

DECLARATION

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Master of Science, is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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To my husband Dawid and my family, thank you for your emotional support and enduring encouragement.

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ABBREVIATIONS

GENERAL ABBREVIATIONS

Ar	aromatic
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
5-FU	5-fluorouracil
HOMO	highest occupied molecular orbital
IR	infra-red
KBr	potassium bromide
K ₂ CO ₃	potassium carbonate
MALDI	matrix assisted laser desorption/ionization
MgSO ₄	magnesium sulfate
MPc	metallo phthalocyanine
MS	mass spectra
NaH	sodium hydride
NaOAc	sodium acetate
NaOH	sodium hydroxide
NMR	nuclear magnetic resonance
Pc	phthalocyanine
PcH ₂	metal-free phthalocyanine

PcLi ₂	lithium phthalocyanine
PcM	metallated phthalocyanine
PDT	photodynamic therapy
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
UV/Vis	ultraviolet-visible
Zn(OAc) ₂	zinc acetate
PcZn	phthalocyaninato zinc (II)

Abstract

This thesis investigates the synthesis of new fluorouracil prodrugs and new prodrug photosensitiser systems for medicinal applications including the design and preparation and attempted preparation of a novel partial cone calixarene porphyrin.

The preparation of a new redox activated prodrug of fluorouracil is described. The biological activity of the new prodrugs was investigated against nitroreductase transfected cells (V79-NTR) and they were successfully activated to fluorouracil as determined by LC-MS. Two non-redox activated prodrugs of fluorouracil were also prepared and these gave activities of 15 and 20 μ M against colon cancer cell lines

.
An attempt was made to prepare a series of porphyrazines that could be used as both PDT agents and carriers for the 'dark' therapeutic nitrous oxide. Unfortunately, purification of these new compounds proved unsuccessful and they were not further developed.

Chapter three outlines the proposed design and synthesis of a new and novel thermally stable partial-cone calix[4]arene porphyrin. The strategy required the selective synthesis of a partial cone O-arylcalixarene nitrile, which was then to be converted to the aldehyde by reduction. The nitrile derivative was successfully prepared by both thermal and microwave conditions, however, the reduction of the nitrile to the aldehyde proved to be problematic. However, it was discovered that only lithium based reducing agents could reduce the nitrile, albeit in low yields. It was discovered that the reduction of the nitrile was sterically hindered due to the presence of calyx[4]arene t-butyl groups in the upper rim. As a consequence of the low yields of the reduction reaction, the condensation of the aldehyde to the target porphyrin could not be achieved.

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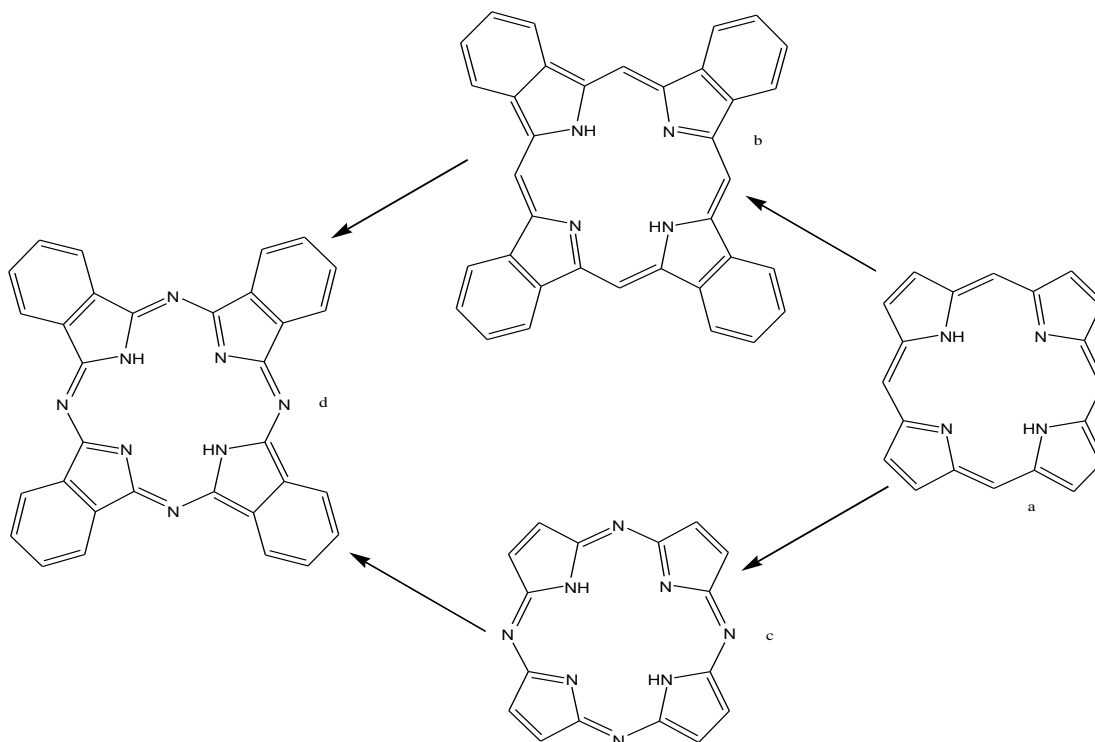
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Chapter 1 *Literature Review*

1.1 Introduction to Phthalocyanines

Phthalocyanines (Pcs) are the most utilised blue and green pigments in industry as they are inexpensive, durable and have an intense absorption at long wavelengths of the visible spectrum.¹ Phthalocyanines have found applications as; dyes, deodorants, as charge generation materials in photocopiers, for removal of sulphur from oil, in photodynamic therapy (PDT) and in data storage for optical disks.² Phthalocyanine was first discovered in 1907 but it wasn't until the 1930's that the structure was elucidated by Sir Reginald Linstead.

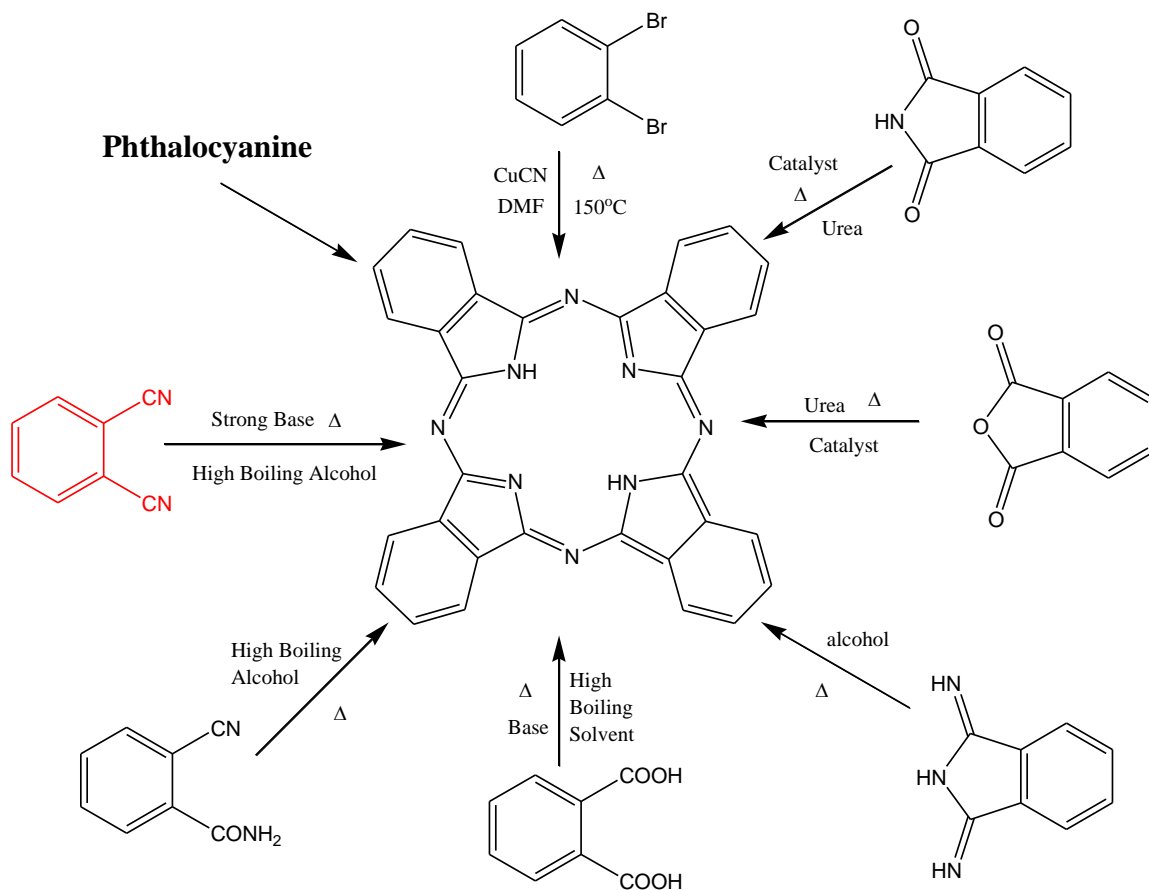


Scheme 1. Relationship between porphyrin and phthalocyanine, a) basic porphyrin, b) a tetrabenzoporphyrin, c) a tetraazaporphyrin or porphyrazine and d) a phthalocyanine.¹

The most prominent methods of preparing phthalocyanines involves the cyclisation of phthalic acid derivatives, e.g. phthalonitrile, o-cyanobenzamide or phthalic anhydride, some of which are shown in scheme 2.²

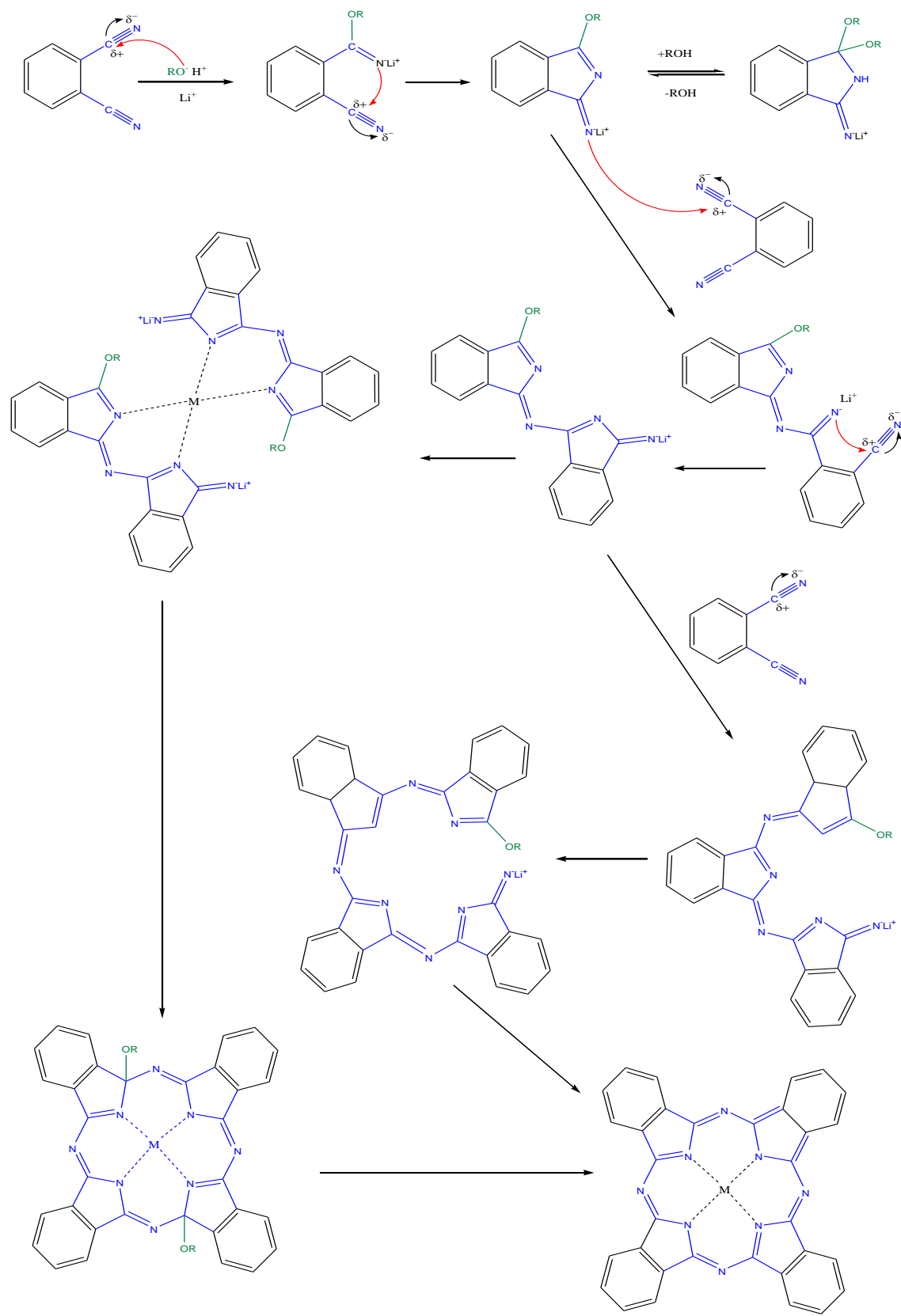
Phthalonitrile, scheme 2, is by and large the most commonly used precursor for the preparation of phthalocyanines. The synthesis involves heating phthalonitrile in a lithium

alkoxide solution to produce the lithiated phthalocyanine. This PcLi_2 can be de-metallated with acid to give the metal-free PcH_2 or treated with a M^{+2} salt to yield the metallated derivative.³



Scheme 2. Phthalocyanine precursor compounds, where the compound in red is phthalonitrile.⁴

A proposed mechanism for the, “Linstead method of phthalocyanine formation” is illustrated in scheme 3.⁵



Scheme 3. Linstead's proposed mechanism for phthalocyanine formation.⁵

1.1.2 Solubility of Phthalocyanines

Phthalocyanine is notorious for its insolubility in water and most organic solvents. This high insolubility is a result of strong intermolecular π - π interactions between the phthalocyanine rings which results in the formation of stable aggregates preventing solubilisation.¹⁻⁵

Pc solubility in organic solvents can be achieved by incorporating bulky substituents such as neopentoxy, t-butyl or long alkyl chains into the periphery benzo groups of the phthalocyanine. This peripheral substitution of phthalocyanines increases solubility by sterically blocking the π - π stacking between phthalocyanine rings. However, even in the presence of these bulky groups aggregation is observed at concentrations above 10^{-5} M. Phthalocyanine solubility in water can be achieved by incorporating sulphonate or carboxylate groups into the peripheral benzo groups of the phthalocyanine. Charge repulsion between these groups prevents aggregation of the phthalocyanine rings.⁴

Unfortunately, the aggregation of phthalocyanines at high concentrations and in the solid state can have a significant detrimental effect for applications that are dependent on the spectral properties of the Pc. Aggregation results in the loss of fluorescence and a broadening of the Pc Q-band (see below).

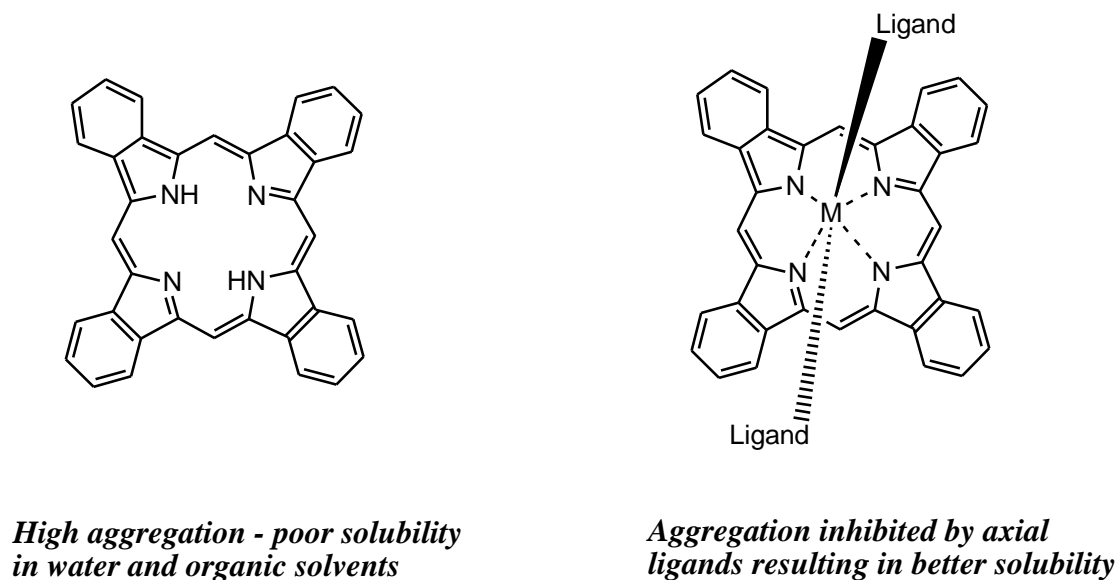
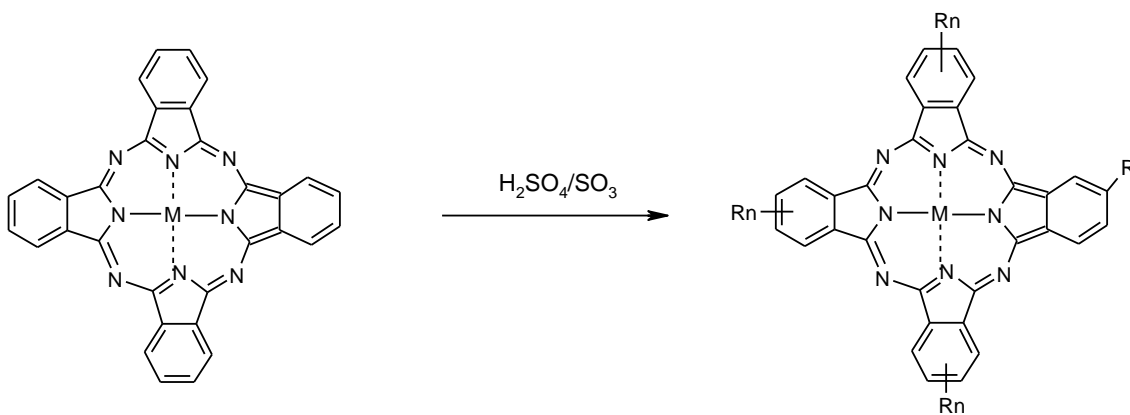


Figure 1. (a) Metal-free phthalocyanine, (b) Axial substituted phthalocyanine.

One approach used to eliminate aggregation is the incorporation of axial ligands that can coordinate with specific metals such as silicon and germanium (figure 1). However, this approach is obviously limited and cannot be applied to other metalated phthalocyanines.⁴

1.1.3 Preparation of substituted phthalocyanines

Perhaps the most direct way of introducing substituents into the periphery benzo groups of a Pc is by direct electrophilic substitution (scheme 4). Substituents such as halogens and sulphonate groups have been introduced in this fashion to yield both phthalocyanine green dye (chlorinated copper Pc) and water soluble phthalocyanines (sulphonated Pc) that are commonly used in inks. However, controlling both the number and positional location of these substituents in the phthalocyanine ring is not possible, and as a result mixtures of products containing 4-16 substituents are typically made. For dye applications this is not a problem since these mixtures give a reproducible colour, however, for more advanced applications, high purity is required, thus controlling both the number and location of substituents within the Pc ring is essential.⁴



Scheme 4. *Electrophilic substitution of PcM.*

To achieve this end it is necessary to prepare the target Pcs from the appropriate substituted phthalonitriles. By using this approach one can control the number and, in some cases, the position of the substituents.

Shown in figure 2 are some commercially available phthalonitriles.

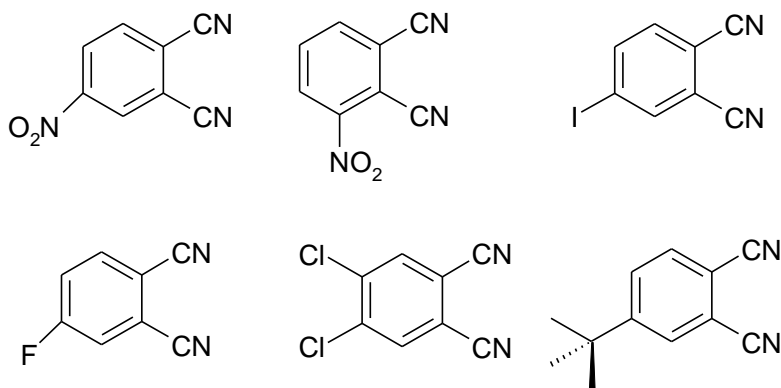
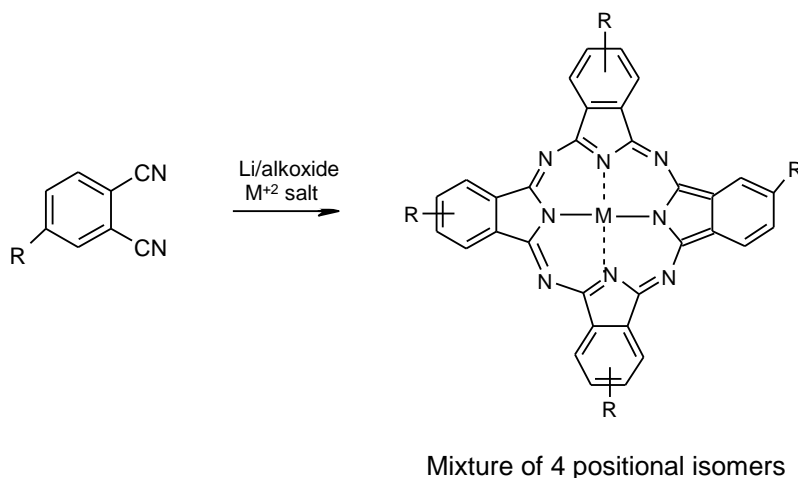


Figure 2. *Commercially available phthalonitriles.*

One key requirement for the substituents introduced into phthalonitrile is that they must be stable to the harsh condensation conditions used to prepare the Pc. This does put a limitation as to the types of functional groups that can be used. However, substituents such as alkoxy, alkyl, alkynyl, alkenyl, and halogen are stable under these conditions and they are the most typically used substituents. Shown in scheme 5 is a typical self-condensation of a substituted phthalonitrile to a tetra substituted Pc. It should be noted that unsymmetrical substituted phthalonitriles (such as 4 or 3-substituted phthalonitrile) will yield mixtures of positional isomers with D_{2h} , D_{4h} , C_{2v} and C_s symmetries; this does give rise to a purity problem. Separation of these isomers has only ever been achieved in a few instances using HPLC. One way of overcoming the positional isomer problem is to cyclise phthalonitriles possessing large substituents (benzyloxy groups) in the 3 position of the phthalonitrile. As a result of steric hindrance only one isomer, the C_s , is made. Unfortunately, this approach is only useful in a limited number of situations.⁶

The most efficient way of overcoming the positional isomer problem is to condense symmetrical phthalonitriles, such as 4,5 or 3,6-substituted phthalonitriles, the resulting Pcs are octasubstituted single isomers.

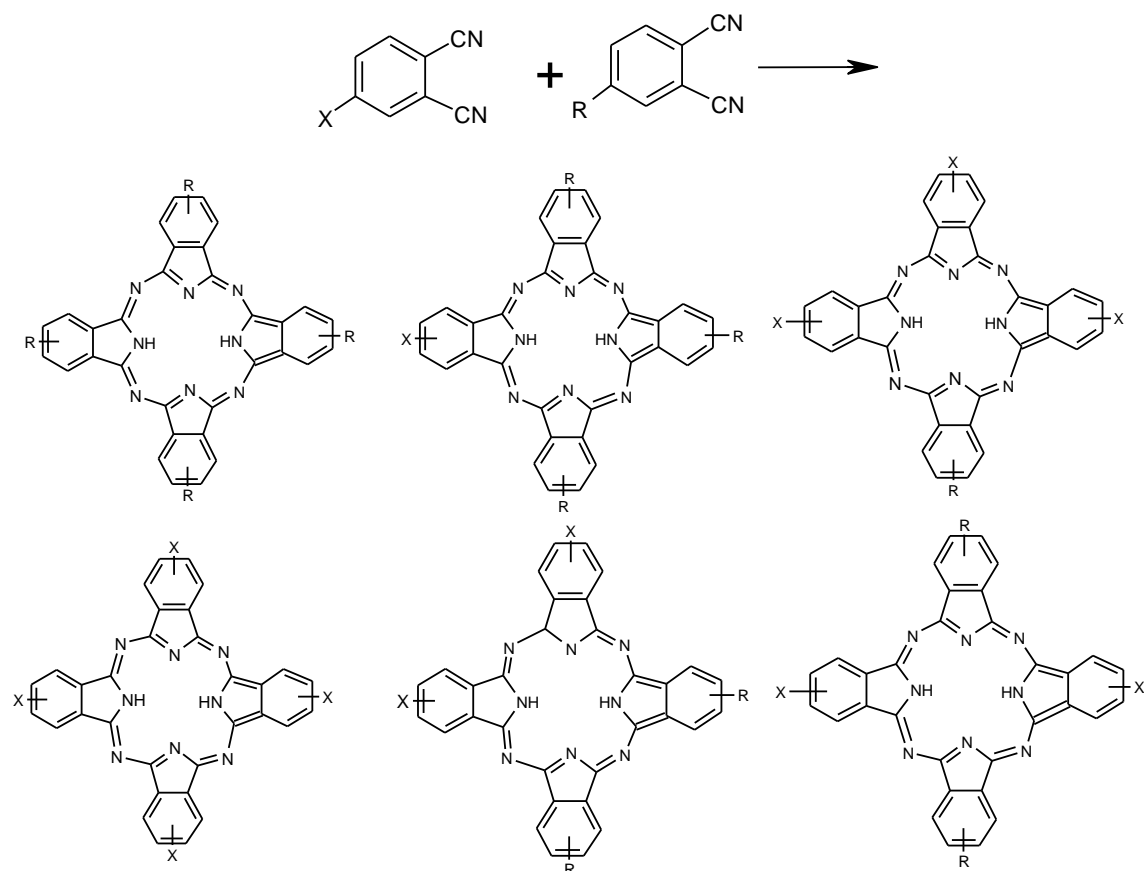


Scheme 5. Self- condensation of a 4-substituted phthalonitrile.

1.1.4 Unsymmetrical substituted phthalocyanines.

Unsymmetrical substituted phthalocyanines have gained much interest over the past three decades, starting with the development of binuclear Pcs for application as solar catalysts for hydrogen generation to the more modern areas of non-linear optics and photodynamic therapy of cancer. Outlined in scheme 6 is a typical methodology for preparing an unsymmetrical Pc, it involves the mixed condensation of two different phthalonitriles (in the example shown the stiochiometry is 1:1). The product obtained is a mixture of six different Pcs (not including positional isomers) however, efficient separation of this mixture is extremely difficult since the Pcs associate very strongly with each other via aggregation.

To enrich one of the Pcs over the rest, specifically PcR_3X_1 , stoichiometric control can be used. Typically a stoichiometry of 4:1 of the R substituted phthalonitrile to the X-substituted phthalonitrile will give an enriched mixture of the target Pc. Unfortunately, purification of this mixture by silica gel chromatography cannot be achieved, there is always trace contamination with by-product Pcs found.⁷ However, it is possible to purify these mixtures using size exclusion chromatography with Biobeads SX-3 (3% crosslinked polystyrene), amounts of up to 50 mg can be purified per run through the column, yielding pure phthalocyanine product.⁸



Scheme 6. Mixed condensation of two different phthalonitriles (stoichiometry of 1:1) and the resultant mixture of phthalocyanines made.

1.1.5 Characterisation – UV-Visible Spectra of phthalocyanines

The intrinsic π -electron conjugated system of phthalocyanines leads to rich and highly informative electronic spectra.⁹ The Q-band arises due to $a_{1u} \rightarrow e_g$, $\pi \rightarrow \pi^*$ transition which gives rise to an orbitally doubly degenerate state 1E_u , in metallated phthalocyanines that have planar symmetry, thus we see one peak for the Q-band between 650 – 750nm (figure 3). In a metal-free phthalocyanine the symmetry is lower (D_{2h}), thus the excited state has two absorptions Q_y and Q_x , and hence two peaks are observed in the absorption spectra. The weaker peaks of the B-band region are vibrational overtones of these and are typically found between 300 – 450nm.^{4,10}

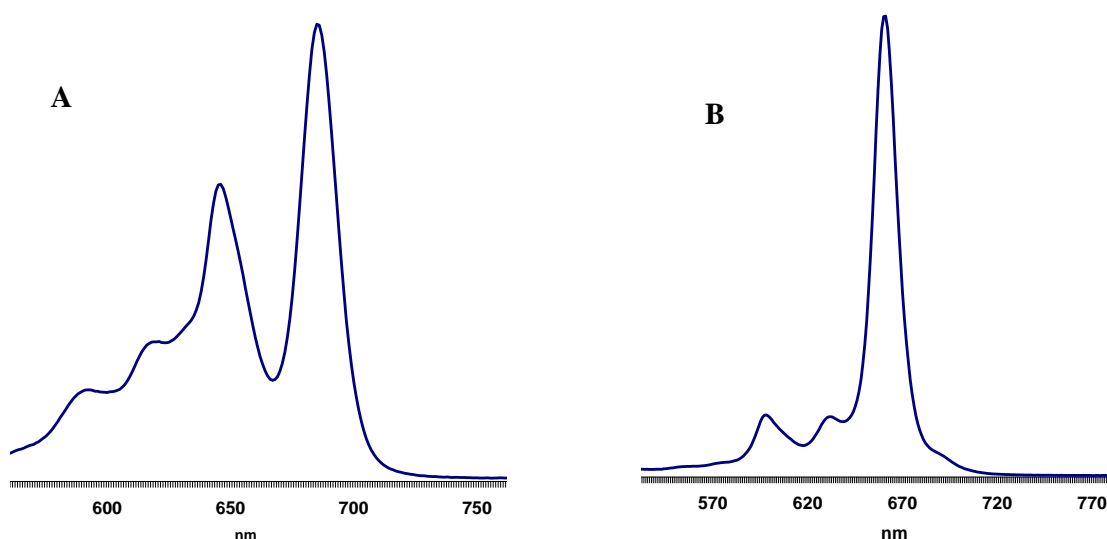


Figure 3. The visible absorption spectra of solutions of (A) metal-free phthalocyanine and (B) a zinc metallated phthalocyanine.¹

The presence and nature of a central metal atom, axial ligation, peripheral substitution, size and symmetry of a phthalocyanine are elements that influence both the absorption maximum and the structure of the Q-band.⁴

The incorporation of electron donating substituents into the 1, 4, 8, 11, 15, 18, 22, 25, positions of the Pc ring, figure 4, increase the energy of the Pc HOMO resulting in a marked red-shift (bathochromic) of the Q-band. Other substituents such as alkynes and benzoannulation will also red-shift the Q-band as a result of extended conjugation.¹¹

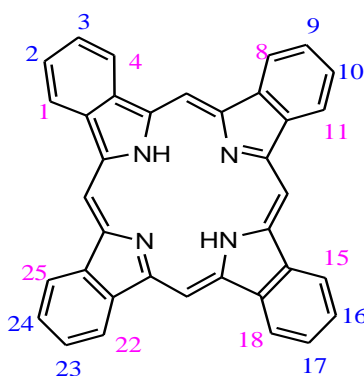


Figure 4. Phthalocyanine showing the peripheral substitution sites in blue and the non-peripheral substitution sites in pink.

