparingly soluble in warm THF. Thus, in order to solubilise both the porphyrin and the amino acid, a mixture of THF and methanol (2:1) was used as the solvent system for the reaction. The reaction was carried out in an analogous manner to the previous attempts, but without any water scavengers present. After being heated for ten hours under an inert atmosphere (N_2), TLC analysis showed a mixture was present which contained a very polar porphyrin spot, which looked promising as the expected imine product. The reaction was cooled to ambient temperature, the reductant solution added and the mixture left to stir for two hours. After aqueous work up, the mixture was separated by column chromatography and the product isolated in 54% yield. In an attempt to improve the yield the reaction was repeated, but this time carried out as a single step process as described above, by adding the starting materials and the reductant together at the beginning of the reaction. After heating for 24 hours, TLC analysis showed what appeared to be a more favourable conversion for the reaction. Indeed, after chromatographic work up the product was isolated in 75% yield. The product was insoluble in many organic solvents, the exception being THF, hence NMR analysis was only possible in d_8-THF. The ^1H spectrum and the peak assignments are shown in Figure 36 and Figure 37. The aromatic region shows a clear pattern of β-proton peaks, characteristic of a β-mono-substituted TPP. The singlet peak 1 was easily assigned to the proton at the 3-position and peak 2 (AB system) is assigned to the β–protons farthest away from the substituted position. The other peaks 3-6 could not be assigned individually, but represent the other four β–protons (shown in blue in Figure 36). The other aromatic signals were assigned using DQF COSY NMR. The substitution of the single β-position breaks the symmetry of the molecule and hence the signals for the phenyl protons split accordingly. The ortho protons split into two signals (peak 7, integral 6H and peak 8, integral 2H) and the meta and para protons overlap in a complex multiplet (peak 10). However, the small multiplet (peak 11, integral 2) is assigned to the two meta protons nearest to the β-substituent, which is corroborated by coupling to peak 8 in the 2D spectrum. Peaks 9 and 12 are typical aromatic doublets from the nitro-

phenyl ring.

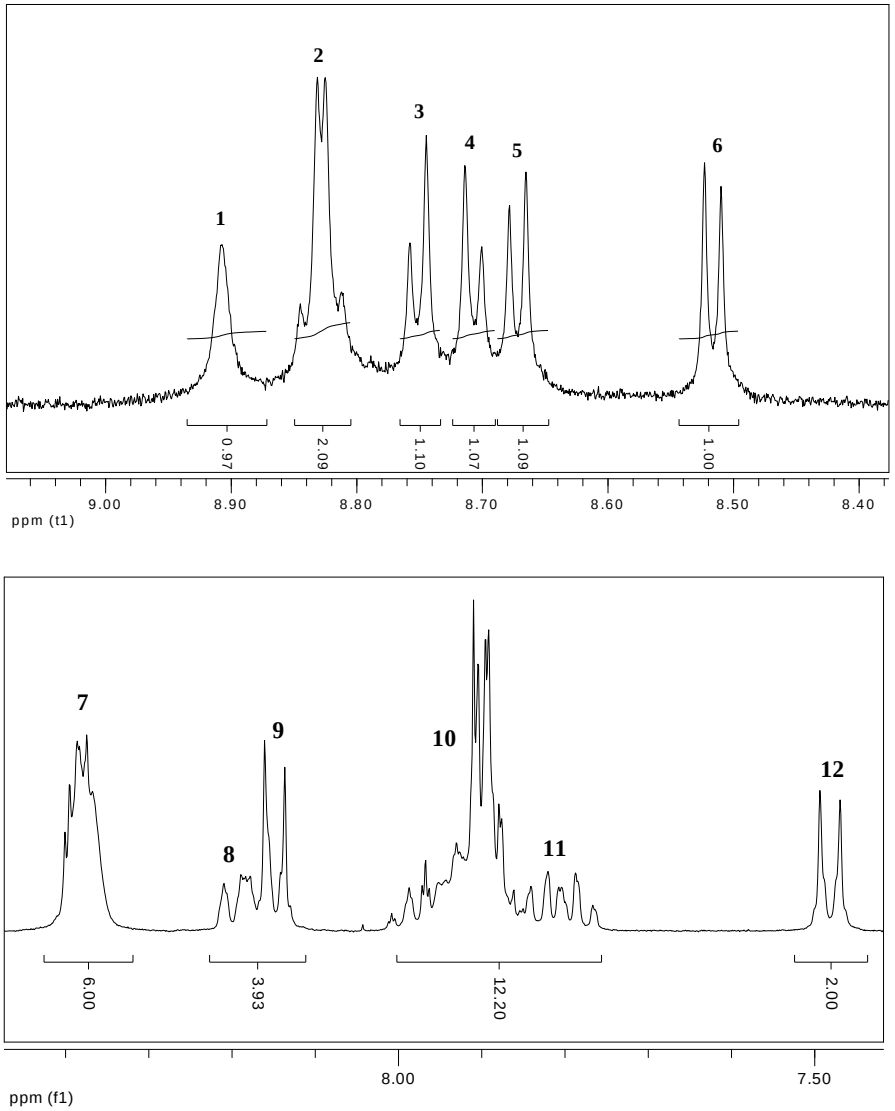


Figure 36: Aromatic ^{1}H peaks and assignments for compound 216

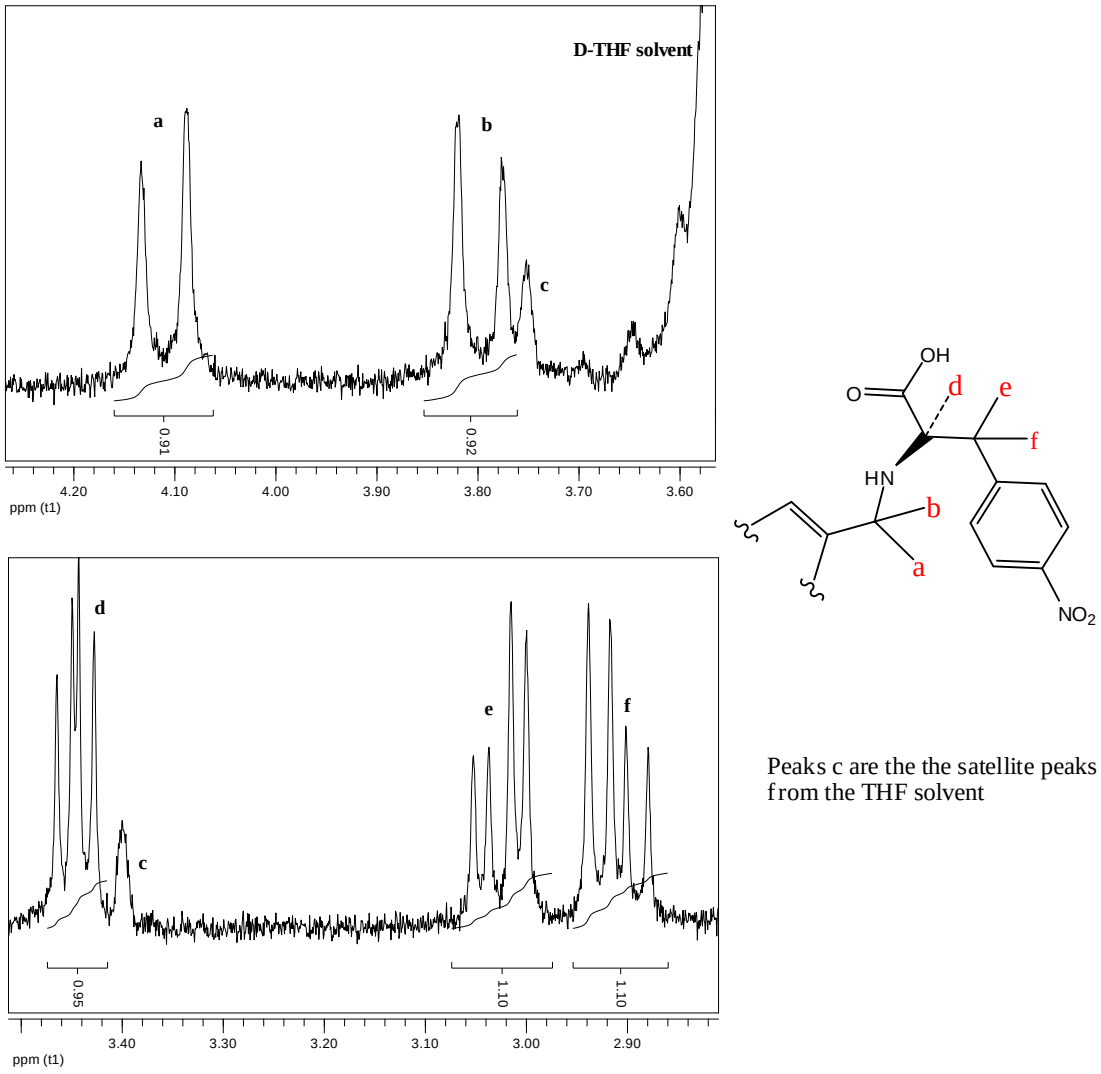


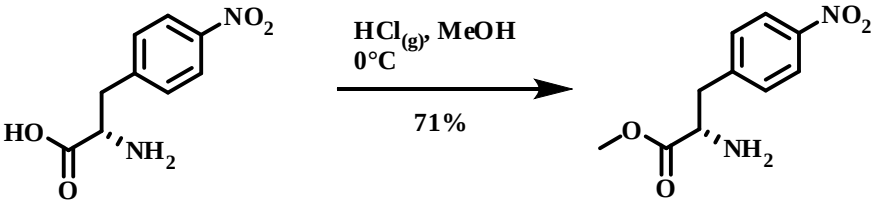
Figure 37: Alkyl peaks and assignments for compound 216

The protons on the amino acid group were also assigned with the aid of the 2D COSY spectrum. The chiral centre confers a diastereotopic relationship onto protons a, b, d, e and f and the splitting patterns reflect this (Figure 37). Peaks a and b both show an integral value of one and couple strongly to each other with a relatively large coupling constant of 15.6Hz (typical of geminal relationship) and are assigned as the CH_2 protons directly attached to the β-carbon on the porphyrin. Peak d couples to both e and f with coupling constants of 7.7 and 5.5Hz, (consistent with vicinal couplings) whereas peaks e and f couple together

with a coupling constant of 13.4Hz, typical of geminal coupling. Both peaks e and f split into a further doublet with the 7.7 and 5.5 Hz constants. Hence peak d is assigned to the proton attached to the chiral carbon, whereas e and f are assigned to the protons bonded to the adjacent carbon. It should be noted that the peak labelled c is a satellite peak from the THF solvent (^{13}C-^{1}H coupling). The peaks for the inner porphyrin NH protons is seen as a broad singlet at -2.80 ppm and the carboxylic acid proton is seen at 11.0 ppm, also as a broad singlet.

3.7.3.5 Reaction using S-4-nitrophenylalanine methyl ester-synthesis of compound 217

The methyl ester of *S*-4-nitrophenylalanine was synthesised from the amino acid by a published procedure[192] and was isolated in 71% yield. (Scheme 70)

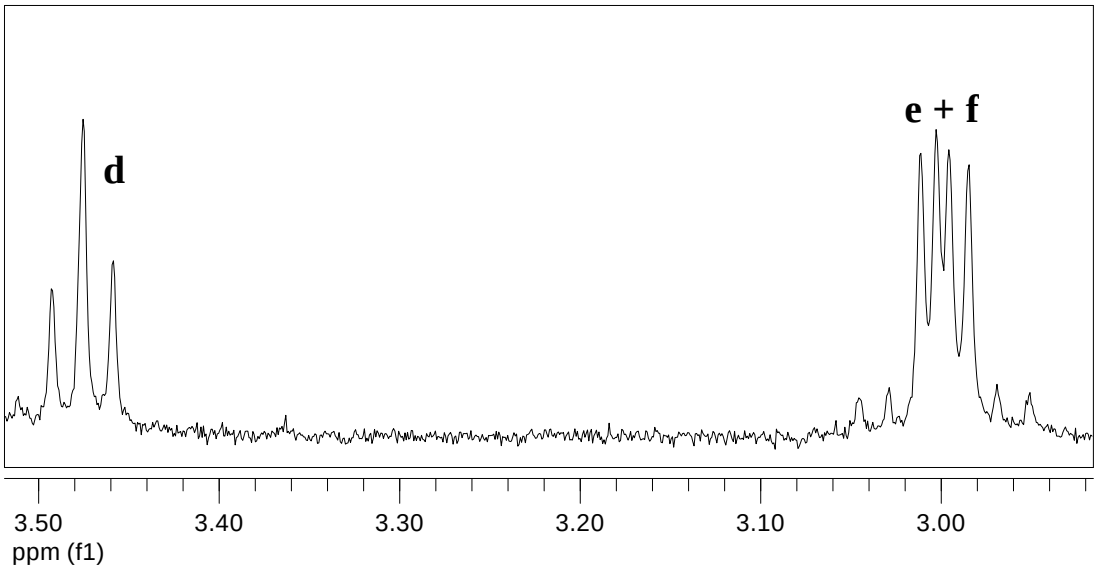


218

Scheme 70: Esterification of *S*-4-nitrophenylalanine[192]

Compound **218** was significantly more soluble in organic solvents than the free amino acid and it was hoped that this would improve solubility of the porphyrin conjugate as well. The reaction between **191** and *S*-4-nitrophenylalanine was carried out using the same one-step method that was used for the free amino acid. Although the reaction appeared to be slower, after 48 hours the starting material had been consumed and after chromatographic work up, the product **217** was isolated in 61% yield. ^{1}H NMR analysis was similar to compound **216** but there was a change to the signals for the proton on the chiral carbon (proton d) and the protons on the carbon adjacent to it (protons e and f in Figure 38). Protons e and f still showed double doublet signals, but their chemical shift values had converged, the overlapping signals giving the complex AB type signal shown.

They coupled together with a ^{2}J value of 13.3 Hz, but coupled with proton d with coupling constants of 6.7 Hz and 7.0 Hz. Due to these similar J values, the signal for proton d was seen as a triplet.



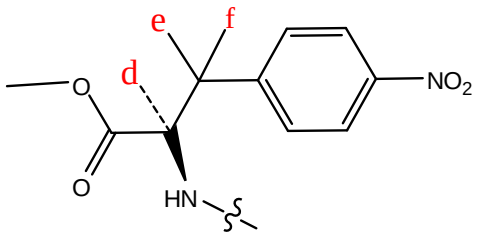
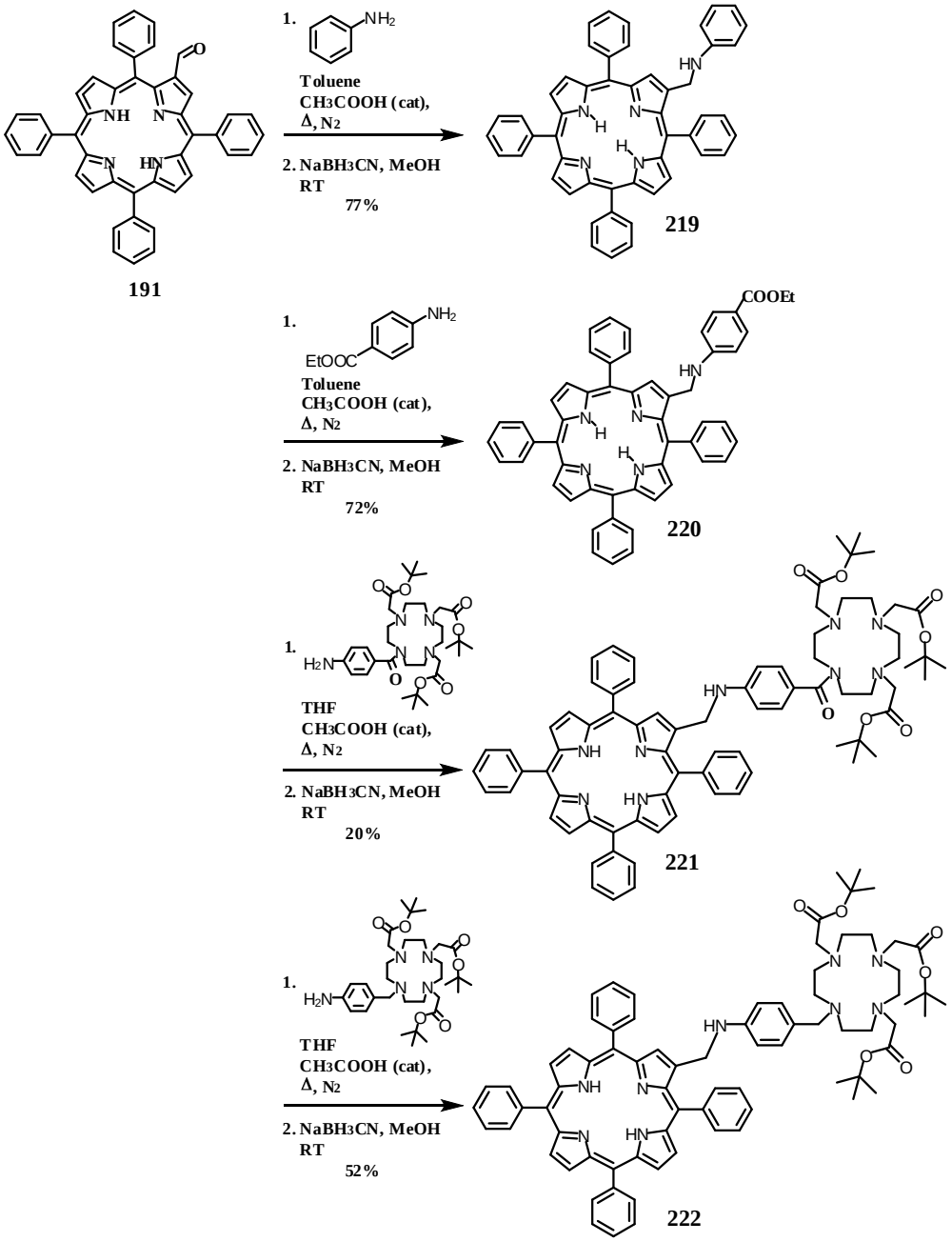


Figure 38: ^{1}H NMR analysis of compound 217

3.7.4 Reductive aminations with aryl amines

The reactions attempted with aryl amines are shown below.



Scheme 71: Reductive aminations with aryl amines

3.7.4.1 Reaction with aniline-synthesis of compound 219

Initially, aniline was reacted with compound **191** using the same one pot, two step method that had been used for the alkyl amines. The two reagents were heated in toluene with acetic acid as catalyst and a Dean Stark trap to drive the equilibrium forward. After 24 hours, TLC analysis of the reaction mixture showed almost all of the porphyrin starting material had been consumed, so the mixture was cooled to ambient temperature before the methanolic reductant solution was added. After stirring for a further 2 hours at room temperature, aqueous work up and chromatography gave the product in 77% yield. In this case there was no apparent degradation of the porphyrin on the silica gel column. The reaction was repeated but adding all the reagents at the start, including the reductant solution, but this gave a lower yield of 60%. Compound **219** gave a clearly resolved ^{1}H NMR spectrum, the relevant parts of which are shown, along with the peak assignments in Figure 39. All assignments were made by reference to the 2D DQF COSY spectrum. It can be seen that the β-substituent splits the ortho protons of the porphyrin phenyl rings into two signals integrating to four protons each (signals a and b). These have been assigned on the basis that the chemical shift of the protons nearest to the substituent will be affected the most, and these are likely to have the most well resolved fine structure. This assignment is also consistent with the assignment for the amino acid porphyrin derivative discussed above (compound **216**). The newly introduced phenyl ring substituent could be seen clearly from the peaks d, e and f and the methylene protons directly attached to the porphyrin β-carbon appear as the singlet g.

3.7.4.2 Reation with ethyl 4-amino benzoate-synthesis of compound 220

Since the one pot, two step procedure described above gave the highest yield of compound **219**, the same method was applied to the reaction with ethyl 4-amino benzoate. The reaction was followed by TLC anlaysis which revealed a high conversion to the imine intermediate after approximately 30 hours. After cooling to ambient temperature, the reductant solution was added and stirred for a further 2 hours. After aqueous work up, once again, there was no degradation of the

product as it was purified by silica gel column chromatography, and compound **220** was isolated in 72% yield. The ^{1}H NMR spectrum was very similar to compound **219** and was assigned again using 2D DQF COSY spectrum and high resolution mass spectrometry was used to confirm the identity of the product.

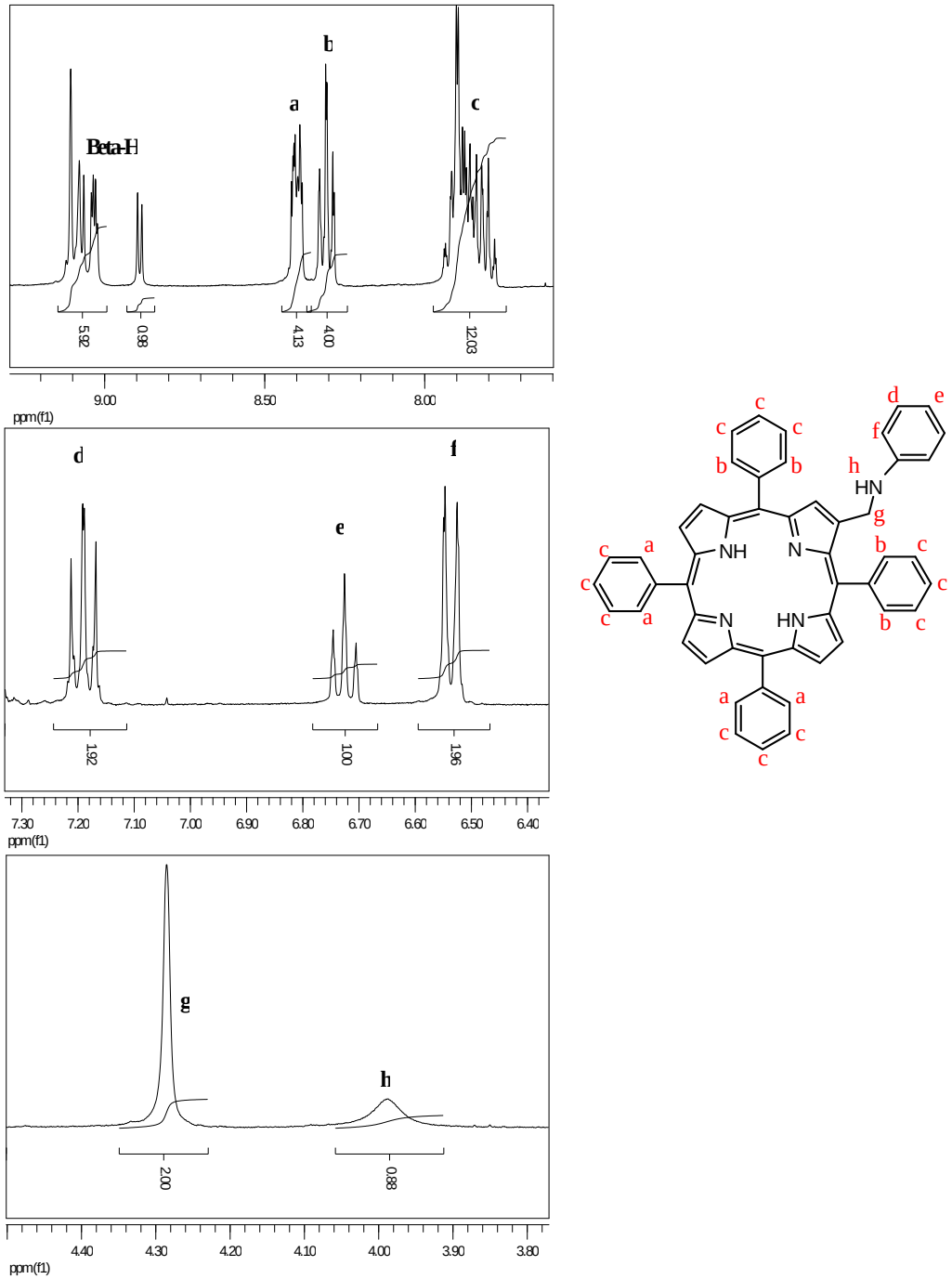


Figure 39 : ^{1}H NMR for compound 219

From the higher yields and the lack of any apparent complications due to degradation during work up and separation of the products, it appeared that aromatic amines were indeed a better choice of reagent for these reactions when compared to the alkyl amines previously tested. Hence, the two cyclen compounds **57** and **134** were chosen as the most likely candidates for direct coupling to the formyl porphyrin in this manner.

3.7.4.3 Reaction with 1,4,7-tris(*tert*-butoxycarbonylmethyl)-10-(4-aminobenzoyl)-1,4,7,10-tetraazacyclododecane, 134 –synthesis of compound 221

Compounds **134** and **191** were dissolved in anhydrous THF and a few drops of acetic acid were added. The choice of THF was due to compound **134** having low solubility in toluene. The reaction was closely monitored by TLC analysis. After heating for 24 hours, a mixture had formed but with a significant amount of starting material still present. The imine intermediate appeared to be present but in very low yield and no further change could be seen occurring. Some activated 4Å molecular sieves were added to the reaction in order to remove water from the equilibrium and hence increase the amount of imine formed. However, this did not have any noticeable effect on the equilibrium mixture. The reaction was allowed to cool, the methanolic reductant solution was added and after 2 hours of stirring, aqueous work up and silica gel chromatography the product was isolated in 15% yield. The reaction was repeated using a double excess of the amine starting material under the same conditions and this led to a lower yield (9%) of product being isolated. The highest yield was obtained when a equimolar ratio of **191** and compound **134**, the acid catalyst and the reductant, were all added at the start of the reaction in a mixture of THF and methanol (3:1 vol:vol). The reaction was allowed to progress for 72 hours, after which time no further change occurred. After aqueous work up, the product was isolated in a yield of only 20%. The low yields of these reactions could be a consequence of the low nucleophilicity of the aromatic amine in **134**. The lone pair on the nitrogen atom can be delocalised through the phenyl ring and onto the carbonyl oxygen atom, as shown in Figure 40, thus making it less available.

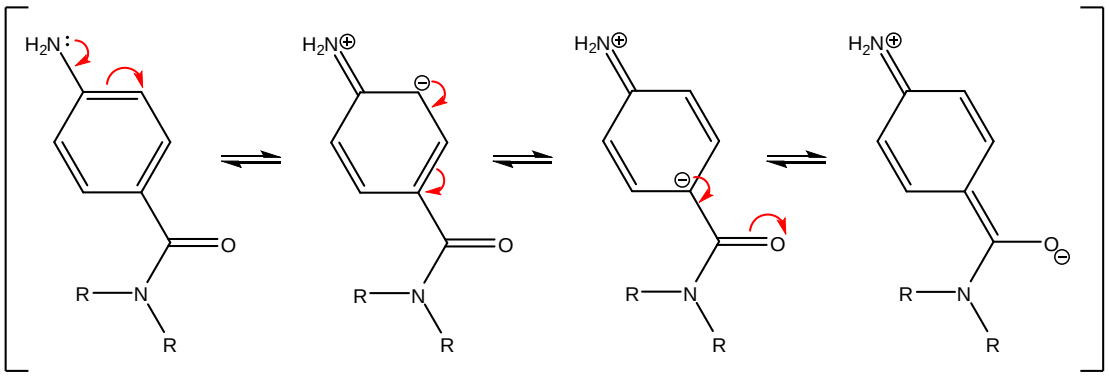


Figure 40: Delocalisation pathway in compound 134

As shown in Figure 41, the aromatic region of the ^{1}H NMR spectrum of compound **221** is very similar to that of compound **219**, which was expected since the porphyrin structures are almost identical. Peaks a and b represent the ortho protons of the phenyl substituents on the porphyrin, peak c is the meta and para protons on the same phenyl rings, and the β-protons appear in exactly the same pattern as compound **219**. The assignment of protons on cyclen / cyclam units is usually very difficult because the flexibility of the ring system leads to the appearance of very broad peaks in the spectrum. Figure 42 shows the ^{1}H NMR cyclen regions for the nitro precursor compound, the reduced amine and compound **221**. In the nitro compound, the electron withdrawing nature of the nitro group confers a visible fine structure to the peaks which is not seen in the other two compounds. 2D COSY NMR shows that peak a couples with peak e, and b couples with f. Protons c and d, as expected, show no coupling partners, and multiplet g shows coupling within the signal. Protons a and b are therefore assigned to the protons nearest to the amide bond, due to having the highest chemical shift. Their coupling partners e and f are assigned to the methylene groups adjacent to the protons a and b. Singlet c integrates to 4 protons and is assigned to the two methylene groups on the pendant arms, while peak d is assigned to the remaining protons on the third pendant arm. These signals are split due to the symmetry of the molecule. Multiplet g represents the remaining eight ring protons which cannot be assigned individually. When this spectrum is compared to the analogous amino compound it can be seen that the electron releasing amino group causes the peaks to converge and they now appear as

broadened signals. Peaks a and b have converged as have e and f leaving two broad peaks integrating to four protons each. The 2D spectrum confirms that these two peaks still correlate. Peak g has become a more broadened signal also, losing the fine structure that could be seen in the nitro compound. The singlet c still remains fairly distinctive, but the singlet d has now collapsed into a broadened signal. The porphyrin derivative, compound **221**, has similar signals which can only be assigned by this retrospective analysis of the precursor compounds.

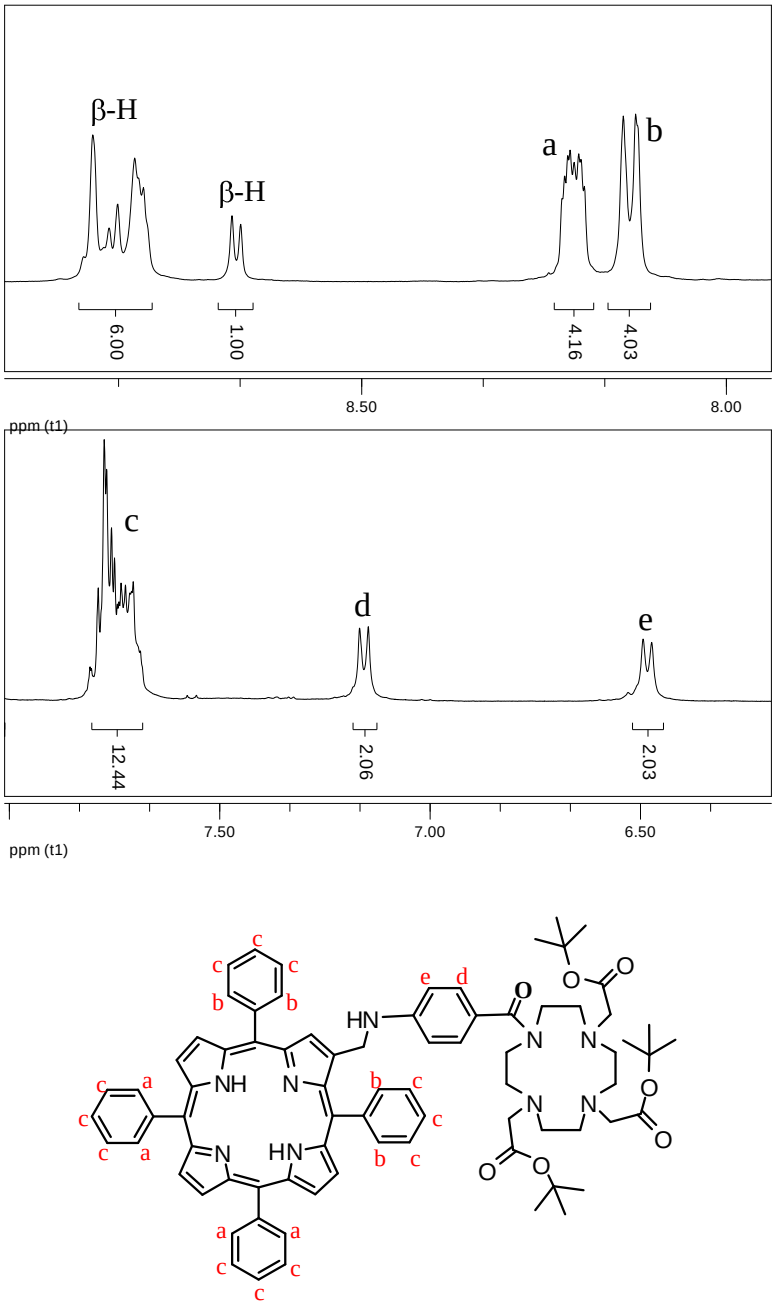


Figure 41: ^{1}H NMR of compound 221

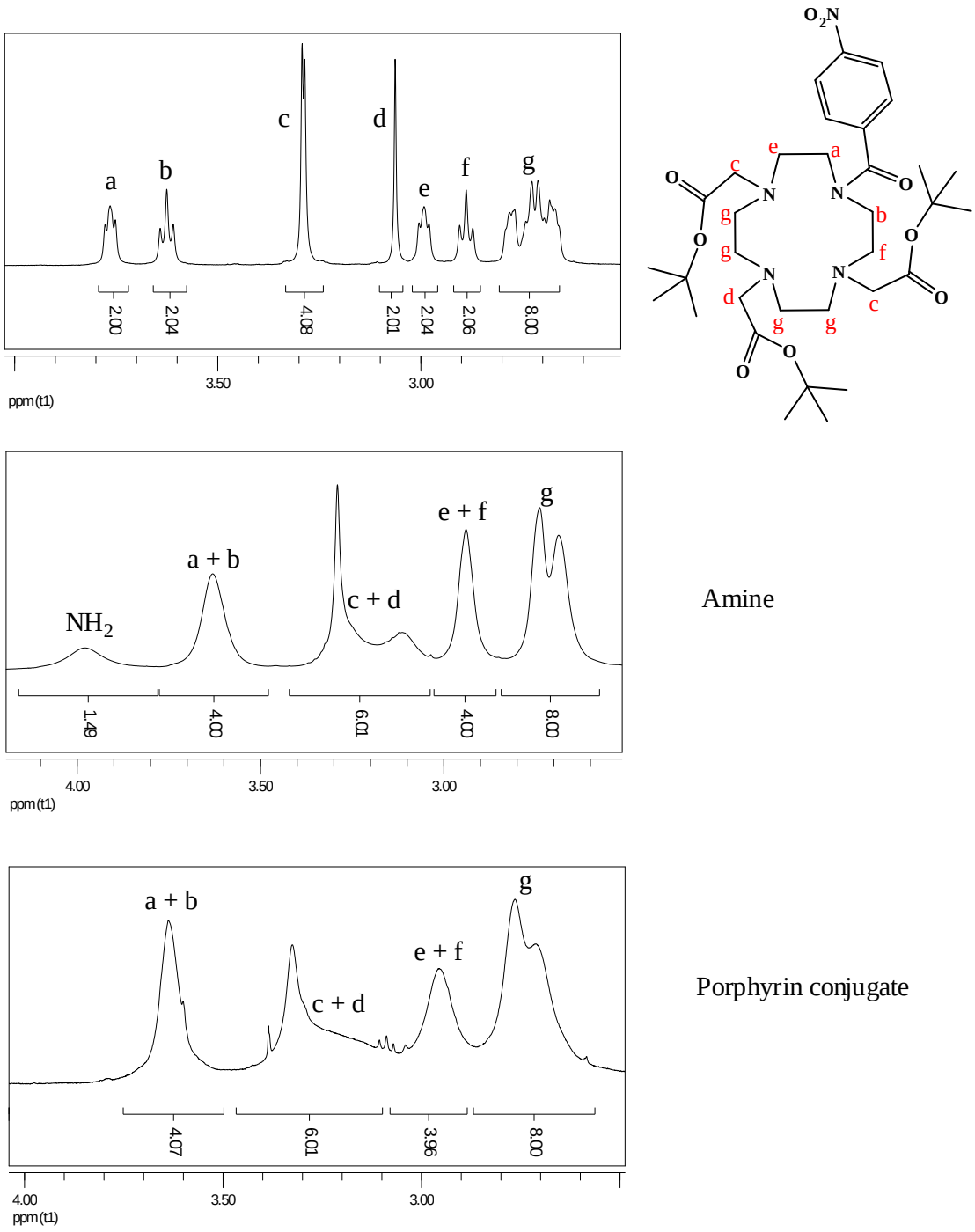


Figure 42: ^{1}H NMR comparison between compound 221 and the analogous precursor compounds

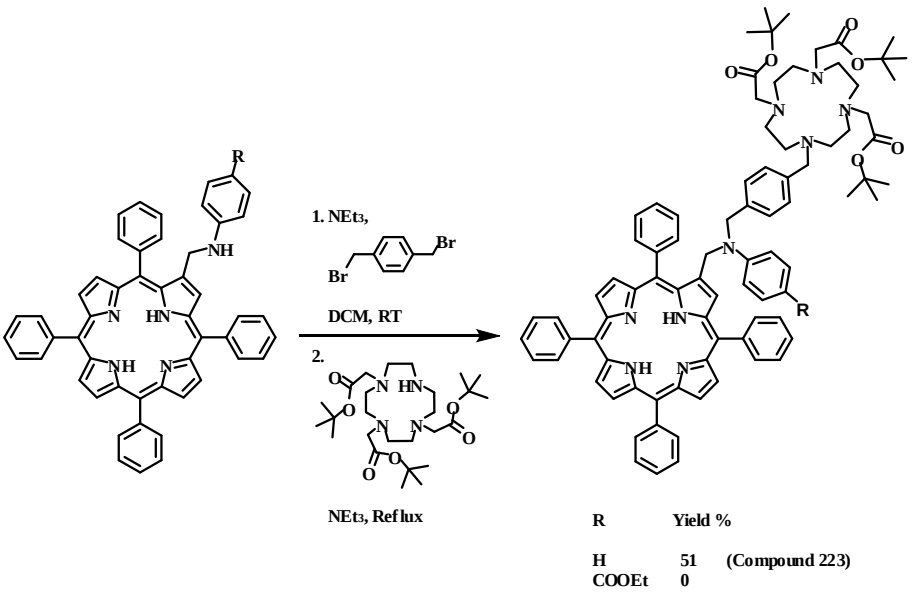
3.7.4.4 Synthesis of compound 222 - Reaction with 1,4,7-tris(*tert*-butoxycarbonylmethyl)-10-(4-aminobenzyl)-1,4,7,10-tetraazacyclododecane –compound 57

The reaction with compound **57** was performed using the same conditions as for the synthesis of compound **221**. An equimolar ratio of compounds **191** and **57** was used and the reductant was added at the start of the reaction. After heating for two days, TLC analysis showed a more promising reaction, with a much higher conversion of starting materials apparent. However, after aqueous work up, significant problems were encountered when isolation of the product was attempted. The purification was carried out by column chromatography on silica gel, using an eluent of 4% MeOH in CH_2Cl_2. A purple band was duly collected which corresponded to the expected fraction, but upon further TLC of this fraction, two overlapping spots appeared. A 2D TLC plate showed that the faster running spot always seemed to split into two spots as it travelled up the plate, indicating that the compound may be unstable on silica. An attempt at purification with alumina (both basic and neutral) did not yield the separation required even after several attempts with different eluent systems. However, after repeating the reaction, it was found that pure product could be obtained by using a silica column which had been pre-eluted with a solution of CH_2Cl_2 and a few drops of triethylamine. Flash chromatography on a very short column allowed access to the pure compound which was identified by NMR analysis. The compound appeared to be stable on this column for short periods of time only, circa ~30 mins. After this purification, the yield obtained was 52% which was an improvement over compound **221**, but was disappointing when compared to the yield anticipated from TLC of the reaction mixture. The ^{1}H and ^{13}C NMR were assigned using 2D correlation spectra and by comparison to precursor compounds as for compound **221**.

3.7.4.5 Further reactions of compounds 219 and 220

Shinkai *et al.*[186] synthesised the copper(II) complex of compound **219** as shown in Scheme 61 and used the 2° aromatic amine to further functionalise the

porphyrin with benzyl substitutents. Thus, it was of interest to investigate whether the free base porphyrins **219** and **220** would react in a similar fashion, allowing incorporation of a cyclen unit onto the porphyrin structure. As shown in Scheme 72, compound **219** was dissolved in CH_2Cl_2, a few drops of NEt_3 were added followed by one equivalent of dibromoxylene. The reaction mixture was stirred at room temperature and, after consumption of the starting material was seen by TLC, a CH_2Cl_2 solution of compound **127** was added and the reaction heated under reflux. TLC analysis indicated several products being formed, one of which showed the correct polarity for the desired product. Another non-polar porphyrin spot was also formed which was consistent with the porphyrin dimer. After aqueous work up and chromatographic separation, the more polar fraction was collected and identified as compound **223** by NMR analysis. The overall yield was 51%.



Scheme 72: Further derivatisation of compounds 219 and 220

However, the result was unsuccessful when the same procedure was undertaken with compound **220**, using the same reaction conditions. TLC analysis showed no consumption of either compound **220** or the dibromoxylene. When the reaction was repeated using K_2CO_3 and DMF, after one hour TLC monitoring showed consumption of the dibromoxylene starting material but no new

porphyrin formation. After cooling, an aliquot was removed and the porphyrin isolated by column chromatography was found to be the starting material. This leads to the conclusion that the electron withdrawing nature of the ethyl ester group in compound **220** reduces the nucleophilicity of the amine to the extent that it does not react with the dibromoxylene.

3.7.4.6 Attempted hydrolysis of compounds 222 and 223

In both compounds **222** and **223**, the *tert*-butyl ester groups needed to be hydrolysed in order to allow synthesis of a metal ion complex of the cyclen based ligand. The two common literature procedures for this involve acidic hydrolysis using TFA in dichloromethane at room temperature or heating under reflux with 6M HCl.[105, 193] The milder TFA procedure was chosen, and compound **222** was dissolved in CH_2Cl_2. After addition of TFA, the reaction was closely monitored by TLC. However, after only 5 mins TLC indicated possible degradation of the porphyrin starting material. One major porphyrinic spot developed with a significantly lower polarity in comparison with the starting material. After aqueous work up, the porphyrin was still soluble in organic solvent. ^{1}H NMR analysis of the crude organic layer showed that although it contained a β-substituted porphyrin, no other peaks confirming the presence of the cyclen moiety could be distinguished. In addition to this, the polarity change indicated that the compound did not contain the cyclen moiety. Several repetitions of the reaction with varying amounts of TFA gave the same outcome, as did heating with 6M $HCl_{(aq)}$.

