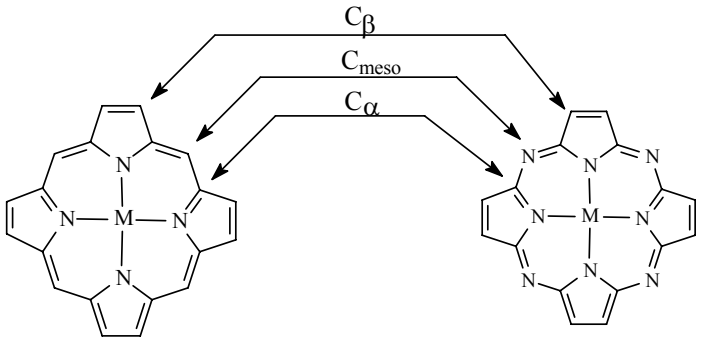
CHAPTER I

INTRODUCTION

Porphyrazines share structural similarities with porphyrins and phthalocyanines. They have diverse applications such as biomedical agents for diagnosis and therapy, precursors to new conducting materials, chemical sensors, ladder polymers and dyes.[1] Of much interest to this class of compounds is the role they play as potential photosensitisers in the treatment of tumours, a process known as photodynamic therapy (PDT).[2-5] A continuing problem in PDT is selectivity of uptake of the porphyrin into cancerous tissue versus healthy tissue. A potential solution to this problem would be to functionalise the photosensitiser with biological molecules such as carbohydrates (e.g. a ribose derivative) to assist in its selective uptake and fluid solubility.

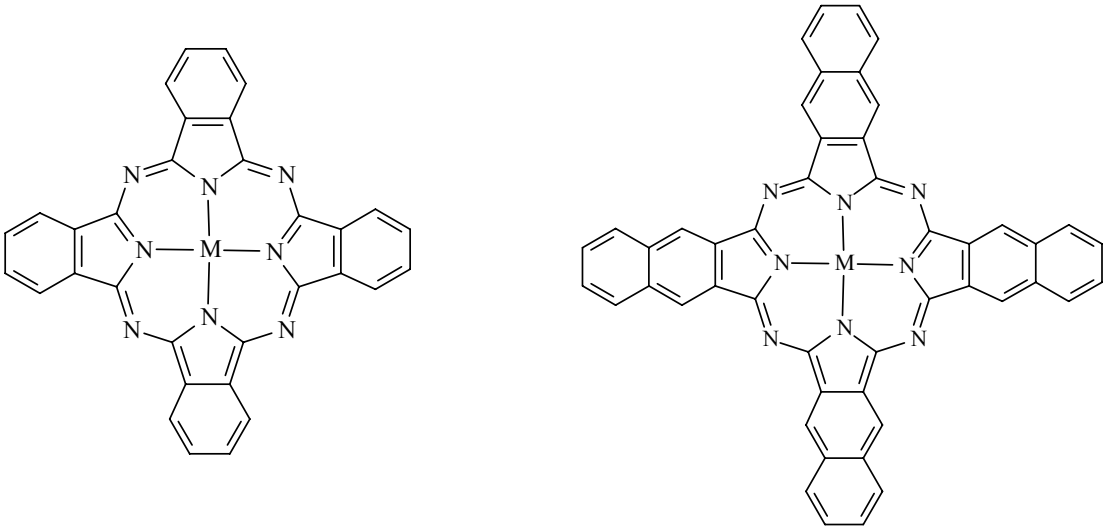
1.1 Tetrapyrrole-macrocycles

The most widely studied tetrapyrrole-macrocycles (photosensitisers) are porphyrins and phthalocyanine derivatives.[6,7] Fig. 1.1 shows the basic structures of porphyrin **1**, porphyrazine **2**, phthalocyanine **3** and naphthalocyanine **4**. Their diverse structures with unique properties and wide distribution in nature are significant factors that govern their importance and potential applications.



1 **2**

porphyrin *porphyrazine*



3 **4**

phthalocyanine *naphthalocyanine*

Figure 1.1: Tetrapyrrole-macrocycles (M=Metal or H_2)

Porphyrazines **2** (Figure 1.1) are distinguished from phthalocyanines (tetrabenzo-porphyrazines) **3** by the presence of benzo rings fused to the pyrrole rings in the latter. On the other hand, naphthalocyanines (tetranaphthalo-porphyrazines) **4** differ from porphyrazines **2** by the presence of naphthalene rings fused to pyrrole rings in the former. All porphyrins, porphyrazines, phthalocyanines and naphthalocyanines share a common macrocyclic substructure which consists of four pyrrolic subunits. Porphyrazines also contain a 22-π electron system present in porphyrins, a feature that is critical for effecting a wide range of extraordinary properties such as the ability to absorb visible light, to mediate the conversion of absorbed light to other forms of chemical and physical energy, and to enhance thermodynamic and kinetic stability. These conjugated systems assume many resonance forms and can accept substituents at a number of positions.

While phthalocyanines and naphthalocyanines are porphyrazine derivatives, porphyrins differ from porphyrazines only in the *meso*-positions, wherein porphyrins have methine bridges and porphyrazines aza bridges. So far, many photosensitisers bearing numerous

and bulky substituents have been synthesized in literature.[6] A porphyrin has twelve positions available for substitution including eight β positions and four *meso*-positions. On the other hand, porphyrazines, phthalocyanines, and naphthalocyanines have 8, 16 and 24 positions available for substitution, respectively.

Tetrapyrrole-macrocycles occur widely in nature with many important biological representatives and these include haems, chlorophylls and vitamin B_{12} for porphyrins amongst several others.[8] Haem is responsible for the transport of oxygen while chlorophyll is useful for the transformation of light into chemical energy in plants during photosynthesis. In the blood, most of the porphyrins are in the red cells in very small quantities as protoporphyrin and coproporphyrin.[9] The analysis of these porphyrinic products in urine and excreta forms the basis for the diagnosis of many liver diseases.

Porphyrins were originally studied for their importance in oxygen transport, photosynthesis, energy production, metabolism and the disease porphyria.[10] There are a number of synthetic porphyrins prepared for several purposes ranging from basic research to functional applications in society.[11-13] Photofrin, for example, is used for treatment of viral infections and cancer.[11,14]

Other porphyrins are used as commercial oxidation catalysts to make fine chemicals and have applications in controlled-polymer synthesis.[15-18] In addition to these current uses, there have been several hundred patents issued in the past few years for the use of porphyrins in molecular electronics and applications in novel functional materials. Metalated porphyrins are able to mediate reactions such as epoxidation, cleavage of amides alkane hydroxylation, Diels-Alder cycloadditions and cyclopropanation.[18,19] There has also been an interest in using porphyrins to make conjugated polymers with unusual electronic properties.[6b]

Conjugated porphyrin polymers are low band-gap organic semi-conductors and this high polarisibility, intense absorption in the near infra-red and strong optical non-linearity make them interesting materials for fabricating ultra-fast telecommunication

switches.[20,21] An exciting challenge in this area is to use non-covalent interactions to control the electronic properties of these polymers, by forming multiple strands and thus restricting torsional freedom.

Since their discovery, phthalocyanines have been mostly used as dyes and pigments because of their blue-green colour, high dying power, chemical inertness, photostability and insolubility in most solvents.[22] Phthalocyanines also show enhanced electrical conductivity, discotic mesophase formation, electrochromism and some sensitivity as PDT agents.[13,23] They are used as catalysts in a number of reactions and as electrocatalysts in the reduction of oxygen at fuel cell electrodes.[22,24] Recently, phthalocyanines have found high-tech applications in ink jet printing and as photoconducting agents in photocopying machines, as laser light absorbers and for optical data storage systems. Others are promising candidates for exploitation in machines such as solar cells, gas sensors and optical limits.[25,26]

Porphyrazines, which are the latest in the discovery of tetrapyrrole-macrocycles among porphyrins and phthalocyanine, are now the subject of increasing importance and interest in their synthesis, properties and application.[27-29] Their applications involve diverse areas such as biomedical agents for diagnosis and therapy, precursors to new conducting materials, chemical sensors, ladder polymers and dyes.[1] However, their physical properties depend on their chemical and geometrical structure. A large amount of theoretical work on these molecules has focused on the understanding of their atomic scale structure, their building blocks, mechanisms of charge transport and optical properties.[30] The ability of peripherally functionalised porphyrazines to bind metals at peripheral functional groups makes them suitable precursors in the assembly of higher order polymetalic arrays.[31]

One *trans*-substituted porphyrazine has been found to display UV-visible absorption spectrum which includes an intense long-wavelength absorption at 800 nm.[1] This is a wavelength at which mammalian tissue is effectively penetrated by light a feature that is

important if deeper seated tumours are to be treated using PDT. Accordingly, porphyrazines can serve an important role as potential photosensitisers in tumour therapy.

1.2 Electronic structure and UV-visible spectra

1.2.1 Porphyrins

In the visible absorption spectra of porphyrins, the highly conjugated aromatic macrocycle shows an intense absorption in the region of about 400 nm which is referred to as the Soret Band (Figure 1.2[32]). Visible spectra of porphyrins also show four weaker bands, the Q bands, at longer wavelengths from about 450-700 nm giving rise to reddish purple colour of porphyrins.[32]

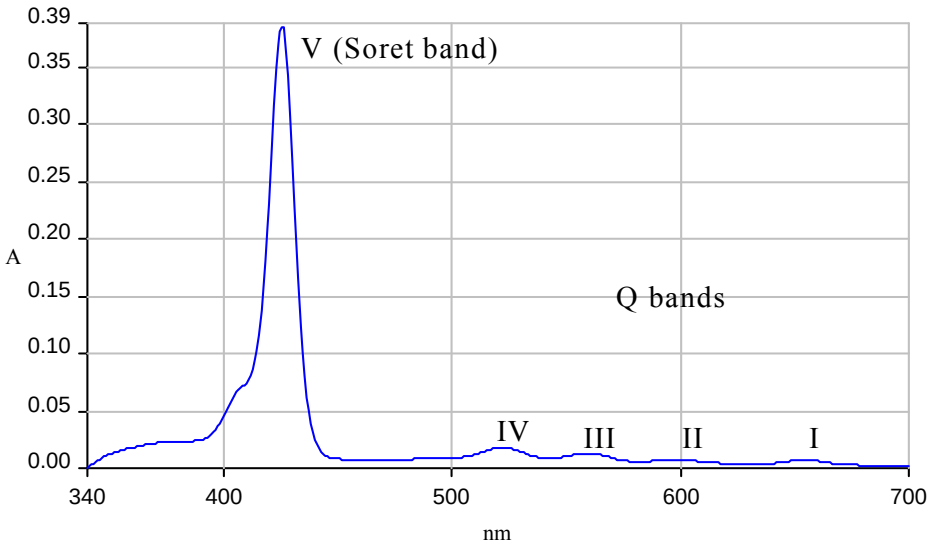


Figure 1.2 : UV-visible spectrum of a porphyrin

1.2.2 Porphyrazines

The UV-spectra in porphyrazines are greatly influenced by substituents and the presence or absence of a metal at the centre. Peripheral substitution greatly influences the UV-visible spectra with *cis*- and *trans*- isomers showing different split in LUMOs. *Trans*-isomers show large splitting compared to *cis*-peripherally substituted porphyrazines. The 1:3 macrocycles, where 1 and 3 represent different substituents, show small splitting of the LUMOs. In non-metalated porphyrazines with reduced symmetry, reduction in symmetry removes degeneracy of e_g LUMO and gives a split Q-band (Figure 1.3).[34] In cases where a lone pair of electrons in peripheral ligating atoms (N) become bonded to metal ions, the Q-band is split into two sharp bands (Figure 1.4) because their interaction with the porphyrazine ring is suppressed.[34]