When Pc rings aggregate (cofacial or tail-to-tail arrangement) the Q-band becomes broadened and blue shifted to ~ 630 nm, which results from intermolecular exciton coupling thereby raising the energy level of the excited state coupling.

Unsymmetrical substituted metalated Pcs containing substituents, which can perturb the molecular orbital (MO) of the Pc, show uniquely split Q-band absorptions. This effect is demonstrated in figure 5 with a series of unsymmetrical substituted Pcs. Each of these Pcs possess conjugating substituents in different symmetrical substitution patterns. Since these substituents conjugate with the Pc macrocycle they cause a perturbation of the Pc molecular orbital resulting in a change in Q-band structure. This change depends on the symmetry of the substitution pattern. To predict the structure of the Q-Band (ie. Single peak, split peak) one must apply ‘Symmetry-adapted perturbation theory’ (SAPT).¹²

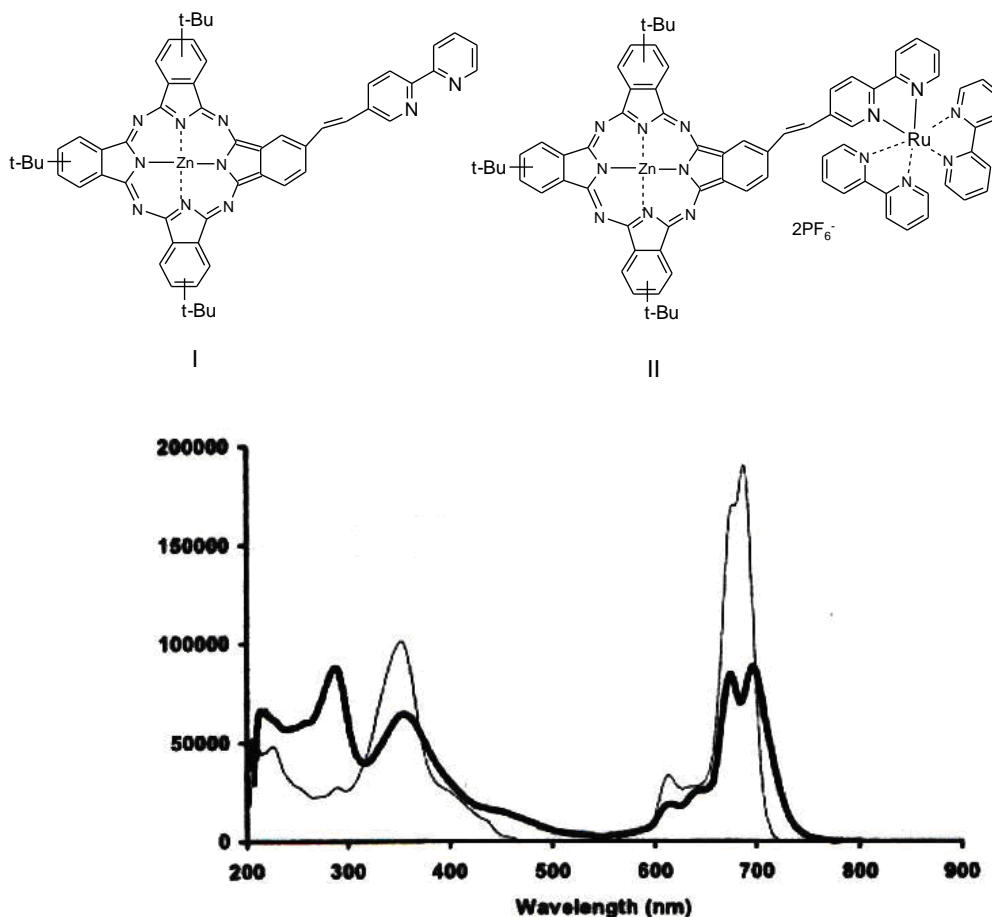


Figure 5. Unsymmetrical substituted Pc's I and II, the UV/Vis spectra of Pc I (thin line), Pc II (solid thick line).

The Hamiltonian of a Pc can be described by a simple equation 1.1.¹²

$$H = H^{(0)} + V \quad (\text{equ 1.1})$$

$H^{(0)}$ is the unperturbed Pc core Hamiltonian, unsubstituted Pc core of D_{4h} symmetry. V is the perturbation caused by the substituents. The V term is a sum of perturbations which span an irreducible representation for D_{4h} symmetry. (Equation 1.2)

$$V = V_{\Gamma 1} + V_{\Gamma 2} + \dots \quad (\text{equ 1.2})$$

$V_{\Gamma 1}, V_{\Gamma 2}, \dots$ are the corresponding symmetry-adapted perturbations.

The V term can be described by symmetry elements: A_{1g} ; B_{1g} ; $E_{u,y}$ and B_{2g} symmetry elements. If the symmetry elements B_{1g} and B_{2g} are present then a perturbation of the Pc molecular orbital will occur resulting in a splitting of the Q-band. All of this can be simplified into a straight forward graphic shown in figure 6. Based on the graphical analysis for Pc II the symmetry elements B_{2g} and B_{1g} are present (their contribution does not equal zero) and as a result a splitting of the Q-band is observed.

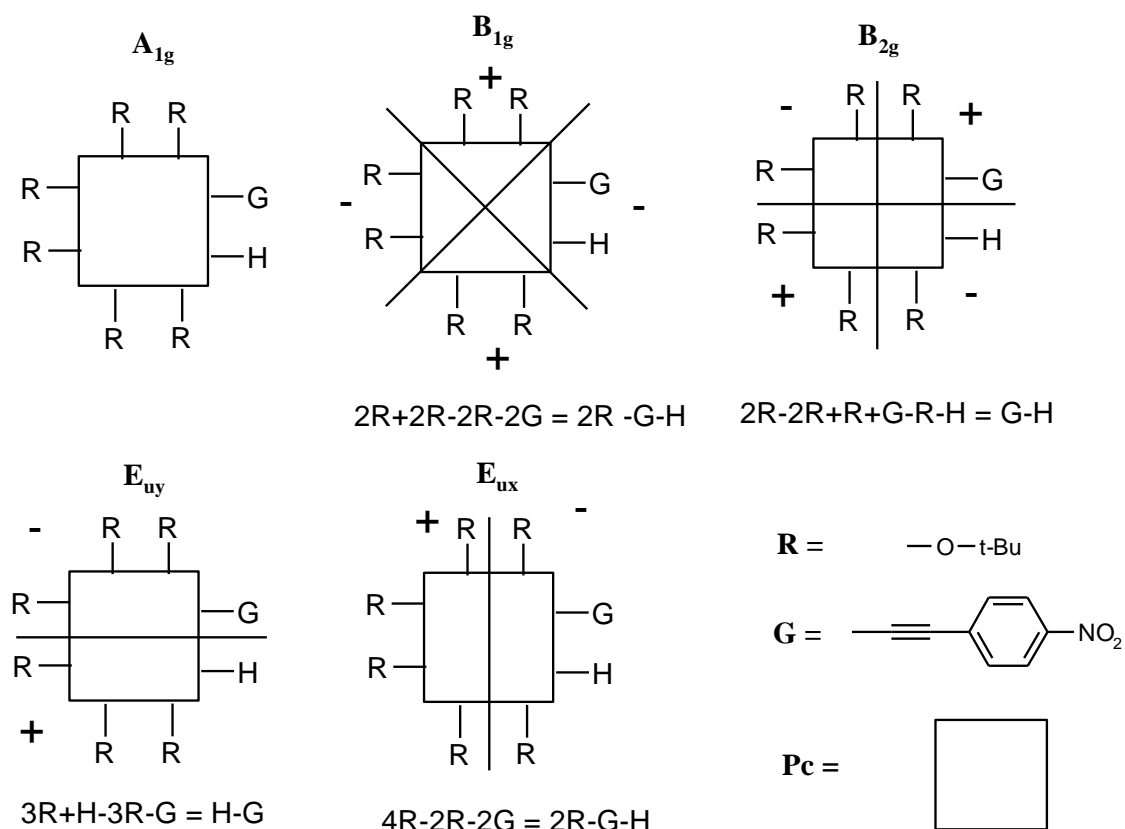
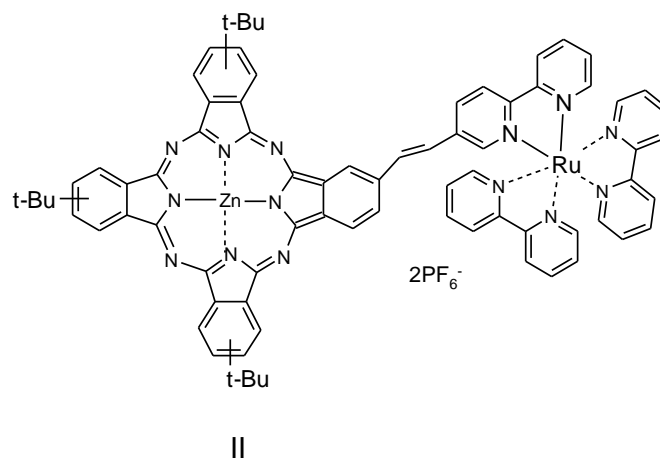


Figure 6. SAPT analysis of a *Cs* symmetry *Pc* II.

The IR spectral characteristics of metal-free phthalocyanines show N-H stretching from 3090 – 3290 cm^{-1} , N-H deformations between 1005 – 1025 cm^{-1} and 720 – 750 cm^{-1} , and C-H stretches appear *c.* 3000 cm^{-1} . Error! Bookmark not defined.

The extinction coefficient of an intense Q-band would generally be $2 \times 10^5 \text{ cm}^2 \text{ mol}^{-1}$ for a phthalocyanine.¹⁻⁵

1.2 Calixarenes.

Calixarenes are basket shaped compounds, composed of repeating phenyl rings with methylene spacers between each ring. They are typically prepared by the condensation of para-substituted phenol with formaldehyde¹³. Calixarenes most commonly reported in the literature are tetramers (composed of four repeating phenolic units and referred to as calix[4]arenes). These calix[4]arenes can assume four different conformations¹⁴: cone, where the four hydroxy groups are positioned on the lower rim of the molecule; partial cone, where one of the phenolic units flips 180° so that its hydroxy groups is positioned on the upper rim; 1,2-alternate, where two adjacent phenolic units flip 180° or 1,3-alternate where two opposite phenolic units flip 180° (see figure 7).

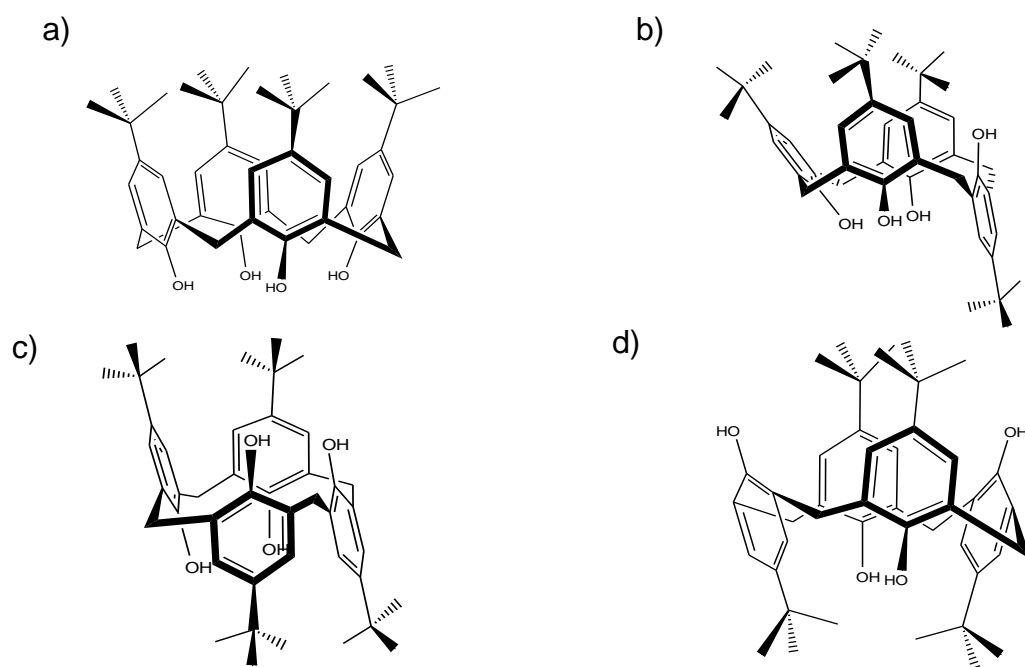


Figure 7. The four conformations of calix[4]arenes, (a) cone, (b) partial cone, (c) 1,2 alternate, (d) 1,3 alternate.

To lock the calixarene into the cone conformation bulky tert-butyl groups are introduced into each of the phenol units at the upper rim, effectively maintaining the basket shape of the tetramer.¹⁵

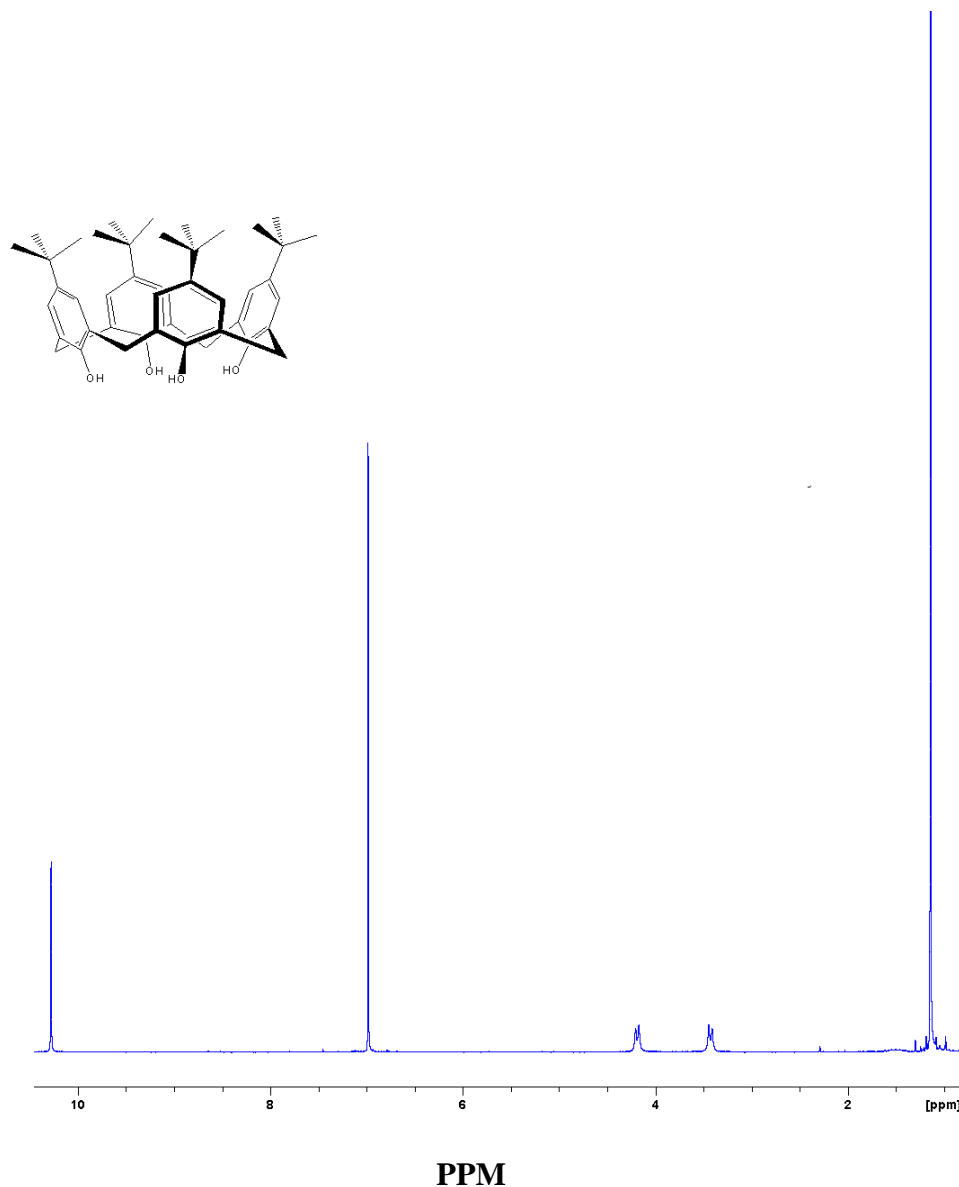


Figure 8. ¹H NMR of *p*-tert-butyl-calix[4]arene.¹⁶

Each conformation of the calix[4]arene has its own unique ¹H NMR spectrum. In the spectrum of the cone conformation of *p*-tert-butylcalix[4]arene the hydroxy protons are found at approximately 10.3 ppm, the aromatic protons appear just above 7 ppm, the methylene bridge protons, are present as doublets at 3.48 ppm and 4.24 ppm (J^2 coupling) and the tert-butyl protons are at 1.2 ppm, as illustrated in figure 8 above. The spectrum of each of the other conformations differ from that of the cone and are most easily elucidated by their methylene bridge features as outlined in table 1.

Conformation	Variation in ^1H NMR Peaks
Cone	One pair of doublets
Partial Cone	Two pairs of doublets of equal intensity and the possibility of a singlet of the same intensity.
1,3-Alternate	One singlet
1,2-Alternate	One singlet and two doublets of equal intensity

Table 1. ^1H NMR patterns for the CH_2 bridging protons in the various conformations of calix[4]arene¹⁶.

Calixarenes and their analogues have been exploited in many areas including, host-guest chemistry, enzyme mimics¹⁷, chiral recognition and optical sensors.¹⁸

1.2.1 Substitution Reactions of Calixarenes.

The selective chemical modification of calixarenes can be achieved making them excellent precursor compounds for more complex molecular assemblies.¹⁹

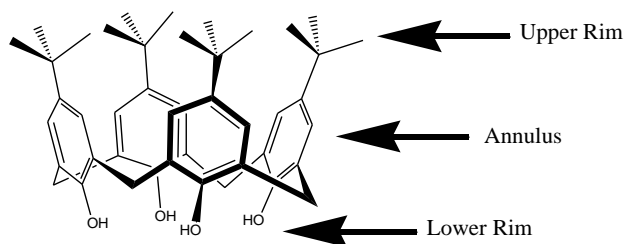


Figure 9. Calixarene segments .

Calixarenes can be readily functionalised via substitution at either the upper rim or lower rim. Substitution at the upper rim, as shown in figure 10, can be used to introduce water solubility if desired.

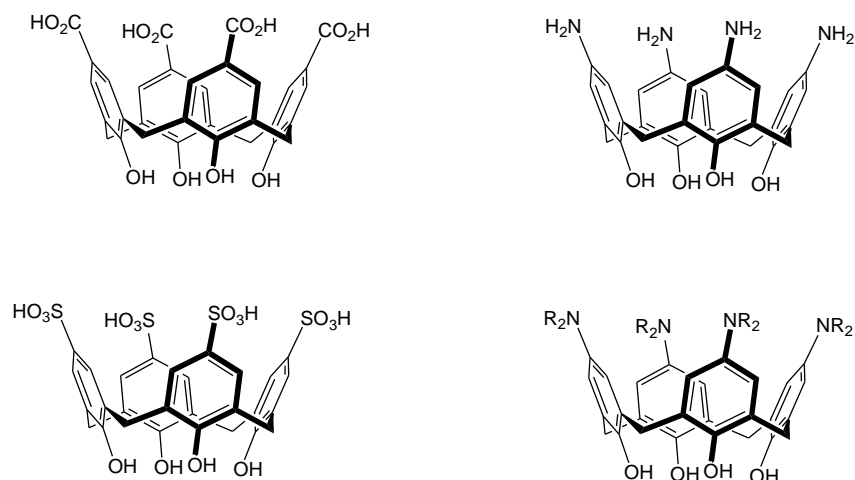


Figure 10. Upper rim functionalised calixarenes.

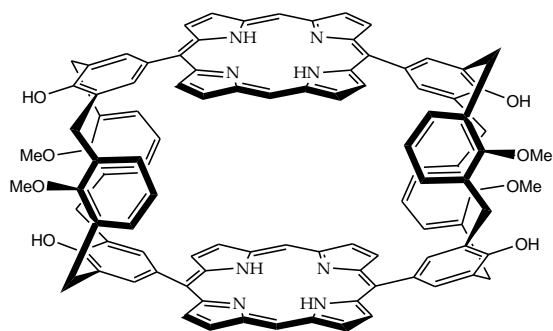
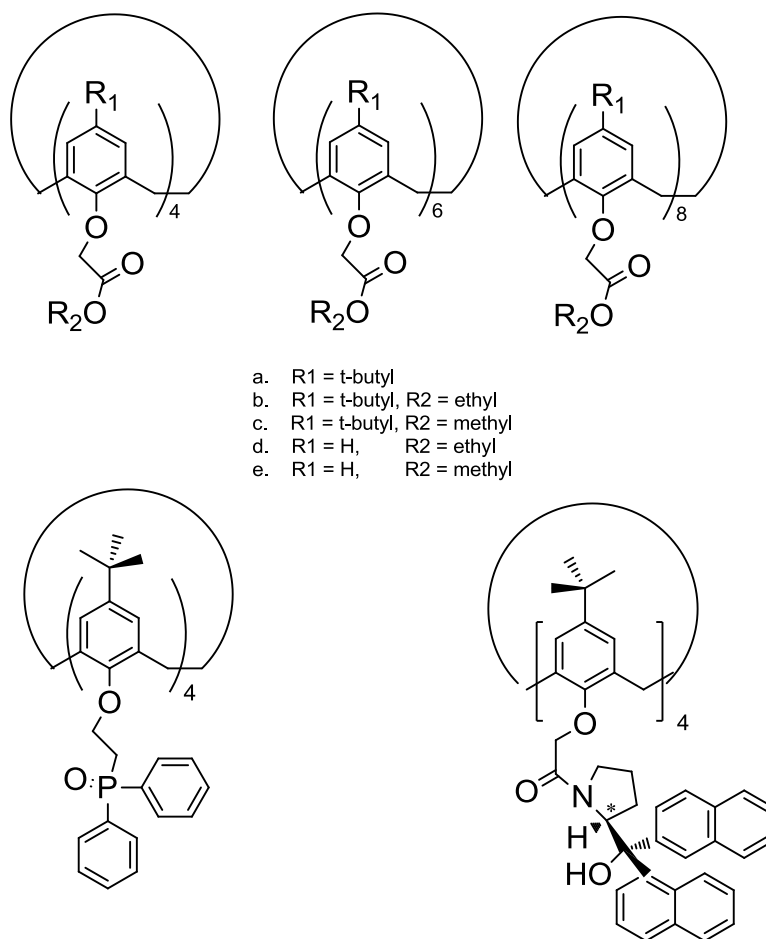


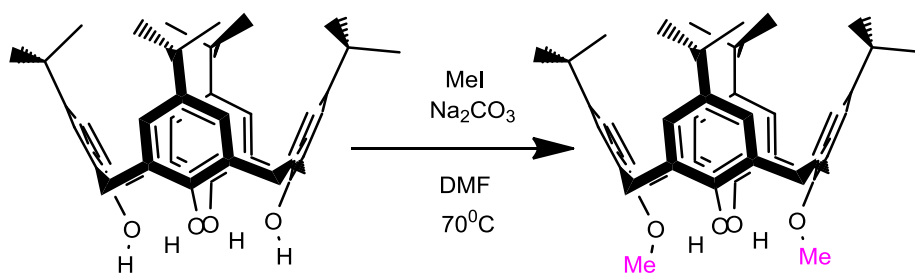
Figure 11. Example of upper rim substitution of a calixarene and the complex structures that can be formed. This is an example of a cofacial bis-porphyrin calixarene.¹⁹

Upper rim substituents that have been employed in the past include acids, tert-butyl groups, amines and porphyrins and have in some cases led to complex and interesting molecular assemblies, figure 11.



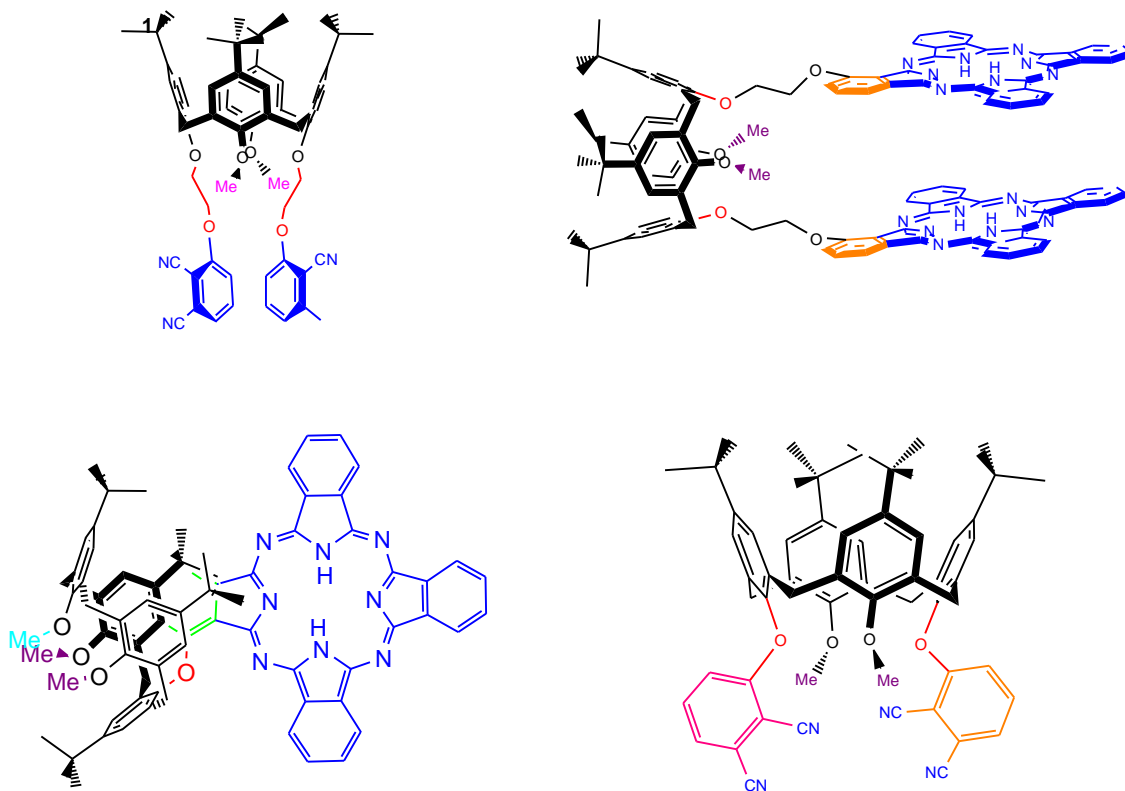
Scheme 7. Some examples of lower rim substituted calix[n]arenes.¹⁸

Lower rim functionalisation has been carried out most commonly with acetates, amides, polyethylene glycol, alkyl groups and benzyl groups (scheme 7). Methods of lower rim modification are most frequently achieved by refluxing calix[4]arene, an appropriate alkylating agent and a base in DMF or acetone. The choice of base (potassium carbonate, sodium carbonate, sodium hydride) will affect both the degree and pattern of substitution. Interestingly, weaker bases with small counter cations give partial substitution of a 1,3-distal pattern (K_2CO_3 , Na_2CO_3) and stronger bases such as NaH (in the presence of an excess of alkylating agent) results in full substitution on the lower rim of the calixarene. Shown in Scheme 7 are selected examples of lower rim functionalised derivatives. The ester derivatives have found application in sodium selective sensors, the phosphine oxide derivatives have excellent potential as nuclear waste extractants.