The reaction with compound **223** using the same conditions as for compound **222** gave similar results. Neither of the porphyrin products could be positively identified by NMR or mass spectrometry analysis, but the lack of any peaks relating to the cyclen moiety leads to the hypothesis that the compounds are cleaving at the benzylic position α to the 2-position of the porphyrin ring. Cleavage at another position would most likely leave an amino substituent on the porphyrin, giving rise to a more polar fraction when analysed by TLC.

3.8 Summary

A methodology has been developed to monofunctionalise tetraphenyl porphyrin, a symmetric molecule, via a known reaction sequence (β-formylation followed by reductive amination) with a variety of substrates, giving access to a range of previously unreported compounds (**216**, **217**, **219**-**223 -** Figure 43).

This work has shown that reductive amination on 2-formyl-TPP can be used to introduce a range of functionality and diversity into the porphyrin core.
As expected, aryl amines seem to be more suited to this type of reaction than alkyl amines, with the notable exceptions of the amino acid derivatives **216** and **217**.
The reactions work with substrates that contain additional reactive groups that may be used to further elaborate the porphyrin structure, and this was demonstrated by the transformation of the aniline derivative **219** into the cyclen containing conjugate, compound **223**.

Direct reductive amination of an amino bearing macrocycle with the formyl porphyrin offers easy access to bismacrocyclic systems, and this was demonstrated by the synthesis of target molecules **221** and **222**.

However, the conjugates **222** and **223** were not stable under the acidic conditions required for deprotection of the *tert*-butyl esters, and so the gadolinium(III) complexes could not be formed.

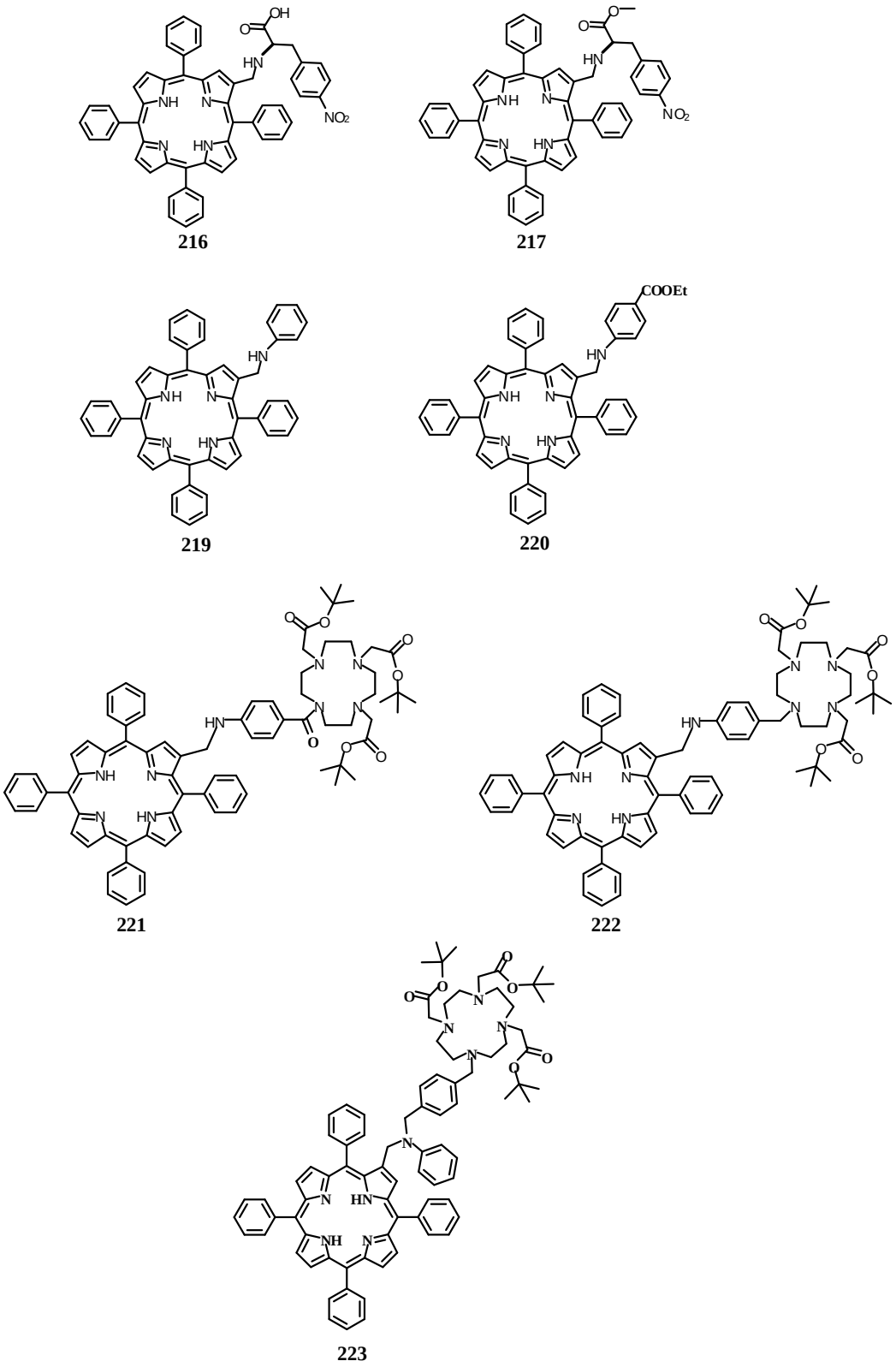


Figure 43: Novel compounds synthesised by the reductive amination of 2-formyl TPP

Chapter 4: Coupling the cyclam/cyclen moieties to a porphyrin via the phenyl ring of a tetraaryl porphyrin

4.1 Introduction

The cyclen/cyclam based compounds synthesised previously (compounds **56**, **57**, **58**, **59**, **60**, **134**, **137** and **138**) are derivatised with amino and aryl bromide groups, so the requirement was for a porphyrin to be synthesised containing a functional group complementary to these.

In the previous chapter, work was described that involved the monofunctionalisation of a symmetric porphyrin with a reactive formyl group, and the subsequent reactions used to couple this porphyrin with the cyclam/cyclen compounds. The alternative option was to incorporate an unsymmetrical substitution pattern at the point of porphyrin formation, a methodology which would allow more diversity to be incorporated into the porphyrin structure.

Hence, the work here describes the syntheses of porphyrins using this approach, and their subsequent coupling reactions with the cyclam/cyclen derivatives.

4.2 Formation of unsymmetrically substituted meso-tetraarylporphyrins

4.2.1 Alder-Longo method[124]

This type of reaction was discussed in the previous chapter in relation to the synthesis of symmetric porphyrins. It can also be used to synthesise unsymmetrically substituted porphyrins by condensing pyrrole with two different aldehydes. In this situation there are, in principle, six porphyrin products obtained as shown in Figure 44. If an equimolar ratio of aldehydes is used and the aldehydes are equally reactive, then the yields of these porphyrins follow a binomial distribution with the expected yields shown in Figure 44. By using approximately a 3:1 ratio of aldehydes, the AB_3 or the A_3B porphyrin yields may be maximised to around 40%. However, it must be remembered that these yields are percentages of the total porphyrin yield which is of the order of 20% of the total reaction yield and in practise the isolated yield of the A_3B porphyrin is often around 5%.

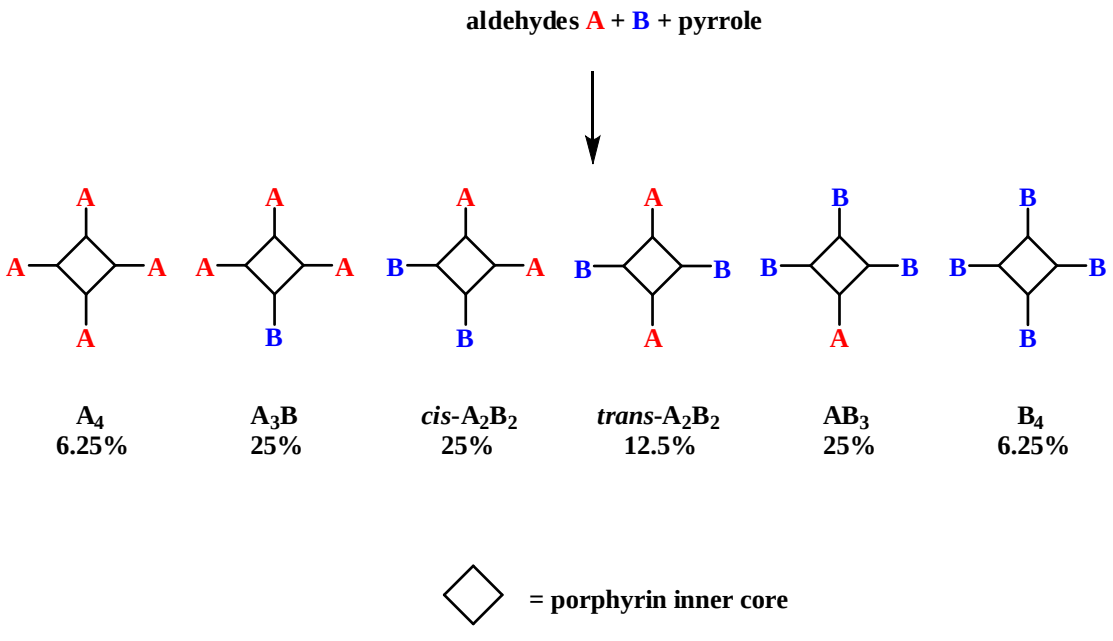
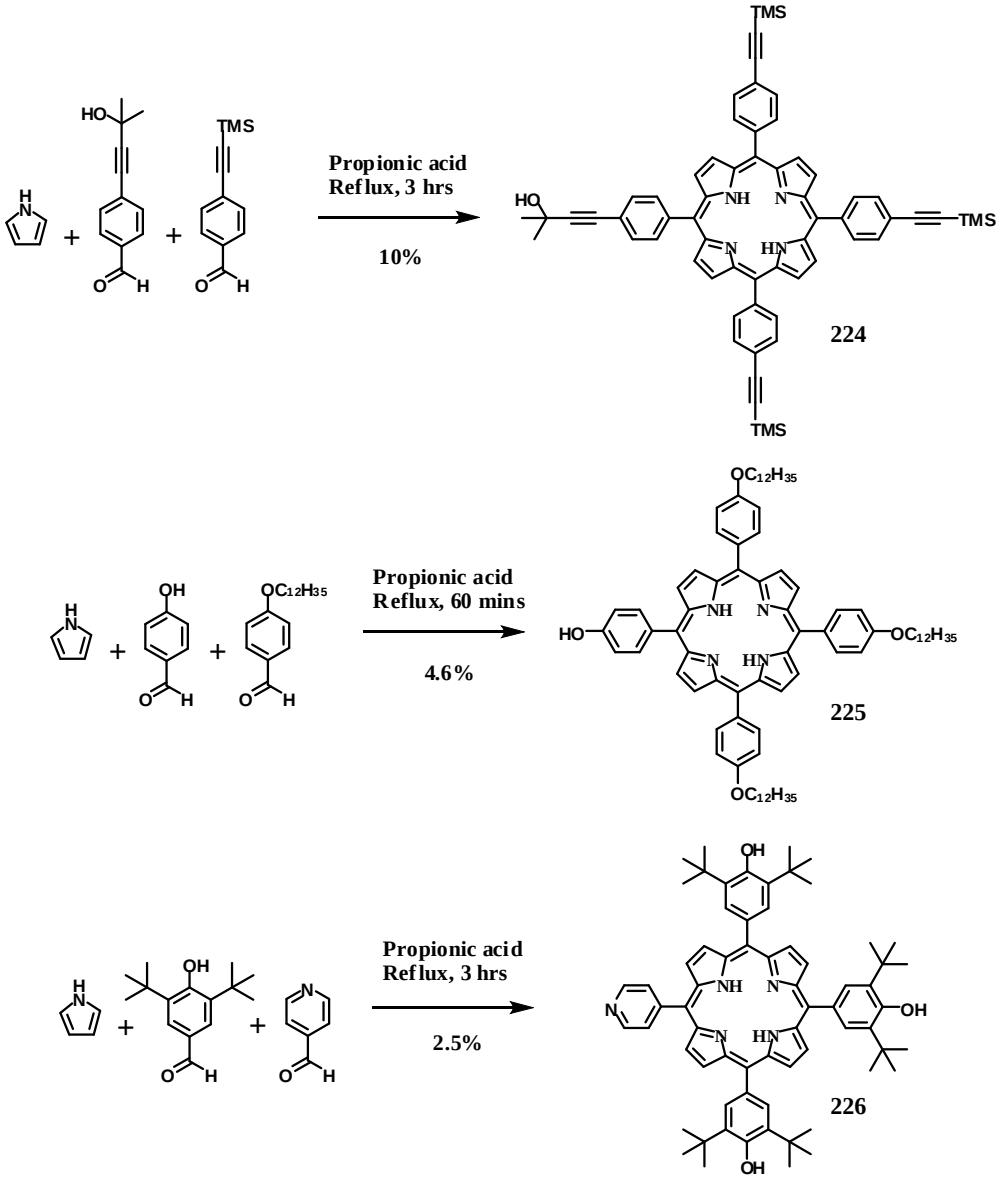


Figure 44: Products formed from a mixed aldehyde porphyrin synthesis

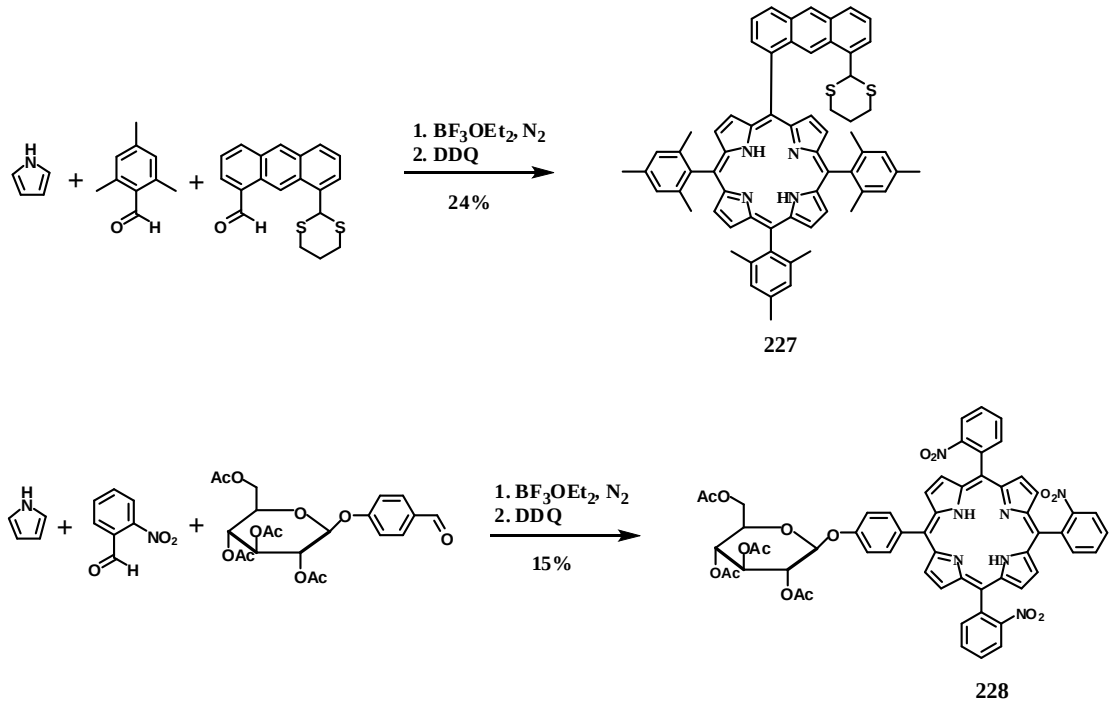
The main drawback to mixed aldehyde condensations using Adler[124] conditions is the amount of tarry side products produced leading to extensive chromatography to isolate the desired porphyrin fraction from the mixture produced. The success of such chromatography may depend on the difference in polarity between the aldehydes used. The relatively wide scope of the Adler reaction is limited by the stability of the aldehydes used under the boiling propionic acid conditions. In spite of this, many porphyrins bearing a wide range of functional groups have been synthesised this way and some examples from literature reports are shown in Scheme 73 (compounds **224**-**226**).[194-196]



Scheme 73: Examples of unsymmetric porphyrins formed by mixed Adler condensations[194-196]

4.2.2 Lindsey method[139, 140]

As discussed in the previous chapter, the Lindsey reaction[139] is more tolerant of sensitive benzaldehydes than the Adler[124] method due to the milder conditions under which the reaction proceeds. By using two different benzaldehydes in the correct ratio, this method can also be used to synthesise asymmetric porphyrins.[150, 197-200] Two examples of porphyrin synthesis using Lindsey conditions which illustrate the advantages of this methodology over the Adler method are shown in Scheme 74. Fish *et al.*[150] exploited the mild Lindsey conditions to synthesise porphyrin **227** which contained the dithianyl group-an acid sensitive moiety which is unlikely to be amenable to the harsher Adler conditions. Krausz *et al.*[197] used the same methodology to obtain the asymmetric porphyrin **228**, containing an acetyl protected hexose, in 15% yield. They also reported the synthesis of this compound using Adler conditions, but the yield was only 4-6%, thus nicely illustrating the advantage of using the Lindsey synthetic route.

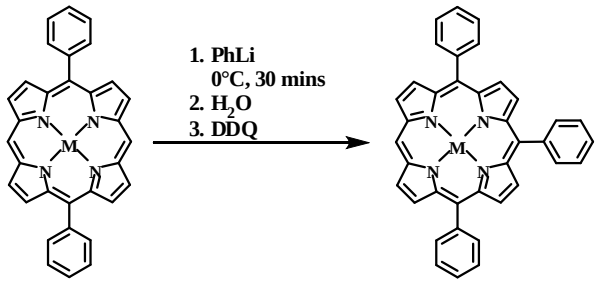


Scheme 74: Examples of unsymmetric porphyrins synthesised by the Lindsey method[150, 197]

The Lindsey conditions fail when the aldehyde is insoluble in either CH_2Cl_2 or $CHCl_3$ and the other main practical drawback is that due to the high dilution conditions required, it is only of realistic value for making porphyrins on a scale of less than 1g.

4.2.3 Unsymmetrically substituted porphyrins via nucleophilic substitution

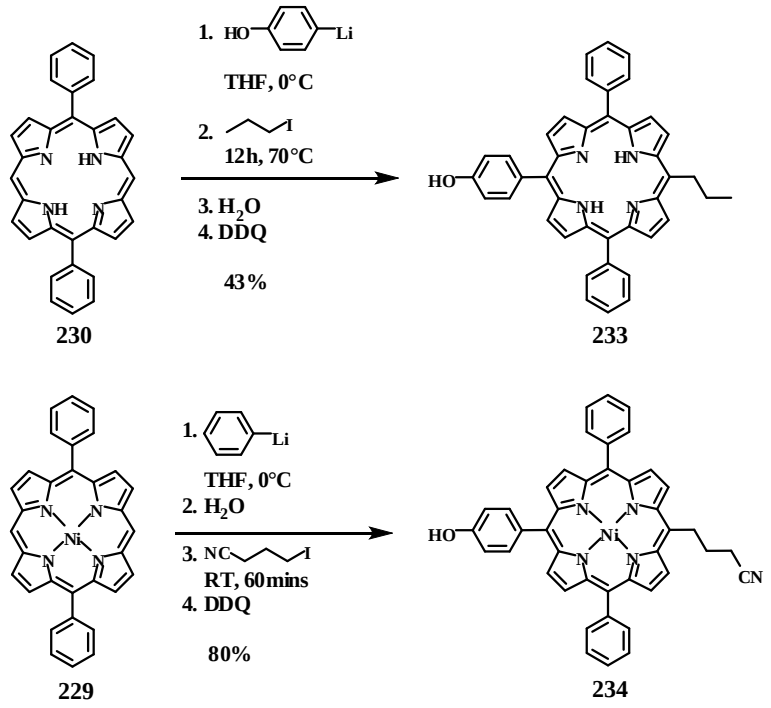
An alternative versatile route to unsymmetric porphyrins has been developed by Senge *et al*.[201] which exploits nucleophilic substitution at the *meso* positions of a preformed porphyrin. As shown in Scheme 75, treatment of 5,15-diphenylporphyrin with phenyllithium followed by hydrolysis and oxidation gave access to the 5,10,15-triphenylporphyrin, both as the nickel(II) complex **231** and in the free base form **232**, in high yields.[202]



Scheme 75: Formation of 5,10,15-Triphenylporphyrin[202]

The reaction is successful with a wide range of substituents on the lithium reagent, such as ethers, alcohols, amines and dioxolane groups.[201] This methodology was subsequently developed to allow two successive substitutions at the meso positions as shown in Scheme 76.[203, 204] As these examples illustrate, the reaction can be achieved using either the free base **230** or the nickel(II) complex of the porphyrin **229**. The order in which the reagents are added and the reaction conditions are, however, different in each case. For the free base porphyrin **230**, successive treatment of a THF solution of the porphyrin with an

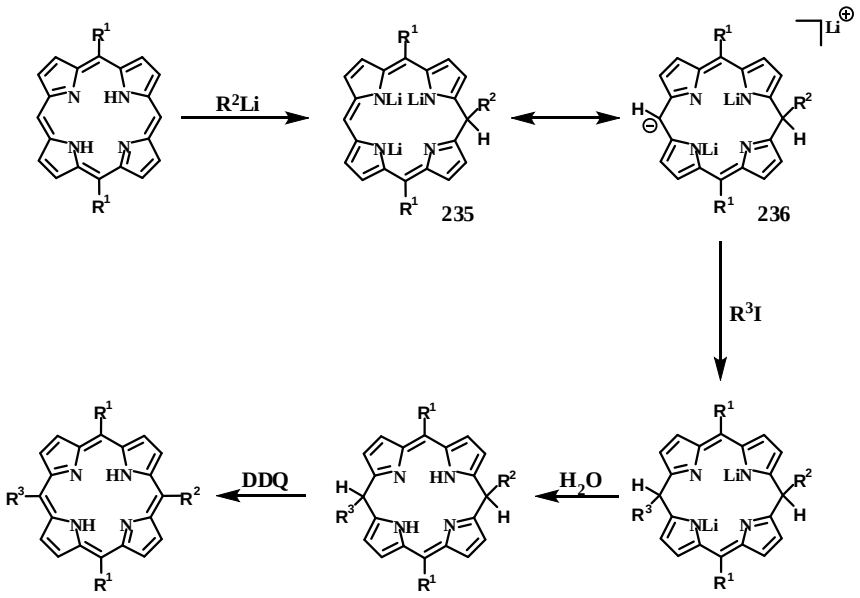
organolithium reagent, organic iodide, water and oxidant (DDQ) gave access to A_2BC type porphyrins eg **233**.[204] Although the lithiation is carried out at 0 °C, after addition of the organic iodide, the reaction mixture is heated to 70 °C for 12 hours before hydrolysis and oxidation. In the case of the nickel(II) complex **229**, the hydrolysis is carried out prior to the addition of the organic iodide after which the reaction is stirred for 60 minutes at ambient temperature before oxidation.[203]



Scheme 76: Synthesis of porphyrins bearing an A_2BC substitution pattern[203, 204]

These differences in the reaction sequence and conditions can be understood by the mechanism of the reaction. Senge *et al.* proposed the mechanism shown in Scheme 77 for the reaction with the free base porphyrin.[205] The initial addition of the lithium reagent gives the phlorin type intermediate **235**, which they believe to have a planar conformation giving an sp^2 character to the carbon on the 20 position of the porphyrin ring.[206] The resonance hybrid **236** carries a negative charge on this carbon, and is thus sp^3 hybridised, giving it nucleophilic character, and it is this form which is thought to attack the organic iodide electrophile. Experiments done by the Senge group showed that the nucleophilic form **236** is

formed when the metal ion complex of the porphyrin is lithiated, whereas the non-nucleophilic form **235** is formed when the free base porphyrin undergoes the same procedure.[205] Hence, the nickel(II) porphyrin can be alkylated at ambient temperature after hydrolysis, whereas this procedure with the free base porphyrin results in no reaction. However, by raising the temperature of the alkylation to 70 °C, the desired product was duty formed after subsequent hydrolysis and oxidation. The conclusion is that **235** is the kinetic intermediate product of the free base reaction, whereas **236** is the thermodynamic product. By giving the reaction mixture extra energy by heating, it allows the nucleophilic inermediate **236** to form which then attacks the electrophile.



Scheme 77: Postulated mechanism of disubstitution of free base 5,15-diarylporphyrins[205]

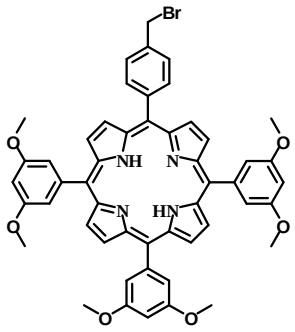
Using this synthetic route, several A_2BC type porphyrins have been reported with a wide range of functionality incorporated onto the meso substituents of the phenyl rings,[201] and these porphyrins are finding uses as building blocks for many types of porphyrin based systems.[207] This methodology gives the advantage of a much higher yield of unsymmetrical porphyrins in comparison with the mixed condensations of the Adler and Lindsey routes. Additionally, the basic conditions used allow diversification of the porphyrin with functional

groups which are not tolerated by the acidic condensation reactions otherwise used.

It should be noted for completeness that several other synthetic routes towards unsymetrically substituted porphyrins exist, whereby the porphyrin macrocycle is built up from its component parts, such as the MacDonald 2+2[208] or rational total syntheses.[209] Taking into account the aims of this project, these are not considered further here.

4.3 Aim

An unsymmetrically substituted porphyrin with an electrophilic group that could react with the amino bearing cyclam/cyclen compounds already synthesised was the initial synthetic target. It was also desirable to have additional functionality on the porphyrin periphery that could be used to attenuate aqueous solubility of the final coupled target compound if required. To these ends, it was decided that compound **237** (Figure 45) was the desired initial target molecule.



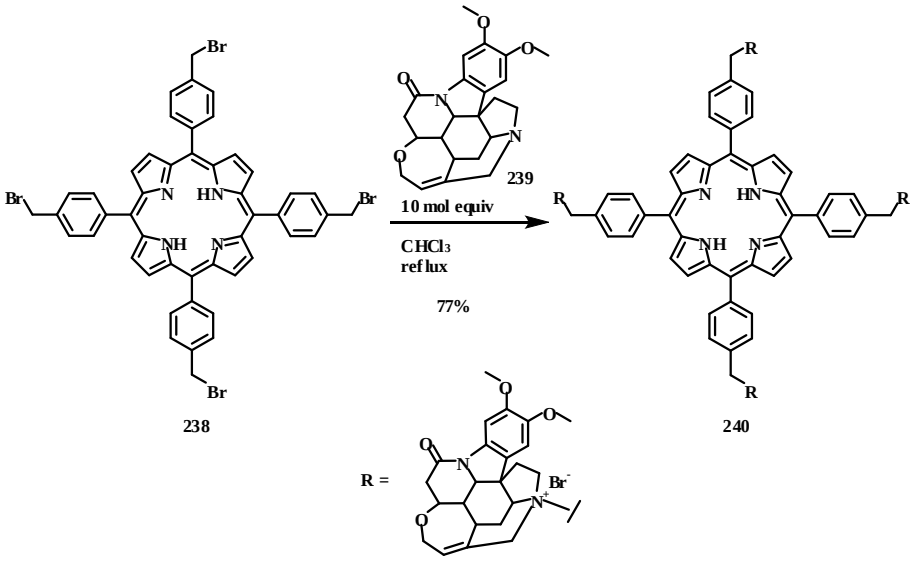
237

Figure 45: Target porphyrin compound containing an electrophilic group

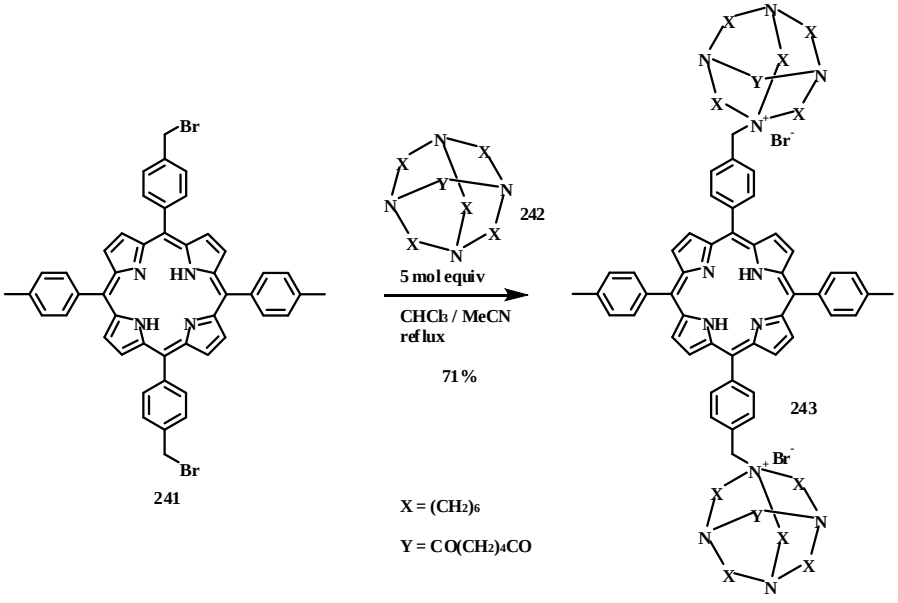
The 1° alkyl bromide group was chosen as it was expected to have the desired reactivity towards the amino bearing cyclam/cyclen macrocycles **56**, **57**, **134**, **137** and **138**. The methoxy substituents are protected hydroxyl groups which give an option for increasing the aqueous solubility of the final coupled compound.

4.4 Literature reports on nucleophilic substitution reactions of halomethyl porphyrins

Substitution reactions of halomethyl porphyrins with various nucleophiles have been reported in the literature[210-212] including two papers where the nucleophile was a sterically bulky amine are shown below in Scheme 78 and Scheme 79.



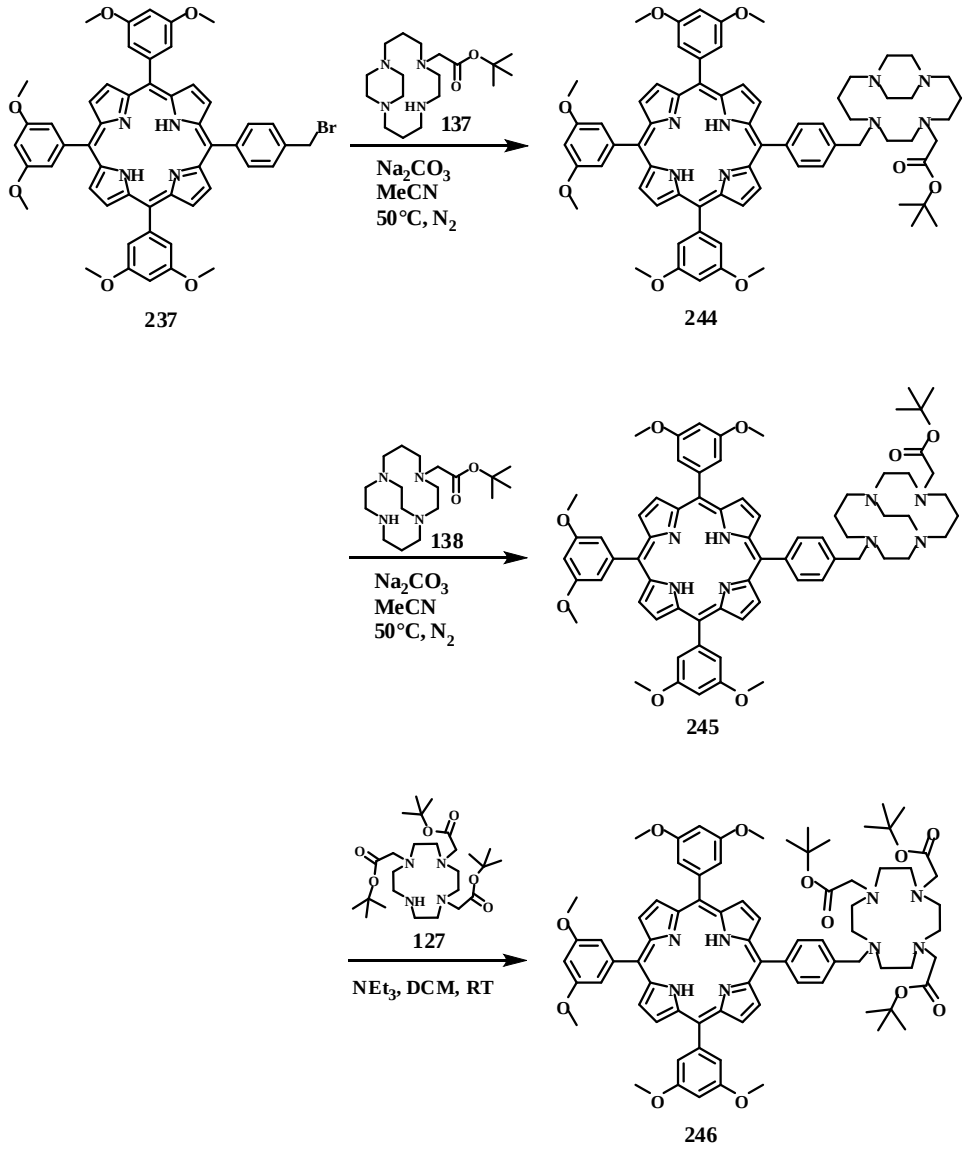
Scheme 78: Reaction of tetra-(4-bromomethyl)phenyl porphyrin with brucine[211]



Scheme 79: Reaction of a dihalomethyl porphyrin with an amido-azonia cryptand[210]

As shown in Scheme 78, Král *et al.*[210] mixed the halomethyl porphyrin **238** and an excess of brucine **239** in chloroform and heated the solution under reflux for 24 hours. No auxiliary base was used and the ionic product **240** precipitated from solution, allowing easy isolation and purification. The same group also reported the dialkylation of porphyrin **241** with the cryptand **242** as shown in Scheme 79.[211] This time the methodology involved the slow addition of a chloroform solution of the porphyrin to an excess of the cryptand in acetonitrile over 8 hours. Once again, no auxiliary base was used and the product **243** was isolated in good yield (71%).

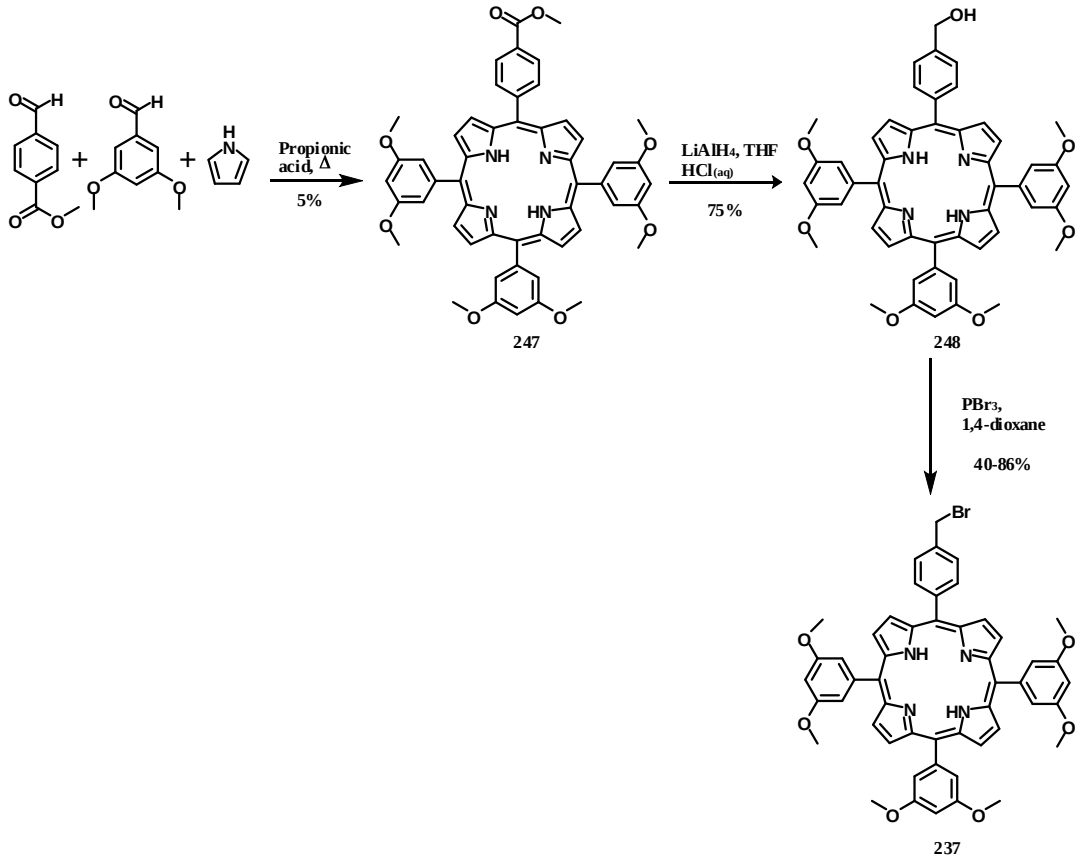
These two reports clearly showed that it was feasible to react the cyclam and cyclen based macrocycles **127, 137** and **138** directly with the bromomethyl porphyrin **237** (Scheme 80). Reaction of these amino containing macrocycles with primary alkyl bromides have been reported in the literature[105, 109] and as discussed in chapter 2, the conditions often used are an equimolar ratio of amine to electrophile and an excess of auxiliary base (usually Na_2CO_3 / K_2CO_3 or NEt_3), and heating to 60-70 °C in anhydrous MeCN solvent.[85, 90, 105] Hence with these two differing scenarios in mind, Scheme 80 shows the reactions which were attempted to couple the porphyrin **237** to the cyclam/cyclen based compounds **127**, **137** and **138**.



Scheme 80: Reactions undertaken between porphyrin 237 and compounds 127, 137 and 138

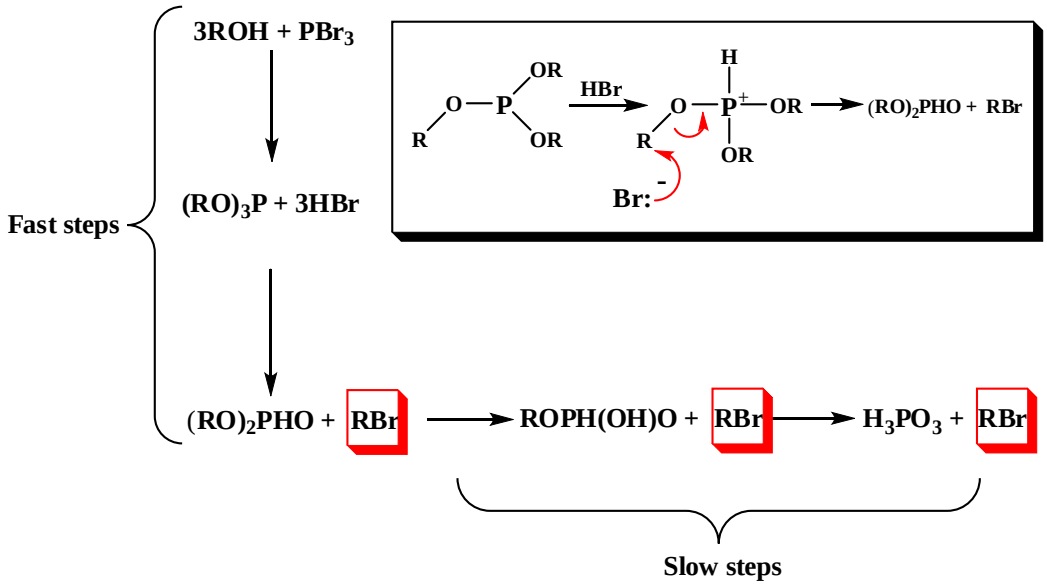
4.5 Results and discussion of coupling reactions via nucleophilic substitution reactions

Porphyrin **247** was synthesised by condensation of methyl(4-formyl) benzoate and 3,5-dimethoxybenzaldehyde with pyrrole under Adler-Longo[124] conditions (Scheme 81). After chromatographic separation, pure **247** was obtained in 5% yield and treated with $LiAlH_4$ in dry THF to give the hydroxy porphyrin **248** in 75% yield. Bromination of porphyrin **248** with phosphorus tribromide proceeded with a large variation of yields, despite using the same reaction conditions. The reaction was performed four times with the lowest yield of 40% and the highest 86% of compound **237**. Each time the side product was collected and it appeared to be a very polar porphyrin species. NMR analysis of the side product proved inconclusive, but a peak of 1616 *m/z* from mass spectrometry suggested a dimeric porphyrin species.