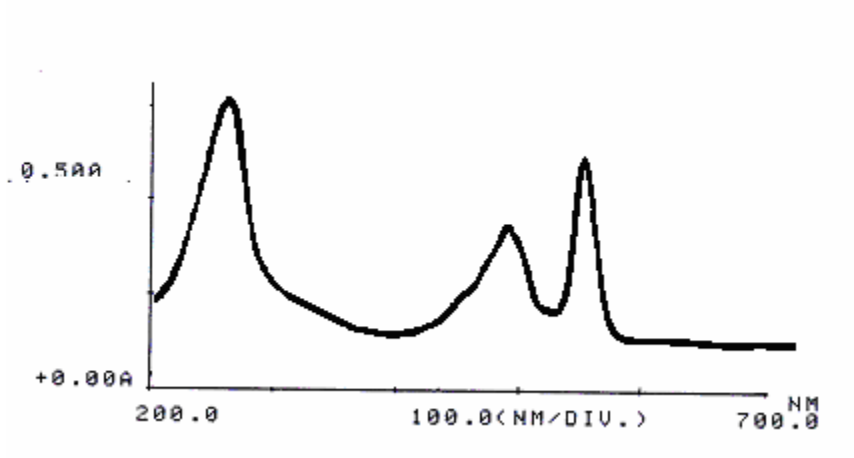


Figure 1.3 : Electronic absorption spectrum of (PzH_2)

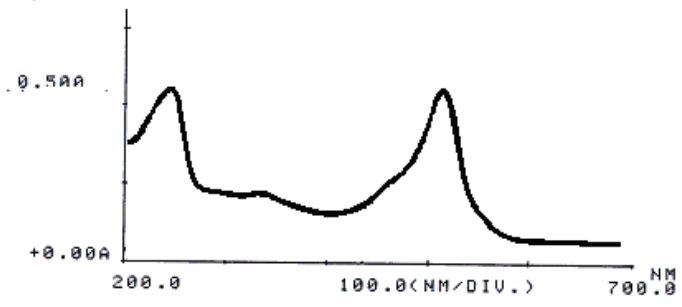


Figure 1.4: Electronic absorption spectrum of [PzNi(II)]

For non-metalated porphyrazines (D_{2h} symmetry), the UV-visible spectra show two lower energy split Q-bands at 550-700 nm and a higher energy Soret (B) band at 300-400 nm, which are assigned to $a_u \rightarrow b_{2g}$ (Q_x), $a_u \rightarrow b_{3g}$ (Q_y) and $b_{1u} \rightarrow b_{2g}$ (B_x), $b_{1u} \rightarrow b_{3g}$ (B_y) transitions (fig. 1.5). These transition bands are assigned to excitations from the two highest-occupied molecular orbitals (HOMO) (a_{1u} and a_{2u}) into lowest unoccupied molecular orbitals (LUMO, e_g).[33-36] The lower energy peak which could be found at 400-500 nm is assigned to n-π* transitions from the lone-pair electrons in external *meso*-nitrogen atoms into a π* ring system.

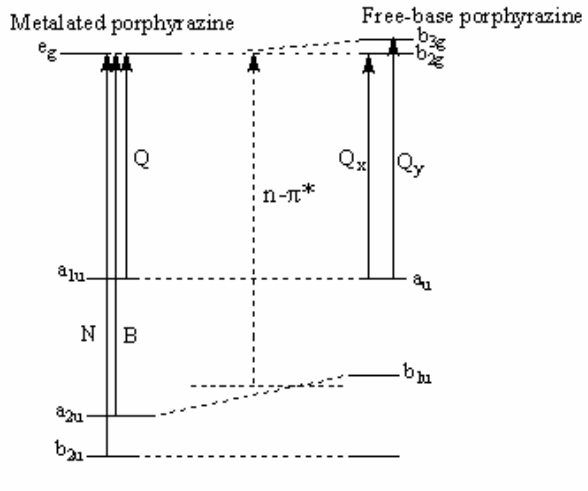


Figure 1.5: Electronic transitions in the visible and close UV regions of metalated and non-metalated porphyrazines

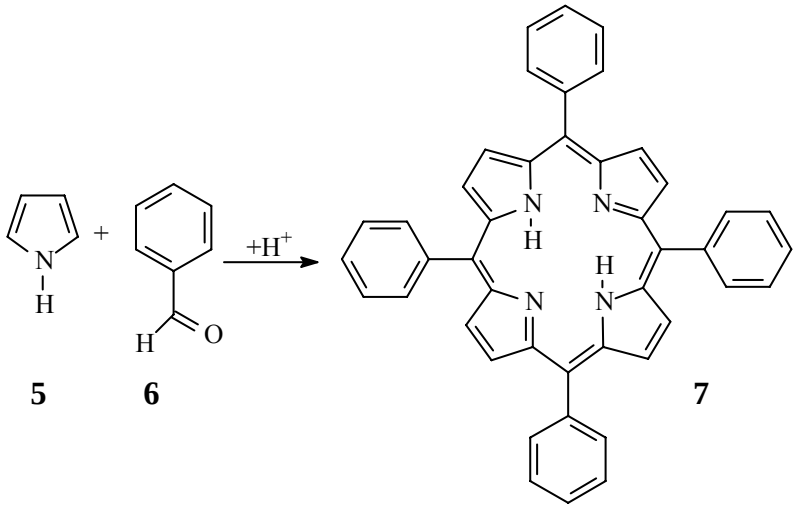
Metalated porphyrazines exhibit two intense π→π* absorbances, a low energy Q band that is accompanied by a slight higher energy shoulder and a higher energy B band. For metalated porphyrazines, the symmetry of the chromophore is D_{4h} with the two LUMOs b_{2g} and b_{3g} giving rise to a two-fold degenerate e_g level resulting to an unsplit Q and B absorptions associated with transitions $a_{1u} \rightarrow e_g$ and $a_{2u} \rightarrow e_g$.[37,38]

1.3 Synthesis

1.3.1 Synthesis of porphyrins

1.3.1.1 Rothemund reaction

The one-step condensation of benzaldehydes with pyrrole to give tetraphenylporphyrins is known as the Rothemund reaction (Scheme 1.1).[39]



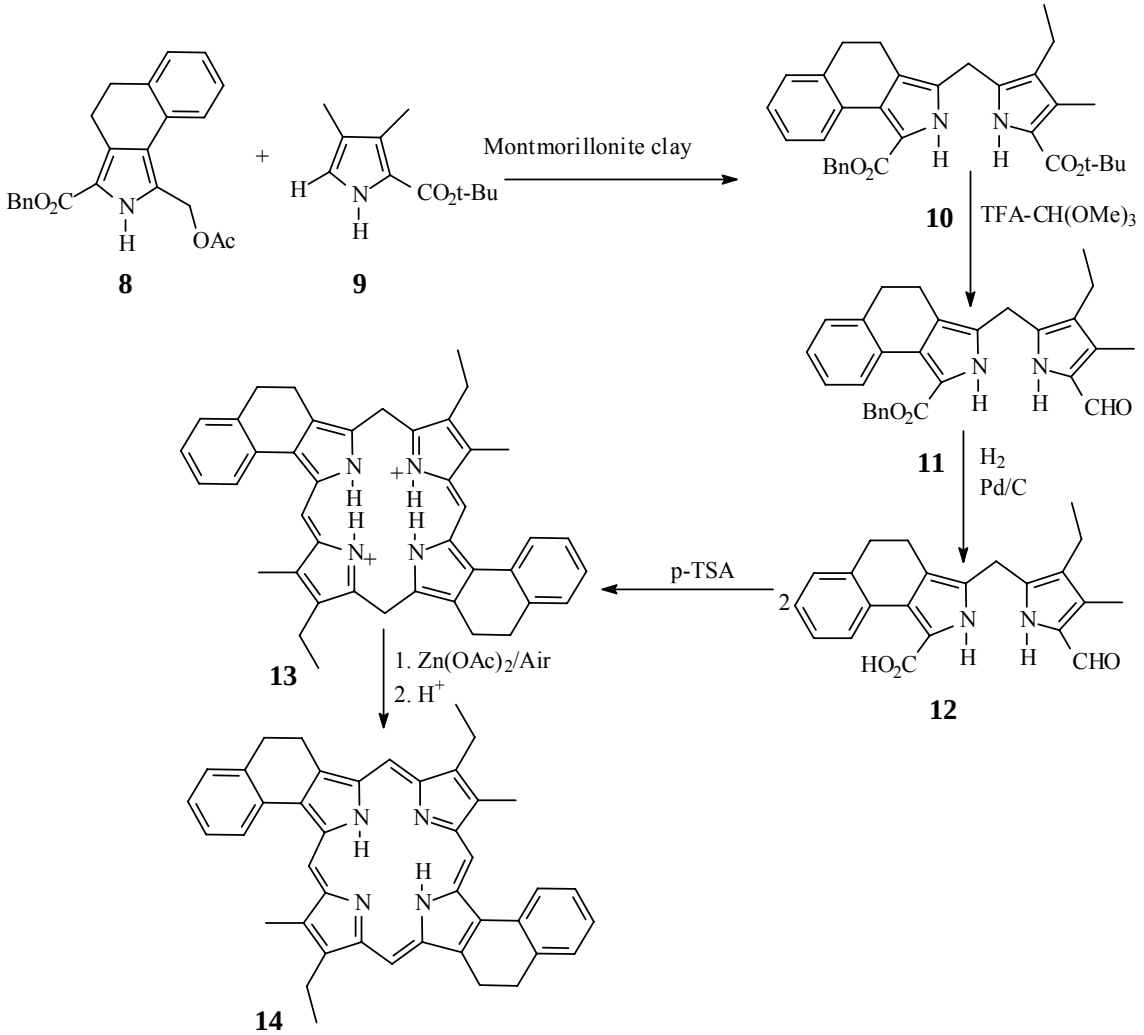
5 **6** **7**

Scheme 1.1

The reaction involves an acid catalysed condensation of pyrrole **5** with the benzaldehyde **6** to give a porphyrin. The Rothemund reaction results in low yields due to the formation of several by-products. In the general procedure of the Adler modification, equal amounts of the pyrrole and the benzaldehyde are added quickly to a refluxing acetic acid, trifluoroacetic acid or propionic acid. The method is mostly used for sterically unhindered and stable benzaldehydes.[39]

1. 3.1.2 Multistep synthesis

Porphyrines can also be synthesised in multistep synthetic routes, which can cause variation in the type as well as the position of the substituents.[40-43] One of the synthetic pathways is shown below in Scheme 1.2.



Scheme 1.2

The system is accessible via a variation on the MacDonald '2+2' condensation which requires compounds that can undergo head-to-tail self-condensation.[43] In this approach, two dipyrrylmethanes **12** undergo head-to-tail self-condensation in the presence of *para*-toluenesulfonic acid to form porphodimethene **13**. Dipyrrylmethane **12** is in turn synthesised from acetoxymethylpyrrole **8** and an α-unsubstituted pyrrole tert-butyl ester **9**

in the presence of montmorillonite clay in dichloromethane (DCM) to yield dipyrrylmethane **10** with benzyl and *tert*-butyl esters.[43]

The *tert*-butyl ester is then cleaved to its corresponding carboxylic acid with trifluoroacetic acid (TFA) and further treated with trimethyl orthoformate-TFA to give aldehyde **11.** The orthoformate acts as a reductant in this conversion. Cleavage of the benzyl ester protecting group by hydrogenolysis gives the carboxylic acid **12** which underwent self condensation. Oxidation of **13** with zinc acetate and air gives porphyrin **14**.[43] In each step, the intermediate can be isolated and fully characterized.

1.3.2 Phthalocyanines

Phthalocyanine was discovered as a crude iron complex in 1928 during dye industrial production by Scottish Dyes Ltd.[44] Since it was unknown whether the product was a single compound or not, various analyses were performed on it. Analytical figures gave an atomic ratio of 4C:1N while decomposition in hot nitric acid gave phthalimide and a ferric salt.[44] It was then suggested that there is a possibility for the preparation of similar pigment by reaction of metals on the dehydration products of phthalimide, *o*-cyanobenzamide and phthalimide.