Scheme 8. *The preparation of a distal 1,3- dimethoxycalix[4]arene.*

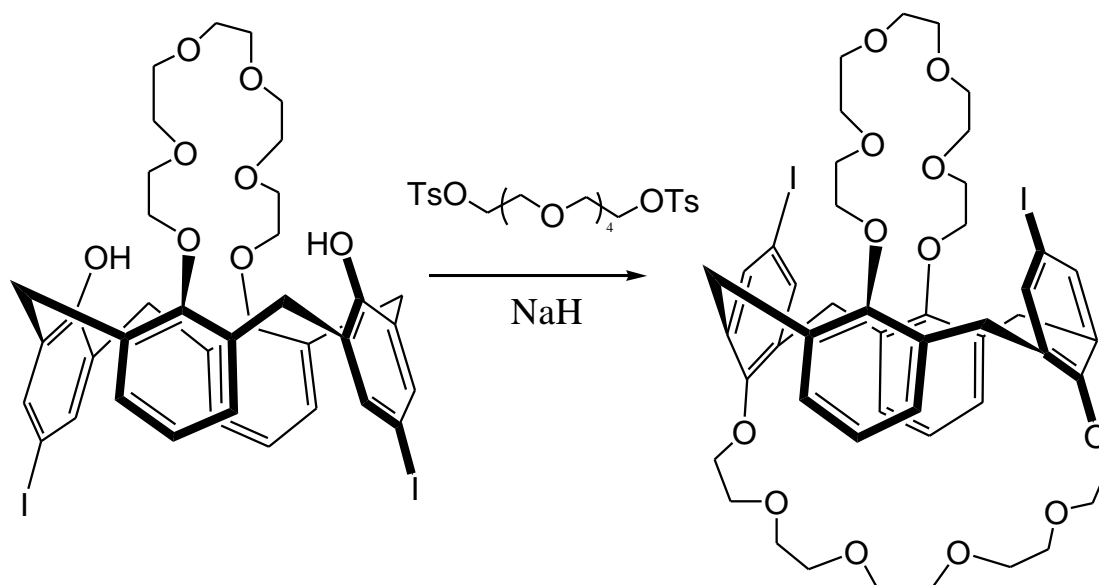
The ability to be able to prepare partial-lower-rim substituted calixarenes such as the distal-1,3-dimethoxycalix[4]arene, scheme 8, give rise to the possibility of creating compounds that are more functionally diverse some recent examples of which are shown in scheme 9.²⁰



Scheme 9. *Examples of novel derivatives that can be prepared from distal-1,3-dimethoxycalix[4]arene.*²⁰

Outlined in Scheme 10 is the preparation of a novel distal substituted calix[4]arene that can be used in the extraction of nuclear waste. The preparation of this novel compound

involves the use of a weak base to achieve partial alkylation of p-tert-butylphenolcalix[4]arene, scheme 10, thus avoiding substitution at all four hydroxy positions. To fully substitute the calix[4]arene a strong base such as NaH in an aprotic solvent with a large excess of alkyl halide would be required.²¹ The distal-1,3-dimethoxycalix[4]arene is of interest in this project as it is a required precursor in the synthesis of our o-arylated calixarenes.



Scheme 10. Preparation of a novel 1,3-distal calixarene used in nuclear waste extraction.

The reason the distal di-substituted calix[4]arene is obtained when using a weak base instead of the completely alkylated calix[4]arene is because the two remaining 2,4-OH groups are stabilised by H-bonding making them less acidic. It is broadly acknowledged that in lower rim oppositely substituted calixarenes in the cone conformation, the hydroxy groups are involved in H-bonding (figure 12).²²

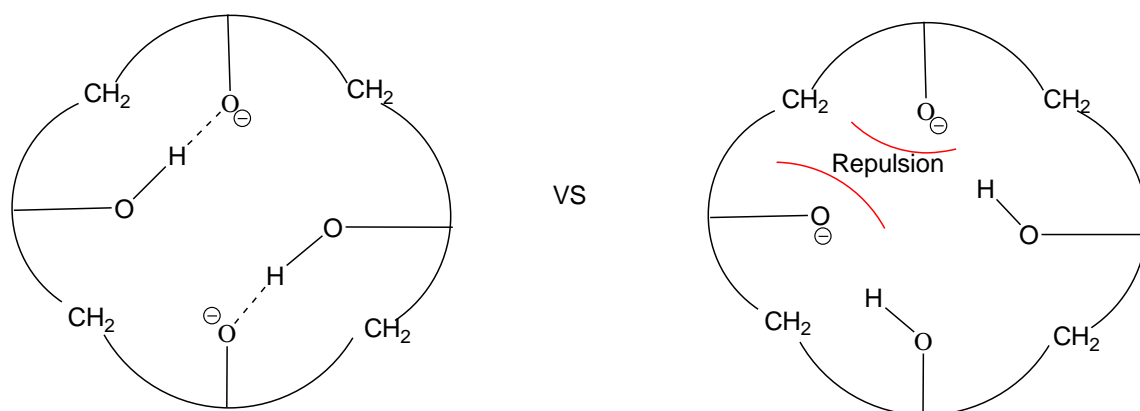
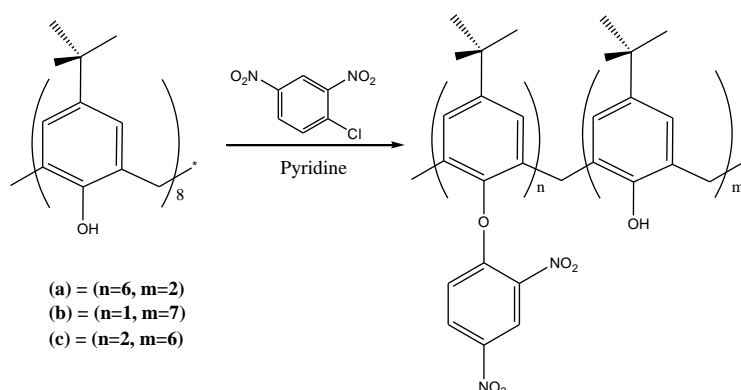


Figure 12. *Hydrogen bond stabilisation of 1,3-disubstituted charged calix[4]arene intermediate vs the 1,2-disubstituted intermediate.*

The use of different alkylating agents to prepare lower rim modifications to the calixarenes can give rise to conformationally different products. Thus, there not only exists the possibility of controlling the substitution pattern on the lower rim of calix[4]arene, but in some cases one can also control the resulting conformation of the calixarene. It should be noted that the resulting conformations shown in scheme 10 can be locked if the substituents on both the upper and lower rim are large enough to prevent rotation of the calixarene phenolic units through the annulus of the calixarene. It has been found that the introduction of alkyl groups, longer than propyl, immobilises the calixarene conformations.

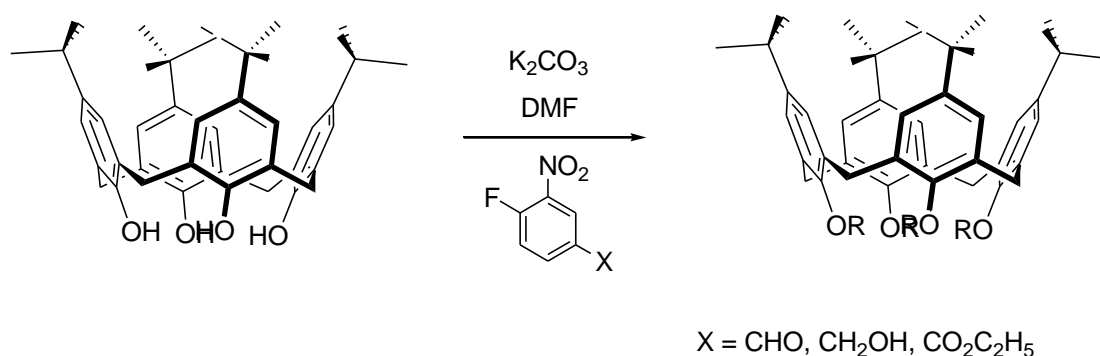
1.2.2 O-arylation of calix[4]arenes

The archives of o-aryl ether calixarenes to date have been sparse, with only one publication prior to 2001 by C David Gutsche in 1979. Gutsche prepared mono-, di- and hexa-2,4-dinitrophenyl ether derivatives of tert-butyl calix[8]arene. These were prepared from p-tert-butylphenol and formaldehyde in the presence of a base to procure calix[8]arene which was then treated with 2,4-dinitrochlorobenzene to obtain the ether derivative, (scheme 11).²²



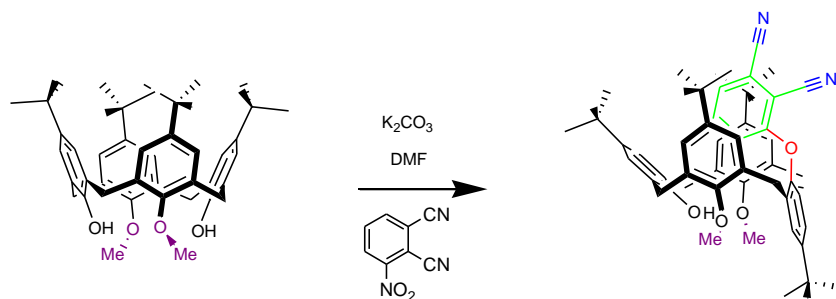
Scheme 11. Preparation of the first partial substituted *o*-arylated calixarenes.²³

Following on from Gutsche, Paris E. Georghiou prepared the first lower rim substituted aryl ethers of *p*-tert-butylcalix[4]arene. Georghiou achieved this through a nucleophilic aromatic substitution reaction of 4-fluoro-3-nitrobenzaldehyde with K_2CO_3 in DMF. Varying the concentration of 4-fluoro-3-nitrobenzaldehyde from two equivalents to an excess, produced mono-, di- and tri-aryl ether products respectively (scheme 12).²³



Scheme 12. Synthesis of *o*-aryl ether calix[4]arene.²³

Finally in 2007 the Nolan group prepared both the di-methoxy and tri-methoxy analogues of *p*-tert-butyl-(dicyanobenzene)calix[4]arene.²⁰ This was accomplished using methyl iodide and K_2CO_3 in DMF to prepare the distal-1,3-di-methoxycalix[4]arene, which was further reacted with nitrodicyanobenzene in K_2CO_3 in DMF producing the *o*-aryl ether calixarene. Further methylation generates the trimethoxy analogue, scheme 13.



Scheme 13. Preparation of self filled partial cone 5,11,17,23,-tetra-*t*-butyl-26,27,28-trimethoxy-25-(-3-[1,2-dicyanobenzene])calix[4]arene.²⁰

Upper rim substituted calix[4]arenes are of significance to this project, and the first ever example is the partial cone 5,11,17,23-tetra-*tert*-butyl-26,27,28-trimethoxy-25-(-3-[1,2-dicyanobenzene])calix[4]arene. These compounds contain an aromatic ring in the upper annulus of the calix[4]arene, they are quite inflexible to conformational change and they have been proven by NMR temperature studies to be conformationally stable to over 140°C (figure 13).

The ¹H NMR spectrum of the partial cone calix[4]arene, (figure 13), reveals the methylene bridge protons are relatively unchanged when compared with the signals of *p*-*tert*-butyl calix[4]arene in figure 8. The additional peaks of the phthalonitrile substituent are found upfield at 3.5 ppm (triplet), 4.5 ppm (doublet) and 6.4 ppm (doublet). The protons of the phthalonitrile group are shifted up-field from their typical values of 7-8 ppm as a result of anisotropic shielding caused by the aromatic annulus of the calix[4]arene (see table 2).²⁰

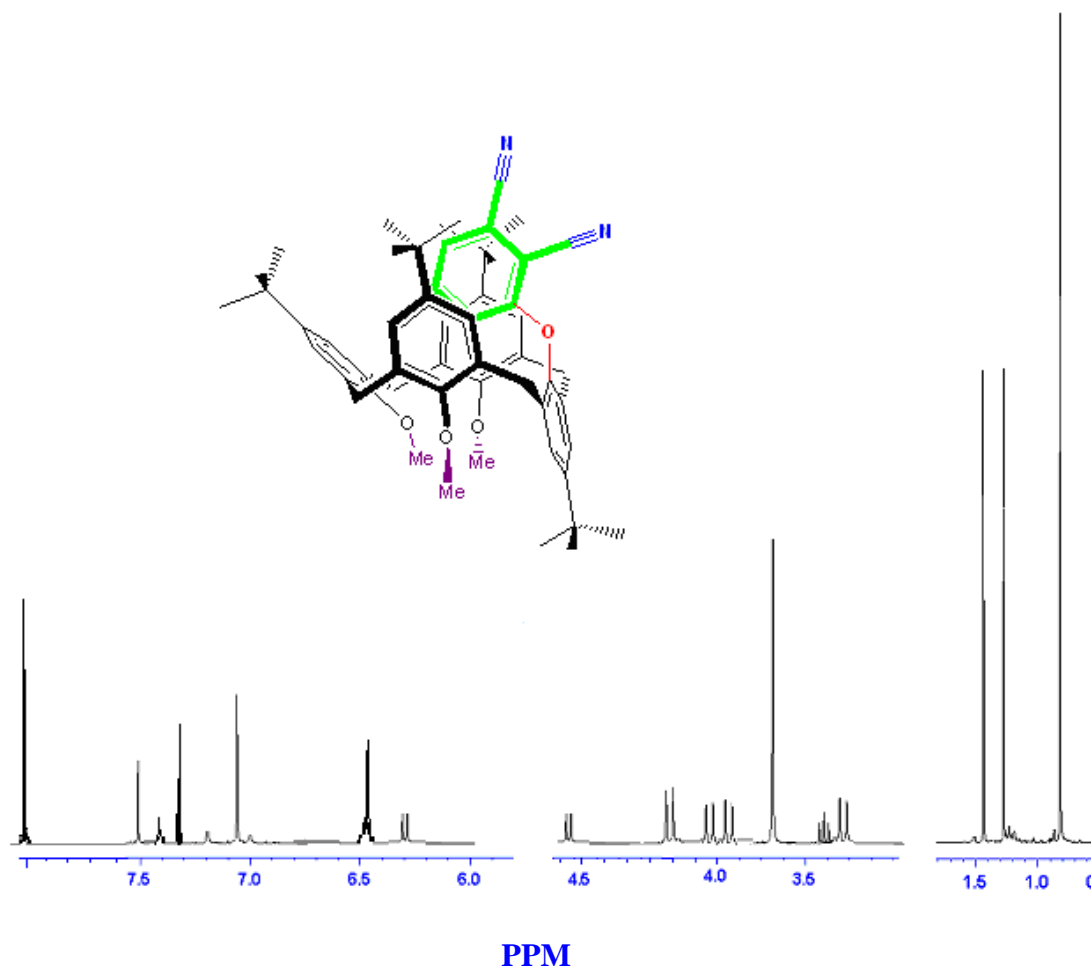


Figure 13. ^1H NMR of the partial cone 5,11,17,23-tetra-tert-butyl-26,27,28-trimethoxy-25-(-3-[-1,2-dicyanobenzene])calix[4]arene.

	Distal Di-methoxy Calixa[4]rene	Partial Cone Calix[4]arene Inclusionary Complex
t-butyl	2 singlets (ratio 1:1)	3 singlets (ratio 1:1:2)
Methylene bridge	Pair of doublets (ratio 1:1)	2 pairs of doublets (ratio 1:1)
Aromatic	2 signals	4 signals

Table 2. Comparison of the ^1H NMR spectra of distal-di-methoxy calixa[4]rene and the partial cone calix[4]arene.

As mentioned earlier, the partial cone conformation is temperature stable to at least 140°C as determined by temperature ^1H NMR, figure 14. One point of note in the

temperature study is that the triplet representing the aromatic phthalonitrile H5 proton is shifted slightly downfield with an increase in temperature, this is thought to be due to the slight movement of the aryl substituent within the calixarene upper rim.

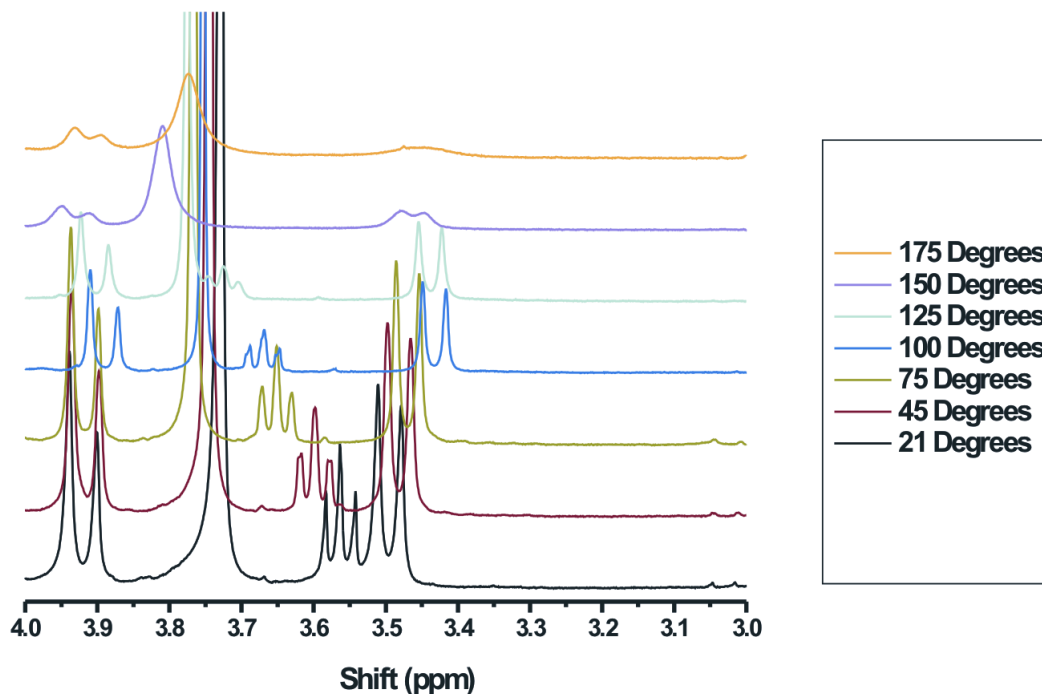


Figure 14. ^1H NMR temperature study of the self filled partial cone 5,11,17,23-tetra-*tert*-butyl-27-hydroxy-26,28-dimethoxy-25-(-3-[-1,2-dicyanobenzene])calix[4]arene.²⁰

1.3 Brief history of Photodynamic Therapy (PDT).

At the beginning of the last century the concept of cell death by an interaction between light and chemicals was discovered by Oscar Rabb, a medical student of von Tappeiner. He serendipitously observed the toxic and deadly outcome of administering acridine red while light was being directed at paramecium *Infusora* during a thunderstorm. Concurrently J. Prime described photosensitivity in an epileptic patient who displayed a reaction to eosin when exposed to the sun and consequently Tappeiner and Jesionek carried out one of the first treatments of skin tumours with white light and eosin, reported

in 1903. Following on from Rabb's innovative breakthrough, in 1907 von Tappeiner and Jodlbauer established that oxygen was fundamental in the cell death process.²⁴

There are two phototherapeutic approaches established for cancer treatment, phototherapy and photochemotherapy. Phototherapy occurs when the application of light to cancerous tissue causes cell death via molecules already present in the cancerous tissue. Photochemotherapy involves the administration of a photosensitizing agent that selectively accumulates in cancerous tissue and becomes activated upon irradiation with light of an appropriate wavelength to cause cell death.^{24,25}

Photodynamic therapy (PDT) requires a photosensitizer, light and oxygen. The photosensitizer is administered and preferentially builds up in cancerous tissue; this is followed with the application of light of the appropriate wavelength (620 nm – 750 nm) thus initiating the photodynamic process. Following light absorption, energy is transferred to molecular oxygen, which yields reactive singlet oxygen as outlined in figure 15 that is capable of causing direct cell death, vascular shutdown and local damage via inflammatory and immune mediators. Two well known PDT photosensitizers in use are the first generation drug Photofrin® and the second generation drug Verteporfin.^{26,27}

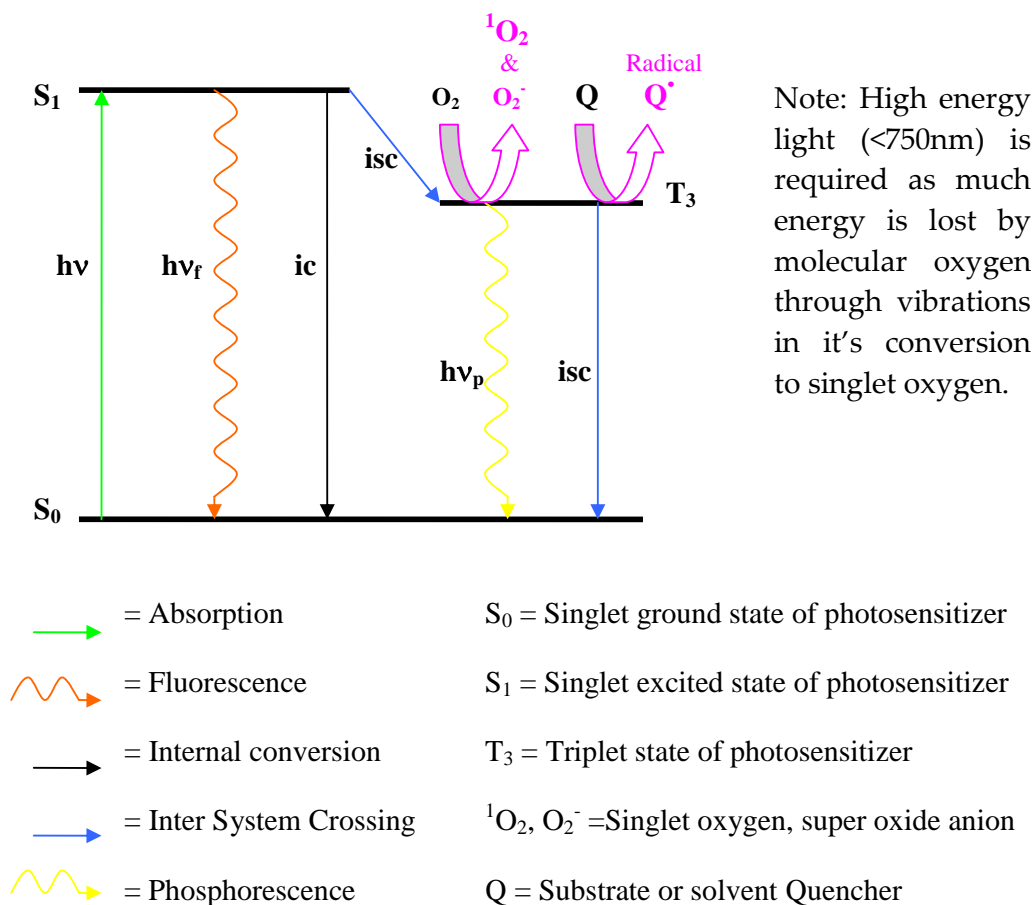


Figure 15. Mechanism of photosensitized singlet oxygen production.^{24,25}

In figure 15, light is applied to the photosensitizer, causing it to become excited from its ground state to its singlet excited state ($S_0 \rightarrow S_1$). The excited photosensitizer can then decay via fluorescence or internal conversion, or it can undergo intersystem crossing (electron spin conversion) to the long lived reactive triplet state ($S_1 \rightarrow T_3$). At this point the triplet state photosensitizer can be quenched via oxygen or substrate to yield the oxygenated products. The generation of singlet oxygen and super oxide anions are the predominant mechanism of cell death and are known as type II reactions. Type I occurs via quenching by solvent or substrate molecules, which yield the corresponding radicals. The cellular targets for PDT are the mitochondria, lysosomes, cell nucleus and cellular membrane.^{27,28,29}

However there are many problems with the current PDT medications on the market. There are many factors to consider in the development of an effective photosensitizer which are listed below:

- 1) The photosensitizer must absorb light in the region 600-750nm with extinction coefficients greater than 100,000 and it should have high quantum yields for the generation of $^1\text{O}_2$,
- 2) The photosensitiser needs to be photostable (i.e not break down or degrade in any way under the application of light) and stable to $^1\text{O}_2$, O_2^- and the radicals or oxygenated species produced *in situ*,
- 3) The photosensitiser must show no “dark toxicity” prior to application of light and before the drug has reached its target, it should be site specific (selectively target cancer tissue),
- 4) The photosensitizer should be a single pure compound (i.e. not a mixture of photosensitizer compounds as is the case with photofrin that is presently used clinically),
- 5) The photosentiser should be easily and quickly expelled from the body after the treatment is finished.

The original PDT sensitizer that came to market in the 1990's, Photofrin[®], had many problems, from impurity and inappropriate absorption wavelength to low extinction coefficient and prolonged lingering of the drug in the body.³⁰ Verteporfin, a second generation photosensitiser, has significantly improved properties with respect to improved extinction coefficient, absorption outside of the biological window and an efficient clearance from the body. However, there are other ways in which PDT photosensitizers can be advanced and progressed. For example, an increase in the potency of the photosensitizer can be achieved through by combining the photosensitiser with other known cancer killers such as NO and 5-fluorouracil.

1.4 Nitric Oxide

Nitric oxide (NO) plays an important role as a cell-signalling molecule, anti-infective agent and as an antioxidant. Also it is well established that nitric oxide is formed as an immune response to pathogen invasion in mammalian biology.³¹ Thus, it is no surprise that NO donating complexes are being investigated for medicinal purposes, with specific applications for the treatment of vascular disorders, cancer and Alzheimer's disease.³² In addition to this, NO has displayed an ability for sensitisation of hypoxic cells to ionizing radiation³³ which has the potential to augment cell death in PDT. Therefore it follows that the development of a PDT sensitizer that can release nitric oxide in cancerous tissue would create a more potent anti-cancer drug. A porphyrin complex that generates nitric oxide upon irradiation has been synthesised and investigated.³⁴

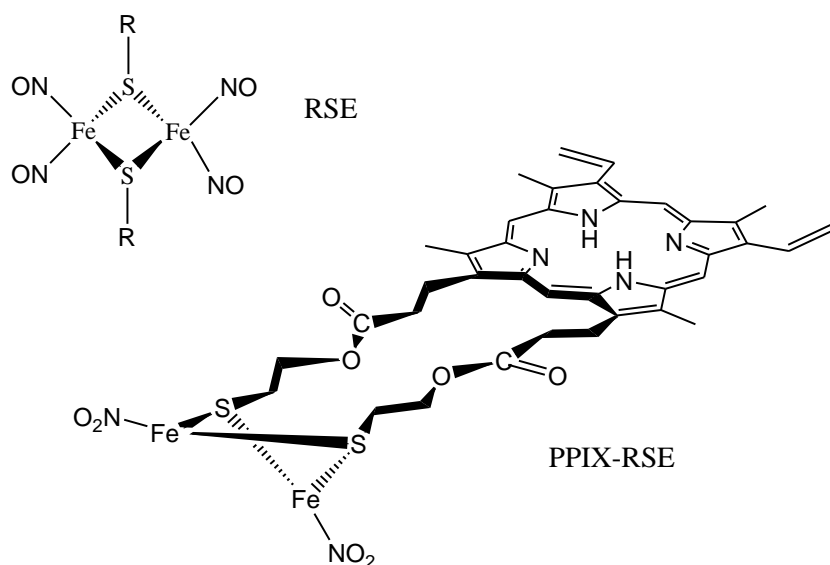


Figure 16. Roussin's Red Salt ester (RSE) and Protoporphyrin IX RSE (PPIX-RSE), which have shown promise as photochemical NO precursors.³⁴

Roussin's red salt (RSE) releases NO upon radiation at 365 nm, however since this wavelength is not suitable for use in PDT of mammalian cells, the protoporphyrin IX chromophore which has higher absorbance at longer wavelengths was investigated (figure 16). It was found that PPIX-RSE is efficient at absorbing light and transferring energy from the porphyrin to the RSE cluster, and thus ultimately leads to the NO

release.³⁴ This example of an NO releasing PDT agent illustrates that such systems are viable prodrug targets.

1.4.2 5-Fluorouracil

5-fluorouracil (5-FU) (figure 17), a fluorinated pyrimidine, has been broadly used in the treatment of cancerous tumours including but not limited to pancreatic, lung, esophageal and gastric cancer.^{35,36,37,38,39} 5-FU kills cancer through the following three actions:

1. DNA synthesis inhibition via thymidylate synthase, which yields 5-fluorodeoxyuracil monophosphate.
2. Integration into DNA as 5-fluorodeoxyuracil triphosphate.
3. Integration into RNA as 5-fluorodeoxyuracil triphosphate.

However, 5-FU brings with it undesirable and non-discriminate toxicity to healthy cells. Therefore many studies and much research have been carried out to develop more site specific methods of delivering this drug to its specific site of action including prodrug development, thus avoiding the healthy cells on its path. In addition work has been done to improve its cytotoxicity to cancerous cells through investigation of more efficient and effective derivatives.

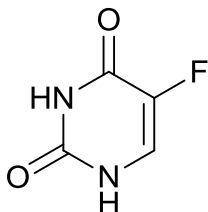
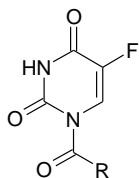


Figure 17. *5-Fluorouracil.*

5-FU prodrugs can be divided into two categories, nucleoside prodrug derivatives and base prodrug derivatives. As base derivative prodrugs are of most relevancy to this project, I will not be discussing the nucleotide class. The base derivatives are most commonly formed through modification at the ¹N position⁴⁰, as this is the more reactive nitrogen synthetically, however alterations at the ³N position have also been reported.²⁸

Improvements in cytotoxicity to cancerous cells and/or reduction in toxicity to healthy cells have been achieved for derivatives with some of the following alterations:

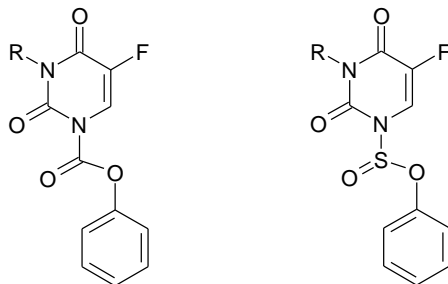
1. Incorporation of a carbonyl group at the ^1N position, as illustrated in figure 18.⁴¹



R = Alkyl or phenyl

Figure 18. Base Derivatives of 5-FU.

2. Introduction of alkoxybenzyl or alkoxyphenylsulfate substituents, again at the ^1N shown in figure 19.⁴²



R = Alkyl

Figure 19. 5-FU derivatives.