Scheme 81: Synthesis of the bromomethyl porphyrin 237

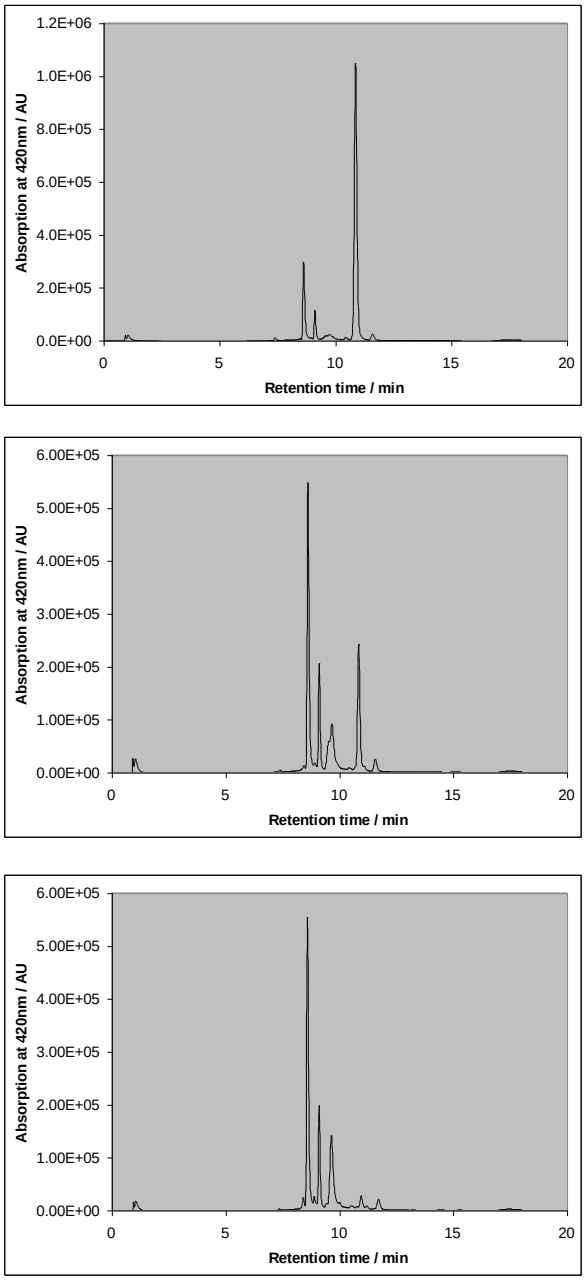
The reaction of phosphorus tribromide with an alcohol requires the alcohol to be in a three fold excess over the brominating agent, and proceeds through creation of an alkoxy tervalent phosphorus species, which is then dealkylated by hydrogen bromide created *in situ* (Scheme 82).[213] One third of the alcohol is converted to the bromide in a fast step, and further reaction converts the further two thirds, but in two slower, rate determining, steps.



Scheme 82: Mechanistic steps in the bromination of an alcohol by phosphorus tribromide[213]

With the electrophilic porphyrin **237** in hand, the next step was to attempt to attach it to a cyclam/cyclen macrocycle. The initial nucleophilic substitution reactions undertaken using porphyrin **237** are shown in Scheme 80.

A typical protocol[105, 109] was adopted for the reaction between compounds **237** and **137**. Both reactants were dissolved in anhydrous MeCN, excess Na_2CO_3 added and the mixture heated at 50 °C under an inert atmosphere. TLC analysis showed the reaction progressed quite slowly, but after 4 hours consumption of the starting material became apparent. The reaction was also monitored by HPLC as shown in Figure 46.



HPLC system : C18 column, linear gradient elution (100% water to 100% MeOH over 20 mins), UV/vis detector 420nm

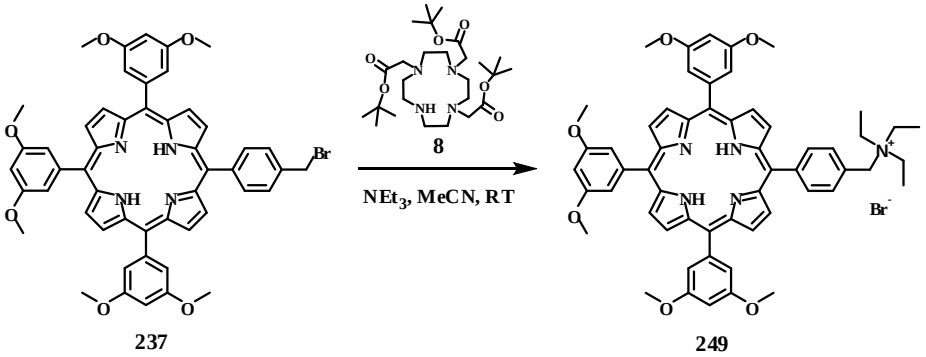
Figure 46: Formation of compound 244 shown by HPLC

The peak showing a retention time of 11 minutes corresponds to the starting material, compound **237**, which is consumed over a period of three days with the production of three new compounds. Upon complete consumption of compound **237**, silica gel chromatography was used to isolate the three products. The first fraction isolated turned out to be the hydroxymethyl porphyrin (compound **248**), indicating nucleophilic attack by hydroxide ion on compound **237**. The second

fraction appeared to decompose on the column and it was only possible to isolate a small amount (~1mg). ^{1}H NMR suggested this was a mixture, but did show the presence of the expected peaks for the desired product **244**. A sample was thus sent for analysis by mass spectrometry and the major peaks present were m/z 1147 and 1160, M^{+} and $(M+Na)^{+}$ respectively, for the desired compound **244**. The third fraction could not be recovered from the column. These results showed that compound **244** was possibly being formed in the reaction, but was too unstable to be isolated in reasonable quantities by chromatography on silica gel.

When the reaction was repeated using the cross bridged cyclam analogue **138** with the same procedure and conditions, the results were similar. A porphyrin fraction of the expected polarity accounted for approximately 50% of the reaction yield by TLC analysis. However, when isolation was attempted, this compound degraded and only a small amount could be collected. Once again, ^{1}H NMR showed this to be an impure fraction, but it contained the desired compound **245**. A significant amount of the hydroxymethyl porphyrin **248** was also recovered.

These two attempts indicated two problems, firstly the products were unstable on silica and a significant side reaction was occurring. The major side product was most likely due to the inorganic base producing hydroxide ions which preferentially attacked the porphyrin, generating compound **248**. The next reaction was attempted using the more readily accessible cyclen derivative **127** and the base used was changed to triethylamine in order to try to solve this problem. An excess of compound **127** was dissolved in CH_2Cl_2 along with compound **237** and one molar equivalent of NEt_3 was added. The reaction appeared to occur readily at room temperature and one major product had formed after 3 hours. However, upon isolation this compound turned out to be the triethylamine adduct of the porphyrin as shown in Scheme 83. The yield of the undesired compound **249** was 65%, so clearly the triethylamine was considerably more nucleophilic than the macrocycle **127**. The minor product was also collected and although analysis by ^{1}H and ^{13}C NMR analysis alongside ESI mass spectrometry confirmed its identity as the desired compound **246**, the isolated yield was only ~1%.

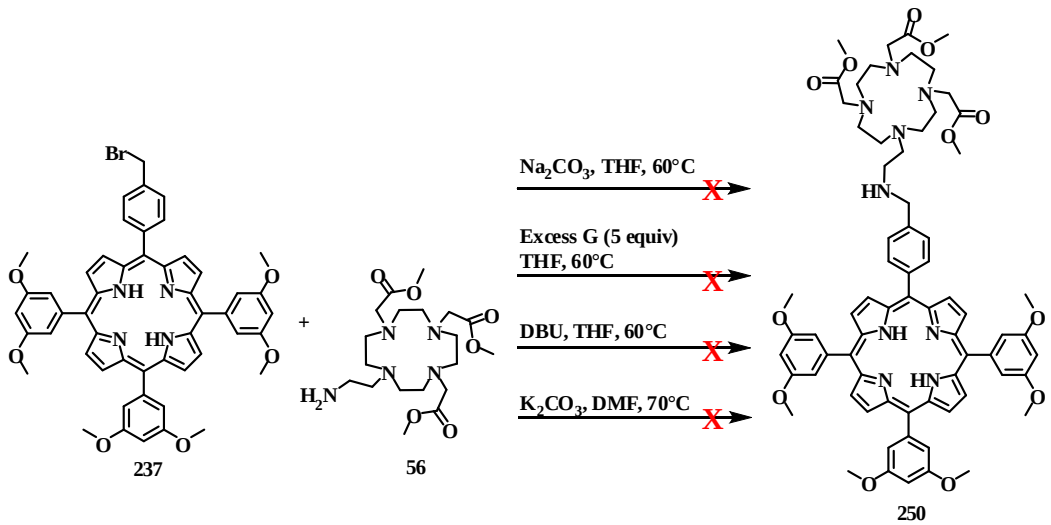


Scheme 83: Formation of triethylamine adduct of compound 237

The reaction was repeated with a 5 times molar excess of compound **127** but without the auxiliary base present. TLC showed no reaction progress after five hours heating under reflux conditions, so one equivalent of inorganic base (K_2CO_3) was added and the porphyrin starting material was quickly consumed. Unfortunately, the main product was again the hydroxymethyl porphyrin, compound **248**. A series of small scale reactions using THF, CH_2Cl_2 and toluene as solvents with inorganic base gave similar results. A final attempt was made using pyridine as solvent with no added base and although this led to some product appearing on TLC, the reaction was very slow and decomposition of compound **127** was seen to occur before a significant amount of product had been formed. These problems seem to imply that the secondary amine in compound **127** has low nucleophilicity which would explain why the auxiliary bases attack the porphyrin preferentially. This type of problem has been reported before as discussed in chapter 2.[85, 90] Although the authors do not discuss where they suspect the problems arise, in this case it seems logical that the steric factor involved in bringing two large macrocycles together in close proximity may be the decisive factor.

Hence, the next attempt to couple the macrocycles together was done using cyclen derivative **56**, containing a free amine on a pendant arm, separated from the parent macrocycle by a two carbon chain (Scheme 84). The working hypothesis was that there would be less steric hindrance in the reaction because the two macrocycles would not be required to approach one another so closely,

hence facilitating a faster reaction. Compound **56** was used in excess (2.5 equivalents) to inhibit multiple alkylation reactions and anhydrous THF was used as the solvent due to its ability to solvate both macrocyclic starting materials. It was decided to use an auxiliary base to ensure the amine was not present in the protonated form and also to consume the HBr produced in the reaction. The initial attempt was made with excess inorganic base (Na_2CO_3) due to the reactivity towards the porphyrin that was shown by NEt_3 in the previous reactions. The coupling was attempted at room temperature but after several hours TLC analysis showed no change to the starting materials, so heat was applied (60 °C) and although the consumption of porphyrin **237** was slow, it was completed over a 4 day period. One main porphyrin product was produced which appeared to have a polarity consistent with the expected product. However, after work up and chromatographic isolation the polar product was again identified as the hydroxymethyl porphyrin **248**. Once more, the base appeared to have preferentially attacked the porphyrin, indicating low nucleophilicity of the amine. A second attempt was made without any additional base, but using five equivalents of compound **56**. Prior to the reaction, a dichloromethane solution of **56** was washed with an aqueous solution of Na_2CO_3 in order to ensure the amine was not protonated. However, despite heating for two days there was no significant change to the starting porphyrin although some degradation of compound **56** was noted.



Scheme 84: Conditions used for the attempted coupling of compounds 237 and 56

The reaction was then attempted using the sterically hindered organic base DBU, with a view to having a base present which would not attack the porphyrin. The other conditions were unaltered and after three days at 60 °C, TLC analysis showed consumption of the porphyrin starting material with one major polar porphyrin spot being produced. After work up this product was isolated by silica gel chromatography and analysed by NMR.

The crucial areas of the NMR spectra for this compound are shown in Figure 47. The aromatic region of the ^{1}H spectum clearly shows the porphyrin is substituted at the para position of one of the phenyl rings. These protons are assigned as shown in Figure 48. The aliphatic peaks are clearly not from a cyclen moiety attached to the porphyrin. Peak a is from the CH_2 group attached directly to the 4-position of the porphyrin phenyl ring and peak b represents the methoxy protons on the porphyrin. The signals c to h seemed consistent with DBU, implying DBU had attacked the porphyrin instead of compound **56**. A comparison of the ^{13}C spectra of the compound and the DBU starting material confirmed this theory (Figure 49). The peaks are labelled according to the ^{1}H-^{13}C correlations shown in the HSQC spectrum, and the peaks in both spectra are shown as CH_2 groups by DEPT except peak b which is the carbon from the methoxy groups on the porphyrin. The main difference, however, is the lack of the imine carbon in the product which is found in DBU at 161ppm (not shown). This clearly indicated that the imine bond is no longer present in the product. Mass spectrometry showed the main peak at m/z 977 which corresponds to the hydroxy compound **251** as shown in Figure 50. The structure is consistent with the ^{1}H spectrum aliphatic signals. There are eight CH_2 groups, of which four would be expected to be at significantly lower ppm, which are indicated by * in Figure 50. So although peaks c-h cannot be assigned they are consistent with the structure given and this assignment is borne out by the four lower signals in the ^{13}C spectrum.

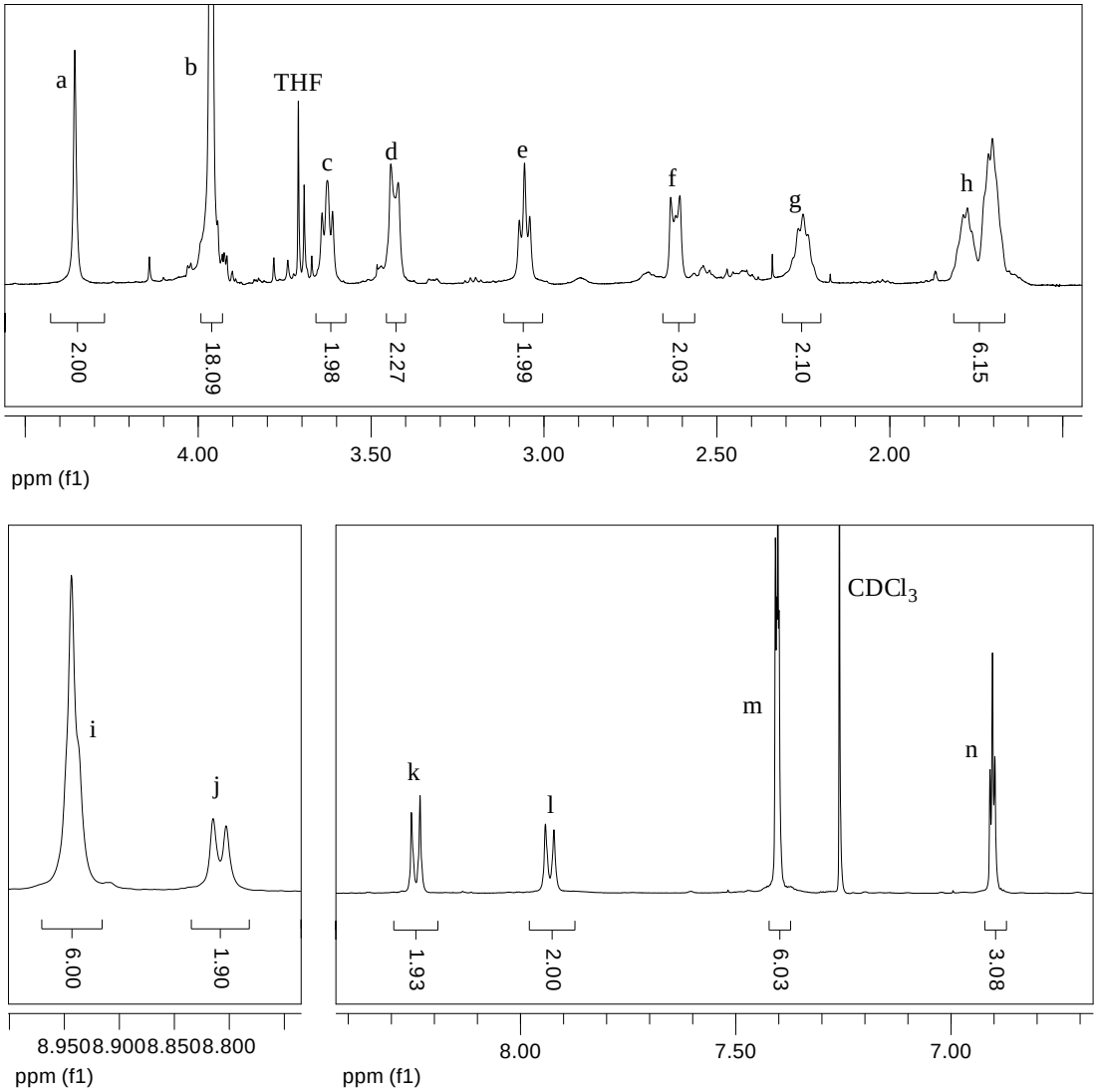


Figure 47: ^{1}H NMR spectrum of compound 251

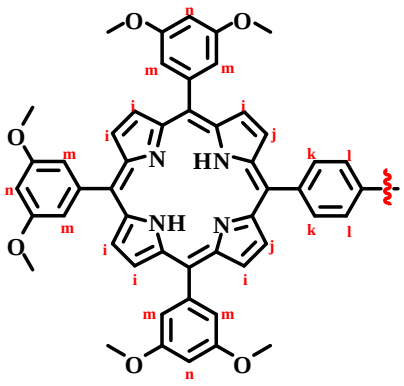


Figure 48: Assignment of aromatic protons in compound 251

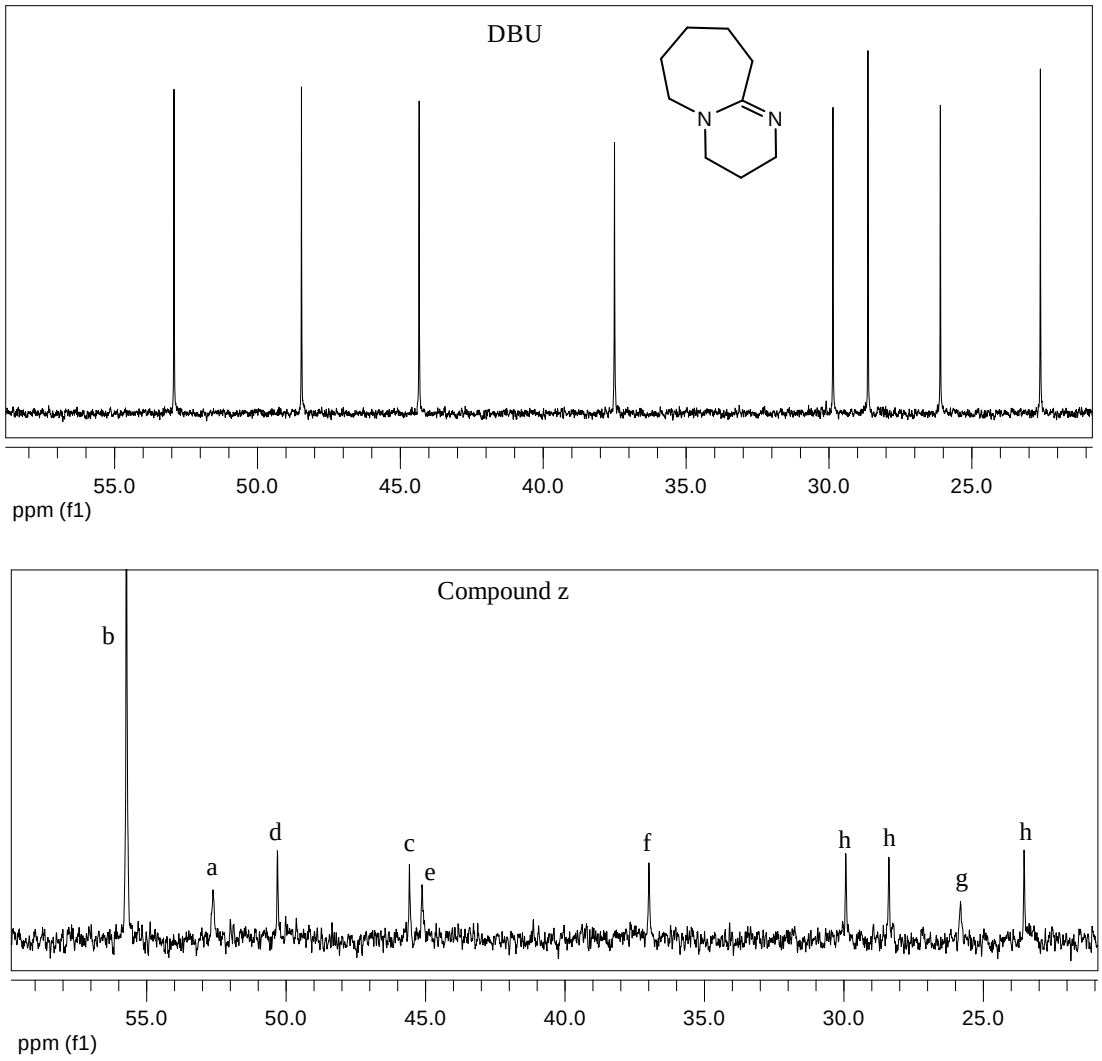


Figure 49: ^{13}C NMR comparison of DBU and compound 251

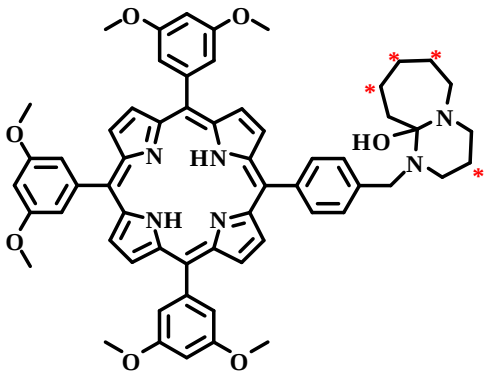
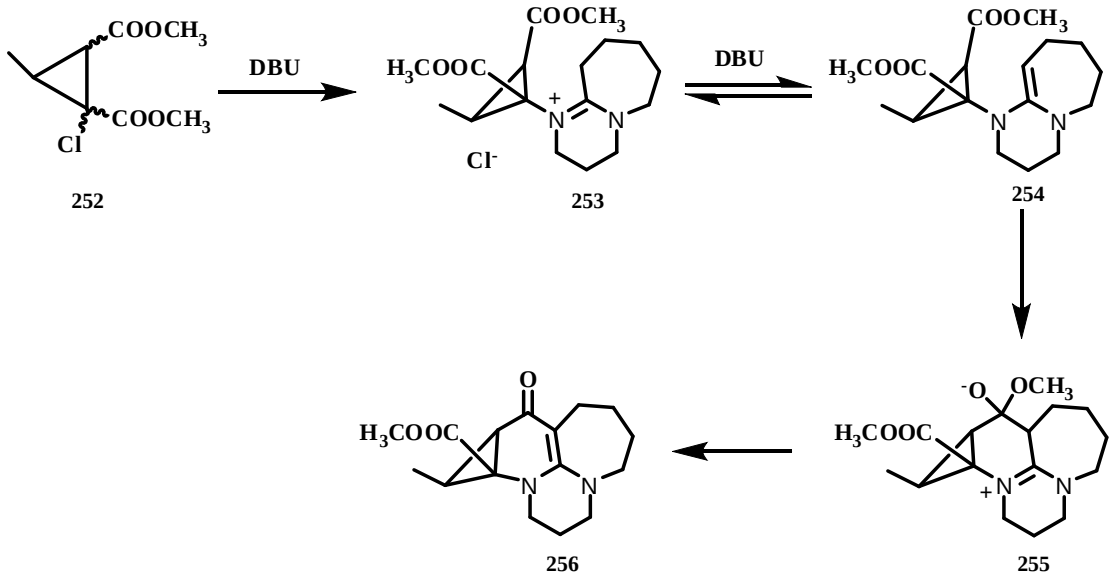
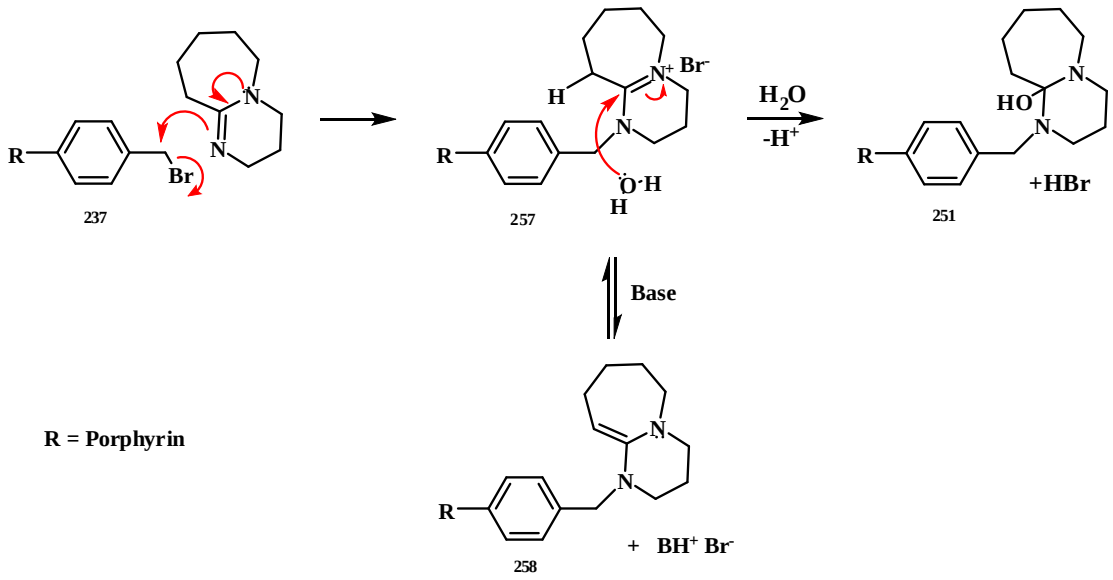


Figure 50: Porphyrin-DBU adduct (proposed structure of compound 251

A search of the literature revealed that although DBU is often used as a non-nucleophilic base, there have been several reports of it acting as a nucleophile.[214-219] In 1981, McCoy and Mal[215] reported unexpected products from the reaction of 1-halocyclopropane-1,2-diesters with DBU. Their proposed mechanism showed DBU acting as a nucleophile, and the enamine **254** subsequently formed undergoing an intramolecular cyclisation reaction with the proximal ester group (Scheme 85). A similar mechanism has been proposed in a paper by Sutherland.[218] Hence, the hydroxyl group could have been formed by nucleophilic attack by water on the iminium ion during the aqueous work up, as shown in Scheme 86.

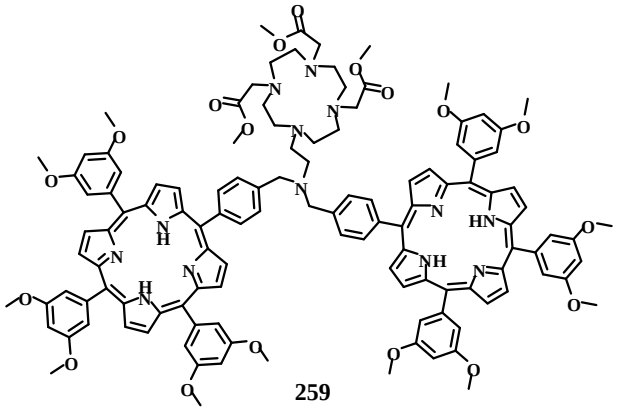


Scheme 85: Nucleophilic behaviour of DBU[215]



Scheme 86: Proposed mechanism of formation of compound 251

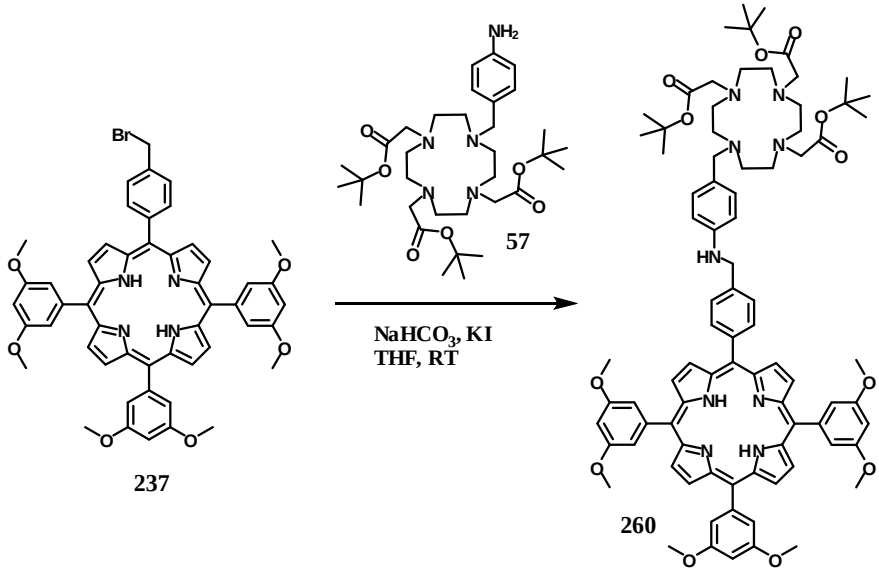
The reaction was attempted a final time, reverting to inorganic base (excess K_2CO_3) but using anhydrous DMF as the solvent. Three equivalents of amine **56** were used and the reaction was heated to 70 °C for 12 hours. After this time TLC analysis showed three new polar porphyrin compounds had formed. After aqueous work up, isolation was attempted by silica gel chromatography. However, the compounds seemed once again to decompose on the column and only small amounts could be obtained. ^{1}H NMR analysis showed that this was clearly a mixture of two porphyrins. After exhaustive TLC analysis, using silica and also neutral and basic alumina, it was not possible to find an eluent system which would separate the mixture. Mass spectrometry results showed a main peak at m/z 2045 which is consistent with the dialkylated compound **259** shown in Figure 51. A smaller peak at m/z 1239 was also present which is consistent with the desired product **250**. Hence, this result combined with the NMR analysis indicate the mixture contained the desired compound but also the dialkylated product.



259

Figure 51: Postulated structure of product formed from coupling compounds 237 and 56

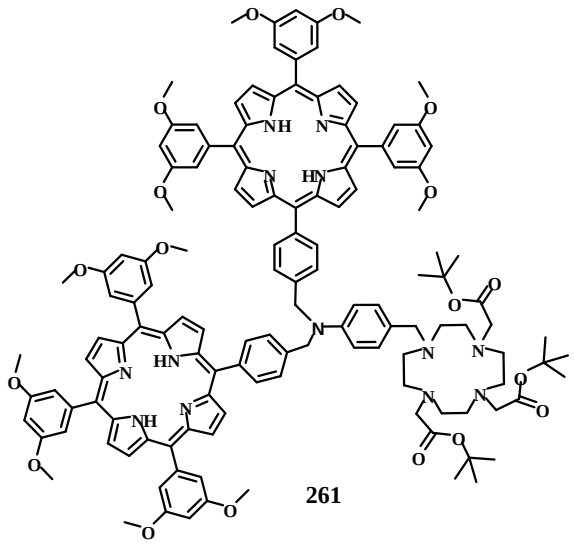
The cyclen derivative was then changed to the aminobenzyl species **57** and the reaction was attempted as shown in Scheme 87.



Scheme 87: Attempted coupling reaction between compounds 237 and 57

Five equivalents of **57** and the porphyrin were dissolved in anhydrous THF and excess $NaHCO_3$ was added along with a catalytic amount of KI. TLC analysis appeared to show the reaction was proceeding at room temperature, so the reaction was not heated. After two days, although there was still a small amount of the porphyrin starting material present, no further change appeared to be taking place, so the reaction was subjected to aqueous work up and the product isolated by column chromatography on silica gel. However, ^{1}H NMR analysis of the fraction collected showed a mixture of two porphyrins. Despite repeated

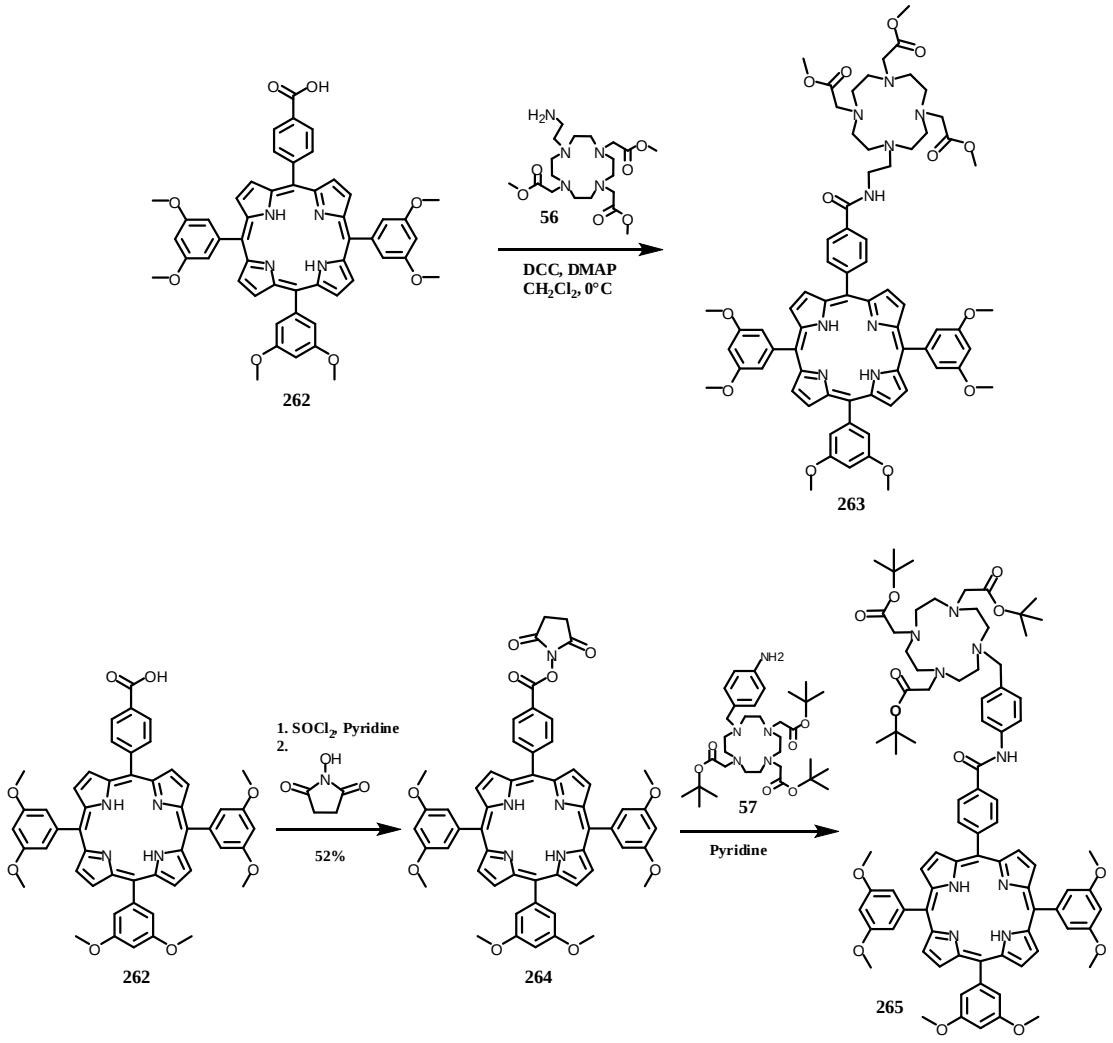
separation attempts using preparative TLC with a range of eluent systems, a pure fraction could not be isolated. Mass spectrometry (ESI) showed a molecular ion peak at 2055 m/z, which corresponds to the Na^+ adduct of the dialkylated species **261** shown in Scheme 87. A peak was also seen at 1449 m/z which corresponds to the Na^+ adduct of the desired product **260**. This information was consistent with the ^1H NMR spectrum which showed the two compounds were approximately a 50:50 mixture. Since the nucleophile was used in a 5x molar excess, this was a surprising result, but one that was consistent with the result for the aminoethyl cyclen derivative **56**. The conclusion that can be drawn is that increased steric hindrance in the 2° amine desired products is not counterbalancing the increased nucleophilicity and they are much more nucleophilic than the starting cyclen derivatives, **56** and **57**.



261

Figure 52: Postulated structure of the product formed by coupling compounds 237 and 57

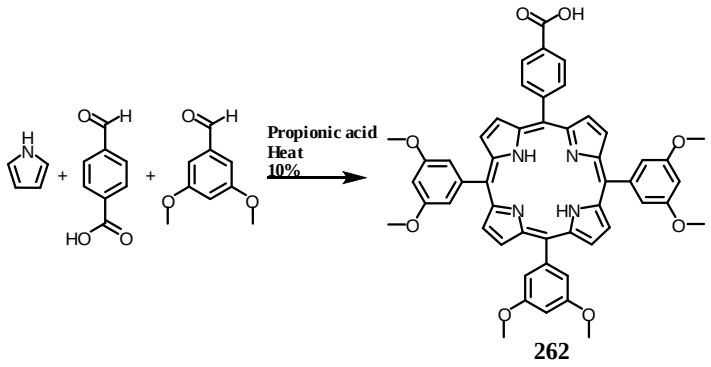
Due to the problems encountered, it was decided to synthesise a new electrophilic porphyrin. To this end, a synthetic route via an activated ester was chosen and the reactions attempted are shown in Scheme 88



Scheme 88: Couplings attempted via activated ester bearing porphyrins

4.6 Results and discussion of coupling reactions via activated esters

The carboxylic acid substituted porphyrin **262** was synthesised by using Adler-Longo[124] methodology in 10% isolated yield (Scheme 89).



Scheme 89: Synthesis of the carboxylic acid appended porphyrin 262

Compound **262** gave a range of options for transformation into an activated ester, two of which were used for attempted coupling to cyclen derivatives **56** and **57** as shown in Scheme 88. Equimolar amounts of porphyrin **262** and DCC were dissolved in CH_2Cl_2 at 0 °C and a solution of excess cyclen **56** was added. After stirring for 5 hours, monitoring by TLC showed two main new porphyrin fractions, one of which showed a polarity consistent with the desired product. All the starting porphyrin **262** had been consumed and after a further three hours, there appeared to be no further reaction taking place. The reaction was stopped and the products were isolated by silica gel chromatography. ^{1}H NMR analysis of the less polar fraction showed it was the DCC-porphyrin intermediate. Unfortunately, once again the polar fraction appeared to degrade rapidly on silica gel. Only a small amount could be collected and a poorly resolved ^{1}H NMR spectrum revealed peaks characteristic of the coupled cyclen-porphyrin product which was confirmed by the ^{13}C NMR spectrum. However, attempts to further purify the product by preparative TLC led to further degradation of the compound. The reaction was repeated and this time the crude product was split into three portions. Isolation and purification was attempted by column chromatography using neutral alumina, basic alumina and silica gel pretreated

with base (NEt_3). However, none of these attempts were successful, with the compound which appeared as one spot by TLC, splitting into several bands as it moved down the column.

The succinimide ester **264** was formed in 52% isolated yield by treatment of porphyrin **262** with thionyl chloride followed by N-hydroxysuccinimide. An equimolar mixture of compounds **264** and **57** was then dissolved in anhydrous pyridine under an inert atmosphere and stirred at ambient temperature. Close analysis by TLC showed no reaction was occurring, so the mixture was heated to 70 °C. However, after 24 hours there was still no change to either of the starting materials, prolonged heating over seven days led to decomposition of the cyclen species **57** with no apparent product formation. A second reaction was undertaken using anhydrous THF as the solvent, with the amine **57** washed thoroughly with base ($NaHCO_{3(aq)}$) prior to addition to the reaction mixture. Once again no reaction appeared to occur, either at room temperature or after prolonged heating at 70 °C.

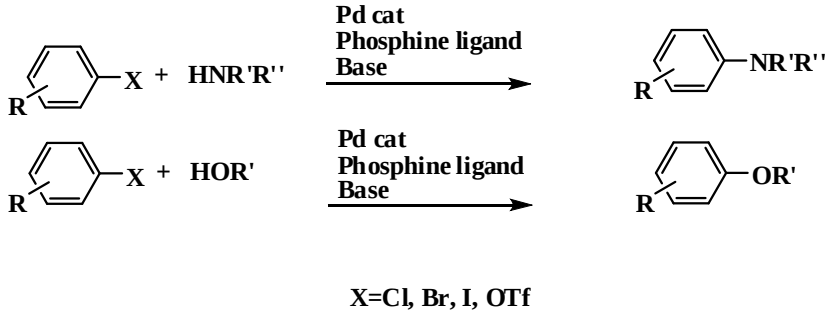
4.7 Buchwald-Hartwig methodology

4.7.1 Introduction

Due to the lack of success attaching the amino bearing cyclam/cyclen derivatives to the bromomethyl porphyrin **237** and the failure of the activated ester route, it was decided to try a new approach using palladium catalysed coupling reactions. Since the amino based cyclen/cyclam macrocycles had already been synthesised, synthesis of an aryl bromide porphyrin would give access to the use of Buchwald-Hartwig chemistry to afford the desired compounds.

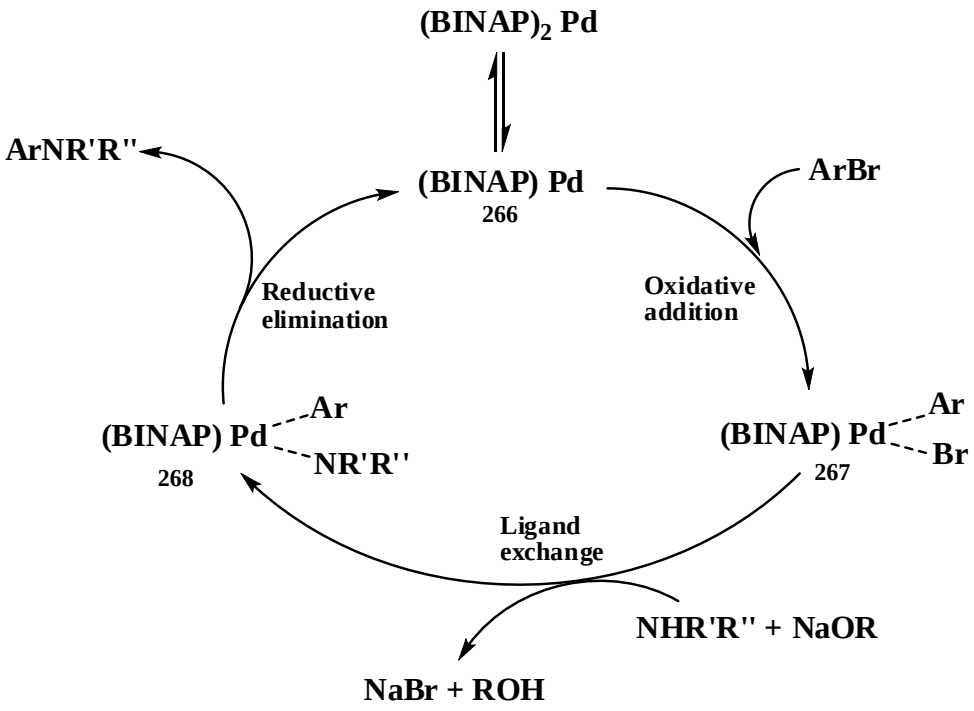
4.7.2 Palladium catalysed aryl amination

In a series of papers beginning in 1994, Buchwald and Hartwig[220, 221] independently discovered and developed new chemistry based on palladium catalysed aryl-nitrogen and aryl-oxygen bond formation using a phosphine based ligand system as shown in Scheme 90. Development of this methodology over the last decade has led to all aspects of the reaction being investigated including different combinations of base, ligand and solvent system as well as the scope of the reaction in terms of substrates used. It has been demonstrated that primary, secondary and aromatic amines can be coupled to aryl chlorides, bromides, iodides and triflates, with a variety of phosphine ligands, of which racemic BINAP is one of the most common. The base originally employed was NaO^tBu, but further studies have shown that the milder bases Cs_2CO_3 and K_3PO_4 can also be utilised to good effect.