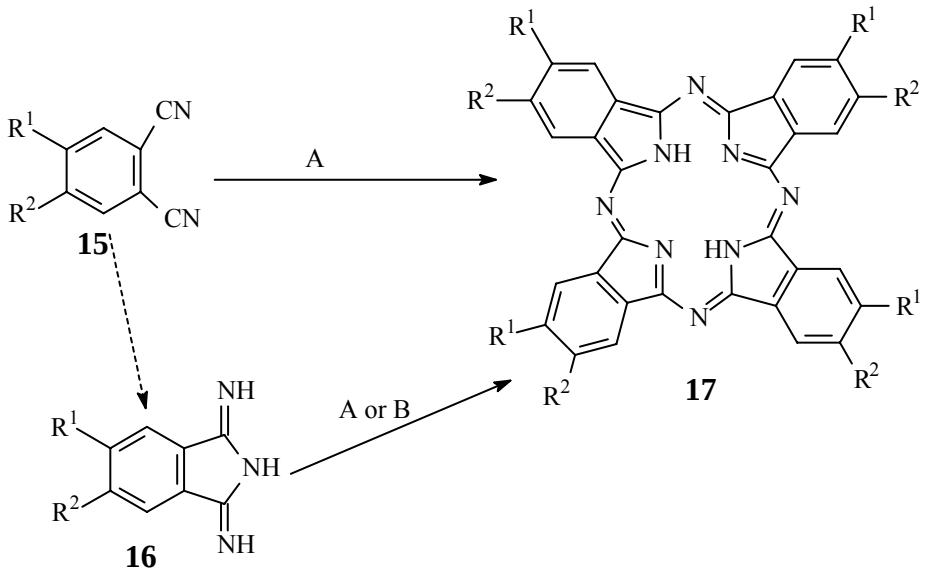
In a series of experiments, it was found that dinitriles were less reactive, while *o*-cyanobenzamide reacted readily with many metals at 250 ^{o}C. Promising results were obtained with the use of elemental magnesium or its oxide, although magnesium phthalocyanine is quite insoluble in most organic solvents. Other elements used were iron (elemental or its oxide), cobalt, nickel and antimony. Phthalocyanines obtained from these metals resemble each other in colour (blue), insolubility (showing that they are metallic derivatives) and stability.

Although none of the intermediates were isolated at the time, the mechanism was proposed to involve the formation of pyrrole ring fused to the aromatic nucleus in the 1 and 2 positions and containing six atoms or less as supported by the formation of phthalic

acid derivatives during hydrolysis and oxidation of phthalocyanines. The synthetic, fission and analysis results indicated the presence of the phthalic dinitrile skeleton. Non-benzenoid carbon atoms were shown to carry no oxygen atom and few or no hydrogen atoms. The intense colour indicated the presence of more than one isoindole unit while analysis of metallic derivatives showed that four isoindole units are combined for every metal atom. This was confirmed by the molecular weight of magnesium phthalocyanine and the results of quantitative oxidation of both free and metallic phthalocyanine.[44]

Metal-free phthalocyanines were also found to be converted into metalated phthalocyanine complexes by treatment with pure metal in boiling quinoline or benzophenone. Magnesium element was found to be most effective in the reaction of phthalimide and ammonia. It was found that many metals and their derivatives react with phthalonitrile to yield compounds of phthalocyanine type.[44]

The first phthalocyanine was reported in literature by Linstead only in 1934.[45] He then synthesised analogous phthalocyanines from *o*-cyanobenzamide and antimony, phthalonitrile and magnesium salt and from phthalonitrile and metallic copper.[45-49] Based on the Linstead synthesis, phthalocyanines are prepared by heating the phthalonitrile or 1,3-diiminoisoindoline in an alcohol in the presence of a transition metal or elemental magnesium or lithium (Scheme 1.3) Paths A and B show conditions under which free-base phthalocyanines can be synthesised starting from a phthalonitrile. Heating the phthalonitrile **15** under basic conditions in pentanol (Path A) results in macrocyclisation to form the corresponding phthalocyanine **17**.[50] Alternatively, phthalonitrile derivatives can be treated with a metal alkoxide in an alcohol at room temperature followed by heating to afford the corresponding phthalocyanine after treatment with a mild acid.[51,52]

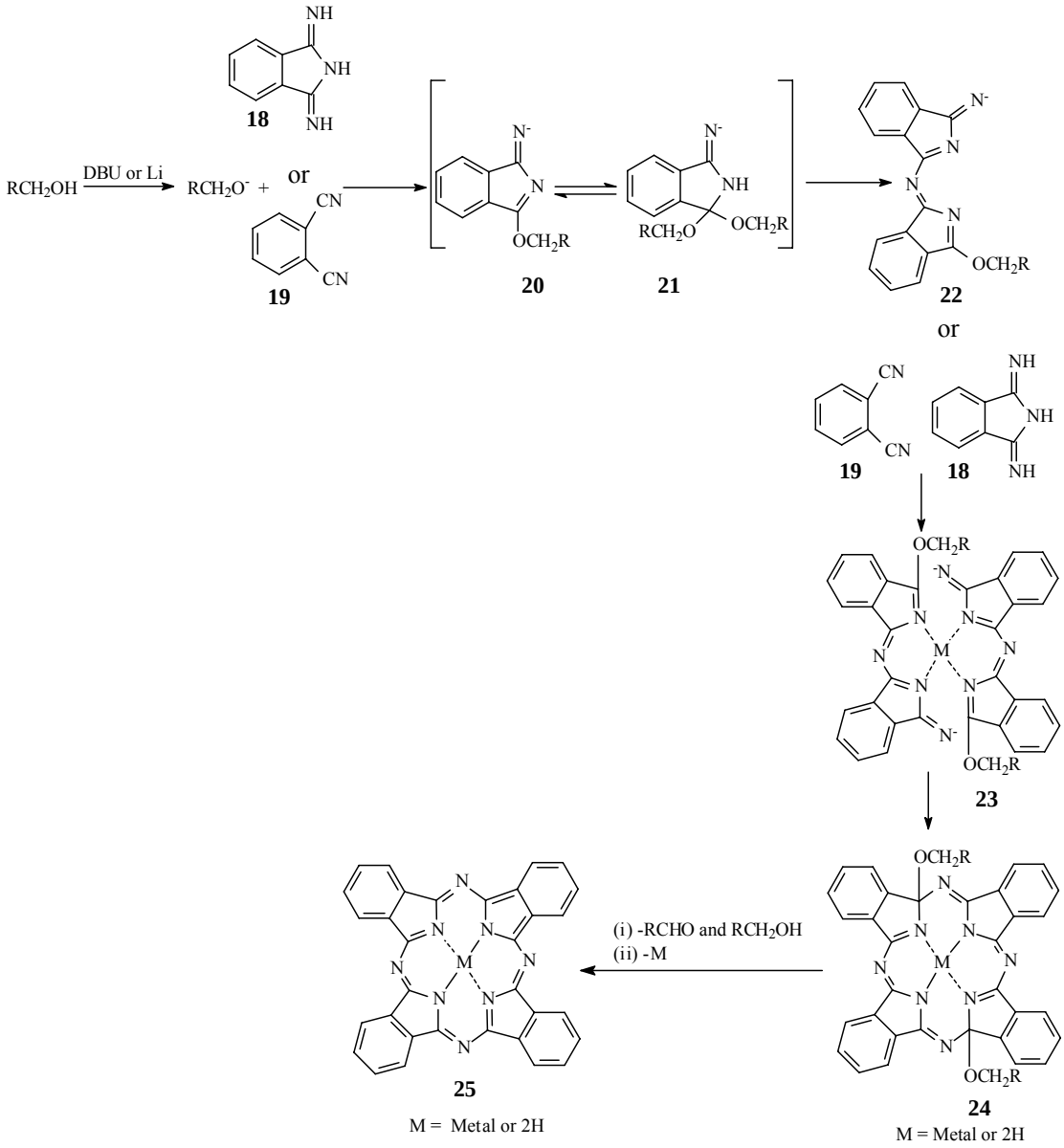


A. (i) Base (NH_3 or DBU or DBN)
(ii) Metal salt (Na^+, Li^+ or Mg^{2+})
B. DMAE, heat
or
DBU(1,8-diazabicyclo[5.4.0]undec-7-ene)
or
DBN (1,5-diazabicyclo[4.3.0]non-5-ene)
or
DMAE (N,N-dimethylaminoethanol

Scheme 1.3

1,3-Diiminoisoindolines **16** derived from **15** can be subjected to basic [Path A (i)] or lithium alkoxide conditions [Path A (ii)] to afford **17**. Compounds **16** can also be cyclised when treated with DME under reflux to yield **17** (Path B).[53]

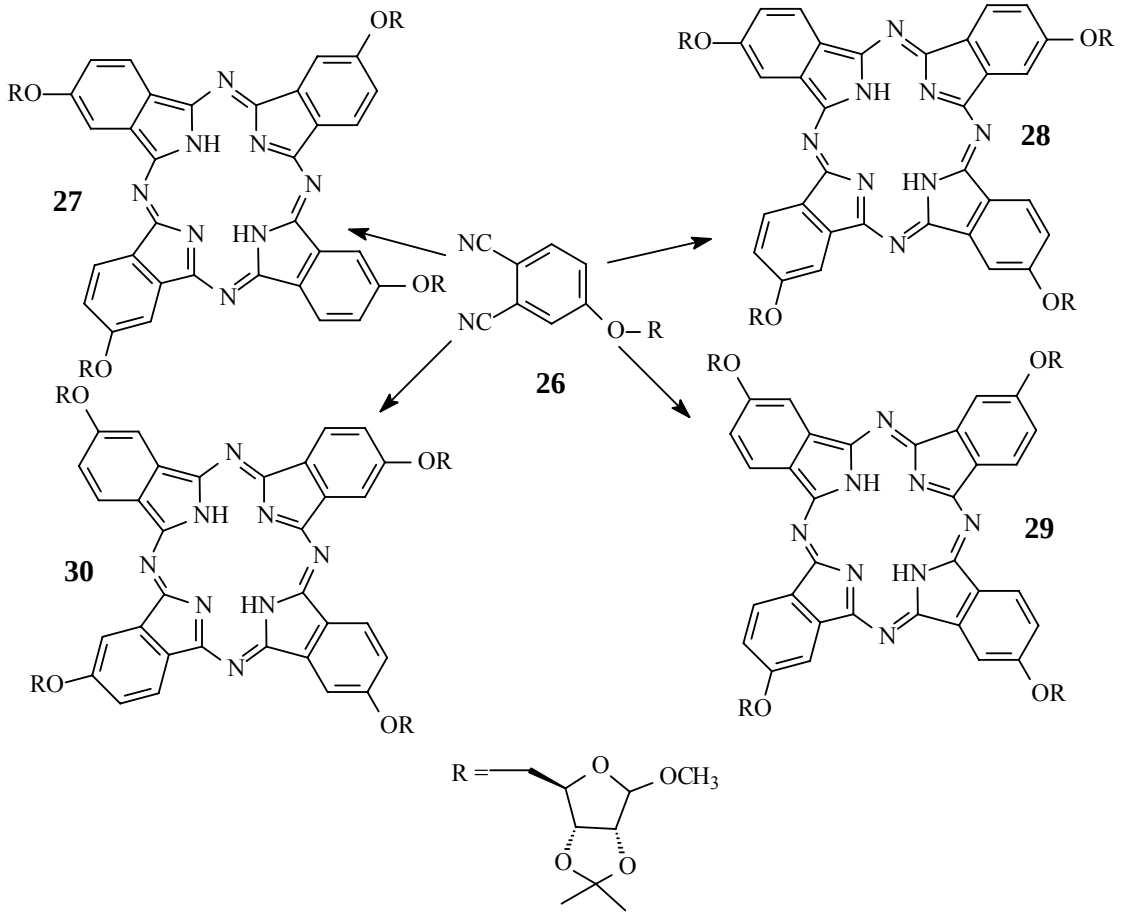
The mechanism of the macrocyclisation reaction is shown below in Schemes 1.4 and is based in some intermediates which have been isolated and characterised.[54a]



Scheme 1.4

The physical, chemical and electronic properties of phthalocyanines are well established. In order to prepare specific derivatives, a regioselective synthetic approach and access to substituted precursors are required.[54a] One of the disadvantages with unsubstituted phthalocyanines is their lack of solubility, which can be attributed to the hydrophobicity of the aromatic core and the planarity of the macrocycle. In order to make them soluble, a number of functional groups have been attached to the periphery of the benzo ring and thus improve the overall properties of the phthalocyanine.[54a]

The substituents can be added to any or all of the 16 available positions of the phthalocyanine. During macrocyclisation of the substituted phthalonitrile to form the phthalocyanine, the method of synthesis leads to isomers. Scheme 1.5 shows four atropisomers formed from a substituted phthalonitrile.