3. Various other derivatives with both open and closed chains added with a 2-oxogroup, shown below in figure 20, have been explored, and as reported by Mori *et al*, the presence of an oxygen in this arrangement aids the release of 5-FU from the prodrug structure through easier dissociation of the C- ^1N bond, thus yielding high anti-tumour activity (47-96%).⁴³

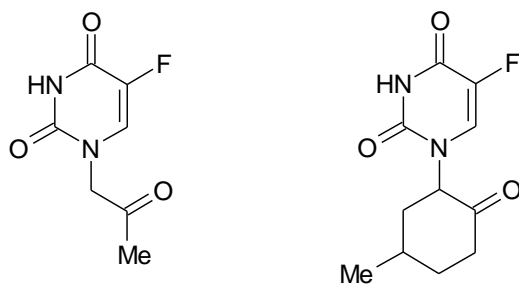


Figure 20. *5-FU-2-coy open chain derivative open chain and 5-Fluorouracil -1-(2'-oxocyclalkyl)uracil derivative.*

4. Nitrobenzyl compounds have shown toxicity to hypoxic cells. Hypoxic cells are often found in cancerous tissue, they occur as a result of a) cells being adequately distant from the nearest blood capillary for long periods of time, b) temporary shutdown of blood vessels causing temporary hypoxia in cells. Essentially hypoxic cells lack oxygen. Thus the inclusion of nitrobenzyl groups into 5-FU (figure 21) creates a bioprecursor prodrug that is activated in hypoxic tissue by reductases to yield two cytotoxic moieties; qiononemine methides and 5-FU a very potent mixture.^{44,45}

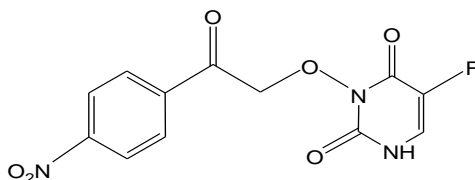
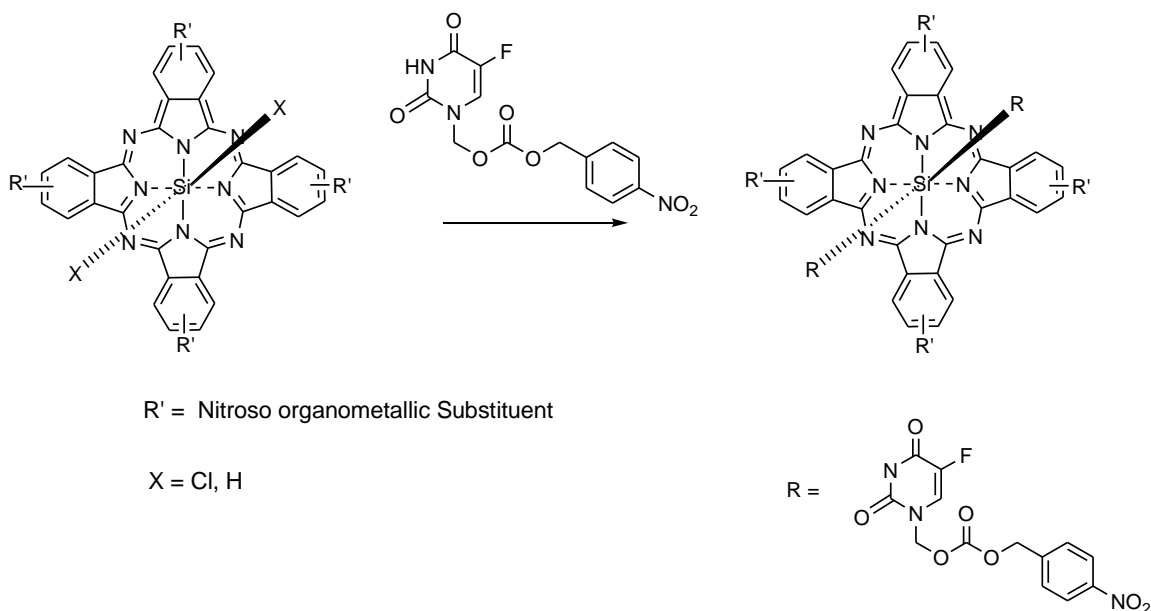


Figure 21. *Prodrug – 3-(p-nitrobenzyloxycarbonyl)-5-fluorouracil.*

1.5 Project Proposal

The principal aim of this work is to design a new anticancer prodrug system that is selective for tumor tissue and which gives reduced cytotoxicity to normal cells. The proposed design involves a novel modification of the established anticancer drug 5-flourouracil, to create a new prodrug. Outlined in Scheme 14 is the proposed strategy. This strategy involves the conjugation of 5-flourouracil to both a *bioprecursor* and to a *carrier linker*. The *bioprecursor* is a nitro aromatic compound that on enzymatic reduction is converted to the cytotoxic quinomethane and releases the fluorouracil (see scheme 15).⁴⁴ The *carrier linker* is a photosensitizer such as a phthalocyanine or porphyrin, which will selectively localize the entire prodrug system toward cancerous tissue and can also be used as a light therapeutic agent if desired.

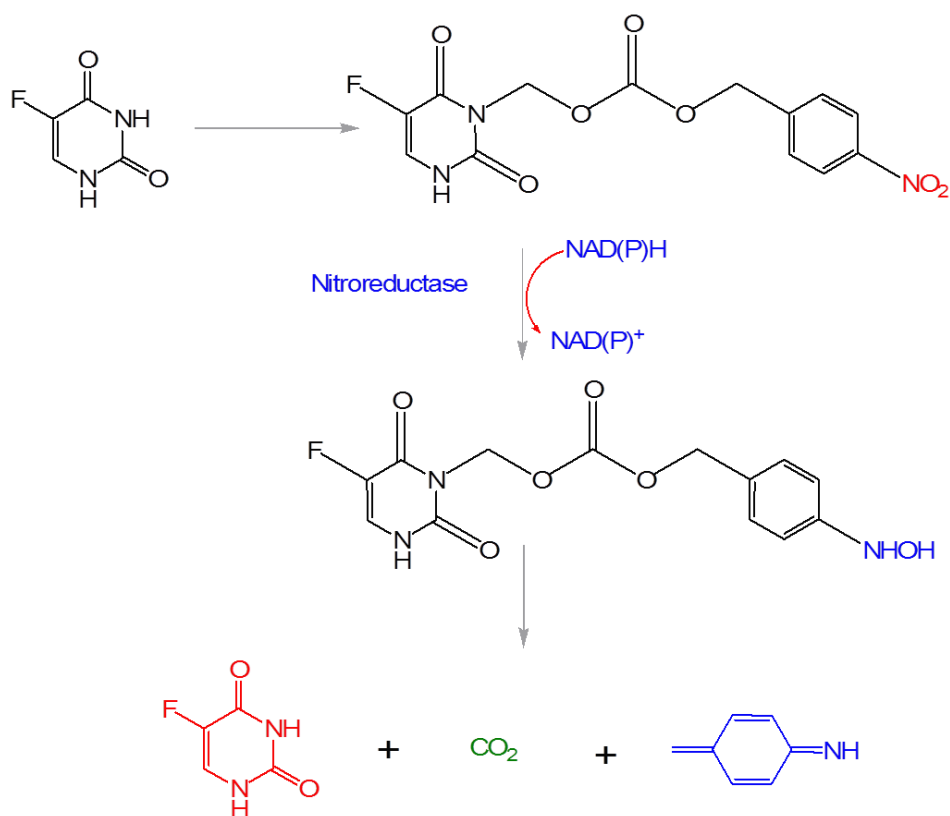
The nitroaromatic system was chosen as the bioprecursor since nitro-aromatic compounds have shown toxicity to hypoxic cells. The toxic compounds, qiononemine methides, are formed when the nitro-aromatic compound is enzymatically reduced (scheme 15). Upon this reduction the 5-flourouracil drug is released and therefore there are two cytotoxic moieties available, thus increasing the potency of the prodrug. It is hypothesised that this reduction will be activated by the reductase produced by a tumour to combat the oxidative stress of Photo Dynamic Therapy.⁴⁴



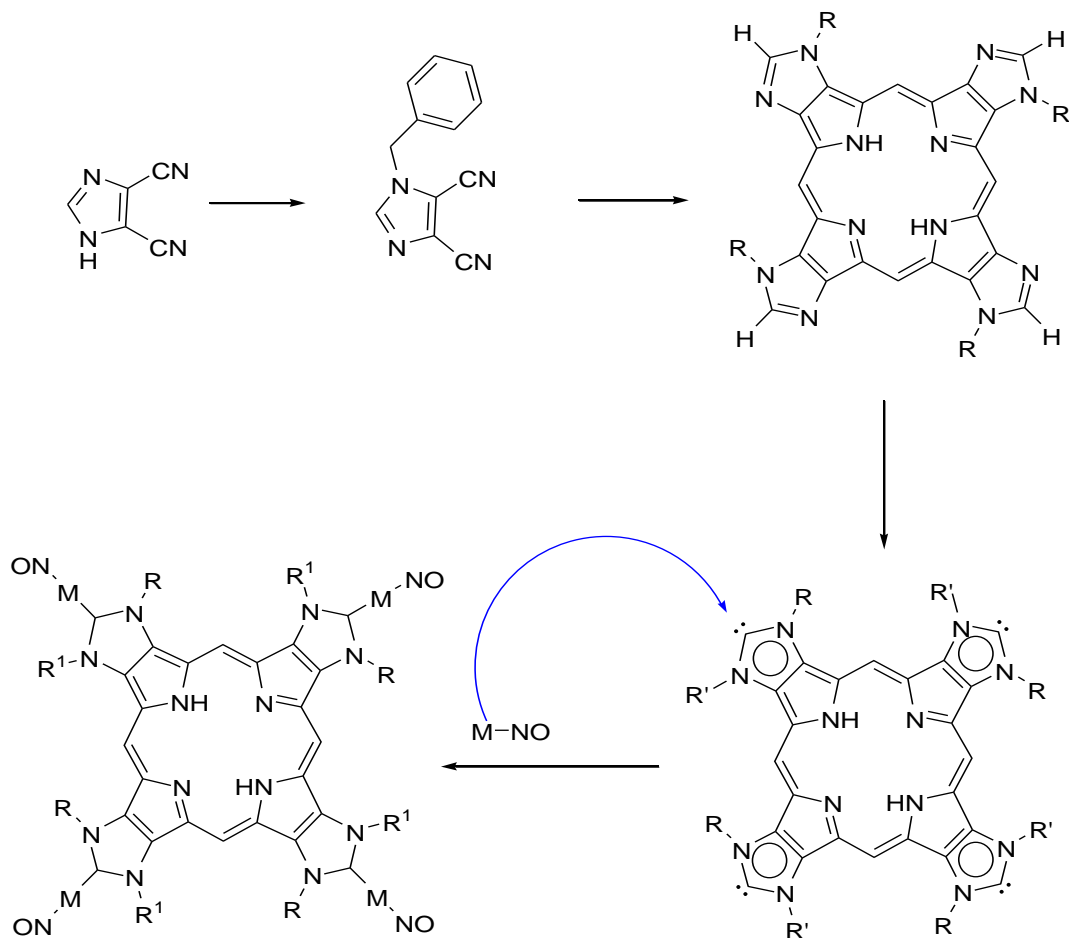
Scheme 14. *Proposed scheme for the incorporation of the bioprecursor protected 5-fluorouracil into the phthalocyanine photosensitizer carrier linker.*

The carrier linker used will also have photocytotoxic effects in cancerous cells, as it is a phthalocyanine photosensitizer. The phthalocyanines have been applied in the PDT of tumours, as outlined in section 1.4. They selectively accumulate in cancerous tissue and are activated by the application of light of a specific wavelength ($< 800 \text{ nm}$).

We are also interested in developing a new prodrug that can incorporate nitrous oxide. Nitrous oxide is a very effective anticancer agent and we believe it is possible to incorporate nitrous oxide organometallic groups into a phthalocyanine via carbene coordination. Outlined in Scheme 16 are the proposed final structures for both prodrug systems.

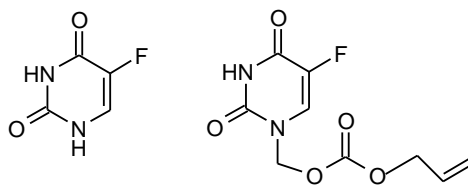


Scheme 15. Reduction of bioprecursor prodrug to drug plus cytotoxic quinonemine methides.



Scheme 16. *The proposed nitrous oxide phthalocyanine prodrug.*

Previous work carried out in the Nolan research group involved the development of new 5-FU prodrugs. Shown in figure 22 is the structure of a new lead hit, this compound possesses a higher bioactivity than 5-FU and was also found to be active against 5-U resistant cell lines

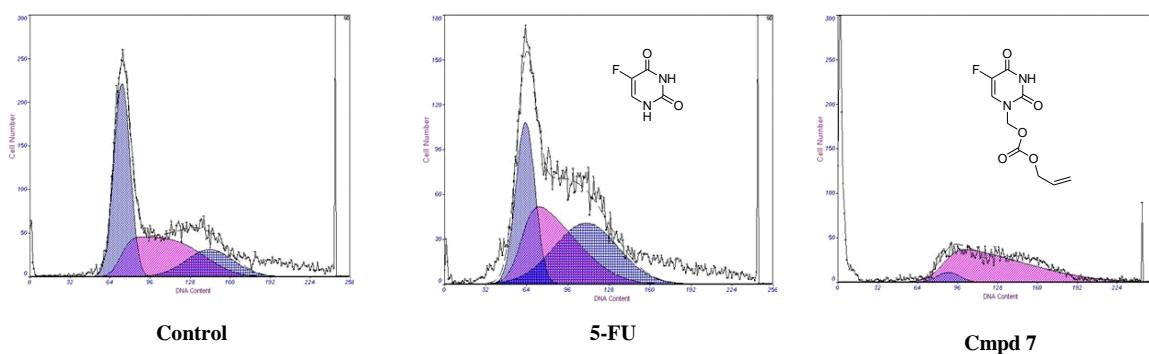


5-FU

7

Comp	IC ₅₀ Values 4T1 cell Line		IC ₅₀ Values SW480 cell Line	
	3 Days µg/ml	5 Days µg/ml	3 Days µg/ml	5 Days µg/ml
5-FU	0.05	0.02	0.80	0.40
1	0.009	0.005	0.40	0.06

Cell cycle analyses of 4T1 cells



Cell cycle analyses of 4T1 cells following 3 days treatment with 0.1mM 5-FU and 5-FU derivatives. X-axis shows DNA content, y-axis represents counts of cell. G1 phase is in light blue, S phase in pink and G2 phase in dark blue.

Figure 22. Biodata for new 5-FU derivative.⁴⁶

It was initially thought that **1** acts simply as a prodrug of 5-FU, that is **1** is converted to 5-FU through hydrolysis of the carbonate substituents. However, cell cycle studies have revealed that the activity of **1** is different from 5-FU, thus **1** does not behave as a prodrug but as a unique new lead.

It is one of the goals of this research to carry out a structure activity relationship (SAR) study on **1** to:

1) determine what structural aspects of the carbonate handle are required for this unique activity ,

2) use the information from this SAR study to develop a potentially better lead.

In tandem with this work it is envisaged to develop an interesting novel calixarene porphyrin. These compounds are to possess a partial cone calix[4]arene platform with a porphyrin (figure 23). The resulting porphyrin should be partially picket fenced, that is the metal centre of the porphyrin will be isolated, as a result such a compounds should act as potential oxidation catalysts and biomimetic sensors (Haemoglobin) with respect to reversible oxygen binding.

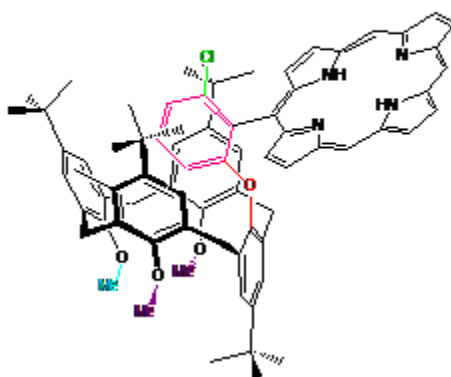


Figure 23. *Proposed calixarene porphyrin.*

Chapter 2 *Synthesis and Characterisation of Novel Third Generation PDT Prodrugs*

Introduction

2.1 Synthesis of 5-Fluorouracil Derivatives

5-fluorouracil (5-FU), as delineated in the literature survey, is a well established and reviewed anti-cancer drug, and as such was considered ideal for study, modification and improvement. Two types of nitroaromatic derivatives and two alkyl chain derivatives were prepared in this work. The logic of the design, synthesis and characterisation of these derivatives are described below.

2.1.1 Trigger-Linker-Effect

The trigger-linker-effect concept was used in the design of the 5-FU derivatives (figure 24).⁴⁷ In the nitroaromatic compounds, the reduction of the nitro group by reductases essentially triggers the release of the active drug from the tripartite prodrug under hypoxic conditions^{48,49}, thus these compounds are expected to have selectivity as they should not become reduced in healthy oxygenated cells.⁵⁰ The choice of linker between 5-FU and the nitro-aromatic trigger will ideally facilitate the release of the drug and cytotoxin from the bio-precursor complex. The formate linker shown in figure 24 below fits these requirements.

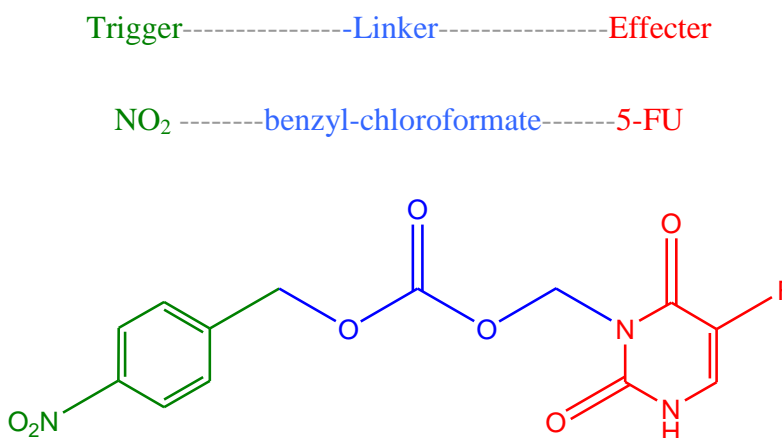
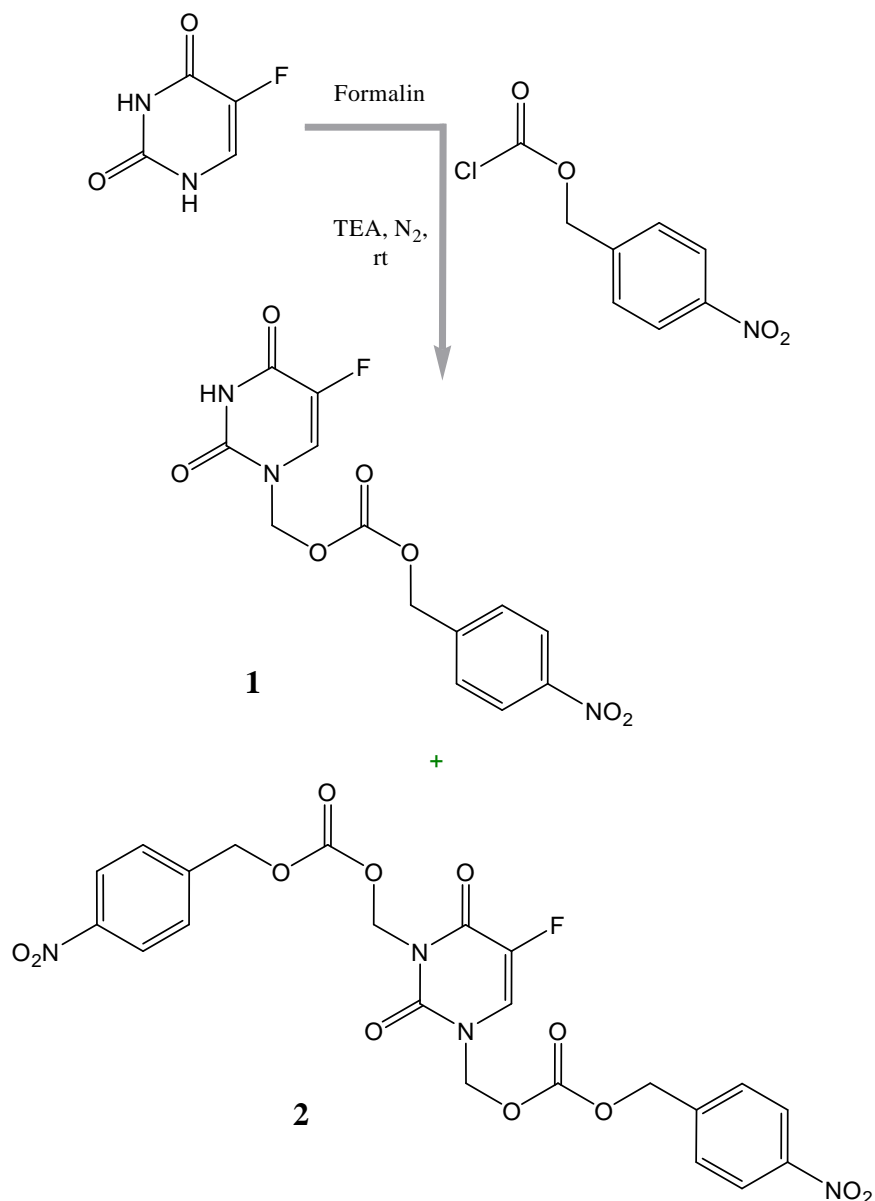


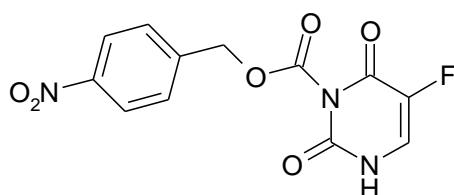
Figure 24. *Trigger-linker-effect concept.*⁵¹

The synthesis of compound **1** and its partner, the di-substituted 5-fluorouracil **2**, was achieved by heating 5-fluorouracil in 37% formalin at 60°C for 45 minutes. The resulting product, an oil, was dissolved in acetonitrile after being dried under vacuum. An equimolar portion of triethylamine and 4-nitrobenzyl chloroformate were reacted with the 5-FU solution resulting in yields of 15% and 37% of **1** and **2** respectively (scheme 17).



Scheme 17. Preparation of 1-nitrobenzyloxycarbonyloxymethyl-5-fluorouracil **1** and 1,3-nitrobenzyloxycarbonyloxymethyl-5-fluorouracil **2**.

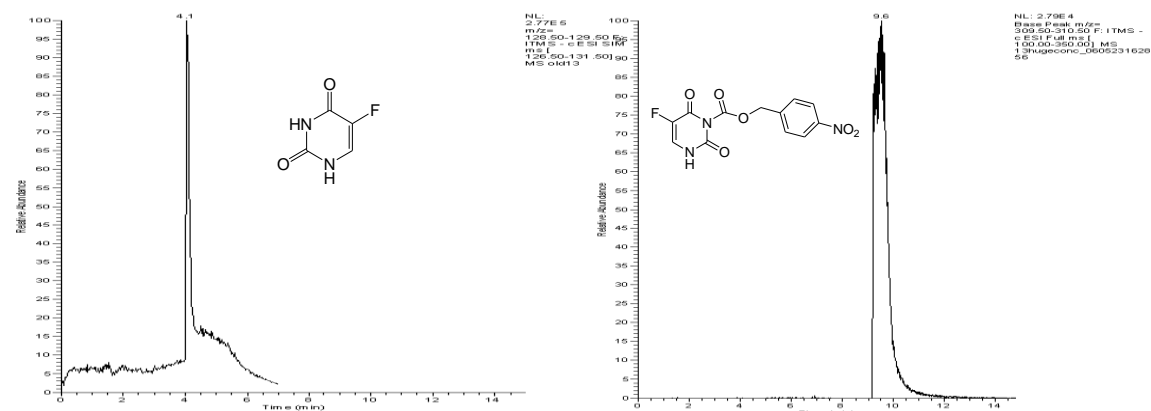
Biological studies on **1** and **2** were carried out at UCD by Dr Aisling Redmond under the supervision of Dr Susan Mc Donnell. The biological assay used was an *in vitro* assay using cancer cell lines that were transfected with bacterial nitroreductase (V79-NTR) and a standard was also run on normal V79 cancer cells as a comparison. It was found that neither compound **1** or **2** showed biological activity with the transfected cells. For activity to be observed it is essential that the nitrobenzyl substituent be reduced to a quinmethide thereby releasing fluorouracil, but this did not occur. This lack of biological activity contrasts with a similar derivative prepared earlier in Dr Nolan's group shown in figure 25.



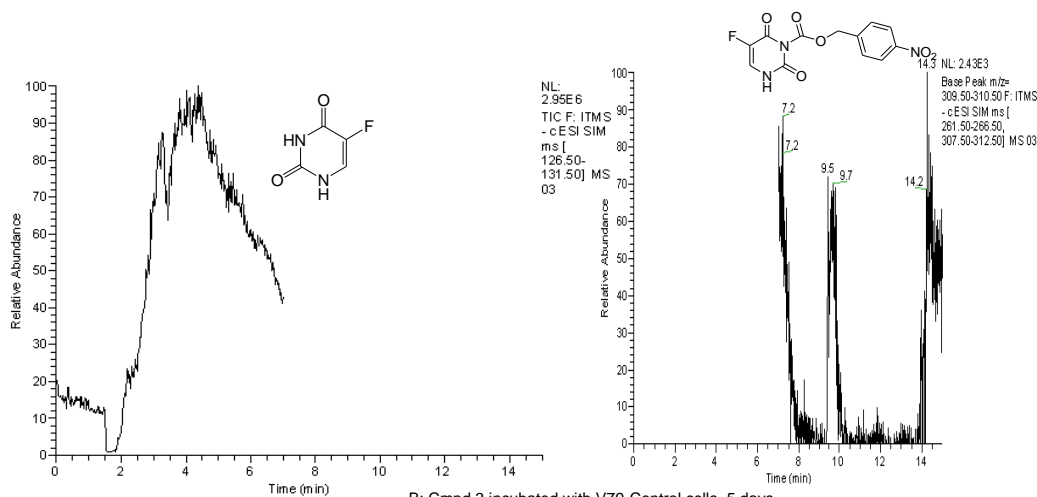
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Figure 25. Redox activated prodrug **3**.

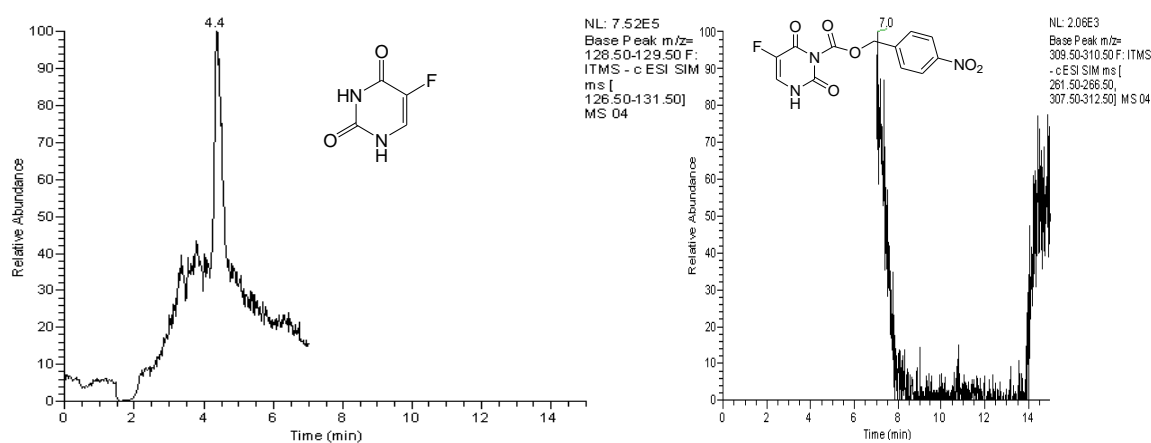
It was proven by both biological assay with nitroreductase transfected cells and metabolite analysis using HPLC-MS (method was developed in the NICB at DCU by Dr Robert O'Connor and Dr Rachel Wall) that release of 5-FU occurred by reduction of the nitrobenzyl group (figure 26). It would appear that the introduction, of a methyl spacer between 5-FU and the nitrobenzyl carbonate has a significant impact on biological activity.



A: 5-FU/Cmpd 3 alone



B: Cmpd 3 incubated with V79-Control cells, 5 days



E: Cmpd 3 incubated with V79-NTR cells, 5 days

Figure 26. HPLC-MS metabolite results of nitroreductase transfected cells (V79-NTR) treated with 5-FU (left side) and the metabolites of prodrug 3 (right side).

2.1.2 5-FU alkyl chain derivatives.

It was previously found that 1-allyloxycarbonyloxymethyl-5-flourouracil **4**, figure 27 has higher bioactivity against 4T1 and SW480 tumour cells than 5-FU. It was of interest to determine the important structural features of this compound that are required for enhanced bioactivity. To achieve this aim a series of new derivatives were prepared to determine the effect of chain length and type, (i.e. the effect of saturating the bond) on biological activity (Figure 28).

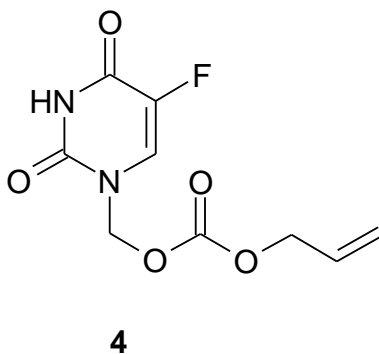


Figure 27. *1-Alloxycarbonyloxymethyl-5-flourouracil (4).*

A propyl chain was chosen to replace the allyl group in **4**, by doing this the effect of the double bond on biological activity can be determined (compound **5** figure 28). A butyl chain was also chosen to determine the effect chain length has, if any, on bioactivity (compound **6**).