Scheme 90: General transformations via Buchwald-Hartwig chemistry[220, 221]

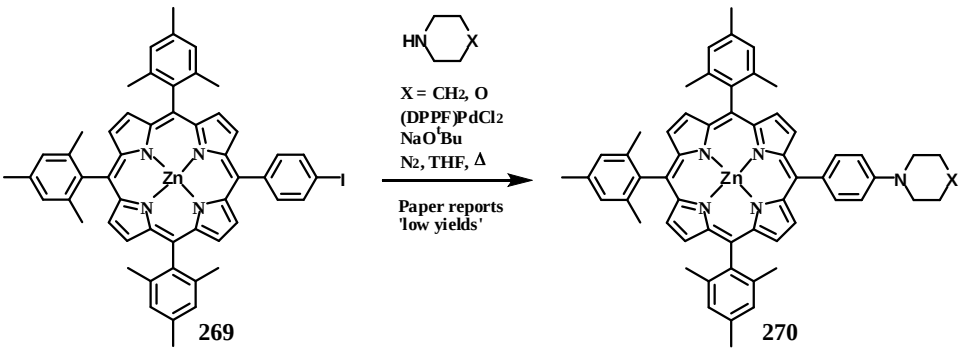
The catalytic cycle is shown in Scheme 91.[222] The active catalytic species in the reaction is palladium(0) which is reductively formed *in situ* from $Pd_2(dba)_3$ or $Pd(OAc)_2$. An equilibrium is established between the four coordinate $Pd(BINAP)_2$ which lies off the catalytic cycle, and the two coordinate Pd(BINAP) **266**. This species undergoes oxidative addition with the aryl bromide substrate giving the four coordinate palladium(II) complex **267**. The amine is deprotonated by the base and subsequently exchanges with the bromide ligand generating **268** which then undergoes reductive elimination, forming the product containing the new aryl-nitrogen bond and regenerating the palladium(0) catalyst **266**.[222]



Scheme 91: Catalytic cycle through which palladium(0) catalysed aminations are believed to proceed[222]

The first report of Buchwald-Hartwig chemistry being used within porphyrin chemistry came in a paper by van Lier *et al.* in 2001.[223] The zinc(II) porphyrin complex **269** was aminated with two different cyclic secondary amines as shown in Scheme 92.[223] Although the reason for using the metal complex of the porphyrin was not discussed in the paper, presumably this was an attempt to prevent the palladium(II) metal ion being encapsulated by the porphyrin under

the basic conditions of the reaction.



Scheme 92: The first example of Pd(0) catalysed aminations involving a porphyrin[223]

A large study of the applicability and scope of this chemistry to porphyrin substrates was done by the group of Zhang and in two papers in 2003 they examined the amination of porphyrins both directly on the porphyrin core and also on the *para* position of the phenyl substituents of both TPP and DPP.[224, 225] Some of their results are shown in Scheme 93.[224, 225] The first paper[224] focussed on the reaction of *meso*-bromo diphenylporphyrin (both free base and zinc(II) complex) with a variety of amino bearing nucleophiles. They reported that DPEphos and *rac*-BINAP (Figure 53) were both equally effective ligand systems and that $Pd(OAc)_2$ and $Pd_2(dba)_3$ were both active catalysts. Their choice of Cs_2CO_3 was due to the occurrence of side reactions when the stronger base NaO^tBu was employed and THF was preferred over toluene due to greater solubility of the porphyrins. Their reactions successfully coupled a wide variety of amines in good yield, including electron rich and electron poor aromatic amines, demonstrating the extensive applicability of this reaction. They did note, however, that although they initially used the zinc(II) ion as an inorganic protecting group for the porphyrin, the results showed it was not required when Cs_2CO_3 was used as base.

The second paper[225] extended this study to multiple aminations on the *para* positions of the phenyl substituents on both diphenyl and tetraphenyl porphyrin. In these cases they discovered that the DPEphos ligand was no longer effective, giving very low conversion of starting material. However, a systematic study of the reaction found that racemic Binap or Xantphos (Figure 53) were the most effective ligands when used in conjunction with $Pd(OAc)_2$ and either Cs_2CO_3 or

NaO^{t}Bu as base. The reaction could be done in toluene or THF successfully at elevated temperatures between 68 °C and 100 °C.

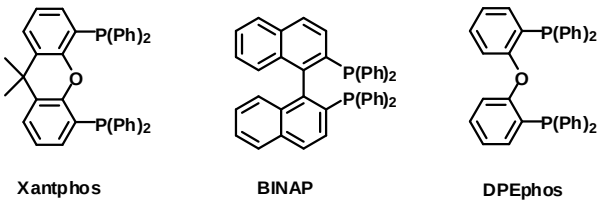
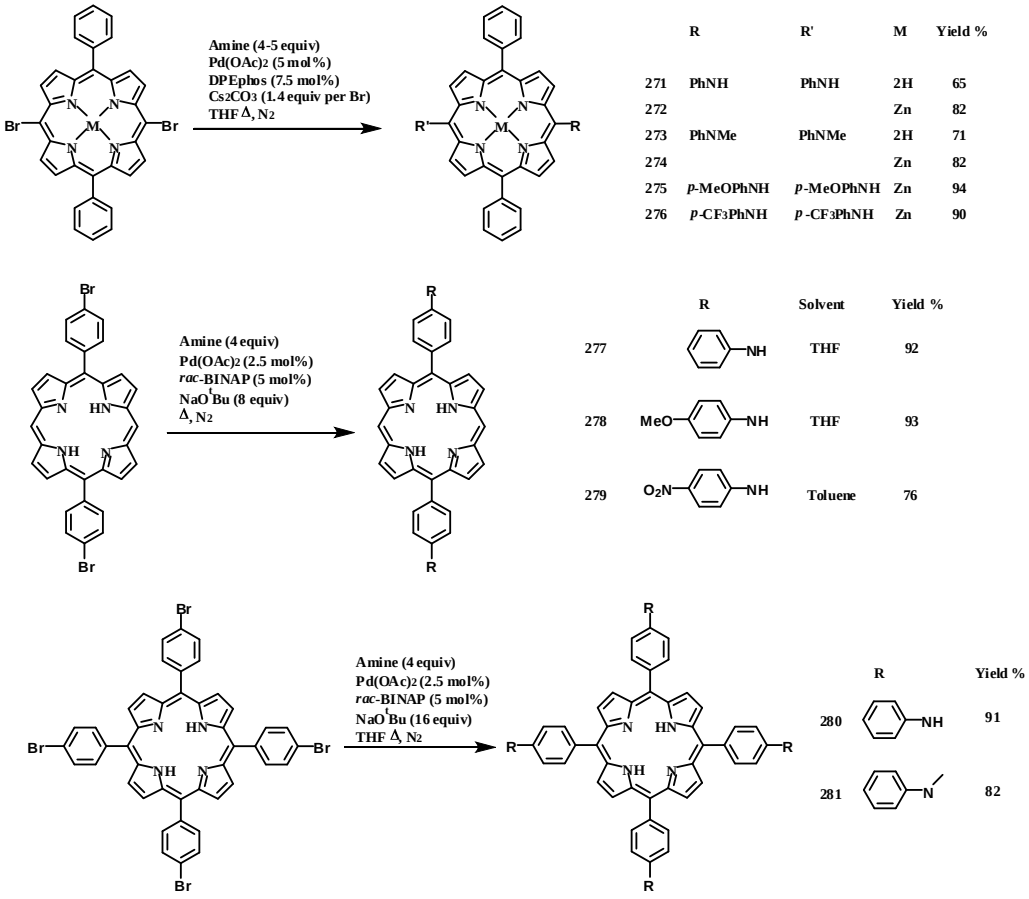


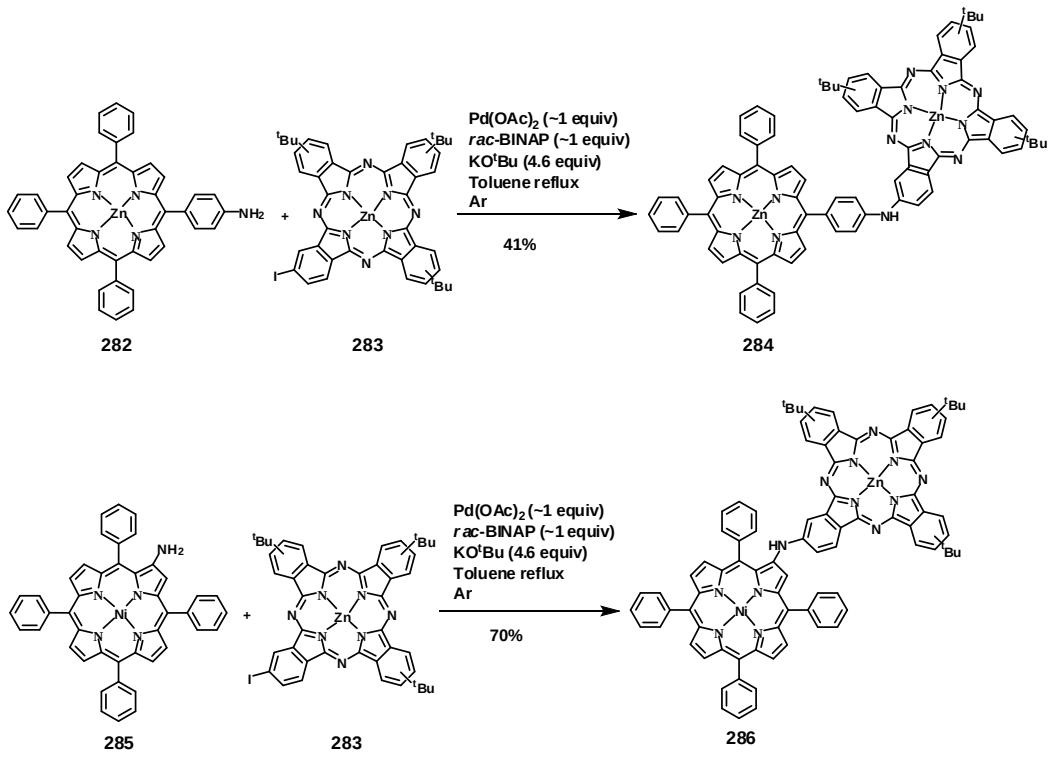
Figure 53: Structures of common phosphine based ligands used in Buchwald-Hartwig chemistry

Both TPP and DPP were aminated using these conditions with a series of amines of varying structure and electronic properties which once again demonstrated the scope of this reaction for use with porphyrin subtstrates. A selection of their results with aryl amines are shown in Scheme 93.



Scheme 93: Examples of the Pd(0) catalysed aminations on porphyrin substrates[224, 225]

Following this work, several reports of the use of Buchwald-Hartwig chemistry to functionalise porphyrin substrates have been published[226-228] and one recent example by the group of Cavaleiro[228] is shown in Scheme 94. They used palladium(0) catalysed amination of porphyrins as a route to the porphyrin-phthalocyanine dyads **284** and **286**. Although in both syntheses the conditions were analogous to those proposed by Zhang *et al*.,[224, 225] they used a stoichiometric amount of both ligand and catalyst, as well as a four fold molar excess of the aryl iodide **283** (phthalocycanine). Metal complexes of both macrocycles were also used and the products were obtained in 41% and 70% for the phenyl coupled and β-coupled dyads respectively (**284** and **286**).



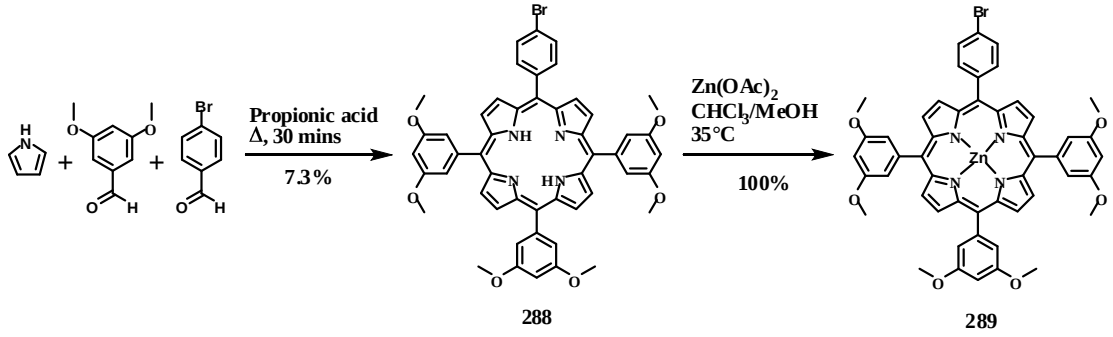
282 283 284

285 283 286

Scheme 94: Porphyrin-phthalocyanine dyads synthesised via palladium(0) catalysed amination reactions[228]

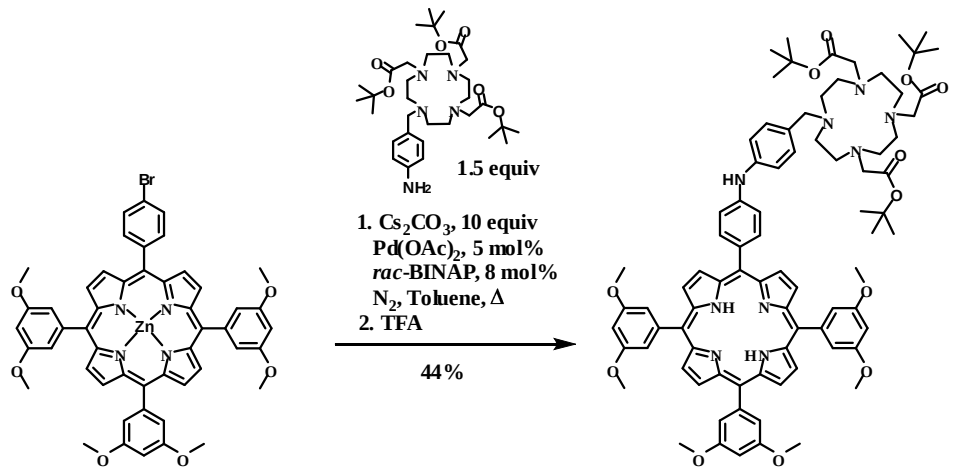
4.7.3 Results and discussion of Buchwald-Hartwig coupling – synthesis of compound 287

An aryl bromide bearing porphyrin was required for the Buchwald-Hartwig coupling attempts and it was decided to keep the methyl ether groups present on the periphery of the porphyrin. Hence, the desired porphyrin was an aryl bromide analogue of the bromomethyl porphyrin **237**. As shown in Scheme 95, the aryl bromide containing porphyrin **288** was synthesised via a mixed condensation using Adler-Longo[124] conditions in 7.3% isolated yield. The zinc(II) complex was then formed by gently warming (35 °C) mixed solutions of **288** in $CHCl_3$ and $Zn(OAc)_2$ in MeOH. As stated previously, the basic conditions of the Buchwald coupling reaction, combined with the nature of the precatalyst, a palladium(II) salt, gave the possibility of the porphyrin sequestering the palladium(II) ion from solution and therefore inhibiting the coupling reaction. Hence, the zinc complex **289** was formed to prevent this.



Scheme 95: Synthetic route to compound 289

The Buchwald-Hartwig type coupling reaction was performed using the conditions shown in Scheme 96. An excess of the aminobenzyl cyclen species **57** was used along with Cs_2CO_3 as base and, after heating for 30 hours, the reaction mixture was cooled to ambient temperature and subjected to aqueous work up. However after careful TLC analysis, it became apparent that isolation by column chromatography would be difficult due to the product fraction streaking on the silica. A brief (10mins) treatment of the crude mixture with TFA removed the zinc(II) ion from the porphyrin and improved the behaviour on silica, allowing isolation of **287** in 44% yield.



Scheme 96: Synthesis of compound 287

Figure 55 and Figure 54 show the aromatic and aliphatic regions of the ^{1}H NMR spectrum of compound **287** respectively, along with the peak assignments in Figure 56.

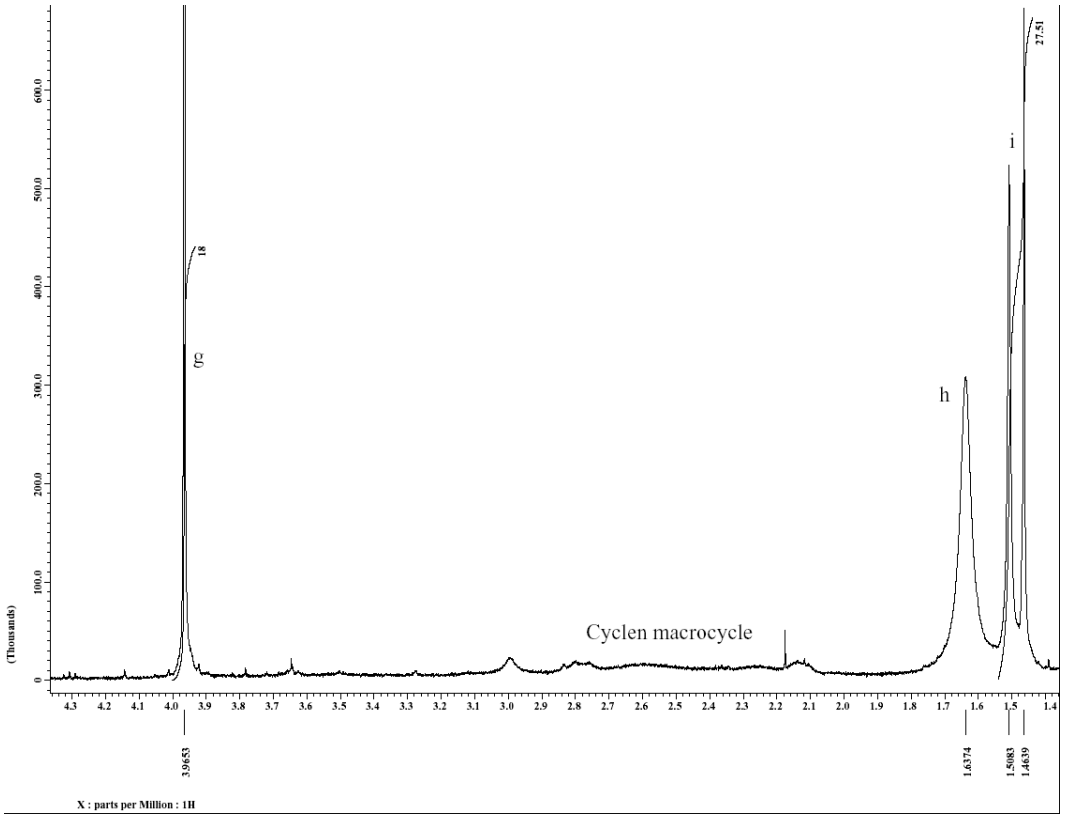


Figure 54: Aliphatic region of ^{1}H NMR spectrum of compound 287

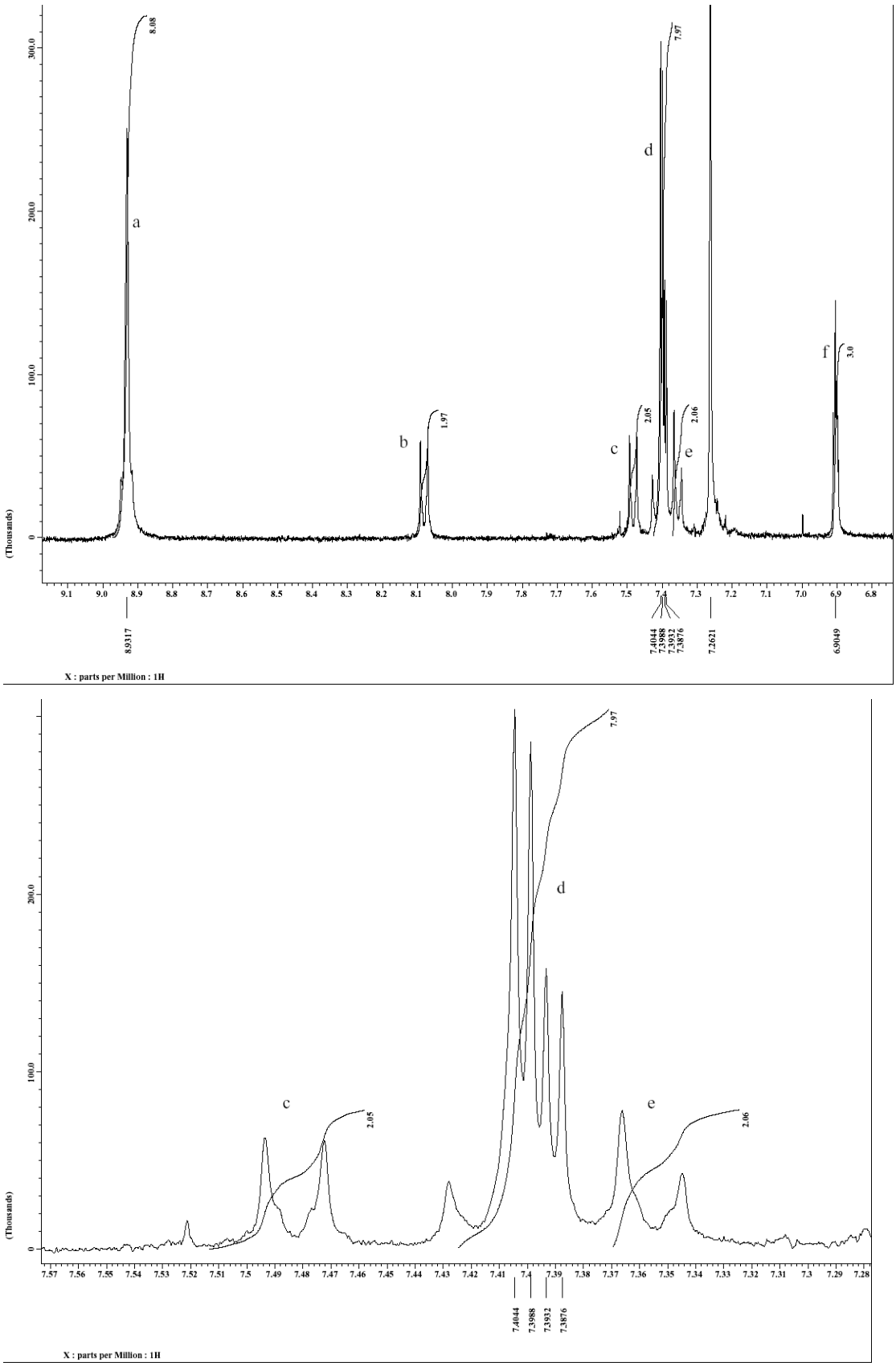


Figure 55: Aromatic region of ^{1}H spectrum of compound 287

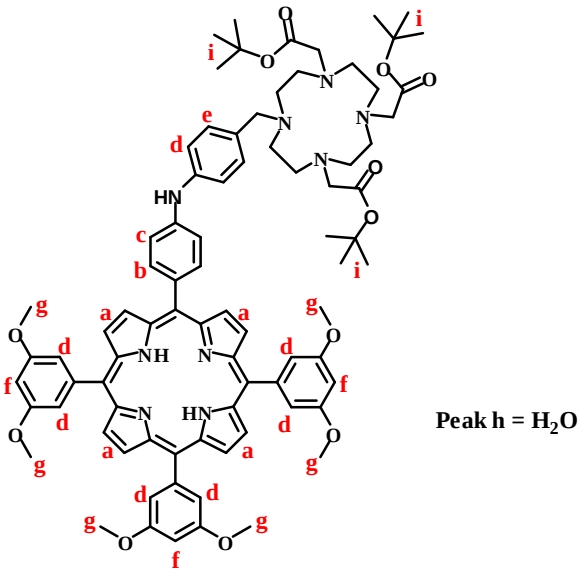


Figure 56: ^{1}H NMR peak assignments for compound 287

Assignment of the aromatic region was quite straightforward, with peak a being assigned to the β–protons from the porphyrin ring and peak f to the three protons on the *para* positions of the phenyl rings on the porphyrin. Four doublets were expected from the two phenyl rings linking the macrocycles and three of these are seen as signals b,c and e. The fourth signal is overlapped by signal d, which is assigned to the *ortho* protons on the porphyrin phenyl rings. The expansion of this area (Figure 55) shows overlapping signals and this is confirmed by the integration of peak d which gives a relative value of 8H (instead of 6H), as well as the 2D DQF COSY spectrum which showed a coupling between peaks d and e. The 2D spectrum also showed coupling between peaks b and c. These coupling interactions alongside the relative chemical shift values allowed assignment of protons b, c and e as shown in Figure 56. The aliphatic region shown in Figure 54 could not be fully assigned due to the broad signals caused by the protons on the cyclen macrocycle and the methylene groups on the pendant arms. However, the methoxy protons g and the *tert*-butyl protons i could be clearly identified. Peak h is due to water in the sample.

The ^{13}C NMR spectrum of compound **287** is shown in Figure 57 and the peak assignments are shown in Figure 58. The characteristic peaks that can be immediately identified are the ester carbons (peaks 1 and 2), the quaternary carbons of the *tert*-butyl groups (peaks 11), the *tert*-butyl carbons (peaks 12) and the methoxy carbons on the porphyrin (peaks 10). Peaks 3, 8 and 9 are the signals generated by the carbons on the methoxy-substituted phenyl rings on the porphyrin. Peak 3 is from the 6 carbons substituted with the methoxy groups, peak 8 is from the 6 carbons in the adjacent *ortho* position and peak 9 is from the three carbons in the adjacent *para* positions. Peak 3 was assigned due to its integration value, chemical shift and DEPT signal alongside its presence in the starting material (porphyrin 19), while peaks 8 and 9 were assigned from the DEPT and ^{1}H-^{13}C correlation spectrum. The ^{1}H-^{13}C correlation spectrum alongside the DEPT spectrum allowed assignment of peaks 4,5,6 and 7 due to their correlations with the protons b,c,d and e. The unassigned peaks in the aromatic region are all quaternary carbon signals comprising of carbons from the porphyrin macrocycle and the phenyl ring adjacent to the cyclen moiety. The DEPT spectrum indicated that the four unassigned signals between 49 ppm and 59 ppm are all CH_2 carbons and are typical of signals found in cyclen macrocycles with pendant arms, and more specifically they are consistent with the starting macrocycle **57**.

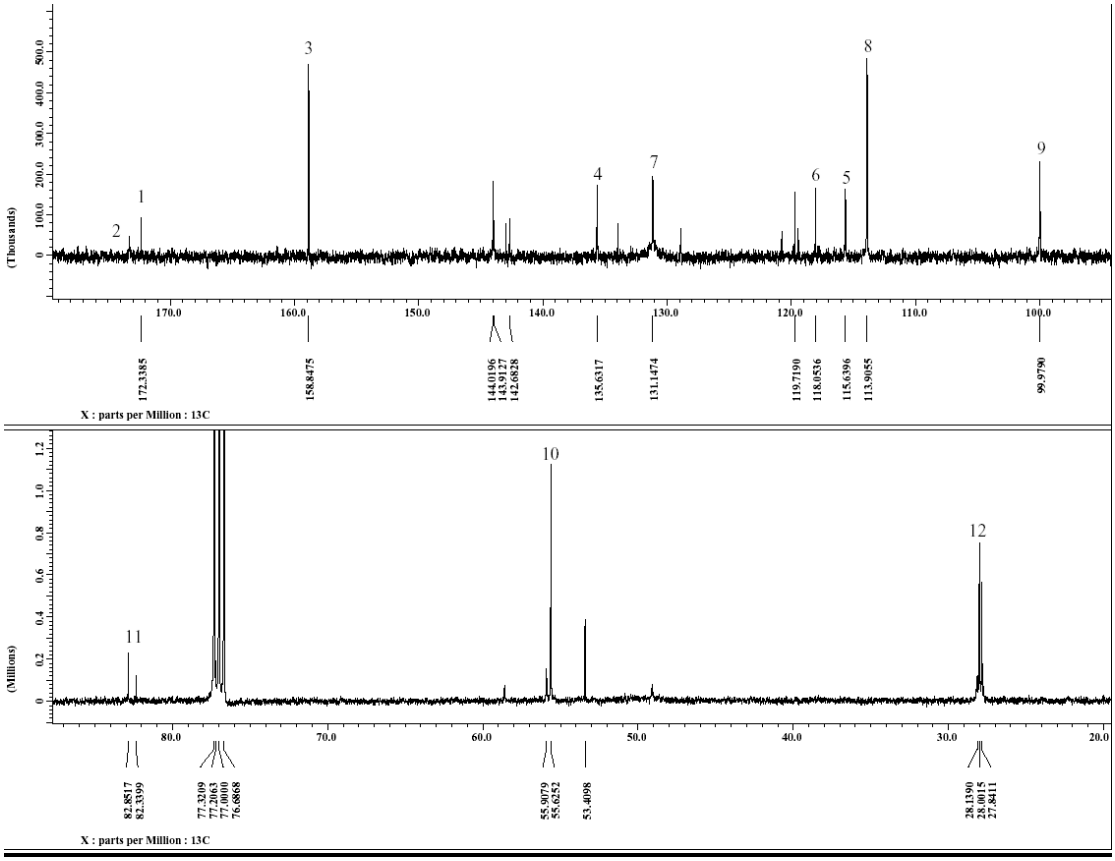


Figure 57: ^{13}C NMR spectrum of compound 287

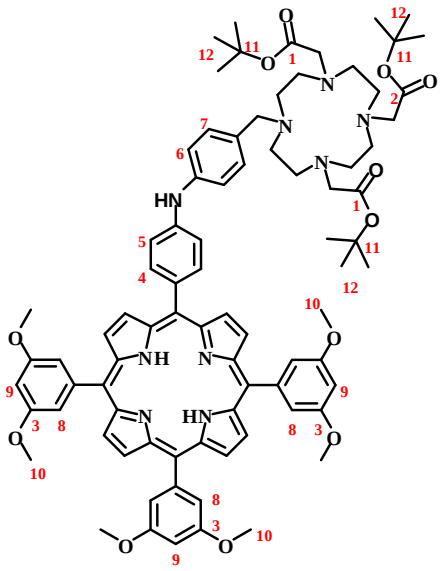


Figure 58: ^{13}C NMR peak assignments for compound 287

Hence, although once again not all the signals can be assigned, the evidence is consistent with both macrocycles being present in this compound, with characteristic signals from both parts of the molecule present and moreover, showing the correct relative integration values in the ^{1}H spectrum. Although NMR analysis of cyclen type macrocycles is fraught with difficulty due to the broadened signals, the ^{13}C spectrum shows enough detail to be confident of the macrocycle being present in the desired form.

Compound **287** was submitted for electrospray mass spectrometry analysis and a solvent system of CH_3CN/H_2O with 0.1% formic acid was used which gave rise to some interesting results. Since ESI usually creates protonated cations, the major peak was expected to be at 1413 m/z corresponding to the species $[M+H]^+$. However, this peak was not seen, but the molecular envelope showed peaks at m/z 1414 (100%), 1415 (85%), and 1416 (45%). The spacing of the peaks one unit apart indicated that these species carried a single positive charge yet had apparently gained two protons to correspond to the signals from the species $[M+2H]^+$, $[M+3H]^+$, and $[M+4H]^+$. This seemed to imply a reduction process had occurred either in the synthesis and isolation of the molecule or in the mass spectrometry analysis. A search of the literature found that this phenomenon had been reported before within mass spectrometry analysis of porphyrins.[229-231] Musselman and co-workers[229] had observed a peak corresponding to the $[M+2H]^+$ species in their analysis of porphyrins using fast atom bombardment. They proposed a mechanism in which the porphyrin was protonated twice, followed by a one electron reduction to give the uni-charged species. The mechanism was consistent with the structure of the analyte molecule in that the presence of basic sites on the porphyrin would allow the initial double protonation and other groups with electron affinity would facilitate the electron capture. Cerny and Gross[232] had already proposed a general mechanism for the formation of $[M+2H]^+$ and $[M+3H]^+$ species in molecules containing several basic sites. They reported that reduction of protonated species should only occur for compounds containing low energy molecular orbitals available to accept the electrons. This is clearly the case for porphyrin macrocycles, which contain an extended system and hence have low energy LUMOs. Cavaleiro *et al.*[230] studied the effect of the matrix in liquid secondary ion mass spectrometry on a range of

porphyrin dimers linked by ester and amide groups. They observed $[M+H]^+$, $[M+2H]^+$, $[M+3H]^+$, and even $[M+4H]^+$ peaks for the dimeric porphyrin species but found that changing the matrix changed the relative abundance of the peaks. They found that in a matrix of 3-nitrobenzyl alcohol the $[M+H]^+$ peak was the most abundant, but in more acidic matrices such as thioglycerol the relative abundance of both the $[M+2H]^+$ and $[M+3H]^+$ peaks increased with the $[M+2H]^+$ peak giving the highest relative abundance. Compound **287** clearly fits the criteria of having several basic sites for protonation and, since it contains a porphyrin subunit, it also has low energy unoccupied orbitals to act as electron acceptors. The acidic solvent used in the ESI analysis also fits well with the conclusions of Cavaleiro *et al.*[230], implying that the peaks seen in ESI are indeed an artefact of the analysis.

Having formed the *bis*-macrocyle **287**, the next step was to attempt insertion of a metal ion into the cyclen ring. This initially required hydrolysis of the *tert*-butyl ester groups which is commonly achieved in literature reports by treatment of a dichloromethane solution of the macrocycle with TFA at room temperature.[105, 193] However, after stirring with TFA for 1 hour, followed by basic aqueous work up, the starting material was still intact. The procedure was repeated and the reaction time extended to 10 hours at room temperature, but TLC analysis showed no other products had formed and once again only starting material was recovered from the reaction mixture. The reaction was then repeated twice at ambient temperature using THF and $CHCl_3$ as solvents. In both cases after 4 hours no reaction was seen to occur, so the reactions were heated to 40 °C for one hour. However, TLC analysis showed degradation of the compound and no starting material was recovered.

Another sample of compound **287** was then dissolved in THF and treated with $HCl_{(aq)}$ (~6M) at ambient temperature. However, once again ^1H NMR analysis showed that the starting material was unchanged by the acid, including the *tert*-butyl ester peaks.

These results showed that surprisingly, compound **287** was stable to Bronsted-Lowry acids at room temperature, so a further attempt at hydrolysis was subsequently made using the reagent trimethylsilyl iodide (TMSI). This reagent

combines a hard acid and weak nucleophile within the same molecule and literature reports show it can be used to cleave esters under neutral conditions, while tolerating a wide range of functional groups.[233-235] Aprotic solvents such as CCl_4 or $CDCl_3$ are used and it has been shown to be especially effective with sterically hindered substrates such as *tert*-butyl esters.[233, 235] However, when compound **287** was dissolved in freshly distilled $CDCl_3$ and treated with TMSI under anhydrous conditions a reaction occurred immediately. TLC analysis showed around eight porphyrin spots had been formed from the starting material. The reaction was left to continue for 1 hour on the basis that partial hydrolysis of the starting material could account for the number of products present. However, after this time, no further change was observed. Preparative TLC was used to attempt to isolate the two most prominent fractions. Only one could be isolated, but ^{1}H NMR analysis did not identify the compound. At this point the reaction was abandoned and it was concluded that the starting material had degraded

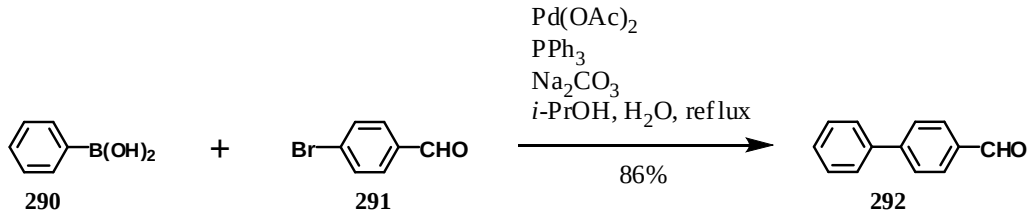
4.8 Suzuki coupling reactions

4.8.1 Introduction

In light of the failed attempts hydrolysing the esters in compound **287**, it was decided to change the coupling reaction in order to create a different linkage between the macrocycles. The palladium(0) catalysed Suzuki reaction was chosen partly due to the success of the Buchwald-Hartwig coupling reaction and also for the direct aryl-aryl linkage that is formed from these couplings. This was expected to be a very stable, robust linkage which would stand up to the acidic conditions of the ester deprotection reaction. Additionally, the cyclen compounds **58**, **59** and **60** each contain an aryl bromide group which is suitable for reaction with a boronic ester appended porphyrin.