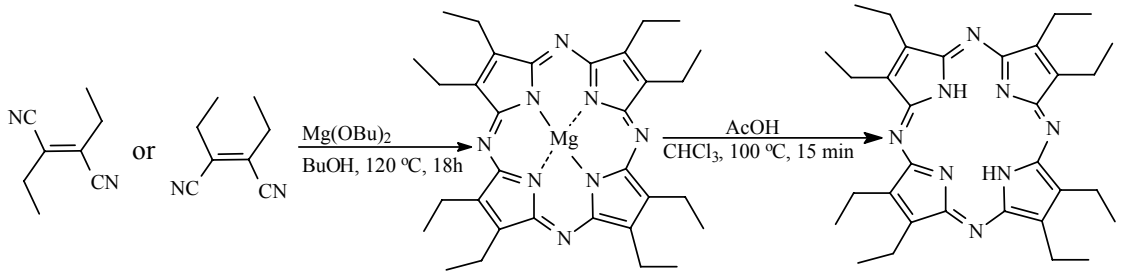
Scheme1. 5

Heating the phthalonitrile **26** in pentanol in the presence of DBU followed by treatment of the crude material with methanol affords the free-base phthalocyanine as a blue-green precipitate. Four isomeric phthalocyanines are expected to be formed, however, these cannot be separated and the mixture leads to poorly resolved ^{1}H-NMR spectrum.

1.3.3 Porphyrazines

This particular class of macrocyclic ligands is composed of four pyrrole rings bridged by aza-nitrogen atoms and is considered to be a structural hybrid of well-known phthalocyanines.[54] Despite structural similarities with phthalocyanines and porphyrins, porphyrazines show several unique and interesting properties. *Tetra*-azaporphyrins (porphyrazines) are relatively poorly studied compared to phthalocynines, most probably due to their fairly recent discovery.

Porphyrazines are synthesised from functionalised nitriles such as maleonitriles, fumaronitriles and phthalonitriles. Like benzoporphyrazines (phthalocyanines), the synthesis of porphyrazines is limited to Linstead macrocyclisation in which the dinitrile **31** or **32** undergoes a macrocyclisation reaction under reflux in the presence of a magnesium alkoxide in the corresponding alcohol (typically 1-butanol or 1-pentanol) or dimethylaminoethanol to yield **33** (Scheme 1.6). Magnesium porphyrazines such as **33** are easily demetalated under acidic conditions to form the free-base porphyrazine such as **34** (Scheme 1.6)[55].



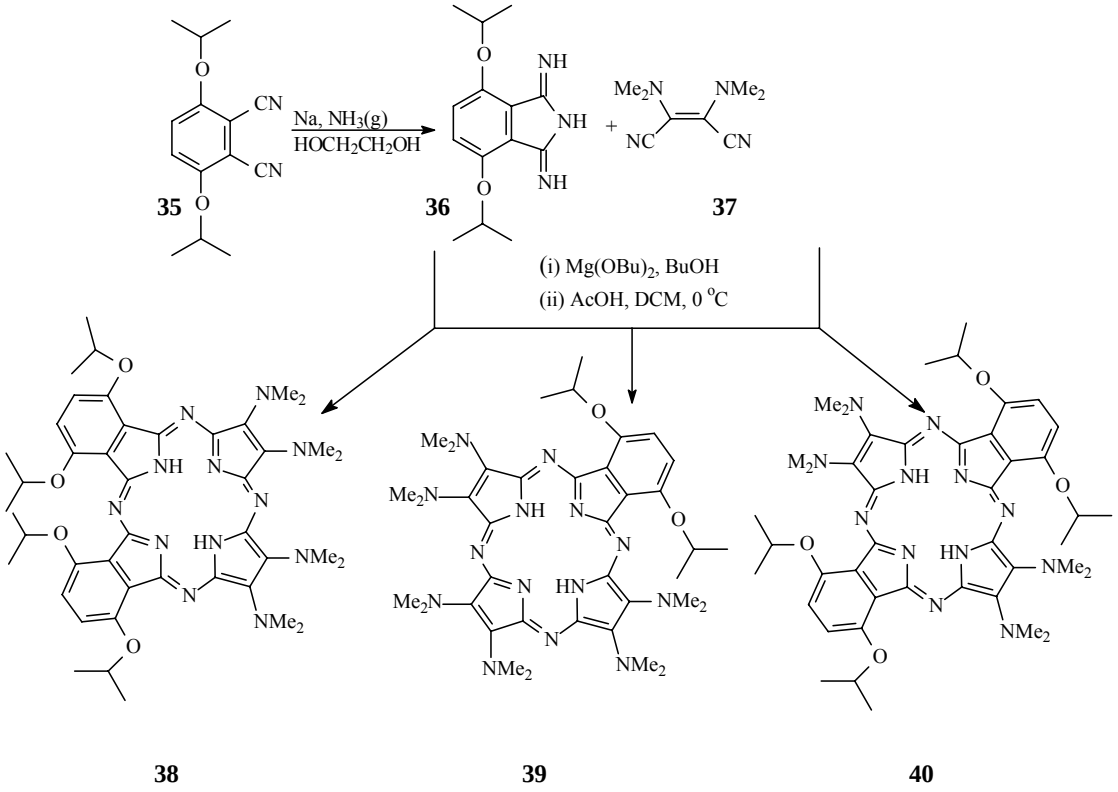
31 **32** **33** **34**

Scheme 1.6

The mechanism of cyclisation is similar to that already shown for phthalocyanines in Scheme 1.4.

Removal of magnesium can be achieved by treating a solution of magnesium octaethyltetraazaporphyrin **33** in tetrachloromethane and acetic acid for 15 minutes, to give free-base porphyrazine **34**.[55] The above synthetic method is the basic Linstead

magnesium template macrocyclisation. The same synthetic method is also applicable if two different precursors are macrocyclised to form different porphyrazine hybrids (Scheme 1.7).[1]



Scheme 1.7

Since phthalonitrile **35** is unreactive in the synthesis of either the phthalocyanine or porphyrazine, it is first converted into its corresponding 1,3-diiminoisoindoline **36** to increase its reactivity. A suspension of phthalonitrile **35** in 1,2-ethanediol is bubbled with ammonia gas in the presence of sodium metal. The 1,3-diiminoisoindoline **36** derived from hydroquinone isopropyl ether **35** is then reacted with dipropylmaleonitrile **37** to give porphyrazine hybrids **38**, **39**, **40** (Scheme 1.7).[1]

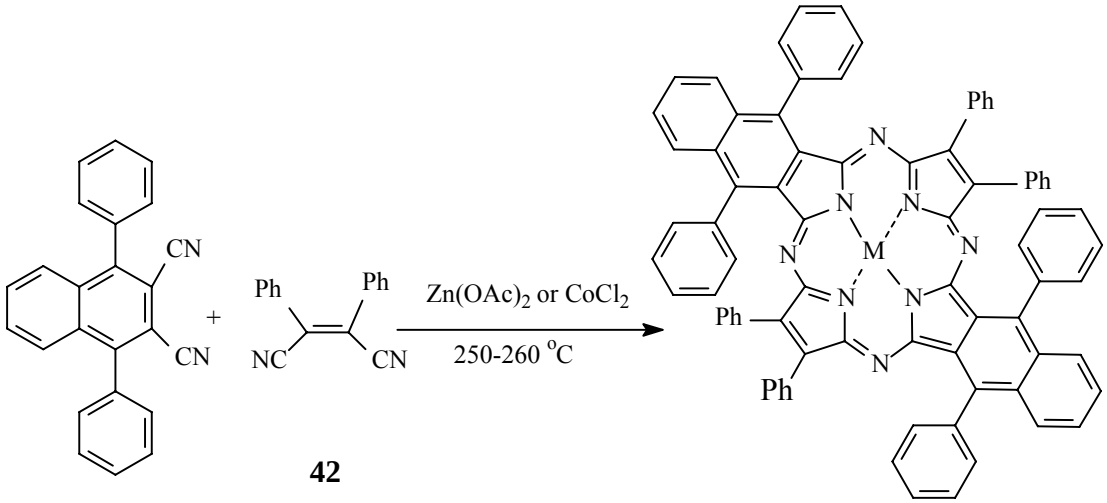
Various substituents provide porphyrazines with substantial organic solubility compared to their porphyrin and phthalocyanine counterparts. Peripheral substitution among porphyrazines is becoming an increasingly popular strategy for the design of functional

dyes and molecular devices. Porphyrazines with peripheral substitution of the form M[Pz-(A_n:B_{4-n})], where A and B are functional groups fused to the β-position of the pyrroles, have the potential to exhibit magnetic and electronic properties.[33,56,57] Due to peripheral substitution, these porphyrazines show unusual UV-visible spectra, redox chemistry and electrochemistry. In addition, they exhibit interesting co-ordination chemistry for binding of metal ions within the macrocyclic cavity and by peripheral ligating groups (N, S, O).[31,60] Substituted amphiphilic and hydrophilic porphyrazines bearing both hydrophilic and hydrophobic moieties can be more potent as photosensitisers in photodynamic therapy.[30,52,54,59,61] With acetylinic units as peripheral substituents, the π-systems of the chromophores are enlarged and bathochromic shifts in the electron absorption and emission spectra are induced. Alkanyl groups also serve as covalent linkers for the assembly of delocalised multichromophore chains or two dimensional polymer networks.[62]

As in the case with the synthesis of substituted phthalocyanines, formation of a mixture of substituted porphyrazines in which different isomeric hybrids are formed, represents one of the greatest problems associated with their synthesis. This limits the synthesis of highly functionalised porphyrazines because of the resulting mixtures which lack distinct structure and the purification of the required product(s) is difficult due to the tendency of the product mixture to aggregate. Substituted porphyrazines can be synthesised by addition or substitution in an already existing porphyrazine. The resulting product(s) can form mixtures since substitution or addition could be at any of the eight positions, and this poses a limitation to the application of this method of functionalisation.[54]

The second method involves the macrocyclisation of substituted precursors, which may lead to a cleaner product of known substitution pattern. Peripheral substitution in porphyrazines is achieved by reacting a substituted phthalonitrile with a maleonitrile, or reacting two maleonitriles. However, as shown above in Scheme 1.9, this method also leads to the formation of isomers due to the symmetry involved in macrocyclisation. While macrocyclisation of substituted precursors has its drawbacks, it is still the method of choice for the synthesis of substituted porphyrazines with improved properties.