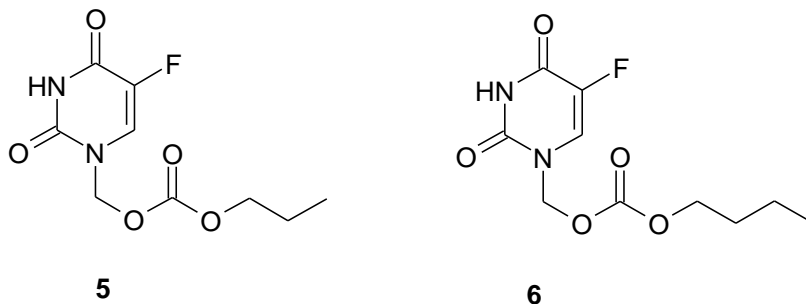
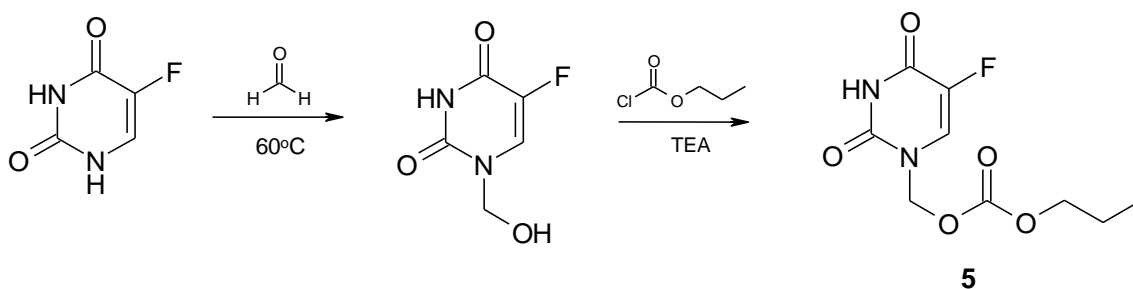
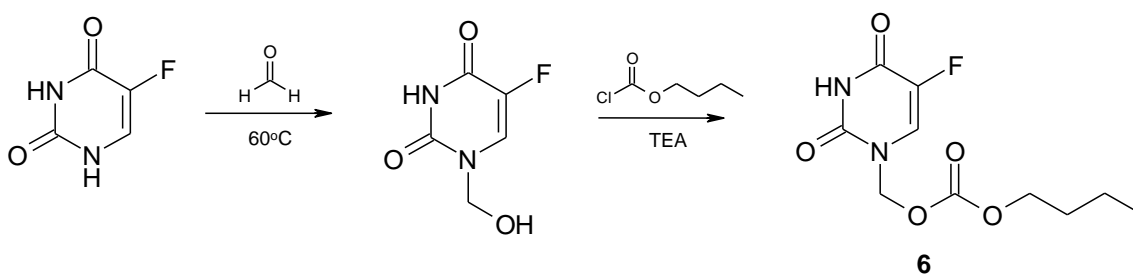


Figure 28. *1-propyloxycarbonyloxymethyl-5-flourouracil 5 and 1-butyloxycarbonyloxymethyl-5-flourouracil 6.*

The alkyl derivatives were prepared using the same method as that used for the nitro derivatives, with the only exceptions being the chloroformate reagent used and a slightly shorter heating time for the hydroxylated 5-FU complex. Propyl chloroformate and butyl chloroformate were employed to produce **5** and **6** respectively, yielding 37% of 1-propyloxycarbonyloxymethyl-5-flourouracil **5** and 40% of 1-butyloxycarbonyloxymethyl-5-flourouracil **6** (scheme 18 and scheme 19).



Scheme 18. Preparation of 1-propyloxycarbonyloxymethyl-5-flourouracil (**5**).



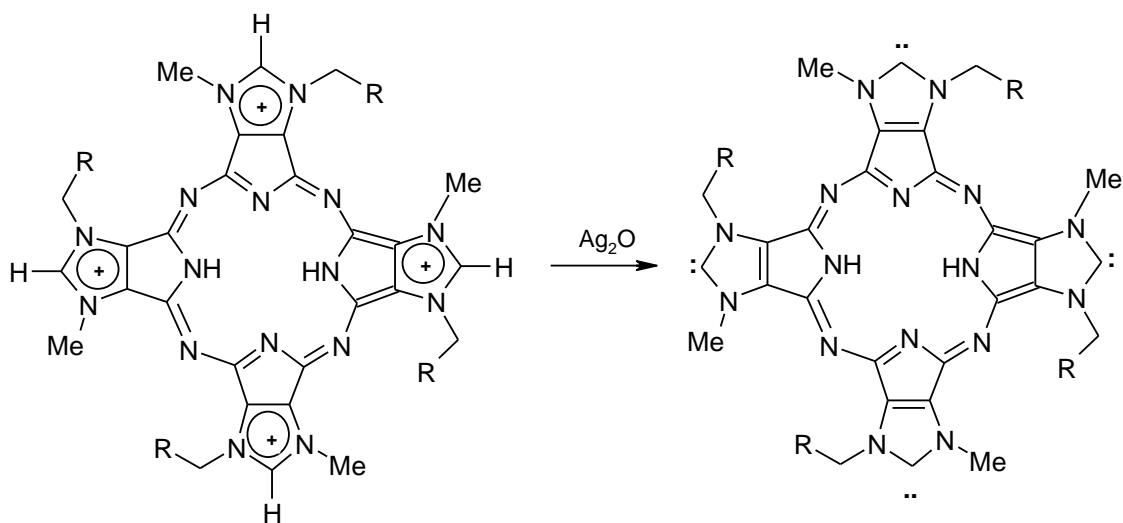
Scheme 19. Preparation of 1-butyloxycarbonyloxymethyl-5-flourouracil (**6**).

Both compounds **5** and **6** have now been evaluated for activity against colon cancer cell lines by Dr Susan McDonnell's group at UCD. Both compounds have shown similar cytotoxicity toward the colon cancer cell lines at values of 15 and 20 μM , which is comparable to the biological activity of 5-FU which typically ranges from 15-20 μM on the same cell lines. These values are an order of magnitude lower than the allyl derivative

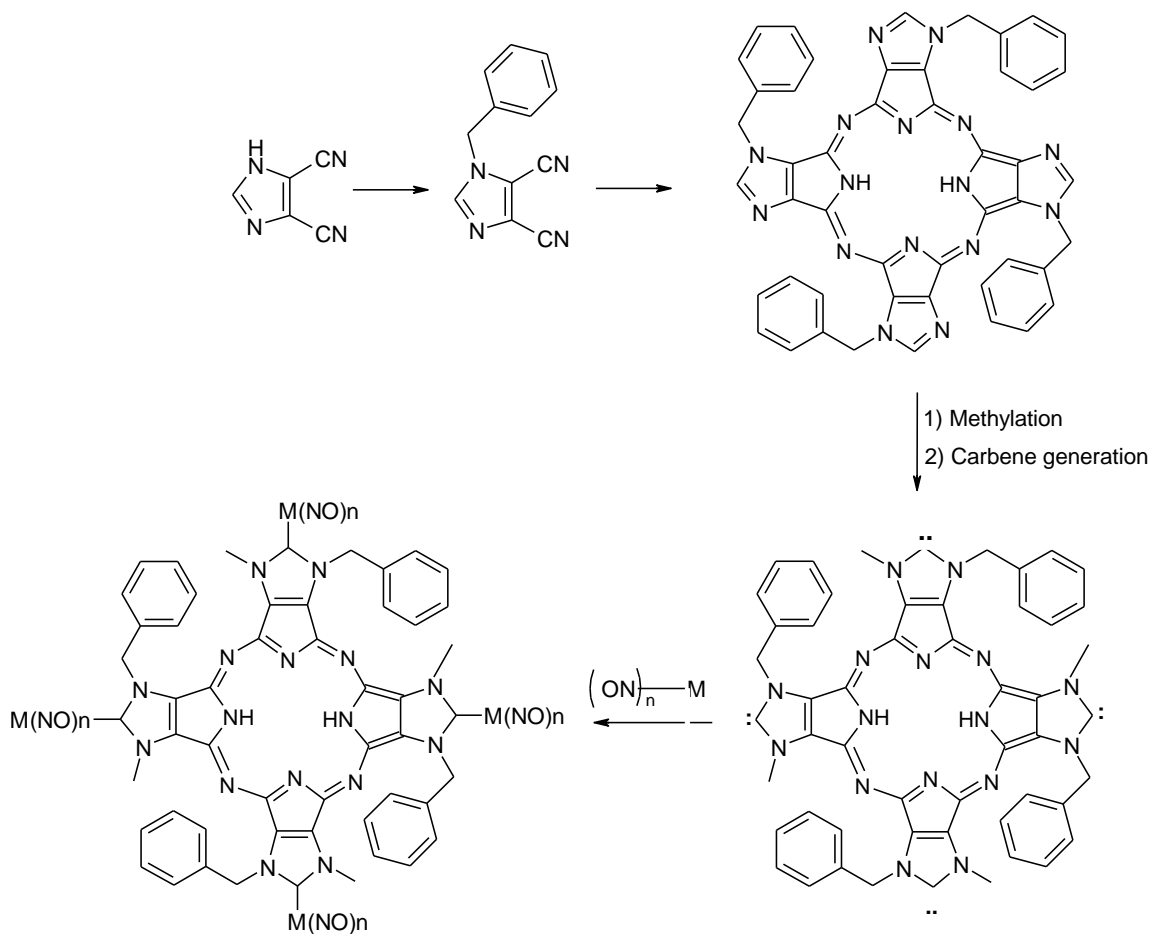
4 (1-5 μM), indicating that the double bond is essential for enhanced activity. Cell cycle studies are not yet available to determine whether the new derivatives behave similar to **4**, or are simply acting as a 5-FU prodrug, (that would be the case if the cell cycles are equivalent to 5-FU). Biological studies are still pending.

2.2 Synthesis of the porphyrazine prodrug carrier.

The preparation of tetra-imidazolium porphyrazine was attempted in order to generate a porphyrazine where four of the peripheral carbons can be converted to their carbene form by removal of the highly acidic proton (scheme 20). Thus, it was hoped to create a platform for linking the organometallic nitric oxide compound to the porphyrazine by carbene co-ordination. Outlined in scheme 21 is the proposed strategy and intended product.



Scheme 20. *Proposed carbene porphyrazine complex via acidic proton removal.*



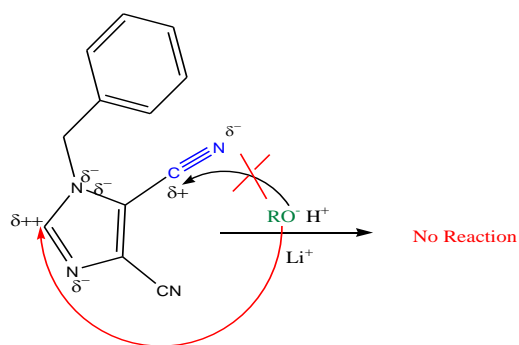
Scheme 21. Preparation of a porphyrazine complex and subsequent binding of NO.

However, the self-condensation of 1-phenyl-4,5-dicyanoimidazole could not be achieved in pentanol at high temperature. At this point it was decided to cross condense 1-phenyl-4,5-dicyanoimidazole with 4,5-dicyanoimidazole in pentanol at high temperature. Various ratios of the reactants were used, from 1:15 moles to ratios of 1:6.66, table 3, (based on ratios taken from the literature) however no product was procured from these efforts.

29:28 mmoles	Reaction Time	Temp
1 : 150	24 hrs	150°C
1 : 9	24 hrs	110°C
1 : 6.67	24hrs	150°C

Table 3. Conditions employed in initial attempted porphyrazine synthesis.

One possible explanation for the failed condensation can be taken from Linstead's proposed mechanism of phthalocyanine formation. In his mechanistic analysis, scheme 3 Chapter 1, the red arrow illustrates that the alkoxy attacks the most electropositive carbon, that of the nitrile group, to initiate the cyclisation and condensation process. However, imidazole possesses an acidic proton at position 2 in the ring (pKa 14.52) (scheme 22). It is quite possible that the base is deprotonating imidazole preventing the formation of porphyrazine.



Scheme 22. Proposed explanation for failed porphyrazine formation, showing proton that is susceptible to alkoxy attack

2.2.1 Synthesis of 1-benzyl-28H,30H-tribenzo[b,g,l]imidazolo[4,5-q]porphyrazine

The preparation of 1-butyl-7,10,14,17,21,24-hexahexyl-28H,30H-tribenzo[b,l,g]imidazole[4,5-q]porphyrazine had been previously reported in the literature by J. Bakboord *et al* (figure 29).⁵² This method involved the cross condensation of dicyanoimidazoles with a partner phthalonitrile to yield a tribenzoporphyrazine.

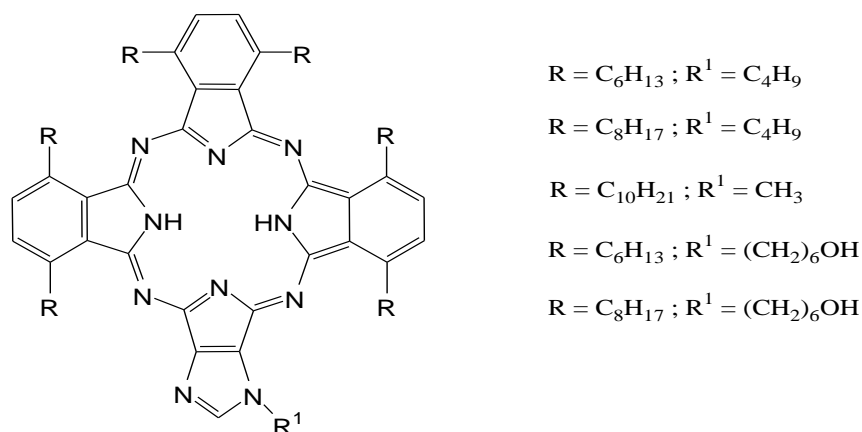
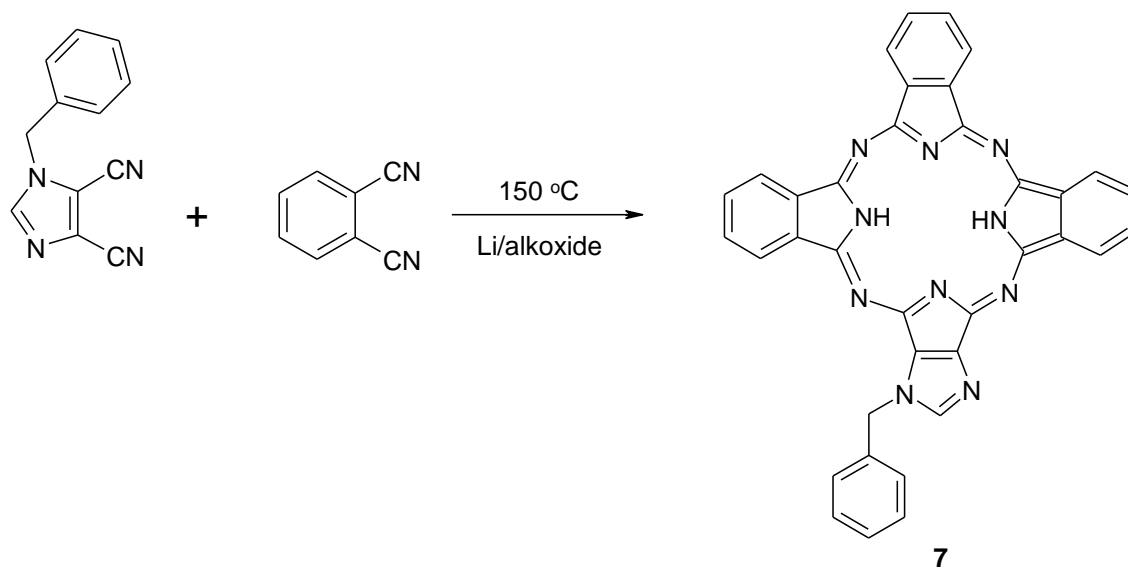


Figure 29. Porphyrazine compounds prepared by Bakboord *et al.*

Bakboord's method was employed using 1-phenyl-4,5-dicyanoimidazole and phthalonitrile in a 1:6 molar ratio respectively in Li/pentanol at 115 °C (Scheme 23). The reaction was allowed to proceed for 24 hours and a deep green pigment product was isolated.⁵³ The compound was first cleaned on a silica gel column and next it was purified on a 3% cross linked polystyrene (SX3) size exclusion column. This separated the porphyrazine from the phthalocyanine by-product.

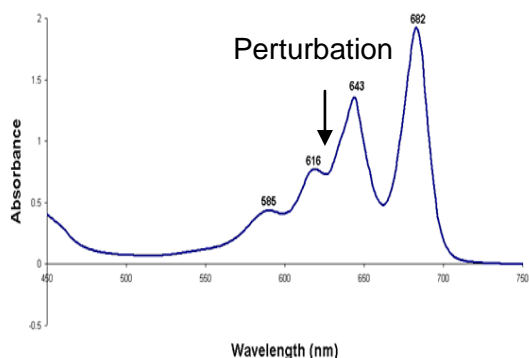


Scheme 23. Preparation of 1-benzyl-28H,30H-tribenzo[b,g,l]imidazolo[4,5-q]porphyrazine **7**.

The isolated unsymmetrical porphyrazine was characterised by UV-Vis spectroscopy, which displayed two principal peaks in the Q-band region of the spectrum, which is indicative of metal-free phthalocyanine. One of the peaks in the spectrum, at 643 nm has a small shoulder of which was perturbed, confirming the presence of an unsymmetrical phthalocyanine (3:1 macrocycle). However, the shoulder peaks at 616 nm and 585 nm are very intense indicating the presence of other phthalocyanine impurities (figure 30).⁵⁴ Further chromatographic separation was attempted using both silica and size exclusion chromatography unfortunately the target phthalocyanine could not be isolated in pure form.

To solve the purification problem a new analogue, 1-butyl-28H,30H-tribenzo[b,g,l]imidazolo[4,5-q]porphyrazine, was prepared as the zinc derivative and not the metal-free. There is previous evidence that metallated unsymmetrical phthalocyanines are easier to separate using SX-3 cross linked polystyrene than the metal-free counterparts.⁵⁵

a)



b)

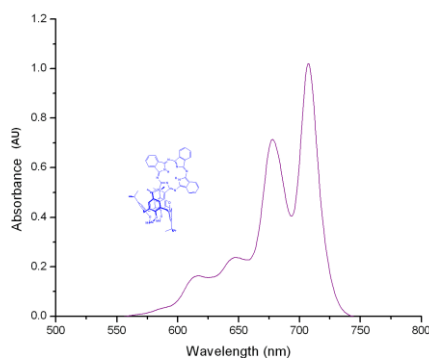
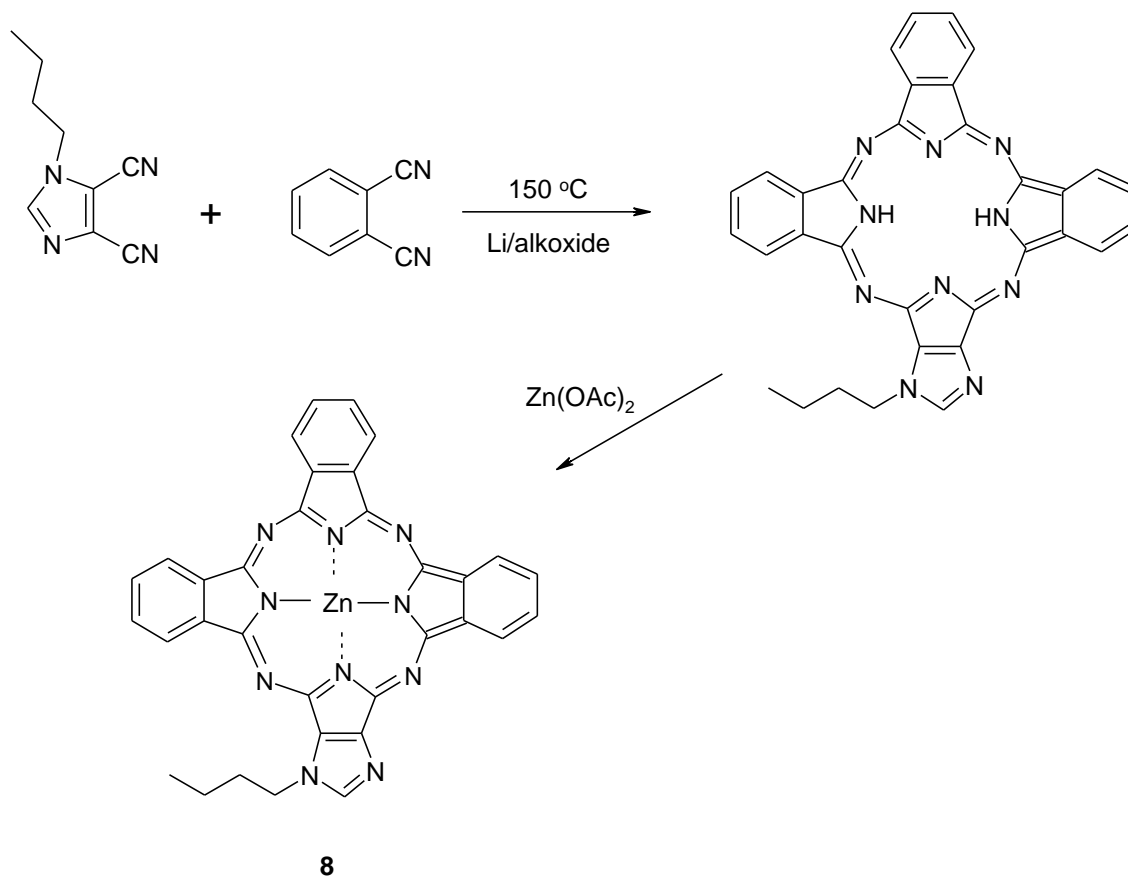


Figure 30. a) UV-Vis spectrum of 1-benzyl-28H,30H-tribenzo[b,g,l]imidazolo[4,5-q]porphyrazine **7** and metal free phthalocyanine. b) An example of a pure metal free phthalocyanine

The preparation of 1-butyl-28H,30H-tribenzo[*b,g,l*]imidazolo[4,5-*q*]porphyrazine is outlined in scheme 24. The required starting material, 1-butyl-4,5-dicyanoimidazole, was prepared using triethylamine, tetrabutylammonium iodide, 4,5-dicyanoimidazole, methyl ethyl ketone and butyl bromide; the reaction was refluxed for four days. Purification was achieved on a silica gel column, with ethyl acetate as eluent. 1-Butyl-4,5-dicyanoimidazole was then employed in the synthesis of the porphyrazine, which was achieved using the same condensation method previously described giving crude Pc product in 74% yield. The crude Pc product was then metallated using zinc acetate. Purification was carried out by initially cleaning the product mixture on a silica gel column, followed by size exclusion chromatography with SX-3 biobeads.



Scheme 24. Preparation of 1-butyl-28H,30H-tribenzo[*b,g,l*]imidazolo[4,5-*q*]porphyrazine **8**.

The UV-Vis spectrum of the isolated porphyrazine, figure 31, displayed two intense peaks at 649 nm and 670 nm, with a small perturbed shoulder peak at 621 nm. Based on SAPT theory (Chapter 1) we would expect there to be two peaks observed for this porphyrazine, since both B_{1g} and B_{2g} symmetry elements are present. The observed UV-Vis spectrum which possesses two peaks at 649 and 670 nm supports this conclusion. Furthermore, the two peaks are separated by 21 nm, this magnitude of separation is typical for this class of phthalocyanine based on previous examples prepared in the past.

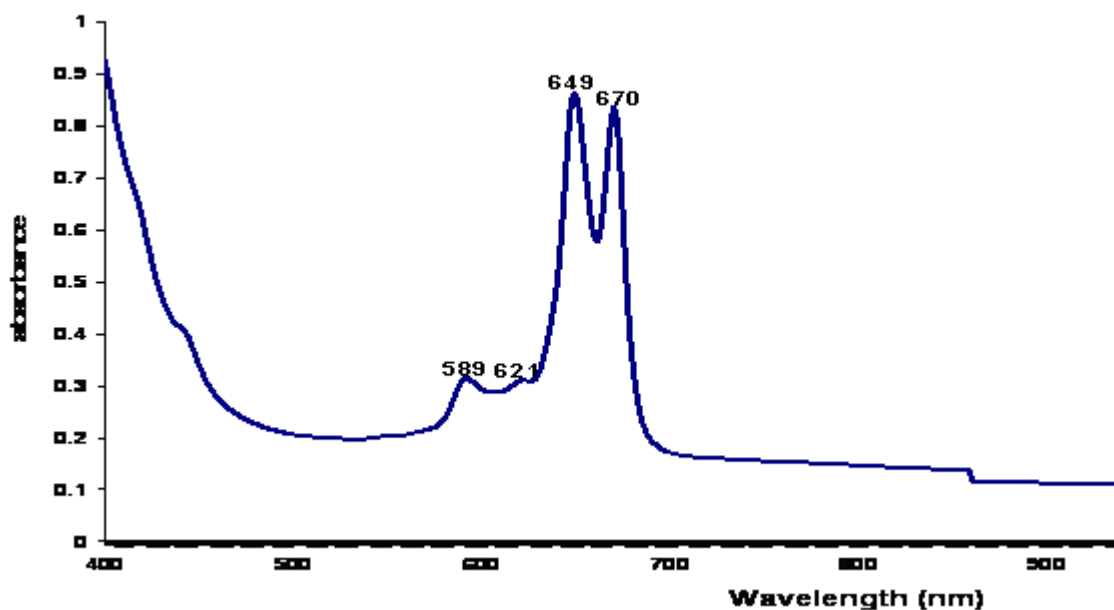


Figure 31. UV-Vis spectrum of 1-butyl-28H,30H-tribenzo[b,g,l]imidazolo[4,5-q-zinc]porphyrazine 8.

To determine if the full 24 hour reaction period was required for formation of the unsymmetrical porphyrazine a time versus reaction progression study was carried out. The reaction was initiated according to the procedure outlined in the experimental section; a green dye formed almost instantaneously after the addition of lithium. An aliquot was taken each hour thereafter to monitor progress in the first six hours of the reaction and then the final aliquot was procured the following morning (figure 32). The results are described in table 4.

Time	TLC Spots	UV-Vis peaks (nm)
1hr	Brown + Green	660 - highly perturbed, 631, 598
2hrs	Brown + Green	655 - highly perturbed, 630, 596
3hrs	Brown + Green	662 - perturbed, 631, 597
4hrs	Brown + Green	660, 631, 597
5hrs	Brown + Pink + Green + Blue	661, 630, 596
6hrs	Brown + Pink + Green + Blue	660, 630, 596
24hrs	Brown + Green + Yellow	660, 629, 596

Table 4. *Time study of the formation of the porphyrazine, where the various colours represent the numerous phthalocyanine and porphyrazine fractions and intermediates that formed.*

From the table above it can be seen that though the colour change was instantaneous after the addition of lithium, the macrocyclic dyes are present but in low concentration.

A UV-Vis assay was performed where a sample from the reaction mixture taken at the appropriate time point was added to a UV-vis cuvette (containing a fixed volume of THF) until an absorption of approximately 4 units was reached (figure 32). Based on this study it would appear that after four hours the concentration of the phthalocyanine product increased relative to the sample taken after 1, 2 and 3 hours. Further reaction aliquots were taken at 5, 6 and 24 hours, however there was no evident change in concentration of product (ie. The same volume of reaction mixture is required at each time point to bring about an absorption of 4 units), indicating that the reaction is complete after four hours.

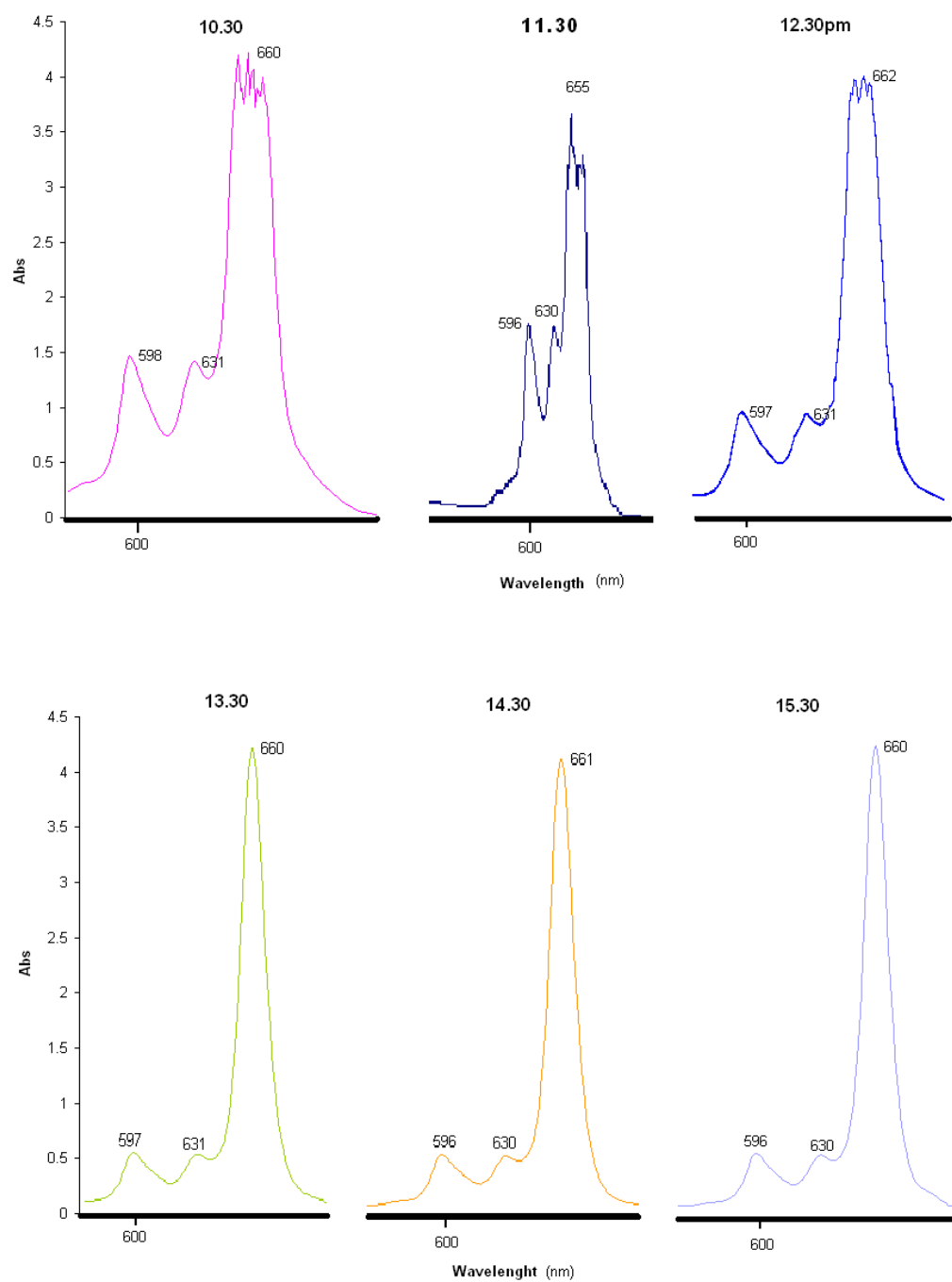


Figure 32. UV-Vis spectra of porphyrazine Pc formation over time, illustrating that the reaction is complete after three hours of reaction.