4.8.2 Aryl-aryl linkages from Pd(0) catalysed reactions

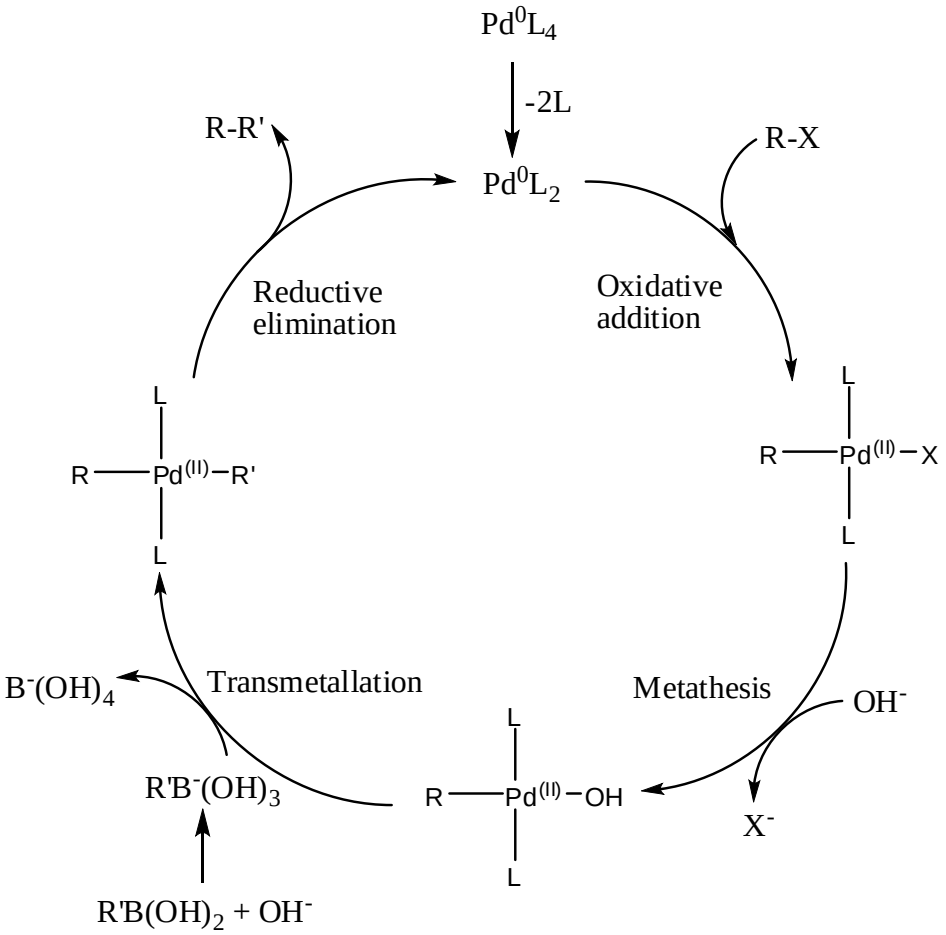
The Suzuki-Miyaura[236] reaction is a palladium catalysed coupling between an aryl/vinyl halide or triflate with an aryl/vinyl boronic acid or ester and is a common methodology used for carbon-carbon bond formation. The reaction is carried out in the presence of base to activate the boronic acid/ester and effective couplings can be achieved in a wide variety of solvent systems.[237-239] The broad scope of the reaction allied to the stability and low toxicity of the organoboron compounds are the main advantages that have led to the widespread use of this chemistry.[237, 238] The active catalytic species is palladium(0) and the reaction is often carried out with a palladium(0) catalyst such as $Pd(PPh_3)_4$ or a palladium(II) precatalyst and a reducing agent (Scheme 97).[240]



290 **291** **292**

Scheme 97: An example of a Suzuki coupling reaction[240]

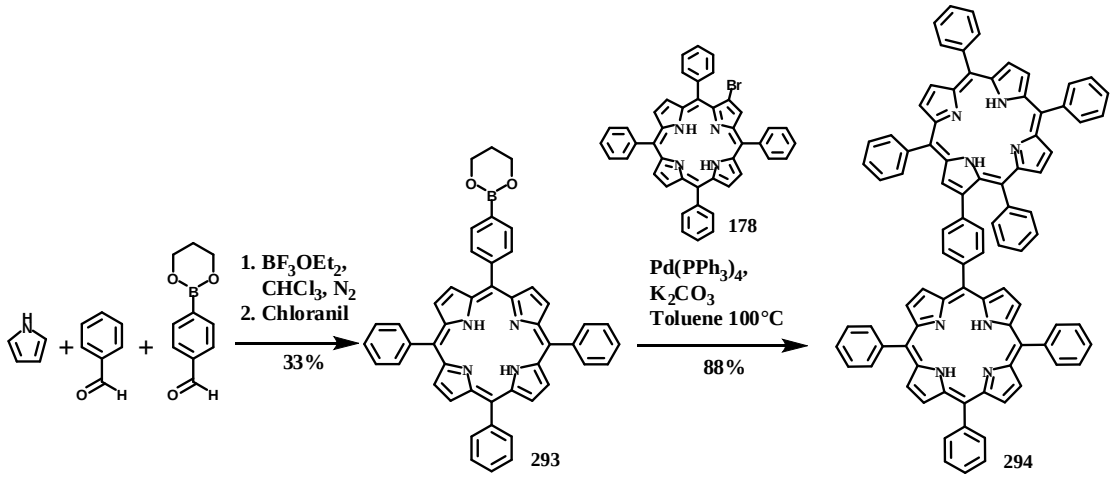
The catalytic cycle is shown below (Scheme 98) and consists of four main steps.[238] The palladium(0) catalyst undergoes oxidative addition to palladium(II) with the organohalide partitioning between the organic residue and the halide ion, which is then substituted by base (metathesis). The base also activates the boronic acid/ester, forming a four coordinate negatively charged boronate species which undergoes transmetallation with the palladium(II) complex. The new carbon-carbon bond is formed by reductive elimination in the last step, which also regenerates the palladium(0) catalyst.[238]



Scheme 98: Catalytic cycle for the Suzuki reaction[238]

4.8.3 Suzuki coupling in porphyrin chemistry

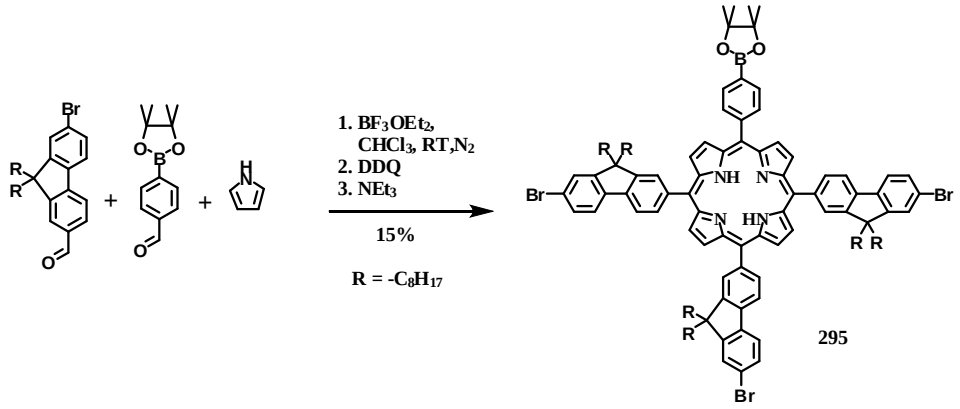
Halide bearing porphyrins have been widely used as the coupling partner to boronic esters in Suzuki couplings.[241-246] Porphyrins with halo groups directly on the porphyrin core (both *beta* and *meso* positions) and on a phenyl ring substituent have all been successfully coupled with a variety of boronic esters.[241, 242, 246] It was not until 1994 however, that a porphyrin with a boronic ester appended group was synthesised and used in a Suzuki coupling reaction.[247] As shown in Scheme 99, Chan and Zhou[165, 247] synthesised the porphyrin **293** by condensation of a statistical mixture of benzaldehyde, pyrrole and *p*-(1,3-dioxaborane)benzaldehyde using Lindsey[139] methodology. They went on to couple this to β-bromo-tetraphenyl porphyrin **178** under Suzuki conditions obtaining the dimer **294** in 88% yield, thus demonstrating the efficacy of porphyrin boronic esters in this type of reaction.[165]



Scheme 99: Porphyrin dimers synthesised via Suzuki coupling [165, 247]

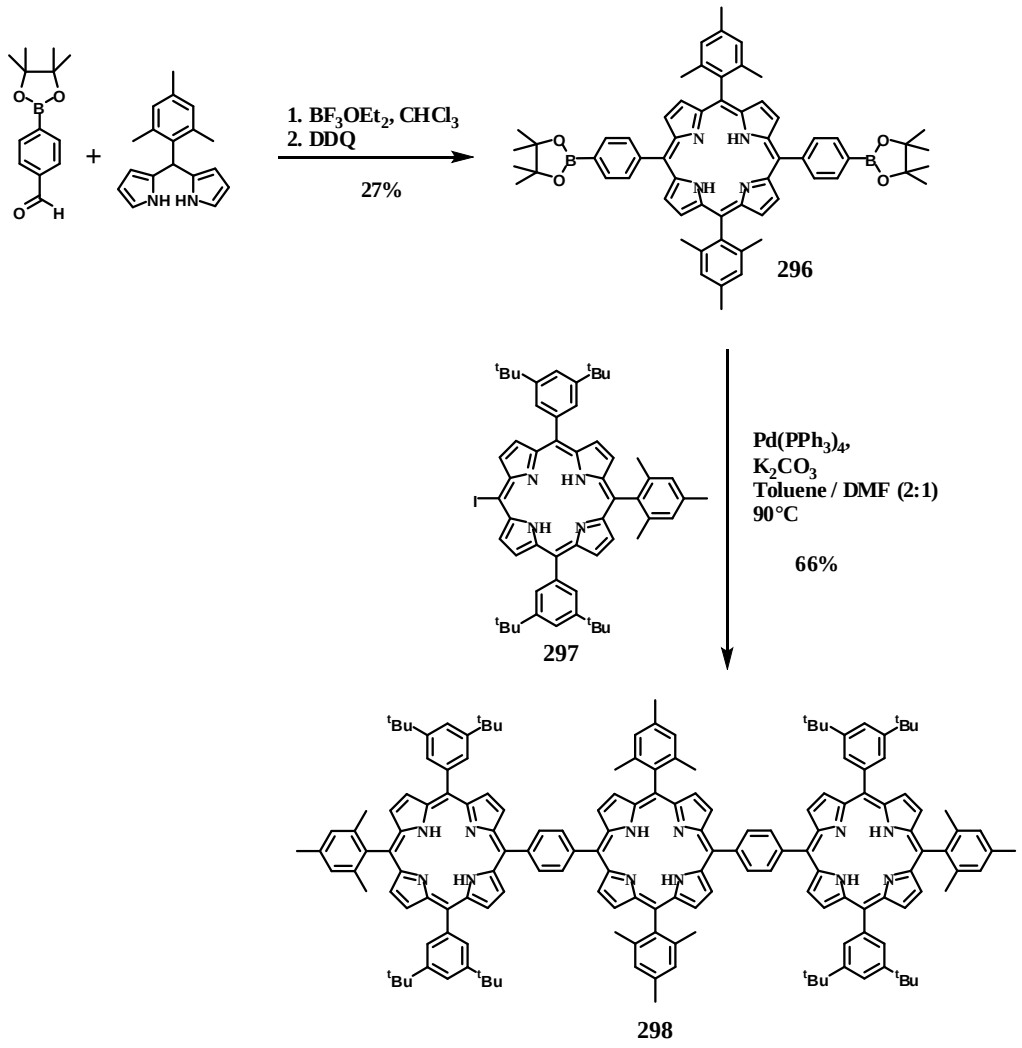
A more recent but similar example was reported by Lu and co-workers[248] who synthesised the pinacol boronate ester porphyrin **295** (Scheme 100) using Lindsey[139] conditions in 15% yield. This porphyrin was then used as the monomer building block from which the authors synthesised hyperbranched

conjugated porphyrin arrays by a one pot Suzuki polycondensation.[248] The alkyl chains were used to aid organic solubility of the polymers.



Scheme 100: Boronic ester appended porphyrin used subsequently to form hyperbranched conjugated porphyrin arrays via Suzuki polymerisation reactions[248]

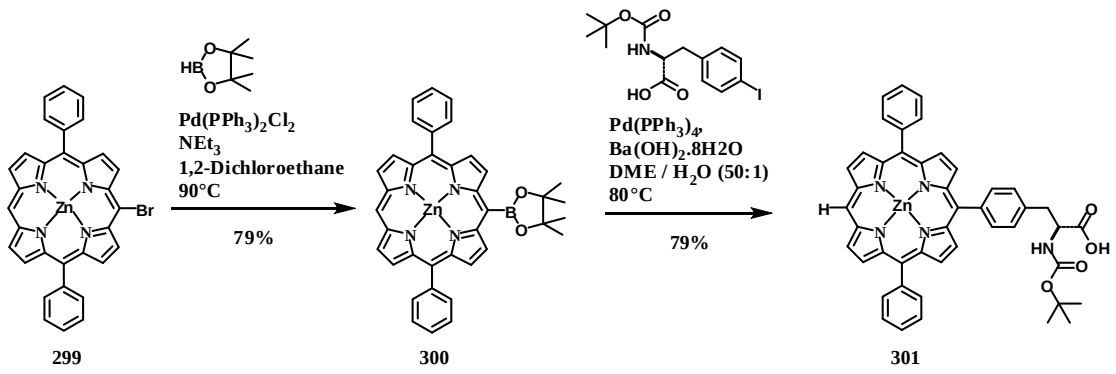
Lindsey *et al.*[209] synthesised the *trans*-A_2B_2 type porphyrin **296** containing two pinacol boronic esters by acid catalysed condensation of 5-mesityldipyrromethane with *p*-(1,3-dioxaborane)benzaldehyde followed by oxidation as shown in Scheme 101. The subsequent Suzuki coupling with the iodo-porphyrin **297** proceeded smoothly to give the porphyrin trimer **298** in 66% yield.[209]



Scheme 101: Synthesis of a porphyrin triad via a Suzuki coupling[209]

In 1998, Therien *et al.*[249] were the first group to report the synthesis and reactions of a boronic ester appended on the *meso* position of a porphyrin. (Scheme 102) The Masuda group had previously reported the use of pinacolborane to transform aryl halides into aryl boronates using a palladium catalysed cross coupling reaction.[250] As shown in Scheme 102, Therien and co-workers[249] extended this methodology to the zinc(II) complex of 5-bromo-10,20-diphenyl porphyrin **299** to synthesise the pinacol boronic ester appended porphyrin **300** in 79% yield. They subsequently coupled **300** to several substrates under Suzuki conditions in good yields and one of these transformations, the reaction with N-Boc protected 4-iodo-*L*-phenylalanine, is shown in Scheme 102. Compound **301** was obtained

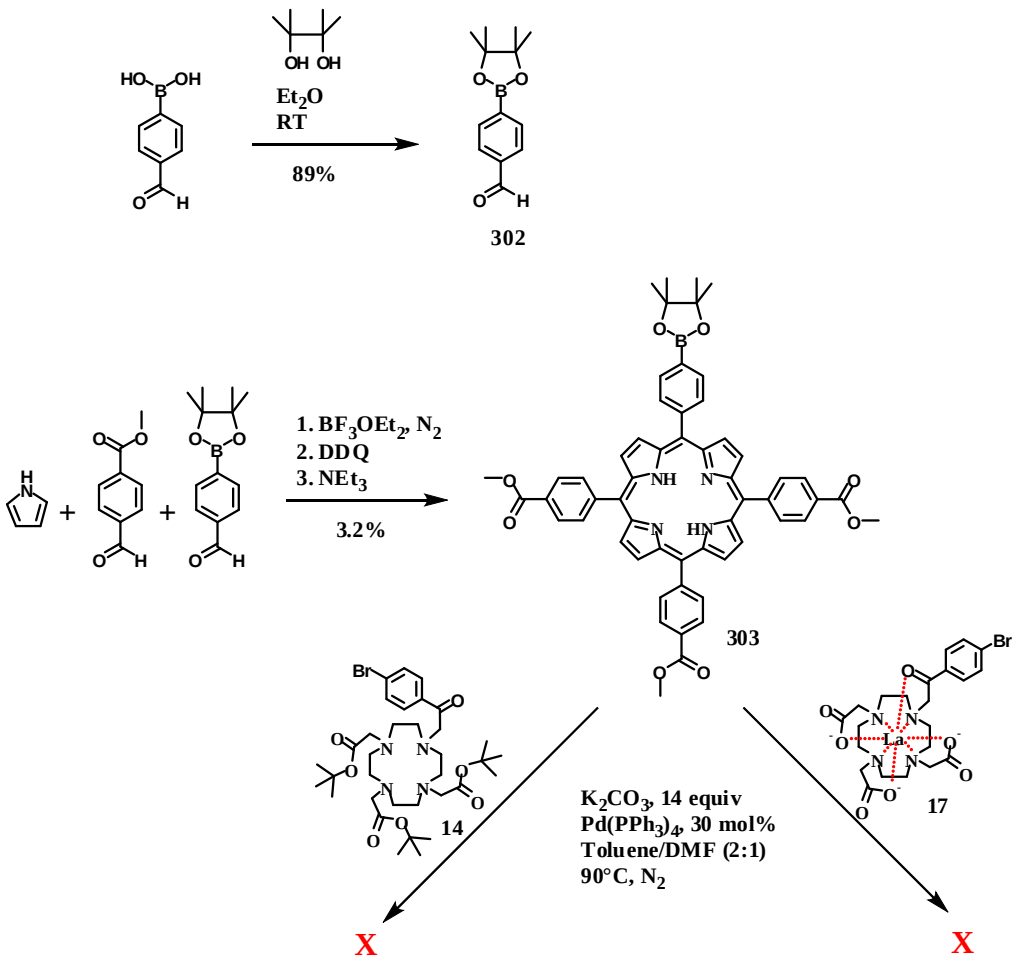
in 79% yield, thus demonstrating that porphyrins with boronic esters on the *meso* positions are also able to undergo these type of coupling reactions . It should be noted that, to date, there have been no examples reported in the literature of porphyrins bearing boronic ester or boronic acid groups on the β-positions of the macrocycle.



Scheme 102: The first example of a porphyrin meso-substituted with a boronic ester[249]

4.8.4 Synthetic strategy

The aryl bromide cyclen derivatives that have already been synthesised directed the need for a boronic ester appended porphyrin. To this end, a boron pinacol ester was chosen as this is known to be stable. It was also decided to substitute the remaining porphyrin phenyl rings with methyl ester groups. When the coupled product has been synthesised, this would facilitate a one step hydrolysis of both the *tert*-butyl esters on the cyclen moiety and the methyl esters on the porphyrin at the same time. Porphyrin **303** was thus chosen as the target molecule (Scheme 103).

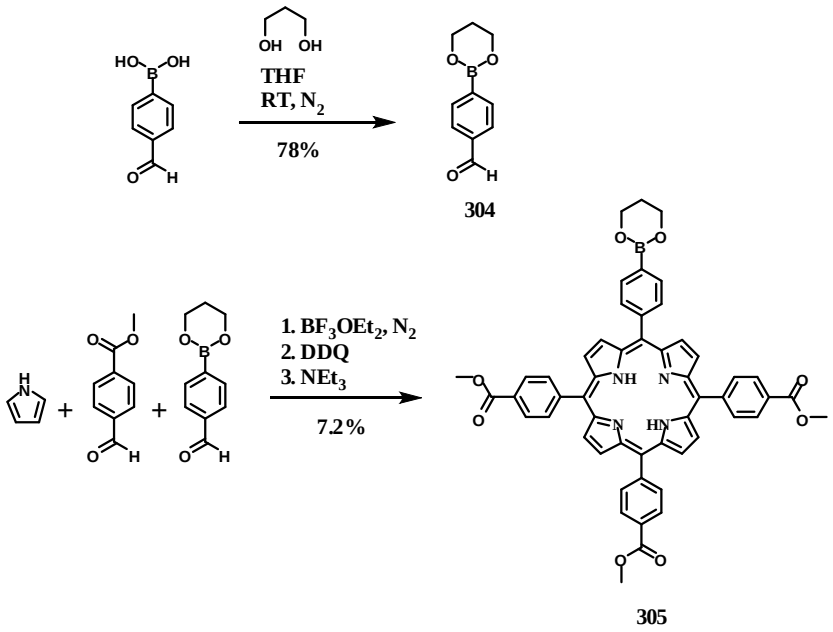


Scheme 103: Synthesis and attempted coupling reactions of pinacol boronic ester porphyrin 303

4.8.5 Results and discussion

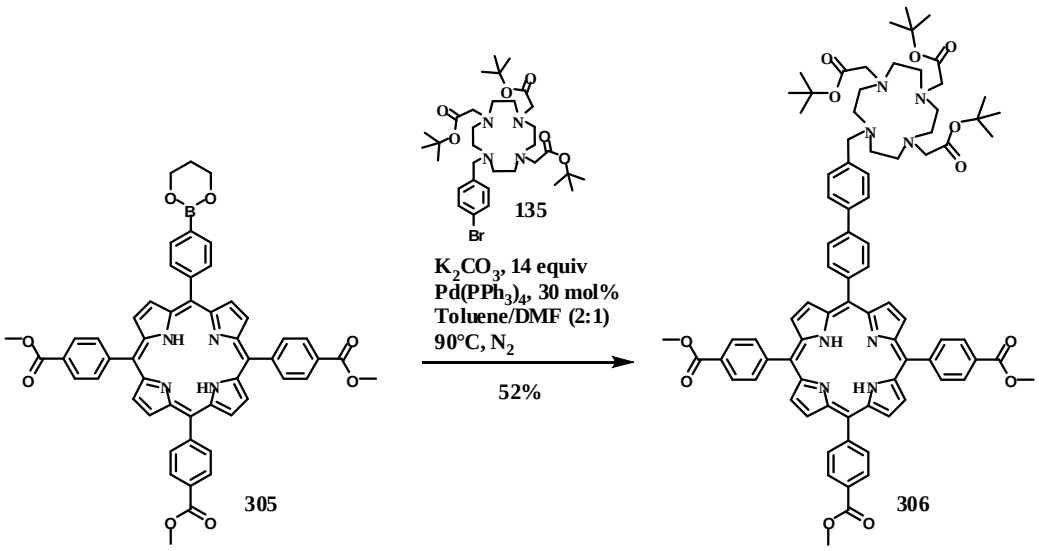
Compound **302** was synthesised using the method reported by Yu *et al.*[209] in good yield (Scheme 103). This was subsequently condensed with pyrrole and methyl(4-formyl)benzoate using Lindsey[139, 230] conditions to form the pinacol boronic ester appended porphyrin **303**. During chromatographic isolation compound **303** appeared to decompose and this led to a very low yield of 3%. This was surprising since the pinacol group is electron releasing and should stabilise the electron deficient boron atom, and analogues of this porphyrin have been reported before, such as compound **296** (Scheme 101). It is feasible that the electron withdrawing ester groups in compound **303** negate the effect of the electron releasing pinacol group and destabilise the porphyrin. In spite of this however, enough compound was collected to use in the following steps of the synthetic route. The initial attempt at Suzuki coupling was carried out with the lanthanum complex **60**. $Pd(PPh_3)_4$ was used as the catalyst, in the presence of K_2CO_3 in a toluene / DMF (2:1) solvent system. The reaction was heated to 90 °C and TLC analysis after 4 hours showed complete consumption of the starting material and a new porphyrin spot. However, when this was isolated, the ^1H NMR did not correlate with the expected compound. The pinacol ester peaks had disappeared, but the porphyrin remained intact and there were no peaks representative of the cyclen species. A ^{11}B NMR showed a strong peak and so this evidence pointed towards hydrolysis of the boronic ester to the boronic acid. Although this species should still be reactive, no coupled product was formed. Since the base in the Suzuki reaction must be strong enough to activate the boronic ester, but must not interfere with any base sensitive groups in the substrate, the reaction was attempted twice more with first K_3PO_4 (a weaker base) and then with Cs_2CO_3 (a stronger base). However, neither of these changes affected the outcome of the reaction. Finally, the cyclen moiety was changed to the free base compound **135** and the coupling conditions from the initial reaction were used, but this was to no avail. The hypothesis at this time was that the stability of the boronic ester may preclude these reactions from taking place. Hence, attention was switched at this point to synthesising an alternative boronic ester substituted porphyrin. As shown in Scheme 104, 1,3-dioxaboranylbenzaldehyde **304** was synthesised in 78% yield using a known

literature method.[251] This was condensed using Lindsey conditions[139] to give porphyrin **305** in 7.2% yield. Although this was again a low yield, the porphyrin did not degrade on the silica column during purification.



Scheme 104: Synthetic route to boronic ester appended porphyrin 305

Compound **305** was subsequently heated to 90 °C with **135** in a mixture of toluene and DMF (2:1) using $Pd(PPh_3)_4$ as catalyst in the presence of K_2CO_3. (Scheme 105) After 17 hours, the product was isolated by column chromatography and identified by NMR and mass spectrometry as the coupled product **306**.



Scheme 105: Suzuki coupling reaction affording cyclen-porphyrin conjugate 306

The ^{1}H NMR spectrum and the proton assignments are shown in Figure 59, and as expected it is very similar to the spectrum for compound **287**. The assignments for the aromatic protons have been made with reference to the 2D DQF COSY correlation spectrum. The cyclen protons appear as a broad signal which cannot be assigned, but which is characteristic of this type of compound.

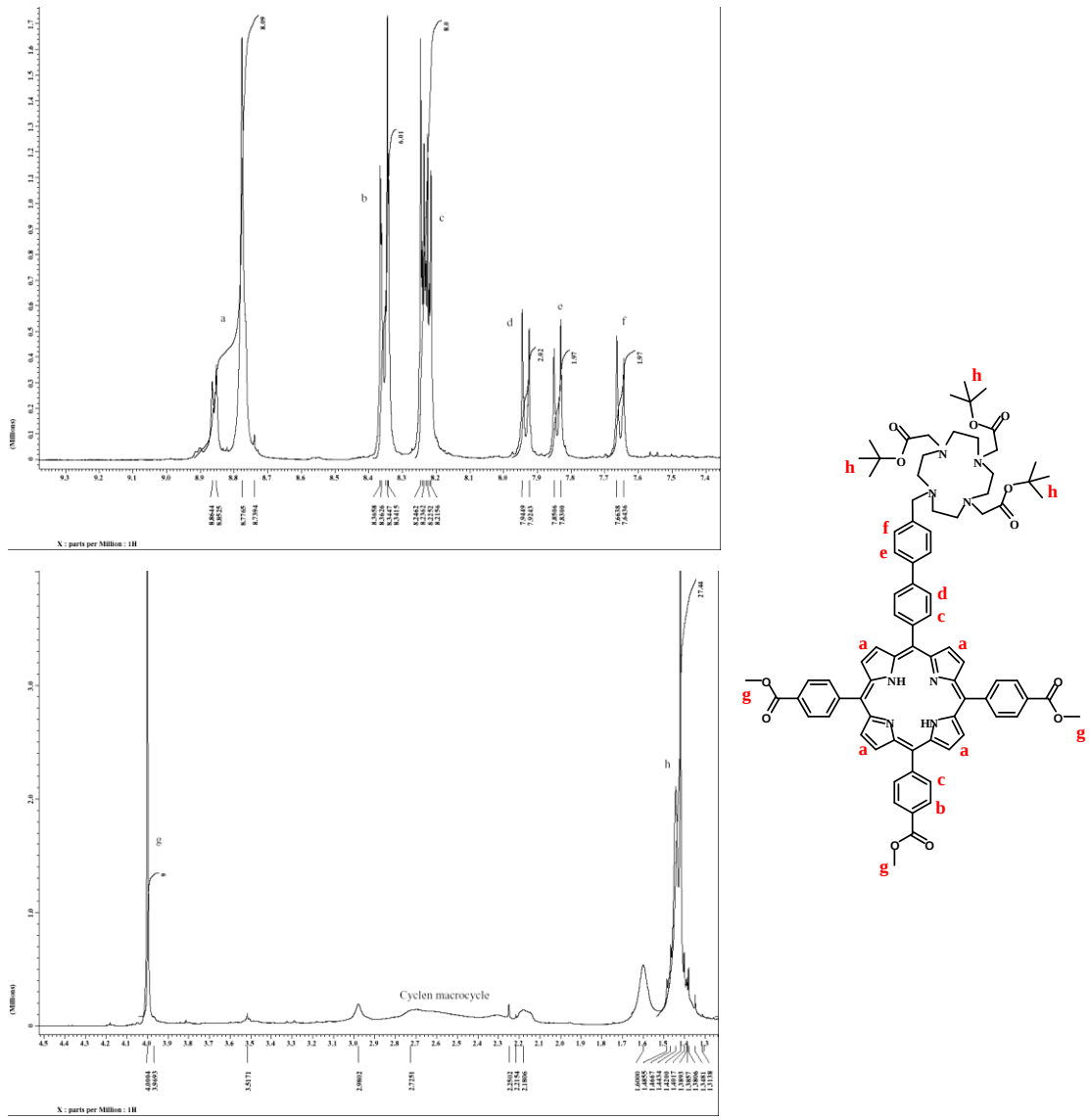


Figure 59: ^{1}H NMR spectrum of compound 306

Hydrolysis of the ester groups within compound **306** was attempted with a dichloromethane solution of compound **306** being treated with concentrated HCl at room temperature. After 1 hour, TLC analysis appeared to show only one very polar spot, indicating this compound did not degrade under strongly acidic conditions. ^{1}H NMR confirmed that all ester groups had been hydrolysed. Water was added to the reaction mixture, excess $GdCl_3.6H_2O$ added and the mixture heated to 60 °C for 8 hours under inert atmosphere. Unfortunately, the compound did not sequester the metal ion and no metal ion complex was formed. Time constraints for this project did not allow for further attempts at formation of the metal ion complex. Although the desired metal complex was not synthesised,

compound **306** was stable to acidic hydrolysis whereas the conjugates that had been synthesised previously, were not. This suggests that, as expected, the aryl-aryl linkage is more robust and that this route is the most promising for the syntheses of these types of bismacrocyclic systems.

4.9 Summary

Nucleophilic substitution of the electrophilic porphyrin **237** with the amino bearing cyclen/cyclam compounds was unsuccessful. The reactions appeared to be limited by the nucleophilicity of the amine groups. In the one reaction where the amine was reactive enough, the reaction could not be controlled and a mixture of the desired product and the dialkylated compound was produced.

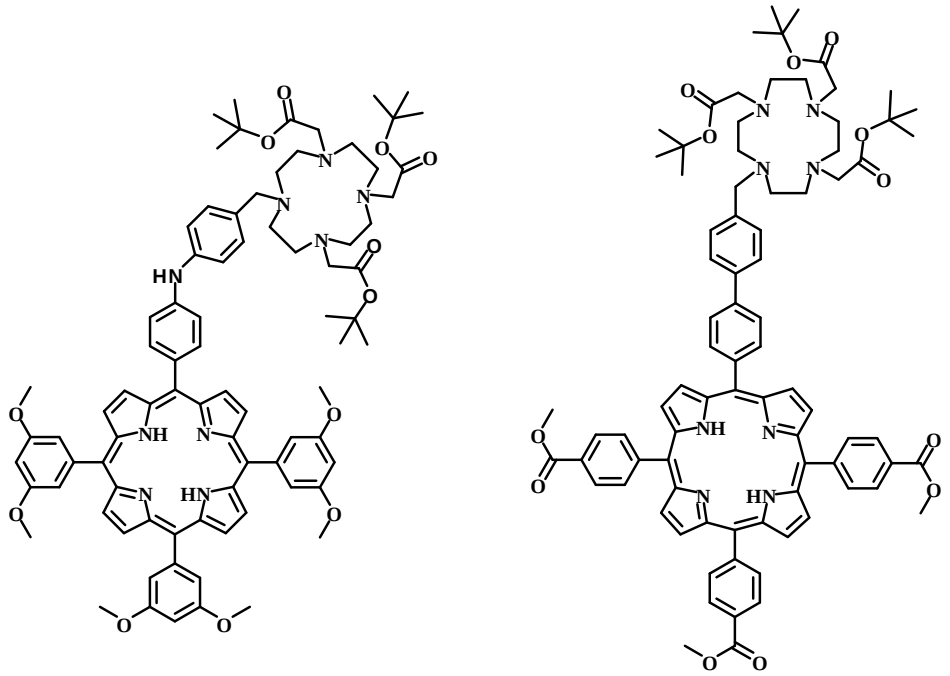
Reactions using activated esters were equally ineffective, with problems again being encountered with the seemingly low reactivity of the amino compounds.

Two successful routes to the desired coupled products have been developed using Pd(0) catalysed reaction sequences, and the compounds produced by these routes are shown in Figure 60.

Buchwald-Hartwig aryl amination afforded the cyclen-porphyrin conjugate **287** in reasonable yield. However, the gadolinium(III) complex could not be formed due to the instability of the compound under the conditions required for deprotection of the *tert*-butyl ester groups.

The second successful route to the coupled cyclen/porphyrin molecules was achieved by using the ubiquitous Suzuki coupling reaction. The effectiveness of this methodology was demonstrated by the synthesis of compound **306** in reasonable yield. The aryl aryl bond created was demonstrated to be robust, as expected, as this compound did not degrade when the ester groups were deprotected. Although the desired metal ion complex was not synthesised, this route fulfils all the necessary requirements for the synthesis of bismacrocycles

for medical imaging and therapy. Access to a large range of analogous compounds can be forseen due to the wide scope of the coupling reaction.



287 **306**

Figure 60: Bismacrocycles successfully synthesised through palladium(0) catalysed coupling reactions

Chapter 5: Concluding remarks

A convergent synthetic methodology has been developed to give access to bis-macrocyclic systems comprising a porphyrin and a cyclen based moiety.

A series of novel macrocyclic chelators based around the cyclen framework have been successfully synthesised, each containing a functional handle for further elaboration of the structure.

Reductive amination reactions of 2-formyl TPP were undertaken with a variety of amino bearing substrates in order to investigate the applicability of this reaction towards obtaining the desired porphyrin-cyclen conjugates. These reactions showed that this methodology can be used to introduce a range of functionality and diversity into the porphyrin core. This was demonstrated by the direct reductive amination of amino bearing cyclen derivatives to give the desired bis-macrocyclic compounds **221** and **222**.

Unfortunately, these compounds were not stable under the acidic conditions required for deprotection of the *tert*-butyl ester groups and so the metal ion complexes could not be formed.

Attempts to couple the amino bearing cyclams with electrophilic porphyrins **237** and **262** were unsuccessful and appeared to be limited by the reactivity of the amine groups.

Buchwald-Hartwig aryl amination afforded the cyclen-porphyrin conjugate **287** in reasonable yield, but once again the gadolinium(III) complex could not be formed due to the instability of the compound under the conditions required for deprotection of the *tert*-butyl ester groups.

Suzuki coupling of the aryl bromide appended cyclen **135** and the boronic ester bearing porphyrin **305** was successful and gave access to the target compound **306**. The aryl-aryl linkage between the two macrocycles in this compound proved more robust and allowed the deprotection of the ester groups without degradation of the rest of the compound.

Since cyclen macrocycles bearing carboxylate pendant arms are known to form stable and inert complexes with gadolinium(III) and porphyrins are known photosensitisers, compound **306** possesses the basic elements required to be investigated as a dual imaging-therapy agent (MRI-PDT).

Moreover, the synthetic route to **306** has wide scope due to the coupling reaction and conditions being tolerant of a range of different functional groups and this will allow many analogues of compound **306** to be synthesised.

5.1 Future work

The initial direction for future development of this work is to resynthesise compound **306** to obtain a larger quantity. The subsequent deprotection and complexation reactions need to be optimized for lanthanide(III) ions and once this has been achieved, a series of experiments could be undertaken to determine the effect that linking the two components has had on their individual properties.

The physical analysis required for the contrast agent would include a relaxivity measurement, which can be compared with the value obtained for the unconjugated gadolinium(III) complex. Synthesis of the europium(III) complex would allow luminescence studies to determine the number of inner sphere bound waters (Q value), which is the main component of relaxivity. Due to the larger size of the conjugate compound, a decrease in the rotational correlation time would be expected with a concomitant increase in relaxivity, and attempts to quantify this effect could also be undertaken. The cyclen derivatised macrocycle would be expected to form a stable complex with the gadolinium(III) ion and any change caused by coupling of the porphyrin could be measured by determination of the stability constant with respect to dissociation of the metal ion. Potentiometric titrations could be used to evaluate competitive binding by the zinc(II) ion, a process which occurs *in vivo*.

In terms of the porphyrin moiety, any changes to the luminescence properties of the molecule on coupling can be probed, for example shifts in the absorption and emission spectra maxima. Measurement of singlet oxygen production as well as light/dark toxicity studies need to be carried out. Cellular uptake can be measured by optical microscopy or MR imaging of cell pellets.

The broad scope of the coupling reaction allows for a series of analogues to be synthesised, and the specific target molecules would be dependent on the results of the aforementioned studies. However, one obvious candidate would be to couple several gadolinium(III) complexes to one porphyrin, in effect increasing the imaging ‘payload’. Relaxivity measurements could be taken to measure any

possible synergistic effects on contrast enhancement. Another option is to change the porphyrin moiety, substituting for one with an absorption band at a longer wavelength to allow more efficient light penetration through tissue, to enhance the effectiveness of PDT.

Chapter 6: Experimental

All starting materials were purchased from Lancaster Synthesis or Sigma Aldrich.

NMR spectra were obtained from a Jeol JNM-ECP 400 MHZ FT-NMR spectrometer, with chemical shifts reported in ppm relative to a tetramethylsilane standard.

Ultra violet/visible absorption spectra were recorded on an Agilent 8453 spectrometer.

Analytical TLC was carried out using aluminium backed silica gel 60 F254 and visualised using UV light (254 / 365 nm). Column chromatography was performed using ICN silica 32-63, 60Å.

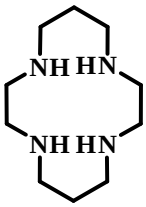
Mass spectrometry results were obtained via services provided by :

EPSRC National Mass Spectrometry Service Centre,
Chemistry Department
University of Wales
Swansea

Analyses were made using the following instrumentation:

MALDI	Applied Biosystems Voyager
Electrospray	Waters ZQ-4000
FAB	Finnigan MAT 95XP

6.01 Synthesis of 1,4,8,11-tetraazacyclotetradecane (5)[122]



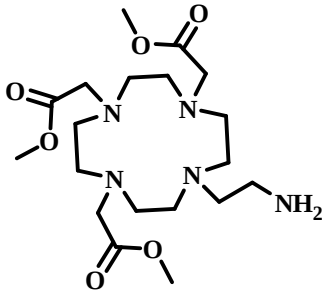
To a solution of nickel (II) perchlorate (54.7g, 0.15mol) in water (400ml) was added 1,5,8,12-tetraazadodecane (26g, 0.15mol) with stirring. The solution was cooled to 5 °C, glyoxal (40% w/v, 22.5ml) added then left to stand at room temperature for 18 hours. The mixture was cooled again to 5 °C and sodium borohydride (11g, 0.3mol) added slowly over 1 hour. Upon addition, the mixture was heated to 90 °C for 15 minutes, filtered through a hot sinter, sodium cyanide (29g, 0.6mol) added and heated under reflux conditions for 2 hours. Upon cooling, sodium hydroxide pellets (15g, 0.38mol) were added and the water removed *in vacuo*. The solid was taken up in chloroform, filtered and dried ($MgSO_4$) before being concentrated *in vacuo* to yield a crude off white solid. This was recrystallised from chlorobenzene (150ml) to yield fine white crystals of compound **5** (10.6g, 35%). m.p. 185-187°C

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.69 (4H, q, ^{3}J=5.3Hz, C*H*$_2$-β-N), 2.32 (4H, br s, N*H*), 2.64 (8H, s, C*H*$_2$-α-N), 2.71 (8H, t, ^{3}J=5.4Hz, C*H*$_2$-α-N)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 29.5 (*C*H_2-β-N), 49.4 (*C*H_2-α-N), 50.8 (*C*H_2-α-N)

MS (ESI) *m/z* 201 $(M+H)^+$

6.02 Synthesis of 1-aminoethyl-4,7,10-(tris-methoxycarbonylmethyl)-(1,4,7,10-tetraazacyclododecane) (56)



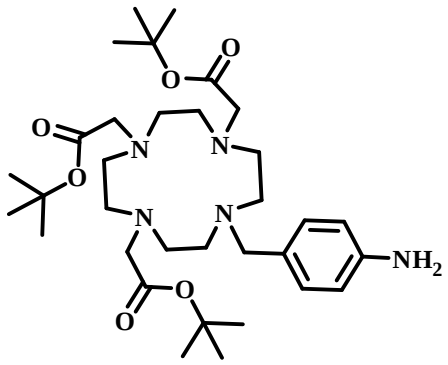
116 (0.5g, 0.94 mmol) was dissolved in anhydrous CH_2Cl_2 (4 mL) under an argon atmosphere. TFA (1mL) and TFA anhydride (1 drop) were added dropwise and the reaction mixture stirred for one hour at ambient temperature. The solvent was then removed *in vacuo* and ethyl acetate (5mL) added. The solvent was then removed carefully by pipette and the washing procedure repeated three times. To the insoluble residue was added a solution of $NaHCO_{3(aq)}$ (10mL) and the mixture stirred for 30 minutes under argon at ambient temperature. The resulting mixture was extracted with CH_2Cl_2 (3 x10mL) and the organic extracts combined and concentrated to give the product as a white semi-solid. (0.40g, 99%)

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 2.31-2.62 (16H, m, NC*H*$_2$ (ring)), 2.63-2.73 (2H, m, NC*H*$_2$$CH_2NH_2$ (pendant arm)), 2.84 (2H, bs, NCH_2C*H*$_2$$NH_2$ (pendant arm)), 3.38-3.42 (4H, m, C*H*$_2$COOMe), 3.46 (2H, bs, C*H*$_2$COOMe), 3.61 (3H, s, COOC*H*$_3$), 3.66 (6H, s, COOC*H*$_3$)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 37.1, 45.9, 49.6, 50.1, 51.1, 51.3, 51.6, 54.96, 55.01, 171.5, 172.1

MS (ESI) *m/z* 432 $(M+H)^+$

6.03 Synthesis of 1,4,7-(tris-*tert*-butoxycarbonylmethyl)-10-(4-aminobenzyl)-1,4,7,10-tetraaza-cyclododecane (57)



Method 1[108]

To a mixture of sulphur (66mg, 2.05mmol) and $NaBH_4$ (26mg, 0.69 mmol) under a nitrogen atmosphere was added anhydrous THF (60mL) and the resulting yellow suspension stirred for one hour. A solution of **132** (0.22g, 0.34mmol) in anhydrous THF (80mL) was added and the mixture heated under reflux for ten hours, during which time the suspension turned a grey/green colour. After cooling to room temperature the solvent was removed *in vacuo* and CH_2Cl_2 (100mL) added to the residue. After several extractions with $NaOH_{(aq)}$ (5% w/v, 5x50mL) to remove the excess sulphur, the organic layer was dried over $MgSO_4$ and after removal of the dessicant, concentrated down to a small volume (~5mL). The product was isolated by gravity column chromatography (silica, eluent : CH_2Cl_2/MeOH, 99:1) as a sticky, pale yellow semi-solid (0.16g, 78%).

Method 2.

Compound **132** (1.2g, 1.86mmol) was dissolved in MeOH (50mL) and Pd/C (10wt%, 64mg) added. H_2 gas was bubbled directly through the suspension and the reaction was followed by TLC (eluent : CH_2Cl_2/MeOH, 99:1). After three hours the solvent was removed *in vacuo* and the residue dissolved in CH_2Cl_2 (40mL). The organics were extracted with $NaHCO_3$ solution several times before being dried over Na_2SO_4. After removal of the dessicant by filtration, the solvent was concentrated down to a small volume (~5mL). The product was isolated by gravity column chromatography (silica, eluent : CH_2Cl_2/MeOH, 98:2) as a

yellow oil which was solidified by trituration with diethyl ether to give a pale yellow hygroscopic solid. Yield : 0.79g, 69.3%

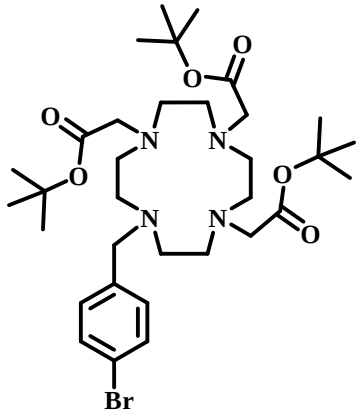
^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.43-1.50 (27H, m, (CH_3)$_3$), 2.18-3.20 (24H, br m, CH_2C(O) x3 , NCH_2 x8, CH_2PhNO_2), 6.63 (2H, d, ^{3}J = 8.4Hz, C(O)-Ar-2,6-*H*), 7.21 (2H, d, ^{3}J = 8.4Hz, C(O)-Ar-3,5-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 27.9, 28.0, 53.4, 55.6, 55.9, 58.9, 82.2, 82.7, 115.1, 126.6, 131.0, 146.1, 171.2, 172.4

MS (ESI) *m/z* 620 $(M+H)^+$

HRMS : calculated for $C_{33}H_{57}N_5O_6$: 620.4382, found 620.4383

6.04 Synthesis of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-10-(4-bromobenzyl)-1,4,7,10-tetraazacyclododecane (58)[105]



To a solution of **127** (1g, 1.9 mmol) and 4-bromobenzyl bromide (0.53g, 2.13 mmol) in acetonitrile (100mL) was added Na_2CO_3 (1.65g, 16mmol) and the mixture heated at 70 °C for 3 days. After this time, TLC (silica, CH_2Cl_2 : MeOH, 95:5) showed complete consumption of starting material **127**. The solids were filtered off, the solvent removed *in vacuo* and the residue dissolved in dichloromethane (100mL). This was washed successively with water (2x100mL) and brine (50mL) and dried over $MgSO_4$. Following removal of the dessicant, the solution was concentrated down to a small volume (~10ml) and purified by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 98:2). Relevant fractions were identified by TLC (silica CH_2Cl_2/MeOH, 95:5), (R_f=0.4), collected and concentrated to give a pale yellow oil. Trituration with diethyl ether gave the product as a sticky, hygroscopic yellow semi-solid (1.2g, 90%)

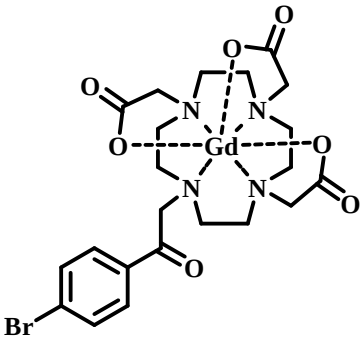
^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.36-1.45 (27H, m, OC(C*H*$_3$)$_3$, 2.1-3.4 (24H, m, NC*H*$_2$, C*H*$_2$COO^{t}Bu, C*H*$_2$PhBr), 7.33 (2H, d, ^{3}J = 8.2Hz, Br-Ar-3,5-*H*), 7.38 (2H, d, ^{3}J = 8.2Hz, Br-Ar-2,6-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 27.7, 27.8, 49.7, 50.5, 53.3, 55.6, 55.8, 58.8, 82.2, 82.8, 121.4, 131.6, 131.7, 136.6, 172.4, 173.4

MS (ESI) *m/z* 683 ^{79}Br(M)$^+$, 685 ^{81}Br(M)$^+$, 705 ^{79}Br(M+Na)$^+$, 707 ^{81}Br(M+Na)$^+$

HRMS : calculated for $C_{33}H_{55}{}^{79}BrN_4O_6$: 683.3378, found 683.3376

6.05 Synthesis of Gadolinium complex of 10-(4'-Bromo-2-acetophenone)-1,4,7,10- tetraazacyclododecane-1,4,7-triacetic acid (59)

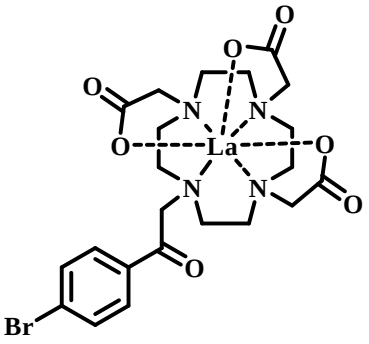


To a solution of **135** (0.51g, 0.73mmol) in dichloromethane (15mL) was added concentrated HCl (2mL) and the reaction was stirred at ambient temperature under an N_2 atmosphere for 2 hours. The solvent was then removed *in vacuo* and water (10mL) added to the yellow residue. This was filtered and the resulting solution adjusted to pH 7 by careful addition of $Na_2CO_{3(aq)}$. An aliquot was removed for NMR analysis and the remainder used straight away in the following procedure.