Trans-substituted porphyrazines can be synthesised by using a phthalonitrile with bulky groups in position 3 and 6 as one of the reactants. In this case, the bulky group appears once or twice due to steric hindrance in which the steric groups cannot be adjacent and co-planar (i.e. bulky groups are prevented from being adjacent to each other) as shown in Scheme 1.8 below.[63]

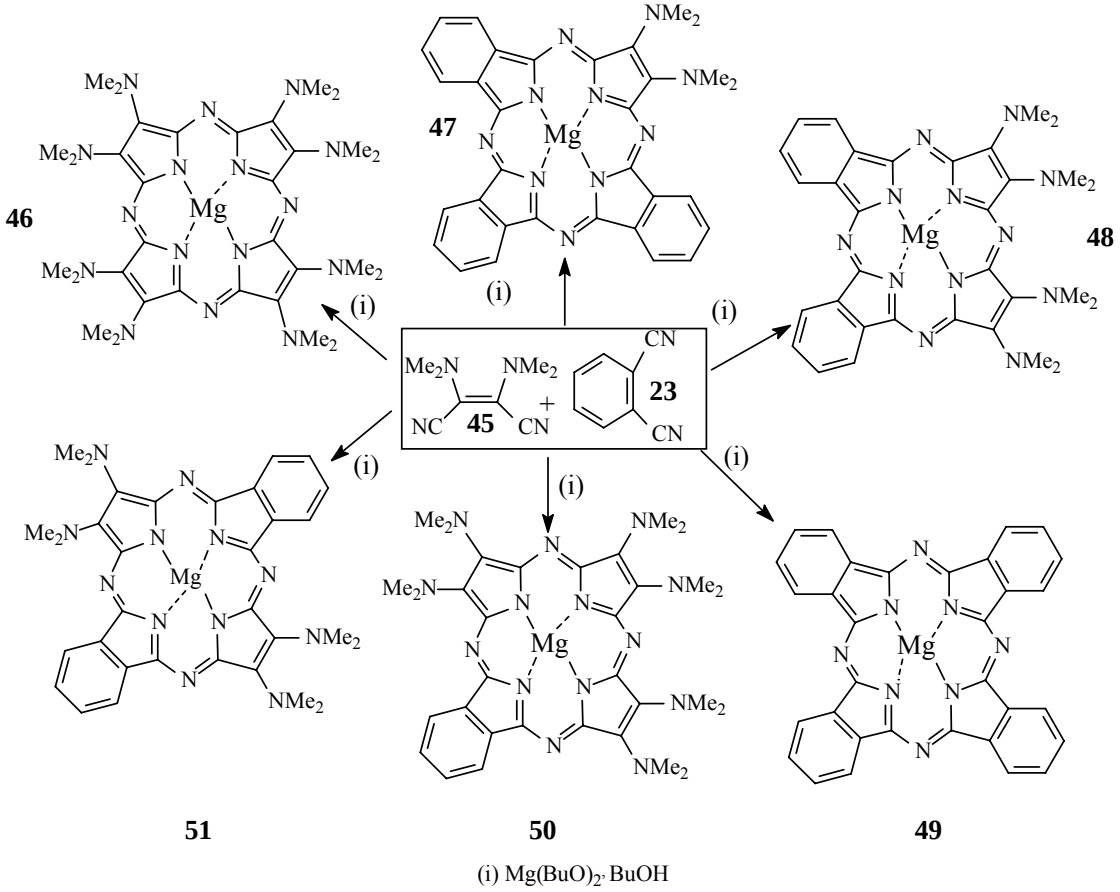


Scheme 1.8

Here, the reaction was performed by reacting one equivalent of diphenylmaleonitrile **42** and three equivalents of 2,3-dicyano-1,4-diphenylnaphthalene **41**, which were fused in the presence of $Zn(OAc)_2$ or $CoCl_2$ (1.21 eq. each) at 250 – 260 °C. In each reaction, a mixture of two products was obtained and product **43** or **44** was isolated.[63]

The most successful pathway of synthesising unsymmetrical substituted porphyrazines is to a use statistical condensation of two substituted precursors A and B in which the desired product can be isolated by column chromatography. Although the method also gives a mixture of six compounds, required compound can be isolated as a major product. If, for example, the reaction is done with the precursors A:B at a ratio of 1:8 or above, the

major product is that in which A:B are incorporated into the pigment in the ratio of 1:3 (Scheme 1.9).



Scheme 1.9

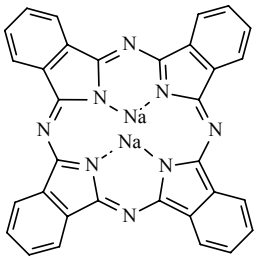
To get **50** in 35 % yield as the second major product, *bis*(dimethylamino)maleonitrile **45** and 1,2-dicyanobenzene **23** are reacted in ratio of 25:1. The mixture, which is dominated by **46** and **50** is easily purified using column chromatography. The same reaction when carried out with *bis*(dimethylamino)maleonitrile **45** and 1,2-dicyanobenzene **23** in the ratio of 3:1, gives **47** (9%), **48** (4.8%) and **51** (0.3%).[5]

1.3.4 Metalation

Like haem, many second generation photosensitisers which are in clinical trials, e.g. texaphyrins and tin etiopurpurin, have co-ordinated metals in their framework.[6a,6b] A metal co-ordinated into the ring may be important in determining the effect of the photosensitiser. For good sensitisers, the metal should increase the intersystem crossing by the sensitiser from singlet to triplet state without reducing the lifetime of the triplet state. The triplet state of the photosensitiser is the most important state in PDT since it transfers excitation energy to the dioxygen molecule, converting it into the singlet state for tumour cell destruction.[64]

The three mostly favoured diamagnetic metals are Al(III), Zn(II), Si(IV) and Sn(IV) ruling out two paramagnetic metals Cu(II) and Fe(II). Tin, which can bind two axial hydroxyl groups as ligands, and aluminium with its affinity to bind one axial hydroxyl group as ligand, add further advantages. Binding of metal to hydroxyl group can make the photosensitiser more soluble for transport in the body fluid.[64, 65]

The normal charge of a porphyrin, porphyrazine or phthalocyanine is -2. Therefore, the metal ion to be accepted in order to form a stable complex is the one with a +2 charge. Metal ions with a charge of +1, requires an additional ion with +1 charge (**52**, Figure 1.6[54]).



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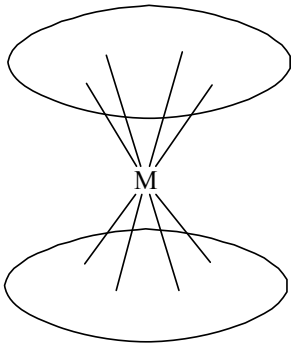


53

Where M = Gd, Lu etc.

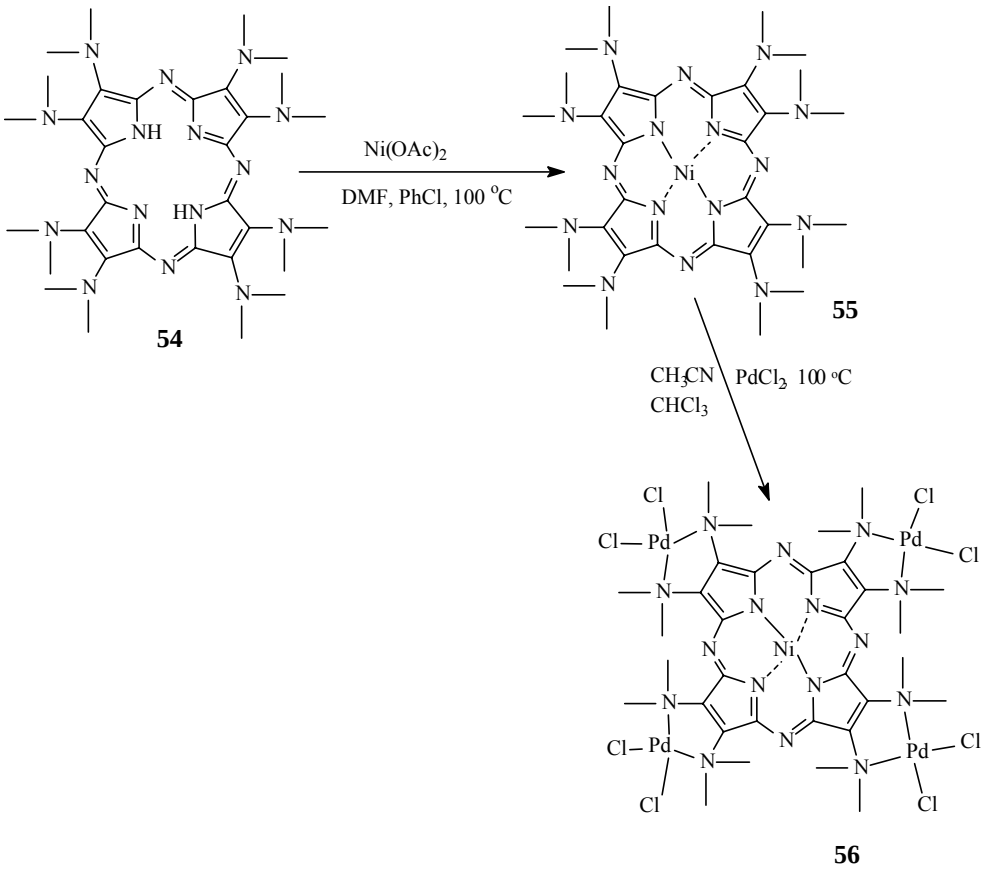
and = porphyrazine

Figure 1.6

Metal ions with a charge of +3 or higher require additional "axial" ligands. In the case of larger ions, the phthalocyanine or porphyrazine or porphyrin cannot fully accommodate

these ions within the core cavity and in this case the metal becomes surrounded by two phthalocyanines, porphyrins or porphyrazines, making a "sandwitch" complex (**53**, Figure 1.6).[54]

Metalation in phthalocyanine and porphyrazine compounds can be incorporated into the synthesis[66,67] as shown in Scheme 1.10. In a metal-free porphyrazine, central metalation is mostly done by heating from 50 ºC to reflux the metal-salt [Zn (OAc_2), $MnCl_2$, etc.] in a mixture of chlorobenzene and DMF or in neat DMF, [68-70] while peripheral metalation can be effected by heating the porphyrazine in a suitable solvent mixture (mostly using acetonitrile-trichloromethane).[59,69]



54 **55** **56**

Scheme 1.10

In the above reaction, central metalation of free-base porphyrazine **54** was achieved by heating it with nickel acetate in DMF-chlorobenzene mixture at 100 ºC to give metalated porphyrazine **55**. Peripheral metalation was achieved by dissolving compound **55** in acetonitrile-chloroform mixture (3:1, v/v) followed by addition of palladium(II) chloride and heating at 100 ºC for 4 hours. The solution was then stirred for 12 hours at 60 ºC, cooled to room temperature and purified using column chromatography to give **56**.[59,69]

1.4 Photochemotherapy

Photochemotherapy involves the use of visible light or ultraviolet light in clinical medicine. In direct photochemotherapy, light is used alone, whereas in indirect photochemotherapy, light is used in conjunction with a photosensitiser. Photoradiation therapy (PRT), or photodynamic therapy (PDT) are some of the alternative names used to describe photochemotherapy.[11,71-73] In this approach visible light is used in combination with light sensitive agents in an oxygen-rich environment. The source of the photons may be an ordinary tungsten lamp, an argon lamp or a laser. A tunable dye laser has the advantage that monochromatic light of a predetermined wavelength can be effectively channeled by way of a fibre optic device into a deeply located tumour.[11]

Photodynamic therapy is the indirect treatment of cancer which involves the selective uptake of the photosensitiser in a much higher concentration in tumour tissues than in normal tissues. It is worth noting that, unlike with X-rays, in the absence of a photosensitiser the visible light is essentially harmless. Basic photochemical properties of porphyrins which have to do with detecting the tumour cells by fluorescence and destroying them by the photodynamic effect, have been reported.[11a]

Photochemotherapy was first reported by a Dane, Niels Finsen, in the 1880s when he showed that a tubercular condition of the skin called *lupus vulgaris* could be treated by direct photochemotherapy.[11] It was also observed in the early years of the 20th century that certain substances called photodynamic agents could photosensitise mammals.

Subsequent research showed that, when porphyrins are injected under the skin, sunburns occur due to photodynamic reactions. Visible light, oxygen and a photosensitiser were found to be necessary for the photodynamic reaction, which destroys living cells.