2.3 Conclusion

Two new 5-FU derivatives, **5** and **6**, were prepared. Initial biological screening on these samples show a lower activity than the allyl derivative **4**, indicating that the double bond is essential for the observed high biological activity of **4**.

Attempts were made to prepare a series of imidazole porphyrazines, however the self-condensation of 1-phenyl-4,5-dicyanoimidazole could not be achieved due to the presence of the acidic proton of the imidazole. 1-Butyl-4,5-dicyanoimidazole was prepared and cross condensed with phthalonitrile. It was found that the target porphyrazine could be separated as the zinc derivative; the metal-free could not be separated from the unsubstituted phthalocyanine byproduct. Characterisation for porphyrazine **8** is based on UV-Visible analysis using SAPT theory.

2.4 Experimental

1,3-Nitrobenzyloxycarbonyloxymethyl-5-fluorouracil, **1**, and 1-Nitrobenzyloxycarbonyloxymethyl-5-fluorouracil, **2**.

5-FU (0.9 g, 7mmol) and formalin (1.16 ml, 15.4 mmol) were stirred at approximately 60°C for 45 mins. The oily product was dried under vacuum and was then dissolved in dry acetonitrile (21 ml) followed by the addition of triethylamine (1.32 ml 9.45 mmol). The solution was then agitated under nitrogen at room temperature, followed by the slow addition of 4-nitrobenzyl chloroformate (1.87 g, 8.68mmol). After stirring at room temperature for 3 hours the mixture was filtered and acetonitrile removed under reduced pressure. The crude product was then dissolved in dichloromethane (21 ml) and washed three times with HCl (1 N), saturated NaHCO₃ aq (21 ml x 2) and water (21 ml x 2). The organic layer was dried over magnesium sulfate overnight, suction filtered and concentrated under vacuum. The product was further purified using silica gel chromatography (5:2 chloroform : ethyl acetate) to yield 15% of compound **1** and 37% of compound **2**.

Characterization of product 1,3-Nitrobenzyloxycarbonyloxymethyl-5-fluorouracil **2**:

¹HNMR $\delta^1\text{H}$ (400 MHz, DMSO – d₆) δ (ppm) 5.347 (s, 2H, O-CH₂-Ph), 5.719 (s, 2H, -¹N-CH₂-O), 5.878 (s, 2H, ³N-CH₂-O), 7.564 – 7.690 (m, 8H, aromatic), 8.30 (d, 1H, CH-CF).

¹³CNMR $\delta^{13}\text{C}$ (100 MHz, DMSO – d₆) δ (ppm) 68.376 (s, 1C, O-CH₂-Ph), 74.374 (s, 1C, N-CH₂-O), 123.894 (s, 4C, aromatic), 129.062 (s, 2C, aromatic), 129.124 (s, 2C, aromatic), 129.878, 130.223 (d, 1C, J_F = 129.7 Hz, N-C=C-F), 137.893, 140.174 (d, 1C, J_F = 867.656 Hz, C-F), 142.968 (s, 1C, N-CO-N), 147.646 (s, 1C, aromatic), 151.140 (s, 1C, aromatic), 153.150 (s, 1C, O-CO-O), 156.32, 156.61 (d, 1C, J_F = 109.04 Hz, N-CO-CF).

¹⁹FNMR $\delta^{19}\text{F}$ (DMSO – d₆) –167.4 (d, 1F).

ESI m/z: 571.2 (M+ Na⁺).

MP: 88 – 91°C

Elemental analysis: C₂₂H₁₇FN₄O₁₂; Calculated: %C = 48.18; %H = 3.12; %N = 10.22
Found: %C = 49.75; %H = 3.62; %N = 9.67

Characterisation of 1-Nitrobenzyloxycarbonyloxymethyl-5-flourouracil **1**:

¹H NMR $\delta^1\text{H}$ (400 MHz, DMSO – d₆) δ (ppm) 5.343 (s, 2H, CH₂-Ph), 5.642 (s, 2H, N-CH₂-O), 7.686 (d, 2H, J = 2.8 Hz, aromatic), 8.160 (d, 1H, CH=CF), 8.272 (d, 2H, J = 2.2 Hz, aromatic), 12.040 (bs, 1H, C-NH-C).

¹³C NMR $\delta^{13}\text{C}$ (100MHz, DMSO – d₆) δ (ppm) 38.427 (s, 1C, O-CH₂-Ph), 73.914 (s, 1C, O-CH₂-N), 123.894 (s, 2C, aromatic), 129.167 (s, 2C, aromatic), 129.555 (d, 1C, J_F = 129.898 Hz, N-C=CF), 140.988 (d, 1C, J_F = 862.92 Hz, C-F), 143.066 (s, 1C, N-CO-N), 147.658 (s, 1C, aromatic), 149.608 (s, 1C, aromatic), 153.667 (1C, O-CO-O), 157.667 (d, 1C, J_F = 97.76 Hz, N-CO-CF).).

¹⁹F NMR $\delta^{19}\text{F}$ (DMSO – d₆) –168.605 (s, 1F).

ESI m/z: 362.0 (M + Na⁺).

MP: 158 - 162°C

Elemental analysis: C₁₃H₁₀FN₃O₇; Calculated: %C = 46.03; %H = 2.97; %N = 12.39
Found: %C = 46.97; %H = 3.36; %N = 11.44

Preparation of a) 1-propyloxycarbonyloxymethyl-5-flourouracil, 5 and b) 1-butyloxycarbonyloxymethyl-5-flourouracil, 6.

5-FU (1.3 g, 10 mmol) and 37 w% formaldehyde in aqueous solution (1.9 mls, 25 mmol) were added to water. The reaction was heated to 60°C under agitation for 30 minutes. The resultant solution was concentrated and dried in a vacuum oven at 60°C for 48 hours. The oily product was then dissolved in dry acetonitrile (30 ml) preceded by the addition of triethylamine (1.89 mls 13.5 mmol). The solution was then agitated under nitrogen at room temperature, followed by the dropwise addition of (reaction a) propyl chloroformate (1.42 mls, 12.4 mmol.) and (reaction b) butyl chloroformate (1.61 mls, 12.4 mmol). After stirring at room temperature for 3 hours the mixture was filtered and acetonitrile removed under reduced pressure. The crude product was then dissolved in dichloromethane (30 ml) and washed three times with HCl (1N), saturated NaHCO₃ aq (30 ml x 2) and water (30 ml x 2). The organic layer was dried over sodium sulfate

overnight, suction filtered and concentrated by rotary evaporation. The product was isolated using silica gel chromatography (methanol-dichloromethane, 1:30, v/v) to yield 37% of compound **5** and 40% of compound **6**.

Characterization of product 1-propyloxycarbonyloxymethyl-5-flourouracil **5**.

¹HNMR $\delta^1\text{H}$ (400 MHz, DMSO – d₆) δ (ppm) 0.963 (t, 3H, J = 7.2 Hz, CH₂-CH₃), 1.684 (h, 2H, J = 6.8 Hz, CH₂-CH₂-CH₃), 4.137 (t, 2H, J = 6.4 Hz, O-CH₂-CH₂), 5.721 (s, 1H, N-CH₂-O), 8.210 (d, 1H, Ch-CF), 12.095 (bs, 1H, NH).

¹³CNMR $\delta^{13}\text{C}$ (100MHz, DMSO – d₆) δ (ppm) 10.359 (s, 1C, CH₃), 21.745 (s, 1C, CH₂-CH₃), 69.984 (s, 1C, O-CH₂-O), 73.535 (s, 1C, N-CH₂-O), 129.912 (d, 1C, J_F = 128.592 Hz, C-CF), 140.964 (d, 1C, J_F = 862.544 Hz, CF), 149.603 (s, 1C, N-CO-N), 153.972 (s, 1C, O-CO-O), 157.653 (d, 1C, J_F = 97.384 Hz, CO-CF).

¹⁹FNMR $\delta^{19}\text{F}$ (DMSO – d₆) –169.046 (d, 1F, Hz).

ESI m/z: 269.1 (M + Na⁺).

MP: 68 - 71°C

Elemental analysis: C₉H₁₁FN₂O₅ Calculated: %C = 43.91; %H = 4.50; %N = 11.38
Found: %C = 43.92; %H = 4.51; %N = 11.08

Characterisation of 1-butyloxycarbonyloxymethyl-5-flourouracil **6**.

¹HNMR $\delta^1\text{H}$ (400 MHz, DMSO – d₆) δ (ppm) 0.899 (t, 3H, J = 7.2 Hz, CH₂-CH₃), 1.343 (h, 2H, J = 7.6 Hz, CH₂-CH₂-CH₃), 1.583 (p, 2H, J = 6.4 Hz, CH₂-CH₂-CH₂), 4.122 (t, 2H, J = 6.4 Hz, O-CH₂-CH₂), 5.586 (s, 2H, N-CH₂-O), 8.155 (d, 1H, CH-CF), 12.055 (bs, 1H, NH).

¹³CNMR $\delta^{13}\text{C}$ (100 MHz, DMSO – d₆) δ (ppm) 13.982 (s, 1C, CH₃), 18.717 (s, 1C, CH₂-CH₃), 30.330 (s, 1C, CH₂-CH₂-CH₃), 38.253 (s, 1C, O-CH₂), 73.534 (s, 1C, N-CH₂-O), 129.583 (d, 1C, J_F = 128.592 Hz, CF-C-N), 140.957 (d, 1C, J_F = 862.544 Hz, CF), 149.603 (s, 1C, N-CO-N), 153.960 (s, 1C, O-CO-O), 157.655 (d, 1C, J_F = 98.136 Hz, N-CO-CF).

¹⁹FNMR $\delta^{19}\text{F}$ (DMSO – d₆) –169.062 (d, 1F,).

ESI m/z: 283.1 (M + Na⁺).

MP: 46 - 48°C

Preparation of 1-benzyl-28H,30H-tribenzo[b,g,l]imidazolo[4,5-q]porphyrizine 7.

1,2-Dicyanobenzene (86 mg, 0.43 mmol) and 1-benzyl-4,5-dicyanoimidazole (21 mg, 0.06 mmol) were dissolved in pentanol (1 ml) and the solution was heated to reflux. Lithium was added and the resulting mixture was refluxed for 6 hrs. Upon cooling, acetic acid (2 ml.) was added and the mixture was stirred for 30 min. The solvent was evaporated off and the residue was taken up in methanol. The mixture was filtered. The product was separated by column chromatography (silica gel, and eluted with chloroform to clean the first fraction and then the eluent was changed to tetrahydrofuran to clean the second fraction). The two fractions were then fully separated by size exclusion chromatography on a bio-bead (SX-3) column using tetrahydrofuran as eluent.

Characterisation of product:

UV-Vis: (Chloroform) 682nm and 643nm.

Synthesis of 1-Butyl-4,5-dicyanoimidazole :

Triethylamine (1.38 ml, 0.01 mol), tetrabutylammonium iodide (5 mg) and 4,5-dicyanoimidazole (1.25 g, 0.011 mol) were added to a solution of butanone (6.25mls) and stirred for 1hr at rt. Butyl bromide (1.2 ml, 0.01 mol) was added drop wise. The solution was refluxed for 2 days. The resulting solution was dissolved in Dichloromethane, washed with dilute ammonium solution followed by water, then dried over MgSO_4 [56]. The solvent was evaporated to yield 69.54% brown oil crude product. This was purified by silica gel chromatography, eluent: Ethyl Acetate. Yielding 46.55% oil product. This was characterised by TLC, ^1H NMR and ^{13}C NMR.

Characterisation of product:

^1H NMR $\delta^1\text{H}$ (400 MHz, $\text{DMSO} - d_6$) δ (ppm) 0.563 (t, 3H, $J = 14.8$ Hz, $\text{CH}_3\text{-CH}_2$), 0.939 (m, 2H, $J = 30$ Hz, $\text{CH}_2\text{-CH}_2\text{-CH}_3$), 1.446 (m, 2H, $J = 29.6$ Hz, $\text{CH}_2\text{-CH}_2\text{-CH}_2$), 3.724 (t, 2H, $J = 14.8$ Hz, $\text{CH}_2\text{-CH}_2$), 6.838 (s, H, N=CH-N).

^{13}C NMR $\delta^{13}\text{C}$ (100 MHz, $\text{DMSO} - d_6$) δ (ppm) 13.707 (s, 1C, CH_3), 19.044 (s, 1C, $\text{CH}_2\text{-CH}_3$), 23.752 (s, 1C, $\text{CH}_2\text{-CH}_2$), 48.321 (s, 1C, N-CH_2), 108.297 (s, 1C, N-C-CN), 111.980 (s, 1C, N-C-CN), 123.402 (s, 1C, N-C-N), 141.404 (s, 2C, CN).

ESI m/z: 197.1 ($\text{M} + \text{Na}^+$).

Elemental Analysis: $\text{C}_9\text{H}_{10}\text{N}_4$ Calculated: %C = 62.05; %H = 5.79; %N = 32.16 Found: %C = 61.52; %H = 5.72; %N = 31.70

Synthesis of the 1-butyl-28H,30H-tribenzo[b,g,l]imidazolo[4,5-q]-zinc-porphyrzine 8.

1,2-Dicyanobenzene (178.84 mg, 1.40 mmol) and 1-benzyl-4,5-dicyanoimidazole (26.81 mg, 0.15 mmol) were dissolved in pentanol (1.5 ml), the solution was heated to reflux (~150 °C). Lithium (20 mg) was added and the resulting mixture was refluxed for 6 hrs. Upon cooling, acetic acid (2 ml.) was added and the mixture was stirred for 30 min. The product was extracted from the solvent by a water/chloroform extraction and the chloroform was then evaporated off. The product was separated by column chromatography (silica gel, chloroform followed by tetrahydrofuran to pre-clean for the bio-bead column). The product was then further separated by size exclusion chromatography on a bio-bead (SX-3) column using tetrahydrofuran as eluent. This had to be repeated to achieve sufficient separation of the product mixture. Next the purified porphyrzine was metallated. To do this pentan-1-ol (1 ml) and lithium were heated to 50°C for 1hr, the Pc was added and after 20 min Zinc Acetate was added. The solution stirred at temperature over night. It was extracted using H₂O/CHCl₃, cleaned on a silica gel column, eluent THF, and purified on an SX-3 bio-bead column, eluent THF. The product was analysed by UV-Vis spectroscopy.

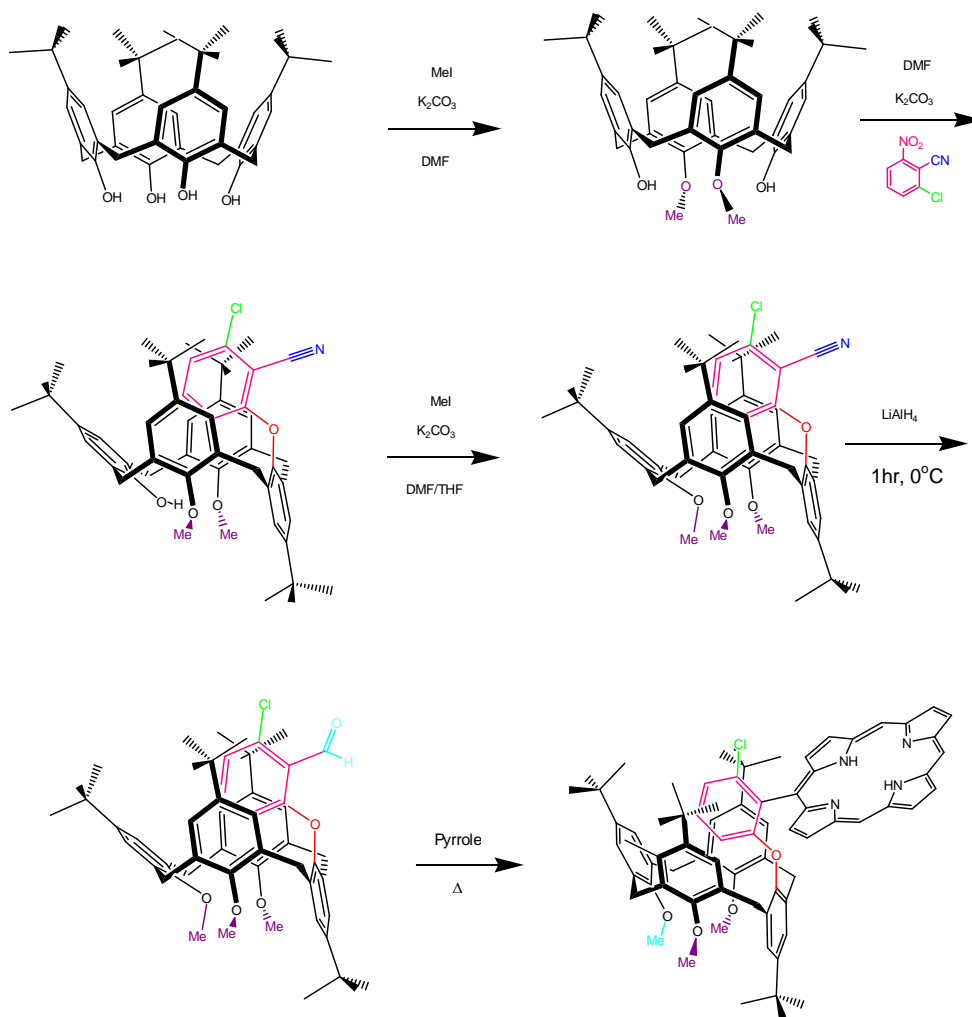
Characterisation of product:

UV-Vis: (Chloroform) 670 nm and 649 nm.

Chapter 3 *Synthesis and Characterisation of a Calix[4]arene Porphyrin*

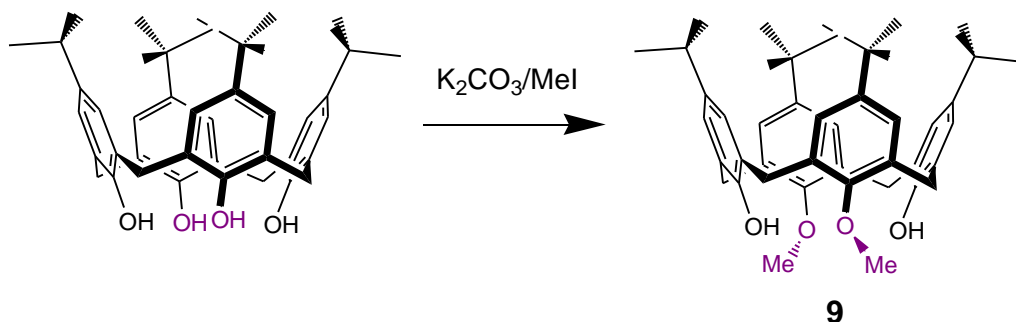
3.1 Introduction

The aim of this work was to prepare the starting material necessary for the preparation of a self-filled partial cone porphyrin derivative outlined in scheme 25 which involved four steps: 1) the methylation of the calixarene, 2) S_NAr reaction of di-methoxy calix[4]arene and 6-chloro-2-nitrobenzonitrile, 3) protection of the remaining hydroxyl group, 4) reduction of the nitrile group to an aldehyde. The last step involves the cross condensation of the partialcone calix[4]arene benzaldehyde with pyrrole and formaldehyde to yield the desired novel partial-cone calixarene porphyrin (scheme 25).



Scheme 25. The synthesis of self filled partial cone 5,11,17,23-tetra-*t*-butyl-27-hydroxy-26,28-dimethoxy-25-(3-[2-chloro-benzaldehyde])calix[4]arene .

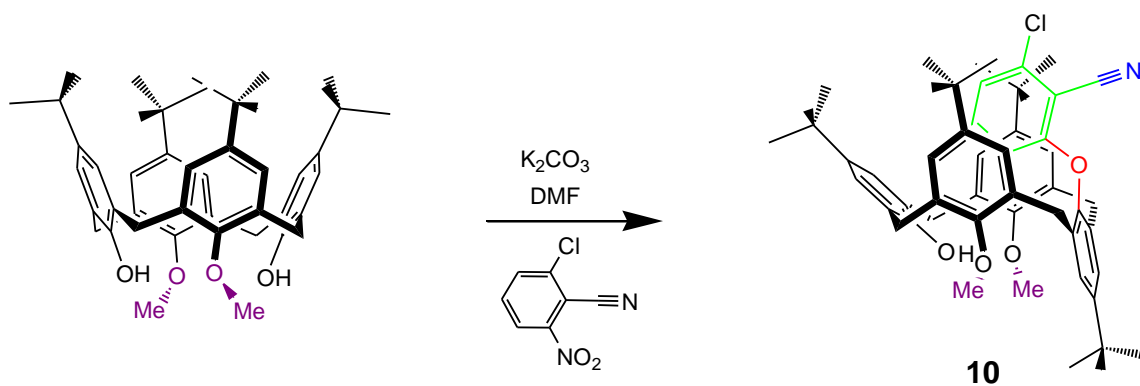
3.2 Synthesis of Self Filled Partial Cone Calix[4]arene



Scheme 26. Preparation of 5,11,17,23-tetra-tert-butyl-25,27-dihydroxy-26,28-dimethoxy calix[4]arene.

The preparation of the self filled partial cone was achieved as previously described, in four stages. In the first step tetra-tert-butyl-calix[4]arene was partially etherified using potassium carbonate and methyl iodide which were dissolved in DMF and refluxed for one hour to produce a mixture of di- and tri-methoxy calix[4]arene (scheme 26). Purification was carried out by flash chromatography on a silica gel column using 9:1 hexane : ethyl acetate as mobile phase to give the distal-1,3-dimethoxy-calix[4]arene in a 30% yield.

Only partial etherification is observed in this reaction since a weak base, K_2CO_3 , was employed, thereby avoiding substitution at all four hydroxy positions. The mechanistic reason that only distal-1,3-di-methoxycalix[4]arene is obtained is because the two remaining OH groups are stabilised by H-bonding making them less acidic. To fully substitute the calix[4]arene a strong base such as NaH in an aprotic solvent would be required.⁵⁷



Scheme 27. Preparation of 5,11,17,23-tetra-tert-butyl-25-hydroxy-27-(2-chloro-benzonitrile)-26,28-dimethoxy calix[4]arene(**10**) .

In the second step, the dimethoxy calix[4]arene , 6-chloro-2-nitrobenzonitrile and potassium carbonate were dissolved in DMF and stirred at room temperature for twenty four hours under vacuum (scheme 27). This brought about an S_NAryl displacement of the nitro group of 6-chloro-2-nitrobenzonitrile by the 1,3-dimethoxycalixarene. The product was purified by silica gel chromatography using hexane : ethyl acetate 10:1 as the eluent to give 5,11,17,23-tetra-tert-butyl-25-hydroxy-27-(2-chloro-benzonitrile)-26,28-dimethoxycalix[4]arene (**10**) in the partial cone conformation in 24% yield. It should be noted that double substitution of the 1,3-dimethoxycalixarene is not observed, only single substitution occurs.

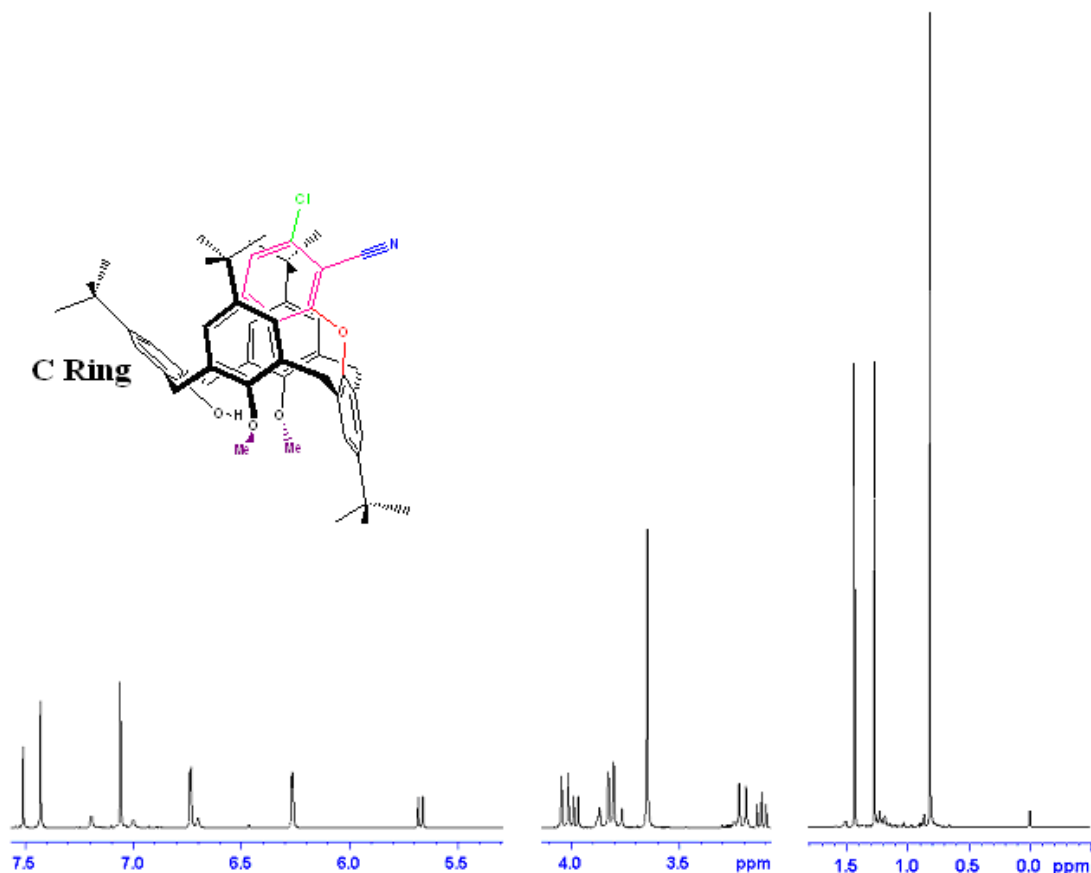


Figure 33. ^1H NMR of Self Filled Partial Cone 5,11,17,23-tetra-*t*-butyl-27-hydroxy-26,28-dimethoxy-25-(3-[2-chloro-benzonitrile])calix[4]arene **10**.

Outlined in figure 33 is the ^1H NMR of compound **10**. The tert-butyl peaks are found at 0.8 ppm, 1.3 ppm and 1.45 ppm. The singlet at 3.65 ppm represents the two methoxy groups while the doublets at 3.2 ppm, 4.05 ppm and the overlapping AB pattern doublets at 3.8 ppm can be assigned to the protons of the calixarene bridging methylene groups. The triplet at 3.1 ppm and the doublets at 3.95 ppm and 5.68 ppm represent the protons of the chlorobenzonitrile substituent. It is important to note the position of these three protons, as they are much further up field than one would normally expect to find aromatic protons. This information confirms that the product is indeed in the partial cone conformation, as part of the benzonitrile substituent lies within the aromatic annulus of the calixarene resulting in the anisotropic shielding of two of the benzonitrile protons

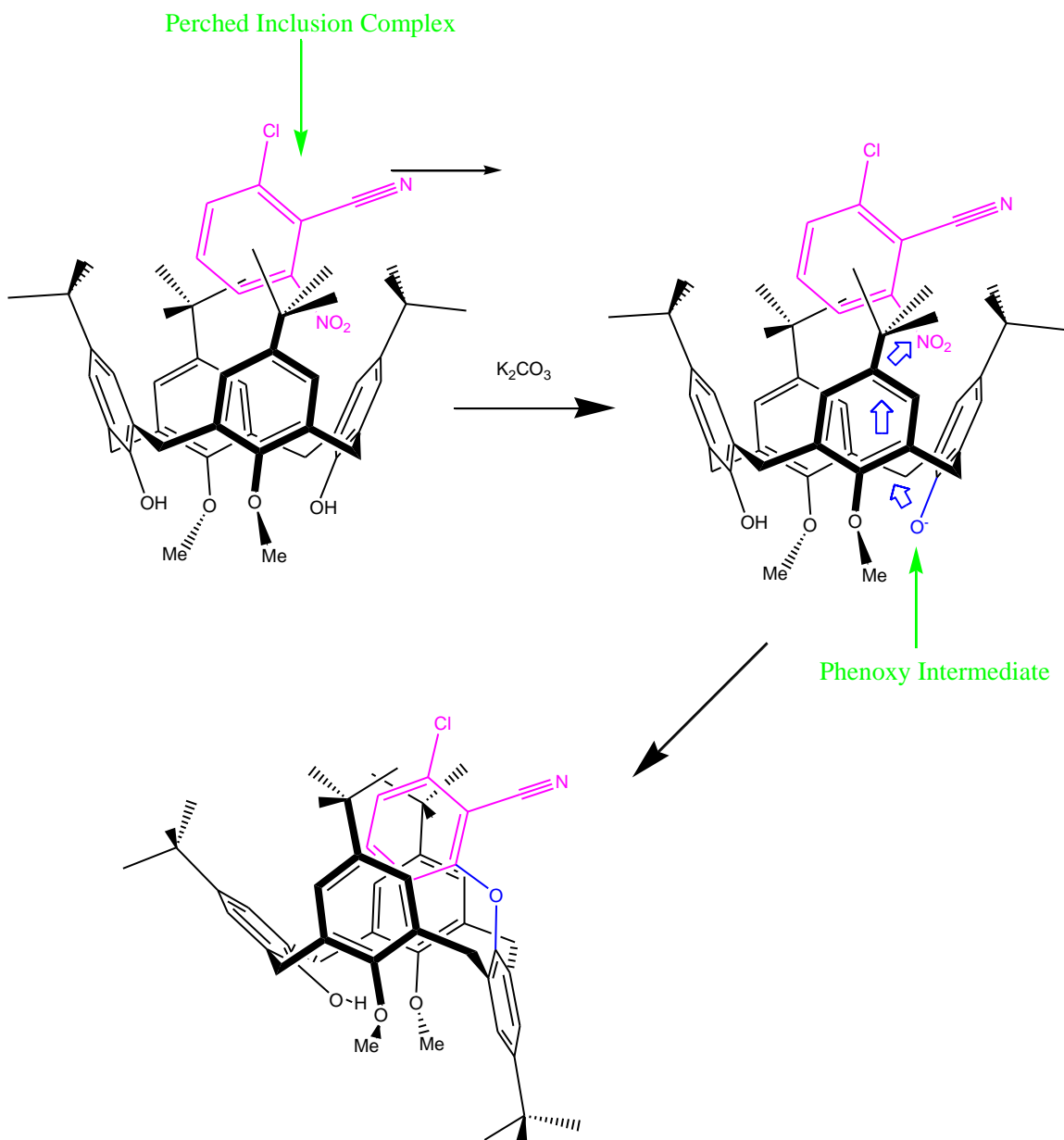
(3.95 ppm and 3.95 ppm). The shielding provided by the calixarene is greatest for the proton para to the cyano group since it points directly into ring C of the calixarene and appears at 3.1 ppm. The proton para to the chloro group is embedded a little less deep in the annulus and is therefore shifted further down field to 3.95 ppm. The third aromatic proton, para to the ether linkage on the benzonitrile substituent, sits at the rim of the calixarene and is therefore influenced only slightly by the shielding effect, thus it is found at 5.58 ppm. The remaining signals from 6.35 ppm to 7.5 ppm can be assigned to the calixarene aromatic protons.