^{1}H N.M.R. [400MHz, D_2O] δ 3.02-3.63 (16H, m, NC*H*$_2$), 3.67 (2H, s, NC*H*$_2$C(O)), 3.84 (4H, s, NC*H*$_2$C(O)), 4.40 (2H, s, NC*H*$_2$C(O)), 7.72 (2H, d, ^{3}J = 8.7Hz) 7.86 (2H, d, ^{3}J = 8.7Hz)
^{13}C N.M.R. [100MHz, D_2O] δ 43.0, 48.3, 48.6, 51.7, 51.8, 54.3, 54.4, 55.9, 57.0, 58.3, 128.8, 129.9, 132.3, 134.2, 170.7, 175.9, 198.8

To the above solution was added dropwise a solution of $GdCl_3.6H_2O$ (0.30g, 0.8mmol) in water (5mL) with vigorous stirring. The resulting solution was heated to 60 °C under an argon atmosphere for 24 hours. After cooling to ambient temperature the solution was concentrated to ~2mL and passed down a size exclusion column (Sephadex LH20, eluent : H_2O). Relevent fractions were collected, combined and concentrated to give the product as a yellow semi-solid. (0.3g, 60%).
MS (FAB) *m/z* 695.04 $(M+H)^+$
HRMS : calculated for $C_{22}H_{28}Br^{155}GdN_4O_7$: 695.0440, found 695.0435

6.06 Synthesis of Lanthanum complex of 10-(4'-Bromo-2-acetophenone)-1,4,7,10- tetraazacyclododecane-1,4,7-triacetic acid (60)

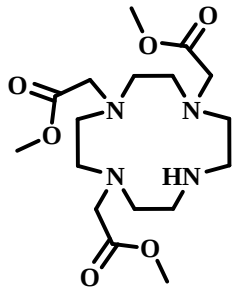


This compound was synthesised in exactly the same way as compound 59. (Yield: 52%)

MS (ESI) *m/z* 696 $(M+NH_4)^+$

HRMS : calculated for $C_{22}H_{32}Br^{139}LaN_5O_7$: 696.0543, found 696.0549

6.07 Synthesis of 1,4,7-(tris-methoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane(90)[101]



To a suspension of cyclen **6** (3g, 17.4 mmol) and $NaHCO_3$ (4.39g, 52.2 mmol) in acetonitrile (800mL) was added dropwise a solution of methylbromoacetate (8g, 52.2 mmol) in acetonitrile (200 mL) over 6 hours. The reaction misture was then stirred for 3 days at ambient temperature under an inert (N_2) atmosphere until TLC (silica, eluent : CH_2Cl_2 / CH_3OH, 95:5) showed complete consumption of the alkylating agent. The mixture was filtered under suction and the solvent removed *in vacuo*. The residual yellow oil was purified by flash chromatography (silica, eluent : CH_2Cl_2 / CH_3OH, 95:5) to yield a yellow oil. (R_f =0.3) Yield : 3.38g, 50%

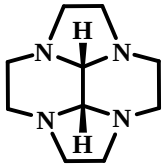
^{1}H N.M.R. [400MHz, $CDCl_3$] δ 2.78-2.98 (12H, m, NC*H*$_2$ (ring), 3.03-3.12 (4H, m, NC*H*$_2$ (ring), 3.39 (2H, s, NC*H*$_2$COOMe), 3.47 (4H, s, NC*H*$_2$COOMe), 3.66 (9H, s, COOC*H*$_3$), 9.90 (1H, bs, N*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 47.2 (N*C*H_2 ring), 48.4 (N*C*H_2COOMe), [49.2, 51.3 (N*C*H_2 ring)], 51.6 (COO*C*H_3), 56.9 (N*C*H_2COOMe x2), [170.6, 171.5 (*C*OOCH_3)]

MS (ESI) *m/z* 389.2 $(M+H)^+$

HRMS : calculated for $C_{17}H_{32}N_4O_6$: 389.2395, found 389.2399

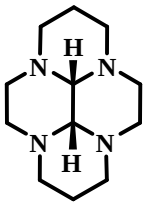
6.08 Synthesis of Perhydro-2a,4a,6a,8a-tetraazacyclopenta[f,g]acenaphthalene (109*)*



A solution of cyclen **6** (5g, 29 mmol) in methanol (100mL) was cooled to 0 °C and an aqueous solution of glyoxal (40% w/v, 4.65 mL, 32mmol) added dropwise over 30 minutes. The resulting solution was allowed to rise to ambient temperature and stirred for three hours. Solvents were subsequently removed *in vacuo* and diethyl ether (50mL) added to the residue. Insoluble solids were removed by filtration and the colourless solution concentrated to give an off white solid. This was triturated several times with diethyl ether to yield the product as a white solid. (5.35g, 97%) m.p. 91-93°C

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 2.52-2.62 (4H, m, NC*H*$_2$), 2.62-2.78 (4H, m, NC*H*$_2$), 2.92-3.00 (4H, m, NC*H*$_2$), 3.00-3.07 (4H, m, NC*H*$_2$), 3.14 (2H, s, C*H*)
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 50.4, 51.1, 77.5,
MS (ESI) *m/z* 195 $(M+H)^+$
HRMS : calculated for $C_{10}H_{18}N_4$: 195.1604, found 195.1605

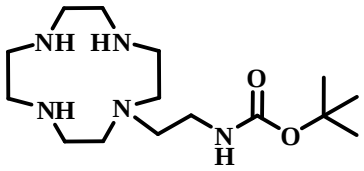
6.09 Synthesis of Decahydro-3a,5a,8a,10a-tetraazapyrene (112)[98]



To a cooled (0 °C) solution of compound **5** (1g, 5mmol) in methanol (30ml) was added a glyoxal solution (40% w/v, 0.8g, 5.5mmol) in methanol (15ml). The reaction mixture was stirred for 3 hours at room temperature after which the solvent was removed *in vacuo*. Diethyl ether (200ml) was added and the resulting suspension filtered. The filtrate was collected and concentrated *in vacuo* to yield compound **112** as a yellow viscous oil (1.05g, 94%).

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.15-1.19 (2H, m, ^{3}J=2Hz, ^{2}J=13.2Hz, C*H*$_2$-β-N), 2.06 (2H, t, ^{2}J=11.5Hz, C*H*$_2$-α-N), 2.10-2.25 (4H, m, C*H*$_2$-β-N, C*H*$_2$-α-N, C*H*$_2$-β-N), 2.27 (2H, d, ^{2}J=10.4Hz, C*H*$_2$-α-N), 2.68 (2H, br d, C*H*$_2$-α-N), 2.88-2.90 (6H, m, C*H*$_2$-α-N), 3.04 (2H, s, NC*H*N), 3.45-3.50 (2H, m, C*H*$_2$-α-N)
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 19.6 (*C*H_2-β-N), 44.7 (*C*H_2-α-N), 52.5 (*C*H_2-α-N), 54.3 (*C*H_2-α-N), 56.0 (*C*H_2-α-N)
MS (ESI) *m/z* 223 $(M+H)^+$

6.10 Synthesis of 1-(2-(*tert*-butoxycarbonyl)-aminoethyl)-(1,4,7,10-tetraazacyclododecane) (115)



Method 1[101]

To a stirred solution of cyclen **6** (3.84g, 22mmol) in toluene (120mL) was added **119** (1.7g, 8mmol) and the solution heated under reflux conditions for 10 hours under a nitrogen atmosphere. Upon cooling to ambient temperature a white solid precipitated out of solution. Water (100mL) was added which caused the precipitate to redissolve and the organic layer was then extracted twice with water (100mL x2) and the aqueous layer separated. This was extracted with CH_2Cl_2 (200mL), and the organic layer separated and dried over $MgSO_4$. After removal of the dessicant by filtration the solvent was removed *in vacuo* to yield a white sticky solid which was triturated several times with cold diethyl ether. Yield 1.16g, 49% based on **119**. m.p. 118-120°C

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.44 (9H, s, (C*H*$_3$)$_3$), 2.50-2.67 (14H, br m, NC*H*$_2$,), 2.77-2.87 (4H, m, NC*H*$_2$), 3.18-3.25 (2H, m, NC*H*$_2$)
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 28.3, 38.4, 46.0, 47.0, 47.7, 52.0, 54.0, 78.7, 155.9
MS (ESI) *m/z* 316 $(M+H)^+$
HRMS : calculated for $C_{15}H_{33}N_5O_2$: 316.2707, found 316.2705

Method 2

Compound **117** (1.2g, 2.9 mmol) was dissolved in hydrazine monohydrate (30mL) and heated to 120 °C under an N_2 atmosphere. After 10 hours $CHCl_3$ (50mL) was added and the chloroform layer separated and concentrated *in vacuo* to yield a yellow oil. This was washed successively with diethyl ether (20mL) and CCl_4 (20mL), however, NMR analysis indicated a small amount of impurity

still present. The solvent was removed *in vacuo* and the resulting solid recrystallised from ethyl acetate to yield a white solid. (0.46g, 51%). m.p. 118-121°C

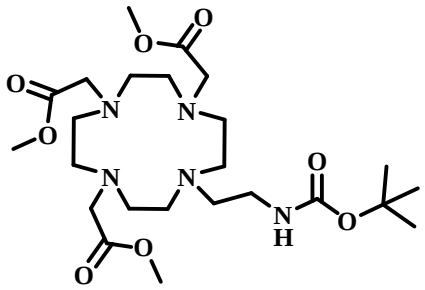
^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.44 (9H, s, (C*H*$_3$)$_3$), 2.50-2.67 (14H, br m, NC*H*$_2$ (ring), NC*H*$_2$ (pendant arm)), 2.77-2.87 (4H, m, NC*H*$_2$ (ring)), 3.18-3.25 (2H, m, NC*H*$_2$ (pendant arm))
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 28.3, 38.4, 46.0, 47.0, 47.7, 52.0, 54.0, 78.7, 155.9
MS (ESI) *m/z* 316 $(M+H)^+$
HRMS : calculated for $C_{15}H_{33}N_5O_2$: 316.2707, found 316.2706

Method 3

Compound **117** (3.0g, 7.6 mmol) was dissolved in $KOH_{(aq)}$ (20% w/v, 50mL) under an N_2 atmosphere and heated to 60 °C. After three days the reaction was cooled to ambient temperature and extracted into $CHCl_3$ (100mL). The organic layer was separated and concentrated to yield a brown oil. A small quantity of CH_2Cl_2 (~10mL) was added and the solution dried over Na_2SO_4. The dessicant was removed and gravity column chromatography (alumina, eluent : CH_2Cl_2/ MeOH 95:5) used to isolate the product as a light brown sticky oil which was solidified by trituration with diethyl ether. Yield 0.92g, 41% m.p. 119-121°C

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.44 (9H, s, (C*H*$_3$)$_3$), 2.50-2.67 (14H, br m, NC*H*$_2$ (ring), NC*H*$_2$), 2.77-2.87 (4H, m, NC*H*$_2$), 3.18-3.25 (2H, m, NC*H*$_2$)
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 28.3, 38.4, 46.0, 47.0, 47.7, 52.0, 54.0, 78.7, 155.9
MS (ESI) *m/z* 316 $(M+H)^+$
HRMS : calculated for $C_{15}H_{33}N_5O_2$: 316.2707, found 316.2708

6.11 Synthesis of 1-(2-(*tert*-butoxycarbonyl)-aminoethyl)-4,7,10-(tris-methoxycarbonylmethyl)-(1,4,7,10-tetraazacyclododecane) (116)



Method 1[101]

Compound **90** (1.5g, 3.86 mmol), compound **119** (0.87g, 3.86 mmol) and Na_2CO_3 (0.41g, 3.86 mmol) were dissolved in acetonitrile (100mL) and stirred under an N_2 atmosphere at ambient temperature. The reaction was monitored by TLC (silica, eluent : CH_2Cl_2 / CH_3OH, 95:5) but after 4 days no reaction was seen to occur. The reaction was subsequently heated to 60 °C and after 8 hours, TLC showed complete consumption of the starting materials. The mixture was filtered under suction and the solvent removed *in vacuo*. The residual brown oil was purified by flash chromatography (silica, eluent : CH_2Cl_2 / CH_3OH, 95:5) to yield a yellow oil. (Yield: 1g). The compound isolated showed a complex, unassignable ^1H NMR spectrum and was clearly not the desired compound.

Method 2

To a solution of **115** (1.15g, 3.65 mmol) in dry acetonitrile (300mL) was added Na_2CO_3 (3.1g, 29 mmol) and the mixture heated to 50 °C. To this was added dropwise a solution of methyl bromoacetate (2.27g, 15 mmol) in dry acetonitrile (50mL). Heating at 50 °C was continued and the reaction progress monitored by TLC (silica, eluent : CH_2Cl_2 / MeOH, 9:1). After 16 hours the reaction showed no further progress and the mixture was cooled to ambient temperature and the solids removed by filtration. The solvent was removed *in vacuo* and the residue dissolved in a minimum of CH_2Cl_2 (~10mL). The product was isolated by flash column chromatography (silica, eluent : CH_2Cl_2 / MeOH, 95:5) as a pale yellow

oil. (Rf =0.4) After being successively triturated with hexane and diethyl ether, the product was obtained as a pale yellow semi-solid. (1.40g, 72%)

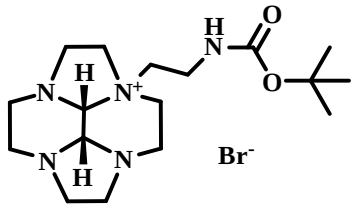
^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.45 (9H, s, C(CH_3)$_3$), 2.31-3.05 (18H, m, NCH_2 x8 (ring), NCH_2 CH_2), 3.20-3.28 (4H, m, NCH_2CH_2, NCH_2COOMe), 3.35 (4H, bs, NCH_2COOMe), 3.79 (3H, s, COOCH_3), 3.80 (6H, s, COOCH_3)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 28.3 ($C$$CH_3$)$_3$, 37.6 (N$C$$H_2CH_2$), 50.4, (N$CH_2$ (ring), 51.1 (N$C$$H_2$ x4 (ring), 52.2 (O$C$$H_3$), 52.5 (O$C$$H_3$), 54.6 (N$CH_2$$C$$H_2$), 55.2 (N$C$$H_2$COOMe x2), 55.7 (N$C$$H_2$COOMe), 79.0 C(CH_3)$_3$), 156.8 (NCO), 173.8 (COOMe), 174.1 (COOMe)

MS (ESI) *m/z* 532 $(M+H)^+$

HRMS : calculated for $C_{24}H_{45}N_5O_8$: 532.3341, found 532.3341

6.12 Synthesis of 2a-(2-*tert*-Butoxycarbonylamino-ethyl)-decahydro-4a,6a,8a-triaza-2a-azonia-cyclopenta[f,g]acenaphthylene bromide (117)



To a solution of **109** (1.3g, 6.7 mmol) in anhydrous acetonitrile (50 mL) was added a solution of **119** (1.5g, 6.7 mmol) in dry acetonitrile (25mL). The resulting solution was stirred under a N_2 atmosphere at ambient temperature. The progress of the reaction was monitored by TLC (silica, CH_2Cl_2 eluent) and was complete after 14 days. Solvents were then removed *in vacuo* and the brown residue washed with diethyl ether (10x 20 mL). After further trituration with cold (0 °C) diethyl ether the product was obtained as an off white solid. (2.06g, 74%) m.p. 80°C decomp.

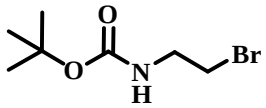
^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.43 (9H, s, C(C*H*$_3$)$_3$), 22.49-2.53 (1H, m, NC*H*$_2$), 2.66-2.73 (1H, m, NC*H*$_2$), 2.74-3.20 (8H, m, NC*H*$_2$, N^+C*H*$_2$), 3.27-3.32 (2H, m, NC*H*$_2$), 3.40-3.46 (1H, m, NC*H*$_2$), 3.68 (1H, bs, C*H*), 3.70-3.88 (4H, m, NC*H*$_2$), N^+CH_2C*H*$_2$), 4.00-4.20 (3H, m, C*H*, N^+C*H*$_2$), NC*H*$_2$), 4.50-4.62 (1H, m, NC*H*$_2$)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 28.4, 35.6, 43.6, 47.8, 48.2, 49.2, 51.9, 56.7, 57.6, 62.2, 72.0, 80.1, 84.1, 156.5

MS (ESI) *m/z* 338.3 $(M\text{-}Br)^+$

HRMS : calculated for $C_{17}H_{32}N_5O_2$ ($-Br^-$) : 338.2551, found 338.2550

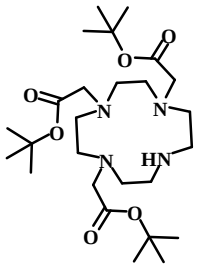
6.13 Synthesis of *Tert*-butyl-2-bromoethylcarbamate(119)[102]



A solution of bromoethylamine hydrobromide (4.7g, 22.9 mmol) in saturated $Na_2CO_{3(aq)}$ (40mL) was extracted with a solution of Boc anhydride (3.2g, 14.7 mmol) in diethyl ether (30mL), followed by two further extractions with diethyl ether (50mL x2). The ethereal extracts were dried over Na_2SO_4 and after removal of the dessicant by filtration, concentrated to ~40mL. This solution was stirred under an N_2 atmosphere at ambient temperature for 6 hours. Solvent was removed *in vacuo*, CH_2Cl_2 (10mL) added and the product isolated by flash column chromatography (silica, eluent: CH_2Cl_2) as a white solid. R_f =0.3 Yield 3.0g, 91% m.p. 33-35°C

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.45 (9H, s, (CH_3)$_3$), 3.46 (2H, t, ^{3}J = 5.6Hz, CH_2), 3.53 (2H, t, ^{3}J = 5.6Hz, CH_2), 5.00 (1H, bs, N*H*)
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 28.3, 32.8, 42.3, 79.8, 155.6

6.14 Synthesis of 1,4,7-(tris-*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraaza-cyclododecane (127)



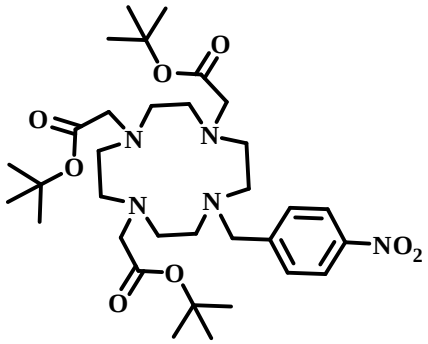
tert-Butyl bromo acetate (3.35g, 17.08 mmol) in CH_2Cl_2 (100 mL) was added slowly over 5 hours to a stirred mixture of cyclen **6** (1.02g, 5.69 mmol) and K_2CO_3 (0.79g, 5.69 mmol) in CH_2Cl_2 (300mL). The reaction mixture was subsequently stirred at ambient temperature for 3 days. The mixture was filtered and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica, eluent: CH_2Cl_2/ MeOH 93:7) (R_f =0.4) and after removal of solvents, the product was isolated as a white semi-solid. Yield 1.51g, 52%

^{1}H N.M.R. [400MHz, $(CD_3)_2CO$] δ 1.38-1.43 (27H, m, $(CH_3)_3$), 2.62-2.75 (8H, m, CH_2CH_2), 2.80-2.88 (4H, m, CH_2CH_2), 2.92-3.02 (4H, m, CH_2CH_2), 3.33 (4H, s, CH_2CO), 3.41 (2H, s, CH_2CO), 8.89 (1H, bs, NH)

^{13}C N.M.R. [100MHz, $(CD_3)_2CO$] δ 27.8, 27.9, 45.5, 48.3, 49.7, 50.7, 51.9, 56.0, 80.5, 80.6, 170.0, 170.6

MS (ESI) *m/z* 515 $(M+H)^+$

6.15 Synthesis of 1,4,7-(tris-*tert*-butoxycarbonylmethyl)-10-(4-nitro-benzyl)-1,4,7,10-tetraaza-cyclododecane (132)[105]



To a solution of **127** (1g, 1.9 mmol) and 4-nitrobenzyl bromide (0.42g, 1.9 mmol) in acetonitrile (100mL) was added Na_2CO_3 (1.65g, 16mmol) and the mixture heated at 70 °C for 5 hours. After this time, TLC (silica, CH_2Cl_2 : MeOH, 95:5) showed complete consumption of starting material **127**. The solvent was removed *in vacuo* and the brown residue was dissolved in dichloromethane (100mL), washed successively with water (2x100mL) and brine (50mL) and dried over $MgSO_4$. Following removal of the dessicant, the solution was concentrated down to a small volume (~10ml) and purified by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 95:5). Relevant fractions were identified by TLC (silica CH_2Cl_2/MeOH, 95:5), R_f =0.3, collected and concentrated to yield compound **132** as a yellow oil (1.2g, 95%)

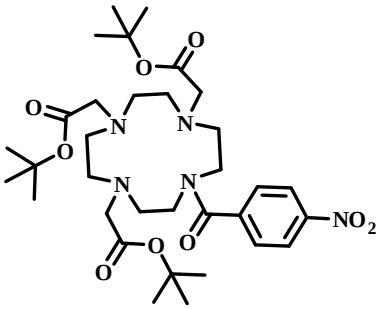
^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.47 (27H, s, OC(C*H*$_3$)$_3$, 2.0-3.2 (24H, m, NC*H*$_2$, C*H*$_2$COO^{t}Bu, C*H*$_2$PhNO$_2$), 7.74 (2H, d, ^{3}J = 8.8Hz, O$_2$N-Ar-2,6-*H*), 8.19 (2H, d, ^{3}J = 8.8Hz, O$_2$N-Ar-3,5-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 27.8, 27.9, 53.4, 55.8, 56.0, 59.1, 82.4, 83.0, 123.6, 131.0, 145.6, 147.2, 172.6, 173.7

MS (ESI) *m/z* 672 $(M+Na)^+$

HRMS : calculated for $C_{33}H_{55}N_5NaO_8$: 672.3943, found 672.3941

6.16 Synthesis of 1,4,7-(tris-*tert*-butoxycarbonylmethyl)-10-(4-nitro-benzoyl)-1,4,7,10-tetraaza-cyclododecane (133)



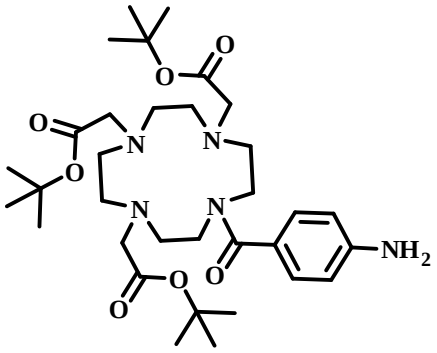
To 4-nitrobenzoic acid (0.07g, 0.4 mmol) was added thionyl chloride (10 mL) and the mixture stirred initially for 1 hour at room temperature, followed by 5 hours heating under reflux conditions. When the evolution of acidic gas had finished the thionyl chloride was removed *in vacuo* and the residue triturated twice with toluene (2x10 mL). To the dark solid residue was added a solution of **127** (0.2g, 0.4 mmol) in anhydrous dichloromethane (25 mL) and triethylamine (0.1 mL). The reaction mixture was stirred at room temperature for 8 hours under an argon atmosphere. After removal of solvent *in vacuo* the crude residue was taken into dichloromethane (100mL) and washed with water (2 x 50mL). The organic layer was separated, dried over $MgSO_4$, concentrated to a volume of ~10 mL and purified by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 97:3). Relevant fractions were identified by TLC (silica CH_2Cl_2/MeOH, 97:3), R_f =0.4, collected and concentrated to yield compound **133** as a yellow oil (0.14g, 54%)

^{1}H N.M.R. [400MHz, CD_2Cl_2] δ 1.31 (9H, s, (C*H*$_3$)$_3$), 1.35 (9H, s, (C*H*$_3$)$_3$), 1.38 (9H, s, (C*H*$_3$)$_3$), 2.63 (8H, br t, NC*H*$_2$ x 4), 2.78 (2H, t, ^{3}J = 6.7Hz, NC*H*$_2$), 2.89 (2H, br t, NC*H*$_2$), 2.92 (2H, s, C*H*$_2$C(O)), 3.21 (4H, s, C*H*$_2$C(O) x 2), 3.52 (2H, t, ^{3}J = 6.7Hz, NC*H*$_2$), 3.67 (2H, t, ^{3}J = 5.0Hz, NC*H*$_2$), 7.51 (2H, d, ^{3}J = 8.9Hz, O_2N-Ar-2,6-*H*), 8.15 (2H, d, ^{3}J = 8.9Hz, O_2N-Ar-3,5-*H*)

^{13}C N.M.R. [100MHz, CD_2Cl_2] δ 28.2, 28.3, 45.1, 49.3, 52.4, 52.8, 52.9, 53.3, 53.5, 53.5, 54.9, 55.5, 58.6, 81.0, 124.0, 128.0, 144.5, 148.2, 169.7, 170.8, 171.0

MS (ESI) m/z 664 $(M+H)^+$, 686 $(M+Na)^+$

6.17 Synthesis of 1,4,7-(tris-*tert*-butoxycarbonylmethyl)-10-(4-aminobenzoyl)-1,4,7,10-tetraaza-cyclododecane (134)

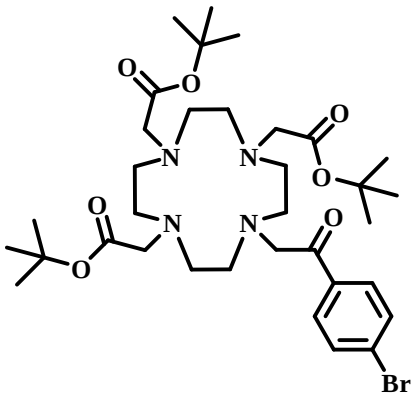


To a solution of **133** (0.5g, 0.75 mmol) in ethanol (25mL) was added 5% wt Pd/C (0.1g) and the mixture stirred at room temperature under a H_2 atmosphere. After 2 hours, TLC (silica CH_2Cl_2/MeOH, 9:1) showed complete consumption of the starting material and the catalyst removed by filtration. After the solvent was concentrated *in vacuo* to a volume of ~5 mL, the crude mixture was separated by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, initially 97:3 rising to 93:7). Relevant fractions were identified by TLC (silica CH_2Cl_2/MeOH, 97:3), R_f =0.3, collected and concentrated to yield compound **134** as a yellow semi-solid (0.34g, 71%).

^{1}H N.M.R. [400MHz, CD_2Cl_2] δ 1.36 (27H, s, $(CH_3)_3$), 2.64 (8H, m, NCH_2 x 4), 2.87 (4H, br s NCH_2 x2), 3.22 (4H, br s, NCH_2 x2), 3.56 (4H, br s, $CH_2C(O)$x2), 3.91 (2H, br s, $CH_2C(O)$), 6.52 (2H, d, ^{3}J = 8.4Hz, O_2N-Ar-2,6-*H*), 7.07 (2H, d, ^{3}J = 8.4Hz, O_2N-Ar-3,5-*H*)

^{13}C N.M.R. [100MHz, CD_2Cl_2] δ 23.9, 24.0, 52.0 (br), 52.7 (br), 55.2, 57.8 (br), 76.6, 109.9, 122.9, 124.3, 144.0, 166.8, 167.9

6.18 Synthesis of 1-(4'-Bromo-2-acetophenone)-4,7,10-tris(*tert*-butoxycarboxymethyl)-1,4,7,10-tetraazacyclododecane (135)

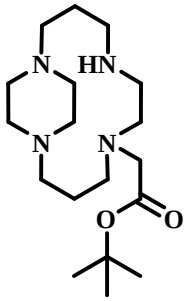


To a solution of **127** (1g, 1.9 mmol) and α-bromoacetophenone (0.59g, 2.1 mmol) in acetonitrile (100mL) was added Na_2CO_3 (1.65g, 16mmol) and the mixture heated at 70 °C for 16 hours. After this time, TLC (silica, CH_2Cl_2 : MeOH, 95:5) showed complete consumption of starting material **127**. The mixture was cooled to room temperature, the solids were filtered off and the solvent was removed *in vacuo* leaving a brown residue. This was dissolved in dichloromethane (100mL), washed successively with water (2x100mL) and brine (50mL) and dried over $MgSO_4$. Following removal of the dessicant, the solution was concentrated down to a small volume (~10ml) and purified by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 95:5). Relevant fractions were identified by TLC (silica CH_2Cl_2/MeOH, 95:5), R_f =0.2, collected and concentrated to yield the title compound as a yellow semi-solid (1.34g, 97%)

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.43 (27H, s, OC(C*H*$_3$)$_3$, 1.9-3.5 (22H, m, NC*H*$_2$, C*H*$_2$COO^{t}Bu, C*H*$_2$PhNO$_2$), 3.9-4.1 (2H, m, NC*H*$_2$C(O)), 7.55 (2H, d, ^{3}J = 8.6Hz, Br-Ar-3,5-*H*), 7.75 (2H, d, ^{3}J = 8.6Hz, Br-Ar-2,6-*H*)
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 27.7, 27.8, 48.7 (br), 52.7 (br), 55.5, 55.8, 60.2, 81.9, 82.0, 128.7, 129.2, 131.9, 134.3, 172.7, 173.0, 198.9

MS (ESI) *m/z* 733 ^{79}Br(M+Na)$^+$, 735 ^{81}Br(M+Na)$^+$
HRMS : calculated for $C_{34}H_{55}{}^{79}BrN_4O_7$: 733.3146, found 733.3150

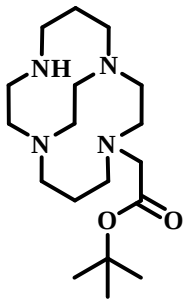
6.19 Synthesis of (1,5,8,12-tetraaza-bicyclo[10.2.2]hexadec-5-yl)-acetic acid *tert*-butyl ester (137)[115]



To a stirred solution of compound **157** (1.02g, 2.4mmol) in ethanol (30ml) was added sodium borohydride (2.3g, 60mmol), dropwise over 30 minutes. The resulting solution was heated under reflux conditions for 1 hour, water (15ml) added and the solvents removed *in vacuo*. The residue was taken up in water (40ml), the solution made strongly basic (pH~14) with potassium hydroxide pellets and extracted with dichloromethane (40ml). The organic layer was separated, dried ($MgSO_4$) and concentrated *in vacuo* to yield compound **137** as a brown viscous oil (0.75g, 91%).

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.35 (9H, s, $C(CH_3)_3$), 1.60-1.75 (4H, m, 2xCH_2-β-N), 2.08-2.14 (2H, m, CH_2-α-N), 2.49-2.54 (6H, m, 3xCH_2-α-N), 2.63-2.66 (2H, m, CH_2-α-N), 2.75-2.85 (6H, m, 3x CH_2-α-N), 2.89-2.92 (2H, t, CH_2-α-N), 3.03-3.10 (2H, dt, CH_2-α-N), 3.24 (2H, s, CH_2CO)
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 23.30 (CH_2-β-N), 25.78 (CH_2-β-N), 28.15 ($C(CH_3)_3$), 47.01 (CH_2-α-N), 48.11 (CH_2-α-N), 50.59 (CH_2-α-N), 50.90 (CH_2-α-N), 51.95 (CH_2-α-N), 54.71 (CH_2-α-N), 55.63 (CH_2-α-N), 56.70 (CH_2-α-N), 77.26 (NCH_2CO), 80.83 ($C(CH_3)_3$), 170.52 ($C(O)O^tBu$)
MS (ESI) *m/z* 342 $(M+H)^+$

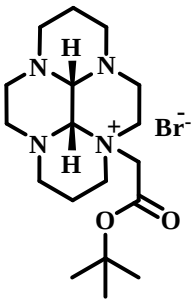
6.20 Synthesis of 4-Carbo-t-butoxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane. (138)[118]



To a solution of **159** (1.8g, 42mmol) in ethanol (120mL) was added palladium on carbon (10 wt%, 100mg) and argon bubbled through the mixture for fifteen minutes. The reaction was then stirred at room temperature under a hydrogen atmosphere (balloon) for five days. When TLC monitoring showed no further change, the reaction mixture was filtered through celite and concentrated in vacuo. The brown residue was dissolved in a small volume of CH_2Cl_2 (10mL) and the crude product isolated by flash column chromatography (silica, eluent: CH_2Cl_2/MeOH, 88:12) as a yellow oil. After trituration with diethyl ether and hexane, the product was isolated as a pale yellow oil (1.1g, 77%).

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.15-1.21 (2H, m, C*H*$_2$-β-N), 1.39 (9H, s, (C*H*$_3$)$_3$), 1.60-1.72 (2H, m, C*H*$_2$-β-N), 1.82-1.97 (1H, m), 2.30-2.50 (1H, m), 2.52-3.08 (16H, m), 3.15-3.29 (2H, m), 3.49-3.50 (1H, m), 3.51-3.60 (1H, m).
20.8 (*C*H$_2$-β-N), 26.6 (*C*H$_2$-β-N), 28.0 (*C*H$_3$)$_3$, [44.1, 47.1, 50.6, 50.7, 52.1, 52.7, 53.3, 54.1, 54.7, 57.4, 58.3] (*C*H$_2$-α-N), 81.06 (*C*(CH$_3$)$_3$), 170.6 (*C*(O)O^{t}Bu)
MS (ESI) *m/z* 341 (M+H)$^+$

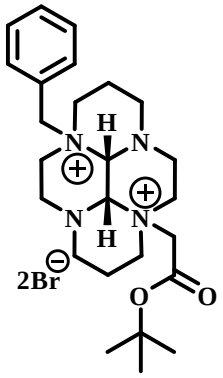
6.21 Synthesis of Decahydro-3a,5a,8a,10a-tetraazapyren-3a-yl)-acetic acid *tert*-butyl ester (157)[103]



To a stirred solution of compound **112** (0.6g, 2.7mmol) in anhydrous acetonitrile (30ml) was added *tert*-butylbromoacetate (2.1g, 11mmol) and the mixture was stirred for 8 hours. The solvent was then removed to yield an off-white solid, which after washing with diethyl ether (4x75ml), afforded compound **157** as a white semi-solid (1.06g, 94%).

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.51 (9H, s, (C*H*$_3$)$_3$), 1.81-5.14 (24H, m, C*H*$_2$C(O)), C*H*$_2$-β-N, C*H*$_2$-α-N, NC*H*N)
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 19.4 (*C*H_2-β-N), 27.8 (*C*H_2-β-N), 28.1 (*C*H_3)$_3$, 42.9 (*C*H_2-α-N), 46.8 (*C*H_2-α-N), 50.1 (*C*H_2-α-N), 52.2 (*C*H_2-α-N), 54.1 (*C*H_2-α-N), 54.9 (*C*H_2-α-N), 56.5 (*C*H_2-α-N), 60.1 (*C*H_2-α-N), 70.7 (N*C*H_2CO), 80.9 (N*C*N), 86.1 (*C*(CH$_3$)$_3$), 163.3 (*C*(O)O^{t}Bu)
MS (MALDI) *m/z* 337 $(M-Br)^+$

6.22 Synthesis of 3a-benzyl-8a-*tert*-butylcarboxymethyl-decahydro-3a,5a,8a,10a-tetraazapyrenium dibromide (158)



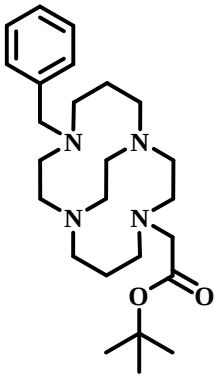
To a stirred solution of **157** (2g, 4.8mmol) in anhydrous acetonitrile (50mL) was added benzyl bromide (1g, 5.8 mmol) and the solution stirred at room temperature under an argon atmosphere for 6 days. The solvent was reduced *in vacuo* to ~30mL and crude product was precipitated by addition of diethyl ether (100mL). The off white solid was collected by filtration, washed with diethyl ether (5x50mL) and dried under vacuum to yield **157** as a white semi-solid (2.5g, 89%).

^{1}H N.M.R. [400MHz, CD_2Cl_2] δ 1.51 (9H, s, $(CH_3)_3$), 1.91-5.86 (26H, m, CH_2C(O), 2xCH_2-β-N, 8xCH_2-α-N, 2xNC*H*N, CH_2Ph, CH_2C(O)O^{t}Bu) 7.46-7.61 (5H, m, CH_2-Ar-2,3,4,5,6-*H*)

^{13}C N.M.R. [100MHz, CD_2Cl_2] δ 22.7, 22.8, 31.6, 49.1, 50.5, 50.6, 50.8, 53.7, 53.8, 61.8, 63.1, 64.4, 66.1, 78.8, 79.2, 90.1, 129.5, 133.2, 134.8, 137.3, 166.9

MS (ESI) m/z 428 $(M\text{-}Br^{-})^{+}$

6.23 Synthesis of (11-Benzyl-1,4,8,11-tetraaza-bicyclo[6.6.2]hexadec-4-yl)-acetic acid *tert*-butyl ester (159)



To a solution of **158** (1.10g, 1.9 mmol) in ethanol (60mL) was added $NaBH_4$ (0.28g, 7.5 mmol) and the mixture stirred at room temperature under argon for 7 days. Water (20mL) was added and the solvent removed *in vacuo*. The residue was dissolved in NaOH(aq) (50mL, 0.05M) and extracted into dichloromethane (50mL). The solvent volume was reduced to ~10mL) and the product **159** isolated by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 96:4), R_f =0.4, as an off white sticky semi-solid (0.5g, 62%).

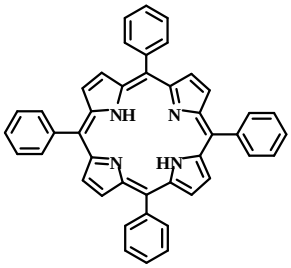
^{1}H N.M.R. [400MHz, CD_2Cl_2] δ 1.31 (9H, s, $(CH_3)_3$), 1.60-3.84 (26H, m, CH_2C(O), CH_2-β-N, CH_2-α-N, NC*H*N, CH_2Ph, CH_2C(O)O^{t}Bu) 7.19-7.30 (5H, m, CH_2-Ar-2,3,4,5,6-*H*)

^{13}C N.M.R. [100MHz, CD_2Cl_2] δ 23.5 (CH_2-β-N), 24.9 (CH_2-β-N), 27.9 (CH_3)$_3$, 46.1 (CH_2-α-N), 48.8 (CH_2-α-N), 49.8 (CH_2-α-N), 50.9 (CH_2-α-N), 51.8 (CH_2-α-N), 53.9 (CH_2-α-N), 55.9 (CH_2-α-N), 56.2 (CH_2-α-N), 56.3 (NCH_2CO), 56.4 (C(CH$_3$)$_3$), 58.8 (N*C*N), 60.3 (N*C*N), 82.0 (CH_2Ph), 127.5 (CH_2-Ar-4-*C*), 128.4 (CH_2-Ar-3,5-*C*), 129.2 (CH_2-Ar-2,6-*C*), 137.0 (CH_2-Ar-1-*C*), 170.2 (C(O)O^{t}Bu)

MS (ESI) *m/z* 431 $(M)^+$

HRMS : calculated for $C_{25}H_{43}N_4O_2$: 431.3383, found 431.3381

6.24 Synthesis of 5,10,15,20-Tetraphenyl-porphyrin (162)[124]



Benzaldehyde (11.1g, 0.1mol) was dissolved in propionic acid (400ml) and the solution heated under reflux conditions. Pyrrole (7g, 0.1mol) was added dropwise and the dark mixture heated for a further 30 minutes. Upon cooling, the black tarry mixture was filtered to yield a purple solid which, after washing with methanol (100ml) and hot water (100ml) afforded compound **162** as purple crystals (3.4g, 22%). m.p. >200 °C

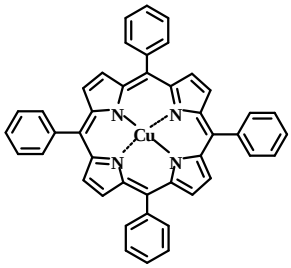
^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.72 (2H, br s, N*H*), 7.75-7.80 (12H, m, 5,10,15,20-Ar-3,4,5-*H*), 8.24-8.26 (8H, m, 5,10,15,20-Ar-2,6-*H*), 8.89 (8H, s, β-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 120.2, 126.7, 127.7, 131.3, 134.6, 142.2

UV-vis (CH_2Cl_2) λmax/nm (log ε) 419 (5.4), 514 (4.3), 549 (4.1), 591 (4.0), 646 (4.0)

MS (ESI) *m/z* 615 $(M)^+$

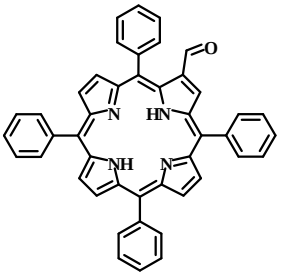
6.25 Synthesis of 5,10,15,20-Tetraphenyl-porphyrinatocopper(II) (183)



To a solution of compound **162** (2g, 3.3mmol) in chloroform (500ml) heated under reflux conditions, was added a suspension of copper (II) acetate (6.5g, 33 mmol) in methanol (100ml). The mixture was heated for 10 hours, cooled and the purple precipitate filtered off. After washing with water (100ml), compound **183** was isolated as a purple solid (1.98g, 90%). m.p. >200°C.