The first chemical substance to show selective retention to tumour tissue while normal surrounding tissues had a low comparable content after intravenous injection is known as hematoporphyrin derivative (HpD).[74] As early as 1936, it was suggested that hematoporphyrin in combination with ultraviolet light could be effective in treatment of skin diseases such as psoriasis. Several reports have been published in which hematoporphyrin derivative in combination with ultraviolet light has been used to treat psoriasis.[75-79]

In 1961 Lipson and Baldes observed that haematoporphyrin derivative had the remarkable property of concentrating with some degree of selectivity in tumour tissue.[11] Due to its selectivity for tumour tissue, they then thought of using it as a photosensitiser for the treatment of cancer. However, there were some reasons why HpD was not an ideal photosensitiser. First, it is a complex mixture of compounds some of which are not PDT active and may cause side effects on patients. Another reason is that it is cleared quite slowly from the body and patients need to remain out of the sun for a long time. HpD is also inefficient at light absorption and therefore produces only a relatively small and shallow photodynamic effect. Subsequently, porfimer sodium (Photofrin) was discovered and was found to be useful in the treatment of psoriasis when brought in contact with suitable light.[11a,14] It is somewhat purified compared to HpD, but it also represents a mixture of porphyrin dimers and higher oligomers derived from HpD, linked primarily by ether bonds and some ester bonds.

Metal-free and metalated photosensitisers emit a bright red fluorescence when irradiated with ultraviolet light. This observation provides a means of detecting small amounts of tumour tissue in a bed of normal tissue. When electronically excited by the absorption of light, the sensitiser may show two sorts of emissions. The emission in the red region, used for the detection of tumour tissue results from the first excited singlet state ($^1S^*$)

which in turn results from the excitation of the electronic ground state (S_o) of the sensitiser when it absorbs light of suitable wavelength, ($S_o \rightarrow {}^1S^*$). The singlet excited state sensitiser can decay back to the ground state with the release of energy in the form of fluorescence and enable identification of tumour tissue.[13,64,80-82]

The singlet excited state is short lived (generally less than 1 μs) and under suitable conditions it undergoes conversion to a longer-lived triplet excited state (${}^3S^*$). This process is known as intersystem crossing, which involves the unpairing of paired electrons in the excited state. Another emission, usually in the near infra-red region, comes from the longer lived excited triplet state of the sensitiser. In fluid solution this emission is usually not observed in organic molecules because the triplet excited state is de-activated in other ways. In the presence of oxygen (${}^3O_2$), which is found in most cells, one of these ways involves the transfer of excitation energy from the photosensitiser triplet species to the dioxygen molecule.[64,80-83] The electronic energy in the singlet electronic excited state can undergo fast conversion into vibrational energy in the excited singlet state of the sensitiser before intersystem crossing to the triplet excited state.[84]

The dioxygen molecule is excited from its triplet ground state to the singlet state dioxygen (${}^1O_2$), provided the energy of the triplet excited state of the photosensitiser is higher than that of the singlet excited state dioxygen.[11,85] There are two types of mechanisms which are thought to be the main path under which singlet excited state oxygen in produced, referred to as Type I and Type II mechanisms.[13] The Type I mechanism involves an hydrogen-atom abstraction or electron-transfer between the excited triplet state of the photosensitiser and a substrate that is either a solvent, another sensitiser or biological substrate to afford free radicals or radical ions that are highly reactive. Free radicals so formed interact with oxygen to generate reactive oxygen species such as superoxide anions or hydroxyl radicals which produce oxidative damage, expressed as biological lesions.[81,86]

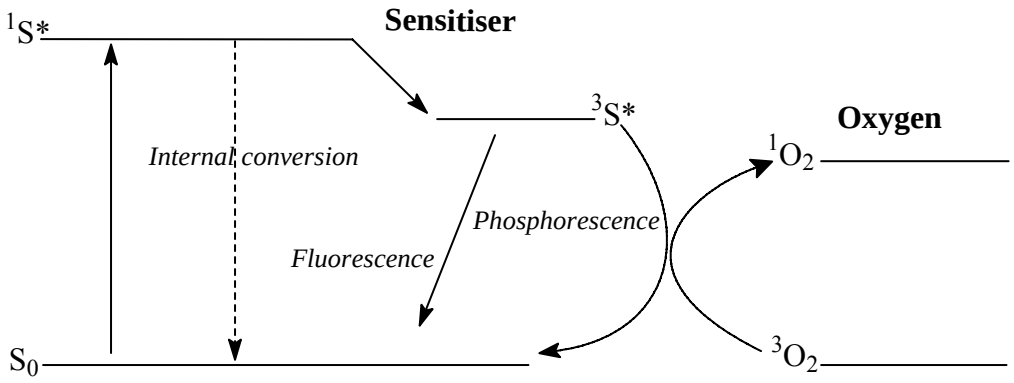


Figure 1.7: Photosensitisation of oxygen via Type II mechanism.

S_0 - Sensitiser in singlet electronic ground state.

$^1S^*$ - Sensitiser in singlet electronic excited state.

$^3S^*$ - Sensitiser in triplet electronic excited state.

$^3O_2$ – Oxygen in triplet electronic ground state.

$^1O_2$ – Oxygen in singlet electronic excited state.

The Type II mechanism involves an interaction between the triplet state photosensitiser ($^3S^*$) and an oxygen molecule to form electronically excited state singlet state oxygen ($^1O_2$) which is highly reactive and is responsible for oxidative damage of many biological systems. It is generally accepted that the Type II mechanism predominates during PDT with singlet oxygen responsible in cell damage, while the Type I mechanism becomes more important at low oxygen concentrations or in more polar environments. Fig. 1.7 illustrates Type II mechanism.[81,86]

The excited singlet state dioxygen is much more toxic and reactive than triplet ground state oxygen, and is known to react with membrane components such as unsaturated lipids, cholesterol and proteins.[81] Therefore just as chlorophyll in plants utilises energy from the sunlight to produce sugars, porphyrins utilise energy from light to produce toxic oxygen species. In photosensitised tissue such a reaction would be expected to lead to membrane damage and eventually to cell death. It is most important to note that when the excited triplet state photosensitiser transfers electronic energy to ground state $^3O_2$ it

returns back to its singlet ground state.[87,88] Singlet oxygen formed in this way is generally thought to be the main pathway leading to photonecrosis in sensitised tissue.

Thus, there is no chemical transformation of the sensitiser when the singlet state oxygen is generated and the cycle may be repeated upon absorption of another photon. For a single photosensitiser molecule, generation of many times its own concentration of singlet oxygen is possible, which is a very efficient way of photosensitisation, provided there is sufficient oxygen supply.[87,88] The transfer of energy from the triplet state of the sensitiser to the triplet oxygen may be so efficient that the yield of the singlet state oxygen may approach the photosensitiser triplet state yield.[87,88]

1.5 How to perform PDT

In a typical treatment of cancer with PDT, the photosensitiser is dissolved in a suitable solvent and intravenously injected to the patient (Figure 1.8).[89,90]

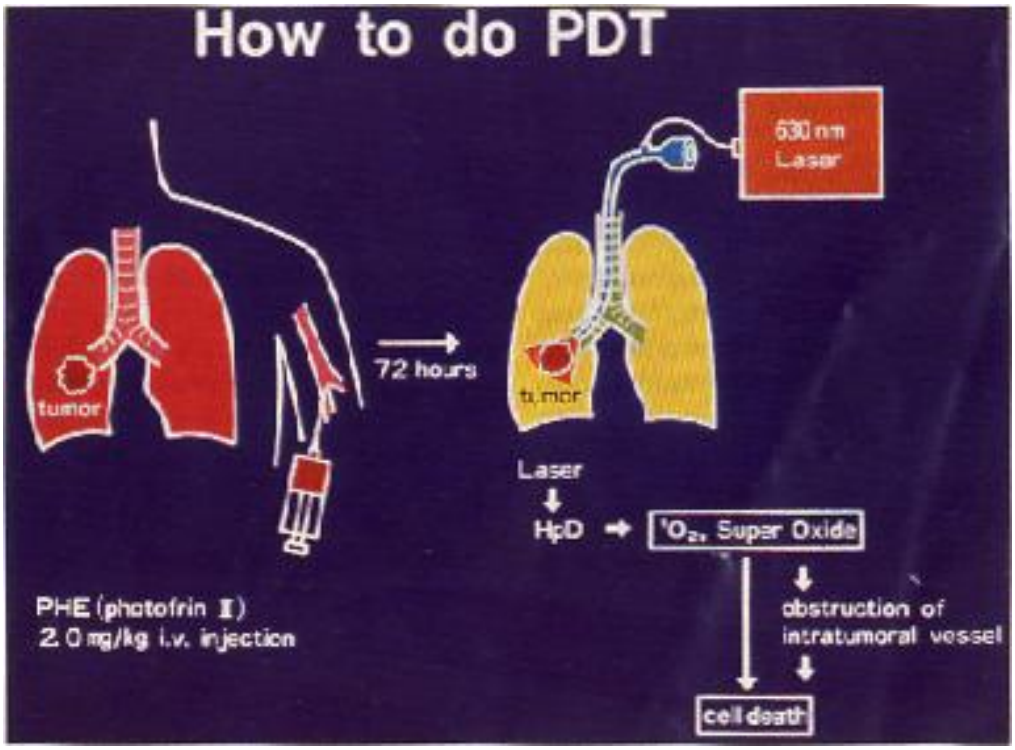


Figure 1.8: How PDT works[90]

The patient is then confined to the dark to allow the photosensitiser to equilibrate (24-72 hours), during which most of the photosensitiser accumulates preferentially in the tumour tissue. The patient is then confined to the dark to allow the photosensitiser to equilibrate (24-72 hours), during which most of the photosensitiser accumulates preferentially in the tumour tissue. The patient is anaesthetised, and a dose of light is administered using an appropriate light delivery technique, such as an optic fibre from a laser source. Tumour cell destruction begins immediately and a period of time is allowed for the photosensitiser and its metabolites to be eliminated from the body, before the patient is allowed back into the light.[89]

1.6 Photosensitiser amphilicity in PDT

1.6.1 Pharmacokinetics

Below is a schematic representation of the membrane bi-layer depicting the two layers (Figure 1.9). The aliphatic portion of the membrane is hydrophobic while the polar portion is hydrophilic. All cellular organelles such as the nucleus, mitochondria, and cytoplasmic reticulum are surrounded by a cell membrane. Entry of extracellular substances into the cell and into all intracellular organelles is controlled by such membranes.

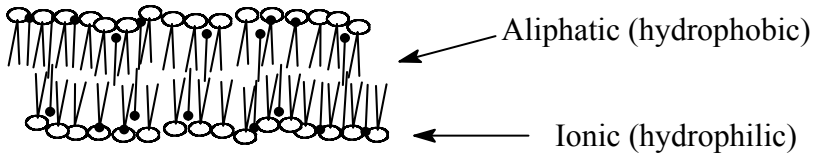


Figure 1.9: Schematic representation of membrane layer

1.6.1.1 Blood and/or plasma transport

The dissolved photosensitiser is transported by the blood or plasma fluid to the tumour cells. Before reaching the target cells, it passes through the different cell environments, a hydrophobic environment in the cell membrane and a hydrophilic environment in the cytoplasm. Blood is mostly composed of water, so for the photosensitiser to be transported by the blood it must be hydrophilic. However, for the photosensitiser to reach the affected cells, it must also be hydrophobic so that it can pass easily through the various cell membranes. Hence a good photosensitiser must posses sufficient hydrophilicity to be transported easily through the blood and enough hydrophobicity to cross the various membranes.

1.6.1.2 Excretion and elimination

If the photosensitiser is too hydrophobic, it will not be excreted from the body and will persist in the tissues. For excretion and elimination, it must be hydrophilic. Studies have shown that hydrophobic photosenitisers take a long time to be excreted out of the body.[74] Consequently, there is a high risk for the patient since a long period is needed for the patient to be in the dark so as to prevent general photosensitivity, while the drug is being cleared from the body. Ideally the photosensitiser needs to possess equal hydrophilicity and hydrophobicity since it needs to pass through both hydrophilic and hydrophobic environments before and after reaching the target cells.

1.6.2 Activity determinant

1.6.2.1 Tumour tissue accumulation

If the photosensitiser is too hydrophilic, much of it will not reach the tumour cells leaving many affected cells having no photosensitiser or with only very low concentrations thereof. This leads to incomplete death of tumour cells since most of the photosensitiser passes out of the body before reaching the target cells, resulting in a small amount of the

drug that accumulates in tumour tissue. Much more of the photosensitiser will accumulate in tumour tissue if it is hydrophobic.