The reaction of di-methoxycalix[4]arene with 6-chloro-2-nitrobenzonitrile to produce **10** was repeated at higher temperature, 60°C, to determine if higher yields could be procured and if higher reaction temperature would affect the conformation of the product. However, the temperature increase had no effect on either yield or reaction time (ie. the same reaction time was required at 60°C) and the product was made solely in the partial cone conformation as determined by ¹H NMR.

The application of microwave assisted synthesis for the preparation of **10** was also explored. The advantages of microwave assisted synthesis include, 1) speed with which the reaction can be completed, 2) this procedure requires a minimal quantity of solvent. The initial method employed involved using a dry mix of the reagents in a 700W microwave oven at atmospheric pressure, however no reaction took place. It was decided to mix the reagents with DMF to form a paste, under these conditions the reaction went to completion in two minutes giving **10** in a 15% yield after chromatography. The resulting product was also found to be solely in the partial cone conformation by ¹H NMR. Since the yield of **10** was lower than the conventional room temperature method, microwave assisted synthesis was not further developed for the preparation of **10**.

At this point a control experiment was carried out where phenol, in place of 1,3-dimethoxycalixarene, was treated with 6-chloro-2-nitrobenzonitrile under the same conditions used to prepare **10**. No product was produced under these conditions. From this result it would appear that for nucleophilic displacement to take place with 2-chloro-6-nitrobenzonitrile the presence of the calixarene is required, or in other words there is a 'calixarene effect' which plays a vital role in the substitution process. It first should be noted that nucleophilic substitution cannot occur on the lower rim of the dimethoxy

calixarene using 2-chloro-6-nitrobenzonitrile as substrate. Based on molecular models the methyl group substituents on the lower rim sterically interact with the ortho substituent to the nitro leaving group of 2-chloro-6-benzonitrile preventing displacement onto the lower rim from occurring. Consequently, the only possible site of reaction is on the upper rim, and this is achieved by the rotation of the phenoxide intermediate through the annulus of the calixarene (scheme 28). When the phenoxide is present within the annulus of the calixarene it becomes more reactive since solvation is absent within the calixarene annulus (a hydrophobic environment). Furthermore, it is possible for 6-chloro-2-nitrobenzonitrile to perch into the upper rim of the calixarene, where it is perfectly oriented for displacement by the calixarene phenoxide. It is for these reasons that a reaction is observed in the case of 1,3-dimethoxycalixarene and not for phenol.



Scheme 28. Mechanistic representation of partial cone formation.⁵⁸

To determine the stability of the partial cone conformation of **10** a sample was analysed by ^1H NMR at temperatures ranging from room temperature to 100°C . The results, figure 33, agreed with those previously found for a similar calix[4]arene prepared using 3-nitrophthalonitrile (as outlined in the literature review, section 1.22).

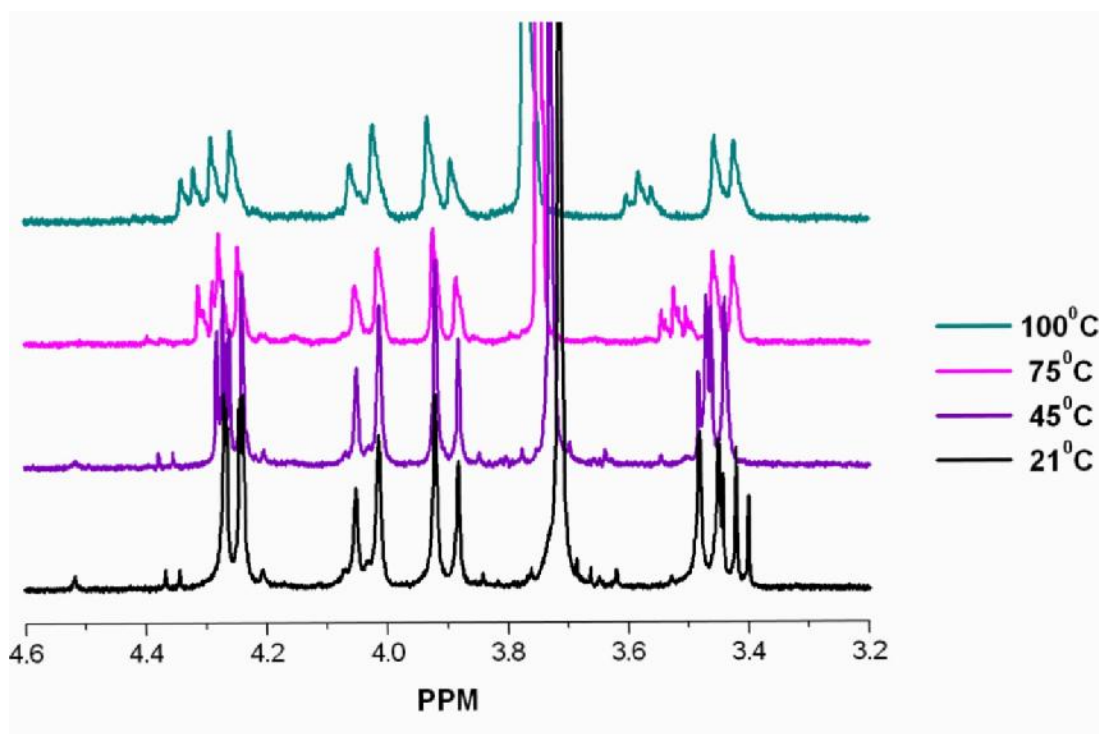
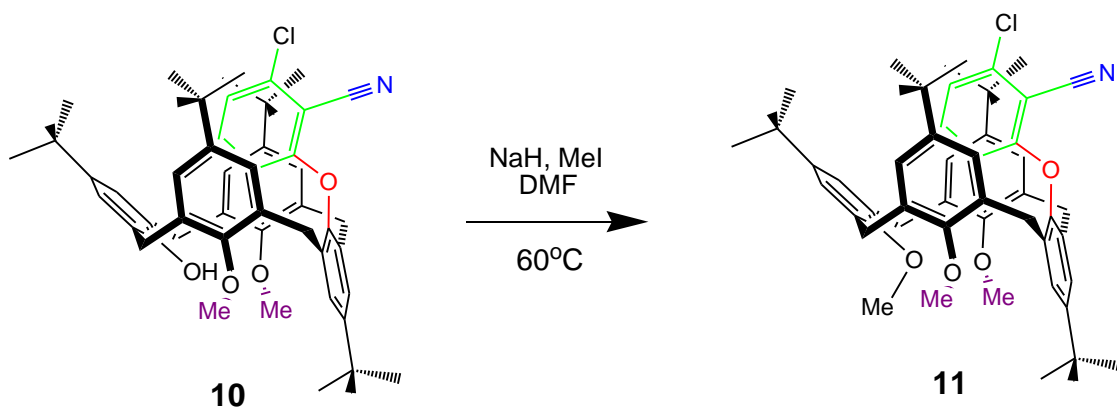


Figure 33. ^1H NMR temperature study of the self filled partial cone 5,11,17,23-tetra-*tert*-butyl-27-hydroxy-26,28-dimethoxy-25-(3-[2-chloro-benzonitrile])calix[4]arene **10**.

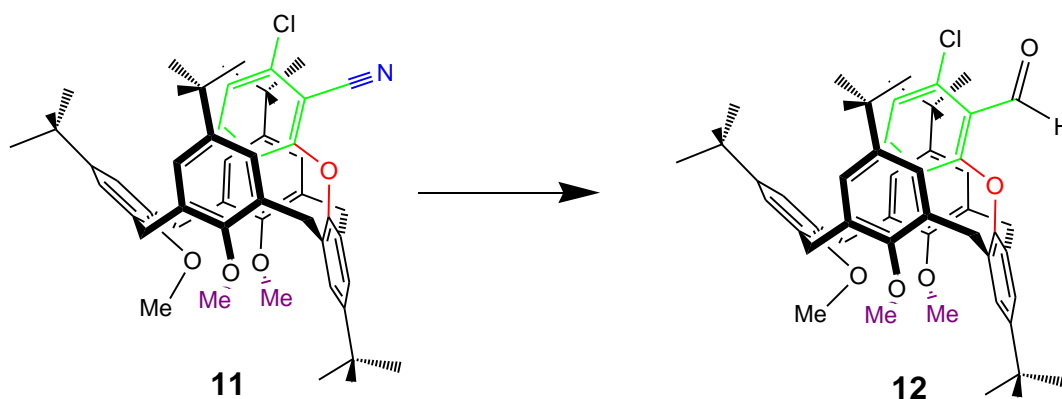
As with the phthalonitrile calix[4]arene discussed in the literature review, in this temperature study the triplet representing the aromatic benzonitrile H5 proton at 3.4ppm (at 21°C) gets shifted slightly downfield with an increase in temperature. This is thought to be due to a slight tilt of the calixarene phenyl ring (C ring).

The third step in this synthesis was the conversion of **10** to the trimethoxy calixarene **11** which was carried to prevent the phenoxy group from interfering with the reduction of the nitrile in the next stage of the synthesis. This methylation reaction required dissolving **10** and sodium hydride in a 50:50 DMF:THF solution and heating to 60°C (scheme 29). Methyl iodide was then added dropwise and the reaction was stirred for twenty four hours. The product was purified by silica gel chromatography, using a mobile phase of 90:10 hexane : ethyl acetate, which yielded 89% of **11**. It should be noted that **11** is prepared solely in the partial cone conformational, that is no conformational change is observed in this reaction.



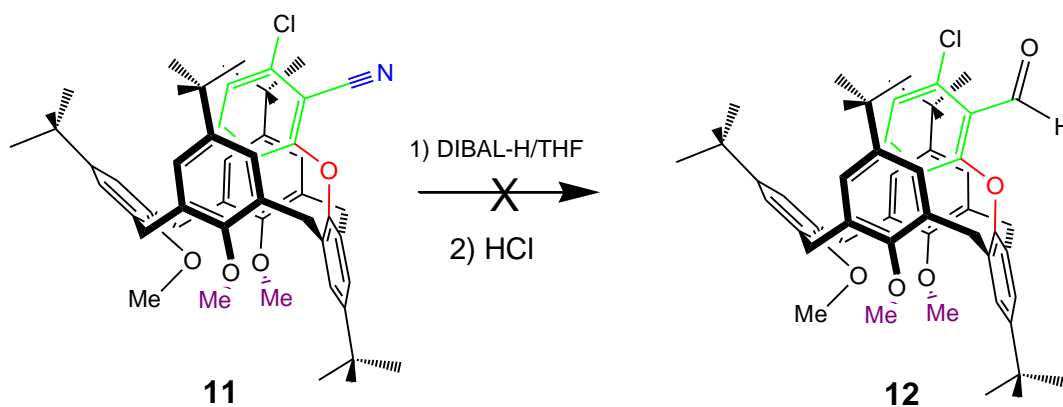
Scheme 29. *Preparation of 11.*

3.3 Reduction of 5,11,17,23-tetra-*t*-butyl-27-hydroxy-26,28-dimethoxy-25-(3-[2-chloro-benzonitrile])calix[4]arene 10 to 5,11,17,23-tetra-*t*-butyl-27-hydroxy-26,28-dimethoxy-25-(3-[2-chloro-benzaldehyde])calix[4]arene 11.



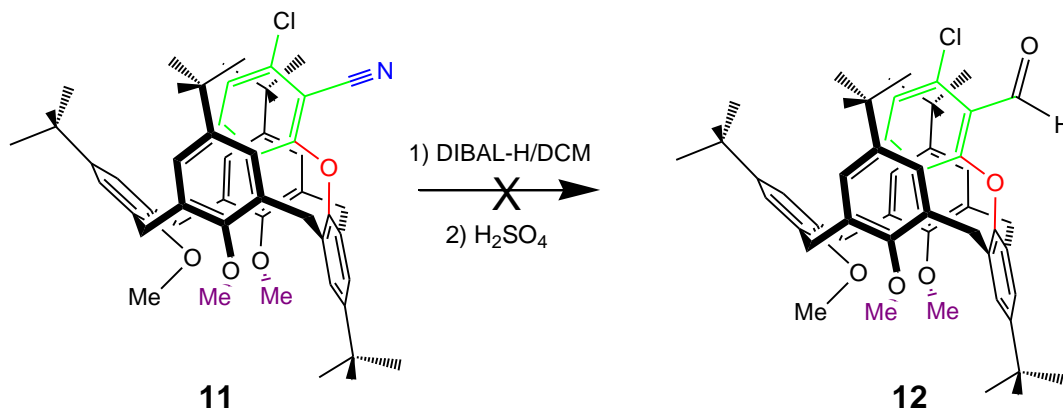
Scheme 30. *Reduction of 11 to benzaldehyde 12.*

This is the fourth and final step in the preparation of **12**. The aim is to reduce the nitrile group of the benzonitrile substituent to an aldehyde, which is required for porphyrin synthesis (scheme 30).



Scheme 31. Failure of DIBAL-H to reduce benzonitrile to benzaldehyde, method 1.

The first method of reducing the nitrile to an aldehyde involved dissolving **11** in anhydrous THF, cooling the reaction mixture to 0°C and slowly adding the reducing agent DIBAL-H, then allowing the reaction to proceed over three hours at room temperature (scheme 31). The reaction was quenched with methanol and aqueous HCl.⁵⁹ However, the ¹H NMR of the crude product showed that only starting material was recovered, that is no reduction took place via this method which was consistent with TLC analysis.



Scheme 32. Attempt using DIBAL-H to reduce benzonitrile to benzaldehyde, method 2.

In the second DIBAL-H method, the calixarene benzonitrile was dissolved in anhydrous DCM. DIBAL-H was added slowly under N₂ and the solution was heated to reflux and stirred overnight (scheme 32). The reaction solution was added to a mixture of 10% H₂SO₄ and ice and finally extracted with DCM.⁶⁰ The resulting crude product was

analysed by ^1H NMR; however once again the spectrum revealed that no reduction took place.

It was concluded that DIBAL-H is physically too large to react with the nitrile within the cavity of the calixarene thus preventing the formation of the aldehyde.

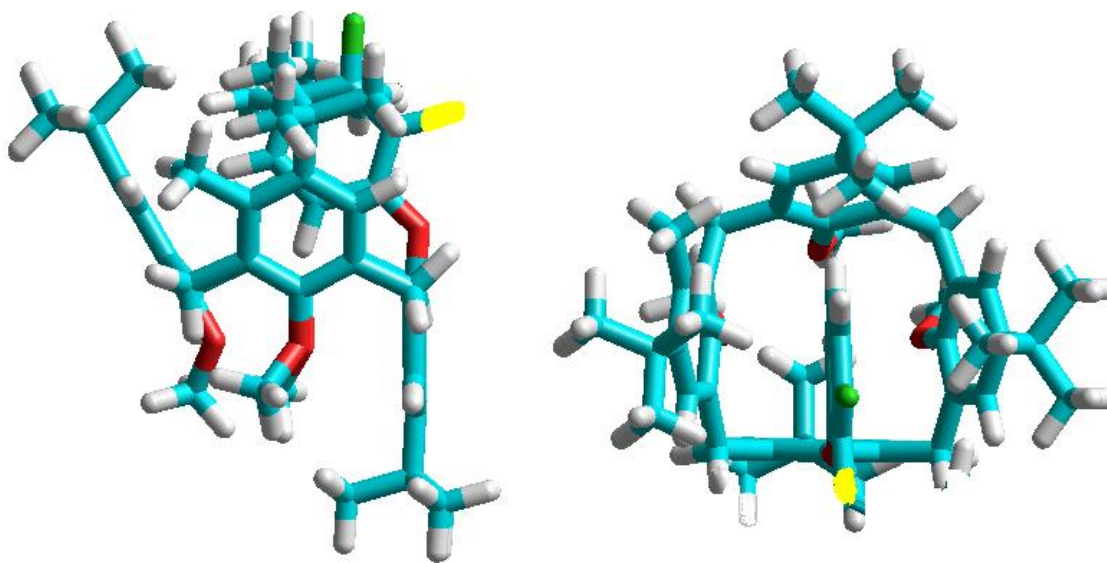
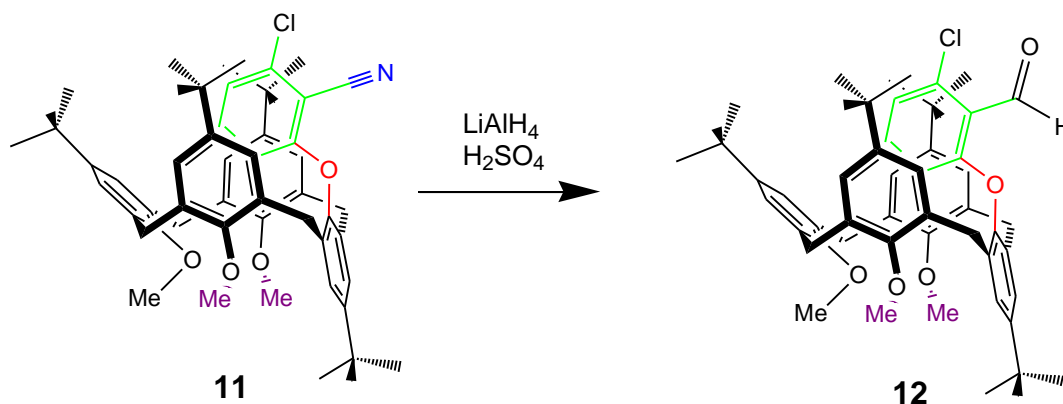


Figure 34. Hyperchem software version 7.51 illustrations of 5,11,17,23-tetra-*tert*-butyl-27-(-6-chloro-benzonitrile)-25,26,28-trimethoxy calix[4]arene **11**, viewing the calixarene basked from the side and from above respectively. The yellow feature represents the nitrile unit.

To illustrate this point the 3-D illustrations (Hyperchem software version 7.51) for **11** are shown in figure 34. It is evident that there is a high degree of congestion around the nitrile group making it difficult for the DIBAL-H to attack, and it is for this reason that the reaction fails.



Scheme 33. *Lithium aluminium hydride reduction of calixarene benzonitrile to calixarene benzaldehyde, method 3.*

A third attempted procedure for the conversion of **11** to **12** was employed using lithium aluminium hydride as the reducing agent (scheme 33). This agent is much smaller than DIBAL-H yet is documented in the literature as being sufficiently strong to reduce a nitrile group to an aldehyde, and has been exploited to this end in many papers. It is worth noting that these reactions were carried out on a small scale, usually <100 mg. The preparation involved dissolving **11** in diethyl ether and reducing the temperature to -8°C. LiAlH₄ was added slowly and the solution temperature was raised to +3°C, at which it was maintained for 1hr with stirring. The reaction solution was then quenched with 5N sulphuric acid, extracted into diethyl ether, washed with water and dried over magnesium sulphate.⁶¹ The crude product was analysed by ¹H NMR and displayed a proton signal in the aldehyde region at 9.71 ppm, indicating that partial reduction had taken place, however starting material was still present. Since both **11** and **12** have the same *rf* value by TLC, making separation impossible by chromatography, it was decided to repeat the reduction on the isolated crude product it was found after the second conversion the aldehyde signal was still present, however starting material was also present. A third attempt, using the crude product from the second attempt, failed to give any reduction, and the originally reduced aldehyde disappeared, thus a clean, isolated sample for analysis was not obtained. One possible cause for the failed reaction attempts may have been due to too much moisture in the system, which would have quenched the LiAlH₄ before it could reduce the nitrile. This effect would have been compounded due to the

small scale of the reactions (100mg). However, eliminating this issue by using new dry solvents, glassware that was oven dried and purging all glassware with N₂ prior to use did not solve the problem and after numerous attempts this preparation could not be repeated.

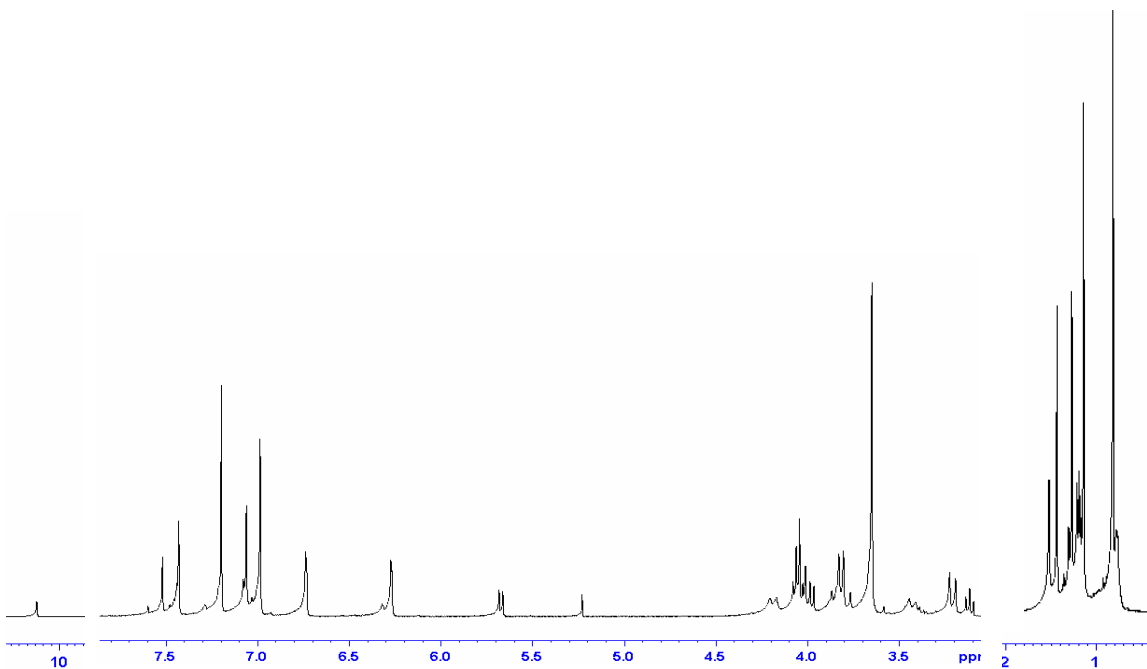
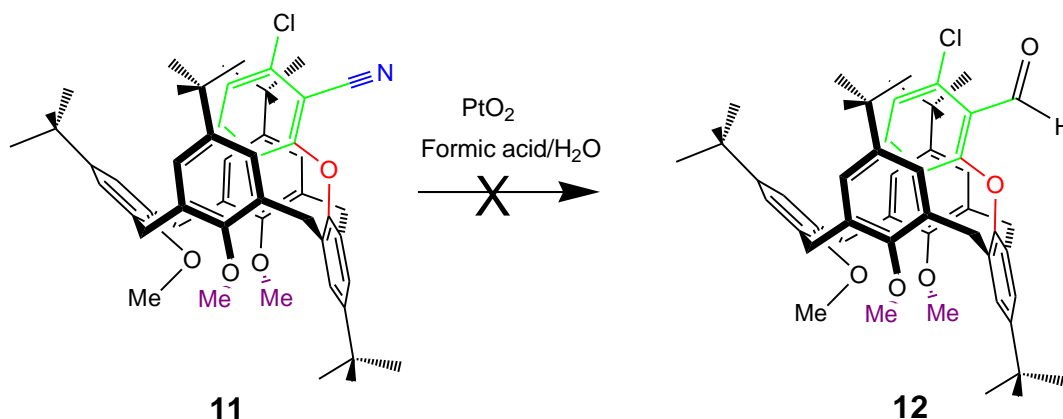


Figure 35. ¹H NMR of 5,11,17,23-tetra-*tert*-butyl-27-(6-chloro-benzaldehyde)-25,26,28-trimethoxy calix[4]arene **12** resulting from LiAlH₄ reduction of **11**.

While the ¹H NMR shown in figure 35 is not as clean as would be ideal, the protons from both the calixarene and the benzaldehyde substituent can be seen. The *tert*-butyl groups are present from 0.5 ppm – 1.5 ppm, the three methoxy units appear at 3.65 ppm, the bridging methylene protons signals appear as doublets at 3.2 ppm and 4.1 ppm and the overlapping pair of doublets appear as expected at 3.8 ppm. The benzaldehyde signals are displayed as a triplet at 3.1 ppm and doublet at 4.2 ppm. The third benzaldehyde doublet signal occurs at 5.7 ppm. The aromatic calixarene protons are present between 6.8 ppm and 7.6 ppm and the aldehyde signal is present at 10.25 ppm.



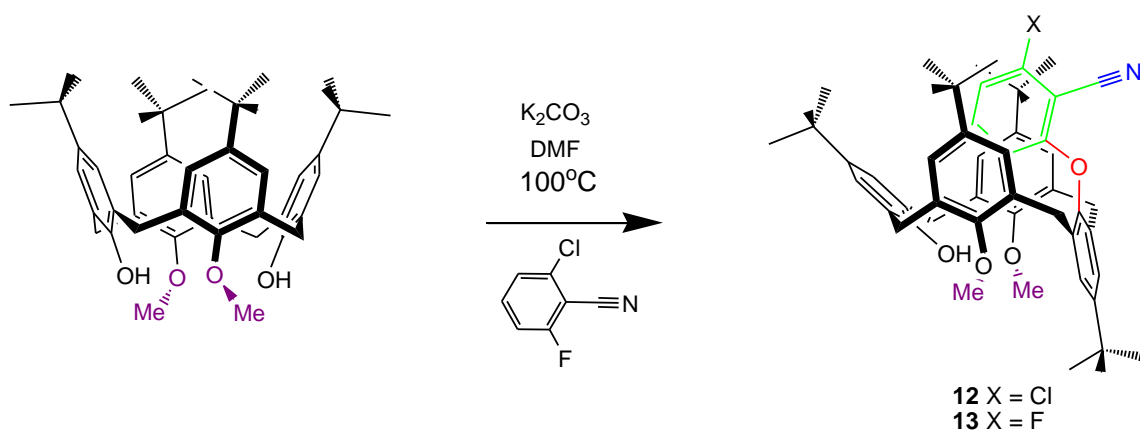
Scheme 34. Attempt using PtO_2 to reduce benzonitrile to benzaldehyde, method 4.

The fourth method explored to prepare **12** involved using platinum oxide dissolved in 80% aqueous formic acid. This method was chosen because unlike the DIBAL-H method or the $LiAlH_4$ method, this preparation was not sensitive to water or moisture. In this method, **11** and platinum oxide are dissolved in 80% formic acid and stirred at 60°C overnight (scheme 34). A water-diethyl ether extraction was carried out and the resulting solution was dried over magnesium sulphate.⁶² The 1H NMR of the crude product initially looked promising, showing a new peak at 8.6 ppm, however the addition of D_2O to the sample followed by a second 1H NMR analysis revealed that the peak at 8.6 ppm disappeared, confirming that the nitrile had been converted to the acid under these conditions.

An alternative synthetic approach to preparing the target partial cone aldehyde is to treat 1,3-dimethoxycalixarene with a series of appropriate aryl aldehydes via an S_NAr reaction. Attempts had been made previously in the Nolan group to prepare partial cone aldehydes under these conditions however. These attempts failed, however, all previous attempts carried out were at room temperature, it may be possible that this synthetic approach may work however higher temperatures may need to be employed.