UV-vis (CH_2Cl_2, nm) λmax/nm (log ε) 418 (5.4), 539 (4.2)
MS (ESI) *m/z* 676 $(M)^+$

6.26 Synthesis of 5,10,15,20-Tetraphenyl-porphyrin-2-carbaldehyde (191)[181]



Phosphorus oxychloride (13.5ml, 0.145mol) was added to dry dimethylformamide (16.8ml, 0.217mol) under a dry argon atmosphere, forming a viscous golden mixture. A cooled (0 °C) solution of compound **183** (1.5g, 2.2mmol) in dry 1,2-dichloroethane (150ml) was then added dropwise, and upon addition, the mixture allowed to rise to room temperature, and subsequently heated under reflux conditions for 7 hours. After cooling, sulphuric acid (18M, 28ml, 0.5mol) was added and the mixture stirred for 6 minutes. The resulting green two phase mixture was washed cautiously with sodium hydroxide (0.72M, 2dm^3), until the colour had turned red. Chloroform (1.5 dm^3) was added, the organic layer separated, dried ($MgSO_4$) and concentrated *in vacuo* to yield a crude purple solid. This was adsorbed onto silica and purified by gravity column chromatography (silica, eluent: CH_2Cl_2). Relevant fractions were identified by TLC (silica CH_2Cl_2), collected and concentrated to yield compound **191** as a purple solid (1.05g, 74%). R*f*=0.60 (silica, CH_2Cl_2); m.p. >200 °C

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.55 (2H, br s, N*H*), 7.71-7.84 (12H, m, 5,10,15,20-Ar-2,4,6-*H*), 8.17-8.26 (8H, m, 5,10,15,20-Ar-2,6-*H*), 8.77 (1H, d, ^{3}J=4.8Hz, β-*H*), 8.78 (1H, AB, ^{3}J=4.8Hz, β-*H*), 8.84-8.94 (4H, m, β-*H*), 9.23 (1H, s, β-*H*), 9.41 (1H, s, C*H*O)

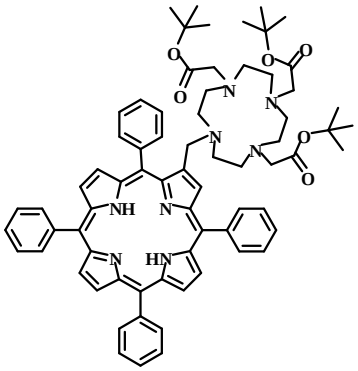
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 120.0, 120.3, 120.6, 122.6, 126.8, 126.9, 127.4, 127.9, 128.1, 129.0, 134.54, 134.57, 134.6, 141.5, 141.7, 142.4, 189.3

UV-vis (THF, nm) λmax/nm (log ε) 431 (5.5), 525 (4.2), 567 (3.8), 604 (3.7), 663 (4.0)

MS (EI) *m/z* 642 $(M)^+$

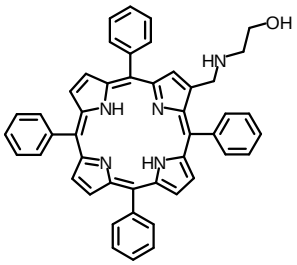
HRMS : calculated for $C_{45}H_{30}N_4O$: 642.2414, found 642.2411

6.27 Attempted synthesis of 1-(5,10,15,20-tetraphenyl-porphyrin-2-ylmethyl)-4,7,10-tris(*tert*-butoxycarboxymethyl)-1,4,7,10-tetraazacyclododecane (212)



A mixture of compounds **191** (0.2g, 0.3mmol) and **127** (0.30g, 0.6mmol) were dissolved in dry toluene (50ml), acetic acid (2 drops) added and the reaction mixture was heated under reflux conditions. After heating for 4 hours, no change to the starting materials could be seen by TLC analysis (silica, CH_2Cl_2), so a Dean Stark head was fitted to the flask and activated molecular sieves (4Å) added to the reaction mixture. The reaction was heated for a further 3 hours and monitored by TLC but no reaction was evident and the reaction was abandoned. The reaction was repeated using anhydrous THF solvent but this also was ineffective.

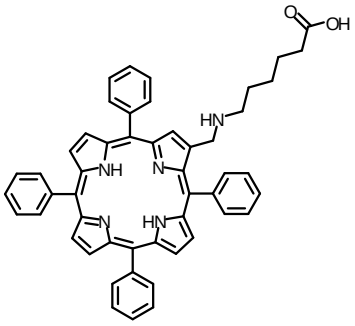
6.28 Attempted synthesis of 2-((5,10,15,20-tetraphenyl-porphyrin-2-ylmethyl)amino)ethanol (213)



Compounds **191** (0.2g, 0.3mmol) and 2-aminoethanol (36mg, 0.6mmol) were dissolved in anhydrous THF (50ml), acetic acid (2 drops) and activated molecular sieves (4Å) were added and the reaction mixture heated under reflux conditions. The reaction was monitored by TLC (silica, CH_2Cl_2), but after heating for 3 hours, no change to the starting materials could be detected. A solution of $NaBH_3CN$ (0.11g, 1.8mmol) in methanol (50mL) was added and the mixture heated for a further 2 hours. However, no reaction progress could be seen by TLC. An aliquot of the solution was removed and the porphyrin fraction isolated by preparative TLC (silica, eluent: CH_2Cl_2), and this was identified as the starting porphyrin **191**.

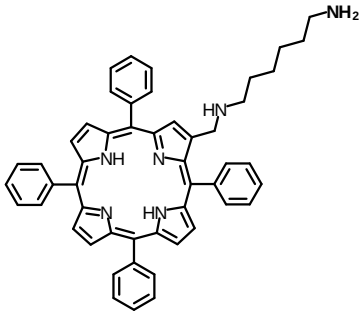
The reaction was repeated using the same amounts of reagents but with a mixed solvent system of toluene / methanol (1:1, 80mL) but no reaction progress could be detected.

6.29 Attempted synthesis of 6-((5,10,15,20-tetraphenyl-porphyrin-2-ylmethyl)amino) hexanoic acid (214)



A mixture of compounds **191** (0.2g, 0.3mmol) and 6-aminohexanoic acid (79mg, 0.6mmol) were dissolved in anhydrous THF (100mL), three drops of CH_3COOH and activated 4Å molecular sieves added, and the solution was heated under an inert atmosphere (N_2). After heating for 5 hours, no further change could be seen by TLC analysis (silica, eluent CH_2Cl_2). The reaction mixture was allowed to cool to ambient temperature while under N_2, followed by addition of a solution of $NaBH_3CN$ (0.11g, 1.8mmol) in methanol (50mL). After stirring for 2 hours, no further change was observed by TLC (silica, eluent CH_2Cl_2). The solvent was removed *in vacuo* and the residue partitioned between water and CH_2Cl_2. The organic layer was separated, washed with brine and dried over $MgSO_4$. An attempt to isolate the more polar porphyrin fraction by column chromatography (silica, eluent CH_2Cl_2 : MeOH, 97:3) failed after the compound appeared to degrade as it moved down the column.

6.30 Attempted synthesis of 6-((5,10,15,20-tetraphenyl-porphyrin-2-ylmethyl)amino) hexylamine (215)



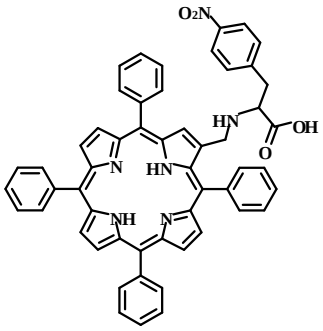
A mixture of compounds **191** (0.2g, 0.3mmol) and 1,6-diaminohexane acid (174mg, 1.5mmol) were dissolved in anhydrous THF (100mL), three drops of CH_3COOH and activated 4Å molecular sieves added, and the solution was heated under an inert atmosphere (N_2). After 5 hours, TLC analysis (silica, eluent CH_2Cl_2) showed consumption of the starting materials and production of a polar porphyrinic spot. The reaction mixture was allowed to cool to ambient temperature while still under N_2 and then a solution of $NaBH_3CN$ (0.11g, 1.8mmol) in methanol (50mL) was added.

After stirring for ten minutes, TLC (silica, eluent CH_2Cl_2) showed a complex mixture of seven porphyrin spots. Column chromatography (silica, eluent CH_2Cl_2 : MeOH, 95:5)was used to try to isolate the fractions, but degradation appeared to occur, with most of the material sticking to the top of the column, and it could not be isolated. The reaction was repeated using a toluene / methanol (1:1) (100mL) mixed solvent system and an aliquot of the reaction mixture was removed prior to addition of the reductant solution, and a solution of $NaBH_3CN$ (0.11g, 1.8mmol) in methanol (50mL) was added to the remainder. The aliquot which had not been reduced was subjected to preparative TLC (silica, eluent CH_2Cl_2 : MeOH, 95:5) but the compounds appeared to degrade very quickly and no pure fraction could be isolated. The remaining reaction mixture was concentrated to a small volume (~5mL) and the residue partitioned between water and CH_2Cl_2. The organic layer was separated, washed with brine and dried over $MgSO_4$. Preparative TLC (silica, eluent CH_2Cl_2 : MeOH, 95:5) was used to separate the porphyrin fractions and although degradation was seen to occur, a

small amount (~1mg) was obtained. Although ^{1}H NMR analysis showed this to be an impure fraction, as described in chapter 3, partial assignment of the ^{1}H NMR spectrum could be achieved :

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.78 (2H, br s, N*H*), 1.83-1.87 (5H, m), 3.72-3.77 (4H,m), 4.00 (1H, br s), 7.58-7.80 (12H, m, 5,10,15,20-Ar-3,4,5-*H*), 8.02 (2H, br d, 5,10-Ar-2-*H*), 8.10-8.22 (6H, m, 15,20-Ar-2,6-*H* and 5,10-Ar-6-*H*), 8.55 (1H, d, β*H*), 8.72-8.91 (6H, m, β*H*)

6.31 Synthesis of 3-(4-Nitro-phenyl)-2-[(5,10,15,20-tetraphenyl-porphyrin-2-ylmethyl)-amino]-propionic acid (216)



To a solution of compound **191** (0.75g, 1.2mmol) and *(S)*-4-nitrophenylalanine (0.2g, 0.9mmol) in THF (100mL) was added a solution of $NaBH_3CN$ (0.11g, 1.8mmol) in methanol (50mL) and the mixture heated under reflux conditions. The reaction was monitored by TLC (silica, CH_2Cl_2 : MeOH, 95:5) until after 24hrs no further change was discernable. Solvent was removed *in vacuo* and the residue partitioned between water and CH_2Cl_2. The organic layer was separated, washed with brine and dried over $MgSO_4$. After removal of solvent, the product was isolated using column chromatography (silica, eluent CH_2Cl_2 : MeOH, 97:3) to yield **216** as a purple solid (0.3g, 41%). m.p. >200°C

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.80 (2H, br s, N*H*), 2.95 (1H, *A*BX, dd, $^2J_{A\text{-}B}$ = 13.4Hz, $^3J_{A\text{-}X}$ = 7.7Hz, O_2NPhC*H*$_2$), 3.06 (1H, A*B*X, dd, $^2J_{B\text{-}A}$ = 13.4Hz, $^3J_{B\text{-}X}$ = 5.5Hz, O_2NPhC*H*$_2$), 3.44 (1H, AB*X*, dd, $^3J_{X\text{-}A}$ = 7.7Hz, $^3J_{X\text{-}B}$=5.5Hz), 3.80 (1H, *A*B, d, $^2J_{A\text{-}B}$ = 15.6Hz, NHC*H*$_2$), 4.1 (1H, A*B*, d, $^2J_{B\text{-}A}$ =15.6Hz, NHC*H*$_2$), 7.39 (2H, d, ^3J = 8.6Hz, O_2N-Ar-2,6-*H*), 7.60-7.86 (12H, m, 5,10,15,20-Ar-3,4,5-*H*), 7.98 (2H, d, ^3J = 8.6Hz, O_2N-Ar-3,5-*H*), 8.02-8.06 and 8.19-8.22 (8H, m, 10,15,20-Ar-2,6-*H*), 8.55 (1H, d, ^3J = 4.7Hz, β*H*), 8.69 (1H, d, ^3J = 4.9Hz, β*H*), 8.72 (1H, d, ^3J = 4.7Hz, β*H*), 8.77 (1H, d, ^3J = 4.9Hz, β*H*), 8.83 and 8.85 (2H, AB, ^3J = 4.6Hz, β*H*), 8.91 (1H, s, β*H*)

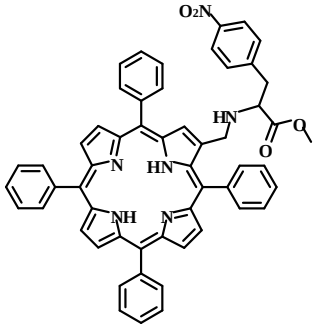
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 38.9, 47.3, 61.8, 118.9, 119.5, 120.0, 120.4, 122.8, 125.1, 126.6, 126.7, 126.88, 126.91, 127.7, 128.0, 128.1, 128.7, 130.4, 133.1, 133.4, 134.3, 134.4, 142.2, 142.3, 142.5, 142.9, 146.6, 146.8, 174.2

UV-vis (THF) λmax/nm (log ε) 418 (5.8), 514 (4.8), 547 (4.2), 590 (4.2), 645 (4.0)

MS (ESI) *m/z* 837 $(M+H)^+$

HRMS : calculated for $C_{54}H_{41}N_6O_4$: 837.3184, found 837.3178

6.32 Synthesis of 3-(4-Nitro-phenyl)-2-[(5,10,15,20-tetraphenyl-porphyrin-2-ylmethyl)-amino]-propionic acid methyl ester (217)



To a stirred solution of compounds **191** (0.16g, 0.25mmol) and **218** (0.056g, 0.25mmol) in a mixture of toluene (100mL) and methanol (30mL) was added acetic acid (0.25mL). The dark coloured solution was heated under reflux conditions and after 1 hour sodium cyanoborohydride (0.2g, 3mmol) was added heating continued for 48 hours. The reaction was monitored by TLC (silica, eluent CH_2Cl_2) and after complete consumption of starting materials the solvent was removed *in vacuo*. The dark solid residue was partitioned between dichloromethane and water, the organic layer separated and dried over $MgSO_4$. After removal of solvents *in* vacuo, the product **217** was isolated by column chromatography (silica, eluent CH_2Cl_2) as a purple solid (0.13g, 61%). m.p. >200°C

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.79 (2H, br s, N*H*), 3.01 (2H, m, NHCH_2), 3.48 (1H, AB*X*, dd, $^3J_{X\text{-}A}$ = 7.0Hz, $^3J_{X\text{-}B}$=6.7Hz), 3.56 (3H, s, COOCH_3), *3*.84 (1H, *A*BX, br d, $^2J_{A\text{-}B}$ = 16.2Hz), 3.98 (1H, A*B*X, br d, $^2J_{B\text{-}A}$ = 16.2Hz), 7.22 (2H, d, ^3J = 8.7Hz, O_2N-Ar-3,5-*H*), 7.75-7.78 (12H, m, 5,10,15,20-Ar-3,4,5-*H*), 7.99 (4H, m, ^3J = 8.7Hz, O_2N-Ar-2,6-*H*, 5-Ar-2,6-*H*), 8.18-8.20 (6H, m, 10,15,20-Ar-2,6-*H*), 8.57 (1H, d, β*H*), 8.76-8.84 (6H, m, β*H*)

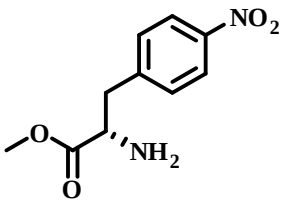
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 39.3, 47.2, 51.8, 61.8, 119.1, 119.2, 120.3, 120.6, 123.4, 126.6, 126.7, 126.9, 127.0, 127.7, 127.8, 128.3, 130.1, 133.2, 133.4, 134.5, 134.6, 141.9, 142.1, 142.2, 142.6, 145.2, 146.8, 174.3

UV-vis (CH_2Cl_2, nm) λmax 420, 517, 549, 583, 646

MS (ESI) *m/z* 851 $(M+H)^+$

HRMS : calculated for $C_{55}H_{43}N_6O_4$: 851.3340, found 851.3329

6.33 Synthesis of (S)-methyl-2-amino-3-(4-nitrophenyl)propanoate[192] (218)



Dry HCl was bubbled through a suspension of *(S)*-4-nitrophenylalanine (1g, 4.4mmol) in anhydrous methanol (30mL) at 0 °C. After dissolution (approx 20mins) the flow of HCl was stoppedand the clear yellow solution stirred at room temperature under N_2 for two hours. The solution was cooled to 0 °C for several hours, but no crystallisation occurred. The crude mixture was separated by column chromatography (eluent CH_2Cl_2 : MeOH, 9:1), relevant fractions combined and solvent removed *in* vacuo to yield **218** as an orange solid (0.7g, 71%). m.p. 210-214°C

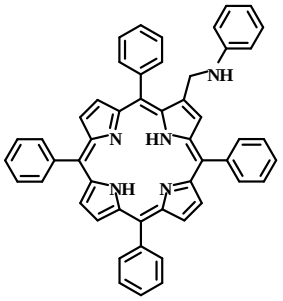
^{1}H N.M.R. [400MHz, $CDCl_3$] δ 3.14 (1H, *A*BX, dd,$^2J_{A\text{-}B}$=14.0Hz, $^3J_{A\text{-}X}$ = 7.1Hz, O_2NPhC*H*$_2$), 3.23 (1H, A*B*X, dd,$^2J_{B\text{-}A}$ = 14.0Hz, $^3J_{B\text{-}X}$=6.5Hz, O_2NPhC*H*$_2$), 3.69 (3H, s, OC*H*$_3$), 4.10 (1H, AB*X*, dd, $^3J_{X\text{-}A}$ = 7.1Hz, $^3J_{X\text{-}B}$=6.5Hz), 7.40 (2H, d, ^{3}J = 8.7Hz, O_2N-Ar-3,5-*H*), 8.13 (2H, d, ^{3}J = 8.7Hz, O_2N-Ar-2,6-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 39.9, 52.1, 54.7, 123.5, 130.0, 144.6, 146.9, 174.1

MS (ESI) *m/z* 225 $(M+H)^+$

HRMS : calculated for $C_{10}H_{13}N_2O_4$: 225.0870, found 225.0867

6.34 Synthesis of Phenyl-(5,10,15,20-tetraphenyl-porphyrin-2-ylmethyl)-amine (219)



A mixture of compound **191** (0.2g, 0.3mmol), aniline (0.03g, 0.3mmol) and acetic acid (2 drops) in dry toluene (50ml) was heated under reflux conditions, using a Dean Stark head to remove water from the reaction. The reaction was monitored using TLC (silica, CH_2Cl_2) until after 24 hours no further reaction change was observed. After cooling, sodium cyanoborohydride (0.02g, 0.32mmol) and methanol (10ml) were added and the mixture stirred for 2 hours. Water (100ml) was added, the organic layer separated, washed with brine (100ml) and dried ($MgSO_4$). After removal of solvent *in vacuo*, the purple solid residue was adsorbed onto silica and separated using gravity column chromatography (silica, eluent: CH_2Cl_2). Relevant fractions were identified by TLC (silica CH_2Cl_2), collected and concentrated to yield compound **219** as a purple solid (0.17g, 77%). R*f*=0.28 (silica, CH_2Cl_2); m.p. >200 °C.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.84 (2H, br s, N*H*), 4.15 (1H, br s, PhN*H*), 4.43 (2H, s, C*H*$_2$NH), 6.46 (2H, d, ^{3}J=7.8Hz, HN-Ar-2,6-*H*), 6.63 (1H, t, ^{3}J=7.8Hz, HN-Ar-4-*H*), 7.05 (2H, t, ^{3}J=7.8Hz, HN-Ar-3,5-*H*), 7.57-7.72 (12H, m, 5,10,15,20-Ar-3,4,5-*H*), 8.03-8.07 and 8.11-8.16 (8H, m, 5,10,15,20-Ar-2,6-*H*), 8.57 (1H, d, ^{3}J=4.8Hz, β-*H*), 8.68-8.79 (5H, m, β-*H*).

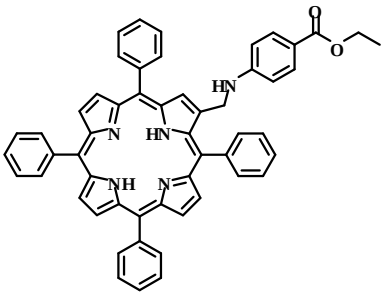
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 44.2, 113.3, 117.6, 119.3, 119.4, 120.3, 120.6, 126.6, 126.7, 127.3, 127.6, 127.7, 128.5, 129.0, 133.2, 134.5, 134.6, 134.7, 141.9, 142.0, 142.2, 142.3, 148.1

UV-vis (CH_2Cl_2, nm) λmax/nm (log ε) 419 (5.6), 516 (4.3), 549 (3.9), 590 (3.8), 646 (3.5)

MS (ESI) *m/z* 720 $(M+H)^+$

HRMS : calculated for $C_{51}H_{38}N_5$: 720.3122, found 720.3109

6.35 Synthesis of 4-[(5,10,15,20-Tetraphenyl-porphyrin-2-ylmethyl)-amino]-benzoic acid ethyl ester (220)



A mixture of compound **191** (0.13g, 0.2mmol), ethyl-*p*-amino benzoate (0.033g, 0.2mmol) and acetic acid (2 drops) in toluene (50ml) was heated under reflux conditions, using a Dean Stark head to remove water from the reaction. The reaction was monitored using TLC (silica, CH_2Cl_2/MeOH, 249:1) until after 30 hours no further reaction change was observed. After cooling, sodium cyanoborohydride (0.02g, 0.32mmol) and methanol (10ml) were added and the mixture stirred for 2 hours. Water (100ml) was added, the organic layer separated, washed with brine (100ml) and dried ($MgSO_4$). After removal of solvent *in vacuo*, the purple solid residue was adsorbed onto silica and separated using gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 249:1). Relevant fractions were identified by TLC (silica CH_2Cl_2/MeOH, 249:1), collected and concentrated to yield compound **220** as a purple solid (0.105g, 66%). R*f*=0.32 (silica, CH_2Cl_2/MeOH, 249:1); m.p. >200 °C.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.84 (2H, br s, N*H*), 1.37 (3H, t, ^{3}J=7.2Hz, C*H*$_3$), 4.33 (2H, q, ^{3}J=7.2Hz, C*H*$_2$CH$_3$), 4.51 (2H, br d, C*H*$_2$NH), 4.58 (1H, br t, PhN*H*), 6.46 (2H, d, ^{3}J=8.8Hz, HN-Ar-2,6-*H*), 7.65-7.78 (12H, m, 5,10,15,20-Ar-3,4,5-*H*), 7.83 (2H, d, ^{3}J=8.8Hz, Ar-3,5-*H*), 8.10-8.13 and 8.20-8.23 (8H, m, 5,10,15,20-Ar-2,6-*H*), 8.66 (1H, d, ^{3}J=4.8Hz, β-*H*), 8.74 (1H, s, β-*H*), 8.78-8.87 (5H, m, β-*H*)

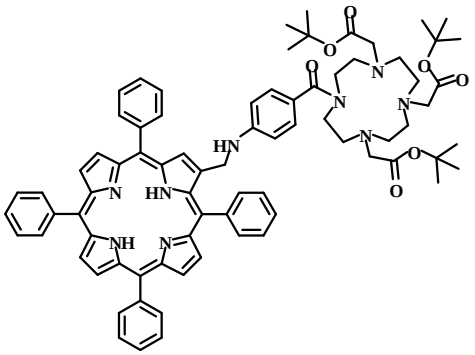
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 14.6, 43.6, 60.3, 111.9, 118.9, 119.3, 119.5, 120.3, 120.7, 126.6, 126.7, 126.8, 127.2, 127.7, 127.8, 128.6, 131.3, 133.2, 134.5, 134.6, 141.8, 141.9, 142.1, 142.2, 151.7, 166.9

UV-vis (CH_2Cl_2, nm) λmax/nm (log ε) 420 (5.4), 516 (4.7), 550 (4.2), 590 (3.9), 646 (4.1)

MS (ESI) *m/z* 792 $(M+H)^+$

HRMS : calculated for $C_{54}H_{42}N_5O_2$: 792.3333, found 792.3321

6.36 Synthesis of (1,4,7-tris-*tert*-butoxycarbonylmethyl-10-[4-[(5,10,15,20-tetraphenyl-porphyrin-2-ylmethyl)-amino]-benzoyl]-1,4,7,10tetraaza-cyclododec-1-yl)-acetic acid *tert*-butyl ester (221)



To a heated solution of compounds **191** (0.20g, 0.31 mmol) and **134** (0.17g, 0.27 mmol) in tetrahydrofuran (30mL) was added acetic acid (0.1mL) followed by a solution of $NaBH_3CN$ (0.1g, 1.5 mmol) in methanol (10mL). The mixture was heated under reflux conditions and monitored by TLC (silica CH_2Cl_2/MeOH, 9:1) until all the starting material **134** had been consumed (3 days). The solvent was removed *in vacuo* and dichloromethane (100mL) added to the dark solid residue. After washing with water (100mL) the organic layer was dried over $MgSO_4$ and the volume reduced to ~10mL. The crude mixture was separated by column chromatography (silica, eluent: CH_2Cl_2/MeOH, initially 97:3 rising to 9:1). Relevant fractions were identified by TLC (silica CH_2Cl_2/MeOH, 97:3), collected and concentrated to yield compound **221** as a purple solid (0.07, 20%). m.p. >150°C decomp.

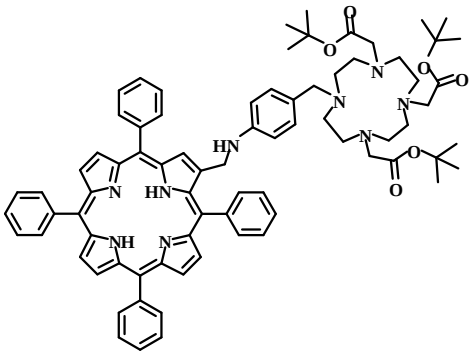
^{1}H N.M.R. [400MHz, CD_2Cl_2] δ -2.91 (2H, br s, N*H*), 1.35 (27H, s, (CH_3)$_3$), 2.50-3.28 (18H, m, NCH_2, CH_2C(O)), 3.51 (4H, br s CH_2C(O)), 4.38 (2H, s, CH_2NHPh), 6.38 (2H, d, ^{3}J = 8.3Hz, O_2N-Ar-2,6-*H*), 7.06 (2H, d, ^{3}J = 8.3Hz, O_2N-Ar-3,5-*H*), 7.58-7.72 (12H, m, 5,10,15,20-Ar-3,4,5-*H*), 8.03 (4H, d, Ar-2,6-*H*), 8.07-8.14 (4H, m, Ar-2,6-*H*), 8.56 (1H, d, ^{3}J = 4.8Hz, β*H*), 8.67-8.79 (6H, m, β*H*)

^{13}C N.M.R. [100MHz, CD_2Cl_2] δ 28.2, 28.3, 43.8, 52.0, 54.1, 55.2, 81.1, 112.5, 119.8, 120.0, 120.6, 120.7, 121.1, 127.1, 127.2, 127.7, 128.10, 128.14, 128.7, 128.9, 133.6, 134.86, 134.95, 134.99, 142.2, 142.3, 142.5, 142.7, 171.0

UV-vis (CH_2Cl_2, nm) λmax/nm (log ε) 419 (5.8), 516 (4.8), 549 (4.3), 591 (4.3), 645 (4.1)

MS (ESI) *m/z* 1260 $(M+H)^+$

6.37 Synthesis of 1,4,7-tris-*tert*-butoxycarbonylmethyl-10-[4-[(5,10,15,20-tetraphenyl-porphyrin-2-ylmethyl)-amino]-benzyl]-1,4,7,10-tetraaza-cyclododecane) (222)



To a heated solution of compounds **191** (0.23g, 0.36 mmol) and **57** (0.20g, 0.32 mmol) in tetrahydrofuran (50mL) under an inert (N_2) atmosphere was added acetic acid (0.1mL) followed by a solution of $NaBH_3CN$ (0.05g, 0.8 mmol) in methanol (20mL). The mixture was heated under reflux conditions and monitored by TLC (silica CH_2Cl_2/MeOH, 95:5) until all the starting material **57** had been consumed (5 days). The solvent was removed *in vacuo* and dichloromethane (100mL) added to the dark solid residue. After washing with water (100mL) the organic layer was separated and dried over $MgSO_4$ and the volume reduced to ~10mL. The crude mixture was separated by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, initially 99:1 rising to 95:5). Relevant fractions were identified by TLC (silica CH_2Cl_2/MeOH, 95:5), collected and concentrated to yield the desired product as a purple solid (0.21g, 52%). m.p. >150°C decomp.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.77 (2H, br s, N*H*), 1.45 (27H, s, (C*H*$_3$)$_3$), 2.08-2.78 (16H, m, NC*H*$_2$ x 8), 2.98 (4H, br s, C*H*$_2$C(O)), 3.58-3.65 (2H, m, NC*H*$_2$Ph, C*H*$_2$C(O)), 3.68 (1H, br s, C*H*$_2$C(O)), 3.72-3.76 (1H, m, NC*H*$_2$Ph), 4.21 (1H, br s, N*H*Ph), 4.47 (2H, s, C*H*$_2$NHPh), 6.46 (2H, d, ^{3}J = 8.3Hz, HN-Ar-2,6-*H*), 7.18 (2H, d, ^{3}J = 8.3Hz, HN-Ar-3,5-*H*), 7.60-7.82 (12H, m, 5,10,15,20-Ar-3,4,5-*H*), 8.13 (4H, d, ^{3}J = 7.0Hz, Ar-2,6-*H*), 8.17-8.23 (4H, m, Ar-2,6-*H*), 8.65 (1H, d, ^{3}J = 4.8Hz, β*H*), 8.73-8.88 (6H, m, β*H*)

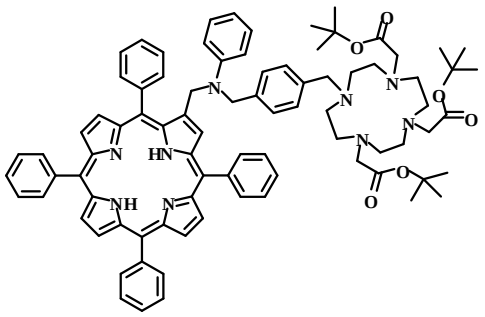
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 27.8, 27.9, 44.1, 49.3 (br), 55.6, 55.9, 58.7,

70.3, 72.5, 82.2, 82.5, 113.3, 119.2, 119.3, 120.4, 120.8, 126.1, 126.6, 126.7, 126.8, 127.2, 127.6, 127.8, 128.4, 130.9, 133.3, 134.5, 134.6, 141.8, 142.1, 142.5, 147.5, 172.6, 173.5

UV-vis (CH_2Cl_2, nm) λmax 419, 516, 549, 593, 646

MS (ESI) *m/z* 1246 $(M+H)^+$

6.38 Synthesis of [4,7-Bis-*tert*-butoxycarbonylmethyl-10-(4-[[phenyl-(5,10,15,20-tetraphenyl-porphyrin-2-ylmethyl)-amino]-methyl]-benzyl)-1,4,7,10tetraaza-cyclododec-1-yl]-acetic acid *tert*-butyl ester (223)



To a solution of compound **219** (0.05g, 0.07mmol) in dichloromethane (5ml) was added triethylamine (2 drops) and α,α-dibromoxylene (0.018g, 0.07mmol), and the mixture stirred at room temperature. Monitoring of the reaction mixture by TLC (silica, CH_2Cl_2) showed consumption of the α,α-dibromoxylene after 4 hours, and a solution of (4,7-Bis-*tert*-butoxycarbonylmethyl-1,4,7,10tetraaza-cyclododec-1-yl)-acetic acid *tert*-butyl ester (0.035g, 0.07mmol) in dichloromethane was added. After heating under reflux conditions for a further 3 days, TLC (silica, CH_2Cl_2/MeOH, 9:1) showed consumption of starting compound **219**. Dichloromethane (100ml) was added and the solution washed with water (100ml), after which the organic layer collected and dried ($MgSO_4$). Following removal of the dessicant, the solution was concentrated down to a small volume (~10ml) and purified by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 9:1). Relevant fractions were identified by TLC (silica CH_2Cl_2/MeOH, 9:1), collected and concentrated to yield compound **223** as a purple solid (0.044g, 52%). R*f*=0.4 (silica, CH_2Cl_2/MeOH, 9:1); m.p. >150 °C decomp.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -3.05 (2H, br s, N*H*), 1.48 (9H, s, C(C*H*$_3$)$_3$), 1.49 (18H, s, C(C*H*$_3$)$_3$), 2.80-2.94 (12H, m, NC*H*$_2$CH$_2$N), 3.01-3.10 (4H, m, NC*H*$_2$CH$_2$N), 3.29 (2H, s, (C*H*$_2$COO^{t}Bu)), 3.38 (4H, s, (C*H*$_2$COO^{t}Bu)), 3.42 (2H, s, NC*H*$_2$-Ph), 4.46 (2H, s, NC*H*$_2$-Ph), 4.59 (2H, s, C*H*$_2$NPh), 6.38 (2H, d, ^{3}J= 7.9Hz, CH$_2$-Ar-*H*), 6.83-6.89 (1H, m, N-Ar-4-*H*), 6.90-6.99 (4H, m, N-Ar-2,6-*H* and CH$_2$-Ar-*H*), 7.24-7.31 (2H, m, N-Ar-3,5-*H*), 7.68-7.84 (12H, m, 5,10,15,20-Ar-3,4,5-*H*), 8.00-8.04 and 8.05-8.10 (4H, m, Ar-2,6-*H*), 8.14-8.20

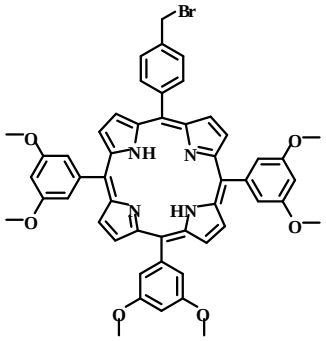
(4H, m, Ar-2,6-*H*), 8.62 (1H, d, ^{3}J=4.8Hz, β-*H*), 8.71 (1H, s, β-*H*), 8.75-8.80 (2H, m, β-*H*), 8.83 (1H, d, ^{3}J=4.8Hz, β-*H*), 8.89-8.91 (2H, m, β-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 28.13, 28.17, 47.4, 48.8 (br), 49.1, 49.7, 51.2, 52.2, 53.4, 55.1, 58.0, 58.4, 81.6, 81.7, 113.5, 117.9, 119.4, 120.3, 120.7, 124.4, 126.7, 126.8, 126.9, 127.4, 128.0, 128.8, 128.9, 129.2, 130.9, 133.1, 134.3, 134.4, 134.5, 141.0, 141.3, 141.6, 141.8, 150.3, 169.6, 170.5

UV-vis (CH_2Cl_2, nm) λmax 420, 516, 551, 589, 646

MS (ESI) *m/z* 1337 $(M+H)^+$

6.39 Synthesis of 5-(4-Bromomethylphenyl)-10,15,20-tris(3,5-dimethoxyphenyl) porphyrin (237)



To a stirred solution of compound **248** (0.28g, 0.34mmol) in anhydrous 1,4-dioxane (100ml) was added phosphorus tribromide (30mg, 0.11mmol), and the mixture stirred for 12 hours under dry nitrogen. Methanol (50ml) was added followed by ethyl acetate (50ml) and the organic solution washed successively with aqueous hydrochloric acid (0.2M, 50ml), aqueous sodium hydrogen carbonate (50ml) and finally brine (50ml). The organic layer was separated and dried ($MgSO_4$), before being concentrated to yield a crude purple solid. This was adsorbed onto silica and purified by gravity column chromatography (silica, eluent: CH_2Cl_2). Relevant fractions were identified by TLC (silica CH_2Cl_2), collected and concentrated to yield compound **237** as a purple solid (0.26g, 86%). R*f*=0.36 (silica, CH_2Cl_2); m.p. >200 °C.

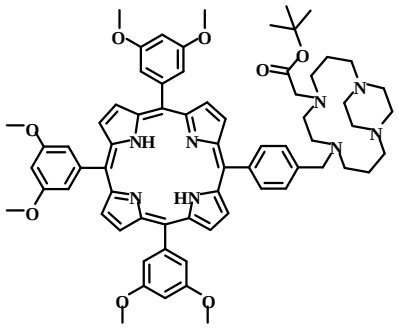
^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.91 (2H, br s, NH), 3.89 (18H, s, OCH_3), 4.78 (2H, s, CH_2), 6.82-6.83 (3H, t, ^{4}J=2.3Hz, 10,15,20-Ar-4-H), 7.32-7.33 (6H, d, ^{4}J=2.3Hz, 10,15,20-Ar-2,6-H), 7.70-7.72 (2H, d, ^{3}J=8.2Hz, 5-Ar-3,5-H), 8.10-8.12 (2H, d, ^{3}J=8.1Hz, 5-Ar-2,6-H), 8.74-8.75 (2H, d, ^{3}J=4.7Hz, β-H), 8.87-8.88 (6H, d, ^{3}J=4.5Hz, β-H)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 55.7, 56.1, 119.3, 119.9, 127.5, 128.0, 130.8, 131.3, 134.9, 137.3, 142.4, 144.0, 158.9

UV-vis (THF, nm) λmax/nm (log ε) 419 (5.6), 513 (4.6), 548 (4.1), 590 (4.0) 647 (4.0)

MS (MALDI) *m/z* 887 ^{79}Br $(M)^+$, 889 ^{79}Br $(M)^+$

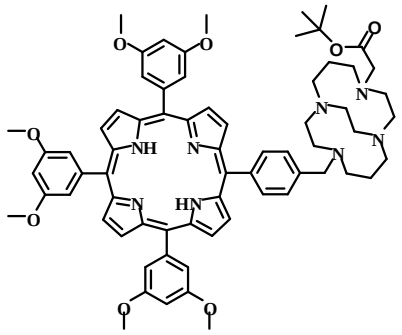
6.40 Attempted synthesis of (8-[4-[10,15,20-Tris-(3,5-dimethoxy-phenyl)-porphyrin-5-yl]-benzyl]-1,5,8,12-tetraaza-bicyclo[10.2.2]hexadec-5-yl)-acetic acid *tert*-butyl ester (244)



To a solution of compound **137** (0.05g, 0.15mmol) in dry acetonitrile (10ml) was added compound **237** (0.13g, 0.15mmol) and sodium carbonate (0.08g, 0.75mmol) and the mixture stirred at 50 °C under a nitrogen atmosphere. Consumption of the starting porphyrin **237** was monitored by taking aliquots of the mixture and separation by HPLC (C18 column, gradient eluent: 100% water to 100% methanol over 20mins), uv-vis detector. After 3 days, complete consumption of compound 3 had taken place. Dichloromethane (10ml) and water (10ml) were added, and the organic layer separated and dried ($MgSO_4$). The solution was concentrated down to a small volume and gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 95:5) was used to separate the porphyrin components of the mixture. The first fraction was identified as compound **248** and the second and third fractions degraded on the silica column. However, enough of the second fraction was recovered for ^{1}H NMR analysis. This indicated that although it was impure, it contained the desired compound.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.84 (2H, br s, N*H*), 1.45-1.51 (9H, m, (CH_3)$_3$), 1.60-3.12 (26H, m, NCH_2), 3.96 (18H, s, OCH_3), 5.07 (2H, s, CH_2), 6.88-6.91 (3H, m, 10,15,20-Ar-4-*H*), 7.36-7.41 (6H, m, 10,15,20-Ar-2,6-*H*), 7.68-8.02 (2H, m, 5-Ar-3,5-*H*), 8.20-8.32 (2H, m, 5-Ar-2,6-*H*), 8.74-8.76 (1H, m, β-*H*), 8.82-8.84 (2H, m, β-*H*), 8.91-8.98 (6H, m, β-*H*)
MS (ESI) *m/z* 1147 $(M)^+$, 1160 $(M+Na)^+$

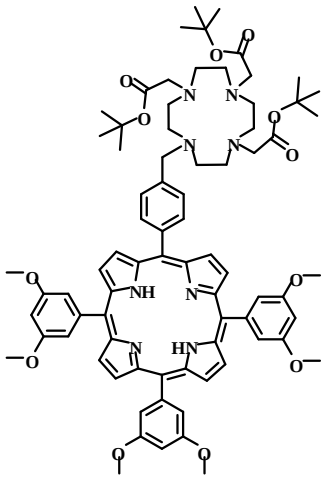
6.41 Attempted synthesis of (8-[4-[10,15,20-Tris-(3,5-dimethoxy-phenyl)-porphyrin-5-yl]-benzyl]-1,4,8,11-tetraaza-bicyclo[6.6.2]hexadec-4-yl)-acetic acid *tert*-butyl ester (245)



To a solution of compound **138** (0.05g, 0.15mmol) in dry acetonitrile (10ml) was added compound **237** (0.13g, 0.15mmol) and sodium carbonate (0.08g, 0.75mmol) and the mixture stirred at 50 °C under a nitrogen atmosphere. Consumption of the starting porphyrin **237** was monitored by TLC (silica, CH_2Cl_2/MeOH, 95:5) After 48 hours, complete consumption of compound 3 had taken place. Dichloromethane (10ml) and water (10ml) were added, and the organic layer separated and dried ($MgSO_4$). The solution was concentrated down to a small volume and gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 95:5) was used to separate the porphyrin components of the mixture. The first fraction was identified as compound **248** and although the second fractions degraded on the silica column, enough (~1mg) was recovered for NMR analysis. The fraction was impure, but appeared to contain the desired compound. Partial assignment of the spectra are given below.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.83 (2H, br s, N*H*), 1.50-1.70 (13H, m, (CH_3)$_3$, NCH_2), 2.50-3.25 (16H, m, NCH_2), 3.96 (18H, s, OCH_3), 6.88-6.92 (3H, m, 10,15,20-Ar-4-*H*), 7.38-7.42 (6H, m, 10,15,20-Ar-2,6-*H*), 7.71 (2H, d, ^{3}J=7.9Hz, 5-Ar-3,5-*H*), 8.18 (2H, m, ^{3}J=7.9Hz, 5-Ar-2,6-*H*), 8.79 (2H, d, ^{3}J=5.0Hz, β-*H*), 8.91-8.99 (6H, m, β-*H*)

6.42 Attempted synthesis of (1,4,7-tris-*tert*-butoxycarbonylmethyl-10-[4-[10,15,20-Tris-(3,5-dimethoxy-phenyl)-porphyrin-5-yl]-benzyl]-1,4,7,10-tetraaza-cyclododecane (246)



Compounds **237** (35mg, 0.04 mmol) and **127** (100mg 0.2 mmol) were dissolved in CH_2Cl_2 (10mL) and NEt_3 (4mg, 6μL, 0.04 mmol) was added. The reaction was stirred at ambient temperature under an N_2 atmosphere. After 3 hours, TLC analysis (silica, CH_2Cl_2/MeOH, 95:5) showed the complete consumption of the starting material and the formation of one major product.