1.6.2.2 Bathocromic electronic spectral shifts

This is the guiding principle that provides chemists with the most difficult problem since they need to ensure that the drug chromophore has the desired long-wavelength of absorption. A red shift or bathocromic effect, is a shift of an absorption maximum towards longer wavelength. It may be produced by the change in the medium or by a substituent in the chromophore which leads to the red shift.[91] Hydrophilic environments tend to cause red shifts.

Red shift has been achieved either by expanding the macrocycle to produce phthalocyanines, texaphyrins, peripherally chromophore functionalised porphyrazines, porphyrin vinylogues etc; or by reducing one or more of the porphyrin's pyrrole rings to give chlorins. Another way of red-shifting and intensifying the long wavelength absorption band of the photosensitiser is to extend the macrocycle conjugation, e.g. with ethynyl groups. The net effect is to give these compounds a green colour, compared to the usual purple/red colour.

These strategies cause a red shift and an increase in the intensity of the long wavelength absorption of the chromophore. In the red region there is the deepest tissue penetration of light, enabling the light to reach even the deepest cells. Since the tumours can be quite deep, red absorption of the photosensitiser is therefore needed for the light to effect maximum damage to cancerous tissue.

1.6.2.3 Singlet oxygen yields

Since singlet oxygen is important in cell destruction by PDT, substantial amounts are needed to maximise tumour cell destruction. The more singlet oxygen produced, the more tumour cells will be destroyed. If the photosensitiser is hydrophilic, much of it will reach the tumour cells resulting in increased production of singlet oxygen. Furthermore, the higher the hydrophilicity of the photosensitiser, the more it will be absorbed in hydroxylic environments where there is a higher concentration of triplet oxygen. Thus there will be an increase in the contact between the photosensitiser and triplet ground oxygen.

Singlet oxygen yield is related to triplet quantum yield of the sensitiser, the lifetime of the triplet quantum yield, the efficiency of energy transfer from the excited triplet state to the ground state oxygen and the ability of the substituents to quench singlet oxygen. It is expected that the higher the triplet quantum yield with efficient energy transfer, the higher the singlet oxygen yield. If there is inefficient quenching of the triplet state by the ground state oxygen, less singlet excited oxygen is formed. Also the longer the lifetime of the triplet state, the higher the amount of singlet oxygen that might be produced since there will be less of the triplet excited state which might be converted back to the ground state. The ground state oxygen to be excited must be sufficient enough in order to have large amount of singlet oxygen produced.[92-94]

1.7 Development of photosensitisers

Haematoporphyrin derivative and photofrin, both complex mixtures of compounds represent the first-generation of photosensitisers. Second-generation photosensitisers on the other hand are pure single compounds that exhibit photosensitisation properties and they include a number of porphyrins and phthalocyanine derivatives. During the early years in the development of photosensitisers for PDT, certain design criteria for a good photosensitiser became apparent.[11,13,95] These can be summarised as follows:

- Lack of toxicity in the dark.

- Selective uptake by tumour tissue.
- Short-term retention to avoid the persistence of photosensitivity.
- Triplet energy greater than 94 kJ/mol.
- Intermediate lipid water partition coefficient.
- Single substance.
- Light absorption in the red or far red range of the visible spectrum.

The substances that meet these criteria include suitably modified porphyrins,[6a] chlorins,[95] porphyrazines[96-100] and phthalocyanines.[6a] So far, many photosensitisers have been synthesised and tested, some of them show little or no activity in photonecrosis, sometimes poisonous.[14] But these efforts have not been wasted, for they have provided the basis for the above design criteria and will continue to do so.

1.7.1 Porphyrin derivatives

Porphyrins were among the first group of compounds to be used as photodynamic therapy agents.[11] Many of the second generation porphyrins are now in clinical trials for different tumour lesions in different locations. They have improved tumour cell concentration compared to normal cells and also have improved fluid solubility and deep tissue penetration.[80,82]

1.7.2 Porphyrazines

Porphyrazine derivatives are showing to be potential candidates as photosensitisers in photodynamic therapy. Recent photophysical studies of some zinc metalated peripherally substituted porphyrazines have shown high triplet quantum yields which are coupled with high oxygen quantum yields. They also absorb strongly in the red region which also

makes them capable of deep tissue penetration and their biomedical application will soon be elaborated.[96-100]

1.7.3 Phthalocyanines

Phthalocyanines and their derivatives have shown great promise in PDT.[101] They are porphyrin-like second-generation sensitisers for photodynamic therapy.[95,101] The most important attribute that makes phthalocyanines suitable for PDT is the intense absorption in the far red and long-lived excited triplet state.

1.8 Drug development in PDT

Drug development in PDT is a much more difficult process than is the case with conventional chemotherapy. Partly because PDT actually requires both a drug and a device, the device being a light source. This two-part procedure makes it possible to avoid the toxicity generally associated with drug based therapy, since only the light-activated sensitiser will yield a cytotoxic result. However, it becomes necessary to consider several new variables, namely the time between sensitiser administration and irradiation, avoidance of skin and eye photodamage, long-term dark toxicity, and appropriate wavelength of irradiation.[7]

During the past few years, a large number of new sensitisers have been suggested for clinical use, on the basis of experimental studies in animal or in cell cultures.[6a] There are several uncertainties and considerable expense related to bringing new agents to the clinic, and these account for the slow incorporation of new sensitisers into medical practice. While adverse reactions are often considered to be of less than major importance in conventional drug therapy of life-threatening disease, PDT will be applied generally to early lesions with curative intent. [7] The occurrence of unexpected toxicity could seriously impair future trials.

A major stumbling block to the use of PDT in the removal of tumour cells is the lack of suitable photosensitisers that can accumulate selectively to the tumour cells and destroy them leaving normal cells unaffected after illumination with light. Although

photosensitisers that can accumulate selectively to the tumour cells have been discovered, their major limitation is that they are activated with wavelengths of light that are not capable of penetrating deeply through tissues or blood.

Other important limitations of current photosensitisers include the difficulty in the functionalisation and purification of the functionalised compounds. Photosensitisers are generally water insoluble, but they can be made to be water-soluble by functionalisation with hydrophilic groups. Clearance from the body is also one of the most important limitations in the use of photosensitisers. Many that have been synthesised are cleared very slowly from the body and therefore cause skin phototoxicity when the patient comes in contact with light. Delayed clearance results in the patient being forced to stay in the dark for long periods of time to give a sufficient period for the photosensitiser to clear from the normal tissue.

PDT is advantageous over standard therapy since it is not painful and is non-invasive. PDT also has the ability to treat multiple lesions at one sitting, good patient acceptance, excellent cosmetic results and apparent lack of major side effects. Acute effects caused by singlet oxygen include severe damage to various cell membranes including plasma, mitochondrial, lysosomal, endoplasmic reticulum, and nuclear membranes; inhibition of enzymes, including mitochondrial, DNA repair and lysosomal enzymes; and inactivation of membrane transport systems. The diseased tissue in which the photosensitiser is localised should preferable be destroyed with either little or no damage to surrounding normal tissue.

Cancer consists of large group of diseases resulting from uncontrolled proliferation of cells. These diseases can originate in almost any tissue of the body. Symptoms and signs of cancer depend on many factors including the location of the tumours, rate of growth, tendency to invade and divide, and some other individual conditions of patients. The most common cancers originate and spread within the chest, abdomen, or pelvis. These sites are difficult to treat because of the close positioning of many overlapping organs. Continued cell division leads to the formation of tumours that invade normal tissues and

organs. In many cases, the cancer cells become dislodged and spread to other locations within the body where they then take up residence and grow, forming secondary tumours. In these situations, the cancer is frequently advanced and not curable by current approaches.

PDT has been used experimentally to treat many types of cancers, cutaneous viral infections, psoriasis and certain diseases of the retina. The selectivity of certain compounds in hyperproliferative tissue,[102] added to the observation that photodynamic therapy can lead to blood vessel stasis, suggested that PDT might be effective in non-tumour diseases. Some of the diseases characterised by tissue hyperproliferation/or neovascularisation, which are being considered for PDT, are menorrhagia, choroidal neovascularisation, cutaneous haemangiomas, benign prostatic hyperplasia and rheumatoid arthritis. These applications of non-tumour PDT, as well as the treatment of various bacterial and fungal conditions, blood sterilisation (PDT kills bacteria and viruses) prior to transfusion, certain viral induced pathologies, immune modulation and bone narrow purging are now under investigation.[102]

1.9 Current perspectives in drug development

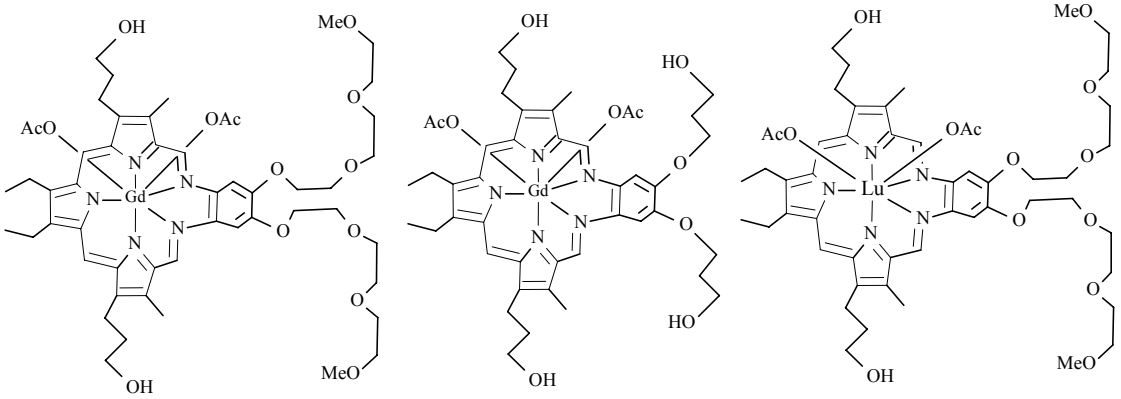
Several new drugs, with a number of promising features for PDT, have come under scrutiny, as pharmaceutical companies enter the race for the development and synthesis of PDT photosensitisers.

1.9.1 Texaphyrins

Texaphyrins are ring-shaped, planar, expanded porphyrin molecules with large metal cations which are commercially available through Pharmacyclics.[6c,103-104] They have been

synthesised with the hope that they can perform specific functions in a number of medical applications and have been aiding to enhance imaging for MRI for many years. Like other porphyrins, texaphyrins accumulate in those parts of the body with high energy usage, such as in cancer cells and are thus useful in the treatment of cancer and other atherosclerotic cardiovascular diseases.[104,106] Texaphyrins have the ability to bind large metal ions, to allow the metal to interact freely with adjacent molecules while still being retained within the texaphyrin structure.

Most texaphyrins contain the paramagnetic metal ion gadolinium.[6c] In the case of porphyrins, this capability is limited only to few smaller metals and in any case, once metalated, porphyrins lose the electronic capabilities required in PDT. Because of their binding effect with metals, texaphyrins can be engineered to mediate a number of biochemical reactions. Pre-clinical testing has shown that there is a 5:1 to 15:1 ratio of texaphyrins in cancerous lesions relative to the surrounding normal tissue. Two of the texaphyrin derivatives which are clinical trials are Lu-Tex and Gd-Tex.[64,80] Since they act like porphyrins in selectively targeting tumour cells, they have the potential to enhance the effects of radiation in the tumour cells and not the surrounding normal tissue.[107,108] Texaphyrins are soluble in water, which makes it safe for them to be used in PDT and simple to be administered to patients, therapy reducing potential toxicity.