The first reaction carried out was the treatment of distal-1,3-dimethoxy calixarene with 6-chloro-2-fluorobenzaldehyde (scheme 36). Reactions were set-up at three different temperature, 60°C, 80°C and 100°C in DMF. No products were observed at either 60°C or

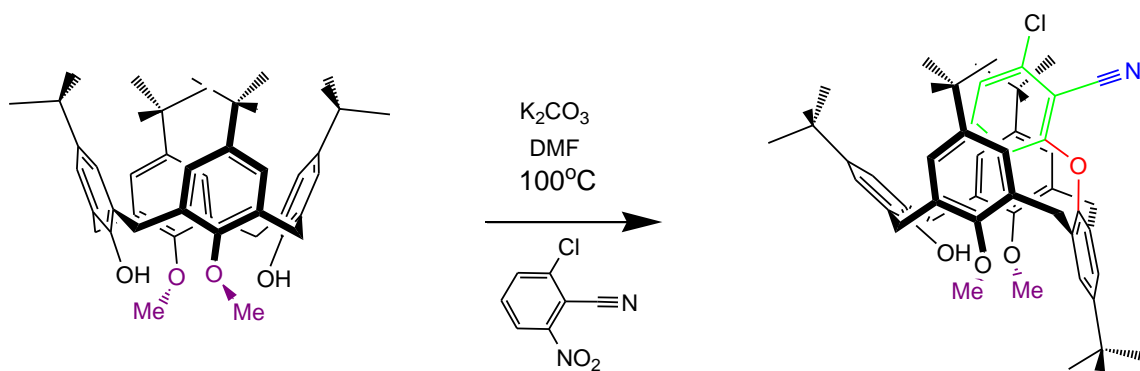
80°C after four days of reaction. However, at 100°C a new spot, albeit faint, was observed by TLC after four days. The product was isolated by silica gel chromatography using 10% ethyl acetate/hexane as eluent. The ^1H NMR of the isolated product showed the presence of three aldehyde peaks, with one of the observed peaks corresponding to the aldehyde proton of 6-chloro-2-fluorobenzonitrile (contaminant). Peaks were also observed between 3-6 ppm which are typical for calixarene, however the spectrum was rather complicated indicating the presence of more than one calixarene product.



Scheme 35. High temperature $\text{S}_{\text{N}}\text{Aryl}$ reaction to prepare aldehyde.

It is possible that this mixture consists of starting material and two calixarene aldehyde products as shown in scheme 35. It should be noted that both fluorine and chlorine are potentially good leaving groups for $\text{S}_{\text{N}}\text{Aryl}$ reactions, it is then possible at high temperature that substitution is occurring at both the 2 and 6 sites of 6-chloro-2-fluorobenzonitrile thereby giving a mixture of products **12** and **13**. Attempts to further separate this mixture were unsuccessful and considering that only 10 mg of this mixture was isolated from a 100 mg scale reaction it was decided not to pursue this approach any further.

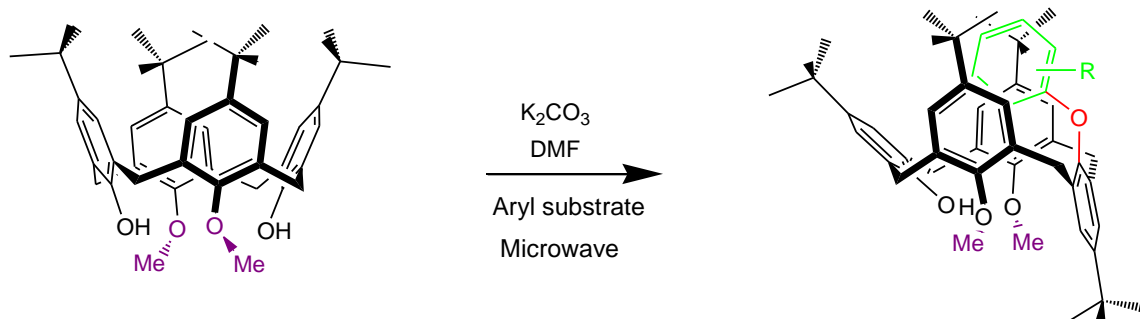
A second attempt to prepare a partial cone aldehyde was carried out using 6-chloro-2-nitrobenzaldehyde (scheme 36) at high temperature. However no observable product was found after 48 hours by TLC, this approach was not further pursued.



Scheme 36. High temperature S_NAr reaction to prepare the aryl aldehyde.

3.4 Microwave assisted synthesis of aldehyde

Finally the possibility of introducing a benzaldehyde into the calixarene by an S_NAr reaction was explored. An S_NAr substitution was attempted between distal-1,3-dimethoxy calixarene and a series of aryl substrates listed in table 5. It was hoped that the variation in the heat profile generated by the microwave assisted technique would promote the reaction to proceed where the thermal methodology had failed.⁶³ However the reactions attempted using the benzaldehyde substrates did not give any substituted calixarene products, only the reaction with 6-chloro-2-nitrobenzonitrile gave product.



Aryl Substrate	Observation
	Reaction observed – compound 10 isolated
	No Reaction
	No Reaction
	No Reaction
	No Reaction
	No Reaction

Table 5. Attempted open atmosphere microwave assisted synthesis of calixarene aldehydes.

3.5 Conclusion

The target of this work was to prepare calixarene **12**. Attempts at converting the nitrile derivative **11** to **12** using various reduction methodologies failed, only one method using $\text{LiAlH}_4/\text{H}_2\text{SO}_4$ yielded an aldehyde product as identified by ^1H NMR. However, this method was not reproducible. A high temperature thermal $\text{S}_{\text{N}}\text{Aryl}$ reaction was attempted to prepare the aldehyde directly from distal-1,3-dimethoxycalixarene, only trace amounts of an inseparable mixture of calixarene products could be isolated.

Microwave assisted $\text{S}_{\text{N}}\text{Aryl}$ displacements were attempted using various aryl aldehyde substrates and distal-1,3-dimethoxycalixarene, unfortunately, none of these reactions yielded any substituted calixarene product. Work on this project was discontinued due to time restrictions.

3.6 Experimental

5,11,17,23-tetra-tert-butyl-25,27-dihydroxy-26,28-dimethoxy calix[4]arene .

Tetra-tert-butyl-calix[4]arene (2g 3.09mmol), methyl iodide (0.75ml, 12.35mmol) and potassium carbonate (4.25g, 30mmol) were dissolved in DMF (25ml) and refluxed at 70° for 1 hr. The reaction mixture was then poured onto iced water (100ml), causing the product to crash out. The crude product was extracted with ethyl acetate (3 x 30ml). The organic layer was washed with water (30ml) then brine (30ml), dried using magnesium sulphate and rotary evaporated, This was followed by recrystallisation from ethanol. The product was adsorbed onto silica and purified by column chromatography, stationary phase silica, mobile phase Hexane: ethyl acetate 90:10, 67% yield (1.405g). The product was analysed by TLC and ¹H NMR.

Characterisation of product:

¹H NMR δ¹H (400MHz, CDCl₃ – d₆) δ (ppm) 0.800 (s, 18H, 2 x t-bu), 1.248 (s, 9H, t-bu), 1.442 (s, 9H, t-bu), 3.223 (d, 2H, J=12.8 Hz, Ph-CH₂-Ph), 3.629 (s, 6H, 2 x OMe), 3.828 (q, 4H, J=15.2 Hz, 2 x Ph-CH₂-Ph), 4.011 (d, 2H, J=9.6 Hz, Ph-CH₂-Ph), 6.266 (s, H, Ar-H), 6.734 (s, H, Ar-H), 7.056 (s, H, Ar-H), 7.508 (s, H, Ar-H).

ESI m/z: 699 (M + Na⁺)

MP: 254 - 256°C

IR: (KBr [cm⁻¹]): 3440cm⁻¹ ν(O-H); 2921cm⁻¹ ν(Bu_t)

5,11,17,23-tetra-tert-butyl-25-hydroxy-27-(2-chloro-benzonitrile)-26,28-dimethoxy calix[4]arene, 9:

5,11,17,23-tetra-tert-butyl-25,27-dihydroxy-26,28-dimethoxy calix[4]arene (0.1g, 0.14mmol), 6-chloro-2-nitrobenzonitrile (0.1g, 0.57mmol) and potassium carbonate (0.08g, 0.57mmol) were dissolved in DMF (7mls) and stirred at room temperature over one day under vacuum. The resulting solution was poured onto ice-water, and purified by silica gel chromatography from hexane : ethyl acetate 10:1, yielding a white solid, 24% yield (0.043g).

Characterisation of product:

¹H NMR δ¹H (400MHz, CDCl₃ – d₆) δ (ppm) 0.8004 (s, 18H, 2 x t-bu), 1.270 (s, 9H, t-bu), 1.432 (s, 9H, t-bu), 3.116 (t, H, J=13.2 Hz, Ar-H-CN), 3.223 (d, 2H, J=13.2 Hz,

Ph-CH₂-Ph), 3.647 (s, 6H, 2 x OMe), 3.827 (q, 4H, J=9.6 Hz, 2 x Ph-CH₂-Ph), 3.966 (d, H, J=8.4 Hz, Ar-H-CN), 4.043 (d, 2H, J=12.8 Hz, Ph-CH₂-Ph), 5.683(d, H, J=8.4 Hz, Ar-H-CN), 6.267 (s, H, Ar-H), 6.734 (s, H, Ar-H), 7.056 (s, H, Ar-H), 7.508 (s, H, Ar-H).

ESI m/z: 834 (M + Na⁺)

IR: (KBr [cm⁻¹]): 3330cm⁻¹ ν (O-H); 2921cm⁻¹ ν (Bu), 2218cm⁻¹ ν (CN)

MP: 249 - 251°C

Elemental analysis: C₅₃H₆₂O₄NCl; Calculated: %C = 78.35; %H = 7.69; %N = 1.72
Found: %C = 78.39; %H = 7.84; %N = 1.63

Microwave Synthesis - General Method:

5,11,17,23-tetra-tert-butyl-25,27-dihydroxy-26,28-dimethoxy calix[4]arene (0.1g, 0.14mmol), 6-chloro-2-nitrobenzonitrile (0.1g, 0.57mmol) and potassium carbonate (0.08g, 0.57mmol) were dissolved in 2 drops of DMF in an open glass vial and placed in a 700W domestic microwave oven on full power, at atmospheric pressure for 1min, 2min and 4min. The resulting paste was purified by silica gel chromatography from hexane : ethyl acetate 10:1, yielding a white solid (15% yield). The isolated product was characterised by ¹H NMR, resulting in the same spectral data as that given above for the conventional thermal method of synthesis.

The same stoichiometric ratios were used in all reactions

5,11,17,23-tetra-tert-butyl-27-(-6-chloro-benzonitrile)-25,26,28-trimethoxy calix[4]arene 10:

A mixture of 5,11,17,23-tetra-tert-butyl-25-hydroxy,27-(-6-chloro-benzonitrile)-26,28-dimethoxy calix[4]arene (0.06g, 0.08mmol) and sodium hydride (0.005g, 0.250mmol) were dissolved in THF:DMF (50:50, 7mls) and heated to 60°C under N₂. Methyl iodide (3.5mls, 6mmol) was added slowly and the reaction solution was stirred over one day. The resulting solution was poured in ice water, extracted with dichloromethane and dried over magnesium sulphate. The solvent was removed and the crude product was purified by silica gel chromatography. Mobile phase hexane : ethyl acetate 90:10, yielding a white solid in 89% yield, (0.049g). The product was analysed by TLC and ¹H NMR.

Characterisation of product:

¹H NMR $\delta^1\text{H}$ (400MHz, $\text{CDCl}_3 - d_6$) δ (ppm) 0.787 (s, 18H, 2 x t-bu), 1.292 (s, 9H, t-bu), 1.442 (s, 9H, t-bu), 3.068 (t, H, $J=13.2$ Hz, Ar-H-CN), 3.284 (d, 2H, $J=13.2$ Hz, Ph-CH₂-Ph), 3.497 (s, 3H, OMe), 3.582 (s, 6H, 2 x OMe), 3.729 (q, 4H, $J=9.6$ Hz, 2 x Ph-CH₂-Ph), 4.022 (d, H, $J=8.4$ Hz, Ar-H-CN), 4.157 (d, 2H, $J=12.8$ Hz, Ph-CH₂-Ph), 5.631 (d, H, $J=8.4$ Hz, Ar-H-CN), 6.163 (s, H, Ar-H), 6.598 (s, H, Ar-H), 7.112 (s, H, Ar-H), 7.435 (s, H, Ar-H).

ESI m/z: 843 (M + NH₃), 848.8 (M + Na⁺)

MP: 237 - 239°C

5,11,17,23-tetra-tert-butyl-27-(-6-chloro-benzaldehyde)-25,26,28-trimethoxy calix[4]arene 11.

Attempted Method #1 ⁶⁴

5,11,17,23-tetra-tert-butyl-27-(-6-chloro-benzonitrile)-25,26,28-trimethoxy calix[4]arene (0.08g, 0.115mmol) was dissolved in anhydrous THF (5mls), cooled to 0°C and DIBAL-H (1.0 M solution in hexane, 0.02ml) was added dropwise with stirring. The solution was allowed to warm to room temperature and stirred for three hours. The solution was again cooled to 0°C and treated with MeOH (0.5mls) and stirred for five minutes. The reaction solution was next poured into 0.5N aqueous HCl (15mls) followed by extraction with ethyl acetate. The organic layer was washed with water and brine and dried over magnesium sulphate. The resulting solution was analysed by TLC and ¹H NMR.

Attempted Method #2 ⁶⁵

5,11,17,23-tetra-tert-butyl-27-(-6-chloro-benzonitrile)-25,26,28-trimethoxy calix[4]arene (0.06g, 0.08mmol) was dissolved in anhydrous DCM, DIBAL-H (1.0M solution in hexane, 0.053mls) was added slowly under N₂ and the solution heated to reflux and stirred over night. The reaction solution was added to a mixture of 10% H₂SO₄ and ice and extraction was carried out with DCM. Magnesium sulphate was used as a drying

agent and the remaining solvent was evaporated off. The resulting solution was analysed by TLC and ^1H NMR

Attempted Method #3 ⁶⁶

5,11,17,23-tetra-tert-butyl-27-(-6-chloro-benzonitrile)-25,26,28-trimethoxy calix[4]arene (0.055g, 0.076mmol) and platinum oxide (0.0018g, 0.008mmol) were dissolved in 80% aqueous formic acid (3mls) and stirred at 60°C over night. A water - diethyl ether extraction was carried out and the resulting solution was dried over magnesium sulphate. The resulting crude product was analysed by TLC and ^1H NMR.

Characterisation of crude product:

^1H NMR $\delta^1\text{H}$ (400MHz, $\text{CDCl}_3 - d_6$) δ (ppm) 0.786 (s, 18H, 2 x t-bu), 1.290 (s, 9H, t-bu), 1.440 (s, 9H, t-bu), 2.589 (t, H, $J=13.2$ Hz, Ar-H-CN), 3.099 (d, 2H, $J=13.2$ Hz, Ph- CH_2 -Ph), 3.740 (s, 9H, 3 x OMe), 3.924 (q, 4H, $J=9.6$ Hz, 2 x Ph- CH_2 -Ph), 4.054 (d, 2H, $J=12.8$ Hz, Ph- CH_2 -Ph), 4.184 (d, H, $J=8.4$ Hz, Ar-H-CN), 5.631 (d, H, $J=8.4$ Hz, Ar-H-CN), 6.162 (s, H, Ar-H), 6.601 (s, H, Ar-H), 7.112 (s, H, Ar-H), 7.438 (s, H, Ar-H), 8.572 (s, H, Ar-COOH).

Attempted Method #4 ⁶⁷

5,11,17,23-Tetra-tert-butyl-27-(-6-chloro-benzonitrile)-25,26,28-trimethoxy calix[4]arene (0.077g, 0.107mmol) was dissolved in diethyl ether (2mls) and brought to -8°C. Lithium aluminium hydride (0.11mls, 0.107mmol) was added and the solution was brought to +12°C and then back down to +3°C, at which it was maintained for 1hr with stirring. The reaction solution was then quenched with 5N sulphuric acid, extracted and washed with diethyl ether and dried over magnesium sulphate. The product was analysed by TLC and ^1H NMR.

Characterisation of crude product:

^1H NMR $\delta^1\text{H}$ (400MHz, $\text{CDCl}_3 - d_6$) δ (ppm) 0.787 (s, 18H, 2 x t-bu), 1.292 (s, 9H, t-bu), 1.442 (s, 9H, t-bu), 3.068 (t, H, $J=13.2$ Hz, Ar-H-CN), 3.254 (d, 2H, $J=13.2$ Hz, Ph-

CH₂-Ph), 3.682 (s, 9H, 3 x OMe), 3.729 (q, 4H, J=9.6 Hz, 2 x Ph-CH₂-Ph), 4.157 (d, 2H, J=12.8 Hz, Ph-CH₂-Ph), 4.222 (d, H, J=8.4 Hz, Ar-H-CN), 5.681 (d, H, J=8.4 Hz, Ar-H-CN), 6.293 (s, H, Ar-H), 6.758 (s, H, Ar-H), 7.112 (s, H, Ar-H), 7.435 (s, H, Ar-H), 10.310 (s, H, Ar-CHO).

High temperature attempt to prepare 125,11,17,23-tetra-tert-butyl-25-hydroxy-27-(-2-chloro-benzonitrile)-26,28-dimethoxy calix[4]arene, 9:

5,11,17,23-tetra-tert-butyl-25,27-dihydroxy-26,28-dimethoxy calix[4]arene (0.1g, 0.14mmol), 6-chloro-2-nitrobenzonitrile (0.1g, 0.57mmol) and potassium carbonate (0.08g, 0.57mmol) were dissolved in DMF (7mls) and stirred at 100°C over four days under vacuum. The resulting solution was poured onto ice-water, and purified by silica gel chromatography from hexane : ethyl acetate 10:1, yielding a trace of white solid, (5 mg).

Bibliography

- ¹ Christie R. M.; *Dyes and Pigments*, **1995**, 27 (1), 35.
- ² *Phthalocyanines - Properties and Applications*, Leznoff, C. C. and Lever, A. P. B., Eds.; VCH: Vol. 1, **1989**; Vol. 2, **1992**; Vol. 3, **1993**; Vol. 4, **1996** New York; C. C. Leznoff;.
- ³ Kadish K. M.; Smith K. M.; Guillard R.; Eds.; *The Porphyrin Handbook, Phthalocyanine Synthesis, 97 Synthesis of Phthalocyanine Precursors*, Elsevier Science – Boston, **2002**, 15, ch1 p5-6, ch2 p64.
- ⁴ Neil McKeown – *Phthalocyanine Materials Synthesis, Structure and Function* – Cambridge University Press, **1998**, pg 19.
- ⁵ Kadish K. M.; Smith K. M.; Guillard R.; Eds.; *The Porphyrin Handbook, Phthalocyanine Synthesis, 97 Synthesis of Phthalocyanine Precursors*, Elsevier Science – Boston, **2002**, 15, ch1 p 6, ch2 p63-64.
- ⁶ Hu, M.; Brasseur, N.; Yildiz S. Z.; Van Lier, J. E.; Leznoff, C.C. *J. Med. Chem.*, **1998**, 41, 1789.
- ⁷ *Syntheses of Metal-free Substituted Phthalocyanines in Phthalocyanines-Properties and Applications*, Leznoff, C. C. and Lever, A. P. B. Eds.; VCH: New York, **1989**, Vol. 1, Chapter 1.
- ⁸ Nevin, W. A.; Liu, W.; Greenberg, S.; Hempstead, M. R.; Marcuccio, S. M.; Melnik, M.; Leznoff, C. C.; Lever, A. B. P. *Inorg. Chem.*, **1987**, 26, 891.
- ⁹ Mamardashvili N. Zh.; Koifman O.I.; *Russian J. Org. Chem.*, **2005**, 41(6), 787.
- ¹⁰ Kobayashi N.; Sasaki N.; Higashi Y.; Osa T.; *Inorg. Chem.*, **1995**, 34, 1636.
- ¹¹ Gonzalez-Cabello A.; Vaquez P.; Torres T; Guldi. D. *J. Org. Chem.*, **2003**, 68, 8635; Nicolau M.; Cabezon B; Torres T. *J. Org. Chem.*, **2001**, 66, 89; Sharman W.M.; Allen C.M.; van Lier J.E. *DDT.*, **1999**, 4, 507.
- ¹² Konami H.; Ikeda Y.; Hatano M.; Mochizuki K. *Mol. Phy.*, **1993**, 153
- ¹³ Gutsche, C. D. *Calixarenes in 'Monographs in Supramolecular Chemistry'*; Stoddart, J. F., Ed.; Royal Society of Chemistry; **1989** ; Gutsche, C. D. *Calixarenes Revisited in 'Monographs in Supramolecular Chemistry'*; Stoddart, J. F., Ed.; Royal Society of

Chemistry; **1998** (c) Scully P. A.; Hamilton T. M.; Bennett J. L.; *J. Org. Lett.*, **2001**, 3, 2741.

¹⁴ Takashi Arimura; Seiji Ide; Yasuhiro Suga; Takuya Nishioka; Shigeo Murata; M. Tachiya; Toshihiko Nagamura; Hiroshi Inoue; *J. Am. Chem. Soc.*, **2001**, 123, 10744.

¹⁵ McMahon G.; Wall R.; Nolan K.; Diamond D.; *Talanta*, **2002**, 57, 1119.

¹⁶ Gutsche C. D.; *Calixarenes in "Monographs in Supramolecular Chemistry"*, **1989**, p108.

¹⁷ Gutsche D. C.; *Calixarenes Revisited*, **1998**, p10.

¹⁸ Diamond D.; Nolan K. *Analytical Chemistry*, **2001**, 73, 23A.

¹⁹ Khoury R. G.; Jaquinod L.; Aoyagi K.; Olmstead M.; Fisher A. J.; Smith K. M.; *Angew. Chem. Int. Ed. Engl.*, **1997**, 36(22), 2497.

²⁰ O'Malley, S.; Alhashimy, N.; O'Mahony, J.; Kieran, A.; Pryce, M.; Nolan, K.; *Tet. Lett.*, **2007**, 48, 681.; Benjamin Schazmann B.; O'Malley S.; Nolan K.; Diamond D. *Supramolecular Chemistry*, **2006**, 18 (6), 515; O'Malley S.; Schazmann B.; Diamond D.; Nolan K. *Tet. Lett.*, **2007**, 48, 9003.

²¹ Gutsche D. C.; *Calixarenes Revisited*, **1998**, p83.

²² Gutsche D. C.; *Calixarenes Revisited*, **1998**, p49.

²³ Chowdhury S.; Georghiou P. E.; *J. Org. Chem.*, **2001**, 66, 6275-6262

²⁴ Mitton D.; Ackroyd R.; *Photodiagnosis and Photodynamic Therapy*, **2005**, 2, 239.

²⁵ Gray I.; *PhD Thesis – Synthesis of New 5-Fluorouracil Photosensitizer Conjugates for use as Prodrugs in the Photodynamic Therapy of Cancer*, DCU, **2004**, p37.

²⁶ Zeitouni N. C.; Oseroff A. R.; Shieh S.; *Molecular Immunology*, **2003**, 39, 1133.

²⁷ Nolan K. J.; *Adv. Med. Chem. Lectures Notes (CS414) - Dublin City University*, **2003**.

²⁸ Gray I.; *PhD Thesis*, DCU, **2004**, p39.

²⁹ Piette J.; Volanti C.; Vantieghem A.; Matroule J. Y.; Habraken Y.; Agostinis P.; *Biomed. Pharmacol.*, **2003**, 66 (8), 1651.

³⁰ Van Dongen G. A. M. S.; Visser G. W. M.; Vrouenraets M. B.; *Adv. Drug Delivery Rev.*, **2004**, 56 (1), 31.

³¹ Foley E.; O'Farrell P. H.; *Genes & Development*, **2003**, (17), 115.

³² N. Y. Ann, *Acad. Sci.*, **2002**, 962, 360-71 12076987.

-
- ³³ Chung P.; Cook T.; Fiu K.; Vodovotz Y.; Zamora R.; Finelstein S.; Billiar T.; Blumberg D.; *Nitric Oxide*, **2003**, 8, 119.
- ³⁴ Conrado C. L.; Weckslar S.; Egler C.; Magde D.; Ford P. C.; *Inorg. Chem., Am Chem. Soc.*, **2004**, 43 (18), 5543.
- ³⁵ Heidleberger C.; Chaudihuri N. K.; Danneberg P.; Mooren D.; Griesbach L.; *Nature*, **1957**, 179, 663.
- ³⁶ Kassam Z.; Lockwood G.; O'Brien C.; Brierley J.; Swallow C.; Oza A.; Siu L.; Knox J. L.; Wong R.; Cummings B.; Kim J.; Moore M.; Ringash J.; *Int. J. Radiation Oncology Bio. Phys.*, **2006**, 65(3), 713
- ³⁷ Li J.; Minnich D. J.; Camp E. R.; Brank A.; MacKay S. L. D.; Hochwald S. N.; *J. Surgical Research*, **2006**, 132 (1), 112
- ³⁸ Hitzman C. J.; Elmquist W. F.; Wattenberg L. W.; Wielmann T. S.; *J. Pharm. Sci.*, **2006**, 96 (5), 1114.
- ³⁹ Chua Y. J.; Cunningham D.; *Best Practice & Best Clinical Gastroenterology*, **2006**, 20 (2), 327.
- ⁴⁰ Rosowsky A.; Kim S. H.; Wick M.; *J. Med. Chem.*; **1981**, 24, 1177.
- ⁴¹ Shibamoto Y.; Zhou L.; Hatta H.; Mori M.; Nishimoto S.; *Int. J. Radiat. Oncol. Biol. Phys.*, **2001**, 49 (2), 407.
- ⁴² Kaldirkyan V.; Geboyany A.; Ter-Zakharyan Z.; Paronikyan V. R.; Garibdzhanyan B. T.; Stepanyan G. M.; Paronikyan G. M.; Akopyan L. G.; *Pharm. Chem. J.*, **1986**, 20 (8), 540
- ⁴³ Mori M.; Hatta H.; Nishimoto S.; *J. Org. Chem.*, **2000**, 65, 4641.
- ⁴⁴ Hay M. P.; Sykes B. M.; Denny W. A.; O'Connor C. J.; *J. Chem. Soc. Perkin Trans.*, **1999**, 1, 2759.
- ⁴⁵ Hu L.; Liu B.; Hacking D. R.; *Bioorg. Med. Chem. Lett.*, **2000**, 10, 797.
- ⁴⁶ Aisling Redmond *PhD Thesis*, June **2007**, UCD.
- ⁴⁷ Hay M. P.; Sykes B. M.; Denny W. A.; O'Connor C. J.; *J. Chem. Soc. Perkin Trans.*, **1999**, 1, 2759
- ⁴⁸ Hay M. P.; Sykes B. M.; Denny W. A.; O'Connor C. J.; *J. Chem. Soc. Perkin Trans.*, **1999**, 1, 2759.
- ⁴⁹ Hu L.; Liu B.; Hacking D. R.; *Bioorg. Med. Chem. Lett.*, **2000**, 10, 797.

-
- ⁵⁰ Hay M. P.; Sykes B. M.; Denny W. A.; O'Connor C. J.; *J. Chem. Soc. Perkin Trans.*, **1999**, 1, 2759.
- ⁵¹ Gray I.; PhD Thesis, Dublin City University, **2004**.
- ⁵² Joan V. Bakboord, Michael J. Cook and Esin Hamuryudan - *Journal of Porphyrins and Phthalocyanines*, **2000**, 4, 510.
- ⁵³ Kadish K. M.; Smith K. M.; Guillard R.; Eds.; *The Porphyrin Handbook, Phthalocyanine Synthesis, 97 Synthesis of Phthalocyanine Precursors*, Elsevier Science – Boston, **2002**, Chapter 1 and 2.
- ⁵⁴ McKeon N. B.; *Phthalocyanine Materials*, **1998**, p89-94.
- ⁵⁵ Kieran Nolan, PhD Thesis, **1996**, York University.
- ⁵⁶ J. V. Bakboord, Michael J. Cook and Esin Hamuryudan - *Journal of Porphyrins and Phthalocyanines*, **2000**, 4, 510.
- ⁵⁷ Gutsche D. C.; *Calixarenes Revisited*, **1998**, p83.
- ⁵⁸ O' Malley S.; *PhD Thesis – Synthesis of Architecturally Novel Calix[4]arene Phthalocyanines*, **2006**, p59.
- ⁵⁹ Murugeasan N.; Gu Z.; Fadnis L.; Tellew J. E.; Baska R. A. F.; Beyer S. M.; Monshizadegan H.; Dickenson K. E.; Valentine M. T.; Griffith Humphries L.; Lan S.-J.; Ewing W. R.; Carlson K. E.; Kowala M. C.; Zahler R.; Macor J. E.; *J. Med. Chem.*, **2005**, 48 (1), 171-179.
- ⁶⁰ Kang Z.; Dykstra C. C.; Boykin D. W.; *Molecules*, **2004**, 9, 158-163.
- ⁶¹ Brown H. C.; Grag, C. P.; *J. Am. Chem. Soc.*, **1964**, 86, 1085-1089.
- ⁶² Xi F.; Kamal F.; Schenerman M. A.; *Tet. Lett.*, **2002**, 43, 1395-1396.
- ⁶³ O'Malley S.; Alhashimy N.; O'Mahony J.; Kieran A.; Pryce M.; Nolan K.; *Tet. Lett.*, **2007**, 48 (4), 681-684.
- ⁶⁴ Murugeasan N.; Gu Z.; Fadnis L.; Tellew J. E.; Baska R. A. F.; Beyer S. M.; Monshizadegan H.; Dickenson K. E.; Valentine M. T.; Griffith Humphries L.; Lan S.-J.; Ewing W. R.; Carlson K. E.; Kowala M. C.; Zahler R.; Macor J. E.; *J. Med. Chem.*, **2005**, 48 (1), 171-179.
- ⁶⁵ Kang Z.; Dykstra C. C.; Boykin D. W.; *Molecules*, **2004**, 9, 158-163.
- ⁶⁶ Xi F.; Kamal F.; Schenerman M. A.; *Tet. Lett.*, **2002**, 43, 1395-1396.

⁶⁷ Brown H. C.; Grag, C. P.; *J. Am. Chem. Soc.*, **1964**, 86, 1085-1089.