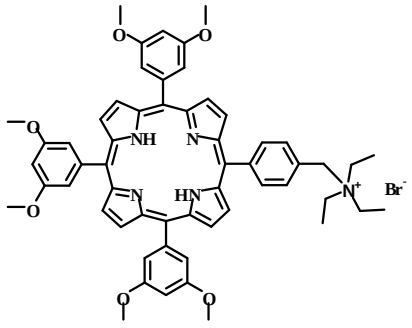
Water (20mL) was added and organic layer was separated and dried ($MgSO_4$), before being concentrated to yield a crude purple solid. This was adsorbed onto silica and purified by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 95:5). The first (minor) fraction was collected and the solvent concentrated to yield a purple solid. Yield <1mg. Analysis by ^{1}H NMR identified the desired compound and this was confirmed by mass spectrometry.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.91 (2H, br s, N*H*), 1.37-1.40 (27H, m, (C*H*$_3$)$_3$), 2.75-3.07 (18H, m, NC*H*$_2$), 3.22 (2H, s, NC*H*$_2$), 3.31 (4H, br s, NC*H*$_2$), 3.89 (18H, s, OC*H*$_3$), 6.82-6.85 (3H, m, 10,15,20-Ar-4-*H*), 7.28-7.32 (6H, m, 10,15,20-Ar-2,6-*H*), 7.79 (2H, d, ^{3}J=7.7Hz, 5-Ar-3,5-*H*), 8.09 (2H, m, ^{3}J=7.7Hz, 5-Ar-2,6-*H*), 8.63 (2H, d, ^{3}J=4.5Hz, β-*H*), 8.85-8.90 (6H, m, β-*H*)

MS (ESI) *m/z* 1344 $(M+Na)^+$

The second fraction was collected and the solvent concentrated to yield a purple

solid. (24mg, 65%). Analysis by ^{1}H NMR identified the compound as **249**, shown below.

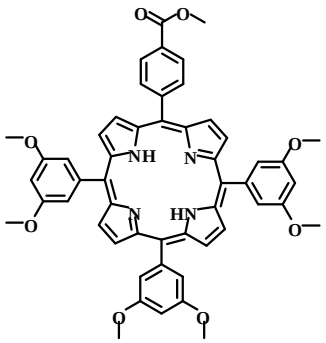


249

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.84 (2H, br s, N*H*), 1.60 (9H, t, ^{3}J=7.3Hz, CH_2CH_3), 3.65 (6H, q, ^{3}J=7.3Hz, CH_2CH_3), 3.96 (18H, s, OCH_3), 5.10 (2H, s, NCH_2Ph) 6.87-6.91 (3H, m, 10,15,20-Ar-4-*H*), 7.37-7.41 (6H, m, 10,15,20-Ar-2,6-*H*), 7.94 (2H, d, ^{3}J=8.1Hz, 5-Ar-3,5-*H*), 8.31 (2H, d, ^{3}J=8.1Hz, 5-Ar-2,6-*H*), 8.63 (2H, d, ^{3}J=4.8Hz, β-*H*), 8.90-9.00 (6H, m, β-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 8.8, 46.1, 53.5, 55.7, 100.2, 114.0, 117.3, 120.2, 120.3, 126.2, 131.0, 135.5, 143.9, 145.0, 159.0

6.43 Synthesis of 5-(4-Carbomethoxyphenyl)-10,15,20-tris(3,5-dimethoxyphenyl) porphyrin[124] (247)



Methyl-4-formyl benzoate (4.94g, 0.03mol) and 3,5-dimethoxybenzaldehyde (15g, 0.09mol) were dissolved in propionic acid (400ml) and the solution heated under reflux conditions. Pyrrole (8.07g, 0.12mol) was added dropwise and the dark mixture heated for thirty minutes. After cooling to room temperature, excess acid was removed *in vacuo* to yield a dark tarry solid. This was purified by gravity column chromatography (silica, eluent: CH_2Cl_2/EtOAc, 99:1). Relevant fractions were identified by TLC, combined and concentrated to yield **247** as a purple solid (1.2g, 5%). R*f*=0.6 (silica, CH_2Cl_2/EtOAc, 99:1); m.p. >200 °C.

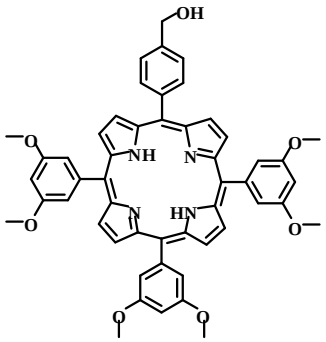
^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.78 (2H, br s, NH), 3.98 (18H, s, OCH_3), 4.13 (3H, s, $COOCH_3$), 6.92-6.93 (3H, t, ^{4}J=2.2Hz, 10,15,20-Ar-4-H), 7.43-7.44 (6H, t, ^{4}J=2.3Hz, 10,15,20-Ar-2,6-H), 8.33-8.35 (2H, d, ^{3}J=8.2Hz, 5-Ar-3,5-H), 8.46-8.48 (2H, d, ^{3}J=8.2Hz, 5-Ar-2,6-H), 8.80-8.83 (2H, m, β-H), 8.98-9.00 (6H, m, β-H)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ55.6, 56.1, 77.3, 77.7, 100.2, 113.9, 117.7, 127.9, 134.6, 144.0, 145.4, 146.1, 158.9, 160.1

UV-vis (CH_2Cl_2, nm) λmax 421, 515, 592, 659

MS (MALDI) *m/z* 852 $(M)^+$

6.44 Synthesis of 5-(4-Hydroxymethylphenyl)-10,15,20-tris(3,5-dimethoxyphenyl) porphyrin (248)



Compound **247** (0.3g, 0.35mmol) was dissolved in anhydrous THF (150ml) under dry nitrogen atmosphere, and the solution cooled to 0 °C. To this was added lithium aluminium hydride (0.5g, 13mmol) and the reaction stirred for 24 hours, protected from light. Upon completion, methanol (10ml) was added, followed by ethyl acetate (50ml). The reaction mixture was washed successively with aqueous hydrochloric acid (0.2M, 150ml), aqueous sodium hydrogen carbonate (150ml) and finally brine (150ml). The organic layer was separated and dried ($MgSO_4$), before being concentrated to yield a crude purple solid. This was adsorbed onto silica and purified by gravity column chromatography (silica, eluent: CH_2Cl_2/EtOAc, 93:7). Relevant fractions were identified by TLC, collected and concentrated to yield compound **248** as a purple solid (0.26g, 90%). R*f*=0.4 (silica, CH_2Cl_2/EtOAc, 93:7); m.p. >200 °C.

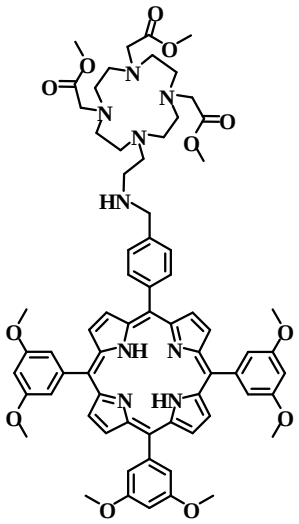
^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.77 (2H, br s, NH), 3.98 (18H, s, OCH_3), 5.02 (2H, s, CH_2), 6.92-6.94 (3H, t, ^{4}J=2.3Hz, 10,15,20-Ar-4-H), 7.44-7.45 (6H, d, ^{4}J=2.2Hz, 10,15,20-Ar-2,6-H), 7.72-7.74 (2H, d, ^{3}J=8.1Hz, 5-Ar-3,5-H), 8.21-8.23 (2H, d, ^{3}J=8.1Hz, 5-Ar-2,6-H), 8.85-8.86 (2H, d, ^{3}J=4.8Hz, β-H), 8.97-8.98 (6H, d, ^{3}J=4.5Hz, β-H)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 55.4, 55.7, 65.3, 100.2, 104.5, 113.9, 119.9, 125.3, 131.1, 134.7, 140.3, 141.5, 144.0, 144.1, 158.9

UV-vis (CH_2Cl_2, nm) λmax 420, 515, 549, 590

MS (MALDI) *m/z* 824 $(M)^+$

6.45 Attempted synthesis of (1,4,7-tris-methoxycarbonylmethyl-10-[-[4-[10,15,20-Tris-(3,5-dimethoxy-phenyl)-porphyrin-5-yl]-benzyl]-1,4,7,10-tetraaza-cyclododecane (250)



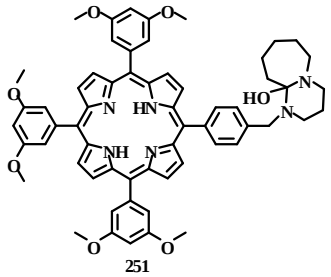
Method 1

Compounds **237** (114mg, 0.13 mmol) and **56** (132mg, 0.31 mmol) were dissolved in anhydrous THF (40mL) and Na_2CO_3 (0.26g, 2.5 mmol) added. The reaction was stirred at ambient temperature under a N_2 atmosphere. Monitoring by TLC (silica, CH_2Cl_2) showed no consumption of the starting materials after 8 hours, so the reaction was heated to 60 °C. After 4 days, TLC (silica, eluent: CH_2Cl_2) showed complete reaction and the reaction mixture was filtered and the solvent removed *in vacuo*. The product was isolated by column chromatography (silica, eluent: CH_2Cl_2/MeOH, 95:5), however NMR analysis identified it as compound **248**.

Method 2

Compounds **237** (40mg, 0.05 mmol) and **56** (54mg, 0.13 mmol) were dissolved in anhydrous THF (25mL), DBU (8mg, 0.05 mmol) added and the reaction mixture was heated to 60 °C under N_2. Monitoring by TLC (silica, CH_2Cl_2) showed consumption of the starting materials after 3 days with formation of one polar porphyrin spot. The solvent was removed *in vacuo* and the residue taken

into a small volume of CH_2Cl_2. The product was isolated by column chromatography (silica, eluent: CH_2Cl_2/MeOH, 95:5), but NMR analysis identified it as compound **251** shown below.



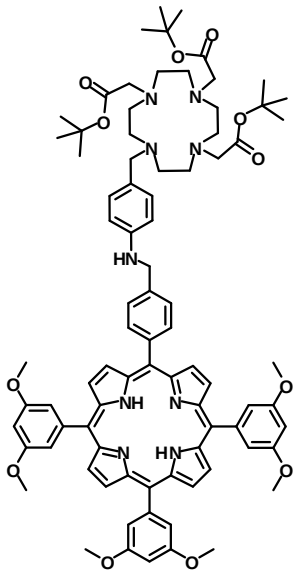
251

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.83 (2H, br s, N*H*), 1.60-1.82 (6H, m, CH_2), 2.20-2.30 (2H, m, CH_2), 2.58-2.63 (2H, m, NCH_2), 3.00-3.09 (2H, m, NCH_2), 3.37-3.48 (2H, m, NCH_2), 3.57-3.65 (2H, m, NCH_2), 3.96 (18H, s, OCH_3), 4.35 (2H, s, NCH_2Ph) 6.88-6.91 (3H, m, 10,15,20-Ar-4-*H*), 7.38-7.42 (6H, m, 10,15,20-Ar-2,6-*H*), 7.93 (2H, d, ^{3}J=8.1Hz, 5-Ar-3,5-*H*), 8.24 (2H, d, ^{3}J=8.1Hz, 5-Ar-2,6-*H*), 8.81 (2H, d, ^{3}J=4.8Hz, β-*H*), 8.94 (6H, br s, β-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 23.4, 25.7, 28.3, 29.8, 36.9, 45.0, 45.5, 50.2, 52.5, 55.6, 100.2, 113.9, 119.2, 119.8, 119.9, 128.1, 135.0, 144.0, 158.8

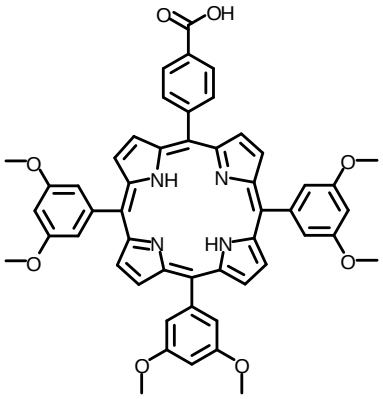
MS (ESI) *m/z* 977 $(M)^+$

6.46 Attempted synthesis of 1,4,7-tris-*tert*-butoxycarbonylmethyl-10-(4-[4-[10,15,20-tris-(3,5-dimethoxy-phenyl)-porphyrin-5-yl]benzylamino]benzyl)-1,4,7,10-tetraazacyclododecane (260)



Compounds **237** (44mg, 0.05 mmol) and **57** (97mg, 0.23 mmol) were dissolved in anhydrous THF (25mL), $NaHCO_3$ (42mg, 0.5 mmol) added and a catalytic amount of KI. The reaction was stirred at ambient temperature under a N_2 atmosphere. After 2 days, TLC (silica, CH_2Cl_2) showed most of the starting porphyrin had been consumed. The reaction mixture was filtered through celite and the solvent removed *in vacuo*. The residue was taken into CH_2Cl_2 and the product isolated by column chromatography (silica, eluent: CH_2Cl_2/MeOH, 95:5). ^{1}H NMR analysis showed this to be a mixture of two porphyrins and the mixture could not be separated by preparative TLC or column chromatography.

6.47 Synthesis of 5-(4-carboxyphenyl)-10,15,20-tris(3,5-dimethoxyphenyl) porphyrin[124] (262)



4-carboxybenzaldehyde (0.45g, 3.0 mmol) and 3,5-dimethoxybenzaldehyde (1.5g, 9 mmol) were dissolved in propionic acid (50ml) and the solution heated under reflux conditions. Pyrrole (0.80g, 12 mmol) was added dropwise and the dark mixture heated for thirty minutes. After cooling to room temperature, excess acid was removed *in vacuo* to yield a dark tarry solid. This was purified by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 94:6). Relevant fractions were identified by TLC, combined and concentrated to yield **262** as a purple solid (0.25g, 10%). R*f*=0.4 (silica, CH_2Cl_2/MeOH 94:6). m.p. >200 °C.

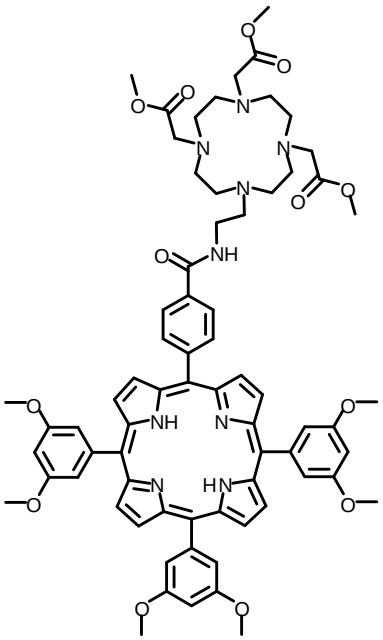
^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.82 (2H, br s, N*H*), 3.97 (18H, s, OCH_3), 6.90-6.93 (3H, m, 10,15,20-Ar-4-*H*), 7.37-7.42 (6H, m, 10,15,20-Ar-2,6-*H*), 8.34 (2H, d, ^{3}J=8.1Hz, 5-Ar-3,5-*H*), 8.50 (2H, d, ^{3}J=8.1Hz, 5-Ar-2,6-*H*), 8.80 (2H, d, ^{3}J=4.8Hz, β-*H*), 8.91-9.00 (6H, m, β-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ55.6, 100.2, 113.9, 118.5, 120.1, 120.2, 128.5, 128.9, 134.6, 143.9, 147.7, 158.9, 171.1

UV-vis (CH_2Cl_2, nm) λmax 421, 516, 591, 657

MS (MALDI) *m/z* 839 $(M+H)^+$

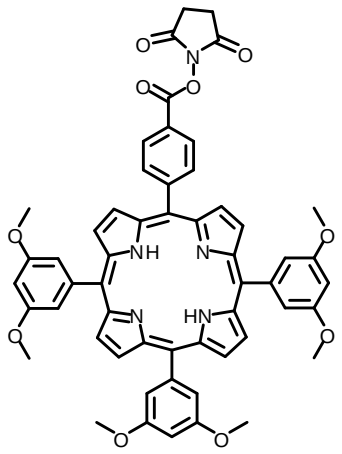
6.48 Attempted synthesis of 1,4,7-tris-methoxycarbonylmethyl-10-(2-(4-[10,15,20-tris-(3,5-dimethoxy-phenyl)-porphyrin-5-yl]benzamido)ethyl))-1,4,7,10-tetraazacyclododecane (263)



To a stirred solution of compound **262** (99mg, 0.12 mmol) and DCC (25mg, 0.12 mmol) in anhydrous CH_2Cl_2 (10mL) at 0 °C under N_2, was added **56** (102mg, 0.24 mmol). The reaction was stirred under N_2 at ambient temperature and after 8 hours, TLC analysis (silica, CH_2Cl_2/MeOH 95:5) showed complete consumption of starting material and two new porphyrin products. The reaction mixture was concentrated and column chromatography (silica, eluent CH_2Cl_2/MeOH 95:5) was used to separate the porphyrin products. The first fraction was collected and although it contained impurities, was identified by NMR as the porphyrin-DCC intermediate of the reaction. The second fraction could only be collected in a very small amount since degradation occurred as it passed down the column.

The reaction was repeated but attempted separation and isolation of the desired compound was not achieved, despite chromatography using neutral alumina, basic alumina and silica gel pretreated with base (NEt_3).

6.49 Synthesis of 4-[10,15,20-Tris-(3,5-dimethoxy-phenyl)-porphyrin-5-yl]-benzoic acid 2,5-dioxo-pyrrolidin-1-yl ester (264)



Compound **262** (219mg, 0.26 mmol) was dissolved in anhydrous pyridine (30mL) under an N_2 atmosphere and $SOCl_2$ added in one portion. After 5 mins, the N_2 flow was removed and a $CaCl_2$ guard tube fitted. The reaction was then heated to 50 °C and after 30 mins, N-hydroxysuccinimide (0.6g, 5.2 mmol) was added in one portion and the reaction stirred at 50 °C for a further 6 hours. The reaction mixture was allowed to cool to ambient temperature and the solvent was removed *in vacuo*. The residue was dissolved in $CHCl_3$ (50mL), washed successively with saturated $NaHCO_3$(aq) and brine before the organic layer was separated and concentrated. The desired compound was isolated by gravity column chromatography (silica, eluent: CH_2Cl_2/MeOH, 96:4). Relevant fractions were identified by TLC, combined and concentrated to yield **264** as a purple solid (0.13g, 52%). R*f*=0.6 (silica, CH_2Cl_2/MeOH 96:4). m.p. >200 °C.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.82 (2H, br s, N*H*), 2.80-3.00 (4H, m, CH_2), 3.96 (18H, s, OCH_3), 6.89-6.93 (3H, m, 10,15,20-Ar-4-*H*), 7.39-7.45 (6H, m, 10,15,20-Ar-2,6-*H*), 8.38 (2H, d, ^{3}J=8.1Hz, 5-Ar-3,5-*H*), 8.54 (2H, d, ^{3}J=8.1Hz, 5-Ar-2,6-*H*), 8.77 (2H, d, ^{3}J=4.8Hz, β-*H*), 8.97 (6H, br s, β-*H*), 9.00 (2H, d, ^{3}J=4.8Hz, β-*H*)

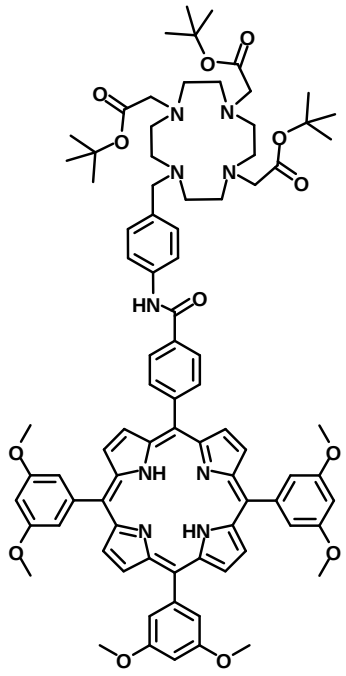
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 25.7, 55.6, 100.1, 113.8, 117.7, 120.2, 120.3,

124.4, 128.9, 134.8, 143.8, 149.3, 158.9, 162.1, 169.3

UV-vis (CH_2Cl_2, nm) λmax 421, 516, 591, 657

MS (MALDI) *m/z* 936 $(M+H)^+$

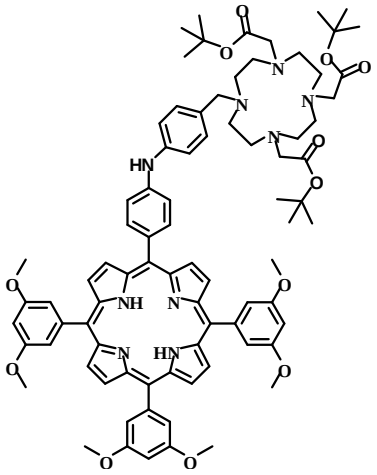
6.50 Attempted synthesis of 1,4,7-tris-*tert*-butoxycarbonylmethyl-10-(2-(4-[10,15,20-tris-(3,5-dimethoxy-phenyl)-porphyrin-5-yl]benzamido)benzyl))-1,4,7,10-tetraazacyclododecane (265)



Compounds **264** (20mg, 0.02 mmol) and **57** (13mg, 0.02 mmol) were dissolved in anhydrous pyridine (5mL) and the reaction stirred under N_2 atmosphere at ambient temperature. After 2 hours, TLC analysis (silica, CH_2Cl_2/MeOH 95:5) showed no change to the starting materials, so the reaction was heated to 70 °C. No change to the reactants could be detected by TLC analysis after 24 hours and prolonged heating only led to decomposition of compound **57**.

The reaction was repeated, but the amine **57** was washed with $NaHCO_{3(aq)}$ prior to being introduced to the reaction vessel. The solvent was also changed to anhydrous THF and the reaction heated at 70 °C. No reaction could be detected once again, and prolonged heating caused decomposition of compound **57**.

6.51 Synthesis of 1,4,7-tris-*tert*-butoxycarbonylmethyl-10-(2-(4-[10,15,20-tris-(3,5-dimethoxy-phenyl)-porphyrin-5-yl]aminophenyl)benzyl))-1,4,7,10-tetraazacyclododecane (287)



Compound **289** (300mg, 0.32 mmol), compound **57** (300mg, 0.48 mmol), Cs_2CO_3 (1.04g, 3.2 mmol), $Pd(OAc)_2$ (3.6mg, 0.02 mmol) and *rac*-BINAP (16mg, 0.03 mmol) were added to a flame dried Schlenk tube and placed under a N_2 atmosphere. Anhydrous toluene (30mL) was subsequently added and a flow of N_2 was bubbled through the mixture for one hour. The reaction was then heated to 90 °C for 24 hours until TLC analysis (silica, CH_2Cl_2/MeOH 99:1) showed no further reaction. The solvent was removed in vacuo, CH_2Cl_2 (100mL) added to the residue and the solution washed with $NaHCO_3$(aq) (100mL) and brine (100mL). The organic extracts were stirred at ambient temperature with TFA (5 drops) for 10 minutes. Saturated $NaHCO_3$(aq) was added dropswise to until the solution was neutral and the organic layer was extracted and dried over Na_2SO_4. The desired compound was isolated by gravity column chromatography (silica, eluent: $CHCl_3$/MeOH 95:5) as a purple solid. (Yield 198mg, 44%). R*f*=0.3 (silica, $CHCl_3$/MeOH 95:5). m.p. >200 °C.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.81 (2H, br s, N*H*), 1.45-1.52 (27H, m, (CH_3)$_3$), 2.08-3.10 (22H, br m, NCH_2), 3.97 (18H, s, OCH_3), 6.89-6.91 (3H, m, 10,15,20-Ar-4-*H*), 7.34-7.37 (2H, m, NCH_2-Ph-2,6-*H*), 7.38-7.44 (8H, m, 10,15,20-Ar-2,6-*H*, NCH_2-Ph-3,5-*H*), 7.48 (2H, d, ^{3}J=8.1Hz, 5-Ar-3,5-*H*), 8.08 (2H, d, ^{3}J=8.1Hz, 5-Ar-2,6-*H*), 8.87-8.95 (8H, m, β-*H*)

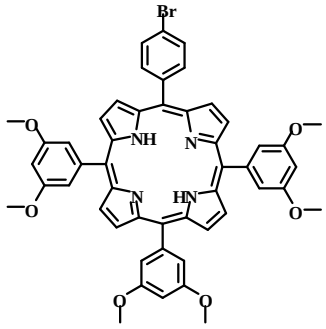
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 27.8, 28.0, 28.1, 49.1 (br), 53.4, 55.6, 55.9,

58.7, 82.3, 82.9, 100.0, 113.9, 115.6, 118.1, 119.5, 119.7, 121.0, 128.9, 131.1, 134.1, 135.6, 142.7, 142.9, 143.9, 144.0, 158.8, 172.3, 173.2

UV-vis (CH_2Cl_2, nm) λmax 421, 515, 552, 590, 648

MS (ESI) *m/z* 1414 $(M+H)^+$, 1415 $(M+2H)^+$, 1416 $(M+3H)^+$

6.52 Synthesis of 5-(4-bromophenyl)-10,15,20-tris(3,5-dimethoxyphenyl) porphyrin[124] (288)



4-bromobenzaldehyde (1.86g, 10 mmol) and 3,5-dimethoxybenzaldehyde (5g, 30 mmol) were dissolved in propionic acid (150ml) and the solution heated under reflux conditions. Pyrrole (2.69g, 40 mmol) was added dropwise and the dark mixture heated for thirty minutes. After cooling to room temperature, excess acid was removed *in vacuo* to yield a dark tarry solid. This was purified by gravity column chromatography (silica, eluent: CH_2Cl_2/Hexane, 9:1). Relevant fractions were identified by TLC, combined and concentrated to yield **288** as a purple solid (0.64g, 7.3%). R*f*=0.4 (silica, CH_2Cl_2/Hexane 9:1). m.p. >200 °C.

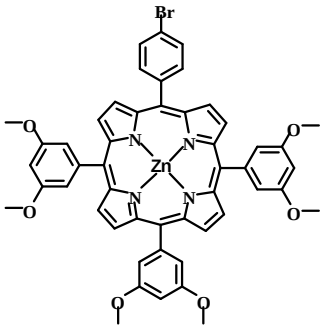
^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.83 (2H, br s, N*H*), 3.96 (18H, s, OCH_3), 6.89-6.91 (3H, m, 10,15,20-Ar-4-*H*), 7.38-7.41 (6H, m, 10,15,20-Ar-2,6-*H*), 7.88 (2H, d, ^{3}J=8.1Hz, 5-Ar-3,5-*H*), 8.07 (2H, d, ^{3}J=8.1Hz, 5-Ar-2,6-*H*), 8.80 (2H, d, ^{3}J=4.8Hz, β-*H*), 8.91-8.98 (6H, m, β-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ55.7, 100.3, 114.0, 118.2, 120.1, 122.3, 130.0, 135.9, 140.6, 144.0, 158.9

UV-vis (THF, nm) λmax/nm (log ε) 419 (5.8), 513 (4.7), 547 (4.2), 590 (4.2), 647 (4.0)

MS (MALDI) *m/z* 872 ^{79}Br $(M+H)^+$, 874 ^{81}Br $(M+H)^+$

6.53 Synthesis of 5-(4-bromophenyl)-10,15,20-tris(3,5-dimethoxyphenyl) porphyrinato zinc(II) (289)



To a solution of compound **288** (0.64g, 0.73 mmol) in $CHCl_3$ (200mL) was added a solution of $Zn(OAc)_2$ (2g, 9.1 mmol) in MeOH (50mL) and the resulting solution was stirred at 35 °C for four hours. Solvents were removed in vacuo, the residue dissolved in CH_2Cl_2 and washed successively with $NaHCO_3$(aq) (100mL) and brine (100mL). After drying over Na_2SO_4, the solvent was removed to give a bright purple solid. (Yield 0.69g, 100%). R*f*=0.5 (silica, CH_2Cl_2). m.p. >200 °C.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 3.92 (18H, s, OC*H*$_3$), 6.81-6.84 (3H, m, 10,15,20-Ar-4-*H*), 7.33-7.37 (6H, m, 10,15,20-Ar-2,6-*H*), 7.87 (2H, d, ^{3}J=8.1Hz, 5-Ar-3,5-*H*), 8.07 (2H, d, ^{3}J=8.1Hz, 5-Ar-2,6-*H*), 8.90 (2H, d, ^{3}J=4.5Hz, β-*H*), 9.02 (4H, br s, β-*H*), 9.04 (2H, d, ^{3}J=4.5Hz, β-*H*)

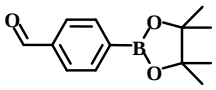
^{13}C N.M.R. [100MHz, $CDCl_3$] δ55.7, 100.1, 113.8, 119.5, 121.1, 122.3, 129.8, 131.7, 132.2, 132.3, 135.8, 141.9, 144.6, 149.9, 150.0, 150.18, 150.22, 158.8

UV-vis (CH_2Cl_2, nm) λmax/nm (log ε) 422 (6.0), 549 (4.5), 593 (3.8)

MS (ESI) *m/z* 934 ^{79}Br $(M)^+$, 936 ^{81}Br $(M)^+$

HRMS : calculated for $C_{50}H_{39}{}^{79}BrN_4O_6Zn$: 934.1339, found 934.1343

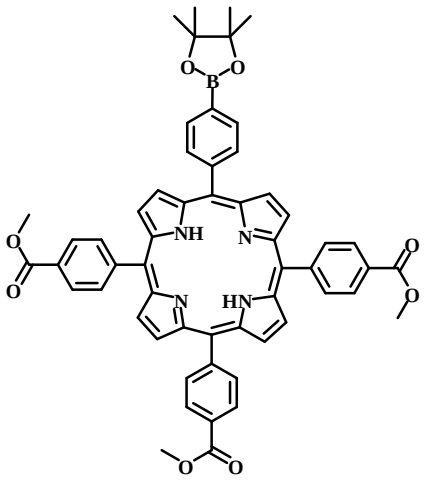
6.54 Synthesis of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde[209] (302)



4-formylphenylboronic acid (4.0g, 26.7 mmol) was suspended in anhydrous diethyl ether (45mL) and pinacol (3.85g, 32.6 mmol) added. The reaction mixture was stirred at room temperature under N_2 for 24 hours after which time the solids had dissolved completely. The organic solution was washed with H_2O (50mL) and the product was isolated by column chromatography (silica, eluent CH_2Cl_2) as a white solid (5.5g, 89%) m.p. 58-59°C

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 1.33-1.36 (12H, m, CH_3), 7.85 (2H, d, ^{3}J=7.9Hz, B-Ar-2,6-*H*), 7.96 (2H, d, ^{3}J=7.9Hz, B-Ar-3,5-*H*), 10.04 (1H, s, C*H*O)
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 24.9, 84.3, 128.7, 135.2, 138.0, 192.7
^{11}B N.M.R. [128.3MHz, $CDCl_3$] δ 30.4

6.55 Synthesis of 5-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl))-10,15,20-tris-(4-carbomethoxyphenyl) porphyrin[139] (303)



Methyl(4-formyl)benzoate (2.59g, 15.7 mmol), pyrrole (1.41g, 21.0 mmol) and compound **302** (1.22g, 5.26 mmol) were dissolved in $CHCl_3$ (2.1 dm^3) and argon was bubbled through the solution for 20 mins. BF_3OEt_2 was added in one portion and the mixture stirred protected from light, under an Ar atmosphere, for 2.5 hours. DDQ (4.76g, 21.0 mmol) was added in one portion, the inert gas flow removed, and the mixture stirred for 6 hours, open to air but protected from light. NEt_3 (0.63g, 0.55mL, 6.3 mmol) was added and the solution stirred for a further 10 mins, before being passed through a short silica pad ($CHCl_3$ eluent) to elute the porphyrins. The solvent was concentrated *in vacuo* to a small volume and silica gel chromatography (silica, eluent: CH_2Cl_2/EtOAc, 97:3) used to isolate the desired compound. Despite obvious degradation as it travelled down the column, after removal of solvent, the product was collected as a purple solid. (144mg, 3%). R*f*=0.6 (silica, CH_2Cl_2/EtOAc 97:3). m.p. >200 °C.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.89 (2H, s, N*H*), 1.48 (9H, s, CH_3), 4.09 (9H, s, COOCH_3), 8.09 (2H, AB, ^{3}J=8.0Hz, 5-Ar-2,6-*H*), 8.12 (2H, AB, ^{3}J=8.0Hz, 5-Ar-3,5-*H*), 8.27, (6H, d, ^{3}J=8.2Hz, 10,15,20-Ar-2,6-*H*), 8.42 (6H, d, ^{3}J=8.2Hz, 10,15,20-Ar-3,5-*H*), 8.73-8.86 (8H, m, β*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 25.0, 52.5, 84.2, 119.0, 119.2, 120.9, 128.0, 129.7, 133.1, 134.1, 134.5, 144.7, 146.7, 167.3, 171.2

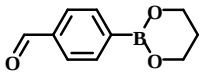
^{11}B N.M.R. [128.3MHz, $CDCl_3$] δ -9.05

UV-vis (CH_2Cl_2, nm) λmax 420, 516, 550, 590, 646

MS (ESI) *m/z* 914 $(M)^+$

HRMS : calculated for $C_{56}H_{47}BN_4O_8$: 914.3481, found 914.3471

6.56 Synthesis of 4-(1,3,2-dioxaborinan-2-yl)benzaldehyde[251] (304)



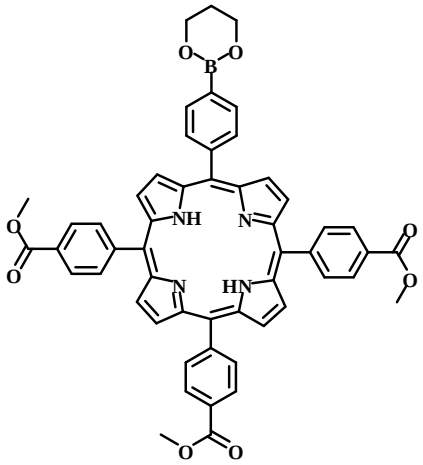
Propane-1,3-diol (3.61g, 47.5 mmol) and 4-formylphenylboronic acid (6.27g, 41.8 mmol) were dissolved in anhydrous THF (60mL) and the solution stirred at ambient temperature under N_2 for 20 mins. The solvent was removed in vacuo and the residue dissolved in CH_2Cl_2 (50mL). The organic solution was washed with H_2O (3x50mL), separated and dried over $MgSO_4$ and concentrated to leave an off white solid. Recrystallisation from CH_2Cl_2/hexane gave a white solid. (Yield: 4.92g, 62%)

^{1}H N.M.R. [400MHz, $CDCl_3$] δ 2.07 (2H, q, ^{3}J=5.4Hz, $CH_2CH_2CH_2$), 4.17 (4H, t, ^{3}J=5.4Hz, CH_2O), 7.82 (2H, d, ^{3}J=7.8Hz, B-Ar-2,6-*H*), 7.90 (2H, d, ^{3}J=7.8Hz, B-Ar-3,5-*H*), 10.02 (1H, s, C*H*O)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 27.3, 62.1, 128.6, 134.1, 137.7, 192.9

^{11}B N.M.R. [128.3MHz, $CDCl_3$] δ 25.7

6.57 Synthesis of 5-(4-(1,3,2-dioxaborinan-2-yl))-10,15,20-tris-(4-carbomethoxyphenyl) porphyrin[139] (305)



Comopund **304** (1.27g, 6.7 mmol), methyl(4-formyl) benzoate (3.28g, 20mmol) and pyrrole (1.79g, 26.7 mmol) were dissolved in chloroform (2.67dm^3) and the solution degassed for 30 mins. BF_3OEt_2 (1.14g, 8mmol) was added and the solution stirred under N_2 whilst protected from light for 2.5 hours. DDQ (6.06g, 26.7 mmol) was added in one portion and the mixture stirred, open to the air, for a further 5 hours. NEt_3 (0.81g, 8.0 mmol) was added and the solution stirred for ten minutes, before being passed through a short silica pad ($CHCl_3$ eluent) to elute the porphyrins. The solvent was concentrated *in vacuo* to a small volume and silica gel chromatography used to isolate the desired compound (eluent: $CHCl_3$: MeOH, 99:1). After removal of solvent, the product was collected as a purple solid. (0.44g, 7.5%). R*f*=0.4 (silica, $CHCl_3$: MeOH, 99:1). m.p. >200 °C.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.88 (2H, s, N*H*), 2.12-2.18 (2H, d, ^{3}J=5.6Hz, $CH_2CH_2CH_2$), 4.04 (9H, s, $COOCH_3$), 4.17 (4H, t, ^{3}J=5.5Hz, CH_2O), 8.08-8.14 (4H, m, 5-Ar-2,6-*H*, 5-Ar-3,5-*H*), 8.22, (6H, d, ^{3}J=8.4Hz, 10,15,20-Ar-2,6-*H*), 8.37 (6H, d, ^{3}J=8.4Hz, 10,15,20-Ar-3,5-*H*), 8.69-8.82 (8H, m, β*H*)

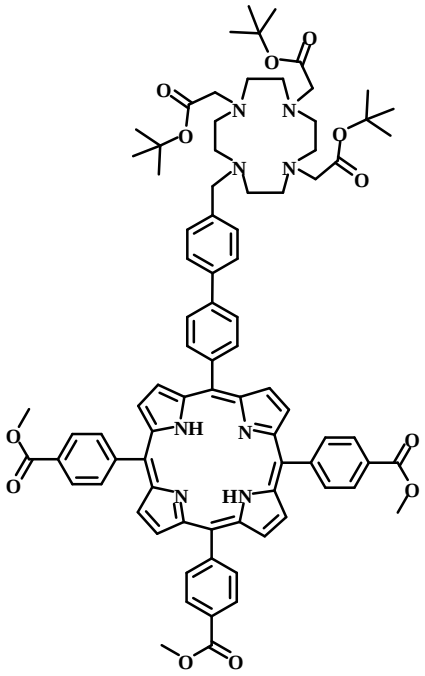
^{13}C N.M.R. [100MHz, $CDCl_3$] δ 27.7, 52.5, 62.3, 113.9, 119.0, 119.2, 121.3, 128.0, 129.8, 132.1, 134.1, 134.6, 144.0, 146.9, 167.3, 171.2

^{11}B N.M.R. [128.3MHz, $CDCl_3$] δ 25.4

UV-vis (CH_2Cl_2, nm) λmax 421, 515, 550, 592, 648

MS (ESI) *m/z* 872 $(M)^+$

6.58 Synthesis of 1,4,7-tris-*tert*-butoxycarbonylmethyl-10-(2-(4-[10,15,20-tris-(4-carbomethoxyphenyl)-porphyrin-5-yl]biphenyl-4-yl)-ethyl))-1,4,7,10-tetraazacyclododecane (306)



Compounds **305** (30mg, 0.034 mmol), compound **58** (23mg, 0.034 mmol), and K_2CO_3 (66mg, 0.48 mmol) were dissolved in a mixture of toluene (2mL) and DMF (1mL) and argon was bubbled through the mixture for 20 mins. $Pd(PPh_3)_4$ (12mg, 0.01 mmol) was added and the mixture pump-purged with Ar 3 times before and heated to 90 °C. After 17 hours, TLC analysis (silica, $CHCl_3$/MeOH 97:3) showed almost complete consumption of the starting material and the production of one major new porphyrin spot. The reaction mixture was cooled to ambient temperature, filtered through celite and the solvents were removed *in vacuo*. The residue was taken into a small amount (~1mL) of CH_2Cl_2 and silica gel chromatography (silica, eluent: CH_2Cl_2/MeOH, 97:3) used to isolate the new porphyrin compound, which was obtained, after removal of solvents, as a purple solid. (Yield 25mg, 52%). R*f*=0.6 (silica, CH_2Cl_2/MeOH 97:3). m.p. >200 °C.

^{1}H N.M.R. [400MHz, $CDCl_3$] δ -2.86 (2H, br s, N*H*), 1.35-1.50 (27H, m, (CH_3)$_3$), 2.08-3.10 (24H, br m, NCH_2), 4.00 (9H, s, COOCH_3), 7.60 (2H, d,

^{3}J=8.1Hz, NCH_2-Ph-2,6-*H*), 7.81 (2H, d, ^{3}J=8.1Hz, NCH_2-Ph-3,5-*H*), 7.91 (2H, d, ^{3}J=8.1Hz, 5-Ar-3,5-*H*), 8.18-8.26 (8H, m, 5,10,15,20-Ar-2,6-*H*), 8.38 (6H, d, ^{3}J=8.1Hz, 10,15,20-Ar-3,5-*H*), 8.72-8.81 (6H, m, β-*H*), 8.82-8.91 (2H, m, β-*H*)

^{13}C N.M.R. [100MHz, $CDCl_3$] δ 28.0, 28.1, 50.1, 52.7, 56.0, 56.2, 59.4, 83.0, 83.5, 119.6, 119.7, 120.7, 125.7, 127.9, 128.3, 130.3, 131.3, 134.9, 135.4, 137.3, 140.2, 140.4, 141.5, 146.8, 146.9, 167.4, 172.9, 173.9

UV-vis (CH_2Cl_2, nm) λmax 421, 516, 551, 593, 646

MS (ESI) *m/z* 1392 $(M+H)^+$

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