57 **58** **59**

Figure 1.10: Texaphyrins

They all possesses a strong broad absorption band centered at 730-732 nm [109] allowing deep light penetration for up to 3-7 mm in thickness and Lu-Tex fluoresces at 750 nm which could allow improved localisation and dosimetry of PDT.[103] Animal studies in which Lu-Tex was injected into tumour-bearing animals demonstrated rapid clearance of the drug from blood and normal tissue, with delayed clearance from tumours, which resulted in up to 8-fold greater concentrations in tumours compared with surrounding tissues.[110] Gd-Tex and other texaphyrins are in clinical trials to evaluate their safety in cancer patients receiving radiation therapy.

1.9.2 BPD verteporfin

BPD verteporfin (benzo-porphyrin-derivative monoacid ring) derived from animal haemoglobin when formulated with liposomes,[64,6c] has shown promising results for use in PDT. Verteporfin (Figure 1.11) asbsorbs at a longer wavelength (690 nm), with about 50% more light tissue penetration compared to Photofrin which absorbs at 630 nm. In addition, verteporfin is rapidly absorbed by the tumour and cleared from the body with minimal skin photosensitivity, within few days.[64,6c]

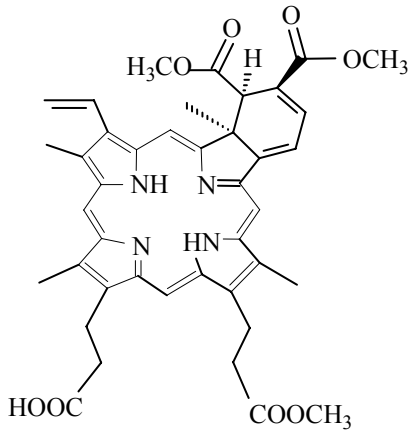


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Figure 1.11: BPDMA, verteporfin

1.9.3 Temoporfin

Temoporfin [*meso*-tetra(*meta*-hydroxyphenyl)chlorin)], which is marketed under the trade name of Foscan^{TM} and is traded by Scotia Pharmaceuticals (UK) as a second generation photosensitiser, is a chlorin member family with interesting clinical characteristics. Although it has some significant drawbacks (patients experience pain during treatment), it offers excellent clinical control of a wide variety of cutaneous lesions, pulmonary, esophageal and especially head and neck tumors in patients considered to be incurable using surgery or radiotherapy. The drug (Figure 1.12) is efficient and affords a short treatment time. Since it activates at 660 nm it allows deeper tissue penetration.[13,6c] Temoporfin [*meso*-tetra(*meta*-hydroxyphenyl)chlorin)] is also in clinical trials in USA and Canada, and is expected to be in the market soon.

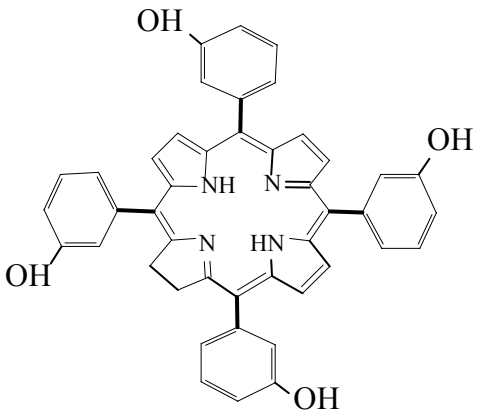

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Figure 1.12: *Meso*-tetra(*meta*-hydroxyphenyl)chlorin

1.9.4 Photofrin

Photofrin (Figure 1.13) is in Phase III clinical trials and has received approval in Canada for use with bladder carcinoma. Photofrin has been shown in other countries to be useful for the treatment of esophageal and lung cancer.[13,6c]

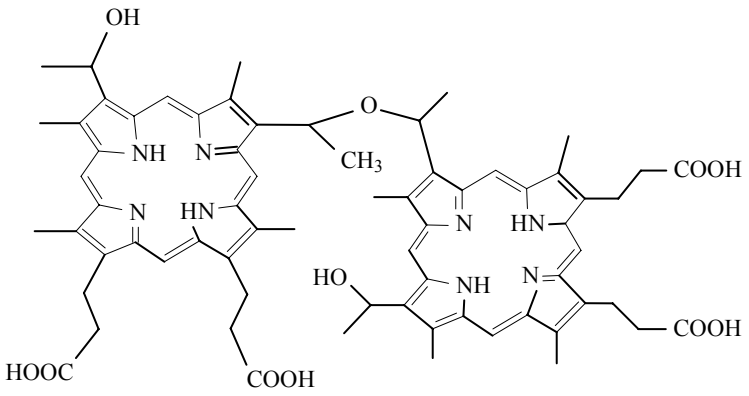

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Figure 1.13: Hematoporphyrin Derivative (Photofrin)

Photofrin has been approved in the U.S.A for treatment of oesophageal cancers, early stage lung cancer and other malignancies.[6c]

1.9.5 5-Aminolaevulinic acid (ALA)

Currently available from DUSA Pharmaceuticals, Inc., 5-aminolaevulinic acid has been shown to be capable of producing reliable photosensitisation when administered orally. ALA, which is a prodrug,[13,6c] is not a photosensitiser but on clinical trials it has shown a promising effect in PDT because upon application, it leads to the biochemical formation and preferential accumulation of protoporphyrin in tumour tissue.

The advantage of ALA (Figure 1.14) compared to other photosensitisers is that it is present in human cells and is a metabolic precursor of the endogenously formed photosensitiser, protoporphyrin IX (PPIX) which is capable of tumour cell destruction.[111] Its synthesis is normally tightly controlled by feedback inhibition of ALA synthetase, presumably by intracellular haem levels. Since it can be synthesised in cells, little or no undesirable side effects are expected in its use as a photosensitier.

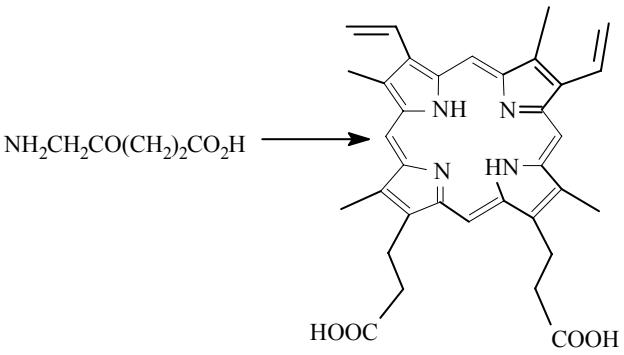

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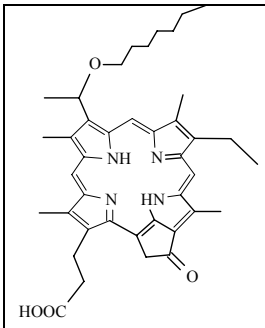
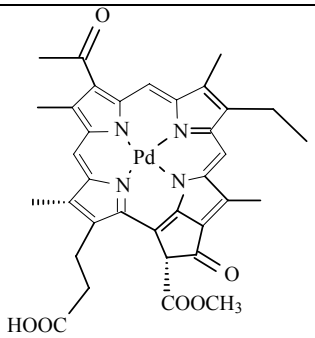
Figure 1.14

Only burning discomfort during light exposure and tolerable stinging are experienced by patients in the clinic. The drug is activated by light at a wavelength of 630 nm, giving sufficient depth of penetration of light for shallow tumours.[6c] Even though there are some promising results obtained with ALA modulated PDT, some significant limitations of its application have been observed, and further improvements are essential.

1.9.6 Other promising photosensitisers.[13,82]

 **64** *Pyropheophorbide-a hexyl ether-HPPH*	 **65** *Palladium bacterio3chlorin-Tookad*

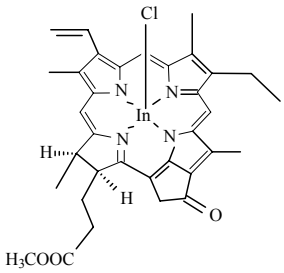
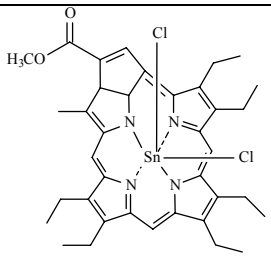
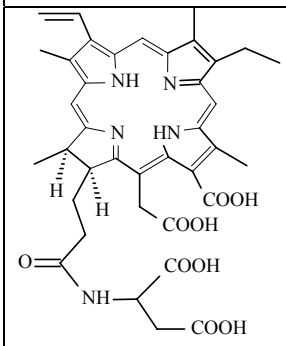
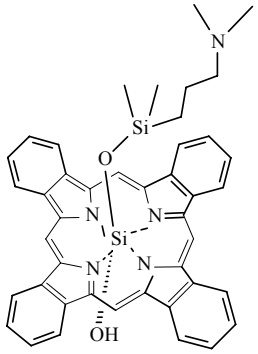
 **66** *Indium pyropheophorbide*	 **67** *Tin etiopurpurin-SnEt2, Purlytin*
 **68** *Monoaspartyl chlorin (e6)-LS11, Npe6*	 **69** *Silicon phthalocyanine-PC4*

Table 1.1

Table 1.1 shows some of the second generation photosensitisers which are promising as photodynamic agents. They are all at different stages of clinical trials.

1.10 Conclusion and objectives

Several new second-generation photosensitisers are now in clinical trials and are showing promising results since their treatment times, typical light-and drug doses are smaller than for HpD. Viewed against Photofrin, the new second-generation sensitisers, plus ALA-PDT, are beginning to prove their worth. An important factor with new sensitisers is their ability to enhance selectivity of tumour damage at lower doses of the sensitiser. Whether photosensitivity is an important issue depends on the treatment. In treatment of

lethal tumours, it doesn't matter how photosensitive patients become: the priority is to ensure maximum tumour destruction.

Today, only a purified form of hematoporphyrin derivative is in therapy, but is not efficient enough for tumour treatment, however, it can be used to treat tumour cells at an early stage. The main disadvantage of Photofrin is its unknown composition for it is still composed of a mixture of porphyrins. Therefore, the synthesis of photosensitisers in which there is certainly regarding the structure and purity thereof is of great importance for the development of new photosensitisers. One of the requirements of new photosensitisers is water solubility, while selective uptake of the photosensitier by the tumour cells versus healthy tissue is also important. This requires that photosensitiser should be soluble in body fluid and be more concentrated in tumour cells.[13,82]

Although some second generation photosensitisers are water soluble and are less toxic, there is still some problem with selective uptake of the photosensitiser by the tumour cells compared to normal cells.[82] As a potential solution, synthesis of carbohydrate substituted porphyrazines is of great interest for cellular recognition and water solubility due to the specific affinity of carbohydrates to energy hungry cells.

A specific objective of this study was to synthesise porphyrazines with carbohydrates as peripheral substituents. This will be addressed by the development of technology in which carbohydrate-functionalised porphyrazines will be produced from the corresponding carbohydrate-substituted phthalonitriles. The rationale behind this use of carbohydrates is two-fold. Firstly, being polar moieties, it is anticipated that the presence of the carbohydrate groups will enhance the water solubility of the porphyrazine. Secondly, depending on the nature of the carbohydrate, this moiety may assist in active uptake of the porphyrazine by cancerous tissue, either by molecular recognition or by, in example, glucose pumps.

Another objective of this study is that deprotection of the synthesised porphyrazines is to be performed. This deprotection will be performed at the site of the carbohydrate moiety, which is to contain removable protective groups. Furthermore, metalation studies of the

free-base pigments is to be addressed. The influence of the carbohydrate substitution (protected and deprotected) on the extraction coefficient will be investigated.

Photophysical studies on the porphyrazines produced form the final part of this study.

The objectives of this study can therefore be listed as follows:

- To synthesise chloro- and nitrophthalonitrile derivatives
- To synthesise glycosyl donors
- To couple the chloro- and nitrophthalonitrile derivatives with monosaccharides and the synthesised disaccharides
- To demetalate the porphyrazine macrocycles
- To incorporate metals (Zn, Ni) into the free-base porphyrazine pigments
- To determine extraction coefficients of carbohydrate-substituted porphyrazines and non carbohydrate-substituted porphyrazines
- To undertake photophysiochemical studies of the free-base and zinc metalated porphyrazines

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